THE EFFECTS OF CORTICOSTERONE ON PERSISTENCE OF ATTENTION IN *MUS MUSCULUS*

By

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When I survey the wondrous cross on which the
Prince of Glory died,
My richest gain I count but loss,
And pour contempt on all my pride.

Were the whole realm of nature mine,
That were an offering far too small,
Love so amazing, So divine,
Demands my soul, my life, my all.

Isaac Watts.

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ABSTRACT

It is widely believed that corticosterone plays a role in psychological, and ultimately behavioural adaptation during stress. The aim of this thesis was to examine the nature of the adaptation brought about by corticosterone.

The hypothesis under test was that corticosterone enhances the persistence of attention. This was primarily suggested by the known actions of the hippocampus - which is the site of the major concentration of the receptors for corticosterone in the brain. The experimental strategy was to examine the effects of exogenous corticosterone on various facets of behaviour believed to relate to the persistence of attention. Both adrenally intact and adrenalectomised animals were used.

In the runway distractability test no effect of corticosterone was detectable, either to a relevant or to an irrelevant stimulus, or to two stimuli presented in succession. Neither did corticosterone produce an effect on the habituation of a response to a novel object or to a hole-poke response. In discrimination problems, corticosterone impaired shifting, regardless of whether the problem was a reversal or a non-reversal shift. Passive avoidance was also impaired, but only as assessed by the comparison between delayed and immediate testing under high doses of corticosterone. Active avoidance responding correlated positively with plasma corticosterone levels, but also only under certain circumstances.

Overall these results are inconclusive, giving only very limited support to the working hypothesis.

CONTENTS

CHAPTER 1

Introduction

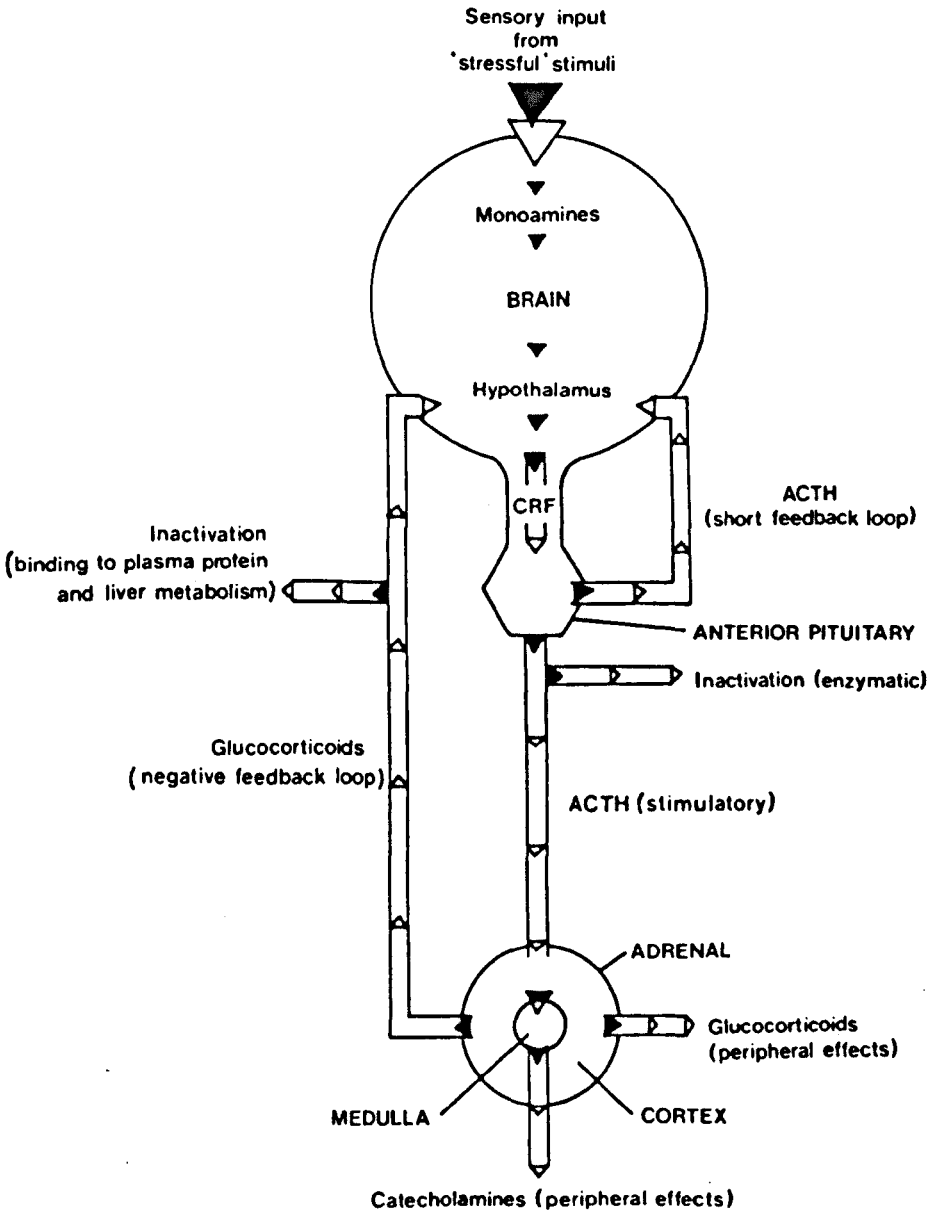
According to the classical concept of hormone functioning hormones act via receptors on peripheral targets to bring about intracellular changes primarily associated with metabolism. The presence of receptors for hormones in the brain suggests that, in addition to these peripheral effects, hormones may act directly on the brain to elicit the so called 'central effects'. This refers to changes in neuroendocrine and/or behavioural adaptation, arising as a result of the effects of hormones on the functioning of the nervous system.

The project reported in this thesis was designed to elucidate the significance of the presence of receptors for the hormone corticosterone (CORT), in the brain for psychological, and therefore ultimately behavioural adaptation under conditions of stress.

Stress is a descriptive term applied to the response accompanying any environmental event that is perceived as at least potentially adverse in nature. The stress response is evoked upon exposure to a stressor, defined as "any anti-homeostatic stimulus" (Sourkes, 1983), in order to re-establish a homeostatic state. The capacity to respond to a stressor is recognised as one of the most basic adaptive mechanisms possessed by animals.

It is important to acknowledge from the outset that when an animal is exposed to an environmental challenge, not one, but several hormonal, neuropeptidergic and neurotransmitter systems are activated (Henry et al., 1976; Ursin, 1982; Sourkes, 1985; Glavin, 1985), and that different stressors act by different pathways (Sourkes, 1983; Odio and Maickel, 1985). Despite the fact that there is no single nonspecific response evoked by all stressful stimuli, a major component of the physiological response to stress is the activation of the hypothalamo-pituitary-adrenal system

Figure 1:1 The dynamics of the hypothalamo - pituitary - adrenal system (after Brain, 1972 a)



(H-PAS). The dynamics of this system are well documented (e.g., Yates et al., 1971), but as a clear understanding of its inter-relationships is essential for the later discussion of the behavioural actions of CORT, they will be briefly reviewed (see Figure 1:1).

The stress response and the dynamics of the hypothalamo-pituitary-adrenal system

As a consequence of the presence of a stressor neural impulses reach neurosecretory cells in the hypothalamus, causing the release of corticotropic releasing factor (CRF), which is transported via the hypophyseal portal system to the anterior pituitary. Here it stimulates the release of adrenocorticotropic hormone (ACTH) (Ganong, 1974). The secretion of both CRF and ACTH is influenced by a number of different neural pathways (Redgate et al., 1973; Sourkes, 1983) and neurotransmitter systems, especially noradrenalin (NA) and serotonin (5-HT) (Kvetnansky et al., 1976; Scapagnini and Nistico, 1978; Weiner and Ganong, 1978; Maickel and Martel, 1983; Glavin, 1985; Kile and Turner, 1985). Stress-induced elevations of CRF and ACTH occur very rapidly: a detectable increase in ACTH levels has been observed within 60-120 secs following stress in rats (Vernikos-Danellis and Heybach, 1980).

ACTH released from the anterior pituitary into the systemic circulation acts upon the adrenal gland which comprises two distinct zones, an inner medulla surrounded by an outer cortex. The latter is composed of 3 cell types arranged in 3 layers: an outer zona glomerulosa - which secretes the mineralocorticoid aldosterone (ALDO); an intermediate zona fasiculata - which secretes glucocorticoids; and an inner zona reticularis - which secretes androgenic steroids. Although mineralocorticoid secretion is slightly affected (Veldhuis, 1982), as is the release of adrenal androgens (Kime et al., 1980), the primary action of ACTH is to stimulate the secretion of glucocorticoids. (In rodents the principal glucocorticoid is

CORT, though in most other mammals it is cortisol).

The adrenal glucocorticoids exert an inhibitory control on the activity of the H-PAS, both under basal and stress activated conditions, by a negative feedback action (review - Keller-Wood and Dallman, 1984). The presently available evidence indicates that the pituitary and hypothalamus are the primary sites of this feedback, two modes of which have been defined. One type is referred to as the rate-sensitive, fast feedback system, and is thought to occur via the inhibition of CRF release at the neurosecretory cells in the hypothalamus. The other type - the so called level-sensitive, delayed feedback system, develops over a relatively longer period of time, and principally involves the blockade of ACTH release from the anterior pituitary; though the release and synthesis of hypothalamic CRF may also be suppressed (Buckingham and Hodges, 1974; Jones et al., 1977; Smelik and Vermes, 1980). Interestingly, synthetic glucocorticoids, such as dexamethasone (DEXA) are more potent suppressors of ACTH release than CORT because of the presence of molecules similar to corticosteroid-binding globulin (CBG) in the pituitary which selectively bind CORT and diminish its efficacy. Presumably, the CBG-like molecules act as a buffering system, ensuring that the pituitary only responds to CORT under conditions of stress. In this context, it is worthwhile noting that the brain does not possess CBG-like molecules. Consequently it may be more responsive to smaller changes in CORT (de Kloet and Veldhuis, 1985).

Whilst the hypothalamus and the pituitary are recognised as the principal sites for the feedback action of CORT it is thought that it can also exert regulatory effects via extrahypothalamic sites, most importantly, the hippocampus (HPC). Since this brain area is also implicated in the behavioural effects of CORT, a point which will be elaborated upon later, its possible involvement in the feedback regulation of the H-PAS deserves some consideration as this raises questions about the degree to which these two functions of the hippocampal CORT receptors are

interrelated.

Although the significance of the HPC in the regulation of the H-PAS is far from certain (Wilson, 1985) any modulatory effect would presumably be through the efferent projections from this neural substrate to the hypothalamus. In support of this, Feldman and Conforti (1980) showed that the suppressive effect of glucocorticoids on H-PAS activity was lacking in dorsally hippocampectomised rats. (Whilst Bohus (1975), agrees in principle with this finding, he claims it is restricted to the ventral, rather than the dorsal HPC). In general, the HPC is considered to have an inhibitory effect. Thus, electrical stimulation of the HPC inhibits the activation of the H-PAS in response to noxious stimuli (Bohus, 1975), and consistent with this is Rotsztejn et al.'s (1975) demonstration of an inverse correlation between the level of CORT binding at the HPC and plasma CORT levels. From an ontogenetical perspective, Sapolsky et al. (1985b) argue that the parallel increases in the number of receptors for CORT in the limbic system and the sensitivity of the H-PAS to this adrenocorticoid during early postnatal life points to the importance of the HPC. Furthermore, the fact that animals with a low hippocampal CORT - receptor capacity are less sensitive to feedback inhibition by glucocorticoids reinforces this (Landfield et al., 1982; Sapolsky et al., 1983a; Sapolsky et al., 1983b; Sapolsky et al., 1985a). However, the picture is complicated by the finding that the effects of hippocampal stimulation depend on its context (Kawakami et al., 1968; Conforti and Feldman, 1976; Osborne et al., 1979) not to mention its intensity and duration (Casady and Taylor, 1976). Moreover, there is diurnal variation in the sensitivity of the H-PAS to hippocampal influences.

Turning aside from the regulatory effects of CORT, the magnitude of response of the H-PAS to a stressor also depends on the circulating levels of mineralocorticoids, which influence the release of ACTH from the pituitary, albeit to a lesser degree than CORT (Veldhuis, 1982). ACTH is

also believed to exert a short auto-regulatory effect on the hypothalamus (Davidson et al., 1968).

In conclusion, the circulating levels of CORT act upon the mechanisms that modulate the release of hypothalamic CRF and/or ACTH from the pituitary and thereby contribute to the regulation of the activity of the H-PAS; though the activity of this endocrine axis is a complex function of all the inhibitory and excitatory influences converging on the hypothalamic CRF cells and the magnitude of the CORT feedback signal. A final point to note is that the activity of the whole system is subject to circadian variation: in nocturnal rodents activity is low in the morning and high in the evening (Nichols and Chevins, 1981). Interestingly, it has been suggested that this circadian rhythmicity interacts with the degree of stress responsiveness in such a way that the magnitude of the response of ACTH to a stressor is depressed at the peak of circadian activity (Ader and Friedman, 1968; Nicholson et al., 1985).

Although this discussion has concentrated on the changes in H-PAS hormones in response to stress, stressors also alter the secretion of many other hormones, including growth hormone, prolactin and adrenal-medullary hormones (Mason, 1968; Brown and Martin, 1974). Furthermore, it is known that exposure to a stressor or CRF not only results in the release of ACTH but also the release of peptides related to ACTH, such as βLPH, MSH, βMSH, and β endorphin, that are derived from the precursor molecule pro-opiomelanocortin (Guillemin et al., 1977; Pelletier et al., 1977; Vale et al., 1981; de Wied and Jolles, 1982; Chretien and Seidah, 1984). For this thesis however the most relevant effect of stress is the release of the glucocorticoid CORT.

The physiological effects of corticosterone

Corticosterone exerts a number of well documented metabolic effects, which are primarily catabolic in nature. In many tissues (notable

exceptions being the brain, heart and red blood cells) it inhibits the uptake of glucose, depresses macromolecular synthesis and enhances gluconeogenesis (de Kloet and Veldhuis, 1985). Furthermore, it has immunosuppressive and antiinflammatory effects (Munck et al., 1984); all of which will have profound physiological consequences for the animal in the adaptation to a stressful stimulus. The traditional view (Selye, 1950) claims that CORT increases the resistance to stress by enhancing defence mechanisms; however, this is clearly incompatible with the immunosuppressive and antiinflammative actions of this adrenocorticoid. Munck et al. (1984) reconcile this paradox by hypothesising that CORT protects the animal against stress by suppressing the primary defence mechanisms that are activated under stress, rather than by protecting the animal against the stressor per se. Thus; as a result of the temporal sequence of the systems responding to stress the influence of CORT is sufficiently delayed in relation to the initial stress respondents to allow these primary defence mechanisms to exert their respective actions (DiGiusto et al., 1971) and the subsequent release of CORT is then well-timed to initiate a process of recovery. According to Munck et al. (1984) this is required because the continued activity of the primary stress respondents would eventually lead to deleterious effects, thereby posing a further threat to homeostasis. Munck et al. (1984) also make the point that since detrimental consequences could result from prolonged exposure to elevated glucocorticoid levels, the adrenocortical response must, itself, be limited and that this is achieved through the negative feedback actions of CORT on H-PAS activity.

The psychological effects of corticosterone

Whilst this discussion has focussed on the physiological significance of glucocorticoids in stress adaptation, this thesis is concerned with the relation between CORT and psychological and behavioural adaptation. This,

however, encompasses two aspects. Firstly, there is the psychological components of a stressor and the implications of this for the level of PAS activity. Secondly, there is the effects of this stress respondent on the psychological state. This discussion will deal with each of these in turn.

Historically, emphasis was initially placed on the release of hormones from the PAS in response to a variety of stimuli which were mainly physical in nature, such as heat or anoxia (Selye, 1950) and it was not until 1968 that the significance of psychological stimuli, such as anxiety, fear and anger (Mason, 1968) was recognised. The demonstration that a supposed stressor fails to elicit an adrenocortical response if the emotional arousal normally associated with it is avoided (Mason, 1971) not only verified the significance of the psychological concomitants of a physiological stimulus in the stress response, but highlighted the fact that psychological stimuli are probably the most potent in affecting the responsiveness of the PAS. The latter is further attested to by the effectiveness of stimuli eliciting hope or disappointment in stimulating the PAS (Levine et al., 1972).

Whilst the significance of the response of the PAS to physical stressors has, at least to some extent, been clarified, the relevance of this response to social and psychological stimuli has proved more elusive. In point of fact, the biological significance of the corticoid response to psychological stimuli that do not signal physical challenge is the subject of fervent debate. It is often concluded that this response is merely a vestige of the response that serves in real conflict. Smith (1973) disputes this, arguing that the increased levels of ACTH and the adrenocorticoids in response to psychological stimuli function to optimise information processing during a stressful experience, and thereby facilitate behavioural adaptation. This is supported by Yates and Maran (1974) who justify their stance by pointing out that it is difficult to envisage a real role for the peripheral metabolic effects of adrenocorticoids under

conditions of psychological stress. The realisation that the stress response is not just a physiological response to physical stressors, but a response resulting from psychological interactions with the physical environment, and that the response evoked by stressors has psychological, as well as physiological consequences, has been very important.

The behavioural effects of corticosterone

Consistent with the psychological functions attributed to the PAS is the reported behavioural activity of the hormones from this endocrine axis. Although substantial evidence has accumulated indicating that both ACTH and the adrenocorticoids affect behaviour, it is often conflictory in nature and/or difficult to interpret unequivocally. In part, this results from the dynamic interrelationships within the PAS, which make it virtually impossible to hold the system constant, whilst manipulating one part of it alone, which inevitably confounds any attempt to conclusively identify the active agent in any behavioural change (DiGiusto et al., 1971; Brain, 1972a) (This can be more fully appreciated if it is considered that the behavioural changes associated with adrenalectomy may be due either to elevated ACTH levels or to lowered CORT levels).

Combined with this, CORT and ACTH tend to exert antagonistic behavioural effects (de Wied, 1967; Bohus and de Kloet, 1979), though admittedly there are exceptions to this generalisation (Leshner et al., 1981; Beckwith et al., 1983). Furthermore, there is the problem of the interactive effects of ACTH and CORT on behaviour. Bohus et al. (1982) demonstrated that the influence of CORT on exploratory behaviour depends on the relative level of ACTH, pointing to the need to be wary of a simplistic approach toward behavioural endocrinology. Yet another problem stems from the properties of the CORT receptor system in the brain. Although this subject will be dealt with in more detail later, at this point it is necessary to be aware that the behavioural effects of CORT often depend on

whether it is given to intact or adrenalectomised animals. This is because, for certain aspects of behaviour, the number of receptors available to mediate the effects of changes in the level of CORT is severely limited, and as a result of the presence of the endogenous hormone in intact animals, the likelihood of a CORT-induced behavioural change being expressed is reduced. Furthermore, the interval between the removal of the adrenals and behavioural testing is often very influential, primarily because the use of chronically adrenalectomised animals is complicated by a variety of changes resulting from the prolonged absence of CORT.

Despite these complications CORT has been shown to possess intrinsic behavioural activity, as suggested by the finding that the magnitude of its effect on a conditioned avoidance paradigm does not correlate with the suppression rate of ACTH (Bohus, 1973); combined with which it is behaviourally active in hypophysectomised animals (de Wied, 1967). Furthermore, the implantation of this adrenocorticoid in the brain, in particular the limbic midbrain, does not influence pituitary ACTH release, yet the behavioural effects resemble those found upon systemic administration of CORT (Bohus, 1968; 1971; 1970).

Bearing in mind the cautionary notes that have been sounded above, the behavioural effects of CORT can be presented, though the following is by no means an exhaustive summary. In view of the importance of the adrenal status of the animal, studies using intact animals will be distinguished from those using adrenalectomised ones:

i Effects of corticosterone on mood and affective state

One of the first indications that CORT might affect behaviour arose from the clinical observation that alterations in glucocorticoid levels, caused by adrenal insufficiency (Addison's disease) or excess (Cushing's syndrome), were associated with changes in mood (Von Zerssen, 1976). Subsequently, changes in the level of CORT have been correlated with affective disorders such as depression (Perini et al., 1984; Rubinow et

al., 1984) and schizophrenia (Oades, 1982). The relationship between CORT and these psychiatric disorders is at present far from understood.

ii Effects of corticosterone on sensory sensitivity

Clinical studies also revealed that both hypo- and hyper-secretion of adrenocortical hormones disrupt sensory processes (Henkin and Barterr, 1966; Henkin et al., 1967; Henkin, 1970a; 1970b). Addison's patients exhibit lowered detection but raised recognition thresholds in a number of sensory modalities, and CORT therapy normalises both. Conversely, Cushing's patients exhibit raised detection and in general lowered recognition thresholds; though the relationship between excessively high glucocorticoid levels and sensory sensitivity is not so clear cut. Cushing's patients may have raised thresholds for both detection and recognition.

Despite this, circadian variations in CORT levels parallel changes in sensory sensitivity, which further points to the role of adrenocorticoids in sensory processes. Sakellaris's (1972) demonstration that adrenalectomy lowers the olfactory threshold, whereas CORT returns this threshold to normal in rats, extends the generality of this finding.

iii Effects of corticosterone on sleep

Glucocorticoids have been shown to influence the occurrence of paradoxical sleep in normal human subjects (Gillin et al., 1972). Similarly, in the rat, adrenalectomy attenuates the circadian variation in paradoxical sleep, as reflected by HPC theta activity: CORT replacement reestablishes this (Johnson and Sawyer, 1971).

iv Effect of corticosterone on feeding and drinking

Recently Yukimara et al., (1978) reported that removal of the adrenals reduces food intake with a corresponding reduction in weight gain; the administration of CORT reverses these effects. However, this study used genetically obese (Zucker) rats, which may have an enhanced responsiveness to CORT. In general the effects of CORT on food and water intake are conflicting (see Rees and Gray, 1984).

v Effect of corticosterone on motor activity

As the circadian rhythm of CORT secretion corresponds with that of locomotor activity, by implication CORT might be expected to influence the level of activity. Despite this, its effects depend on the type of activity measured, and even then they are often inconsistent.

Thus, adrenalectomy decreases running wheel activity and CORT replacement reinstates it. Surprisingly, CORT also decreases running wheel activity in intact rats (Leshner, 1971).

By contrast, adrenalectomy increases general activity levels, as measured by gross body movements (Katz and Carroll, 1978).

Corticosterone also affects activity in an open-field, elevating the levels in intact animals. (Despite this, the plasma CORT levels do not correlate reliably with open field behaviour, Ader et al., 1967; Brain and Nowell, 1969a; Stern et al., 1973).

vi Effects of corticosterone on exploratory behaviour

File (1978) reports that CORT fails to influence the level of exploration in intact rats as measured in the hole board. However, long-term adrenalectomy affects certain components of exploratory behaviour in a novel environment (Veldhuis et al., 1982a and 1982b; Veldhuis and de Kloet, 1983b). Specifically, rearing and ambulation are reduced, and both are normalised by CORT treatment. These effects contrast with those reported by Micco and McEwen (1980), who found that neither adrenalectomy nor CORT altered exploratory behaviour.

vii Effects of corticosterone on habituation

According to File (1978) intact rats treated with CORT do not exhibit alterations in the rate of habituation of an orienting response (OR) or an exploratory response. Removal of the adrenals is also without effect (Davis and Zolovick, 1972).

viii Effects of corticosterone on aggression

Corticosterone facilitates some types of intermale aggression: a single dose to intact rats increases the level of shock-induced attack behaviour, adrenalectomy has the opposite effect (Rees and Gray, 1984). Despite this, chronic CORT treatment does not affect aggression in intact male mice, but it reduces the aggression-promoting effects of testosterone (T) in castrated mice (Simon and Gandleman, 1978). Submissiveness to attack, which is affected independently of aggressiveness, is promoted by CORT (Leshner and Politch, 1979).

ix Effect of corticosterone on induced amnesia

Corticosterone administered to intact mice antagonises induced amnesia in a dose-dependent fashion, such that higher doses facilitate retention (Flood et al., 1978).

x Effects of corticosterone on conditioned avoidance responding

Aversive stimuli are by definition stressful and therefore result in the release of CORT (Coover et al., 1973). This presumably prompted the extensive studies that have been conducted on the effects of CORT on conditioned avoidance responding. In the early studies CORT was administered to intact rats and little or no effect on the learning of active or passive avoidance responses (AAR and PAR respectively) was found, however performance was improved and the retention of these behaviours profoundly altered.

Acquisition

Whereas learning of a one way AAR in intact animals is not affected by CORT (Bohus and Lissak, 1968), administration of this steroid after partial acquisition of a response in a two-way active avoidance task enhances the rate of acquisition (de Wied et al., 1972). Acquisition of a PAR is attenuated by adrenocorticoids: pretraining treatment with glucocorticoids in intact rats shortens passive avoidance latencies (Bohus et al., 1970; Bohus 1971; 1973).

In general, adrenalectomy does not affect the acquisition of a conditioned avoidance response (CAR), though under certain conditions, such as high shock intensity, it results in an improvement (Beatty et al., 1970).

Performance

The performance of various conditioned behaviours is usually improved by CORT. Thus it reduced intertrial responding during the acquisition of a one-way active avoidance task without reducing the number of avoidance responses (Bohus and Lissak, 1968).

Maintenance

The retention of learned behavioural responses in both active and passive avoidance situations is affected by CORT, often in a dose-dependent fashion. It facilitates the extinction of a one way AAR in intact rats (de Wied, 1967; Van Wimersma Greidanus, 1970). Kovacs et al. (1976) demonstrated a biphasic effect, with low doses facilitating and high doses delaying extinction of this behavioural response. PAR retention is also influenced by CORT in a dose dependent fashion: it is suppressed by higher but facilitated by lower doses of this adrenocorticoid (Kovacs et al., 1977).

Corticosterone has also been shown to affect the maintenance of conditioned avoidance behaviour in adrenalectomised animals. Indeed, they continued responding for longer than their intact counterparts during the extinction of a one way AAR, and CORT-replacement facilitated the extinction of this conditioned response (Bohus and Lissak, 1968, Weiss et al., 1970). Similarly, adrenalectomy increases the resistance to extinction of a two-way AAR, whereas CORT facilitates extinction (de Wied, 1967). In addition, adrenalectomised rats performed better than intact rats in PAR retention tests, exhibiting longer response latencies, whereas replacement with CORT impaired the retention of this behavioural response (Bohus et al., 1970; Weiss et al., 1970). Bohus et al. (1970) also demonstrated that

CORT facilitates the extinction of a PAR.

Forced extinction

CORT is necessary for the phenomenon of forced extinction. Short-term adrenalectomy (1 hour prior to the confinment to the shock chamber) prevents the usual forced extinction of an avoidance response, whereas CORT administered at the time of surgery normalises this behaviour (Bohus et al., 1982).

xi Effects of corticosterone on conditioned appetitive responses

Adrenal influences on the conditioning of appetitively motivated tasks have been much less extensively studied than conditioning on aversively motivated tasks, however the acquisition of an appetitively motivated response is not affected by CORT, except where the motivation level is low due to a short deprivation regime (Bohus, 1973).

As with aversively motivated tasks CORT tends to enhance performance; improving differential responding on a low rate of reinforcement, and enhancing performance on a delayed response learning (DRL) task, both on fixed and variable interval schedules (Levine, 1968). In addition, it improves the reversal of an appetitively motivated discrimination response by differentially enhancing the extinction of responding to the non-reinforced stimulus (Bohus, 1973). Conflict surrounds the effect of CORT on the extinction of an appetitively motivated task: it has been demonstrated to facilitate extinction of a food reinforced runway response in intact rats by Garrud et al. (1974), though Micco et al. (1979) and Micco and McEwen (1980) claim that this is not the case. Indeed, the latter authors found that whilst adrenalectomy facilitates extinction, and CORT replacement has a normalising effect, CORT administered to intact rats was ineffective.

If any generalisations can be made from the mass of complex evidence on the behavioural effects of CORT, it is perhaps that it affects adaptive behaviour, whether controlled by positive or negative motives; and that its

strongest effects are on the maintenance of a learned response. Despite this generalisation a number of variables influence the nature of the CORT effect. Firstly, the motivational stimulus is important, as suggested by the opposite effects of CORT on aversively and appetitively motivated tasks. Secondly, the level of arousal is important, as suggested by the finding that CORT often fails to affect behaviour if the level of task activation is too low (Micco and McEwen, 1980), or too high (Bohus, 1973). It has also been suggested that the sensitivity of behaviour to modification by CORT may depend on the kind or degree of learning involved. Yongue and Roy (1985) claim that the behavioural effects of CORT emerge under changing conditions only: which is consistent with the fact that this adrenocorticoid is very responsive to changes in environmental contingencies (Levine et al., 1972; Hennessy and Levine, 1978).

The mechanism of the behavioural effects of corticosterone

When confronted with the diversity of the behavioural effects of CORT, and the numerous variables influencing these effects, it is tempting to conclude that the complexity of the situation defies any attempt to establish a unitary theory. Whilst it is plain that different behaviours may have different neural mechanisms and that this poses a problem for the development of a unitary theory, there are certain findings which point to the existence of a common psychological substrate, and this will be the next subject of discussion.

The primary mechanism by which CORT influences behaviour has been the subject of much debate. However, in this thesis, it is believed to be involved in attention; this belief stems from two lines of evidence, which will be discussed in turn. The first is the known attentional effects of ACTH; and the second, the locations of the receptors for CORT in the brain, and the function of these brain areas in attention.

The effects of ACTH on attention

As the majority of studies relevant to the mechanism by which the PAS hormones affect behaviour concentrate almost exclusively on ACTH, and as this peptide is strongly implicated in attention (the evidence for which is presented below), it has been assumed that CORT may also be involved in this process. Whilst it is acknowledged that this assumption may be too simplistic, it is considered to be a useful starting point. The potential value of extrapolating from the known mechanism of ACTH to that possibly used by CORT was suggested by the fact that ACTH and CORT tend to exert opposite effects. Thus, at the behavioural level, ACTH enhances the retention of both active and passive avoidance responses (de Wied, 1964; de Wied and Bohus, 1966; Weiss et al., 1970; Garrud et al., 1974; Bohus and de Wied, 1981), whilst CORT enhances the extinction of these responses (de Wied, 1967; Bohus and Lissak, 1968; Bohus, 1970; Bohus et al., 1970; de Wied et al., 1972; - for review see Bohus and de Wied, 1980). Similarly, they exert opposite electrophysiological effects: ACTH has an excitatory effect on brain activity in contrast to the inhibitory effect of CORT (Pfaff et al., 1971). This is in harmony with the finding that the receptors for ACTH are concentrated in the ascending reticular activating system (ARAS) - a brain area implicated in excitatory processes, whereas CORT's receptors are predominantly located in the HPC - an inhibitory area of the brain (McEwen and Weiss, 1970). As ACTH and CORT tend to exert opposite effects, CORT may exert an opposite effect on attention to that of ACTH, and through their opposite action, the PAS hormones may act to finely tune attention.

Although the above has attributed a role to ACTH in attention, mention has already been made of the controversy surrounding the primary mechanism by which the behavioural effects of the PAS hormones are brought about. The following discussion of the mechanism of ACTH is intended to illustrate this, and justify an attentional role.

The controversy associated with the mechanism of the behavioural effects of the PAS hormones arises because it is usually not clear whether these hormones act via arousal, attentional, motivational, learning or memory processes directly. However, in view of the close relationship between these processes, an alteration in one unavoidably affects the others. Consequently, it is inevitably difficult to isolate that principally responsible for the behavioural activities of the PAS hormones. In view of the vast range of psychological constructs that have been invoked to account for the actions of the hormones from the PAS, this discussion is necessarily limited to the 4 major areas of debate; fear, general motivation, memory and attention (review - de Wied, 1977a; Bohus, 1979; Sandman et al., 1981).

1. Fear

The earliest hypothesis invoked a role for the PAS hormones in fear processes. Although the fear idea may have had its origins in the idea that stress is equivalent to fear, and stress activates the PAS, it was primarily proposed as a result of the initial studies into the effects of the PAS hormones which exclusively involved situations that were aversive in nature. On the basis that ACTH retards, whereas CORT facilitates extinction of a CAR (de Wied, 1967) it was suggested that ACTH enhances fear, whilst CORT reduces it. However, this hypothesis has several weaknesses: firstly, assuming that fear takes time to develop, glucocorticoids should exert differential effects on immediate and delayed avoidance responding, yet this does not happen (Bohus, 1971). Additionally, if the PAS hormones affect fear they should influence the acquisition of a CAR, but this is not the case. Finally, PAS hormones are behaviourally active in appetitively motivated tasks. Since the actions of these hormones are not confined to situations involving fear motivation the hypothesis is seen to provide only an incomplete explanation of the actions of these hormones (Beckwith and Sandman, 1978).

Succeeding the fear hypothesis, opinion was divided between those favouring a general motivational effect and those prefering a memory effect.

2. General motivation

The central tenet of the theory invoking a role for the PAS hormones in general motivation held that it acted upon the motivational significance of a stimulus, thereby altering the probability of the generation of a stimulus-specific response (Bohus, 1973; de Wied, 1977b and 1977c; Bohus and de Wied, 1981). However, ACTH retains its behavioural effectiveness in situations, such as a PAR task, in which the influence of motivational components has been minimised (Sandman et al., 1971), pointing to the inadequacy of this hypothesis.

3. Memory

The finding that ACTH administration during the acquisition of a CAR was ineffective, whereas ACTH administered during extinction prolonged the extinction of avoidance behaviour, was taken to indicate a role for the PAS hormones in the modulation of memory processes (de Wied and Bohus, 1966). More specifically, ACTH was implicated in retrieval rather than consolidation processes as it only attenuated induced amnesia if it was administered prior to the retrieval test (Rigter and Van Riezen, 1975). (However Gold and Van Buskirk's, 1976a and b, finding that ACTH administered immediately after learning improved retention 24 hours later suggests that it may also affect consolidation under certain conditions.) According to de Wied, the main proponent of the memory theory, ACTH activates the reticulo-limbic system, thereby facilitating retrieval from memory. This in turn promotes motivational and/or attentional phenomena aimed towards enhancing the probability of a stimulus eliciting a stimulus-specific response (review - de Wied and Jolles, 1982). De Wied's suggestion stems from the claim that attention to, and recognition of, a stimulus requires the retrieval of information from memory (Van Riezen et

al., 1977). However, attempts to extend investigations into the effects of the PAS hormones on memory have not generated any further support for this hypothesis. Indeed, Ward et al. (1979) demonstrated that ACTH/MSH decreased the reaction time in a recognition task by almost equal amounts in four sizes of memory set, which suggests that the peptide does not influence memory processes.

4. Attention

Sandman et al. (1972) effectively disproved the memory hypothesis by their demonstration that ACTH/MSH 4-10 improved performance in a reversal task. Once rats had acquired a brightness discrimination task they were either given a reversal task (in which the previously non-rewarded response was reinforced) or a memory task (in which performance on the original discrimination was retested some time after the acquisition of the discrimination). The results revealed that although acquisition of the discrimination was not affected, ACTH/MSH 4-10 facilitated the reversal of the discrimination but failed to bring about differences in the memory for the original discrimination. This undermined the theory of the role of ACTH in memory enhancement as subsequent reversal of a learned response should be more difficult if ACTH improves memory. This is because interference theory holds that the greater the memory for a particular response, the more difficult it is to learn a different, but similar response. Rather this indicates that ACTH influences attentional processes, as improved performance on a reversal discrimination has been convincingly argued to reflect enhanced attention to relevant environmental stimuli (Mackintosh, 1965; Mackintosh and Holgate 1969; Sutherland and Mackintosh, 1971). Further support for the influence of ACTH on attention comes from the differential nature of its effect on intra- and extra-dimensional shifting in rats. It improves the former whilst impairing the latter (Sandman et al., 1974), revealing an enhancement of attention towards the initially reinforced dimension.

The attentional role of ACTH has received yet more support from its influence on various behavioural and physiological indices of attention. Thus ACTH/MSH increases the averaged evoked potentials during attention, and these are reduced by the presentation of a distractant (Kastin et al., 1971); and increases heart rate during the OR (Sandman et al., 1977). Behaviourally, it improved performance on the Rod and Frame test such that the ACTH treatment group was better able to overcome a distracting background (Sandman et al., 1977). Moreover, ACTH improved performance on the Benton Visual Retention test by reducing the number of errors of ommission rather than commission (Broverman et al., 1968). In a clinical context, mentally retarded adults with severe attentional deficits show marked improvements upon treatment with ACTH (Sandman et al., 1976; 1980a and 1985). Similarly, this peptide improves cognitive functioning, especially with respect to attentional processes, in senile elderly patients (Ferris et al., 1976). In connection with this, ACTH induces alterations in sensory sensitivity. It is well established that it raises the detection threshold and lowers the recognition threshold (which surprisingly resembles the effects of CORT) and in so doing impairs simple detection but improves recognition. Based on this, ACTH has been implicated in the mechanism of neural gating, which is an integral component of the process of selective attention. Without this, animals would be bombarded with distracting 'perceptual noise', with deleterious consequences for cognitive functioning. The adaptive significance of the action of this peptide in raising the absolute detection threshold whilst facilitating the processing of information once it had passed this filter is that, as a consequence, the selection of relevant and the rejection of irrelevant information is enhanced and the efficiency of cognitive functioning improved (Sandman et al., 1977; Kastin et al., 1977).

Consistent with this attentional role that ACTH is posited to play is the finding that it improves performance on a task guided by a single

informative event, which suggests that it improves the use of information (Isaacson et al., 1976). Based on the evidence reviewed above it is concluded that the behavioural effects of ACTH are best explained in terms of attention. In view of the closely interwoven nature of the PAS, combined with the tendency for ACTH and CORT to exert antagonistic effects, it is inferred from this that CORT is also likely to be involved in this process.

Arousal and the pituitary-adrenal system

Whilst the role of the hormones from the PAS in attention is considered to have the most explanatory power, any discussion of the significance of this endocrine axis would not be complete without at least a passing reference to its possible effects on arousal. In recognition of the considerable disagreement over the mechanism by which the PAS hormones influence behaviour, Bohus (1979) asserts that ACTH may act via the common mechanism of arousal to affect attention, learning, motivation and memory processes. The similarity between a number of the behavioural effects of ACTH-related peptides and the psychostimulant amphetamine (McGaugh, 1973) supports this non-specific arousal hypothesis, as does the finding that the changes in the activity of the brain induced by ACTH are similar to the increase in averaged somatosensory evoked potentials when the stimulus intensity is increased (de Wied et al., 1976). The presence of receptors for ACTH in the ARAS strengthens the association between the PAS and arousal as this brain area is recognised as the neural substrate responsible for 'setting' the level of arousal (Moruzzi and Magoun, 1949*). In the psychoendocrine hypothesis of arousal (Hennessy and Levine 1979) the PAS is considered to play a fundamental part in the feedback system regulating the effects of arousal on behaviour. Moreover, the behavioural effects of the PAS hormones are thought to be the result of their effects on systems mediating arousal (ARAS) and inhibition (HPC). Accordingly, the PAS both influences and is itself influenced by arousal. Whilst the

relationship between the PAS and arousal, and the implications that this has for behavioural adaptation has support, the problem of defining arousal (Hinde, 1970; Andrew, 1974) is usually considered to reduce the usefulness of this concept as an explanation of the behavioural role of the PAS. Indeed, although Davies (1983) claims that arousal relates variations in behavioural intensity to variations in psychophysical activity, Broadbent (1971) argues that whilst

> "the physiological concept of arousal is certainly of interest and of ultimate relevance to the one we have found from behaviour, at this stage the connection of any physiological measure with the psychological state is too remote to make it practical to attach one concept directly to the other" p.413.

Confronted with such criticism it was felt that the arousal hypothesis should not be adopted. Despite this, the potential relevance of arousal as the psychological construct upon which the PAS hormones may act should not be unduly dismissed, especially in view of the close relationship between the level of arousal and PAS activation (Levine et al., 1972).

Furthermore, whilst CORT is here thought to affect attention, the potential involvement of arousal in these effects is supported by the intimate connection between arousal and attention. At a most simplistic level, it is obvious that attention to a stimulus depends on the level of general arousal (Deutsch and Deutsch, 1963), though a short digression into the detailed relationship between these two processes reveals that they are likely to be interrelated in a complex fashion.

Arousal and attention

The interactional relationship between arousal and attentional selectivity is a very complicated, not to say contentious issue. According to Easterbrook (1959) the inverted U-shaped function (Yerkes-Dodson Law) relating the level of arousal and behavioural performance is due to the

effects of arousal on attention. Under conditions of low arousal attentional selectivity is thought to be correspondingly low, and as a result irrelevant cues are likely to be accepted for further processing. With increasing levels of arousal, attentional selectivity is increased, resulting in the rejection of a greater proportion of irrelevant cues and the reallocation of attentional resources, such that an increased proportion of the available resources is devoted to important stimuli, thereby improving performance. However, once the optimum level of arousal has been reached, any further increases in arousal are considered to bring about a deterioration in performance, as further increases in attentional selectivity result in the rejection of relevant cues. Thus, up to a certain level of arousal, the corresponding increase in attentional selectivity is regarded as adaptive, however once this level has been exceeded further increases are likely to be maladaptive. Easterbrook regards the increased attentional selectivity emerging under conditions of heightened arousal as a 'coping response' aimed at increasing the efficiency of information processing under conditions that are likely to be of significance to the animal. Although Easterbrook receives support (e.g., Hasher and Zacks, 1979*), Mandler (1975*) disagrees, suggesting that under high levels of arousal there is a reduction in attentional selectivity resulting from a lowering of the filter threshold. This increases the amount of sensory information receiving further processing, which in turn increases competition for limited processing resources. This overloading results in allocation of processing resources to irrelevant stimuli, further decreasing the efficiency of information processing.

Eysenck (1983) also disputes Easterbrook's stance, arguing that high arousal levels are associated with increased rather than decreased levels of distractability. According to Eysenck, whilst at low arousal levels an increase brings about an increase in attentional selectivity, at high arousal levels any further increase is associated with a decrease in

attentional selectivity. Whether the position advocated by Eysenck or Easterbrook is the correct one is unknown; however both agree that moderate arousal levels are optimal for information processing. Finally, as Eysenck (1983) asserts that many, though not all, of the effects of arousal are mediated by attentional processes, and in view of the close relationship between arousal and the PAS, this tends to support the examination of the PAS hormones in the process of attention irrespective of the difficulty over the definition of arousal.

At this juncture it is necessary to discuss the concept of attention in a little more detail, though it should be emphasised that this thesis is primarily concerned with the physiological influences upon attention rather than the theoretical aspects of this process.

The concept of attention

Attention is the "selective aspect of perception" (Treisman, 1969). For the present purposes the definition put forward by Oades (1979) has been adopted, according to whom attention refers to

> "the ability to select some sensory input channels over others by the central nervous system for further processing and behavioural organisation".

From this definition it is apparent that the central problem addressed by attention is the way in which certain stimuli are selected for registration and response, whilst others are rejected.

Krechevsky's (1932*) observation that rats will learn about the significance of some but not all cues whilst trying to solve a complex discrimination problem indicates that the capacity to process information is limited. As a result of this, the ability to select relevant information has profound implications for the efficiency of information processing.

Broadly speaking attention theories fall into two main classes -

early, and late selection theories (Posner and Boies, 1971; Posner, 1982; Johnston and Dark, 1986). According to the early selection theories, there is only one locus of selection, which is thought to occur early in the sequence of information processing, before the stimulus reaches the stage at which recognition occurs. Selection must, therefore, take place on the basis of physical cues alone (Broadbent, 1958). In contrast, late selection theories (such as Deutsch and Deutsch, 1963) hold that all incoming information is identified, but only some is responded to, as the limitation is imposed at a later stage in the processing sequence.

Late selection theories are supported by the evidence from dichotic shadowing tasks in which messages in the "unattended" channel have been shown to receive some processing (Gray and Wedderburn, 1960*; Moray, 1969). Treisman (1969) modified Broadbent's original theory; and in her filter amplitude theory, unattended inputs are not rejected but "attenuated", and as a consequence are less likely to receive the full processing required for recognition and response. This theory retains early selection on the basis of physical characteristics, but also incorporates an additional point of selection. This occurs at a later stage in the information processing sequence, and is based on the "pertinence" of the information. The concept of "pertinence" is derived from Norman (1968) who proposed that the central threshold for a particular stimulus depends not only on its physical intensity but also on its relevance or pertinence for ongoing activities. Treisman's filter amplitude theory is the most widely recognised attentional theory, and serves to illustrate that selection of information for processing occurs at several points. Indeed, selection may involve a variety of different mechanisms ranging from receptor reflex responses, direct interaction between input channels (collateral inhibition), to central matching processes distinguishing between stimuli on the basis of elaborate criteria.

Whereas these theories relate to studies on attention in humans,

Andrew (1976) addresses the problem in animals. Andrew's model of attention draws on the principles of Sokolov's (1963*) theory of the habituation of the orientation response. According to Sokolov (see Andrew, 1972a and 1978), habituation depends on a process of matching. A novel stimulus elicits an OR, initiating the development of a neuronal model corresponding to the stimulus and repeated presentations of this stimulus result in the elaboration of its neuronal model. If the presentation of the stimulus is followed by a comparison with a fully matching model, the stimulus no longer evokes an OR, indicating that habituation has occurred. However, if the stimulus is changed, so that it no longer corresponds to its neuronal model, the OR reappears. However, Andrew's theory greatly extends upon the basic premise put forward by Sokolov, and a central tenet of this theory is that animals employ 'rules of selection' in information processing. These rules determine which stimuli are to be selected for further processing, either on the basis of a single stimulus characteristic ('stimulus-set') or on the basis of several complex stimulus characteristics ('response-set') (Broadbent, 1970). Once selected, recognition only ensues if the stimulus activates a 'recognition unit' (Kahneman, 1973*), which is a central representation or 'neuronal model' corresponding to the particular stimulus. Recognition units are formed on the basis of previous experience of that category of stimulus. For activation of a recognition unit to occur there must be a certain degree of match between the stimulus and its recognition unit. Activation also depends on the threshold for activation, which varies along with the criterion for match. An important point for the understanding of this theory is that recognition units differ in their thresholds for activation as a result of past experience and immediate prior experience. Moreover, all other things being equal, a stimulus included under current rules of selection is more likely to activate a recognition unit than an unselected one. Activation is thought to be followed by a further phase of comparison between the stimuli and a

recognition unit, and on the basis of this one of three responses is evoked. Under conditions of match, depending on which is most appropriate, the animal will either emit a specific response to the stimulus, or fail to respond. The latter indicates that the stimulus is familiar, and on the basis of past experience no response is required. However, under conditions of mismatch a variety of behaviours, ranging from further examination to fleeing, may occur, depending on the degree of mismatch.

In this model sustained attention to a stimulus, or prevalent control of behaviour by a particular stimulus, arises as a result of persistence in the use of one set of rules of selection. A shift in attention corresponds to a change in these rules. This may occur for one of two reasons: firstly, they may be changed because the rules for certain stimuli, presumably those conveying survival significance to the animal, have priority, and can override the current ones. Alternatively, since these rules are not perfect, attenuation of other stimuli may not be complete, and they may 'slip' through and activate their recognition unit, especially if the activation threshold is low.

Measurement of attention

Although attention is not an overt phenomenon capable of direct observation, it can be studied at a number of different levels (Posner, 1982). At the behavioural level it is possible to observe it indirectly on the basis of those stimuli controlling behaviour. However, there is a problem associated with the study of attention at this level which relates to the distinction between persistence and perseveration. Persistence refers to the continued use of specific central specifications in the control of stimulus selection, whereas perseveration refers to the repetitive performance of a specific motor response. Empirically, however, it is not usually possible to distinguish these phenomena, as both result in the repetition of behaviour. A further problem with attention, as

recognised by Moray (1969), is that this general term encompasses a variety of related processes, such as attention focussing, attention switching, retrieval of specifications etc., and it is possible that each of these may be susceptible to independent modification. This highlights an important point; namely, although extrapolation from the probable mechanism of ACTH implicated CORT in attentional processes, the specific role of this adrenocorticoid remains unknown. In connection with this, an examination of the function of CORT's neural substrate, not only reinforced its involvement in attention, but also provided a deeper understanding of its specific role in this psychological process. This discussion will now, therefore, consider the neural substrate for CORT in more detail.

The neural substrates of corticosterone

Autoradiographic techniques revealed that the receptors for adrenocorticoids are predominantly localised in extrahypothalamic structures, in particular, neurons of the HPC - especially the CAI and CAII neurons of the Ammon's horn, and the granular neurons of the dentate gyrus, the subiculum and the induseum griseum. The dorsolateral septum also possesses a high density of receptors, as does the amygdala and the cortex - especially the entorhinal, suprarhinal, pyriform and cingulate cortex. Other brain regions, including the olfactory nucleus, habenular nucleus and the red nucleus, have a more diffuse distribution of receptors. Finally, motor neurons of the cranial nerve nuclei and spinal cord possess large numbers of receptors (McEwen and Weiss, 1970; Gerlach and McEwen, 1972; McEwen et al., 1972; 1975). Thus, whilst the receptors are concentrated in limbic areas, they nonetheless have a widespread distribution throughout the brain. Surprisingly, the hypothalamus possesses only a relatively low number of receptors.

The interaction between these receptors and adrenocortiocoids may bring about both genomic and non-genomic changes, primarily in proteins,

which affect a wide variety of cellular functions, e.g., additional steroid receptors, components or modulators of membrane transport mechanisms, enzymes involved in neurotransmitter or neurohormone metabolism, protein kinases and protein hormones. Typically, genomic effects are delayed in onset and prolonged in duration, whilst non-genomic effects have a rapid onset and short duration. The latter tend to be confined to the period of the steroids presence; though this is not necessarily so for genomic effects, however they may begin as early as 15-20 mins after steroid application (Pfaff et al., 1971; McGowann-Sass and Timiras, 1975). In addition to these receptor-mediated effects, it is possible that steroids evoke direct cellular responses. Although such effects are poorly understood, they may involve action on pre- and post- synaptic membranes, alterations in electrolyte balance and membrane permeability to neurotransmitters or their precursors, and/or the functioning of transmitter receptors. (As an aside, it should be noted that in addition to these direct effects, glucocorticoids may exert indirect ones by permitting or amplifying the actions of other hormones - de Kloet and Veldhuis, 1985).

The hippocampus as the primary site of the actions of corticosterone

Since receptors for the adrenocorticoids are localised predominantly, though admittedly not exclusively, in the HPC, by inference, CORT is most likely to mediate its behavioural actions via this brain area though the possibility remains that other areas of the brain are involved. However, as the HPC has been implicated in the feedback regulation of the PAS, it is possible to argue that it is solely involved in the neuroendocrinological effects of CORT. Whilst the regulatory feedback effects of CORT at the HPC, and the involvement of brain areas other than this one in the behavioural effects of CORT cannot be denied, the behavioural significance of this limbic site is attested to by the correspondence between the regional distribution of the CORT receptors and early brain implantation studies.

These revealed that the facilitatory effect of CORT upon the extinction of a CAR depended on the site of implantation, with the most pronounced effect emerging with hippocampal implants (Bohus, 1970; Bohus, 1973). The role of the HPC is further supported by the correlations between the capacity of the hippocampal CORT receptor system and behaviour. There is great individual variability in the CORT receptor capacity in the HPC, and those individuals with higher capacities have impaired PAR combined with improved two way AAR performance (Angelucci et al., 1981). Yet further support comes from the finding that CORT also elicits numerous neurochemical and neurophysiological changes within this brain area (review - Luttge, 1983; Meyers, 1985), some of which correlate with behavioural alterations, and a selection of these will now be reviewed.

Neurochemical and neurophysiological actions of corticosterone at the hippocampus

Corticosterone has been shown to reduce single unit activity in the HPC (Pfaff et al., 1971), though the evidence is conflicting (Woodbury, 1958; Feldman and Dafny, 1970). Several transmitter systems have been identified within the HPC, including the dopaminergic (DA) and adrenergic (A) systems; the septal - cholinergic input, the ascending serotonergic input from the raphe nuclei, and the noradrenergic (NA) input from the locus coeruleus. In addition there are excitatory amino acids, such as glutamate from the entorhinal cortex and aspartate from the HPC commissural fibers, and inhibitory transmitters such as ɣ-aminobutyric acid (GABA). Several effects of CORT on the neurotransmitter systems in the HPC have been described though these are mainly indirect. Thus there are no reported effects on the metabolism of NA, A or DA. Rather, the evidence is of changes in the transmitter's receptor systems: Mobley and Susler (1980) demonstrated that the sensitivity of the NA-receptor coupled adenylate cyclase is regulated by CORT. Corticosterone also influences the number of

β-adrenergic receptor sites. Similarly, the number of high affinity binding sites for GABA has been shown to be controlled by the levels of CORT: though Miller et al. (1978) report a reduction, Majewska et al. (1985) report an increase in response to CORT.

A reciprocal relationship is thought to exist between the central 5-HT system and the H-PAS. CORT acts on many aspects of 5-HT metabolism, in general exerting a stimulatory effect (Van Loon et al., 1981). This is supported by the close correlation between the turn over rate of 5-HT and plasma CORT levels (de Kloet et al., 1982). Further evidence in support of this relationship comes from the correspondence between the diurnal rhythms of CORT and 5-HT (Azimitia and McEwen, 1974), though this is a controversial issue (Krieger, 1974). In spite of the well documented positive nature of this correlational relationship, the effects of stress on 5-HT are far from straightforward: the ensuing increased levels of CORT produce different patterns of change in the levels of 5-HT depending on the nature of the stressor (Palkovitz et al., 1976; Telegdy and Vermes, 1976). Confirmation of the positive relationship between 5-HT and CORT is provided by the finding that adrenalectomy decreases, whereas glucocorticoid replacement increases, the formation of 5-HT from its precursor tryptophan (Azimita et al., 1970). This effect is thought to be due to the enhancement by CORT of the activity of tryptophan hydroxylase, the rate limiting enzyme in 5-HT biosynthesis (Azimitia and McEwen, 1974). Not surprisingly, the increased levels of 5-HT arising in rèsponse to CORT result in a reduction of the number of hippocampal 5-HT receptors (Biegon et al., 1985; de Kloet et al., 1986).

Although this discussion has concentrated on the effects of CORT on the 5-HT system it is worth noting that 5-HT induces corresponding changes in the level of CORT (Van de Kar et al., 1985). In view of the close relationship between 5-HT and CORT it has been suggested that some of the behavioural alterations attributable to CORT may be caused by changes in

5-HT neurotransmission. Kovacs et al. (1976; 1977) disclosed that the administration of CORT to intact rats produces a dose-dependent effect upon 5-HT levels: with low doses of CORT, 5-HT content and turn over increase, while with high doses both decrease. Furthermore, this paralleled the biphasic effect of different doses of CORT on both PAR and AAR tasks. Thus, in the case of PAR, a low dose of CORT improves PAR and increases the levels of 5-HT, whereas a high dose of CORT impairs PAR and decreases 5-HT levels. These parallel effects of CORT on hippocampal 5-HT levels and on HPC-dependent behaviour, all attest to the potential mediatory role of this transmitter in CORT's effects upon the HPC.

In summary, then, it emerges that the HPC is most likely to be the primary neural structure mediating the behavioural effects of CORT. Accordingly, in order to further assess the influence of this adrenocorticoid in attention and to deepen the understanding of this influence, the role of the HPC must be examined.

The role the the hippocampus in psychological processes

Unfortunately there is little agreement on the specific function of this part of the brain. Schmajuk (1984) reviewed the most influential theories, such as spatial mapping (Nadel and O'Keefe, 1974); contextual retrieval of cues (Hirsh, 1974; Winocur and Olds, 1978; Winocur, 1980; Winocur and Gilbert, 1984); working memory (Olton et al., 1978); and attentional inhibition (Douglas, 1967; Douglas and Pribram, 1969; Gray, 1982), and concluded that no single theory correctly predicts all the empirical evidence. Nonetheless, even though these theories apparently deal with disparate behavioural effects, the psychological constructs that have been invoked are not necessarily mutually exclusive. More importantly, if the HPC is regarded as a polyfunctional structure, it is feasible that each contains an element of the truth. Indeed, it is probably fallacious from the outset to expect to be able to ascribe a single function to a structure

which is part of a complex distributed system. Despite this, in this thesis emphasis is placed on those attributing an attentional role to this structure, more specifically, in attentional inhibition. This is not intended to say that this is the only correct theory, or that others are false. Rather, as it has been argued that control of attention is an important function of CORT on the basis of the behavioural effect of ACTH, and as the HPC has a high density of receptors for this adrenocorticoid, attentional theories are the most relevant in the present context. Support for the specific involvement of the HPC in attentional inhibition is given below.

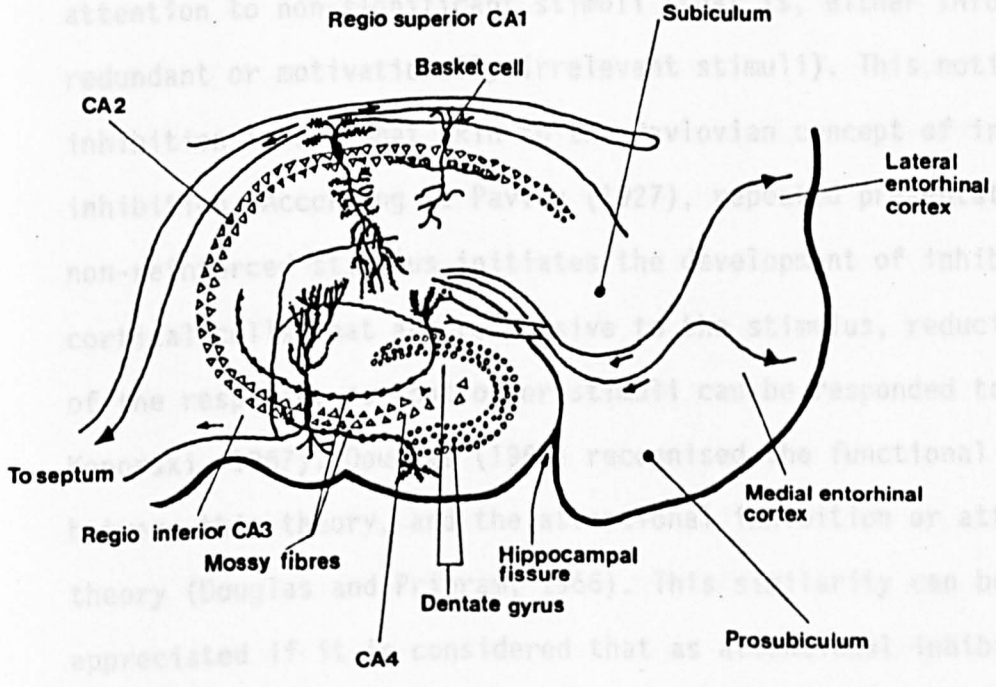
The hippocampus and attentional inhibition

Although theories of hippocampal function are legion, and generate much disagreement, there tends to be consensus on one point: the HPC is inhibitory. The real dispute concerns the nature of this inhibitory influence, whether it be inhibition of sensory systems, motor responses or cognitive processes. This dispute primarily originates from the lack of distinction between whether the brain mechanisms involved are responsible for response inhibition - which specifically refers to the suppression of motor responses and does not presuppose the involvement of underlying attention mechanisms; or attentional inhibition - which refers to the inhibition of attention to non-relevant stimulis. Although response inhibition and attentional inhibition theories are related, they are conceptually distinct, in a similar fashion to the processes of perseveration and persistence. Indeed, if inhibition refers to response inhibition, then the disruption of inhibition will result in perseveration of motor responding, or the inability to inhibit a motor response. Whereas, if attentional processes are being invoked, then the disruption of inhibition will result in persistence in the use of a set of central specifications, or the inability to switch attention. However, this only

occurs once attention has been focussed on a particular stimulus. If no stimulus has been established as prepotent, then the acquisition of a new response will proceed as normal. Since attentional persistence is observed as behavioural perseveration, it is difficult, though not impossible to determine at a behavioural level which of these two mechanisms is primarily responsible for the effects attributed to the disruption of inhibition. Herein lies the source of the controversy over the nature of the inhibitory influence of the HPC.

Discussion of the inhibitory nature of the HPC is helped by prior consideration of selected neuroanatomical and neurophysiological aspects of this area of the brain. The hippocampal complex consists of 4 principal areas: the entorhinal cortex, the subicular complex and the HPC proper - comprising the dentate gyrus and Ammon's horn (which is itself divided into the CAI, CAII, CAIII and CAIV regions), (see Figure 1:2).

Figure 1:2 Schematic diagram of the hippocampal formation (after O'Keefe and Nadel, 1978)



Neuroanatomically, of particular relevance to the nature of the function of the HPC that is proposed in this thesis, is the strong reciprocal connections between this limbic site and the ARAS, and the fact that the HPC has no direct connections with either the primary sensory or motor structures. It is, however, connected with the entorhinal cortex and the septum/cingulate cortex, and is thereby capable of exerting indirect effects upon both sensory and motor processes.

Electrophysiologically, the HPC is predominantly inhibitory in its action. Kimble (1968) cites the discovery that stimulation of this area of the brain has a depressive effect on the amplitude of cortical evoked potentials (Redding and Siegfried, 1962*), which has been taken to indicate that the HPC inhibits the ARAS.

Assuming that the HPC is involved in attention, and that it exerts an inhibitory influence, a central tenet of this thesis is that this brain area is involved in the process of attentional inhibition. It has already been mentioned that this is considered to be a mechanism for reducing attention to non-significant stimuli (that is, either informationally redundant or motivationally irrelevant stimuli). This notion of attentional inhibition is somewhat akin to the Pavlovian concept of internal inhibition. According to Pavlov (1927), repeated presentation of a non-reinforced stimulus initiates the development of inhibition in those cortical cells that are responsive to the stimulus, reducing the strength of the response, so that other stimuli can be responded to (see also Konorski, 1967). Douglas (1967) recognised the functional similarity between this theory, and the attentional inhibition or attention - shift theory (Douglas and Pribram, 1966). This similarity can be better appreciated if it is considered that as attentional inhibition develops, the degree of control exerted by the initially dominant stimulus is reduced and the animal is as a result able to decouple its attention from this stimulus to a new or more relevant one.

Although for the purposes of this thesis the HPC is considered to be primarily involved in attentional inhibition, in view of the controversy surrounding the nature of the inhibitory influence of the HPC, this stance needs to be justified. Accordingly, the evidence appertaining to the inhibitory influence of the HPC will now be examined in a little more detail.

Studies into the nature of this inhibitory influence make extensive use of animals bearing lesions to the HPC in behavioural tests requiring the development of inhibition, (e.g., extinction, spontaneous alternation, habituation and discrimination reversal, to name but a few). The underlying rationale being that if the HPC is involved in inhibition, performance of hippocampectomised animals on these tasks should be impaired. Although a considerable amount of research has been conducted on the behavioural effects of hippocampal lesions, for the purposes of the present argument it is only necessary to outline the major generalisations that can be made (a more detailed behavioural profile can be obtained from Table 1:1). It should be noted that there are numerous inconsistencies within the literature (see Nadel et al., 1975). Moreover, there are a number of important considerations that must be recognised when attempting to construct a theory of hippocampal function based primarily on lesion data. These include lesion size, especially as the HPC may not be functionally homogeneous (Kohler, 1976; Bohus et al., 1982) and other brain areas may be disrupted; the nature or complexity of the task; and the level of arousal, as this affects the demands on attentional selectivity (Deutsch and Deutsch, 1963). In addition, there is the potential logical fallacy in inferring the role of the HPC from the behaviour of hippocampectomised animals, as the behaviour observed may reflect the properties of the remainder of the brain upon release from the control of the HPC.

Table 1:1 The effects of hippocampectomy on behaviour

Behavioural paradigm	Effect of hippocampectomy (HPCX=hippocampectomised animal)	References
Acquisition classical conditioned response	Normal Impaired to CS- in Pavlovian fear conditioned paradigm	Schmajuk, 1984 Micco and Schwartz, 1971
Extinction classical conditioned response	Retarded	Schmajuk, 1984
Acquisition instrumental response (runway response)	Normal	*Douglas and Pribram, 1966 Jarrard et al., 1964
Extinction instrumental response	Retarded	*Douglas and Pribram, 1966 Jarrard et al., 1964
Acquisition discrimination task	Normal	Kimble, 1968 Silveira and Kimble, 1968
Acquisition simultaneous discrimination	Normal	Kimble, 1963
	Impaired	Winocur and Mills, 1970
Aquisition successive discrimination	Impaired	Kimble, 1963
Acquisition successive go/no-go discrimination	Impaired	Buerger, 1970
Reversal discrimination task	Retarded	*Douglas and Pribram, 1966 Silveira and Kimble, 1968
	Normal	Harley, 1979
NRS shift	Normal	Harley, 1979
Aquisition of one way AAR	Impaired	Niki, 1962 Olton and

		Isaacson, 1968
Retention one way AAR	Impaired	Olton and Isaacson, 1968
Acquisition two-way AAR	Facilitated	Olton and Isaacson, 1968 Antelman and Brown, 1972 Isaacson, 1974
Acquisition of PAR	Impaired	Kimble, 1963
Retention of PAR	Impaired	Kimble, 1963
Orienting reaction:	Normal	Hendrickson et al., 1969
depends on background activity	Impaired	Hendrickson et al., 1969 Silveira and Kimble, 1968
Distractability: irrelevant distractant	Reduced	Wicklegren and Isaacson, 1963 Riddell et al., 1969 *Douglas and Pribram, 1969
relevant distractant	Normal	*Douglas and Pribram, 1969
Exploration of novelty: novel environment	Increased	Gustafson, 1975
familiar environment	Decreased	
Habituation	Retarded	*Douglas and Pribram, 1969 Gustafson and Koenig, 1979 Riddell et al., 1969 Leaton, 1965 Lalonde and Botez, 1985
	Task specific	Kohler, 1976
General activity	Increased Differences in general activity may reflect differences	Leaton, 1965 Nadel and O'Keefe, 1975

	in habituation and level exploration	
Open field activity	Increased number of squares entered	Kimble, 1963 Douglas, 1967 Nyakas et al., 1983
Spontaneous alternation	Decreased	Roberts et al., 1962
Performance on DRL task: no pretraining	Normal	Schmaltz and Isaacson, 1966
CRF pretraining	Impaired	Clark and Isaacson, 1965
Alteration in level of responding to changes in reinforcement schedule	Impaired	*Douglas and Pribram, 1966
Radial maze performance (spatial discrimination test)	Impaired	Olton et al., 1978
Maze learning	Impaired HPCX repeatedly re-enter blind alleys	Leaton, 1965 Roberts et al., 1962 Kimble, 1963
Food search	Impaired development of food search strategy	Oades and Isaacson, 1978
Latent inhibition	Impaired - do not exhibit pre-exposure effect	Solomon, 1979
Differential partial reinforcement effect	Impaired do not exhibit maximisation of responding	*Douglas and Pribram, 1966
Memory recall	Anterograde amnesia	**Scoville and Milner, 1957 **Penfield and Milner, 1958

Unless otherwise stated the species was the rat.

* Rhesus monkey; ** Humans

Typically, there is no learning or memory impairment per se at least in simple tasks, rather the behavioural deficits of hippocampectomised animals are most satisfactorily characterised by the inability to withhold a prepotent response, even in the face of changing environmental contingencies. Thus, there is a greater resistance to extinction; difficulty in altering response rates with changed reinforcement schedules; impaired discrimination reversal; retarded habituation; indistractability to novel stimuli; poorer inhibition of a previously learned approach response in a passive avoidance situation; reduced alternation; absence of latent inhibition and impaired maximisation in responding in a differential partial reinforcement task (see Table 1:1 for references).

After reviewing the effects of hippocampal disruption Douglas (1967) concludes that it is the degree of inhibition within a task that determines the susceptibility of hippocampectomised animals to behavioural impairment, as they

> "excel over normals on tasks in which a disruptive inhibitory tendency is present, are normal on tasks in which no inhibition is involved and are inferior on tasks demanding an inhibitory tendency", p.428.

Although there is evidence for the disinhibition of motor behaviour in hippocampectomised animals (Micco and Schwartz, 1971), which accords with theories attributing a direct role in motor inhibition for the HPC e.g., 'response braking' theory (Altman et al., 1973), support for a response inhibition mode of action is at best fragmentary. This controversy has been neatly resolved by assessing the performance of animals bearing hippocampal lesions in a DRL task. This task requires a high degree of response inhibition, as a response before the DRL interval has elapsed resets the timer, and in so doing delays reinforcement. If the HPC is involved in response inhibition hippocampectomised animals should be slow in acquiring a schedule that requires low rates of responding. Conversely, if the HPC is

involved in attentional inhibition, acquisition of a DRL should be unimpaired, provided no response schedule has already been established. By contrast, in hippocampally lesioned animals that have received previous training on a continuously reinforced task (CRF), which establishes a high response rate, acquisition of a DRL would be predicted to be retarded. Schmaltz and Isaacson (1966) demonstrated that the acquisition of a DRL is only disrupted after preliminary CRF training. This indicates that hippocampectomised animals are able to suppress responding, which is inconsistent with theories claiming that the HPC is primarily involved in response inhibition (see also Winocur and Mills, 1969). Additionally, the evidence that the acquisition of a go/no-go successive discrimination is unaffected by hippocampectomy poses a further problem for the response inhibition stance (Buerger, 1970). Rather, this evidence points to the involvement of the HPC in attentional inhibition. According to the attentional theories, the HPC exerts its inhibitory effects via the ARAS; and in response to the presentation of a non-relevant stimulus it is thought to inhibit this system, decreasing its responsiveness to the stimulus, which is as a consequence excluded or gated out from further attentional control. Ultimately this lessens the degree of control exerted by the stimulus on behaviour. The outcome of this is that attention can be decoupled from one stimulus and shifted to others (Douglas and Pribram, 1966; Kimble, 1968; Pribram and McGuinness, 1975; Solomon, 1979). This hypothesis that the HPC, in coordination with the arousal systems, is involved in attentional inhibition is consistent with the known neuroanatomical and neurophysiological relationships between these two brain structures.

There has, however, been some disagreement over the means by which the HPC acts to exclude stimuli from attentional control. According to Silveira and Kimble (1968) a mechanism exists which is responsible for focussing attention on a stimulus and inhibiting attention switching, and the HPC is

thought to inhibit this, thus allowing attention switching. By contrast, the HPC has been attributed a direct inhibitory effect on attention, which once again results in attention switching (Kimble, 1968). A further problem is whether the HPC excludes stimuli in a global or a specific fashion. In the former, the exclusion of irrelevant stimuli is widespread such that the level of stimulus sampling is affected; whereas in the latter, exclusion is restricted to those stimuli that have already gained attentional control, affecting the shifting of attention. Relevant to this, Hendrickson et al. (1969) found that hippocampally lesioned animals were able to orient to a novel stimulus in a similar fashion to their control counterparts unless it was competing for attention with a motivationally relevant, or a novel neutral stimulus, which had already captivated attention, in which case the level of orienting was reduced. Since orienting per se was unaffected by hippocampectomy, this suggests that the ability to switch attention was impaired but only once it had been captivated by a stimulus. From this it can be inferred that the HPC is more involved in the exclusion of specific stimuli. There is, however, a qualification to this, as although the HPC may be primarily involved in the exclusion of specific stimuli, it may also make a contribution to non-specific exclusion. Evidence supporting this comes from studies into the role of the HPC in memory. Alot of the early research on the HPC was prompted by the reports of anterograde amnesia in humans with temporal lobe lesions (Penfield and Milner, 1958; Scoville and Milner, 1957) which were thought to be due to a storage or consolidation deficit. Animal research inspired by these findings did not corroborate this, as hippocampectomised rats were able to learn and recall a task (Kimble, 1963). Douglas (1967) reconciled this apparent contradiction by suggesting that the memory deficit observed in man was a secondary effect of a primary dysfunction in the process of attentional inhibition. This rested on the speculation that under normal conditions the HPC acting in its capacity as a gating

mechanism excludes irrelevant, interfering stimuli, and thereby protects the memory trace from the disruptive effects of interference during the process of consolidation. If this is the case, animals bearing hippocampal lesions would be more susceptible to interference by irrelevant stimuli. As a result, even though the ability to store and recall information might be unaffected by HPC lesions, a memory deficit emerges so long as the normal mechanism for excluding irrelevant stimuli is aberrant. This is consistent with interference theory which holds that forgetting arises primarily as a function of interference. Once the significance of the effects of interference upon memory had been realised the discrepancy between the human and animal investigations was attributed to the lower levels of interference in the animal test situation (Weiskrantz and Warrington, 1975). In support of this, hippocampectomised rats trained on tasks incorporating a large interference component exhibit impaired recall (Winocur, 1979; Winocur, 1980).

This increased susceptibility to interference in hippocampally lesioned animals points to a possible role of the HPC in the non-specific exclusion of stimuli.

Factors influencing the activity of the hippocampus

Thus far the HPC has been considered in isolation from all the factors that under normal circumstances would be acting to influence its activity. It is important to be aware of the drawbacks inherent in such an approach, especially since the role of this area of the brain in attentional inhibition will undoubtedly be subject to numerous modulatory influences. For example, NA modulates hippocampal activity in a context-dependent fashion. This transmitter is released from the locus coeruleus via the dorsal noradrenergic bundle (DNB) into the HPC in response to irrelevant stimuli and has the effect of reducing the level of responsiveness. On this basis NA has been ascribed a 'screening' function towards the HPC (Segal

and Bloom, 1976a; 1976b), inhibiting attention to irrelevant stimuli via its influence on this brain area. As a short aside, it is interesting to note that NA appears to be closely associated with the process of attention; though it is not clear whether it has an attentional role per se or whether its role arises through its modulation of the HPC. Despite this, as NA has a screening function towards the HPC, this would suggest that decisions about relevance/irrelevance are taken prior to the involvement of the HPC. The influence of NA in attention is supported by the behavioural evidence that lesions to the DNB (DNBX) result in increased levels of distractability to irrelevant stimuli (Mason and Fibiger, 1978; Mason and Iversen, 1979; Mason and Lin, 1980). Although Mason and Iversen (1979) claim that NA acts to lower the level of stimulus sampling by signalling that stimuli are unimportant, Gray (1982) argues that it signals that a stimulus is important. Whatever the role of this transmitter proves to be, it highlights the problem of trying to devise a satisfactory account of the HPC without considering the effects of the myriads of influences that impinge upon this brain structure and alter its activity.

The working hypothesis

As it has been established that there is good evidence that CORT is likely to influence attention, that this is also, at least one of the roles of the HPC, and that the HPC contains a high proportion of the CORT receptors in the brain, it is possible to adopt with some confidence the working hypothesis that this adrenocorticoid influences attention. It is presumed that this is achieved through CORT modulating the activity of the HPC, though this is not tested. Moreover, by extrapolation from the particular attentional effects of the HPC it seems likely that CORT would affect the process of attentional inhibition. More specifically, it is predicted that CORT would suppress this inhibitory influence, resulting in an increased level of attentional persistence with a reduction in attention

switching. The justification for this prediction is derived from the fact that CORT has an inhibitory effect upon the HPC and the evidence for this is given below.

The inhibitory effect of corticosterone upon the hippocampus

Whilst the relationship between CORT and the HPC is likely to be complex, it tends to inhibit the HPC. Evidence for this stems prima facie from the behavioural similarities between animals bearing hippocampal lesions and those treated with CORT. (As changes in the level of CORT, result in the inhibition of an already inhibitory structure and this is potentially confusing, it is helpful to point out that as CORT produces effects resembling those found upon hippocampectomy; adrenalectomy enhances hippocampal activity). Thus, Micco et al. (1979) established that adrenalectomy facilitates the extinction of an appetitively motivated runway response and that CORT has a normalising effect, which is tantamount to CORT retarding extinction. Similarly, lesions to the HPC retard the extinction of this response (Jarrard et al., 1964; Douglas and Pribram, 1966). As a further illustration of the similarity between the behavioural effects of CORT and HPC lesions, it has been reported that whereas hippocampectomy exerted an opposite effect to adrenalectomy on exploratory activity in a novel environment, the behaviour of these brain lesioned animals resembled that exhibited by adrenalectomised rats with replacement levels of CORT (Nyakas et al., 1983). The electrophysiological effects of CORT on the HPC provide further support for its postulated suppressive influence: CORT inhibits single unit activity within the HPC (Pfaff et al., 1971). (Interestingly this effect occurs within one hour of steroid administration, which is consistent with the time course for the behavioural actions).

The neurochemical changes induced by CORT within the HPC are also consistent with the proposal that this adrenocorticoid inhibits the HPC.

For example: CORT lowers the uptake of the inhibitory neurotransmitter, GABA (Miller et al., 1978), and in so doing potentiates the inhibitory effect of GABA on neurotransmission within the HPC. (Although this is in keeping with the known effects of CORT on HPC activity, there is a dissociation between the time course of the effects of CORT on neural activity and GABA uptake, which weakens this line of argument). Corticosterone also brings about changes in the activity of the inhibitory transmitter 5-HT, the timing of which is more compatible with the other changes associated with this adrenocorticoid. It has already been noted that the CORT-induced changes in the activity of the 5-HT system parallel behavioural changes, suggesting that this transmitter may be responsible for mediating the effects of CORT at the HPC (Kovacs et al., 1976; Angelucci et al., 1980; de Kloet et al., 1982). The discovery that CORT not only brings about behavioural effects resembling those found with hippocampectomy, but that it also induces electrophysiological and neurochemical changes within the HPC that are likely to suppress the activity of this neural substrate led McEwen (1982) to conclude that

> "corticosterone may act to suppress the role of the hippocampus in filtering out behaviourally irrelevant sensory stimuli". p.17

Thus, in inhibiting the HPC, CORT is likely to disrupt the process of inhibition, i.e., CORT will exert a disinhibitory effect. As a consequence it would be expected to enhance the persistence of attention, and thereby promote behavioural perseveration.

In spite of the evidence supporting this inhibitory relationship, qualification is needed because the effects of CORT on the HPC are selective in nature. For example, Micco and McEwen (1980) demonstrated that CORT failed to affect the level of spontaneous alternation or locomotor activity, both of which are sensitive to hippocampal disruption. These dissociations may point to the need to adopt a cautious approach when

attempting to infer the behavioural significance of CORT from its effects on the HPC, drawing heavily on the behavioural similarities between those animals bearing hippocampal lesions and those given steroid treatment. Nonetheless, they do not undermine the theory that CORT is capable of exerting behavioural effects through its suppression of the functioning of the HPC, as there are a number of explanations for them.

Firstly, as this failure to find a CORT effect appears to be restricted to low arousal conditions, the inhibitory effect may only emerge under moderate levels of arousal. Secondly, the kind or degree of learning may determine the effects of CORT upon the HPC, especially considering that the most pronounced effects of this adrenocorticoid tend to be exerted under conditions of conflict or change (Younge and Roy, 1985). More importantly, whilst McEwen (1982) holds that CORT inhibits the HPC in a simplistic fashion, Bohus et al. (1982) advocate a modulatory role for CORT. Accordingly it would only be expected to affect a subset of the behavioural functions of the HPC. Finally, the crux of the matter may simply be that CORT affects brain areas outside the HPC and/or that hippocampal activity may be changed without the involvement of CORT.

Whilst this thesis adopts as its working hypothesis the notion that CORT, in general, acts upon the HPC to suppress its function in the development of inhibition, this position is not without opposition. Based on the finding that CORT facilitates the extinction of a CAR (e.g., Bohus and Lissak, 1968), this adrenocoritcoid has been claimed to exert a potentiatory rather than suppressive effect (Levine et al., 1968; Bohus, 1970). Indeed, this suggests that CORT eliminates behaviour that is no longer relevant. However, as CORT exerts opposite effects upon the extinction of appetitively and aversively motivated responses (McEwen and Micco, 1980), the nature of the stimulus may be crucially important.

In spite of this inconsistency, the postulated suppression of the inhibitory activity of the HPC by CORT is accumulating increasing support

(Bohus et al., 1982; de Kloet and Veldhuis, 1985). As a final point in connection with the attentional effects of CORT, it is noteworthy that this adrenocorticoid exerts opposite effects to those of the DNB (McEwen, 1982). Since the DNB is believed to facilitate selective attention (Mason and Iversen, 1979) by inference CORT suppresses mechanisms which facilitate this attentional process.

Two-receptor types for corticosterone in the hippocampus

Having established most of the background necessary to understand the postulated psychological effects of CORT there remains one final point to be considered; which is that within the brain there are two receptor systems for CORT, the characteristics of which complicate any investigation into the significance of this adrenocorticoid. The presence of two receptor systems was first suggested by the differential neuroanatomical uptake pattern exhibited by DEXA and CORT within the brain (de Kloet et al., 1975; McEwen, 1977). Whereas the binding of CORT was largely restricted to the HPC, DEXA displayed only a low level of binding to limbic structures. Instead its binding was localised in neurons near the ventricles, glial cells, endothelial cells, epithelial cells of the choroid plexus, cells of the meninges and the circum-ventricular organs and neurons in the medial basal hypothalanus. Subsequently this differential pattern of binding was shown to be due to receptor heterogeneity, (Veldhuis et al., 1982b; Veldhuis and de Kloet, 1983a; de Kloet and Velhuis, 1985; Reul and de Kloet, 1986).

The two receptor systems that were recognised have been termed the glucocorticoid-receptor system (GR) and the corticosterone - receptor system (CR) and they differ with respect to their steroid specificity, regional localisation and binding capacity.

Steroid specificity

Veldhuis et al. (1982b) studied the relative specificity of the receptor systems and found that the CR has a 6-10 fold greater affinity for CORT than the GR, and that CORT is preferentially bound by CR. By contrast, the GR does not exhibit the steroid specificity found in the CR. Indeed, it preferentially binds other glucocorticoids, such as DEXA. This difference in steroid specificity accounts for the names given to the two receptor systems.

Regional localisation

Whereas receptors in the CR system are found almost exclusively in the neurons of the HPC and septum, receptors belonging to the GR system display a much wider distribution throughout the brain. The highest density of GRs has been recorded in the lateral septum, dentate gyrus, n.tractus solitarii and amygdala, though a substantial number also exist in the paraventricular nucleus and the locus coeruleus. Glucocorticoid-receptors have also been located in areas of the HPC other than the dentate gyrus, though in lower numbers (Reul and de Kloet, 1986).

Binding capacity

It has been demonstrated that the level of occupation of the CR system by CORT is 80-90% during diurnal trough levels of PAS activity, though this increases to 90-99% under conditions of elevated CORT, such as the diurnal peak of PAS activity or exposure to a stressor. By contrast, the GR system is only 10% occupied at trough levels of CORT, and reaches only 50-60% under physiological conditions of elevated levels of CORT.

In addition, it has been shown that whilst 1µg/100g CORT administered subcutaneously to adrenalectomised rats results in 80% occupation of the CR, the GR remains almost totally unoccupied. A replacement dose of 50-100µg CORT/100g b.w. is required to achieve 50% occupation of the GR

(McEwen et al., 1974; Reul and de Kloet, 1986).

From this it emerges that the GR and CR systems are differentially occupied by increasing plasma levels of CORT and that under any natural circumstance only 10-20% of the CR are available to interact with increasing levels of CORT. The situation with the CR contrasts sharply with that found in the GR, which are only occupied to a large extent upon marked elevations in the plasma levels of CORT. These differences in receptor occupancy presumably reflect the differential affinity for CORT exhibited by the two receptor systems.

Although Rees and Gray (1984) disputed the existence of two distinct classes of glucocorticoid receptor, invoking differences in steroid permeability at the blood brain barrier as the underlying cause of the differences in the pattern of steroid binding, it is difficult to account for the differences in receptor affinity without resorting to the presence of receptor heterogeneity. Veldhuis and de Kloet (1983a) acknowledge that factors such as penetration to brain cells through the blood brain barrier, clearance from circulation and binding to transcortin, may influence the pattern of binding. Even so, the concept of receptor heterogeneity is supported (e.g., Meyer, 1985).

Problems introduced by the properties of the hippocampal-corticosterone receptors

The presence of a receptor system in the HPC that responds exclusively to CORT reinforces the significance of this steroid in HPC dependent functions. Nonetheless, if the CR system is primarily responsible for the effect of CORT on the HPC it is difficult to envisage its functional significance in responding to changing levels of CORT, as under normal circumstances it is approximately 80% occupied. This point is highlighted by the finding that the administration of CORT to intact animals often fails to influence HPC - dependent behaviour (Micco et al., 1979), combined

with which certain CORT-induced changes only emerge if the criteria prescribed by the properties of the CR system are fulfilled. Thus, several aspects of behaviour and neurochemistry are disrupted by adrenalectomy and selectively reinstated by physiological levels of CORT. Moreover, this adrenocorticoid is the sole agonist, as steroid replacement with DEXA, PROG or ALDO proved ineffective, and under certain circumstances even antagonistic (reviews - Veldhuis and de Kloet, 1984; de Kloet, 1984), e.g., exploratory behaviour - Veldhuis et al. (1982a); Veldhuis and de Kloet (1983b); extinction of an appetitively reinforced reponse - Micco et al., (1979); Micco and McEwen (1980); forced extinction of a PAR - Bohus and de Kloet (1981). Among the neurochemical responses - the synthesis rate of 5-HT is subject to the same stringent specificity for CORT (de Kloet et al., 1982; de Kloet et al., 1983).

Permissive and regulatory influences of corticosterone on the hippocampus

In order to resolve the problem surrounding the CR system, Reul and de Kloet (1986) speculate that the differential responsiveness of the CR and GR to changes in the levels of CORT may have far reaching consequences for its control of brain functions. Indeed, through its action with these two receptor system, they suggest that it is possible that this adrenocorticoid is involved in two fundamentally different functions. More specifically, actions mediated via the CR may be more related to the permissive/tonic influences of CORT on the brain, whereas those mediated by the GR may be more involved in regulatory feedback effects on stress-activated brain mechanisms.

Ingle (1954) introduced the concept of permissive hormone action, and although the meaning of this term often seems to be far from clear, Leshner's (1979) discussion of the dichotomy of the activational effects of hormones provides a clearer definition of these two types of hormone action. Leshner (1979) distinguishes between two qualitatively distinct

ways in which the hormonal state at or around the time at which a behaviour is displayed can affect that behaviour:

i. The baseline hormonal state, which refers to the effects of the hormone before exposure to the behaviourally relevant stimulus on the response that is elicited by that stimulus. According to Leshner, the baseline hormonal state prepares or pre-sets the neural mediating mechanisms that are involved in the behavioural response to the stimulus, in a somewhat similar fashion to Ingle's (1954) notion of permissive effects.

ii. The feedback effects, which refers to the behavioural consequences of there being a hormonal response to a behavioural experience, somewhat akin to Ingle's (1954) notion of regulatory effects.

This distinction complements Munck et al.'s (1984) recognition that the CORT response to stress may comprise both permissive and regulatory actions on physiological processes. However, if the CR is exclusively concerned with permissive actions, this raises doubts as to whether short term changes in the levels of CORT can alter attentional processes mediated by the HPC; rather, any effects would be expected to be restricted to the establishment of the baseline of attentiveness. Whilst the implications of the presence of a receptor system that is almost maximally saturated under basal conditions should not be minimised, there are alternative means by which CORT can affect the HPC, and a hasty rejection of the potential significance of CORT at the HPC with respect to short term attentional changes should, therefore, be guarded against.

The mediation of short-term changes in response to corticosterone

In connection with this, it must firstly be pointed out that if the CR system is primarily responsible for most of the behavioural effects of CORT, this introduces a discrepancy with many of the early studies, in which it elicited changes in HPC-sensitive behaviour in intact animals. Obviously these changes are at variance with the properties of this particular receptor system on two accounts:-

i. They do not conform with the strict steroid specificity required - as a dissociation between the effects of CORT and DEXA was rarely reported, even though it was tested, (Henkin, 1970a; Van Wimersma Greidanus, 1970).
ii. They do not conform with the limited binding capacity of the CR system, presuming steroid application produces levels of CORT falling within an upper physiological or supraphysiological range.

This indicates that the influence of CORT on behaviour need not fulfil the strict specificities prescribed by the CR, pointing to the prospect of it inducing short-term attentional changes mediated via the HPC.

To achieve this, it is possible that its effects are not receptor-mediated. Indeed, it will be recalled that CORT is able to exert direct membrane effects. However, if receptor-mediated events are involved its effects may arise through the GR system. The presence of GRs in the limbic system emphasises their possible involvement. This, however, is not intended to deny the potential significance of an interaction between CORT and its receptors in extra hippocampal sites. Corticosterone may act via the GR in different brain structures to elicit direct effects. Alternatively, it may act via the GR present in structures related to the HPC, and thereby bring about indirect alterations in this area of the brain. Finally, in spite of the high level of receptor occupancy in the CR

system, there remain two possible means by which transient elevations of CORT can influence the HPC through this system. Firstly, the presence of 20% of these receptors in an unoccupied state may be sufficient to mediate its effects. Secondly, mediation of the CORT signal via the CR may be achieved by changes in the capacity of this system. Thus, a response occurring in the presence of CORT may not only depend upon the extent of receptor occupation but on the number of receptors available. Indeed, since changes in the number of receptors for CORT will affect the sensitivity of the HPC to modulation by CORT, the magnitude of the HPC response to the steroid will be determined by this. Consequently, changes in the number of receptors for CORT will have considerable functional significance.

It has been shown that various factors influence receptor number (though affinity is unaffected). Of particular relevance to this thesis is the finding that CORT is capable of exerting a regulatory effect upon its own receptor capacity. In recognition of this, and in view of the limited capacity of the CR system, Angelucci et al. (1980) advocate a change in receptor number as the mechanism by which CORT exerts its effects via the CR. In general, there is an inverse relationship between changes in plasma CORT levels and the density of receptors for CORT in the brain (though, see Angelucci et al., 1980). In response to adrenalectomy there is a biphasic increase in the number of receptors (McEwen et al., 1974). This up-regulation is thought to involve either de novo synthesis of receptors, or the unmasking of cryptic receptors (Luttge, 1983). Conversely, elevated levels of CORT arising through the administration of exogenous CORT or as a result of stress, reduce receptor number (Sapolsky et al., 1974; Sapolsky and McEwen, 1985). Down-regulation arises as a reduction in the total number of receptors. The finding that the receptor capacity for CORT in the HPC alters during an individual's lifespan and that these changes correlate with changes in the activity of the PAS further points to the occurrence of autoregulation (Landfield, 1978; Roth, 1979; Landfield, 1981; Sapolsky et

al., 1983a and 1983b; Sapolsky et al., 1985a). However, autoregulation is absent during neonatal life (Meaney et al., 1985a). It is worthwhile noting that partial destruction of the HPC results in the compensatory upregulation of the receptors for CORT in the remaining neurons of this brain area (Nyakas et al., 1983).

Other factors which have been shown to exert regulatory influences on the CORT receptor capacity include the neuropeptides ACTH and vasopressin (de Kloet and Veldhuis, 1980; Veldhuis and de Kloet, 1982a; Veldhuis and de Kloet, 1982b; Sapolsky et al., 1985c).

Finally, neurotransmitter systems may also have a regulatory effect. For example, changes in the level of 5-HT have dramatic effects on receptor number (Angelucci et al., 1982).

The functional significance of changes in the CORT receptor capacity is attested to by the finding that individual variability in this capacity correlates with differences in behaviour (Angelucci et al., 1980; Angelucci et al., 1981). Furthermore, age-related changes in this capacity have behavioural correlates. The HPC CORT receptor system is disrupted in senescent rats and numerous psychological dysfunctions arise, including severe attentional deficits (Landfield, 1978; 1981; Sapolsky et al., 1983b; 1984; 1985a). Treatment with the ACTH4-9 analog ORG2766, which normalises this also improves age-related behavioural deficits (de Kloet, personal communication).

Typically changes in the CORT receptor capacity, tend to be long-term in nature, however there is also evidence that receptor regulation may take place within one hour (Angelucci et al., 1982). As this is roughly the span of the normal CORT stress response all of the arguments advanced earlier for the action of CORT which were thrown into doubt by the apparent inability of the adrenocorticoid to effect changes through receptor occupancy can now be advanced again, this time invoking mediation through changes in receptor number, though there still remains the possibility that

any of the other receptor mechanisms that have been described also make a contribution towards the behavioural effects of CORT.

The experimental strategies

To restate, then, it is the purpose of this thesis to systematically examine the effects of CORT on attention. This was achieved by examining the effects of experimentally induced elevations of CORT on aspects of behaviour known to depend on the normal functioning of the HPC and which are generally regarded to involve attentional inhibition. In addition, aspects of behaviour reflecting attentional inhibition, but which have not been screened in hippocampectomised animals were examined. Examples of behaviour fulfilling this criterion include distractability (Chapter 3); the response to a novel object under conditions of attentional competition (Chapter 4); habituation (Chapter 5); discrimination shifting (Chapter 6); and conditioned avoidance learning (Chapter 7). The experimental animal was the mouse.

The initial strategy was to administer CORT to adrenally intact animals, though this was, without exception, ineffective. As these early studies were conducted in ignorance of the limited receptor capacity for CORT, the consistency of this failure was at the time perplexing, especially as it was known that the various situations under investigation were likely to activate the PAS, which by intuition suggested that CORT should have been behaviourally effective. However, with the knowledge that the retention of CORT at the HPC is limited, this puzzle seemed to be resolved, as it could be argued that any exogenously administered CORT would as a consequence be unable to interact with the already occupied receptors of the HPC. However, despite the logical appeal of this argument, after some thought it was difficult to accept, primarily because if CORT is involved in psychological and behavioural adaptation its elevations should be capable of bringing about behavioural changes. It was, speculated,

therefore, that the absence of an effect of exogenous CORT might have been related to the confounding elevations of endogenous CORT. Such elevations might have arisen as a result of various factors within the experimental situation, such as the stress of injection and handling, not to mention the testing procedure itself - especially in the case of tasks involving a high arousal provoking component. Accordingly, it is possible that the levels of endogenous CORT reached sufficiently high levels to cause all the receptor-mediated mechanisms to operate close to their maximum capacity thereby rendering any exogenous CORT ineffective, because there were no receptor mechanisms available to it. Assuming this to be correct, the use of intact animals was not considered to be the most suitable design for studying the interaction between CORT and the HPC. The experimental strategy was, therefore, revised and adrenalectomised animals used instead, thereby circumventing any problems introduced by elevations in the levels of endogenous CORT. Although studies into the effects of CORT usually adopt a classical endocrine approach, as this thesis was especially concerned with the effects of short-term increases in CORT, such as occurs under conditions of stress, on psychological functioning, this was unsuitable. This is because under normal circumstances the effects of stress-induced elevations of CORT would occur against a CORT-background, and obviously, a classical endocrine approach would not provide an equivalent context. In an attempt to simulate the situation found normally, adrenalectomised animals were placed on a maintenance dose of CORT. This was intended to reinstate moderate levels of this steroid. Subsequently, graded doses of CORT were superimposed on this baseline, and the resulting behavioural effects were evaluated. (In some experiments, the permissive effects were also assessed. This was accomplished by comparing the behaviour of CORT-replaced adrenalectomised animals with their non-replaced counterparts). In order to obtain replacement levels of CORT approximating to those found in mice under non-stress conditions a maintenance dose of 20μg CORT/ml drinking

water was selected initially. This was based on van Dijk et al.'s (1981) demonstration that an equivalent dose administered to adrenalectomised rats prevented adrenalectomy-induced changes in pituitary and plasma ACTH levels, though it failed to avert the depletion of hippocampal ACTH levels. However, this dose produced higher circulating levels of CORT than those found under non-stress conditions. The maintenance dose was, therefore, reduced to 5µg CORT/ml. Unfortunately there was a long delay between the experiments and assaying for CORT, and this meant that a number of experiments had been conducted before it transpired that the levels of CORT were undesireably high.

This gradual refinement in technique from the use of adrenally intact animals to the use of adrenalectomised animals with appropriate CORT replacement in manipulating the circulating levels of CORT took place over most of the 3 year span of this project, and, in consequence, could only be applied to the last experiments that were done. Time allowed for only one or two experiments to be repeated using the improved technique, namely those on distractability and on habituation, which are reported in Chapters 3 and 5 respectively. The order in which the experiments are presented is that in which it now seems most logical to discuss them, and not that in which they were carried out.

Finally, in addition to providing a suitable physiological background, the use of CORT-replaced adrenalectomised animals conferred several supplementary advantages, though one or two disadvantages as well. It is well recognised that removal of the adrenals introduces a number of problems, not least of which is the elevation of ACTH, arising as a result of the removal of adrenocortical feedback (Beatty et al., 1970; Bohus et al., 1982). In connection with this, the removal of the adrenals may also elevate the level of a variety of peptides related to ACTH and in view of the powerful behavioural effects of these peptides it was obviously desirable to avert such changes, and the reinstatement of CORT should have

achieved this.

Related to its effect on ACTH, CORT replacement should also minimise the hypertrophy of accessory adrenal nodules which become activated after adrenalectomy in response to the elevated levels of ACTH. This point acquires especial significance in the light of Hawkins et al.'s (1974) claim that it is difficult to successfully eliminate CORT in mice because they are notorious for the presence of accessory adrenal nodules. A further undesirable adrenalectomy - induced change that would be prevented by the replacement of CORT relates to the up-regulation of the number of receptors for CORT in response to lowered levels of this steroid in the circulation. It has been demonstrated that the administration of maintenance levels of CORT in the drinking water successfully averted changes in receptor density in the HPC (Angelucci et al., 1980; Tornello et al., 1982).

Although the use of adrenalectomised animals with replacement levels of CORT avoids interference by the endogenous steroid, and on the whole controls for the usual unwanted consequences of adrenalectomy, any procedure involving the removal of the adrenal gland not only gets rid of the cortex, but also the medulla. The ensuing reduction in the level of the adrenomedullary catecholamines, especially adrenaline, is an important consideration, as it inevitably confounds the study of the effects of CORT (Di Giusto et al., 1971; Brush and Froelich, 1975; Mileusnic et al., 1986) and this must be borne in mind when interpreting the behaviour of adrenalectomised animals.

CHAPTER 2

Methods and materials

Animals

The experimental animals were outbred "TO" male albino mice, originally obtained from A. Tuck and Son Ltd., Battlesbridge, Essex, and bred at the University of Keele, (2nd - 4th generation). The mice were housed under a reversed lighting regime (red lights on 12.00-22.00hrs) at 20±2^{o}C.

Prior to behavioural testing the mice were housed in same sex groups - comprising litter mates and non litter mates, with approximately 10 mice in each group - in a large cage (42x25x11cm). Food (Pilsbury's small animal diet) and water were available ad libitum. All the cages contained sawdust bedding (source: Pilsbury's Ltd.) which was changed weekly, or when necessary. During behavioural testing the mice were singly housed in a small cage (30x13x11cm). Both the large and the small cages were plastic with stainless steel tops (source: North Kent Plastics Ltd.). Relatively strict controls over housing conditions were employed, with single housing of animals throughout behavioural testing being adopted, in order to minimise the variability in pituitary-adrenal and pituitary-gonadal activity that arises under the social conditions associated with group-housing (Brain and Nowell, 1969b). The mice were aged between 10-25 weeks at the time of testing, and all were sexually inexperienced.

Experimental treatments

1. Chronic testosterone administration

In experiments necessitating chronic T administration castrates were implanted with a silastic:T pellet, 20mm in length (see APPENDIX 6). A 1:5.4 by weight mixture of the steroid and silastic was extruded through the nozzle of a 2ml syringe (2mm diam.) under water. The silastic: T rope

was allowed to cure at 37°C for at least 24 hours, after which 20mm lengths were cut and used as implants (Gurney and Konishi, 1980). The dosage of T was 5mg/cm. For implantation the animals were lightly anaesthetised using ether. Fur was shaved from an area of the nape of the neck, and the area swabbed with hibitane. An incision was made into the skin with scissors (approximately 1cm in length), the connective tissue was cleared and a subcutaneous channel was formed into which the pellet was inserted. The incision was closed with one wound clip, and dusted with cicatrine antibiotic powder.

2. Bilateral castration

The surgical equipment was sterilised using hibitane. The animals were anaesthetised under ether, and an ether nose-cone regulated the level of anaesthesia. The ventral suface was shaved, swabbed with hibitane, and a mid-line incision made through the skin and body wall. The testes were located, isolated, ligatured, and then cut free. The body cavity was sutured using 1 or 2 stiches. The primary incision was closed by 3-4 wound clips, and dusted with cicatrine. 7 to 10 days later the wound clips were removed under light ether anaesthesia. Sham castrates received primary and secondary incisions and the testes were located.

Post-operatively the animals were singly housed with paper towelling rather than sawdust covering the floor.

3. Bilateral adrenalectomy

The surgical equipment was sterilised using hibitane. Sagatal anaesthesia (60mg/ml diluted in a 1:9.1 ratio in 10% ethanol), injected intra-peritoneally, was used. The dosage regime was linear for mice weighing above 30gms. A mouse of this weight was given 0.365ml of the diluted sagatal solution and for every additional gram body weight, the dose was increased by 0.015ml. The animals were weighed on an electronic

balance (Sartorius 1404MP8).

The dorsal surface was shaved, swabbed with hibitane, and a mid dorso-lateral incision made through the skin and the body wall. Once located the adrenal fat capsule was clamped between a pair of small curved forceps and using a second pair of forceps the adrenal was teased clear of the kidney. The body cavity was sutured. The primary incision was closed by 4 wound clips and dusted with cicatrine.

Sham adrenalectomised animals received primary and secondary incisions and the adrenals were located. Post-operatively the mice were singly housed with paper towelling bedding. Since the use of phenobarbitones with small rodents is often complicated by the problem of hypothermia (Green, 1979), the mice were allowed to recover in a warm room ($28\pm1^{o}C$) for 24 hours.

Blood sampling

Retro-orbital puncture was used to obtain all the blood samples (Riley, 1960*). This method allows repeated blood sampling from a single animal, and the plasma CORT levels obtained by this method do not differ from those obtained by other methods, such as the commonly used method of decapitation (Nichols, 1980).

The animal was lightly anaesthetised with ether, a small glass tube was inserted into the orbital sinus, and the blood that flows along the glass tube was collected in 400µl plastic tubes. Approximately 300µl of blood was collected, which was heparinised by means of heparin capillary tubes. The blood was then centrifuged for 3-5 minutes at approximately 3,500 rpm, frozen and stored at $-20^{o}C$ until assaying. As a general rule, since there is no significant increase in CORT until 4 minutes following a stressor (Davidson et al., 1968), the blood samples were drawn within 3 minutes from the time at which the animals were first exposed to ether, unless resting levels were required when the samples were drawn within 3 minutes of disturbing the cage. All blood sampling was performed in an

adjacent room to where the mice were housed or tested.

Plasma corticosterone levels

A radioimmunoassay, originally developed by Nichols (1980) and subsequently modified by Bishop (1987) to incorporate an extracted standard curve, was used to determine the total plasma concentrations of CORT. Initially the samples were washed with 2,2,4 trimethyl pentane, extracted into ethyl acetate and then incubated with radioactive (3H) labelled CORT, and antibody specifically raised against this adrenocorticoid. In this assay rabbit anti-corticosterone-21-thyroglobulin serum, supplied by Miles - Yeda Ltd., was used, which only cross reacts significantly (>10%) with progesterone and deoxycorticosterone, and not with cortisol, T, or any other important steroid. Since progesterone is removed during the wash, and since deoxycorticosterone does not occur in significant quantities in mouse plasma (Gross et al., 1972*), the assay has good specificity for CORT in this species. Subsequently, the free and bound fractions were separated and the amount of bound 3H CORT was counted by a Packard Tri-Carb 300 scintillation counter. The counts obtained from the standards were used to construct a standard curve against which the values of CORT were determined. These values were converted to concentrations (ng/ml) using a log transformation incorporated into a computer programme. The recovery of CORT averaged 94% and the mean inter assay variation was 12.9%. The least detectable concentration was less than 5ng/ml.

Where necessary, if the values of the known concentrations indicated that a fault had developed within an assay, the recorded levels of CORT were adjusted in accordance with the degree of error.

The technical assistance of Mr. I. Wright with these assays was greatly appreciated.

Plasma testosterone levels

Total plasma concentrations of T were determined using a radioimmuno assay developed by Wheeler and Luther (1983*). The reagents for the assay were supplied in kit form by St. Thomas's Hospital, London.

Samples were firstly extracted in diethyl ether, without any prior wash or chromatographic purification. Following the evaporation of ether, the extract was redissolved in a buffer solution and incubated together with radioactive (^{125}I) labelled T and sheep antibody, specifically raised against T (Guildhay antisera, University of Surrey, Guildford). This antiserum cross reacts significantly with only 5α-dihydrotestosterone (20%) and less than 0.1% with other steroids.

The bound T was separated by the addition of a second antibody (donkey-antisheep) which adheres to the antibody with its bound T. Testosterone was then counted by a Packard Autogamma Scintillation Spectrometer. A standard curve was constructed using a set of standards, against which the values of T were determined. These values were converted to concentrations (ng/ml) using a least-squares fit incorporated into a computer programme (Amersham Radioassay Curve Fit Program).

The recovery of T averaged 97.6% and the mean interassay variation was 9.7%. The least detectable concentration was less than 1.5ng/ml.

Miss M.J. Bishop kindly performed the T assays and in view of the small number involved the quality controls are by necessity based on a larger number of assays than reported in this thesis.

Data presentation and statistical analysis

If normally distributed, the data is presented as means with standard errors, otherwise it is presented as medians with 95% confidence limits. Parametric statistical tests, including the one way unrelated ANOVA, two way unrelated ANOVA, two way mixed ANOVA and the Tukey test, were applied to data that met the criteria for their use - derived from a random sample,

approximating to a normal distribution - as assessed by the Kolmogorov - Smirnov test, equal variance within groups - as assessed by the F-Max test, and interval or ratio scale. Where necessary the data was transformed, though unless otherwise stated the transformed values are not presented. All the % data was arcsine transformed.

Non parametric statistics were applied to data that did not fulfil all the above criteria, and included the Kruskal-Wallis analysis of variance (KWANOVA) and the Mann-Whitney U test (MWU). Proportional data was analysed by Fisher's Exact Probability Test. All correlational analysis used Spearman's Rank Correlation Coefficent.

$P < 0.05$ was taken as the criterion of statistical significance. Useful statistical references included Siegel (1956); Sokal and Rohlf (1969; 1973); Meyers and Grossen (1978); Greene and D'Oliveira (1982).

Sources of materials

Material	Supplier
Testosterone(17β,Hydroxy-3-oxo-4-androstene)	SIGMA
T oenanthate(4,Androsten-17β-ol-3-one enanthate)	"
T propionate(4,Androsten-17β-ol-3-one propionate)	"
ACTH1-39(Porcine,Grade V 71ius/mg)	"
ACTH4-10	"
Metyrapone(2-Methyl-1,2-di-3-pyridyl-1-propanone)	"
Corticosterone(Δ^4-Pregnene-11β,21-diol-3,20-dione)	"
Olive oil	"
Sesame oil	"
Dimethyl sulfoxide	"
Propylene glycol(Propane-1,2-diol)	B.D.H.
Ethyl alcohol	"
Diethyl ether	"
Hibitane(Chlorohexidine gluconate solution)	"
Sodium chloride(Analar for saline)	"
Cyproterone acetate	Schering Chemicals Ltd.
Tego disinfectant	T.H.Goldschmidt Ltd.
Silastic 738 Dow Corning	Farnell Electronic Components Ltd.
1ml syringes and needles(25gX5/8)	Becton-Dickinson & Gillette Surgical
2ml syringes	Everette Medical Products Ltd.
Cicatrine antibiotic powder	Wellcome Laboratories
Whites torsion balance (range 0.01-5.00mg)	
Fur clippers	Oster International
Stainless steel dissecting equipment	Richardsons of Leicester Ltd.
Michel clip forceps and 7.5mm Michel wound clips	Arnold R. Horwell Ltd.
Sutures (plain sterilised surgical suture. Catgut 1.5 metric 5/0 B.P.)	Arnold Young and Son Ltd.
Suture needles (round bodied Fistula No.4)	MaCarthys Surgical Ltd.
Sagatal (phenobarbitone sodium B.P.) 60mg in 0.1ml	May and Baker Ltd.

CHAPTER 3

The effects of corticosterone on runway distractability

Introduction

The runway distractability test is one of the most commonly used measures of the persistence of attention. Accordingly, this chapter describes the effects of CORT on the level of distractability in the runway. Firstly, the theoretical basis of this test will be discussed, followed by a review of the effects of hippocampectomy on runway distractability and the relevance this has for CORT. Finally, the experimental strategy will be described.

In this test an animal is trained to run a runway for a food reward and upon reaching an asymptotic running time it is exposed to two types of distracting stimuli. Typically, one involves a change in the runway wall, such as the placement of white panels along the black walls. This is regarded as 'irrelevant', as it is unconnected with the goal. The second usually involves a change in the food dish, such as its colour, and is regarded as 'relevant' because of its goal associations. The effect of these distractants is reflected by the 'distraction interval', which is operationally defined as the difference in feeding latency on the distraction trial as compared with the mean latency for the preceeding control trials. According to Gustafson (1975) this provides a sensitive measure of distractability.

The significance of the runway test in the study of persistence was recognised by Archer (1974; 1977). This arose from studies on the effects of T on search behaviour in chicks. Testosterone-treated chicks showed longer runs of pecks on their preferred type of food on a 'pebbled' floor than controls, and longer runs of pecks in a particular area on a 'plain' floor. Moreover, with a 'clustered' floor, once a T-treated chick had eaten all the preferred food in a cluster comprising both preferred and non

preferred food, it often made a single peck towards the non preferred food before moving to another cluster. Despite this, unlike their oil-controls, they rarely changed to search for non-preferred food (Andrew and Rogers, 1972; Andrew, 1972a and 1972b). From these findings Andrew (1972a, p.193) concluded that T' increased persistence of activation or availability of search specifications, once they had come into use'. (This increased persistence refers to the search specifications for both the stimulus and the spatial location). In order to assess the validity of this conclusion, which was based exclusively on search tests, Archer (1974) examined the effects of T on distractability in chicks. Using the runway test he found that T-treated chicks exhibited a decreased level of distractability to irrelevant stimuli, whereas relevant stimuli produced the opposite effect. This was taken as further support for the enhancement of persistence by T; and the finding that T produced a similar effect in mice, pointed to the generality of its influence (Archer, 1977).

Attribution of the effects of T on distractability to an enhancement of persistence relies heavily on the model of attention advanced by Andrew (1976), which is discussed in detail in Chapter 1. It will be recalled that in this model, the rules of selection specify those stimuli that are selected for attention and which thereby come to control behaviour. Furthermore, those stimuli that are not included in these rules are either partially or completely exluded from attention. This is exploited in the runway test as follows. The basic principle is that through training it is possible to determine which stimuli come to control selection: stimuli associated with the goal (i.e., food-dish) captivating attention. As a result, the rules of selection come to specify these 'relevant' stimuli to the exclusion of others ('irrelevant'). Once this has occurred, changes in relevant stimuli would be expected to bring about behavioural changes more easily than changes in irrelevant stimuli. This is because relevant stimuli are more likely to be selected and thus to activate 'recognition units'

corresponding to the original goal; the resulting mismatch between this recognition unit and the altered stimulus causing sustained attention to it (Sokolov, 1963*). The outcome of this is an increased latency to perform the response, which in the case of the runway test is to feed from the food dish. In contrast, since changes in irrelevant stimuli are not likely to be noticed, the final response is unaffected. Thus, the latency to resume the final response following a change in a stimulus reflects the degree to which the rules of selection for that particular stimulus are included in the selection process.

Having established the principles of the test, it is easier to understand Andrew's (1976) claim that if the degree of persistence in the use of the rules of selection is increased, then a greater exclusion of irrelevant stimuli would be expected, and changes in these stimuli should be less effective in disrupting behaviour. Conversely, changes in relevant stimuli should exert a correspondingly more marked effect. This relies on the contention that an increase in the level of persistence results in an increased resistance to shift in the use of central specifications once activated, with a facilitation of the reinstatement of the current rules after a brief interruption (Andrew, 1976). It is important, however, to recollect that the exclusion of irrelevant stimuli is rarely absolute, and that these stimuli may 'slip' through and activate their recognition unit. As a result, examination of unselected stimuli may follow. Whilst this represents a change in the rules of selection, if the level of persistence is high, the response to unselected stimuli is usually only temporary as it tends to be followed by an immediate reinstatement of the original rules. From this it is clear that the relative effectiveness of changes in irrelevant or relevant stimuli in catching and maintaining an animal's attention can be measured by the duration of the interruption of ongoing behaviour and that this will, in turn, reflect the degree of persistence.

Having established the runway distractability test as a suitable

measure of persistence, it is significant that hippocampectomised animals show reduced distraction to irrelevant stimuli placed in the runway compared with their controls, whether the distractant is visual, auditory (Riddell et al., 1969) or tactile (Wicklegren and Isaacson, 1963). Although the effect of hippocampectomy on the level of distractability to a relevant stimulus has not been studied in the runway, hippocampectomised animals are as distracted as their controls by the presentation of a relevant distractant during a sequential task. Furthermore, as in the runway test, they are less distracted by irrelevant stimuli (Douglas and Pribram, 1969). These findings suggest that animals with hippocampal lesions are less able to disengage their attention from those stimuli on which they have learned to focus. This is consistent with the description of the lesion effect as an enhancement of the persistence of attention arising through an impairment of attentional inhibition.

It is important to note that although the effect of hippocampectomy on this phenomenon is relatively consistent, it is nonetheless governed by a number of parameters, other than the relevance of the stimulus, and these may have bearings on the study of the effects of CORT on distractability. The intensity of the distractant, for instance, is important: hippocampectomised monkeys exhibit a reduced level of distractability to loud auditory distractants, but not to less intense ones (Douglas and Pribram, 1969). However, with very loud ones, the level of responsiveness is actually increased above control levels (Coover and Levine, 1972). The effect of a distractant may depend on its intrinsic salience to the animal (Mason and Fibiger, 1978); which is not too surprising, as animals would be expected to possess innate or previously conditioned tendencies to attend to certain stimuli more than others. The response may also be affected by the animal's history, as animals reared in 'enriched' environments exhibit enhanced novelty responsiveness (though this may simply reflect lowered levels of fear). In addition, the characteristics of the background against

which the distracting stimulus is presented are influential: Gustafson and Koenig (1979) found that hippocampectomised rats displayed lowered levels of distractability with intense background noise, but not with less intense background noise.

Despite the existence of these potentially confounding variables, the effect of hippocampectomy on distractability is surprisingly consistent, tending to confirm the role that the HPC is posited to play in attentional inhibition. This view is, however, not without opposition. Harley (1972) argues that the failure to respond to a stimulus does not preclude the possibility that it has been attended to. In support of this, Harley (1972) demonstrated that rats bearing lesions to the HPC were able to learn about superfluous cues in a discrimination task, claiming that this indicates that these animals are not inattentive in a simple fashion. Although this interpretation is tenable, the finding that animals with hippocampal lesions attend to redundant cues does not deny the possibility that such lesions affect attentional inhibition. This is because an increase in the level of persistence does not preclude irrelevant stimuli from gaining attentional control, rather it reduces the likelihood of this occurring. Thus, attending to redundant cues may simply represent a temporary shift in the rules of selection. This, therefore, does not pose a severe threat to the persistence hypothesis advocated here.

Central to this thesis is the proposition that the behavioural effects of CORT arise through its suppression of attentional inhibition at the HPC, which is expressed as an enhancement of persistence. The foregoing discussion was intended to justify the use of the runway test in an examination of this. The hypothesis under investigation being that CORT increases the level of persistence in a similar fashion to hippocampectomy. To date, an investigation into the effects of this adrenocorticoid on distractability is without precedent. Nonetheless, Birke et al. (1979) examined the changes in distractability during the oestrous cycle in rats

and found that it was reduced in females at oestrus, as compared with those at dioestrus. This bears on the effects of CORT, as it is well established that there is a peak of CORT at oestrus (Resko, 1969*), pointing to the possibility that these attentional changes were actually dependent upon CORT.

Any study into the effects of CORT is, however, complicated by the influence of T and ACTH: accordingly both must be controlled. The primary reason for controlling ACTH relates to its intrinsic behavioural activity, which could obscure a CORT effect. The reason for controlling T is three-fold.

i. In view of the role played by T in distractability (Archer, 1974; 1977), the known variations in endogenous T between individuals (Batty, 1978; Bishop, personal communication) could confound experimentally induced changes in distractability.

ii. T may directly, or indirectly influence the circulating levels of CORT, again complicating the experiments beyond interpretation.

iii. Conversely, CORT can influence the circulating level of T, so that observed changes in distractability could not be unequivocally attributed to either hormone.

These latter two possibilities arise because of the interaction between the pituitary-gonadal system (PGS) and the PAS, though the nature of this interaction is unclear. At first it was thought to be mutually inhibitory, at least in males (Brain, 1972a; Mann et al., 1982). Thus, CORT directly inhibited testicular androgen production (Collu et al., 1984). Similarly, stress-induced elevations of CORT inhibited the secretion of T (Armario and Castellarios, 1984). Reciprocally, T inhibited ACTH and adrenocortical activity (Kitay, 1963), in addition to reducing adrenal weight and the production of glucocorticoids (Brain, 1972a).

Nonetheless, the assumption that this negative relationship holds under all circumstances has been questioned. Doerr and Pirke (1976)

demonstrated a diurnal variation in responsiveness: whereas cortisol flattened the nocturnal rise of T, it failed to affect T when administered during the day. Another variable affecting the interrelationship between these hormone axes is the route of administration: central, unlike peripheral ACTH, elevates T (Haun and Haltmeyer, 1975). The recent finding that ACTH exerts a biphasic effect on T, with an initial transitory increase (60-120 mins post-injection) followed by a decrease of T, may resolve some of this apparent conflict (Pitzel et al., 1984a; 1984b; Goncharov et al., 1984). Moreover, Vreeburg (1984) points out that whereas acute ACTH increases the levels of T, chronic ACTH has the opposite effect. Thus, the PAS-PGS interrelationship is not always negative; rather, many variables interact to determine its nature. Whatever the truth of the matter, since changes in the level of adrenocortical activity alter the levels of T and vice versa, it is important to control for this undesirable source of variability.

Although it would have been possible simply to use castrated animals, T-replaced castrates were used for the following reasons. Firstly, as T directly influences distractability it was thought best to work with animals with circulating T levels in the normal range. Secondly, there is some evidence that T and CORT may, under certain circumstances, act synergistically (Heller, 1979; 1984). Also, in a pilot study on the effect of a number of PAS manipulations on distractability (summarised in APPENDIX 1) T appeared to alter the influence of these manipulations on behaviour. However, it is difficult to know whether it was acting directly or by altering CORT levels. Having decided to use T-replaced castrates, experiments were devised to determine an effective dose. At the same time this verified the suitability of the runway and distractants for TO mice, as the performance of this strain of mice in the runway test has never before been reported.

Although initial studies failed to demonstrate a significant effect of

T on distractability, with non-replaced castrates displaying similar levels of distractability to intact controls (EXPERIMENT 1), high levels of T exerted the predicted effects (EXPERIMENT 2). In an attempt to account for the finding that an absence of T did not affect distractability, the possibility of adrenal compensation of androgens was explored. Both the levels of T in non-replaced castrates (EXPERIMENT 3) and the effects of an anti-androgen upon distractability (EXPERIMENT 4) were assessed.

Once an appropriate replacement dosage of T had been determined the effects of CORT on runway distractability were examined. Initially, various PAS manipulations were used in an attempt to independently vary the levels of CORT and ACTH, and in so doing to distinguish those effects that were truly attributable to CORT from those for which ACTH was responsible (EXPERIMENT 6). The subjects of this experiment were adrenally intact with controlled levels of T.

Despite the adoption of these lengthy measures, none of the PAS manipulations were found to exert a significant effect. This may have been because the animals were adrenally intact, and endogenous CORT was present in high quantities, effectively preventing the exogenous CORT exerting an effect. This seemed especially likely as the test involves food deprivation, and the daily CORT peak entrains to the presentation of food under these conditions (Krieger, 1974; Levine and Coover, 1976). In order to circumvent this, CORT was examined in mice with controlled baseline levels of the steroid (EXPERIMENTS 7 and 8). The gonads were present in the subjects of these experiments for two reasons. First and foremost, in view of the difficulty in removing the adrenals, combining this with castration seemed impractical. It would have heightened the surgical trauma, increasing the risk of the loss of animals. Secondly, as fluctuations in CORT levels cannot occur in the absence of the adrenals, any problems connected with T affecting the levels of this adrenocorticoid are precluded. However, as the possibility still remains of T affecting the

levels of ACTH (Colby, 1978) and of CORT altering the levels of T, this approach is really a pragmatic compromise.

In connection with the experiments concerned with CORT: although its effect on food intake is a controversial issue (Rees and Gray, 1984), and any such effect would have profound consequences for the degree to which an animal focusses on the food dish and excludes stimuli unassociated with it, CORT does not appear to affect the sensitivity to appetitive motivation. APPENDIX 5 shows that the amount eaten under both ad lib. and 12 hour deprivation conditions is independent of the circulating levels of CORT.

A brief overview of the experimental procedure is now given in the general method section. This procedure retained its essential form throughout, though various modifications were introduced and are detailed where appropriate. It is important to note that the early experiments used an irrelevant distractant only, after Wicklegren and Isaacson (1963). Initially this was believed to suffice in assessing attention. Only later was it realised that it was difficult to place any confidence in an interpretation based on the response to one class of distractant. The reason for this is that apparent differences in distractability might simply reflect differences in novelty responsiveness rather than attention per se. In order to exclude a novelty interpretation, the relative level of distractability to both irrelevant and relevant stimuli must be assessed: if an animal exhibits differential responding to the two classes of distractant, then a simple change in novelty responsiveness cannot be invoked as the explanation. Accordingly in later experiments both an irrelevant and a relevant distractant were used.

General Method

Subjects

As a general rule intact males were transferred from group to single housing conditions 3 days prior to the start of testing. Castrated and sham castrated mice were singly housed for 2 weeks post surgery, after which they were returned to group housing conditions for 7 days, before being singly housed as above. In studies using adrenalectomised subjects, these animals were singly housed from the day of surgery until testing was complete. Their intact controls were housed in a similar fashion. The mice were maintained on a 12 hour food deprivation regime throughout the course of behavioural testing.

Apparatus

A straight runway made of black perspex was used with sliding doors, held by magnetic catches, at each end enclosing the start/goal box (70x11x11cm) - see Figure 3:1. A clear perspex food dish with crushed food pellets was introduced through a hole in the side wall of the goal box. A clear perspex lid, enabling observation, enclosed the runway. The runway stood on a detachable wooden base, with melamine finish, which was covered with a fresh sheet of absorbent paper for each animal.

Two identical runways were used: whilst observations were being made from one, a mouse was habituating in the other. Two distractants were used:

1. Two white panels (7x11cm) which were placed half way along the runway walls.
2. A black food dish

Procedure

On the third day of single housing the mice were placed on a 12 hour food deprivation regime. The mice were weighed beforehand and daily thereafter (using a Sartorious 1404MP8 electronic balance) to ensure that

they remained within 80% of their original body weight.

The distractability test comprised 3 phases: pretraining, training and testing.

1. Pretraining - consisted of 10 minutes in the runway with food present at either of its ends.

2. Training - commenced the following day. The mice were given 6 trials a day for 5 days. At the beginning of each session the mouse was placed in the runway, with both doors open, but without food. After 7 minutes habituation it was enclosed at one end of the runway, which then acted as the start box, and food was introduced into the goal box. After 15 secs the door of the start box was raised, and the time taken to leave the start box, traverse the runway and feed uninterruptedly for 4 secs was recorded. A stopwatch was started as the door was raised - if 60 secs elapsed without the mouse leaving the start box, the door was closed, and the next trial was started, the first being recorded as 60 secs. When the mouse left the start box the stop watch was reset. However, if the mouse failed to eat within 60 secs, this trial was also recorded as 60 secs, and the next trial was started. Both the start box and goal box door were closed after the mouse to prevent retracing.

In order to ensure that the mouse fed for 4 secs, a second stopwatch was started when the mouse commenced feeding. Both stop watches were stopped together when this feeding criterion was reached. If necessary the second stopwatch was restarted if feeding was interrupted before the feeding criterion had been reached. (A feeding criterion was implemented because a distractant may lead to an interruption of feeding rather than a delayed onset of feeding - Archer, 1977). The 4 secs feeding time was subtracted from the trial duration to obtain the actual running time. Once the feeding criterion had been reached the mouse was allowed to feed for an additional 10 secs before the food dish was removed. This point constituted the end of the trial. The mouse remained in the goal box, which then acted

as the start box for the following trial. The intertrial interval was 15 secs.

On the fifth day of training those mice reaching the criterion of less than an average of 8 secs running time were tested for their distractability the following day; those exceeding it were excluded from further testing.

3. Testing

There were 2 testing procedures.

1. White panels only

The mice were allowed 7 minutes habituation, as before, followed by 5 'control' trials, which were the same as those during training. These control trials were followed by a single distraction trial, in which the panel distractant was introduced into the runway. The time taken to leave the start box and feed was recorded.

2. White panels and black food dish

In this procedure there were two test trials, which were given on different days. On the first, the mice were allowed 7 minutes habituation, followed by 3 control trials, after which a single distraction trial was given. Either the panel or the dish distractant was presented during this trial. Finally, 2 further control trials were given. On the second, the other distractant was presented. The presentation of the two distractants was counterbalanced within each condition so that half the subjects in any one condition were presented with the panels first, whereas the other half were first presented with the dish. In order to minimise any effects that the presentation of one distractant might have on the level of responsiveness to the second, the two days of testing were separated by a training day, comprising 6 control trials.

In this procedure both testing trials were video-recorded. For those distraction trials in which the dish was presented the animals' behaviour toward the dish was recorded for 30 secs, once the mouse had approached it.

The animals were coded to minimise experimenter bias during the analysis of the video data and the following measures were analysed:

i. Dish distractant:-

a. time spent examining dish } in 30 secs

b. time spent eating }

ii. Panel distractant:-

a. time spent examining panels

Subsequently, the proportion of time spent in examination of the panel distractant was computed, and this is expressed as a percentage of the duration of the distraction trial. (The rationale behind this is that it provides a means of assessing the relative contribution made to the distraction interval by the time spent investigating the distractant as opposed to the time spent in general exploration of the runway).

In both the "white panels only", and the "white panels and black food dish" procedures the distraction time was obtained by subtracting the mean running time for the control trials preceding the distraction trial from the running time in the distraction trial.

Any mouse that failed to reach the running criterion during testing was excluded and compensatory animals were run at a later date. All training and testing was carried out between 12.00-4.00 pm.

Hormone treatments

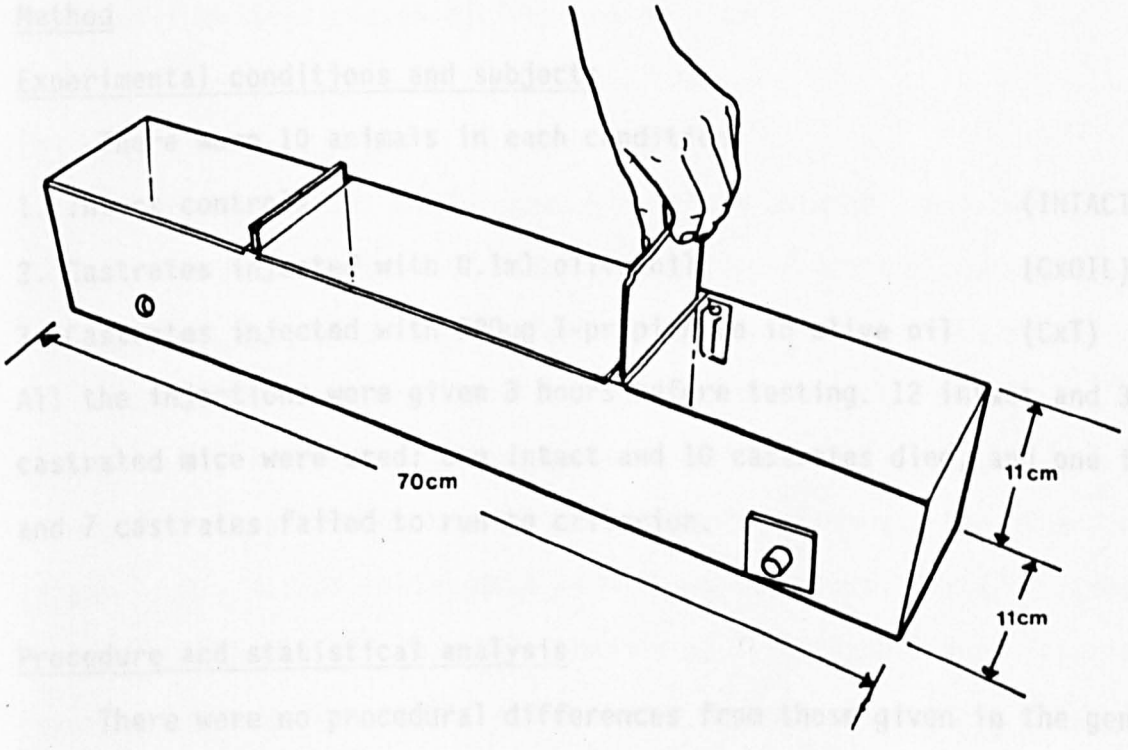
The details of the various hormone treatments are given in the relevant sections.

As a general rule, long-acting compounds were given immediately after the last session of training (approximately 24 hrs pre-test), whereas shorter acting compounds were administered on the day of testing at a specific interval prior to testing.

All the injections were given subcutaneously. A further generalisation is that the mice were assigned to one of the treatment conditions on an

arbitrary basis. As the order of training and testing was determined by a pre-set sequence, this controlled for the order in which the various treatment conditions were tested: more precise details are given where necessary. Finally, the treatments were coded, unless it is specified otherwise, so distractability testing was performed blind with respect to the injected groups.

Figure 3:1 Diagram of runway.



EXPERIMENT 1

The effects of castration and testosterone replacement on distractability

Whereas Archer (1977) administered 7.5mg T-oenanthate to male mice 48 hours prior to testing, the effects of 500µgT- propionate administered 3 hours pre-test were examined. This dose was based on Samuels et al. (1981). A short-acting T ester was employed because unlike the testing regime adopted by Archer (1977), which extended over 2 days, there was only a single testing session.

Method

Experimental conditions and subjects

There were 10 animals in each condition:

1. Intact controls (INTACT)
2. Castrates injected with 0.1ml olive oil (CxOIL)
3. Castrates injected with 500µg T-propionate in olive oil (CxT)

All the injections were given 3 hours before testing. 12 intact and 37 castrated mice were used: one intact and 10 castrates died, and one intact and 7 castrates failed to run to criterion.

Procedure and statistical analysis

There were no procedural differences from those given in the general method. White panels only were used. The data is expressed as means ±S.E., and was analysed by the KWANOVA.

Results

Table 3:1 The effects of castration and testosterone replacement on distractability (Means ±S.E.)

CONDITION N=10	DISTRACTION TIME-SECS
INTACT	5.4±1.0
CxT	12.7±5.3
CxOIL	18.7±6.3

KWANOVA: H=3.7

There were no significant differences detected.

Discussion

As no significant differences were found between treatments, the formal conclusion must be that the prediction of greater distractability in non replaced castrates was not confirmed. However, the trend of the results was in the predicted direction, and the differences, though not statistically significant, were quite large. The fact that the distraction time for the replaced castrates was intermediate between the other two values suggests that replacement with T was only partially effective. The failure to find an effect of the absence of T was surprising. There are, however, at least 4 possible explanations for this:-

1. It is possible that the panel distractant was unsuitable, though this is unlikely, as the level of distractability observed was similar to that found previously in mice (Archer, 1977). (The importance of selecting the appropriate distractants is considered more fully in the general discussion).

2. In view of the known strain differences in T sensitivity (Poggioli et al., 1984), and the finding that it has a dose-dependent effect on attentional phenomenon - with moderately high levels possessing the greatest behavioural activity (Andrew and Rogers, 1972) - it is possible

that its effects are confined to higher levels in TO mice (see EXPERIMENT 2).

3. Although castration removes the major source of androgens, the effects of removing the gonads on T levels are complicated. This is because other sources of androgens exist. For instance, the adrenal cortex is recognised to secrete weak androgens (Odell and Parker, 1985; de Kloet and Veldhuis, 1985), though there is little agreement over the extent to which this occurs. Even though Kime et al. (1980) acknowledge the potential of the adrenal cortext to release androgens, they claim that the level of androgens from this source following castration is very low. By contrast, Ando et al. (1986) report that whereas the level of T was low 5 days post-castration, its level 47 days after the removal of the gonads was markedly elevated. This was attributed to an increased secretion of androstenedione from the adrenals, which was subsequently converted to T. The capacity of the adrenal to secrete androgenic steroids has been corroborated by the report that in addition to acting directly on the testes, ACTH acts on the adrenals in gonadectomised animals to raise androgen output (Chambers, 1982). In view of this, it is possible that adrenal androgen secretion is increased in castrates in order to compensate for the absence of gonadal androgens. If this occurs, it would readily explain the difficulty that has been encountered in trying to demonstrate an effect of castration on distractability. Furthermore, although it is possible that the weaker androgens originating from the adrenal are sufficient to reinstate the behavioural deficiencies, it is also possible that they are converted into more potent androgens, with enhanced behavioural activity (Odell and Parker, 1985; Ando et al., 1986). The plausibility of this 'compensation hypothesis' as an explanation of the failure to find an effect of the absence of T on distractability can be explored in two ways. Firstly, the levels of T in non-replaced castrates can be assayed (see EXPERIMENT 3). Secondly, the level of distractability

in animals in which the effect of T, regardless of its source, have been nullified by treatment with an antiandrogen can be assessed (see EXPERIMENT 4).

4. Last, but by no means least; since TO mice are an outbred strain, the inability to detect a statistically significant difference in distractability may simply relate to the increased level of variance in outbred strains that effectively obscures any real effect that might be present.

EXPERIMENT 2

The effects of a high replacement dose of testosterone on distractability

The objective of this experiment was to determine whether a high replacement dose of T produced the expected effects on distractability.

Method

Experimental conditions and subjects

There were 10 animals in each condition:

1. Intact controls (INTACT)
2. Sham castrates (SHAM Cx)
3. Castrates injected with 500µg T-propionate immediately after the last training session, followed by a second injection of 500µg T-propionate 3 hours prior to testing. The vehicle was 0.1ml olive oil (CxT)

10 intact, 10 sham castrates and 12 castrates were used, though 2 castrates failed to run to criterion.

Procedure and statistical analysis

There were no procedural differences from those given in the general method; white panels only were used. The mice were blood sampled immediately after testing. These samples were subsequently assayed for CORT, and the results of this are presented in EXPERIMENT 5.

The data is expressed as means ±S.E.

The level of distractability of the intact controls run in EXPERIMENT 1 was compared with that of their counterparts run in this experiment using the MWU test. As there were no significant differences, the distractability data from the non-replaced castrates from EXPERIMENT 1 was analysed in conjunction with the data from EXPERIMENT 2 using the KWANOVA, followed by the MWU test.

Results

Table 3:2 The effects of castration and a high level of testosterone replacement on distractability (Means±S.E.)

CONDITION N=10	DISTRACTION TIME-SECS
INTACT[b]	8.6±2.4
SHAM Cx	7.6±4.3
CxT	3.2±1.0
CxOIL[+a]	18.7±6.3

+MWU INTACT from EXPERIMENT 1 as compared with INTACT from EXPERIMENT 2: U=41 Not significant.

KWANOVA: H=12.5, $p<0.05$

a. MWU different from CxT: U=12, $p<0.001$

b. MWU different from CxT: U=21, $p<0.05$

Discussion

The report of a dose-dependent effect of T (Andrew and Rogers, 1972) was substantiated by the present finding. Those animals with very high levels of T were significantly less distractable than either their intact controls or their non-replaced counterparts. Since this effect is confined to high levels this may imply that it is pharmacological. Nonetheless, since there was, once again, a trend in the data at the lower range of T, with non-replaced castrates displaying the highest distraction intervals, the explanation may simply be that high T levels are needed to magnify the behavioural effects and thereby to overcome the confounding effect of individual variance. The possibility remains, however, that the failure to find an effect of castration reflects the presence of adrenal androgens: this is examined in more detail in the following experiments.

EXPERIMENT 3

The levels of testosterone in non-replaced castrates

Method

Subjects and procedure

4 TO mice were castrated at 12 weeks of age. At 25 weeks, following 4 days of individual housing, they were blood sampled. All samples were taken at 11.00 a.m., within 3 minutes of disturbing the cage. Care was taken to ensure that the animals were not otherwise disturbed prior to sampling.

Results and discussion

The mean plasma level of T was 3.75±1.2ng/ml. This compares with values in the range of 5-20 ng/ml for intact males of a similar age and identical strain, recorded under the same sampling conditions in this laboratory (M. Bishop, personal communication). Even though only 4 mice were used, it seems justified to draw the conclusion that 13 weeks after castration even the low levels of T might be sufficient to prevent an effect of castration on distractability. However, it is important to realise that these T levels were obtained from long-term castrates whilst the behavioural studies were carried out on shorter term castrates. Consequently, these T levels may provide a conservative measure of the degree to which the adrenal compensates for the absence of gonadal steroids. Indeed, Ando et al. (1986) suggest, adrenal compensation for castration takes time.

EXPERIMENT 4

The effects of the antiandrogen cyproterone acetate on distractability

Cyproterone acetate (CYPA) is one of the most commonly used antiandrogens. By definition an antiandrogen is a substance that acts to prevent an androgen from expressing its activity at its target sites, rather than to reduce the synthesis or release of releasing factors, gonadotrophins or androgens (Neumann and Steinbeck, 1974). Cyproterone acetate not only acts in this way, but has 'progestational' activity, suppressing gonadotrophin release, thus also lowering endogenous T (Neumann and Steinbeck, 1974; Prove and Immelmann, 1982). However, the latter authors claim it is also a partial androgen agonist. Despite this, Thompson and Wright (1979) showed that its effects on distractability resemble those of surgical castration, and on this basis it was selected for use here.

Method

Experimental conditions and subjects

There were 10 animals in each condition:

1. Intact controls (INTACT)
2. Intacts injected with 0.1ml vehicle (INTACT + (80% sesame oil: 20% dimethylsulfoxide-DMSO) (VEHICLE)
3. Intacts injected with 2mg CYPA in the vehicle (INTACT + (after Jones and Nowell, 1974) CYPA)

(The CYPA was firstly dissolved in the DMSO, then diluted in the sesame oil, forming a fine emulsion).

The treatments were given daily, commencing 5 days prior to the onset of behavioural testing and continuing throughout. 36 intact mice were used, though one intact control, 2 CYPA and 2 vehicle controls did not run to criterion and one animal treated with CYPA died. The animals were singly housed 7 days prior to the start of testing.

Procedure

There were no procedural differences from those given in the general method, except that the training and testing was conducted between 5.00-8.00pm. Both white panels and black food dish were used. The mice were blood sampled immediately after the second day of testing and the samples were subsequently assayed for T.

Statistical analysis

The behavioural data is presented as means ±S.E. The dish and panel data were analysed separately throughout. The distraction times were log transformed (LOGX+1) prior to being analysed by the two way unrelated ANOVA (treatment x order of presentation). As the order of presentation proved to be insignificant, data was subsequently combined with respect to this variable and the time spent examining the dish and panel distractants, and the %time examining the panels were analysed using a one way unrelated ANOVA. The time spent feeding from the food dish during a 30 sec period was also analysed by a one way unrelated ANOVA.

The T data is presented as means ±S.E and was analysed using a one way unrelated ANOVA. Spearman's Rank Correlation Coefficient assessed the degree to which the levels of T correlated with the level of distractability to the dish, and the panel distractants, both within conditions, and when the data from the different conditions had been combined.

Results

Table 3:3 The effects of CYPA on distractability (Means±S.E.)

CONDITION N=10	DISTRACTION TIME-SECS DISH	PANEL
INTACT	1.36±0.7	3.2±1.03
INTACT + VEHICLE	2.67±1.3	4.8±0.8
INTACT + CYPA	0.84±0.4	6.8±1.7

TWO WAY UNRELATED ANOVA: DISH

Treatment F(2,24)=0.62, p=0.55

Order of presentation F(1,24)=0.20, p=0.66

Interaction F(2,24)=0.67, p=0.52

TWO WAY UNRELATED ANOVA: PANEL

Treatment F(2,24)=1.97, p=0.16

Order of presentation F(1,24)=1.19, p=0.28

Interaction F(2,24)=0.45, p=0.64

There were no significant differences to either distractant.

Table 3:4 The effect of CYPA on the time spent feeding (Means±S.E.)

CONDITION N=10	TIME FEEDING-SECS
INTACT	20.6±1.2
INTACT + VEHICLE	19.0±1.9
INTACT + CYPA	18.9±1.3

ONE WAY UNRELATED ANOVA: F(2,27)=0.367, p=0.696

There were no significant differences detected.

Table 3:5 The effect of CYPA on the time spent examining the panels (Means±S.E.)

CONDITION N=10	TIME INVESTIGATING-SECS
INTACT	2.5±0.92
INTACT+VEHICLE	2.55±0.68
INTACT+CYPA	4.2±0.86

ONE WAY UNRELATED ANOVA: F(2,27)=1.481, p=0.245

There were no significant differences detected.

Table 3:6 The effect of CYPA on the % time spent examining the panels (Means±S.E.)

CONDITION N=10	%TIME INVESTIGATING
INTACT	28.7±7.5
INTACT+VEHICLE	30.9±6.1
INTACT+CYPA	42.8±6.8

ONE WAY UNRELATED ANOVA: F(2,27)=1.431, p=0.257

There were no significant differences detected.

Table 3:7 The effect of CYPA on the time spent examining the dish (Means±S.E.)

CONDITION N=10	TIME INVESTIGATING-SECS
INTACT	2.56±0.53
INTACT+VEHICLE	3.4±0.88
INTACT+CYPA	1.4±0.44

ONE WAY UNRELATED ANOVA: F(2,27)=1.847, p=0.18

There were no significant differences detected.

Table 3:8 The effect of CYPA on testosterone levels (Means±S.E.)

CONDITION N=10	T ng/ml
INTACT	1.5±0.5
INTACT+VEHICLE	2.0±0.6
INTACT+CYPA	1.3±0.2

ONE WAY UNRELATED ANOVA: F(2,27)=0.689, p=0.511

There were no significant differences detected.

Table 3:9 The results of the correlational analysis between testosterone and distractability - within conditions

	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos	
CONDITION N=10	DISH	PANEL
INTACT	0.34	-0.08
INTACT+VEHICLE	-0.05	-0.24
INTACT+CYPA	0.15	0.32

There were no significant within condition correlations. Also, no significant correlation was detected between T and distractability to the dish and panel distractant when the conditions were combined to give N=30. The observed value of rho being 0.16 and -0.09 respectively.

Discussion

As no significant differences were found, it must be concluded that even when all sources of T have been rendered ineffective, its predicted effects upon distractability do not emerge. Consequently, the hypothesis that the adrenal increases its output of androgenic steroids in response to castration, and that this compensatory increase obscures an effect of castration on distractability must be rejected.

As the levels of T in the intact controls were well below the predicted 5-20ng/ml range - which was probably due to the change in the time of sampling - this may explain why no effect on distractability emerged.

Although it was surprising to find that the T levels were unchanged after CYPA treatment, this does not necessarily undermine its effectiveness. After all, it is primarily an antiandrogen, and therefore acts to block the effects of T at its receptors, rather than to reduce its levels in the plasma.

The conclusions from the experiments reported so far can be summarised as follows:-

1. The effect of castration on runway distractability could not be confirmed to the level of statistical significance, although a clear trend was shown.
2. Low levels of T, presumed to be of adrenal origin, are present in castrates. Even so, as CYPA was no more effective than castration at altering distractability, this is probably not important. However, as CYPA is thought to act as a partial agonist, there remains the possibility that

it reinstated some of the behavioural effects of T, and therefore that adrenal compensation might be of some importance.

3. A single dose of 500µg T was without a clear effect: two successive 500µg doses significantly reduced distractability.

4. The difficulty in showing the effects of castration and T is probably due to individual variation in T levels and/or behavioural responsiveness to this androgen, perhaps associated with the use of an outbred strain.

Although the effects of T on distractability have not been as easy to demonstrate as first anticipated, with high levels being needed to bring the effect to statistical significance, the present series of experiments not only confirms that T does influence distractability but also that this test is useful under our conditions.

As the main objective of this thesis is to study the role of CORT in attention, the effects of the T replacement regime upon CORT levels are important, and this will now be examined, before the main experiments are reported.

EXPERIMENT 5

The effects of changes in the level of testosterone on plasma corticosterone levels

The objective of this experiment was to determine the effect of two successive doses of 500µg T-propionate on the plasma levels of CORT. The controversy surrounding the PAS-PGS inter-relationship has already been alluded to.

Method

The blood samples taken from the animals in EXPERIMENT 2 were used for this study.

Statistical analysis and results

The CORT data is expressed as means ±S.E and was analysed by the KWANOVA, followed by the MWU test.

Table 3:10 The effects of testosterone on plasma corticosterone levels (Means±S.E.)

CONDITION N=10	CORT ng/ml
INTACT	105.2±9.9
SHAM Cx[a]	199.9±25.2
CxT[b]	352.9±53.5

KWANOVA: H=18.00, $p<0.05$

a. MWU different from INTACTS: U=7, $p<0.005$

b. MWU different from SHAM Cx: U=22, $p<0.05$

Discussion

The finding that sham castrates had significantly higher CORT levels than intacts was at first difficult to explain. Brain (1971) cites evidence

that the experience of defeat in a fight (regarded as a short-term stressor) increased adrenal weight and elevated plasma CORT levels. Furthermore, these defeated animals responded to subsequent attack by a greater increase in CORT than usual. These changes were attributed to a 'carry-over' or proactive effect of the original stressor on subsequent stress responses. In their discussion of the proactive effects of stress, Murison et al. (1986) conclude that although the degree of cross-tolerance is a debatable issue, it is well established that prior exposure to a stressor potentiates the response elicited by subsequent stressors. However, the various indices of the stress response respond differentially to pre-exposure (Murison and Isaksen, 1980), and the nature of these proactive effects is determined by a number of variables, not least, the species of the animal (Armario and Castellarios, 1984). In this light, elevated levels of CORT in sham castrates may reflect the proactive effects of the stress of surgery on subsequent responses to stressful experiences.

As an aside, proactive effects are claimed to be confined to the responsivity of the PAS, and to be without effect on its basal level of activity (Sakellaris and Vernikos-Danellis, 1975; Hennessy et al., 1977). A comparison of CORT levels under resting conditions in chronic castrates and intact males reported in APPENDIX 2 confirmed this. Two indices of PAS activity were examined: revealing similar CORT levels (one way unrelated ANOVA $F(1,18)=0.094$, $p=0.76$) and adrenal weights (one way unrelated ANOVA $F(1,18)=1.769$, $p=0.2$).

Whilst it is difficult to present an unequivocal explanation of the elevated levels of CORT in the T-replaced castrates, as they had higher CORT levels than the sham castrates, it is clear that the explanation can not simply be found in the proactive effects of the stress of surgery. Rather the high levels of T may have been stimulating the release of CORT from the adrenals. Nonetheless, since there was no vehicle control, the elevated levels of CORT may have arisen as a result of the stress

associated with the injection procedure, especially as the proactive effects of surgery might be expected to potentiate this. The relationship between CORT and T would have been clearer if the levels of CORT present in non-replaced castrates had been determined.

The possibility that T stimulates the release of CORT has received tentative support from an early series of distractability experiments, which examined the effects of a number of different PAS manipulations (see APPENDIX 1). Although the behavioural data is not discussed here, a number of points relevant to the effects of T on CORT levels emerged from these experiments. For instance, T-replaced castrates (2x500µg T-propionate) treated with 200µg CORT appeared to possess higher levels of CORT than their non-replaced counterparts treated with the same dose of CORT. There remained, however, the possibility that this was due to the stress of repeated injection involved in the T-replacement regime, as this was not controlled for. Nonetheless, this is unlikely as intact males treated with 200µg CORT also appeared to possess higher levels of CORT. Despite this, it is difficult to arrive at a definite conclusion concerning the effects of T on CORT levels from these results, as there were a number of methodological shortcomings which made it impossible to compare the different conditions directly.

A further point that deserves to be mentioned is that the effects of T would appear to be confined to conditions of PAS activity, as it has been shown that non-replaced castrates and intact males have similar CORT levels under conditions of rest (see APPENDIX 2).

To conclude; although the evidence is suggestive of a stimulatory effect of T on the PAS, this is a very contentious subject. Whatever the relationship between the PAS and the PGS is proven to be, this will have profound implications for any examination concerned with CORT.

EXPERIMENT 6

The effects of manipulations of the pituitary-adrenal system on distractability in adrenally intact mice

According to Di Giusto et al. (1971) there are basically 4 conditions that can occur within the PAS i.e., high ACTH-high CORT; low ACTH-low CORT; low ACTH-high CORT; high ACTH-low CORT, and from a comparison of the effects emerging from each of these it should be possible to distinguish the effects of CORT. In this light, it seemed appropriate to implement the following conditions:-

i. Administration of ACTH - this results in high levels of both ACTH and CORT.

ii. Administration of CORT - this results in low ACTH and high CORT. Corticosterone through the negative feedback system depresses the levels of ACTH within an hour of its administration (Klein, 1975).

iii. Administration of metyrapone - this results in high ACTH and low CORT levels.

Metyrapone blocks the activity of the enzyme 11-β-hydroxylase in the adrenal and thereby prevents the conversion of 11-deoxycortisol to cortisol (Jenkins et al., 1958). ACTH levels are elevated by virtue of the low levels of CORT which release the pituitary from the negative feedback influence of CORT.

An alternative means of achieving high levels of ACTH in conjunction with low levels of CORT that was adopted involved the administration of the heptapeptide ACTH 4-10. This peptide fragment corresponds to the behaviourally active core of the ACTH molecule, yet it is devoid of adrenocorticotropic activity (de Wied, 1969). The extra-adrenal nature of the actions of this peptide enable the examination of the effects of ACTH in isolation of its influence on the adrenal cortex. (This is however an over simplification, as the presence of the adrenals confers the capability to respond to any arousing aspect of the experiment, whereupon increased

levels of CORT would coincide with high levels of ACTH).

This approach also provided the opportunity to examine the possibility that ACTH and CORT exert interactive effects upon behaviour following Bohus et al. (1982). As an extension of this interactive approach the effect of the various PAS manipulations were examined both in the presence, and in the absence of T. The rationale for this derived from the possible behavioural significance of an interaction between the PGS and PAS, both at physiological and behavioural levels, alluded to earlier.

Finally, as CORT is known to exert a dose-dependent effect on numerous facets of behaviour (e.g., Kovacs et al., 1976; 1977) the possibility that different doses of CORT influence distractability differentially was also examined.

Method

Experimental conditions and subjects

(see Table 3:11 for details).

There were 10 animals in each condition.

NOTE: + = injected with

1. INTACT CONTROLS	(I)
2. SHAM CASTRATES	(SCx)
3. CASTRATES + OIL	(CxOIL)
4. CASTRATES + 7.5mg T-OENANTHATE	(CxT)
5. CxT + 50µg CORT	(CxT CORT 50)
6. CxT + 100µg CORT	(CxT CORT 100)
7. CxT + 150µg CORT	(CxT CORT 150)
8. CxOIL + 100µg CORT	(CxOIL CORT 100)
9. CxT + 0.5 iUS ACTH1-39	(CxT A0.5)
10. CxT + 1.0 iUS ACTH1-39	(CxTA1.0)
11. CxT + 2.0 iUS ACTH1-39	(CxTA2.0)
12. CxOIL + 1.0 iUS ACTH1-39	(CxOILA1.0)
13. CxT + 0.002mg ACTH4-10	(CxTA4-10)
14. CxOIL + 0.002mg ACTH4-10	(CxOILA4-10)
15. CxT + 5mg METYRAPONE	(CxT MY)
16. CxOIL + 5mg METYRAPONE	(CxOIL MY)
17. CxT + CORT VEHICLE	(CxT CORTV)
18. CxT + NaCl	(CxTNaCl)

Table 3:11 Summary of experimental treatments

TREATMENT	DOSE	REFERENCE
TESTOSTERONE OENANTHATE	7.5mg in 0.1ml olive oil. Single dose immediately after last training session.	Archer (1977) APPENDIX 4
CORTICOSTERONE	CORT50=50µg CORT in 0.1ml vehicle CORT100=100µg CORT in 0.1ml vehicle CORT150=150µg CORT in 0.1ml vehicle Corticosterone administered one hour prior to distractability testing.	Leshner et al., (1980) Klein (1975)
ACTH1-39	A0.5=0.5ius in 0.1ml vehicle (0.9%NaCl) A1.0=1.0ius in 0.1ml vehicle A2.0=2.0ius in 0.1ml vehicle ACTH1-39 administered one hour prior to distractability testing.	Leshner et al., (1973)
ACTH4-10	0.002mg in 0.1ml vehicle (0.9%NaCl) ACTH4-10 administered on hour prior to distractability testing.	Brain (1972b).
METYRAPONE	5mg in 0.1ml vehicle (0.9%NaCl) Single dose administered immediately after last training session.	Based on Bialik (1984) APPENDIX 3
CORTVEHICLE	0.1ml 1 96% ethanol: 9 0.9% NaCl Administered one hour prior to distractability testing.	Van Wimersma Greidanus (1970)
NaCl	0.1ml 0.9% saline. administered one hour prior to distractability testing.	

203 castrates, 14 sham castrates and 11 intact mice were used, though 14 castrates and 2 sham castrates died and 29 castrates, 2 sham castrates and 1 intact did not run to criterion.

Procedure

There were no procedural differences from those given in the general method; white panels and black food dish were used.

The mice were run in 18 batches, with only one subject from each condition present in any one batch. This also applied where it was necessary to run compensatory animals. Within repeated runs the order of

testing was rotated, so that if the intact condition was run first in batch 1, it was run second in batch 2, and eighteenth in batch 18. The mice were blood sampled immediately after the second distraction test, and the samples were subsequently assayed for CORT.

Statistical analysis

All the behavioural data is presented as means ±S.E. The dish and panel data was analysed separately throughout. The distraction times for both the dish and panel distractants were log transformed (LOG X+1) prior to analysis using a two way unrelated ANOVA (treatment x day of testing). In the event of the order of testing proving to be without effect, the data obtained from the video analysis was combined irrespective of this variable and analysed by a one way unrelated ANOVA.

As there were a large number of experimental treatment groups run together, the data was sub-grouped into the most appropriate combination of conditions and analyses were carried out within these groupings.

For CORT:-

The effects of CORT in the presence of T

CxT, CxTCORT50, CxTCORT100, CxTCORT150, CxTCORTV

The effects of CORT in the absence of T

Cx OIL, CxOILCORT100

The interactive effects of CORT and T- this was assessed by comparing the effects of the same dose of CORT in the presence of T and in its absence.

CxTCORT100, CxOILCORT100

For ACTH1-39

The effects of ACTH1-39 in the presence of T

CxT, CxTA0.5, CxTA1.0, CxTA2.0, CxTNaCl

The effects of ACTH1-39 in the absence of T

CxOIL, CxOILA1.0

The interactive effects ofACTH1-39 and T

CxTA1.0, CxOILA1.0

For ACTH4-10

The effects of ACTH4-10 in the presence of T

CxT, CxTA4-10, CxTNaCl

The effects of ACTH4-10 in the absence of T

CxOIL, CxOILA4-10

The interactive effects of ACTH4-10 and T

CxTA4-10, CxOILA4-10

For Metyrapone

The effects of MY in the presence of T

CxT, CxTMY

The effects of MY in the absence of T

CxOIL, CxOIL MY

The interactive effects of MY and T

CxTMY, CxOIL MY

The effects of T were assessed by comparing the following conditions:-

INTACT, SHAMCx, CxT, CxOIL

The CORT data is presented as means ±S.E. It was sub-grouped as above, and analyses were carried out within these groupings. The rationale for analysing those conditions in which T was present separately from those in which it was absent relates to the effects T might have on the levels of CORT. The CORT data was analysed using a one way unrelated ANOVA, followed

by the Tukey test.

Spearman's Rank Correlation Coefficient was used to assess the degree of correlation between the levels of CORT and distractability. The correlations were computed on those groups that had been subjected to similar treatments:

e.g., CxT, CxTCORT50, CxTCORT100, CxTCORT150

and which therefore, would be expected to possess similar endocrine states. The CxT condition acted as the baseline in all the correlational analyses. By combining treatment groups which differed in one respect only, the sample size was increased and it was hoped that the presence of a significant correlation would be more easily detected as a result.

Although it is likely that the levels of CORT would be very similar on the two days of testing, since this assumption may not be valid, the correlational analysis was restricted to the level of distractability to whichever distractant was presented immediately prior to blood sampling and the levels of CORT obtained at this point. This, however, resulted in small samples sizes (N=5).

The time spent feeding was analysed using a one way unrelated ANOVA.

Results

Table 3:12 The effects of manipulations of the pituitary adrenal system on distractability (Means±S.E.)

CONDITION (N=10)	DISTRACTION TIME-SECS DISH	PANEL
INTACT	4.7±1.6	7.6±2.0
SHAM Cx	9.7±5.4	5.5±1.1
CxT	8.9±5.4	5.2±1.2
CxOIL	2.7±0.9	6.9±1.8
CxTCORT50	2.5±0.9	6.6±2.1
CxTCORT100	5.0±2.1	10.0±5.3
CxTCORT150	8.3±5.5	7.6±3.7
CxOILCORT100	6.8±5.7	3.8±0.9
CxTA0.5	4.6±1.9	4.8±2.6
CxTA1.0	4.8±2.6	7.0±2.2
CxTA2.0	4.2±1.7	5.4±1.0
CxOILA1.0	1.1±0.5	10.9±3.7
CxTA4-10	4.6±1.8	6.2±1.0
CxOILA4-10	2.6±1.2	6.3±1.6
CxTMY	9.2±5.8	3.5±0.8
CxOILMY	6.5±5.4	10.7±3.6
CxTCORTV	2.9±1.2	4.5±0.9
CxTNaCl	3.8±1.6	5.1±1.4

Table 3:13 The results of the two-way unrelated ANOVAs for distractability to the dish distractant

COMPARISON		RESULT	
T INTACT, SHAMCx, CxT, CxOIL	HORM ORDER INTERACTION	F(3,32)=0.724 F(1,32)=1.453 F(3,32)=1.031	p=0.545 p=0.237 p=0.392
CORT in presence T CxT, CxTCORT50, CxTCORT100, CxTCORT150, CxTCORTV	HORM ORDER INTERACTION	F(4,40)=0.611 F(1,40)=0.048 F(4,40)=2.36	p=0.657 p=0.828 p=0.07
CORT in absence T CxOIL, CxOILCORT100	HORM ORDER INTERACTION	F(1,16)=0.225 F(1,16)=0.291 F(1,16)=0.806	p=0.642 p=0.597 p=0.382
ACTH in presence T CxT, CxTA0.5, CxTA1.0, CxTA2.0, CxTNaCL	HORM ORDER INTERACTION	F(4,40)=0.255 F(1,40)=0.007 F(4,40)=1.957	p=0.905 p=0.934 p=0.120
ACTH in absence T CxOIL, CxOILA1.0	HORM ORDER INTERACTION	F(1,16)=2.263 F(1,16)=0.07 F(1,16)=0.064	p=0.150 p=0.795 p=0.804
A4-10 in presence T CxT, CxTA4-10, CxTNaCl	HORM ORDER INTERACTION	F(2,24)=0.369 F(1,24)=2.614 F(2,24)=1.161	p=0.695 p=0.119 p=0.330
A4-10 in absence T CxOIL, CxOIL A4-10	HORM ORDER INTERACTION	F(1,16)=0.135 F(1,16)=0.059 F(1,16)=0.045	p=0.718 p=0.812 p=0.834
MY in presence T CxT, CxTMY	HORM ORDER INTERACTION	F(1,16)=0.254 F(1,16)=1.242 F(1,16)=1.094	p=0.621 p=0.281 p=0.311
MY in absence T CxOIL, CxOILMY	HORM ORDER INTERACTION	F(1,16)=0.020 F(1,16)=0.113 F(1,16)=0.527	p=0.889 p=0.741 p=0.478

No significant effects of treatment, order of presentation or interaction were found.

Table 3:14 The results of the two-way unrelated ANOVAs for distractability to the panel distractant

COMPARISON	RESULT		
T	HORM	F(3,32)=0.396	p=0.757
	ORDER	F(1,32)=0.981	p=0.329
	INTERACTION	F(3,32)=0.227	p=0.877
CORT in presence T	HORM	F(4,40)=0.283	p=0.887
	ORDER	F(1,40)=0.307	p=0.583
	INTERACTION	F(4,40)=0.151	p=0.962
CORT in absence T	HORM	F(1,16)=0.580	p=0.457
	ORDER	F(1,16)=0.704	p=0.414
	INTERACTION	F(1,16)=0.006	p=0.938
ACTH in presence T	HORM	F(4,40)=0.289	p=0.921
	ORDER	F(1,40)=1.328	p=0.256
	INTERACTION	F(4,40)=1.008	p=0.415
ACTH in absence T	HORM	F(1,16)=0.550	p=0.469
	ORDER	F(1,16)=0.767	p=0.394
	INTERACTION	F(1,16)=0.048	p=0.829
ACTH4-10 in presence T	HORM	F(2,24)=0.553	p=0.582
	ORDER	F(1,24)=0.009	p=0.924
	INTERACTION	F(2,24)=0.018	p=0.982
ACTH4-10 in absence T	HORM	F(1,16)=0.106	p=0.749
	ORDER	F(1,16)=0.044	p=0.836
	INTERACTION	F(1,16)=0.925	p=0.350
MY in presence T	HORM	F(1,16)=0.486	p=0.496
	ORDER	F(1,16)=0.768	p=0.394
	INTERACTION	F(1,16)=1.023	p=0.327
MY in absence T	HORM	F(1,16)=1.010	p=0.330
	ORDER	F(1,16)=2.139	p=0.163
	INTERACTION	F(1,16)=0.519	p=0.482

No significant effects of treatment, order of presentation, or interaction were found.

Table 3:15 The results of the one way unrelated ANOVAs to determine the interactive relationship between the various pituitary-adrenal manipulations and testosterone on the dish distractant

COMPARISON	RESULT	
CxT CORT100 vs CxOIL CORT100	F(1,18)=0.573	p=0.459
CxTA1.0 vs CxOIL A1.0	F(1,18)=2.189	p=0.156
CxTA4-10 vs CxOIL A4-10	F(1,18)=0.491	p=0.492
CxTMY vs CxOIL MY	F(1,18)=0.167	p=0.687

No significant interactive effects were found.

Table 3:16 The results of the one way unrelated ANOVAs to determine the interactive relationship between the various pituitary-adrenal manipulations and testosterone on the panel distractant

COMPARISON	RESULT	
CxTCORT100 vs CxOILCORT100	F(1,18)=0.498	p=0.490
CxTA1.0 vs CxOILA1.0	F(1,18)=0.945	p=0.344
CxT A4-10 vs CxOIL A4-10	F(1,18)=0.120	p=0.733
CxT MY vs CxOIL MY	F(1,18)=7.625	p=0.013

Whilst the presence/absence of T failed to influence distractability to the panels under most PAS manipulations, those animals treated with MY in the absence of T were more highly distracted than their T-replaced counterparts.

Table 3:17 The effects of pitutary-adrenal manipulations on the time spent feeding (Means ± S.E.)

CONDITION N=10	TIME FEEDING - SECS
I	18.8 ± 1.9
SHAM Cx	18.2 ± 2.0
Cx T	20.3 ± 2.3
Cx OIL	17.5 ± 1.6
CxT CORT50	20.6 ± 2.0
CxT CORT100	19.8 ± 2.1
CxT CORT150	17.5 ± 1.6
Cx OIL CORT100	16.6 ± 1.8
Cx TA0.5	22.0 ± 1.3
Cx TA1.0	18.0 ± 1.6
Cx TA2.0	18.0 ± 2.1
Cx OIL A1.0	16.8 ± 1.1
Cx TA4-10	18.9 ± 2.1
Cx OIL A4-10	18.7 ± 1.7
CxTMY	17.8 ± 2.1
Cx OILMY	17.0 ± 1.8
CxT CORTV	18.5 ± 1.3
CxT NaCl	21.9 ± 1.6

Table 3:18 The results of the one way unrelated ANOVAs for the effects of pituitary-adrenal manipulations on the time spent feeding.

COMPARISON	RESULT	
T	F(3,36) = 0.346	P = 0.792
CORT in presence T	F(4,45) = 0.476	P = 0.753
CORT in absence T	F(1,18) = 0.157	P = 0.696
ACTH1-39 in presence T	F(4,45) = 1.161	P = 0.341
ACTH1-39 in absence T	F(1,18) = 0.144	P = 0.709
A4-10 in presence T	F(2,27) = 0.530	P = 0.595
A4-10 in absence T	F(1,18) = 0.229	P = 0.638
MY in presence T	F(1,18) = 0.634	P = 0.436
MY in absence T	F(1,18) = 0.061	P = 0.808

No significant effects were detected.

Table 3:19 The effects of pituitary-adrenal manipulations on the time spent examining the panels (Means ± S.E.)

CONDITION N=10	TIME EXAMINING PANELS - SECS
I	4.01 ± 1.14
SHAM Cx	3.26 ± 0.53
Cx T	3.94 ± 1.11
Cx OIL	4.00 ± 0.87
Cx T CORT50	4.48 ± 1.47
Cx T CORT100	3.59 ± 0.56
Cx T CORT150	3.38 ± 0.89
Cx OIL CORT100	3.49 ± 0.80
Cx T A0.5	2.90 ± 0.76
Cx T A1.0	4.80 ± 1.46
Cx T A2.0	3.04 ± 0.42
Cx OIL A1.0	6.82 ± 2.66
Cx T A4-10	5.24 ± 0.84
Cx OIL A4-10	4.48 ± 1.08
Cx T MY	2.34 ± 0.64
Cx OIL MY	4.77 ± 1.20
Cx T CORT V	3.13 ± 0.83
Cx T NaCl	3.35 ± 1.05

Table 3:20 The results of the one way ANOVAs for the time spent examining the panels

COMPARISON	RESULT	
T	F(3,36) = 0.146	P = 0.931
CORT in presence T	F(4,45) = 0.26	P = 0.9
CORT in absence T	F(1,18) = 0.17	P = 0.68
ACTH1-39 in presence T	F(4,45) = 0.58	P = 0.68
ACTH1-39 in absence T	F(1,18) = 1.02	P = 0.33
A4-10 in presence T	F(2,27) = 0.93	P = 0.41
A4-10 in absence T	F(1,18) = 0.127	P = 0.73
MY in presence T	F(1,18) = 1.559	P = 0.228
MY in absence T	F(1,18) = 0.276	P = 0.606

No significant effects were detected.

Table 3:21 The results of one way unrelated ANOVAs to determine the interactive relationship between the various pituitary-adrenal manipulations and testosterone on the time spent examining the panels

COMPARISON	RESULT	
Cx T CORT100 VS Cx OIL CORT100	F(1,18) = 0.009	P = 0.924
Cx T A1.0 VS Cx OIL A1.0	F(1,18) = 1.021	P = 0.33
Cx T A4-10 VS Cx OIL A4-10	F(1,18) = 0.308	P = 0.586
Cx T MY VS Cx OIL MY	F(1,18) = 2.060	P = 0.168

No significant interactive effects were detected.

Table 3:22 The effects of the pituitary-adrenal manipulations on the % time spent examining the panels (Means ± S.E.)

CONDITION	% TIME EXAMINING PANEL
I	41.1 ± 9.1
SHAM Cx	41.4 ± 5.4
Cx T	42.6 ± 5.8
Cx OIL	42.3 ± 5.4
Cx T CORT50	50.6 ± 7.6
Cx T CORT100	46.4 ± 7.3
Cx T CORT150	32.0 ± 7.9
Cx OIL CORT100	44.6 ± 7.2
Cx T A0.5	37.3 ± 6.1
Cx T A1.0	42.9 ± 6.6
Cx T A2.0	40.4 ± 4.9
Cx OIL A1.0	44.8 ± 5.8
Cx T A4-10	57.5 ± 5.6
Cx OIL A4-10	47.5 ± 4.0
Cx T MY	34.3 ± 7.5
Cx OIL MY	42.1 ± 7.9
Cx T CORT V	38.2 ± 6.7
Cx T Na Cl	36.2 ± 8.2

Table 3:23 The results of the one way unrelated ANOVAs for the % time spent examining the panels

COMPARISON	RESULT	
T	F(3,36) = 0.063	P = 0.979
CORT in presence T	F(4,45) = 1.36	P = 0.26
CORT in absence T	F(1,18) = 0	P = 1.0
ACTH1-39 in presence T	F(4,45) = 0.396	P = 0.81
ACTH1-39 in absence T	F(1,18) = 0.017	P = 0.897
A4-10 in presence T	F(2,27) = 2.928	P = 0.071
A4-10 in absence T	F(1,18) = 0.321	P = 0.578
MY in presence T	F(1,18) = 1.013	P = 0.327
MY in absence T	F(1,18) = 0.07	P = 0.794

No significant effects were detected, though T-replaced castrated treated with ACTH4-10 tended to spend a greater proportion of the time examining the panels than their controls.

Table 3:24 The results of the one way unrelated ANOVAs to determine the interactive relationship between the various pituitary-adrenal manipulations and testosterone on the % time spent examining the panels

COMPARISON	RESULT	
Cx T CORT100 VS Cx OIL CORT100	F(1,18) = 0.003	P = 0.957
Cx T A1.0 VS Cx OIL A1.0	F(1,18) = 0.168	P = 0.687
Cx T A4-10 VS Cx OIL A4-10	F(1,18) = 2.094	P = 0.165
Cx T MY VS Cx OIL MY	F(1,18) = 0.609	P = 0.445

No significant interactive effects were found.

Table 3:25 The effects of pituitary-adrenal manipulations on the time spent examining the dish (Means ± S.E.)

CONDITION *	TIME EXAMINING DISH - SECS
I	2.8 ± 1.0
SHAM Cx	3.2 ± 0.9
Cx T	3.6 ± 1.1
Cx OIL	3.8 ± 0.7
Cx T CORT50	4.1 ± 1.1
Cx T CORT100	3.7 ± 0.9
Cx T CORT150	3.2 ± 0.5
Cx OIL CORT100	3.1 ± 0.7
Cx TA0.5	3.5 ± 0.8
Cx TA1.0	3.4 ± 0.6
Cx TA2.0	3.6 ± 0.5
Cx OIL A1.0	3.8 ± 0.8
Cx T A4-10	3.3 ± 0.8
Cx OIL A4-10	4.5 ± 1.2
Cx T MY	4.5 ± 0.9
Cx OIL MY	3.4 ± 0.5
Cx T CORT V	5.1 ± 0.7
Cx T NaCl	3.7 ± 1.0

* N = 9 in some instances as video-tape damaged before behaviour fully analysed.

Table 3:26 The results of the one way ANOVAs for the time spent examining the dish

COMPARISON	RESULT	
T	F(3,34) = 0.194	P = 0.9
CORT in presence T	F(4,40) = 0.611	P = 0.657
CORT in absence T	F(1,17) = 0.428	P = 0.522
ACTH1-39 in presence T	F(4,42) = 0.022	P = 0.999
ACTH1-39 in absence T	F(1,17) = 0.00	P = 0.986
A4-10 in presence T	F(2,24) = 0.039	P = 0.962
A4-10 in absence T	F(1,16) = 0.259	P = 0.617
MY in presence T	F(1,16) = 0.365	P = 0.554
MY in absence T	F(1,16) = 0.191	P = 0.668

No significant effects were detected.

Table 3:27 The results of the one way unrelated ANOVAs to determine the interactive relationship between the various pituitary-adrenal manipulations and testosterone on the time spent examining the dish

COMPARISON	RESULT	
Cx T CORT100 VS Cx OIL CORT100	F(1,17) = 0.30	P = 0.591
Cx T A1.0 VS Cx OIL A1.0	F(1,18) = 0.165	P = 0.689
Cx T A4-10 VS Cx OIL A4-10	F(1,16) = 0.670	P = 0.425
Cx T MY VS Cx OIL MY	F(1,16) = 1.115	P = 0.307

No significant interactive effects were found.

Table 3:28 The effects of the testosterone manipulations on the plasma levels of corticosterone (Means ± S.E.)

CONDITION N=10	CORT ng/ml
I	181.9 ± 19.6
SHAM Cx a.	276.6 ± 23.3
Cx T	241.5 ± 20.8
Cx OIL b.	320.7 ± 30.1

ONE WAY UNRELATED ANOVA: $F(3,36) = 6.056$, $P = 0.002$

TUKEY TEST: HSD = 90.7

a. different from INTACT: mean difference = 94.7, $p < 0.05$

b. different from INTACT: mean difference = 138.8, $p < 0.05$

Intact mice had similar levels of CORT to T-replaced castrates, but significantly lower levels than both sham castrates and non-replaced castrates.

Table 3:29 The effects of the corticosterone manipulations in the presence of testosterone on the plasma levels of corticosterone (Means ± S.E.)

CONDITION N=10		CORT ng/ml
Cx T		241.5 ± 20.8
Cx T CORT V		300.3 ± 34.6
Cx T CORT50	a.	199.3 ± 17.5
Cx T CORT100		234.9 ± 23.4
Cx T CORT150	b.	343.1 ± 24.1

ONE WAY UNRELATED ANOVA: $F(4,45) = 5.345$, $P = 0.001$

TUKEY TEST: HSD = 99.66

a. different from Cx T CORT V: mean difference = 101.0, $P < 0.05$

b. different from Cx T: mean difference = 101.6, $P < 0.05$

Cx T CORT50: mean difference = 143.8, $P < 0.05$

Cx T CORT100: mean difference = 108.2, $P < 0.05$

Neither 50µg nor 100µg CORT produced significant elevations in the levels of CORT relative to injected controls, though the highest dose significantly elevated CORT. The vehicle controls had higher levels of CORT than those animals given 50µg CORT, but similar levels to their non-injected controls.

Table 3:30 The effects of the corticosterone manipulations in the absence of testosterone on the plasma levels of corticosterone (Means ± S.E.)

CONDITION	N=10	CORT ng/ml
Cx OIL		320.7 ± 30.1
Cx OIL CORT100		293.3 ± 29.1

ONE WAY UNRELATED ANOVA: $F(1,18) = 3.776$, $P = 0.07$

No significant differences were detected.

Table 3:31 The effects of ACTH1-39 in the presence of testosterone on the plasma levels of corticosterone (Means ± S.E.)

CONDITION	N=10	CORT ng/ml
Cx T		241.5 ± 20.8
Cx T NaCl		307.0 ± 24.5
Cx T A0.5	a.	487.4 ± 57.1
Cx T A1.0		555.3 ± 45.5
Cx T A2.0		616.6 ± 38.5

ONE WAY UNRELATED ANOVA: $F(4,45) = 16.52$, $P < 0.001$

TUKEY TEST: HSD = 159.4

a. different from Cx T NaCl: mean difference = 180.4, $P < 0.05$

All the doses of ACTH1-39 produced a significant increase in the levels of CORT with respect to both the vehicle and non-injected controls. There were no differences in the levels of CORT between the 3 doses of ACTH1-39. The vehicle did not elevate CORT levels significantly.

Table 3:32 The effects of ACTH1-39 in the absence of testosterone on the plasma levels of corticosterone (Means ± S.E.)

CONDITION N=10	CORT ng/ml
Cx OIL	320.7 ± 30.1
Cx OIL A1.0	690.3 ± 55.8

ONE WAY UNRELATED ANOVA: $F(1,18) = 33.961$, $P < 0.001$

Table 3:33 The effects of ACTH4-10 in the presence of testosterone on the plasma levels of corticosterone (Means ± S.E.)

CONDITION N=10	CORT ng/ml
Cx T	241.5 ± 20.8
Cx T NaCl	307.0 ± 24.5
Cx T A4-10	243.6 ± 29.0

ONE WAY UNRELATED ANOVA: $F(2,27) = 2.212$, $P = 0.129$

ACTH4-10 did not alter plasma CORT significantly.

Table 3:34 The effects of ACTH4-10 in the absence of testosterone on the plasma levels of corticosterone (Means ± S.E.)

CONDITION N=10	CORT ng/ml
Cx OIL	320.7 ± 30.1
Cx OIL A4-10	316.9 ± 29.2

ONE WAY UNRELATED ANOVA: $F(1,18) = 0.008$, $P = 0.929$

ACTH4-10 did not alter plasma CORT significantly.

Table 3:35 The effects of metyrapone in the presence of testosterone on the plasma levels of corticosterone (Means ± S.E.)

CONDITION N=10	CORT ng/ml
Cx T	241.5 ± 20.8
Cx T MY	226.3 ± 16.8

ONE WAY UNRELATED ANOVA: $F(1,18) = 0.320$, $P = 0.579$

Metyrapone did not alter plasma CORT significantly.

Table 3:36 The effects of metyrapone in the absence of testosterone on the plasma levels of corticosterone (Means ± S.E.)

CONDITION N=10	CORT ng/ml
Cx OIL	320.7 ± 30.1
Cx OIL MY	264.3 ± 48.7

ONE WAY UNRELATED ANOVA: $F(1,18) = 0.933$, $P=0.347$

Metyrapone did not alter plasma CORT significantly.

Table 3:37 The results of the one way unrelated ANOVAs on the effects of the presence of testosterone on the plasma levels of corticosterone resulting from the various pituitary-adrenal manipulations

COMPARISON	RESULT
Cx T CORT100 VS Cx OIL CORT100	$F(1,18) = 0.014$, $P = 0.91$
Cx T A1.0 VS Cx OIL A1.0	$F(1,18) = 3.511$, $P = 0.08$
Cx T A4-10 VS Cx OIL A4-10	$F(1,18) = 3.16$, $P = 0.09$
Cx T MY VS Cx OIL MY	$F(1,18) = 0.569$, $P = 0.469$

No significant effects were detected.

Table 3:38 The results of the correlation analysis between the levels of corticosterone and distractability to the dish

CONDITION	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos
HIGH CORT: LOW ACTH Cx T, Cx T CORT50, Cx T CORT100, Cx T CORT150	-0.083
HIGH CORT: HIGH ACTH Cx T, Cx T A0.5, Cx T A1.0 Cx T A2.0	-0.54*
LOW CORT: HIGH ACTH Cx T, Cx T A4-10	-0.142

* $P < 0.05$

High levels of distractability correlated with lower levels of CORT, but only when the levels of ACTH were also high.

Table 3:39 The results of the correlation analysis between the levels of corticosterone and distractability to the panels

CONDITIONS	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos
HIGH CORT: LOW ACTH Cx T, Cx T CORT50, Cx T CORT100, Cx T CORT150	-0.35
HIGH CORT: HIGH ACTH Cx T, Cx T A0.5, Cx T A1.0, Cx T A2.0	0.06
LOW CORT: HIGH ACTH Cx T, Cx T A4-10	-0.079

No significant correlations were detected.

Discussion

The finding that neither CORT, ACTH1-39, ACTH4-10 nor MY, regardless of the dose delivered, affected any index of distractability to either an irrelevant or a relevant distractant, not only fails to support the hypothesis that CORT influences this phenomenon, but also undermines the likelihood that the entire PAS is involved in attentional processes, or at least those reflected by distractability.

Although this is surprising as there is a wealth of data implicating the PAS in attentional processes, there are a number of explanations for this finding and on this basis the hypothesis should not be ruled out at this juncture.

Firstly, the fact that the circulating levels of CORT were relatively high in the controls suggested that this high baseline level may have confounded the identification of a behavioural effect. This is because it would saturate most of the available receptor systems, and in so doing render any experimentally induced elevations of CORT ineffective. This problem arises from the use of adrenally intact animals, which are capable of responding adrenocortically to any arousing aspect of the experiment, with obvious consequences for the number of receptors remaining unoccupied. In connection with this, as both ACTH1-39 and ACTH4-10 are involved in the control of the number of receptors for CORT in the HPC (de Kloet and Veldhuis, 1980; Veldhuis and de Kloet, 1982a and 1982b), any attempt to elucidate a role for CORT in the level of distractability would be further complicated in those conditions in which these peptides are present.

Secondly, there was a wide variation in the levels of CORT between individuals within a condition, which may have reduced any effect to statistical insignificance. However, if this was the case, correlational analysis should have uncovered a consistent and clearly defined relationship between CORT and behaviour. The absence of such a relationship may simply relate to the fact that plasma levels of a hormone do not always

provide an accurate measure of brain levels, or the degree to which the hormone binds to its receptors, though a fuller consideration of this point is deferred to the general discussion. Despite this generalisation, high levels of CORT correlated with reduced distractability to the relevant distractant, though distractability to the irrelevant distractant was unaffected. From the specificity of this effect it could, at first, be inferred that high levels of CORT impaired attentional processes. However, in this instance, the effect might not have been exclusively due to CORT, but to its interaction with ACTH. This is because the correlation was restricted to those conditions in which both ACTH and CORT were present in high levels. It was not found when the levels of ACTH were high but CORT were low, or when CORT alone was present in high quantities. Despite the apparently interactive nature of this correlation it was still difficult to reconcile the association between high CORT levels and low distraction times to the relevant distractant with the role that this adrenocorticoid is hypothesised to play in persistence. Rather, the level of distractability to the relevant stimulus should be increased rather than decreased if the persistence of attention is enhanced (Andrew, 1972a; Mason and Iversen, 1979). However, as the correlation was only found when the circulating levels spanned a very wide range and extended to higher levels than those found normally, the possibility remains that it was pharmacological.

Having discussed the behavioural effects of the PAS manipulations this discussion will now turn to the effects of the manipulations of the PGS. Once again changes in the levels of T failed to exert a significant effect on distractability. This suggests that the T levels resulting from the replacement regime resembled those occurring in intact males. As before there was a trend within the data in the predicted direction: T-replaced castrates displayed the highest level of distractability to the dish, but the lowest to the panels.

There was no apparent interaction between the PAS and T, with the exception that animals treated with MY in the absence of T were more distracted by the panels than their T-replaced counterparts. It is difficult to put forward a satisfactory explanation for this, especially as it was not confirmed by the behaviour exhibited by animals presumed to be of a similar hormonal status. However, since MY has been claimed to exert direct brain effects (Shire, 1979; Bialik, 1984), the most likely explanation is that this effect reflects an interaction between MY and T, rather than the PAS and T.

Despite the fact that the behavioural findings were, on the whole, disappointing, some very interesting data emerged from the analysis of the plasma levels of CORT. For instance, it was surprising to find that neither 50µg nor 100µg CORT markedly elevated CORT relative to their controls. Furthermore, as the levels of CORT in the former conditions would have been expected to be raised, if only in response to the stress associated with injection, it was difficult to provide a satisfactory account for this finding. It was speculated that the presence of relatively low levels of CORT may have arisen from the negative feedback effects of the exogenous CORT on the release of ACTH, which would effectively prevent the release of endogenous CORT that normally occurs under these stressful conditions. As an extension of this; with the administration of increasingly high doses of CORT, a point would eventually be reached at which the negative feedback influence of exogenous CORT would be exceeded by the amount injected, so that there would be a net increase in the circulating level. If such a system operates, it would account for the presence of significantly higher levels of CORT upon the administration of the highest dose of CORT only. The administration of increasing amounts of ACTH1-39 also failed to produce a statistically significant graded increase in the circulating levels of CORT. As the capacity of the adrenal to release CORT in response to ACTH is limited, this may have been due to the 'nonlinearity' of the responsiveness

of the adrenal gland to ACTH (Wilson, 1985). Indeed, as the levels of CORT ensuing from the lowest does of ACTH were fairly high, it is possible that a 'ceiling effect' had been introduced. However, since there was an upward trend in the levels of CORT from ACTH0.5 - ACTH2.0, it may simply have been the case that the doses were not sufficiently different in magnitude to produce a statistically significant gradation in CORT.

As expected ACTH4-10 did not elevate the levels of CORT above those found in control animals. This confirms the extra-adrenal nature of its actions, and counters doubts raised about its effectiveness (Brain, 1972b).

Contrary to expectations MY failed to depress the levels of CORT below those found under control conditions. Since MY inhibits the activity of one of the enzymes involved in the biosynthesis of CORT, it was reasoned that a relatively long period of time would be needed for the release or degradation of existing stores of CORT to be completed, and on this basis, it was administered at least 24 hours prior to testing. Accordingly, the presence of appreciable quantities of this adrenocorticoid after this period was initially attributed to the stores of either the enzyme 11β hydroxylase or the steroid itself requiring a longer period to be fully depleted. However, MY has been shown to act within a surprisingly short period of time. Jenkins et al. (1958) demonstrated that CORT was depleted to below 10% of its initial value within 10 minutes in dogs treated with 75mg/kg MY, though it returned to 75% within 3 hours. This evidence of the rapid, short-lived nature of the effects of MY offers the most plausible explanation of the high levels of CORT that were found. Nonetheless, in view of the fact that the half life of CORT is 20 minutes (Levine and Coover, 1976), it is still difficult to fully envisage how MY exerts its actions within such a short period.

The present findings concerning the effects of T on the levels of CORT were inconsistent with those reported earlier (see APPENDIX 1 and Table 3:10) in which high levels of T appeared to stimulate the release of CORT

from the adrenals. Here it was found that non-replaced castrates tended to possess the highest levels of CORT. Although their CORT levels were not significantly greater than those found in their T-replaced counterparts, they were significantly higher than in intact mice (see Table 3:28). This may, therefore, represent an oil injection effect. As the interaction between hormonal axes is often biphasic (Pitzel, 1984a), this discrepancy may have arisen as a consequence of the different intervals elapsing between the administration of T and blood sampling: the time lag in the present experiment being much greater than previously.

The discovery that the levels of CORT resulting from the various manipulations of the PAS were independent of the presence of T, further contributed to the confusion surrounding the nature of the PAS-PGS relationship, especially as preliminary studies had hinted that CORT tended to be elevated in the presence of T, regardless of the treatment condition (see APPENDIX 1). Once again this apparent inconsistency may simply highlight the importance of the administration - sampling interval, and therefore need not invalidate the observation that T under certain conditions stimulates the adrenal gland.

Having discussed the behavioural and physiological data, there are some methodological points that deserve further explanation before any conclusions can be reached. Firstly, the T replacement regime was modified, such that the mice were given 7.5mg T-oenanthate. This dose was selected because it had already been shown to reinstate the behavioural effects of T in a number of different strains of mice (Archer, 1977). The oenanthate rather than the propionate form of T was used as the latter was considered to be unsuitable when testing extended over a period greater than 48 hours (Turner, 1979). Whilst the oenanthate ester enters the circulation at the same rate as other esters upon subcutaneous administration, it is metabolised more slowly, and therefore maintains T at a higher level over a longer period (Junkman, 1952). Blood samples from castrated mice treated

with 7.5mg T-oenanthate, taken at 24 and 72 hours post-injection confirmed this, though the levels reached were fairly high for "TO" males (see APPENDIX 4). This also indicates that the T levels were relatively similar on the 2 days of testing.

In a similar fashion, a single dose of MY also produced equivalent hormonal states on the two days of testing as revealed by blood samples taken 24, 48 and 72 hours after the administration of 5mg MY. There was, however, a tendency for the levels of CORT to fall with time (see APPENDIX 3).

As one of the most frequently raised criticisms of studies using ACTH4-10 as a means of assessing the independent effects of ACTH relates to the dose of the peptide fragment administered relative to the usual dose of the parent molecule (Sandman et al., 1980b), in the present experiment the dose chosen was the molar equivalent of the 2iu dose of ACTH1-39, taking into consideration the relative purity of the two preparations.

To conclude: although the behavioural evidence was not supportive of the postulated role of CORT in distractability, this should not be taken as disproving the hypothesis. Indeed, the extent to which endogenous elevations obscured the detection of behavioural effects in the present experiment must firstly be estimated before any firm conclusions can be reached, and this, therefore, is the subject of the following experiment.

EXPERIMENT 7

The effects of corticosterone on distractability under controlled baseline levels (20µg CORT/ml)

For reasons given above it was considered essential to ensure that the baseline level of CORT remains moderately low. Accordingly, long term adrenalectomised mice were placed on a maintenance dose of CORT, which was designed to reinstate levels of CORT that were sufficiently low to enable subsequent experimentally induced elevations of CORT to be expressed at the level of the receptors. In this experiment a maintenance dose of 20µg CORT/ml drinking water was adopted (van Dijk et al., 1981), which was intended to produce levels approximating to those found in intact males under resting conditions. (The value of controlling the baseline level of CORT is discussed in greater detail in Chapter 1).

Unlike previous runway experiments, this was restricted to the effects of CORT. The problems inherent in this approach are appreciated, especially the potential confounding effects of changes in ACTH on behaviour. Finally, for reasons explained earlier (see p. 75), the animals were not castrated.

Method

Experimental conditions and subjects

There were 8 animals in each condition:

1. Intact controls (INTACT)
2. Sham adrenalectomised injected with 0.1ml propylene glycol (SHAM+VEHICLE)
3. Adrenalectomised maintained on the saline solution (1% ethanol in 0.9% NaCl solution) (ADX-SALINE)
4. Adrenalectomised maintained on the CORT solution (20µg CORT/ml saline solution) (ADX-CORT)
5. Adrenalectomised maintained on the CORT solution injected with 300µg CORT in the vehicle (ADX - CORT+300)

All the injections were given one hour before testing. 10 intact, 10 sham adrenalectomised, 11 non-replaced adrenalectomised and 25 CORT-replaced adrenalectomised mice were used, though 2 non-replaced adrenalectomised and 3 CORT-replaced adrenalectomised died, and 2 intacts, 2 sham-adrenalectomised, 1 non-replaced adrenalectomised and 6 CORT-replaced adrenalectomised failed to run to criterion. The mice were behaviourally experienced as they had previously served in a novel object experiment. All the operations were carried out 10-15 days prior to the start of runway testing. Immediately after surgery the adrenalectomised mice were either placed on the CORT solution or the control saline solution and they remained on the appropriate drinking solution throughout the period of behavioural testing.

Procedure

There were no procedural differences from those given in the general method. Both white panels and black food dish were used. Three batches of mice were run, with as far as was possible, an equal number of animals from all the conditions present in each batch. The mice were blood sampled immediately after the second day of testing and the samples were subsequently assayed for CORT.

Statistical analysis

All the behavioural data is presented as means ± S.E., and where necessary was transformed (LOG X + 1) to reduce heterogeneity of variance before analysis. Distraction times to the dish and panels were analysed by a two way unrelated ANOVA (treatments X order presentation). As the order of presentation proved to be without effect, the video data was combined irrespective of this variable and analysed by a one way unrelated ANOVA. The Tukey test was used to identify the source of any difference. The CORT data is presented as means ± S.E., and was analysed by the KWANOVA followed

by the MWU test.

Spearman's Rank Correlational Coefficient was employed to assess the degree of correlation between the levels of CORT and distractability. The data was combined over conditions, and as before this analysis was confined to the distractant presented on the second day of testing. To control for any secondary effects of adrenalectomy, such as the absence of the adrenal medulla, a second combined conditions analysis was conducted, excluding adrenally intact animals.

Results

Table 3:40 The effects of corticosterone on distractability (Means ± S.E.)

CONDITION N=8	DISTRACTION TIME - SECS	
	DISH	PANEL
INTACT	0.8 ± 0.4	6.8 ± 2.8
SHAM + VEHICLE	0.6 ± 0.4	3.1 ± 1.4
ADX - SALINE	1.7 ± 0.8	7.2 ± 2.3
ADX - CORT	0.9 ± 0.7	7.5 ± 2.7
ADX - CORT + 300	4.1 ± 3.5	5.6 ± 1.3

TWO WAY UNRELATED ANOVA : DISH

Treatment $F(4,30) = 1.570$, $P = 0.208$

Order $F(1,30) = 2.137$, $P = 0.154$

Interaction $F(4,30) = 1.267$, $P = 0.305$

TWO WAY UNRELATED ANOVA : PANELS

Treatment $F(4,30) = 0.642$, $P = 0.637$

Order $F(1,30) = 0.124$, $P = 0.727$

Interaction $F(4,30) = 0.570$, $P = 0.687$

No significant effect of treatment or order of presentation and no interaction effect was detected.

Table 3:41 The effects of corticosterone on the time spent feeding (Means ± S.E.)

CONDITION N=8	TIME FEEDING - SECS
INTACT	22.2 ± 1.6
SHAM + VEHICLE	25.9 ± 1.6
ADX - SALINE	20.8 ± 2.0
ADX - CORT	20.3 ± 2.2
ADX - CORT + 300	20.6 ± 2.3

ONE WAY UNRELATED ANOVA: $F(4,35) = 1.374$, $P = 0.263$

No significant effects were detected.

Table 3:42 The effects of corticosterone on the time spent examining the panels (Means ± S.E.)

CONDITION N=8	TIME EXAMINING PANELS - SECS
INTACT	4.8 ± 1.9
SHAM + VEHICLE	1.6 ± 0.6
ADX - SALINE	5.2 ± 1.3
ADX - CORT	4.6 ± 1.5
ADX - CORT + 300	4.0 ± 0.8

ONE WAY UNRELATED ANOVA: $F(4,35) = 1.218$, $P = 0.321$

No significant effects were detected.

Table 3:43 The effects of corticosterone on the % time spent examining the panels (Means ± S.E.)

CONDITION N=8	% TIME EXAMINING PANELS
INTACT	44.4 ± 8.1
SHAM + VEHICLE a.	20.4 ± 6.2
ADX - SALINE	49.5 ± 3.8
ADX - CORT	39.2 ± 7.0
ADX - CORT + 300	43.2 ± 3.7

NOTE: values in table untransformed.

ONE WAY UNRELATED ANOVA: $F(4,35) = 3.722$, $P = 0.013$

(ARCSINE TRANSFORMED DATA)

TUKEY TEST: HSD = 19.1

a. different from ADX - CORT + 300: Mean difference = 19.3, $P < 0.05$

different from INTACT: Mean difference = 19.5, $P < 0.05$

different from ADX - SALINE: Mean difference = 23.0, $P < 0.05$

With the exception of the CORT-replaced mice, those acting as the sham adrenalectomy-vehicle controls spent a significantly smaller proportion of the time examining the panels than all the other conditions.

Table 3:44 The effects of corticosterone on the time spent examining the dish (Means ± S.E.)

CONDITION N=8	TIME EXAMINING DISH - SECS
INTACT	2.6 ± 0.7
SHAM + VEHICLE a.	0.5 ± 0.3
ADX - SALINE	4.6 ± 1.5
ADX - CORT	1.8 ± 0.6
ADX - CORT + 300	1.9 ± 0.5

NOTE: values in table are untransformed

ONE WAY UNRELATED ANOVA: $F(4,35) = 3.065$, $P = 0.029$

(LOG TRANSFORMED DATA)

TUKEY TEST: HSD = 0.932.

a. different from ADX - SALINE: Mean difference = 1.099, $P < 0.05$

Table 3:45 The effects of treatments on the plasma levels of corticosterone (Means ± S.E.)

CONDITION N=8		CORT ng/ml
INTACT		207.8 ± 46.9
SHAM + VEHICLE	a.	460.2 ± 108.1
ADX - SALINE	b.	23.2 ± 5.1
ADX - CORT	c.	190.5 ± 49.5
ADX - CORT + 300		3883.6 ± 1634.6

KWANOVA: $H = 31.4$, $P < 0.001$

a. MWU different from INTACT: $U = 15$, $P < 0.05$

b. MWU different from INTACT: $U = 0$, $P < 0.001$

c. MWU different from ADX - CORT + 300: $U = 0$, $P < 0.001$

There were significant differences between all the conditions, except INTACT and ADX-CORT.

Table 3:46 The results of the correlation analysis between the levels of corticosterone and distractability

	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos	
CONDITIONS	DISH	PANEL
Including adrenally intact mice. N=20	-0.087	-0.31
Excluding adrenally intact mice. N=12	-0.316	-0.54

There were no significant correlations detected.

Discussion

As the maintenance dose resulted in approximately 190ng/ml CORT in the circulation and the CORT levels in male mice under resting conditions fall within the range of 50-100ng/ml at the circadian peak, this dose was too high for its intended purpose. Furthermore, as the levels of CORT arising from the administration of 300µg CORT can be assumed to exceed those found under physiological conditions, these were also unacceptably high. The presence of such high levels is undesirable, for reasons that have already been elaborated. EXPERIMENT 8 was designed to correct this. Since the fundamental aim of reinstating a moderately low baseline level was not achieved, there is little value to be gained from examining the behavioural data in any detail. Rather, it is sufficient to point out that the hypothesis that CORT increases the level of distractability to a relevant and decreases it to an irrelevant distractant was not supported. This is most clearly illustrated by the fact that despite marked differences in the circulating levels of CORT between non-replaced adrenalectomised animals at one extreme and their CORT-replaced counterparts treated with 300µg CORT at the other, the level of distractability was remarkably similar. If anything, the finding that non-replaced animals spent the greatest amount of time examining the dish would run counter to the working hypothesis. Moreover, the failure to detect a correlational relationship further weakens this hypothesis, (though the drawbacks associated with plasma levels of a hormone are considered in greater detail in the general discussion).

There are a number of other points of interest: firstly, the relatively high level of investigation of a relevant distractant by the non-replaced adrenalectomised animals (Table 3:44) was possibly related to the presence of elevated levels of ACTH, resulting from adrenalectomy, rather than the low levels of CORT (Beatty et al., 1970). If this is the case, it supports the role attributed to this peptide in the enhancement of

attentional selectivity (Sandman et al., 1981; Sandman and Kastin, 1981). However, as the non-replaced adrenalectomised animals also spent a greater proportion of the time examining the panels, this suggests that they were more distracted regardless of the relevance of the stimulus. This implies that the level of general responsiveness, rather than the level of selective attentiveness, is elevated in these animals. Furthermore, any attempt to explain these differences in terms of ACTH fails as non-replaced adrenalectomised animals and their CORT-replaced counterparts treated with 300µg CORT would be expected to possess widely different levels of ACTH (high, low respectively), yet the level of distractability toward the panels was very similar.

One useful result of this experiment was the confirmation of successful adrenalectomy provided by the low CORT levels in the ADX - SALINE group (Table 3:45). It had been feared that regeneration of adrenocortical tissue (Hawkins et al., 1974) might have occurred, giving higher circulating levels of CORT.

Two further points worthy of mention relate to the controls. In the interest of minimising the number of animals, the SHAM + VEHICLE group was used as a combined control for both surgery and injection. Their high CORT levels (Table 3:45) may thus have been due to 'proactive effects' (Murison et al., 1986) causing hyper-reactivity of the PAS to the injection, the task, or both: it is not possible to say which. Finally, sham adrenalectomised animals differ fundamentally from their counterparts in which the adrenals have been removed. They possess the adrenal medulla and as a result any differences may reflect the activity of adrenomedullary catecholamines. In addition, they tend to display an increased corticoid response to stressful experiences, which adrenalectomised animals are incapable of showing. Taken together, this undermines the value of using these animals as controls, a point that is pursued in greater depth in the following experiment.

EXPERIMENT 8

The effects of corticosterone on distractability under controlled baseline levels (5µg CORT/ml)

The present experiment examines the effects of CORT on distractability against a lower replacement regime than that used previously. The replacement dose adopted was 5µg CORT/ml drinking water.

Method

Experimental conditions and subjects

There were 8 animals in each condition:

1. Intact controls. (INTACT)
2. Intact injected with 0.1ml propylene glycol. (INTACT + VEHICLE)
3. Sham adrenalectomised. (SHAM ADX)
4. Adrenalectomised maintained on the saline solution (1% ethanol in 0.9% NaCl solution) (ADX-SALINE)
5. Adrenalectomised maintained on the CORT solution (5µg CORT/ml saline solution) (ADX-CORT)
6. Adrenalectomised maintained on the CORT solution injected with 50µg CORT in the vehicle. (ADX - CORT + 50)

All the injections were given one hour prior to testing. 18 intact, 10 sham-adrenalectomised, 19 non-replaced adrenalectomised and 24 CORT-replaced adrenalectomised were used, though 9 non-replaced adrenalectomised and 6 CORT-replaced adrenalectomised died, and 2 intact, 2 sham-adrenalectomised, 2 non-replaced adrenalectomised and 2 CORT-replaced adrenalectomised failed to run to criterion. All the mice were behaviourally naive. The operations were carried out 2 days prior to the start of testing. Immediately after surgery the adrenalectomised mice were either placed on the CORT solution or the control saline solution, and they

remained on the appropriate drinking solution throughout the period of behavioural testing.

Procedure and statistical analysis

There were no procedural differences from those given in the general method. Both white panels and black food dish were used. As with EXPERIMENT 7, 3 batches of mice were run. The statistical analysis was identical to EXPERIMENT 7.

Results

Table 3:47 The effects of corticosterone on distractability (Means ± S.E.)

	DISTRACTION TIME - SECS	
CONDITION N=8	DISH	PANEL
INTACT	1.3 ± 0.5	4.9 ± 1.5
INTACT + VEHICLE	1.3 ± 0.8	6.0 ± 1.9
SHAM ADX	1.7 ± 0.9	9.1 ± 3.2
ADX - SALINE	2.8 ± 1.3	6.0 ± 1.9
ADX - CORT	1.4 ± 0.9	8.8 ± 3.7
ADX - CORT + 50	4.3 ± 2.2	3.9 ± 0.8

TWO WAY UNRELATED ANOVA: DISH

Treatment $F(5,36) = 0.675$, $P = 0.645$

Order $F(1,36) = 0.108$, $P = 0.744$

Interaction $F(5,36) = 1.266$, $P = 0.299$

TWO WAY UNRELATED ANOVA: PANEL

Treatment $F(5,36) = 0.693$, $P = 0.632$

Order $F(1,36) = 0.103$, $P = 0.750$

Interaction $F(5,36) = 1.568$, $P = 0.194$

No significant effect of treatment or order of presentation, and no interaction effect was detected.

Table 3:48 The effects of corticosterone on the time spent feeding (Means ± S.E.)

CONDITION N=8	TIME FEEDING - SECS
INTACT	21.4 ± 1.5
INTACT + VEHICLE	19.7 ± 1.6
SHAM ADX	23.8 ± 1.6
ADX - SALINE	19.7 ± 1.7
ADX - CORT	21.2 ± 2.5
ADX - CORT + 50	21.1 ± 2.1

ONE WAY UNRELATED ANOVA: $F(5,42) = 0.653$, $P = 0.661$

No significant effects were detected.

Table 3:49 The effects of corticosterone on the time spent examining the panels (Means ± S.E.)

CONDITION N=8	TIME EXAMINING PANELS - SECS
INTACT	3.6 ± 1.3
INTACT + VEHICLE	3.3 ± 1.1
SHAM ADX	5.3 ± 1.1
ADX - SALINE	2.7 ± 0.7
ADX - CORT	1.9 ± 0.5
ADX - CORT + 50	2.3 ± 0.7

ONE WAY UNRELATED ANOVA: $F(5,42) = 1.652$, $P = 0.168$

No significant effects were detected.

Table 3:50 The effects of corticosterone the % time spent examining the panels (Means ± S.E.)

CONDITION N=8	% TIME EXAMINING PANEL
INTACT	38.2 ± 7.5
INTACT + VEHICLE	32.3 ± 5.8
SHAM ADX	47.6 ± 6.3
ADX - SALINE	29.9 ± 6.6
ADX - CORT	19.2 ± 4.7
ADX - CORT + 50	27.6 ± 7.9

ONE WAY UNRELATED ANOVA: $F(5,42) = 1.823$, $P = 0.129$

No significant effects were detected.

Table 3:51 The effects of corticosterone on the time spent examining the dish (Means ± S.E.)

CONDITION N=8	TIME SPENT EXAMINING DISH - SECS
INTACT	2.7 ± 0.9
INTACT + VEHICLE	3.2 ± 0.9
SHAM ADX	1.8 ± 0.8
ADX - SALINE	5.0 ± 1.4
ADX - CORT	2.3 ± 0.8
ADX - CORT + 50	1.4 ± 1.1

ONE WAY UNRELATED ANOVA: $F(5,42) = 1.610$, $P = 0.179$

No significant effects were detected.

Table 3:52 The effects of treatments on the plasma levels of corticosterone (Means ± S.E.)

CONDITION N=8	CORT ng/ml
INTACT b.	175.4 ± 26.0
INTACT + VEHICLE	195.2 ± 36.2
SHAM ADX	205.2 ± 27.3
ADX - SALINE a.	10.4 ± 2.1
ADX - CORT d.	61.8 ± 15.9
ADX - CORT + 50 c.	595.3 ± 40.9

KWANOVA: H = 38.65, P < 0.001

a. MWU different from INTACT: U = 0, P < 0.001

b. MWU different from ADX - CORT: U = 4, P < 0.001

c. MWU different from ADX - CORT: U = 0, P < 0.001

d. MWU different from ADX - SALINE: U = 15, P < 0.05

Whilst the INTACT, INTACT + VEHICLE and SHAM ADX groups did not differ from one another, each of the remaining groups differed from all others.

Table 3:53 The results of the correlation analysis between the levels of corticosterone and distractability

	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos	
CONDITIONS	DISH	PANEL
Including adrenally intact mice N=24	-0.096	-0.026
Excluding adrenally intact mice N=12	-0.02	-0.09

There were no significant correlations detected.

Discussion

An analysis of the circulating levels of CORT in those animals placed on the revised replacement regime revealed that a dose of 5µg CORT/ml produced levels within the desired range. Moreover, although the levels reached when 50µg CORT were superimposed upon this baseline were relatively high for a male mouse, they were similar to those found under highly stressful situations and were, therefore, considered to be acceptable. As CORT levels fell within the predicted range, the gradation between controls and experimentals should have afforded an ideal opportunity to assess the extent to which transient elevations in CORT do have the hypothesised effect on the HPC on attentional processes such as distractability. Despite this, distractability appeared to be unaffected by CORT. Furthermore, whilst it is recognised that plasma levels of a hormone do not always provide a reliable index of its activity within the brain - a point covered in greater detail in the general discussion - CORT levels did not correlate with the degree of distractability to either distractant.

Although there were no significant effects, it is interesting to note that some trends in the data support the hypothesis (acute CORT - treated animals showed the lowest distraction intervals to the irrelevant and highest to the relevant stimulus), but others do not (the same animals spent the least time examining the food dish).

The tendency for non-replaced adrenalectomised animals to spend a short period examining the panels in this experiment contrasts with EXPERIMENT 7, in which animals belonging to this condition displayed the opposite tendency. The amount of time spent examining the dish was, however, similar in both. Differences in the interval between surgery and testing may account for this. Indeed, Borrell et al. (1983) found that behavioural deficits emerging 1-2 days after adrenalectomy may disappear 10 days later.

Turning to the levels of CORT present in the various conditions:

surprisingly, neither the sham-adrenalectomised nor the intacts injected with the vehicle solution had significantly higher levels of CORT than their controls. It will be recalled that sham adrenalectomised mice injected with the vehicle have been shown to possess elevated levels (see Table 3:45). In this experiment, the sham adrenalectomy and vehicle controls were separated in order to assess the significance of the independent effects of the test and the injection procedure. To this end, although the differences in the levels of CORT were insignificant, there was a definite upward trend in both conditions relative to controls, suggesting that the elevated CORT levels in the sham adrenalectomised mice injected with the vehicle probably represents the summation of stress responses to the injection and the test; presumably, both being potentiated by the proactive effects at work in the shams. Furthermore, the fact that the vehicle controls tended to have elevated CORT levels, indicates that an interval of one hour is insufficient to allow CORT to return to its baseline level - contrary to the claim made by Levine and Trieman (1969).

Finally, it should be noted that the non-replaced adrenalectomised mice in this experiment had lower CORT levels than the corresponding animals in EXPERIMENT 7 (see table 3:45). The shorter interval between adrenalectomy and blood sampling in this experiment probably accounts for this. Whilst the implementation of a shorter interval had its advantages, it may have led to higher mortality. Presumably a longer interval allows a fuller recovery, and the animals are as a consequence better able to withstand conditions of food deprivation.

General Discussion

It had been reasoned that transient elevations in CORT induce short-term changes in attention via the HPC resembling those that emerge under conditions of hippocampal inactivity. As the behaviour of hippocampectomised animals is consistently affected by distracting stimuli, the finding that CORT failed to exert a similar effect was disappointing.

The early findings that distractability was unaffected by CORT in intact animals was attributed to the unavailability of receptor mechanisms because of high endogenous CORT. This explanation, however, received no support from experiments designed in accordance with the properties of the hippocampal receptor systems. Taken together, the evidence does not support the hypothesis that CORT influences the role subserved by the HPC in attentional inhibition, or at least that reflected by distractability. As the HPC is known to be limited in its capacity to respond to CORT, the absence of an effect of this adrenocorticoid may point to the inherent weakness in an hypothesis attributing any significance to its transient elevations in the functions of this neural substrate. Notwithstanding this, CORT would, if it affects this behavioral phenomenon at all, be expected to affect distractability in a permissive fashion. However, non-replaced adrenalectomised animals did not exhibit a distractability deficit, and by implication CORT does not possess a permissive effect on the functioning of the HPC in distractability.

In spite of this evidence, a dismissal of the working hypothesis, on the basis of a single experimental series, is considered to be unjustified. This is because there are a number of shortcomings that can be recognised with the design of these experiments and these may account for the failure to obtain the predicted effect. Firstly, the distracting stimuli that were used may not have been suitable. The significance of the nature of the distractants has already been alluded to. As a general rule, they must be neither too feeble, so as to go unnoticed, nor too powerful, so as to evoke

a pronounced startle response. The problem of selecting appropriate stimuli is compounded by strain differences. Archer (1977), disclosed that BALB/c and PORTON mice exhibit marked differences in the level of distractability to the same distractants. The dish and panel distractants used in the present experiments were selected on the basis of preliminary studies, in which they were found to evoke distraction intervals of a similar order of magnitude to those previously found in mice. Even so, the possibility remains that these stimuli were not optimum for 'TO' mice. It is possible, for instance, that the degree to which distractability is sensitive to hormonal modulation may differ between strains.

Secondly, there appeared to be a slight tendency for the distraction times to the panels to become shorter over successive runs. In addition, mice often reached an asymptotic level of running by the end of the third training session in the latter runs, unlike the earlier runs, in which running performance was still improving at this point. This suggests that an overtraining effect may have come into play. (If so, it was probably the result of a practice effect on the behalf of the experimenter rather than a change within the animals per se). This may be important for the effects of hormones on distractability as overtraining is thought to enhance persistence. It increases distractability to a relevant, and decreases it to an irrelevant distractant (Archer, 1977). Presumably, this effect arises because as an animal is trained on a task it attends more to stimuli associated with reinforcement and less to others. Thus, after a greater period of training, features of the runway wall (unassociated with the goal) will be progressively excluded, and therefore less effective in distracting the animal, whereas the converse applies to goal-associated stimuli. In view of the effects of overtraining on persistence, if the mice were overtrained, this would probably obscure any effect of CORT on this attentional process.

An additional factor that might have contributed to the failure to

detect a hormone effect relates to the considerable individual variance between 'TO' mice. This strain of mouse is outbred, and therefore, likely to be less uniform, both behaviourally and physiologically, than inbred strains typically used in behavioural studies. However, the problem of variability should have been overcome by examining the extent to which plasma levels of CORT correlated with the level of distractability. The fact that this series of experiments relied heavily on a correlational approach, yet with one exception such a correlational relationship was not found, was very disappointing. Even so, it is important not to overlook the fact that any correlation between CORT and behaviour based on peripheral levels of this adrenocorticoid has its shortcomings. The degree of convergence between peripheral and central hormone levels is a fervently debated subject. Some claim they are proportionally related, whilst others claim that the relationship is inverse. This conflict is likely to have arisen from the lack of control over a number of different factors which are known to affect the levels of a hormone in the periphery relative to the brain. These factors include the specific brain area measured and the interval between administering the hormone and sampling for it. Nicolini et al. (1984) simultaneously measured the concentration of a number of adenohypophyseal hormones in the serum and CSF under basal and activated conditions and found a direct correlation under both conditions, though the levels tended to be greater in the plasma. Despite this, they acknowledge that negative correlations do exist. Moreover, they claim that the nature of the correlation depends on the molecular weight of the hormone and the degree to which it penetrates the blood-brain barrier. This is especially relevant in the light of the recent finding that CORT lowers the permeability of the blood-brain barrier (Izquierdo, 1985; Long and Holaday, 1985). Angelucci et al. (1980) measured the levels of CORT in the peripheral circulation and in the brain one hour after administering the steroid to adrenalectomised mice, and found that with a rapid increase in

plasma levels, there was an inverse correlation with brain levels, though more direct correlations are found when the levels of CORT increase in a steady fashion. In view of these factors it may be too naive to expect to find a correlation between plasma CORT levels and distractability.

Even if plasma levels provided an accurate reflection of brain levels, the degree to which a hormone is bound to its receptors in the brain may be independent of its levels there. Patacchioli et al. (1983) observed an inverse relationship between brain levels of CORT and the capacity of the brain to bind it, especially in the HPC. This presumably arises through the down-regulation of receptors by CORT. Since other factors are known to regulate the number of CORT-receptors e.g., ACTH1-39 (de Kloet and Veldhuis, 1980); ACTH4-10 (Veldhuis and de Kloet, 1982a and 1982b), the likelihood of finding a correlation between plasma levels of CORT and the degree to which this adrenocorticoid occupies its receptors becomes increasingly remote. As the degree of binding is the most important determinant of behavioural activity this reinforces the contention that it may be too simplistic to expect plasma levels to coincide with the behavioural effects of CORT.

A final problem which may account for the failure to find an effect of CORT on distractability is the possible complications introduced by the existence of interactions between the effects of different hormones on behaviour. Thus, the influence of CORT may only gain expression under certain specific conditions. The fact that the only correlation between CORT and distractability that was found appeared to depend on the level of ACTH is suggestive of an interaction (see Table 3:38).

To summarise: as there are a number of plausible explanations for the present failure to find an effect of CORT, it is first necessary to screen its effects on a variety of HPC sensitive behaviours that are considered to require the development of attentional inhibition, before attempting to reach any conclusions regarding the validity of the working hypothesis.

CHAPTER 4

The effects of corticosterone on attention switching as reflected by the degree of responsiveness to a stimulus under conditions of attentional competition

Introduction

In the previous chapter it was concluded that the failure to demonstrate an effect of CORT on distractability could not be taken as proof that this adrenocorticoid is not involved in attentional inhibition. Rather other behavioural tests reflecting this inhibitory influence must firstly be screened. Accordingly this chapter is concerned with its effects on the ease with which attention is disengaged from an established point of fixation, under conditions of attentional competition.

Interest in attention switching originated from the role that the HPC is claimed to play in attentional inhibition. The functional similarity between Pavlovian internal inhibition (Pavlov, 1927) and attention shifting (Douglas and Pribram, 1966) is well established (Douglas, 1967), and has already received detailed discussion in Chapter 1.

Thus, assuming that the HPC is involved in attentional inhibition, disruption of this brain area would be expected to impair the development of this inhibitory influence, and as a result initially prepotent stimuli would continue to control attention. This would reduce the ability to decouple attention from an established point of fixation. However, there are surprisingly few studies dealing directly with the HPC and attention switching. Nonetheless, the indistractability to an irrelevant stimulus (e.g., Riddell et al., 1969), combined with the normal level of distractability to a relevant stimulus (e.g., Douglas and Pribram, 1969) after hippocampal lesions in rats and Rhesus monkeys respectively, may indicate a reduced ability to disengage attention from stimuli that have assumed attentional control. Furthermore, the finding that

hippocampectomised monkeys experience greater difficulty in learning to respond in sequence to two stimuli presented simultaneously has also been attributed to a reduction in the capacity to switch attention (Kimble and Pribram, 1963). This is based on the observation that the cue immediately preceding reward tends to control attention.

More direct evidence has been provided by the discovery that hippocampectomised animals display a reduced level of responding to a dishabituating stimulus. Gustafson (1975) demonstrated that when the presentation of a distractant which appeared to have undergone habituation was immediately preceded by a second distracting stimulus, the response to the latter was less marked. In addition, this stimulus was less effective in dishabituating the response. There are, however, two possible explanations of this.

One holds that the process of habituation was incomplete in the hippocampectomised animals: the second, that the attention of these animals was still captivated by the initial distractant, albeit not in an overtly observable fashion, so that the presentation of the second stimulus passed unnoticed. (The plausibility of this suggestion is supported by the finding that habituation of overt responding may occur in the absence of habituation of an observing response to a stimulus - Douglas and Pribram, 1969). The latter interpretation agrees well with a lesion-induced disruption of the processes involved in the shifting of attention.

The most direct indication of an attention switching deficit was the demonstration by Hendrickson et al. (1969) that orientation was absent or greatly reduced in hippocampectomised animals when either a motivationally relevant or novel neutral stimulus had previously captured attention, but not otherwise. As this occurred regardless of the motivational significance of the competing stimulus, it suggests attention, rather than motivation is the process altered by hippocampectomy.

It is important to note that although hippocampectomised animals are

characterised by an impaired capacity to switch attention, attention shifts do occur given time. Presumably, the control over behaviour exerted by any one stimulus wanes as a function of a number of factors e.g., satiation, even in these animals. As the capacity to switch attention is not completely absent, this indicates that the HPC is not essential. Rather, the normal functioning of this area of the brain is believed to be most crucial when rapid shifts of attention are needed.

As CORT is predicted to induce effects resembling those associated with hippocampectomy, it would be expected to impair attention switching, especially under circumstances necessitating rapid shifting. However, there are no known studies dealing directly with this subject. Having said this, Birke (1979) studied the effects of the oestrous cycle on the pattern of investigation of four novel objects presented in an arena, and found that female rats and guinea pigs displayed longer bouts of investigation at oestrus than at dioestrus. Furthermore, oestrus females tended, once they had finished investigating one object, to move directly to a different object. Taken together this suggests that if attention has been focussed, shifts of attention are less likely to occur at oestrus. Whilst this may point to a direct effect of the principal hormones associated with the oestrus cycle, namely oestrogen and progesterone, Birke (1979) acknowledges that CORT may be involved. Indeed, CORT is elevated at oestrus (Resko, 1969*), and it may, therefore, be responsible for the observed reduction in attention switching.

In order to assess the effects of CORT on attention switching the level of responding to the presentation of a second novel stimulus was examined under various levels of CORT. In principle the design of the experiment was similar to that adopted by Hendrickson et al. (1969), though the testing situation differed greatly. Most importantly, unlike Hendrickson et al. (1969) who presented the novel stimulus once the animals had ceased to exhibit any overt behaviour, novelty responsiveness was

examined whilst the animal was running along a runway. It will be recalled that in the runway test an animal is trained to run for a food reward until an asymptotic level of running has been reached, whereupon a distracting stimulus is presented. In this study, however, two stimuli were presented in rapid succession. As before, their effect was reflected by the distraction interval.

It was of fundamental importance to ensure the suitability of the stimuli that were used: each had to exert a similar effect on the degree of novelty responsiveness. In addition, the successive presentation of the 2 stimuli had to evoke a greater response than either presented by itself on the controls (see EXPERIMENT 1). Only when this had been established was it possible to proceed with the experiment proper (see EXPERIMENT 2). The theoretical premise upon which this experiment was based holds that if persistence is enhanced the likelihood of responding to the second stimulus would be diminished. This is because the presentation of the first novel stimulus would result in the activation of the central specifications corresponding to this stimulus, and these specifications would continue to control attention. Thus, if CORT suppresses the development of attentional inhibition with the ensuing potentiation of persistence, the likelihood of responding to the second stimulus would be reduced and its presentation would not produce a significantly greater effect upon the distraction interval than the first distractant alone. By contrast; in controls, as the development of attentional inhibition proceeds normally, the efficiency of attention switching should be greater and animals should respond to both stimuli. Accordingly, the distraction interval should be greater, because it would represent the cumulative effect of both stimuli. (This premise relies heavily on my inability to demonstrate an effect of CORT on the level of distractablity to a panel distractant in Chapter 3).

Finally, the subjects of this experiment were adrenalectomised and placed on a maintenance dose of 5µg CORT/ml drinking water. A strategy that

had emerged to be the most suitable for examining the effects of short-term changes in CORT on processes subserved by the HPC.

EXPERIMENT 1

The effects of single distracting stimuli as compared with the effects of two distractants presented in succession on the distraction interval in the runway

The purpose of this experiment was to ensure that the distracting stimuli gave similar distraction intervals when presented singly, but a greater distraction interval when presented successively.

Method

Subjects and procedure

The procedures for pretraining and training were identical to those in Chapter 3, though that for distractability testing was modified. Following 3 control trials there was a single test trial, in which 2 white panels (7 X 11cm) placed half way along the runway walls (distractant 1), a strip of sandpaper (7cm wide) lying halfway along the runway floor (distractant 2), or 2 white panels a quarter of the way along the runway followed immediately by the strip of sandpaper (distractant 3), were presented. The time taken to leave the start box and feed from the food dish was recorded. The distraction interval was determined by subtracting the mean running time for the 3 control trials from the running time on the test trial.

6 animals were presented with each of the distractants. 20 mice were used, though 2 did not run to criterion.

Statistical analysis and results

The distraction data is presented as means ± S.E. and was analysed by the KWANOVA followed by the MWU test.

Table 4:1 The effects of stimuli presented singly and successively on the distraction interval (Means ± S.E.)

STIMULUS N = 6	DISTRACTION TIME - SECS
PANELS	4.5 ± 1.2
SANDPAPER	4.8 ± 1.2
PANELS + SANDPAPER a.	16.4 ± 4.4

KWANOVA: H = 8.9, P < 0.05

a. MWU different compared with either panels or with sandpaper alone, P < 0.001

Distractability to the stimuli presented singly did not differ, however the successive presentation of these stimuli resulted in a significantly greater distraction interval.

Discussion

The heightened response in control animals to the presentation of two stimuli, which in themselves evoke a similar magnitude of responding, shows that their successive presentation has a cumulative effect on the level of responsiveness. These stimuli were, therefore, considered suitable for the subsequent experiment.

It is worthwhile pointing out that several pilot studies were carried out prior to the one reported here, and that the apparent fortuitous selection of stimuli fulfilling the necessary criteria conceals the reality of the situation.

EXPERIMENT 2

The effects of corticosterone on the response to the presentation of a second distracting stimulus in the runway

The purpose of this experiment was to examine the effects of transient elevations of CORT on the responsiveness to the presentation of a distractant under conditions of attentional competition.

Method

Experimental conditions and subjects

There were 10 animals in each condition:

1. Intact controls (INTACT)
2. Adrenalectomised maintained on the CORT solution (5µg CORT/ml saline solution - 1% ethanol in 0.9% w/v NaCl) (ADX-CORT)
3. Adrenalectomised maintained on the CORT solution injected with 0.1ml propylene glycol (ADX-CORT+VEHICLE)
4. Adrenalectomised maintained on the CORT solution injected with 50µg CORT in the vehicle (ADX-CORT+50)
5. Adrenalectomised maintained on the CORT solution injected with 100µg CORT in the vehicle (ADX-CORT+100)

All the injections were given subcutaneously one hour prior to testing. The treatments were coded.

64 adrenalectomised and 12 intact mice were used; though 6 adrenalectomised and 2 intact failed to reach criterion and 18 adrenalectomised died. All the operations were carried out 5 days prior to the start of testing. Immediately after surgery, the adrenalectomised mice were placed on the CORT solution and were maintained on this throughout the period of behavioural testing.

Procedure

There were no procedural differences from those given in EXPERIMENT 1, panels followed by sandpaper were presented.

Animals were adrenalectomised in batches of approximately 20 and after recovery were allocated at random to treatments. The intacts were selected, also at random. Within a batch, animals were tested from each condition in a pre-set sequence until the batch was completed. Three such batches were needed for this experiment. The animals were blood sampled immediately after the test trial, and the samples were subsequently assayed for CORT.

All behavioural testing was carried out between 12.00 - 3.30 pm.

Statistical analysis

Both the data for the distraction interval and the CORT data is presented as means ± S.E., and was analysed by the KWANOVA followed by the MWU, where this was necessary.

Spearman's Rank Correlation Coefficient was used to assess the degree of correlation between the levels of CORT and the distraction interval, within conditions and over conditions (both including and excluding adrenally intact animals).

Results

Table 4:2 The effects of corticosterone on the distraction interval to the panel-sandpaper stimuli (Means ± S.E.)

CONDITION N=10	DISTRACTION TIME-SECS
INTACT	15.8±4.2
ADX-CORT	10.3±1.2
ADX-CORT+VEHICLE	10.4±1.7
ADX-CORT+50	9.0±1.6
ADX-CORT+100	6.9±0.8

KWANOVA: H = 7.49

No significant differences were detected.

Table 4:3 The effects of treatments on the plasma levels of corticosterone (means ±S.E.)

CONDITION N=10	CORT ng/ml
INTACT[a]	186.3±27.2
ADX-CORT	71.6±10.2
ADX-CORT+VEHICLE[b]	91.0±12.7
ADX-CORT+50[c]	543.1±38.1
ADX-CORT+100	820.0±37.6

KWANOVA: H = 42.01, $p<0.001$

a. MWU different from ADX-CORT: U=8, $p<0.001$

b. MWU different from ADX-CORT+50: U=0, $p<0.001$

c. MWU different from ADX-CORT+100: U=0, $p<0.001$

There were significant differences in the levels of CORT in all conditions, except the ADX-CORT and ADX-CORT+VEHICLE conditions.

Table 4:4 The results of the correlation analysis between corticosterone and the distraction interval to the panel-sandpaper stimuli-within conditions

CONDITION N=10	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos
INTACT	-0.188
ADX-CORT	-0.367
ADX-CORT+VEHICLE	-0.139
ADX-CORT+50	0.779*
ADX-CORT+100	0.054

* $p<0.05$

There was a positive correlation in the ADX-CORT+50 condition, but not in any other condition. No significant correlations were detected when the data from the various conditions was combined, regardless of whether the analysis included adrenally intact animals, or not. The observed value of rho being -0.262 (N=50) and -0.279 (N=40) respectively.

General discussion

Despite the presence of markedly different circulating levels of CORT there were no significant differences in the distraction interval evoked in response to the successive presentation of two novel stimuli. This finding was disappointing, especially as there appeared to be a very suggestive trend within the data: animals possessing lower levels of CORT tending to exhibit longer distraction intervals. Thus, intacts displayed the greatest level of responsiveness to the successive presentation of two novel stimuli; though the distraction interval of these animals was not markedly dissimilar from either the maintenance controls or the vehicle controls. In contrast, those animals treated with CORT appeared to be relatively less affected by the second novel stimulus. This was especially the case upon the administration of 100µg CORT. The progressive decline in the level of responsiveness to the second stimulus under conditions of attentional

competition as the levels of CORT increased, points to a possible dose-dependent effect on attention switching.

As there is considerable individual variability within TO mice this had at first been put forward to account for the failure to detect a significant difference. If this was the case the correlational analysis should have been significant. As it was not, the hypothesis that CORT impairs attentional switching is weakened. As a matter of fact, the discovery of a positive correlation, even though it was confined to those animals possessing relatively high levels of CORT, indicates that within a specific range, those animals with higher levels were more responsive to the presentation of a second novel stimulus. This suggests that CORT acted to enhance attention switching. It is difficult to offer a satisfactory explanation of this, especially as it stands opposed to the observation that animals with higher levels of this adrenocorticoid were inclined to be less responsive to stimuli under conditions of attentional competition.

The levels of CORT present in the circulation following chronic treatment with 5µg CORT/ml were similar to those found under resting conditions at the circadian peak in adult male mice. Surprisingly, intact mice had higher CORT levels than those maintained on the replacement therapy. (As no similar difference had been found in the previous runway experiments, this may mean that the presentation of two novel stimuli is a more stressful experience than the presentation of a single stimulus).

It was difficult to account for the presence of the high levels of CORT that arose upon the administration of 50µg, and in particular 100µg CORT, especially as they were higher than had previously been found with these doses. In view of the presence of such high CORT levels after the administration of 100µg CORT, it seems unlikely that the associated behavioural effects would be of physiological significance. When the limitations of the receptor mechanisms responsible for mediating CORT's effects on behaviour are recalled, this can be more fully appreciated.

Similarly, though to a lesser degree, as the level of CORT reached after a dose of 50μg CORT is at least equivalent to, if not in excess of, that occurring under extremely stressful conditions, the physiological significance of the behavioural effects emerging in this condition are dubious. Recognition of this casts doubt on the true meaning of the positive correlation between CORT and the distraction interval.

As hippocampectomy reduces the level of responsiveness to novel stimuli when attention is captured by a different stimulus (Hendrickson et al., 1969), implicating the HPC in attentional switching, the failure to uncover a similar effect with CORT undermines the contention that it suppresses this function of the HPC. Moreover, the doubts that this raise extend beyond the degree of equivalence between the effects of hippocampectomy and CORT in attention switching to the fundamental significance of the adrenocorticoid in attentional inhibition. Indeed, if CORT impairs the development of this inhibitory influence, the degree to which the central specifications for a particular stimulus persist in controlling attention should be enhanced. The inevitable result of which should be a reduction in the ease with which attention is decoupled from one stimulus and shifted onto another. Accordingly, the hypothesis that CORT suppresses hippocampal attentional inhibition may need to be revised. Beforehand two alternatives should be considered:

Firstly, some treatment conditions were far from ideal as the circulating titres of CORT were likely to be in excess of the capacity of the receptor-mediated mechanisms responsible for its actions at the HPC. Secondly, with the benefit of hindsight, the experimental design may have been flawed. It depended on the assumption that the first stimulus of the pair gained the animals attention, and whilst it most certainly did so, what emerged so clearly in Chapter 3 was the great variation between even control animals in the length of time their attention was held by this distractant. This means that by the time the second stimulus was presented

some would have already switched their attention back to the goal, whilst others would still be attending to the first stimulus. The unfortunate consequence of this is that there was no uniform baseline established for the controls. Furthermore, as it is difficult to resolve the point of fixation at the presentation of the second stimulus, the possibility remains that attention had reverted to the food dish. Therefore, this experiment could have been assessing the level of distractability to two irrelevant distractants in sequence, rather than the ease with which attention is switched between these two stimuli. If this was the case, as Chapter 3 failed to demonstrate an effect of CORT on the level of distractability to an irrelevant stimulus, the present finding may no longer prove so surprising.

In retrospect, it would have been more appropriate to have presented the novel stimuli against a neutral background as this would have increased the likelihood of attention remaining on the first novel stimulus. In connection with this, Hendrickson et al. (1969) found that the performance of a behavioural response towards a motivationally relevant stimulus at the time of the presentation of the novel stimulus produces an OR deficit in hippocampectomised animals, intimating that this response introduces an element of competition. Despite this, Gustafson (1975) demonstrated that the OR to visual and auditory stimuli was only slightly affected by hippocampectomy when these stimuli were presented during ongoing behaviour, indicating that the occurrence of a behavioural response does not invariably compete with the novel stimulus.

Finally, several methodological points need to be discussed. Firstly, as the stimuli were balanced with respect to their intensity and relevance, it was reasoned that the need to counterbalance their order of presentation was negligible. However, as their modality differed, and the effectiveness of a distractant is modality dependent (Mason and Fibiger, 1978), it could be argued that they should have been counterbalanced.

A further criticism is that as both stimuli were present throughout the test trial, it is possible that they acted as a compound stimulus. This argument assumes that the sandpaper was treated as a visual stimulus. Whilst this possibility can not be denied, and whilst it would offer yet another explanation of the failure to find an effect of CORT on attention switching, it is unlikely as the sandpaper was the same colour as the runway base.

Lastly: although this point was mentioned earlier it is important to reiterate that dysfunctions in the capacity to switch attention may only emerge under specific conditions. This is highlighted by the evidence that the disengagement of attention from an established point of fixation does occur in animals bearing hippocampal lesions, despite their impaired attentional inhibition. Thus, they still learn about the presence of redundant cues, whilst focussing on a stimulus in a discrimination task (Harley, 1972). Similarly, they do not always have an OR deficit when a novel stimulus is presented during the performance of a behavioural response (Gustafson, 1975).

In the light of the criticism that can be raised against this experiment, combined with the apparent sensitivity required to reveal alterations in attention switching, the lack of success in showing an effect of CORT need not imply a total rejection of the working hypothesis. Any conclusions relating to the interaction between this adrenocorticoid and the HPC, and the implications this has for its influence in attentional inhibition must, therefore, be deferred until the experimental foundations necessary for these conclusions have been more firmly established.

CHAPTER 5

The effects of corticosterone on habituation

Introduction

Novelty responsiveness and its subsequent habituation are regarded as attentional phenomena (Deutsch and Deutsch, 1963; Lynn, 1966; Mason and Iversen, 1979). Habituation refers to the decrement in response amplitude as a function of the repeated presentation of the stimulus that originally evoked the response (Sokolov, 1963*). The HPC is thought to be important for normal habituation (Douglas, 1967; Kimble, 1968). In view of the hypothesis that CORT suppresses the attentional functions subserved by the HPC, the experiments reported in this chapter ascertain CORT's influence on habituation. The following discussion first reviews the documented effects of hippocampectomy on habituation, pinpointing and clarifying some of the confusion surrounding these effects. Several of the most plausible explanations of the observed habituation deficit are then presented, and their relevance to the study of the effects of CORT on attention assessed. Finally, previous studies exploring the relationship between CORT and habituation are detailed, concluding with a brief description of the experimental strategy adopted for the experiments reported in this chapter.

It is widely upheld that the HPC is essential for normal habituation, and that hippocampal damage reduces the rate of habituation. The effects of hippocampectomy on behavioural habituation have been examined using several different response categories (e.g., spontaneous activity in a novel environment and the OR). The contexts also vary greatly, from an open-field to a complex runway situation. The contention that hippocampectomy retards habituation has principally been derived from reports that it prolonged activity in a novel environment (Roberts et al., 1962; Douglas and Isaacson, 1964). Despite this, the literature is fraught with inconsistences, to the extent that behavioural differences have been

reported even under similar testing situations. Thus, Jarrard (1968) found that the habituation of activity in a novel environment proceeded at a normal rate in hippocampectomised animals.

Kohler (1976), in a comprehensive analysis of the effects of 2 different hippocampal lesions on the habituation of a variety of behavioural responses in the rat, identified 3 factors that might account for this inconsistency:-

i. Size and type of lesion.
ii. Nature of the specific class of response undergoing habituation.
iii. Level of task-associated arousal.

These will be discussed in turn.

Lesion type 1 retarded the habituation of the OR, though the rate of habituation of an arrest-reaction to the same stimulus was increased! Habituation of locomotor and rearing responses, as well as the hole-poke response proceeded normally. In contrast, only across-session habituation of the OR and the hole-poke response were affected by lesion type 2. The other responses habituated normally. On the basis of this evidence Kohler (1976) concluded that hippocampectomy does not produce a general dysfunction in habituation, and where present it depends in part on the specific nature of the lesion.

Furthermore, the nature of the response to be habituated is important. This suggests that habituation is not a unitary phenomenon, and that different neural mechanisms underlie the habituation of different responses. Moreover, the HPC may only be involved in specific classes of response: Gustafson and Koenig (1979) revealed that the OR and the subsequent behavioural reactivity towards a novel object were differentially affected by hippocampectomy. Habituation of the latter was retarded whilst the former habituated normally. Similarly, whilst

hippocampectomised monkeys failed to habituate to a distractant presented during a sequential task, this depended on the measure used, as habituation of the overt response was normal, whereas the 'observing response' was retarded (Douglas and Pribram, 1969).

The finding that the habituation of an arrest-reaction was facilitated is at direct variance with the notion that lesions to the HPC induce a deficit in this process. The level of task-associated arousal, and its implications for the relative importance of stimuli connected with the task, are invoked to account for this. Kohler (1976) points out that this finding was restricted to conditions of deprivation, which are likely to induce a high level of arousal. Furthermore, the novel stimulus was presented against a motivationally relevant response. Consequently, the faster habituation of the arrest-reaction may reflect an enhanced capacity to 'block-out' stimuli competing with motivationally relevant behaviour, especially if this behaviour is well established and the level of arousal is high.

Although each of the above may obscure the effects of hippocampectomy on habituation, its effects are generally considered to reflect a genuine disruption of habituation, though this is not without opposition. These effects have also been attributed to changes in the level of activity or exploration, rather than a change in the process of habituation per se. The problem this introduces is accentuated by the difficulty in disentangling cause and effect. Furthermore, exploration which implies the acquisition of information from the environment (Barnett and Cowan, 1976) is often equated with activity. However, as activity may change for a variety of reasons, other than a true change in the level of exploration - such a measure of exploration is considered to be of limited use and may even be misleading (Glickman and Hartz, 1964; Birke, 1979).

Leaton (1965) attributed the slower rate of habituation by hippocampally lesioned rats in a T-maze to a reduction in the inhibition of

exploratory tendencies. Furthermore, Leaton regarded any apparent changes in activity as reflecting changes in the level of exploration, supporting this by the disclosure that there were no initial differences in the level of activity between lesioned and non-lesioned animals. Conversely, Nadel et al., (1975) argue that hippocampally lesioned animals are hyperactive and not hyperexploratory, as they display highly repetitive behaviour which is not directed toward any specific aspect of the environment. They cite as further support the evidence that a novel object presented in the home cage is explored less, despite a high level of activity (Glickman et al., 1970). Although Nadel et al., (1975) present a fairly convincing case for their position, the effect of hippocampectomy on activity is inconclusive. Whilst hippocampectomised animals enter more squares in an open field (Kimble, 1963), the level of activity in these animals depends on the measure of activity, the level of arousal and the specific conditions under which it is expressed (Isaacson, 1974).

Douglas (1967) asserts that any apparent differences in either the level of activity or exploration reflect a direct lack of habituation. This is supported by the demonstration that even though hippocampectomised animals exhibited a reduced tendency to spontaneously alternate and maintained a high level of locomotor activity in a novel environment for longer periods than their controls; neither the latency to leave the start box in the spontaneous alternation task, nor the initial level of activity in the free locomotor task were affected (Roberts et al., 1962). These findings are, however, not without exception. Kohler (1976) observed an initial elevation in the level of free locomotor activity in hippocampectomised animals, yet by the end of the testing session the level of activity of these animals had fallen to that found in their controls. In view of the similarities in the duration of the test session (15 mins), and the testing procedure, these differences were at first difficult to explain. Roberts et al., (1962) noted a correlation between the amount of

hippocampal destruction and the rate of habituation and this suggests that variations in the lesion might provide the explanation. In conclusion, despite the qualifications reviewed above, the evidence indicates that hippocampectomy tends to impair the rate at which habituation proceeds.

Turning to the possible explanations of this: reference has already been made to the possibility of ascribing an attentional interpretation to an alteration in the rate of habituation. However, as is often the case within an attentional framework, there exist a number of different, though not necessarily mutually exclusive, explanations for such an alteration. The first relies heavily on Sokolov's (1963*) theory of habituation of the OR. This is discussed in greater detail in Chapter 1; in brief, habituation depends on the progressive elaboration of the central model for the stimulus evoking the response, and is complete when the presentation of the stimulus is followed by its comparison with a fully matching model. Both the OR and the exploration that follows serve to increase the acquisition of stimulus - information, and thereby elaborate the recognition unit. Thus, an animal may continue to respond to a stimulus because it fails to extract as much information about it, at any one time, as an animal exhibiting a normal rate of habituation. This results in residual uncertainty over its identity, and the animal must continue responding for longer in order to gain the information required to construct an internal representation sufficiently detailed for a match to occur, and for the response to habituate.

The fact that hippocampectomised animals do not lack the ability, but rather take a longer time to habituate is consistent with the suggestion that the processing of stimulus information differs upon hippocampal disruption. This is substantiated by the finding that these animals tend to be unresponsive to the presence of additional cues in the environment (Winocur and Mills, 1970), though the degree to which this occurs depends on the significance of the stimuli (Douglas and Pribram, 1969). Similarly,

Isaacson (1974) showed that animals bearing hippocampal lesions do not gain as much information from the environment as controls: pre-exposure of lesioned animals to a maze did not improve subsequent maze learning. In spite of this, such animals are able to respond to redundant cues (Harley, 1972). The explanation is not, therefore, simply to be found by regarding them as less able to process stimulus information.

An alternative explanation is based on Pavlov's (1927) claim that habituation involves internal inhibition. If this is so, the rate of habituation would be expected to be retarded if the development of this inhibition is impaired. Assuming that the HPC is the site of attentional inhibition, the lesion-induced retardation of habituation would be attributable to the failure to develop this inhibitory influence.

The retardation of habituation associated with hippocampectomy may, therefore arise through a disruption of the processing of information, with less or different information being taken in; and/or through an increased tendency to persist in attending to stimuli that already control attention, as a result of the failure to develop attentional inhibition.

If, as hypothesised, CORT suppresses the attentional functions of the HPC, it should retard habituation in a similar fashion to hippocampal lesions. In addition, the absolute level of responsiveness to a stimulus should not be affected. However, studies into the effects of CORT on habituation, though limited in number, leave the impression that this process is not retarded. For instance, even though Davis and Zolovick (1972) found that adrenalectomy was without effect on the habituation of a startle response, Levine (1971) demonstrated that it was retarded - a finding which is opposed to the hypothesised influence of CORT. Furthermore, the report that CORT given to intact rats failed to affect the habituation of an OR to an auditory stimulus and of a hole-poking response (File, 1978) is especially disturbing, as both these measures of habituation are known to be retarded by hippocampectomy (Kohler, 1976).

As habituation is a 'test-dependent' phenomenon (Kohler, 1976; File, 1978), this chapter examines the effects of CORT under two different testing situations: habituation of the investigatory response towards a novel object in a familiar environment, and habituation of a hole-poke response. Examination of the former situation was supported by the finding that T, which is also claimed to increase persistence (see Chapter 3), retards the habituation of this response. Testosterone-treated chicks responded to a novel object for longer, and the resumption of maintenance behaviour was delayed (Andrew, 1976). In order to explain this, it has been assumed that T, through its enhancement of persistence, acts upon the rules of selection specifying the novel object, increasing their persistence of activation, so that attention is sustained to a greater extent than usual. Surprisingly the effects of hippocampectomy on the habituation of this particular response has not been subject to direct investigation. Nonetheless, it is known that animals bearing hippocampal lesions do tend to display a low level of responding to a novel object presented in a familiar environment (Douglas and Pribram, 1966; Glickman et al., 1970). The nature of the environment is relevant because the level of responsiveness elicited by a novel stimulus depends on the background against which it is presented. Presumably the degree of contrast between an object and its background influences the perceived novelty of the stimulus, and thereby the response it evokes. This is suggested by the finding that an avoidance reaction is elicited by a novel object in a familiar environment, whereas the same object in an unfamiliar environment elicited an investigatory reaction (Misslin and Ropartz, 1981a; 1981b). The fact that the degree of reponsiveness to a novel object depends on its background points to the importance of selecting the appropriate background when studying the effects of CORT. Whilst studies using "TO" mice have confirmed the occurrence of differential reponsiveness to novelty as a function of the background conditions, this strain of mice tend to exhibit

a greater level of investigation to a novel object in a familiar environment (Creighton, 1985; personal observations). In order to avoid the potential complications introduced by a low level of responding, CORT's effects were examined with the novel object being presented in a familiar environment, where the level of responsiveness would be high. The mice were housed in large cages. This was because preliminary studies using small cages had indicated that, by virtue of the confined area of these cages, mice were forced to be in close proximity to the object, producing unrealistically high measures of novelty responsiveness. Additionally, under these conditions it was often difficult to determine whether behaviour involving the object was truly investigatory. As before, CORT was initially given to intact mice and subsequently to adrenalectomised mice placed on a maintenance dose of either 20 µg or 5µg CORT/ml. Although adrenally intact mice had controlled levels of T, adrenalectomised mice did not, for reasons given in Chapter 3.

With respect to the habituation of a hole-poke response: it has been shown that the number of hole-pokes is unaffected by hippocampectomy. However, habituation of hole-poking across sessions, though not within a session, is retarded, albeit in a lesion-dependent manner (Kohler, 1976). Accordingly, CORT would be expected to retard the habituation of this response across sessions, though not necessarily within sessions. As File (1978) found that it did not affect the rate at which this response habituates in intact rats, the response was examined in adrenalectomised animals placed on a maintenance dose of 5µg CORT/ml drinking water.

EXPERIMENT 1

The effects of corticosterone on the habituation of responding to a novel object presented in a familiar environment in adrenally intact mice

Method

Experimental conditions and subjects

There were 10 animals in each condition:

1. Castrates injected with 7.5mg T-oenanthate in 0.1ml olive oil (CxT)
2. T-replaced castrates injected with 0.1ml propylene glycol (CxT+VEHICLE)
3. T-replaced castrates injected with 50µg CORT in the vehicle (CxT+CORT50)
4. T-replaced castrates injected with 100µg CORT in the vehicle (CxT+CORT100)

All the injections were given subcutaneously. With the exception of T, which was administered 48 hours prior to testing, the other treatments were given one hour prior to testing. The CORT treatments were coded.
42 mice were used. They were castrated at 12 weeks of age and left to recover for 3 weeks. 2 mice died.

Procedure

Once the recovery period had elapsed, the mice were individually housed in a large cage for 48 hours prior to testing. On the day of testing, 55 minutes after injection, a mouse, in its cage, was taken into an adjacent laboratory. Five minutes later it was placed in a small bare cage for 30 secs, while the novel object (a metal boss - see Figure 5:1)

was placed in the centre of the home cage. The mouse was then returned to its cage.

Observations were made over 5 successive one minute periods, and the following were recorded, using a pen and paper:-

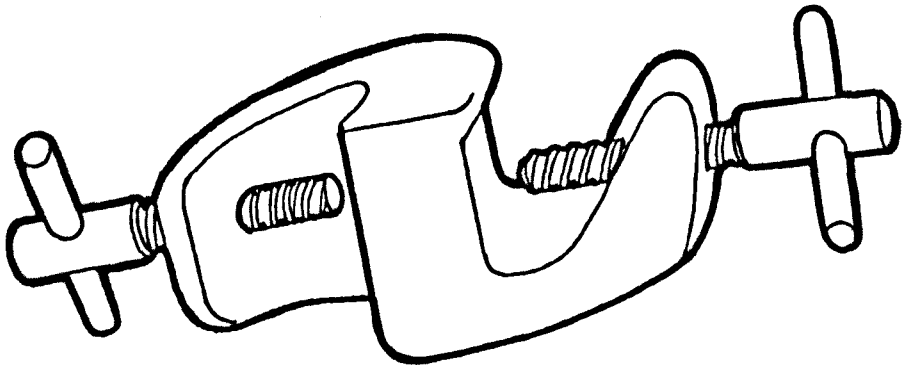
i. time investigating the object
ii. number of investigations

Investigation of the object comprised sniffing the object with nose not more than 2cm away from it, touching it, or climbing onto it.

At the end of 5 minutes, the mouse was blood sampled. These samples were subsequently assayed for CORT.

Mice were castrated in batches, and after recovery were ascribed at random to treatments. Within any one batch there were an equal number of mice from each treatment condition, and the mice were tested from every condition in a pre-set sequence until the batch was completed. 4 such batches were needed for this experiment. All behavioural testing was carried out between 12.00-4.00pm.

Figure 5:1 The novel object (actual size)



Statistical analysis

The behavioural data is presented as means ± S.E. Unless otherwise stated it was transformed (LOG X + 1) to reduce heterogeneity of variance. The following were analysed, minute by minute, using a two-way mixed ANOVA:-

i. number of investigations
ii. time investigating
iii. mean bout length

Any significant difference was further analysed by a one way unrelated ANOVA at each minute interval. If a difference was detected at any one minute, the Tukey test was implemented to explore this.

The data was then combined over the 5 minutes and analysed by a one way unrelated ANOVA:-

i. total number of investigations
ii. total time investigating
iii. overall mean bout length

The CORT data is presented as means ± S.E. and was analysed by a one way unrelated ANOVA, followed by the Tukey test.

Spearman's Rank Correlation Coefficient was used to assess the degree of correlation between CORT levels and the difference from minute 1 to 5 in all 3 measures of novelty responsiveness. Both within and combined conditions correlations were determined.

Results

Table 5:1 The effects of corticosterone on the number of investigations. (Means ± S.E.)

CONDITION N=10	NUMBER MINUTE 1	2	3	4	5
CxT	7.7 ± 1.1	5.5 ± 1.0	4.3 ± 0.9	4.4 ± 1.2	3.5 ± 0.8
CxT+VEHICLE	7.6 ± 0.7	5.5 ± 1.0	4.4 ± 0.7	2.5 ± 0.5	3.0 ± 0.7
CxT+CORT50	8.0 ± 1.1	4.6 ± 1.2	3.8 ± 0.8	2.9 ± 0.5	2.7 ± 0.6
CxT+CORT100	7.0 ± 1.1	4.9 ± 0.8	3.8 ± 0.7	3.5 ± 0.5	2.7 ± 0.6

TWO WAY MIXED ANOVA:

Treatment $F(3,36) = 0.138$, $P = 0.94$

Time $F(4,144) = 26.62$, $P < 0.001$

Interaction $F(12,144) = 0.533$, $P = 0.89$

(see Figure 5:2)

Table 5:2 The effects of corticosterone on the time investigating. (Means ± S.E.)

CONDITION N=10	TIME - SECS MINUTE 1	2	3	4	5
CxT	9.1 ± 1.8	7.3 ± 1.8	5.7 ± 1.7	5.0 ± 1.0	5.3 ± 2.3
CxT+VEHICLE	15.3 ± 1.6	16.1 ± 3.0	11.3 ± 2.6	3.2 ± 1.4	5.5 ± 2.3
CxT+CORT50	11.3 ± 1.9	7.8 ± 2.2	10.7 ± 3.0	8.7 ± 3.7	3.7 ± 1.4
CxT+CORT100	10.1 ± 1.7	10.1 ± 3.0	7.5 ± 2.2	4.8 ± 1.9	2.0 ± 0.5

TWO WAY MIXED ANOVA:

Treatment $F(3,36) = 0.41$, $P = 0.75$

Time $F(4,144) = 20.11$, $P < 0.001$

Interaction $F(12,144) = 1.67$, $P = 0.08$

(see Figure 5:3)

Table 5:3 The effects of corticosterone on the mean bout length. (Means ± S.E.)

CONDITION N=10	MEAN BOUT LENGTH - SECS MINUTE 1	2	3	4	5
CxT	1.2 ± 0.2	1.1 ± 0.2	1.2 ± 0.4	1.6 ± 0.6	1.3 ± 0.4
CxT+VEHICLE	2.1 ± 0.2	3.3 ± 0.9	4.0 ± 2.0	1.2 ± 0.5	1.4 ± 0.3
CxT+CORT50	1.4 ± 0.2	1.5 ± 0.4	2.1 ± 0.5	2.4 ± 0.9	0.9 ± 0.3
CxT+CORT100	1.4 ± 0.2	1.8 ± 0.4	1.9 ± 0.6	1.2 ± 0.4	0.7 ± 0.1

TWO WAY MIXED ANOVA:

Treatment $F(3,36) = 1.02$, $P = 0.39$

Time $F(4,144) = 5.41$, $P < 0.001$

Interaction $F(12,144) = 1.82$, $P < 0.05$ a

(see Figure 5:4)

a. ONE WAY UNRELATED ANOVA:

Minute 1 $F(3,36) = 2.69$, $P = 0.06$

Minute 2 $F(3,36) = 2.68$, $P = 0.06$

Minute 3 $F(3,36) = 1.24$, $P = 0.31$

Minute 4 $F(3,36) = 0.40$, $P = 0.75$

Minute 5 $F(3,36) = 1.09$, $P = 0.37$

No significant treatment effect was detected for any index of responsiveness to a novel object.

The number of investigations, time investigating and mean bout length all diminished with time. However, with the exception of the latter, there was no interaction between the hormone treatments and the rate of habituation. The interaction effect was probably due to the vehicle controls as they displayed a much steeper increase in the mean bout length, upto and including minute 3, which rapidly declined thereafter. By contrast, the other conditions were steady or increased the mean bout length upto minute 3 or 4, before declining.

Figure 5:2 The effects of corticosterone on the number of investigations (means).

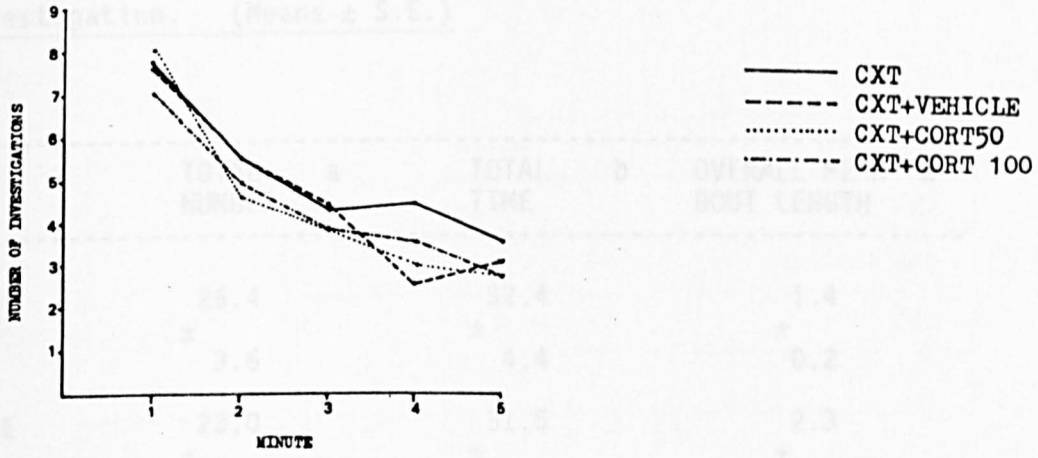


Figure 5:3 The effects of corticosterone on the time investigating (means).

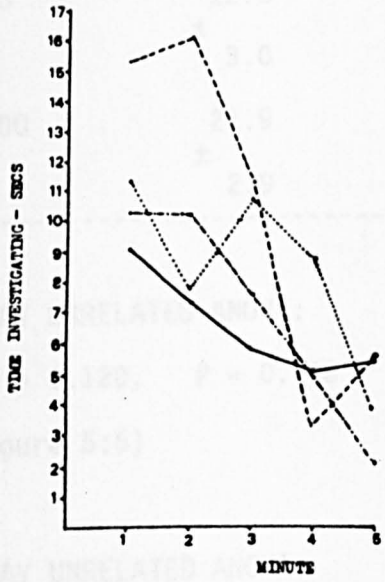


Figure 5:4 The effects of corticosterone on the mean bout length (means).

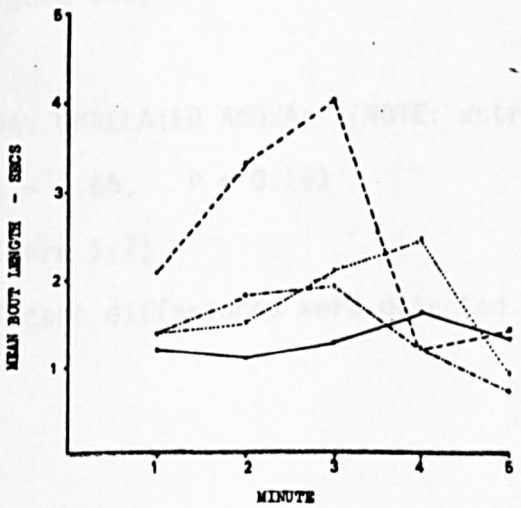


Table 5:4 The effects of corticosterone on the total measures of novel object investigation. (Means ± S.E.)

CONDITION N=10	TOTAL NUMBER a	TOTAL TIME b	OVERALL MEAN BOUT LENGTH c
CxT	25.4 ± 3.6	32.4 ± 4.4	1.4 ± 0.2
CxT+VEHICLE	23.0 ± 2.5	51.5 ± 7.8	2.3 ± 0.4
CxT+CORT50	22.8 ± 3.0	46.7 ± 11.9	1.8 ± 0.4
CxT+CORT100	21.9 ± 2.9	34.4 ± 6.3	1.5 ± 0.2

a. ONE WAY UNRELATED ANOVA:

$F(3,36) = 0.120$, $P = 0.948$

(see Figure 5:5)

b. ONE WAY UNRELATED ANOVA:

$F(3,36) = 0.576$, $P = 0.635$

(see Figure 5:6)

c. ONE WAY UNRELATED ANOVA: (NOTE: untransformed)

$F(3,36) = 1.65$, $P = 0.193$

(see Figure 5:7)

No significant differences were detected.

Figure 5:5 The effects of corticosterone on the total number of investigations (means and upper S.E.)

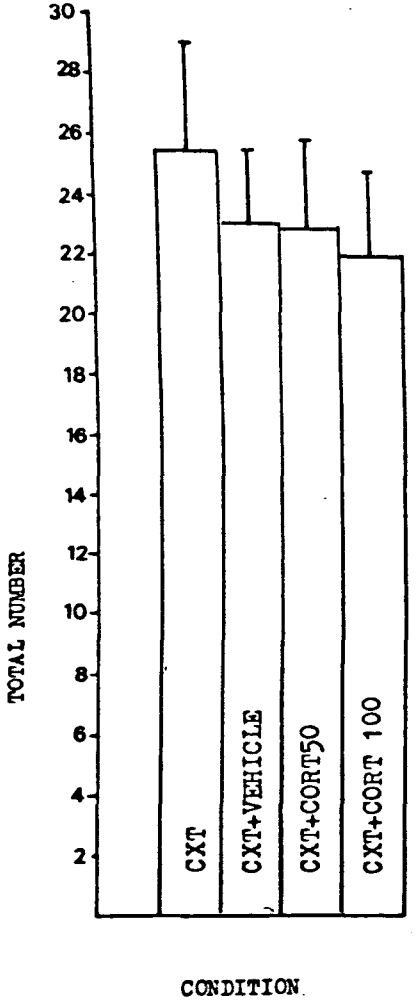


Figure 5:6 The effects of corticosterone on the total time investigating (means and upper S.E.)

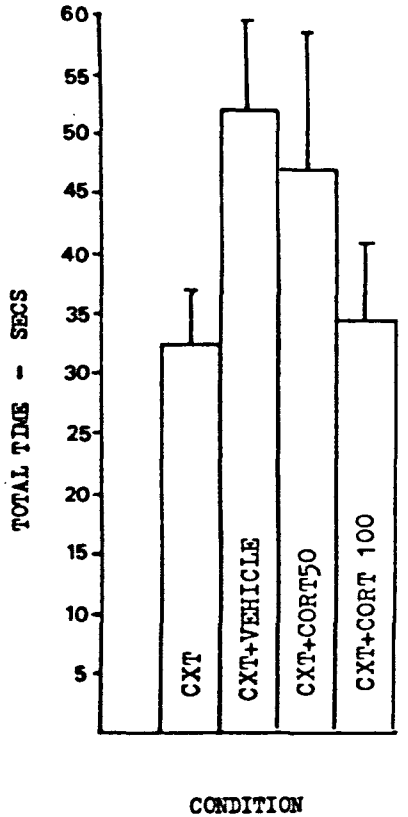


Figure 5:7 The effects of corticosterone on the overall mean bout length. (means and upper S.E.)

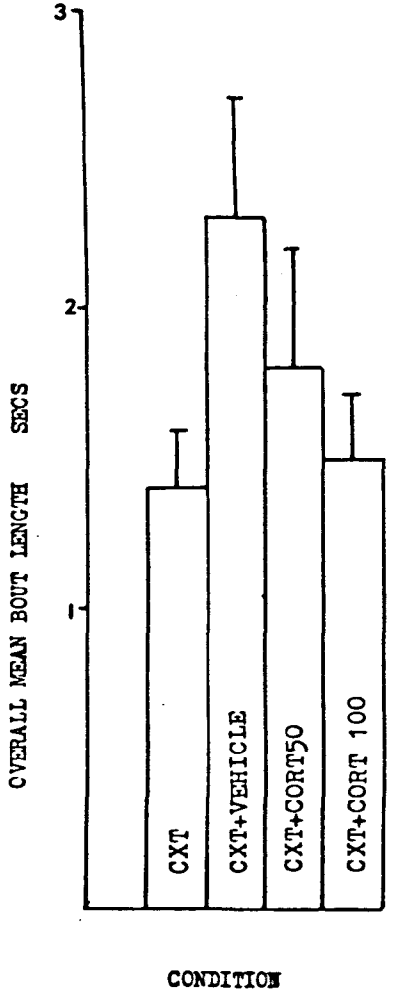


Table 5:5 The effects of treatments on the plasma levels of corticosterone. (Means ± S.E.)

CONDITION N=10		CORT ng/ml
CxT		114.0 ± 22.8
CxT+VEHICLE		213.2 ± 42.8
CxT+CORT50	a.	396.5 ± 33.0
CxT+CORT100		502.1 ± 49.9

ONE WAY UNRELATED ANOVA:

$F(3,36) = 20.66, \quad P < 0.001$

TUKEY TEST: HSD = 182.5, $P < 0.01$

a. Different from CxT+VEHICLE: Mean difference = 281.9, $P < 0.01$

The vehicle controls had similar CORT levels to the controls proper. The administration of 50 µg and 100 µg CORT elevated these levels. However, these two conditions did not differ from one another.

Table 5:6 The results of the correlation analysis between corticosterone and the change in novelty responsiveness from minute 1 to 5 - within conditions

CONDITION N=10	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos			
	CxT	CxT+VEHICLE	CxT+CORT50	CxT+CORT100
NUMBER OF INVESTIGATIONS	-0.306	0.136	-0.112	-0.191
TIME INVESTIGATING	-0.176	0.209	-0.127	-0.079
MEAN BOUT LENGTH	0.285	0.067	0.345	0.445

No significant correlations were detected.

Table 5:7 The results of the correlation analysis between corticosterone and the change in novelty responsiveness from minute 1 to 5 - combined conditions

CONDITIONS N=40	SPEARMAN'S RANK CORRELATION COEFFICIENT rhos
NUMBER OF INVESTIGATIONS	-0.047
TIME INVESTIGATING	0.097
MEAN BOUT LENGTH	0.178

No significant correlations were detected.

Discussion

There had been no expectation that the initial level of responsiveness to a novel object would be affected by CORT, and consistent with this, it was similar in all conditions. Although it had been hypothesised that CORT would reduce the rate of habituation, this was not supported. Even though all the behavioural parameters declined with time, the rate of this decline was similar regardless of marked differences in circulating levels of CORT. Furthermore, no correlation could be found between circulating levels of CORT and any measure of investigation. Correlational analysis was restricted to the differences in the behavioural measures at minute 1 as compared with minute 5, as this was considered to provide the best indication of the change in investigatory behavior with time.

The results, therefore, lend no support to the working hypothesis. The lack of statistical effect may be explained in one of 3 ways: the hypothesis may be wrong. Alternatively, as discussed in Chapter 3, circulating CORT levels may be so unrepresentative of brain levels and receptor occupation that the hypothesis has not been effectively tested. Lastly, and again as in earlier discussion: with the benefit of hindsight, the use of adrenally intact animals was probably ill-advised. The next experiment repeats this experiment using adrenalectomised mice with controlled baseline levels of CORT to clarify this.

EXPERIMENT 2

The effects of corticosterone on the habituation of responding to a novel object presented in a familiar environment under controlled baseline levels (20 µg CORT/ml)

Method

Experimental conditions and subjects

There were 10 animals in each condition:

1. Intact controls (INTACT)
2. Sham adrenalectomised injected with 0.1ml propylene glycol (SHAM ADX+VEHICLE)
3. Adrenalectomised maintained on the saline solution (1% ethanol in 0.9% $^{w}/_{v}$ NaCl) (ADX-SALINE)
4. Adrenalectomised maintained on the CORT solution (20µg CORT/ml saline solution) (ADX-CORT)
5. Adrenalectomised maintained on the CORT solution injected with 100 µg CORT in the vehicle (ADX-CORT+100)
6. Adrenalectomised maintained on the CORT solution injected with 300 µg CORT in the vehicle (ADX-CORT+300)

All the injections were given subcutaneously, one hour prior to exposure to the novel object. The treatments were coded.
10 intact, 51 adrenalectomised and 11 sham adrenalectomised animals were used, though 11 adrenalectomised and 1 sham adrenalectomised died.

All the operations were carried out 72 hours prior to testing. Immediately afterwards the adrenalectomised animals were placed on the appropriate drinking solution. Sham adrenalectomised animals remained on tap water.

Procedure

There were no procedural differences from those given in EXPERIMENT 1. The mice were tested in 4 batches. Within a batch they were allocated at random to treatments, and were tested from each condition in a pre-set sequence, until the batch was completed.

Statistical analysis

The behavioural data is expressed as means ± S.E. and was analysed as in the previous experiment. The CORT data is expressed as means ± S.E. and was analysed by the KWANOVA, followed by the MWU test.

Spearman's Rank Correlation Coefficent was used to assess the degree of correlation between CORT levels and the difference from minute 1-5 for all 3 measures of novelty responsiveness. Both within and combined (including and excluding adrenally intact animals) condition correlations were determined.

Table 5:9 The effects of corticosterone on the number of investigations (Means ± S.E.)

CONDITION N=10	NUMBER 1	2	3	4	5
INTACT	7.1 ± 1.0	6.3 ± 0.9	4.4 ± 0.9	3.0 ± 0.5	3.9 ± 0.5
SHAM ADX + VEHICLE	6.5 ± 1.5	3.6 ± 0.9	3.3 ± 1.0	3.0 ± 0.9	3.4 ± 0.9
ADX-SALINE	3.7 ± 1.1	2.7 ± 1.0	4.0 ± 1.0	2.8 * ± 0.7	4.2 ± 0.6
ADX-CORT	5.9 ± 1.1	5.3 ± 1.0	4.9 ± 0.8	5.8 ± 1.1	3.1 ± 0.8
ADX-CORT+100	5.4 ± 1.2	5.3 ± 1.3	5.6 ± 1.2	5.3 ± 1.1	3.8 ± 0.9
ADX-CORT+300	5.4 ± 1.2	5.2 ± 1.1	6.6 ± 1.2	6.1 ± 0.8	4.5 ± 0.8

TWO WAY MIXED ANOVA:

Treatment	$F(5,54) = 1.16$,	$P = 0.34$
Time	$F(4,216) = 4.68$,	$P = 0.001$
Interaction	$F(20,216) = 1.73$,	$P = 0.03$ a.

(see Figure 5:8)

a. ONE WAY UNRELATED ANOVA:

Minute 1	$F(5,54) = 0.93$,	$P = 0.47$
Minute 2	$F(5,54) = 1.58$,	$P = 0.18$
Minute 3	$F(5,54) = 1.35$,	$P = 0.26$
Minute 4	$F(5,54) = 3.01$,	$P = 0.02$*
Minute 5	$F(5,54) = 0.43$,	$P = 0.83$

* TUKEY TEST at minute 4: HSD = 3.3, $P < 0.05$

different from ADX-CORT+300: Mean difference = 3.3

Table 5:10 The effects of corticosterone on the time investigating. (Means ± S.E.)

CONDITION N=10	TIME - SECS MINUTE 1	2	3	4	5
INTACT	10.1 ± 1.5	13.4 ± 3.1	9.6 ± 2.5	7.7 ± 4.2	5.5 ± 1.8
SHAM ADX + VEHICLE	6.9 ± 1.9	5.1 ± 1.6	6.4 ± 2.4	6.5 ± 2.6	6.8 ± 2.9
ADX-SALINE	5.1 ± 1.5	4.9 ± 2.1	10.3 ± 2.7	8.9 ± 2.8	13.1 ± 3.6
ADX-CORT	7.8 ± 1.8	8.5 ± 2.1	6.7 ± 1.9	10.7 ± 2.8	6.2 ± 2.0
ADX-CORT+100	7.3 ± 2.0	7.9 ± 2.3	9.9 ± 2.4	10.4 ± 2.2	8.3 ± 2.5
ADX-CORT+300	6.8 ± 2.2	10.9 ± 3.4	17.5 ± 4.6	13.1 ± 3.2	11.3 ± 3.8

TWO WAY MIXED ANOVA:

Treatment $F(5,54) = 0.81$, $P = 0.55$

Time $F(4,216) = 1.34$, $P = 0.12$

Interaction $F(20,216) = 1.87$, $P = 0.02$

(see Figure 5:9)

Table 5:11 The effects of corticosterone on the mean bout length. (Means ± S.E.)

CONDITION N=10	MEAN BOUT LENGTH - SECS				
	MINUTE 1	2	3	4	5
INTACT	1.4 ± 0.1	2.2 ± 0.5	2.5 ± 0.9	1.9 ± 0.8	1.3 ± 0.4
SHAM ADX + VEHICLE	0.8 ± 0.2	1.0 ± 0.3	1.1 ± 0.4	1.3 ± 0.4	1.3 ± 0.5
ADX-SALINE	1.4 ± 0.4	0.9 ± 0.3	2.3 ± 0.7	2.4 ± 0.7	3.1 ± 1.0
ADX-CORT	1.1 ± 0.2	1.3 ± 0.3	1.1 ± 0.2	1.6 ± 0.4	1.5 ± 0.5
ADX-CORT+100	1.1 ± 0.2	1.1 ± 0.2	1.5 ± 0.3	1.7 ± 0.3	1.6 ± 0.5
ADX-CORT+300	1.0 ± 0.2	1.7 ± 0.4	2.6 ± 0.8	2.0 ± 0.4	2.0 ± 0.6

TWO WAY MIXED ANOVA:

Treatment $F(5,54) = 1.17$, $P = 0.34$

Time $F(4,216) = 3.95$, $P = 0.004$

Interaction $F(20,216) = 1.09$, $P = 0.36$

(see Figure 5:10)

Figure 5:8 The effects of corticosterone on the number of investigations. (means).

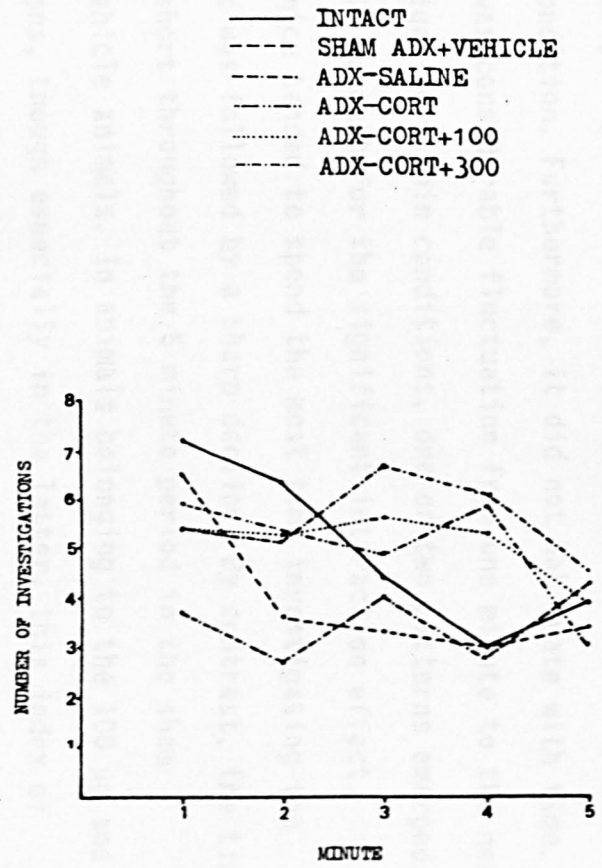


Figure 5:9 The effects of corticosterone on the time investigating (means)

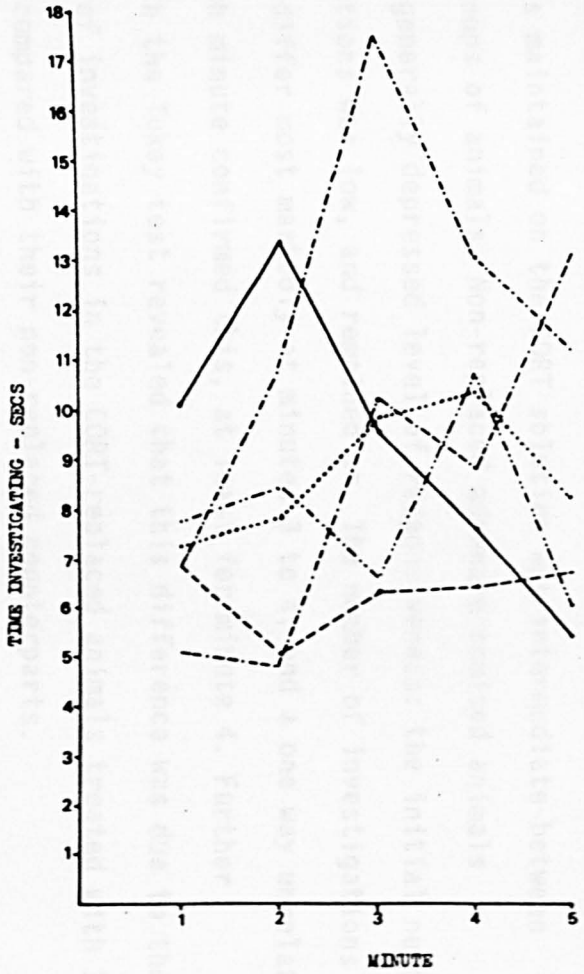
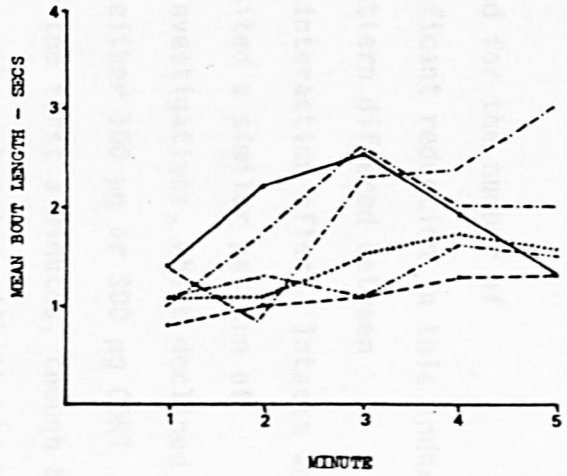


Figure 5:10 The effects of corticosterone on the mean bout length (means).



No significant treatment effect was detected for the number of investigations. There was, however, a significant reduction in this index of novelty responsiveness over time. The pattern differed between conditions, accounting for the significant interaction effect. Intacts and sham adrenalectomised-vehicle animals exhibited a similar pattern of behaviour, with an initial high number of investigations, which declined sharply. By contrast, animals treated with either 100 µg or 300 µg CORT increased the number of investigations over the first 3 minutes, though by minute 5, the number had decreased. Moreover, those treated with the higher of the two doses of CORT consistently investigated the object on a greater number of occasions. The rate of decline in the number of investigations in those animals maintained on the CORT solution was intermediate between these two groups of animals. Non-replaced adrenalectomised animals exhibited a generally depressed level of responsiveness: the initial number of investigations was low, and remained so. The number of investigations appeared to differ most markedly at minutes 3 to 4; and a one way unrelated ANOVA at each minute confirmed this, at least for minute 4. Further analysis with the Tukey test revealed that this difference was due to the high number of investigations in the CORT-replaced animals treated with 300 µg CORT, as compared with their non-replaced counterparts.

The time investigating the novel object was not significantly affected by any treatment condition. Furthermore, it did not habituate with time. Even though there was considerable fluctuation from one minute to the next in the time investigating within conditions, one or two patterns emerged, and these presumably account for the significant interaction effect. Initially, intact mice tended to spend the most time investigating the object, though this was followed by a sharp decline. By contrast, the time investigating was short throughout the 5 minute period in the sham adrenalectomised-vehicle animals. In animals belonging to the 100 µg and 300 µg CORT conditions, though especially in the latter, this index of

novelty responsiveness increased steadily over successive minutes upto minute 3, after which it declined. Time investigating the novel object fluctuated over the 5 minutes in those animals placed on the maintenance dose of CORT, though by minute 5 they spent a similar amount of time as intacts, but less than those treated with either 100 µg or 300 µg CORT. The non-replaced adrenalectomised animals spent a very short time investigating at minute 1, and unlike all the other conditions, the time investigating increased over the following minutes. (A one way unrelated ANOVA at each minute failed to reveal any significant differences).

The treatment conditions did not exert either a main or an interaction effect upon the mean bout length, though it increased significantly with time.

Table 5:12 The effects of corticosterone on the total measures of novel object investigation. (Means ± S.E.)

CONDITION N=10	TOTAL NUMBER a.	TOTAL TIME b.	OVERALL MEAN BOUT LENGTH c.
INTACT	24.7 ± 2.1	46.4 ± 9.6	1.7 ± 0.3
SHAM ADX + VEHICLE	19.8 ± 5.0	31.8 ± 9.3	1.2 ± 0.2
ADX-SALINE	17.4 ± 3.1	42.3 ± 9.3	2.3 ± 0.3
ADX-CORT	25.0 ± 3.5	39.9 ± 9.1	1.4 ± 0.3
ADX-CORT+100	24.7 ± 4.1	43.8 ± 9.0	1.6 ± 0.2
ADX-CORT+300	27.8 ± 3.2	59.7 ± 14.0	2.0 ± 0.3

a. ONE WAY UNRELATED ANOVA:

$F(5,54) = 1.131$, $P = 0.355$

(see Figure 5:11)

b. ONE WAY UNRELATED ANOVA:

$F(5,54) = 0.807$, $P = 0.550$

(see Figure 5:12)

c. ONE WAY UNRELATED ANOVA:

$F(5,54) = 1.849$, $P = 0.119$

(see Figure 5:13)

No significant differences were detected; though animals treated with 300 µg CORT tended to exhibit a greater total number of investigations, as well as a greater total time investigating, than all other conditions.

Figure 5:11 The effect of corticosterone on the total number of investigations (means and upper S.E.)

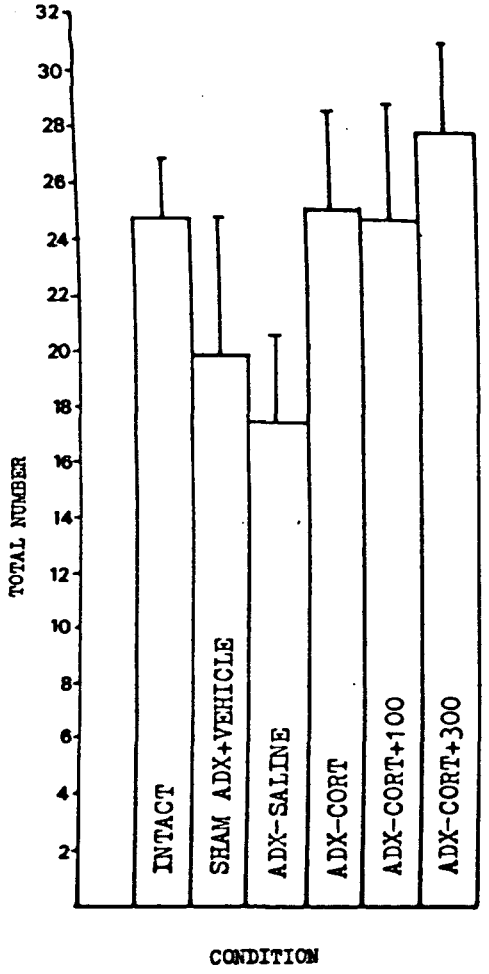


Figure 5:12 The effects of corticosterone on the total time investigating (means and upper S.E.)

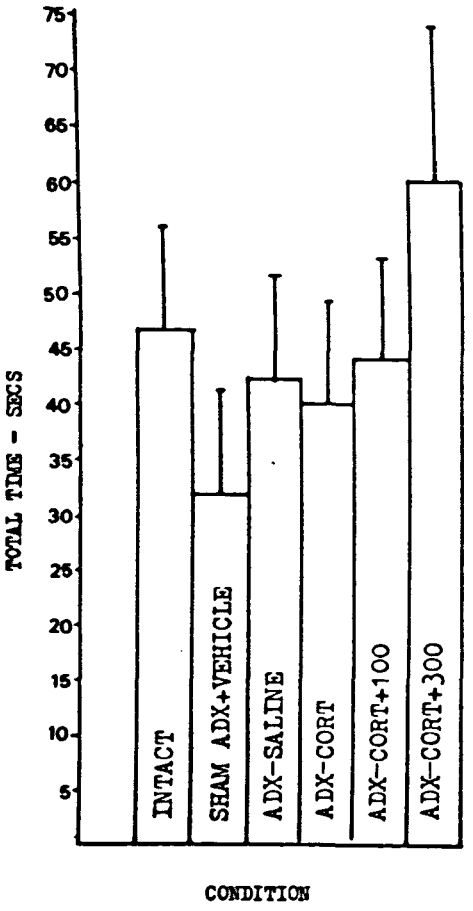


Figure 5:13 The effects of corticosterone on the overall mean bout length (means and upper S. E.)

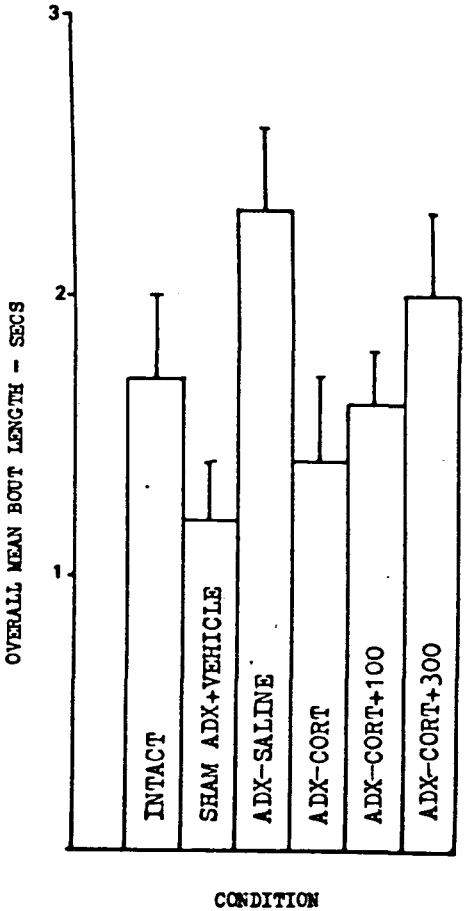


Table 5:13 The effects of treatments on the plasma levels of corticosterone. (Means ± S.E.)

CONDITION N=10		CORT ng/ml
INTACT	b.	87.0 ± 11.9
SHAM ADX + VEHICLE		182.8 ± 54.7
ADX-SALINE	a.	19.4 ± 5.6
ADX-CORT		208.4 ± 35.2
ADX-CORT+100	a.	513.1 ± 48.8
ADX-CORT+300	a.	1285.7 ± 196.8

KWANOVA: $H = 50.68$, $P < 0.01$

a. MWU different from all other conditions, $P < 0.005$

b. MWU different from ADX-CORT: $U = 12$, $P < 0.005$

Non-replaced adrenalectomised animals had significantly lower CORT levels than intacts. These, in turn, had similar levels to animals belonging to the SHAM ADX+VEHICLE condition, but lower levels than those in the ADX-CORT condition. The administration of 100 µg or 300 µg CORT produced dose-dependent elevations.

Table 5:14 The results of the correlation analysis between corticosterone and the change in novelty responsiveness from minute 1 to 5 - within conditions

	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos					
CONDITION N=10	INTACT	SHAM ADX +VEHICLE	ADX-SALINE	ADX-CORT	ADX-CORT +100	ADX-CORT +300
NUMBER OF INVESTIGATIONS	0.009	*-0.712	-0.457	-0.185	*0.836	0.109
TIME INVESTIGATING	0.067	-0.503	-0.018	-0.199	0.394	-0.067
MEAN BOUT LENGTH	0.115	-0.509	0.188	-0.306	-0.018	*-0.673

*$p<0.05$

A negative correlation was found in the SHAM ADX+VEHICLE condition for the number of investigations, such that those animals with high CORT levels showed a smaller drop in the number of investigations from minute 1-5. The reverse relationship was found for animals belonging to the ADX-CORT+100 condition. Animals treated with 300µg CORT displayed a negative correlation between CORT levels and the difference in the mean bout length from minute 1-5. No other within condition correlations were detected.

Table 5:15 The results of the correlation relationship between corticosterone and the change in novelty responsiveness from minute 1-5 - combined conditions

CONDITIONS	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos: INCLUDING ADRENALLY INTACT MICE N=60	EXCLUDING ADRENALLY INTACT MICE N=40
NUMBER OF INVESTIGATIONS	0.042	0.173
TIME INVESTIGATING	-0.034	0.087
MEAN BOUT LENGTH	-0.017	0.106

No significant correlations were detected, regardless of the presence of the adrenals.

Discussion

Contrary to expectations the maintenance regime reinstated levels of CORT exceeding those found in intact animals under conditions of rest, calling into question its suitability for its intended purpose of producing a moderate baseline level. Indeed, by virtue of this, the availability of receptor mechanisms to respond to elevations of CORT would be minimised, concealing any behavioural effects. Furthermore, both the 100µg and 300µg CORT doses were too high; though the levels associated with both of these may have arisen, in part, on account of the baseline level upon which they were superimposed. Whatever the reason, the circulating levels of CORT most certainly exceeded the limits of the normal receptor mechanisms. In view of the fact that this experiment did not fulfill its fundamental purpose, there is little value to be gained from a detailed examination of the results. Accordingly only those providing a deeper insight into the significance of CORT in habituation will be discussed.

Notwithstanding these shortcomings, it was encouraging to find that CORT appeared to retard habituation. Thus, even though there was a general reduction in the number of investigations from minutes 1 to 5, animals with

higher levels of CORT, namely those treated with 100µg or 300µg CORT, displayed an increase from minutes 1 to 3. Moreover, a dose-dependent effect was suggested. Animals treated with 300µg CORT consistently investigated the novel object on a greater number of occasions than their 100µg CORT counterparts. Similarly, whereas those animals treated with 100µg or 300µg CORT spent increasingly more time investigating the novel object over successive minutes, up to minute 3, especially those treated with the higher dose, animals belonging to the other conditions tended to show a reduction in the time investigating, at least after minute 2. Also, by minute 5, animals treated with 100µg and 300µg were apt to spend the most time investigating the novel object. Furthermore, although the totals data did not reveal any significant differences, there was, once again, a tendency for animals treated with 300µg CORT to be relatively more responsive to the novel object over the 5 minute testing period. Unfortunately, the non-replaced adrenalectomised mice were characterised by a low level of reactivity - as reflected by the low number of investigations and the initial tendency to spend very short periods of time investigating the novel object. As a result it is difficult to draw any conclusions regarding the effects of low levels of CORT on habituation, and therefore to confirm the role it is suggested to play by the other treatment conditions. Although these results lend support to the working hypothesis, the absence of a consistent and clearly defined correlation between the levels of CORT and the change in investigatory behaviour over 5 minutes, weakens the likely involvement of this adrenocorticoid in habituation. Indeed, even though a negative correlation was found between the levels of CORT and the difference from minute 1 to 5 in the number of investigations and the mean bout length, suggesting that high levels of CORT coincide with a retarded rate of habituation, a positive correlation was found between CORT and the difference in the time investigating from minute 1 to 5. Furthermore, these correlations only emerged in isolated

treatment conditions. Nonetheless, in view of the shortcomings of any correlation based on peripheral CORT levels, this evidence may tentatively suggest that CORT affects the rate of habituation. However, it is difficult to reach any firm conclusions until its effects have been re-examined against a lower baseline level, and this is the subject of the following experiment.

Before turning to this it is interesting to note that despite previous evidence indicating that sham operations and the delivery of an injection independently activate the PAS, animals belonging to the SHAM ADX+VEHICLE condition had very similar levels of CORT to their controls. The low level of task-associated arousal in the present experiment may account for this. It is possible, even though Hennessy et al. (1977) claim that the proactive effects of stress potentiate the level of PAS activity in a generalised manner, that these effects are actually restricted to more intense stimulation. In support of this: the presence of elevated CORT levels in sham operated animals tends to be confined to tasks comprising a high level of task activation, such as the runway distractability test. Admittedly, even here, CORT levels are only elevated if the shams also acted as the vehicle controls. Presumably, this reflects the cumulative effect of the stressful aspects of the test situation and the injection procedure.

The problems associated with the use of sham operated animals as the controls for the effects of surgery, especially if it involves removal of the adrenals, has already been commented upon. As the true comparison to be made in order to assess the effects of transient elevations of CORT on behaviour is between those animals maintained on replacement levels of CORT, and those in which an acute dose of CORT is superimposed upon these levels, it is argued that the incorporation of a sham adrenalectomy condition is superfluous. Accordingly, this condition is ommitted from here onwards.

EXPERIMENT 3

The effects of corticosterone on the habituation of responding to a novel object presented in a familiar environment under controlled baseline levels (5µgCORT/ml)

Method

Experimental conditions and subjects

There were 10 animals in each condition:

1. Intact controls (INTACT)
2. Adrenalectomised maintained on the saline solution (ADX-SALINE)
   (1% ethanol in 0.9% w/v NaCl)
3. Adrenalectomised maintained on the CORT solution (ADX-CORT)
   (5µg CORT/ml saline solution)
4. Adrenalectomised maintained on the CORT solution injected with 0.1ml propylene glycol (ADX-CORT+VEHICLE)
5. Adrenalectomised maintained on the CORT solution injected with 50µg CORT in the vehicle (ADX-CORT+50)
6. Adrenalectomised maintained on the CORT solution injected with 100µg CORT in the vehicle (ADX-CORT+100)

All the injections were given subcutaneously, one hour prior to exposure to the novel object. The treatments were coded. 10 intact and 77 adrenalectomised mice were used, though 17 of the latter died. The operations were carried out 72 hours prior to testing. Immediately afterwards the adrenalectomised animals were placed on the appropriate drinking solution.

Procedure and statistical analysis

The procedure and statistical analysis were as in the previous experiment, though only 3 batches of animals were used in this experiment.

Results

Table 5:16 The effects of corticosterone on the number of investigations. (Means±S.E.)

CONDITION N=10	NUMBER MINUTE 1	2	3	4	5
INTACT	7.0 ± 0.8	7.1 ± 1.2	7.1 ± 0.9	4.7 ± 0.8	4.2 ± 0.8
ADX-SALINE	4.0 ± 1.2	3.2 ± 0.9	2.5 ± 0.9	2.7 ± 0.7	2.9 ± 0.7
ADX-CORT	3.6 ± 0.7	3.8 ± 0.8	4.4 ± 0.7	3.8 ± 1.0	4.6 ± 0.9
ADX-CORT+VEHICLE	4.1 ± 1.2	3.1 ± 1.4	3.2 ± 1.6	3.3 ± 1.1	3.9 ± 1.2
ADX-CORT+50	3.6 ± 0.8	4.0 ± 1.4	3.0 ± 0.9	2.9 ± 0.6	3.6 ± 0.8
ADX-CORT+100	2.5 ± 0.9	3.0 ± 1.3	3.3 ± 0.9	3.1 ± 1.1	2.9 ± 1.1

NOTE: analysis was computed on untransformed data.

TWO WAY MIXED ANOVA:

Treatment $F(5,54) = 2.16$, $p = 0.07$

Time $F(4,216) = 0.93$, $p = 0.45$

Interaction $F(20,216) = 0.98$, $p = 0.49$

(see Figure 5:14)

Table 5:17 The effects of corticosterone on the time investigating (Means±S.E.)

CONDITION N=10	TIME-SECS MINUTE 1	2	3	4	5
INTACT	6.4 ± 1.2	10.9 ± 3.3	12.4 ± 3.8	11.7 ± 3.3	5.8 ± 1.7
ADX-SALINE	4.7 ± 1.3	9.9 ± 3.6	10.6 ± 4.7	7.2 ± 2.4	8.2 ± 3.0
ADX-CORT	3.2 ± 0.8	7.6 ± 2.9	9.1 ± 2.5	11.8 ± 3.6	7.3 ± 2.0
ADX-CORT+VEHICLE	4.4 ± 1.2	4.8 ± 2.6	6.6 ± 3.3	8.2 ± 2.9	10.0 ± 3.2
ADX-CORT+50	5.3 ± 1.6	11.4 ± 4.5	7.0 ± 2.6	8.8 ± 3.5	10.4 ± 3.1
ADX-CORT+100	3.3 ± 1.7	3.9 ± 1.5	3.7 ± 1.0	5.4 ± 2.5	4.7 ± 2.3

TWO WAY MIXED ANOVA:

Treatment $F(5,54) = 0.97$, $p = 0.44$

Time $F(4,216) = 0.87$, $p = 0.48$

Interaction $F(20,216) = 0.79$, $p = 0.72$

(see Figure 5:15)

Table 5:18 The effects of corticosterone on the mean bout length (Means±S.E.)

CONDITION N=10	MEAN BOUT LENGTH-SECS MINUTE 1	2	3	4	5
INTACT	0.94 ± 0.2	1.28 ± 0.3	1.5 ± 0.4	2.17 ± 0.5	1.28 ± 0.2
ADX-SALINE	1.4 ± 0.4	2.5 ± 0.9	2.4 ± 0.9	1.8 ± 0.5	1.7 ± 0.6
ADX-CORT	0.8 ± 0.1	1.6 ± 0.5	1.7 ± 0.4	2.1 ± 0.5	1.6 ± 0.4
ADX-CORT+VEHICLE	1.2 ± 0.2	0.8 ± 0.5	0.8 ± 0.4	1.3 ± 0.5	1.9 ± 0.7
ADX-CORT+50	1.0 ± 0.2	1.9 ± 0.8	2.3 ± 1.3	2.2 ± 0.9	2.1 ± 0.5
ADX-CORT+100	0.9 ± 0.3	0.7 ± 0.2	1.0 ± 0.3	1.1 ± 0.4	1.1 ± 0.4

TWO WAY MIXED ANOVA:

Treatment $F(5,54) = 0.68$, $p = 0.64$

Time $F(4,216) = 2.63$, $p = 0.03$

Interaction $F(20,216) = 0.63$, $p = 0.88$

(see Figure 5:16)

There were no significant treatment effects detected on any of the behavioural measures. There was, however, a strong trend towards an effect on the number of investigations. Presumably, this resulted from the greater number of investigatory responses observed in the intacts over the first 3 minutes.

Whilst the number of investigations and the time investigating did not habituate, there was a significant effect of time on the mean bout length, which tended to increase over successive minutes. There were no interaction effects.

Figure 5:14 The effects of corticosterone on the number of investigations (means).

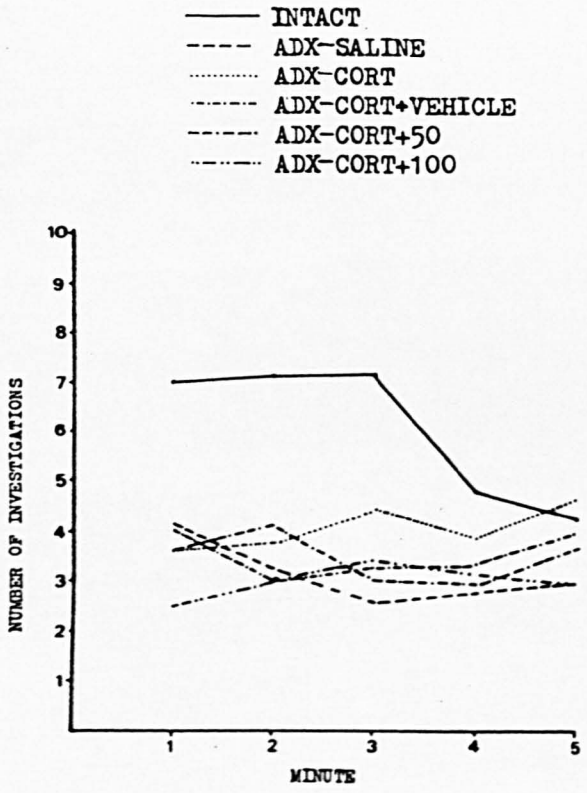


Figure 5:15 The effects of corticosterone on the time investigating (means).

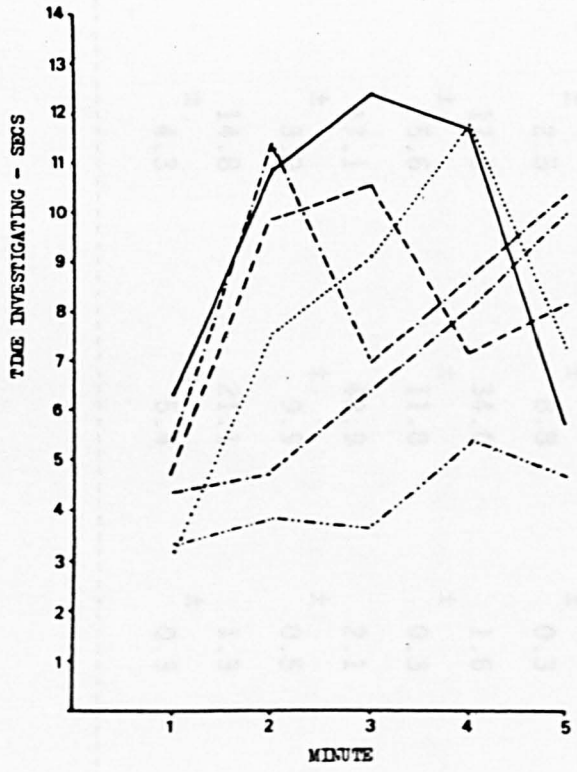


Figure 5:16 The effects of corticosterone on the mean bout length. (means).

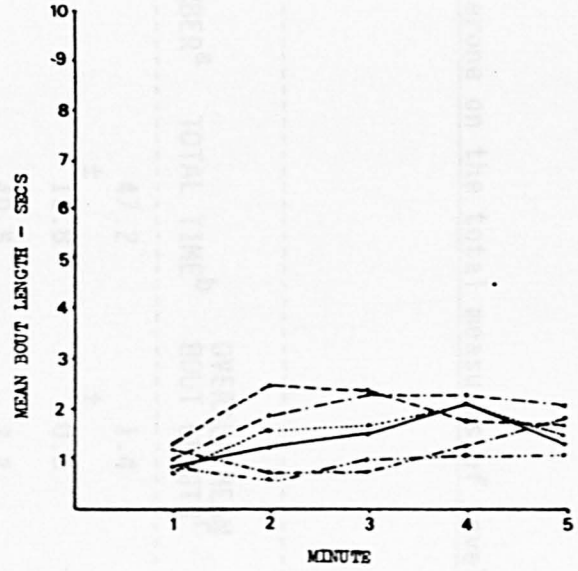


Table 5:19 The effects of corticosterone on the total measures of novel object investigation. (Means±S.E.)

CONDITION N=10	TOTAL NUMBER[a]	TOTAL TIME[b]	OVERALL MEAN BOUT LENGTH[c]
INTACT	30.1 ± 2.8	47.2 ± 10.8	1.4 ± 0.3
ADX-SALINE	15.3 ± 3.2	40.5 ± 12.8	2.2 ± 0.6
ADX-CORT	20.2 ± 2.5	34.5 ± 8.8	1.6 ± 0.3
ADX-CORT+VEHICLE	17.6 ± 5.6	34.0 ± 11.8	1.6 ± 0.3
ADX-CORT+50	17.1 ± 3.9	42.9 ± 9.9	2.1 ± 0.5
ADX-CORT+100	14.8 ± 4.3	21.1 ± 5.4	1.3 ± 0.3

[a] ONE WAY UNRELATED ANOVA:

$F(5,54)=2.16$, $P=0.07$

(see Figure 5:17)

[b] ONE WAY UNRELATED ANOVA:

$F(5,54)=0.80$, $p=0.56$

(see Figure 5:18)

[c] ONE WAY UNRELATED ANOVA:

$F(5,54)=0.91$, $p=0.48$

(see Figure 5:19)

No significant differences were detected; though differences in the total number of investigations only just failed to reach significance. Intacts were inclined to exhibit a greater number of investigations in total than the other conditions.

Figure 5:17 The effects of corticosterone on the total number of investigations (means and upper S.E.)

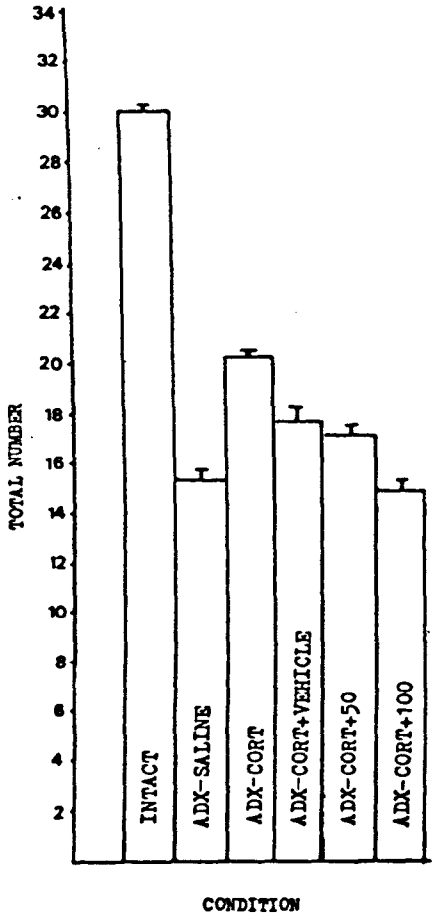


Figure 5:18 The effects of corticosterone on the total time investigating. (means and upper S.E.)

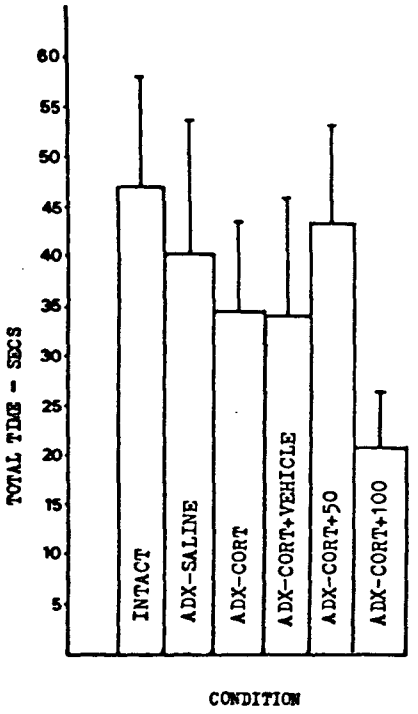


Figure 5:19 The effects of corticosterone on the overall mean bout length (means and upper S.E.)

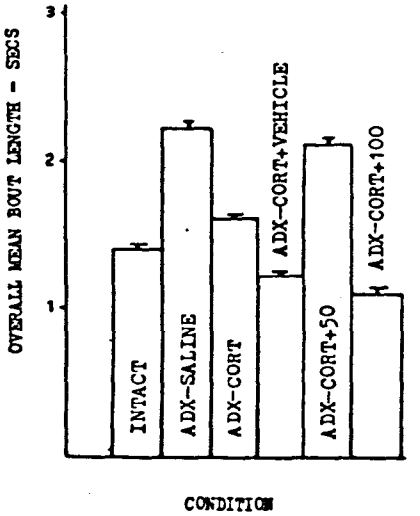


Table 5:20 The effects of treatments on plasma levels of corticosterone. (Means±S.E.)

CONDITION N=10		CORT ng/ml
INTACT		84.9±14.3
ADX-SALINE	a	7.0± 2.0
ADX-CORT		92.1±16.7
ADX-CORT+VEHICLE		77.7± 8.8
ADX-CORT+50	a	484.3±58.1
ADX-CORT+100	a	765.2±53.5

KWANOVA: H=49.87 , p<0.01

a. MWU different from all other conditions, p<0.005

Non-replaced adrenalectomised animals had lower CORT levels than either their replaced counterparts or intact controls. The maintenance dose produced circulating levels resembling those found in intacts, and the delivery of the vehicle did not have any effect. The administration of 50µg or 100µg CORT produced dose-dependent elevations in the levels of CORT.

Table 5:21 The results of the correlation analysis between corticosterone and the change in novelty responsiveness from minute 1 to 5 - within conditions

	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos					
CONDITION N=10	INTACT	ADX-SALINE	ADX-CORT	ADX-CORT +VEHICLE	ADX-CORT +50	ADX-CORT +100
NUMBER OF INVESTIGATIONS	0.17	-0.539	0.397	0.054	-0.003	-0.524
TIME INVESTIGATING	-0.321	*-0.673	0.276	0.309	-0.064	-0.503
MEAN BOUT LENGTH	*-0.612	-0.527	0.261	0.224	0.167	-0.221

*p<0.05

A negative correlation was found in the ADX-SALINE condition for the time investigating, such that those animals with high CORT levels displayed a smaller drop in the time investigating the object from minute 1-5.

A negative correlation was also found between the levels of CORT and the difference in the mean bout length from minute 1 to 5 in intact animals. No other within condition correlations were detected.

Table 5:22 The results of the correlation analysis between corticosterone and the change in novelty responsiveness from minute 1 to 5- combined conditions

	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos	
CONDITIONS	INCLUDING ADRENALLY INTACT MICE N=60	EXCLUDING ADRENALLY INTACT MICE N=50
NUMBER OF INVESTIGATIONS	-0.090	-0.085
TIME INVESTIGATING	0.015	0.078
MEAN BOUT LENGTH	-0.014	0.014

No significant correlations were detected, regardless of the presence of the adrenals.

Discussion

On the whole the levels of CORT fell within the predicted range. Thus: the revised maintenance dose reinstated levels resembling those found in intact male mice under conditions of low arousal. Furthermore, the levels reached when 50µg CORT were superimposed upon this baseline were acceptable because they corresponded to those found under stress conditions. However, as 100µg CORT produced levels exceeding even those under highly stressful conditions, they were considered to be unacceptably high. Putting the data from this condition to one side, the gradation between the baseline controls and those animals treated with 50µg CORT should have been

appropriate for an examination of the effects of short term elevations of CORT on HPC-dependent processes. However, the habituation of responding to a novel object was unaffected, suggesting that CORT does not retard habituation. The inconsistency of the correlational relationship between these two parameters supports this. Indeed, even though a negative correlation was found between the levels of CORT and the difference in the time investigating and the mean bout length with time, suggesting that CORT retards habituation, these correlations emerged in different treatment conditions, and were not confirmed when the data from different conditions was combined. The significance of these correlations is, therefore, difficult to ascertain.

As the various indices of novelty responsiveness did not habituate, this may account for the failure to detect a differential rate of habituation under markedly different levels of CORT. Indeed, with the exception of the intact controls, which tended to exhibit a decline, albeit nonsignificant, in the level of responsiveness to the novel object, the reverse tendency was observed in animals from all other conditions. At first this suggested that the observation period was not long enough to reveal any differences in the rate of habituation. This, however, is unlikely, as 5 minutes had been sufficient to allow the development of habituation in the past. It is difficult to account for this discrepancy as the experimental procedure was, to all intents and purposes, identical throughout. Moreover, the pattern of habituation displayed by the control animals was remarkably similar from one experiment to the next.

EXPERIMENT 4

The effects of corticosterone on the habituation of a hole-poke response under controlled baseline levels (5µg CORT/ml).

The hole-board test was introduced by Boissier and Simon (1962) and has subsequently been used extensively in the assessment of the effects of various drugs on behaviour (e.g., File, 1977). Typically, the hole-board consists of a large arena with a variable number of holes in the floor, through which the animal may poke its head - though not its whole body. Head-dipping, as assessed by the number of hole-pokes, is taken as a measure of exploration. Moreover, the hole-board is believed to provide a reliable measure of exploration that is uncontaminated by any changes in motor activity (File and Wardhill, 1975a; 1975b).

In this experiment the hole-poke response is used to assess the effects of CORT on exploration and its subsequent habituation, both across- and within-sessions (after Lalonde and Botez, 1985).

Method

Experimental conditions and subjects

There were 10 animals in each of the 5 conditions:

1. Intact controls (INTACT)
2. Adrenalectomised maintained on the CORT solution (5µg CORT/ml saline solution - 1% ethanol in 0.9% w/v NaCl) (ADX-CORT)
3. Adrenalectomised maintained on the CORT solution injected with 0.1ml propylene glycol (ADX-CORT+VEHICLE)
4. Adrenalectomised maintained on the CORT solution injected with 50µg CORT in the vehicle (ADX-CORT+50)

5. Adrenalectomised maintained on the CORT solution injected with 100µg CORT in the vehicle (ADX-CORT+100)

All the injections were given subcutaneously, one hour prior to testing throughout the testing period. The treatments were coded.

10 intact and 58 adrenalectomised mice were used, though 18 of the latter died. All the operations were carried out 72 hours prior to testing. Immediately afterwards, the mice were placed on the CORT solution.

Apparatus

An aluminium chamber (18x18x18cm), suspended at a height of 600cm within a large plastic container, was used. In the centre of the floor of this chamber there was a single hole (18mm diam.).

Procedure

The animal to be tested was placed in the testing chamber at a specific corner and the number of hole-pokes - defined as the animal poking its nose through the hole below the base of the platform - was observed and manually recorded, minute by minute, during a 4 minute session, on each of 5 consecutive days. Immediately after the fifth session the mice were blood sampled. These samples were subsequently assayed for CORT. The mice were tested in 3 batches. Within a batch they were allocated at random to treatments, and were tested from each condition in a pre-set sequence, until the batch was completed. The apparatus was cleaned with mild disinfectant between animals. All testing was carried out between 12.00-3.30 pm.

Statistical analysis

The behavioural data is expressed as means ±S.E. and was analysed as follows:-

i Total number of hole-pokes - one way unrelated ANOVA.

ii Habituation within a session - the number of hole-pokes in minutes 1-2 was compared with the number in minutes 3-4 within sessions by analysing each session separately using a two way unrelated ANOVA.

iii Habituation over sessions for minutes 1-4. If there was no significant within-session habituation, the data for minutes 1-2 and 3-4 was combined for each session and analysed over sessions using a two way mixed ANOVA.

iv Habituation over sessions for minutes 1-2 and 3-4 - two way mixed ANOVA.

The CORT data is presented as means ±S.E. and was analysed by the KWANOVA, followed by the MWU test.

Spearman's Rank Correlation Coefficient was used to assess the degree of correlation between CORT levels and the difference in the number of hole-pokes from session 1 to 5. Both within and combined (including and excluding adrenally intact animals) conditions correlations were computed.

Results

Table 5:23 The effects of corticosterone on the total number of hole-pokes during a 4 minute session over 5 days. (Means±S.E.)

CONDITION N=10	NUMBER OF HOLE-POKES
INTACT	58.1±4.6
ADX-CORT	64.8±7.6
ADX-CORT+VEHICLE	57.7±6.8
ADX-CORT+50	49.8±5.7
ADX-CORT+100	51.5±9.6

ONE WAY UNRELATED ANOVA:

$F(4,45)=0.711$, $p=0.589$

(see Figure 5:20)

No significant difference was detected.

Figure 5:20 The effects of corticosterone on the total number of hole-pokes during a 4 minute session over 5 days (means ± S.E.)

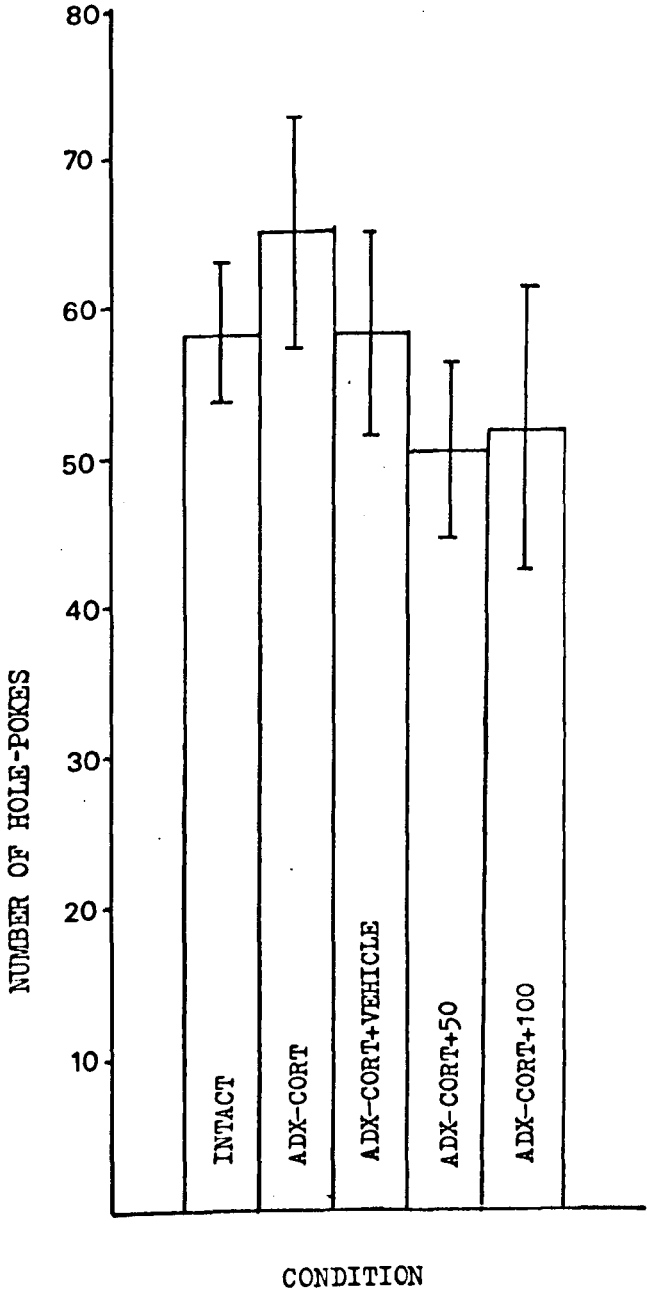


Table 5:24 The effects of corticosterone on the number of hole-pokes during the first two minutes and last two minutes of a 4 minute session over 5 days. (Means±S.E.)

CONDITION N=10	SESSION 1 *a		2 *b		3 *c		4 *d		5 *e	
	1-2	3-4	1-2	3-4	1-2	3-4	1-2	3-4	1-2	3-4
INTACT	6.4 ± 1.9	9.3 ± 1.8	6.5 ± 1.0	7.4 ± 0.9	3.8 ± 0.9	4.9 ± 1.0	4.3 ± 1.0	4.9 ± 0.7	4.3 ± 0.8	5.3 ± 0.9
ADX-CORT	12.0 ± 1.2	10.2 ± 0.9	7.3 ± 1.0	6.2 ± 1.1	5.5 ± 1.0	6.2 ± 0.9	3.8 ± 0.9	4.5 ± 0.8	4.2 ± 1.0	4.9 ± 1.2
ADX-CORT+VEHICLE	7.4 ± 1.8	8.9 ± 2.3	6.0 ± 1.2	6.7 ± 1.8	6.3 ± 1.7	4.9 ± 1.3	4.1 ± 1.1	4.3 ± 1.0	4.3 ± 0.8	4.8 ± 0.8
ADX-CORT+50	6.5 ± 1.4	7.8 ± 1.5	6.4 ± 1.5	5.6 ± 1.3	4.0 ± 1.0	5.0 ± 1.3	3.8 ± 0.6	4.8 ± 0.8	3.6 ± 1.1	3.3 ± 0.7
ADX-CORT+100	6.8 ± 1.8	7.0 ± 1.8	5.6 ± 1.1	7.5 ± 1.2	4.4 ± 1.2	4.7 ± 1.2	2.6 ± 1.1	3.4 ± 1.1	4.3 ± 1.0	5.2 ± 1.2

(see Figures 5:21-5:25)

*Within session habituation - TWO WAY UNRELATED ANOVA:

a. Session 1

Treatment	$F(4,90) = 1.904$, $p=0.116$
Minute 1-2: 3-4	$F(1,90) = 0.329$, $p=0.568$
Interaction	$F(4,90) = 0.381$, $p=0.822$

b. Session 2

Treatment	$F(4,90) = 0.177$, $p=0.950$
Minute 1-2: 3-4	$F(1,90) = 0.168$, $p=0.683$
Interaction	$F(4,90) = 0.513$, $p=0.726$

c. Session 3

Treatment	$F(4,90) = 0.695$, $p=0.597$
Minute 1-2: 3-4	$F(1,90) = 0.206$, $p=0.651$
Interaction	$F(4,90) = 0.371$, $p=0.828$

d. Session 4

Treatment	$F(4,90) = 0.856$, $p=0.493$
Minute 1-2: 3-4	$F(1,90) = 1.244$, $p=0.268$
Interaction	$F(4,90) = 0.05$, $p=0.995$

e. Session 5

Treatment	$F(4,90) = 0.645$, $p=0.632$
Minute 1-2: 3-4	$F(1,90) = 0.824$, $p=0.366$
Interaction	$F(4,90) = 0.141$, $p=0.967$

No treatment effect was found on the number of hole-pokes, and therefore the level of exploration within any session. Furthermore, the number of hole-pokes during the first 2 minutes as compared with the last 2

of any one session did not differ, indicating that habituation of this response had not occurred within sessions. Rather the number of hole-pokes tended to increase in the latter 2 minutes of each session.

There were no interaction effects.

Figures 5:21-25 The effects of various treatment conditions on the number of hole-pokes during the first two minutes (E) and the last two minutes(L) of a 4 minute session over 5 days (means ± S.E.)

Figure 5:21 INTACT

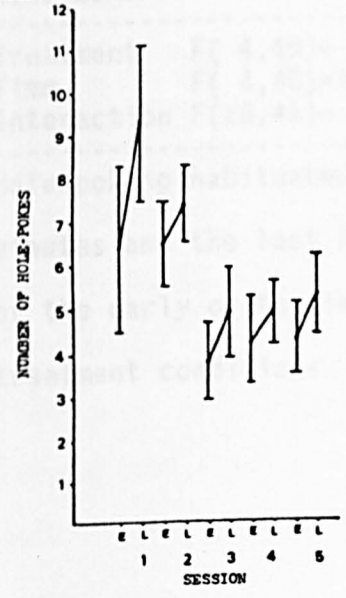


Figure 5:22 ADX - CORT

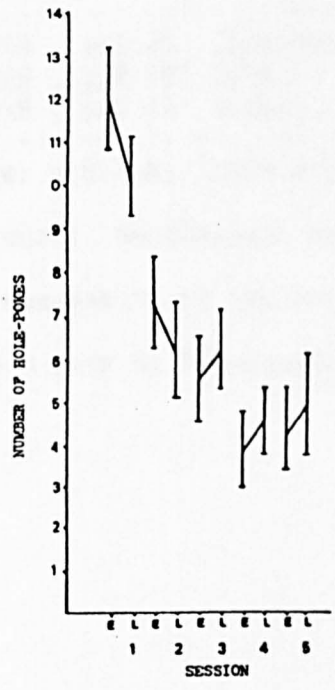


Figure 5:23 ADX -CORT + VEHICLE

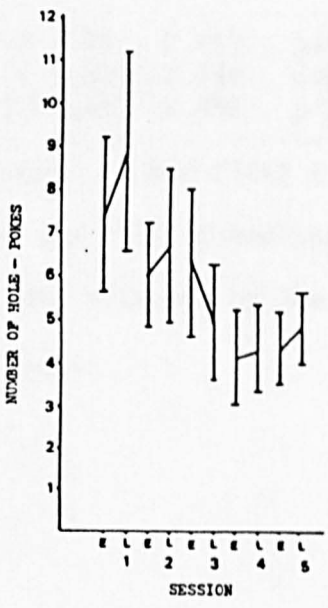


Figure 5:24 ADX -CORT +50

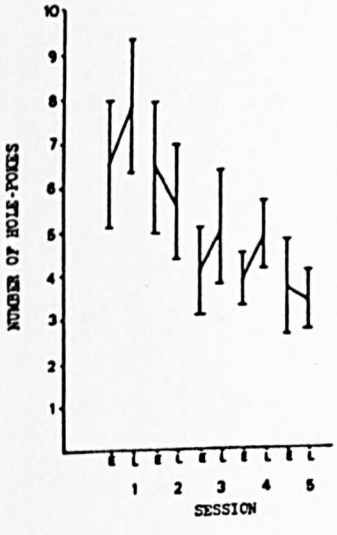


Figure 5:25 ADX -CORT + 100

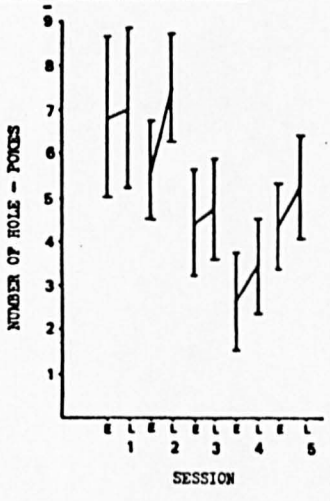


Table 5:25 The results of the analysis of the effects of corticosterone on between-session habituation - TWO WAY MIXED ANOVA

MINUTES 1-2		MINUTES 3-4	
Treatment	F(4,45)= 0.868 , p=0.49	Treatment	F(4,45)= 0.413 , p=0.798
Time	F(4,45)=14.219 , p<0.001	Time	F(4,45)=12.379 , p<0.001
Interaction	F(16,45)= 1.018 , p=0.44	Interaction	F(16,45)= 0.484 , p=0.952

Hole-poking habituated over sessions, both with respect to the first 2 minutes and the last 2 minutes. Nonetheless neither the rate of habituation of the early or the late components of the session was affected by the treatment conditions. There were no interaction effects.

Table 5:26 The effects of corticosterone on the number of hole-pokes during a 4 minute session over 5 days. (Means±S.E.)

CONDITION N=10	SESSION 1	2	3	4	5
INTACT	16.7±2.9	13.9±1.6	8.7±1.7	9.2±1.4	9.6±1.6
ADX-CORT	22.2±1.7	13.5±2.0	11.7±1.8	8.3±1.4	9.1±2.1
ADX-CORT+VEHICLE	16.3±3.7	12.7±2.4	11.2±2.9	8.4±2.0	9.1±1.4
ADX-CORT+50	14.3±2.8	12.0±2.7	9.0±2.3	8.6±1.1	6.9±1.7
ADX-CORT+100	13.8±3.5	13.1±2.2	9.1±2.2	6.0±2.0	9.5±2.2

TWO WAY MIXED ANOVA:

Treatment $F(4,45) = 0.659$, $p=0.624$

Time $F(4,45) = 16.618$, $p<0.001$

Interaction $F(16,45) = 0.656$, $p=0.834$

(see Figures 5:26-5:30)

Since there was no within session habituation, the number of hole-pokes over the first 2 and last 2 minutes of each session was combined. Although the number of hole-pokes significantly decreased over session, no significant main effect of the treatments on habituation over sessions was detected. Also, there were no interaction effects.

Figures 5:26 - 5:30 The effects of the various treatment conditions on the number of hole-pokes (combined for minutes 1-2 and 3-4) over 5 days. (means ± S.E.)

Figure 5:26 INTACT

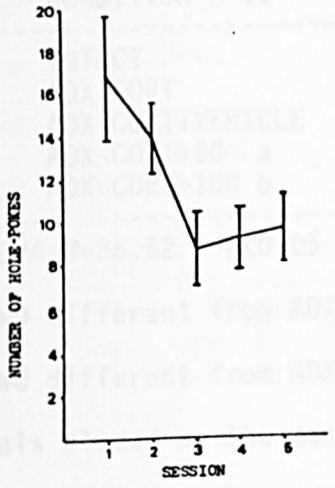


Figure 5:27 ADX - CORT

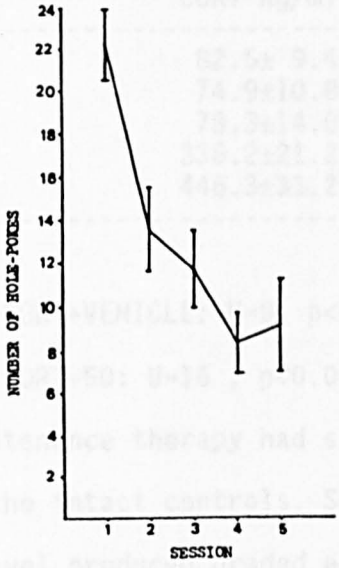


Figure 5:28 ADX - CORT + VEHICLE

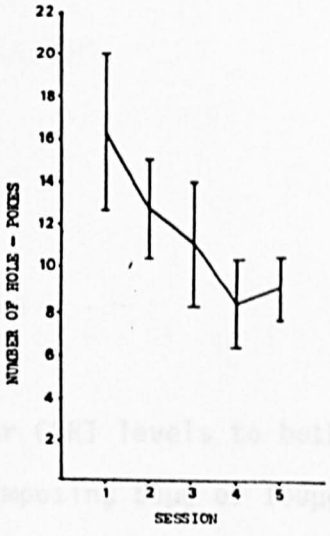


Figure 5:29 ADX -CORT + 50

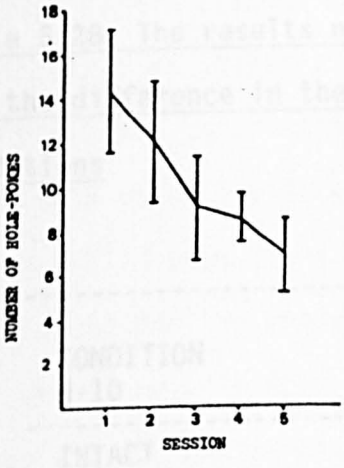


Figure 5:30 ADX -CORT + 100

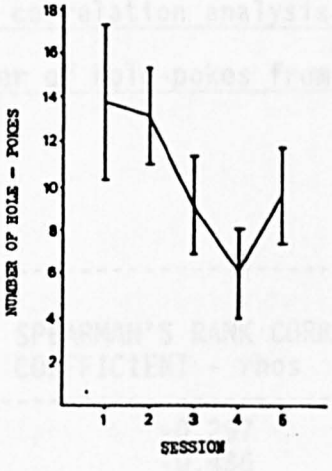


Table 5:27 The effects of treatments on the plasma levels of corticosterone. (Means±S.E.)

CONDITION N=10	CORT ng/ml
INTACT	82.5± 9.4
ADX-CORT	74.9±10.8
ADX-CORT+VEHICLE	78.3±14.0
ADX-CORT+50 a	336.2±21.2
ADX-CORT+100 b	446.3±33.2

KWANOVA:H=36.52 , $p<0.05$

a. MWU different from ADX-CORT+VEHICLE: U=0, $p<0.005$

b. MWU different from ADX-CORT+50: U=16 , $p<0.05$

Animals placed on the maintenance therapy had similar CORT levels to both the vehicle controls and the intact controls. Superimposing 50µg or 100µg CORT upon this baseline level produced graded elevations of CORT.

Table 5:28 The results of the correlation analysis between corticosterone and the difference in the number of hole-pokes from minutes 1-5- within conditions

CONDITION N-10	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos
INTACT	-0.297
ADX-CORT	-0.436
ADX-CORT+VEHICLE	0.212
ADX-CORT+50	0.164
ADX-CORT+100	0.561

No significant correlations were detected.

Table 5:29 The results of the correlation analysis between corticosterone and the difference in the number of hole-pokes from minutes 1-5- combined conditions

CONDITIONS	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos
INCLUDING ADRENALLY INTACT MICE N=50	-0.166
EXCLUDING ADRENALLY INTACT MICE N=40	-0.171

No significant correlations were detected, regardless of the presence of the adrenals.

Discussion

A maintenance dose of 5µg CORT/ml has been established to reinstate baseline levels resembling those found in intact male mice under resting conditions (see Chapters 3 and 4). Although the levels of CORT reached when 50µg CORT were superimposed upon this baseline were similar to those found previously with this dose, 100µg CORT resulted in relatively lower levels. Consequently, both doses produced acceptable levels of CORT in the circulation and the gradation between those animals with baseline levels of CORT and those with 50µg and 100µg CORT was well suited to an examination of the effects of transient changes in this adrenocorticoid on HPC-sensitive processes, such as habituation.

There were no differences in the total number of hole-pokes, indicating that the absolute level of exploration was unaffected by CORT. Although this is consistent with the findings reported by File (1978), it contrasts with the demonstration that adrenalectomy reduced certain components of exploratory behaviour, which were specifically reinstated by CORT (Veldhuis et al., 1982a). Contrary to expectations, habituation did not occur within sessions. Conversely, the number of hole-pokes tended to

increase in the latter 2 minutes of each session. Despite this, habituation did occur across sessions, both when the first 2 minutes and the last 2 minutes of each 4 minute session were analysed separately, and when combined. Nonetheless, CORT did not retard its rate. Furthermore, this finding of habituation across- but not within- sessions, contrasts with the effects of hippocampectomy (Kohler, 1976).

The absence of habituation within-sessions may indicate that the sessions were too short for it to occur; though this is unlikely as the number of hole-pokes actually increased in the latter 2 minutes of each session. Whilst this may indicate that the animals found the testing chamber novel - such that general exploratory tendencies predominated in the first 2 minutes, or the hole aversive, observations of the animals in the chamber did not support either of these. Whereas mice exhibited a high inclination to explore the testing chamber, combined with characteristic 'wall hugging' behaviour, in the first 2 minutes of session 1, this was not so marked subsequently. Furthermore, even though there was across-session habituation, hole-poking in the last 2 minutes of a session remained higher than in the first 2, even at session 5. It is difficult to explain this, though it possibly reflects the influence of some other factor, such as 'boredom'. This is suggested by the observation that exploration of the testing chamber was greatly reduced in the later sessions, and as the testing chamber was relatively homogeneous, the hole offers the only incentive to explore.

In conclusion: as CORT failed to affect the rate at which a hole-poke response habituates, despite the implementation of conditions suitable for uncovering such an effect, it is improbable that it alters the functions of the HPC that contribute to the development of habituation. The absence of a reliable correlational relationship between the levels of CORT and the change in the number of hole-pokes over sessions corroborates this. However, since the effects of hippocampectomy on hole-poking depend on the

nature of the lesion (Kohler, 1976); and since CORT has already been shown to be without effect on the habituation of this behavioural response (File, 1978), the possibility remains that the selection of this particular response was inappropriate.

General discussion

Although the capacity of the receptor systems to respond to CORT is known to be limited, the finding that it did not affect the rate of habituation in intact mice was, at first, still surprising. This is because the presentation of a novel object was assumed to evoke only a low level of task-associated arousal, so endogenous CORT would remain at a low level. If so, provided the doses of CORT that were selected resulted in plasma levels equivalent to those found normally, they should have been capable of expressing their effects via the usual receptor-mediated mechanisms. However, as there is evidence that mice are very responsive to novelty (Levine and Trieman, 1964; Ader et al., 1967), it seemed possible that increases in endogenous CORT, as a function of the arousal associated with the task, may have obscured the expression of the effects of the exogenously induced elevations of CORT. Other factors may also have acted to stimulate the release of endogenous CORT, reducing the receptor mechanisms available to the adrenocorticoid even further. It is possible, therefore, despite the initial reservations, that the failure to detect an effect of CORT on habituation may have been due to the unavailability of receptor mechanisms to interact with it. However, as it failed to affect habituation even when it was examined against controlled baseline levels, the conclusion that it has no real effect is hard to avoid.

Nonetheless, there was some indication in EXPERIMENT 2 of the retardation of habituation. Unfortunately, this result was produced with what turned out to be unphysiologically high circulatory levels of CORT, and was not repeated under more realistic conditions in either EXPERIMENT 3 or 4. Furthermore, the correlational relationship between CORT and the rate of habituation of both the responsiveness to novelty and hole-poking was inconsistent.

However, as the role of the HPC in habituation is known to be a 'test-dependent phenomenon' (Kohler, 1976) these classes of response may

have been unsuitable. The problem of selecting appropriate responses is compounded by the fact that the habituation dysfunction that occurs in hippocampectomised animals often depends on the nature of the lesion. Detecting the involvement of the HPC will, therefore, depend on combining the appropriate lesion with the appropriate task. Moreover, if it is considered that CORT interacts with different regions of the HPC to differing degrees, bringing about differential effects as a function of its specific site of action (Bohus, 1975), this problem can be more fully appreciated.

Notwithstanding the possibility that the classes of response that were selected were unsuitable, as no discernible effect was found when non-replaced adrenalectomised animals were compared with their CORT-replaced counterparts, following the logic of Reul and de Kloet (1986), by implication this adrenocorticoid does not exert a permissive effect upon the functioning of the HPC in the process of habituation. Conceding this undermines yet further the significance of CORT in habituation. This is because, as CORT is believed to inhibit the HPC in a tonic fashion under normal conditions (Pfaff et al., 1971), HPC-sensitive behaviour should undergo some change in its absence.

In addition to undermining a role for CORT in habituation, the findings presented in this chapter may have more profound implications, as the retardation of habituation assumes a central position in the operational definition of increased persistence (Kohler, 1976). Even so, as the retardation of habituation may arise as a result of an inefficiency in information processing rather than an increased level of persistence, the hypothesised role of CORT need not be abandoned yet.

CHAPTER 6

The effects of corticosterone on a reversal and a non-reversal shift discrimination

Introduction

Discrimination tests are used extensively in attention research. Their primary use is to assess the selectivity of attention and the degree of dimensional control over attention. They can also be used to evaluate the persistence of attention. Disruption of the HPC induces discrimination deficits, the nature of which has been interpreted to indicate an enhancement of persistence. In view of this, the present series of experiments is concerned with the effects of CORT on discrimination behaviour.

In a discrimination task an animal learns to give consistently different responses to different stimuli. The finding that the more an animal learns about one dimension of a two-dimensional discrimination, with both dimensions relevant, the less is learned about the other (Sutherland and Holgate, 1966), indicates that animals can not respond to all cues with equal effectiveness at the same time (modified non-continuity theory - Mackintosh, 1965). Moreover, as the solution of a discrimination only occurs when the animal learns to respond to the relevant cue, this limited capacity for stimulus processing inevitably introduces the problem of selection and by necessity implies the involvement of selective attention for efficient discrimination learning. In essence, discrimination tasks examine the efficiency with which animals learn to select the correct cue, and this reflects something of the attentional processes that are involved.

There are different types of discrimination tasks and the emergent behaviour depends upon the nature of the task, with ramifying implications for the attentional theory accounting for discrimination behaviour. Accordingly, the different types of discrimination task will be discussed

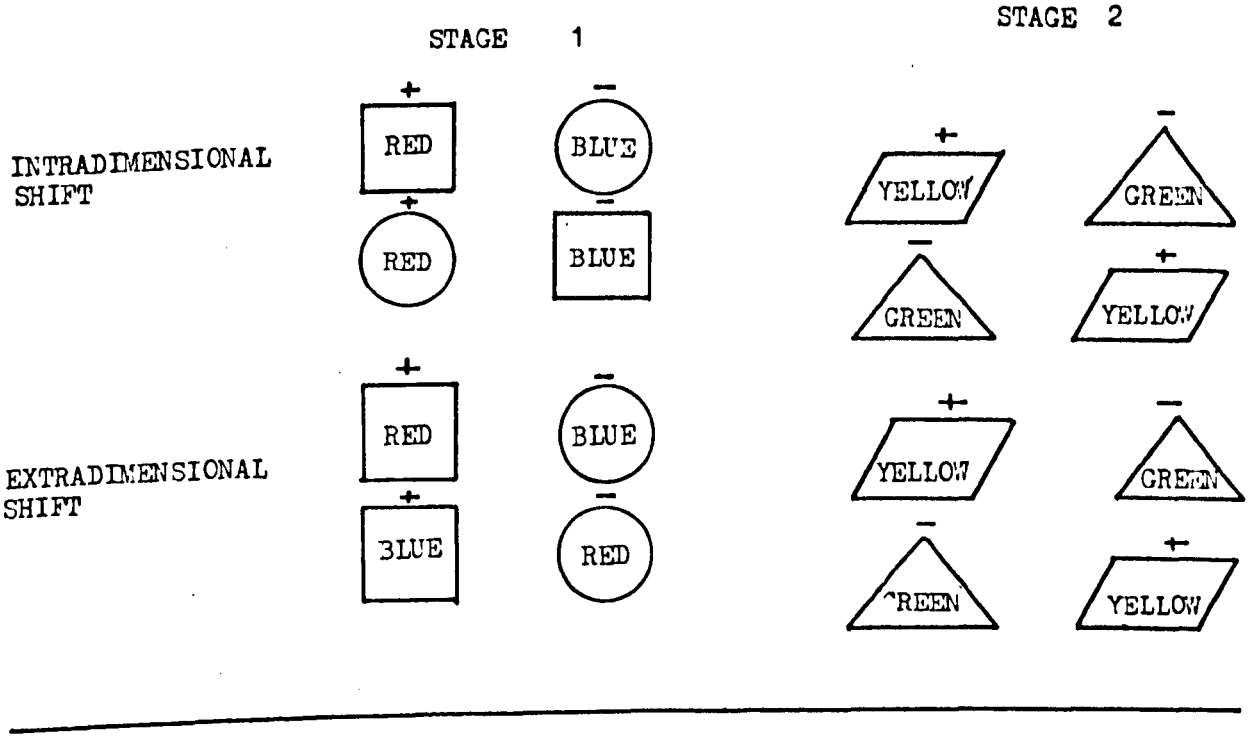
first, followed by their attentional significance, focussing on their value in the study of the persistence of attention. The effects of hippocampectomy on discrimination shifting will then be described. Finally, the known effects of CORT on discrimination behaviour will be reviewed.

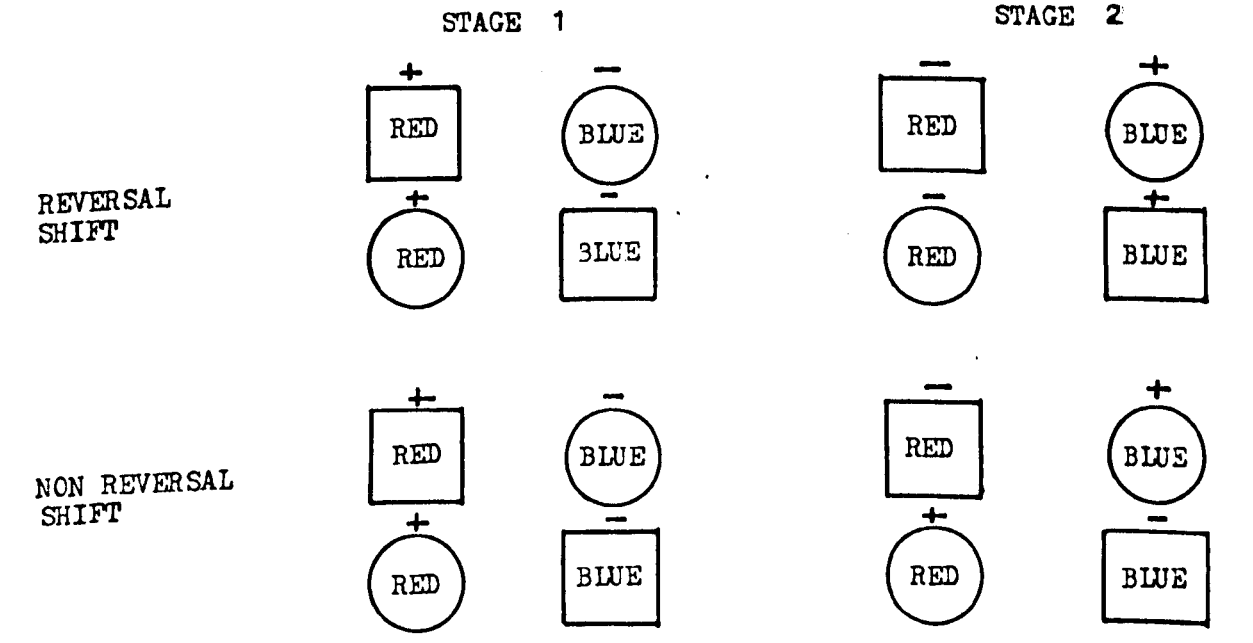
Basically there are 4 types of discrimination shift:- (see Figure 6:1).

1. Intradimensional shift (IDS) - in which the relevant dimension remains the same from one problem to the next.
2. Extradimensional shift (EDS) - in which the relevant dimension changes.
3. Reversal shift (RS) - in which the reinforcement contingencies of the original discrimination problem are reversed.
4. Non-reversal shift (NRS) - in which the relevant dimension is changed.

It is important to note that in both the IDS and EDS, the actual stimuli presented change between the first and second problem, whereas in the RS and NRS the same stimulus pairs presented in the original discrimination problem are present in the shift problem.

Figure 6:1 Schematic illustration of the design of an intra-dimensional and extra-dimensional shift, and a reversal and a non-reversal shift (after Sutherland and Mackintosh, 1971 ; and Mackintosh, 1974).





\+ indicates that the stimulus was positively reinforced.

The two-stage theory of discrimination learning (Sutherland, 1964; Sutherland and Mackintosh, 1971) holds that learning a discrimination task involves two processes. The first - learning to attend to the relevant stimulus dimension, is a perceptual process in which one stimulus dimension is selected from all those available. It is assumed that the input from a stimulus goes to a number of different analysers, each of which analyses the stimulus along a particular dimension, e.g., brightness, orientation. Fundamental to this theory is the assumption that all the analysers appertaining to a particular stimulus can not be used simultaneously. If this is correct, efficient discrimination depends on learning to engage or 'switch in' the most relevant i.e., that corresponding to the stimulus dimension that most reliably predicts reinforcement. It is only when an analyser is switched in that its output determines which response occurs.

This leads to the second process, which involves learning the correct response attachments, i.e., which stimuli within the dimension are associated with reinforcement. It is implicit in the theory that the strength of response associated with a particular analyser can only be modified if that analyser is switched in. A further important point is that the strength of learning of the relevant dimension and the strength of the response attachments are governed by different rules, and as a consequence, these strengths are accumulated at different rates and have different maxima.

The value of this theory is attested to by its success in explaining a number of diverse and previously inexplicable phenomena. As an examination of its explanatory power not only clarifies the significance of discrimination behaviour in attention research, but also brings to light some important points connected with the use of discrimination tests, a number of these phenomena will now be discussed.

Firstly, the paradoxical effect of overtraining on RS and NRS discriminations: Mackintosh (1962) found that overtraining facilitated the

former, but impaired the latter. At first it was difficult to account for these results, as according to the logic of the classical stimulus-response theory overtraining would add additional strength to the correct solution of the original discrimination problem, retarding its extinction and impairing a shift, whether of a reversal or a non-reversal type. However, according to the two-stage theory of discrimination learning, this effect could be explained by assuming that overtraining increases the strength of attention to the relevant dimension to a greater degree than the strength of the choice response. If this assumption is correct, during an RS, response attachments will extinguish before attention to the relevant dimension. As a result the overtrained animal will continue to attend to the relevant dimension, and will only have to acquire the new response attachments within this dimension in order to solve the discrimination shift. By contrast, the non-overtrained animal, with its lower strength of attention to the relevant dimension, will cease attending to this dimension more readily. This increases the likelihood of attending to irrelevant dimensions, retarding the acquisition of the new response attachments within the original dimension.

Similarly, during a NRS, as the theory of discrimination learning holds that only one analyser can be switched in at any one time, if the dimension of relevance in the original discrimination is more firmly switched in as a result of overtraining, the likelihood of switching to other analysers is diminished. Consequently, discrimination shifting involving transfer to a different stimulus dimension is impaired. By contrast, because a non-overtrained animal is more likely to switch to other analysers, a NRS proceeds with relative ease.

Further empirical support for the two-stage theory of discrimination learning has been provided by the finding that an IDS is learned faster than an EDS (Tighe et al., 1971; Mackintosh, 1974). It is claimed that since these tasks involve a change in the specific values of the stimulus

dimensions, it is impossible to account for the differential discrimination behaviour in terms of the establishment of approach and avoidance responses to specific stimuli, rather:

> "the implication is that differential reinforcement correlated with one set of stimuli increases the rate at which subjects associate other stimuli differing along the same dimension with reinforcement, while if a set of stimuli is not correlated with reinforcement, subjects are less able to associate stimuli differing along that dimension with reinforcement". (Mackintosh, 1974 p.595).

Thus, in effect, Mackintosh (1974) is maintaining that reinforcement differentially affects the dimensional control of attention: increasing it to relevant stimulus dimensions, and decreasing it to those that are irrelevant. The situation is, however, more complicated than this, as the differential IDS and EDS performance also arises because reinforcement accumulates strength for the stimulus dimension over and above that for the response. Nonetheless, the overall effect is that responding continues along the originally relevant dimension, regardless of the nature of the discrimination shift, and this is responsible for the differential discrimination behaviour.

It is, at this point, important to emphasise that whilst a comparison of the relative ease of ID- and ED- shifting is claimed to reflect the degree of dimensional attentiveness, a similar comparison under RS and NRS conditions does not. The reason for this stems from the fact that the same stimulus pairs are presented in both the original and shift problems. As a result, it is possible, in the NRS to continue to make a response that was correct in the original discrimination, and be rewarded on 50% of the trials for doing so. This partial reinforcement - which does not occur in the RS - is likely to interfere with the course of learning on the NRS, reducing the value of a comparison between the rate of learning on RS and

NRS problems for the purpose of evaluating the degree of dimensional attentiveness. However, in the experiments which follow, the comparison between the performance on these two discrimination shifts is not being used for this purpose, but to assess the persistence of attention. If persistence is enhanced, all stimuli other than the one upon which attention is being fixated should be excluded, and changing the point of fixation should be difficult whether the stimulus belongs to the same or a different dimension. Moreover, in this situation the occurrence of partial reinforcement in the NRS should be without effect.

A RS-NRS comparison also differentiates between the possibility of an influence upon attentional selectivity as opposed to attentional persistence in the following fashion. Whilst an effect on both of these would be expected to impair a RS, if the selectivity of attention is specifically affected, a NRS would not necessarily be impaired, and might even be improved; whereas if the persistence of attention is affected, a NRS would be expected to be impaired (Mason and Iversen, 1979). It may clarify this to point out that a reduction in selective attention is usually attributed to an increase in the number of cues sampled. As the number of cues associated with reinforcement will be increased, each will, by virtue of the total number of cues, be less firmly attached to the reinforcement. Furthermore, as the rate of extinction depends on the strength with which a stimulus is associated with reinforcement, responding to these cues will be extinguished faster. Thus: although attention to the relevant dimension will cease earlier which impairs reversal shifting - as both the relevant dimension and the correct response have to be re-learnt, these changes will facilitate non-reversal shifting. This is because by reducing the strength with which each cue is associated with reinforcement, attention to the previously relevant cue will cease earlier, and more importantly attention to the relevant dimension of the new discrimination will start.

Even though performance on a RS as compared with a NRS is of value in studying the persistence of attention a comparison of the effects of hippocampectomy on these two discrimination problems has only been carried out on one occasion and neither was affected (Harley, 1979). This is surprising, as the influence of hippocampectomy on reversal shifting has been the subject of extensive investigation and there is compelling evidence that it is usually retarded. Unfortunately, as with most areas of research connected with the HPC, controversy surrounds both the nature of its effects and the correct interpretation of them. As a detailed consideration of these is beyond the scope of this discussion, only the most important areas of debate will be detailed.

It is generally claimed that hippocampectomised animals acquire an initial response readily, but have problems reversing it; however this is an over-simplification. Although the acquisition of the original discrimination response proceeds normally in a simple 2-choice simultaneous discrimination (rats - Kimble, 1963; Rhesus monkeys - Douglas and Pribram, 1966), it is deficient in a successive discrimination (Kimble, 1963), a successive go, no-go discrimination (Buerger, 1970), and in discriminations involving differential partial reinforcement (Douglas and Pribram, 1966). Regarding the reversal of a response: although it is usually retarded by hippocampal lesions (spatial task - Thompson and Langer, 1963; Kimble and Kimble, 1965; non-spatial task - Douglas and Pribram, 1966), this is not always the case (e.g., Samuels, 1972; Harley, 1979; Munoz and Grossman, 1980). As there has been only a one investigation into the effects of hippocampal lesions on a NRS (Harley, 1979), it is obviously much more difficult to arrive at a general conclusion from this. Indeed, although no effect was found any conclusions based on a solitary findings would be premature.

In view of the uncertainty surrounding the nature of the effects of hippocampectomy it is difficult to adopt any one interpretation with

confidence. Whilst no one can make a definitive statement as to how the HPC functions, especially as this brain area is likely to be functionally heterogeneous, the attentional theory is advocated here, as it has throughout this thesis. This theory states that efficient shift behaviour necessitates the development of attentional inhibition towards the originally positive cue, and because this is lacking in hippocampectomised animals, the decrement of both attention and response strength can not occur. As a consequence, these animals persist in attending to the originally prepotent stimulus, which impairs RS and NRS behaviour. In support of the attentional role of the HPC is the finding that the hypothesis behaviour of hippocampectomised animals differs from their non-lesioned counterparts, both during the acquisition and extinction of a RS. They displayed fewer and longer bouts of hypothesis testing than their controls, suggesting that they persist in a particular hypothesis (Isaacson, 1974).

Although the attentional theory is favoured here, for the sake of completeness it is worthwhile noting that Nadel et al., (1975) argue that the discrimination behaviour of hippocampectomised animals supports their spatial information hypothesis of hippocampal function. This is because reversal deficits emerge most consistently in tasks involving spatial cues. However, deficits also arise in tasks containing no obvious spatial components (Douglas and Pribram, 1966; Silveira and Kimble, 1968; Gray, 1982). The discrimination behaviour of these animals has also been taken to support a role for the HPC in the processing of information (Winocur, 1979; 1980; and Winocur and Gilbert, 1984). Winocur and Olds (1978) found that changes in background stimuli facilitate reversal behaviour in hippocampectomised animals, even though the previously relevant cue is still present. On this basis, hippocampectomy is held to limit the processing capacity, resulting in an increased reliance upon contextual information. Thus, only if the context is changed can re-evaluation of the

test situation and the appropriate behavioural adjustments occur. Otherwise, the contextual information serves as a retrieval cue for the previously correct response (Winocur and Mills, 1970; Winocur and Breckenbridge, 1973). (As an aside, if there is an increased dependence upon contextual cues it is possible that the dissociations in the effects of the HPC on discrimination behaviour may be explained by a change in the test conditions from the original acquisition of a discrimination to its reversal).

Having established the potential value of discrimination tests for evaluating the working hypothesis that CORT via its suppression of the HPC enhances persistence, it was disappointing to discover that studies concerned with the effects of CORT on discrimination behaviour are limited in number. Furthermore, they tend to conflict with one another. Differences in strain, task, identity of the glucocorticoid , its dose, route of administration and time of administration, the level of motivation, not to mention its nature may underlie this. Beckwith et al., (1983) examined the effects of CORT, administered throughout both acquisition and reversal, on the reversal of an appetitively motivated brightness discrimination using an operant paradigm, and found that reversal was impaired. In contrast, Bohus (1971) demonstrated an enhancement of reversal in a signal - discriminative conditioned response; though the glucocorticoid under investigation was DEXA rather than CORT, and it was given during reversal only. DEXA - treated rats exhibited more conditioned responses over the first 3 days of reversal training than controls, with fewer responses to the negative stimulus, and an accelerated responding to the newly reinforced stimulus.

Recently, evidence has been produced pointing to the importance of the receptor system for CORT in the HPC for the normal functioning of this area of the brain in reversal behaviour. Unilateral ablation of the HPC, which brings about compensatory changes in the number of receptors for CORT in

the contra-lateral lobe with time (Nyakas et al., 1983), has been associated with impaired reversal shifting when the receptor number was low. Restoration of normal discrimination behaviour occurred when the hippocampal CORT-receptor capacity was at its highest. Interestingly, adrenalectomy accelerated the recovery of the lesion-induced deficit and from this it can be inferred that CORT acts to retard normal reversal behaviour in unilaterally hippocampectomised animals (de Ronde et al., 1986). Presumably this arises through CORT's inhibitory effect on the remaining HPC. There is, however, the possibility that the down-regulation of receptors by CORT underlies the retardation of the normalisation of reversal behaviour. If the HPC has an inhibitory role in the regulation of the PAS, as was intimated in Chapter 1, then this effect would be even more marked. Partial loss of this brain area would result in the release of the PAS from its inhibitory control, elevating the circulating levels of CORT. Despite this, Landfield et al. (1981) found that whilst adrenalectomy retards the age-related morphologic changes within the HPC, the impaired performance on reversal tasks, which tends to occur with age, is not averted.

In view of the lack of consistency within the empirical data relating to the effects of CORT on reversal shifting, and as its effects on non-reversal shifting have not, to date, been subjected to investigation, it is obviously difficult to decide whether the hypothesis that this adrenocorticoid enhances persistence is supported. Since this inconsistency may have arisen through the implementation of a variety of different behavioural paradigms, especially as CORT is known to exert opposite effects depending upon the nature of the motivational stimulus (e.g., Micco et al., 1979), the experiments reported here examined the effects of this adrenocorticoid on both a RS and a NRS in a water 'T' maze, under conditions that remained essentially the same for both. A water maze was selected because early attempts to train mice to learn a spatial

discrimination for food reinforcement in a Y-maze were surprisingly unsuccessful. The report that guinea-pigs, which are notoriously difficult to use in behavioural tests, readily acquire a position discrimination in a T-water maze (Adlard et al., 1974; Smart and Adlard, 1974) pointed to the greater likelihood of success with this type of maze. A review of the literature supported this: discrimination studies with mice, though few in number, invariably use a water maze (e.g., Goodlett et al., 1983). Presumably the motivation to escape from water is sufficiently powerful to eliminate any other behavioural tendencies of the mouse, which would otherwise hinder the acquisition of the discrimination response.

Firstly CORT's influence on the reversal of a spatial discrimination was evaluated; it was given either throughout the original discrimination (EXPERIMENT 1a) or throughout the reversal (EXPERIMENT 1b). This was followed by an examination of its effects on a NRS, which involved shifting from a brightness cue to a position cue, with the brightness cue present but irrelevant (EXPERIMENT 2): CORT was given throughout the discrimination shift. All the animals were adrenally intact, with controlled levels of T. As these experiments extended over a relatively long period, silastic - T pellets were used, providing relatively stable replacement levels of the androgen over this period. A pilot study, which is reported in APPENDIX 6, examining the circulating concentrations of T over a 4 week period following the implantation of different silastic - T pellets, was carried out in order to ascertain the most appropriate pellet for these experiments.

EXPERIMENT 1

The effects of corticosterone on reversal shifting in adrenally intact mice

To date, the majority of reversal studies have employed either brightness or spatial discrimination tasks. The advantage of the latter is that they are more easily learnt, presumably because other discrimination problems tend to be complicated by marked spatial preferences. Accordingly, the present experiments examined the effects of CORT on the reversal of a spatial discrimination.

The details of the experimental procedure for the RS is now given in the general method section. This procedure was the same in EXPERIMENTS 1a and 1b, the only difference being the timing of the CORT treatment.

General method

Experimental conditions and subjects

There were 4 experimental conditions:

1. T-replaced castrate controls (CxT)
2. T-replaced castrates injected with 0.1ml propylene glycol (CxT+VEHICLE)
3. T-replaced castrates injected with 50 µg CORT in the vehicle (CxT+CORT50)
4. T-replaced castrates injected with 100µg CORT in the vehicle (CxT+CORT100)

All the injections were given subcutaneously, one hour prior to behavioural testing, regardless of whether the treatments were given during acquisition or reversal. The treatments were coded, so that the experiments were conducted blind with respect to those conditions requiring an injection. Specific details of the number of animals used will be given in

each experiment. All the mice were castrated and after a 3 week recovery period implanted with a silastic - T pellet, as described in Chapter 2. The mice were individually housed.

Apparatus

The apparatus consisted of a wooden 'T'-maze painted black, two aluminium platforms and a sliding door (see Figure 6:2). Mice were introduced into the water via an aluminium funnel (this prevented them seeing the position of the escape platform and orienting to it).

The water was sufficiently deep to prevent the mice touching the floor of the maze, and was maintained at 22 ± 1^{o}C.

Procedure

Behavioural testing commenced later on the day on which the T - pellets were implanted. Mice were tested in numerical order, which was determined before they were assigned to the different experimental conditions.

No attempt was made to control for, or eliminate extra-maze cues. The position of the maze with respect to these cues varied from day to day. There were 3 phases of behavioural testing: establishment of side preference, original position discrimination and reversal position discrimination.

1. Establishment of side preference

The mice were given 3 trials in the water maze with escape possible from both arms. Position preference was operationally defined as the side most frequently chosen.

2. Original position discrimination

Each mouse was introduced into the water at the start point via a funnel; the sliding doors were open and the escape platform was situated at the end of the non-preferred arm. A non-correction procedure was used: once the mouse had entered either arm the doors were closed, thereby preventing retracing. Entries into the incorrect arm were scored as an error, and the animal was retained in the water for a further 15 seconds before being removed to a holding cage, where it spent the intertrial interval. If the animal entered the correct arm escape was possible via the platform. Mice were retained in the water for a maximum of 60 secs; if any mouse had not entered either arm within this period, the trial was scored as a balk and the animal was transferred to the holding cage until the next trial commenced.

The mice were given 6 trials a day with a 15 second intertrial interval. Immediately after the last trial of a session the mice were towel dried and returned to their home cages.

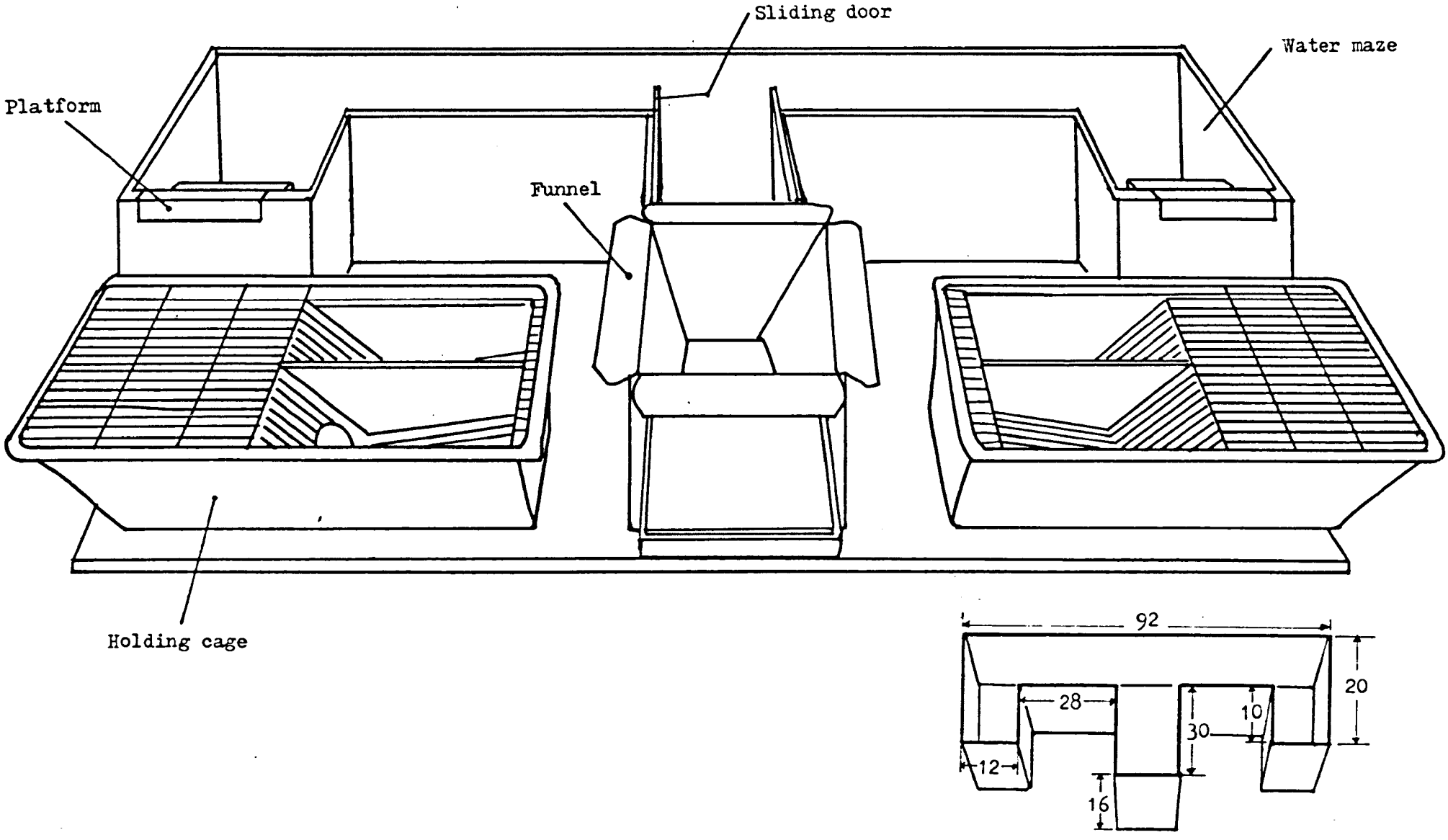
Training on the original discrimination continued until the mice reached a criterion of 5/6 correct responses on two consecutive days. If this criterion was not reached within 10 days, behavioural testing was terminated. Once criterion had been reached the mice were required to learn the reversal discrimination. Testing on this commenced the following day.

3. Reversal position discrimination

In order to escape from the water the mice were required to reverse the learned response. Training on the reversal discrimination continued until a criterion of 5/6 correct on 2 consecutive days was reached. Immediately following the last trial the mice were blood sampled. These samples were subsequently assayed for CORT. All behavioural testing was carried out between 12.00-4.30pm.

The following behavioural parameters were used to assess the effects of CORT on the reversal of a learned spatial discrimination:- trials to criterion on the original discrimination; trials to criterion on the reversal discrimination; initial errors - defined as the errors preceding the first correct choice during the reversal; perseverative errors - defined as consecutive recurrence of a particular erroneous response; total errors; and residual errors - defined as the total errors minus initial errors.

Figure 6:2 Diagram of the water T-maze (dimensions in centimetres).



EXPERIMENT 1a

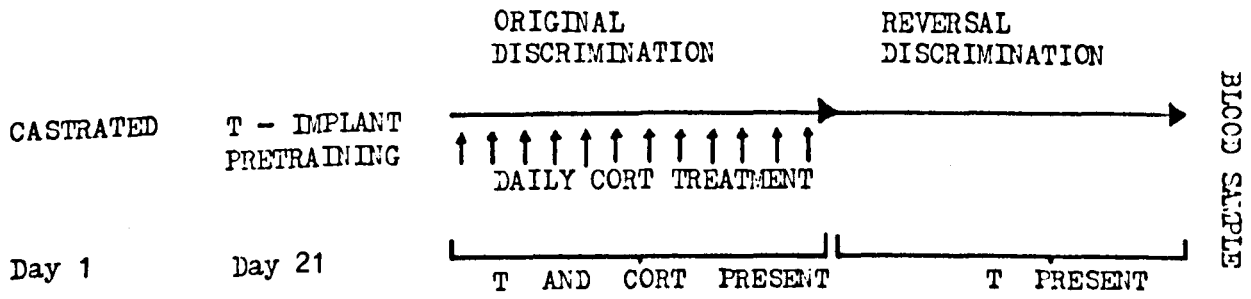
The effects of corticosterone administered during the acquisition of a discrimination on reversal shifting

Method

There were no methodological differences from those given in the general method. The treatments were given throughout the acquisition of the original discrimination response. There were 10 animals in each condition. 50 mice were used, though 4 died and 6 were excluded from further testing, either because they did not reach criterion or because an adequate number of animals had been obtained for each condition.

The mice were tested in a single batch, and were arbitrarily assigned to one of the experimental conditions prior to the onset of behavioural testing.

SUMMARY OF METHOD



Statistical analysis

The behavioural data is expressed as medians and 95% confidence limits and was analysed by the KWANOVA. Some animals did not reach criterion on the reversal discrimination within 60 trials, though for the purpose of statistical analysis a value of 60 was used. The degree of correlation

between the number of trials to criterion on the original and reversal discrimination within a condition was measured by Spearman's Rank Correlation Coefficient.

The CORT data is presented as means ± S.E. and was analysed by a one way unrelated ANOVA.

Results

Table 6:1 The effects of corticosterone on discrimination behaviour. (Medians and 95% confidence limits)

CONDITION N=10	TRIALS TO * ORIGINAL CRITERION	TRIALS TO * REVERSAL CRITERION	INITIAL ERRORS	PERSEVER-ATIVE ERRORS	TOTAL ERRORS	RESIDUAL ERRORS
CxT+CORT100	18.0 18-24	27.0 18-36	4.0 2-7	6.0 3-14	11.0 5-16	6.0 1-12
CxT+CORT50	18.0 18-18	24.0 18-30	4.0 1-6	4.0 2-7	9.0 6-10	5.0 2-7
CxT+VEHICLE	18.0 12-30	30.0 24-36	6.0 2-9	7.0 3-14	13.0 8-17	7.0 3-9
CxT	18.0 12-24	27.0 18-30	4.0 3-7	5.5 2-11	9.0 5-13	4.0 2-9
KWANOVA: H	1.8	5.1	2.2	2.4	4.3	2.2

* = see Figure 6:3

No significant effects were detected.

Figure 6:3 The effects of corticosterone given throughout acquisition on the number of trials to criterion on the original and reversal discriminations (medians).

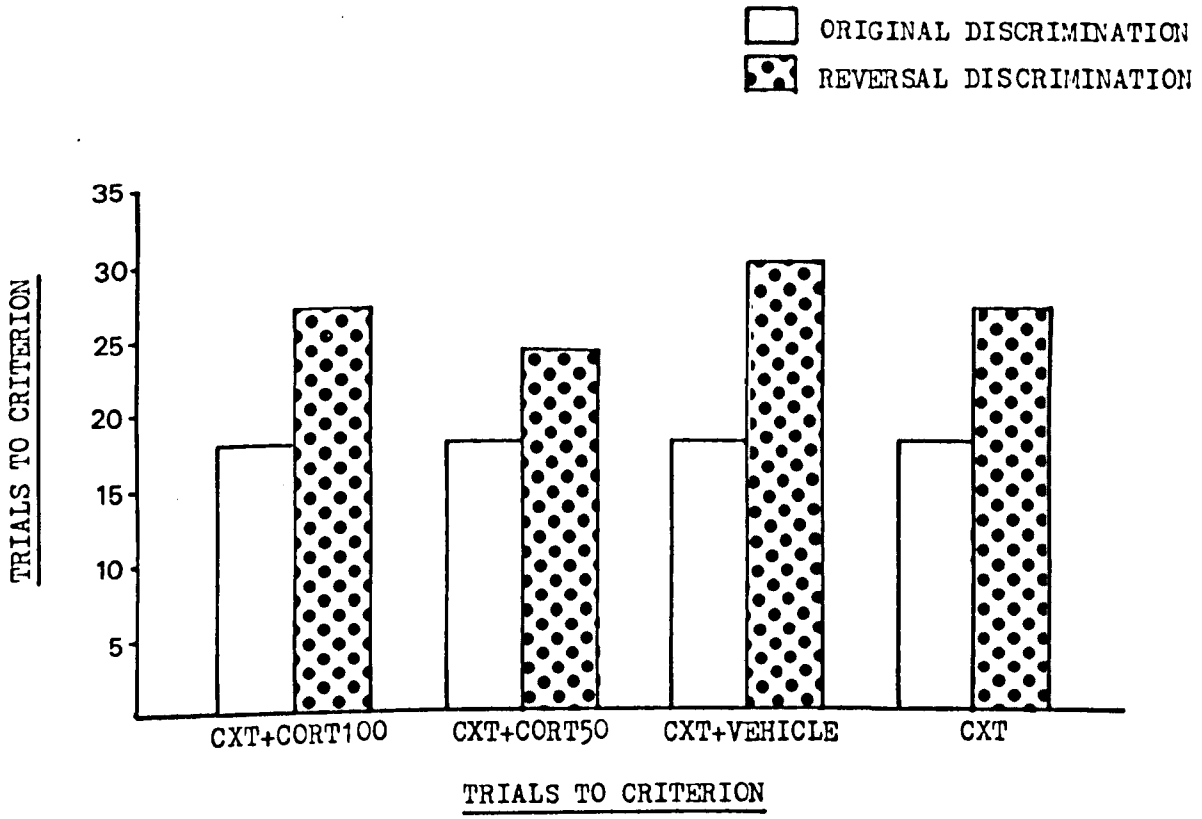


Table 6:2 The results of the correlation analysis between the number of trials to criterion on the original and reversal discriminations

CONDITION N=10	SPEARMAN'S RANK CORRELATION COEFFICIENT rhos
CxT+CORT100	0.085
CxT+CORT50	0.148
CxT+VEHICLE	0.227
CxT	0.70 *

* $p < 0.05$

Those T-replaced castrates that acquired the original discrimination rapidly tended to reverse it rapidly. A similar relationship was not found in the other treatment conditions.

Table 6:3 The effects of treatments on the plasma levels of corticosterone (Means ± S.E.)

CONDITION N=10	CORT ng/ml
CxT+CORT100	62.9 ± 9.8
CxT+CORT50	82.3 ± 14.4
CxT+VEHICLE	76.4 ± 10.7
CxT	79.9 ± 9.2

ONE WAY UNRELATED ANOVA: $F(3,36) = 0.605$, $P = 0.616$

There were no significant effects detected.

Discussion

As expected there were no significant differences in the number of trials required to reach criterion on the original discrimination. This alleviated fears that differences in the levels of CORT during the acquisition of the original response might have influenced the rate at which this response was acquired. Contrary to expectations CORT failed to affect any measure of reversal behaviour. Indeed, the only indication that it might influence this behaviour was provided by the finding that the speed of learning on the original and reversal discriminations did not correlate in those animals treated with CORT, unlike their controls. However, since such a relationship was not found in the vehicle controls its significance is dubious.

It was necessary to determine the degree of correlation between the number of trials on the original and reversal discriminations in order to clarify whether the speed at which a learned discrimination response is reversed simply reflects the ability to learn rather than an attentional phenomenon *per se*. This is because, if a positive correlation exists, this might be argued to indicate that the speed of learning of the original

discrimination is the sole determinant of the speed at which the reversal discrimination is learnt. Whereas, if no correlation exists, this might reinforce the use of discrimination tests as a means of examining attentional processes.

As there was evidence that the number of trials required to reach criterion on the original and reversal discriminations were positively correlated in the controls, this might, according to the above rationale, undermine the value of discrimination tasks in the study of attentional processes. Notwithstanding this, the failure to find a similar correlation in the CORT conditions suggests that exogenous elevations of CORT disrupt the transfer process responsible for this correlation. Furthermore, although the level of CORT in response to the injection of the vehicle is unknown in this experiment, previous experiments have shown it to be elevated, and as the correlation between the number of trials to criterion on the original and reversal discriminations was also absent in these animals, elevations of CORT, both endogenous and exogenous would seem to disrupt the transfer process. This, in turn, suggests that CORT impairs the ability to reverse a discrimination. Interestingly, Silveira and Kimble (1968) found that the number of trials to criterion on the original and reversal discriminations were negatively correlated in hippocampectomised animals. Thus, hippocampectomy also appears to disrupt the process involved in the transfer from one discrimination problem to the next. Though speculative, this might reflect an increased level of persistence, as it suggests that these animals continued to respond to the cue that was correct originally.

Finally, there are two practical points that need to be commented upon, both of which are connected with the time of blood sampling:-

i) Whilst it is recognised that a terminal blood sample is far from ideal, the only alternative would have been to take the sample when criterion had been reached on the original discrimination. This, however,

was considered to be even more unsatisfactory, as it may have disrupted reversal behaviour.

ii) Even though no differences were anticipated in the circulating levels of CORT, this was analysed all the same, in order to ascertain whether chronic treatment with CORT exerts long lasting effects upon the dynamics of the PAS - fortunately this did not appear to be the case. In connection with this, because of the delay between the hormone manipulations and the time of blood sampling, the value to be gained from assessing the possible correlation between CORT and reversal shifting was difficult to envisage, and it was, therefore, not assessed.

EXPERIMENT 1b

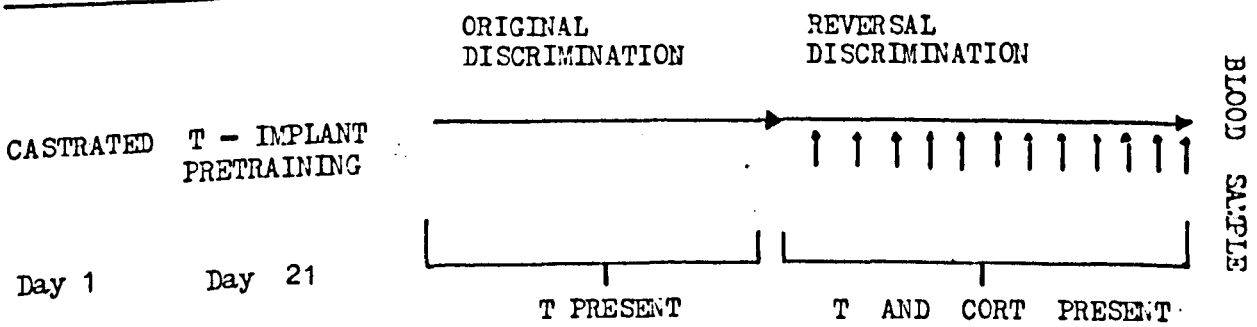
The effects of corticosterone administered during the reversal of a discrimination on reversal shifting

Method

There were no methodological differences from those given in the general method. The treatments were given throughout the reversal of the discrimination response. There were 12 animals in each condition. 60 mice were used, though 5 died and 7 were excluded from testing because they did not reach criterion.

Upon reaching criterion on the original discrimination the mice were alternately assigned to one of the experimental conditions. (This resulted in subgroups with highly similar mean scores on the original learning task, thereby controlling for the level of learning on this task). The mice were tested in 2 batches, with an equal number of individuals being assigned to each condition from both batches.

SUMMARY OF METHOD



Statistical analysis

The statistical analysis of the behavioural data was identical to that in EXPERIMENT 1a.

The CORT data is expressed as means ± S.E. and was analysed by the KWANOVA followed by the MWU test.

Spearman's Rank Correlation Coefficient was used to assess the relationship between the levels of CORT and the number of trials to criterion on the reversal discrimination, both within conditions, and when the data from the different conditions had been combined.

Results

Table 6:4 The effects of corticosterone on discrimination behaviour (medians and 95% confidence limits)

CONDITION N=12	TRIALS TO * ORIGINAL CRITERION	TRIALS TO * REVERSAL CRITERION	INITIAL ERRORS	PERSEVER-ATIVE ERRORS	TOTAL ERRORS	RESIDUAL ERRORS
CxT+CORT100	18.0 12-30	36.0 30-48	4.0 2-16	17.5 8-31	21.0 14-34	13.5 6-17
CxT+CORT50	18.0 12-24	36.0 24-48	8.0 2-10	16.0 12-30	20.0 10-34	9.0 4-20
CxT+VEHICLE	18.0 12-24	30.0 24-42	7.0 4-11	10.0 5-21	13.5 8-25	5.5 2-18
CxT	24.0 18-24	30.0 24-36	1.5 1-5	8.5 5-16	12.0 7-19	7.5 2-18
KWANOVA: H	1.6	3.5	7.0	4.3	4.3	1.7

* = see Figure 6:4

No significant effects were detected.

Table 6:5 The results of the correlation analysis between the number of trials to criterion on the original and reversal discriminations

CONDITION N=12	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos
CxT+CORT100	0.15
CxT+CORT50	*0.46
CxT+VEHICLE	*0.55
CxT	0.58

* $p < 0.05$

Unlike the animals treated with CORT, the controls that acquired the original discrimination rapidly, reversed it rapidly also.

Table 6:6 The effects of treatments on the plasma levels of corticosterone (Means ± S.E.)

CONDITION N=12		CORT ng/ml
CxT+CORT100		772.1 ± 89.4
CxT+CORT50	b.	398.3 ± 27.3
CxT+VEHICLE	a.	123.3 ± 9.5
CxT		128.5 ± 6.1

KWANOVA: $H = 37.7$, $P < 0.001$

a. MWU different from CxT+CORT50: U=0, $P < 0.001$

b. MWU different from CxT+CORT100: U=19, $P < 0.05$

The vehicle controls possessed similar CORT levels to their non-injected counterparts. 50 and 100 µg CORT resulted in dose-dependent elevations.

Figure 6:4 The effects of corticosterone given throughout reversal on the number of trials to criterion on the original and reversal discriminations (medians).

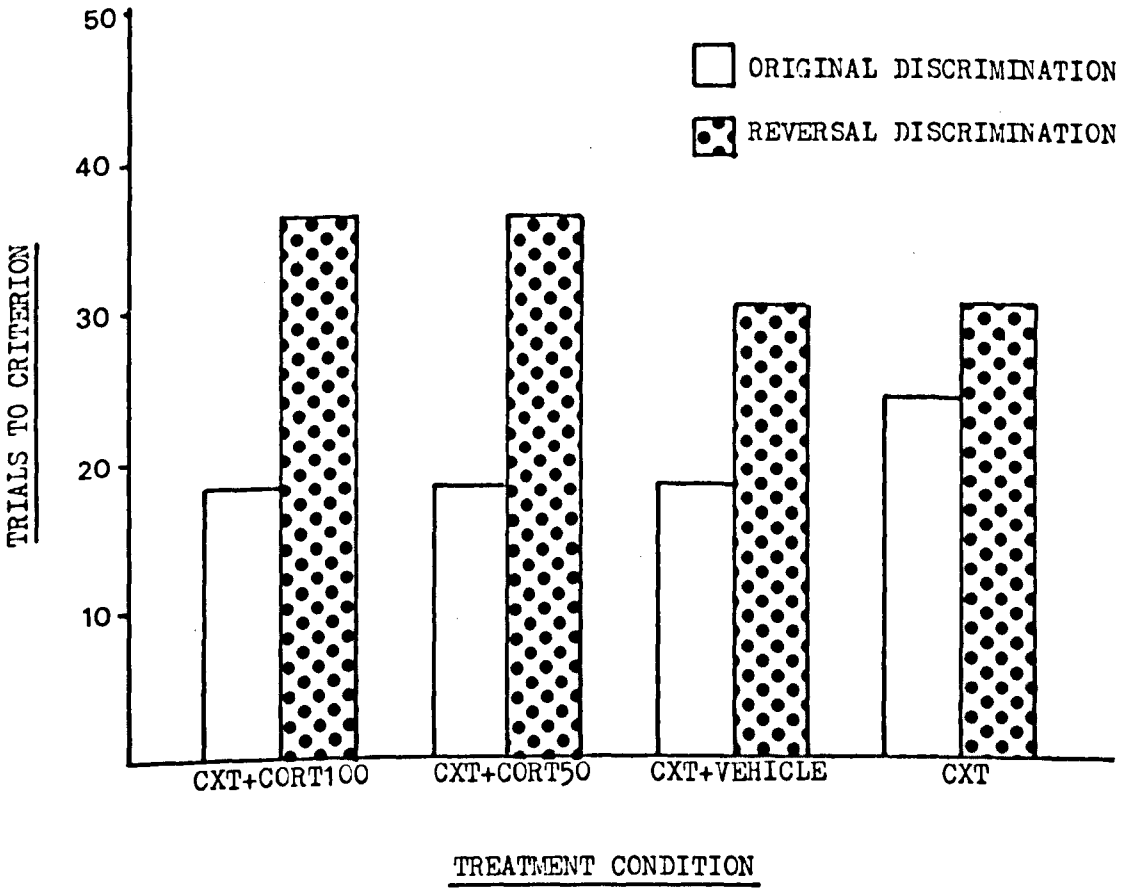


Table 6:7 The results of the correlation analysis between the levels of corticosterone and the number of trials to criterion on the reversal discrimination - within conditions

CONDITION	N=12	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos
CxT+CORT100		- 0.162
CxT+CORT50		- 0.502
CxT+VEHICLE		- 0.416
CxT		- 0.126

There were no significant within condition correlations. Neither was any significant correlation found when the conditions were combined to give N=48; the observed value of rho being 0.08.

Discussion

As expected there were no significant differences in the number of trials required to reach criterion on the original discrimination. However, the absence of any significant differences in reversal behaviour, despite the presence of markedly different levels of CORT was unexpected and disappointing. Even so, there was a tendency for those animals treated with CORT to exhibit an inferior level of performance on the reversal discrimination. Corticosterone increased the number of trials required to reach criterion on this discrimination, and increased the number of perseverative errors, total errors and residual errors. Moreover, there appeared to be a dose-dependent effect: those animals treated with the higher dose of CORT requiring more trials to reach the reversal criterion, and exhibiting more errors than those treated with the lower dose.

At this point it is appropriate to comment upon the significance of the different types of error that were used. Initial errors are considered to reflect the ease with which responding is shifted to a new stimulus.

Perseverative errors indicate the degree to which an animal perseverates in responding to a stimulus, and by inference the extent to which it persists in attending to the stimulus. Finally, residual errors are believed to be potentially useful in clarifying the nature of persistence. Thus, if the enhancement of persistence increases the resistance to shift in the use of a particular set of central specifications, and facilitates the reinstatement of these specifications following a change in the rules of selection, the number of residual errors would be higher than if the reinstatement of the central specifications corresponding to the originally prepotent stimulus is not facilitated. In this light, CORT treated animals would appear to be more persistent, as the greater number of perseverative errors suggests that they persisted in testing a particular hypothesis once they had started; furthermore, the greater number of residual errors indicates that, following a brief interruption of the rules of selection, they tended to revert to the original stimulus and continued responding to it for longer. In view of the consistency of these findings it was surprising to find that the number of initial errors was not elevated.

That CORT may be influencing the reversal of a learned discrimination was further suggested by the evidence that those animals treated with this adrenocorticoid did not exhibit the correlational relationship between the number of trials to criterion on the original and reversal discriminations that was found in their controls. A similar finding was reported in the previous experiment, and whilst this is encouraging, it is difficult to account for the present demonstration of a positive correlation within the vehicle controls, as this was not found previously (see Table 6:2).

Taken together this evidence suggests that the persistence of attention is enhanced by CORT. The insignificant nature of the results may, therefore, simply indicate that the attentional effect of this adrenocorticoid is very subtle. However, there are at least two possible reasons for the difficulty in demonstrating a stronger effect. The first

relates to the nature of the discrimination problem. Mason and Iversen (1979) point out that an attentional effect only emerges if the task requires the use of one specific cue which does not innately captivate the animal's attention. This is because, if a cue is used which has a high natural salience, the animal will not have to learn to attend to it, and more importantly, it will not have to learn to ignore other cues in order to attend to it. Moreover, Mason and Iversen (1979) contend that rodents, especially rats, have an innate tendency to attend to spatial cues, and on this basis they argue that the use of these cues in a discrimination task is inappropriate.

An additional problem with spatial tasks is that they usually involve far fewer irrelevant stimuli than other discrimination tasks (Mackintosh, 1965). In support of this is the finding that the beneficial effects of overtraining on reversal shifting are often only found in spatial tasks if additional irrelevant stimuli are included. Presumably in the absence of these stimuli the task is too simple for an overtraining effect to emerge, but in their presence, which renders the task more difficult, the animal benefits from a period of extended training. Whilst these criticisms are accepted, and whilst they point to factors that may have acted to reduce the likelihood of a stronger CORT effect emerging, their significance is difficult to estimate. Indeed, despite the reservations that may be raised against spatial tasks, they are frequently used, and marked deficits do occur in hippocampectomised animals when they are required to reverse a discrimination involving spatial cues (e.g., Kimble and Kimble, 1965). Nonetheless, in order to minimise the problems associated with spatial tasks, no attempt was made to control for the presence of extra-maze cues, and the position of the maze with respect to these cues was varied from day to day. This was intended to increase the number of extra-maze cues, and by altering the position of the maze any tendency to select these cues as opposed to spatial cues would be controlled.

The second possible reason for the failure to detect a stronger CORT effect relates to the circulating levels of CORT. Although the controls possessed CORT in levels which fell in the non-stressed physiological range, they inclined towards the upper end of this range. As a consequence, it is likely that there was only a limited capacity available to any elevations of CORT to exert their effect at the receptor level. Thus, even though the lower dose of CORT produced circulating levels corresponding to those found under highly stressful conditions, the limited availability of receptor mechanisms to respond to it may have been insufficient for a behavioural effect to be expressed. This may explain the failure to detect behavioural differences between treatment groups with progressively elevated levels of CORT as this problem is obviously compounded with even higher CORT levels.

Finally, if the results of the present experiment are compared with those from the previous one, a number of interesting points emerge. For instance, the number of trials required to reach criterion on the original discrimination was strikingly similar in both experiments, and the behaviour of the uninjected and vehicle controls was relatively consistent throughout. In spite of this, a number of differences appeared at reversal in those animals treated with CORT, depending on whether the treatment was given during the acquisition (ACQ) or the reversal (REV) phase of the discrimination. One of the most pronounced of which was that they required more trials to reach criterion than their controls in the REV experiment but slightly less in the ACQ experiment. Furthermore, in the REV experiment they displayed considerably more errors (perseverative, total and residual) than in the ACQ experiment. Since an increase in the number of errors indicates a lowered level of performance, this suggests that the administration of CORT during reversal exerts a greater deleterious effect on discrimination behaviour at reversal than if it is administered at an earlier stage in training. Mason and Iversen (1979) hold that if a hormone

affects the selective aspects of attention, such an effect emerges if it is present during the original acquisition of the response, even though the consequences of its effects only emerge when the behavioural contingencies have been altered. By contrast, if a hormone affects the persistence of attention, such an effect only emerges if it is present when the behavioural contingencies are changed. In this light, as CORT's effects were most marked when it was present during reversal, by implication it affects the persistence of attention.

To conclude: whilst the evidence from both experiments on the reversal of a learned discrimination does not offer conclusive support for the working hypothesis, CORT's involvement in the persistence of attention as opposed to its selectivity emerges as the more convincing alternative. Even so, it is not possible to attribute a role to CORT in this particular attentional phenomenon on the basis of its effects upon the behaviour displayed during a RS alone, for reasons that have already been elaborated. In order to clarify the situation, and to confidently reject the possibility that CORT influences the selective aspects of attention it is necessary to examine its effects on a NRS, and this is the subject of the next experiment.

EXPERIMENT 2

The effects of corticosterone on non-reversal shifting in adrenally intact mice

Introduction

Examining the effects of CORT on a NRS provides an assessment of its influence on persistence in two ways. Firstly, it differentiates between its effects on the persistence of attention as opposed to its selectivity, in the way that has already been described. Secondly, by applying the principles of sub-problem analysis, a NRS allows for a more refined assessment of the degree to which CORT affects the continued responding to the originally correct stimulus.

Indeed, although sub-problem analysis was originally designed for the assessment of dimensional attentiveness (Tighe et al., 1971; Sandman et al., 1974; Beckwith et al., 1976), it can be used to assess the persistence of attention in the following way. Basically, exploiting the fact that a NRS involves transfer between different stimulus dimensions, with the same stimulus pairs being presented in both the original and the shift problems, such that there are trials during the shift when the animal is reinforced for making a response that was correct during the original discrimination and be rewarded for doing so, it separates this discrimination task into 2 problems:

1. Unchanged dimension problem - in which the reinforced stimulus of the previously relevant dimension coincides with the correct stimulus of the dimension of relevance in the shift.
2. Changed dimension problem - in which the new cue is paired with the non-reinforced stimulus of the previously relevant dimension. Once separated, it examines for differences in the pattern in which these two problems are learnt and this reflects the degree of attentional persistence. To be more explicit: if during a NRS, animals are trained to respond first to dimension A e.g., bright-dark (B-D), with dimension B

e.g., left-right (L-R) irrelevant. The positively reinforced stimulus, dark, is presented equally often on the left or right side of the maze. In the second (shifted) phase of the experiment, animals are trained to respond to the L-R dimension, with the B-D dimension irrelevant, although it is still alternated between left and right on an equal (though irregular) basis. Animals which have learned the B-D discrimination and which persist in using it should score 50% correct responses. This would consist of 100% correct on the 'unchanged dimension' and 0% on the 'changed'. From this it is clear that if CORT enhances the level of attentional persistence to prepotent stimuli, animals treated with this adrenocorticoid should display a high level of responding on the unchanged dimension, but a low level on the changed. In addition, it should take longer for these animals to start responding on the changed dimension problem.

In the light of the potential value that the performance on the subproblems of a NRS has for an assessment of the role of CORT in the persistence of attention, the behaviour of CORT-treated animals on changed and unchanged problems was compared with their controls.

Furthermore, as with a RS, the effects of CORT on the acquisition of a NRS was measured by the number of trials required to reach criterion on this discrimination shift. Moreover, the type of errors exhibited during this shift afforded a more detailed examination of the nature of any discrimination deficit.

Finally, in the experiment that follows, all the animals were reinforced if they responded to the dark stimulus during the original brightness discrimination. The reason for this being that preliminary studies had revealed a marked preference for the lighted arm. Presumably, this reflects some intrinsic adaptive significance of light in an aquatic environment. It might, for example, signal safety in a flooded burrow. Alternatively, it may be attractive in a cold, wet environment, because of

its association with warmth (though there were no actual differences in temperature in the light and dark arms of the maze). However, as Howard and Granoff (1968) do not mention that their mice displayed a preference for the lighted arm in a water maze, its true significance is difficult to determine.

Method

Experimental conditions and subjects

There were 8 animals in each condition:

1. T-replaced castrate controls (CxT)
2. T-replaced castrates injected with 0.1ml propylene glycol (CxT+VEHICLE)
3. T-replaced castrates injected with 100µg CORT in the vehicle (CxT+CORT100)
4. T-replaced castrates injected with 300µg CORT in the vehicle (CxT+CORT300)

All the injections were given subcutaneously one hour prior to behavioural testing throughout the shift phase of the task. The treatments were coded so that the experiment was conducted blind with respect to those conditions requiring an injection.

45 mice were used, though one died and 12 failed to reach criterion. All the mice were castrated, and after a 3 week recovery period, implanted with a silastic-T pellet, in the manner described in Chapter 2. The mice were individually housed.

Apparatus

The water T-maze has already been described. For the purpose of this experiment the only modification was the placing of a bench lamp, with a 25w white light bulb, above the choice point. It had a flexible head which could be pointed toward the left or the right arm of the maze. In order to accentuate the differences in brightness in the light and dark arms, a

piece of clear perspex was placed along the top of the light arm, whereas a piece of black perspex was placed on the top of the dark arm (see Figure 6:5).

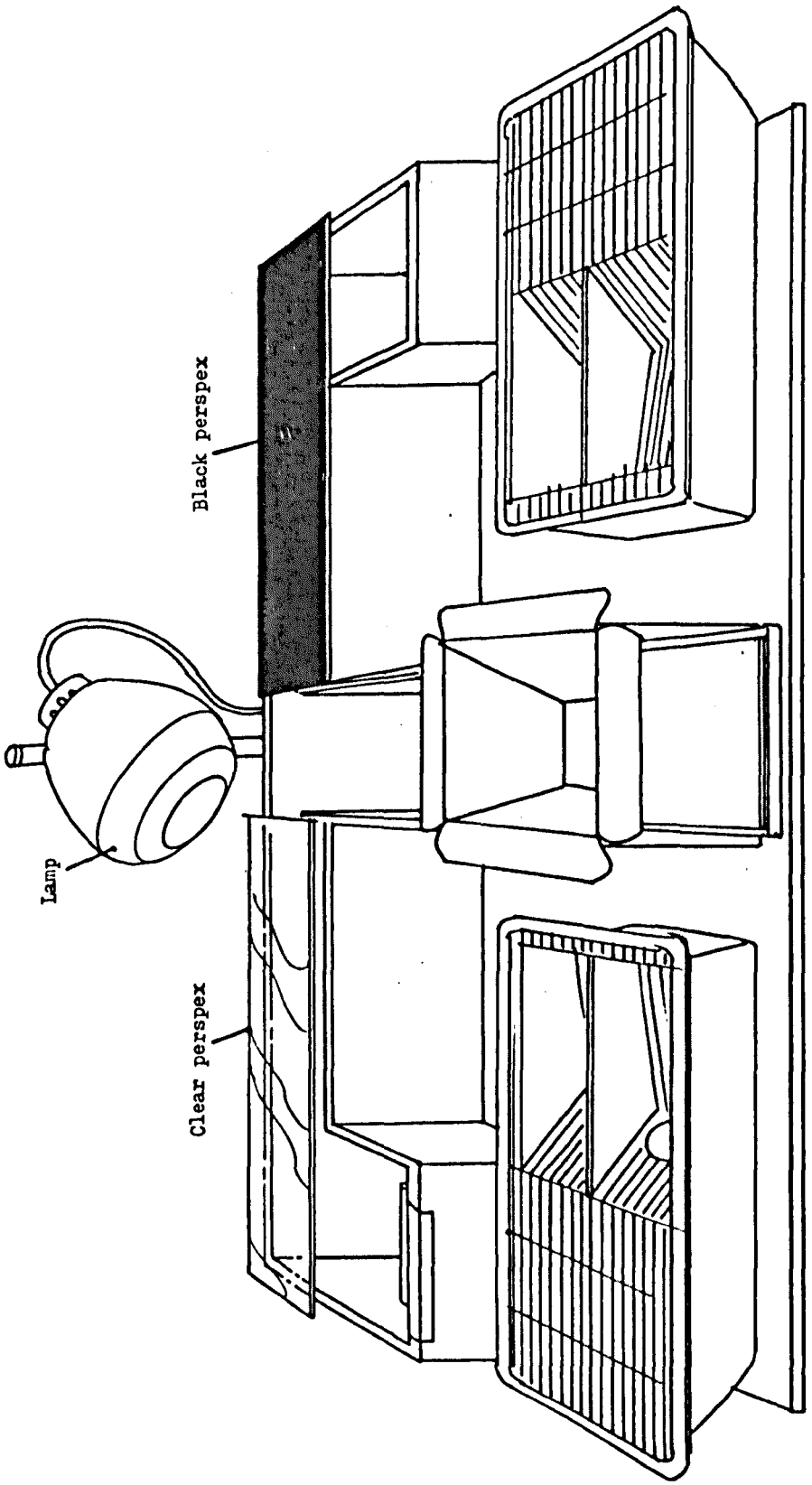


Figure 6:5 Diagram of the water T - maze for the nonreversal shift.

Procedure

Behavioural testing commenced later on the day on which the T-pellets were implanted. The discrimination task comprised 3 phases: pretraining, original brightness discrimination training and non-reversal shift position discrimination training.

1. Pretraining

With platforms present at both arms of the maze, the mice were given 2 free choice trials, followed by 4 forced choice trials: 2 to the left arm and 2 to the right. If during the free choice trials a mouse selected the same arm on both trials, then the following pair of trials were given to the opposite arm. The light was not present during pretraining.

2. Original brightness discrimination training

The mice were given 6 non correction trials a day, separated by a 15 second inter-trial interval, which was spent in an adjacent holding cage. A modified Gellerman series (Fellows, 1967) determined the position of the light, so that each arm was lighted on 3 trials of any session, and no arm was lighted on more than 2 consecutive trials. The following series were employed and they were alternated every two sessions: LRLLRR or RLRRLL. All the mice were trained with dark positive. The procedure was identical to that described in EXPERIMENT 1a.

Training on the original brightness discrimination continued until a criterion of 5/6 correct responses on two consecutive days had been reached. If any animal failed to reach this criterion within 20 days it was excluded from further testing. Once a mouse had reached criterion on the original brightness discrimination it was shifted to the position discrimination which commenced the following day.

Alternate assignment of animals to different conditions upon reaching criterion resulted in subgroups which were balanced for their performance

on the original discrimination.

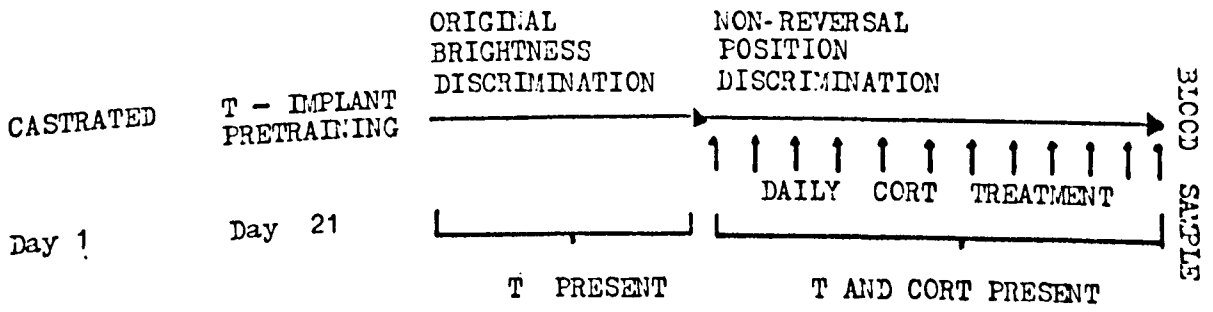
3. Non-reversal shift position discrimination training

During this phase, half the animals from any one condition were reinforced for selecting the right arm of the maze whilst the other half were reinforced for responding to the opposite maze arm, irrespective of the position of the light, which was varied, as before. Training on the NRS was continued until a criterion of 5/6 correct responses on two consecutive days was reached. The mice were then blood sampled. The samples were subsequently assayed for CORT.

Training on the NRS was terminated if an animal failed to reach criterion within 10 days. Animals were trained and tested in numerical order which was determined prior to them being assigned to the various experimental conditions. 2 batches of animals were run, with an equal number of animals from every condition in both. All behavioural testing was carried out between 12.00-4.00p.m. The following parameters were used to assess the effects of CORT on a NRS:-

Trials to criterion on the original discrimination; trials to criterion on the NRS discrimination; initial errors on the changed dimension - defined as the number of trials preceding the first correct choice on the changed dimension; responses to irrelevant cues - defined as 5 consecutive responses to either cue of the previously relevant dimension; and total errors.

SUMMARY OF METHOD



Statistical analysis

The behavioural data is expressed as medians and 95% confidence limits, with the exception of the sub-problem data, which is presented in its raw form. Firstly, the MWU test was used to check that the left-right variable introduced at the shift phase had not affected discrimination shifting. Subsequent behavioural analysis was carried out by the KWANOVA. The degree of correlation between the number of trials to criterion on the original and shift discriminations within a condition was measured by Spearman's Rank Correlation Coefficient.

For subproblem analysis the total number and % correct responses within each session for the changed and unchanged dimension are presented. A criterion of 2/3 correct responses on 2 consecutive days was adopted. (It was assumed that once an animal had reached criterion, performance would remain at this high level, and a score of 100% was allocated on subsequent sessions). Fisher's Exact Probability test (2x2) was used to analyse the number of animals reaching criterion on the unchanged dimension by the end of session 2, and on the changed dimension by the end of session 5, between conditions.

The CORT data is presented as means ±S.E. and was analysed by the KWANOVA, followed by the MWU test.

Spearman's Rank Correlation Coefficient was used to assess the degree of correlation between the levels of CORT and the number of trials to criterion on the NRS, both within conditions and when the data from all the conditions had been combined. Similar correlational analysis was carried out between the levels of CORT and the number of trials to criterion on the changed and unchanged dimensions.

Results

Table 6:8 The effects of the left-right variable on the number of trials required to reach criterion on the non-reversal shift discrimination. (Medians and 95% confidence limits)

CONDITION N=4	NUMBER OF TRIALS	
	LEFT	RIGHT
CxT+CORT300	39.0 (15.4-56.6)	21.0 (5.8-62.8)
CxT+CORT100	27.0 (6.1-81.1)	30.0 (7.7-58.2)
CxT+VEHICLE	30.0 (16.5-40.5)	21.0 (4.3-49.7)
CxT	21.0 (10.4-28.6)	24.0 (16.2-31.8)

MWU: U=38

The left-right variable did not exert a significant effect. Accordingly, in all further analyses the data was combined irrespective of this.

Table 6:9 The effects of corticosterone on discrimination behaviour.

(Medians and 95% confidence limits)

CONDITION N=8	TRIALS TO* ORIGINAL CRITERION	TRIALS TO* NRS CRITERION	INITIAL ERRORS	RESPONSES TO IRRELEVANT CUES	TOTAL ERRORS
CxT+CORT300	57.0 30-78	30.0 12-60	10.0 0-47	2.0 0-9	9.5 1-25
CxT+CORT100	54.0 30-90	27.0 18-54	5.0 1-63	2.0 0-13	8.5 2-32
CxT+VEHICLE	60.0 36-96	27.0 18-48	4.0 0-15	1.5 1-3	7.5 3-24
CxT	51.0 30-84	24.0 12-30	6.0 0-16	1.0 0-3	4.5 1-10
KWANOVA: H=	1.2	2.4	1.5	1.3	2.5

* see Figure 6:6

No significant effects were detected.

Figure 6:6 The effects of corticosterone on the number of trials to criterion on the original and nonreversal discriminations (medians).

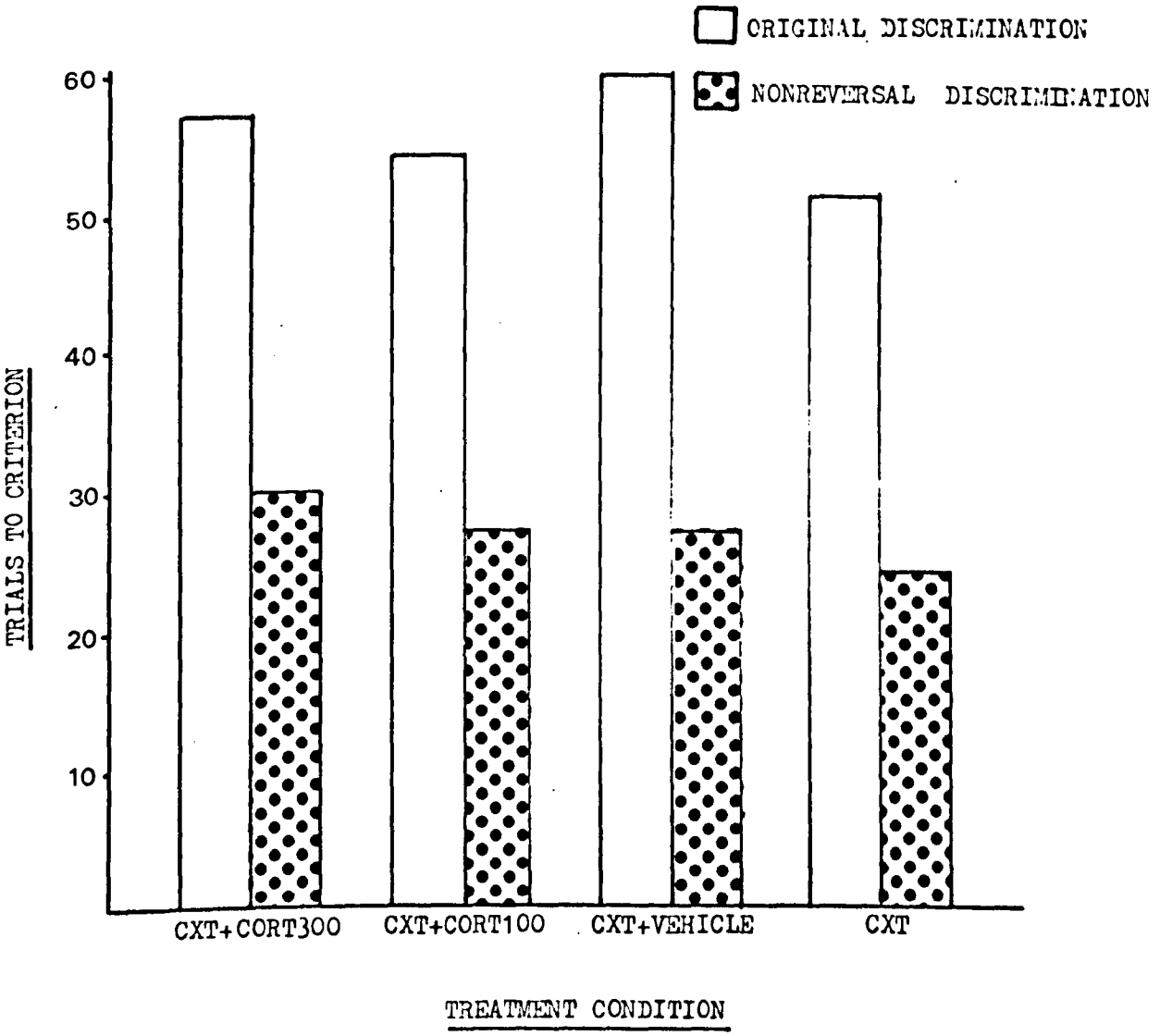


Table 6:10 The results of the correlational analysis between the number of trials to criterion on the original and non-reversal shift discriminations

CONDITION N=8	SPEARMAN'S RANK CORRELATION COEFFICIENT rhos
CxT+CORT300	0.47
CxT+CORT100	0.32
CxT+VEHICLE	0.41
CxT	0.31

No significant correlations were detected within any condition.

Table 6:11 The effects of treatments on the plasma levels of corticosterone. (Means±S.E.)

CONDITION N=8		CORT ng/ml
CxT+CORT300	a	1653.3±68.1
CxT+CORT100	a	607.8±73.9
CxT+VEHICLE	a	229.3±48.8
CxT	a	81.6±10.7

KWANOVA: H=27.5 , $p<0.001$

a. MWU different from all other conditions, $p<0.001$.

Table 6:12 The results of the correlation analysis between the levels of corticosterone and the number of trials to criterion on the non-reversal shift discrimination - within conditions

CONDITION N=8	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos
CxT+CORT300	0.470
CxT+CORT100	0.321
CxT+VEHICLE	0.411
CxT	0.315

There were no significant within condition correlations. Also, no significant correlation was detected when the conditions were combined to give N=32, the observed value of rho being 0.318. It is, however, worthwhile pointing out that the probability of significance for the observed relationship between the levels of CORT and the number of trials to criterion on the NRS, actually fell between 0.07-0.08.

Subproblem analysis

The effects of the various treatment conditions on the total number of correct responses and the % correct responses on the changed and unchanged dimensions over sessions (total possible correct is 24)

Tables 6:13a and 6:13b CxT+CORT300

(see Figure 6:7)

(a)

UNCHANGED N=8	SESSION 1	2	3	4	5	6	7	8	9	10
TOTAL NUMBER CORRECT RESPONSES	21	21	22	24	24	24	24	24	24	24
% CORRECT RESPONSES	87	87	91	100	100	100	100	100	100	100

(b)

CHANGED N=8	SESSION 1	2	3	4	5	6	7	8	9	10
TOTAL NUMBER CORRECT RESPONSES	6	9	11	15	15	17	20	21	23	24
% CORRECT RESPONSES	25	37	45	62	62	70	83	87	95	100

Tables 6:14a and 6:14b CxT+CORT100

(see Figure 6:8)

(a)

UNCHANGED N=8	SESSION 1	2	3	4	5	6	7	8	9	10	11	12	13
TOTAL NUMBER CORRECT RESPONSES	22	21	23	23	23	24	24	24	24	24	24	24	24
% CORRECT RESPONSES	91	87	95	95	95	100	100	100	100	100	100	100	100

(b)

CHANGED N=8	SESSION 1	2	3	4	5	6	7	8	9	10	11	12	13
TOTAL NUMBER CORRECT RESPONSES	4	8	12	14	19	20	19	21	21	21	22	24	24
% CORRECT RESPONSES	16	33	50	58	79	83	79	87	87	87	91	100	100

Table 6:15a and 6:15b CxT+VEHICLE

(see Figure 6:9)

(a)

UNCHANGED N=8	SESSION 1	2	3	4	5	6	7	8
TOTAL NUMBER CORRECT RESPONSES	21	22	21	22	22	24	24	24
%CORRECT RESPONSES	87	91	87	91	91	100	100	100

(b)

CHANGED N=8	SESSION 1	2	3	4	5	6	7	8
TOTAL NUMBER CORRECT RESPONSES	5	9	13	18	20	21	23	24
%CORRECT RESPONSES	20	37	54	75	83	87	95	100

Table 6:16a and 6:16b CxT

(see Figure 6:10)

(a)

UNCHANGED N=8	SESSION 1	2	3	4	5
TOTAL NUMBER CORRECT RESPONSES	23	23	23	24	24
%CORRECT RESPONSES	95	95	95	100	100

(b)

CHANGED N=8	SESSION 1	2	3	4	5
TOTAL NUMBER CORRECT RESPONSES	6	14	18	23	23
%CORRECT RESPONSES	25	58	75	95	95

The vehicle controls and the controls proper displayed a similar pattern of learning. Performance was high on the unchanged dimension and increased sharply on the changed dimension, stabilising for both at the same time. By contrast, those animals treated with CORT showed a lengthened presolution period for the changed dimension, combined with which, the rate at which performance on this dimension improved was much slower than in the controls.

Figure 6:7 Percentage of correct responses in CXT+CORT300.

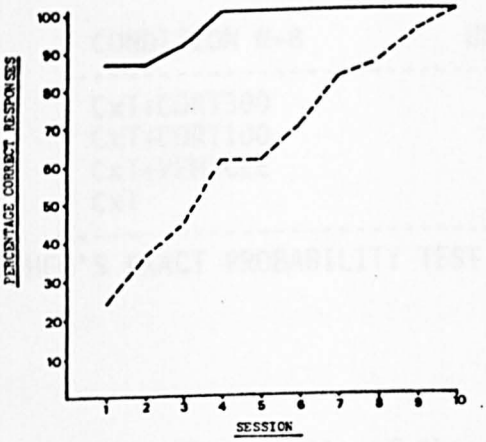


Figure 6:8 Percentage of correct responses in CXT+CORT100.

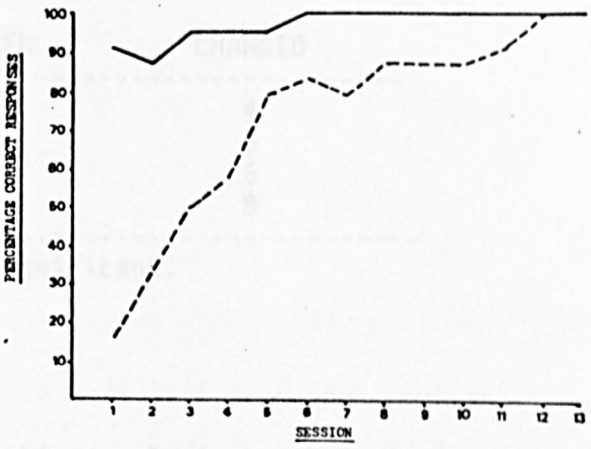


Figure 6:9 Percentage of correct responses in CXT+VEHICLE.

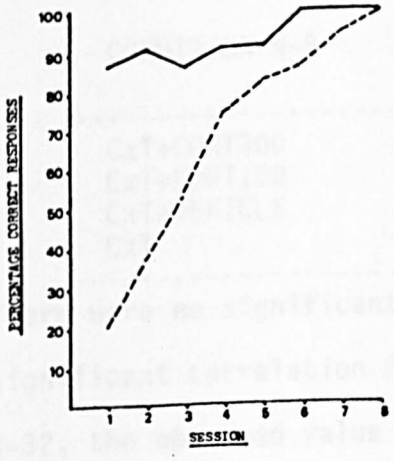


Figure 6:10

Percentage of correct responses in CXT.

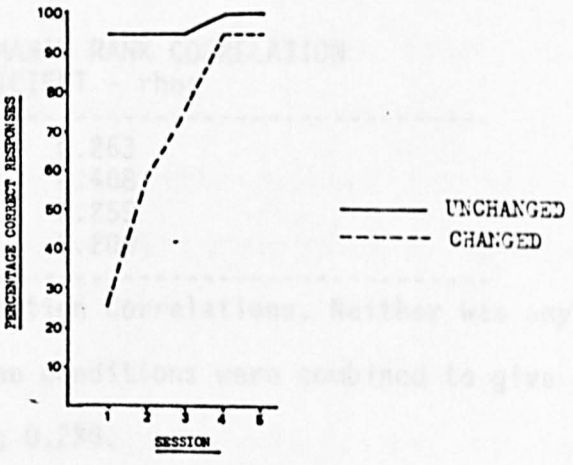


Table 6:17 The effects of corticosterone on the number of animals reaching criterion by session 2 on the unchanged dimension and by session 5 on the changed dimension

CONDITION N=8	UNCHANGED	CHANGED
CxT+CORT300	6	4
CxT+CORT100	7	5
CxT+VEHICLE	8	6
CxT	8	8

FISHER'S EXACT PROBABILITY TEST Not significant.

Table 6:18 The results of the correlation analysis between the levels of corticosterone and the number of trials to criterion on the unchanged dimension - within conditions

CONDITION N=8	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos
CxT+CORT300	0.263
CxT+CORT100	0.408
CxT+VEHICLE	0.259
CxT	0.209

There were no significant within condition correlations. Neither was any significant correlation found when the conditions were combined to give N=32; the observed value of rho being 0.236.

Table 6:19 The results of the correlation analysis between the levels of corticosterone and the number of trials to criterion on the changed dimension - within conditions

CONDITION N=8	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos
CxT+CORT300	0.470
CxT+CORT100	0.321
CxT+VEHICLE	0.411
CxT	0.428

There were no significant within condition correlations. However, when the conditions were combined to give N=32, a significant positive correlation emerged, the observed value of rho being 0.356 ($p<0.05$). This indicates that animals with higher CORT levels required more trials to reach criterion on the changed dimension.

Discussion

As expected there were no significant differences between conditions in the number of trials required to reach criterion on the original brightness discrimination, indicating that the different conditions were balanced with respect to their performance on this discrimination. However, contrary to expectations, CORT did not exert a significant effect, direct or correlational, on any measure of NRS behaviour, despite its presence in markedly different quantities. Nonetheless, those animals treated with the higher dose of CORT were inclined to require a greater number of trials on the NRS, combined with which, they displayed a greater number of initial and total errors. Furthermore, when the NRS was divided into 2 problems, animals with higher levels of CORT required more trials to reach criterion on the changed dimension (though the number of days required to reach criterion on it was unaffected). This is encouraging because it suggests that CORT acts to increase the tendency to respond to the previously

relevant dimension, which, in turn, lends support to the contention that it disrupts the process of attentional inhibition required for efficient non-reversal shifting.

It is important to note that several methodological shortcomings can be recognised, and these may have acted against a more pronounced CORT effect emerging in this experiment. The first relates to the small number of subjects in each treatment condition. In part, this arose because many animals failed to reach criterion on the original brightness discrimination, and had therefore to be excluded. Smart et al. (1977) encountered a similar problem with rats trained on a visual discrimination in a water T-maze, and speculated that visual discriminations may be intrinsically difficult in a water environment. Howard and Granoff (1968), however, did not report any difficulties in training mice to learn a brightness discrimination in a water maze.

Additionally, since the number of trials per day was limited to 6 by the nature of the task, and since in the sub-problem analysis these were divided equally between the changed and unchanged problems, this restricts the criterion connected with the subproblems to a relatively low level of responding. This, too, may have masked any differences. Before turning aside from the criterion implemented for the subproblem analysis, it should be noted that it was less stringent than usual because performance on the changed dimension rarely reached perfection. Admittedly, this was far from ideal, as the animals were, therefore, displaying a relatively low level of performance, and yet were considered to have reached criterion which usually implies a high level of performance. The problem this introduces would have been averted if training had continued until a 100% performance level had been reached, though this would have necessitated a considerably extended period of training. An alternative solution would have been to increase the number of trials per session, though due to the practical consideration of physical exhaustion in the mice, this was not possible.

Another reason for the failure to detect a stronger CORT effect relates to its levels in the plasma. Although the controls possessed CORT in levels which fell in the non-stressed range, they were still quite high. Accordingly, only a limited receptor capacity would be available to any elevations of CORT, and this may not have been sufficient for a CORT effect to be expressed at the receptor level.

General discussion

Transient elevations in the levels of CORT are hypothesised to suppress the HPC in the inhibition of attention; thereby enhancing attentional persistence. Corticosterone's effects should, therefore, resemble those associated with hippocampectomy.

It was encouraging to find that CORT, like hippocampectomy did not affect the acquisition of a discrimination response, and that its effects were restricted to the reversal of the response. Whilst the hypothesised role of CORT in attentional inhibition was not conclusively proved, some supporting evidence was found. Firstly, both reversal and non-reversal shifting tended to be inferior in CORT-treated animals, relative to their controls. The value of comparing the performance on a RS and a NRS has already been extoled, and in this light, it can be concluded that CORT is likely to be involved in the persistence of attention rather than its selectivity. This is corroborated by the nature of the errors displayed in both tasks by CORT-treated animals. Furthermore, the fact that CORT exerted an effect when administered during reversal, but not at an earlier stage of training reinforced this conclusion.

In view of the consistency of this trend, the failure to find statistically significant differences may simply indicate that CORT's effects upon the persistence of attention are weak; alternatively there may have been factors present masking its true effect. A number of such factors have already been identified. The most likely of which was the use of adrenally intact animals. Indeed, as the circulating levels of CORT were relatively high in controls, and still higher in the vehicle controls, the number of receptors available to interact with any further elevations of this adrenocorticoid was inevitably very limited. Thus, the presence of endogenous CORT in high quantities may have obscured a CORT effect.

Despite this, there was a trend within the data. As this emerged with high levels of CORT it poses a problem, as it is not easy to reconcile it

with the properties of CORT's principal receptor system in the HPC. Similarly, the earlier reports of CORT inducing changes in discrimination behaviour upon administration to intact animals (e.g., Bohus, 1971; Beckwith et al., 1983) are also incompatible with the limitations of this receptor system, and therefore difficult to explain. The possibility that CORT's effects on discrimination behaviour are primarily mediated by the GR system provides a partial solution to this discrepancy, as this receptor system has a much greater capacity to respond to CORT than the CR system (Reul and de Kloet, 1986). Recently, de Ronde et al. (1986) presented evidence attesting to the probable significance of the GR system in the effects of CORT on discrimination behaviour. Even so, as this receptor system has its own limitations, it is still difficult to envisage the mechanism(s) underlying the behavioural effects of the very high levels of CORT that were present in these experiments. Whilst the involvement of the GR system is accepted as the most plausible explanation of the observed tendency of animals possessing high levels of CORT to display impaired discrimination shifting, it is possible that a more pronounced effect would have emerged if CORT had fallen within more reasonable levels. In order to overcome the problems arising through the presence of high levels of CORT, and to reach any conclusions on its role in attentional inhibition, an experimental design more compatible with the known properties of its receptor mechanisms would have to be implemented. Unfortunately, time did not permit this for the purposes of this thesis.

An additional, though admittedly more speculative reason for the failure to find a stronger effect of CORT relates to the possibility that it emerges when the levels of attention deviate from normal. This was suggested by the evidence that ACTH4-10 exerts a greater effect on the reversal of a discrimination response in attentionally deficient rats (Sandman et al., 1971). Similarly, Sandman (1985) showed that hormone therapy with MSH/ACTH produced a relatively greater improvement in the

cognitive and social functioning of mentally retarded adults, whereas those individuals with normal or superior attentional capabilities tended to be unaffected. Thus, in a similar but converse way, it is possible that CORT only acts on attention under conditions of hyper attentiveness in order to reduce attentional inhibition. This notion is consistent with the homeostatic function attributed to CORT by Munck et al. (1984).

Given these factors, and the other factors recognised to introduce problems in elucidating the psychological and behavioural effects of CORT, such as the influence of ACTH, not to mention the presence of receptors for CORT outside the HPC whose behavioural effects may differ from those mediated by the HPC, the effects of CORT on a RS and a NRS are encouraging for the working hypothesis.

CHAPTER 7

The effects of corticosterone on conditioned avoidance responding

Introduction

Performance in passive avoidance and two-way active avoidance problems is claimed to reflect the process of attentional inhibition (Douglas, 1967). This, therefore, forms the subject of this chapter.

The most commonly used two-way active avoidance task was first described by Warner (1932) and involves a 'shuttlebox', which comprises two compartments divided by a barrier, with an electrified floor. When the electric shock is applied, the animal is required to cross the barrier in order to terminate the shock. An avoidance response consists of crossing the barrier in response to a conditioned stimulus signalling that shock is imminent. As trials in the shuttlebox are repeated, the animal is required to return to the compartment in which it has just been shocked in the preceding trial.

In a passive avoidance task, the animal is usually trained to make a certain response. Once this response is established, the animal is shocked when it makes the response, which causes it to cease making this response for a time (passive avoidance), after which it usually resumes the reponse. The latency to resume responding is indicative of the strength of the passive avoidance tendency.

An appreciation of the relevance of conditioned avoidance tasks to the study of the inhibition of attention stemmed from the effects of the HPC upon them. Accordingly, the effects of hippocampectomy on conditioned avoidance responding will be presented, followed by an examination of their significance for the role that the HPC is believed to play in the inhibition of attention, and therefore their usefulness in assessing attentional inhibition.

In general, hippocampectomy enhances the acquisition of a two-way AAR

(Olton and Isaacson, 1968; Antleman and Brown, 1972), and attenuates the acquisition and retention of a PAR (Isaacson and Wicklegren, 1962; Kimble, 1963; Winocur, 1980) - though this generalisation is not without exceptions (Nadel et al., 1975). If the HPC is assumed to be the site of attentional inhibition, then a reduction in the capacity to inhibit attention from an established point of fixation should offer a satisfactory explanation for these effects of hippocampectomy. However, whilst it readily explains the poorer performance in a passive avoidance situation - as successful performance demands the inhibition of a previously established stimulus-induced response, its ability to explain the superior performance in a two-way active avoidance situation proved a little more elusive.

The reason for this is that this avoidance problem involves a strong conflict component necessitating behavioural flexibility. If the HPC is involved in attentional inhibition, hippocampectomy would remove the animal's ability to inhibit attention, with a concomitant reduction in behavioural flexibility. This would be expected to attenuate rather than accentuate performance. Nonetheless, the counterintuitive behaviour of hippocampectomised animals in a two-way active avoidance problem can be explained by their inability to generate attentional inhibition in a normal fashion. The power of the attentional inhibition hypothesis in explaining the somewhat anomalous behaviour of hippocampectomised animals in a two-way AAR was first recognised by Douglas (1967). It will be recalled that Douglas (1967) after reviewing a large number of studies relating hippocampal function and behaviour concluded that the degree of inhibition involved in a task determines the extent to which hippocampectomised animals are behaviourally impoverished relative to controls (see p. 41). Moreover, Douglas (1967) surmised that it is possible to explain the superior two-way active avoidance performance of these animals by recognising that this task incorporates an "intrinsic disruptive inhibitory tendency", which under normal circumstances acts to interfere with

performance. This tendency is presumed to arise from the nature of the task, as an animal is required to return to a compartment in which it has recently been shocked. Presumably inhibition develops towards re-entry and this impairs performance. The fact that control animals frequently freeze during a two-way active avoidance problem substantiates this. However, since the development of inhibition is impaired in hippocampectomised animals, the interference that would normally arise is averted, and as a consequence these animals excel in this task.

Whilst this explanation receives substantial support (e.g., Kimble, 1968; Silveira and Kimble, 1968), others have been put forward, though they are not necessarily incompatible with the above. One of the most plausible of these is based on the contention that spatial cues tend to be important in conditioned avoidance problems, yet hippocampectomised animals are deficient in their ability to associate stimuli with specific spatial locations (Olton and Isaacson, 1968; Isaacson, 1974). Thus, according to this theory controls associate both sides of the chamber with shock, and therefore have conflicting hypotheses regarding the location of a 'safe-place', which retards the acquisition of a two-way AAR. By contrast, hippocampally lesioned animals, because they are less likely to learn to avoid a place where they have been shocked, do not experience conflict in returning to such an area, which facilitates the acquisition of this response. The finding that hippocampectomy reduces spontaneous alternation - which represents a tendency to avoid previously visited places and not a reduced tendency to alternate per se (Douglas, 1966) - has been taken to support an impairment of the use of spatial cues advocated by this explanation (Roberts et al., 1962; Douglas and Isaacson, 1964).

It is also possible that hippocampally lesioned animals adopt different hypotheses from their controls. If they are less able to use spatial information, they may, for example, learn to run at the sound of a warning signal, rather than to run to a specific place (Isaacson, 1974). In

connection with this, as hippocampally intact animals tend to learn a two-way active avoidance problem as if it comprises two separate problems, unlike their lesioned counterparts which treat the task as a single problem, this supports the possibility that hippocampectomised animals may learn the problem in a different manner (Olton and Isaacson, 1968).

Lastly, Altman et al. (1973), in the true tradition of response-inhibition theories, suggest that the facilitated acquisition of a two-way AAR may simply represent an increased tendency to repeat a previous response.

Having established the value to be derived from assessing performance on a two-way active avoidance and a passive avoidance task, the potential usefulness of these tasks in elucidating the role of CORT in the process of attentional inhibition can be more clearly appreciated. There already exists a substantial amount of literature on the effects of CORT on these two types of conditioned avoidance behaviour, however none of the experiments were conducted with the objective of assessing the effects of this adrenocorticoid on attentional processes. Neither have the effects of CORT on a PAR and a two-way AAR been discussed in relation to one another.

The existing literature will now be reviewed. It should be noted that various glucocorticoids have been used, in addition to CORT, and it will become apparent that this complicates matters. Additionally, as it is often argued that those avoidance effects that are attributed to CORT are actually due to changes in ACTH, where possible the independent effects of CORT will be delineated, so that its significance in conditioned avoidance behaviour can be more accurately defined. A final point to bear in mind is that whilst the effects of CORT on the extinction of a CAR are well established, its effects on the acquisition of these reponses are not. Moreover, where they exist they tend to be less pronounced than those exerted upon the maintenance of an avoidance response. In spite of this, CORT alters the acquisition of a PAR. However, it has been reported to both

enhance (intact rats - Endroczi, 1972; adrenalectomised rats - Endroczi and Nyakas, 1972) and attenuate acquisition. In support of an attenuation of a PAR several studies have demonstrated that the administration of glucocorticoids to intact animals during pretraining shortens passive avoidance latencies immediately after training (acquisition) as well as 24 hours thereafter (retention). For instance, either DEXA or cortisone administered before the learning session in an aversive-aversive or an approach-aversive situation suppressed both immediate and delayed passive avoidance (Bohus et al., 1970; Bohus, 1971). Similarly, water-deprived rats treated with CORT or cortisol resumed drinking in a chamber where they had recently been shocked more readily than controls (Bohus, 1973; 1975). Interestingly, CORT, unlike cortisol, only attenuated passive avoidance responding 24 hours after the learning trial, whilst the immediate avoidance latency was only slightly shorter. Cortisol, however, induced a passive avoidance deficit at both time points. This dissociation serves to highlight the point that different glucocorticoids may exert differential effects upon avoidance behaviour.

The finding that adrenalectomised animals display longer response latencies than their intact controls, whereas CORT replacement shortened these latencies, has been taken as additional support for the suppressive role of CORT in passive avoidance behaviour (Weiss et al., 1969; 1970). Studies involving intracerebral implantation of CORT have also shown an impairment of the acquisition and the retention of a PAR, though the nature of the effect depends on the specific site of implantation. Implants in the HPC or septum had a suppressive effect, whereas implants in the frontal or orbital cortex were ineffective (Bohus, 1968; 1970; 1973).

Initially it was difficult to account for the discrepancies in the literature, though recognition of the importance of variables such as the intensity of shock and the dose of CORT resolved this. Bohus (1973) found that the degree of which CORT modifies avoidance behaviour is reduced under

high shock intensities relative to moderate intensities; and high doses impaired avoidance responding, while lower doses were ineffective. Further evidence for the dose-dependent nature of CORT's effects was provided by Kovacs et al. (1977) who also found that high doses were associated with inferior passive avoidance, though lower doses actually exerted a facilitatory effect.

Although the effects reviewed above have been attributed to CORT, they may in fact be due to a direct effect of ACTH. Despite the controversy surrounding the effects of this peptide on a PAR, the general consensus holds that it exerts a facilitatory effect (e.g., Levine, 1968). If this is so, the above findings could be attributed to the effects of changes in the levels of ACTH in response to the negative feedback actions of CORT. In this context, Levine and Levin (1970) found that mice displaying superior passive avoidance behaviour tend to be more adrenally active. At first this may suggest that CORT facilitates avoidance behaviour, however as DEXA exerted a deleterious effect, these authors regarded ACTH to be of primary importance. Although the potential involvement of ACTH in passive avoidance behaviour can not be denied, if it is considered that DEXA is a glucocorticoid as well as a potent suppressor of ACTH, the possibility of a direct glucocorticoid effect remains. Despite this, Weiss et al. (1970) claim to have effectively disproved the significance of CORT by their demonstration that hypophysectomy attenuates, whilst ACTH reinstates normal passive avoidance behaviour. It is, however, difficult to conclusively ascribe this effect to lowered levels of ACTH as studies based on hypophysectomy are confounded by the multiple deficiencies associated with this surgical procedure. The fact that substitution therapy with either thyroxine, CORT, and T, or growth hormone, normalises behaviour points to the importance of concomitant changes in sensory, motor, and/or metabolic functions. Obviously, it is difficult to reach any conclusions on the specific involvement of ACTH in this particular avoidance paradigm.

Whatever the nature of its effects eventually turns out to be, this does not necessarily invalidate the independent involvement of CORT.

In support of this, the magnitude of CORT's effect on passive avoidance responding does not correlate with the suppression of ACTH release, though admittedly there is a tendency for the attenuation of this response to be positively correlated with CORT's suppressive potency on ACTH (Bohus, 1973). Moreover, although the HPC is implicated in the neuroendocrinological regulation of the H-PAS (Casady and Taylor, 1976), CORT implants in this area of the brain do not greatly influence the release of ACTH from the pituitary, yet the behavioural effects that emerge upon systemic administration are still mimicked (Bohus, 1968; 1973). Also, CORT has been shown to be behaviourally active in the absence of the pituitary. Drawing these lines of evidence together, the majority of the behavioural effects of CORT would appear to be due to a direct interaction with the brain, rather than the inhibition of pituitary ACTH release (Bohus et al., 1982).

Recently, the effects of CORT on passive avoidance retention have been complicated even further: Borrell et al. (1983; 1984) showed that whereas short-term adrenalectomy produced an avoidance deficit, which was not normalised by CORT, this did not occur with long-term adrenalectomy. Moreover, in this instance CORT impaired passive avoidance retention. As the removal of the adrenal medulla also caused a retention deficit, which was restored by NA or A, the deficit was attributed to adrenomedullary catecholamines, and not CORT. Moreover, the absence of a retention deficit associated with long-term adrenalectomy was attributed to ACTH compensating for the absence of these catecholamines. Following adrenalectomy the levels of ACTH increase progressively, thereby explaining the differences between short and long term adrenalectomy. Furthermore, the impairment of passive avoidance responding upon the administration of CORT to animals in which the adrenals had been absent for a relatively long period was ascribed to a

reduction in the sensitivity to NA and A as a result of CORT. This was confirmed by the finding that, following pretreatment of adrenalectomised animals with CORT, a higher dose of catecholamines was required to reinstate normal retention of a PAR. That the influence of CORT on passive avoidance behaviour depends on its interaction with the NA-system is supported by the demonstration that the impairment of avoidance behaviour was greatest when adrenalectomy was combined with lesions to the dorsal noradrenergic bundle (Ogren and Fuxe, 1977). However, whether it is correct to make inferences about peripheral catecholamines on the basis of central systems in this way is questionable.

Obviously, the effects of CORT on passive-avoidance behaviour are complex, and appear to depend on the interaction that this adrenocorticoid has with a variety of factors, both peripheral and central. Nonetheless, there is evidence which convincingly points to CORT possessing intrinsic behavioural activity in this particular avoidance situation, and where present, it appears to be deleterious in nature.

As with passive avoidance, it is generally contended that the effects of CORT on the acquisition of a two-way AAR are minimal, rather it is more strongly involved in the maintenance of this response. In point of fact, it is well established that adrenalectomy increases the resistance to extinction, whilst CORT has the opposite effect. Interestingly, these effects are found even in the absence of the pituitary (de Wied, 1967). Despite this generalisation, CORT has been found to alter the rate at which a two-way AAR is acquired, though its effects are conflicting.

Although de Wied et al. (1972) demonstrated an enhancement of acquisition when CORT was administered to intact rats once the response had been partially acquired; its administration throughout the acquisition of this response did not affect avoidance behaviour, neither did adrenalectomy, unless very high shock levels were delivered (de Wied, 1977b). This discrepancy is primarily due to the confounding influence of

ACTH, which is claimed to exert a facilitatory effect. De Wied (1964) reported that whereas the acquisition of this avoidance response was unaffected by the removal of the adrenals, it was impaired by hypophysectomy, and ACTH exerted a restorative effect. However, as the response of the hypophysectomised animals was, once again, restored by substitution therapy, the impaired learning may have been due to deficiences in sensory, motor and/or metabolic functions, rather than a depletion of ACTH per se. Despite this, a facilitatory role for ACTH receives support from the evidence that ACTH - related peptides promote the acquisition of a shuttlebox response (Drago et al., 1984). Furthermore, since some of these peptides are devoid of adrenocortical activity, the peptide effect would appear to be independent of CORT. Beatty et al.'s (1970) discovery that both ACTH and adrenalectomy facilitated the acquisition of a two-way AAR provided the most damaging evidence for the possible significance of CORT in this avoidance behaviour, as it seemed to conclusively point to the significance of ACTH and deny CORT. However, as DEXA was found to be without effect this complicated matters. Indeed, this finding not only weakens the probable significance of ACTH, but more importantly, it may actually point to a facilitatory role of CORT. This is because, if ACTH facilitates the acquisition of a two-way AAR, then its depletion - through the actions of DEXA - should have retarded the rate of acquisition. As this was not the case, it is possible that the increased levels of glucocorticoids counteracted the effects of decreased levels of ACTH. Thus, both ACTH and glucocorticoids may facilitate the acquisition of this particular response.

Although the literature relevant to the effects of the PAS hormones on CAR is extensive, it is hoped that this brief review has conveyed the essence of the conflict that surrounds it. The lack of success in finding a consistent CORT effect is probably due to inadequate methodology in an area that requires especially efficient and sensitive techniques. It is well

recognised that variables such as the specific requirements of the task, the level of motivation, the identity of the steroid and its dose, not to mention the intensity of the shock, all influence conditioned avoidance behaviour. The absence of adequate controls may, therefore, provide a satisfactory explanation of the inconsistency within the literature. Despite this, it was encouraging to discover that CORT, like hippocampectomy tended to attenuate passive avoidance but accentuate two-way active avoidance behaviour. This correspondence is, in turn, encouraging for the hypothesis that CORT affects avoidance behaviour through its suppression of the activity of the HPC. The importance of the HPC in the behavioural activity of CORT in conditioned avoidance situations is further attested to by the evidence that intracerebral implants of this adrenocorticoid exert their most pronounced effects on avoidance behaviour if situated in the HPC (Bohus, 1968; 1970; 1973). The fact that animals with higher CORT receptor capacities in the HPC display superior avoidance performance - that is, longer response latencies in a passive situation, and a faster rate of learning in a shuttlebox situation (Angelucci et al., 1980; 1981) - emphasises, even further, the significance of the HPC with respect to the modulation of avoidance behaviour by CORT.

In the light of the evidence presented during this discussion, an examination of the effects of CORT on passive-avoidance and two-way active avoidance behaviour should prove valuable in assessing the validity of the working hypothesis. Accordingly, the effects of CORT on conditioned avoidance responding were determined in adrenalectomised animals with controlled baseline levels (5 µg/ml drinking water) of CORT.

EXPERIMENT 1

The effects of corticosterone on a passive avoidance response

A conflict one trial learning step-through paradigm was used. Corticosterone was manipulated prior to the learning trial and its effects on immediate and delayed passive avoidance behaviour were measured.

Method

Experimental conditions and subjects

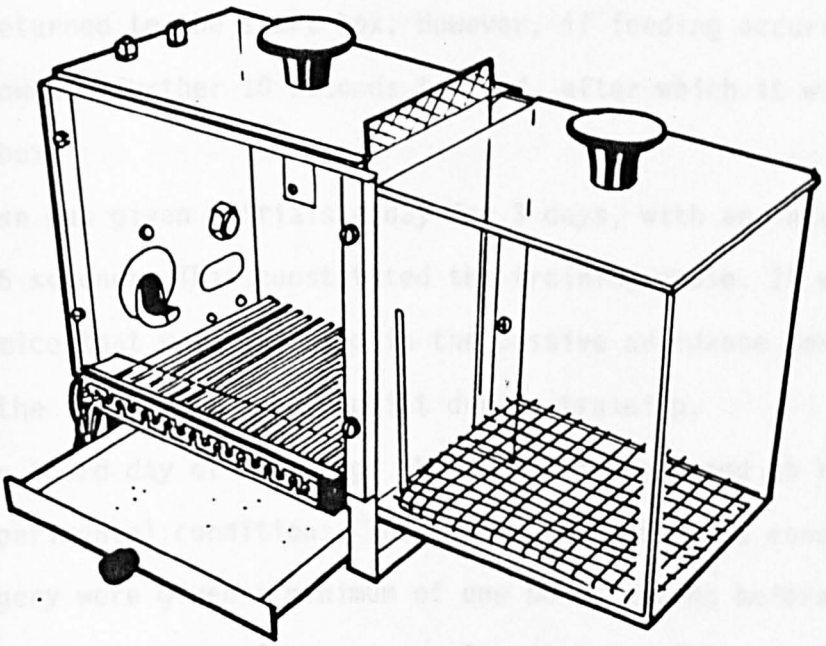
There were 10 animals in each condition:-

1. Intact controls. (INTACT)
2. Intact injected with 0.1ml propylene glycol (INTACT+VEHICLE)
3. Adrenalectomised maintained on the saline solution (1% ethanol in 0.9% $^{W}/_{V}$ NaCl) (ADX-SALINE)
4. Adrenalectomised maintained on the CORT solution (5µ g CORT/ml saline solution) (ADX-CORT)
5. Adrenalectomised maintained on the CORT solution injected with 50 µg CORT in the vehicle (ADX-CORT+50)

All the injections were given subcutaneously, one hour prior to the learning trial.

20 intact and 52 adrenalectomised mice were used, though 12 adrenalectomised mice died and 10 were excluded from avoidance testing because they displayed a marked reluctance to enter the goal box. The mice were individually housed 3 days prior to the start of testing and were maintained on a 12 hour food deprivation regime.

Figure 7:1 Diagram of passive avoidance chamber.



Apparatus (see Figure 7:1)

The apparatus was adapted from a Skinner box (suppliers: Techserv. Inc., Maryland, U.S.A.) and consisted of 2 adjoining chambers: an outer (12x8x12cm) and an inner (13x10x12.5cm). Both chambers had a metal grille base, though only that of the inner was electrified: it was connected to a control box, which regulated the shock that was delivered. A doorway (8x4cm) in the wall common to both chambers linked them, and a sliding door enabled the animal to be enclosed in either chamber. The inner chamber had a food dish projecting through one of its walls.

Procedure

Following a 10 minute period of habituation to the goal box, with food present in the food dish, the mouse was removed to the outer chamber, where it was enclosed for 30 secs. The sliding door was then opened and the mouse was allowed to enter the goal box, where food was available from the food

dish. If the mouse did not enter the goal box within 60 seconds it was removed from the start box, and then replaced in this chamber. Similarly, if the mouse entered the goal box, but did not start feeding within this time it was returned to the start box. However, if feeding occurred, the mouse was allowed a further 10 seconds to feed, after which it was returned to the start box.

Each mouse was given 6 trials a day for 3 days, with an intertrial interval of 15 seconds. This constituted the training phase. It was ensured that all the mice that were included in the passive avoidance test phase had fed from the food dish at some point during training.

After the third day of training, the mice were assigned at random to one of the experimental conditions. Those mice ascribed to a condition involving surgery were given a minimum of one hour feeding before they were anaesthetised in preparation for surgery. Immediately after surgery the mice were placed on either the CORT or the saline solution, depending on the treatment condition to which they belonged.

On the following day, for the first trial, no food was present in the goal box. Each mouse was enclosed in the start box for 30 seconds, the door was then raised and the latency to enter the goal box was recorded. (Any mouse taking longer than 60 seconds was excluded from avoidance testing at this point). Upon entering the goal box the mouse was subjected to unavoidable foot shock for 3 seconds (LEARNING TRIAL). The voltage setting had previously been determined in a pilot study to that which gave an overt distress response in most mice. This corresponded to a setting of 70 arbitrary units. It was increased as necessary if no response was observed. The mouse was returned to the outer chamber 10 seconds after the shock had terminated. Once the intertrial interval had elapsed, the door was opened and the latency to enter the inner chamber was measured (ACQUISITION or IMMEDIATE RETENTION TRIAL). A criterion of 'four paws in' was adopted, and a maximum of 180 seconds was allowed. Food was present in the goal box

during this trial. Immediately after this trial the mouse was blood sampled. The samples were subsequently assayed for CORT.

Passive avoidance latencies were retested 24 hours later, using the same criteria as before (DELAYED RETENTION TRIAL).

As the mice were numbered, they were trained and tested in accordance with their numerical order. Several batches of animals were run. As far as was possible, an equal number of individuals was ascribed to any one condition from a single batch. Where necessary additional mice, to compensate for those lost through surgery, or excluded during the course of behavioural testing, were run at a later date. The apparatus was cleaned with mild disinfectant between animals throughout training and testing. All behavioural testing was carried out between 12.00-4.00pm.

Statistical analysis

As several mice displayed avoidance latencies greater than 180 seconds, the behavioural data was treated non-parametrically. All behavioural measures are presented as medians with 95% confidence limits, and were analysed by the KWANOVA. In addition, the number of animals within each condition that reduced their re-entry latency from the immediate to the delayed retention trial relative to the number that did not show such a reduction, was compared between conditions using Fisher's Exact Probability test.

The CORT data is presented as means ± S.E. and was analysed by the KWANOVA, followed by the MWU test. Spearman's Rank Correlation Coefficient was used to assess the degree of correlation between CORT levels and delayed avoidance latencies within a condition. A similar analysis was carried out combining the data from different conditions, both including and excluding adrenally intact animals.

Results

Table 7:1 The effects of corticosterone on the latency to enter the goal box. (Medians and 95% confidence limits)

CONDITION N=10	LATENCY - SECS LEARNING	IMMEDIATE RETENTION	DELAYED RETENTION
INTACT	10 5 - 28	88 24 - 140	178 6 - 180
INTACT+VEHICLE	8 4 - 40	85 33 - 180	127.5 9 - 180
ADX-SALINE	11 3 - 46	128.5 16 - 180	180 40 - 180
ADX-CORT	27 7 - 52	180 31 - 180	180 16 - 180
ADX-CORT+50	28.5 7 - 51	164 112 - 180	70 8 - 180
KWANOVA: H	7.9	11.3	3.8

(see Figure 7:2)

There were no significant differences detected.

Figure 7:2 The effects of corticosterone on the latency to enter the goal box (medians).

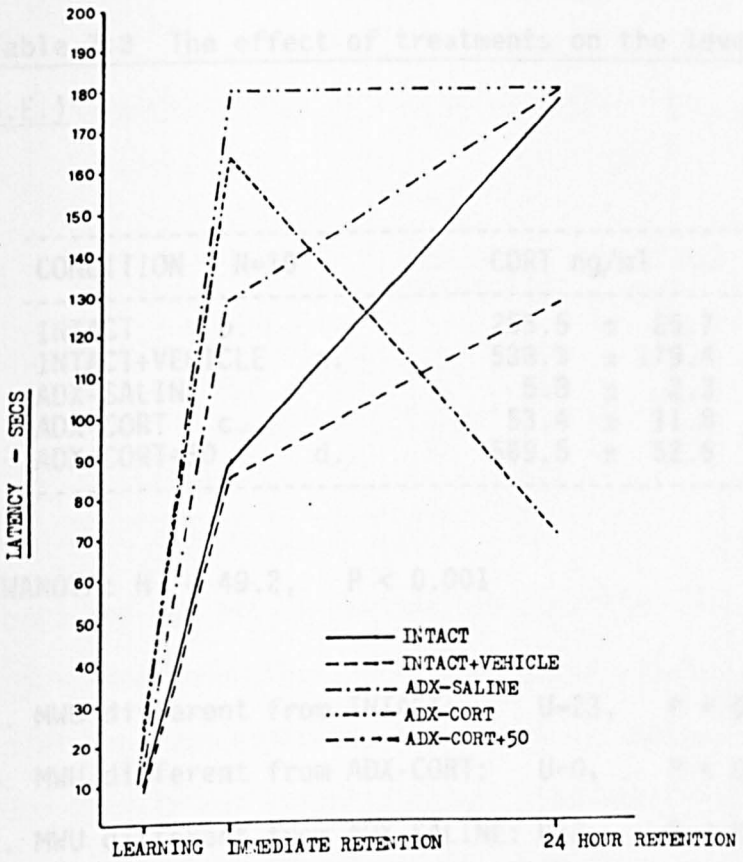


Table 7:2 The effects of corticosterone on the reduction of the avoidance latency from the immediate to the delayed retention test

CONDITION N=10	NUMBER ANIMALS REDUCING LATENCY	NUMBER ANIMALS NOT REDUCING LATENCY
INTACT	4	6
INTACT+VEHICLE	5	5
ADX-SALINE	2	8
ADX-CORT	3	7
ADX-CORT+50 *	8	2

* FISHER'S EXACT PROBABILITY TEST - different from ADX-CORT, $P < 0.03$

The tendency to reduce the avoidance latency from the immediate to the delayed retention test was greater in those animals treated with CORT than their controls, but similar in all other conditions.

Table 7:3 The effect of treatments on the level of corticosterone (Means ± S.E.)

CONDITION N=10		CORT ng/ml
INTACT	b.	253.5 ± 25.7
INTACT+VEHICLE	a.	538.3 ± 179.4
ADX-SALINE		5.8 ± 2.3
ADX-CORT	c.	53.4 ± 11.8
ADX-CORT+50	d.	569.5 ± 52.6

KWANOVA: $H = 49.2$, $P < 0.001$

a. MWU different from INTACT: U=23, $P < 0.05$

b. MWU different from ADX-CORT: U=0, $P < 0.001$

c. MWU different from ADX-SALINE: U=2, $P < 0.001$

d. MWU different from ADX-CORT: U=0, $P < 0.001$

All the conditions possessed significantly different levels of CORT from one another, except INTACT+VEHICLE and ADX-CORT+50.

Table 7:4 The results of the correlation analysis between corticosterone and re-entry latency at the delayed retention test - within conditions

CONDITION N=10	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos
INTACT	0.248
INTACT+VEHICLE	-0.661
ADX-SALINE	-1.360
ADX-CORT	0.209
ADX-CORT+50	0.276

There were no significant within condition correlations. Also, no significant correlations were detected when the data from the various conditions was combined, regardless of whether the analysis included adrenally intact animals, or not. The observed value of rho being -0.217 (N=50) and -0.28 (N=30) respectively.

Discussion

According to the rationale of Anlezark et al. (1973), the fact that there were no significant differences in the latency to enter the goal box on the learning trial indicates that CORT did not affect motor capabilities. Although no direct effects of CORT were found on the re-entry latency at either the immediate or the delayed retention tests, as animals treated with 50 µg CORT displayed shorter latencies at the delayed as compared with the immediate retention test, whereas the reverse was true for all other animals, this suggests that CORT increases persistence. The reason for this is that by implication, attention is still controlled by the previously relevant stimulus, so that the tendency to respond to this stimulus is increased. The result of which is an inability to modify behaviour with respect to the recent aversive stimulus, thereby impairing passive avoidance conditioning.

It is, however, possible to put forward an alternative explanation of

this finding, invoking memory rather than attentional processes. For instance, CORT-treated animals may have been less reluctant to re-enter a chamber where they recently received a shock because CORT impairs the consolidation of the memory for the aversive experience. In support of this, immediate retention is believed to provide a clearer reflection of attentional processes than delayed retention: the latter being clouded by possible memory interpretations. Accordingly, if CORT is involved in attentional processes, it should have exerted its most marked effects at immediate retention and the fact that it only affected performance at delayed, relative to immediate retention casts doubts on a pure attentional interpretation. Obviously, it is difficult to identify the true psychological substrate of CORT on the basis of a single experiment. Moreover, there are a number of reasons why CORT may have failed to affect immediate retention, some of which are outlined below. On this basis, the possibility remains that it is involved in attentional processes.

The short delay between shock and immediate retention testing (25 seconds) may have introduced a number of confounding factors. The most obvious of which is the possibility that the surgical procedure that the animals were subjected to caused sufficient discomfort or debilitation in the animal's physical state to lower their incentive to enter the goal box, even though their attention may still have been focussed on its previously positive associations. The fact that these animals tended to take longer to enter the goal box on the learning trial supports this. The resumption of behavioural testing shortly after surgery had not been anticipated to be problematic because experimenters implementing intervals as short as 12 hours had not reported any difficulties (e.g., Micco, 1979). Also, observations on mice 24 hours after adrenal removal had not revealed any signs of physical impoverishment. However, as the mice in this experiment had been maintained on a food-deprivation regime for several days previously, this probably weakened them; an effect which would have been

compounded on the day of surgery, as the anaesthetic was active over several hours, during which feeding was not possible. In an effort to avert any such effect the animals were allowed to feed prior to surgery. In retrospect, it would probably have been better to have returned the animals to ad lib. feeding for several days following surgery before testing them; though this would have introduced its own problems, as performance on the learning trial would probably have declined as a result.

An additional reason for the lack of a CORT effect on the immediate retention of a passive avoidance response relates to the confounding effects that arise in the absence of the adrenal medulla. As adrenalectomy usually involves the removal of the adrenal medulla, as well as the cortex, and catecholamines originating from the medulla are strongly implicated in behavioural arousal, adrenalectomised animals would be expected to be less responsive (Di Giusto et al., 1971). However, two markedly different consequences of this can be envisaged, though which is correct is not known:-

i. The tendency to respond might be lowered, thereby increasing the latency to enter the goal box.

ii. The responsiveness to shock might be lowered, thereby increasing the tendency to re-enter the goal box, and in so doing decreasing the re-entry latency. (Although it is predicted that a lowered level of arousal is most likely to reduce the responsiveness to shock, it is possible that shock delivered to a quiescent animal might actually have a greater effect than in a highly aroused animal. In this case, the absence of adrenomedullary catecholamines might increase the re-entry latency).

A change in the levels of catecholamines upon adrenalectomy introduces a further problem as adrenalin is implicated in the acquisition of a

response. Demedullation leads to a retardation in the rate at which a response is acquired, which inevitably complicates any examination into the effects of CORT on acquisition involving adrenalectomised animals (Di Giusto et al., 1971).

Finally, even though all the animals responded to the shock and the levels of CORT fell within a suitable range for an examination of its effects upon HPC-sensitive behaviour, the failure to detect a significant relationship between CORT and immediate passive avoidance behaviour may have arisen because of the considerable amount of intrinsic variability in mice. This exists not only in their conditioned avoidance behaviour, but also in the influence that CORT has upon it. Furthermore, this is combined with individual differences in shock sensitivity. Indeed, when the numerous sources of variability are taken into consideration the failure to find a stronger effect of CORT is hardly surprising, and the evidence when viewed as a whole is fairly encouraging for the hypothesis that it suppresses passive avoidance behaviour. In spite of this, two criticisms of this experiment can be advanced, both of which potentially undermine the significance of this evidence, and these will now be discussed. The first relates to the possibility that CORT alters the motivation to seek food, and in so doing the degree to which a passive avoidance response in the conflict situation involving appetitive motivation is suppressed. Whilst this criticism is valid, and would serve to question the significance of a CORT effect, experiments summarised in APPENDIX 5 show that food intake is unaffected by this adrenocorticoid.

The second criticism relates to the fact that the appropriate control for a possible state-dependent effect of CORT was neglected. The phenomenon of state-dependency was first recognised by Pappas and Gray (1971), who found that animals trained <u>and</u> tested under the influence of DEXA performed better than those trained in its presence, but tested in its absence (or

vice-versa). In the light of this, as the CORT-treated animals reduced their re-entry latency at the delayed retention test relative to the immediate retention test, and CORT was only present in elevated levels at the latter, it is possible that the shorter avoidance latencies reflect a state-dependent effect. Assessing the effects of administering CORT prior to both the immediate and delayed tests would have clarified this. Notwithstanding the plausibility of this criticism, CORT has never, to date, been demonstrated to exert a state-dependent effect on avoidance behaviour.

Finally, a number of problems arose in connection with the methodology of this experiment. One of the most difficult of these was posed by the considerable individual differences in shock sensitivity that exist (Beatty and Fessler, 1976; Lovely et al., 1972). This was compounded by the fact that as a one trial learning passive avoidance situation involves a single exposure to shock, it is imperative to deliver the appropriate intensity of shock to each animal. In selecting this, it must be borne in mind that the shock must be sufficiently intense for the animal to perceive it as noxious, without being of such a high intensity that all the animals avoid the situation in which they experienced it, regardless of the incentives to return to that situation. Obviously, the latter is undesirable because it would be difficult to obtain a CORT effect on the integration of the conflict experience. Indeed, Joffe (1965) reported that differences in the latency to re-enter a goal box after shock do not occur if the shock is very severe. Similarly, it will be recalled that the effect of CORT on passive avoidance responding is reduced under high intensity stimulation (Bohus, 1973). In appreciation of the importance of selecting the correct level of shock a pilot study was conducted to determine the most appropriate shock regime (intensity and duration). Additionally, as the effects of CORT on shock sensitivity are controversial, a pilot study was also carried out to establish whether different levels of CORT affect this

parameter. These pilot studies revealed that animals with different CORT levels did not differ consistently in their shock sensitivity (non-replaced adrenalectomised mice as compared with their CORT-replaced counterparts). However, individuals within a condition (of the same age, sex and approximately the same weight) displayed considerable variability. Some responded to as little as 20 voltage units, while others only responded at higher intensities, in some cases as high as 130 units. It was, therefore, difficult to select a single shock intensity and deliver this to all animals and still be confident that each had been subjected to a similarly aversive experience. Consequently, it seemed more appropriate to adjust the intensity of shock delivered to each animal. A full discussion of this is deferred until the general discussion.

A further problem concerned the position of the learning trial. It was placed at the start of the testing session because pilot studies had shown that if several food reinforced trials were given prior to this trial, subsequent avoidance of the goal box was negligible, even though the animals were observed to be distressed by the shock during it. This suggested that a positive experience in the goal box immediately prior to a negative one reinforces the positive aspects of the goal box, overcoming the influence of a single subsequent aversive experience. By contrast, if a single learning trial was given, the animals were reticent to enter the goal box.

Finally, although blood samples were taken before behavioural testing was completed, and this is acknowledged to be far from ideal, it was essential in this particular instance, in order to arrive at any meaningful correlations between avoidance behaviour and CORT. Any effects introduced by this would, however, have been present in every condition, reducing the significance of this problem.

EXPERIMENT 2

The effects of corticosterone on a two-way active avoidance response

The effects of CORT on the acquisition of a two-way AAR were examined in a shuttlebox over 10 consecutive days. Corticosterone was manipulated throughout the experiment.

Method

Experimental conditions and subjects

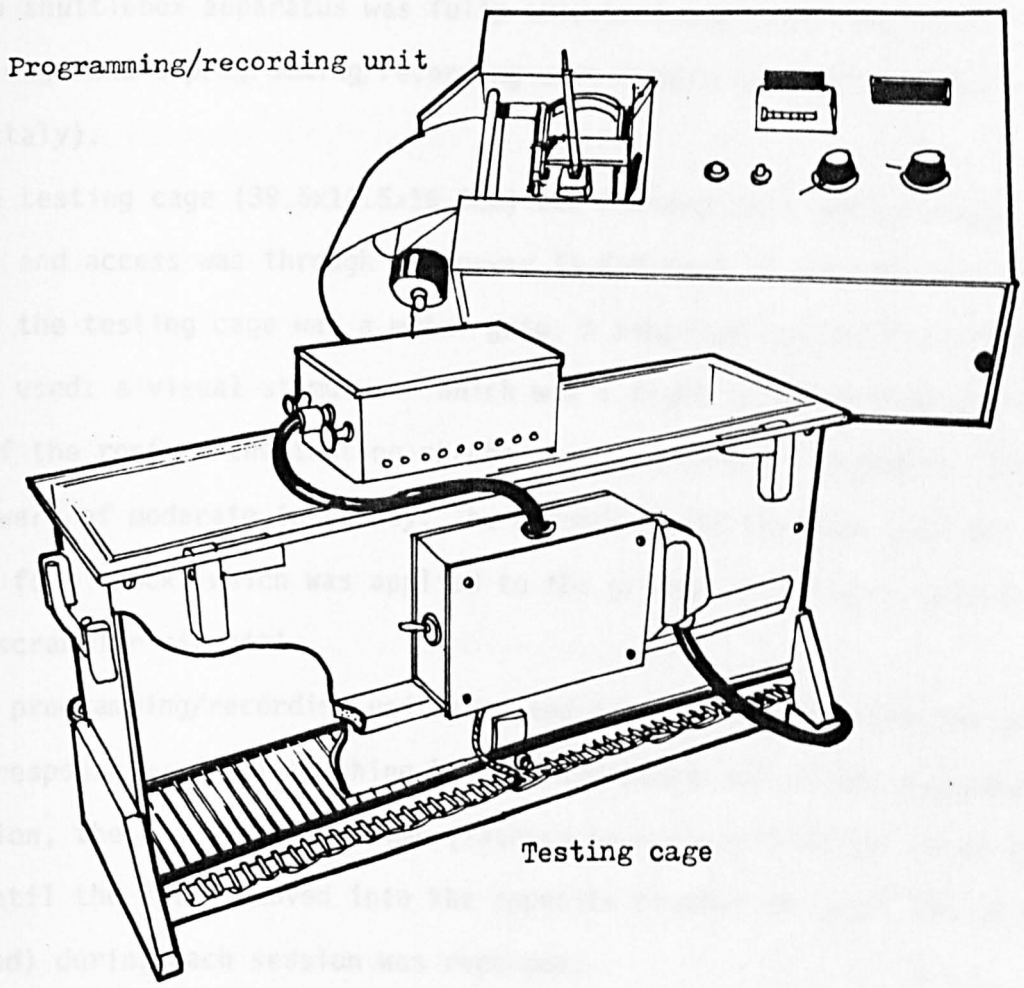
There were 9 animals in each condition:-

1. Intact controls (INTACT)
2. Adrenalectomised maintained on the CORT solution (5µg CORT/ml saline solution - 1% ethanol in 0.9% w/v NaCl) (ADX-CORT)
3. Adrenalectomised maintained on the CORT solution injected with 0.1ml propylene glycol (ADX-CORT+ VEHICLE)
4. Adrenalectomised maintained on the CORT solution injected with 50µg CORT in the vehicle (ADX-CORT+50)
5. Adrenalectomised maintained on the CORT solution injected with 100µg CORT in the vehicle (ADX-CORT+100)

All the injections were given subcutaneously, one hour prior to shuttlebox training, each day throughout acquisition. The treatments were coded.

9 intact and 36 adrenalectomised mice were used. Adrenalectomy was performed 4 days before the start of avoidance testing, and these mice were maintained on the CORT solution from that point. The mice were not behaviourally naive as they had been exposed to a novel object 24 hours prior to the onset of avoidance testing.

Figure 7:3 Diagram of the active avoidance apparatus.



Apparatus (see Figure 7:3)

The shuttlebox apparatus was fully automatic and consisted of a testing cage and a programming/recording unit (suppliers: Ugo Basile - Milan, Italy).

The testing cage (39.5x10.5x16.0cm) was divided into two by a high barrier, and access was through a doorway (3.0x3.6cm) in this barrier. The floor of the testing cage was a metal grid. A compound conditioned stimulus (CS) was used: a visual stimulus - which was a light (15w) located at the centre of the roof of the testing chamber, and an acoustic stimulus. Both stimuli were of moderate intensity. The unconditioned stimulus (US) was electric foot shock, which was applied to the gridded floor by a special 'static scrambler circuit'.

The programming/recording unit supplied the stimuli and recorded the animals responses - distinguishing between avoidance and escape responses. In addition, the total waiting time (latency to respond from the onset of the CS until the animal moved into the opposite chamber or until the US was terminated) during each session was recorded.

The intertrial interval was fixed at 13 secs, and the CS-US interval was 3 secs. The CS remained on when the US was delivered. The CS or, the CS and the US, were terminated when the animal crossed the barrier, or 4 secs after the initiation of the US, depending on which occurred first.

-Procedure

On the first day of avoidance training, each mouse was allowed to habituate to the shuttlebox for 10 mins before the start of the conditioning session. By using two testing cages, which were connected alternately to the programming/recording unit, it was possible to test one animal in cage A, whilst a second animal was habituating in cage B. On completion of the avoidance session in cage A, a fresh animal was placed in this cage for habituation and cage B was then connected to the

programming/recording unit for testing.

During avoidance conditioning each mouse was initially subjected to a shock intensity of 27V, however where necessary this was increased until an overt distress response had been evoked. This intensity was then used in subsequent avoidance testing for this particular animal, though it was verified daily, and where necessary it was readjusted. Each mouse was given 10 trials a day for 10 days. At the end of the tenth session the mice were blood sampled. The samples were subsequently assayed for CORT.

Three batches of animals were run with 15 animals in each. The adrenalectomised animals were allocated at random to treatments and the intacts were also randomly selected. Animals were tested in accordance with a pre-set sequence, until the batch was exhausted.

The apparatus was cleaned with mild disinfectant between animals. All behavioural testing was carried out between 12.00-4.30pm.

Statistical analysis

The behavioural data is presented as means ±S.E. The data for the waiting time and the number of conditioned avoidance responses, over sessions was analysed by a two way mixed ANOVA (treatment x session), though in the latter instance the data was log transformed (LOG x +1).

The total number of conditioned avoidance responses and the total waiting time, over the 10 sessions was analysed by the KWANOVA. The CORT data is presented as means ±S.E. and was analysed by the KWANOVA, followed by the MWU test.

Spearman's Rank Correlation Coefficient was used to assess the degree of correlation within each condition between CORT and avoidance behaviour, as reflected by the number of avoidance responses and the waiting time, at session 10, as well as the correlation between CORT and the total measures of avoidance behaviour. Similar correlation analyses were computed combining the data from different conditions, both including and excluding

adrenally intact animals.

Results

Table 7:5 The effects of corticosterone on the number of conditioned avoidance responses per session over 10 sessions (Means±S.E.)

CONDITION N=9	NUMBER OF AVOIDANCES SESSION 1	2	3	4	5	6	7	8	9	10
INTACT	0.33 ± 0.23	0.33 ± 0.23	0.78 ± 0.46	1.22 ± 0.62	1.22 ± 0.66	1.55 ± 0.6	3.11 ± 1.16	3.44 ± 1.03	3.33 ± 1.17	2.22 ± 0.89
ADX-CORT	0 ± 0	0.22 ± 0.15	0.22 ± 0.15	0.33 ± 0.17	1.11 ± 0.39	0.33 ± 0.23	0.44 ± 0.24	1.11 ± 0.26	1.33 ± 0.37	0.78 ± 0.28
ADX-CORT+VEHICLE	0.22 ± 0.15	0.44 ± 0.24	0.78 ± 0.52	1.67 ± 0.85	2.1 ± 1.0	1.78 ± 0.91	2.55 ± 1.11	2.89 ± 1.24	1.22 ± 0.57	2.11 ± 0.96
ADX-CORT+50	0.44 ± 0.44	0.55 ± 0.38	0.78 ± 0.46	0.33 ± 0.17	1.0 ± 0.67	1.78 ± 0.76	2.55 ± 0.99	2.89 ± 1.08	1.33 ± 0.62	4.11 ± 1.31
ADX-CORT+100	0.44 ± 0.34	0.22 ± 0.15	1.22 ± 0.4	0.78 ± 0.36	1.11 ± 0.51	1.89 ± 0.79	2.78 ± 0.95	3.0 ± 1.07	2.78 ± 0.78	3.22 ± 1.08

TWO WAY MIXED ANOVA:

TREATMENT $F(4,40)= 1.146$, $p=0.35$

SESSION $F(9,40)=14.007$, $p<0.001$

INTERACTION $F(36,40)=1.021$, $p=0.44$

Corticosterone did not exert a significant effect upon the number of conditioned avoidance responses either as a main or an interaction effect. Avoidances increased significantly over sessions.

Table 7:6 The effects of corticosterone on the total waiting time per session over 10 sessions (Means±S.E.)

CONDITION N=9	WAITING TIME - SECS SESSION 1	2	3	4	5	6	7	8	9	10
INTACT	59.8 ± 5.1	72.1 ± 5.8	68.6 ± 4.4	63.3 ± 4.4	62.1 ± 5.1	67.4 ± 4.4	68.8 ± 7.3	66.2 ± 6.0	62.9 ± 6.6	62.7 ± 6.2
ADX-CORT	58.2 ± 2.7	80.5 ± 4.1	75.1 ± 4.9	69.9 ± 4.0	74.2 ± 5.1	83.8 ± 6.7	87.1 ± 4.2	81.3 ± 5.5	81.6 ± 4.6	73.0 ± 5.1
ADX-CORT+VEHICLE	69.8 ± 4.4	70.1 ± 5.1	72.5 ± 4.2	63.4 ± 4.4	64.2 ± 5.3	80.1 ± 6.1	76.9 ± 7.7	76.7 ± 8.3	78.7 ± 5.5	63.1 ± 6.6
ADX-CORT+50	72.4 ± 3.7	67.4 ± 2.8	74.9 ± 3.8	70.3 ± 3.4	72.0 ± 6.7	77.9 ± 6.2	70.8 ± 6.1	72.1 ± 6.8	75.2 ± 6.3	57.9 ± 6.8
ADX-CORT+100	66.9 ± 3.8	73.0 ± 4.4	69.0 ± 4.0	70.5 ± 3.5	67.3 ± 3.2	71.5 ± 4.3	73.5 ± 6.2	70.6 ± 5.7	65.3 ± 4.1	55.0 ± 4.0

TWO WAY UNRELATED ANOVA:

TREATMENT $F(4,40) = 1.29$, $p=0.29$

SESSION $F(9,40) = 6.35$, $p<0.001$

INTERACTION $F(36,40) = 1.28$, $p=0.14$

Corticosterone did not exert a significant effect upon the total waiting time, either as a main or an interaction effect. Waiting times altered significantly over sessions.

Table 7:7 The effects of corticosterone on the number of conditioned avoidance responses over 10 sessions (Means±S.E.)

CONDITION N=9	NUMBER OF AVOIDANCE RESPONSES
INTACT	17.5±5.6
ADX-CORT	5.9±1.1
ADX-CORT+VEHICLE	15.8±5.7
ADX-CORT+50	15.8±3.8
ADX-CORT+100	17.4±4.4

KWANOVA: H=5.17 Not significant

Table 7:8 The effects of corticosterone on the total waiting time over 10 sessions (Mean±S.E.)

CONDITION N=9	WAITING TIME - SECS
INTACT	653.8±39.8
ADX-CORT	764.7±31.7
ADX-CORT+VEHICLE	715.6±39.5
ADX-CORT+50	710.9±42.3
ADX-CORT+100	682.7±25.5

KWANOVA: H=5.5 Not significant

Table 7:9 The effects of treatments on the plasma levels of corticosterone (Means±S.E.)

CONDITION N=9		CORT ng/ml
INTACT	a	116.1±19.5
ADX-CORT		64.8±14.8
ADX-CORT+VEHICLE		82.6± 9.5
ADX-CORT+50	b	377.8±22.2
ADX-CORT+100	c	510.7±38.6

KWANOVA: H=34.4 , p<0.001

a. MWU. different from ADX-CORT: U=21 , p<0.05

b. MWU. different from ADX-CORT+VEHICLE: U=0 , p<0.001

c. MWU. different from ADX-CORT+50: U=11 , p<0.001

With the exception of the ADX-CORT condition and the vehicle controls, which had similar levels of CORT, all the other conditions had significantly different CORT levels.

Table 7:10 The results of the correlation analysis between corticosterone and avoidance behaviour at session 10 - within conditions

	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos	
CONDITION N=9	NUMBER OF AVOIDANCE RESPONSES	WAITING TIME
INTACT	-0.571	0.404
ADX-CORT	0.146	0.217
ADX-CORT+VEHICLE	0.571	-0.560
ADX-CORT+50	0.183	-0.099
ADX-CORT+100	0.329	-0.083

No significant within condition correlations were detected.

Table 7:11 The results of the correlation analysis between corticosterone and avoidance behaviour combining the data over 10 sessions - within conditions

CONDITION N=9	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos NUMBER OF AVOIDANCE RESPONSES	WAITING TIME
INTACT	-0.592	0.537
ADX-CORT	-0.171	-0.317
ADX-CORT+VEHICLE	0.571	-0.583
ADX-CORT+50	0.517	-0.433
ADX-CORT+100	0.133	0.183

No significant within condition correlations were detected.

Table 7:12 The results of the correlation analysis between corticosterone and avoidance behaviour at session 10 - combined conditions

CONDITION	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos NUMBER OF AVOIDANCE RESPONSES	WAITING TIME
INCLUDING ADRENALLY INTACT ANIMALS N=45	0.343*	-0.308*
EXCLUDING ADRENALLY INTACT ANIMALS N=36	0.459*	-0.392*

*$p<0.05$

Regardless of whether adrenally intact animals were included in the analysis, or not, animals with higher levels of CORT displayed a greater number of conditioned avoidance responses and lower waiting times at session 10.

Table 7:13 The results of the correlation analysis between corticosterone and avoidance behaviour combining the data over 10 sessions - combined conditions

CONDITION	SPEARMAN'S RANK CORRELATION COEFFICIENT - rhos NUMBER OF AVOIDANCE RESPONSES	WAITING TIME
INCLUDING ADRENALLY INTACT ANIMALS N=45	0.295*	-0.241
EXCLUDING ADRENALLY INTACT ANIMALS N=36	0.488*	-0.405*

* $p<0.05$

Animals with higher levels of CORT displayed the greatest number of avoidance responses, irrespective of the presence of the adrenals.

High levels of CORT correlated with lower waiting times, though this was only statistically significant when adrenally intact animals were excluded.

Discussion

At first the acquisition of a two-way AAR appeared to be completely unaffected by CORT. Indeed, despite the fact that the treatment conditions, with the possible exception of that in which 100μg CORT was given to CORT replaced animals, should have afforded an ideal opportunity to assess the extent to which CORT modulates functions subserved by the HPC, neither the number of avoidance responses, nor the waiting time during the acquisition of the avoidance response was altered. However, the correlation analysis revealed that animals with higher levels of CORT displayed a greater number of conditioned avoidances, combined with lower waiting times (both at session 10, and over the 10 sessions), suggesting that this adrenocorticoid facilitates acquisition. Presumably, individual variability within each condition obscured these differences with between condition comparisons.

Although this is encouraging for the hypothesised role of CORT on the HPC, as hippocampectomy also facilitates the acquisition of a two-way AAR (e.g., Olton and Isaacson, 1968), this effect may have been due to ACTH rather than CORT. The involvement of ACTH was suggested by the opposite direction of the correlations in the intacts as compared with their adrenalectomised counterparts, and the fact that these two groups of animals were likely to possess different hormonal states. Thus, in the adrenalectomised animals (combined - see Tables 7:12 and 7:13), high levels of CORT were positively correlated with the number of avoidance responses, and negatively correlated with waiting time, whereas the reverse occurred in adrenally intact animals (see Tables 7:10 and 7:11). Whilst high CORT levels would be expected to coincide with low levels of ACTH in the former, they are more than likely to coincide with high levels of ACTH in the latter. Extrapolating from this, the following relationships would be expected to occur: in adrenalectomised animals - high CORT and low ACTH would be associated with superior avoidance behaviour (i.e., an increased number of avoidance responses and a decreased waiting time); and by

inference low CORT and high ACTH would be associated with inferior avoidance behaviour. By contrast, in intact animals - high CORT and high ACTH would be associated with inferior avoidance behaviour, whilst it would be superior under conditions of low CORT and low ACTH. From this it would seem that the correct interpretation of the correlational evidence is that low levels of ACTH, rather than high levels of CORT, facilitate the acquisition of a two-way AAR. If so, this contrasts with the evidence that high levels of this peptide exert a facilitatory effect (de Wied, 1964; Drago et al., 1984). It is, however, not possible to accurately predict the circulating levels of ACTH at any instant. Furthermore, if the correct interpretation of this data is in terms of ACTH, the effect should have shown up in the analysis excluding the intacts, yet this was not so. Moreover, as the direction of the correlations did reverse in some conditions (see Table 7:11), any conclusions relating to the roles of ACTH and CORT must be reserved until firmer evidence is available. Nonetheless, the possibility remains that the opposite direction of these correlations was due to the presence of adrenomedullary catecholamines in the intacts. Demedullation alters the rate at which a response is acquired, and A exerts a restorative effect (Di Giusto et al., 1971). However, as intacts did not acquire the shuttlebox response at a different rate from those animals lacking the adrenal medulla, the significance of the catecholamines originating from this source is unclear. If they enhance the acquisition of a response, the finding that low levels of CORT correlate with superior avoidance behaviour in intacts is difficult to reconcile with the fact that CORT is responsible for the conversion of NA to A: rather lowered CORT levels would be expected to be associated with low levels of A, with obvious behavioural implications (Di Giusto et al., 1971).

Although it is difficult to reach any firm conclusions regarding the effects of CORT on the acquisition of a two-way AAR, this may have been compounded by a number of factors within this experiment which were likely

to reduce the prospect of a CORT effect emerging. For instance: the 10 day period of avoidance conditioning may have been too short. Initially this was considered to be sufficient, even though mice were known to experience great difficulty acquiring a two-way AAR. This is because CORT was hypothesised to exert a facilitatory effect and it was reasoned that such an effect would emerge even when the animals had not reached a high level of avoidance responding.

Although it can not be denied that a two-way active avoidance problem is intrinsically difficult to acquire - a fact attested to by the low level of responding even at the end of the tenth training session - this may have been unnecessarily accentuated in this experiment by the avoidance apparatus. The major drawback of this apparatus stemmed from the fact that it was fully automatic, and the CS and US were only terminated when the full weight of the animal was in the chamber designated 'safe' for that particular trial. As mice tended to cross from one chamber to the other in a rather hesitant manner, this resulted in them continuing to receive shock as they entered the 'safe' chamber, until they had fully entered it. The distinction between the two chambers was thereby reduced, which inevitably hindered acquisition. It is possible that this also accounts for the observation that some animals tended to remain immobile, usually huddled in a corner, when shock was delivered. Presumably, they were unable to distinguish between the chambers, and regarding the shock as inescapable, no longer attempted to escape from it. Similar findings have been reported by Seligman (1975*).

One final point relates to the shock intensity. Preliminary studies screening animals for their sensitivity to shock confirmed that whilst there were no consistent differences between animals possessing markedly different levels of CORT, there was considerable inter-individual variability. Consequently, it was difficult to adopt one level of shock for all animals. Instead it was adjusted in accordance with each animal's

sensitivity: a fuller discussion of this point is deferred until the general discussion. Daily verification of the suitability of the intensity of shock delivered to each animal was necessary in order to ensure that they continued to respond to this level of shock throughout the period of conditioning. This takes into consideration any possible day to day variations, as well as the possibility of a change in shock sensitivity as a result of training.

General Discussion

The experiments reported here attempt to determine the effects of CORT on passive and two-way active avoidance responding, with the objective of assessing the degree of correspondence between its effects and those of hippocampectomy, and from this to extrapolate to its possible significance in attentional inhibition. It is well established that destruction of the HPC results in inferior passive avoidance (e.g., Kimble, 1963), but superior active avoidance responding (e.g., Olton and Isaacson, 1968), and that this has been attributed to an impaired capability to inhibit attention (e.g., Douglas, 1967). In the experiments described in this chapter, CORT also tended to impair a PAR and to facilitate a two-way AAR. As the results are as predicted, the working hypothesis is not falsified, and this is encouraging for the role that CORT is believed to play in attentional inhibition. Even so, other interpretations (e.g., ACTH, adrenomedullary hormones) are inevitably possible.

The fact that these results were not more clearly defined was a little disappointing. The presence of intrinsic inter-individual variability at several different levels may have obscured CORT's effects. Levine (1968) recognised 'good' and 'bad' avoiders, and these behavioural differences were subsequently correlated with differences in the capacity of the HPC to bind CORT (Angelucci et al., 1980; 1981). Thus, not only does avoidance behaviour vary between individuals, but CORT's capacity to influence this behaviour is also variable! It is possible, therefore, that the existence of two definite subpopulations of animals introduced a serious confounding variable into the experiments reported in this chapter, and CORT's effects might have been more apparent if the examination had been confined to one or the other of these populations. In connection with this, the fact that it proved so difficult to uncover within condition correlations between plasma CORT levels and avoidance responding was probably due to the variability in the capacity of the brain to interact with CORT, as this is

relatively independent of its levels in the peripheral circulation.

The problem of inter-individual variability was compounded even further by the tendency of different mice to respond differently to a standard level of shock. As a consequence of this, it was difficult to determine the most appropriate level of shock for each animal. Yet this is very important as the modulation of shock motivated behaviour by CORT depends on its interaction with the degree to which shock motivates an animal to respond (Bohus, 1973). In order to ensure that each animal was exposed to an appropriate intensity of shock, the shock was adjusted from one individual to the next in both the passive and the active avoidance tasks. All the animals were initially subjected to an intensity that had been established to evoke an overt distress response (the 'jump-flinch threshold' - Leshner, 1978), from the majority of animals. Whilst this was suitable for most mice, occasionally it had to be adjusted in accordance with the sensitivity of the particular mouse. The underlying assumption was that animals displaying a similar distress response have experienced a similarly aversive stimulus. Most studies concerned with shock-motivated behaviour deliver a constant voltage or constant current. However, current density is the physical factor which determines the discomfort of the stimulus, together with the region of the animal's body being stimulated. The presence of urine or faeces on the electrified grid results in variable electrical resistance, so that constant current or voltage devices are rendered ineffective. In this light, it seemed most appropriate to adjust the shock intensity from one individual to the next until the behavioural threshold had been reached. Indeed, Sprott (1975) claims that this "removes one of the major contaminants of avoidance research".

An additional problem connected with shock intensity stems from the possibility that CORT influences shock responsiveness. Although any such effect would have widespread significance for avoidance behaviour involving shock motivation, this area of research is highly contentious.

Adrenalectomy has been claimed to both increase shock sensitivity (Pare and Cullen, 1971), and to decrease it (Gibbs et al., 1973). Acknowledging this conflict, Leshner (1978) concludes that whilst it is possible that the hormones from the PAS affect avoidance behaviour because they alter the sensitivity to shock, these sensory effects are not very dramatic and can not account for the marked effects of CORT that have been reported. Rather, CORT modulates avoidance behaviour through its direct interaction with the brain. This is supported by my own pilot investigations: shock sensitivity of non-replaced adrenalectomised animals as compared with their CORT-replaced counterparts did not differ consistently. Thus, although the possibility remains that CORT affects shock sensitivity, this is regarded with some scepticism, at least for the present.

Given these factors, and the other factors that are recognised to pose problems in any investigation into the significance of CORT, such as the influence of ACTH, and in the case of studies involving adrenalectomised animals - the complications that arise in the absence of the adrenomedullary catecholamines, not to mention the intrinsic difficulty in demonstrating an effect upon conditioned avoidance behaviour by virtue of the special requirement for sensitive techniques, the fact that CORT influenced both a PAR and a two-way AAR lends support to the working hypothesis. Despite this, it is possible to argue that the evidence did not reflect a true change in attentional inhibition, but a change in the level of general activity, as an increase in this would bring about similar changes in conditioned avoidance behaviour. However, as the appropriate controls were overlooked, it is impossible to resolve this. Nonetheless, whilst it is admitted that the effects of CORT on general activity are controversial (see Chapter 1), in those avoidance studies that have bothered to incorporate the relevant activity controls, its influence was negligible (Weiss et al., 1970; Beatty et al., 1970).

In conclusion; even though the failure to run the appropriate controls

may interfere with the interpretation of the effects of CORT on conditioned avoidance behaviour, it seemed unlikely that alterations in the level of activity were responsible for the differences in avoidance behaviour, rather CORT was more likely to have affected the inhibition of attention.

CHAPTER 8

Summary and general discussion

The capacity to respond to stress is regarded as one of the most basic adaptive mechanisms possessed by animals. In rodents, the secretion of CORT is a central feature of this response. However, the specific purpose of stress-induced elevations of this adrenocorticoid has been a fervently debated subject. Initially, they were believed to be exclusively involved in physiological adaptation at the periphery. Despite this, Yates and Maran (1974) claim that the relatively rapid rise of CORT under stressful conditions (Davidson et al., 1968) cannot be adequately explained in terms of its known peripheral actions. This is because there is typically a long delay (2-4 hours) between an increase in the levels of CORT and the response in the peripheral tissues, yet the elevation in CORT's levels under conditions of stress is very fast (2 minutes). Thus stress-induced elevations of CORT occur faster than most peripheral tissues appear to respond to them. The finding that psychological stimuli are the most potent in effecting the adrenocortical stress response (Mason, 1971) has been argued to point to CORT's probable importance in psychological adaptation (Smith, 1973).

In spite of the appeal of this line of argument, the response of the PAS to psychological stimuli does not necessarily imply that CORT is involved in psychological adaptation. The presence of receptors for CORT in the brain is more convincing. However, as discussed in Chapter 1, the brain may simply be a site for the negative feedback effects of CORT. Receptors in the brain for this adrenocorticoid may, therefore, influence ACTH and CRF release rates, rather than the psychological state of an animal. Even though it can not be denied that the brain does act in this way (e.g., Wilson, 1985), the evidence that CORT exerts behavioural effects is impressive (see review in Chapter 1). Also, the demonstration that implants

of CORT directly into the brain bring about the same alterations in behaviour as systemic administration reinforces the behavioural significance of this adrenocorticoid. Consequently, the conclusion that CORT is involved in psychological adaptation, and ultimately the control of behaviour under conditions of stress, seems unavoidable.

Nonetheless, the significance of CORT in stress adaptation is complicated by the fact that plasma and brain levels of this hormone do not always correlate, combined with which the number of receptors for CORT in the brain is subject to autoregulation. As a consequence, the very rapid rise of plasma CORT in response to a stressor is not necessarily reflected so rapidly at the receptors in the brain. Obviously, this raises doubts over CORT's significance in the process of adaptation under conditions of stress.

Assuming that CORT is involved in psychological and behavioural adaptation in response to a stressor, the question of whether these effects are of overriding importance, or subsidiary to the effects in physiological adaptation, and the possible relationship between the two, remains unresolved. Furthermore, the controversy surrounding the nature of the psychological effects looms as large as ever.

In an attempt to pinpoint a more specific action of CORT, this thesis examined the effects of this steroid on attentional processes. Impetus for this was provided by the known effects of ACTH-whose actions tend to be opposite to those of CORT - and the HPC - the neuroanatomical focal point of CORT's actions - as both have been implicated in attention. More specifically, based on the fact that CORT suppresses the activity of hippocampal neurons (Pfaff et al., 1971), and exerts behavioural effects resembling those found upon hippocampectomy (e.g., Micco et al., 1979), CORT was hypothesised to retard the development of attentional inhibition, and in so doing to enhance the persistence of attention. The end result would be the perseveration of a behavioural response once it had been

established.

In order to test this the effects of transient elevations of CORT on several facets of behaviour were examined. If correct, the hypothesised reduction of attentional inhibition by CORT would have been manifested in the following ways:- a reduced level of distractability to an irrelevant distractant (Chapter 3); an inability to switch attention (Chapter 4); a retarded rate of habituation (Chapter 5); an impaired ability to shift a learned discrimination response (Chapter 6); combined with an impairment of passive avoidance responding and a facilitation of the acquisition of a two-way AAR (Chapter 7).

The evidence presented in Chapters 6 and 7 provided some support for the working hypothesis. Corticosterone-treated animals tended to display an impaired ability to shift a learned discrimination on both a reversal and non-reversal problem. They also tended to enter a compartment where they had previously experienced shock more readily than their controls, and acquired a two-way AAR faster. Apart from this, however, support for the working hypothesis was hard to find.

Indeed, as CORT did not influence distractability or habituation, yet these are probably the strongest tests of attention, this casts serious doubts on an hypothesis advocating a role for CORT in attention. Moreover, if it is considered that all the experiments reported in this thesis were conducted on a single strain of mouse - using mice of the same sex and of a similar age - under standard conditions of housing, testing etc., and that comparable and carefully worked out doses of CORT were used, the failure to find a consistent effect of CORT should be regarded, to all intents and purposes, as a falsification of the working hypothesis.

Even though the argument has repeatedly been advanced that it is possible that none of the experimentally induced elevations of CORT matched with complete fidelity those occurring under stress, and that this might preclude rigorous testing of the hypothesis, this argument may be void on

two accounts. Firstly, the CORT-receptor systems may be capable of responding to higher levels of CORT than was originally envisaged. Recently, this has been hinted at by McEwen et al., (1986). Thus, although the capacity of CORT's principal receptor system in the HPC is known to be strictly limited, the limitations of the remaining receptor mechanisms at CORT's disposal are unknown. It is usually assumed that they are exceeded at CORT levels surpassing those found normally, though this assumption may be invalid. Secondly, it is difficult to determine the upper physiological limit of CORT with any degree of certainty. This is illustrated by the fact that it has been shown, through the administration of large doses of exogenous ACTH, that the secretory capacity of the adrenal cortex exceeds the levels of CORT that are usually found in the plasma (Harvey, 1984). This suggests that under certain circumstances CORT may occur in quantities that have, up until recently, been regarded to be unphysiological. If these two points are viewed together, it is possible that the CORT levels may not have deviated from those found under physiological conditions, after all.

As the attentional hypothesis would predict the observed changes in discrimination behaviour and conditioned avoidance behaviour, some explanation of these results is required. Whilst it would be wrong to dismiss the potential significance of these changes for an attentional interpretation, as extensive training was involved in both types of test this introduces the possibility that processes such as learning, memory consolidation or retrieval, rather than attention, are influenced by CORT. Having said this, the manner in which an effect on these processes could bring about the observed changes is difficult to conceive.

As the evidence presented in this thesis goes against an attentional effect of CORT, it now becomes difficult to explain the known alterations in sensory sensitivity that are associated with this adrenocorticoid (e.g., Henkin, 1970a; 1970b; Sakellaris, 1972; Doty et al., 1982). This is because these imply a role in attention.

The truth of the matter may be that these results are more directly related to arousal, and that CORT thereby influences attention, as well as learning, memory and motivation processes. The diffuse distribution of CORT's receptors throughout the brain suggests a more widespread involvement in psychological adaptation than was first anticipated. Moreover, the fact that the pronounced circadian peak of CORT precedes the activity peak in a number of different species (Krieger, 1974; Nichols and Chevins, 1981) points strongly to a role for CORT in arousal. This is not the first time that CORT has been ascribed such a role: according to Hennessy and Levine's psychoendocrine hypothesis, arousal activates the PAS, which in turn regulates arousal and its effects on behaviour. In this hypothesis ACTH and CORT are held to exert opposite effects on arousal through their interaction with excitatory and inhibitory brain structures. Consistent with this, both ACTH and CORT have receptors in the ARAS and the HPC, and are, therefore, capable of modulating excitatory and inhibitory processes.

As yet, however, the problems associated with the definition of arousal (Hinde, 1970; Andrew, 1974) - which I do not pretend to be able to resolve - preclude an effective examination of CORT's possible role in this process. Consequently, any attempt to determine its effects on arousal must be deferred until there is greater consensus on the precise meaning of the term.

To conclude: whilst CORT is believed to be involved in brain processes underlying adaptation to stress, the specific nature of its involvement remains unresolved, though a direct effect on attention has effectively been refuted by the work reported here. An appreciation of the difficulties in manipulating CORT, especially in view of the complications introduced by the fluid nature of its receptor systems, and of the confounding influence of other hormones and transmitters, together with the difficulties in reaching an unequivocal and testable definition of the psychological

process under investigation, is essential for this to be resolved.

APPENDIX 1

The effects of pituitary-adrenal manipulations on distractability to an irrelevant distractant

Introduction

The series of runway studies presented here were a preliminary attempt to examine the effects of a number of different manipulations of the PAS on distractability. The mice used were usually either T-replaced or non-replaced castrates; though on occasion they were gonadally intact. The use of both T-replaced and non-replaced castrates enabled an assessment of the possible interaction between the PAS and PGS in distractability. Since blood samples were taken and the levels of CORT were determined, insight into the potential interaction between these two hormonal axes at an endocrinological level was also possible.

Method

Experimental conditions and subjects

(see Table A1:1 for details of treatments)

There were 10 animals in each of the 6 conditions:

1. T-replaced castrates injected with 0.1 ml 95% ethanol in 0.9% NaCl (1:9) (CxT+CORTVEHICLE)
2. T-replaced castrates injected with 200 µg CORT in the vehicle (CxT+CORT200)
3. Non-replaced castrates injected with 200 µg CORT in the vehicle (Cx+CORT200)
4. Intacts injected with 200 µg CORT in the vehicle (INTACT+CORT200)

5. T-replaced castrates injected with 2ius ACTH1-39 in 0.1 ml 0.9% NaCl solution (CxT+ACTH)
6. Non-replaced castrates injected with 2ius ACTH1-39 in the vehicle (Cx+ACTH)

All the injections were given subcutaneously.

73 mice, 61 of which were castrated, were used; though 3 castrates died and 8 castrates and 2 intact mice did not run to criterion.

Table A1:1 Summary of the experimental treatments

HORMONE	DOSE	REFERENCE
CORT	200µg CORT in 0.1ml vehicle 1 hour pre-test	Moyer and Leshner, 1976
CORT VEHICLE	0.1ml 95% ethanol in 0.9% NaCl (1:9) 1 hour pre-test	van Wimersma Greidanus, 1970
ACTH1-39	2ius ACTH in 0.1ml 0.9% NaCl 1 hour pre-test	Moyer and Leshner, 1976. Leshner et al., 1973
T-REPLACEMENT	500µg T-propionate 24 hours pre-test, followed by 500µg T-propionate 3 hours pre-test. The vehicle was 0.1ml olive oil	

Procedure and statistical analysis

There were no procedural differences from those given in Chapter 3. White panels only were used. The mice were blood sampled after the distraction trial, and the samples were subsequently assayed for CORT.

It is important to note that each condition was run separately.

Both the behavioural and CORT data are presented as means ± S.E.

Results

Table A1:2 The effects of pituitary adrenal manipulations on distraction times to an irrelevant distractant and plasma corticosterone levels (Means ± S.E.)

CONDITION	DISTRACTION TIME-SECS	CORT - ng/ml
CxT+CORT200	31.2 ± 7.2	608.5 ± 65.2
Cx+CORT200	19.0 ± 6.7	283.1 ± 41.7
INTACT+CORT200	9.2 ± 1.3	686.1 ± 53.8
CxT+CORT VEHICLE	4.4 ± 1.7	301.3 ± 37.0
CxT+ACTH	4.4 ± 1.4	578.2 ± 44.2
Cx+ACTH	3.8 ± 1.0	582.5 ± 30.1

Discussion

As the different conditions were run at widely different times a thorough analysis of this data was not valid. Keeping this criticism in mind, it is interesting to note that in those animals treated with CORT, the circulating levels of this adrenocorticoid were higher when T was present. This trend did not extend to those animals treated with ACTH, as CORT levels were similar regardless of the presence of T.

Regarding the level of distractability: on the whole CORT appeared to increase responsiveness to the panels; though in the absence of appropriate controls it is impossible to reach any meaningful conclusions.

APPENDIX 2

The effects of castration on pituitary-adrenal activity under resting conditions

Introduction

In order to assess the claim that the effects of castration (i.e., absence of T, but also the proactive effects of the surgery involved) are restricted to the responsiveness of the PAS rather than its basal level of activity (Hennessy et al., 1977), the level of activity of the PAS under resting conditions was compared in chronic castrates and intact male mice. Two indices of PAS activity were used: plasma levels of CORT - which reflect the present level of activity, and left adrenal weight - which reflects the recent past level of activity (Brain, 1972a).

Method

Experimental conditions and subjects

There were 10 animals in each condition:

1. Intact (INTACT)
2. Non-replaced castrates (CASTRATES)

10 intact and 10 castrated mice were used, all had previously served in the runway distractability test. Following behavioural testing they had been group housed for approximately 9 weeks, after which they were singly housed for 5-7 days.

Procedure

All samples were drawn one hour prior to lights-off, within 3 minutes of disturbing the case. Care was taken to ensure that the animals were not disturbed for at least 2 hours prior to blood sampling. Immediately

afterwards the mice were sacrified, then weighed. The left adrenal was removed, dissected clean and weighed wet to the nearest 0.01mg using a Calin 29 automatic electrobalance (Calin Instruments Inc.).

Statistical analysis

The adrenal weights are given as a % of the body weight. The data is expressed as means ± S.E. and was analysed by a one way unrelated ANOVA.

Results

Table A2:1 The effects of castration on plasma corticosterone levels (Means ± S.E.)

CONDITION N=10	CORT ng/ml
CASTRATES	44.7 ± 5.9
INTACTS	42.0 ± 6.3

ONE WAY UNRELATED ANOVA: $F(1,18) = 0.094$, $P = 0.763$

No significant effects were detected.

Table A2:2 The effects of castration on the weight of the left adrenal as a % of body weight (Means ± S.E.)

CONDITION N=10	% L.ADRENAL WEIGHT g
CASTRATES	0.0060 ± 0.00091
INTACTS	0.0073 ± 0.00043

ONE WAY UNRELATED ANOVA: $F(1,18) = 1.769$, $P = 0.20$

No significant effects were detected.

Discussion

The absence of T did not result in adrenal hypertrophy, undermining the suggestion that T exerts a chronic inhibitory influence on the PAS. Furthermore, it can be inferred that the stress associated with surgery does not augment the basal level of activity of the PAS.

APPENDIX 3

The effects of single dose of 5mg metyrapone on the levels of corticosterone 24, 48 and 72 hours post-administration

This experiment aimed to verify that the administration of a single dose of metyrapone produces similar CORT levels over the following 72 hours.

Method

Subjects and procedure

6 intact males, which had served in the runway distractability test 10 days previously, were injected with 5mg metyrapone in 0.1ml vehicle (0.9% NaCl) subcutaneously. Blood samples were taken 24, 48 and 72 hours later. Blood sampling was carried out one hour prior to lights off, within 3 minutes of disturbing the cage.

Results and discussion

The mean plasma level of CORT was 69.6 ± 9.0 ng/ml after 24 hours; 51.8 ± 3.7 after 48 hours and 32.7 ± 5.0 after 72 hours. This indicates that CORT levels tend to fall with time following a single dose of metyrapone. This may, however, be an artefact, as repeated blood sampling is known to produce distortions in the recorded plasma levels of a hormone (Frankel and Ryan, 1981).

APPENDIX 4

The effects of a single dose of 7.5mg testosterone-oenanthate on testosterone levels in castrated mice 24 and 72 hours post-administration

This experiment aimed to verify that the administration of a single dose of 7.5mg T-oenanthate to castrated male mice reinstates constant levels of T over the following 72 hours.

Method

Subject and procedure

8 castrated mice which had served in the runway distractability test 4 weeks previously were injected subcutaneously with 7.5mg T-oenanthate in 0.1ml olive oil. 4 mice were blood sampled 24 hours later, and the remaining 4 were sampled after 72 hours. Blood sampling was carried out one hour prior to lights off, within 3 minutes of disturbing the cage.

Results and discussion

The mean plasma level of T was 38.2 ± 11.6 ng/ml after 24 hours and 27.9 ± 6.3 ng/ml after 72 hours. Although it is, by virtue of the small sample sizes (N=4), difficult to draw any firm conclusions, the level of T was similar over a 72 hour period, though there was a tendency for it to decline with time. As "TO" male mice typically possess T within the range of 5-20 ng/ml (Bishop - personal communication) 7.5mg T-oenanthate produces relatively high levels of T.

APPENDIX 5

The effects of corticosterone on food intake under ad lib. and 12 hour food-deprivation conditions

Introduction

The effects of glucocorticoids on food intake is a controversial issue. Dexamethasone administered to intact rats has been claimed to reduce food intake and lower body weight (Beatty et al., 1970). By contrast CORT has been shown to increase food intake, at least in rabbits (Rees and Gray, 1984). To complicate the picture further Yukimara et al. (1978) suggested that these contradictory findings may reflect species or strain differences in sensitivity to glucocorticoids. This arose from their finding that whereas adrenalectomy reduced food intake and weight gain in both genetically lean and obese rats, CORT caused a much greater increase in these parameters in the obese rats, suggesting a hypersensitivity to the effects of glucocorticoids.

In view of the implications that differences in the motivation to seek food in response to CORT would have for behavioural tests involving appetitive motivation, the present study aimed to clarify the effects of this adrenocorticoid on food intake under ad lib. and deprivation conditions. Although Andrew and Rogers (1972) and Bohus (1973) have demonstrated that different durations of deprivation bring about different behavioural effects, suggesting that the effects of various hormonal manipulations may depend on the level of deprivation, the present study was confined to an examination of the effects of a 12 hour period of food deprivation. This is because this period of deprivation was implemented in all the behavioural tests involving appetitive motivation.

In the present experiment the food consumption of CORT replaced and non-replaced adrenalectomised mice, sham adrenalectomised and intact mice was examined.

Method

Experimental conditions and subjects

There were 8 animals in each condition:

1. Intact controls (INTACT)
2. Sham adrenalectomised (SHAM ADX)
3. Adrenalectomised maintained on a saline solution (1% ethanol in 0.9% $^{W}/_{V}$ NaCl) (ADX-SALINE)
4. Adrenalectomised maintained on a CORT solution (20µg CORT/ml saline solution) (ADX-CORT)

8 intact, 8 sham adrenalectomised, 8 non-replaced adrenalectomised and 8 CORT-replaced adrenalectomised mice were used. All the mice were behaviourally experienced. They had served in the runway test, and prior to that they had been exposed to a novel object.

The operations were carried out 19-24 days prior to the start of the food intake study. Throughout this period and for the duration of the food intake study the adrenalectomised mice were maintained on the appropriate drinking solution.

Procedure

Food intake under a 12 hour deprivation regime

The food intake study commenced immediately after the last day of runway testing. Following 12 hours food deprivation the mice were presented with a weighed quantity of food and allowed to feed for 12 hours before the next period of food deprivation. The amount of food remaining after 12 hours was weighed, and the quantity of food eaten over 12 hours was calculated. This procedure was repeated over 3 consecutive days, and the mean quantity of food consumed over these 3 days represents food intake under conditions of deprivation.

Food intake under ad lib. conditions

The mice were then allowed to feed ad lib. for 2 days, after which the amount of food eaten in 24 hours was determined, using a similar procedure as above. Ad lib. food intake was determined over 3 consecutive days.

The amount of food eaten under both ad lib. and deprivation conditions was measured at 11.00pm using a Sartorius 1404MP8 electronic balance. Two batches of animals were run with an equal number of animals from every condition in both batches.

Statistical analysis

The data is presented as means ± S.E. The mean amount of food consumed over 3 days under ad lib. and 12 hour deprivation conditions was analysed using a one way unrelated ANOVA.

Results

Table A5:1 The effects of corticosterone on food intake under ad lib. conditions (Means ± S.E.)

CONDITION N=8	FOOD INTAKE g
INTACT	9.0 ± 0.4
SHAM ADX	9.3 ± 0.3
ADX-CORT	8.1 ± 0.3
ADX-SALINE	9.1 ± 0.5

ONE WAY UNRELATED ANOVA: $F(3,28) = 1.91$, $P = 0.15$

Table A5:2 The effects of corticosterone on food intake under 12 hour deprivation conditions (Means ± S.E.)

CONDITION N=8	FOOD INTAKE g
INTACT	6.8 ± 0.3
SHAM ADX	7.3 ± 0.3
ADX-CORT	6.1 ± 0.2
ADX-SALINE	6.8 ± 0.3

ONE WAY UNRELATED ANOVA: $F(3,28) = 2.74$, $P = 0.06$

Although there were no significant differences in the amount of food consumed under either ad lib. or deprivation conditions, CORT-replaced adrenalectomised animals tended to eat less food when they were subjected to a 12 hour period of food deprivation.

Discussion

As there were no significant differences in the amount of food consumed under ad lib. or deprivation conditions the conclusion must be that CORT does not affect food intake. However, CORT-replaced adrenalectomised animals tended to eat less food under deprivation conditions. This is difficult to explain, especially as these animals did not possess widely different plasma levels of CORT from their intact controls. Indeed, although CORT was not measured in this experiment, blood samples had been taken immediately prior to the start of the food intake study and subsequent determination of the levels in circulation at that time had revealed that intacts possessed very similar levels to CORT-replaced adrenalectomised animals (207.8 and 190.5 ng/ml respectively - see Table 3:45). By contrast, non-replaced adrenalectomised animals had significantly lower levels (23.2 ng/ml see Table 3:45), yet their food consumption bore close resemblance to their adrenally intact controls.

As CORT does not affect food intake its effects on behaviour involving appetitive motivation can be examined, and so long as a 12 hour period of deprivation is used, any behavioural effects can confidently be ascribed to CORT. Nonetheless, the possibility remains that CORT affects appetitive motivation under different deprivation regimes as suggested by Bohus (1973).

One possible criticism of this study is that the animals were adrenalectomised approximately 20 days prior to the start of the food intake study, which is a sufficiently lengthy period for adrenal hypertrophy to occur. Moreover, since the animals were not blood sampled at the end of this study it is possible that the failure to find differences in the amount of food consumed arose because the various conditions actually had similar levels of CORT. However, it has already been mentioned that these animals had very different levels of CORT at the start of the food intake study, and since this was 19-24 days after the removal of the

adrenals, there is no reason to presuppose that these levels would be greatly different at the end of the food intake study.

APPENDIX 6

Determination of the most effective testosterone replacement regime using a modified silastic implant technique

Introduction

The objective of this experiment was to select an appropriate chronic T-replacement regime. Silastic is known to offer good steroid permeability, combined with good tissue biocompatibility. Furthermore, it is relatively well established that silastic-steroid implants provide controlled release of the steroid over long periods of time (Smith et al., 1977). Accordingly, silastic-steroid implants provide the ideal solution to the problem of a chronic T-replacement regime.

As Turner (1979) demonstrated that the daily release rates of T from silastic capsules of different lengths both *in vivo* and *in vitro* were directly proportional to the length of the capsule, the present study examined the efficiency of various lengths of silastic - T implants in reinstating a steady plasma level of T in castrated mice over a 4 week period. Following Barkley and Goldman (1977) and Simon and Gandleman's (1978) finding that a 1cm silastic implant, containing 5mg T, maintained both normal levels of aggressive behaviour and the weight of the accessory glands in castrated mice, a similar dose of T was used in this experiment.

Method

Subjects and procedure

3 weeks after castration 12 mice were implanted with a silastic-T pellet containing 5mg T/cm (see Chapter 2). 3 pellet lengths were used: 5mm, 10mm and 20mm, and there were 4 animals in each condition. Blood samples were taken 24 hours, 7,14,21 and 28 days later. Blood sampling was carried out at 11.00-12.00 am, within 3 minutes of disturbing the cage. The samples were assayed for T. As the T-assay requires a small volume of

plasma only about 150µl of blood was taken. (This reduces any effects that are recognised to arise as a result of repeated blood sampling - Ryan and Frankel, 1981).

Results and discussion

Table A6:1 The effects of various lengths of silastic - testosterone pellets on the plasma levels of testosterone over a 4 week period. (Means±S.E.)

IMPLANT LENGTH N=4	T ng/ml 24 HOURS	7 DAYS	14 DAYS	21 DAYS	28 DAYS
5mm	7.4 ±2.7	38.1 ±25.2	8.9 ±0.8	5.3 ±0.8	5.2 ±0.3
10mm	12.9 ±3.8	22.8 ±10.7	9.2 ±0.8	7.4 ±1.2	8.7 ±1.6
20mm	21.7 ±2.5	19.8 ±3.2	15.2 ±2.2	15.7 ±2.1	9.0 ±0.6

Discussion

As expected the plasma T levels depended on the length of the pellet, with the longest reinstating the highest. Moreover, this pellet also produced the most stable levels of T, both at any one sampling point, as well as across samplings, at least up to 21 days post-implantation. From this it was deduced that a 20mm implant was the most suitable for the chronic replacement of T; though the levels of T were slightly higher than those found usually in intact male mice.

*not read in the original

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