AVOIDANCE BEHAVIOR IN RATS WITH HEREDITARY HYPOTHALAMIC DIABETES INSIPIDUS

BY

WILLIAM BAILEY

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1975

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy

Jan 23, 1975
date

Jack Rabin
Chairman of Examining Committee

Jan 27, 1975
date

Executive Officer

Supervisory Committee

The City University of New York

ACKNOWLEDGEMENTS

I would like to thank my advisor Dr. Jack Orbach and the other members of my committee, Drs. Doreen Berman and Sandra Shapiro, and Drs. Gerard Smith and Jay Weiss who served as outside readers, for their advice and helpful criticism. Special appreciation is expressed to Dr. Jay Weiss who generously provided space in his laboratory at Rockefeller University, equipment, a stipend and supervision for this project.

Many people at Rockefeller University contributed to this research: Dr. Larissa Pohorecky provided training and assistance in biochemical techniques; Dr. Michael Draper synthesized a substantial quantity of ACTH 4-10; Ken Garay measured the urine osmolarities; Lee Jaffe and Hope Berkeley assisted with the assay of norepinephrine; and Marjorie McMiniman cared for the Brattleboro rats.

Sincere thanks are expressed to Dr. Carl Hanson for donating the original Brattleboro breeding stock, to Dr. Tong Joh for performing the assays for dopamine beta hydroxylase and to Dr. Heinz Valtin for advice on the care and breeding of Brattleboro rats.

Most of all, I would like to thank my wife for her patience and encouragement and her many contributions to this manuscript, which included typing all the drafts.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Nature of the Problem

The pioneering work of Selye (1950) on the role played by the pituitary-adrenal system as part of an organism's response to stress has stimulated psychologists and neuroendocrinologists to investigate whether pituitary and adrenal hormones affect the behavior as well as the physiology of animals in stressful situations. Research on this problem has focused on the relationship of the pituitary hormones, adrenocorticotrophic hormone (ACTH), melanocyte stimulating hormone (MSH) and vasopressin, and the adrenal corticosteroids to the performance of rats on a variety of aversively motivated conditioning tasks. Largely through the systematic research of de Wied and his colleagues (de Wied, Bohus & Greven, 1968), it has been established that pituitary and adrenocortical hormones affect avoidance performance in opposite ways, particularly during extinction. When given exogenously, ACTH, MSH and vasopressin prolong the process of extinction while corticosteroids shorten it. Most important is the fact that these hormones seem to exert their effect directly on specific brain structures rather than via peripheral endocrine systems (for comprehensive reviews see Di Guisto, Cairncross & King, 1971; de Wied, 1969a, 1969b; de Wied, van Delft, Gispen, Weijnen & van Wimersma Greidanus, 1972b; Levine, 1968; Smith, 1973).

There are a few clues as to how pituitary hormones influence avoidance behavior. For instance, it is known that fragments of the ACTH molecule increase the incorporation of ^{3}H-leucine into brain proteins (Reading, 1972; Versteeg, Gispen, Schotman, Witter & de Wied, 1972) and increase the polysome content of the brain stem (Gispen, de Wied, Schotman & Jansz, 1971) in hypophysectomized rats. In normal rats, ACTH fragments increase the levels of gamma-amino butyric acid and serotonin in the midbrain and increase the turnover of norepinephrine, while decreasing the turnover of serotonin (Leonard, 1974). MSH has been reported to increase the occurrence of EEG frequencies in the theta range (Sandman, Denman, Miller, Knott, Schally & Kastin, 1971). However, it has not been possible to identify which physiological effects of these peptides are related to avoidance behavior. Neither is it known how or why ACTH and MSH, hormones of the anterior and intermediate lobes of the pituitary, should have effects on avoidance similar to those produced by vasopressin, a posterior lobe hormone. MSH and ACTH have similar effects on behavior presumably because of their structural similarity and the resulting similarity of biological activity, but their structure and known biological functions are quite unlike that of vasopressin.[1]

The purpose of this investigation was to study the avoidance behavior of rats of the Brattleboro strain. These rats, derived from Long-Evans (LE) stock, inherit hypothalamic diabetes insipidus; rats homozygous for this trait (DI) have a total deficiency of vasopressin, while heterozygous rats (HE) demonstrate only a partial vasopressin

---

[1]The amino acid sequence of selected peptides is given in Appendix I. This includes pituitary hormones or their synthetic analogues.

deficiency (Valtin, Sawyer, & Sokol, 1965). It was thought that the performance of DI rats on active and passive avoidance would provide an indication of the role of endogenous vasopressin in normal rats. HE rats of the same age served as controls, but in several experiments normal LE rats were also included. In addition, the effect of ACTH on the passive avoidance of these rats was investigated to test the hypothesis that the effects of ACTH on avoidance are mediated by the release of endogenous vasopressin. The effect of vasopressin replacement therapy on the passive avoidance and the physiological condition of Brattleboro rats was also observed.

The remainder of the introductory section is divided into three parts. First, the role of ACTH and vasopressin in avoidance behavior is reviewed to indicate similarities and differences in their action. Second, the endocrine and physiological data on the Brattleboro strain are reviewed in order to evaluate the hypothesis that the hypothalamic defect in Brattleboro rats directly affects only the synthesis and release of vasopressin and not other pituitary hormones. Third, an outline of the experiments and methodological considerations are presented.

The Role of ACTH and Vasopressin in Avoidance Behavior

ACTH

Hypophysectomized and adenohypophysectomized rats are profoundly impaired in the acquisition of conditioned avoidance in the shuttlebox. De Wied discovered that ACTH, or a mixture of cortisone, testosterone and thyroxin improved the avoidance learning of these rats (de Wied, 1964, 1969a) and that growth hormone was similarly effective in

hypophysectomized rats (de Wied, 1969b). It might be argued that the beneficial influence of these hormones could be explained by their ability to improve the physical condition of these animals. However, alpha- and beta-MSH or fragments of the ACTH molecule, in small doses, all facilitate acquisition by hypophysectomized rats (de Wied, 1969a; de Wied, Witter & Lande, 1970; Greven & de Wied, 1973) and are without significant corticotrophic or general metabolic effects (de Wied, 1969b).

In intact rats ACTH, MSH or ACTH fragments usually do not enhance acquisition performance of shuttlebox or pole jump avoidance, but they dramatically delay extinction of these tasks when administered during extinction (Bohus & de Wied, 1965; de Wied, 1966a; de Wied & Bohus, 1966). An extra-adrenal site of action for these peptides seems certain, for the ability of ACTH to delay extinction in the shuttlebox is not reduced in adrenalectomized rats (Bohus, Nyakas & Endröczi, 1968). In addition, intracerebral implants of ACTH 1-10 are able to prolong the extinction of a pole jumping response only when placed into specific brain regions (van Wimersma Greidanus & de Wied, 1971). The amino acid sequence of ACTH and MSH which possesses the same potency as the parent molecules in inhibiting the extinction of pole jump avoidance is the core heptapeptide ACTH 4-10 (Greven & de Wied, 1967).

A role for endogenous ACTH in passive avoidance has been suggested by an experiment in which hypophysectomized rats, which lack ACTH and other pituitary hormones, showed shorter passive avoidance latencies than normal rats, whereas adrenalectomized rats which have high basal and stress levels of ACTH and lack adrenal hormones showed longer passive avoidance latencies than normal rats (Weiss, McEwen, Silva & Kalkut, 1969, 1970a). The hypothesis that ACTH or a similar

peptide was involved in this experiment is further supported by the finding that injections of ACTH or MSH in saline prolong passive avoidance when given to normal rats before either training or extinction trials (Dempsey, Kastin & Schally, 1972; Guth, Seward & Levine, 1971; Sandman, Kastin & Schally, 1971).

These findings are in contrast to those found in experiments in which ACTH in gelatin has been used. ACTH gel shortens passive avoidance latencies when given before training or extinction trials, although this effect is blocked if ACTH is given before both training and testing (P. Gray, 1975). J. Gray (1971b) reported that the prolonged extinction produced by ACTH gel in a food rewarded runway task was blocked by treatment with the ACTH gel prior to training on the first day. Since the ACTH gel in the P. Gray (1975) study had a similar effect on passive avoidance to that reported for dexamethasone (Pappas & P. Gray, 1971), a potent synthetic corticosteroid, it is likely that the effect of the ACTH gel on extinction behavior in these studies is related to the release of corticosteroids from the adrenal. The pattern of effects for dexamethasone and ACTH in gel is characteristic of drugs which induce state-dependent learning (Overton, 1966).

Results of experiments using synthetic ACTH fragments are much more significant than those using the full ACTH molecule, for the following reasons. First, the use of ACTH fragments directly rules out the participation of adrenal corticosteroids because these fragments do not have significant corticotrophic activity (de Wied, 1969b). Adrenal steroid hormones have been shown to have opposite actions to that of ACTH on avoidance behavior (Bohus & Lissák, 1968; de Wied, 1966b; Weiss et al., 1969). Second, the effect of 'purified' ACTH on

behavior in one study has been traced to the presence of vasopressin in the preparation (Lande, Flexner & Flexner, 1972). Finally, the N-terminal fragments of ACTH are less likely to initiate non-specific changes in arousal or motivation through peripheral influences on blood pressure (Bailey, 1973), fat metabolism or blood glucose (Engel & Lebovitz, 1966) than the full ACTH molecule because most extra-adrenal actions of ACTH, except for its melanocyte stimulating activity, increase as the peptide chain is lengthened from the N-terminal. With respect to non-specific arousal, the peptide fragments ACTH 1-10 and ACTH 4-10 do not affect open field activity (Bohus & de Wied, 1965; Weijnen & Slangen, 1970) in contrast to ACTH (Ley & Corson, 1973), nor do they increase the sensitivity of rats to footshock (Gispen, van Wimersma Greidanus & de Wied, 1970) as has been reported for ACTH (Paré & Cullen, 1971).

In summary, the anterior pituitary hormones ACTH and MSH, as well as a number of ACTH fragments, overcome the impaired active avoidance performance of hypophysectomized rats and consistently prolong the extinction of active and passive avoidance in intact rats, presumably independent of adrenal steroidogenesis. These findings are compatible with the hypothesis that the release of ACTH in response to the avoidance situation is necessary for normal acquisition and extinction.

Vasopressin

In contrast to the severe acquisition deficit imposed by adenohypophysectomy or hypophysectomy, removal of the intermediate and posterior lobes of the pituitary in combination (neurohypophysectomy)

does not affect acquisition of shuttlebox avoidance, but does produce rapid extinction (de Wied, 1965). Since the lesion is a combined one, it is not possible to conclude that the rapid extinction is a consequence of MSH deficiency or vasopressin deficiency, or both. Treatment with alpha-MSH delays extinction, and replacement therapy with pitressin tannate, a long acting posterior pituitary extract, or purified lysine vasopressin, either during acquisition or extinction, normalizes extinction in these animals (de Wied, 1965). A small pharmacological dose, .06 U, of synthetic lysine vasopressin[2] during training improves the shuttlebox avoidance of hypophysectomized rats (Bohus, Gispen & de Wied, 1973) and prolongs the normally rapid extinction of hypophysectomized rats that are given cortisone, testosterone and thyroxin during acquisition (de Wied, 1969b).

The treatment of normal rats with lysine vasopressin or a synthetic vasopressin analogue also affects avoidance. One U of pitressin tannate every two days prolongs extinction in the shuttlebox equally well whether it is given during acquisition or during extinction (de Wied & Bohus, 1966). Lysine vasopressin also prolongs extinction of pole jump (Bohus, Ader & de Wied, 1972; de Wied, 1971; de Wied, 1973) and passive avoidance tasks (Ader & de Wied, 1972; Bohus et al., 1972, Lissák & Bohus, 1972; Thompson & de Wied, 1973). The active component of a porcine pituitary extract which restores the ability of hypophysectomized rats to perform in the shuttlebox has been identified as desglycinamide lysine vasopressin (Lande, Witter & de Wied,

---

[2]The estimated concentration of vasopressin in rat eye plexus blood after passive avoidance testing is between .00034 and .00063 U/ml (Thompson & de Wied, 1973).

1971). Desglycinamide lysine vasopressin (D-LVP) has been synthesized and also found to prolong the extinction of pole jump (de Wied, Greven, Lande & Witter; 1972a; Wang, 1972). It is noteworthy that D-LVP has little of the antidiuretic, pressor or corticotrophic releasing factor potency of lysine vasopressin (de Wied et al., 1972a).

For vasopressin to be most effective in prolonging the extinction of passive avoidance or pole jump avoidance it must be administered within one hour before or after training or extinction trials; when administered six hours before or after a trial it has no effect (Bohus et al., 1972; de Wied, 1971). If vasopressin is administered before either passive or pole jump avoidance extinction sessions on the same day, there is no generalization of its effect from one task to the other (Bohus et al., 1972).

These findings suggest that vasopressin and D-LVP affect active and passive avoidance in much the same way as do ACTH, ACTH fragments, and MSH. This similarity of action would seem to indicate that these hormones influence behavior through a common mechanism. It has been argued, however, by several investigators that anterior and posterior pituitary hormones affect behavior in different ways (Ader & de Wied, 1972; Lissák & Bohus, 1972). They note that the change in performance produced by ACTH or MSH on acquisition persists only a short time after treatment, 4-48 hours, while pitressin or lysine vasopressin delay extinction for long periods, 72 hours-35 days, whether given during acquisition or extinction (Bohus et al., 1973; de Wied & Bohus, 1966; Thompson & de Wied, 1973). Moreover, bilateral lesions of the para-fascicular area of the medial thalamus abolish the prolongation of extinction in the shuttlebox by alpha-MSH or very low doses of ACTH

4-10, while that produced by lysine vasopressin is little affected by these lesions (Bohus & de Wied, 1967; van Wimersma Greidanus, Bohus & de Wied, 1974). Evidence from stereotaxic implantations of ACTH 1-10 also implicate the parafascicular area as the main site of action of ACTH peptides on avoidance (van Wimersma Greidanus & de Wied, 1971). The possibility that vasopressin mimics the effects of ACTH merely because of its ability to stimulate the release of ACTH[3] is contradicted by the finding that lysine vasopressin facilitates acquisition (Bohus et al., 1973) and prolongs extinction in hypophysectomized rats (de Wied, 1969b). While this evidence suggests that ACTH and vasopressin act independently, it is still possible that the effect of exogenous ACTH is mediated by the release of endogenous vasopressin. Although direct evidence for this latter hypothesis is scanty, several points suggest its consideration. First, hypophysectomized rats or rats minus only the posterior and intermediate lobes of the pituitary, are still capable of producing and releasing small quantities of vasopressin and oxytocin. Vasopressin and oxytocin are synthesized in the supraoptic and paraventricular nuclei, respectively, and transported down the neurohypophyseal tract to the posterior pituitary where they are stored prior to release. Within a short time after removal of the posterior lobe a new minature neurohypophysis is formed which is sufficiently functional that only a mild diabetes insipidus develops (Nagy, Czakó, Durszt & László, 1972). Therefore, ACTH could influence avoidance responding via release of vasopressin or similar peptides

---

[3]Vasopressin is very similar in structure to corticotrophic releasing factors found in the hypothalamus (Guillemin, 1964).

even in hypophysectomized, adenohypophysectomized, or neurohypophysectomized rats. The shorter duration of action of exogenous ACTH relative to exogenous vasopressin could be accounted for by the limited quantity of vasopressin available for endogenous release by ACTH. Second, there is evidence that both ACTH 4-10 and D-LVP facilitate the release of vasopressin in a passive avoidance test (Thompson & de Wied, 1973). Rats shocked after entering a darkened box received ACTH 4-10, D-LVP or placebo injections one hour before an extinction trial the following day. Groups of control rats that were similarly handled and exposed to the apparatus but received no shock were given comparable injections and also tested for extinction. Passive avoidance latencies and antidiuretic activity of eye plexus blood sampled after the first passive avoidance extinction trial were greater in ACTH 4-10 treated rats than in placebo injected rats only if the rats had received shock on the training trial the previous day. In non-shocked rats, ACTH 4-10 affected neither antidiuretic activity nor passive avoidance latency. Although D-LVP increased the antidiuretic activity of both shocked and non-shocked rats, the antidiuretic activity and extinction latencies were significantly higher in the shocked rats that had received D-LVP than in similarly treated non-shocked rats. In other experiments it was shown that both the antidiuretic activity of blood and passive avoidance retention latencies increased monotonically in response to increases in the shock intensity on the training trial (Thompson & de Wied, 1973). Thus, the antidiuretic material, presumably vasopressin, released by footshock or by ACTH and D-LVP may be responsible for changes in passive avoidance latency.

ACTH β 1-39, β 1-24, and β 1-16 have been found to show depressor effects similar to vasopressin, using the cock's blood pressure assay for detecting posterior pituitary contaminants in preparations of ACTH (Jaques, 1965). The release by these peptides or a non-specific action may be responsible for this finding.

The only data that do not fit the above analysis are that D-LVP was found not to affect extinction of running for food in a straight runway, although porcine ACTH, ACTH β 1-24 and ACTH 4-10 did prolong extinction (Garrud, Gray & de Wied, 1974). If D-LVP and ACTH both stimulate the release of vasopressin or if D-LVP acts like endogenous vasopressin, both peptides should prolong extinction. Furthermore, the effect of D-LVP might be expected to be greater or more long lasting than that of ACTH peptides.

In summary, deficiencies of anterior pituitary hormones caused by adenohypophysectomy or hypophysectomy impair active and passive avoidance, presumably because of the absence of ACTH. Neurohypophysectomy shortens shuttlebox extinction, but the relation of this finding to a vasopressin deficiency is in doubt because this operation also removes the intermediate lobe which secretes MSH. The literature suggests that injections of ACTH, MSH and vasopressin affect active and passive avoidance in a similar but not identical fashion. The argument that ACTH and vasopressin affect behavior in different ways is based upon differences in the length of time over which they influence performance and in their ability to overcome the effect of medial thalamic lesions. Other findings, however, support the hypothesis that ACTH may affect behavior indirectly, even in hypophysectomized, neurohypophysectomized, or adenohypophysectomized rats, by

stimulating the release of endogenous vasopressin. Thus, injections of ACTH and vasopressin may differ in the magnitude but not in the final mechanism of their effects on behavior.

Endocrine Physiology of the Brattleboro Rat

The Brattleboro strain of rats is of LE ancestry[4] and has been studied by physiologists and endocrinologists because of the heritability in this strain of hypothalamic diabetes insipidus. The defect appears to be an autosomal recessive trait occurring with homozygosity of recessive genes at either locus of a single pair of loci, and may be associated in some pedigrees with albinism and the frequent occurrence of runts, stillbirths, and newborn mortality (Saul, Garrity, Benirschke & Valtin, 1968). Assays of the hypothalamus and pituitary in DI rats homozygous for this trait indicate that these structures contain no biologically active or immunoreactive vasopressin (Miller & Moses, 1973; Valtin, Sawyer & Sokol, 1965); and their urine output is 16 times that of normal rats (Swabb, Boer & Nolten, 1973). Pituitary levels of oxytocin are about 1/3 that of normal animals but this depletion is brought about by the continual release of oxytocin from the pituitary in response to the mild but chronic state of dehydration found in these animals, and it is reversed by vasopressin therapy (Valtin et al., 1965). HE rats have normal levels of oxytocin in the pituitary, while the levels of vasopressin in the hypothalamus and pituitary are 2/3 to 1/7 of those found in normal rats, indicating a partial impairment in the synthesis (Valtin et al., 1965) and release of vasopressin (Miller &

---

[4]LE rats are not known to have any disorders of hypothalamo-pituitary function. Unless otherwise noted, references to normal rats will designate LE rats.

Moses, 1971; Moses & Miller, 1970). Despite the partial vasopressin defect in HE rats their urine volume is only 1 1/2 to 2 times greater than normal and serum osmolarities are not different from normal (Moses & Miller, 1970; Swabb et al., 1973).

The hypertrophy of the hypothalamo-neurohypophyseal system which occurs in DI rats together with histological (Scott, 1968; Sokol & Valtin, 1962) and enzymatic evidence of enhanced neurosecretory activity (Swabb et al., 1973) might indicate the synthesis of a defective vasopressin molecule. Harrington & Valtin (1965) originally proposed this hypothesis because DI rats required 28 days of vasopressin replacement therapy before they were able to produce urine as concentrated as that of normal rats, and the production of an inactive analogue of vasopressin competing for binding sites in the kidney might inhibit the antidiuretic effect of injected vasopressin. Later, however, Valtin discovered that the pituitary of DI rats does not contain any substance other than oxytocin capable of inhibiting the kidney response to vasopressin (Sawyer & Valtin, 1965). It is more likely, then, that the hyperactivity of the hypothalamo-neurohypophyseal system in DI rats is related to the production and rapid release of neurophysin, the carrier-protein for vasopressin. Neurophysin is not found in the pituitary of DI rats, but the serum concentration is much higher than in normals, while the serum neurophysin levels of HE rats are intermediate between those of DI and normal rats (Cheng, Friesen & Martin, 1972). The hypertrophy and evidence of enhanced neurosecretory activity of the hypothalamo-neurohypophyseal system might therefore reflect an inability to produce biologically active vasopressin in spite of continued osmoreceptor stimulation (Valtin, 1967).

The most plausible explanation for the refractoriness of DI rats to vasopressin therapy is that their kidneys have been damaged by a chronic deficiency of potassium. Möhring, Dauda, Haack, Homsy, Kohrs & Möhring (1972a) have shown that DI rats are hypokalemic, and that the occurrence and severity of kaliopenic nephropathy increased with age. Pitressin tannate injections increase the retention of potassium by DI rats but do not affect potassium balance in normal rats (Möhring, Schömig, Brekner & Möhring, 1972b).

While no explanation was offered for the potassium deficiency of DI rats in the above investigations it is almost certainly caused by the loss of salts associated with the large volumes of urine excreted, and perhaps, by changes in the extra-renal distribution of sodium, potassium and water associated with the relative dominance of adrenal mineralocorticoids in the absence of vasopressin (Friedman, Scherrer, Nakashima & Friedman, 1958). Just as the behavioral changes which follow ablations of a specific brain structure do not reflect the function of the excised tissue, so it is with an experimentally or genetically induced deficiency of vasopressin which is more adequately characterized as a syndrome of adrenocortical hyperactivity. In rats with diabetes insipidus, hypokalemia, excessive renal loss of potassium (Möhring et al., 1972a), slight hypernatremia (Valtin & Schroeder, 1964) and diminished extracellular fluid volume (Harrington & Valtin, 1968), suggest aldosteronism or the relative dominance of similar mineralocorticoids (Tepperman, 1968).

Although DI rats are not known to have any genetic neurophypophyseal deficiency other than that affecting vasopressin, some studies suggest that deficiencies of adenohypophyseal hormones may be present

in these animals. Most important to the present investigation is the observation that Brattleboro DI rats as well as neurohypophysectomized rats show a diminished pituitary-adrenal response to weak but not strong stresses. For instance, DI rats show normal increases in plasma corticosterone after histamine, hypoxia or large doses of ACTH, but a diminished response to noise or brief ether exposures (Yates, Russell, Dallman, Hedge, McCann & Dhariwal, 1971). Arimura, Saito, Bowers and Schally (1967) found no difference between HE rats and DI rats in their corticosterone response to ether, histamine, vasopressin or acetylcholine, but in response to an i.p. injection of saline, DI rats did not secrete as much corticosterone as normals. McCann found that restraint and etherization for blood sampling elicited subnormal cortisterone responses from DI rats one minute after the initiation of stress, and similar results were obtained on later testing even after dehydration was reduced with several weeks of pitressin tannate treatment (McCann, Antunes-Rodrigues, Nallar & Valtin, 1966). These experiments suggest that the diminished corticosterone response of DI rats to mild stresses may be related either to a subnormal corticotropin releasing factor (CRF) response, or to a reduced pituitary ACTH response to CRF. It has been suggested that another reason why the stress response of the DI rat may be reduced under some circumstances is that vasopressin may normally sensitize the ACTH producing cells of the pituitary to CRF (Yates et al., 1971).

The adrenal glands of DI rats are less sensitive to small quantities of ACTH than normal controls, but after chronic administration of vasopressin beginning at four days of age, the corticosterone responses of the two groups were not significantly different.

Both normal and DI rats given chronic vasopressin treatment, however, showed only small corticosterone increases to stress in comparison to untreated rats (Wiley, Pearlmutter & Miller, 1974). These data imply that the inability of McCann et al. (1966) to reverse the deficient stress response of DI rats with vasopressin can be explained by the lack of a normal vasopressin treated control group and an insufficient dose and duration of treatment with vasopressin.

In order to reconcile these disparate findings it should be noted that neurophypophysectomized rats also show a subnormal corticosteroid response to weak stresses (Arimura, Yamaguchi, Yoshimura, Imazeki & Itoh, 1965; de Wied, 1961, 1968; Miller, Yueh-Chien, Wiley & Hewitt, 1974). Therefore, if a CRF or ACTH deficit does exist in DI rats it is probably secondary to a deficiency of vasopressin rather than genetic in origin. Second, in both DI and neurohypophysectomized rats, the production of corticosterone by the adrenal might be expected to be reduced because of the demands placed upon the adrenal to produce mineralocorticoids, and both glucocorticoid and mineralocorticoid hormones require the same steroid precursors. Thus, the decreased output of corticosterone in response to mild stresses in DI and neurohypophysectomized rats might not be related to a defect at the hypothalamic or pituitary level, but to a decrease in adrenal sensitivity caused by the chronic diversion of precursor resources into the production of mineralocorticoids.

A further pituitary deficiency of the DI rat which is almost certain to be confirmed by future experimental studies is that of growth hormone. DI rats are reported to be smaller than similar aged normal or HE rats (Valtin et al., 1965). Chronic vasopressin therapy

to DI rats for two months after weaning increased body weight relative to peanut oil injected DI rats but did not bring their weight up to normal nor did it increase tail length. Growth hormone treatment, however, did normalize both body weight and tail length after five months, without affecting water intake (Sokol, 1973). As thyroid function is reported to be normal in DI rats (Galton, Valtin & Johnson, 1966) a genetic abnormality of growth hormone secretion may be responsible for the smaller size of DI rats. Another possibility is that pre- and post-natal malnutrition associated with an unusual drain of the mother's metabolic resources and body salts by DI rats, and to a lesser extent by their HE littermates could produce irreversible stunting of growth and lowered growth hormone levels (Nitzan & Wilber, 1974). Regardless of the cause of the stunted growth of DI rats, impairment of growth hormone release is not likely to affect behavior insofar as the response to acute stress associated with behavioral testing is concerned, as it has been shown that in the rat, unlike man, growth hormone levels decrease in response to stressful situations (Schalch & Reichlin, 1968; Takahashi, Daughaday & Kipnis, 1971; Mitchell, Smyrl, Hutchins, Schindler & Critchlow, 1972).

This review of the endocrine physiology of the Brattleboro strain indicates that DI rats have disturbances of other hormonal systems in addition to an absolute deficiency of vasopressin. A reduced pituitary-adrenal response to mild stress seems to be characteristic of DI rats, but this may be attributed to a decreased sensitivity of the adrenal to ACTH. DI rats are hypokalemic and this and other alterations in electrolyte distribution are in part related to the relative dominance of mineralocorticoid hormones in the absence of vasopressin. The

stunted growth of DI rats is likely to be caused by a deficiency of growth hormone, but it is not known whether a genetic factor is involved. Finally, the synthesis and release of oxytocin may be increased in DI rats but can be normalized by vasopressin administration.

HE rats have often been considered as normal controls for DI rats in physiological studies even though they are known to have a slight defect in the synthesis and release of vasopressin. The use of the HE rat as a control in behavioral studies is perhaps even more important as it is not known what genetic factors other than those responsible for a vasopressin deficiency may be peculiar to this strain. Thus, until further data are available on the significance of a slight disturbance in vasopressin capability or other characteristics of the HE rat, it is desirable to use both HE and normal rats as controls wherever possible.

Although the DI rat may not be a perfect model of a selective deficiency of vasopressin, it is still the best available. Other models have greater limitations. Neurohypophysectomy produces a relatively minor vasopressin defect, removes the source of MSH, and results in alterations of pituitary-adrenal function like those found in DI rats. Complete lesions of the supraoptic nucleus where vasopressin is synthesized are difficult to obtain and lesions of the hypothalamo-neurohypophyseal tract at the level of the median eminence produce incidental damage to hypothalamic centers regulating the secretion of other pituitary hormones including ACTH. Thus, the DI rat has disturbances of hormonal systems other than vasopressin but most seem to be a direct or indirect consequence of a deficiency of vasopressin.

Experimental Plan and Methodological Considerations

If the presence or release of vasopressin plays an important role in establishing or maintaining avoidance, then alterations of performance might be expected in DI rats. It is not easy to predict, however, whether a deficiency of vasopressin will result in a change in avoidance opposite to that produced by the administration of exogenous vasopressin. Since changes in other hormonal systems occur in response to a deficiency of vasopressin, the difference between a normal rat and a DI rat is probably much more than the difference in their ability to synthesize vasopressin. One of the most important of these secondary changes, in terms of the interpretation of avoidance data, is the reduced sensitivity of the pituitary-adrenal system to stress. Therefore, careful consideration was given throughout the experiments to the relevance of this as well as to changes in other hormonal systems. Consideration was also given to the possibility that HE rats were not the best controls by which the performance of DI rats should be judged. Because HE rats have a partial vasopressin defect, normal LE rats would seem preferable; however, it is not known what genetic differences exist between Brattleboro and LE rats other than those relating to the synthesis and release of vasopressin. Therefore, both HE and LE rats were tested with DI rats whenever possible.

It is on extinction that the effects of pituitary hormones on avoidance are most evident (de Wied et al., 1972b). Therefore, DI and HE rats were compared on the extinction of passive avoidance (Chapter II). Brattleboro rats and LE rats of the same age were observed on a single open field test to determine whether general activity levels were related to passive avoidance (Chapter III). The effect of

potassium supplementation on the open field activity of another group of Brattleboro rats is also presented in Chapter III. Since the vasopressin deficiency of the neurohypophysectomized rat has been reported to affect the extinction but not the acquisition of shuttlebox avoidance, DI and HE rats were compared on active avoidance in the shuttlebox (Chapter IV).

To test the hypothesis that ACTH affects avoidance indirectly by stimulating the release of vasopressin, the effect of ACTH 4-10 on the passive avoidance of Brattleboro and LE rats was observed (Chapter V). If this hypothesis is true, then the passive avoidance of DI rats should be unaffected by ACTH 4-10. In the last experiment vasopressin was administered to DI rats and its effect on passive avoidance was measured (Chapter VI). Additional data were gathered on the rats in this experiment including the levels of dopamine beta hydroxylase and corticosterone in serum and norepinephrine in brain.

The difficulty of breeding and maintaining a large number of Brattleboro rats made it necessary to use these animals in more than one experiment. The sequence of experimentation was planned, however, so that interaction between experiments was minimized. For instance, one group of Brattleboro females was observed in the open field (Chapter III - Open Field Test 2), tested 2 1/2 months later on passive avoidance (Chapter II), and finally retested on passive avoidance 4 1/2 months later (Chapter V - Experiment ACTH 1). Except for the open field (Chapter III - Open Field Test 1) and passive avoidance experiments (Chapter V - Experiment ACTH 2) on age matched Brattleboro and LE rats which were separated by only two weeks, all experiments on the same animals were conducted at least two months apart.

Some data were amenable to standard parametric statistical analyses, e.g. open field activity. Other data, however, like latency scores in passive avoidance experiments and defecation measures were summarized by medians and analysed with non-parametric tests described by Siegel (1965). This was necessary because of the occurrence of skewed distributions, indeterminate values, and the small number of rats in each condition. In some cases the power of these tests was apt to be low with standard confidence levels, and often the importance of a Type I and a Type II error was judged approximately equal. Winer (1962, p. 13) has advised that lower confidence levels are desirable in such circumstances, and therefore confidence levels as low as .20 were given consideration as indicated in the text.

CHAPTER II

PASSIVE AVOIDANCE

The influence of pituitary hormones on active avoidance in normal rats is most evident during extinction (de Wied et al., 1972b). It is, therefore, not surprising that exogenous ACTH 4-10 and vasopressin are able to prolong extinction of passive avoidance (Bohus et al., 1973; Thompson & de Wied, 1973). On the other hand, the hypophysectomized rat, with deficiencies of all pituitary hormones, has been reported to extinguish faster than normal rats in passive avoidance (Weiss et al., 1969, 1970a). Since DI rats are similar in several respects to hypophysectomized rats in that they have disturbances of vasopressin, ACTH or corticosteroids, and probably growth hormone, it might be expected that DI rats would show rapid extinction of passive avoidance. In this experiment DI rats were compared to HE rats on a step-through passive avoidance task. In addition, two groups of LE rats, one similar in weight to the DI rats, the other similar in weight to the HE rats, were tested separately from the Brattleboro rats to determine if differences in weight per se would affect avoidance performance.

Method

Subjects. Seven month old male (9 DI & 7 HE) and female (11 DI & 13 HE) rats were used in this study. They were bred from HE females and DI males obtained from the National Institutes of Health. At one

month of age the pups were separated from the mother and housed in wire cages, 4-5 rats in each cage. Food and water were available ad libitum. Overhead fluorescent lighting was on from 0800 to 2000 hours each day. At two months of age DI rats were identified on the basis of their 24 hour water intake, and all rats were marked by ear punches. One HE male, three HE females and one DI female were albino mutants. At 4 1/2 months of age all participated in an open field experiment which will be described in Chapter III. Passive avoidance training was started at age seven months.

Two groups of LE female rats weighing 130-140 g and 190-200 g, respectively, were obtained from Blue Spruce Farms, Altamont, N.Y., so that after 7-10 days in the animal colony their weights would approximate those of the Brattleboro females. Unlike the Brattleboro rats, these LE rats were not tested in the open field.

Apparatus. Passive avoidance conditioning was carried out in the step-through apparatus described by Weiss et al. (1970a) and is shown in Fig. 1. The apparatus was located in a small semi-darkened room a short distance from the animal colony.

Procedure. On the first day of the experiment each rat was placed in the small chamber facing away from the larger adjoining compartment. As the rat turned around, the door between the small and large compartment was opened and a timer started. As soon as the rat was within the large compartment, the timer was stopped, the door closed, and a 1.0 ma a.c. scrambled grid shock was administered for 2 sec. The rat was removed from the compartment 15 sec after termination of shock and returned to its cage. Single retention trials

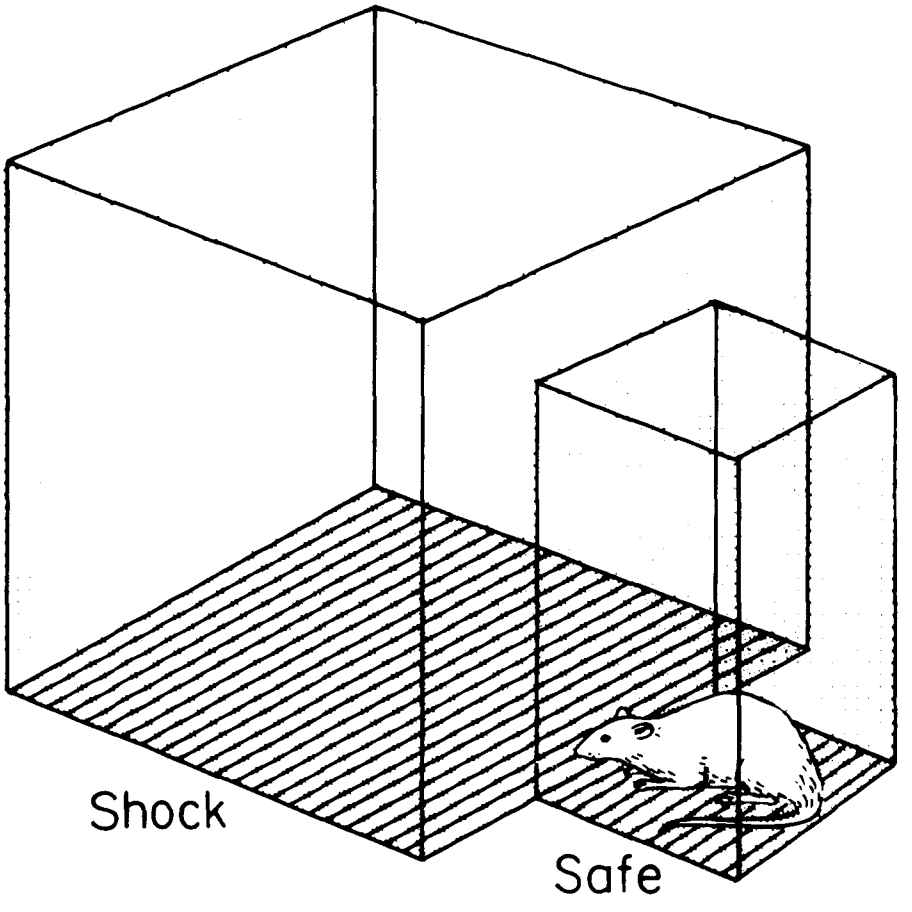


Fig. 1. Passive avoidance apparatus.

were given on the second and third day. On these and subsequent trials no shock was administered and rats not entering the large compartment within 360 sec were returned to their cages. Retention was tested in the same way on the fourth and fifth days except that the three day old sawdust underneath the grids was removed, the clear plexiglas walls were covered with white paper and the grid floor was covered with white paper over cardboard. This procedure has been shown to increase the sensitivity of the passive avoidance test to differences in hormonal status (Weiss et al., 1969) and was used in all subsequent passive avoidance experiments. Step-through latencies were timed to the nearest 1/10 sec with a stopwatch and the boluses excreted by each rat were counted and removed. After completion of the experiment on day 5, the animals were weighed. About 2 1/2 weeks after the experiment the Brattleboro rats were transferred to individual cages in the colony room and water intake, estimated from changes in weight of the water bottles, and urine volume were measured. Urine osmolarity was determined with a Fiske osmometer.

Results

The median step-through latencies on all five days for 15 Brattleboro males and 22 females are shown in Fig. 2 and Fig. 3, respectively. One DI male and two HE females were not included in the data analysis because of procedural error or equipment failure. On the first day all animals quickly entered the large compartment and there were no significant differences between the latencies of HE and DI rats, but on the following days both male and female DI rats displayed passive avoidance extinction deficits in comparison to HE

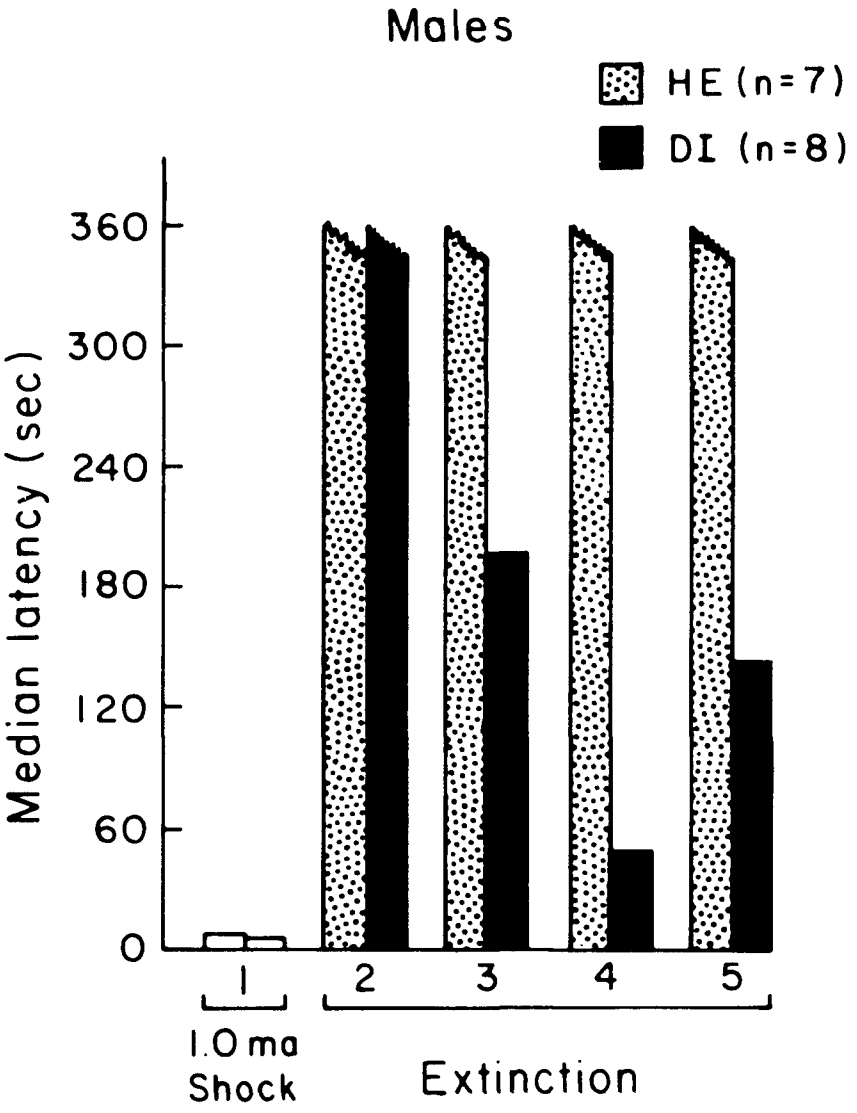


Fig. 2. Passive avoidance of Brattleboro males.

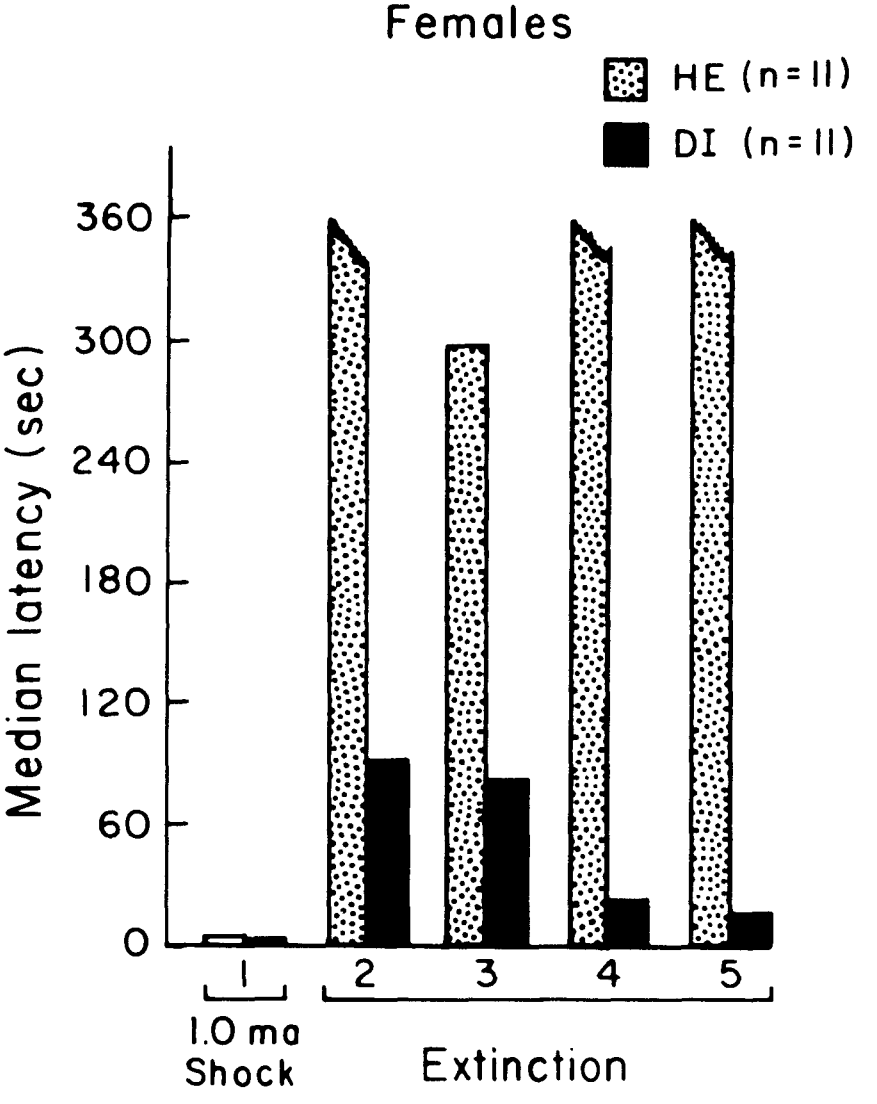


Fig. 3. Passive avoidance of Brattleboro females.

rats. On days 2 to 5 only one of the seven HE males entered the compartment within the cutoff latency of 360 sec. Although half of the DI males did not step out on days 2 and 3, on days 4 and 5 all but one or two entered the compartment and their latencies were shorter than HE rats as determined by two-tail Mann-Whitney tests (day 4, $p = .02$; day 5, $p = .014$).

The data for the females are similar to those for the males except that the median latencies of the DI females appear shorter than DI males. On each of the four days of extinction, DI females stepped into the shock compartment faster than the HE females ($p = .02$ on each trial, Mann-Whitney tests). Male and female DI rats excreted fewer boluses than HE rats on day 4 and day 5, and fewer DI females defecated on day 5 than HE females ($p = .05$, Fisher-Yates test).

There were significant differences in body weight between HE and DI rats of the same sex and between sexes, as can be seen in Table 1. Males were heavier than females, and HE rats were heavier than DI rats. There was little overlap in the distribution of male HE and DI weights--only one HE male weighed less than 325 g, while only on DI male weighed more than 291 g. No overlap was observed in the weight distributions of HE and DI females. Sokol (1973) has also observed that DI rats are smaller than HE rats. Table 1 also shows that DI rats consistently drank more water, and excreted larger and less concentrated amounts of urine than did HE rats. The 6:1 ratios of urine osmolarities for HE and DI rats were similar to those reported by Valtin et al. (1965) and Swaab et al. (1973). The absolute osmolarities observed in this study were higher than they reported, however, because of urine evaporation during the 24 hour collection period and because of a two week delay

TABLE 1

PHYSICAL CHARACTERISTICS OF DI AND HE RATS

	Body Weight (g ± s.e.m.)	Water intake (g/day ± s.e.m.)	Urine volume (ml/day ± s.e.m.)	Urine osmolarity (mOsm/kg ± s.e.m.)
Males				
HE (n=7)	363 ± 23	38 ± 5	7 ± 1	3985 ± 237*
DI (n=7)	270 ± 11	233 ± 15	196 ± 15	607 ± 40
Females				
HE (n=11)	219 ± 5	41 ± 2	8 ± 1*	5483 ± 284*
DI (n=11)	160 ± 4	200 ± 6	156 ± 7	941 ± 30*

*Data of one subject were lost.

before the osmolarity of the samples could be measured.

The mean weights of the two LE groups were 186 ± 4 g and 230 ± 7 g which are to be compared to the DI animals weighing 160 ± 4 g and to the HE animals weighing 219 ± 5 g in the previous experiment. Both LE groups were somewhat heavier than the two Brattleboro groups but there was approximately the same range of body weight between them as between DI and HE rats. The median latencies of the 186 g group appeared to be slightly longer than those of the heavier 230 g group on days 3 and 4 of extinction (Fig. 4). Mann-Whitney tests (two-tail), however, revealed that there were no significant differences. The median bolus count for each group was zero on all days.

Comparisons of the median latencies of the six lightest animals in the 186 g group (mean weight = 178 g) vs the six heaviest animals in the 230 g group (mean weight = 239 g) did not reveal differences any greater than those obtained with the whole group comparisons, even though the mean weight difference between the six lightest and the six heaviest LE females was similar (61 g) to that observed between the DI and HE females (59 g).

Discussion

The data show that both male and female DI rats are more likely to enter the compartment where they previously received shock than HE rats. The shorter passive avoidance latencies of DI rats cannot be explained by the hypothesis that lighter rats are less sensitive to shock, as lighter rats have been found to be more sensitive to foot-shock than heavier ones (Gibbs, Sechzer, Smith & Weiss, 1973; Paré, 1969). HE rats tend to defecate more than DI rats, suggesting that HE rats are more fearful (J. Gray, 1971a; Weiss et al., 1970a), but

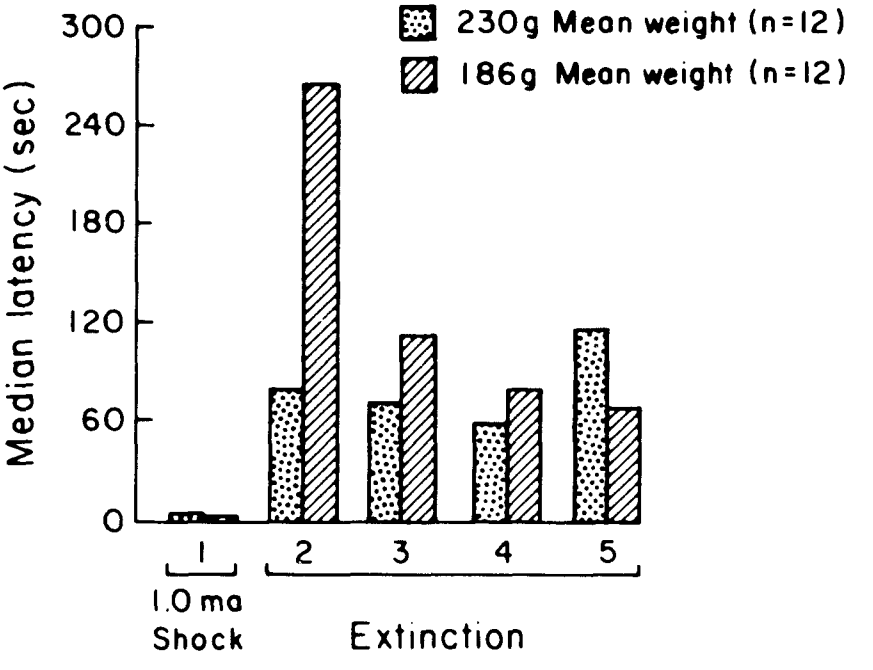


Fig. 4. Passive avoidance of Long-Evans females as a function of body weight.

this may be a necessary consequence of the fact that HE rats spent more time in the apparatus (Pinel, Malsbury & Corcoran, 1971). Since the differences in water intake, urine volume, and urine osmolarity between DI and HE rats were similar to those reported by Valtin et al. (1965), it was assumed that DI rats in the present study were also unable to synthesize vasopressin.

The passive avoidance latencies of DI females (Fig. 3) appeared shorter than LE females (Fig. 4) on the last two days of extinction, as might have been expected; however, the latencies of HE females were much greater than those of LE females. If HE and LE rats are similar except for the slight vasopressin defect in HE rats, then it would seem that the latencies of HE females should be slightly below those of LE females. Although part of the difference between HE and LE females might be explained by the fact that HE females were four or five months older than LE females, the magnitude of the differences in latency suggests that HE rats differ in more than one way from normal LE rats.

CHAPTER III

ACTIVITY IN THE OPEN FIELD

The previous experiment demonstrated that DI rats returned to a compartment in which they received footshock much sooner than did HE rats. Although differences in body weight per se did not explain this difference in performance, it is still possible that differences in activity or exploratory tendencies could account for the findings. If DI rats show stronger exploratory or activity tendencies than HE rats they are more likely, therefore, to enter the shock compartment on extinction trials. In this regard, Endröczi (1972) reported that activity levels in a maze-type open field were inversely related to the rate of learning of a passive avoidance response. To evaluate the possibility that differences in passive avoidance performance of DI and HE rats were related to differences in their characteristic levels of activity or exploratory tendencies, observation was made of their behavior during a single open field test. The behavior of the rat in response to a brief exposure to an open area, particularly ambulation and defecation, is interpretated as indicating either activity, 'emotionality' (Whimby & Denenberg, 1967) or fearfulness (J. Gray, 1971a).

Two open field studies were carried out. Open Field Test 1 was designed to compare HE and DI rats with each other and with age-matched normal LE rats. The comparison of HE and LE rats was of interest because the synthesis and release of vasopressin in HE rats is partially

impaired (Miller & Moses, 1971; Moses & Miller, 1970; Valtin et al., 1965). The purpose of Open Field Test 2 was to determine the role of hypokalemia in the open field activity of DI rats. If it is hypokalemia rather than a primary deficiency of vasopressin that affects the activity of the DI rat, then it should be reversed by potassium supplementation.

Open Field Test 1

Method

Subjects. Ten DI (4 male & 6 female) and twelve HE (6 male & 6 female) Brattleboro rats were obtained from the National Institutes of Health at two months of age. At the same time, 12 male and 12 female LE rats of the same age (± one day) were obtained from Blue Spruce Farms, Altamont, N.Y. All animals were housed under the same conditions previously described. Both strains had been used in passive avoidance experiments with ACTH 4-10 10 days previously (males), or 14 days previously (females) described in Chapter V. At the time of open field testing the rats were a little over three months old.

Apparatus. The open field was a 120 cm square plywood box painted flat black with walls 39 cm high. The floor of the field was divided into 24 cm squares outlined in black enamel. The apparatus was placed at one end of the animal colony room to avoid unnecessary handling of the rats during transport to and from the cage.

Procedure. Each rat was placed in the center of the open field, and the number of squares crossed and rearings on hind legs were recorded at one minute intervals. A stopwatch was used for timing, and

tally counters for cumulating crossings and rearings. After three minutes of observation the rat was returned to its cage, the number of boluses recorded and the field wiped clean with paper towels. An effort was made to minimize the influence of one rat on another: males were tested three days after females; before each experiment the field was prepared by a DI, HE and LE rat of the same sex from the animal colony; and the rats were run in randomly ordered groups with the constraint that each group contained one HE, one DI and one or two LE rats.

Results

Activity. The mean number of crossings and rearings for HE, DI and LE groups of each sex is shown in Fig. 5. Both DI and HE groups of

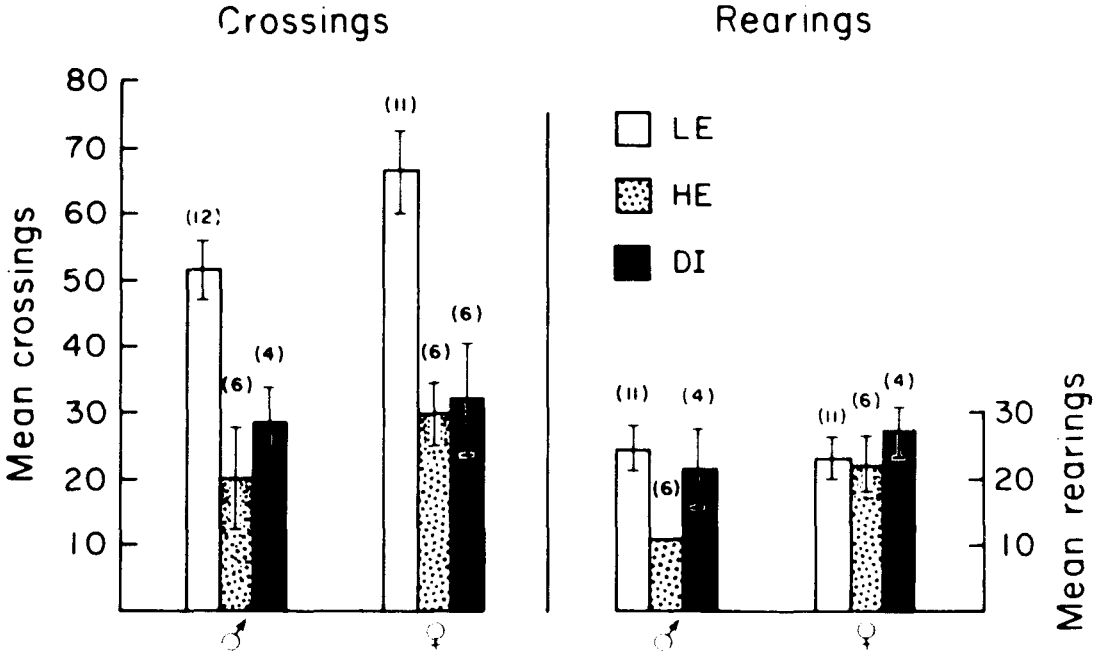


Fig. 5. Open field activity of Brattleboro and Long-Evans rats. The bars represent mean crossings and rearings ± s.e.m. observed in these groups. Numbers in parentheses indicate the number of animals included in each mean.

each sex were very much less active than the LE groups, making about half as many crossings. Comparisons of the effects of genotype and sex on crossings were made by analysis of variance (Table 2). Genotype but not sex differences were significant and Newman-Keuls comparisons indicated that while DI and HE rats did not differ between themselves in the number of crossings, each made significantly fewer crossings than the LE rats.

Table 2

ANALYSIS OF VARIANCE OF CROSSINGS: GENOTYPE VS SEX

Source of variation	Sum of squares	df	Mean square	F	p
Between subjects					
Genotype (G)	8666.87	2	4333.43	15.42	<.01
Sex (S)	860.93	1	860.93	3.06	n.s.
GS	216.45	2	108.22	<1	n.s.
Within cell	10962.31	39	281.08		

An analysis of the crossings by male rats of each group during each of the three minutes of the test indicated that over-all differences between the LE and Brattleboro groups were in part related to a decline in the activity of Brattleboro groups on the second and particulary the third minute while a constant level of activity was exhibited by LE rats during the entire test.[5]

[5]See additional open field data, Appendix II.

The failure to find significant sex differences for the DI and HE rats is not surprising, given the overlap in their standard errors, but this was not the case for LE rats. The F statistic, however, is known not to be as powerful as the method of individual comparisons when the means obtained from treatment distributions are not normally distributed, e.g. the means for several treatments fall close together, at some distance from other treatment means (Winer, 1962, p. 78). Because the mean number of crossings for DI and HE rats were similar and considerably below those of the LE rats, pairwise comparisons of all group means were made using the Newman-Keuls procedure. The results are exactly the same as with the above analysis except that the LE females were found to have made significantly more crossings than the LE males ($p < .05$).

Except for the HE males, who made relatively few rearings, Brattleboro and LE rats of both sexes reared about equally often (Fig. 5). No differences between groups or between males and females were statistically significant (Table 3).

Table 3

ANALYSIS OF VARIANCE OF REARINGS: GENOTYPE VS SEX

Source of variation	Sum of squares	df	Mean square	F	p
Between subjects					
Genotype (G)	496.87	2	248.43	2.46	n.s.
Sex (S)	257.89	1	257.89	2.55	n.s.
GS	232.39	2	116.20	1.15	n.s.
Within cell	3836.45	38	100.96		

Defecation. HE males excreted slightly more boluses (Mdn = 4) than either the DI (Mdn = .5) or LE (Mdn = 0) groups, but these differences did not reach statistical significance. Among females, the HE rats (Mdn = 2.5) excreted more boluses than DI rats (Mdn = 2.0) and LE rats (Mdn = 0) but Mann-Whitney tests (two-tail) indicated that only the difference between DI and LE rats was significant ($p = .05$).

Open Field Test 2

Method

Subjects. Ten DI and seven HE male, and twelve DI and fourteen HE female rats, all 4 1/2 months old and previously described in Chapter II were employed in this experiment. For a two week period prior to open field testing, one half of both groups of rats were supplemented with 1% potassium chloride (KCl) solution ad libitum.

Apparatus. The open field was described earlier.

Procedure. The general procedure was like that of Open Field Test 1 with the following exceptions. Male and female rats were all tested on the same day in the early afternoon. The males were observed first and the field was thoroughly cleaned with water and dried before the females were observed. HE and DI rats were run in random order but no special preparation of the field was made before testing.

Results

Activity in KCl supplemented rats. Analysis of the data indicated that there was no consistent effect of KCl administration on the mean number of crossings by HE or DI rats. KCl treated rats seemed,

however, to rear slightly less often than control rats (Table 4).

Table 4

OPEN FIELD PERFORMANCE OF DI AND HE RATS:

EFFECT OF KCl SUPPLEMENTATION

Group	Crossings $\bar{x}$ ± s.e.m.		Rearings $\bar{x}$ ± s.e.m.	
	Control	KCl	Control	KCl
Males				
DI (n=5)	54 ± 10	66 ± 3	25 ± 6	16 ± 3
HE (n=3)	35 ± 11	62 ± 4	27 ± 4*	20 ± 7
Females				
DI (n=6)	58 ± 5	62 ± 4	25 ± 4	20 ± 4
HE (n=7)	55 ± 2	52 ± 6	26 ± 4	23 ± 2

*Rearings not recorded for one animal in this group.

Analyses of variance on total crossings and rearings of female rats did not reveal any significant differences even with a p value of .10 between HE and DI rats, or between rats who received or did not receive KCl supplementation (Tables 5, 6).

The data for male rats were not similarly analysed because only three of six HE males received KCl, and rearings were not recorded for one HE male receiving KCl. The number of crossings made by each of the three HE-KCl males were all higher than those recorded for the HE-control males. The number of rearings of the two HE-KCl males was within the range of rearings observed in the HE-control males. Among

TABLE 5

ANALYSIS OF VARIANCE OF CROSSINGS: KCl EFFECT--FEMALES

Source of variation	Sum of squares	df	Mean square	F	p
Between subjects					
Genotype (G)	347.63	1	347.63	2.25	n.s.
KCl (K)	12.33	1	12.33	<1	n.s.
GK	158.54	1	158.54	1.02	n.s.
Within cell	3404.19	22	154.74		

TABLE 6

ANALYSIS OF VARIANCE OF REARINGS: KCl EFFECT--FEMALES

Source of variation	Sum of squares	df	Mean square	F	p
Between subjects					
Genotype (G)	46.79	1	46.79	<1	n.s.
KCl (K)	125.42	1	125.42	1.83	n.s.
GK	10.29	1	10.29	<1	n.s.
Within cell	1508.47	22	68.57		

DI males there was no significant difference between KCl and control animals in the mean number of crossings or rearings (crossings, $t = 0.26$; rearings, $t = 0.57$, df = 8). As no effect of KCl supplementation on open field activity was demonstrated, except in HE males, the data for control and KCl supplemented animals in DI and HE groups were combined

in the remaining analyses.

Activity in KCl and control groups combined. Fig. 6 shows the open field activity of male and female HE and DI rats. Analysis of

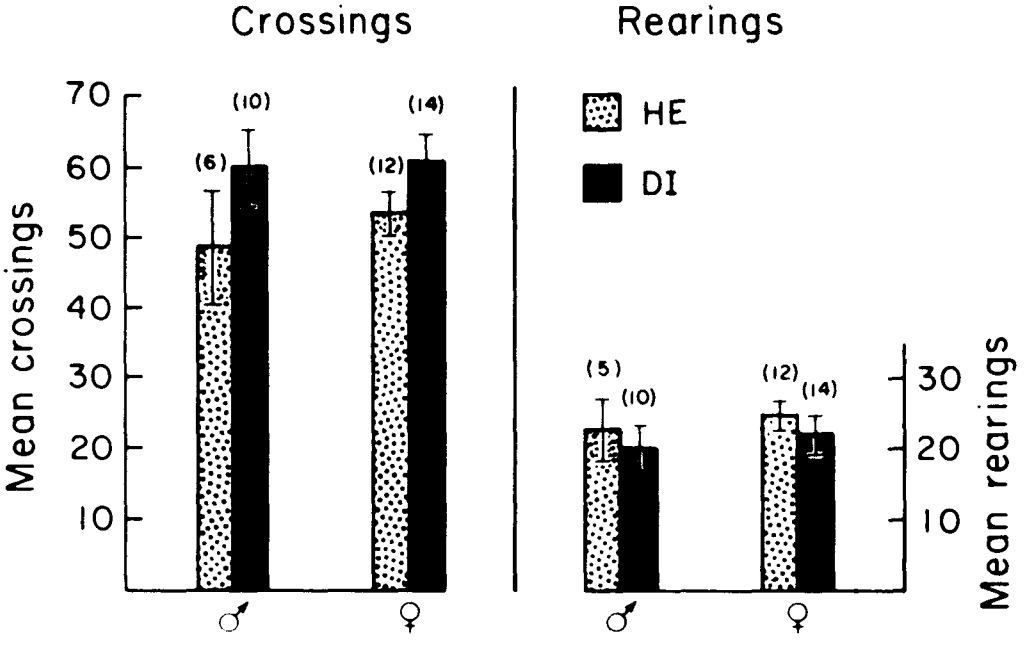


Fig. 6. Open field activity of Brattleboro rats. The bars represent mean crossings and rearings ± s.e.m. observed in these groups. Numbers in parentheses indicate the number of animals included in each mean.

variance comparisons of genotype (HE or DI) and sex effects on crossings were not significant (Table 7). In the analysis of variance of rearings the mean square for genotype was significantly less ($p < .01$) than the within cell mean square (Table 8). As genotype did not have a significant simple effect in either males or females considered separately,

the explanation for this might lie in some difference in the experimental situation between the female and male open field tests which might have decreased the size of the mean square for genotype relative to the mean square for the interaction.

TABLE 7

ANALYSIS OF VARIANCE OF CROSSINGS: GENOTYPE VS SEX

Source of variation	Sum of squares	df	Mean square	F	p
Between subjects					
Genotype (G)	823.77	1	823.77	3.83	n.s.
Sex (S)	86.40	1	86.40	<1	n.s.
GS	37.34	1	37.34	<1	n.s.
Within cell	8174.27	38	215.11		

TABLE 8

ANALYSIS OF VARIANCE OF REARINGS: GENOTYPE VS SEX

Source of variation	Sum of squares	df	Mean square	F	p
Between subjects					
Genotype (G)	.19	1	.19	1/F=454	<.01
Sex (S)	39.23	1	39.23	<1	n.s.
GS	56.99	1	56.99	<1	n.s.
Within cell	3024.18	37	81.73		

Minute by minute comparisons of crossings and rearings over three minute test period showed that there were no significant differences between DI and HE rats either in the over-all level or in the distribution of open field activity over time.[6]

Defecation. The median number of boluses recorded for DI males (3) was higher than for HE males (0; $p = .001$, Mann-Whitney test, two-tail). Among the females, the bolus count was greater for DI rats than for HE rats (Mdn = 1), but this difference was not significant.

Discussion

The behavior of DI and HE rats in the open field was quite similar in both Open Field Tests 1 and 2. This suggests that the marked differences in passive avoidance behavior observed in Chapter II cannot be explained indirectly by characteristic differences between DI and HE rats in activity level or exploratory drive. The ambulation of DI and HE rats in Open Field Test 1 was much lower than in Open Field Test 2. The indication from the second open field test that DI males excreted more boluses than HE males was not evident in the first test. These differences between the open field tests are probably not related to the differences in the ages of the rats in these experiments, because defecation and ambulation in the open field have been reported not to change appreciably between the age of two and six months (Candland & Cambell, 1962).

The main finding of the open field experiments was that DI and HE rats made less than half the number of crossings made by normal LE

---

[6] See additional open field data, Appendix II.

rats, but reared equally often (Open Field Test 1). When approached at the end of the testing period the Brattleboro rats froze or remained quiet in contrast to the LE rats that ran away from the experimenter's hand. The lower activity level of Brattleboro rats may reflect diminished active escape behavior or a weaker exploratory tendency. The lower activity and generally higher defecation rate of Brattleboro rats also might indicate that they are more fearful or emotional than LE rats. Gray and others have argued that fear inhibits exploration (J. Gray, 1971a; Montgomery, 1955; Russell, 1973b), and a review of many open field studies concluded that ambulation and defecation in the open field are negatively correlated (Archer, 1973). Whatever the nature of the difference between the response of the LE and Brattleboro rats to the open field, it is important to consider what variable may be responsible. One possibility is that genetic differences unrelated to those responsible for the defect in vasopressin metabolism are involved. Another, is that the open field activity of the LE and Brattleboro rats may reflect differences in their early handling and life history (Denenberg, 1967). For instance, the greater urine volume of HE and DI pups required that the breeding cage be frequently changed, so that they may have received more handling than LE pups.

Except for HE males, the provision of a 1% potassium chloride solution to DI and HE rats did not affect open field activity. Potassium treated HE males made more crossings than HE control males but the small number of animals in these groups and the substantial variation in the scores of both DI and HE male rats suggests that further verification of an effect of potassium chloride in HE males is necessary. Potassium supplements to hypokalemic DI rats did not, however, affect

their open field behavior. This indicates that the difference between DI and LE rats observed in Open Field Test 1 may not be directly related to differences in their potassium balance. It is possible, however, that extra potassium provided to DI rats may not be effectively absorbed or utilized in the absence of vasopressin.

CHAPTER IV

ACTIVE AVOIDANCE

Hormonal factors are believed to affect the acquisition and extinction of shuttlebox avoidance. From the work of de Wied it appears that the impaired acquisition of shuttlebox avoidance by adenohypophysectomized and hypophysectomized rats may be related to a deficiency of ACTH (de Wied, 1964, 1969a, 1969b). On the other hand, the rapid extinction of neurohypophysectomized and hypophysectomized rats treated with cortisone, testosterone and thyroxin during acquisition, as compared to normal or adenohypophysectomized rats, suggests that posterior or intermediate lobe hormones may be important in maintaining the avoidance response once it has been acquired. The differential involvement of anterior pituitary hormones in shuttlebox acquisition, and posterior or intermediate lobe hormones in extinction is also suggested by the fact that the acquisition of shuttlebox avoidance by neurohypophysectomized rats is not different from normal animals (de Wied, 1965).

In this experiment, DI and HE rats were compared on shuttlebox avoidance to determine whether these rats would show similar acquisition and extinction.

Method

Subjects. Subjects were 8 DI and 7 HE male rats aged 8 1/2 months who had participated in the passive avoidance experiment (Chapter II).

Apparatus. The shuttlebox was a clear plexiglas box 30 x 30 x 25 cm divided into two halves by a barrier 5 cm high. The unconditioned stimulus (US) was a 0.6 ma d.c. shock which was passed through a mechanical scrambler and delivered to the animal through the stainless steel grid floor of the shuttlebox. The floor of each half of the box was independently hinged so that whenever the animal stepped on it a microswitched closed; a response was recorded when one microswitch opened and the other closed as the animal moved from one side of the box to the other. The conditioned stimulus (CS) was a 1000 Hz tone of 80 db intensity.[7] Stimuli were presented and responses recorded automatically by an electromechanical control system.

Procedure. Just before the first trial, the animal was allowed a three minute adaptation period in the box; thereafter only 30 sec were allowed each day before training. If the rat did not cross to the opposite side of the box within five sec after the onset of the CS, the US was administered and both the CS and US remained on until escape occurred or until 23 sec had elapsed. Ten trials per day were given until a criterion of 80% avoidance on three consecutive days was reached. Animals that did not reach criterion between 120-140 trials were not tested further. For the others, 10 extinction trials were given on each of four days following criterion performance. The procedure was the same as during acquisition except that the CS was terminated after five sec and no US was given.

---

[7] Sound pressure level (SPL) measurements re 20 uN/m^2 were made with a sound survey meter (Type 1555-A, General Radio Co.). Ambient SPL was about 50 db and the variation of the SPL of the CS at various locations within the box was less than 10%.

Results

Only two out of eight DI rats reached criterion within 140 trials. In contrast, five of seven HE rats were able to reach this criterion. The difference in the number of animals reaching criterion was not significant (Fisher-Yates test, $p > .05$). Although DI rats made slightly fewer avoidances than HE rats during the first days of acquisition (Fig. 7), analyses of variance on the number of avoidances

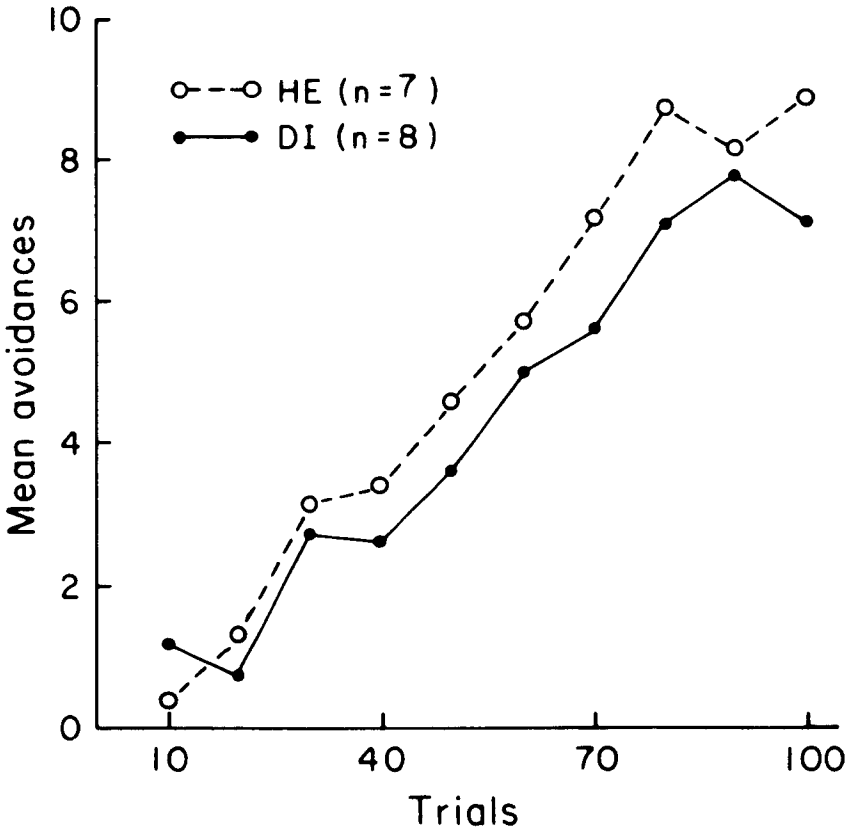


Fig. 7. Acquisition of shuttlebox avoidance by Brattleboro males.

(Table 9), number of consecutive avoidances (Table 10) or number of intertrial responses (Table 11) using the transformation $\sqrt{x} + \sqrt{x+1}$

TABLE 9

ANALYSIS OF VARIANCE: ACQUISITION OF SHUTTLEBOX AVOIDANCE

Source of variation	Sum of squares	df	Mean square	F	p
Between subjects					
Genotype (G)	1.86	1	1.86	<1	n.s.
Subjects w.g.	80.65	13	6.20		
Within subjects					
Days (D)	374.96	9	41.66	26.88	<.01
GD	8.04	9	.89	<1	n.s.
D x subjects w.g.	181.68	117	1.55		

TABLE 10

ANALYSIS OF VARIANCE: CONSECUTIVE AVOIDANCES

Source of variation	Sum of squares	df	Mean square	F	p
Between subjects					
Genotype (G)	5.96	1	5.96	<1	n.s.
Subjects w.g.	99.59	13	7.66		
Within subjects					
Days (D)	437.43	9	48.60	10.92	<.01
GD	15.80	9	1.76	<1	n.s.
D x subjects w.g.	521.05	117	4.45		

TABLE 11

ANALYSIS OF VARIANCE: INTERTRIAL RESPONSES

Source of variation	Sum of squares	df	Mean square	F	p
Between subjects					
Genotype (G)	7.75	1	7.75	1.37	n.s.
Subjects w.g.	73.36	13	5.64		
Within subjects					
Days (D)	427.82	9	47.54	19.02	<.01
GD	15.85	9	1.76	<1	n.s.
D x subjects w.g.	292.48	117	2.50		

to achieve homogeneity of variance did not reveal any significant differences between them on these measures. Examination of the performance of individual animals in each group, however, suggested that the performance of the DI rats deteriorated, did not continue to improve or became quite variable after 10 or 11 days. The median escape latencies of both DI and HE rats were in the range of 1.0 - 1.5 sec on all but the first three days of training. On these three days the escape latencies of HE rats, 12.5, 8.0 and 7.5 sec; and DI rats, 9.5, 6.5 and 6.0 sec were long but not significantly different (Mann-Whitney tests).

During the course of 40 extinction trials there was no deterioration of avoidance responding for the five HE (mean avoidance/day = 8.5) or the two DI rats (mean avoidance/day = 9.1) that reached criterion.

Discussion

It was difficult to decide whether the avoidance performance of the DI rats resembled that of HE rats. Only a few DI rats were eventually able to reach criterion, but their performance during the first 100 trials was not very much below that of HE rats. No conclusion could be reached about extinction as avoidance by both DI and HE rats continued at criterion level for 40 trials; a difference may well have appeared if more extinction trials had been run. The data do suggest, nevertheless, that under the conditions of this study, the avoidance capability of rats entirely without vasopressin is more like that of normal or neurohypophysectomized rats (de Wied, 1965), than hypophysectomized or adenohypophysectomized rats (de Wied, 1964, 1969a, 1969b).

It is tempting to speculate that the failure of most DI rats to improve in the last days of training could be related to difficulty in developing sufficient motivation from the presentation of the CS alone, without the stronger stress provided by UCS presentations. Thus, sufficient release of ACTH and corticosteroids with the stress of shock would allow adequate performance until the point where the rat would not be receiving enough shocks to maintain pituitary-adrenal activation. Another possibility is that the less robust and weaker DI rats--muscular weakness is a symptom of potassium deficiency--were not able to withstand the exertion and stress associated with daily active avoidance training over long periods of time. Because the escape latencies of DI and HE rats, however, were so similar it is reasonable to assume that the responsiveness of DI rats to shock was not a major factor in accounting for their failure to reach criterion.

CHAPTER V

EFFECTS OF ACTH 4-10 ON PASSIVE AVOIDANCE

The short passive avoidance latencies of DI rats described in Chapter II may have been related either to a deficiency of vasopressin or to changes in the activity of other hormonal systems in the absence of vasopressin. For instance, if the release of ACTH was impaired, then short passive avoidance latencies might also be predicted, as Weiss et al. (1969, 1970a) have suggested that the relatively short passive avoidance latencies of hypophysectomized rats are mainly related to a deficiency of ACTH. A straightforward method of determining the hormonal basis for the passive avoidance behavior of the DI rat would be to compare the effects of ACTH and vasopressin treatment; the hormone that increases DI latencies to the level of HE rats would presumably indicate the relevant hormone deficiency. However, in normal rats ACTH 4-10, MSH and vasopressin all prolong passive avoidance in a similar fashion when administered before training or extinction trials (Ader & de Wied, 1972; Bohus et al., 1972; de Wied, 1973; Dempsey et al., 1972; Guth et al., 1971; Sandman et al., 1971b), so it is conceivable that any of these hormones would be able to raise the passive avoidance latencies of DI rats to the level of HE rats.

The passive avoidance response of DI rats to ACTH may, nevertheless, be relevant to the hypothesis that ACTH influences avoidance by stimulating the release of vasopressin, which in turn affects performance. As ACTH 4-10 in a dose of 30 µg/100 g has been reported to

increase the antidiuretic activity of eye plexus blood and to prolong passive avoidance when administered one hour prior to an extinction trial (Thompson & de Wied, 1973), it is possible that the release of endogenous vasopressin rather than a direct effect of ACTH 4-10 was involved. If ACTH 4-10 increases passive avoidance latencies via the release of vasopressin, then the latencies of DI rats that are unable to synthesize vasopressin should be unaffected by ACTH.

In Chapter II it was suggested that the longer passive avoidance latencies of HE females relative to control LE females of similar or smaller size might indicate that HE rats are not 'normal' in spite of the fact that their water balance is in some respects similar to that of normal LE rats (Moses & Miller, 1970). The hypothesis was further justified by the marked difference in the response of the Brattleboro and LE rats to the open field (Chapter III). This raised the possibility that DI and HE rats might not respond to ACTH like normal LE rats, and in fact, a number of studies have reported 'paradoxical' effects of ACTH or MSH in animals that are 'hypoactive' or when moderate to high footshock levels are used (Korányi, Endröczi, Lissák & Szepes, 1967; Ley & Corson, 1971a, 1971b; Stratton & Kastin, 1974).

In this chapter two experiments are described in which ACTH 4-10 was administered to Brattleboro rats. In the first experiment ACTH 4-10 or vehicle was given to DI and HE rats before each extinction trial. The second experiment was a replication of the first experiment except that ACTH 4-10 or vehicle was administered before the first trial as well as before the extinction trials, and two groups of LE rats were also tested. One group consisted of LE rats matched in age with the Brattleboro rats. Injections before both training and

extinction were given in order to determine whether the effects of ACTH 4-10 in the first experiment would be reversed by the administration of ACTH 4-10 before the training trial on day 1, as has been reported by P. Gray (1975) for large doses of 'purified' ACTH administered in a gelatin vehicle.

ACTH Before Extinction Trials (Experiment ACTH 1)

Method

Subjects. The subjects were 14 DI and 17 HE female rats that had prior experience on open field and passive avoidance tests. All rats were 11 1/2 months of age except for four HE and two DI rats that were 10 months of age. The mean weights of the DI and HE rats were 183 ± 4 g and 230 ± 5 g, respectively.

Apparatus. The passive avoidance apparatus described in Chapter II was used.

Procedure. To insure that previous passive avoidance experience would not interfere, all rats were handled daily for one week and then given a two minute exposure to the shock compartment of the apparatus on three successive habituation days, followed by a single measurement of their step-through latencies into the shock compartment without administering shock (pre-test). Except as noted below, the testing procedure was the same as in Chapter II. An extra day of testing was given before changing the apparatus on the last two days, so that extinction trials were given on days 2 through 6. On the first day of the experiment each rat received a two sec 0.5 ma shock in series with a one megaohm resistor after stepping into the large compartment. The resistor was added to the shock circuit to further reduce the effect

of variations in resistance between rats on the current actually received. One hour before testing on the second through sixth days, 8 HE and 7 DI rats selected at random were injected subcutaneously with 30 μg/100 g of ACTH 4-10. The remaining 9 HE and 7 DI rats were injected with an acidified saline vehicle prepared as described by Orias & McCann (1972).

The ACTH 4-10 was synthesized by the solid-phase method and thoroughly purified and assayed for *in vitro* lipolytic activity according to the procedures of Draper, Merrifield & Rizack (1973). One batch of ACTH 4-10 was prepared for the first three days of the experiment and another for the remaining three days. Loss of activity with dilution was minimized by preparing the injection solutions at a concentration of 400 μg/ml. Because ACTH 4-10 is readily adsorbed to glass surfaces it was prepared in a plastic beaker and injected with plastic disposable syringes. Between injections the ACTH 4-10 was protected from light and both ACTH 4-10 and vehicle were kept on ice. The solutions were stored in the dark at 3°C overnight.

Results

The mean number of rearings and median defecation counts recorded during the habituation days were similar for both DI and HE rats (Table 12). On the passive avoidance pre-test the distribution of their step-through latencies was quite similar (*Mdn* = 3.0 sec for both groups).

Whereas on the first day of the passive avoidance experiment the median latencies of the treatment groups were not significantly different from one another, differences between treatment groups did

TABLE 12

REARING AND DEFECATION DURING HABITUATION

TO THE SHOCK COMPARTMENT

Habituation day	Rearings $\bar{X} \pm$ s.e.m.		Boluses Mdn	
	HE (n=17)	DI (n=16)	HE (n=17)	DI (n=16)
1	15 ± 4	19 ± 5	0	0
2	16 ± 5	17 ± 5	0	1
3	13 ± 3	17 ± 5	0	0

occur during the five days of extinction testing (Fig. 8). The latencies of DI-ACTH rats appeared shorter than DI-VEH rats on every day of extinction, and Mann-Whitney tests (two-tail) indicated that confidence levels approached significance on day 2 (p = .10), day 5 (p = .13) and day 6 (p = .18). However, the true difference between the latencies of DI-ACTH and DI-VEH rats may well have been greater if a longer cut-off latency had been used. The latencies of the DI-ACTH rats were shorter than HE-ACTH rats on day 2 (p = .04), day 4 (p = .07) and day 5 (p = .01), but this may in part be due to a slight but not significant elevation of latencies by ACTH 4-10 in HE rats, compared to HE-VEH rats, on the first three days of extinction.

The median number of boluses left in the apparatus each day was one or less for all groups except on day 2, when the DI-VEH animals left more boluses (Mdn = 3) than the HE-ACTH (Mdn = 2, p = .05), the DI-ACTH (Mdn = 1, p = .06), or the HE-VEH rats (Mdn = 0, p = .05) as determined by Mann-Whitney tests (two-tail).

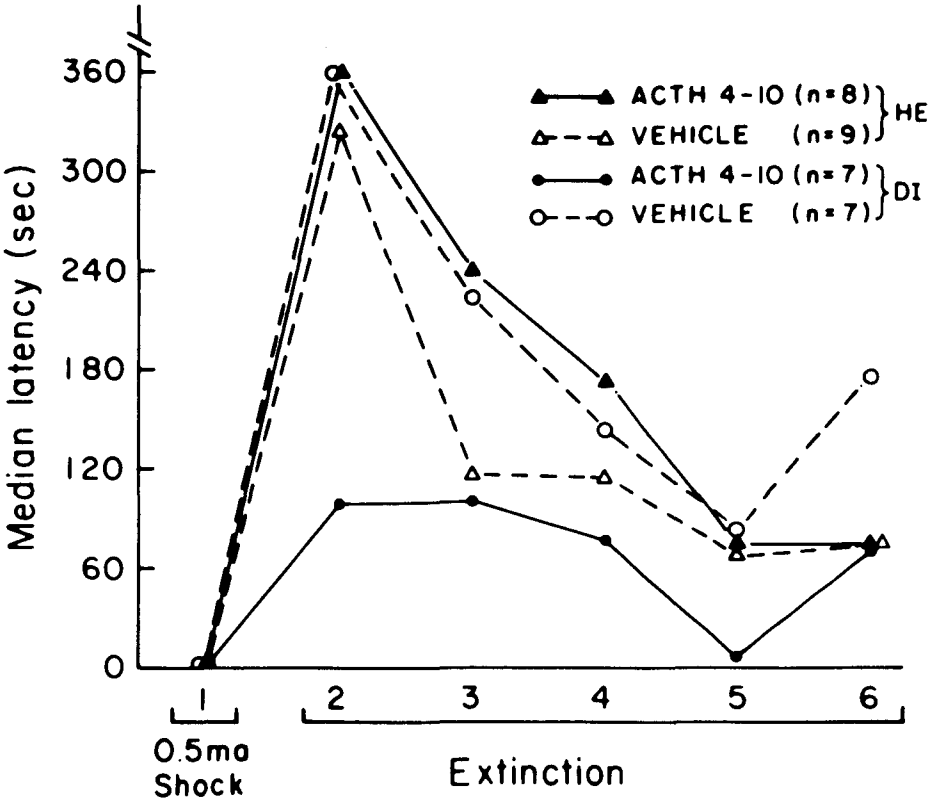


Fig. 8. Effect of ACTH 4-10 on passive avoidance of Brattleboro females. One hour before extinction trials on days 2-6 rats received injections of 30 µg/100 g of ACTH 4-10 or saline vehicle.

ACTH Before Training and Extinction Trials

(Experiment ACTH 2)

Method

Subjects. Two groups of rats were tested: the first consisted of 10 three month old LE females obtained from Blue Spruce Farms, Altamont, N.Y.; the second consisted of 4 DI, 6 HE and 12 LE males, and 6 DI, 6 HE and 12 LE females previously described in Chapter III. The mean weight of the LE females was 250 ± 5 g. In the second group,

male DI, HE and LE rats weighed 187 ± 14 g, 274 ± 7 g and 374 ± 7 g, and the female DI, HE and LE rats weighed 127 ± 2 g, 183 ± 4 g and 218 ± 4 g, respectively. These rats were just under three months of age and had not yet been run in the open field test. During the week prior to the experiment all rats were handled every other day.

Apparatus. The passive avoidance apparatus described in Chapter II was used.

Procedure. The rats in the first and second groups were tested as described in Experiment ACTH 1 except that: half of each group was injected with ACTH 4-10 or vehicle before the training trial on day 1 as well as before each of the four extinction trials; the dosage of ACTH 4-10 was the same as before, 30 µg/100 g for the first group; but slightly lower, 28 µg/100 g for the second group and the rats in the second group were run each day in randomly constituted groups containing one HE, one DI and one or two LE rats. Male rats were tested one week after the females

Results

Group 1--LE females. ACTH 4-10 treated female rats exhibited longer median passive avoidance latencies than females receiving vehicle (Fig. 9). Step-through latencies on day 1 were not affected by ACTH 4-10, but avoidance latencies on the first three extinction trials were longer in rats than had received ACTH 4-10. The latency distributions overlapped slightly so that confidence levels between .075 - .111 were obtained (Mann-Whitney tests, one-tail). There was no difference between ACTH 4-10 or vehicle injected rats in the number of boluses excreted.

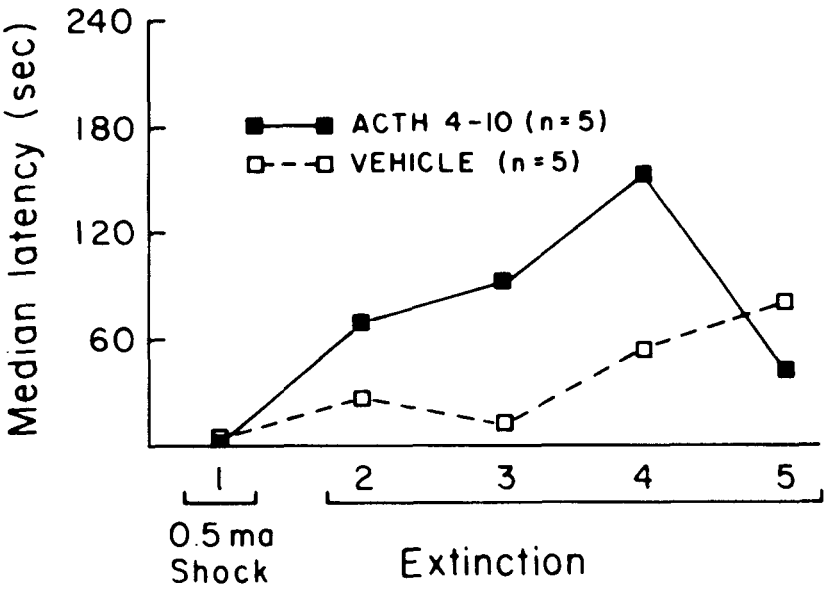


Fig. 9. Effect of ACTH 4-10 on passive avoidance of Long-Evans females. Rats received daily injections of 30 µg/100 g of ACTH 4-10 or saline vehicle one hour before testing.

Group 2--HE, DI and LE females. ACTH 4-10 reduced the passive avoidance latencies of DI females especially on day 3 (p = .20) and day 4 (p = .20). The latencies of HE females appeared slightly lower when treated with ACTH 4-10. It should be noted, however, that the differences between ACTH 4-10 and vehicle treated females in HE and DI groups were obscured by the ceiling effect of the cutoff latency, so that the magnitude of the effect of ACTH 4-10 in DI and HE groups is probably greater than is suggested by the latencies shown in Fig. 10. Another indication that ACTH 4-10 shortened the latencies of DI females was that the latencies of DI-ACTH females were shorter than those of HE-ACTH females on day 4 and day 5 (Mann-Whitney tests, two-

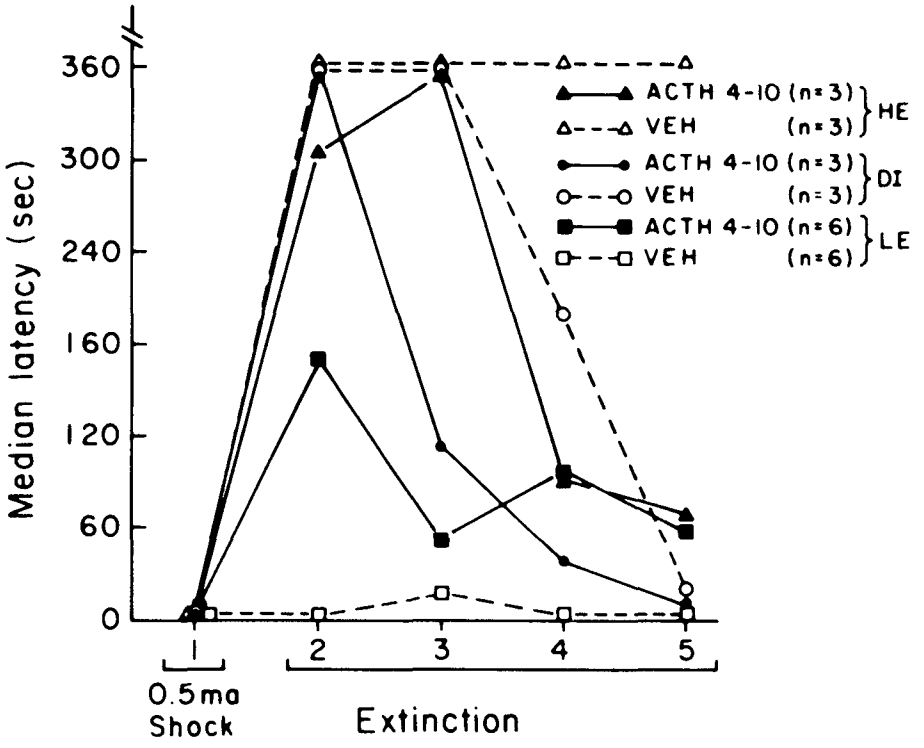


Fig. 10. Effect of ACTH 4-10 on passive avoidance of Brattleboro and Long-Evans females. Rats received daily injections of 28 µg/100 g of ACTH 4-10 or saline vehicle one hour before testing.

tail, $p = .05$). The effect of ACTH 4-10 on the latencies of LE females was opposite to its effects in Brattleboro rats. LE females treated with ACTH 4-10 displayed longer passive avoidance latencies on day 2 ($p = .039$), 3 ($p = .047$), 4 ($p = .013$) and 5 ($p = .008$) as well as on day 1 ($p = .047$), the training day (Mann-Whitney tests, one-tail).

Differences were found between vehicle and ACTH treated HE, DI and LE groups, independently of the effect of ACTH 4-10; HE-VEH rats seemed to have longer latencies than DI-VEH rats, particularly on the last two extinction trials; and both DI-VEH and HE-VEH rats had

longer latencies than LE-VEH rats on the last four days of extinction testing (Mann-Whitney tests, two-tail, $p = .10$). It should be noted that the LE-VEH rats did not really avoid the shock compartment on extinction trials as their latencies were similar on most days to those on the training day before they received shock.

The median number of boluses excreted by ACTH 4-10 and vehicle treated females within each group were quite similar on all days. There were, however, marked differences between Brattleboro and LE rats. The median number of boluses excreted by LE rats was zero on all five days, but the median number of boluses excreted by DI and HE females was higher and quite similar for both groups (overall Mdn = 4, range 1-6). Fisher-Yates calculations of the probabilities associated with the number of rats defecating each day in the HE and LE groups indicated that proportionally more DI than LE females defecated on day 2 ($p = .005$) and on day 4 ($p = .025$). Mann-Whitney tests suggested similar conclusions.

Group 2--HE, DI and LE males. In contrast to the relatively long latencies displayed by female rats in this passive avoidance situation, the latencies of male rats were surprisingly short (Fig. 11). Because of the small number of DI males and because the range of latencies for ACTH 4-10 and vehicle treated DI males overlapped considerably, these rats have been grouped together in Fig. 11 and in the statistical analyses of the data. ACTH 4-10 did not significantly increase the latencies of HE males or seem to affect DI or LE males. The reason why ACTH 4-10 did not affect the performance of LE males is not clear, but it may be that the level of footshock was not high enough to affect rats of this size.

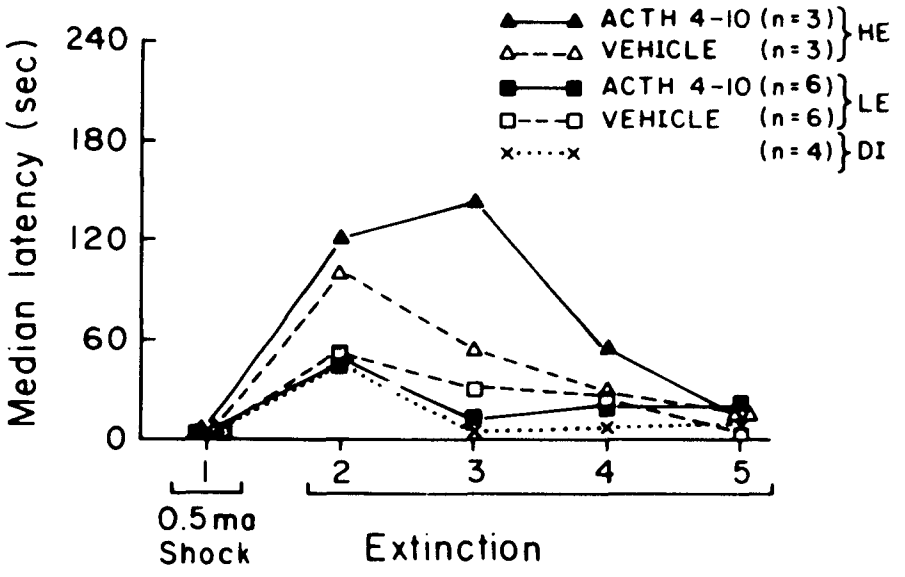


Fig. 11. Effect of ACTH 4-10 on passive avoidance of Brattleboro and Long-Evans males. Rats received daily injections of 28 µg/100 g of ACTH 4-10 or saline vehicle one hour before testing.

The passive avoidance latencies of DI and LE-VEH males, or HE-VEH and LE-VEH males were not significantly different on any day, but the latencies of DI males appeared shorter than those of HE-VEH males on day 1 (p = .056), 3 (p = .11), and 4 (p = .11).

No differences were noted in the number of boluses excreted by ACTH 4-10 or vehicle injected rats or in the number of rats in each group that defecated. A greater number of Brattleboro rats defecated during testing than LE rats; more HE rats defecated than LE rats on day 2 (p = .025), day 3 (p = .05), and day four (p = .025), while more DI rats defecated on day 5 (p = .06) than LE rats.

Discussion

In both experiments the passive avoidance latencies of DI females injected with ACTH 4-10 were shorter than either those of DI females injected with vehicle or those of HE females injected with ACTH 4-10. Although the confidence levels were not high because of the limitation set by the cutoff latency, the fact that the same result was obtained with rats of different ages and experimental histories and even different ACTH 4-10 treatment conditions is noteworthy. While ACTH 4-10 did not seem to greatly affect HE females, it did increase the passive avoidance latencies of both groups of LE females (Experiment ACTH 2). This confirms the findings of Thompson & de Wied (1973) and in addition demonstrates that an ACTH peptide affects behavior in female as well as male, and hooded (LE) as well as albino rats. An effect of ACTH 4-10 was observed in LE females in Group 2 even before they received shock; the latencies of the LE-ACTH group (Mdn = 7.6 sec) were significantly greater than those of the LE-VEH group (Mdn = 3.3 sec). Although the slight effect of ACTH 4-10 in these rats on the first trial was not great enough to explain its effect on postshock extinction latencies, it is consistent with the report of Weiss et al. (1969) that adrenalectomized rats that have high circulating levels of ACTH show greater reluctance to enter a compartment where rats have been shocked than normal or hypophysectomized rats, although there is no difference between these groups if no animal had previously been shocked in the compartment.

If ACTH 4-10 treatment of the DI rat is regarded as substitution therapy for a deficiency of ACTH, then the expected effect of exogenous ACTH on passive avoidance will vary depending on whether its action in

DI rats is compared to that in HE or LE controls. If HE rats are considered the relevant control, then ACTH 4-10 should have increased latencies; on the other hand, if normal LE rats are considered to be relevant controls, then ACTH 4-10 should have decreased latencies, as was observed. It is helpful to consider that the effect of ACTH 4-10 on passive avoidance seemed to depend upon the general level of performance of the rats to which it was administered. When given to rats with low to moderate latencies (LE females in Experiment ACTH 2; HE females in Experiment ACTH 1) as judged by vehicle controls, it increased latencies; but when given to rats with long latencies (HE & DI females in Experiment ACTH 2; DI females in Experiment ACTH 1), ACTH 4-10 decreased latencies. Although no effect of ACTH 4-10 could be demonstrated in male rats in Experiment ACTH 2, it may be that this level of footshock was not strong enough to establish passive avoidance in animals of this size.

Since ACTH 4-10 modified the performance of DI rats, that have no vasopressin, it cannot be argued that this peptide requires the presence or release of vasopressin to affect avoidance. On the other hand, the 'vasopressin competence' level of the rat does seem to influence the behavioral action of ACTH 4-10. As the 'vasopressin competence' levels of the rats are placed in descending order, normal, HE, and DI; ACTH 4-10 increases, does not affect, and decreases passive avoidance latencies in that order.

The fact that the effect of ACTH 4-10 on DI rats was similar whether injected before extinction trials (Experiment ACTH 1) or before both training and extinction trials (Experiment ACTH 2), suggests: (a) that the effects of ACTH 4-10 or similar fragments of ACTH do not

depend upon what stage of the experiment the peptide is administered (Dempsey et al., 1972; Guth et al., 1974; Sandman et al., 1971b) and (b) the state-dependent learning effects reported for large doses of purified ACTH gel (P. Gray, 1975) on a similar passive avoidance task, are probably related to the corticotrophic effects of this preparation.

An unexpected finding was that the passive avoidance latencies of LE-VEH rats were quite short. In fact, the LE-VEH females did not really demonstrate any passive avoidance at all. Although the 0.5 ma shock used in these experiments is quite low in comparison to many other passive avoidance studies, it has been reported that passive avoidance can be established with a 0.25 ma shock under similar conditions (Ader, Weijnen & Moleman, 1972). The failure to establish passive avoidance in LE-VEH females in the present study was not because the shock was below detection threshold, because all rats reacted noticeably to the shock. A similar failure to obtain noticeable conditioning in vehicle injected rats, but large increases in latency in vasopressin treated rats has been reported by Bohus et al. (1972).

It was also found that the avoidance latencies of LE-VEH females were shorter than those of both DI-VEH and HE-VEH females, and that the latencies of DI males were shorter than those of HE-VEH males (Experiment ACTH 2). It might be that the shorter latencies of LE females reflect the reduced sensitivity to shock of heavier rats (Gibbs et al., 1973; Paré, 1969), but the ranking of HE, DI and LE females in order of passive avoidance latencies did not correspond to the ordering of their respective body weights. For example, HE females had slightly longer latencies than DI females and yet they were on the average 56 g heavier. Furthermore, Gibbs et al. (1973)

have shown that there are no differences in the flinch, jump or vocalization thresholds of male Sprague-Dawley rats weighing 177 g or 243 g, a range which includes the 47 g spread in Experiment ACTH 1, and the 35 g spread in Experiment ACTH 2 between DI and HE females.

Since explicit controls for differences in body weight were not run in these experiments, however, it is possible that some of the groups differed slightly in their shock sensitivity for this reason.[8] As the weight differences between HE and DI females, or HE and LE females were relatively small, 50 g, the only group differences which might have been affected were females, LE-DI = 91 g, and males: HE-DI = 87 g; DI-HE = 100 g; and LE-DI = 187 g. Thus, the short passive avoidance latencies of males in comparison to females might in part be accounted for by higher shock thresholds associated with their greater body weight. The low shock level used in these experiments may not have been sufficient to elicit substantial passive avoidance in rats of this size.

The poor passive avoidance of the LE strain relative to the Brattleboro strain may be linked to the great differences in their activity in the open field situation (Chapter III). One may hypothesize that the much greater activity level, exploratory drive or escape tendency displayed by the LE rat in the open field was also manifested on passive avoidance extinction trials. The LE rat was more active than the Brattleboro rat and was therefore more likely to cross into the shock compartment.

---

[8] A check on the resistance between the hind feet of HE, DI and LE female rats did not result in significant differences and what differences there were, were not in keeping with the results of the passive avoidance tests.

The observation that HE and DI rats are perhaps more similar to one another than to LE rats in their passive avoidance strengthens the hypothesis that neither DI or HE rats are like normal LE rats in this regard. Other data supporting this hypothesis are that the latencies of HE rats in these experiments and in a previous experiment (Chapter II) were considerably longer than those of LE rats. Finally, HE rats and DI rats were both considerably smaller than LE rats of the same age. Although DI rats have proved to be smaller than HE rats in previous studies, the finding that HE rats are smaller than LE rats has not been noted before.

The 'paradoxical' effect of ACTH 4-10 on passive avoidance that was observed in DI and to a lesser extent in HE rats can be related to the data of Korányi et al. (1967). They found that the passive avoidance of mice classified as 'hypoactive' or 'hyperactive' in the open field was differentially affected by ACTH; the latencies of 'hypoactive' mice were reduced, but those of 'hyperactive' mice were increased. In view of these findings it may be helpful to consider that the factor(s) that account for differences in passive avoidance between DI and HE rats, which seem not related to activity level, may not be the same factor(s) which account for differences between Brattleboro and normal LE rats.

The passive avoidance latencies of DI-VEH females were not shorter than HE-VEH females as was observed in Chapter II, possibly because of their prior passive avoidance testing. However, in Experiment ACTH 2 the DI-VEH rats of both sexes had slightly shorter passive avoidance latencies than HE-VEH rats.

The amount of defecation by DI, HE and LE groups on extinction trials seemed to be related to their passive avoidance latencies. Although rats spending the most time in the apparatus tend to defecate more, it was observed in this study that most defecation occurs during the first minute or two after the rat is placed in the apparatus and that differences in bolus counts were noted even when the latencies of the groups were almost identical, e.g. in Experiment ACTH 2 the DI rats defecated more than LE rats on day 5 and their latencies were similar. An increase in defecation with an increase in passive avoidance latency in this experiment may indicate that Brattleboro rats are more fearful than LE rats.

CHAPTER VI

EFFECTS OF VASOPRESSIN REPLACEMENT THERAPY ON THE PASSIVE AVOIDANCE AND PHYSIOLOGY OF DI RATS

In previous experiments it was shown that DI rats have shorter passive avoidance latencies than HE rats (Chapter II, Chapter V), and that the latencies of DI rats were further reduced by the administration of ACTH 4-10 (Chapter V). In this experiment DI rats were injected daily with pitressin tannate starting three days before and continuing a few days after passive avoidance testing, to determine how vasopressin replacement therapy affected their passive avoidance. Pitressin tannate contains partially purified vasopressin in a tannate complex, but ACTH and MSH are present as impurities. Although it would have been better, for this reason, to use a synthetic vasopressin preparation, long acting tannate complexes of synthetic vasopressin are not available. Vasopressin is only effective for a few hours unless slowly released from a depot vehicle.

Following passive avoidance testing, water intake was measured. Body weight and tail length were also measured in order to confirm the observation of Sokol (1973) that the body structure of DI rats is smaller than that of HE rats. The rats were then sacrificed to obtain data on levels of corticosterone and dopamine beta hydroxylase (DBH) in serum, and norepinephrine (NE) in brain. The corticotrophic effect of vasopressin therapy was estimated by measuring serum corticosterone.

The fraction of serum corticosterone which is not bound to serum proteins was measured, as it is this unbound steroid which is physiologically active (Daughaday, 1967).

When NE is released from peripheral sympathetic nerves the enzyme DBH, which catalyses the formation of NE from dopamine, is also released (Weinshilboum, Thoa, Johnson, Kopin & Axelrod, 1971). Since the serum levels of DBH are not affected by adrenomedullectomy, it is thought that serum DBH primarily measures the release of NE from blood vessels (Weinshilboum & Axelrod, 1971). The high level of DBH found in the serum of hypophysectomized and DI rats probably reflects an increase in peripheral sympathetic activity in response to a diminished extracellular fluid volume (Lamprecht & Wooten, 1973). It has also been shown that pitressin tannate therapy is effective in lowering DBH levels in hypophysectomized rats when administered in doses of 2.5 - 5 U b.i.d. for four days (Lamprecht & Wooten, 1973). In the present experiment a 0.5 U dose of pitressin was administered daily for 10 days to determine what relationship, if any, there was between the effect of this dose of pitressin on passive avoidance and its ability to reduce peripheral sympathetic activity in DI rats as measured by serum DBH.

It was decided to measure the level of NE in the brain as Weiss et al. (1970a) found that the turnover of NE was increased in adrenalectomized rats and decreased in hypophysectomized rats. These investigators hypothesized that alterations in the NE metabolism of these animals were related to the prolonged passive avoidance latencies observed in adrenalectomized rats and the shorter passive avoidance latencies of hypophysectomized rats. If changes in passive avoidance are related to alterations of NE metabolism, then the activity of NE

neuronal systems might be affected in DI rats and would manifest itself as a decreased turnover of NE. ACTH 4-10 increases and glucocorticoids decrease the turnover of NE in the brains of normal rats (Fuxe, Hökfelt, Jonsson & Lindbrink, 1973; Leonard, 1974), and it has been postulated that changes in avoidance behavior produced by manipulation of the pituitary-adrenal system are mediated by central noradrenergic activity (Fuxe et al., 1973). In the experiment described here the NE level, not turnover, was measured because of the small number of animals available.

Method

Subjects. The subjects were seven HE and eight DI males aged 13 months that had participated in passive avoidance tests 8 1/2 months (Chapter II) and shuttlebox avoidance 5 1/2 months before (Chapter IV).

Apparatus. The passive avoidance apparatus described in Chapter II was used.

Behavioral procedure. Before beginning passive avoidance testing all rats were given two two-minute periods of habituation to the shock compartment two days apart. The number of rearings and the number of boluses excreted were recorded each day. On the day following the second habituation period, each rat was placed into the small chamber and step-through latencies into the shock compartment were measured. Passive avoidance testing began the next day as previously described in Chapter V.

Beginning on the first habituation day and continuing until two days after passive avoidance testing, half of the DI rats received

subcutaneous injections of 0.5 U of pitressin, while the remaining DI and all HE rats received an equivalent volume of peanut oil (.1 ml). Injections were given two to three hours after testing each day. It was decided to give the injections after testing so as to prevent any immediate effects of the injection, e.g. vasopressin pressor effects, from affecting performance. After the last extinction trial the rats were weighed and transferred to individual cages for measurements of 24 hour water intake.

Biochemical procedure. On the third day after testing (18 hours after the last injection) the rats were quickly removed from their cages in random order and decapitated. Trunk blood was collected in centrifuge tubes and stored on ice for DBH and corticosterone measurements. The brain was rapidly removed from the skull and the hypothalamus, brain stem and mesencephalon, and telencepalon[9] were dissected on a chilled plate, and frozen in dry ice for later determination of NE. Tail length was measured to the nearest mm.

Serum was separated from the clotted blood by centrifugation for 10 minutes at 10,000 r.p.m. at 4°C, and stored at -20°C. A 50 µl aliquot of serum was assayed for DBH by Dr. T. Joh at Cornell University Medical School according to a new method which he has developed (Joh, Ross & Reis, 1974).

The method used for the determination of brain NE was that described by Pohorecky (1974).

---

[9]Telencephalon consisted of whole brain minus hypothalamus brainstem, mesencephalon and cerebellum.

Results

Behavioral data. The median number of rearings on the first and second days of habituation are shown in Table 13. DI rats given

TABLE 13

MEDIAN REARINGS DURING HABITUATION TESTS

Group	Habituation day	
	1	2
HE-vehicle (n=7)	4	9
DI-vehicle (n=4)	9	17.5
DI-vasopressin (n=4)	10	12.5

vehicle or vasopressin reared more frequently than HE rats, and these differences proved to be significant on both days (Mann-Whitney tests, two-tail, $p = .06$) or better. Pre-test step-through latencies were similar for HE (Mdn = 5.6 sec), (DI-VEH (Mdn = 5.2 sec) and DI-VP (Mdn = 5.3 sec) groups. Few rats defecated on either habituation day or on the pre-test day, and no group differences were noted. The habituation data did not correlate with later passive avoidance performance.

The results of the passive avoidance experiment are shown in Fig. 12. On the first day of the experiment all rats had short step-through latencies as on the pre-test except for one DI-VEH rat that had an extremely long latency, 340 sec, and its data had to be discarded for this reason. The DI-VP rats had shorter passive avoidance latencies than HE rats on day 2 ($p = .024$) and day 3 ($p = .024$). In

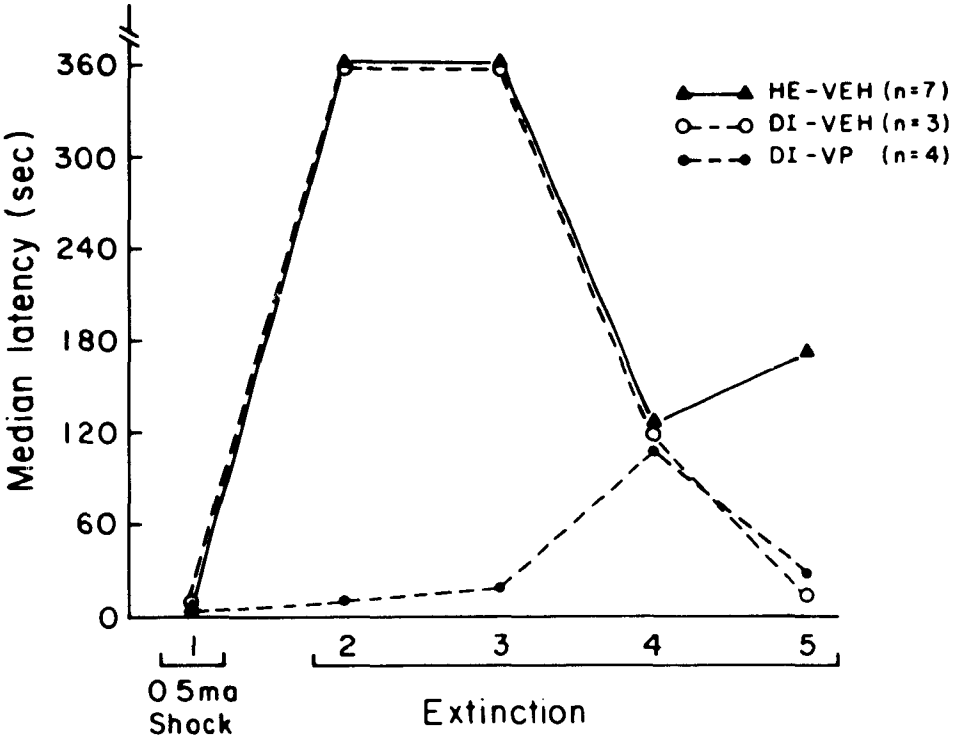


Fig. 12. Passive avoidance of DI males receiving vasopressin replacement therapy.

fact, passive avoidance was not observed in DI-VP rats; only one of these four rats showed any increase in step-through from the training trial on day 1 to the first extinction trial on day 2. There was no overlap in the distribution of DI-VEH and DI-VP latencies on day 3 (p = .056), but on day 2, 4, and 5 there was overlap in the observed latencies.

On day 2, no boluses were left by DI-VP rats, but two of the three DI-VEH rats left boluses (Mdn = 3.5) and five of the seven HE rats left boluses (Mdn = 3) in the apparatus. In fact, no DI rat injected with vasopressin excreted any boluses except on day 4 when three of the four rats defecated (Mdn = 2.5). None of the DI-VEH rats

and only one of the seven HE rats defecated that day. Too few boluses were excreted by any group to be analysed on any other day.

Biochemical data. As shown in Table 14, vasopressin was effective in reducing the water intake of DI rats, but not to the level of HE rats. DI-VEH and DI-VP rats were of similar weight but both were lighter than HE rats. The tail lengths of the DI-VEH rats were shorter than those of the HE rats ($t = 2.30$, $p < .025$, one-tail) as were those of DI-VP rats. The latter group, however, contained one rat with a tail longer than any other DI or HE rat.

TABLE 14

PHYSICAL AND BIOCHEMICAL DATA

	Groups		
	HE (n=7)	DI-VEH (n=4)	DI-VP (n=4)
Body weight (g) $\bar{x} \pm$ s.e.m.	424 ± 28	333 ± 12	337 ± 14
Water intake (g) Mdn ± s.i.q.r.	35 ± 8	251 ± 46	75 ± 20
Tail length (cm) $\bar{x} \pm$ s.e.m.	20.9 ± .5	19.3 ± .4	20.7 ± .8
Serum dopamine beta hydroxylase (μmoles/prod/hr) Mdn ± s.i.q.r.	18.0 ± 8.6*	29.8 ± 9.9	31.1 ± 4.8
Serum corticosterone (μg/100 ml) Mdn ± s.i.q.r.	1.8 ± 1.2	2.1 ± 1.4	10.2 ± 9.1

*Data from three animals were discarded--see text.

The serum DBH levels were higher in DI rats than HE rats (p = .017, one-tail). Two HE rats had to be excluded because of their abnormally low DBH values and another because of a reagent induced artifact. Large quantities of lipids were observed in the serum of the first two animals. Other animals may have had, however, significant but less noticeable quantities of serum lipids. Such quantities of lipids interfere with the assay, producing lower DBH values, and therefore the absolute DBH values, may not be accurate, although the DBH values of HE rats were in the normal range (T. Joh, personal communication).

Resting levels of unbound cortisterone in the rat have been reported by Knigge and Hoar (1963) to be close to zero. The levels of unbound corticosteroid in HE and DI-VEH rats in this experiment were quite low, but it is possible that they were above zero because the rats were sacrificed in the afternoon when resting levels begin to rise, towards the end of the light period. The steroid level of DI-VP rats appeared higher than HE rats (p = .06) or DI-VEH rats (p = .10).

The NE assay data are shown in Table 15. The concentration of NE in the hypothalamus of DI-VEH rats was lower than in HE-VEH rats (p = .034). In the telencephalon, the NE levels of DI-VEH rats were higher than HE-VEH rats (p = .072). Pitressin treatment of DI rats appeared to slightly increase the NE levels of the hypothalamus, and decrease the levels in the telencephalon towards those of HE rats, but these effects were not statistically significant. In the mesenecephalon and brainstem regions the NE levels of all three groups were similar. The brain weights of DI-VEH, DI-VP and HE-VEH rats were 1.44 ± .03 g, 1.55 ± .02 g and 1.57 ± .04 g, respectively. As expected the brains of the small DI-VEH rats were lighter than HE rats (t = 2.26, df = 9,

TABLE 15

CONCENTRATION OF NOREPINEPHRINE (ng/g) IN BRAINSTEM + MESENCEPHALON, HYPOTHALAMUS AND TELENCEPHALON

Group	Region		
	BS + Mes Mdn (range)	Hypothal Mdn (range)	Tel Mdn (range)
HE-VEH (n=7)	449 (379-561)	1407 (792-1637)	387 (298-427)*
DI-VP (n=4)	417 (376-516)	926 (838-1253)*	395 (356-424)
DI-VEH (n=4)	453 (449-544)*	838 (648-916)*	437 (398-532)

*Data from one animal discarded--inadequate recovery of NE.

$p < .025$, one-tail), but vasopressin treatment increased the weight of the DI-VP rat brains ($t = 2.28$, $df = 6$, $p < .05$, two-tail) possibly because of an increase in brain water. It might be argued that group differences in the concentration of NE expressed as ng/g of tissue could have arisen because of differences in their regional brain weights. However, this was not found to be true, for group differences were also evident in the total amount of NE recovered. Unless otherwise noted, all comparisons were made with two-tail Mann-Whitney tests. The results of NE determinations on several samples had to be discarded because of the inadequate recovery of NE.

Discussion

Pitressin treatment dramatically reduced the step-through latencies of DI rats on all retention trials. In fact, these rats did not seem to display any passive avoidance. Several explanations were considered. The most obvious was that pitressin therapy was effective

in normalizing the physiological condition of these rats which in turn might have led to an increase in their vigor and activity. The short latencies of the DI-VP rats seemed similar to those of normal LE rats in previous experiments, and it was suggested that the poor passive avoidance of LE rats might be associated with their higher activity level. Other data, however, may not be consistent with this explanation. Pitressin treatment did not seem to affect the rearings of DI rats during the habituation days. If a dramatic increase in general activity had been produced by pitressin, an increase in rearings should have been observed. In addition, there was little evidence that pitressin improved the physical condition of DI rats. Pitressin reduced their water intake, but water intake was still more than twice that of HE rats. This was not unexpected; other investigators have found that 1.0 U/day of pitressin may be required to return the water intake of DI rats to normal (Harrington & Valtin, 1965). The high serum DBH levels observed in the DI rats reflect intense peripheral sympathetic activity. This confirms the observation of Lamprecht & Wooten (1973). The 0.5 dose of pitressin used in the current experiment, which only slightly ameliorated the water balance of DI rats, was not effective in reducing sympathetic vascular activity, as the serum DBH levels in treated and untreated DI rats are the highest yet reported (T. Joh, personal communication). It should be noted that a small part of the increased serum concentration of DBH in DI rats can be attributed to a smaller extracellular fluid volume associated with dehydration.

It is interesting to note that the passive avoidance of DI rats was not directly related to their characteristically high level of peripheral sympathetic activity, as pitressin reduced passive avoidance

latencies without affecting serum levels of DBH. It is possible, however, that pitressin may have affected peripheral tissues without necessarily reducing serum levels of DBH. If the elevated passive avoidance latencies of Brattleboro rats relative to LE rats were associated with an increased sensitivity to shock, pitressin might have returned sensitivity to normal. A greater release of catecholamines from the adrenal in Brattleboro rats might be expected to increase their shock sensitivity, as both Gibbs et al. (1973) and Paré & Cullen (1971) have suggested that epinephrine increases shock sensitivity.

DI rats were found to be lighter in weight and have shorter tails than HE rats. The difference in weight between DI and HE rats is partially explained by the fact that DI rats are leaner and this is correctible by long term vasopressin therapy from birth. The smaller body structure of these animals is not affected by pitressin therapy but can be corrected by long term treatment with growth hormone (Sokol, 1973). In the present experiment no effect of pitressin on tail length was found and the dose and duration of pitressin were insufficient to grossly affect body weight. However, the retention of only a few grams of water would be expected to improve their water balance. In summary, the administration of 0.5 U/day of pitressin to DI rats for 10 days did not dramatically improve their general physical condition; water intake was still twice that of HE rats and peripheral sympathetic vascular activity was not reduced. Although the data indicate that pitressin did not significantly reverse abnormalities of vascular and kidney function, it is possible that the brain and other tissues may have responded to the dose of pitressin given in this experiment or to

even smaller doses.

Since pitressin therapy elevated serum levels of unbound corticosterone in DI rats, it is possible that the corticotrophic effects of the injections were responsible for the observed suppression of passive avoidance. Corticosteroids have been shown to shorten passive avoidance. Corticosteroids have been shown to shorten passive avoidance latencies, and a state-dependent learning effect has been proposed to explain this finding (Pappas & P. Gray, 1971). On the other hand, prolonged stimulation of the adrenal by vasopressin or peptide impurities like ACTH in the pitressin may have rendered the adrenal less responsive to stress. This kind of pharmacological effect may also be the reason that DI and HE rats chronically treated with pitressin show a smaller pituitary-adrenal response to stress than would be ordinarily expected. A reduction of adrenal steroid synthesis by cyclohexamide to the stress of a single passive avoidance training trial has been shown to produce severe passive avoidance defecits which can be normalized by the administration of hydrocortisone at the same time as cyclohexamide (Nakajima, 1973).

Another explanation for the poor passive avoidance performance of pitressin treated DI rats was suggested in this study by the data on brain NE levels. It is admittedly speculative in nature but is presented because it may be of value in interpreting and integrating some of the puzzling findings of this experiment and those reported by others.

The level of NE in the telencephalon of DI-VEH rats was higher than that of HE-VEH rats, but in the hypothalamus NE levels were lower. Since data on the turnover of NE in these regions were not available,

the assumption was made that low levels of NE were associated with an increase in the activity of NE neurons, while higher levels reflect reduced activity of NE neurons, as measured by turnover.[10] Given this assumption we can speculate that the low levels of NE in the DI hypothalamus are associated with an increase in the activity of NE neurons such as might be expected if these neurons are relaying osmoreceptor activity to the supraoptic nucleus (Bridges & Thorn, 1970), or if they are involved in the inhibitory regulation of peripheral sympathetic activity, as suggested by studies on the spontaneously hypertensive rat (Yamori, Lovenberg & Sjoerdsma, 1970). Thus, the relatively weak effect of pitressin in reducing the water intake and peripheral sympathetic activity of DI-VEH rats is reflected in the small increase in the concentration of NE in the hypothalamus which occurred in pitressin treated DI rats.

According to the previous assumption, the high levels of NE in the DI telencephalon reflect the reduced activity of NE neurons in this region. Weiss et al. (1970a) found that the activity of NE neurons, as measured by the turnover of NE, was lowered in the brain of hypophysectomized rats. Since the tissue considered as telencephalon in this experiment comprised about 65% of the tissue analysed, the NE level of this region is probably similar to that which would have been obtained for the whole brain. It is reasonable, then, to state that the assumed lowered turnover of NE in the telencephalon of DI rats in the present study is comparable to the finding by Weiss et al. (1970a) of a reduced turnover of NE in whole brain samples of hypophysectomized rats. That vasopressin therapy would reduce the telencephalic NE levels of DI rats

---

[10] J. Weiss and L. Pohorecky have found this relationship in rats exposed to stressful situations (personal communication, 1974).

towards those of HE rats is what might be expected if the ACTH and MSH contaminants of the pitressin increased the turnover of NE as has been shown for ACTH 4-10 and ACTH β 1-24 in normal rats (Fuxe et al., 1973; Leonard, 1974). However, it seems that the reduced turnover of NE in hypophysectomized rats is not affected even by large doses of ACTH (Fuxe et al., 1973). Therefore, we might hypothesize that the reduced turnover of NE in hypophysectomized rats, and assumed for DI rats in the present study, is related to a deficiency of vasopressin, not NE, and that vasopressin would be able to affect the turnover of NE in hypophysectomized rats in a manner similar to that suggested in this study for the telencephalon of DI rats.

The above speculations can be summarized as follows. The lower NE level in the hypothalamus of DI rats may primarily reflect the peripheral disturbances of these animals, e.g. decreased extracellular fluid volume and plasma hyperosmolarity, which were little affected by the small dose and short duration of vasopressin therapy in this study. Elevated levels of NE in hypophysectomized rats and the telencephalon of DI rats are also related to a vasopressin deficiency. Although one might expect that the turnover of NE in the hypothalamus and the telencephalon of DI rats should be similarly affected by vasopressin, it is known that these structures are innervated by anatomically separate ascending NE pathways arising in the reticular formation; the cortex and hippocampus receive fibers from the dorsal NE bundle, while the hypothalamus and subcortical limbic areas receive fibers from the ventral NE pathway (Ungerstedt, 1971).

As pitressin could be more effective in altering brain NE levels than peripheral sympathetic activity, it is possible that the reduction

of passive avoidance latencies by pitressin was associated with an alteration of NE activity or possibly other neurotransmitters. If this is true, then the poor passive avoidance of pitressin treated DI rats might be related to a change in their level of activity, arousal, or stress. The regulation of arousal and psychomotor activity has been associated with NE and dopamine (Chan & Webster, 1971; Gordon & Shellenberger, 1974; Moore & Rech, 1969; Rech, Borys & Moore, 1966; Rech & Moore, 1971), while both physical and psychological stress have been shown to affect the metabolism of NE (Thiérry, Javoy, Glowinski & Kety, 1968; Weiss, Stone & Harrell, 1970b).

CHAPTER VII

GENERAL DISCUSSION

Passive Avoidance

Comparison of HE and DI rats

The passive avoidance of Brattleboro rats homozygous for diabetes insipidus (DI) was shown to be inferior to that of heterozygous (HE) rats (Chapter II; Chapter V). A number of explanations were considered. Since DI rats were found to be considerably lighter than HE rats, it seemed possible that their poor passive avoidance may have been a function of their reduced body weight--perhaps lighter rats are less sensitive to footshock. Control experiments, however, indicated that the difference between DI and HE females in passive avoidance was not a simple function of body weight, as weight differences of similar magnitude did not affect the passive avoidance of LE females (Chapter II). Moreover, several studies have shown that lighter rats are more sensitive to footshock (Gibbs et al., 1973; Paré, 1969) so that one would have expected better, not poorer, passive avoidance by DI rats.

It has been shown that the poor passive avoidance of female rats relative to male rats is largely accounted for by the higher levels of exploratory activity of female rats (Denti & Epstein, 1972), and that the exploratory activity of males in a maze-type open field is inversely related to their passive avoidance learning (Endröczi, 1972). However, the poor passive avoidance of DI rats could not be accounted for on the basis of a higher level of exploratory activity

as the activity of DI and HE rats was similar on open field tests (Chapter III).

Since Weiss et al. (1969, 1970a) have attributed the shortened passive avoidance latencies of hypophysectomized rats to a deficiency of ACTH, it might be argued that the reduced pituitary-adrenal response to weak but not strong stress (Arimura et al., 1967; McCann et al., 1966; Wiley et al., 1974; Yates et al., 1971) is involved. If this is so, DI rats should demonstrate a weaker pituitary-adrenal response and shorter passive avoidance latencies to a weak than to a strong footshock on the training day. A comparison of the responses of DI females to a 1.0 ma (Chapter II) and a 0.5 ma footshock (Chapter V) suggests that this is not the case.[11] In fact, the reverse was true--the latencies of DI females were shorter when trained with strong as compared to weak footshock. Another indication that DI rats release normal quantities of ACTH is the finding by Wiley et al. (1974) that the decreased pituitary-adrenal response to weak stresses may derive not from a hypothalamo-pituitary defect, but from the insensitivity of the DI adrenal to ACTH. Finally, if the poor passive avoidance was related to a deficiency of ACTH, then the administration of ACTH 4-10 should have increased rather than decreased passive avoidance latencies of DI rats (Chapter V).

It seems unlikely, therefore, that the poor passive avoidance of DI rats, as compared to HE rats, observed in these experiments could be attributed to weight related differences in shock sensitivity, general

---

[11] A similar comparison for male DI rats was difficult to make because of indeterminate latency scores in Chapters II and VI, and the unusually short latencies of all male rats in Chapter V.

level of activity, or a pituitary deficiency of ACTH. Instead, the weight of the evidence points to the deficiency of vasopressin as the factor responsible. DI rats are totally deficient in vasopressin, whereas HE rats have only a mild deficiency of vasopressin. It must be remembered, however, that a deficiency of vasopressin over a period of time may lead to secondary changes in the activity of other endocrine glands such as the adrenal. For instance, DI rats show hypokalemia and other symptoms of aldosteronism (Möhring et al., 1972a). Although potassium supplementation did not influence the open field activity of DI rats, it is possible that a quite indirect disturbance of metabolism initiated by vasopressin deficiency may affect the passive avoidance of DI rats.

Comparison of Brattleboro to LE rats

The better passive avoidance of Brattleboro rats, especially HE rats, as compared to LE rats in Chapter V was totally unexpected. How was it possible that rats partially or totally deficient in vasopressin could outperform normal animals? On the behavioral level, a likely explanation is that Brattleboro rats engaged in less exploratory activity as compared with LE rats and therefore were not as likely to enter the shock compartment on extinction trials. A related interpretation of the better passive avoidance of Brattleboro rats in terms of the open field data is based upon the hypothesis that Brattleboro rats are generally more fearful than LE rats (Chapter III). There is considerable evidence that fear inhibits exploratory behavior (J. Gray, 1971a) and thus the low level of open field activity of Brattleboro rats may indicate that they are more fearful than LE rats. Since

defecation by the rat under these conditions represents an unconditioned response to fear stimuli (J. Gray, 1971a), this hypothesis is also supported by the observation that during both open field (Chapter II - Open Field Test 2) and passive avoidance (Chapter V - Experiment ACTH 2) both male and female Brattleboro rats tended to defecate more often and excrete more boluses than LE rats. Although Brattleboro rats may have defecated somewhat more often than LE rats in the passive avoidance apparatus because they were usually in the apparatus for a longer period of time, this was not the case in the open field experiments. The low defecation level of LE rats was perhaps all the more surprising in view of the fact that heavier rats might be expected to have a higher basal defecation rate as they have more fecal material available (Russell, 1973a).

In spite of the above analysis which suggests the usefulness of concepts such as activity or fearfulness in explaining the differences between the passive avoidance of Brattleboro and LE rats, the possibility exists that both DI and HE rats may be more sensitive to footshock than LE rats because they are lighter, or for reasons that are unrelated to differences in body weight. One possibility is that Brattleboro rats release greater amounts of catecholamines from the adrenal which renders them more sensitive to shock.

Active Avoidance

In the shuttlebox no great differences were observed between DI and HE rats until the late stages of training when the avoidance performance of DI rats waned and few were able to reach criterion. This may have been due to weakness associated with potassium deficiency and

the stress of prolonged testing. An additional consideration is that since DI rats were lighter than HE rats and therefore, perhaps, more sensitive to shock, their performance may have been impaired; it has been shown that acquisition of shuttlebox avoidance by normal rats becomes more difficult as the intensity of the footshock is increased above 0.4 ma (Stratton & Kastin, 1974). In view of the observation that the passive avoidance latencies of DI females were shorter at 1.0 ma than at 0.5 ma, it may be that a severe acquisition deficit would have been apparent earlier in shuttlebox training if a more intense footshock had been employed.

The impaired acquisition of DI rats might seem to contrast with the normal acquisition of shuttlebox avoidance by neurohypophysectomized rats (de Wied, 1965), but the severity of the vasopressin deficiency may be important--the deficiency of vasopressin is absolute in DI rats, while in neurohypophysectomized rats it is relatively mild (Nagy et al., 1972). The avoidance deficit, however, did not seem to be as great as that reported for hypophysectomized or adenohypophysectomized rats (de Wied, 1964, 1969a, 1969b). Unfortunately, too few DI rats reached criterion, and extinction was not continued long enough to determine whether the extinction of DI rats would have been shortened as reported for neurohypophysectomized rats (de Wied, 1965).

Effect of ACTH 4-10 on Passive Avoidance

In LE females, ACTH 4-10 was found to increase passive avoidance latencies (Chapter VI). This confirms the report by Thompson & de Wied (1973) who used male Wistar rats. On the other hand, ACTH 4-10 seemed to have little effect on HE females and actually reduced the passive avoidance latencies of DI rats relative to vehicle injected DI rats

and HE rats injected with ACTH 4-10. This finding mitigates against the hypothesis that ACTH 4-10 exerts its behavioral effects only in the presence or through the release of endogenous vasopressin. Nevertheless, it seems that ACTH 4-10 does not have the same behavioral action in rats deficient in vasopressin as it does in normal rats.

This is not the first time that ACTH or MSH has been reported to exert paradoxical effects. Stratton & Kastin (1974) compared rats injected with MSH to saline injected controls. The rats injected with MSH performed better in the shuttlebox with a low intensity footshock of 0.3 ma, but more poorly with footshock of moderate intensity, 0.9 ma. On a shock motivated brightness discrimination task in a Y-maze, rats injected with 1.0 U of ACTH made more avoidances if they were classified as 'hypoactive' when tested with a 0.2 ma footshock, but those classified as 'hyperactive' were not affected. At a higher shock level of 0.4 ma, ACTH increased the errors of 'hyperactive' rats without affecting their avoidance performance (Ley & Corson, 1971a). A similar effect was reported by Korányi et al. (1967) who noted that ACTH increased the passive avoidance of mice who were 'hyperactive' in the open field but decreased that of mice who were 'hypoactive'. It has been proposed that the effects of ACTH of MSH in these and other experiments involve an increase in behavioral excitability or arousal (Korányi et al., 1967; Kastin, Miller, Nockton, Sandman, Schally & Stratton, 1973; Weiss et al., 1969, 1970a). This is consistent with the evidence reviewed by Kastin et al. (1973) which indicated that MSH increases arousal in both rats and humans. If ACTH 4-10 influenced passive avoidance in the present experiments by increasing arousal, then its seemingly paradoxical effects in DI rats could be explained

by the deleterious effects of 'overarousal'. The relationship between arousal and performance on a variety of tasks has been described by an inverted U-function (Duffy, 1962). The tendency of LE rats to actively explore the open field, to show short passive avoidance latencies, and defecate infrequently in these stressful situations suggested that they were not especially fearful. An increase in arousal induced by ACTH 4-10 in the presence of fear stimuli might have raised their low fear levels and improved passive avoidance (Weiss et al., 1969, 1970a). In HE rats, whose low ambulation and high defecation scores suggested that they were more aroused or fearful than LE rats, a further increase in arousal by ACTH 4-10 had little effect on their passive avoidance--perhaps because their fear level, even at a low shock intensity, placed them close to the top of the inverted U-function. A further increase in arousal might slightly increase their latencies to a maximum or even decrease their latencies slightly if greater than optimal arousal were produced. If the arousal level of DI rats was above that of HE rats during these experiments, then their poor passive avoidance may be related to the descending portion of the arousal-performance function. Thus, treatment of DI rats with ACTH 4-10 might well be expected to shorten their latencies as was demonstrated in Chapter V.

The effect of ACTH 4-10 in the passive avoidance experiments may also be related to the "law of initial value," which relates the initial value of a physiological function to the magnitude of its response to stimulation. When initial levels are low, stimulation can produce large increases, but when initial levels are high, small changes or even decreases may occur in response to the same stimulation

(Wilder, 1957).

Effect of Vasopressin on Passive Avoidance

The poor passive avoidance of DI rats treated with pitressin is difficult to explain. It may be that a corticotrophic effect of vasopressin or other peptide impurities in vasopressin adversely affected performance through a state-dependent decrement in performance such as described by P. Gray (1975) and Pappas & P. Gray (1971). Or, the administration of a dose of vasopressin which was relatively large with respect to tissues other than the kidney, may have produced an overarousal like that suggested as an explanation for the paradoxical effect of ACTH 4-10 in DI rats. Several points argue against this interpretation. First, the latencies of vasopressin treated DI rats were more like those of normal LE rats than those of DI rats injected with ACTH 4-10 (Chapter V). Second, no vasopressin treated rat defecated in the apparatus, except on the fourth day when fear cues were reduced by a change in the visual and olfactory characteristics of the apparatus. Again, the low defecation scores of these rats are more like those of LE rats than DI rats injected with ACTH 4-10. Although it could be that vasopressin induced a much greater arousal than ACTH 4-10 because it was administered in a preparation which was active for a longer period of time, there is another possible explanation. It was proposed that vasopressin therapy might be effective in lowering the high levels of NE and increasing the turnover of NE in the telencephalon of DI rats relative to that of HE rats. However, the passive avoidance latencies of DI rats injected with vasopressin were shorter than HE or DI rats injected with vehicle. Thus, the

levels and turnover of NE in HE rats might also be greater than in normal LE rats. If we take the levels of NE in the cortex of male albino rats, 290 μg/g,[12] as a benchmark then the NE levels of both DI and HE rats are high. By lowering NE levels and increasing turnover, vasopressin might be able to shorten the passive avoidance latencies of both HE and DI rats to the level of normal LE rats. This interpretation would be consistent with the hypothesis that vasopressin reduces the passive avoidance latencies of DI rats by reducing their arousal or fearfulness.

Without further behavioral and biochemical data, however, it is difficult to decide whether an alteration in the NE levels of DI rats and their passive avoidance deficiencies produced by pitressin would be associated with an increase or decrease in arousal. In view of this problem, the suggestion that ACTH 4-10 produced an 'overarousal' in DI rats must be considered a tenetative explanation, it is possible that while ACTH 4-10 and similar peptides may increase arousal in normal rats, the abnormal functioning of neuronal systems involving NE or other neurotransmitters in Brattleboro rats may be such that these peptides decrease arousal. Nevertheless, the data permits the speculation that the marked reduction of passive avoidance in DI rats by pitressin may be associated with alterations in the level and possibly the turnover of brain NE in the telencephalon or hypothalamus.

---

[12] W. H. Bailey, unpublished observations, 1974.

CHAPTER VIII

SUMMARY AND CONCLUSIONS

It is known that exogenous ACTH, MSH and vasopressin improve the impaired active avoidance of hypophysectomized rats and prolong extinction of both active and passive avoidance (Bohus et al., 1972; Bohus et al., 1973; Dempsey et al., 1972; de Wied, 1969b ; Guth et al., 1971). While there is evidence that endogenous levels of ACTH may play a role in acquisition and extinction of avoidance (de Wied et al., 1972b) it is not known whether endogenous levels of vasopressin are of similar importance.

In the present investigation, the active and passive avoidance of rats of the Brattleboro strain, with a total genetic deficiency of vasopressin associated with hypothalamic diabetes insipidus (DI rats) were compared to rats heterozygous for this trait (HE rats). Since HE rats also have a slight defect in the synthesis and release of vasopressin (Moses & Miller, 1970), rats of the Long-Evans (LE) strain from which Brattleboro rats were derived were included in some experiments as an additional control.

Passive avoidance of DI rats was found to be impaired when compared to HE rats at shock levels of 0.5 and particularly at 1.0 ma. A control experiment indicated that differences in passive avoidance latency were not directly related to weight differences between DI and HE rats. Nor was the difference in passive avoidance

related to a difference in the general activity level of DI & HE rats, as their behavior during open field tests was quite similar.

Passive avoidance latencies of both HE and DI rats were longer than those of age matched LE rats, when a 0.5 ma shock was used and the latencies of HE rats were longer than those of weight matched LE rats when a 1.0 ma shock was used. The superior passive avoidance of Brattleboro rats might in part be related to an increased sensitivity to shock as Brattleboro rats are considerably lighter than LE rats and therefore, more sensitive to shock--Gibbs et al., 1973; Paré, 1969. The comparison of Brattleboro and LE rats on open field tests, however, indicated that Brattleboro rats defecated more, were considerably less active and seemed more fearful than LE rats. Thus, the less active and more fearful Brattleboro rats might not be as likely to re-enter the shock compartment on extinction trials as would LE rats, independent of considerations of shock intensity.

The effect of ACTH 4-10 on the passive avoidance behavior of Brattleboro rats was also studied. The hypothesis that the effect of ACTH on avoidance is mediated by the release of endogenous vasopressin was disproved, since ACTH 4-10 affected the passive avoidance of DI rats. However, in two experiments ACTH 4-10 had a 'paradoxical' effect on the passive avoidance of DI females. When administered prior to both training and extinction trials, or just prior to extinction trials, ACTH 4-10 shortened passive avoidance latencies in DI rats and did not affect HE rats. This is in contrast to the longer passive avoidance latencies which ACTH 4-10 produced in LE females.

Although most passive avoidance studies have reported that ACTH 4-10 or MSH treated rats have longer passive avoidance latencies

(Dempsey et al., 1972; Guth et al., 1971; Sandman et al., 1971b; Thompson & de Wied, 1973), it has been noted that ACTH or MSH may have paradoxical effects on avoidance at moderate to high shock levels when given to hypoactive rats or mice (Korányi et al, 1967; Ley & Corson, 1971a, 1971b; Stratton & Kastin, 1974). These studies suggest that the arousal or activity level of the animal affects its response to ACTH. Thus, Brattleboro rats might not be expected to respond to ACTH in the same way as LE rats in these experiments.

When compared on active avoidance in the shuttlebox, DI rats did not perform as well as HE rats, particularly during the last stages of training. This suggests that the avoidance impairment of DI rats may well be a general one, as both active and passive avoidance were affected. While the weight of the evidence suggests that the differences between DI and HE rats on active and passive avoidance were related to the absence of vasopressin in the DI rat, it is likely that other hormonal or physiological changes secondary to a vasopressin deficiency were also involved. For instance, it has been shown that a reduced pituitary-adrenal response to stress is associated with deficiencies of vasopressin in the DI and neurohypophysectomized rat (de Wied, 1961; Wiley et al., 1974). Although a reduced pituitary-adrenal response might have affected the performance of the DI rat, the results suggest that other aspects of vasopressin deficiency were involved.

When pitressin injections were given to DI rats as vasopressin replacement therapy, short passive avoidance latencies were observed, hardly greater than those observed before shock, while DI and HE rats receiving vehicle injections displayed quite long passive avoidance

latencies. The NE levels in the telencephalon of DI rats were higher than in HE rats, but in the hypothalamus of DI rats the levels of NE were lower than in HE rats. Since the 0.5 U dose of vasopressin did not normalize water intake or reduce the increased peripheral sympathetic activity of the DI rat, the hypothesis was advanced that the effect of vasopressin on passive avoidance was associated with alterations in the level and turnover of brain NE or other neurotransmitters.

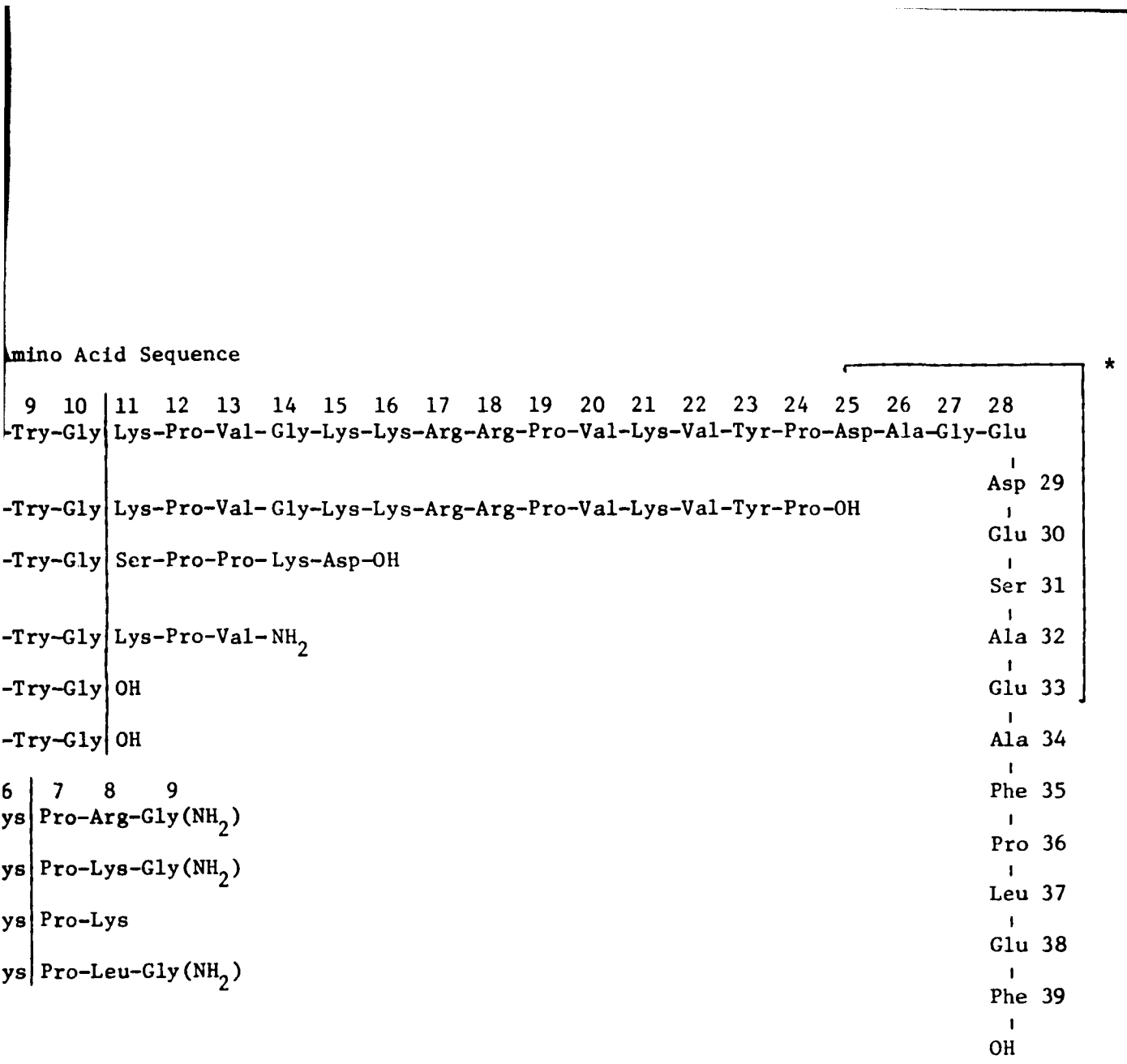
The fact that the behavior and size of HE rats was quite different from LE rats indicates that HE rats are not normal, or that there are differences between Brattleboro and LE rats apart from those involving vasopressin. It should be considered in future investigations that differences between Brattleboro and LE rats may be genetic in origin, or may be related to nutritional or environmental factors differentially affecting their early development.

APPENDIX I

AMINO ACID SEQUENCE OF SELECTED PEPTIDES

Peptide	Amino Acid Sequence
	1 2 3 | 4 5 6 7 8 9 10 | 11 12 13
ACTH 1-39 (Human)	H-Ser-Tyr-Ser | Met-Glu-His-Phe-Arg-Try-Gly | Lys-Pro-Va
ACTH β 1-24	Ac-Ser-Tyr-Ser | Met-Glu-His-Phe-Arg-Try-Gly | Lys-Pro-Va
β-MSH (Bovine)	H-Asp-Ser-Gly-Pro-Tyr-Lys | Met-Glu-His-Phe-Arg-Try-Gly | Ser-Pro-Pr
α-MSH	CH_3CO-Ser-Tyr-Ser | Met-Glu-His-Phe-Arg-Try-Gly | Lys-Pro-Va
ACTH 1-10	H-Ser-Tyr-Ser | Met-Glu-His-Phe-Arg-Try-Gly | OH
ACTH 4-10	H | Met-Glu-His-Phe-Arg-Try-Gly | OH
	1 2 3 4 5 6 | 7 8 9
Arginine Vasopressin	Cys-Tyr-Phe-Glu(NH_2)-Asp(NH_2)-Cys | Pro-Arg-Gly(NH_2)
Lysine Vasopressin	Cys-Tyr-Phe-Glu(NH_2)-Asp(NH_2)-Cys | Pro-Lys-Gly(NH_2)
Desglycinamide Vasopressin	Cys-Tyr-Phe-Glu(NH_2)-Asp(NH_2)-Cys | Pro-Lys
Oxytocin	Cys-Tyr-Ileu-Glu(NH_2)-Asp(NH_2)-Cys | Pro-Leu-Gly(NH_2)

*All interspecies differences occur in this sequence.


mino Acid Sequence
*
9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
-Try-Gly Lys-Pro-Val- Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-Asp-Ala-Gly-Glu
Asp 29
-Try-Gly Lys-Pro-Val- Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-OH
Glu 30
-Try-Gly Ser-Pro-Pro- Lys-Asp-OH
Ser 31
-Try-Gly Lys-Pro-Val- NH2
Ala 32
-Try-Gly OH
Glu 33
-Try-Gly OH
Ala 34
6 7 8 9
ys Pro-Arg-Gly(NH2)
Phe 35
Pro 36
ys Pro-Lys-Gly(NH2)
Leu 37
ys Pro-Lys
Glu 38
ys Pro-Leu-Gly(NH2)
Phe 39
OH

APPENDIX II

Open Field Test 1

Tabulations of crossings for each of the three minutes of the open field test were only made for male rats (Table 16). Unfortunately, the distribution of crossings in each minute was not recorded for one of the DI rats. DI rats made slightly more crossings than HE rats

TABLE 16

CROSSINGS DURING EACH MINUTE OF THE OPEN FIELD TEST

Minute	Crossings $\bar{x} \pm$ s.e.m.		
	DI (n=3)	HE (n=5)	LE (n=11)
1	14 ± 3	8 ± 1	19 ± 1
2	8 ± 2	6 ± 1	16 ± 1
3	7 ± 2	0 ± 0	16 ± 1

during all three minutes, and in both groups their crossings declined over time. LE rats made the most crossings in each of the three minutes and their crossings had not declined very much at the end of the test. Because the data from only three DI rats were available, these rats were not included in the analysis of variance comparisons of genotype vs minutes (Table 17). As both factors as well as the type x minutes interaction were significant, differences between HE and LE rats were evaluated at each of the three minutes, and differences

TABLE 17

ANALYSIS OF VARIANCE OF CROSSINGS OVER TIME: MALES

Source of variation	Sum of squares	df	Mean square	F	p
Between subjects					
Genotype (G)	1590.31	1	1590.31	12.67	<.01
Subjects w.g.	1757.84	14	125.56		
Within subjects					
Minutes (M)	128.17	2	64.08	13.10	<.01
GM	72.38	2	36.19	7.40	<.01
M x subjects w.g.	136.78	28	4.89		

between minutes evaluated separately for HE and LE rats. Only in the third minute did the LE rats make significantly more crossings than HE rats (p <.01) and that was because only one of the six HE rats made any crossings in that minute. Significant differences between minutes were found only for HE rats (p <.01); Newman-Keuls comparisons indicated that the crossings made by HE rats in each of the first two minutes were significantly greater than in the third minute (p <.01).

Open Field Test 2

As can be seen in Table 18, the mean number of crossings declined over the three minute period for all groups. An analysis of variance confirmed that there was no significant effect of sex or genotype but there were differences between minutes (Table 19). Individual comparisons using the Neuman-Keuls procedure revealed that

the number of crossings made by all animals in the first minute were significantly greater than the second ($p < .01$ or third minute ($p < .01$).

TABLE 18

CROSSINGS AND REARINGS DURING EACH MINUTE OF THE OPEN FIELD TEST

Minute	Crossings $\bar{x} \pm$ s.e.m.				Rearings $\bar{x} \pm$ s.e.m.			
	Males		Females		Males		Females	
	HE (n=6)	DI (n=10)	HE (n=13)	DI (n=12)	HE (n=5)	DI (n=10)	HE (n=13)	DI (n=12)
1	19 ± 1	27 ± 2	24 ± 1	26 ± 2	7 ± 1	5 ± 1	8 ± 1	6 ± 1
2	18 ± 4	18 ± 3	15 ± 2	18 ± 2	9 ± 2	8 ± 1	10 ± 1	8 ± 1
3	11 ± 4	16 ± 3	16 ± 2	16 ± 1	7 ± 1	7 ± 1	8 ± 1	8 ± 1

TABLE 19

ANALYSIS OF VARIANCE OF CROSSINGS OVER TIME

Source of variation	Sum of squares	df	Mean square	F	p
Between subjects					
Genotype (G)	28.95	1	28.95	<1	n.s.
Sex (S)	25.30	1	25.30	<1	n.s.
GS	23.83	1	23.83	<1	n.s.
Subjects w.g.	42445.81	37	1147.18		
Within subjects					
Minutes (M)	1655.40	2	827.70	11.11	<.01
GM	165.13	2	52.56	<1	n.s.
SM	128.93	2	64.46	<1	n.s.
GSM	44.69	2	22.34	<1	n.s.
M x subjects w.g.	5513.58	74	74.51		

Rearings increased from the first to the second minute and then declined for all groups except for the DI females. An analysis of variance (genotype vs sex vs minutes) did not indicate any significant differences between DI and HE rats but the minutes factor and the sex factor were significant (Table 20). Since the F-ratio for sex

TABLE 20

ANALYSIS OF VARIANCE OF REARINGS OVER TIME

Source of variation	Sum of squares	df	Mean square	F	p
Between subjects					
Genotype (G)	14.00	1	14.00	<1	n.s.
Sex (S)	.09	1	.09	1/F = 2226	<.01
GS	19.66	1	19.66	<1	n.s.
Subjects w.g.	7213.12	36	200.36		
Within subjects					
Minutes (M)	74.30	2	37.15	5.15	<.01
GM	1.65	2	.82	<1	n.s.
SM	15.57	2	7.78	1.08	n.s.
GSM	12.53	2	6.26	<1	n.s.
M x subjects w.g.	519.42	72	7.21		

was significantly less than unity and none of the Newman-Keuls comparisons between minutes were significant although the overall F-ratio for minutes was significant, analysis of variance comparisons between HE and DI rats were carried out separately on males and females to determine the source of the constriction (Tables 21, 22). In

TABLE 21

ANALYSIS OF VARIANCE OF REARINGS OVER TIME: MALES

Source of variation	Sum of squares	df	Mean square	F	p
Between subjects					
Genotype (G)	6.39	1	6.39	<1	n.s.
Subjects w.g.	461.61	13	35.50		
Within subjects					
Minutes (M)	46.53	2	23.26	8.25	<.01
GM	2.08	2	1.04	<1	n.s.
M x subjects w.g.	73.39	26	2.82		

TABLE 22

ANALYSIS OF VARIANCE OF REARINGS OVER TIME: FEMALES

Source of variation	Sum of squares	df	Mean square	F	p
Between subjects					
Genotype (G)	16.69	1	16.69	<1	n.s.
Subjects w.g.	545.23	23	23.70		
Within subjects					
Minutes (M)	39.68	2	19.84	2.63	n.s.
GM	21.41	2	10.70	1.42	n.s.
M x subjects w.g.	346.91	46	7.54		

confirmation of the previous analysis, there were no significant differences between DI and HE rats of either sex. There was no significant difference in the number of rearings made by female rats over the three minutes, but the minutes factor was significant for males and Newman-Keuls comparisons revealed that the number of rearings by males was significantly greater in the second ($p < .01$ and third minutes ($p < .05$) than in the first minute.

BIBLIOGRAPHY

Ader, R., & de Wied, D. Effects of lysine vasopressin on passive avoidance learning. Psychonomic Science, 1972, 29, 46-48.

Ader, R., Weijnen, J.A., & Moleman, P. Retention of a passive avoidance response as a function of the intensity and duration of electric shock. Psychonomic Science, 1972, 26, 125-218.

Archer, J. Tests for emotionality in rats and mice: A review. Animal Behavior, 1973, 21, 205-235.

Arimura, A., Saito, T., Bowers, C.Y., & Schally, A.V. Pituitary-adrenal activation in rats with hereditary hypothalamic diabetes insipidus. Acta Endocrinologica, 1967, 54, 155-165.

Arimura, A., Yamaguchi, T., Yoshimura, K., Imazeki, T., & Itoh, S. Role of the neurohypophysis in the release of adrenocorticotrophic hormone in the rat. Japanese Journal of Physiology, 1965, 15, 278-295.

Bailey, W.H. Effect of ACTH and related peptides on cardiovascular activity in the rat. Unpublished manuscript, 1973.

Bohus, B., Ader, R., & de Wied, D. Effects of vasopressin on active and passive avoidance behavior. Hormones and Behavior, 1972, 3, 191-197.

Bohus, B., & de Wied, D. Inhibitory and facilitatory effect of two related peptides on extinction of avoidance behavior. Science, 1965, 153, 318-320.

Bohus, B., & de Wied, D. Failure of α-MSH to delay extinction of conditioned avoidance behavior in rats with lesions in the parafascicular nuclei of the thalamus. Physiology and Behavior, 1967, 2, 221-223.

Bohus, B., Gispen, W.H., & de Wied, D. Effect of lysine vasopressin and ACTH 4-10 on conditioned avoidance behavior of hypophysectomized rats. Neuroendocrinology, 1973, 11, 137-143.

Bohus, B., & Lissák, K. Adrenocortical hormones and avoidance behavior in rats. International Journal of Neuropharmacology, 1968, 7, 301-306.

Bohus, B., Nyakas, C., & Endröczi, E. Effects of adrenocorticotrophic hormone on avoidance behavior of intact and adrenalectomized rats. International Journal of Neuropharmacology, 1968, 7, 307-314.

Bridges, T.E., & Thorn, N.A. The effect of autonomic blocking agents on vasopressin release in vivo induced by osmoreceptor stimulation. Journal of Endocrinology, 1970, 48, 265-276.

Candland, D.K., & Campbell, B.A. Development of fear in the rat as measured by behavior in the open field. Journal of Comparative and Physiological Psychology, 1962, 55, 593-596.

Chan, O., & Webster, R.A. Importance of noradrenaline found in a functional pool in maintaining spontaneous locomotor activity in rats. British Journal of Pharmacology, 1971, 41, 700-708.

Cheng, K.W., Friesen, H.G., & Martin, J.B. Neurophysin in rats with hereditary hypothalamic diabetes insipidus (Brattleboro strain). Endocrinology, 1972, 90, 1055-1063.

Daughaday, W.H. The binding of corticosteroids by plasma protein. In A.B. Eisenstein (Ed.) The adrenal cortex. Boston: Little, Brown and Co., 1967.

Dempsey, G.L., Kastin, A.J., & Schally, A.V. The effects of MSH on a restricted passive avoidance response. Hormones and Behavior, 1972, 3, 333-337.

Denenberg, V.H. Stimulation in infancy, emotional reactivity, and exploratory behavior. In D.H. Glass (Ed.), Biology and behavior: neurophysiology and emotion. New York: Russell Sage Foundation & Rockefeller Univ. Press, 1967.

Denti, A., & Epstein, A. Sex differences in the acquisition of two kinds of avoidance behavior in rats. Physiology and Behavior, 1972, 8, 611-615.

de Wied, D. The significance of the antidiuretic hormone in the release mechanism of corticotropin. Endocrinology, 1961, 68, 956-970.

de Wied, D. Influence of anterior pituitary on avoidance learning and escape behavior. American Journal of Physiology, 1964, 207, 255-259.

de Wied, D. The influence of the posterior and intermediate lobe of the pituitary and pituitary peptides on the maintenance of a conditioned avoidance response in rats. International Journal of Neuropharmacology, 1965, 4, 157-167.

de Wied, D. Inhibitory effect of ACTH and related peptides on extinction of conditioned avoidance behavior in rats. Proceedings of the Society for Experimental Biology and Medicine, 1966, 122, 28-32. (a)

de Wied, D. Opposite effects of ACTH and glucocorticoids on extinction of conditioned avoidance behavior. In Proceedings of the Second International Congress on Hormonal Steroids (Vol. 132). Amsterdam: Excerpta Medica, 1966. (b)

de Wied, D. Influence of vasopressin and of a crude CRF preparation on pituitary ACTH-release in posterior-lobectomized rats. Neuroendocrinology, 1968, 3, 129-135.

de Wied, D. The anterior pituitary and conditioned avoidance behavior. In Proceedings of the Third International Congress of Endocrinology (Vol. 184). Amsterdam: Excerpta Medica, 1969. (a).

de Wied, D. Effects of peptide hormones on behavior. In W.F. Ganong & L. Martini (Eds.), Frontiers in neuroendocrinology. New York: Oxford University Press, 1969. (b)

de Wied, D. Long term effect of vasopressin on the maintenance of a conditioned avoidance response in rats. Nature, 1971, 232, 58-60.

de Wied, D. The role of the posterior pituitary and its peptides on the maintenance of conditioned avoidance behavior. In K. Lissák (Ed.), Hormones and brain function. New York: Plenum Press, 1973.

de Wied, D., & Bohus, B. Long term and short term effects on retention of a conditioned avoidance response in rats by treatment with long acting pitressin and α-MSH. Nature, 1966, 212, 1484-1486.

de Wied, D., Bohus, B., & Greven, H.M. Influence of pituitary and adrenocortical hormones on conditioned avoidance behavior in rats. In R.P. Michael (Ed.), Endocrinology and human behavior. London: Oxford University Press, 1968.

de Wied, D., Greven, H.M., Lande, S., & Witter, A. Dissociation of the behavioral and endocrine effects of lysine vasopressin by tryptic digestion. British Journal of Pharmacology, 1972, 45, 118-122. (a)

de Wied, van Delft, A.M., Gispen, W.H., Weijnen, J.A., & van Wimersma Griedanus, Tj.B. The role of pituitary-adrenal system hormones in active avoidance conditioning. In S. Levine (Ed.), Hormones and behavior. New York: Academic Press, 1972. (b)

de Wied, D., Witter, A., & Lande, S. Anterior pituitary peptides and avoidance acquisition of hypophysectomized rats. In D. de Wied & J.A.W.M. Weijnen (Eds.), Pituitary, adrenal & brain (Progress in Brain Research, Vol. 32). Amsterdam: Elsevier, 1970.

Di Giusto, E.L., Cairncross, K.D. & King, M.G. Hormonal influences on fear-motivated responses. Psychological Bulletin, 1971, 75, 432-444.

Draper, M.W., Merrifield, R.D., & Rizack, M.A. Lipolytic activity of Met-Arg-His-Phe-Arg-Trp-Gly, a synthetic analog of the ACTH (4-10) core sequence. Journal of Medical Chemistry, 1973, 16, 1326-1330.

Duffy, E. Activation & behavior. New York: John Wiley & Sons, 1962.

Engel, F.L., & Lebovitz, H.E. Extra-target organ actions of anterior pituitary hormones. In G.W. Harris & B.T. Donovan (Eds.), The pituitary gland (Anterior Pituitary, Vol. 2). Berkeley, California: University of California Press, 1966.

Endröczi, E. Limbic system, learning and pituitary-adrenal function. Budapest: Akadémiai Kiadó, 1972.

Friedman, S.M., Scherrer, H.F., Nikashima, M., & Friedman, C.L. Extra-renal factors in diabetes insipidus in the rat. American Journal of Physiology, 1958, 192, 401-404.

Fuxe, K., Hökfelt, T., Jonsson, G., & Lidbrink, P. Brain-endocrine interaction: Are some effects of ACTH and adrenocortical hormones on neuroendocrine regulation and behaviour mediated via central catecholamine neurons. In K. Lissák (Ed.), Hormones and brain unction. New York: Plenum Press, 1973.

Galton, V.A., Valtin, H., & Johnson, D.G. Thyroid function in the absence of vasopressin. Endocrinology, 1966, 78, 1224-1229.

Garrud, P., Gray, J.A., & de Wied, D. Pituitary-adrenal hormones and extinction of rewarded behavior in the rat. Physiology and Behavior, 1974, 12, 109-119.

Gibbs, J., Sechzer, J.A., Smith, G.P., Conners, R., & Weiss, J.M. Behavioral responsiveness of adrenalectomized, hypophysectomized and intact rats to electric shock. Journal of Comparative and Physiological Psychology, 1973, 82, 165-169.

Gispen, W.H., de Wied, D., Schotman, P., & Jansz, H.S. Brain stem polysomes and avoidance performance of hypophysectomized rats subjected to peptide treatment. Brain Research, 1971, 31, 341-351.

Gispen, W.H., van Wimersma Greidanus, Tj.B., & de Wied, D. Effects of hypophysectomy and ACTH 1-10 on responsiveness to electric shock in rats. Physiology and Behavior, 1970, 5, 143-146.

Gordon, J.H., & Shellenberger, M.K. Regional catecholamine content in the rat brain: Sex differences and correlation with motor activity. Neuropharmacology, 1974, 13, 129-137.

Gray, J. The Psychology of fear and stress. New York: McGraw Hill Co., 1971. (a)

Gray, J.A. Effect of ACTH on extinction of rewarded behaviour is blocked by previous administration of ACTH. Nature, 1971, 229, 52-54. (b)

Gray, P. Effect of adrenocorticotrophic hormone on conditioned avoidance in rats interpreted as state-dependent learning. Journal of Comparative and Physiological Psychology, 1975, 88, 281-284.

Greven, H.M., & de Wied, D. The active sequence in the ACTH molecule responsible for inhibition of the extinction of conditioned avoidance behaviour in rats. European Journal of Pharmacology, 1967, 2, 14-16.

Greven, H.M., & de Wied, D. The influence of peptides derived from corticotropin (ACTH) on performance: structure activity studies. In E. Zimmerman, W.H. Gispen, B.H. Marks & D. de Wied (Eds.), Drug effects on neuroendocrine regulation (Progress in Brain Research, Vol. 39). Amsterdam: Elsevier, 1973.

Guillemin, R. Hypothalamic factors releasing pituitary hormones. Recent Progress in Hormone Research, 1964, 20, 89-130.

Guth, S., Seward, J.P., & Levine, S. Differential manipulation of passive avoidance by exogenous ACTH. Hormones and Behavior, 1971, 2, 127-138.

Harrington, A.R., & Valtin, H. Vasopressin effect on urinary concentration in rats with hereditary hypothalamic diabetes insipidus. Proceedings of the Society for Experimental Biology and Medicine, 1965, 118, 448-450.

Harrington, A.R., & Valtin, H. Impaired urinary concentration after vasopressin and its gradual correction in hypothalamic diabetes insipidus. Journal of Clinical Investigation, 1968, 47, 502-510.

Jaques, R. Non-specific effects of synthetic corticotrophin polypeptides. International Archives of Allergy, 1965, 28, 221-230.

Joh, T.H., Ross, R.A., & Reis, D.J. A simple and sensitive assay for dopamine-β-hydroxylase. Analytical Biochemistry, 1974, 62, 248-254.

Kastin, A.J., Miller, L.H., Nockton, R., Sandman, C.A., Schally, A.V., & Stratton, L.O. Behavioral aspects of MSH. In E. Zimmerman, W.H. Gispen, B.H. Marks & D. de Wied (Eds.), Drug effects on neuroendocrine regulation (Progress in Brain Research, Vol. 39). Amsterdam: Elsevier, 1973.

King, A.R., & de Wied, D. Localized behavioral effects of vasopressin on maintenance of an active avoidance response in rats. Journal of Comparative and Physiological Psychology, 1974, 86, 1008-1018.

Knigge, K.M., & Hoar, E.M. A procedure for measuring plasma binding of adrenal corticoids: effect of acute and chronic stress. Proceedings of the Society for Experimental Biology and Medicine, 1963, 113, 623-627.

Korányi, L., Endröczi, E., Lissák, K., & Szepes, E. The effect of ACTH on behavioral processes motivated by fear in mice. Physiology and Behavior, 1967, 2, 439-445.

Lamprecht, F., & Wooten, G.F. Effect of hypophysectomy on serum dopamine-beta-hydroxylase activity in rat. Endocrinology, 1973, 92, 1543-1546.

Lande, S., Flexner, J.B., & Flexner, L.B. Effect of corticotrophin and desglycinamide-lysine vasopressin on suppression of memory by puromycin. Proceedings of the National Academy of Science, 1972, 69, 558-560.

Lande, S., Witter, A., & de Wied, D. An octapeptide that stimulates conditioned avoidance acquisition in hypophysectomized rats. Journal of Biological Chemistry, 1971, 246, 2058-2062.

Leonard, B.E. The effect of two synthetic ACTH analogues on the metabolism of biogenic amines in the rat brain. Archives Internationales de Pharmacodynamie et de Therapie, 1974, 207, 243-251.

Levine, S. Hormones and conditioning. In W.J. Arnold (Ed.), Nebraska symposium on motivation (Vol. 18). Lincoln: University of Nebraska Press, 1968.

Ley, K.F., & Corson, J.A. ACTH; Differential effects on avoidance and discrimination. Experientia, 1971, 27, 958-959. (a)

Ley, K.F., & Corson, J.A. ACTH and zinc-phosphate vehicle interact with UCS parameters to influence shuttlebox CAR. Hormones and Behavior, 1971, 2, 149-160. (b)

Ley, K.F., & Corson, J.A. Effects of ACTH, adrenalectomy and time of day on emotional activity of the rat. Behavioral Biology, 1973, 9, 111-115.

Lissák, K., & Bohus, B. Pituitary hormones and avoidance behavior of the rat. International Journal of Psychobiology, 1972, 2, 103-115.

McCann, S.M., Antunes-Rodrigues, J., Nallar, R., & Valtin, H. Pituitary-adrenal function in the absence of vasopressin. Endocrinology, 1966, 79, 1058-1064.

Miller, M., & Moses, A.M. Radioimmunoassay of urinary antidiuretic hormone with application to study of the Brattleboro rat. Endocrinology, 1971, 88, 1389-1396.

Miller, R.E., Yueh-Chien, H., Wiley, M.K., & Hewitt, R. Anterior hypophysial function in the posterior-hypophysectomized rat: Normal regulation of the adrenal system. Neuroendocrinology, 1974, 14, 233-250.

Mitchell, J.A., Smyrl, R., Hutchins, M., Schindler, W.J., & Critchlow, V. Plasma growth hormone levels in rats with increased naso-anal lengths due to hypothalamic surgery. Neuroendocrinology, 1972, 10, 31-45.

Möhring, J., Dauda, G., Haack, D., Homsy, E., Kohrs, G., & Möhring, B. Increased potassium intake and kaliopenic nephropathy in rats with genetic diabetes insipidus. Life Sciences (Part I), 1972, 11, 679-683. (a)

Möhring, J., Schömig, A., Brekner, H., & Möhring, B. ADH-induced potassium retention in rats with genetic diabetes insipidus. Life Sciences (Part I), 1972, 11, 65-72. (b)

Montgomery, K.C. The relation between fear induced by novel stimulation and exploratory behavior. Journal of Comparative and Physiological Psychology, 1955, 48, 254-260.

Moore, K.E., & Rech, R.H. Behavioral and norepinephrine-depleting effects of disulfiram in resperpine pretreated rats. Archives of Internationales Pharmacodynamie et de Therapie, 1969, 180, 413-422.

Moses, A.M., & Miller, M. Accumulation and release of pituitary vasopressin in rats heterozygous for hypothalamic diabetes insipidus. Endocrinology, 1970, 86, 34-41.

Nakajima, S. Amnesic effect of cyclohexamide antagonized by corticosteroids. Paper presented at the meeting of the American Psychological Association, Montreal, 1973.

Nagy, V., Czakó, L., Durszt, F., & László, F. Antidiuretic hormone release in the neurohypophysectomized rats. Acta Physiologica Academiae Scientiarum, Hungaricae, 1972, 41, 321.

Nitzan, M., & Wilber, J.F. Effect of postnatal malnutrition on plasma proteins and growth hormone in the rat. Hormone Research, 1974, 5 167-172.

Nyakas, Cs. Influence of corticosterone and ACTH on the postnatal development of learning and memory functions. In K. Lissák (Ed.), Hormones and brain function. New York: Plenum Press, 1973.

Orias, R., & McCann, S.M. Natriuresis induced by alpha and beta melanocyte stimulating hormone (MSH) in rats. Endocrinology, 1972, 90, 700-706.

Overton, D.A. State-dependent learning produced by depressant and atropine-like drugs. Psychopharmacologia, 1966, 10, 6-31.

Pappas, B.A., & Gray, P. Cue value of dexamethasone for fear-motivated behavior. Physiology and Behavior, 1971, 6, 127-130.

Paré, W.P. Age, sex and strain differences in the aversive threshold to grid shock in the rat. Journal of Comparative and Physiological Psychology, 1969, 69, 214-218.

Paré, W.P., & Cullen, J.W. Adrenal influences on the aversive threshold and CER acquisition. Hormones and Behavior, 1971, 2, 139-147.

Pinel, J.P.J., Malsbury, C.W., & Corcoran, M.E. The incubation effect in rats: Skin resistance changes after footshock Physiology and Behavior, 1971, 6, 111-114.

Pohorecky, L.A. Effects of ethanol on central and peripheral noradrenergic neurons. Journal of Pharmacology and Experimental Therapeutics, 1974, 189, 380-391

Reading, H.W. Effect of some adrenocorticotrophin analogues on protein synthesis in the brain. Biochemical Journal, 1972, 127, 7p. (Abstract)

Rech, R.H., Borys, H.K., & Moore, K.E. Alterations in behavior and brain catecholamine levels in rats treated with α-methyltyrosine. Journal of Pharmacology and Experimental Therapeutics, 1966, 153, 412-419.

Russell, P.A. Open-field defecation in rats: Relationships with body weight and basal defecation level. British Journal of Psychology, 1973, 64, 109-114. (a)

Russell, P.A. Relationships between exploratory behavior and fear: A review. British Journal of Psychology, 1973, 64, 417-433. (b)

Sandman, C.A., Denman, P.M., Miller, L.H., Knott, J.R., Schally, A.V., & Kastin, A.J. Electroencephalographic measures of melanocyte-stimulating hormone activity. Journal of Comparative and Physiological Psychology, 1971, 76, 103-109. (a)

Sandman, C.A., Kastin, A.J., & Schally, A.V. Behavioral inhibition as modified by melanocyte-stimulating hormone (MSH) and light-dark conditions. Physiology and Behavior, 1971, 6, 45-48. (b)

Saul, G.B., Garrity, E.B., Benirschke, K., & Valtin, H. Inherited hypothalamic diabetes insipidus in the Brattleboro strain of rats. Journal of Heredity, 1968, 59, 113-117.

Sawyer, W.H., & Valtin, H. Inhibition of vasopressin antidiuresis by extracts of pituitaries from rats with hereditary hypothalamic diabetes insipidus and by oxytocin. Endocrinology, 1965, 76, 999-1001.

Schalch, D.S., & Reichlin, D. Stress and growth hormone release. In A. Pecile & E.E. Muller (Eds.), Growth hormone. Amsterdam: Excerpta Medica, 1968.

Scott, D.E. Fine structural features of the neural lobe of the hypothalamus of the rat with homozygous diabetes insipidus (Brattleboro Strain). Neuroendocrinology, 1968, 3, 156-176.

Selye, H. Stress. Montreal: Acta Medical Publishers, Inc., 1950.

Siegel, S. Non parametric statistics for the behavioral sciences. New York: McGraw-Hill Book Co., Inc., 1956.

Smith, G.P. Adrenal hormones and emotional behavior. In E. Stellar & J.M. Sprague (Eds.), Progress in physiological psychology (Vol. 5). New York: Academic Press, 1973.

Sokol, H.W. Skeletal elongation stimulated by exogenous growth hormone, but not vasopressin, in growth-retarded rats having diabetes insipidus. Program of the 55th meeting of the Endocrine Society, 1973. (Abstract)

Sokol, H.W., & Valtin, H. The morphology of the neurosecretory system in a strain of rats with familial hypothalamic diabetes insipidus. American Zoologist, 1962, 2, 560. (Abstract)

Stratton, L.O., & Kastin, A.J. Avoidance learning at two levels of shock in rats receiving MSH. Hormones and Behavior, 1974, 5, 149-155.

Swaab, D.F., Boer, G.J., & Nolton, J.W.L. The hypothalamic-neurohypophyseal systems (HNS) of the Brattleboro rat. Acta Endocrinologica, 1973, 80, 80. (Abstract)

Takahashi, H., Daughday, W.H., & Kipins, D.M. Regulation of immunoreactive growth hormone secretion in male rats. Endocrinology, 1971, 88, 909-917.

Tepperman, J. Metabolic and endocrine physiology (2nd edition). Chicago: Year Book Medical Publishers, Inc., 1968.

Thiérry, A., Javoy, F., Glowinski, J., & Kety, S.S. Effects of stress on the metabolism of norepinephrine, dopamine and serotonin in the central nervous system of the rat. I. Modifications of norepinephrine turnover. Journal of Pharmacology and Experimental Therapeutics, 1968, 163, 163-171.

Thompson, E.A., & de Wied, D. The relationship between the antidiuretic activity of rat eye plexus blood and passive avoidance behavior. Physiology and Behavior, 1973, 11, 377-380.

Ungerstedt, U. Sterotaxic mapping of the monoamine pathways in the rat brain. Acta Physiologica Scandinavica, 1971, Suppl. 367, 1-48.

Valtin, H. Hereditary hypothalamic diabetes insipidus in rats (Brattleboro strain). American Journal of Medicine, 1967, 42 814-827.

Valtin, H., & Schroeder, H.A. Familial hypothalamic diabetes insipidus in rats (Brattleboro strain). American Journal of Physiology, 1964, 206, 425-430.

Valtin, H., Sawyer, W.H., & Sokol, H.W. Neurohypophyseal principles in rats homozygous and heterozygous for hypothalamic diabetes insipidus (Brattleboro strain). Endocrinology, 1965, 77, 701-706.

van Wimersma Greidanus, Tj.B., & de Wied, D. Effects of systemic and intracerebral administration of two opposite acting ACTH-related peptides on extinction of conditioned avoidance behavior. Neuroendocrinology, 1971, 7, 291-301.

van Wimersma Greidanus, Tj.B., Bohus, B., & de Wied, D. Differential localization of the influence of lysine vasopressin and ACTH 4-10 on avoidance behavior: A study in rats bearing lesions in the parafascicular neculei. Neuroendocrinology, 1974, 14, 280-288.

Versteeg, D.H.G., Gispen, W.H., Schotman, P., Witter, A., & de Wied, D. Hypophysectomy and rat brain metabolism: Effects of synthetic ACTH analogs. In E. Costa, L.L. Iverson & R. Paoletti (Eds.), Advances in biochemical psychopharmacology (Vol. 6). New York: Raven Press, 1972.

Wang, S.S. Synthesis of desglycinamide lysine vasopressin and its behavioral activity in rats. Biochemical and Biophysical Research Communications, 1972, 48, 1511-1515.

Weijnen, J.A.W.M., & Slangen, J.L. Effects of ACTH-analogues on extinction of conditioned behavior. In D. de Wied, & J.A.W.M. Weijnen (Eds.), Pituitary adrenal and the brain (Progress in Brain Research, Vol. 32). Amsterdam: Elsevier, 1970.

Weinshilboum, R., & Axelrod, J. Serum dopamine-β-hydroxylase: decrease after chemical sympathectomy. Science, 1971, 173, 931-933.

Weinshilboum, R.M., Thoa, N.B., Johnson, D.E., Kopin, J.J., & Axelrod, J. Proportional release of norepinephrine and dopamine-β-hydroxylase from sympathetic nerves. Science, 1971, 174, 1349-1351.

Weiss, J.M., McEwen, B.S., Silva, M.T.A., & Kalkut, M.F. Pituitary-adrenal influences on fear responding. Science, 1969, 163, 197-199.

Weiss, J.M., McEwen, B.S., Silva, M.T.A., & Kalkut, M.F. Pituitary-adrenal alterations and fear responding. American Journal of Physiology, 1970, 218, 864-868. (a)

Weiss, J.M., Stone, E.A., & Harell, N. Coping behavior and brain norepinephrine. Journal of Comparative and Physiological Psychology, 1970, 72, 153-160. (b)

Whimbey, A.E., & Denenberg, V.H. Two independent behavioral dimensions in open field performance. Journal of Comparative and Physiological Psychology, 1967, 63, 500-504.

Wilder, J. The law of initial value in neurology and psychiatry: facts and problems. Journal of Nervous and Mental Disease, 1957, 125, 73-86.

Winer, B.J. Statistical principles in experimental design. New York: McGraw-Hill Book Co., 1962.

Yamori, Y., Lovenberg, W., & Sjoerdsma, A. Norepinephrine metabolism in brainstem of spontaneously hypertensive rats. Science, 1970, 70, 544-546.

Yates, F.E., Russell, S.M., Dallman, M.F., Hedge, G.A., McCann, S.M., and Dhariwal, A.P.S. Potentiation by vasopressin of corticotropin release induced by corticotropin-releasing factor. Endocrinology, 1971, 88, 3-15.