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The Effects of Lysine Vasopressin on Conditioned Behaviour in Rats

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The Effects of Lysine Vasopressin on Conditioned Behaviour in Rats

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DECLARATION

I declare that during the course of this research I have not been registered for another award of the CNAA or of any University and that the material contained in this thesis has not previously been submitted for any academic award.

James Joseph Hagan.

James Joseph Hagan

To Carole

To my parents

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ABSTRACT

This thesis is concerned with lysine vasopressin's (LVP's) behavioural activity. Chapter One describes vasopressin's synthesis, secretion, metabolism, pressor, antidiuretic and putative corticotrophic functions with emphasis on behaviourally significant aspects. Chapter Two reviews behavioural data showing that manipulations of endogenous vasopressin levels alter subsequent avoidance performance. Although these data have predominantly been interpreted in favour of vasopressin altering memory formation (consolidation) results reported from an experiment combining response prevention trials and vasopressin injections failed to support the consolidation hypothesis. Chapter Three reviews the response prevention literature and confirms the feasibility of using prevention trials with automated shuttle box training (Experiment One). LVP (1 µg/rat) injected immediately after training increased subsequent extinction responding (Experiment Two). Experiment Three showed that LVP (1 µg/rat) increased responding when injected immediately after prevention trials but decreased extinction responding when injected after 30 minutes of post training retention in the home cage or 30 extinction trials. LVP injections 30 minutes after training and immediately after prevention trials increased suppression of concurrent lever press responding 24 hours later (Experiment Four). Manipulating the training-injection interval after automated training yielded maximal response reductions with a 60 minute interval (Experiment Five) with indications of a negative dose response curve for higher (2-4 µg/rat) doses (Experiment Six). Manual shuttle box tests showed that with a 30 minute training-injection interval subsequent extinction responding varied as an inverted "U" shaped function of the LVP dose (Experiment Seven). Opposite effects of 0.11 µg/rat and 2.97 µg/rat were confirmed with higher training shock levels (Experiment Nine). A further experiment (Experiment Eight) revealed a complex interaction between dose and injection interval. Extinction responding was also reduced by some doses of DG-LVP (Experiment Ten). Post training manipulation of cholinergic activity did not alter LVP's response reducing effects in well trained rats (Experiment Eleven) although some cholinergic involvement was indicated (Experiment Fourteen) in the response increasing effects of LVP (1 µg/rat) injected 30 minutes after training in poor avoidance learners (Experiments Twelve and Thirteen). Tests on the suitability of appetitive responding for exploring vasopressin's behavioural effects showed that both a variable interval (60 seconds) schedule and differential reinforcement of low response rates (DRL) schedule were sensitive to high LVP doses (3-4 µg/rat). The implications of these data for our understanding of vasopressin's behavioural effects are discussed.

CONTENTS

ABSTRACT

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ABBREVIATIONS

General

Adrenocorticotrophic hormone	ACTH
Alpha-methyl-para-tyrosine	AMPT
Antidiuretic hormone	ADH
Arginine vasopressin	AVP
Arginine vasotocin	AVT
Catecholamine(rgic)	CA
Central nervous system	CNS
Cerebrospinal fluid	CSF
Corticotropin releasing factor	CRF
Cyclic adenosine monophosphate	cAMP
Des-glycinamide	DG (Des-Gly)
Diabetes insipidus	DI
Dopamine(rgic)	DA
Grammes per body weight	gbw
Heterozygous diabetes insipidus	HE-DI
Home cage	HC
Homozygous diabetes insipidus	HO-DI
Intra peritoneal	IP
Intraventricular	IV
Interquartile range	IQR
Lysine vasopressin	LVP
Neurosecretory granules	NSG
Neurosecretory material	NSM
Noradrenaline (noradrenergic)	NA
Physostigmine sulphate (eserine)	Phy
Response prevention	RP
Saline	Sal
Scopolamine hydrobromide	Scop
Subcutaneous	SC

Units

International units	IU
Milli units	mU
Kilogrammes	kg
Grammes	g
Milligrammes	mg (10^{-3})

Units (continued)

Microgrammes	µg (ug) (10^{-6})
Micromolar	µmol
Molar	mol
Nanogrammes	ng (10^{-12})
Picogrammes	pg (10^{-9})

LIST OF FIGURES

Page

LIST OF TABLES

INTRODUCTION

An important development in our understanding of the relationship between brain and behaviour in the last two decades has been the realization that peptide hormones play an important role in regulating physiological and behavioural processes. Current biological and physiological research in this area depends heavily on recent technical developments; however, much of the initial impetus came from the behavioural studies of de Wied and his colleagues. They showed that both adrenocorticotrophic hormone (ACTH) and melanocyte stimulating hormone (MSH) could alter levels of conditioned avoidance responding in adenohypophysectomised, hypophysectomised and intact rats independently of endocrine target organ effects (see de Wied and Gispen (1977) for review). They also produced extensive evidence implicating vasopressin in behavioural processes independent of its pressor and antidiuretic functions (see Chapter Two). In addition recent evidence implicates oxytocin (Schulz et al 1974; Bohus et al 1978a) endorphins and enhephalins in modulating avoidance extinction rates (de Wied et al 1978).

Psychological theories and constructs have been used extensively to explain these findings, and it has been argued that as behavioural procedures are not only sensitive to,but can differentiate pharmacological responses from structurally related peptides, these are necessarily affecting different mechanisms. These results have clearly stimulated expectations that peptide hormones will prove useful in the treatment of mental illness; ACTH and MSH appear to affect memory (Flood, Jarvik, Bennet and Orme 1976; Rigter, Jansenns-Elbertse and van Reizen 1976) and attention processes in rats (Champney, Sahley and Sandman 1976; Beckwith; Sandman and Kastin 1976), normal adult males (Miller, van Reizen and Kastin 1976; Dornbush and Nikolovski 1976), mentally retarded adult males (Sandman, George, Walker, Nolan and Kastin 1976) and the elderly (Ferris et al 1976). The fragment des-tyrosine-gamma-endorphin may prove therapeutic in treatment of schizophrenia (de Wied 1979) and vasopressin has been tested with amnestic patients (Legros et al 1978, Oliveros et al 1978) and may prove beneficial in treating lesh-nyhans disease (Anderson et al 1979).

Discussionsin this thesis focus on vasopressins and the widely accepted hypothesis that these peptides play a physiological role in regulating memory formation (consolidation). Attention has been focussed on vasopressins, rather than comparing different classes of peptides as is common in the literature, in order to avoid the methodological difficulties inherent, though rarely discussed, in the latter

approach. These problems are particularly acute with systemic injections. Multiple sites may be involved with differential accessibility; blood/brain barrier permeability, metabolic and behavioural half life and susceptibility to enzymatic degradation will vary between peptides. In addition, each peptide will activate central and peripheral endocrine target organs differentially, altering the animal's physiological conditions and triggering compensatory mechanisms.

The behavioural effects of vasopressins form part of a wider spectrum of pharmacological responses, some of which are thought to reflect physiological roles for the endogenous peptide. Chapter One briefly describes aspects of vasopressin's synthesis in the anterior hypothalamus, transport along the hypothalamo-hypophyseal tract, secretion into the periphery and evidence suggesting direct secretory routes into the cerebrospinal fluid (CSF) and blood vessels supplying the anterior lobe of the pituitary gland. Several aspects of the data, including peripheral metabolism rates, secretion under stress, presence of vasopressin in the CSF, its putative role as corticotropin releasing factor (CRF) and capacity, at least in high doses, to stimulate the pituitary-adrenal axis bear directly on the peptide's involvement in behavioural regulation.

The hypothesis that vasopressin plays a physiological role in the formation of memory (consolidation) derives from experiments which show that manipulations of endogenous vasopressin levels and post training pharmacological challenge with exogenous vasopressin result in altered performance levels when responding is subsequently tested in the absence of reinforcement (extinction). These data, with supportive evidence from experiments using amnestic treatments and studies which explore the neuroanatomical and neurochemical for vasopressin's behavioural effects, are reviewed in Chapter Two.

A result which appears to contradict the consolidation hypothesis was reported by King and de Wied (1974) using vasopressin injections coupled with response prevention trials, a behavioural procedure which reduces avoidance responding in extinction. This important negative result forms the basis of some of the experiments described in later chapters; therefore a brief review of the response prevention literature, highlighting important variables, together with an experiment demonstrating the feasibility of using prevention trials after shuttle box avoidance responding in a delayed testing procedure is presented in Chapter Three.

In subsequent chapters a number of experiments are reported, all using post training injections, some with and some without response

prevention trials, which show that, although vasopressin quite clearly alters the status of an aversively conditioned stimulus, as measured by both avoidance extinction and suppression of a concurrent operant baseline, the effects appear to be independent of any "informational" or "associational" changes. In addition, as both magnitude and direction of performance changes in extinction varied as a function of peptide dose and the interval between training and injection, it was concluded that the data could not be explained by the consolidation hypothesis alone.

CHAPTER ONE

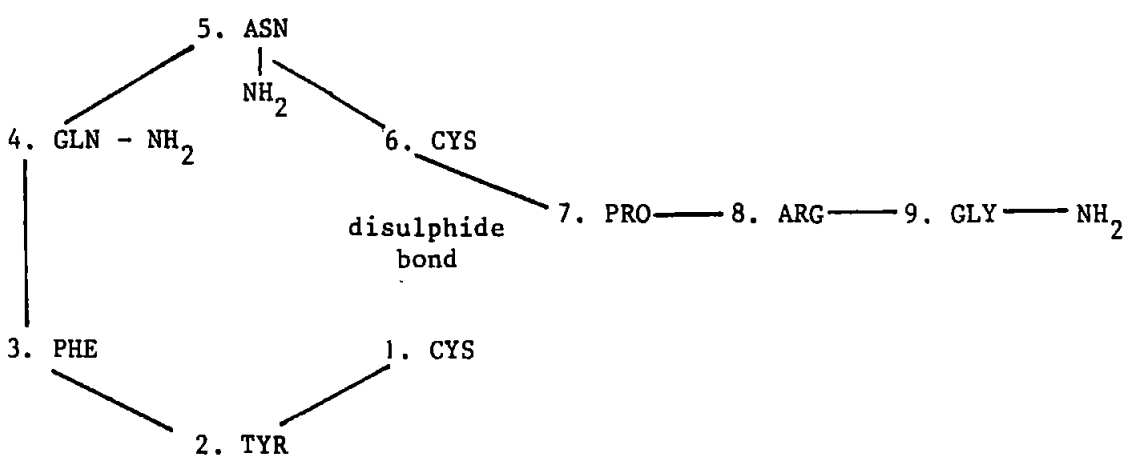
THE VASOPRESSINS: ANATOMICAL, FUNCTIONAL, PHYSIOLOGICAL AND PHARMACOLOGICAL CONSIDERATIONS

This chapter describes aspects of the structure, synthesis and secretion of vasopressin; additional consideration is given to factors which affect secretion, neurochemical control of secretion, effects of the peptide on target organs and the role which vasopressin plays in activating the pituitary adrenal axis.

1.1 Structures and Evolution of Posterior Lobe Peptide Hormones

The vasopressins are classified as octapeptides or nonapeptides and are formed by a ring of six and tail of three amino acids linked by peptide bonds (Bennett and Freiden 1972). This structure is illustrated in Figure One, a disulphide bond between the two cysteine residues in positions one and six produce the characteristic ring of the structure (Conn and Stumpf 1972).

Figure One: Structure of Arginine Vasopressin (AVP)



The pressor effects of bovine pituitary extract were first discovered by Oliver and Schafer (1895) but it remained for Du Vigneaud (1955) to identify the molecular structure of the active principle. Vertebrate neurohypophyseal extracts have yielded seven biologically active octapeptides, three of which exhibit strong antidiuretic and pressor action (Sawyer 1967). These three are arginine vasotocin (AVT), arginine vasopressin (AVP; cf Figure One) and lysine vasopressin (LVP). The four remaining posterior lobe peptides are oxytocin, mesotocin, isotocin and glumotocin. These exhibit low antidiuretic and pressor action (Walter et al 1967). Oxytocin is secreted from the mammalian pituitary and stimulates milk ejection, from the mammary glands, and uterine contractions (Bennett and Frieden 1972). The amino acid sequences of these peptides are presented in Figure Two.

Figure Two: The amino acid sequences of posterior lobe peptide hormones

(1) Arginine Vasotocin (AVT)

Cys	Tyr	Ile	Gln	Asn	Cys	Pro	Arg	Gly (NH_2)

(2) Arginine Vasopressin (AVP)

Cys	Tyr	Phe	Gln	Asn	Cys	Pro	Arg	Gly (NH_2)

(3) Lysine Vasopressin (LVP)

Cys	Tyr	Phe	Gln	Asn	Cys	Pro	Lys	Gly (NH_2)

(4) Oxytocin

Cys	Tyr	Ile	Gln	Asn	Cys	Pro	Leu	Gly (NH_2)

(5) Isotocin

Cys	Tyr	Ile	Ser	Asn	Cys	Pro	Ile	Gly (NH_2)

(6) Mesotocin

Cys	Tyr	Ile	Gln	Asn	Cys	Pro	Ile	Gly (NH_2)

(7) Glumitocin

Cys	Tyr	Ile	Ser	Asn	Cys	Pro	Gln	Gly (NH_2)

* ——— denotes ring structure

AVT has been found in all major non-mammalian vertebrates including reptiles, amphibians, teleost fish, cyclostomes and birds. Structural variation from AVT does not appear until the mammals, indicating structural and functional stability over some four hundred million years of evolutionary development (Sawyer 1967). Most vertebrates have two neurohypophyseal hormones, one in the vasopressin series (cf structures 1-3, Figure Two) and one in the oxytocin series (cf structures 4-7, Figure Two). These two separate series may have developed as a result of doubling the gene controlling AVT synthesis (Sawyer 1964). The common predecessor hypothesis is supported by comparisons of biological potency between the peptides. AVT, the proposed predecessor, is principally found in non-mammalian vertebrates although reports also suggest it to be present in foetal mammalian neurohypophyses (Viszolyi and Perks 1969) and in pineal tissue from rats (Rosenbloom and Fisher 1975). AVT is equipotent on uterine, mammary, pressor and antidiuretic activity (Walter et al 1967). In contrast oxytocin and the vasopressins, which are found in the mammals, show greater specificity of action with vasopressins showing high activity on the antidiuretic and pressor assays and low activity on the uterine and mammary assays whilst oxytocin exhibits high activity on the mammary and uterine assays but low activity on antidiuretic and pressor assays (Walter et al 1967).

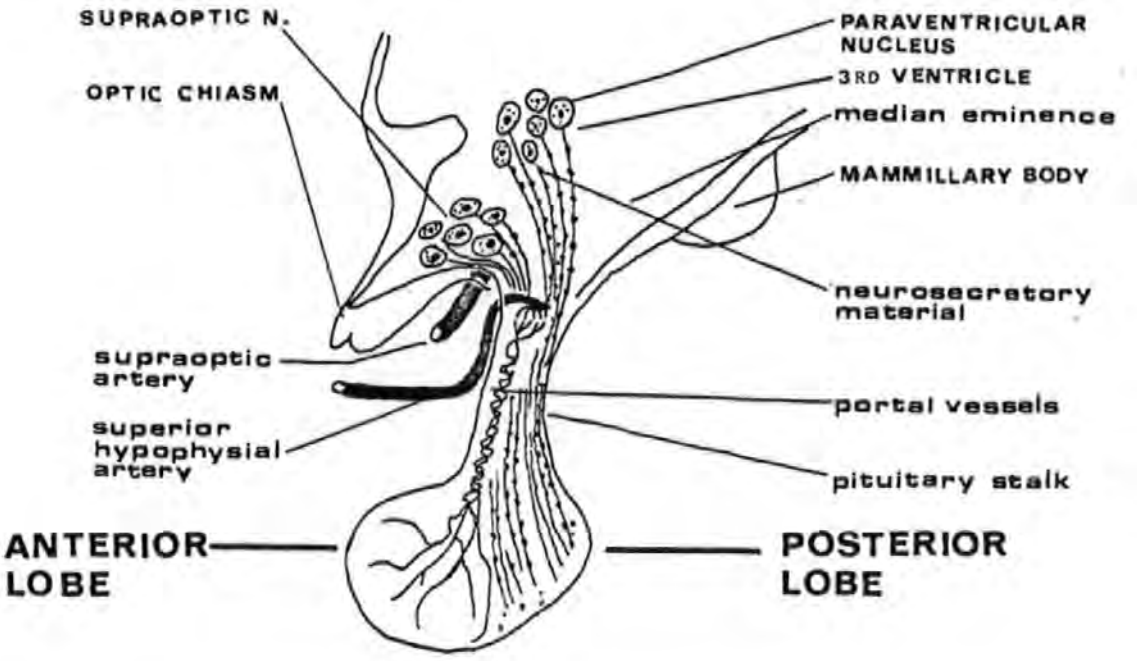
Comparing within the vasopressin series, AVP appears to be more potent than LVP in both pressor and antidiuretic action. These peptides also have different distributions. AVP is far more widespread than LVP in the mammals, this latter being specific to members of the Suina including the wild boar (sus scrofa), wart hog (phacochoerus aethiopicus), bush pig (potomochoerus porcus) and hippopotamus (hippopotamus amphibius) (Ferguson and Heller 1965). Evidence of AVP has been found in the wild boar, the probable ancestor of the domestic pig, suggesting that the capacity to synthesise AVP may have been lost as a result of selective breeding (Sawyer 1967).

1.2 Synthesis

The evidence available suggests that although vasopressin and oxytocin are secreted from the posterior lobe of the pituitary gland (neurohypophysis/pars nervosa) this is not the site of synthesis. Two remote production sites have been identified, the supraoptic and paraventricular nuclei in the anterior hypothalamus. The peptide is actively transported between these sites of synthesis and secretion. Evidence on these points will be considered in the following three

subsections. The key structures and anatomical relationships are described in Figure Three.

Figure Three: Simplified schematic reconstruction of the hypothalamic-hypophyseal nerve tracts and associated pituitary structures



1.2.1 The site of biosynthesis

A wealth of evidence supports the existence of a fibre tract connecting the supraoptic nucleus in the anterior hypothalamus with the posterior lobe of the pituitary gland (Magoun and Ranson 1939, cited in Melville and Hare 1945). Silver staining clearly distinguishes the tract (Melville and Hare 1945) and pituitary stalk transection leads to fibre degeneration at sites distal to the lesion, posterior lobe atrophy and degeneration of 80-95% of supraoptic nuclei neurons.

The supraoptic neurons were evidently involved in the secretion of antidiuretic hormone (ADH) (Scharrer and Scharrer 1945, cited in Melville and Hare 1945). Indeed Melville and Hare (1945) confirmed that the supraoptic neurons contained ADH equivalent to 1.5-5 units of pituitrin (crude extract from the posterior pituitary). In addition hypophysectomy and pituitary stalk transection induced a degeneration of the supraoptic nuclei accompanied by loss of the antidiuretic principle (Melville and Hare 1945). This evidence, coupled with the view expressed by O'Connor (1947) (cited in Bargmann and Scharrer 1951) that the cells in the pars nervosa have little in common with secretory cells

elsewhere in the body, led to the suggestion that production sites for the posterior lobe hormones were remote from the secretion sites in the posterior pituitary. The evidence reviewed by Bargmann and Scharrer (1951) suggested that the synthesis of ADH was restricted to the supraoptic and paraventricular nuclei in the anterior hypothalamus.

Furthermore, Mirsky, Stein and Paulisch (1954a) found that ADH secretion occurred in the absence of the neurohypophysis. It was apparent that the supraoptic and paraventricular nuclei, not the neurohypophysis were the source of ADH. These nuclei are formed by the cell bodies of neurons whose axons form the hypothalamo-hypophyseal tract and terminate in the posterior pituitary gland where they act as storage vessels for ADH and from which release occurs on demand (Scharrer and Scharrer 1954; Sachs 1967). Vasopressin cells are principally found in the ventral and caudal supraoptic nucleus whilst oxytocin containing cells predominate in the dorsal, medial and caudal areas (Antunes and Zimmerman 1978). Cytoarchitectural studies, using a Golgi-Cox staining method, have revealed three cell types in the supraoptic nucleus - long multipolar neurons, bipolar neurons and smaller interneurons (Felten and Cashner 1979). These latter are probably not involved in neurosecretion. Oxytocin and vasopressin cells could not be distinguished structurally (Felten and Cashner 1979).

Dense granular structures are found within the cells of the hypothalamo-hypophyseal tract. Evidence for the active transport of neurosecretory granules (NSG) aggregated into neurosecretory material (NSM) was first provided by Hild (1951, cited in Bargmann and Scharrer 1951). Transection of the frog pituitary stalk resulted in a marked accumulation of NSM in the tract between the lesion and the nucleus preopticus. The relationship between ADH and NSM is supported by observations of a good correlation between the amount of stained NSM in the tract and the animal's state of hydration. Dehydration induces high plasma ADH levels and depletion of NSM within the tract, conversely hydrated rats have low plasma ADH levels and an accumulation of NSM in the tract (Bargmann and Scharrer 1951). These data suggest that cytoplasm bearing ADH flows from the cell bodies of the supraoptic nucleus along the axons in the supraopticohypophyseal fibre tract to the posterior pituitary. As a note of caution, Sachs (1967) points out that NSM stainability may not accurately reflect ADH levels in the posterior pituitary gland. Indeed, Krisch (1979) has recently visualised a form of vasopressin in the rough endoplasmic reticulum of the supraoptic perikarya and in the intercellular clefts of immobilisation stressed rats. Krisch (1979) suggests that under stressful conditions vasopressin may be rapidly released in a

more soluble and freely available form than that which is granule bound.

In summary, ADH is synthesised in the cells of the supraoptic and paraventricular nuclei and transported to the posterior pituitary by axonal flow in association with NSM. This conclusion is supported by observations that NSG are formed by golgi bodies in the anterior hypothalamic nuclei, in keeping with the role of golgi bodies in other secretory tissues, eg pancreas (Sachs 1967). In addition, lesions to the supraopticohypophyseal tract results in diabetes insipidus (DI), the severity of which is directly related to the degree of interruption inflicted upon the tract (Ranson and Magoun 1939). DI is characterised by primary polyuria and secondary polydipsia due to abnormally low ADH levels resulting from nephrogenic or neurogenic failure, or from genetic inability to synthesise vasopressin (see Section 1.10.1).

1.2.2 The process of biosynthesis

Recent work by Sachs and his colleagues (Sachs 1967; Gainer et al 1977a) has clarified the steps involved in vasopressin synthesis with radioactive tracer techniques. These authors used radioactively labelled cysteine, administered by various routes, allowing the experimenter to monitor incorporation of the label into pituitary peptides and proteins. Cysteine, one of the amino acids, is widely distributed in peptides and proteins. In particular it occupies positions one and six in the vasopressin structure.

Ventricular infusion of [^{35}S] cysteine into dogs followed by centrifugation of hypophyseal tissue did not reveal radioactive vasopressin in association with the ribosome rich fractions, as would be expected if nucleic acids within the ribosomes were involved in synthesis. Two interpretations seemed likely; either nucleic acids were not involved in synthesis or synthesis did involve nucleic acids and the product was not vasopressin but an inert precursor molecule. If an inert precursor was involved then there should be a considerable time lag between the infusion of labelled cysteine and the emergence of labelled vasopressin. Sachs and Takabatake (1964) infused radioactive amino acid into dogs and observed that no radioactive vasopressin emerged within 1.5 hours of infusion; however, if the dogs were spared for an additional 4.5 hours then substantial amounts of radioactive vasopressin were detectable. They hypothesised that if an inert precursor was involved and its synthesis was nucleic acid dependent then puromycin, a protein synthesis inhibitor, should inhibit vasopressin synthesis. Additional infusion experiments revealed that vasopressin was not synthesised when puromycin was present from the start of

infusion; however, if puromycin presence was restricted to the post infusion period then radioactive vasopressin was synthesised.

The ventricular infusion technique involves difficulties in interpretation. The amino acid used is ubiquitous in brain proteins and peptides; it is difficult to determine the degree of cysteine uptake into brain tissue or to what extent breakdown of the amino acid structure occurred, thereby releasing the radioactive label and allowing incorporation into other structures. These objections are to some extent answered by reports that identical results are found following bilateral injection of [^{35}S] cysteine into the supraoptic nucleus of ether anaesthetised cats (Gainer et al 1977b) and using in vitro preparations (Sachs 1967).

The data suggested that [^{35}S] cysteine was initially incorporated into an inert precursor molecule, the synthesis of which was probably dependent upon RNA in the ribosomes. This stage involves protein synthesis and is puromycin sensitive. After synthesis the inert precursor is bound into secretory granules, probably by the golgi bodies (Sachs 1967). The subsequent elaboration into vasopressin appears not to involve further protein synthesis, as indicated by the lack of puromycin sensitivity. Inert prohormone synthesis may be restricted to the perikarya of the cells. Leclerc and Pelletier (1974) used an immunohistochemical technique and found that vasopressin was restricted to the axons of the cells in the supraoptic and paraventricular nuclei; no reaction was found in the perikarya. However, Krisch (1979) has found evidence of vasopressin in the endoplasmic reticulum of neurosecretory perikarya.

Labelling techniques have also yielded data on axonal transport following elaboration into NSM. Gainer et al (1977b) injected [^{35}S] cysteine bilaterally into the supraoptic nuclei of ether anaesthetised rats and then killed the animals at various times after injection to elucidate the time course of labelled proteins emerging at the supraoptic nucleus, median eminence and posterior pituitary gland. Incorporation at the supraoptic nucleus was very rapid, appearing 30 minutes after injection. Substantial incorporation appeared at the median eminence after one hour, rapidly increased between one and two hours and slowly reached a peak after twelve hours. Labelled proteins were not detected at the posterior pituitary until two hours after injection. Thereafter incorporation increased steadily to a peak at twelve hours. For all areas the detection of labelled proteins was maximal twelve hours after injection and was maintained until the final test 24 hours after injection, confirming that proteins are synthesised in the

supraoptic nucleus and transported to the posterior pituitary in the hypothalamo-hypophyseal tract which traverses the median eminence (cf Figure Three) (Gainer et al 1977b). The rate of detection following injection is consistent with the original hypothesis proposed by Bargmann and Scharrer (1951) that transportation from the site of synthesis to the site of secretion in the posterior pituitary is by axonal flow.

1.2.3 Neurophysins

Vasopressin is found in close association with specific proteins, neurophysins which have a molecular weight of approximately 20,000. Kurtzman and Boonjarern (1975) and Zimmerman et al (1973b) provide evidence for two such proteins. In contrast, Burford and Pickering (1972) found three neurophysins in the rat hypothalamo-hypophyseal tract and only one of these appeared in association with vasopressin. Gainer et al (1977a), however, could not confirm the presence of three neurophysins using an identical technique. Neurophysin is distributed throughout the neurons of the tract and in the supraoptic and paraventricular nuclei (Zimmerman et al 1973b).

The close association between vasopressin and at least one species of neurophysin has been taken to indicate that neurophysin and vasopressin are bound together possibly for the purposes of transport along the axons (Kurtzman et al 1975; Wimersma et al 1977). This is uncertain in view of findings that the neurohypophyseal vasopressin content does not always bear a constant relationship to the neurophysin content as would be expected if neurophysins acted solely as transport molecules in a one to one relationship with vasopressin (Bakker et al 1975).

Results obtained with radioactive labelling techniques suggest the presence of four neurohypophyseal hormones in addition to vasoprssin and oxytocin (Gainer et al 1977a). These authors argue for the existence of a precursor which is common to neurophysin and the other neurohypophyseal hormones. Following the suggestion of Sachs (1967) that an inert precursor molecule is produced by translation from RNA at the ribosomes and elaborated into NSG they proposed that the precursor may be cleaved in the NSG to produce oxytocin, vasopressin, neurophysin and possibly the four other peptides which have been detected. This is an attractive hypothesis of considerable biological economy, a common precursor protein undergoes post translational cleavage within the transport granule whilst in transit in the hypothalamo-hypophyseal tract. The peptide which is produced would depend upon the particular combination of proteolytic enzymes packaged with the precursor.

1.2.4 Summary

Vasopressin synthesis starts in the supraoptic and paraventricular nuclei of the anterior hypothalamus. The peptide is transported to the posterior pituitary gland via the axons of the cells which constitute the hypothalamo-hypophyseal tract. Synthesis may occur during the process of transport within the neurosecretory granule deriving vasopressin and/or other peptides from a common protein precursor.

1.3 Secretion

The secretory process is described and evidence for vasopressin's primary peripheral route of secretion is briefly presented. Evidence for two additional routes of secretion, to the hypophyseal portal blood supply and to the cerebrospinal fluid (CSF) is discussed. These two routes are important in considering the behavioural effects of vasopressins as the hypophyseal portal blood supply provides access to the adenohypophysis, and may serve as a route through which vasopressin affects the release of behaviourally active adrenocorticotrophic hormone (ACTH) and corticosteroids. Furthermore, if vasopressin is secreted into the CSF, then the peptide may be acting directly on central nervous system (CNS) structures using the CSF as the transport medium.

1.3.1 Exocytosis

Through the mechanism of axoplasmic flow (Bargmann and Scharrer 1951; Gainer et al 1977b) granules (NSM) stream down the hypothalamo-hypophyseal tract into the posterior pituitary and are released into the capillary blood supply by exocytosis (Holmes and Ball 1974). During this process the enclosing membrane of each granule fuses with the cell membrane simultaneously rupturing to exude the granule contents into the perivascular space and from there into the capillary blood supply. Surplus membrane fragments may persist in the perivascular space or be digested by the lysosomes which are evident in the posterior pituitary nerve endings (Whitaker, Labella and Sanwal 1970). In vitro vasopressin release can be stimulated by potassium (K^+) and calcium (Ca^{++}) ions (Douglas 1963). The release mechanism may involve the destruction of binding between vasopressin and neurophysin by Ca^{++} following its entry into the cell after depolarisation (Holmes and Ball 1974). Vasopressin is secreted into the capillary blood supply and through this route affects its physiological role of water reabsorption in the kidney (Rydin and Verney 1938; Verney 1947).

1.3.2 Secretion into the hypophyseal portal blood vessels

In view of the close association between vasopressin and neurophysin (Section 1.3.3) the presence of neurophysin in a structure may indicate the presence of vasopressin. Neurophysin has been found in the supraoptic and paraventricular nuclei, in the axons of the supraoptico hypophyseal tract and in the external layer of the median eminence (Zimmerman 1973a,b). The axons of the tract pass close to the wall of the third ventricle in the region of the median eminence (see Figure Two , Section 1.3) and a small number of axons appear to terminate in this area (Zimmerman 1973a,b). Considered together, these findings provide circumstantial evidence for a secretory route from the supraoptic nucleus to the median eminence, an area from which capillary blood vessels drain into the portal blood vessel.

In order to test this hypothesis, Zimmerman et al (1973a) cannulated monkey portal veins to collect hypophyseal portal blood for comparison with systemic venous blood. Neurophysin and vasopressin concentrations were measured using a radioimmunoassay. The portal blood contained an average neurophysin level of 61.5 ng/ml compared with 2.5 ng/ml in the systemic blood; the mean portal blood level for vasopressin was 13,000 pg/ml compared to 42 pg/ml in the systemic blood. The authors suggested that the source of the higher vasopressin and neurophysin levels was probably the axons of the supraoptico hypophyseal tract terminating in the median eminence and draining into the portal blood vessels. Zimmerman et al (1975) confirmed these observations and in addition reported that electron microscopy of the median eminence area revealed large osmophillic granules (Herring bodies) in the perivascular space, granules which are typically found in the supraopticohypophyseal neurons (see Section 1.2.1). These data are compatible with the hypothesis that vasopressin and indeed neurophysin gain access to the anterior lobe of the pituitary gland and its behaviourally active hormones via the portal blood supply.

1.3.3 Secretion into the cerebrospinal fluid (CSF)

Anatomical evidence supports the existence of a secretory link between the cells of the supraoptic and paraventricular nuclei and the CSF. Rodriguez (1970) has observed that many of the neurons in the ventral preoptic nucleus of the toad are bipolar. Short ventricular processes terminate on the ependymal lining of the preoptic recess giving direct access to the ventricular fluid. However, an examination of 200 of these endings, using gomori stain, revealed no evidence of

neurosecretory material which would be expected if these processes act as secretory routes. In contrast, the ventricular processes in the trout are rich in neurosecretory material (Muller 1969). These data indicate one possible mode of access to the CSF. Felten and Cashner (1979) have reported bipolar and multipolar neurons in the supraoptic nucleus of an adult rabbit. Axons of the nultipolar neurons were seen to course towards the median eminence, supporting the hypothesis that neurons of the hypothalamo hypophyseal tract terminate in the median eminence (Section 1.3.2). Robinson and Zimmerman (1973) have suggested that neurophysin and vasopressin first gain access to the CSF via axons terminating in the median eminence and from there are taken by the tanycytes of the ependymal layer in the infundibular recess back to the hypophyseal portal blood system. This single route could explain the presence of vasopressin in the portal system and in the CSF. Involvement of the tanycytes is a recent suggestion, but the idea that secretion into the CSF occurs via the infundibular recess has a long history. The hypothesis was first suggested by Herring (1908, cited in Cushing 1931) who observed that secretory granules moved towards and into the infundibular recess, indicated by the presence of hyaline bodies (Herring bodies) which he believed to be secretory products. The hypothesis has recently been supported by data from Wittowski (1968). Electron microscopy confirmed that axons from the supraoptico-hypophyseal tract terminated in the infundibular recess of the third ventricle; these were rich in neurosecretory material which appeared to egress into the ventricular fluid. Furthermore, Rodriguez (1970), using electron microscopy, has confirmed the presence of neurosecretory granules (1,300-1,500 A^{o} diameter) in the ependymal layer of the infundibular recess in toads. Therefore posterior lobe peptides may gain access to the CSF by two routes, via short ventricular processes into the preoptic recess - this route has little supportive evidence - or into the infundibular recess via the median eminance - this route is well supported by the evidence.

The evidence for a secretory route for vasopressin into the CSF must meet three criteria. The first has been discussed and demands sufficient anatomical evidence for the route; the second is that the CSF contains vasopressin; the third and most difficult to satisfy is that CSF vasopressin originates by direct secretion and not by "leakage" across the blood/CSF barrier or by other means of indirect access (Rodriguez 1970).

Many experiments between 1915 and 1930 (Cushing 1931) indicate the presence of vasopressin in the CSF but Van Dyke et al (1929) argued

that these findings were artefacts of a non-specific assay technique. More recently Heller et al (1968) found that CSF, withdrawn by cisternal puncture from experimental rabbits, inhibited diuresis in assay rats following stimulation of the vagal nerve under pentabarbitone anaesthesia, or treatment with pentabarbitone anaesthesia alone. Both these treatments stimulate the release of vasopressin (Ginsburg and Brown 1956). In contrast the inhibition was not seen after a local anaesthetic. The assay was performed using ethanol anaesthetised hydrated rats, a preparation in which endogenous vasopressin release is blocked in the assay rat. The destruction of antidiuretic activity in the CSF of vagal and pentabarbitione stimulated rabbits by incubation with trypsin or sodium thioglycollate confirmed that the antidiuretic activity was due to vasopressin and was not a non-specific effect. The data indicate that CSF contains vasopressin following the stimulation of endogenous secretion, assay levels were greater following pentabarbitone anaesthesia than following local anaesthesia and were also higher in vagal stimulated pentabarbitone rats than in non-vagal stimulated pentabarbitone anaesthetised controls. The source of vasopressin in either case could be direct secretion into the CSF or leakage from the plasma across the plasma/CSF barrier; this could occur naturally or be caused by pentabarbitone distorting the normal barrier permeability. The vasopressin stimulating effect of pentabarbitone may be restricted to the period immediately after injection as Heller et al (1968) found that the barbiturate increased both peripheral and CSF levels of vasopressin when samples were taken two to three minutes after anaesthetic, but when Vorherr et al (1968) delayed the collection of samples for half an hour after anaesthetisation only very low levels of plasma and CSF vasopressin were found. In both studies rabbits were used; the assay preparations were similar and the dose of pentabarbitone was identical (30 mg/kg). Vorherr et al (1968) confirmed that haemorrhage massively increased plasma vasopressin levels (Ginsburg and Brown 1956), and also increased CSF vasopressin, although these never exceeded 25% of plasma levels. The specificity of the CSF antidiuretic principle was confirmed by destruction of antidiuretic activity following incubation with vasopressinase from human pregnancy plasma; however, the source of vasopressin was not specified.

Determining the source of CSF vasopressin involves measuring to what extent the blood/CSF barrier is permeable to vasopressin and similar peptides. If the barrier is impermeable then the source of CSF vasopressin is more likely to be a direct secretory pathway. These studies have used peripheral administration followed by examination of the CSF

to determine whether or not vasopressin levels have increased after administering the exogenous peptide. This approach is complicated by the fact that uptake of vasopressin by the kidney is rapid and efficient even for large exogenous doses (Section 1.8.1). If a small dose does not change CSF vasopressin levels this may be due to peripheral enzymatic degradation preventing sufficiently high concentrations from reaching the blood CSF barrier. If on the other hand the dose is in excess of the normal physiological range, then the subsequent changes of vasopressin levels in the CSF may be due to distortion of normal barrier permeability brought about by the increase in blood pressure which characteristically accompanies large vasopressin doses (Section 1.8.2). Thus Heller et al (1968) found that CSF vasopressin levels increased in response to a non-physiological dose of 50 mu of AVP/kg within two minutes of the peripheral injection. In contrast Vorherr et al (1968) used a constant infusion of AVP (5 mu/min) for 40 minutes, or 25 mu/min for two hours; neither affected CSF vasopressin levels. However, these tests by Vorherr et al (1968) were run after animals had previously been subjected to haemorrhage which itself may have distorted barrier permeability. More recently, Zaidi and Heller (1974) injected radioactively labelled oxytocin (^{3}H oxytocin) or vasopressin (^{3}H lysine vasopressin) intravenously into urethane anaesthetised rats and studied the appearance of radioactivity in plasma and CSF sampled by cannulation and cisternal puncture. In oxytocin treated rats the subsequent plasma radioactivity count (849 counts/ml/min) was far in excess of the CSF level (22 counts/ml/min) and the plasma count for vasopressin treated rats (1753 counts/ml/min) was far in excess of the CSF level (35.5 counts/ml/min). The authors concluded that barrier permeability for the peptides was low, supporting the hypothesis that the high CSF levels seen after the stimulation of endogenous secretion (Vorherr et al 1968; Heller et al 1968) originated from direct secretion into the CSF and not from the plasma. However, Zaidi et al (1974) had also used a non-physiological dose of 117 mu of labelled vasopressin, which may have distorted barrier permeability. A further possibility is that enzymes degraded the structure of labelled vasopressin very rapidly but released the breakdown products, including the tritium label back into the plasma, in which case the radioactive counts do not necessarily reflect the fate of the exogenous peptide or its capacity to cross the barrier. Similar objections may apply to the study by Greenberg et al (1976) who reported that, following intracarotid injection of labelled vasopressin (^{14}D AVP), radioactivity was detected, after 15 seconds and ten minutes, in all major brain areas, including the cerebral cortex,

hypothalamus, caudate nucleus, brain stem and cerebellum in roughly equal proportions. The pineal and pituitary glands exhibited levels ranging from 5x to 8x that found in other areas. Binding appeared to be non-specific and passive, the increased levels observed for the pituitary and pineal glands may reflect increased binding at these sites or the fact that the glands are located outside the blood brain barrier. Alternatively, the labelled peptide may have been rapidly degraded or synthesised in which case the distribution of radioactivity after injection may not reflect the distribution of peptide uptake but simply the distribution of amino acids or their fragments following degradation.

1.3.4 Extrahypothalamic vasopressinergic pathways

The development and widespread application of microdissection techniques coupled with the use of sensitive radioimmunoassays have revealed the presence of peptide hormones in brain tissue remote from the hypothalamus. The earliest evidence for this was published by Barry (1963), using Gomori stain which was incapable of distinguishing individual peptides. The presence of vasopressin and oxytocin in the supraoptic and paraventricular nuclei has been confirmed (George and Jacobowitz 1975; Dogterom et al 1978). Fibres coursing from the supraoptic nucleus to the posterior pituitary were found to contain large amounts of vasopressin in the region of the median eminence (see Figure Three) (George and Jacobowitz 1975). Vasopressin levels were higher in the retrochiasmatic nucleus, lying adjacent to the supraoptic nucleus, than in the supraoptic nucleus itself which may indicate transformation of the peptide in transit to a form recognisable to the vasopressin antibody (George and Jacobowitz 1975). This confirms the suggestion by Gainer et al (1977) that synthesis is completed in transit (see Section 1.2.3 for discussion).

A number of fibres emanate from the paraventricular nucleus and enter the dorsal hippocampus and subiculum after traversing the ventral fornix commissure (Buijs 1978). These fibres pass close to the subfornical organ which contains vasopressin fibres (Buijs 1978) and vasopressin (Summy-Long 1978) and continue to the ventral hippocampus via the fimbria (Buijs 1978). Dogterom et al (1978) have confirmed the presence of vasopressin in the anterior and dorsal hippocampus. Additional fibres originating in the paraventricular nucleus course rostrally to the medial and lateral septum. Dogterom et al (1978) have confirmed the presence of vasopressin in the septum. From the paraventricular nucleus two pathways course to the substantia nigra, one

via the infundibular recess and arcuate nucleus and the other caudally through the substantia nigra to the medulla oblongata (Buijs 1978). Finally, a series of fibres pass via the stria terminalis to the nuclei of the amygdala (Buijs 1978), the presence of vasopressin in the amygdala has been confirmed by Dogterom et al (1978). The hypothalamus has long been considered to be the highest centre of autonomic control, mediated via its anatomical connections with the lower brain stem. Recent evidence from experiments using the horseradish peroxidase technique to track individual neurons has challenged this concept by demonstrating the existence of a direct pathway from segments 7 and 9 of the rat spinal cord to the paraventricular nucleus (Ono et al 1978).

Another fibre system, characterised by its fine fibres (Sofroniew and Weindl 1978, originates in the suprachiasmatic nucleus and projects to the lateral septum (Sofroniew and Weindl 1978; Buijs 1978), medial dorsal thalamus and solitary tract (Sofroniew and Weindl 1978). Finally these authors described a pathway to the lateral habenular nucleus which coursed under the ependyma of the third ventricle. In the projection areas described the fibres make numerous axosomatic contacts and do not appear to contact the capillary blood vessels suggesting that their primary function in these brain areas is not secretory (Sofroniew and Weindl 1978; Krisch 1978). Furthermore the vasopressin pathways are absent in rats with a genetical absence of vasopressin (Sofroniew and Weindl 1978; Buijs 1978) (see Section 1.10 for discussion of genetic DI). The functional significance of these pathways remains to be established.

1.3.5 Summary

The primary route for vasopressin secretion, from the posterior pituitary gland into the circulation is well established. Evidence has been presented supporting the hypothesis that vasopressin is secreted into the hypophyseal portal blood supply and from there to the anterior lobe of the pituitary. Anatomical evidence supports the existence of a fibre tract linking the cells of the supraoptic and paraventricular nuclei with the CSF via the median eminence and the infundibular recess. In support of the direct secretory route, observations confirm the presence of vasopressin in the CSF using a specific assay technique. However, it has not been unequivocally demonstrated that the source of CSF vasopressin is not plasma borne peptide crossing the blood/CSF barrier. Recent studies have shown vasopressin to be present in numerous extrahypothalamic pathway originating from the paraventricular and suprachiasmatic nuclei.

1.4 Neurotransmitter Control of Vasopressin Secretion

Evidence is presented which indicates that adrenergic, cholinergic and histaminergic neurons play integrated roles in regulating vasopressin secretion.

1.4.1 Adrenergic involvement

Histological studies have indicated that noradrenergic fibres are present in structures which are involved in vasopressin secretion. Carlsson et al (1962) reported that the supraoptic, paraventricular and periventricular but not the pars nervosa stained heavily for noradrenaline; later Shute and Lewis (1966) confirmed these observations. Furthermore, Fuxe and Hokfelt (1970) reported that fibres which stained for noradrenaline in the supraoptic and paraventricular nuclei were activated by hypo-osmotic and hyperosmotic stimuli, which are known to affect vasopressin release (Section 1.6.1).

Early physiological experiments had suggested adrenergic involvement in vasopressin secretion. O'Connor and Verney (1945) reported that intravenous injections of adrenaline (20 ug) prevented the antidiuretic response normally induced by mild shock in dogs when given before but not after shock, adrenaline was ineffective when posterior pituitary extract was used instead of shock to induce antidiuresis. Adrenaline did not mediate its effects by altering arterial blood pressure and Verney (1947) suggested adrenergic involvement in ADH secretion. Additional support for this hypothesis was provided in a study by Abrahams and Pickford (1956) who found that 200 ug of acetylcholine blocked diuresis in hydrated bitches when injected into the carotid artery. The effect of acetylcholine was in turn blocked by pretreatment with 2 ug of adrenaline and occasionally by 1 ug or 0.5 ug provided that adrenaline pretreatment preceded the acetylcholine injection by not more than 45 seconds and not less than eight seconds. Doses of 3 ug, 4 ug and 5 ug of adrenaline were found to be less effective than the lower doses and 10 ug was completely inactive. In a later study, Mills and Wang (1964b) elicited vasopressin secretion by electrically stimulating the ulnar and vagal nerves and areas in the medulla, pons and midbrains of anaesthetised dogs. Low doses of hydergine (hydrogenated ergot alkaloids) and phenoxybenzamine, an α adrenergic blocker which inhibits noradrenaline reuptake, blocked vasopressin secretion elicited by ulnar but not vagal stimulation (Mills and Wang 1964a). Higher doses of phenoxybenzamine and hydergine reduced the effects of vagal stimulation and prevented vasopressin secretion

following electrical stimulation of the central tegmental tract. High doses were required in order to block secretion following stimulation of the pons or medulla. More recently Guzek et al (1978) used severe dehydration, extending over four days, to deplete vasopressin levels and found that depletion was partially prevented by pretreatment with phenoxybenzamine. Although these studies suggest that blocking α adrenergic activity prevents vasopressin secretion following a variety of stimuli, there is contradictory evidence from Corson (19) who reported that ephidrine, an adrenergic mimetic, inhibited vasopressin secretion in hydrated bitches, indicated by increased urine volume and decreased urine osmolality. This apparent conflict may be resolved by studies reported by Kulsrethra et al (1976) who examined the effects of injecting adrenergic, cholinergic, dopaminergic, tryptaminergic and histaminergic drugs on urine flow, and plasma vasopressin levels in the jugular vein of anaesthetised dogs when injected introcerebroventricularly. The α adrenergic agonists phenylephrine and noradrenaline (10-200 ug) decreased urine flow and increased plasma vasopressin levels, the log dose response curves were linear and the response was blocked by pretreatment with phenoxybenzamine. In contrast the β adrenergic mimetic isoprenaline increased urine flow and decreased plasma vasopressin levels; this response was blocked by pretreatment with propanolol, a β blocker. Urano and Kobayashi (1978) have reported similar results following microinjections of phenoxybenzamine and dichlorisoproterenol (β adrenergic blocker) into the supraoptic nucleus of hydrated rats. Therefore increased α adrenergic activity appears to be associated with facilitated vasopressin release whereas increased β adrenergic activity inhibited release. Bi-directional effects were reported with adrenaline, low doses (1-5 ug), increased urine flow and decreased plasma vasopressin whereas large doses (50-500 ug) had the opposite effects (Kulsrethra et al 1976); this dual effect appears to be due to the sensitivity of β adrenoreceptors to low doses of adrenaline as phenoxybenzamine reversed the antidiuretic response to large adrenaline doses without altering the diuretic response to low doses (Kulsrethra et al 1976). Furthermore the diuretic response seen after blocking the effect of high adrenaline doses with phenoxybenzamine was itself blocked by pretreating the animals with the β adrenergic blocker propanolol. Therefore, vasopressin secretion appears to be under dual control with α adrenergic neurons involved in facilitating whilst β adrenergic neurons inhibit release.

The hypothesis of 'dual control' proposed by Kulsrethra et al (1976) may explain why Mills and Wang (1964a) found that various doses

of phenoxybenzamine blocked vasopressin secretion following electrical stimulation of brain areas. Furthermore the differential sensitivity of β receptors to low doses of adrenaline may explain why O'Connor and Verney (1945) found that 20 ug of adrenaline blocked the antidiuretic response to mild shock if it is assumed that use of the intravenous route would have permitted peripheral uptake, degradation and binding to effectively reduce the concentration of adrenaline at the receptor site to the range reported by Kulsrethra et al (1976) to stimulate β adreno-receptors following intracerebroventricular injections. In addition to its role in the regulation of secretion adrenaline has been reported to produce short term diuresis probably mediated by changes in renal blood flow (Pickford 1939; O'Connor and Verney 1945; Abrahams and Pickford 1956).

1.4.2 Cholinergic involvement

The results from histochemical studies indicate that cholinergic neurons are found in those areas which are associated with vasopressin secretion. Feldberg and Vogt (1948) reported that the level of acetylcholine synthesis in the supraoptic nucleus was considerably higher than in the neural lobe. Similarly, Abrahams et al (1957) detected the presence of acetylcholinesterase in the supraoptic nucleus and in the supraoptico-hypophyseal tract but not in the neural lobe. Acetylcholinesterase is present in the cell bodies but not the axons of cells constituting the supraoptic and paraventricular nuclei (Shute and Lewis 1966). Tracing the degeneration which followed thermal lesions revealed a cholinergic pathway which originated in the ventral tegmental area, traversed the lateral preoptic area and terminated in the supraoptic nucleus (Shute and Lewis 1966). More recently Rotter et al (1979) have described an autoradiographic technique for localising the distribution of muscarinic receptors using [3H] propylbenzilycholine mustard, a potent and irreversible muscarinic antagonist, they reported high grain counts in the supraoptic nucleus, indicating a dense muscarinic receptor distribution.

A large number of studies have shown that acetylcholine stimulates the release of vasopressin. Molitor and Pick (1924, cited in Pickford 1939) reported that diuresis was inhibited by treatment with choline; later Pickford (1939) found that intravenous acetylcholine induced dose dependent antidiuresis in hydrated dogs, beginning approximately five minutes after treatment. Large doses also induced muscular weakness, panting and paling of the lips and vulva; however, these effects were absent with smaller doses which still induced considerable antidiuresis,

suggesting that the antidiuretic response was not secondary to the peripheral effects of the drug. Furthermore, the antidiuretic response was blocked by posterior lobectomy, indicating pituitary involvement (Pickford 1939). Antidiuresis was also seen after direct injection of acetylcholine or the acetylcholinesterases physostigmine and diisopropylfluoride into the supraoptic nucleus and this too was abolished by posterior lobectomy (Pickford 1947). More recently Kulsrethra et al (1976) reported an antidiuretic response after intracerebroventricular injections of acetylcholine in anaesthetised dogs and Urano et al (1978) found antidiuresis following microinjections into the supraoptic nucleus. In vitro studies have confirmed that acetylcholine stimulates the release of vasopressin but not from the isolated neural lobe. In addition microiontophoretic application of acetylcholine was found to accelerate the electrical activity of cells in the supraoptic nucleus (Barker et al 1971).

Although there is strong supportive evidence that acetylcholine stimulates vasopressin secretion, the evidence from experiments which use cholinergic blocking agents is contradictory. A number of authors have found these not to be effective in preventing secretion. Pickford (1939) reported that atropine did not block the antidiuretic response to acetylcholine; similarly Mills and Wang (1964a) found that neither atropine or ethylbenztropine blocked vasopressin secretion in response to electrical stimulation of the ulnar or vagal nerves and the central tegmental pathway. No effect was found using crystalline atropine implanted in the hypothalamus (Hedge and de Wied 1971) and intracerebroventricular atropine was only weakly effective in blocking the antidiuretic response to acetylcholine (Kulsrethra et al 1976). In contrast Bridges and Thorn (1970) successfully used atropine to block the release of vasopressin in response to a hypertonic solution; this was recently confirmed by Sobczak (1978). In addition Urano et al (1978) reported that the antidiuretic response to acetylcholine injected directly into the supraoptic nucleus was blocked by pretreatment with 20 ug of atropine. Finally in a paper published by Guzek et al (1978) atropine was found to partially prevent the severe depletion of vasopressin seen when rats were dehydrated for four days; however, vasopressin was significantly depleted by atropine itself when given to non-dehydrated rats.

The contradictory results from these experiments may partly reflect the wide range of experimental procedures used. In addition recent data from Sladek and Joynt (1979a,b) suggest that cholinergic control of vasopressin secretion is nicotinic rather than muscarinic. Atropine, which is a muscarinic antagonist, did not block secretion in

response to acetylcholine (Sladek and Joynt 1979a) and osmotic stimulation (Sladek and Joynt 1979b); this agrees with many of the results previously discussed. However, the nicotinic antagonists hexamethonium, tetraethylammonium chloride and trimethaphan successfully blocked release in response to both stimuli. Furthermore Rotter et al (1979) have described an autoradiographic technique which revealed high density muscarinic receptor distribution in the supraoptic nucleus. Failure to block secretion with atropine may in some cases reflect the nicotinic nature of the pathways involved.

In summary, data from histochemical, physiological and pharmacological experiments strongly implicate cholinergic neurons in the regulation of vasopressin secretion. Furthermore, Kulsrethra et al (1976) reported that secretion of vasopressin in response to acetylcholine could be blocked with an α-adrenergic blocker, suggesting a functional relationship between adrenergic and cholinergic neurons; this is discussed in more detail in Section 1.4.4.

1.4.3 Histaminergic involvement

The evidence which suggests a role for histaminergic neurons in regulating vasopressin secretion comes mainly from pharmacological experiments. Early observations from Dale and Laidlaw (1910, cited in Blackmore and Cherry 1955) suggested considerable parallelism between reduced urine flow and decreased arterial blood pressure following an intravenous injection of histamine. This observation was confirmed by Blackmore et al (1953) using an intravenous infusion of histamine (2.5 ug/kgbw/min) over a two hour test period. The antidiuretic response was apparently mediated by vasopressin and was not secondary to blood pressure changes as Mirsky et al (1954a) found that histamine (1 mg/100 gbw IP) induced peak plasma vasopressin levels after five minutes gradually declining to control levels after 30 minutes. In addition prolonged intravenous histamine infusion reduced the urine flow in control group hydrated bitches but not in those suffering from diabetes insipidus induced by neurohypophysectomy (Blackmore and Cherry 1955) and finally De Wied (1960) reported that carotid artery plasma ADH levels were significantly elevated over control levels after an IP injection of histamine (5 mg/100gbw).

Although these data indicate that histamine induced antidiuresis due to facilitated vasopressin secretion the physiological importance of this mechanism is uncertain. Kulsrethra et al (1976) reported variable results with intracerebroventricular injections of histamine in comparatively low doses (1-20 mg). In contrast, doses in the range from

25-500 ug increased plasma vasopressin levels and decreased urine flow; the poor response to low doses suggests that the response to histamine may be of pharmacological and not of physiological importance.

One interesting possibility is that histamine induced antidiuresis is mediated by catecholaminergic neurons (Kulsrethra et al 1976). These authors reported that the specific antihistamine mepyramine blocked the antidiuretic response to intracerebtoventricular histamine (400 ug); however, the response was also blocked by pretreatment with tetrabenazine (3 mg/kg IP) but not atropine (2 mg). Furthermore, the α-adrenergic blocker phenoxybenzamine (2 mg) converted the histamine induced antidiuresis to diuresis and this response could itself be blocked by propanolol (β-adrenergic blocker). These data suggest that histamine induced antidiuresis requires the participation of central adrenergic pathways.

1.4.4 Neurotransmitter integration in the control of vasopressin secretion

Evidence for the involvement of other putative neurotransmitters in regulating vasopressin secretion is conflicting. Bridges et al (1975) reported that intraventricular injections of Y-aminobutyric acid elicited antidiuresis when a low dose (5 ug) was used but not when the doses were higher (10-100 ug). Dopamine, delivered by the same route, elicited vasopressin secretion and pronounced antidiuresis (Bridges et al 1975). The effect of dopamine was confirmed by Urano et al (1978) using microinjections into the supraoptic nucleus (10-20 ug) but could not be confirmed by Kulsrethra et al (1976) using a wide range of doses (10-100 ug) injected directly into the cerebral ventricles. Similarly Kulsrethra et al (1976) reported that 5-hydroxytryptamine (100-500 ug) did not induce antidiuresis when injected intracerebroventricularly but Urano et al (1978) reported antidiuresis after microinjections of 5-hydroxytryptamine directly into the supraoptic nucleus when much lower doses were used (5-15 ug). Clearly these data allow no firm conclusions as to the involvement of dopaminergic or serotonergic neurons in regulating vasopressin secretion.

The data discussed in the preceding sections implicate cholinergic, α-adrenergic and histaminergic pathways in regulating the secretion of vasopressin. Kulsrethra et al (1976) have proposed a model for integrating the pharmacological data from their experiments suggesting multiple control of secretion. Principally secretion is facilitated by α-adrenoceptive neurons and inhibited by β-adrenoceptive neurons. As the antidiuretic responses

to histamine and acetylcholine could be blocked by phenoxybenzamine (α blocker) α-adrenergic neurons appear to be the final excitatory neurotransmitter link although these must also be cholinoceptive. This would explain the absence of acetylcholine in the cells of the neural lobe (Feldberg and Vogt 1948; Abrahams et al 1957) and why direct stimulation of the neural lobe with acetylcholine failed to stimulate vasopressin secretion (Douglas and Poisner 1964). Furthermore Mills and Wang (1964a) reported that subthreshold doses of adrenergic antagonists potentiated the diuretic action of subthreshold doses of a muscarinic antagonist, suggesting a functional relationship between the two systems. Kulsrethra et al (1976) argued that the cholinoceptive cells were muscarinic on the basis of the partial blocking of antidiuresis seen after atropine; however, more recent data from Slobek et al (1979a,b) suggest that these neurons may be nicotinic.

1.5 Feedback in the Control of Vasopressin Secretion

Feedback control refers to a mechanism whereby vasopressin may regulate its own secretion in response to circulating levels. There is little direct evidence for such a mechanism operating in the case of peripheral vasopressin levels and in view of the findings discussed in section 1.8.1 indicating removal rate at the kidney to be approximately equal to secretion rate at the pituitary; such a mechanism would appear superfluous. Evidence against the existence of a feedback loop was reported by Bakker et al (1975). They injected 0.5 IU of vasopressin or control vehicle into rats over a period of eight days and although this dose raised urine osmolality and reduced water intake bioassays of the posterior pituitary revealed no changes in the vasopressin or oxytocin levels. However, three neurophysins were significantly increased as a result of treatment. Although the evidence does not favour the existence of a feedback loop, the authors pointed out that the result could be explained by postulating an inhibition of secretion from the lobe followed by less rapid breakdown of the neurophysins.

Contradictory evidence has been reported by Kulsrethra et al (1976) using a pharmacological preparation. Graded doses of vasopressin (0.001-1 IU) injected directly into the ventricles of hydrated dogs under α-chloralose anaesthesia increased urine flow and decreased plasma vasopressin levels. This response could be blocked by pretreating the animals with tetrabenazine (30 mg/kg) or propanolol (2 mg) but not by α-adrenergic blockers or atropine. These data indicated the existence of feedback loop mediated via the CSF and involving β-adrenergic

inhibitory neurons.

1.6 Factors Affecting the Secretion of Vasopressin

A number of physiological factors affect the secretion of vasopressin, including dehydration, blood volume, sexual stimulation, angiotensin and stress. These factors must be considered when evaluating data from behavioural experiments involving procedures which may directly or indirectly stimulate vasopressin secretion.

1.6.1 Dehydration

Vasopressin plays an essential role in maintaining the organism's fluid balance (Section 1.8.1) by stimulating water reabsorption at the kidney and is sensitive to changes in the animal's state of hydration. Increased antidiuretic activity in the urine of dehydrated rats was reported by Gilman and Goodman (1937). Later Mirsky et al (1954a) found that plasma antidiuretic levels in rats following 24 hours of water deprivation were 29.7 mu/100 ml compared to 18.4 mu/100 ml in control rats maintained on ad lib water. Furthermore, Little and Radford (1964) have observed that sustained dehydration for one to three days significantly increased vasopressin levels. Czackes, Kleeman and Koenig (1964a) have confirmed that three days of dehydration increased the plasma concentration of vasopressin and the turnover rate. Conversely, three days of overhydration decreased the turnover rate and reduced the plasma concentration to zero (below the lower sensitivity level of the assay). Czackes et al (1964b) confirmed these observations and reported that dehydration increased disposal rates at the kidney. Therefore the build-up of plasma vasopressin levels following dehydration was due to increased secretion, not decreased elimination, at the kidney (Sawyer et al 1966). More recently it has been confirmed that severe dehydration leads to depletion of neurohypophyseal vasopressin levels as measured by bioassay (Guzek et al 1978) and radioimmunoassay (Rougoun-Rapuzzi et al 1978). Clearly an animal's state of hydration is a critical factor in determining the level of vasopressin secretion. Furthermore the regulation of secretion is mediated by changes in plasma osmolality. Verney (1947) used 40 min carotid infusions of hypertonic saline or dextrose to demonstrate that the liberation of vasopressin was affected by the osmotic pressure of the extracellular fluid. These observations were confirmed by Kovacs et al (1951) and by Zuidema et al (1956) who found that isotonic saline did not invoke the response. De Wied (1960) has also reported increased plasma vasopressin following intracarotid

administration of hypertonic saline. The osmoreceptors which monitor osmolality changes may be located in the internal carotid arteries (Jewell and Verney 1947) although more recent observations from Hayward and Vincent (1970) suggest a location within 1 mm of the supraoptic nucleus in the perinuclear zone. Osmoreceptor sensitivity is such that a 2% change in the plasma osmotic pressure is sufficient to stimulate vasopressin release. Recording from single cells in the supraoptic nucleus of rats, Bennet (1973) has observed increased firing rates following 23.5 hours of water deprivation. In addition, intragastric water infusion sufficient to induce a 3% decrease in plasma osmolality decreased the firing rates observed in water deprived rats. Conversely, intracarotid injection of 16% saline solution induced a 3% increase in plasma osmolality plus a significant increase in the firing rates of supraoptic neurons, corresponding well to the threshold values required to stimulate vasopressin secretion (Verney 1947). Furthermore, the electrophysiological response to intracarotid saline was biphasic; the author suggested that the initial increase in firing rates were the result of painful stimulation caused by the hypertonic saline whereas the secondary response corresponded to the osmolality changes. Wakerley et al (1978) have also confirmed that supraoptic neuron firing rates increase in response to dehydration. The evidence suggests that firing rates of supraoptic cells are responsive to the same changes in conditions which excite and inhibit vasopressin secretion. However, data from Kannan and Yagi (1978) suggest that the situation may be considerably more complex. A carotid injection of hypertonic Locke's solution reduced the firing rates of only two antidromically identified neurosecretory neurons. In contrast, 32 such neurons showed increased firing rates. Of these 32 neurons, 23 showed a monophasic excitatory response whilst the remaining nine exhibited a biphasic response in which an excitatory phase was followed by an inhibitory phase during which firing rates were reduced. The physiological significance of these different populations of neurosecretory cells is not clear; however, it is clear that changes in plasma osmolality such as those induced by dehydration or overhydration affect the firing rates of neurosecretory cells and the secretion of vasopressin from the posterior pituitary gland.

Much recent research has indicated the presence of vasopressin in brain structures which are structurally and functionally remote from the pituitary gland (see Section 1.2.4). Summy-Long et al (1978) have reported that vasopressin levels in the sub-fornical organ and the hippocampal commissure-fornix were increased following dehydration

whereas the levels in the anterior commissure and fornix were unaffected. The functional significance of vasopressin in these structures is unknown.

Little attention has been paid to processes associated with rehydration. Recently Rougoun-Rappuzzi et al (1978) have reported that increased vasopressin levels in the posterior lobe of the pituitary are evident after one minute of drinking; levels increased to a maximum 15 minutes then again three hours later.

1.6.2 Angiotensin and renin

Angiotensin plays a vital role in the regulation of fluid balance. In response to a drop in blood volume, pressure or a decrease in sodium ion concentration the kidney liberates renin which is converted to angiotensin and finally angiotensin II in the liver. This peptide causes a constriction in blood vessels thereby compensating for pressure loss. In addition the peptide stimulates the release of aldosterone from the adrenal glands which in turn stimulates the reabsorption of sodium in the kidney, and prevents further sodium loss (Myers 1974). Furthermore, both angiotensin II and renin have dipsogenic effects following intracranial injection (Fitzsimmons 1971). Thus both renin/angiotensin and vasopressin are involved in the regulation of fluid balance. A number of experiments suggest that centrally administered angiotensin II may stimulate the release of vasopressin. Malvin (1971) used peripheral intravenous infusions of 10 ng/kg/min into unanaesthetised dogs and observed an increase in plasma vasopressin levels 30 minutes after the onset of infusion. A lower infusion concentration of (5 ng/kg/min) angiotensin was ineffective. The effect could have been the artifactual result of angiotensin increasing blood pressure; however, a ventriculocisternal perfusion elevated plasma vasopressin levels in the absence of a pressor response, or changes in plasma osmolality. Yamamoto, Share and Schade (1978) have confirmed that ventriculo cisternal perfusion (19 ng/min) of angiotensin II increased plasma vasopressin levels. The site at which angiotensin II evokes vasopressin release is not well understood; Nicholl and Barker (1971) reported that the iontophoretic application to single neurons in the cat supraoptic nucleus resulted in rapid increases in the firing rate of these cells. On the basis of this result and considering previous indications that angiotensin stimulated the release of vasopressin, Myers (1974) has suggested that the activation of these cells by angiotensin may result in the release of vasopressin. However, this seems unlikely in view of the fact that Nicoll et al (1971) could not find any change in firing

rates of antidromically identified supraoptic neurosecretory neurons in response to the systemic administration of angiotensin II. In addition Malvin (1976) has reported that angiotensin II failed to stimulate vasopressin release in isolated posterior lobes. In a review of the relationships between vasopressin and the angiotensin-renin systems, Share (1979) has concluded that although centrally administered angiotensin stimulates vasopressin secretion the physiological significance of the effect is uncertain. Furthermore, although vasopressin may inhibit renin secretion from the kidney at levels likely to be achieved physiologically there appears to be little correlation between levels of vasopressin and renin under a variety of conditions known to affect both systems.

The E series prostaglandins are endogenous to the CNS (Barker 1977) and may be involved in mediating dipsogenic activity following intraventricular angiotensin (Epstein and Kennedy 1976). In addition, Andersson and Leksell (1975) and Yamamoto et al (1976) (cited in Yamamoto et al 1978) reported that ventriculocisternal perfusion with E series prostoglandins increased the plasma vasopressin titer. When indomethacin, an inhibitor of prostoglandin synthesis, was infused, vasopressin release was partially inhibited although indomethacin alone was incapable of affecting the vasopressin plasma levels. These data may suggest that the E series prostaglandins are involved in mediating the effects of angiotensin on vasopressin secretion (Barker 1977). However, when prostaglandin EI was injected directly into the lateral ventricle of the rat brain it was found to exert powerful antidipsogenic effects blocking the dipsogenic activity of angiotensin II, carbachol and polyethylene glycol (Kenney and Epstein 1978). These findings preclude a straightforward conclusion as to the physiological role which prostoglandins play directly or with angiotensin in mediating vasopressin regulation.

1.6.3 Changes in blood volume

Reductions in blood volume stimulate vasopressin secretion (Heller et al 1968). Ginsburg and Heller (1953) examined variations in the ADH potency of rat plasma as a function of the volume of blood withdrawn from the external jugular vein or from the common carotid artery. In both cases the plasma ADH potency depended upon the amount of blood withdrawn. The 5th ml of venous plasma (approximately 30% of the total circulating plasma had been withdrawn) contained 3.6 mu/ml plasma, representing approximately 20 x the level of ADH present in the 1st ml withdrawn. Similarly the 6th ml of arterial plasma contained 1 mu/ml,

approximately 20 x the level found in the 1st ml withdrawn. At a comparable stage of haemorrhage the ratio of ADH potency between the venous and arterial samples varied from between 4:1 to 8:1. De Wied (1960) has confirmed these results using a different assay technique. Furthermore Noble and Taylor (1953) found similar effects in humans following fainting induced by venesection. In order to reduce the confounding effects of massive blood withdrawal, Share (1967) used gradual small changes in the extracellular fluid volume and found that a 15% reduction in the fluid volume induced a 6 x increase in the blood ADH titer despite the maintenance of constant blood pressure. However, the method used to reduce extracellular fluid and maintain blood pressure (intraperitoneal dialysis) also increases plasma osmotic pressure thereby possibly stimulating ADH secretion (Sawyer et al 1966) (also cf Section 1.6.1). The posterior pituitary origin of the activity was confirmed by Moll and De Wied (1962) who observed that the effects of haemorrhage on ADH secretion could be blocked by posterior lobectomy.

Neurosecretory neurons also receive inputs from baroreceptors; Kannan et al (1978) found that antidromically identified neurosecretory neurons in the posterior pituitary responded to carotid occlusion or a pressure pulse with reduced firing rates. Similar results were reported by Yamashita and Koizumi (1979) who found a linear relationship between sinus pressure and the level of inhibition observed in the firing rates of neurons in the supraoptic nuclei of anaesthetised cats. This inhibitory effect was accompanied by decreased blood pressure and the supraoptic neurons appeared to be extremely sensitive to baroreceptor excitation; the authors argued that the cells of the supraoptic nucleus played a physiological role in the barostatic reflex. The pressor effects of vasopressin are discussed in more detail in Section 1.8.2 .

1.6.4 Stress

A number of early studies in the literature suggest that vasopressin is secreted under stressful conditions; indeed procedures such as handling, exposure to footshock, loud noises, strange environments and anaesthetics, eg ether, have commonly been used to stress animals and elevate plasma vasopressin levels. Mild electrical stimulation applied "until the animal (dog) showed signs of annoyance" produced antidiuresis (Verney 1947) which was diminished by posterior lobectomy (O'Connor and Verney 1942). Similarly Dempster and Joekes (1955) confirmed the antidiuretic response to electrical stimulation in dogs with denervated kidneys. The plasma vasopressin response to extended footshock (120 secs) was found to be maximal five minutes later, returning

to normal after 15 minutes (Mirsky et al 1954a). Histological changes have been reported following painful stimulation. Rothballer (1953) reported evidence for three phases of neurohypophyseal change in rats in response to pinpricks to the tail. Within two minutes vasodilation occurred and NSM moved towards the capillary vessels, between four and six mins there was considerable loss of NSM, presumed into the dilated capillary vessels. Restoration of NSM, although evident after one hour, was not complete until three hours after stimulation. Similar changes were apparent in the pituitary stalk and median eminence. Antidiuresis has been reported to occur during the extreme stress of bladder canulation and exposing the jugular vein in rats (De Wied 1960) and in response to ischaemic forearm muscle pain in humans (Kelsall 1949). Mirsky et al (1954a) reported that loud noises elevated plasma vasopressin levels but not to the extent seen after footshock. Furthermore, although one minute of handling or exposure to an unfamiliar environment was ineffective, a marked elevation of plasma vasopressin levels was seen in response to longer periods of stimulation (Mirsky et al 1954a). The studies suggest that vasopressin is secreted during stress; this conclusion is supported by a number of experiments described in Section 1.9 .

These studies relied exclusively on indirect measures of vasopressin release (antidiuresis) and bioassays; the concensus achieved with these methods has been challenged by more recent studies using radioimmunoassays to directly measure plasma vasopressin levels. Keil and Severs (1977) found that ether exposure for one minute did not affect subsequent plasma vasopressin levels in normal rats. When basal levels were elevated by dehydration ether exposure resulted in significant declines in plasma levels. Similarly centrifugation for periods up to two hours did not alter plasma vasopressin levels in normal rats but dehydrated rats responded to the longest period of stress with a decline in plasma vasopressin levels. The absence of any effect in normal rats under ether has been confirmed by Huzain et al (1979). In addition these authors reported that forced exercise, swimming, continuous loud noise and restraint in a strange environment did not elevate plasma vasopressin levels. Only electric shock (45 secs, 58 v), mild manual restraint for three minutes or body compression for 60 secs significantly elevated plasma AVP. Resting levels were in the region of 1.69 pg/ml rising to 42.4 pg/ml under restraint and 283 pg/ml after compression. Impaired breathing, associated hypoxia or hemodynamic changes during compression may account for the increases in AVP. Regardless of the actual mechanism the negative results obtained after the stress of ether, forced exercise, swimming and centrifugation

suggest that stress per se does not stimulate AVP secretion. The conflict between these and earlier studies may be due to a number of factors, bioassays and indirect measures may be influenced by non-specific factors; excessive manual restraint may have been used prior to decapitation to hold animals excited by stressful procedures, an antidiuretic substance other than vasopressin may be released during stress or finally the radioimmunoassays used may be insensitive to the form of vasopressin secreted during stress.

1.6.5 Sexual stimulation

Circumstantial evidence suggests that sexual stimulation may excite vasopressin secretion. A slight but significant decrease in the volume of urine voided by hydrated male rats following copulation has been reported by Eranko et al (1953). This effect did not appear in females; one hour after copulation the voiding rates of both male and female subjects were higher than controls. Friberg (1953) observed a significant reduction in the urine flow following coitus in humans. Neither experiment assayed directly for vasopressin. Stronger evidence comes from Peeters et al (1963) who reported antidiuresis in sexually mature hydrates rams following massage of the seminal vesicles and ampullae or coitus with oestrous ewes. The antidiuresis was accompanied by increased urinary K^+, Na^+ and Cl^-. In addition, the time course and form of the antidiuresis were identical to that obtained with physiological doses of pitressin (2-4 mu) suggesting posterior pituitary origin.

1.6.6 Summary

The principle factors affecting the rate of vasopressin secretion have been described. Many experiments have demonstrated that water conservation during dehydration is mediated by increased vasopressin secretion increasing the level of water reabsorption at the kidney. Small changes in plasma osmolality trigger this mechanism. Angiotensin and renin also play important roles in the conservation of body salts during dehydration and although some experimental evidence suggests that vasopressin may inhibit the release of renin from the kidney and angiotensin II may stimulate vasopressin secretion the physiological significance of this relationship is uncertain. Changes in blood volume and pressure stimulates vasopressin secretion and recent electrophysiological evidence implicates cells of the supraoptic nucleus in the barostatic reflex. Copulation and sexual stimulation may also increase vasopressin secretion.

Finally Hayward and Jennings (1973) suggest that the population of vasopressin secreting neurons in the supraoptic nucleus may be differentiated in terms of their baseline activity levels reflecting functional differentiation. They have identified three types of neurons, silent neurons showed no activity, continuously active neurons showed rhythmic activity during slow wave sleep which changed to irregular discharges during the waking hours and burster cells which were characterised by 5-10 seconds of discharge followed by 7-12 seconds of silence. Furthermore, an intracarotid infusion of hypertonic saline or noxious stimuli disrupted this pattern of responding. The role of hypertonic saline in stimulating the release of vasopressin has been discussed; the possibility that noxious stimuli are also effective in this respect is discussed in Chapter Two. The authors reported that the noxious stimuli (pin pricks) appeared to be capable of stimulating cell activity in a manner similar to a conditioning effect, ie performing the actions leading up to pin pricking was sufficient to effect the electrical responses of these cells.

1.7 Physiological Levels of Vasopressin in the Plasma

This section examines the normal physiological range of vasopressin plasma concentration. These data are important for assessing physiological significance of the behavioural effects discussed in the following chapter. Accurate estimates of baseline levels may often be confounded by stimulating secretion during the collection of samples, either by handling (Hu ain et al 1979) or other stressors (Huzain et al 1979; Keil and Severs 1979). Furthermore, the advent of radioimmunoassay techniques with increased sensitivity suggest that earlier estimations based on bioassays may have been too high.

The earliest reports were from Shannon (1942) who infused pituitary lobe extract into the ear vein of freely moving diabetes insipidus dogs (10-15 kg) and found graded antidiuresis with infusion rates in the range of 1-5 mu (pressor assay) per hour; this was verified in normal dehydrated dogs. In addition Verney (1947) found that a 1% rise in the arterial blood osmotic pressure stimulated vasopressin secretion equivalent to 3.6 mu/hour of posterior lobe extract. Lauson (1967) has estimated the rate of vasopressin liberation to lie in the range of 7.5-50 mu/hour for a normal 70 kg man. When calculated according to body weights, these estimates agree well. The data on vasopressin levels in men under varying states of hydration has been reviewed by Sawyer and Mills (1966). Subjects with DI (Yoshida et al 1963) and

normal subjects with induced watery diuresis (Sawyer, cited in Sawyer and Mills 1966) registered no blood vasopressin using an assay technique in which the lower sensitivity level was 1 µg/ml of blood. Normal hydration (Yoshida et al 1963) produced a mean value of 1.9 µg/ml (range 1-2.7) whereas overnight thirsting produced mean values of 6.5 mu/ml (Yoshida et al 1963) and 6.0 mu/ml (Sawyer, cited in Sawyer and Mills 1966). These values compare well with the minimal predicted antidiuretic limits from Lauson (1960) of 1-5 µg/ml. On the basis of these estimates Lauson (1967) has suggested that the secretion rate in a normal man after overnight thirsting should be approximately 800 µg/min (48 mu/hour). In the normal long evans rat, Miller and Moses (1971), using a radioimmunoassay technique, have estimated the normal rate of vasopressin secretion to be approximately 4.5 mu/24 hours in rats with ad lib access to food and water. In contrast, rats which were heterozygotic for genetic vasopressin deficiency (brattleboro strain; cf Section 1.9.2) yielded a secretion rate of 2.3 mu/24 hours and those homozygous for the strain yielded 0.16 mu/24 hours. In response to four days of dehydration, normal rats responded with peak values of 18.2 mu/24 hours, heterozygotic brattleboros responded with peak values of 5.5 mu/24 hours and homozygotes exhibited no change. Laszlo and De Wied (1966) reported urinary vasopressin levels of 0.46 mu/24 hours increasing by a factor of 14 in response to hypertonic saline. The differences between the absolute levels reported by Laszlo et al (1966) and Miller et al (1971) probably reflects the use of different extraction and assay procedures. More recently, Huzain et al (1979) have estimated basal plasma levels to be approximately 1.69 pg/ml (plasma); this agrees well with estimates from a number of authors (see Huzain et al 1979) and confirms the data from Keil and Severs (1976). Direct measurements of hormone levels by the radioimmunoassay technique eliminates the influence of non-specific factors inherent in bioassays and measurement of the antidiuretic response.

The rate of irreversible removal of vasopressin by all routes (µg/ml) is proportional to the arterial plasma concentration of vasopressin (µg/ml) (Lauson 1967). "Clearance" may therefore be defined as follows:

$$\text{Clearance (mls)} = \frac{\text{Total rate of vasopressin removal (µg/min)}}{\text{Plasma vasopressin concentration (µg/ml)}} .$$

Under steady state conditions, ie when no changes occur in the plasma vasopressin concentration, the rate of clearance equals the rate of secretion or infusion. Using infusions of vasopressin in the physiological range Czaczkes et al (1964a) estimated a total clearance of

8.5 ml/min/kg; one quarter of the total vasopressin content of the plasma was irreversibly removed every minute. The estimated half life for vasopressin under these conditions was 2.77 minutes. For higher concentrations of AVP Sawyer (1963) (cited in Lauson 1967) calculated a much higher total clearance of 52 ml/min/kg, yielding a half life of 45 seconds for vasopressin; under these conditions the total plasma vasopressin content was cleared nearly three times every two minutes. These figures emphasise the point made by Lauson (1967) that large doses of LVP are removed with great efficiency from the circulation. This author found that high concentrations of vasopressin had a clearance of 21 ml/min/kg and a half life of 1.10 minutes. Thus well over half of the total vasopressin in the plasma was removed every minute. The higher clearance found with AVP (Sawyer 1963) compared to LVP (Lauson 1967) may reflect the role of the former as being the natural anti-diuretic principle in rats which were also used for the assay.

Estimates for the half life of intravenously administered AVP in ethanol anaesthetised hydrated rats (Czaczkes et al 1964b; Smith et al 1965b, cited in Lauson 1967) yield values of 1.5 mins, 1.92 mins, 3.46 mins and 1.1 mins. In the rat virtually all clearance occurs at the kidney (Ginsburg and Heller (1953). These half life estimates have important implications for understanding the behavioural effects of vasopressin following peripheral injections (Chapter Two). In view of the rapid elimination of the peptide it is clear that behavioural effects do not stem from the long term presence of abnormally high peptide concentrations.

1.8 Target Organ Effects

Vasopressin derives its name from its effect on the peripheral blood vessels and plays a role in mediating pressor responses. In addition the peptide plays a key role in regulating water reabsorption at the kidney.

1.8.1 Regulation of water reabsorption

Early observations indicated that the isolated perfused dog kidney excreted a large volume of dilute urine which could be reduced either by adding posterior pituitary extract to the perfusate or by passing the perfusate through an isolated dog's head containing an intact pituitary (Verney 1926). A time lag of 15 mintues was observed between the onset of maximum hydration and the onset of maximal antidiuresis. Verney argued that this was the time required to reduce the secretion of

antidiuretic principle from the posterior pituitary and remove it from the blood. Verney (1947) demonstrated that osmotic pressure controlled the release of ADH and matched the response thus elicited with the response to posterior pituitary extract.

The mammalian kidney, with its myriads of microscopic nephrons, presents a formidable organ for study; the technical problems have forced researchers to examine similarly responsive but not more accessible tissues, eg the toad bladder. This organ regulates fluid balance in a similar manner to the distal convoluted tubule in the kidney (Leaf 1967). Vasopressin, when applied to the serosal surface, increases the permeability of the isolated toad bladder in the absence of an osmotic gradient. Linear increases in the osmotic gradient in the presence of the hormone produces a linear increase in the nett water flux (Leaf 1967). Therefore, when applied to the serosal but not to the mucosal (urinary) surface of the membrane the peptide alters permeability with a resultant change in the rate of water flux across the membrane. Koefoed-Johnsen and Ussing (1953) argued that this effect is mediated by increasing the pore sizes in the membrane. Hays et al (1971) favour an explanation in terms of an increase in membrane diffusion permeability. Elements of both explanations may be correct (Leaf 1967). He has suggested that the membrane is composed of two layers in which a porous membrane is overlaid by a diffusion barrier.

The toad bladder actively pumps Na^+ from the urinary to the body fluids against a concentration gradient. This action is stimulated by applying pitressin to the serosal surface of the bladder, is highly specific to Na^+ and requires energy, as demonstrated by the increased oxygen consumption seen after simultaneous application of Na^+ to the mucosal and vasopressin to the serosal surface of the membrane.

Evidence suggests that these effects are mediated by 3'5' cyclic amp (cAMP) (Orloff and Handler 1967) which has been found to mimick the effects of vasopressin on water flow. Furthermore, Takahashi et al (1966) have found excreted 3'5' cAMP in the urine following antidiuresis induced by exogenous vasopressin. Homozygous DI rats have reduced excretory 3'5' cAMP and Johnsen and Nielsen (1978) have reported that arginine vasotocin, the frog antidiuretic principle increased cAMP levels in isolated frog epithelia. These data implicate cAMP in mediating vasopressin's membrane effects. Orloff and Handler (1967) have suggested that the peptide increases the conversion of adenosine triphosphate (ATP) to 3'5' cAMP by increasing adenyl cyclase. Dousa (1973) reviewed the evidence that peptide receptors associated with adenylate cyclase were located on the basilar and lateral plasma

membranes (serosal) whilst the barrier controlling water and solute permeability were located on the luminal or mucosal surface. Adenylate cyclase is activated by vasopressin analogues. The mechanism by which cAMP may affect membrane changes is unknown but could involve varying the pore size. The administration of cAMP is associated with an increase in the mechanical deformability of the membrane and Dousa (1973) has suggested that 3'5' cAMP may affect a reversible modification of specific membrane proteins.

Evidence has accumulated suggesting that the E series prostaglandins play a role in regulating the intracellular effects of vasopressin in the toad bladder (for review see Orloff and Zusman 1978). Prostaglandin EI blocks the action of vasopressin but not cAMP at the toad bladder and prevents vasopressin induced accumulation of cAMP. Furthermore, indomethacin, a prostaglandin synthesis inhibitor, has been reported to enhance the effects of vasopressin in rats, dogs and men (see Orloff and Zusman 1978 for references). Thus vasopressin appears to increase water permeability by increasing cAMP synthesis and simultaneously trigger increased synthesis of prostaglandin E which then diminishes adenylate cyclase activity thereby diminishing the response to vasopressin.

1.8.2 Effects on blood pressure

Much of the earliest research and clinical usage of posterior lobe extracts was related to their capacity to induce transient increases in arterial blood pressure (Oliver and Schafer 1895; see also Erwald and Weichel 1978 for references). Changes in blood pressure have been reported in response to peripheral injections with vasopressin or posterior lobe extracts in excess of that required to produce antidiuresis. Statt and Chenoweth (1966) demonstrated a triphasic pressor response following intravenous injections of vasopressin (300 mu/kg) into rats anaesthetised with pentabarbitone, which is known to stimulate vasopressin secretion (Ginsburg and Brown 1956). Blood pressure reached a maximum of 180 mm Hg compared to the pretreatment mean of 123 mm Hg. Pressure increases were accompanied by increased catecholamine release into the cavernous sinus and occlusion of the CNS circulation removed the source of catecholamines and either diminished or eliminated the pressor response. These data suggested a relationship between vasopressin and catecholamines in mediating the pressor response to large vasopressin doses. Chenoweth et al (1958) reported a synergistic relationship between vasopressin and catecholamines in eliciting the pressor response, they reported that pretreatment with L norepinephrine,

dopamine or hydroxyamphetamine potentiated the pressor effects of vasopressin. Experiments with lower doses (10 mu/kg) have successfully elicited the pressor response in rats with isolated and denervated carotid sinuses; furthermore, the response was blocked by pretreatment with phentolamine (2 mg/kg) or phenoxybenzamine (5 mg/kg) (Traber et al 1968).

Vasopressin levels in the physiological range have variable effects on blood pressure. Traber et al (1968) reported that endogenous vasopressin secreted in response to intra-carotid injections of hypertonic solutions did not affect arterial pressure in intact rats but increased pressure in rats with isolated and denervated carotid sinuses. Intravenous infusions of vasopressin at much lower doses (235-300 μu/min) did not affect blood pressure but potentiated the pressor response to norepinephrine and epinephrine in pithed rats (Bartelstone et al 1965). Pretreatment with 280 μu/min doubled the pressor response to 50 ng of norepinephrine. Infusion rates in excess of 500 μu/min were required to elevate arterial blood pressure in the absence of norepinephrine treatment. Potentiated pressor responses were affected within ten minutes of vasopressin application or withdrawal and were detectable in isolated aortic strips as well as in pithed rats.

Intravenous infusions of LVP in men exert a dose dependent increase in systemic and arterial blood pressure with peak values occurring approximately five minutes after the onset of infusion (Erwald and Wiechel 1978). Peak pressure occurred earlier after higher doses and was accompanied by bradycardia. In general, intact animals show much less sensitivity to the pressor effects of vasopressin than do isolated or denervated preparations; thus the predominant use of high doses to achieve effects. Cowley et al (1974) have argued that the baroreceptor reflex system plays a major role in buffering the pressor action of vasopressin. Mohring et al (1979) have extended this argument to account for their observations that plasma levels of AVP are strongly correlated with the severity of hypertension in spontaneously hypertensive rats, by suggesting that in these animals there exist deficiencies in the reflex mediated buffering of the pressor response to vasopressin. A causal role for vasopressin in mediating chronic blood pressure elevation in these rats was suggested by the observation that systemic injections of anti-vasopressin serum reduced blood pressure temporarily. Angiotensin and renin do not appear to be involved in mediating chronic hypertension. The hypothesis is supported by Crofton et al (1979) who have found that vasopressin plays an essential role as a pressor agent in mediating and maintaining DOC salt (deoxycorticosterone) hypertension.

They demonstrated that unilateral nephrectomy followed by DOC salt and maintenance on salinated drinking water induced hypertension in normal long evans rats but not in rats with a hereditary lack of vasopressin (HO-DI; see Section 1.9.2). Furthermore, blood pressure was reduced by vasopressin analogues which block the pressor response, indicating the specificity of vasopressin as the pressor agent.

An additional role for vasopressin in cardiovascular regulation has been proposed by Bohus (1980). Intravenous LVP was found to reduce the pressor response to posterior hypothalamic stimulation, reaching its maximum inhibitory effect approximately 60 minutes after the onset of infusion, long after the initial pressor response had disappeared (Bohus 1974). Dose dependent reductions in the magnitude of the pressor response to stimulation of the mesencephalic reticular formation have been confirmed by Versteeg et al (1979) following intracerebroventricular injections of nanogram quantities of AVP. This apparently central effect may be mediated by exciting central noradrenergic mechanisms involved in the regulation of cardiovascular function. Centrally administered vasopressin has been found to increase noradrenaline turnover in specific brain regions, particularly the nucleus tractus solitari, which appear to be involved in regulating cardiovascular functions (Tanaka et al 1977; see Section 2.6.1).

1.9 Vasopressin Involvement in the Release of Adrenocorticotrophic Hormone (ACTH)

It was suggested by Harris (1955) that ACTH secretion was regulated by a humoral agent released into the portal blood vessels. Saffran coined the term corticotrophin releasing factor (CRF) the identity of which has remained elusive despite extensive research. Many authors have proposed that vasopressin is the CRF but the evidence for this is contradictory. In order to interpret the behavioural effects of vasopressin it is necessary to consider the extent to which treatment with the peptide is likely to stimulate the secretion of behaviourally active ACTH.

1.9.1 Preliminary evidence

Early experiments relied extensively on indirect measures of ACTH secretion. Nagareda and Gaunt (1951) monitored changes in adrenal ascorbic acid (AAA) following injections of pitressin (IP) in intact rat and reported that although 5 mu elicited antidiuresis without changing AAA levels, both 100 mu and 400 mu depleted AAA suggesting that pitressin

activated the adrenal gland. In addition a number of studies claim to have demonstrated simultaneous increases in ACTH and vasopressin during stress; however, the reliability of these observations has recently been questioned (see Section 1.6.4 for discussion). Surgical lesions of the supraopticohypophyseal tract at the level of the median eminence disrupted both vasopressin and ACTH secretion and the severity of secondary polydipsia was inversely proportional to depletion of plasma ACTH and AAA (McCann and Brobeck 1954); in addition AAA levels were not affected by the acute stresses of surgery, histamine or epinephrine.

The results from Nagareda and Gaunt (1951) and McCann and Brobeck (1954) suggest that vasopressin may stimulate ACTH release thereby affecting AAA levels. There are three principle difficulties with this interpretation. Pitressin is a crude extract from the posterior lobe of the pituitary and may be contaminated with genuine CRF from surrounding tissue. Second, increased ACTH secretion, as measured by AAA changes, was achieved only at very high doses. Thirdly, ACTH was not directly assayed; changes in secretion were inferred from AAA levels.

In a review of the literature concerning the role of vasopressin in ACTH secretion, Nicholls (1961) listed 25 experiments using different preparations and measures which demonstrated that the adrenal system was activated by vasopressin or posterior lobe extracts. However, all the studies had used high doses and it was argued by Nicholls (1961), echoing the doubts of Nagareda and Gaunt (1951), that under normal physiological conditions the concentration of vasopressin was unlikely to reach the level required to stimulate ACTH secretion; in support of this argument Nicholls and Guillemin (1959) could find no correlation between diuresis and 17 hydroxycorticosteroid levels following stimulation by hydration, hypertonic saline or vasopressin in low doses. Over a wide range of doses (10-300 mu/rat iv) Doepfner et al (1963) found a linear positive dose response relationship between plasma corticosteroid levels and the log of the vasopressin dose in rats treated with morphine to block endogenous secretion.

Recent evidence suggests that the argument against vasopressin as the CRF based on its capacity to reach the required concentrations under physiological conditions is invalid. McCann et al (1954) has argued that the volume of blood in the portal vessels is very small and under these conditions vasopressin could attain local concentrations which in the periphery would be considered pharmacological. Zimmerman et al (1973a) reported that vasopressin concentrations in the hypopyseal blood (13000 pg/ml) was approximately 300 times higher than in the systemic blood (42 pg/ml).

Interpretation of indirect measures of ACTH secretion is hampered by conflicting evidence on the capacity of vasopressin to directly stimulate the adrenal gland. Arimura (1955) has reported that even large doses of pitressin did not affect AAA levels in hypophysectomised rats; this was confirmed by Lipscombe et al (1960). However, Hilton et al (1959) reported elevated plasma hydroxycortisone levels in the adrenal venous blood following infusion of the adrenals of pentabarbitone anaesthetised, hypophysectomised dogs with synthetic vasopressins in the dose range 0.001 - 0.4 pressor units/ml/min. The increase showed a degree of dose dependency and higher infusion doses induced longer responses. Furthermore Anderson and Egdahl (1964) reported that 17 hydroxycorticosteroid levels in the adrenal venous blood were increased by 25 mu of vasopressin administered into the adrenal artery but not into the internal carotid artery. Much higher vasopressin doses were required to stimulate the adrenals via the internal carotid artery; 50 mu produced a response in 50% of the animals and 100 mu increased levels in all the animals.

1.9.2 Deficient stress responses in Brattleboro rats

The use of surgical procedures to induce a vasopressin deficit by lesioning the supraopticohypophyseal tract in rats (McCann and Brobeck 1954) may leave some axons intact, thereby complicating interpretation of the effects of surgery. The discovery of a strain of rats which were genetically incapable of synthesising vasopressin (Valtin and Schroeder 1964) stimulated many experiments aimed at determining the capacity of these animals to respond to stress and the importance of vasopressin in regulating pituitary adrenal functions. Diabetes Insipidus (DI) arose spontaneously in a colony of Long Evans rats maintained at Brattleboro, USA, and is characterised by polyuria, polydipsia decreased urine osmolality and body weight with increased Na^+ and K^+ content in the urine of animals homozygous for the defect (HO-DI). Urine characteristics were not altered by dehydration, stress or hypertonic saline, but responded to treatment with vasopressin. Assays of pituitary and hypothalamic tissue from HO-DI rats revealed minimal pressor and antidiuretic activity and reduced oxytocic activity. Rats heterozygous for the strain (HE-DI) showed normal oxytocic activity but reduced pressor and antidiuretic activity compared to normal Long Evans adults (Valtin 1967). The condition stems from autosomal recessive genes at a single pair of loci and is associated with a higher than normal frequency of runts, stillborns and newborn deaths (Valtin 1967).

Many studies have since confirmed the absence of vasopressin in

HO-DI rats with a number of methods. Miller and Moses (1971) using a radioimmunoassay found secretion rates of 0.16 mu/24 hours in HO-DI rats compared to 2.3 mu/24 hours for HE-DI rats and 4.5 mu/hour for normal long evans stock. In response to four days of dehydration normals responded with an increase to 18.2 mu/24 hours, HE-DI rats increased to 5.5 mu/24 hours and HO-DI rats failed to respond. Similar findings were reported by van Wimersma Greidanus et al (1974) and De Wied et al (1975a). Rosenbloom and Fisher (1975) and Chateau et al (1979). Furthermore, Leclerc et al (1974) used an immunohistochemical process and confirmed that the supraoptic nucleus, paraventricular nucleus, median eminence and posterior pituitary of HO-DI rats were deficient in NSM which is associated with vasopressin secretion (see Section 1.3) and gave no positive reactions to antivasopressin serum.

In addition to the absence of vasopressin HO-DI rats exhibit hypokalemia, rapid renal loss of K^+ (Mohring et al 1972a,b) diminished extracellular fluid volume (Harrington and Valtin 1968), reduced adrenal responsiveness to stress (see below) and ACTH and a possible defect in growth hormone synthesis. The low levels of K^+ and extra-cellular fluid suggest a preponderance of mineralocorticoids, as these and glucocorticoids derive from common precursors there may be deficient glucocorticoid synthesis therefore accounting for the deficient plasma corticosterone response to mild stress reported for HO-DI rats (McCann et al 1966; Arimura et al 1967; Wiley et al 1974).

HO-DI rats respond to the stress of manual restraint and ether with lower plasma steroid levels than normal controls (McCann et al 1966); after three minutes in an ether jar controls had steroid concentrations of 75 ug/100 ml compared to 55 ug/100 ml in HO-DI rats. Arimura et al (1967) could not confirm this finding using ether, histamine, acetylcholine or nembutal as stressors, whilst HO-DI rats gave consistently lower responses the differences were insignificant; however, the steroid response to epinephrine hydrochloride (0.02 mg/100 mg) or saline (0.2 ml, 0.9%) was significantly lower in HO-DI rats than controls, suggesting that these animals were deficient in their steroid response to stress as McCann et al (1966) had proposed. Furthermore, in the case of the ether experiment, Arimura et al (1967) used 75 seconds exposure, compared to three minutes in the McCann et al (1966) study, and found much lower baseline steroid levels in the control animals (32.5 ug/100 ml); therefore the failure to replicate may be ascribed to procedural differences, particularly in the severity of the ether stress. Some of these findings have been replicated by Wiley et al (1974). Resting plasma corticosterone levels in HO-DI and HE-DI rats

were equivalent; furthermore, steroid responses to a low dose of histamine (18 ug/100 gbw) were the same. In contrast a higher dose (36 ug/100 gbw) injected through a jugular cannula differentiated the two groups; HO-DI rats responded with significantly lower steroid levels than HE-DI rats. In addition the response to ether and haemorrhage and to the milder stresses of bell ringing and cage shaking resulted in lower steroid responses in HO-DI rats.

The data suggest that the steroid response to stress in HO-DI rats although present is somewhat deficient, as these animals lack vasopressin it appears that vasopressin plays a role in mediating the steroid response to stress. There are two difficulties with the hypothesis that vasopressin is the CRF raised by the data. First, although the response is deficient it is present which would not be the case if vasopressin alone regulated ACTH secretion. Secondly, Wiley et al (1974) reported that HO-DI rats showed reduced steroid responsiveness to ACTH using studies in vivo and in vitro, a deficit which was reversed by long term replacement therapy with vasopressin starting at four days old. Therefore the deficient steroid response to stress may reflect reduced adrenal sensitivity to ACTH resulting from long term vasopressin depletion.

Reduced adrenal sensitivity to ACTH following long term vasopressin depletion also poses problems for the hypothesis that vasopressin acts as CRF under limited conditions of mild stress. Smelik et al (1962) reported that handling and exposure to a strange environment depleted posterior lobe CRF activity; furthermore, mild electric shock produced an attenuated steroid response in posterior lobectomised rats compared to sham operated controls whereas the response to severe shock was the same in both groups. Similarly De Wied et al (1961) reported that posterior lobectomy attenuated the steroid response to loud noises, pain and exposure to a strange environment but did not alter the response to ether, histamine or nicotime tartrate. Five days of pitressin treatment alleviated the deficient response to mild stress. Similar results were found when vasopressin was depleted by maintaining the rats on 2.5% salinated drinking water for seven days; depletion was indicated by loss of NSM from the posterior lobe. The attenuated steroid responses observed during mild stress may reflect the lack of vasopressin exerting its role as CRF; alternatively it may reflect decreased adrenal sensitivity resulting from vasopressin depletion accompanied by non-specific steroid release triggered by the application of severe footshock and the various drugs used.

1.9.3 Vasopressin as the CRF

The identity of CRF has remained elusive; some of the data suggesting a role for vasopressin in this respect has been described above. Animals with a genetical absence of vasopressin show deficient adrenal responses to stress and although a number of studies have shown that vasopressin, particularly in high doses, stimulates ACTH secretion, the evidence for this is conflicting.

Arimura et al (1965) reported that neurohypophysectomised rats did not increase their blood corticosterone levels in response to LVP (100 mu/100 gip or 400 mu/100 giv), but did respond to posterior lobe extract. Moreover, Hedge et al (1966) found that relatively low doses of vasopressin (2 mu/100 gbw) did not stimulate ACTH secretion in rats treated with dexamethasone and morphine whereas pituitary extract did. Similar results have recently been reported by Yasuda and Greer (1976). Using an in vitro system of cultured adenohypophyseal cells followed by direct measurements of ACTH levels by radioimmunoassay they found that synthetic LVP or AVP neither stimulated ACTH secretion nor potentiated the action of CRF contained in hypothalamic extract even in doses up to 4 ug/ml. Furthermore, using a potent bioassay for CRF, Krieger et al (1977) found the highest concentrations of CRF to be in the median eminence. Very small amounts of activity were found in the supraoptic or paraventricular nuclei, which would not be the case if vasopressin was the CRF. In addition HO-DI rats had reduced but distinct CRF activity in the median eminence. Pearlmutter et al (1980) have confirmed the presence of CRF like activity in the median eminences of HO-DI rats although these rats are known to lack AVP and its associated neurophysins. The CRF potency of HO-DI stalk median eminence extract was over 95% of normal rats and Pearlmutter et al (1980) argued that the structure of CRF was closely related but not identical to vasopressin. They attributed the reduced CRF activity reported for HO-DI stalk median eminence extract by Gillies and Lowry (1979; 1980) and Krieger et al (1977) to the use of a dispersed pituitary cell assay system. The argument that CRF may be structurally similar to AVP gains some support from much earlier experiments. Saffran et al (1955) reported that whilst chromatographically identified vasopressin increased ACTH secretion in vitro they were also able to isolate a vasopressin free preparation from the posterior lobe capable of stimulating ACTH secretion. Furthermore, Schally and Guillemin (1963) identified a CRF which was composed of all the amino acid groups of vasopressin plus serine and histidine.

On the other hand, Yates et al (1971) argued that vasopressin

potentiates the CRF. They reported that a dose of AVP or LVP, not by itself capable of stimulating ACTH secretion, potentiated the ACTH response to crude pituitary extract in intact rats; a similar effect was found when endogenous AVP levels were increased by dehydration.

The argument has been reversed by Gillies and Lowry (1979) who found that the major peak of CRF activity in chromatographed stalk median eminence extract from normal male rats is identical to AVP chromatographically, immunologically and biologically in a dispersed pituitary cell assay system. In addition, they have reported ACTH secretion in response to low doses of AVP (100 pg/ml). Two smaller CRF peaks were also identified (Gillies and Lowry 1979) which potentiated the ACTH agonist properties of the AVP peak to yield the full biological activity characteristic of stalk median eminence extract and on the basis of this finding they suggested that AVP is the CRF but that its activity is modulated by synergistic factors with reduced CRF activity. In support of this argument, Gillies and Lowry (1980) reported that stalk median eminences from HO-DI rats lacked AVP after chromatography but contained approximately 20% normal CRF activity and the two synergistic peaks of the chromatogram. Full agonist activity was restored by adding small amounts of AVP. Although these data strongly support the hypothesis that vasopressin is the CRF, they clearly conflict with the reports discussed earlier and a concensus remains to be achieved on this question.

1.10 Electrophysiological Characteristics of Vasopressin Secreting Cells and Electrophysiological Effects of Vasopressin

In vitro studies of the electrical activity of cells from the supraoptic nucleus revealed the presence of units which maintained spontaneous discharge in the presence of synaptic blockade (Gahwiler and Dreifuss 1979). A number of authors have linked the activity of such pacemaker cells to the tonic regulation of vasopressin secretion (see Gahwiler and Dreifuss 1979 for references).

Microelectrophoretic application of LVP to cells of the supraoptic nucleus inhibited firing rates in 80% of cases (Nicholl and Barker 1971) and the authors suggested that vasopressin mediated inhibition of its own release. In support of this hypothesis, Vincent and Arnauld (1975) reported that when injected into the carotid artery of monkeys vasopressin (5 x 10 - 9 moles) decreased the firing rates of cells in the supraoptic nucleus which had been identified as responsive to osmotic stimuli. Inhibition lasted 15 to 20 seconds after intracarotid

injection but intravenously injected LVP was inactive even in doses four times as high. Chronic dehydration also inhibited firing rates and was accompanied by the characteristic rise in plasma osmotic pressure. However, after five days of dehydration firing resumed its normal rate, which the authors suggested reflected chronic vasopressin depletion. Barker (1976) has suggested that presynaptic vasopressin receptors could modulate the electrical activity of supraoptic neurons by altering the release of excitatory or inhibitory transmitters involved in regulating secretion (see Section 1.5). Indeed some of the pharmacological evidence discussed in Section 1.5 suggests that vasopressin secretion is regulated by a negative feedback loop. In contrast, Dreiffus et al (1974) reported recurrent inhibition in HO-DI rats. In this case AVP is unlikely to be the transmitter and suggests that in the case of microelectrophoretic (Nicholl and Barker 1971) and intracarotid application inhibition of firing rates may reflect membrane effects or effects mediated at sites distal to the supraoptic nucleus.

In order to avoid the complexities inherent in trying to analyse peptide hormone effects in intact mammalian nervous systems a number of studies have used invertebrate systems. LVP, AVP, hemolysine vasopressin and oxytocin induce specific effects on an identified cell from the land snail (Otala lactea) (Barker and Gainer 1974). The responsive cell was inactive during the snail's dormant period and responded to acetylcholine with a typical transient depolarisation. In contrast, LVP and its analogs (10^{-9} moles) rapidly induced bursting pacemaker activity from the cell extending long after the period of application. Four hours of washing was required to normalise electrical activity. Cell sensitivity was restricted to the axon hillock. The mechanism by which vasopressin analogues exerted their effects appears to differ from that of neurotransmitters. Exposure of the responsive cells to a peptide bath induced changes in the steady state properties of the membrane including the development of voltage dependent Na^+ and K^+ conductance whereas putative transmitters changes voltage independent conductance; the intact vasopressin molecule was required for the effects as desglycinamide analogs were inactive (Barker 1977). The data suggest that in the invertebrate nervous system vasopressin may modulate cell firing characteristics and in support of this hypothesis Ishfin et al (1975) has reported evidence indicating the presence of the hormone in Otala lactea.

1.11 Summary

The aim of this chapter has been to establish that the consequences of treating intact animals with vasopressin are far reaching and involve many physiological systems, although this does not by itself establish physiological roles for the endogenous peptide. Increased vasopressin levels may stimulate water reabsorption accompanied by changes in osmotic pressure and ion concentrations; increase blood pressure with activation of the barostatic reflex; stimulate secretion of behaviourally active ACTH and corticosteroids and increase the transit of vasopressin across the blood/CSF barrier to gain direct access to CNS structures. Furthermore, experimental procedures commonly used in behavioural studies, eg handling, electric footshock and manual restraint whilst injecting the animals are liable to alter endogenous levels and trigger compensatory mechanisms in a number of systems. Finally pharmacological manipulations of neurotransmitter systems carried out for behavioural reasons are liable to interfere directly with neurochemical systems involved in controlling secretion, with consequences for baseline secretion rates.

CHAPTER TWO.

THE EFFECTS OF VASOPRESSIN ON BEHAVIOUR.

2.0 INTRODUCTION.

Studies discussed in chapter one relate to aspects of vasopressin's synthesis , secretion and putative physiological and pharmacological roles which bear directly or indirectly on our understanding of behavioural roles for the peptide. Additional experimental evidence indicates distinct effects on conditioned behaviour and this chapter describes and discusses the evidence for this in detail.

Hypotheses for explaining the behavioural activity of vasopressins refer to inferred processes such as memory , arousal , fear etc rather than to the behavioural changes per se. This reflects preferences for concepts which provide unitary and parsimonious explanations of morphologically diverse behaviours.

Despite extensive research we still lack a coherent model of the physiological events presumed to underlie the behaviourally defined process of memory. In its absence the most fruitful approach in the animal literature has been to operationalise definitions. The post training period is defined as the consolidation phase whilst subsequent tests measure retention (usually 24 to 48 hrs later.) The concept and terminology derive from models of human memory which postulate that information is first stored in short term labile stores then transferred or "consolidated" to long term permanent store for subsequent retreival (Muller and Pilzecker 1900: Hebb 1949).

The theory explains aspects of human amnesia successfully (Russell and Nathan 1946 ; Warrington and Weiskrantz 1973). Post training treatment within the consolidation phase eg. anoxia , concussion , drugs , electroconvulsive shock (ECS) etc., which alter subsequent retention are said to interact with consolidation processes at the physiological level. When combined with training procedures in which stimulus , response and reinforcement events are easily specified the model is a powerful analytical tool for studying the effects of post training treatments without affecting sensory or motor capacities during training. However , without a well grounded physiological theory of memory there are no a priori criteria for establishing the

length of the consolidation phase. In addition there is considerable disagreement in estimates of the length of post training sensitivity. Using ECS different authors have reported the labile phase to be destructible up to a few seconds , 6 hours or 3 days (Deutsch 1973). Puromycin has yielded estimates varying from less than 1 hour up to 3 weeks (John 1967). Furthermore , on the basis of human neuropsychological data indicating that long term recall may remain intact when short term recall is disrupted (Warrington and Shallice 1969; Shallice and Warrington 1970) it has been argued that consolidation failure is an inadequate explanation of the amnesic syndrome (Warrington and Weiskrantz 1973). Recall improvements seen after cueing suggested a role for retreival deficits. Application of the consolidation model to the analysis of animal experiments may therefore be misleading. Finally , in the case of the vasopressin literature the use of standardised behavioural procedures has increased the replicability of findings but the interaction between peptide and behavioural variables has remained relatively unexplored partially as a result of the wide acceptance of the consolidation hypothesis.

In the experimental studies discussed below a number of main themes can be identified. First , attempts to establish that reduced avoidance responding in extinction following surgical manipulations of the endocrine system were in part due to vasopressin deficits . Second , attempts to demonstrate that intact rats showed increased extinction responding after vasopressin injections corresponding to then current theories on the nature of memory storage. Third , attempts to show that these changes reflect a physiological role for the endogenous peptide and are not pharmacological artifacts and finally to correlate vasopressin's behavioural effects first with specific brain regions and circuits and then with catecholaminergic neurotransmitter systems.

2.1. Effects on Avoidance Responding.

Removal of the posterior and intermediate lobes of the pituitary gland disrupts learning and extinction of two way shuttle box avoidance responding (de Wied 1965). One week after lobectomy rats were trained to avoid shock (40v 1.8 ma) preceded by 5 secs of buzzer

as the conditioned stimulus (CS) for 10 trials per day on 14 successive days. Rats which failed to make 80% correct responses over the last 3 days were dropped from the experiment (0% lobectomised; 17% shams). During 9 days of extinction testing in which shock was omitted the lobectomised rats made significantly fewer responses than shams, a deficit attributed to the absence of posterior and intermediate lobe hormones (de Wied 1965), ruling out general debilitation and gross sensory or motor deficits as a result of surgery on the basis that lobectomised rats did not differ significantly from shams during training to escape shocks of unspecified intensity in a runway. Furthermore, avoidance response rates in lobectomised rats were significantly increased by peripheral injections of pitressin tannate (crude posterior pituitary extract 1 ml 10 IU) 2 hours before test sessions on alternate days starting on the first day of training.Responding during extinction was maintained in excess of 90% and water intake was reduced to normal . In order to determine the behavioural activity of pitressin's principal hormonal constituents ACTH in a zinc phosphate complex (0.5 or 1.5 IU/48 hrs) ,MSH in zinc phosphate (2 ug or 6 ug/48/hrs) and LVP in zinc tannate (0.33 or 1 U/48 hrs) were tested in posterior lobectomised rats. All three peptides increased avoidance responding in extinction compared to their vehicle control groups. However only LVP normalised water intake suggesting that the behavioural deficit associated with posterior lobectomy is not due to abnormal water regulation. Increased responding in extinction after higher doses indicated a degree of dose dependency. Evidence from hypophysectomised rats, maintained on hormone replacement therapy of thyroxin, corticosterone and testosterone in order to counter the debilitating effects of surgery, showed that both LVP (1 U/48 hrs) and MSH (6ug/48 hrs) significantly increased avoidance responding in extinction compared to saline controls and this apparently was not due to stimulating the secretion of endogenous ACTH. Furthermore, adenohypophysectomised rats maintained on replacement therapy did not show acquisition or extinction deficits compared to sham operated controls, suggesting again that endogenous ACTH deficits were not the cause of low avoidance response rates in posterior lobectomised rats.

The data were interpreted as indicating a role for posterior lobe peptides in the maintenance of avoidance responding, independent of effects on water regulation, ACTH secretion or motor and sensory deficits (de Wied 1965).There are a number of difficulties with this

interpretation. Escape responding is of doubtful value in detecting subtle motor or sensory defects. In later experiments (Bohus et al 1973) much lower response rates were reported for hypophysectomised rats not maintained on hormone replacement therapy , in the absence of tests on intact rats a contributory role for replacement therapy cannot be excluded. Sham operated controls were not tested for the effects of pitressin or peptides.

In subsequent experiments some of these difficulties and the problems inherent in using surgical procedures to examine endocrinological effects on behaviour were overcome by using intact animals. De Wied and Bohus (1966) were the first to show an effect of pitressin on avoidance responding in intact rats and to distinguish the effects of pitressin (1 IU/rat) from a-MSH in zinc phosphate (10 ug/rat) using the shuttle box procedure previously described. Rats were injected prior to and on alternate days during training. Responding in training was not affected by treatment but during 10 trials of extinction on each of 14 consecutive days both placebo and a-MSH animals made significantly fewer responses than pitressin treated rats. When tested again 7 days later these differences were maintained, suggesting a long lasting effect of pitressin on avoidance responding. However , the behavioural requirements for the effect were uncertain as injections during training had elevated responding in extinction . Therefore in a subsequent experiment the peptides were injected on alternate days during 14 days of extinction testing. In this case placebo rats extinguished rapidly but both pitressin and a-MSH groups maintained high response rates (in excess of 90%). Treatment was discontinued and 21 days later 3 extinction sessions revealed that a-MSH rats responded at control levels but pitressin treated rats continued to make significantly more responses than control. Pitressin therefore exerted a long term effect on extinction responding whether injected during training or extinction.

In contrast the effect of a-MSH was restricted to the period of treatment during extinction. The long term active component of pitressin was therefore not α MSH and probably not ACTH, earlier evidence (de Wied 1965) suggested a role for LVP. The long term nature of the effect led de Wied and Bohus (1966) to suggest the involvement of processes related to long term memory formation.

The identity of vasopressin as the active constituent of pitressin was confirmed by de Wied (1971) using a pole jump avoidance task. Intact rats were trained to avoid shock (0.2 ma) preceded by a

light as the CS for 10 trials per day on 3 consecutive days. Those rats which made more than 10 correct responses were then given 10 extinction trials , animals making 8 or more correct responses in the first extinction test were injected with either saline, ACTH 4-10 (100 ug) or LVP (1 ug ;60 IU/mg) sc. Extinction tests were repeated at 2,4,24,48 and 72 hrs. Saline controls extinguished rapidly , ceasing to respond after the 4 hr test, ACTH 4-10 increased extinction responding up to the 4 hr test. In contrast LVP maintained responding at 90% over all 5 tests. A number of other peptides were tested and found to be ineffective including oxytocin (1 ug;60 IU/mg), angiotensin II (1 ug), insulin (1 ug) and growth hormone (1 ug). Some aspects of the procedure may restrict the generality of the results, two behavioural criteria restricted the test population, in addition very small groups were used and no statistical tests were reported. Despite these points many subsequent experiments have confirmed the effects of both LVP and ACTH 4-10 on avoidance responding. More recent studies have shown that oxytocin affects avoidance extinction when injected peripherally (Schulz et al 1974) and directly into the ventricles of the brain (Bohus,Kovacs and de Wied 1978; Bohus,Urban,van Wimersma Greidanus and de Wied 1978).

The behavioural activity of LVP in hypophysectomised rats (de Wied 1965) was confirmed by Bohus (1973) using the two way shuttle box avoidance task with 5 secs of buzzer as the CS and 0.12 ma of shock, other aspects have been descibed (de Wied 1965, de Wied and Bohus 1966). During the first 7 days of training both LVP (1 ug/rat) and ACTH 4-10 (20 ug/ml) significantly elevated response rates compared to saline. When treatment was discontinued on day 7 the response rates of ACTH 4-10 treated rats declined to control levels but those of the LVP rats remained significantly higher. Hypophysectomised rats injected with saline had very low response rates, contrasting with the earlier report from de Wied (1965). The difference may be attributed to lower shock level, termination of shock after a maximum of 20 secs on each trial or the omission of hormone replacement therapy. Low response rates in control groups confound interpretation in terms of learning related processes , a problem found also in some early passive avoidance studies on the effects of vasopressin in intact rats (Ader et al 1972; Bohus et al 1972; Wang 1972). In addition, increased inter-trial responding in hypophysectomised rats treated with LVP compared to saline (Bohus 1973) suggest a relative lack of stimulus control when response rates

are elevated by vasopressin.

The effect of vasopressin cannot be attributed to a general excitation as intact rats injected SC with LVP have increased re-entry latencies on a step through passive avoidance task (Ader and de Wied 1972). Rats were placed on an elevated, well illuminated platform and latencies to enter a dark chamber with a grid floor were recorded.If the sum of the latencies on 3 succesive trials did not exceed 30 secs then on entering the chamber on the 4th trial the rat received footshock for 2 secs, (0 ma, 0.125 ma or 0.25 ma). Re entry latencies 24 and 48 hrs after shock increased as a function of the training shock. LVP (0.3 ug/rat, 0.9 ug/rat 2.7 ug) or saline was injected immediately after shock.LVP significantly increased re-entry latencies 48 but not 24 hrs after training. Higher doses of LVP yielded higher re-entry latencies,indicating a dose dependent effect but this was not tested statistically. LVP did not affect responding after either 0 ma or 0.125 ma in control rats. Krejci and Kupkova (1978) have confirmed these findings using the step through passive avoidance task with 0.35 ma shock. LVP (1 ug) injected after the learning trial significantly increased re-entry latencies 2 and 3 but not 7 and 13 days after training.

2.2. Time Dependent Effects.

The effects of vasopressin on extinction responding diminish as the interval between the end of training,first extinction session or retention test increases. This was first shown by de Wied (1971) using pole jump avoidance responding (see sect. 2.1 for procedures and criterion). When LVP (1 ug) was injected immediately after the first extinction test response rates in extinction 24 and 48 hrs later were significantly elevated. When treatment was delayed for 60 mins the effect was reduced but when delayed for 6 hrs response levels in extinction were comparable to saline controls.

Subsequently it was shown that to be effective the injection may either follow or precede the first pole jump extinction session by up to 60 mins (de Wied 1973). Behavioural potency diminished as a function of increased intervals. Furthermore Bohus et al (1972) have shown that when LVP (1 ug) precedes the final training session of pole jump avoidance by 60 mins then extinction response

levels are significantly higher than saline controls 48 hrs but not 24 hrs later, failure to observe an effect 24 hrs after injection is probably due to high baseline responding in saline controls in this test. When the injection preceded the final training session by 6 hrs LVP did not affect extinction . The results indicate that vasopressin increases extinction responding when injected within 60 mins before or after training or the first extinction session. King and de Wied (1974) have shown a time dependent LVP effect after a single correct avoidance response. Rats were removed from the apparatus after the first correct pole jump response and returned to the home cage for injecting after the appropriate interval. During subsequent training session rats were give the balance of 30 training trials. LVP (lug) increased responding in extinction 48 hrs later when injected 60 mins but not 6 hrs after the first correct response. In addition these authors have shown that under the influence of LVP classical conditioning trials alone are sufficient to act as behavioural substrate for the peptide's effects when followed by avoidance training and extinction (King and de Wied 1974). Time dependent decreases in behavioural potency , thought to reflect interactions with time dependent consolidation processes , coupled with numerous observations that effects persist long after the time of injection form the basis for interpreting vasopressin's behavioural effects in terms of consolidation.

This explanation alone is insufficient however as a number of studies have shown that vasopressins increase passive avoidance re-entry latencies when injected one hour before the retention test (Ader and de Wied 1972; Rigter et al 1974 ;1975 ; Raemakers et al 1977 Bookin and Pfeifer 1977; Pfeifer and Bookin 1978 see section 2.4.2). Krejci and Kupkova (1978) have reported that DG LVP, DC DAVP an analog without sedative effects and DG-Trigly-LVP an analog with low pressor and antidiuretic activity were effective when injected 20 but not 120 or 180 mins prior to the retention test,confirming that the effects of pre retention injections are also time dependent and do not appear to be mediated either by pressor and antidiuretic activity or by reduced motor activity. However certain behaviourally active AVP analogs reduced mobility in open field tests in low doses (1-3 ug sc) and produced sleep like immobility with higher doses (10-30 ug sc). Pre test injections may therefore exert subtle but confounding influences on responding, thereby increasing the difficulties of interpretation. Bookin and Pfeifer (1977) have argued

that the effects of vasopressin on two apparently distinct mechanisms calls into question the validity of the two stage model , this reflects trends in the human literature , furthermore the extent to which animal procedures may be argued to distinguish these putative stages is doubtful.

2.3. Structure Activity Studies.

Previous experiments had shown that the behaviourally active sequence of ACTH was located in residues 4-10, neither the entire structure nor its endocrine effects were necessary for behavioural activity (de Wied and Gispen, 1977). A similar strategy has been applied to locate the behaviourally active sequence of vasopressin. Removal of glycinamide from position 9 of LVP or AVP produces des-glycinamide LVP (DG LVP) and DG AVP which retain 50% of the behavioural potency associated with the parent molecule but appear to lack endocrine activity. Lande et al (1971) showed that DG LVP (20 ug in zinc phosphate) restored shuttle box avoidance responding in hypophysectomised rats. Subsequently de Wied et al (1972) confirmed the behavioural activity of DG LVP in intact rats. Ten training trials per day for 3 days were given on the pole jump avoidance task, 5 secs of light as the CS preceded shock, rats which made more than 10 correct avoidances were injected with either LVP (12,36 or 108 mu) or DG LVP (0.1, 0.3 or 0.9 ug) immediately after training. Neither saline controls or non shock peptide controls were included and no statistical analysis was reported. Extinction responding was tested at 24, 48, 120 and 268 hrs after injection. The high dose of each peptide maintained responding in excess of 80% in all tests. Intermediate doses showed high response levels in the first test therafter declining to intermediate levels. The low doses showed high response levels in the first test declining to low levels in the second test and no subsequent data was reported. The results suggest a relationship between dose and both magnitude and duration of effects, however this conclusion is equivocal in the absence of the proper control groups and statistical tests. Furthermore, DG LVP showed very low activity when assayed for pressor, antidiuretic , oxytocic and CRF activity using bioassays. It was argued that removal of the glycinamide destroyed normal endocrine activity but retained

behavioural activity (de Wied et al 1972) indicating that vasopressin's behavioural effects are not mediated by its endocrine target organs. Krejci and Kupkova (1978) confirmed the effects of DG LVP on avoidance responding and also reported that another analog with low pressor and antidiuretic activity DG-Trigly-LVP increased passive avoidance retention latencies after post training injections.

The behavioural potency of DG LVP has been confirmed by Wang (1972) in active and passive avoidance tasks. DG LVP (0.125 ug SC) injected after pole jump training increased responding to more than 75% on extinction trials 24 and 72 hrs later compared to 10% for saline controls. However only 4 rats were used per group and no statistical tests were made, as in the de Wied et al (1972) study. Post training DG LVP injections (0.0625 ug ;0.25 ug SC) increased passive avoidance retention. However only 5 rats were used per group and no statistical tests were made. If DG LVP retains behavioural activity in the absence of endocrine activity this may indicate functional disocciation for different parts of the vasopressin molecule (de Wied et al 1972). However Rigter (personal communication) has found that DG LVP (8 ug) administered over a number of days using either minipumps to acheive constant infusion or repeated injections reduces water intake and urine flow in DI rats suggesting that DG LVP retains endocrinological activity.

The physiological significance of DG-analogs is uncertain and largely speculative. Glass et al (1969) have isolated an enzyme from the toad bladder which cleaves glycinamide from AVP and LVP, a similar system may operate in the kidney. Lande et al (1971) isolated an octapeptide from hog pituitaries which they identified as DG LVP. This may have been an artifact of tryptic digestion used in the early stages of isolation although the authors argued that this was unlikely on the basis of high yields of ACTH and LVP from the same source despite their susceptibility to tryptic digestion. Many subsequent studies (see below) support the conclusion that DG LVP is behaviourally active.

The behavioural potency of smaller vasopressin fragments has been studied using the pole jump avoidance (de Wied et al 1975;1976). Up to 5 ug of peptide were injected sc after training in order to establish the amount of each fragment required to elevate responding to 6 or more correct responses during the third and final extinction test. AVP and LVP were most potent and their DG analogs retained approximately 50% activity. The tail sequence (H-CYS PRO LEU

GLY NH2) showed no behavioural activity and Pressinoic acid, the ring structure (see figs 1,2) showed behavioural activity equal to 10% of the parent molecule. De Wied et al (1975) suggested that the C terminal fragment may play a significant role in modulating the behavioural effects of vasopressin by protecting against enzymatic degradation after peripheral injections. This is supported by two types of observation. Direct injection of a small dose of AVP (25 pg) into the lateral ventricles of the brain significantly increased pole jump avoidance responding in extinction (de Wied 1976). Furthermore, pressinoic acid retained 50% activity and PRO ARG GLY, a tail structure analog, showed less than 1% activity of the parent structure. The differences in potency when pressinoic acid was injected centrally and peripherally was attributed to reduced enzymatic breakdown associated with the central route and suggested that in the case of peripheral injections the tail fragment served a protective role (de Wied 1976). Krecji and Kupkova (1978) reported that behavioural activity was enhanced in vasopressin analogs which were resistant to various forms of enzymatic breakdown. Peptides were injected immediately after the learning trial in a step through passive avoidance test (0.35 ma). LVP (lug) increased re-entry latencies relative to saline when tested 2 and 3 but not 7 and 13 days after injection. Analogs which were resistant to amino peptidases and reductive ring cleavage, de-amino-6-carba-arginine-8-vasopressin, de-amino-6-carba-ornithine-8-vasopressin and an analog resistant to aminopeptidases and trypsin, de-amino-(8-D-arginine)-vasopressin all increased re-entry latencies up to 13 days after training.

2.4. Effects of Vasopressin on Experimental Amnesia.

Peripheral and central injections of vasopressin and its analogs causes a long term elevation of response rates during active avoidance extinction, and increased re-entry latencies during passive avoidance tests. The peptides are most effective when injected within an hour of training or the first extinction test. Treatment is ineffective when delayed for 6 hrs. The long term time dependent elevation of response rates led de Wied and Bohus (1966) to suggest that vasopressin affected mechanisms involved in memory formation

independently of its endocrinological roles in antidiuresis and the pressor response (de Wied et al 1972). This hypothesis has been tested in a number of experiments using laboratory techniques to produce retrograde amnesia for learned responses.

2.4.1. Puromycin.

Early theories of the mechanisms underlying memory formation suggested that protein synthesis played a central role. Puromycin is known to block protein synthesis for approximately 6 hrs after injection (John 1967) and to result in subsequent behavioural deficits resembling retrograde amnesia.

Lande et al (1972) reported that vasopressin antagonised puromycin amnesia. Mice were trained to avoid shock in a Y maze by choosing the correct arm on successive trials. Response retention levels were calculated on the basis of trials required to re-achieve criterion a week later. Puromycin di-hydrochloride (90-129 ug) injected intra-cerebrally one day after training resulted in total loss of retention. When DG LVP (0.1 mg) was injected either 1,5 or 20 hrs before training the pooled data for all intervals showed significant savings in both trials and errors whilst re-acheiving criterion compared to puromycin treated controls. Similarly DG LVP (0.1 mg) improved retention when injected 12 hrs after training. Saline controls were omitted from both pre and post training injection experiments. Savings may therefore have been due either to peptide or a factor related to the injection routine. Recent evidence has shown that behavioural responses to drug treatments are modified by pre injection routines (Riffee, Wilcox and Smith 1979). When injection was delayed for 24 hrs after training DG LVP was ineffective. Lande et al (1972) suggested that DG LVP afforded protection of the response against puromycin amnesia. The absence of saline controls, pooling of data across treatment intervals and the extremely high doses of DG LVP ,some 100x larger than the dose reported by de Wied (1971) to exert maximal behavioural effects in intact rats, renders this conclusion equivocal . These methodological problems have been overcome in more recent studies showing that AVP (0.07 uM) and LVP (0.2 uM) protect against puromycin amnesia when injected 1 but not 3 days prior to training; post training injections

were effective with delays of 0 ,6 and 16 but not 24 hrs (Flexner et al 1978) . The period of pre and post training sensitivity in these studies is considerably longer than in intact rats (see section 2.2), no explanation was given for this discrepancy. The effectiveness of immediate post training injections was confirmed by Flexner et al (1978) who also showed that dose response functions were similar for AVP,LVP and DG LVP although AVP was the most potent at low doses.

Structure activity studies using identical training, treatment and test procedures confirmed that both AVP and LVP (0.1 mg) injected sc after training protected against the amnestic effects of puromycin (90 ug) injected 24 hrs after training (Walter et al 1975). Pressinoic acid was ineffective, in contrast de Wied et al (1975) reported that pressinoic acid retained approximately 10% of the activity of the parent structure after peripheral injections in intact rats and 50% after central injections. In addition the C terminal fragment PRO-LEU-GLY-NH2 (PLG) and its analog PRO-LYS-GLY-NH2 protected against puromycin amnesia (Walter et al 1975). In contrast de Wied et al (1975) found that the isolated tail fragment was inactive in intact non drugged rats. The C terminal dipeptide LEU-GLY-NH2 and its cyclic analog (CYCLO), derived from oxytocin, were extremely potent anti amnesics. Subsequent studies have demonstrated a positive significant correlation between the degree of protection afforded by cyclo against puromycin amnesia and the concentration of peptide present in synaptosomal fractions from mouse brain tissue (Rainbow et al 1979).

The biochemical mechanisms underlying puromycin amnesia are not well understood, the role of protein synthesis blockade is not clear. Evidence suggests that the behavioural effects of puromycin may be mediated by the formation of peptidyl puromycin which persists in the synaptosomes long after injection (Gibbs and Mark 1973). In addition amnesia was reversed by saline injections (Gibbs and Mark 1973), highlighting the importance of adequate saline control groups which were omitted in the Lande et al (1972) study. Furthermore there is conflicting evidence as to the onset of the puromycin sensitive stage of consolidation. Flexner et al (1963) reported sensitivity up to 3 weeks after training ,Davis and Agranoff (1966) reported amnesia when puromycin was injected immediately but not 60 mins after training. In addition the period of susceptibility to puromycin was increased by up to 3 hrs by retaining the animals in the training environment for 3 hrs after training (Davis and Agranoff

1966). Although this may suggest that environmental cues trigger the start of consolidation (John 1967) it is unlikely that protein synthesis inhibition is similarly triggered.

2.4.2. Anoxia.

Post training exposure to a carbon dioxide (CO_2) rich atmosphere results in respiratory arrest,anoxia and retrograde amnesia for the previously learned response (Rigter et al 1974;1975). Rats were trained in the step through passive avoidance task using 0.35 ma of shock for 3 secs, then removed from the training apparatus and placed in an enclosed chamber with a CO_2 atmosphere until respiratory arrest occurred, approximately 30-35 secs later. Passive avoidance of the shock compartment in the training apparatus was tested after 24 hrs, shocked animals which had short re-entry latencies after anoxia were considered amnesic for the passive avoidance response (Rigter et al 1974;1975). ACTH 4-10 reversed amnesia when injected 60 mins before the retention test but not 60 mins before training. In contrast DG LVP (10 ug sc) reversed amnesia when given 60 mins before training, retention testing or both (Rigter et al 1974). Re entry latencies for amnestic animals were higher than for non shocked controls indicating that the reversal of amnesia after DG LVP was partial. Rigter et al (1975) confirmed these findings and reported that the effect of DG LVP was time dependent. Amnesia was partially reversed when DG LVP (10 ug) was injected 60 mins but not 2,4 or 6 hrs before training. The same dose partially reversed amnesia when injected up to 6 hrs before the retention test. However Rigter et al (1975) could not reverse CO2 amnesia using post training injections of DG LVP. This contrasts with the results from many studies (see above) which did not use amnestic treatment and found that post training vasopressin injections increased subsequent extinction responding and with the results obtained with puromycin induced amnesia (see section 2.4.1) in which it has been generally found that post training vasopressin injections exert a powerful influence on subsequent responding. The differential time course for pre-acquisition and pre retention DG LVP effects on behaviour were interpreted as indicating that different biological mechanisms underlie the effects of vasopressin injections at these times (Rigter

et al 1975). Informal observations of rats injected after shuttle box avoidance training (expt One Chapter Five) suggest that LVP treated rats are less active than saline controls an hour after injection. Similarly Krecji and Kupkova (1978) have reported reduced mobility in the open field following vasopressin analogs injected SC in the dose range 1-3 ug. Higher doses (10-30 ug) induced "sleep like" immobility in the absence of catalepsy or ataxia. These effects were noted up to 4 hrs after injection. This may confound the interpretation of increased passive avoidance latencies in terms of antagonised amnesia, especially in view of the high dose of DG LVP used and indications that it may retain peripheral endocrinological effects (see sect 2.3). A further difficulty is the use of inappropriate control groups. Respiratory arrest takes up to 35 secs to develop , by itself this constitutes a considerable stress. Control groups were confined in a normal atmosphere under no comparable stress. Therefore disrupted retention may not be unequivocally attributed to amnestic effects of anoxia, motivational and other non specific effects may be involved. Leukel and Quinton (1964) have shown that 60 secs of exposure to a CO2 atmosphere acts as a negative reinforcer.

The biological basis of CO_2 amnesia is poorly understood. Leonard et al (1975) reported that hippocampal and brain stem serotonin levels were elevated after passive avoidance training whereas hippocampal noradrenaline decreased. When training was followed by CO2 exposure serotonin levels remained unchanged and noradrenaline levels were increased. Rigter et al (1975) confirmed the results for serotonin but not for noradrenaline. Furthermore they reported that the effectiveness of the amnestic treatment decreased as a function of the interval between training and anoxia and this was paralleled by increasing serotonin levels in the hippocampus. Ramaekers et al (1977) confirmed the antiamnesic effects of DG-LVP injected prior to step through passive avoidance training and retention and confirmed that increased serotonin levels following footshock was prevented in CO2 amnesia. Furthermore , pretreatment with DG-LVP (5ug/rat) elevated hippocampal serotonin levels and prevented the decrease in serotonin levels associated with CO2 amnesia. Similar but less pronounced effects were reported for DG-LVP injected 23 hrs after training and 1 hr prior to decapitation, at the usual time for retention testing. Although the data show changes in

hippocampal serotonin and possibly noradrenaline following anoxia and of serotonin following DC-LVP the measurement of transmitter levels does not allow a conclusion as to the nature of synaptic changes involved, although increased serotonin was correlated with decreased 5 Hydroxyindolacetic acid levels suggesting effects on serotonin turnover (Leonard et al 1975). Furthermore the data suggest a correlation but not a causal link between serotonin, noradrenaline and response retention.

Post training injections of convulsive doses of pentylenetetrazol (50 mg/kg) have been reported to induce amnesia for the passive avoidance response which could be reversed by LVP (lug) injections one hour prior to either training or retention (Bookin and Pfeifer 1977). These findings have been confirmed by Conti and Bohus (personal communication). Pfeifer and Bookin (1978) have confirmed their results using ECS.

2.5. Physiological Significance of Vasopressin Effects on Avoidance Responding.

Changes in active avoidance response rates and passive avoidance retention after vasopressin injections constitute one amongst many pharmacological responses to the peptides. Taken in isolation these effects do not prove that endogenous vasopressin plays a physiological role in maintaining learned behaviour , although the presence of vasopressin in the perpiphery ,CSF and in extrahypothalamic pathways coupled with the behavioural potency of central injections (section 2.3) , prolonged behavioural effects despite a short metabolic half life (section 1.7) and deficient responding in hypophysectomised rats suggest that this may be so. The studies discussed below have tackled this problem directly by quantifying vasopressin changes associated with behavioural changes and behavioural changes associated with vasopressin deficits.

2.5.1. Variations in Vasopressin Levels during Avoidance Responding.

Thomson and de Wied (1973) claimed to have found evidence that passive avoidance retention was directly related to plasma AVP levels. Twenty four hours after a single shock (0,0.25 , 0.5 , 1 ma for 3 secs) in the step through passive avoidance apparatus (Ader et al 1972) rats were returned to the apparatus for retention testing and immediately after the test were anaesthetised with ether and 1-2 ml of eye plexus blood withdrawn. Antidiuretic activity of eye plexus blood was determined by bioassay. Vasopressin was confirmed as the antidiuretic principle using sodium thioglycollate. Non shocked control rats showed 0.34 mu/ml ADH activity, levels of 0.42 mu/ml, 0.53 mu/ml and 0.63 mu/ml were found in rats given 0.25, 0.5 and 1 ma shock respectively. The differences between successive levels were significant. Re -entry latencies were not statistically analysed, the reported medians tended to increase as a function of shock but the reliability of these figures is doubtful as the values at 0.5 and 1 ma were considerably lower than in many other reports using identical training parameters. Furthermore, exposure to ether is widely used in experiments to stimulate vasopressin secretion (see section 1.9.2) and therefore constitutes a source of uncontrolled artifact.

Van Wimersma Greidanus et al (1979) failed to confirm the findings of Thompson and de Wied (1973), no significant changes were found in AVP levels of trunk blood , as measured by radioimmunoassay , collected prior to pole jump avoidance training and extinction . In passive avoidance tests only rats trained with the highest shock level (1 ma) and showing the longest re-entry latencies (300 secs) had a slight but significant elevation of plasma AVP levels. In addition CSF was withdrawn via a polyethylene cannula inserted prior to experiments in the right lateral ventricle of the brain. Samples taken after passive avoidance retention showed that although basal AVP CSF levels were much higher than plasma levels (>10.4) and re-entry latencies increased as a function of shock intensity in training there were no significant effects of training on AVP levels in the CSF. The results suggest that neither plasma nor CSF AVP levels bear a direct relationship to response retention as Thompson and de Wied (1973) suggested.

The effect of peripherally and centrally injected vasopressin is not therefore to enhance normal fluctuation in peripheral and CSF vasopressin levels during behaviour even though peripheral secretion is well established (section 1.2) and the evidence for direct secretion into the CSF is favourable (section

1.3.3). As endocrine target organ mechanisms had been ruled out on the basis of low endocrine activity in behaviourally active derivatives of vasopressin, particularly DG-LVP and DG-AVP (section 2.3) these findings were interpreted as supporting a role for extrahypothalamic vassopressinergic pathways in mediating the behavioural response to exogenous AVP , assuming that access to the limbic region is via the CSF (van Wimersma Greidanus et al 1979) (see also section 1.3.4). There are three difficulties with the argument; DG LVP shows endocrine activity under certain test conditions ; peripheral mechanisms other than endocrine target organ effects have not been considered and there is no direct evidence to implicate extrahypothalamic vasopressin fibres in behavioural regulation.

2.5.2. Behavioural Deficits in HO-DI rats.

If endogenous vasopressin plays a physiological role in regulating learned responding then rats with a genetical absence of vasopressin (HO-DI) should show deficient avoidance responding. Experiments discussed below have tested this hypothesis. Characteristics of HO-DI rats have been described in section 1.9.2. These studies have established that HO-DI rats lack vasopressin.

Behavioural experiments with these animals have produced conflicting results. Bohus et al (1975) have reported that both HO-DI and HE-DI rats maintain higher levels of ambulation, rearing and grooming than Wistar controls in the open field test and make fewer faecal boli during the first test session . Bailey (1975) confirmed that HO-DI and HE-DI rats were not significantly different from each other but that both had lower ambulation scores and made fewer faecal boli than Long Evans control rats. Bohus et al (1975) reported that HO-DI rats had a significantly higher threshold for eliciting flinches in response to footshock and both HO-DI and HE-DI rats had higher thresholds for eliciting jerking, jumping and running than istar controls. In contrast Celestian et al (1975) reported that HO-DI ,HE-DI and normal Long Evans control rats had identical shock sensitivity. The discrepancies may reflect the use of different control strains in different experiments and highlights the problems

of establishing reliable baselines for these endocrinologically abnormal rats.

2.5.3. Passive Avoidance Responding in HO-DI rats.

A number of experiments show that HO-DI rats have deficient passive avoidance retention. De Wied et al (1975) trained normal wistars, HO-DI and HE-DI rats in the step through passive avoidance task using 0, 0.25, 0.5 and 1 ma. Re-entry latencies were measured immediately, 3, 24, 48 and 120 hrs after training. Median re-entry latencies for HE-DI rats increased as a function of the training shock, 1 ma caused total passive avoidance (300 secs) up to 120 hrs after training. In contrast HO-DI rats passively avoided only immediately and 3 hrs after training, re-entry was rapid during subsequent tests. Even 1 ma was insufficient to increase latencies 120 hrs after training. These findings were confirmed by Bohus et al (1975) . HE-DI rats showed passive avoidance after 3 secs of 1 ma shock at the immediate ,3 hr and 24 hr test. HO-DI rats showed significant avoidance when tested immediately, and after 3 hrs but not after 24 hrs.

In contrast Bailey (1975) reported that HO-DI rats avoided on the first day of testing but not on subsequent days, whereas HE-DI rats maintained avoidance throughout testing. The results of testing 24 hrs after training reported by de Wied et al (1975) and Bohus et al (1975) conflict with those of Bailey (1975), the decay in avoidance retention seen in HO-DI rats may therefore be related to repeated testing. Reduced shock sensitivity in the HO-DI and HE-DI rats compared to wistar controls (Bohus et al 1975) may contribute to retention differences between these groups although this is clearly not the sole factor as HO-DI rats differed substantially from HE-DI rats on avoidance retention but differences in shock sensitivity were relatively small.

The importance of shock levels during passive avoidance training is highlighted by additional data from Bailey (1975). When the shock level for passive avoidance training was reduced to 0.5 ma both HO-DI and HE-DI rats maintained avoidance responding when tested 24 and 48 but not 72 or 96 hrs after training. Contrary to de Wied et al (1975) and Bohus et al (1975) the performance of these groups was

virtually indistinguishable. Furthermore daily pitressin (0.5U) injections during training and testing abolished the avoidance response in HO-DI rats. Unfortunately Bailey (1975) did not establish the active component of pitressin and therefore the significance of this finding for assessing the hypothesis that endogenous vasopressin plays an essential role in maintaining responding is unclear.

2.5.4.Active Avoidance Responding in HO-DI rats.

As in the passive avoidance studies the results of active avoidance tests using HO-DI rats are conflicting. Bohus et al (1975) have reported consistent findings from pole jump and shuttle box avoidance tests. Rats were trained to avoid shock (0.2 ma) preceded by 5 secs of CS (light) during 10 trial on each of 6 consecutive days in the pole jump apparatus. Wistar controls made significantly more responses in training than either HO or HE DI groups, however all rats made more than 75% correct responses after 6 days of training. During extinction HO-DI rats made significantly fewer responses than HE-DI rats or wistar controls . HE-DI rats also made significantly fewer responses than wistars. A similar pattern was found during shuttle box training (5 secs CS; 0.16 ma) and extinction. Miller et al (1976) repeated the Bohus et al (1975) shuttle box experiment and confirmed poor learning in HO-DI rats at 0.16 ma but higher response levels at 0.25 ma. Rats with greater than 18% correct responses in training were tested in extinction HO-DI rats made fewer responses than HE-DI controls, this was attributed to the absence of vasopressin and intermediate responding in HE-DI rats was attributed to abnormally low vasopressin levels (41% normal; van Wimersma Greidanus et al 1977, Dogterom 1978). Reduced shock sensitivity in HE and HO DI rats compared to wistars (Bohus et al 1975) was ruled out as a significant factor on the basis that large differences in response levels between these groups were paralleled by small differences in shock sensitivity.

The results reported by Bohus et al (1975) and Miller et al (1976) could not be confirmed by Bailey (1975) using the shuttle box. The CS (5 secs 80 db tone) was followed by a 0.6 ma shock, training continued for 10 trial per day until rats had made 80% correct responses on 3 consecutive days. Only 2 out of 8 HO-DI rats and 5 out

of 7 HE-DI rats acheived this criterion but between these groups there were no significant differences during training. During 4 days of extinction testing all rats responded at criterion levels. The data suggest that HO-DI rats can learn and maintain avoidance responding despite the absence of vasopressin. However as the number of HO-DI rats acheiving criterion was low this is a dificult result to interpret.

Celestian et al (1975) have reported that HO-DI rats have higher response rates than controls during extinction of shuttle box responding. Those rats which made >80% correct responses on the final day of training were used for the experiment. Acquistion levels were lower for HO-DI rats (30%) than for either HE-DI (78%) or controls (64%) , confirming Bailey's (1975) observations of poor learning. Those rats which achieved criterion were tested in extinction and the HO-DI rats made significantly more responses when tested 72,168,336 and 504 but not 48 hrs after training compared to HE-DI rats and controls. This result is the opposite to that reported by Bohus et al (1975) and Miller et al (1976). Comparing across shuttle box experiments suggests that shock level in training is an important variable in determining extinction response levels in HO-DI rats. Response levels were lower that control when training shock was low (0.16 ma Bohus et al 1975), equal to control at intermediate shock levels (0.6 ma Bailey 1975) and greater than control at high shock levels (1 ma Celestian et al 1975). In addition Bailey (1975) found no differences between HO-DI and HE-DI rats during passive avoidance retention when trained with 0.5 ma.

The evidence for extinction response deficits in HO and HE-DI rats is clearly conflicting, furthermore , where deficits have emerged these are confounded by differences during learning. Bohus et al (1975) reported that during pole jump avoidance training wistars reached criterion by day 3 compared to day 6 for HO and HE-DI rats, during shuttle box training wistars and HE-DI rats reached 80% correct responses by day 3 compared to day 12 for HO-DI rats. Bailey (1975) reported that after 10 days of training only 2 out of 8 HO-DI rats acheived criterion compared to 5 out of 7 HE-DI's. Furthermore , active avoidance responding becomes unstable in HO-DI rats after 3 days (Celestian et al 1975) or after 10 days (Bailey 1975; Miller et al 1976).

Analysis of covariance using terminal acquisition levels as covariates showed that the extinction performance of HO-DI, HE-DI

and normals was not significantly different when learning differences were taken into account , in fact the significantly higher regression slopes for the extinction performance of normals showed that HO-DI rats actually extinguished responding more slowly than normals (Miller et al 1976).

The conflicting results of avoidance extinction tests and the confounding influence of learning deficits suggests that where behavioural differences are found between normals and HO-DI rats these cannot be unequivocally interpreted in terms of memory deficits due to the absence of vasopressin but are more likely to reflect a number of factors including differential shock sensitivity and emotionality, general debilitation, growth hormone deficiency (Arimura et al 1968), potassium deficiency (Bailey 1975) or corticosteroid deficiency (see section 1.9.2).

Recordings from the dorsal hippocampus indicate that HO-DI rats have a lower mean frequency of rhythmic slow wave activity (7.6 +/- 0.1 hz) than HE-DI rats (8.1 +/- 0.04 hz) (Urban and de Wied 1975). The difference was partially corrected with DG-AVP (1,2 ug SC) although the peak frequency for HO-DI's remained significantly lower. The significance of EEG waves in this frequency range is uncertain although several authors have suggested a role in memory formation (Adey et al 1967). Similarly Landfield et al (1972) reported that cortical EEG observed 30 mins after passive avoidance training followed by electroconvulsive shock was significantly correlated with subsequent response retention. Urban and de Wied (1975) suggested that the lower peak and mean frequencies in HO-DI rats may reflect disrupted consolidation.

2.5.5. Avoidance Responding after Anti-Vasopressin serum.

An alternative method for examining the role of endogenous vasopressin in the maintenance of avoidance responding is to destroy the centrally and peripherally available hormone in normal rats by injecting a serum containing antibodies raised against the peptide (van Wimersma Greidanus et al 1975, 1976). Post training intracerebroventricular (icv) injections of antivasopressin serum (1 ul) reduced re-entry latencies following step through passive avoidance training (0.75 ma 3 secs) when tested 4, 24 or 48 hrs after

injection but not when tested after 2 mins or 1 hr. The antivasopressin serum was equally effective when injected 30 mins before training. Subsequent experiments showed that the retention deficit did not emerge until 3 hrs after injection. In contrast rats injected with either control or antioxytocin serum showed maximal retention at all tests (300 secs). Central injections of the antivasopressin serum did not alter urine flow or osmolality whereas peripheral injections of much higher doses (100 ul) increased flow and decreased the vasopressin content of the urine but did not alter passive avoidance retention (van Wimersma Greidanus et al 1975). The authors argued that centrally available vasopressin played an essential role in memory consolidation independent of pressor and antidiuretic activity , supporting conclusions from earlier studies with hypophysectomised rats (section 2.1) and vasopressin derivatives (section 2.3).

In order to examine time dependency of the anti-vasopressin serum effect on passive avoidance retention the serum (0.1 ul) was injected at various intervals before and after training and retention was tested 24 and 48 hrs later (van Wimersma Greidanus et al 1976). When injected 30 , 60 or 120 mins prior to training re-entry latencies 24 and 48 hrs later were significantly lower than normal serum controls. Injections 3 hrs before training decreased re-entry latencies only at the 48 hr test and when injected 6 hrs prior to training the serum was ineffective. Similar effects were reported when lul of serum was injected at these intervals after training but injections were ineffective with intervals equal to or exceeding 6 hrs. However control injections of normal serum were only given 30 mins before training and not at each injection interval. This increases the difficulties of interpretation by failing to control for the behavioural effects of injection per se at different intervals before and after training. The data presented in experiment (see section 5.1 fig 19) show that avoidance response levels in extinction vary as a function of the post training injection interval even with saline injections. Similarly Riffee et al (1979) have reported that pre injection routines per se alter behavioural responses to drug treatments.

Dose response studies have shown that lower antivasopressin serum doses (0.1, 0.033 ul) significantly reduced re-entry latencies 24 and 48 hrs later when injected immediately after training (van Wimersma et al 1976). The lowest dose tested (0.01 ul) reduced

re-entry latencies only at the 48 hr test. The effects on passive avoidance retention cannot be attributed to increased motor activity as treatment with anti-vasopressin serum during pole jump avoidance training also facilitated extinction (van Wimersma Greidanus et al 1975). The absence of any effects on learning performance confirmed previous observations that vasopressin does not affect learning per se.

When injected 60 mins prior to the first retention test antivasopressin serum (1ul) significantly reduced re-entry latencies during the subsequent test but not during the second test 24 hrs later, lower doses (0.1, 0.033 ul) significantly reduced re-entry latencies in both tests. It was concluded that information retreival as well as consolidation was disrupted by destroying centrally available vasopressin, as evidenced by lower re-entry latencies at the 24 hr test for all doses. Re-entry latencies were also lower than control levels 48 hrs after injection in the case of the two lower doses but not the high dose. It was argued that the high dose prevented consolidation of information gained on the first re-entry test when re-entry was rapid therefore these rats were behaving in the second test as if their behaviour was normal on the first. This is an elegant explanation but not entirely consistent with previous results which showed that low doses of serum also disrupted consolidation.

Disrupted passive avoidance retention following immediate post training destruction of centrally available vasopressin agrees well with the findings of studies which used vasopressin injections. However the use of antiserum arguably constitutes a pharmacological manipulation , ie vasopressin content is reduced below the normal physiological level . Subsequent behavioural changes may not reflect the normal physiological functions of the peptide. In addition , the specificity of antisera to their target compounds is difficult to establish. Cross reaction with unknown but structurally similar compounds cannot be ruled out. Lack of pharmacological agonists and antagonists for vasopressin leaves the possibility of receptor mediation of behavioural effects unresolved and prevents the use of standard psychopharmacological techniques for identifying normal physiological functions.

2.6. Mid-Brain and Limbic Sites of Action.

A number of studies have attempted to determine the brain areas involved in mediating vasopressin's behavioural activity. That these effects are centrally mediated has been assumed on the basis that peripheral target organ effects appeared not to be involved (section 2.3) that central injections were more potent than peripheral (section 2.3) ; that antivasopressin serum disrupted responding when injected centrally but not peripherally (section 2.5.5) and that vasopressin could be demonstrated in high quantities in the CSF (section 1.3.3) and in extrahypothalamic fibres (section 1.3.4). The results from lesion studies described below suggest an important role for mid - brain structures.

Micro injections of LVP (0.1 ug) increased subsequent resistance to extinction of pole jump avoidance responding when injected into the posterior thalamus (van Wimersma Greidanus et al 1973) but not the venteromedial and anteromedial thalamus, posterior hypothalamus, substantia nigra, substantia grisea, reticular formation, putamen and dorsal hippocampus. Subsequent studies showed the parafascicular nucleus of the thalamus to be sensitive to vasopressin microinjections (van Wimersma Greidanus et al 1974 b). In addition, electrolytic lesions in this region reduce the behavioural activity of the peptide (van Wimersma Greidanus et al 1974 b). After post operative recovery rats were trained to avoid shock (unspecified) in the pole jump apparatus on 4 consecutive days then tested in extinction on days 5 and 8 . Lesioned rats showed poorer learning and extinction performance than sham operated controls. On day 9 rats were retrained for 10 trials then on the 10th day 10 extinction trials were followed by injections of saline or LVP (1.8, 5.4 ug sc). Both doses increased extinction responding in sham operated controls during 3 subsequent extinction tests. Extinction responding in lesioned rats was elevated over 3 days by the highest LVP dose and over the first 2 days by the lower dose . However, despite these increases the response levels of lesioned rats did not match those of controls. It was concluded that although destruction of the parafascicular nucleus reduced sensitivity to vasopressin the area was not essential for the behavioural effects of the peptide (van Wimersma Greidanus et al 1974 a,c).

Lesions of the rostral septum completely disrupted the effects of LVP on pole jump avoidance extinction (van Wimersma

Greidanus et al 1974 a). Lesioned rats showed deficient learning but during extinction saline treated shams and lesioned rats responded similarly. LVP (3 ug) retarded extinction in shams but neither 3 or 9 ug's affected the performance of lesioned rats. In addition small lesions to the anterodorsal hippocampus prevented the effects of low (1,3 ug) but not a high dose (9ug) of LVP.

Lesions to the amygdaloid complex also prevented the extinction effects of vasopressin injections. Post operative pole jump avoidance training revealed no significant differences between sham operated controls and rats with extensive bilateral electrolytic lesions in the amygdala during learning or extinction. LVP (3 ug SC) enhanced subsequent extinction response levels when injected immediately after the first extinction session in sham operated rats. However, neither 3 nor 5 ug SC exerted any influence in lesioned rats, (van Wimersma Greidanus et al 1979a).

Pre and post commisural fornix lesions , which effectively disrupt septo-hippocampal pathways , did not prevent the effects of LVP on pole jump extinction responding (van Wimersma Greidanus et al 1979 b) thereby contradicting earlier conclusions that an intact Limbic system is required for the behavioural effects of the peptide (van Wimersma Greidanus et al 1974). In summary , the results from lesion studies indicate that although destruction of the parafascicular nucleus and dorsal hippocampus reduce sensitivity to the behavioural effects of vasopressin their anatomical integrity is not essential. In contrast destruction of both the rostral septum and amygdaloid complex block the behavioural effects of LVP. The functional relationship between these structures with respect to the behavioural activity of vasopressin is unclear , lesion studies alone cannot completely characterise the regional basis for vasopressin's behavioural effects. Although the effects of lesions may reflect damage to vasopressinergic fibres found in the limbic system (see section 1.3.4) there is no direct evidence to support this conclusion.

Subsequent experiments using post training microinjections of AVP into brain nuclei have indicated a role for the dorsal / medial septal nuclei, hippocampal dentate gyri and dorsal raphe nucleus in mediating the effects of AVP on step through passive avoidance responding (Kovacs et al 1979a). Bilateral injections of AVP (25-25 pg) , a dose insufficient to affect behaviour when injected into the ventricles (Bohus et al 1978) , directly into the

dorsal septal nucleus immediately after passive avoidance training significantly increased re-entry latencies 24 and 48 hrs later compared to saline (Kovacs et al 1979 a). Oxytocin , which is structurally related to vasopressin (see section 1.2) injected bilaterally into the dorsal septal nuclei (25-25 pg) also increased re-entry latencies 24 and 48 hrs later. Although experiments using ICV (Bohus et al 1978) and peripheral (Shultz et al 1974) injections have reported opposite effects for oxytocin and AVP on passive avoidance retention and hippocampal theta rhythm (Bohus et al 1978). In contrast , microinjections of AVP into the hippocampal dentate gyri increased , whilst oxytocin decreased , subsequent re-entry latencies. AVP injections into the adjacent subiculum were ineffective (Kovacs et al 1979a). Both AVP and oxytocin increased re-entry latencies 24 but not 48 hrs after a midline injection of 50 pg into the dorsal raphe nucleus. Neither peptide affected behaviour when injected bilaterally (25-25 pg) into the amygdaloid complex although previously van Wimersma et al (1979a) reported that amygdaloid lesions blocked the effects of systemic AVP (see above) on pole jump avoidance responding. This discrepancy may reflect methodological differences such as the injection route and behavioural task , alternatively the disruptive effects of amygdaloid lesions may have been due to damage inflicted on nearby structures or fibres of passage

2.7. Catecholaminergic Involvement.

Catecholaminergic (CA) pathways in the CNS may play an important role in mediating the behavioural effects of vasopressin. LVP (300 mu/kg) injected 10 mins prior to training did not affect the step up response to shock , but significantly increased subsequent step down latencies in a passive avoidance task (Kovacs et al 1977) Pretreatment with the CA synthesis blocker alpha-methyl-para-tyrosine (AMPT) (30 mg/kg) , which did not itself alter responding , blocked the effects of LVP. Analysis of the CA content of brain regions showed that 10 mins after LVP injections dopamine (DA) levels in the hypothalamus , septum and striatum were elevated compared to saline injected controls whereas noradrenaline (NA) levels remained unchanged. In order to determine effects on turnover AMPT (250 mg/kg)

was injected with LVP (300 mu) and 4 hrs later rats were decapitated. This higher dose of AMPT reduced CA levels by 60-70% but did not affect passive avoidance responding (Kovacs et al 1977). LVP increased the rate of NA disappearance in the septum but not the hypothalamus or striatum and of DA in the septum and striatum but not the hypothalamus (Kovacs et al 1977).

A more exact regional characterisation was attempted by Tanaka et al (1977a). AMPT (300 mg/kg ip) injections were followed after 30 mins by AVP (10,30,100 ng icv) injections and 3 hrs later rats were decapitated and brains dissected for subsequent analysis of NA and DA content. 10 ng AVP did not alter CA disappearance , 30 ng increased the disappearance of NA in the medulla oblongata and of DA in the preoptic area. 100 ng of AVP increased NA disappearance only in the thalamus and hypothalamus. NA levels in the septal region , preoptic area , amygdala and hippocampus and DA levels in the septal region , basal ganglia and amygdala were unaffected. The absence of significant effects of 10 ng of AVP on CA metabolism , despite the behavioural activity of much lower doses injected by the same route (1 ng pg; Bohus et al 1978) may indicate either that CA metabolism changes are not involved or that the assay technique is insensitive and the analysis of large areas of tissue is inappropriate. In view of CA metabolism changes seen in HO-DI rats and in normal rats treated with anti-vasopressin serum the latter interpretation has been widely accepted (see below).

Lack of effects on CA metabolism in areas which microinjection studies suggested to be sensitive to AVP , particularly the hippocampus , prompted measurements in discrete nuclei on the basis that changes within major anatomical areas are likely to be relatively restricted. Tanaka et al (1977b) measured changes in CA levels in 35 selected nuclei following injections of AVP (30 ng icv). AMPT was injected IP 30 mins prior to peptide injections. Rats were decapitated 3.5 hrs later and CA levels measured in nuclei dissected by tissue punching. NA levels were depleted in the dorsal septal nucleus, medial forebrain bundle, anterior hypothalamic nucleus parafascicular nucleus and dorsal raphe nucleus after AVP injections. Decreased steady state levels after synthesis inhibition was interpreted as indicating accelerated CA disappearance due to elevated neural activity. AVP increased NA levels in the supraoptic nucleus and nucleus ruber , suggesting decreased neural activity. DA levels were decreased in the caudate

nucleus, median eminence, dorsal raphe and region A8 of the mesencephalon following AVP. Furthermore there were no significant changes in NA levels in the nuclei of the amygdaloid complex, subiculum, dentate gyrus or CA2 region of the hippocampus following AVP injections. DA was undetectable in the cortical or medial amygdaloid nuclei or in the hippocampus.

Accelerated NA disappearance in the dorsal septal nucleus and of NA and DA in the dorsal raphe nucleus is in accord with the suggestion that the behavioural effects of microinjections of vasopressin into these areas may involve CA nerve terminals. Furthermore , NA depletion in the parafascicular nucleus supports the conclusion from lesion studies that this area is sensitive to the effects of AVP on pole jump avoidance responding (see section 2.6.1). The absence of CA changes in the amygdaloid complex is in accord with the insensitivity of the area to AVP microinjections (Kovacs et al 1979) but conflicts with the report that bilateral amygdaloid lesions block the effects of AVP on pole jump extinction (van Wimersma Greidanus et al 1979a). If the complex is essential for the effects of LVP on avoidance then this may not involve CA neurons. If the behavioural effects of AVP are mediated by noradrenergic nerve terminals in the dentate gyrus then the absence of CA metabolism changes in the area after larger AVP injections conflicts with the report that this area is behaviourally sensitive to AVP microinjections. Changes in NA metabolism in the supraoptic nucleus following AVP injections may reflect altered neural activity as a result of inhibited AVP secretion from the posterior pituitary although previous studies using AVP and its antiserum injected ICV did not report alterations in peripheral AVP levels (van Wimersma Greidanus et al 1975;1976). In addition the existence of an inhibitory feedback loop for regulating vasopressin secretion is not well supported (see section 1.5).The functional significance , if any, of decreased NA in the nucleus ruber and of altered DA metabolism in the median eminence and region A8 is not clear.

Behavioural deficits in HO-DI rats , which lack the capacity to synthesise vasopressin (see section 1.9.2) have been used in support of the argument that effects on avoidance extinction reflect a physiological role for the endogenous peptide. The evidence for this has been discussed (section 2.5.2,3,4) and found to be equivocal , however acceptance of this conclusion coupled with the evidence that AVP altered CA metabolism in discrete brain regions

(Kovacs et al 1977; Tanaka et al 1977 a,b) prompted an examination of CA levels in HO-DI rats compared to non DI littermates (Versteeg et al 1978). Rats were decapitated 3.5 hrs after an injection of AMPT and nuclei dissected out using a tissue punch technique. Steady state levels of NA were higher in the dorsal septal nucleus and supraoptic nucleus of HO-DI rats , turnover was markedly increased in these regions. HO-DI rats showed lower NA rate constants in the arcuate and parafascicular nuclei , the rostral nucleus tractus solitari and slightly increased turnover in the periventriclar nucleus , medial forebrain and anterior hypothalamic bundles. DA levels were unaffected but in HO-DI rats the rate constants were reduced in the caudate nucleus , median eminence , the A2 region and CA2 of the hippocampus. Adrenaline levels and rate constants were lower in the paraventricular nucleus.

Similarly , antivasopresin serum injected ICV 30 mins after AMPT injections has been reported to decrease NA disappearance in the dorsal septal and parafascicular nuclei and in the nucleus of the solitary tract 3 hrs later. DA disappearance was decreased in the caudate nucleus and region A2 of the medulla oblongata (Versteeg et al 1979).

To evaluate the hypothesis that the opposite effects of exogenous vasopressin and endogenous deficiencies of the peptide on behaviour are mediated by opposite effects on CA activity requires comparison across the experiments of Tanaka et al (1977) and Versteeg et al (1978 ,1979). The significance of such comparisons for understanding the biochemical basis for the behavioural actions of vasopressin is limited by the fact that at 30 ng the dose used by Tanaka et al (1977) was considerably larger than that normally used to elicit behavioural effects when injected ICV and the behavioural effects of this high dose have not been reported. Opposite effects on NA metabolism were reported for the dorsal septal and parafascicular nuclei when the effects of AVP injections (Tanaka et al 1977) are compared with endogenous AVP deficits in HO-DI rats (Versteeg et al 1978) and artificial deficits after antivasopressin serum injections (Versteeg et al 1979). Opposite effects on NA metabolism were also reported in the supraoptic nucleus , and medial forebrain and anterior hypothalamic bundles when levels after AVP injection are compared to the effects of endogenous deficits in HO-DI rats. CA metabolism in these areas remained unaffected by anti-vasopressin serum. This may be due to reduced accessability to these areas for

the serum , alternatively , the effects of AVP injections may reflect pharmacological effects at these nuclei rather than normal physiological involvement. Opposite changes in DA metabolism were reported for the caudate nucleus following AVP injections compared to both HO-DI and anti serum treated rats. DA was also opositely affected in the median eminence when AVP injected rats are compared to HO-DI rats. Anti serum did not affect DA metabolism in this region. AVP also altered CA metabolism in the nucleus ruber , dorsal raphe nucleus and regions A1 , A6 and A8 of the mesencephalon whereas no changes were observed in these regions in HO-DI rats or following anti serum. The discrepancy in region A6 (locus coeruleus) raises the question of the extent to which NA changes in the cell bodies of fibres forming the ascending noradrenergic system participate in mediating the behavioural effects of exogenous AVP. Kovacs et al (1979 , 1980) have argued that these cell bodies do not participate on the basis of microinjection studies (see below) , though clearly the neurochemical data described indicate possible involvement.

Areas which appear sensitive to the behavioural effects of vasopressin receive noradrenergic input from fibres of the dorsal noradrenergic bundle originating in the locus coeruleus. Destruction of this system using bilateral injections of the specific neurotoxin 6-Hydroxydopamine (6-OHDA) injected into the dorsal noradrenergic bundle blocked the effects of AVP (5 ug sc) injected immediately after passive avoidance training on re-entry latencies 24 and 48 hrs later. NA depletion was confirmed in the dentate gyrus and A6 regions, DA levels in these structures were unaffected. During the first test lesioned rats injected with saline showed no retention effects although 24 hrs later they had significantly lower re-entry latencies than sham saline controls , indicating only a very minor effect of the lesion itself on passive avoidance responding. The results indicated that an intact dorsal noradrenergic bundle was required as a substrate for the effects of vasopressin. However microinjections of the peptide (25 pg bilaterally) into the locus coeruleus itself did not affect subsequent re-entry latencies in otherwise intact rats. As the noradrenegic cell bodies appeared to be insensitive it was concluded that the effect was mediated at the fibre terminals (Kovacs et al 1979). Post training microinjections of AVP into the dorsal raphe nucleus, which receives inputs fom the locus coeruleus, also facilitated subsequent retention except in rats with lesions to the nucleus induced by either 6-OHDA or 5,6

Dihyroxytryptamine (5,6,DHT) which elevated 5HT uptake in the mesencephalon and dorsal hippocampus. However, 5,6-DHT lesions did not block the effects of systemic AVP (5 ug sc). This may suggest that an intact dorsal raphe serotonergic system is not required for mediating systemic effects but as comparable tests on rats with 6-OHDA lesions to this structure were not carried out the role of noradrenergic fibres in the dorsal raphe nucleus is uncertain.

Schulz et al (1979) have argued that the effects of AVP on striatal DA are mediated presynaptically. Unilateral destruction of the substantia nigra using 6-OHDA results in ipsilateral fibre degeneration , reduced striatal DA levels and supersensitivity at post synaptic striatal DA receptors. Activation of presynaptic receptors induces ipsilateral rotation in rats due to the predominant influence of presynaptic terminals on the intact side. Conversely , postsynaptic activation results in contralateral rotation due to supersensitivity of postsynaptic receptors on the side of the lesion. LVP (50 ng ICV) induced significant increases in ipsilateral rotation indicating a presynaptic effect. Similar results were reported for oxytocin and PLG. Direct microinjections of LVP into the substantia nigra of intact rats did not induce assymetrical rotation and Schulz et al (1979) concluded that as the effects of LVP appeared not to be mediated either at the level of the cell body or postsynaptically the influence of the peptide was probably at DA terminals in the striatum.

To summarise , the data show that in the absence of CA synthesis AVP alters CA metabolism in discrete brain nuclei when injected into the brain ventricles in doses which are in excess of those required to demonstrate behavioural effects in intact rats. No changes in CA metabolism were reported following 10 ng , therefore the effects of AVP are either not mediated by CA neurons in which case CA metabolism changes are pharmacological artifacts , or alternatively the behavioural effects of AVP involve CA neurons but the procedures are insensitive to metabolism changes after low AVP doses. Evidence from studies of HO-DI rats and following the destruction of endogenous AVP by antiserum support the second conclusion. However in the absence of pharmacological agonists and antagonists questions as to the mechanism underlying the interaction between CA neurons and vasopressin remained unanswered. Some of the evidence supports the argument that the peptide acts presynaptically on NA and DA neurons although the existence of presynaptic receptors remains speculative.

With regard to the brain nuclei involved the evidence from lesion , microinjection and CA metabolism studies is contradictory ,with the exception of NA in the dorsal septal nucleus and DA in the caudate nucleus the effects of manipulating AVP levels are not consistent across studies , however these differences may reflect methodological factors. Delanoy et al (1973) reported that following ICV injections of AVP, LVP and AVT (0.1 1 ug) mice showed hyperactivity and excessive foraging and grooming. This response was not altered by a wide range of drugs known to affect CA and cholinergic transmission. There are no reports of similar reactions in rats, the underlying mechanisms are not well understood but may indicate changes in membrane characteristics rather than at receptors , evidence from invertebrate cells indicating that vasopressin alters membrane responses to stimulation in vivo have been discussed previously (section 1.10).

2.8 Vasopressin's effects on Morphine and Alcohol Tolerance.

Vasopressin and related analogues have been reported to alter the development of morphine tolerance and self administration . Studies by Krivoy et al (1974) showed that vasopressin enhances the development of morphine analgesia in mice. Chronic administration three times daily of increasing doses of morphine sulphate (5-20 mg /kg bw) increased response latencies on a hot plate compared to saline controls. Tolerance developed over 5 days on this regime ,as indicated by decreasing latencies. When morphine injections were followed by DG-LVP (50 ug) reaction times were further reduced , indicating facilitation of tolerance development. The same dose of DG-LVP was ineffective in saline controls . In addition Cools et al (1977) reported that DG-LVP accelerated the development of tolerance when injected directly into the nucleus linearis intermedius raphe in freely moving cats. Conversely the development of tolerance in HO-DI rats occurs more slowly than in their HE-DI littermates (De Wied and Gispen 1976). Furthermore as Terenius et al (1975) reported that DG-LVP showed no affinity for dihydromorphine binding sites normal binding of morphine to its receptors appears not to be disrupted by the peptide.

Similarities between the effects of drugs on learning

tasks and the development of morphine tolerance led to the suggestion that similar mechanisms may underlie these processes at a cellular level. The action of puromycin , which blocks protein synthesis (see section 2.4.1), on avoidance retention is blocked by DG-LVP (Lande et al 1971), Furthermore puromycin , actinomycin and cycloheximide all block the development of tolerance to morphine (Cohen et al 1965; Cox et al 1970) suggesting a common mechanism involving protein synthesis in learning and tolerance development. Seigel (1975) argued that tolerance to small morphine doses is a learned response involving compensatory physiological responses initially elicited by morphine , which come to be elicited by environmental stimuli associated with morphine administration. However the failure to observe extinction of tolerance to large morphine doses (Sklar and Amit 1978) and failure to replicate Seigel's original findings (Shearman et al 1979) suggests that learning may not be involved . In addition the importance of puromycin's effect on protein synthesis per se for understanding its effects on behaviour are equivocal (see section 2.4.1).

That narcotic analgesics have distinct stimulus properties has been shown by Colpaert (1979). Van Ree and de Wied (1977) reported that pretreatment with DG-AVP suppressed heroin self administration compared to saline injections , an effect which the authors argued was due to reduced reinforcing stimulus properties of the narcotic after the peptide mediated by interfering with dopaminergic transmission. In contrast Mello and Mendelson (1979) failed to find an effect of DG-AVP (25 ;125 ug/kg) on morphine self administration in dependent rhesus monkeys or on food self administration. Although the discrepancies may reflect methodological differences between the studies , particularly species and schedules of reinforcement, the findings of Mello et al (1979) argue against a role for vasopressin or related analogues in modulating the stimulus properties or narcotic analgesics. More recent theories of opiate dependence and withdrawal stress the importance of events at the cellular level such as reduction of opiate receptor populations following prolonged morphine exposure (Shulz et al 1980) and hypertrophy of second messenger systems (Collier et al 1980).

Tolerance also occurs to the effects of alcohol following prolonged consumption and withdrawal symptoms are seen when consumption is prevented. The importance of learning as a factor in the alcohol dependency syndrome (Crabbe and Rigter 1980) coupled with the possibility that tolerance development and learning may involve

analagous processes at the cellular level have prompted experiments on the effects of vasopressin derivatives on the development of alcohol tolerance and withdrawal (Crabbe and Rigter 1980). A number of factors hamper the development of satisfactory animal models for the alcohol dependency syndrome . Strong aversion to alcohol and higher alcohol metabolism rates in rodents force experimenters to use special strains of rats , intragastric feeding or inhalation of alcohol fumes , high doses and prolonged intoxicatication in order to demonstrate tolerance and withdrawal phenomena. Hoffman et al (1979) have reported that repeated AVP injections (10 ng) slowed down the rate at which ethanol tolerance disappeared in mice measured by changes in body temperature and sleep time. They postulated a central mechanism on the basis that ethanol metabolism per se was unaffected by AVP. Crabbe and Rigter (1980) have confirmed these findings using constant infusions of DG-AVP via minipumps , peptide treatment also exacerbated withdrawal symptoms e.g convulsions. In the absence of discrete stimulus response events these results do not easily lend themselves to interpretation in terms of learning. More convincing data has been reported by Mucha and Kalant (1979) who found that DG-LVP injections enhanced the increase of alcohol intake seen with a forced consumption design although it was ineffective when alcohol intake was stable at the maximum level accepted by each rat. Although this resembles facilitated response acquisition the relevance of the comparison is uncertain as LVP was completely ineffective even with high doses (42 ug).

2.9. Summary and Conclusions.

Early studies showed (section 2.1) that removal of the posterior and anterior lobes of the pituitary gland induced a deficit in active avoidance responding which could be corrected by replacement with posterior lobe extract , ACTH ,MSH and LVP independently of pressor and antidiuretic functions. Subsequently it was shown that synthetic vasopressins increased active and passive avoidance in extinction when injected peripherally in intact rats . Although some of the early studies were methodologically marred by the use of small groups , restrictive behavioural criteria , omission of statistical tests (see section 2.1) and failure to establish

conditioned passive avoidance in control groups (Ader et al 1972 ; Bohus et al 1972 ; Wang 1972) the evidence firmly supports the conclusion that vasopressin injections increase responding in avoidance extinction. As similar effects have been reported in experiments using avoidance of trained fighter mice (Leshner and Roche 1977; Roche and Leshner 1979) and sexually rewarded behaviour (Bohus 1977) the effects of vasopressin injections are not restricted to shock motivated responding .

Evidence that the behavioural potency of peripherally injected vasopressins decreased, as the interval between training , first extinction session or first retention test and vasopressin injection increased, suggested time dependent changes in the substrate with which the peptides interact. Of particular importance are those results indicating that potency diminishes as a function of the interval when the peptides were injected after training, thereby eliminating the possibility of disrupting normal learning. Time dependent reductions in potency fit well with the hypothesis that time dependent physiological changes underlying memory consolidation are affected by vasopressins. Increased responding in subsequent extinction tests ,according to this hypothesis , reflect facilitated consolidation. The effects of vasopressins appear to be highly specific to the extinction phase of behaviour , with the exception of de Wied (1973) who reported facilitated learning with ornithine vasopressin and a transient facilitation with lysine vasopressin there are no reports of effects on learning per se although a number of studies have injected the peptides during and prior to training.

The consolidation hypothesis alone is insufficient to account for all the data. A number of studies have reported increased passive avoidance responding when vasopressins were injected 1 hour prior to extinction tests (23 hrs after training). Retrograde activity spanning such a long period has been considered unlikely and several authors have postulated an additional effect on response retreival, however interpretation of results from pre test injections may be confounded by subtle influences on motor or sensory capacities.

Pretreatment with vasopressin analogs partially reverses the response deficits caused by anoxia, ECS, pentylenetetrazol and puromycin, deficits normally interpreted in terms of retrograde amnesia. However in the absence of a coherent theory of the physiological mechanisms involved in these effects it is difficult to draw any conclusions as to the mechanisms involved in mediating the

effects of vasopressin in these experiments.

Direct measurement of catecholaminergic metabolism changes has revealed altered metabolism in a number of discrete brain nuclei following vasopressin injections into the lateral ventricles of the brain, supporting a role for CA neurons in mediating vasopressin's behavioural effects. However these changes were seen after a dose which was some 10x higher than that normally required for behavioural effects via this route . The discrepancy probably reflects methodological difficulties associated with measuring small quantities in restricted tissue samples although the extent to which CA metabolism changes may represent pharmacological artifacts is difficult to evaluate.

Considerable experimental effort has been directed towards establishing whether or not vasopressin's behavioural effects and its effects on CA metabolism reflect a normal physiological role for the endogenous peptide. Evidence from HO-DI rats which lack the capacity to synthesise vasopressin is conflicting, discrepancies may reflect methodological differences or the confounding effects of the severely abnormal endocrinology of these rats. Studies using specific antivasopressin serum show that the destruction of centrally but not peripherally available vasopressin reduces subsequent passive avoidance retention , this contrasts with the effects of vasopressin injections. Attempts to demonstrate time dependency in this effect are methodologically marred and when injected prior to passive avoidance extinction the dose response relationships were inconsistent with previous findings. Furthermore antisera may destroy unidentified compounds with structural similarities to the vasopressin molecule. Studies from intact , HO-DI rats and antiserum treated rats agree on the sensitivity of NA metabolism in the Dorsal septal nucleus , parafascicular nucleus and of DA metabolism in the caudate nucleus to altered vasopressin levels. Vasopressins also alter the development of morphine tolerance and alcohol consumption and withdrawal symptoms, the relationship between these effects and altered conditioned responding is unclear but may involve CA neurons also . Attempts to draw parallels between learning and tolerance effects have met with only partial success.

The mechanism underlying putative interactions between CA neurons and vasopressins remain to be determined. Receptor populations for the peptide in the CNS have not been identified. Electrophysiological data from invertebrate preparations (see section

1.10) indicate effects of the peptides quite distinct from putative transmitter effects. These studies may provide clues to the action of vasopressins in the mammalian CNS , the activity of neuropetides is now discussed by many authors in terms of modulating transmitter functions (Dismukes 1980).

CHAPTER THREE

THE EFFECTS OF RESPONSE PREVENTION ON AVOIDANCE EXTINCTION.

3.0 INTRODUCTION.

In contrast to the effects of post training vasopressin injections response prevention trials , i.e. thwarting the avoidance response in the presence of the CS , decreases subsequent extinction responding (see below). Behaviourally this has been interpreted in terms of the additional 'information' conveyed during confinement leading either to facilitated fear extinction ,learning of an alternative response or disruption of the expectation that failure to respond is followed by shock (see below). If the increased avoidance extinction response levels seen after vasopressin injections reflect enhanced information storage then vasopressin injections in conjunction with prevention trials should result in further reductions in extinction response levels. However , King and de Wied (1974) found that when LVP (1 ug SC) preceded prevention trials by one hour the effect was to increase extinction response levels 48 hrs later. The authors argued that vasopressin did not invariably enhance consolidation.

This observation , coupled with the effects of pre-retention vasopressin injections (section 2.2) are the only indications in the literature that the consolidation hypothesis alone is insufficient to explain the effects of vasopressins on avoidance extinction. As this result may alter our understanding of the behavioural actions of vasopressin, a number of experiments were performed to replicate and extend the finding that vasopressin enhanced avoidance extinction when given in conjunction with prevention trials (King and de Wied 1974). The purpose of the present chapter is to briefly review the response prevention literature and to report an experiment which replicates the effect with shuttle box avoidance behaviour.

3.1 Response Prevention.

Methods for hastening avoidance extinction have attracted attention since Miller (1948) suggested that anxiety reduction motivated phobic and neurotic behaviour , avoidance responding has since been used as an animal model for exploring the elimination of persistent responding and response prevention or 'flooding' developed as a technique for facilitating extinction. In the literature the terms response prevention and flooding are used interchangeably and may refer to one of three closely related procedures ;

a) continuous presentation of the conditioned stimulus (CS) (Shearman 1970 ;Bankart and Eliot 1971) also referred to as flooding type 1 (Baum 1973).

b) discrete or continuous CS presentations with the response thwarted by a barrier (Solomon , Kamin and Wynne 1953 ; Page and Hall 1953 ; Carlson and Black 1959 ; Polin 1959 ;) also referred to as flooding type 2 (Baum 1973).

c) continuous CS exposure with part of the apparatus removed to prevent responding (Baum 1973 ; Bankart and Eliot 1973) referred to as flooding type 3 (Baum 1973).

Tests of the relative efficiency of each procedure have produced conflicting results. Whilst some authors found CS exposure and CS exposure with responding prevented to be equally effective (Shearman 1970 ; Baum 1973) and superior to CS presentations with responding prevented by removing part of the apparatus (Oler and Baum 1968 ; Baum 1973 ; Lawson 1976) others have found CS exposure alone more effective than CS exposure with responding prevented (Polin 1959) and vice versa (Berman and Katzev 1972 ; Bankart and Eliot 1974). Conflicting results probably reflect the wide range of methodological differences between studies.

Reduced responding in extinction following response prevention trials has been demonstrated using a number of different behavioural baselines including passive avoidance (Page 1953 ; Page and Hall 1955) , one way active avoidance (Baum 1966 ; 1973; Bankart and Eliot 1974 ; Marrazo and Riccio 1974) , shuttle box avoidance (Solomon , Kamin, Black 1953 ; Carlson and Black 1959 ; Polin 1959 ; Benline and Simmel 1967 ; Weinberger 1965 ; Shearman 1970 ; Berman and Katzev 1972), and escape responding (Franchina et al 1975 ; Franchina , Hauser and Agee 1975 ; Franchina and Myers 1976)

3.1.1. Additional Variables.

Variables which have been shown to affect the outcome of response prevention trials include shock level in training , length of confinement , social facilitation , movement facilitation and positively reinforcing intracranial stimulation.

In the ledge jump apparatus Baum (1969a) showed that the effect of a fixed period (5 mins) of confinement on the grid floor of the shock chamber decreased as a function of increased shock levels in training. Tortura and Denny (1973) using mixed passive and active avoidance reported similar findings. Furthermore , a single shock during extended overtraining trials reduced the effectiveness of 5 mins of confinement (Baum 1968).

Extended confinement in the presence of the CS during shuttle box extinction testing reduced extinction responding as a function of the length of confinement (Denny and Weisman 1964 ; Weinberger 1965). However in these studies CS exposure was confounded with test duration and the treatment and test phases were not independent. Subsequently Benline and Simmel (1967) reported that when variable numbers of prevention trials followed 50 shuttle box avoidance training trials extinction response levels were inversely proportional to the length of confinement, although after 3 test days the significant effect of prevention trials were eliminated. Similar findings have been reported using the ledge jump avoidance task (Baum 1969a), one way avoidance (Bersh and Keltz 1971; Schiff et al 1972) and mixed active and passive avoidance (Tortura and Denny 1973), although paradoxical effects have been reported when high training shock levels (1.8 ma) are combined with short confinement periods (Tortura and Denny 1969a).

These findings show that total CS exposure or the number of response prevention trials is a key variable determining subsequent extinction response levels. However, in several studies these variables are confounded with total treatment time (Benline and Simmel 1967; Baum 1969 ; Bersh and Keltz 1971 ; Tortura and Denny 1973 ; Schiff et al 1972). Using one way shuttle box responding Ward (1976) deconfounded these variables by varying the number of response prevention trials then equating treatment times across groups by retaining the rats in the apparatus with the barrier removed for the balance of their treatment period. Ten extinction trials followed

immediately and showed a strong trend ($p < 0.06$) for an overall response prevention effect , therefore confounding treatment time with CS exposure appears to be relatively unimportant.

Evidence concerning the relative importance of suppressing the response and degrading response contingent CS termination in determining the efficacy of response prevention trials is conflicting. Shearman (1970) argued that degrading the learned relationship between responding and CS termination was a key variable. Bankart and Eliot (1974) tested this hypothesis in the ledge jump apparatus but could only confirm that response prevention procedures were always more effective than procedures in which response contingent CS termination alone was degraded. Cassady et al (1971) have argued that CS termination may only be an informative cue in complex tasks such as two way shuttle box avoidance, the failure of Bankart and Eliot (1974) to replicate the findings of Shearman (1970) may therefore reflect methodological differences.

The presence of rats , previously habituated to the apparatus , during prevention trials facilitates the effects of response prevention in one way shuttle box (Hall 1955) and ledge jump avoidance tasks (Baum 1969 b). Increased movement per se rather than other aspects of social interaction may be the important variable as Lederhandler and Baum (1970) reported that mechanical facilitation of movement during confinement also increased the efficacy of a fixed period of confinement. Similar results have been reported with movement induced by electrical stimulation of the capsule crus cerebri (Hunsicker et al 1973). Conversely , restricted movement reduced the effects of confinement (Baum and Myran 1971).

Positively reinforcing intracranial stimulation (+ICS) of the medial forebrain bundle (Paxton et al 1974) or lateral posterior hypothalamus (Baum et al 1973) is a potent counterconditioner (Reid 1971) and adjunct to response prevention trials. Gordon and Baum (1971) reported that although neither 5 mins of confinement nor +ICS reduced pole jump avoidance extinction when given alone a combination of both was effective. This has been confirmed using the ledge jump task by Voss et al (1974) , using overtrained rats by Paxton et al (1974) who showed that +ICS was most effective when combined with confinement on the grid floor, and using signalled lever press avoidance by Monico (1971) and Stone (1971). Furthermore , the effects were found with an interval of 72 hrs between treatment and testing (Becker et al 1977). Aversive stimulation from electrodes in

the reticularis pontis caudalis was ineffective (Baum et al 1973), suggesting that counterconditioning takes place with +ICS , in support of this argument Prado-Alcala et al (1973) have found that +ICS is most effective when given whilst rats were moving away from the safety ledge during confinement on the grid floor. However counterconditioning is not necessarily the mechanism underlying response prevention itself.

3.1.2. Theories of Response Prevention.

Four theories attempt to account for the effect of response prevention trials on subsequent extinction responding. Relaxation theory argues that during prevention trials animals learn "relaxational responses". The frequency of 'emotional' responses such as abortive avoidance attempts and freezing decreases during confinement following avoidance training with dogs (Solomon Kamin and Wynne 1953) and rats (Baum and Bindra 1968). Conversely general mobility and grooming increase (Baum and Bindra 1968; Baum 1969a; Spring et al 1974). Furthermore variables which alter the efficacy of prevention trials also alter the occurrence of 'emotional' and 'relaxational' responses e.g. shock levels in training (Baum 1969 b) , social facilitation (Baum 1969 b), delayed prevention trials (Baum 1972) and loud noise during prevention (Baum and Gordon 1970).

However , Morokoff and Timberlake (1971) could not confirm these behavioural changes during prevention trials, despite significant effects on extinction. The term "relaxational" has been applied to many of the responses typically made by rats in relatively novel environments with the exception of avoidance attempts and freezing. The rationale for this classification is not clear neither is it clear why the occurrence of such behaviour should cause reduced extinction responding.

According to two factor theory fear initially becomes classically conditioned to the CS. Operant avoidance responding is then maintained by fear reduction (Mowrer 1947 ; Rescorla and Solomon 1967). Extinction of the pavlovian contingency predicts reduced avoidance responding therefore it has been argued that reduced avoidance responding after response prevention trials reflects extinction of fear of the CS. A number of experiments have attempted

to establish that fear of the CS is reduced after prevention trials.

Abortive avoidance and freezing decreases in frequency during prevention trials (Baum 1969 b; Baum and Gordon 1970; Baum 1972). Following prevention trials in a one way avoidance task rats enter the shock compartment more rapidly than controls (Shipley et al 1971; Bersh and Paynter 1972) and food deprived rats ate more free food following prevention trials (Bersh and Paynter 1972) although these authors did not test extinction responding. Bankart and Eliot (1974) confirmed these findings but food consumption was measured after extinction trials thereby confounding total CS exposure across groups. When tested after prevention trials and before extinction trials food intake was unchanged as a result of prevention trials.

Brief shocks during prevention increased extinction responding compared to prevention trials alone except in the case of long shock exposure (Bersh and Miller 1975; see also Marrazo et al 1974). Conversely , when prevention trials were paired with safety signals , established in training, extinction responding was further reduced (Hawk and Riccio 1977). In addition Mineka (1976) reported that when rats were trained in the shuttle box and ledge jump apparatus using the same CS then response prevention in either apparatus reduced subsequent shuttle box extinction . Although prevention trials in the shuttle box did not reduce ledge jump responding , suggesting that factors other than fear extinction contribute to the effects of prevention trials. Suppression of appetitively motivated lever pressing (VI 60 secs) by the CS was reduced when prevention trials followed shuttle box training (Monti and Smith 1976). However this was only apparent during the first 3 CS presentations and in subsequent trials the reverse was found. Finally , multivariate analysis of behaviour in the ledge jump apparatus showed that response prevented rats approached the grid floor sooner and more frequently, spent more time on the grid floor and safety tested less and sooner than non prevented controls (approach but not alight onto grid floor) (Corriveau and Smith 1978).

Principal component analysis indicated that one factor , which the authors concluded was fear, accounted for 52% of the total variance.

Counterconditioning theory argues that during prevention trials an alternative response is adventitiously paired with the CS and shock omission. This response, which is incompatible with the original response then becomes the operant for fear reduction.

In support of this hypothesis a number of studies have reported increased passive avoidance of the shock chamber in a one way avoidance task following prevention trials. Food deprived response prevented rats were slower than controls in approaching free food in the shock compartment (Page and Hall 1953 ; Page 1955 ; Coulter et al 1969). In these experiments passive avoidance was tested after extinction trials thereby confounding total CS exposure across groups, however, Linton et al (1970) reported similar findings when this confounding was removed. Marrazo and Riccio (1974) and Bersh and Miller (1975) found that prolonged shocks during prevention enhanced rather than reduced the effects of prevention trials, a result which they argued was incompatible with the fear extinction hypothesis but supported the counterconditioning hypothesis. Further support for the counterconditioning theory is found in those experiments which show that +ICS acts as a potent adjunct to prevention trials (see above).

The evidence in favour of counterconditioning is based almost entirely on passive avoidance studies using identical training procedures followed by 5 mins of response prevention and as such may reflect a specific effect of short confinements. In support of this argument Rorbaugh and Riccio (1970) reported that fear conditioned water deprived rats showed increasingly long latencies to approach free water in the shock compartment with confinement times up to 5 mins. In contrast approach latencies decreased with confinement times from 5 to 50 mins. In addition Eyesenck (1967) has reported that unreinforced CS exposures do not always lead to enhanced extinction and may have the contrary effect of enhancing fear (Napalkov 1963 ; Eyesenck 1968).

An alternative theory of avoidance responding argues that responding is maintained not by fear reduction (Miller 1948: Mowrer 1947) but by preferences and expectancies which determine the rat's behaviour during learning and extinction (Seligman and Johnston 1973). Exposure to the CS-UCS contingency may activate responding and condition fear but in terms of the 'cognitive' theory this simply reflects an animal's preference for not being shocked and allows the subsequent development of 'expectancies' that (a) responding leads to the preferred omission of shock and (b) not responding leads to the non-preferred shock. Having been established during training the normal shock avoidance extinction procedure , in which shock is simply switched off, barely disrupts these established expectancies

as the animal will not normally detect any change in the contingencies until it fails to respond, at which point the expectancy that no response leads to shock is disconfirmed, thus explaining why avoidance responding may be resistant to extinction (Solomon et al 1953; Seligman and Campbell 1965; Shearman 1970; Wilson 1973). In contrast, response prevention immediately leads to disconfirmation of expectancies by forcibly detaining the rat in the presence of the CS and omitting shock and therefore leads to more rapid extinction. This account does not depend on fear reduction for the maintenance of responding therefore experiments which apparently show that measures of fear and response rates may vary independently (see above) do not contradict the theory.

3.2 Experiment One: The Effect of Response Prevention on Shuttle Box Avoidance Extinction

Introduction

Although extensively used in the literature, three factors render ledge jump responding unsuitable as a baseline for the present studies. The emergence of the escape ledge from the wall of the apparatus serves as the conditioned stimulus (CS), and does not permit presentations of the CS off the baseline (see Experiment 4). Furthermore, response prevention in this apparatus usually involves removing the escape ledge, even though this is probably the least efficient method of prevention (see above). Finally, the ledge jump apparatus has not been used to study vasopressins effects on behaviour.

These considerations prompted the choice of two way shuttle box avoidance as offering several advantages; the compound CS may be presented off the baseline (see Experiment 4); response prevention may be accomplished without removing part of the apparatus. Finally, it has been extensively used in studying the behavioural effects of vasopressins (see Section 2.1).

Whilst the shuttle box offers advantages over the ledge jump apparatus, it also makes different behavioural demands. Bolles (1971) suggests that the contingencies in an avoidance schedule vary in their relative contributions towards establishment and maintenance of responding depending upon the apparatus used. Bi-directional avoidance responding in the shuttle box introduces an element of passive avoidance for the side in which shock was last experienced.

The first objective of this experiment is to establish that shuttle box avoidance responding is sensitive to the effects of response prevention, defined as forced exposure to the CS with prevention of the

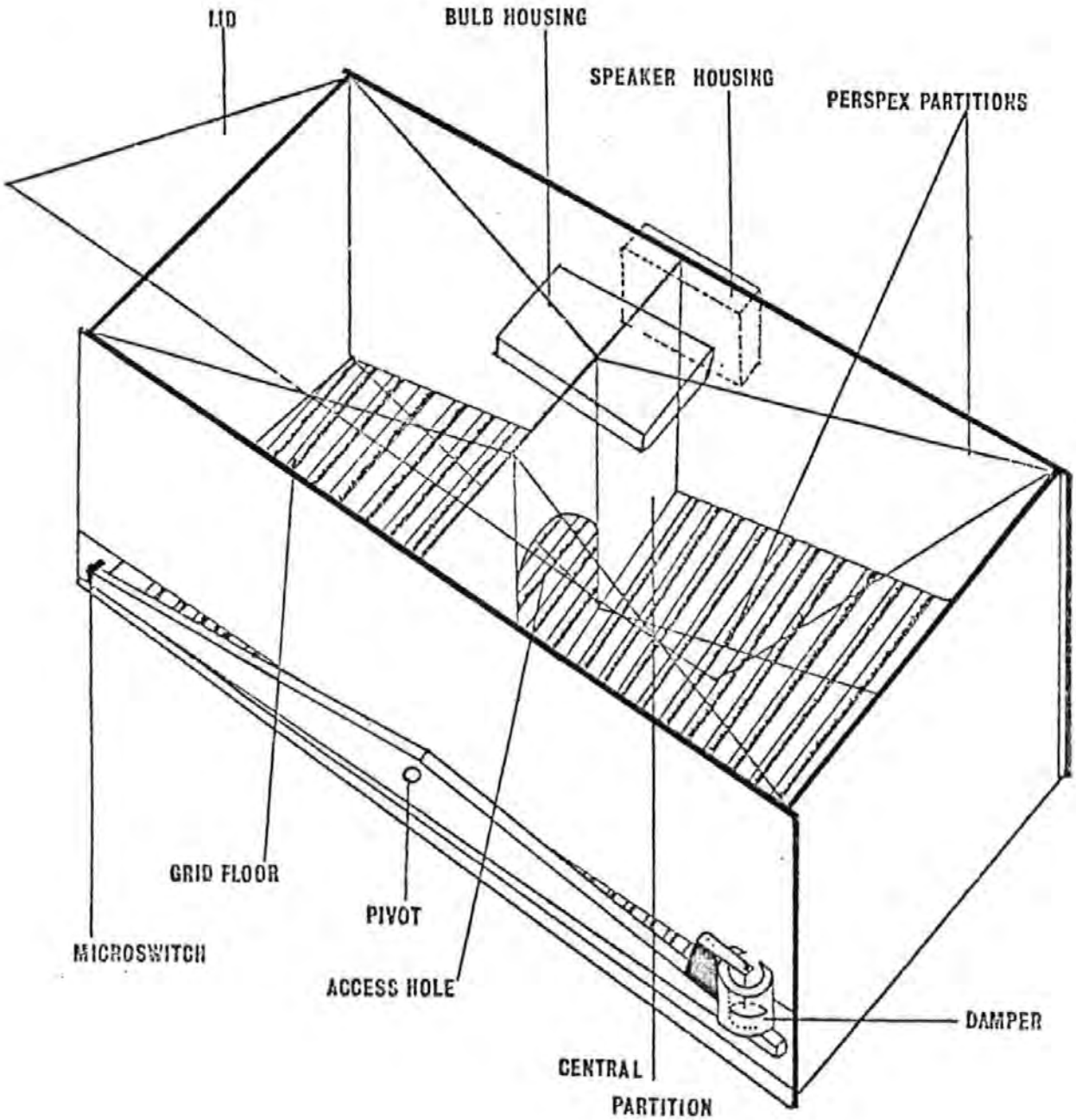
avoidance response in the absence of shock. Solomon et al (1953) failed to find an effect of prevention using dogs and high shock. In contrast, Carlson and Black (1959) reported a marked effect using dogs with lower shock and massed prevention trials. Similarly, Polin (1959) found prevention trials effective with rats in the shuttle box. However, Polin (1959) used extinction trials in which CS termination was not contingent upon responding. Benline and Simmel (1967) reported that prevention trials exerted a temporary response reduction in extinction; however, in this study total treatment time and the duration of response prevention were confounded. The present experiment tested the effects of prevention trials on rats trained with low shock levels and using response contingent CS termination during extinction testing. In order to equate total treatment time across groups, a control group spent a period equivalent to that required for response prevention in the home cage. Thirty prevention trials were used as extensive evidence in the literature (see above) suggested prolonged prevention to be more effective than short periods in reducing extinction responding.

The second objective of the experiment is to establish for how many extinction test trials after treatment the effects of prevention are evident. Polin (1959) and Benline and Simmel (1967) using the shuttle box and Crawford (1977) using the ledge jump apparatus have reported that the effects of prevention trials do not persist throughout extended extinction trials.

The third objective is to determine whether or not the effects of prevention are found when extinction testing is delayed for 24 hours after prevention trials. Rats were tested either immediately (IMM) or 24 hours after prevention. The consolidation hypothesis proposed to explain the behavioural effects of LVP (see Chapter Two) should be tested using a post training injection (Dawson and McGaugh 1973) in order to separate memory effects from confounding influences on other aspects of behaviour. Sufficient time must elapse between peptide treatment and extinction testing to allow the dissipation of short term effects. Therefore, it is necessary to demonstrate that the effects of prevention are seen 24 hours later.

The final objective of the experiment is to establish the effects of 30 extinction trials on subsequent extinction responding in order to confirm that response prevention reduces extinction responding compared to an equivalent period of extinction treatment (Bankart and Elliot 1974). However, extinction is not a well-controlled procedure; with a fixed number of extinction trials the experimenter cannot control the total CS exposure to each animal - this may prove to be an important

Figure4 Two way shuttle box.



source of variability.

Methods

Subjects

Forty-eight adult male CFHB Wistars (300-500 g) from a closed colony at Plymouth Polytechnic (derived from stock supplied by Anglia Laboratory Animals Company Limited) were housed three or four to a cage with ad lib food and water.

Apparatus

Two standard commercial shuttle boxes supplied by Ugo Basille Company Limited and measuring 48 cm long by 21 cm high and wide (internal dimensions) were used. Each box was divided into two equal compartments by a black metal partition (21 cm x 21 cm) with a hole of 9.5 cm radius in the base of the partition to allow access between the two halves. In addition two transparent perspex partitions (3.2 mm thick) were placed vertically between the floor and the roof of each compartment. These were positioned to form a 'v' shaped compartment with the base of the 'v' opening to the access hole (see Figure 4). Pilot studies showed that this facilitated learning. The floor of the chamber was formed by 40 stainless steel rods (1.25 cm (centres)) through which shock was delivered. The floor was pivoted at the centre and a response was detected when the rat moved across the centreline tilting the floor and activated a reed microswitch. A speaker was housed adjacent to the side of the chamber at the midline. Two bulbs were mounted on the roof of the chamber straddling the midline dividing partition. Both shuttle boxes were housed in sound and light attenuating chambers.

Schedule

There was a fixed interval of 60 secs between the start of successive CS presentations. The inter trial interval (ITI) had a minimum duration of 40 secs and a maximum of 60 secs. Each ITI was followed by 10 secs of the CS alone, then 10 secs of CS plus shock.

A compound CS was used, consisting of a mixed frequency tone (5 dbs above background noise of 62 dbs measured using International Scale C) accompanied by the illumination of two 10 watt clear bulbs mounted one each side of the central partition. Footshock (UCS) produced by the Basille control box (1.5 setting) was sufficient to induce mild flinching, occasionally but not usually accompanied by vocalisation. A static pattern of voltage differences was produced within each group of four consecutive bars and repeating throughout the grid floor (mean voltages: 0.925 v ac, 1.3 v ac, 1.87 v ac, 1.15 v ac). Shock was delivered in

bursts of 0.5 seconds with 1.5 seconds of no shock. Pilot studies indicated faster learning when shocks were spaced in this way.

A response was detected when the animal crossed the midline whilst the CS was on. An avoidance response during acquisition was defined as a response during the CS only period which cancelled shock and returned the schedule to the ITI. Responses made during the CS plus UCS period constituted an escape response, terminated shock and returned the schedule to the ITI. If the subject did not respond during either the CS along or the CS plus UCS period this was a failure to respond, thereby preventing excessive shock exposure. Data from each shuttle box was recorded on a pen tracer which registered the occurrence of an avoidance, an escape or a failure plus the number of shocks received in each trial.

Procedure

Subjects were housed in an animal house separate from the laboratory at a constant temperature, in darkness from 6 pm to 6 am and were transported to the laboratory before each session. All experiments were run between 9 am and 6 pm.

At the start of training subjects were allowed to adapt to the shuttle box for ten minutes. During training each rat received a maximum of 50 trials on each of three consecutive days. Training was to a criterion of ten consecutive correct avoidance responses which has been widely used in response prevention experiments reported in the literature (see Section 3.1). Thirteen rats failed to achieve criterion and were dropped from the study. Those animals which attained criterion were randomly assigned to one of six treatment groups in a 3 x 2 design. Three treatment conditions, retention in the home cage for 30 minutes, 30 extinction trials and 30 response prevention trials, were first tested either immediately or 24 hours after treatment. These conditions are described below.

(1) Home cage retention plus immediate extinction test (HC IMM). Rats were removed from the shuttle box and retained in the home cage for 30 minutes then returned to the shuttle box for the 50 extinction trials of Extinction Test 1 (T1). Twenty-four hours later there were 50 more extinction tests (T2).

(2) Home cage retention plus extinction testing 24 hours later (HC 24). Rats were trained to criterion then removed to the home cage. Twenty-four hours afterwards rats were given the first extinction test (T1) followed 24 hours later by Extinction Test 2 (T2).

(3) Extinction treatment plus an immediate extinction test (EXT IMM). Having reached criterion shock was disconnected and rats given 30 trials of normal extinction with response contingent CS termination. Immediately

afterwards each rat was tested on 50 extinction trials (T1) followed 24 hours later by the second extinction test (T2).

(4) Extinction treatment plus the first extinction test 24 hours later (EXT 24). Having reached criterion shock was disconnected and rats were given 30 extinction trials. Rats were returned to the home cage and 24 hours later returned to the shuttle box for 50 extinction trials (T1) followed 24 hours later by the second extinction test (T2).

(5) Response prevention followed immediately by the first extinction test (RP IMM). Having reached the criterion rats received 30 trials of response prevention, during which shock was disconnected and an opaque black barrier placed across the access hole between the two compartments, preventing the rat from shuttling. Twenty seconds of CS were presented every 60 seconds for 30 minutes. After this the barrier was removed and rats began the first batch of 50 extinction test trials (T1) followed 24 hours later by the second extinction test (T2).

(6) Response prevention followed 24 hours later by the first extinction test (RP 24). After response prevention trials rats were returned to the home cage and 24 hours later returned to the shuttle box for the first extinction test (T1), followed 24 hours later by the second extinction test (T2).

Summary of the experimental procedure

Training days	Testing days One	Two	Three
Training of 50 trials per day for a maximum of three days. Ss trained to criterion of ten consecutive correct avoidance responses.	HC IMM, RP IMM, EXT IMM groups given Extinction Test 1 (T1).	HC IMM, RP IMM, EXT IMM groups given Extinction Test 2 (T2).	
	HC 24, RP 24, EXT 24 groups return to home cage.	HC 24, RP 24, EXT 24 groups given Extinction Test 1 (T1).	HC 24, RP 24, EXT 24 groups given Extinction Test 2 (T2).

Results

Acquisition

Acquisition performance was measured using five indices; trials to criterion, escapes to criterion, total failures to respond and total shock pulses. The data for each subject is contained in Table A1 (the prefix A denotes that a table is to be found in Appendix A). Acquisition data were analysed with analysis of variance Winer (1962) which are

Figure 5 Total avoidance responses during extinction. (mean and s.e.m.)

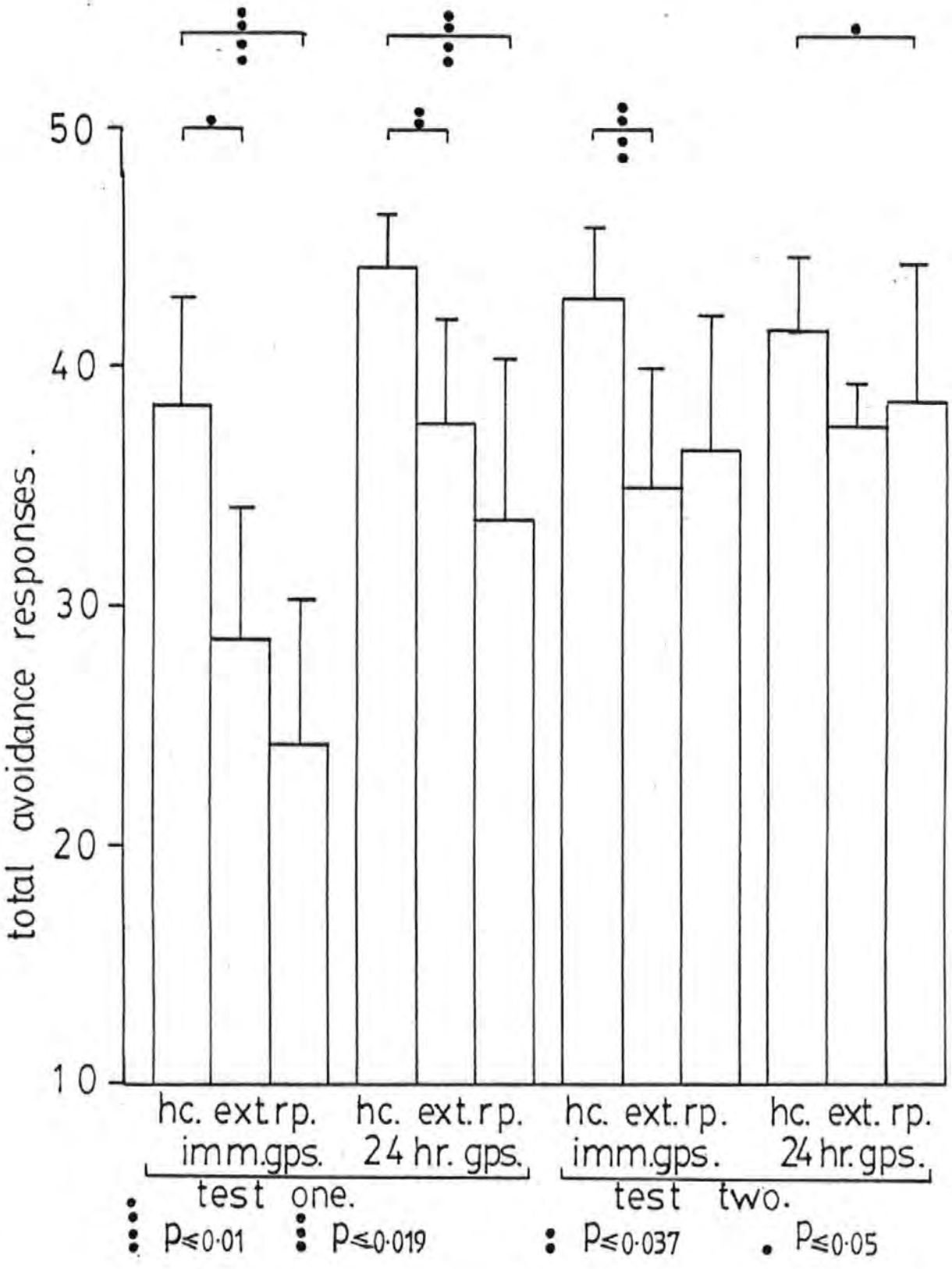


Figure 6 Short avoidance responses during extinction. (mean and s.e.m.)

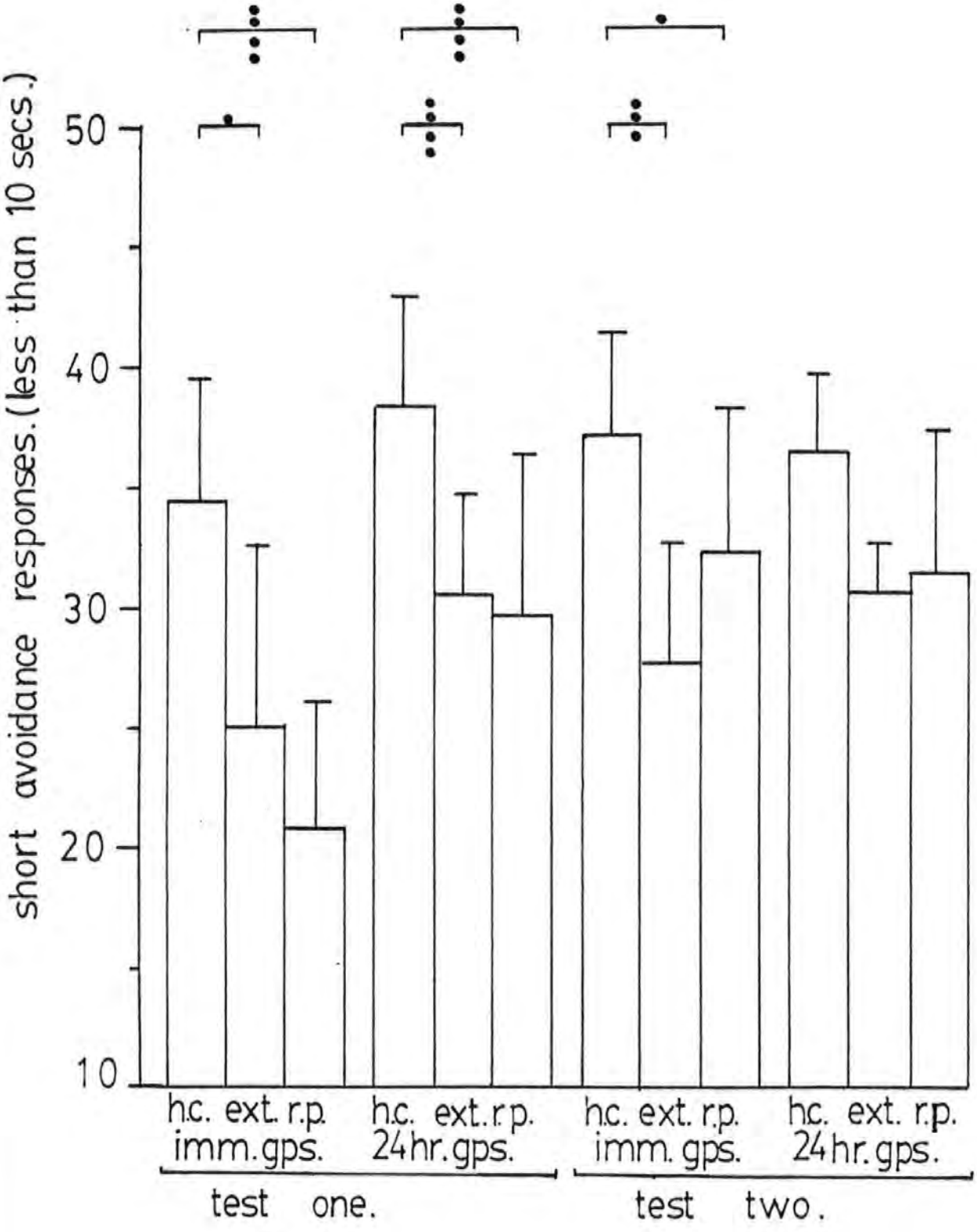


Figure 7 Long avoidance responses during extinction. (mean and s.e.m.)

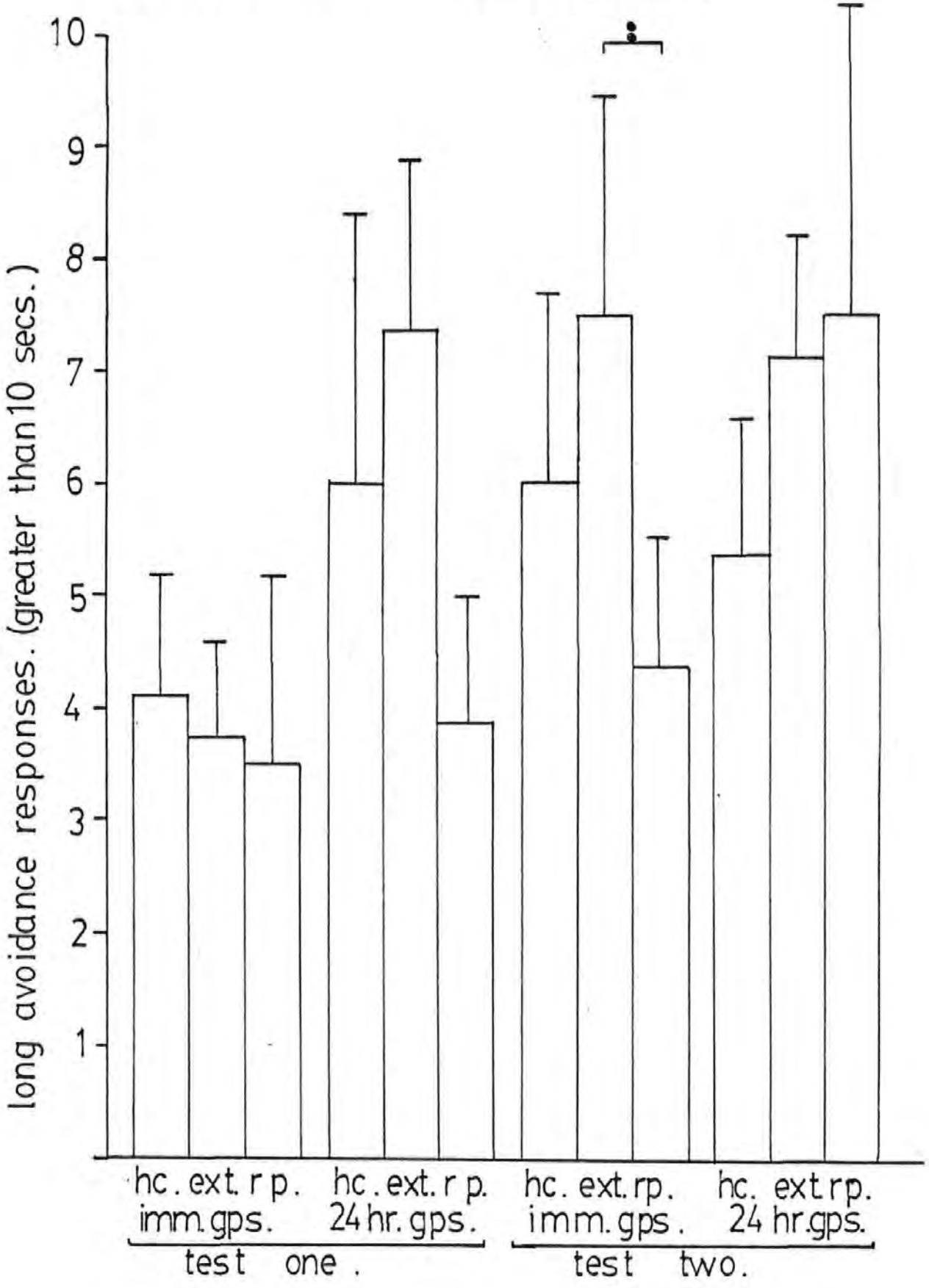
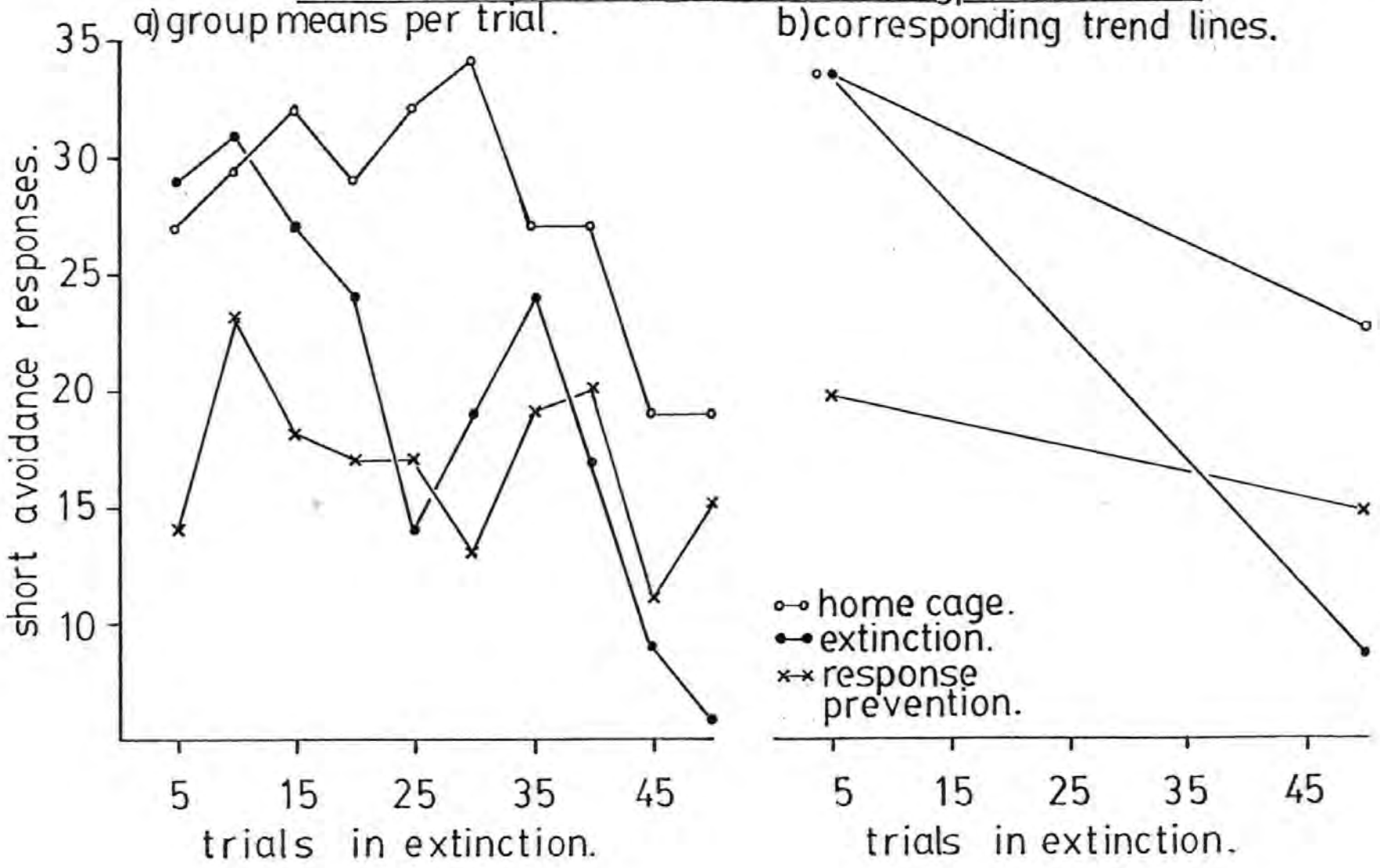


Figure 8 The mean number of short avoidances as a function of trial blocks in extinction. (data for imm. gps. test one.)



summarised in Table A2. No significant differences were found between groups on any of these measures.

Extinction

During extinction testing the CS could remain on, in the absence of shock, for a maximum period of 20 seconds, the same value used during training. An avoidance response was defined as one which occurred during these 20 seconds. Subsequently a distinction was made between short avoidances, ie those occurring within ten seconds of CS onset and long avoidances, ie those occurring between the tenth and twentieth seconds after CS onset.

The performance of each subject during extinction is summarised in Table A3 and in Figures 5 , 6 and 7 . Analysis of covariance (Winer 1962) showed that there was no significant covariance between short avoidances in extinction and either trials to acquisition criterion ($F = 1.36$, df 5,41) or the number of avoidances to acquisition criterion ($F = 1.34$, df 5,41) (see Table A4). It was concluded that differences in extinction response levels were not due to different acquisition performance levels.

Extinction data was recorded as the total number of each type of response made in each of the ten blocks of five trials contained in both Test 1 and Test 2 (see Table A5) and was analysed using Friedman's two way non-parametric analysis of variance (Siegel 1956). The outcome of these analyses is summarised in Table A6. There were significant differences in the total number of avoidance responses made by those groups tested immediately after treatment during Test 1 ($p < 0.001$) and Test 2 ($p = 0.012$). Similarly, there were significant differences between groups tested 24 hours after treatment during Test 1 ($p < 0.001$) and Test 2 ($p = 0.05$). Pairwise comparisons were made between groups (Table A7) using the method outlined by Hollander and Wolfe (1973).

In the immediate test groups, response prevention reduced the total number of avoidances made during Test 1 ($p < 0.01$) compared to home cage controls. Extinction treated rats had reduced responding relative to home cage controls during both Test 1 ($p < 0.05$) and Test 2 ($p < 0.01$).

When the first extinction test was postponed for 24 hours, response prevention reduced responding during Test 1 ($p < 0.01$) and Test 2 ($p < 0.05$) relative to home cage controls. Extinction treatment reduced responding relative to home cage controls only during Test 1 ($p < 0.037$). There were no significant differences between response prevented and extinction treated rats on either Test 1 or Test 2.

Analysis of short avoidance responses (Table A6) revealed significant differences between groups in both Test 1 ($p < 0.01$) and Test 2

($p < 0.02$) when the first extinction test followed immediately, and when the first test was postponed for 24 hours after treatment there were significant differences during Test 1 only ($p < 0.001$) (see Table A6). Pairwise comparisons (Hollander and Wolfe 1973) (Table A7) indicated that with immediate testing response prevention reduced the number of short avoidances relative to home cage controls during both Test 1 ($p < 0.01$) and Test 2 ($p = 0.05$). Similarly, extinction treatment reduced the number of short avoidances relative to home cage controls during both Test 1 ($p = 0.05$) and Test 2 ($p < 0.019$).

When testing was postponed for 24 hours after treatment, response prevention ($p < 0.01$) and extinction treatment ($p < 0.01$) reduced the number of short avoidances made during Test 1 but not Test 2. There were no significant differences between response prevented and extinction treated rats in the number of short avoidance responses.

The analysis of long avoidance responses (Table A6) revealed significant group differences in Test 2 ($p < 0.05$) when tested immediately and when the first test was postponed for 24 hours differences approached, but did not reach, significance during Test 2 ($p < 0.1$). Pairwise comparisons (Hollander and Wolfe 1973) (Table A7) revealed that in the case of the immediate test groups, the Test 2 difference was between extinction treated and response prevented rats ($p < 0.037$). For those groups in which the first test was postponed for 24 hours there was a trend for extinction and response prevented rats to make more long responses than response prevented rats ($p < 0.1$). There was also a trend ($p < 0.1$) for response prevented rats to make more long avoidance responses than home cage controls when the first test was postponed until 24 hours after treatment.

These analyses established differences between groups in responding after treatment. Additional comparisons were made to determine the treatment effects upon rates of change of responding within each of the 50 trial extinction tests. Group data (Table A5) were used to compute regression lines. The slope co-efficients are shown in Table A8. In order to fulfil the minimum requirements of the Kruskal Wallis analysis of variance (Siegel 1956) data for immediate and 24 hour test groups were combined and showed significant differences in the slopes of regression lines for short avoidances over trial blocks during Test 1 ($p = 0.067$) (Table A9). Pairwise comparisons (Hollander and Wolfe 1973) showed that extinction treated rats had greater negative slopes than response prevented rats ($p = 0.067$) (see Figure 8).

Discussion

There were significant differences between groups in the total number of avoidances during extinction. However, when a distinction was made between short (< 10 seconds) and long (> 10 seconds) responses it became clear that the short avoidances alone provided a more sensitive measure, as a result of removing the confounding influences of the long avoidance responses which in general were insensitive to treatment effects.

Response prevention significantly reduced the number of short avoidance responses made in extinction compared to retention in the home cage for 30 minutes. Reduction in responding was greatest when the first extinction test was made immediately after treatment and was also evident when these rats were retested 24 hours later. This result confirms the effect of response prevention which has been widely reported in the literature (see Section 3.1). The effect was restricted to short avoidances and there were no differences between response prevented and home cage control rats in the long avoidance data.

Similar results were found when the first extinction test was delayed for 24 hours after response prevention. Under these conditions response prevented rats made fewer short avoidances than home cage controls during the first but not the second extinction test 24 hours later. Again, there were no differences between these groups in the long avoidance data. The response prevention procedure used therefore affected responding after an interval of 24 hours between treatment and test and is suitable as a baseline in experiments using post trial injections allowing dissipation of short term effects of vasopressin. The data also suggest that the effects of prevention are temporary; there were no effects during the second extinction test in animals for whom the first extinction test had been delayed for 24 hours after treatment. This finding supports those of Polin (1959), Benline and Simmel (1967) and Crawford (1977) (see Sections 3.1 and 3.2).

Response prevention did not produce significant differences in the number of short avoidances made during extinction compared to 30 trials of extinction treatment. However, long latency responses were significantly greater in extinction treated rats compared to response prevented rats during Test 2 of the immediately tested groups. Similarly, there was a trend for long avoidance responses made by extinction treated rats to exceed those made by response prevented rats during Test 1 for the 24 hour test groups. Long latency responses may become reinstated more rapidly after extinction treatment than after response prevention, suggesting differences in the behavioural effects of each treatment.

The lack of any difference between response prevention and extinction treatment in the short avoidance data may be explained by the observation that for Test 1 avoidance data the extinction treated rats had significantly higher negative slopes than response prevented rats, ie within these tests extinction treated animals extinguished at a significantly faster rate than response prevented rats (see Figure 8). At the beginning of the extinction test, extinction treated rats responded like home cage rats but towards the end of the test their response rate resembled that of response prevented rats. This crossover in the avoidance rates for these groups may explain why no differences were found in the absolute number of short avoidances.

The effects of prevention were evident as reduced responding throughout the session whereas extinction treatment produced a higher within session rate of extinction and more rapid reinstatement of long latency avoidance responses. This distinction may reflect procedural differences such as longer CS exposure, non-contingent CS termination, thwarting of the response or a combination of these factors. The greater within session response rate stability seen after response prevention coupled with the fact that prevention places the schedule contingencies under the experimenter's control render response prevention a more reliable procedure than extinction for achieving reduced responding in extinction.

In theoretical terms the reduced extinction responding seen after response prevention may be interpreted in terms of enhanced extinction of conditioned fear, greater disconfirmation of expectancies, counter-conditioning of an alternative and incompatible response or the development of relaxation responses. All four theories (see Section 3.2) may account for the result.

CHAPTER FOUR

THE EFFECTS OF LVP AND RESPONSE PREVENTION ON AVOIDANCE EXTINCTION AND CONDITIONED SUPPRESSION

4.0 Introduction

Three experiments are reported; the first shows that immediate post training LVP (1 µg) injections increase subsequent extinction responding. The second experiment shows that when injected after either 30 minutes in the home cage or 30 extinction trials LVP (1 µg) reduced extinction responding but increased extinction responding after 30 response prevention trials. The third experiment shows that LVP (1 µg) increases suppression of operant level press responding by concurrent presentations of the aversive CS. Response prevention trials, although altering operant baselines, did not alter suppression but delayed the suppressant effects of LVP injections.

These results show that under different experimental conditions, possibly due to timing of injections, LVP may either increase or decrease extinction responding. Furthermore, under conditions conducive to decreased responding the effect can be reversed by preceding LVP injections with response prevention trials. Subsequent experiments (Chapter Five) explore the variables controlling the direction of vasopressin's effects on avoidance extinction. Exploration of the interaction between prevention trials and LVP injections both on avoidance extinction and using CS presentations concurrent with operant responding showed that neither the effects of LVP or prevention trials could be explained in terms of simple psychological constructs such as "fear" or "memory"

4.1 Experiment Two: The Effects of Post Training LVP on Shuttle Box Avoidance Extinction

Introduction

Chapter Two reviewed the behavioural effects of vasopressins and their analogues. Avoidance extinction responding increased when peptides were injected after training or before extinction testing. The object of this experiment was to establish whether or not shuttle box avoidance extinction in an automated apparatus was sensitive to the effects of LVP injected immediately after training.

Methods

Subjects

Twenty-two adult male cfhb Wistars rats (400-450 g) from the closed colony maintained at Plymouth Polytechnic were housed four to a cage with ad lib food and water.

Apparatus

The apparatus and data recording has been described in Section 3.2.

Procedure

Prior to training, rats were placed in the apparatus for ten minutes adaptation. Training continued to the criterion of ten correct consecutive avoidance responses and was restricted to a maximum of 50 trials per day on two consecutive days. Two rats failed to achieve criterion within the limit and were discarded. A further three were discarded as a result of experimenter error. Responses made during the CS were not counted as avoidances unless the animal had received at least one footshock.

Treatment

Immediately after attaining criterion rats were randomly allocated to one of two treatment groups, saline or LVP. All injections were made subcutaneously into the rats' rear flank. The control group was injected with 0.5 ml of physiological saline and experimental rats with 0.5 ml of physiological saline containing LVP (2 μg/ml), provided by Sigma Chemicals Company Limited as a crystalline solid with a pressor assay potency of 75 IU/mg. Solutions were stored at 1-5^{o}C and injected at room temperature.

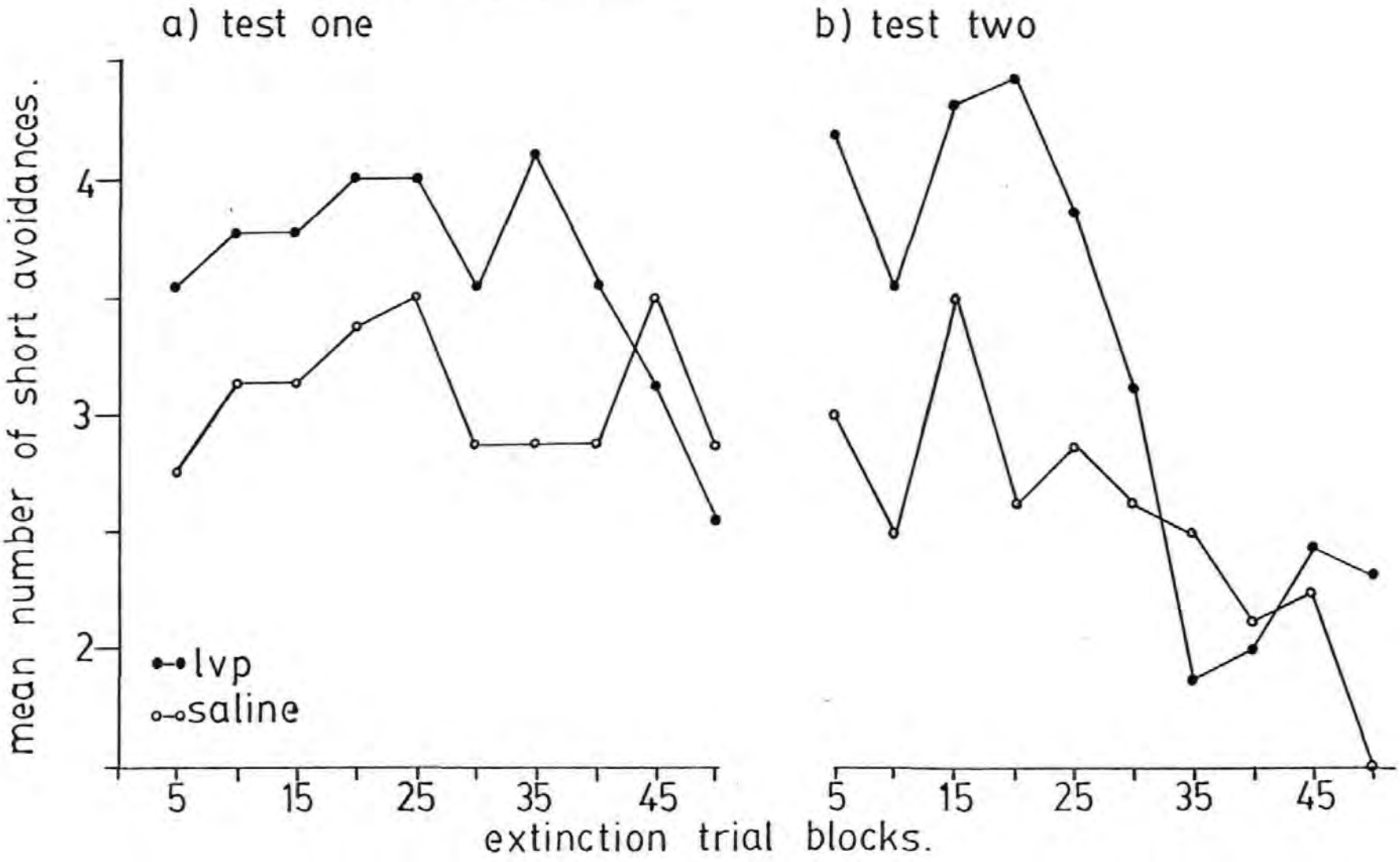
Testing

Twenty-four hours after treatment, rats were returned to the apparatus for 50 trials of extinction and this procedure was repeated on the following day. The extinction schedule was identical to that used in acquisition but shock was omitted (see also Experiment 1).

Summary of the experimental design

Training	Treatment	Testing
S trained to a criterion of ten correct consecutive avoidance responses within a maximum of two training days with 50 trials per day.	Saline or LVP injected immediately after S had attained the learning criterion.	S was given 50 extinction trials per day. Tests were run at approximately the same time on each day.

Figure 9 The mean number of short avoidances as a function of trial blocks in extinction.



Results

Acquisition

Acquisition performances were recorded using five indices, trials to each criterion, the number of avoidances, escapes and failures to respond and shocks received whilst attaining criterion (Table A10). Groups were compared using the two tailed independent 't' tests (Winer 1962). Results are shown in Table A11. There were no significant differences between groups.

Extinction

Data for each subject in extinction are presented in Table A12. Analyses of covariance (Winer 1962) showed no significant covariance between the number of short avoidance responses in extinction and either the number of trials to criterion ($F = 0.35$; df 1,14) or the number of avoidances to criterion ($F = 0.46$; df 1,14) (Table A13).

The mean number of avoidances responses (short plus long) per block of five trials in extinction for each group were compared using Wilcoxon's signed ranks test (Seigel 1956) (see Table A14). LVP treated rats made significantly more responses in Extinction Test 1 ($p < 0.0098$) and Test 2 ($p < 0.005$) than saline controls. When a distinction was made between short avoidance responses (< 10 seconds) and long avoidance responses (> 10 seconds) (Table A14) Wilcoxon's signed ranks test (Seigel 1956) revealed that LVP treated rats made significantly more short avoidance responses during Test 1 ($p < 0.009$) and Test 2 ($p < 0.0137$) than saline controls and there were no significant differences between groups in the number of long avoidance responses. These analyses are summarised in Table A15.

Regression lines were calculated using the method of least squares for the short avoidance data as a function of trials in extinction (Table A14). During Test 1 the saline group yielded a slope coefficient of 0.018 whereas LVP treated rats during Test 1 yielded a slope coefficient of -0.812. These slopes were compared using the Hollander test for parallelism (Hollander and Wolfe 1973). The slopes were not significantly different ($T+ = 10$, $n = 5$, $p = 0.31$), indicating that within Test 1 extinction occurred at similar rates in both groups. During Test 2, saline treated rats yielded a slope coefficient of -1.163, lower than that of the LVP group (-2.448). These Test 2 trend lines tended not to be parallel ($T+ = 14$, $n = 5$, $p = 0.062$) indicating that extinction occurred at a slightly higher rate in LVP treated rats than in the saline controls in Extinction Test 2. Saline performance in Test 1 was compared with performance in Test 2 and Hollander's test for parallelism indicated that these lines were not parallel ($T+ = 15$, $n = 5$,

p = 0.062) indicating that extinction occurred at a slightly higher rate in LVP treated rats than in the saline controls in Extinction Test 2. Saline performance in Test 1 was compared with performance in Test 2 and Hollander's test for parallelism indicated that these lines were not parallel ($T+ = 15$, $n = 5$, $p = 0.031$). Similar differences were seen when the data for LVP treated rats in Test 1 was compared with performance in Test 2 ($T+ = 15$, $n = 5$, $p = 0.031$). Therefore the rate of extinction was higher for both groups in Test 2 than in Test 1.

Discussion

LVP significantly increased the total number of avoidance responses during Extinction Tests 1 and 2 compared to saline treated controls. Furthermore, these differences were due primarily to changes in the number of short avoidance responses with no significant differences in the number of long avoidance responses made by each group. These results support those reported in the literature (Chapter Two). In addition, the absence of any effects on the number of long avoidances together with the peptide effect 48 hours after treatment, despite evidence for a short metabolic half life (see Section 1.), argue against an explanation of vasopressin's effect by short term motor effects.

Increased avoidance responding seen after vasopressin treatment is greatest during the early portion of each extinction test and this leads to the higher rate of extinction seen in LVP treated rats, especially during Test 2. The effect of vasopressin therefore fades within each extinction test but is reinstated in the 24 hour interval between tests, an effect not previously reported. The data are compatible with the consolidation of memory hypothesis (Chapter Two), proposed to account for the effects of LVP, if it is assumed that enhanced consolidation of information into long term memory should lead to an increase in the avoidance response level during extinction.

4.2 Experiment Three: The Effects of LVP and Response Prevention on Shuttle Box Avoidance Responding

Introduction

In the preceding experiments, two procedures were described which have opposite effects upon the extinction of avoidance responding. In Experiment One, 30 response prevention trials reduced responding during subsequent extinction whilst in Experiment Two a post training injection of LVP (1 µg) increased responding during subsequent extinction testing. The interaction between these two procedures has been studied by King

and De Wied (1974) using the pole jump avoidance who showed that LVP injected before response prevention increased subsequent extinction responding. If decreased extinction responding after prevention trials can be explained by associative changes then this result cannot be explained by the consolidation of memory hypothesis proposed to explain the effects of LVP (Chapter Two). Indeed, if LVP facilitated consolidation of recently acquired behavioural information it would be expected that when given in conjunction with prevention trials extinction responding should be reduced even further. In the King and De Wied (1974) study LVP was injected before response prevention and pretreatment may have confounded an effect on consolidation with motor or motivational changes; Dawson and McGaugh (1973) have suggested that compounds which affect time dependent consolidation processes should be effective when injected after behavioural procedures. The first object of this experiment is therefore to EXTEND the findings of King and De Wied (1974) using post training injections to maximise the chances of detecting and minimise the chances of confounding a consolidation effect.

The second object of the experiment is to examine the effect of the peptide given after extinction treatment. In view of the similar effects which 30 extinction trials and 30 trials of prevention had on the responding during extinction testing in Experiment One, it was of interest to determine whether or not these procedures were identically affected by LVP (1 µg).

The third object of the experiment is to determine whether or not the effects of LVP could be detected during extended extinction testing. Results in the literature (Section 2.) indicate that a post training injection of the peptide may exert an increase in extinction responding which extends long after treatment.

Methods

Subjects

Forty-eight adult male cfhb rats (350-450 gms) from the closed colony maintained at Plymouth Polytechnic were housed three or four to a cage, with ad lib access to food and water.

Procedure

The apparatus and the training schedule have been described in detail in Experiment One. Briefly, the animals were placed in the shuttle box for five minutes in order to adapt and then received 50 training trials per day on each of a maximum of two days. Training stopped when the subject had reached the learning criterion of ten correct consecutive avoidance responses. Fourteen animals which failed to attain this

criterion within two days were discarded from the experiment. During the early stages of training a small number of rats responded to the CS without having received footshock; in order that these responses should not bias the acquisition data a response was only included as an avoidance if the animal had received a shock on a previous trial. When each rat had attained the criterion it was randomly allocated to one of the following six treatment groups.

Treatment

(1) Home cage retention plus saline injection (HCS). Having attained learning criterion the rat was returned to the home cage for 30 minutes and was then given an injection of saline.

(2) Home cage retention plus LVP injection (HCL). Having attained the criterion each rat was returned to the home cage for 30 minutes and then given an injection of LVP (1 µg SC).

(3) Extinction treatment plus saline injection (EXT S). Having attained criterion, each rat remained in the shuttle box for 30 extinction trials. During extinction the schedule remained the same as for acquisition but the shock source was disconnected.

(4) Extinction treatment plus LVP injection (EXT L). Having attained the criterion each rat remained in the shuttle box for 30 extinction trials followed by LVP (1 µg SC) injection.

(5) Response prevention plus saline injection (RP S). Having attained criterion each rat remained in the shuttle box for 30 trials of response prevention during which a black barrier blocked the access between compartments preventing the shuttle response and retaining the animal in the presence of the CS. On each trial the CS remained on for 20 seconds and at the end of these trials each rat received a saline injection.

(6) Response prevented plus LVP injection (RP L). These rats were treated in the same manner as those in group RP S but were injected with LVP after the prevention trials.

Peptide treatment

All rats were injected SC with a constant volume of solution, at room temperature. Lysine vasopressin was supplied as a crystalline powder by Sigma Chemicals Limited (lot number 65c-0156) with a pressor potency of approximately 75 IU mg and was injected dissolved in 0.5 ml of physiological saline (0.09%) in a dose of 2 µg/ml. Saline controls received 0.5 ml of physiological saline. Solutions were stored at 1-5^{o}C.

Testing

After training and treatment each subject was tested during three sessions, T1, T2 and T3. Each consisted of 50 extinction trials on each

of three consecutive days. Testing began approximately 24 hours after injection and was repeated at approximately the same time on each day. During extinction the schedule remained the same as in acquisition but the shock source was disconnected. Training and testing spanned either four or five days depending on whether the subject had reached criterion on the first or second day of acquisition. In addition to T1, T2 and T3, subjects were given two short extinction tests, T4 and T5, each of ten extinction trials. For subjects which attained criterion on the first day of training T4 and T5 were run 168 and 192 hours respectively after T3. However, if criterion was reached on the second day of training then T4 and T5 followed T3 at 144 and 168 hours respectively. This complication was unavoidable in view of the number of animals involved in the study and the limited time available.

Results

Acquisition

Performance during acquisition is summarised in Table A16. Five indices were recorded; avoidances to criterion, trials to criterion, escapes to criterion, shocks to criterion and failures to respond whilst attaining criterion. Data from acquisition was compared using one way analysis of variance (Winer 1962) and outcomes from these analyses are contained in Table A17. There were no significant differences between groups on any of these indices.

Extinction

During extinction testing a distinction was made between short avoidances responses, made within ten seconds of the CS onset, and long avoidances, made between ten and 20 seconds after CS onset. When added together, these two categories yield the total number of responses made during extinction. These data are presented in Table A18 and in Figures 10, 11 and 12. In order to test the hypothesis that differences in extinction responding could be the result of differences in acquisition performance, two analyses of covariance were run (Winer 1962). No significant covariance was found between the number of trials to criterion and the number of short avoidances in Extinction Test 1 ($F,df\ 5,41 = 0.83$) or between the number of avoidances to criterion and the number of short avoidances in Extinction Test 1 ($F,df\ 5,42 = 0.85$). These analyses are summarised in Table A19.

Data from extinction tests were reduced by dividing each animal's test performance into blocks of five trials and counting the total number of responses, the number of short avoidance responses and the number of long avoidance responses in each block of five trials. In Table A20

Figure10 Total avoidance responses during extinction testing. (mean and s.e.m.)

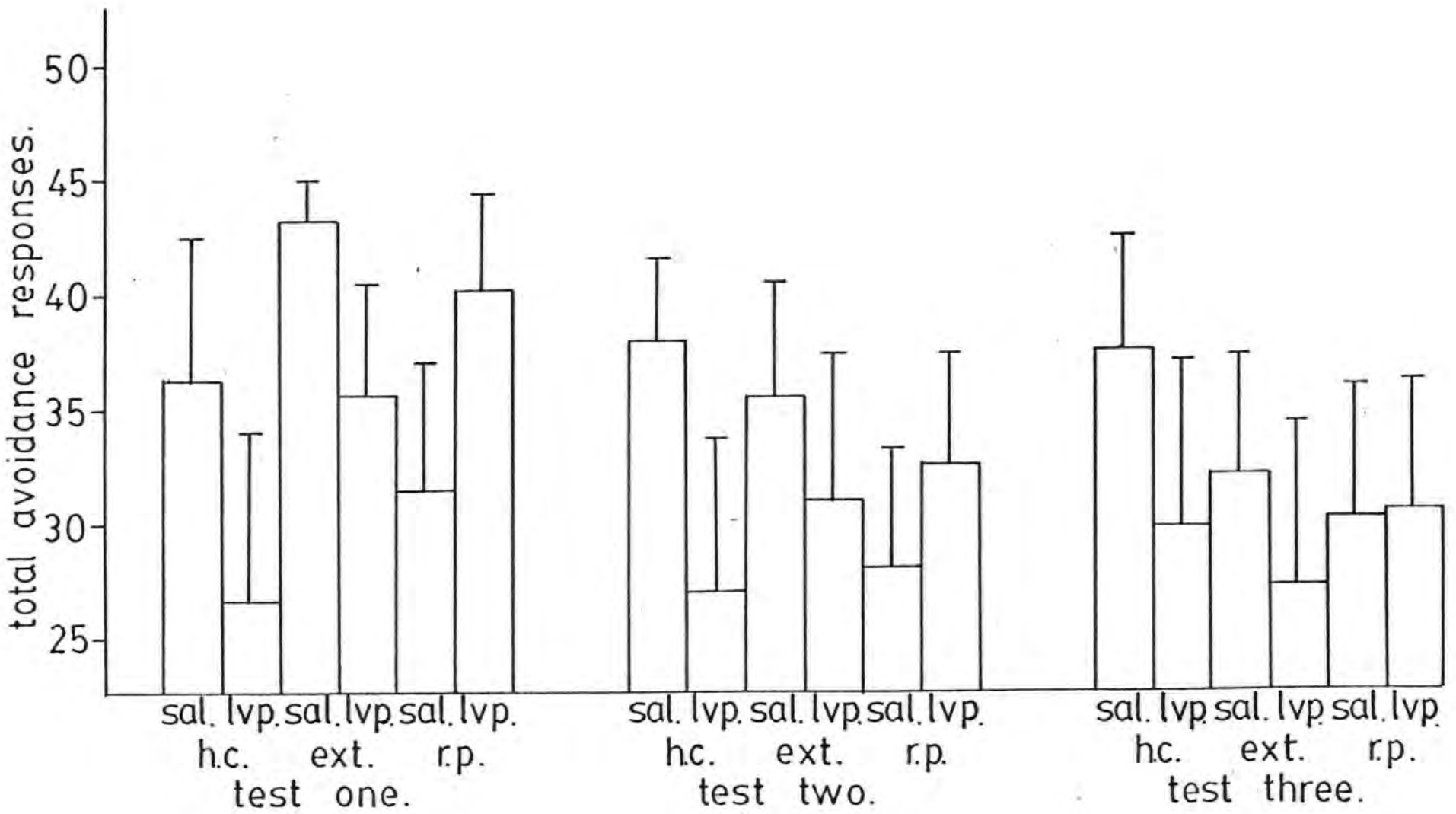


Figure11 Short avoidance responses during extinction testing. (mean and s.e.m.)

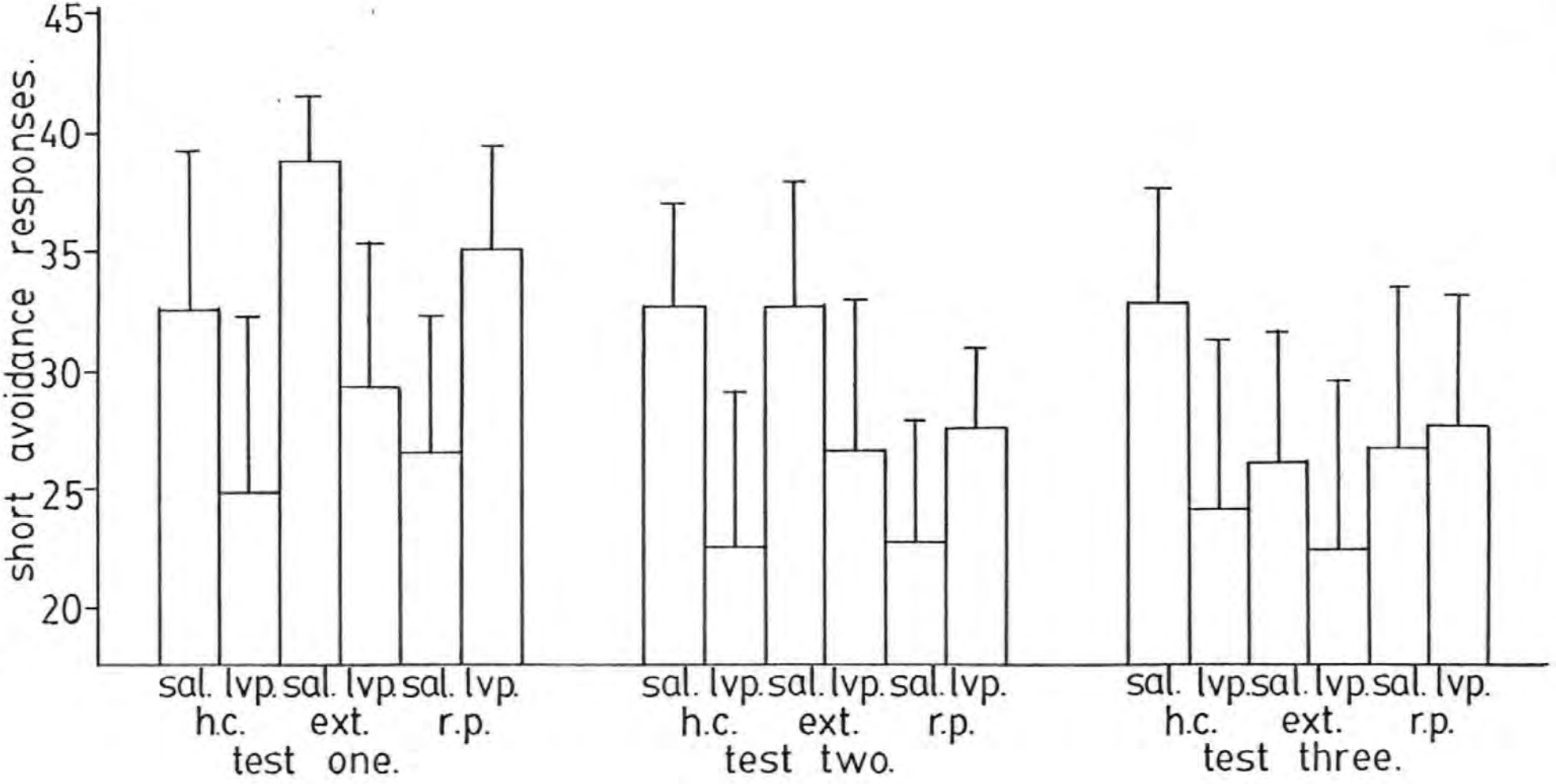
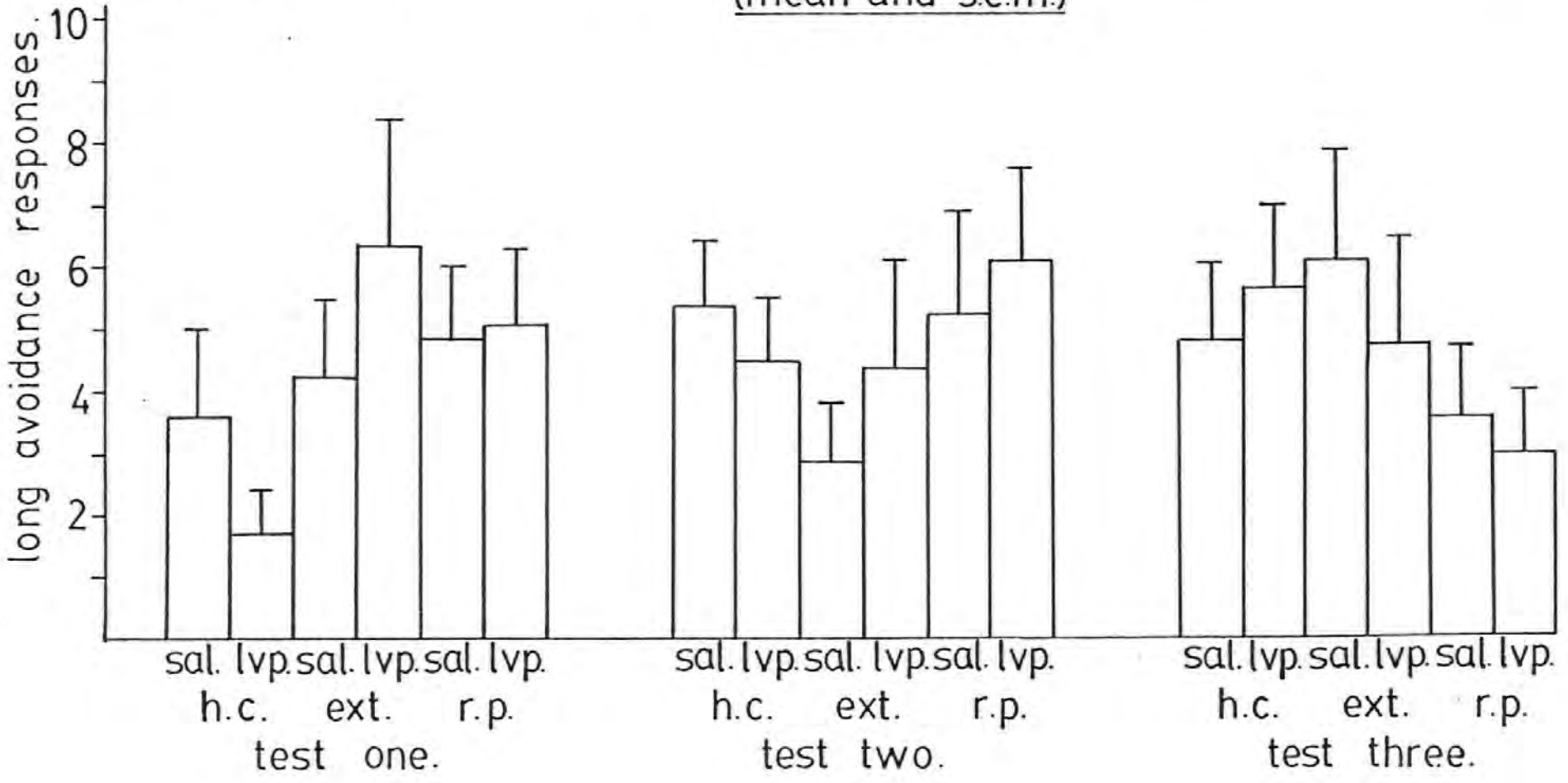


Figure12 Long avoidance responses during extinction testing.
(mean and s.e.m.)



extinction data are presented as the mean number of each response type per animal per block of five trials. These data were analysed using Friedman's non-parametric two way analysis of variance (Seigel 1956) and outcomes are presented in Table A21. During Test 1 there were significant differences between groups in the total number of avoidances ($p < 0.001$), short avoidances ($p < 0.001$) and long avoidances ($p < 0.02$). During Test 2 also there were significant differences between groups in total avoidances ($p < 0.001$), short avoidances ($p < 0.001$) and long avoidances ($p < 0.05$). During Test 3 there were significant differences between groups in the total number of responses ($p < 0.001$) and short avoidances ($p < 0.02$). There were no significant differences between groups during Tests 4 and 5. Breakdown analyses were made in order to locate significant differences between groups within each extinction test (Hollander and Wolfe 1973); selected outcomes are presented in Table A23.

Response prevention followed by saline tended to reduce the number of short avoidance responses in Test 1 ($p < 0.1$) compared to animals which were retained in the home cage and given saline. During Test 2 this difference was significant ($p < 0.009$) and there was a strong trend to reduce the total number of responses ($p < 0.1$). During Test 3 the total number of responses was reduced by response prevention ($p < 0.023$) and short avoidances showed a strong trend towards being reduced ($p < 0.1$). There were no significant effects of response prevention on the number of long avoidance responses during any of the extinction tests.

Extinction trials followed by a saline injection significantly increased the total number of responses made in Extinction Test 1 ($p < 0.047$) compared to home cage saline rats. During this test there were no significant differences between these two groups in the number of short or long avoidance responses. During Test 2 there was a trend ($p < 0.1$) for extinction saline rats to make fewer long avoidance responses than home cage saline rats. During Test 3 there were no significant differences between these groups.

Extinction trials followed by saline significantly increased the total number of avoidance responses made during Extinction Test 1 compared to response prevented saline treated rats ($p < 0.009$). Furthermore, this difference was due to the greater number of short avoidance responses made by the extinction saline rats ($p < 0.009$) as there was no significant difference in the number of long avoidance responses made by these two groups. Similarly, extinction treatment plus saline significantly increased the total number of responses made during Test 2

compared to response prevented rats treated with saline ($p < 0.009$). This increase was also due to increased short avoidance responses ($p < 0.008$) with no change in the number of long avoidances. During Test 3 there were no significant differences between these two groups.

LVP given after 30 minutes of retention in the home cage tended to reduce the total number of responses made during Extinction Test 1, compared to a saline injection given after retention in the home cage ($p < 0.08$). During this test LVP significantly reduced the number of short avoidances ($p = 0.05$) and did not affect the number of long avoidance responses. During Extinction Test 2 LVP significantly reduced total number of avoidance responses ($p < 0.009$) and the number of short avoidance responses ($p < 0.009$) compared to saline when given to rats which had been retained in the home cage for 30 minutes before injection. There were no effects of the peptide on long avoidances during Test 2. During Test 3 the total number of responses ($p < 0.023$) and the number of short avoidance responses ($p < 0.023$) were significantly reduced in the LVP treated rats. Again, there was no effect upon the number of long avoidance responses.

LVP given to extinction treated rats significantly reduced the total number of responses ($p < 0.047$) and the number of short avoidances made during Test 1 ($p < 0.009$) compared to extinction treated rats given saline. There was no effect upon long avoidance responses. During Test 2 there was no effect of the peptide on responding by extinction treated rats but there was a strong trend for LVP to decrease the number of short avoidances ($p < 0.1$) and increase the number of long avoidances ($p < 0.1$). During Test 3 there were no significant effects of the peptide on either short or long avoidance responses although there was a strong trend for LVP to reduce the total number of responses compared to saline ($p < 0.1$).

During Test 1 LVP tended to increase the total number of responses compared to saline ($p < 0.1$) when given to response prevented rats and significantly increased the number of short avoidance responses compared to saline in response prevented rats ($p < 0.009$) but did not affect the number of long avoidances. During Test 2, LVP significantly increased the total number of avoidance responses made compared to saline in response prevented rats ($p = 0.05$). There were no significant effects on either the short or long avoidances. During Test 3 there were no significant differences between the LVP and saline response prevented groups.

When animals which had been retained in the home cage then given LVP were compared with animals which had been response prevented then given LVP, it was found that in Test 1 response prevention significantly

increased the total number of responses ($p < 0.009$), the number of short avoidances ($p < 0.009$) and the number of long avoidance responses ($p < 0.023$). During Test 2 response prevention significantly increased the total number of avoidance responses ($p < 0.023$) but did not significantly affect either short or long avoidance responses when considered alone. There were no significant differences between these groups during Test 3.

Trend lines were calculated for the performance of each group during each extinction test using the method of least squares with the short avoidance data (Table A22). Slopes were compared across groups using the Kruskall Wallis non-parametric analysis of variance (Seigel 1956) but there were no significant differences between groups ($H = 6.39$, $df = K - I = 5$, $p < 0.3$).

Discussion

Control animals retained in the home cage for 30 minutes then injected with saline showed stable response levels throughout the three major extinction tests (T1, T2 and T3). Response prevented rats injected with saline made consistently fewer responses than home cage saline controls. During Test 1, response prevention tended to reduce the number of short avoidance responses, during Test 2 there was a trend to reduce the total number of responses and a significant reduction in the number of short avoidance responses. In Test 3 response prevention significantly reduced the total number of responses and there was a strong trend to reduce the number of short avoidance responses. Failure to observe changes in regression lines fitted to the within test data indicated that no treatment affected the within test pattern of response change.

These results confirm the effects of prevention which have been widely reported in the literature (Section 3.1) and the results of Experiment One. In addition, failure to observe an effect of response prevention during Tests 4 and 5 support the findings of Experiment One and those of the literature (Polin 1959, Benline and Simmel 1969, Crawford 1977) which suggest that the effects of prevention diminish with repeated trials , although the duration of the effect varies between studies.

Thirty trials of extinction followed by saline injection significantly increased the total number of responses made during Test 1 compared to home cage saline controls without affecting within test regression slopes, but this effect did not persist through the later extinction tests. This result contrasts to the effects seen in Experiment One where it was found that extinction trials reduced response levels and increased

the rate of within test response change. Furthermore, extinction treated rats given a saline injection made significantly more responses than response prevented saline treated rats during Tests 1 and 2 without altering regression slopes. These increases in the total number of responses were due to increases in the number of short avoidances made by the extinction treated rats relative to response prevented rats as there were no changes in the long avoidances. These results contrast to the effects of extinction trials compared to response prevention in Experiment One where it was found that these two treatments both reduced the absolute response level relative to home cage controls although extinction treatment resulted in a higher within test extinction rate.

Differences between Experiment One and the present experiment suggest that extinction trials are not a reliable way of reducing responding during extinction tests. In the present experiment, animals were given a saline injection after 30 trials of extinction and this may account for the differences between the results of these two experiments. Indeed, Riffee et al (1979) have observed changes in behaviour as a result of saline injections. An additional factor may be that the experimenter has little control over experimental contingencies during extinction trials, CS exposure and termination are related to individual response rates and are uncontrolled variables. The effect of the treatment is therefore likely to vary between batches of animals depending on response levels during treatment.

Response prevention did not reduce responding in extinction, relative to home cage saline controls, when followed by LVP instead of saline. In Test 1 response prevented rats given LVP tended to make a greater total number of responses and made significantly more short avoidances than response prevented rats given saline. Similarly, in Test 2 response prevented rats given LVP made significantly greater number of total responses than their saline treated counterparts. There were no significant differences between these groups during Extinction Test 3.

This effect of the peptide was only evident, therefore, during the first two extinction tests; indeed during the third extinction test the usual response reducing effect of prevention trials was evident as these animals were responding at the same level as response prevented saline treated rats and both these groups made significantly fewer responses than home cage saline controls during Extinction Test 3. This result confirms the effect reported by King and De Wied (1974) and differs only in that these authors found the effect of the peptide to be strongest on the second day of extinction testing whereas in the present experiment

the effect was most pronounced during early extinction tests. Therefore it may be argued, as King and De Wied (1974) did, that the peptide does not invariably enhance consolidation of recently acquired behavioural information. If such were the case, then responding should have been further reduced after response prevention. This interpretation assumes, as do all the peptide experiments reviewed in Chapter Two, that in an experiment which uses post learning peptide treatment an increased response rate in extinction represents enhanced memory storage and decreased responding reflects disrupted consolidation. In addition, it is assumed that new behavioural information is conveyed during response prevention which accounts for decreased responding after prevention (see Section 3.1).

Two further aspects of the present data point away from the consolidation hypothesis. When 30 trials of extinction were followed by LVP rats made a significantly fewer total avoidance responses and short avoidances during Test 1 than their saline treated counterparts. Furthermore, there was a strong trend for LVP to reduce the number of short avoidances during Test 2 and the total number of responses during Test 3. In addition, when LVP was given to rats detained in the home cage for 30 minutes, it significantly reduced the total number of responses and the number of short avoidance responses made during Tests 1, 2 and 3. Therefore, the effects of LVP injected after either extinction trials or home cage retention is to reduce extinction responding, a novel finding which stands in sharp contrast to data discussed in Chapter Two.

If the results from response prevented and extinction treated rats, under saline and under vasopressin, are considered together, then an interesting set of effects is apparent. After saline extinction treated rats make more responses than response prevented rats during Tests 1 and 2. However, both groups react to LVP in opposite ways. Avoidance responding goes down in extinction treated rats and up in response prevented rats. The normal effects of each treatment are reversed to the extent that there are no significant differences between them after the peptide. It is possible to argue that these opposite effects of the peptide reflect the presence of some contingency in one behavioural procedure and not in the other. This could be greater CS exposure, non-contingent CS termination or the thwarting of the avoidance response, all factors present in response prevention but not in the extinction procedure. In theoretical terms, the difference in reaction to LVP could reflect the fact that response prevention induces a counterconditioned response not present in the extinction treated group (cf Section 3.1.2), or disconfirms the rat's expectancy that shock follows no response and no shock

follows a response (Seligman and Johnston 1973). It seems unlikely, from the present result that LVP increases fear of the CS, at least in the terms of two factor avoidance theory; if such was the case then LVP should have increased responding in both extinction treated rats and response prevented rats.

Rats retained in the home cage and those given extinction trials are similar in as much as neither experience any radical change in the contingencies of the avoidance schedule. Extinction treated rats maintained a high response rate during the actual extinction treatment; these data are summarised in Table A25 (saline mean + sem = 27.875 ± 1.63 LVP mean + sem = 26.25 ± 1.971). It is possible to argue, therefore, that the opposite effects of LVP with response prevented rats compared to both extinction treatment and home cage retention may be accounted for in terms of the schedule changes (changes in behavioural information) which occur during response prevention but not during either extinction treatment or retention in the home cage. These contingency changes may alter the animal's cognitive expectancies or induce a counterconditioned response (see Section 3.1.2). In view of the limited conditions under which this latter effect has been demonstrated, it appears that the data most strongly support an explanation in terms of cognitive expectancies. However, there is a very strong proviso which must be considered before accepting an explanation of this nature. The explanation is based upon comparisons involving effects of vasopressin which have not previously been reported, ie responding was reduced following both home cage retention and extinction treatment. Therefore for both empirical and theoretical reasons it is necessary to investigate the reasons for this reversal of the normal and widely reported effects of vasopressin which were confirmed in Experiment Two. One difference between the design of the present experiment and that of Experiment Two is that LVP was injected after a 30 minute interval of retention in the home cage; in Experiment Two LVP was injected immediately after training. According to the results from De Wied (1973) and King and De Wied (1974) (see Section 2.2) this time lag should not affect the outcome of vasopressin treatment which has an estimated behavioural half life of one hour at the dose used in the present experiment. The effect of varying the time of the injection was therefore examined in later experiments (see Chapter Five).

4.3 Experiment Four: The Effect of LVP on Suppression of Lever Pressing by the CS Following Response Prevention or Confinement in the Home Cage

Introduction

Behavioural effects of LVP and its analogues have been extensively studied using aversive conditioning procedures, in particular two way shuttle box avoidance (De Wied 1971), the pole jump task (King and De Wied 1974) and step through passive avoidance task (Ader and De Wied 1972) (see Chapter Two). In these procedures animals were trained, treated with the peptide at the appropriate time, then returned to the apparatus in order to measure the change in response probability as a result of peptide treatment. The results reported in the literature indicate that LVP and its analogues increase the probability of responding during active avoidance extinction and increase the latency to re-enter the shock compartment in the passive avoidance task. The results of Experiment Two confirm these findings in the shuttle box but it is clear from the results of Experiment Three that this effect of LVP cannot be found under all experimental conditions. The results of Experiment Three, combined with the consideration that previous studies had concentrated on test situations which required the animal to perform the trained response in extinction, prompted the design of the present experiment. This study was designed to measure disruption of lever press responding caused by concurrent presentation of the compound CS previously used in training the avoidance task and examined whether LVP and response prevention affected this variable in the same way as they affected avoidance extinction.

Garrud (1974) failed to see an effect of LVP (2 µg) on operant responding during concurrent CS paired with footshock; this may have been due to the use of pre-test injections combined with a procedure which elicited strong stimulus control, rendering the procedure rather insensitive. The present experiment was based on a design used by Kamin, Brimer and Black (1963) in which the CS was presented in the absence of shock. The training and treatment schedules of Experiment 3 were repeated using rats which had also been trained to lever press for food on a variable interval schedule in which a pellet was delivered on average every 60 seconds (VI 60 secs). Twenty-four hours after the post training injection of LVP or saline subjects were placed in the lever press box and the conditioned stimulus used in the avoidance schedule was presented during lever pressing. Changes in the operant response rate were evaluated as a function of treatment.

Experiments using concurrent classical and operant schedules form the background against which the present experiment should be viewed. Since its experimental inception by Estes and Skinner (1941) the approach has fostered a huge literature and diverse theoretical accounts. In a recent review, Henton (1978) points to two broad classes of theories proposed to account for changes in the operant baseline during classical trials.

Inductive and experimental approaches have stressed the importance of interactions which occur between the responses controlled by each schedule. Thus Brady and Hunt (1955) proposed a research strategy, based on results from Estes and Skinner (1941), which led to the competing response hypothesis (Brady 1971). Changes in the operant baseline during CS presentations were ascribed to the elicitation of responses conditioned to the CS but not necessarily compatible with the execution of the lever pressing response. Similarly, Lyon (1968) proposed that changes in the operant baseline could either be the result of interference from competing responses or to punishment of the operant by adventitious pairing of the lever press response with shock. Henton and Iversen (1978) extended the competing response hypothesis proposed by Brady and Hunt (1955), arguing that experiments which use aversive classical trials superimposed on an appetitive baseline should be viewed as part of a wider class of procedures in which simultaneous schedules interact, producing local changes in the response pattern elicited by either schedule in a manner dependent on the controlling variables of both schedules.

In contrast to inductive approaches stand a number of deductive or inferential models. Kamin (1965) has suggested that alterations in the operant rate may serve as an indirect quantification of classical conditioning processes. In support of this hypothesis Annau and Kamin (1961) reported systematic changes in the index of suppression as a function of UCS intensity. Similarly, Kamin, Brimer and Black (1963) observed systematic changes in a suppression index during training and extinction of an avoidance response. These changes were attributed to alterations in the level of conditioned fear. Rescorla and Solomon (1967) have also proposed that changes in the operant rate during the CS could be an indirect measure of conditioned emotions. Azrin and Hake (1969) explained operant rate changes as a function of a general emotional state, which stemmed from pairing a stimulus with a strong positive or negative reinforcer, accompanied by both overt and covert, autonomic and cardiac, responses. Although the present experiment is not intended to unravel the complexities of opposing theories, the

results will be discussed in terms of compatibility with these major theoretical positions.

Methods

Subjects

Thirty-two adult male cfhb Wistar rats (200-250 g) from the closed colony maintained at Plymouth Polytechnic were housed three or four to a cage with ad lib access to water. Rats were reduced to approximately 80% of their free feeding weight. Body weight was routinely checked at the start of each lever pressing session.

Apparatus

Avoidance responding was established using the apparatus described in detail in Experiment One. A compound CS was provided by the illumination of two 10 watt clear bulbs, mounted on the roof of the cage, coupled with a mixed frequency tone (90 dbs) measured on international Scale A, mounted on the rear wall panel of the apparatus.

Lever pressing was trained in a standard two lever skinner box (Grason Stadler model number 1111) housed in a ventilated, sound and light attenuating cabinet (Grason Stadler model number 1101). Events were programmed and recorded using Grason Stadler Series 1201 programming equipment. In order to present the CS from the avoidance schedule whilst rats were lever pressing the rear wall and lid of a Basille shuttle box, with the speaker housing used to generate the tone CS and the light housing was strapped to the rear wall and roof of the Skinner box. Background noise, originating mainly from the ventilating fan in the Skinner box, was rated at 74 dbs measured on international Scale A. Tone volume in the shuttle box and the avoidance apparatus were equated.

Procedure

Lever press training pilot studies confirmed the conclusion of Blackman (1968) that the absolute response rate contributed to the magnitude of the suppressive effect of a concurrent CS. The VI 60 second schedule was selected in order to produce stable response rates which were comparable across subjects. In addition the limited hold was included to stabilise the number of reinforcements available throughout the 30 minute test sessions independently of the response rate. The intervals were chosen from Clark (1958) and yielded an inter-reinforcement interval with an arithmetic mean of 60 seconds.

At the start of the experiment rats were reduced to 80% of their free feeding body weight and during this period were fed at midday on each day with approximately 15 g of standard laboratory food with ad lib

water. Having been reduced to their target body weight, they were stabilised on the feeding regime for seven days and then response shaping began. During the period of response shaping the bulk of each rat's daily food requirement was met with 45 mg pellets (Campden Instruments Company Limited) dispensed as reinforcement for approaching and then pressing the lever. Additional standard lab pellets were provided for individuals as required to maintain their target weights. Having been shaped to press the lever rats were then established on a continuous reinforcement schedule (CRF) and those which failed to acquire the response were dropped from the experiment.

Schedule control was programmed in such a way that the interval between the availability of reinforcements could be reduced to less than one second, ie shorter than the time required to retrieve and consume the previously delivered pellet. At such a low inter reinforcer availability interval the programme therefore mimicked a CRF schedule. By gradually extending this interval it was possible to transfer each subject to the final goal of a VI 60 second schedule, at a rate suited to each individual subject. Responding on this schedule was stabilised for 15 experimental days (three calendar weeks) with one 30 minute session per day. At the end of each session subjects were fed with sufficient food to maintain their body weight.

Avoidance training

After 15 days on the VI 60 schedule, rats were trained to avoid footshock in the shuttle box up to a criterion of ten correct consecutive avoidance responses. This training procedure has been described in detail in Experiment One. Lever pressing sessions were maintained throughout avoidance training.

Treatment

Having attained the learning criterion rats were randomly allocated to one of four groups in a 2 x 2 design. Two groups were returned to the home cage for 30 minutes and two groups received 30 trials of response prevention, as described in Experiment One. Following these behavioural treatments subjects were given an injection of either saline or LVP (1 µg/0.5 ml) SC. Batch details and the method of preparing the solution have been described in Experiment Two. Following injection, each rat was returned to the home cage.

Testing

Approximately 24 hours after injection, rats were returned to the Skinner box for 30 minutes of lever pressing. The first ten minutes were used as a warm-up period; during the subsequent 20 minutes the

compound CS, used in avoidance training, was presented ten times (trials) for 20 seconds on each occasion. These trials were distributed throughout the 20 minutes of the test session according to a VI two minute schedule. Intervals were selected from Clark (1958) to yield the arithmetic mean interval of 120 seconds. Twenty-four hours after this first test (Test 1) the procedure was repeated (Test 2). The control apparatus was programmed to count the number of lever presses made during the 20 seconds immediately preceding a presentation of the CS (Period A) and during the 20 seconds of CS presentation (Period B) on a digital print-out unit. It was then possible to compute an index of disruption of baseline responding (suppression ratio: SR) as a result of CS presentation, according to the formula from Kamin, Brimer and Black (1963):

$$sr = \frac{\text{responses during Period B}}{\text{responses during Period A + responses during Period B}}$$

This formula yields values ranging from 0 (maximum suppression) to 1 (maximum facilitation). A value of 0.5 indicates that the CS did not affect lever press responding relative to pre-CS levels. Clearly the ratio may fluctuate as a function of changes during either Period A or B; however, given a constant response rate during Period A on a series of trials then a steady recovery of responding in Period B will yield a decelerating curvi-linear function with an asymptotic value of 0.5 (Henton 1978). This formula was chosen to provide maximal comparability with the study of Kamin, Brimer and Black (1963) in describing the rapid extinction of suppression which was anticipated.

Extinction of the avoidance response

Approximately 24 hours after the second suppression test, subjects were returned to the shuttle box for 50 trials of extinction testing; this was repeated 24 hours later. The details of extinction testing have been described in Experiment One.

Summary of the experimental design

(1) Establish and maintain VI 60 sec schedule (five to six weeks);
(2) train the avoidance response to a criterion of ten consecutive correct responses; having attained the criterion, subjects were immediately given
(3) behavioural treatments; rats were either retained in the home cage for 30 minutes or received 30 trials of response prevention; this was followed immediately by
(4) vasopressin or a saline injection;
(5) 24 hours after the injection each rat was returned to the Skinner box for the first suppression test;

Figure13 The mean number of lever presses made by each group during period A of each suppression trial.

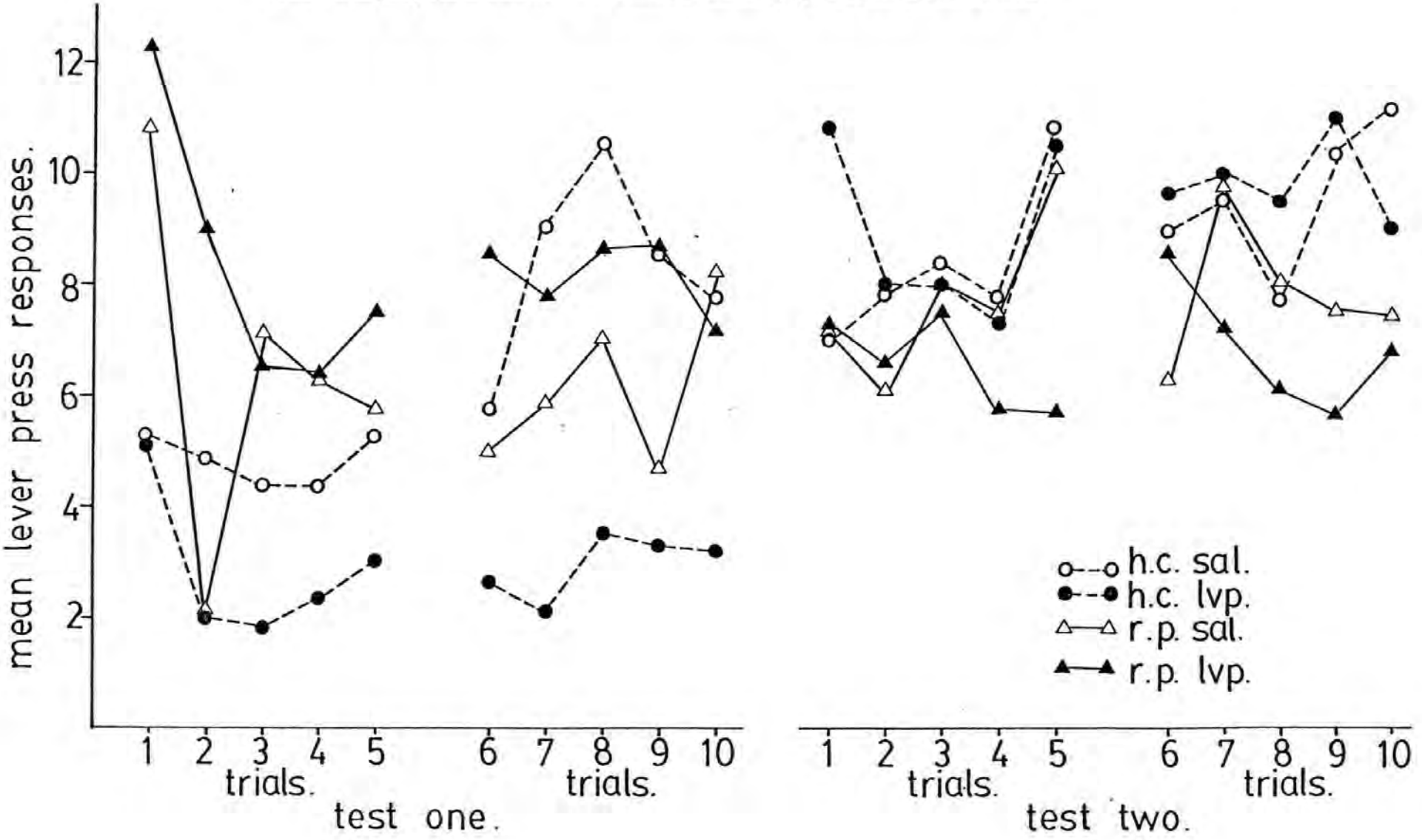


Figure 14 The mean number of lever press responses made by each group during period B of each suppression trial.

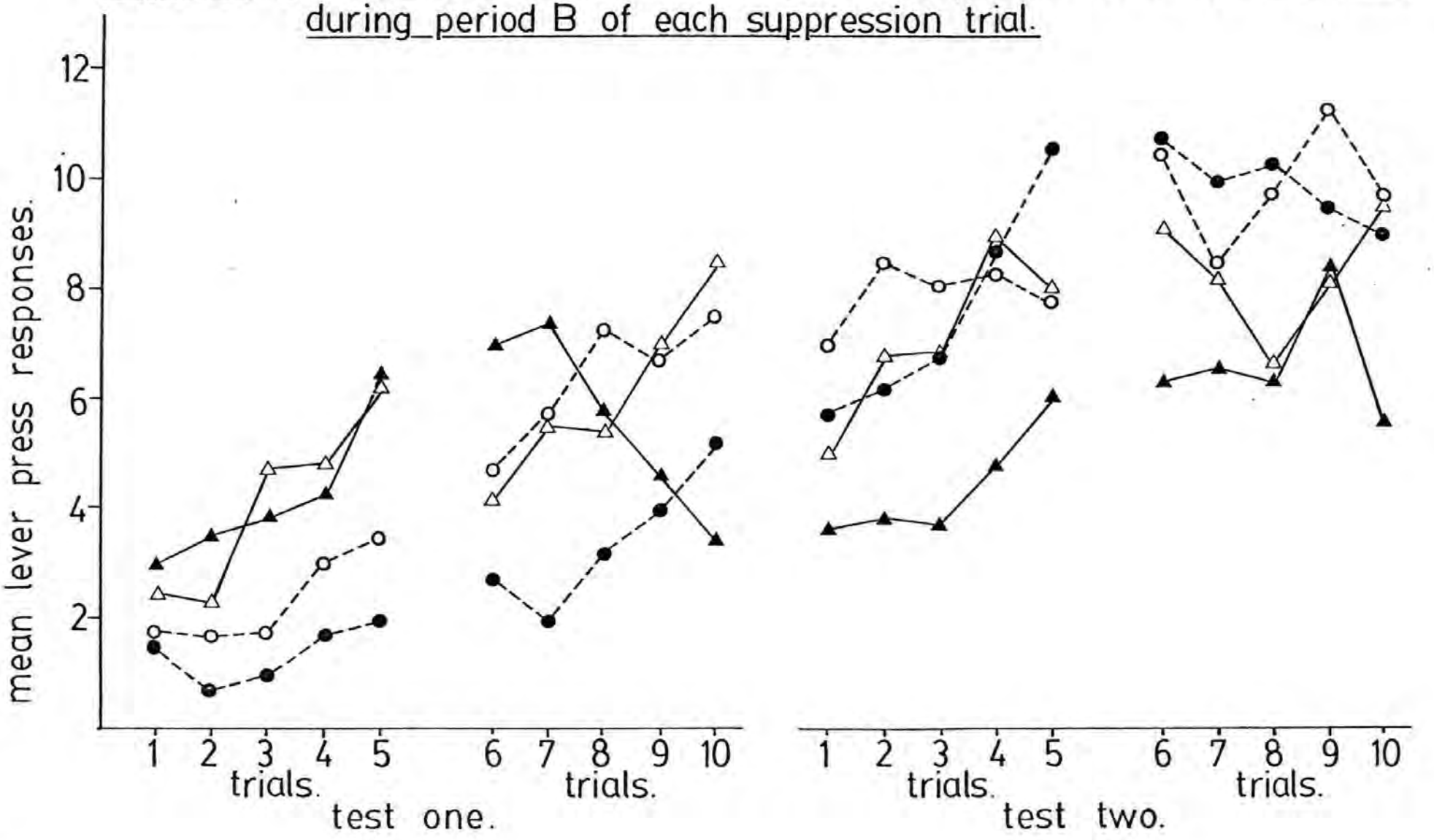
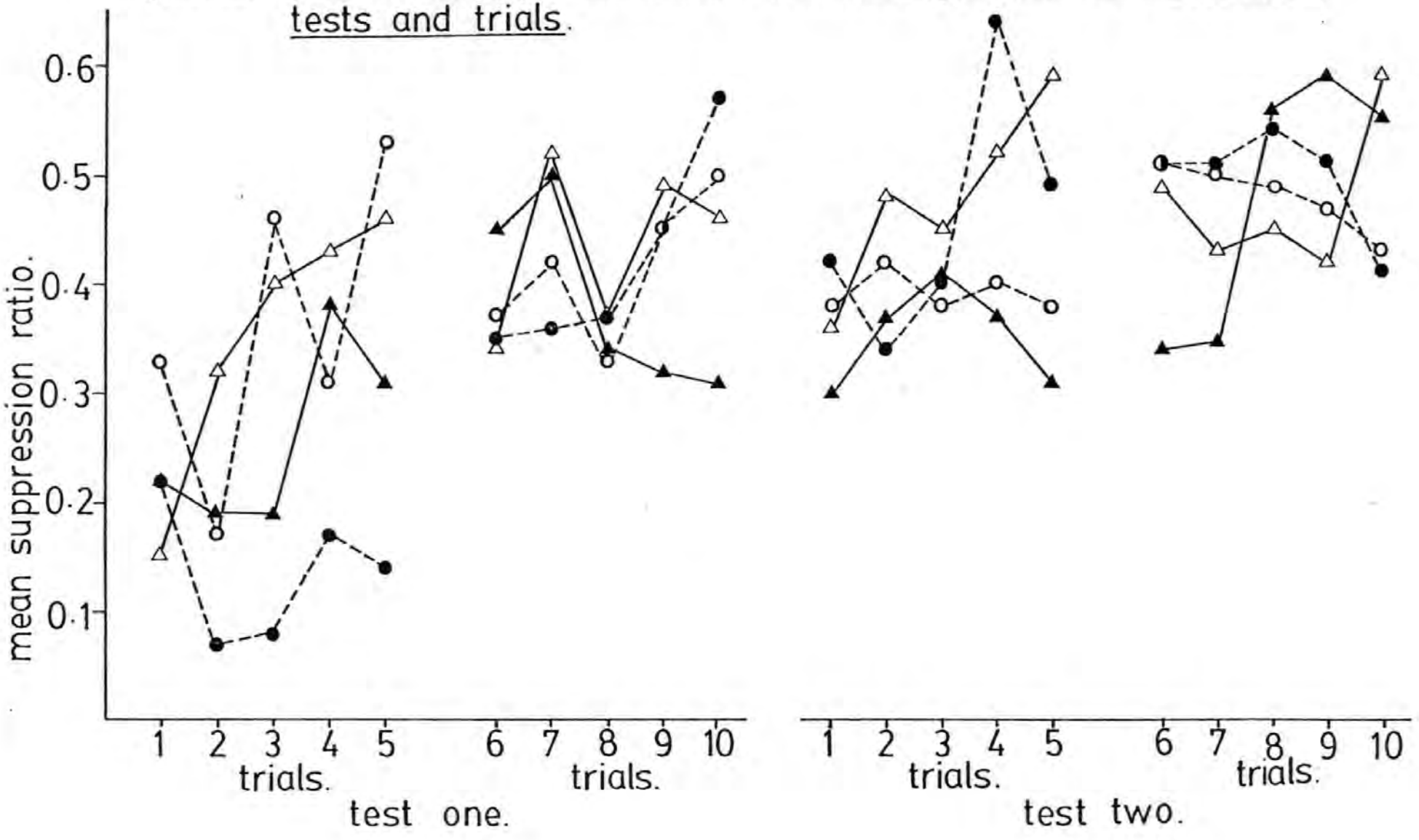


Figure 15 The mean suppression ratio for each group as a function of tests and trials.



(6) 24 hours after the first suppression test each rat was returned to the Skinner box for the second suppression test;

(7) 24 hours later each rat was tested for extinction of the avoidance response in the shuttle box;

(8) the extinction test was repeated 24 hours later.

Results

Acquisition of the avoidance response

The performance of animals during acquisition was recorded using five measures. These were: the number of trials taken to reach criterion, the number of avoidance responses, escape responses and failures to respond whilst reaching criterion and the number of foot-shocks received. The data from each subject are presented in Table A25. Data were analysed using a parametric one way analysis of variance (Winer 1962) and the outcomes are presented in Table A26. There were no significant differences between groups during acquisition.

Lever pressing performance

Table A27 and Figures 13, 14 and 15 show the number of lever presses made by each rat during periods A and B on each suppression trial. The data for each group were summed across subjects to give the sum, mean, standard deviation and standard error of responses during each period on every trial.

To examine comparability of response rates across groups at the beginning of the first suppression test the number of responses made during Period A of the first suppression trial were compared. Home cage saline rats ($\bar{X}$ = 5.25) did not differ significantly from home cage LVP rats ($\bar{X}$ = 5.125) ($t = 0.0602$, $df = 14$). Response prevented saline rats ($\bar{X}$ = 10.86) made significantly fewer responses than home cage saline controls ($t = 2.3016$, $df = 13$, $p < 0.05$) and response prevented LVP rats (12.33) did not differ significantly from response prevented saline rats ($t = 0.6599$, $df = 14$).

For the main statistical comparisons the suppression tests were divided into blocks of five trials (see Table A28). The mean number of responses per subject during Period A were analysed as a function of trials and groups using analysis of variance (see Table A29). Significant overall F ratio was followed by multiple comparisons using Neuman Keuls test (Table A32) (Winer 1962).

In Test 1, trials one to five, there was a significant effect of treatments ($p < 0.010$) and trials ($p < 0.05$). During trials 6-10 of Test 1, there was a significant effect of treatments ($p < 0.01$) but not of trials. In Test 2, trials 1-5, there was no effect of either

treatments or trials, but during trials 6-10 of Test 2 there was a significant effect of treatment ($p < 0.01$).

Responding during the B periods of each suppression trial (Table A28) was analysed in an identical way (Table A30). During Test 1, trials 1-5, there was a significant effect of treatment ($p < 0.01$) and trials ($p < 0.01$). In Test 1, trials 6-10, there was a significant effect of treatment ($p < 0.05$) but no effect of trials. In Test 2, trials 1-5, there was a significant effect of treatment ($p < 0.01$) and trials ($p < 0.01$). In Test 2, trials 6-10, there was an effect of treatment ($p < 0.01$) but not trials.

A suppression ratio (sr) was calculated for each animal on each trial using the formula described previously ($sr = \frac{B}{A+B}$) (see Table A28). The mean ratio for each trial was then analysed as a function of trials and treatments (see Table A31) as described for period A and B data. There was a significant effect of treatments ($p < 0.01$) in Test 1, trials 1-5, and in Test 2, trials 1-5 ($p = 0.05$).

Selected comparisons are given below in Table 1 (see also Table A32).

Trials effects can be seen in Figures 13, 14 and 15. During Test 1, trials 1-5, Newman Keuls comparisons showed more period A responses during trial 1 than on trials 2, 3, 4 or 5 (all p's < 0.05). There were also fewer period B responses during trials 1 and 2 than during trial 4 (p's < 0.05). Similarly there were fewer period B responses during trials 1, 2, 3 and 4 than in trial 5 (p's < 0.05). In Test 2, trials 1-5, there were fewer period B responses made during trial 1 than in either trial 4 or 5 (p's < 0.05). There were also fewer responses made during trial 3 than trial 5 ($p < 0.05$).

Extinction of avoidance responding

The data from extinction tests were analysed in the manner described in Experiment One. Data from each subject are presented in Table A33 and in Figures 16, 17 and 18. The data were summed across subjects to obtain the mean number of responses made on each extinction trial per group (see Table A34) and were analysed using Freidman's two way analysis of variance (Seigel 1956). In Extinction Test 1 there were significant effects on the total responses ($p < 0.01$) and short avoidances ($p < 0.01$). During Test 2 there were significant effects on total responses ($p < 0.001$), short avoidances ($p < 0.05$) and long avoidances ($p < 0.05$). Multiple comparisons (Hollander and Wolfe 1973) (see Table A35) indicated that all significant effects were due to differences between home cage animals and response prevented animals with no significant effects of peptide treatment. Response prevented LVP treated rats made significantly fewer total avoidance responses than home cage

Table 1: Selected outcomes from Newman Keuls comparisons on lever pressing data. Table shows the relative response rate between selected groups where differences are significant ($p < 0.05$)

	Responses	Trials 1-5	Trials 6-10	Trials 1-5	Trials 6-10
Home cage saline	A	ns	Hcs > Rps	ns	ns
versus	B	Hcs < Rps	ns	ns	Hcs > Rps
Response prevented saline	SR	ns	ns	ns	ns
Home cage saline	A	ns	Hcs > Hcl	ns	ns
versus	B	Hcs > Hcl	Hcs > Hcl	ns	ns
Home cage LVP	SR	Hcs > Hcl	ns	ns	ns
Response prevented saline	A	ns	Rps < Rpl	ns	ns
versus	B	ns	ns	Rps > Rpl	Rps > Rpl
Response prevented LVP	SR	ns	ns	Rps > Rpl	ns
Home cage LVP	A	Hcl < Rpl	Hcl < Rpl	ns	Hcl > Rpl
versus	B	Hcl < Rpl	Hcl < Rpl	Hcl > Rpl	Hcl > Rpl
Response prevented saline	SR	Hcl < Rpl	ns	ns	ns

Figure 16 Total avoidance responses during extinction testing. (mean and s.e.m.)

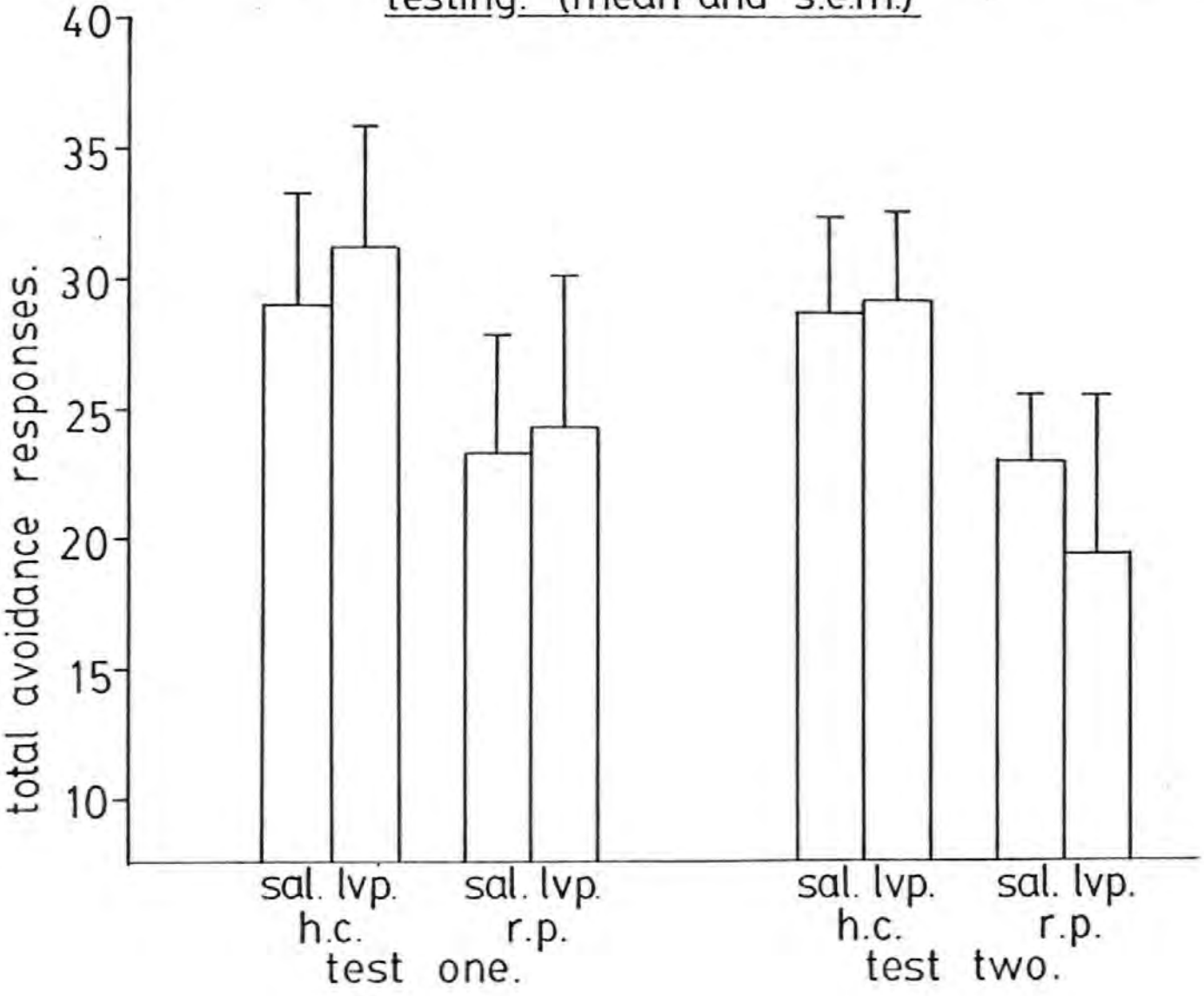


Figure17 Short avoidance responses during extinction testing. (mean and s.e.m.)

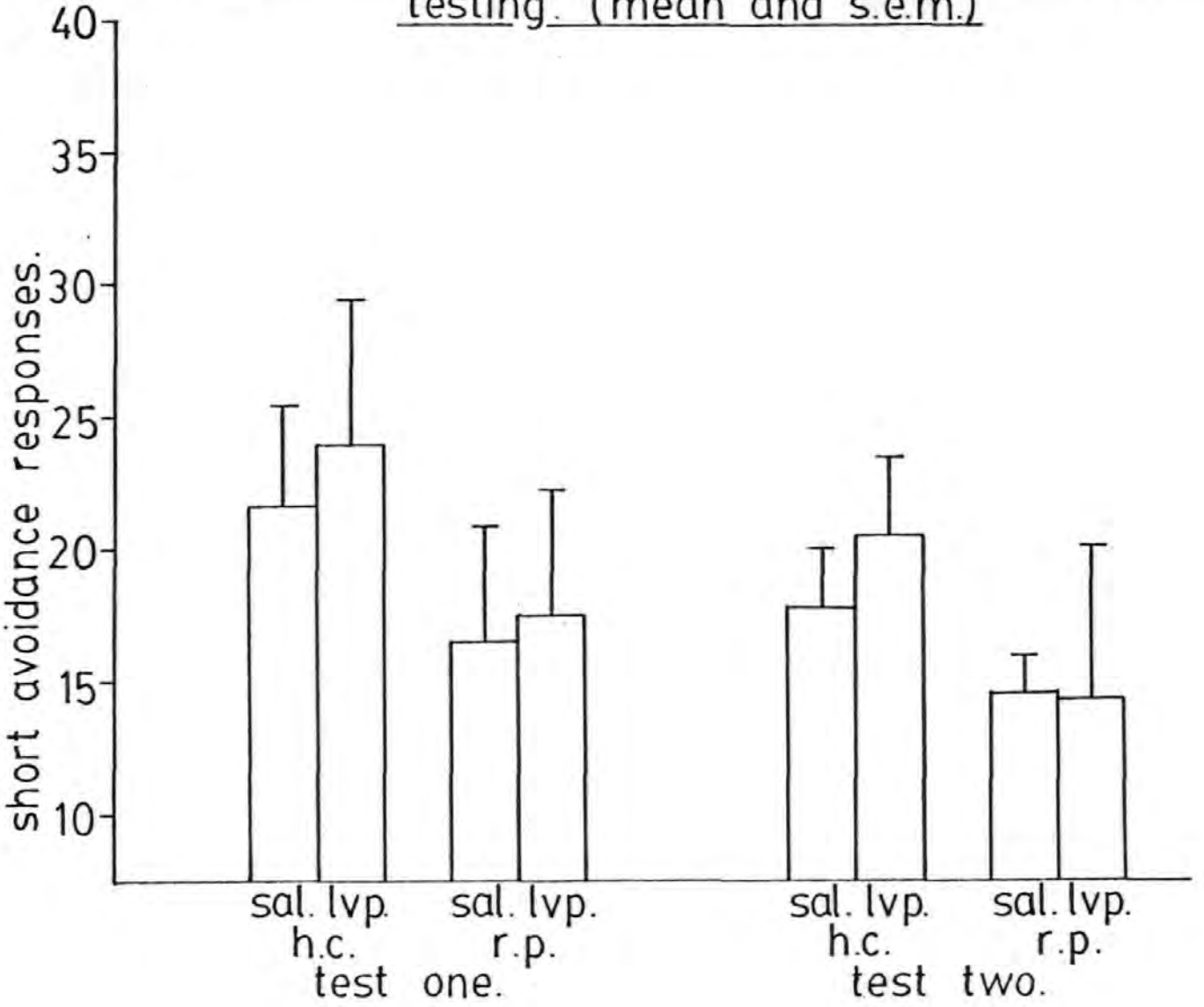
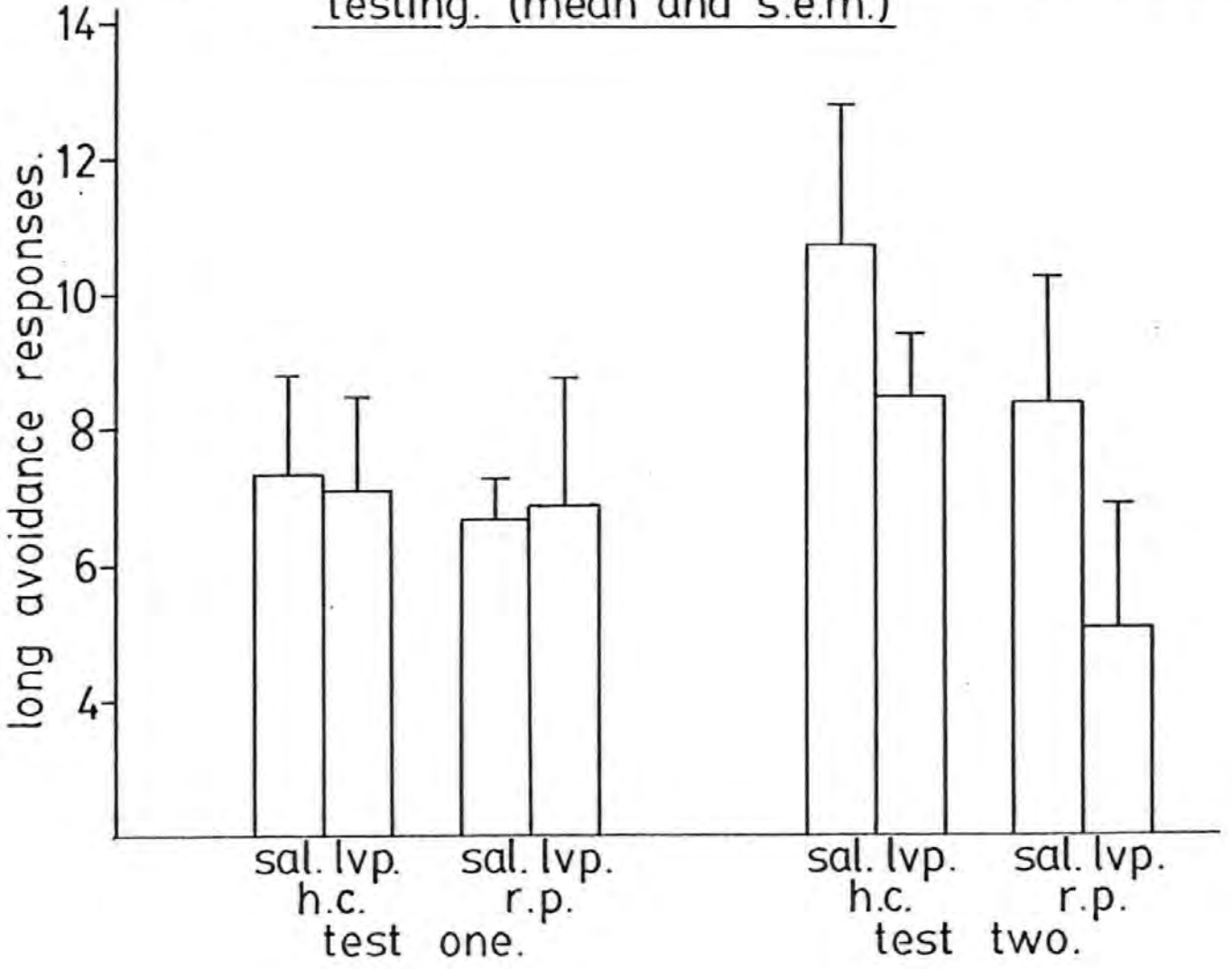


Figure 18 Long avoidance responses during extinction testing. (mean and s.e.m.)



LVP treated rats in Test 1 ($p < 0.029$) and Test 2 ($p < 0.01$). Furthermore, they made fewer short avoidances in Test 1 ($p < 0.01$) and Test 2 ($p < 0.029$) and fewer long avoidances in Test 2 ($p < 0.029$). Response prevented saline rats made fewer total responses than home cage LVP rats in Test 1 ($p < 0.029$). In Test 2 response prevented LVP rats made fewer total avoidance responses than home cage saline rats ($p < 0.01$).

Discussion

Figure 14 shows that during the initial CS presentation (period B) the response rate was low and gradually increased as a function of repeated CS presentations; this conclusion is supported by the significant trials effects in the period B data. In Test 1, trials 1-5, the response rate during trial 4 was significantly higher than on trials 1 and 2 ($p's < 0.05$). Similar effects were found in the first five trials of Test 2, period B responding was greater on trial 4 than on trial 1 ($p < 0.05$) and greater on trial 5 than on either trials 1 or 3 ($p < 0.05$).

Reduced responding was not restricted to periods in which the CS was superimposed. The data in Figure 13 clearly show that period A responding in all treatment groups dropped markedly after the first CS trial. During Test 1 responding on trial 1 was significantly greater than on trials 2, 3, 4 and 5. This contrasts with response rats in the B periods during these trials which did increase with repeated CS presentations. Figure 13 shows a slight though non-significant increase in period A responding. Therefore changes in responding were not restricted to the periods of CS presentation but generalized to the inter CS periods. The response reduction during CS presentations tended to extinguish more rapidly than the changes during the inter CS periods (A).

Comparison of lever press response rates in the 20 seconds which immediately preceded the first CS presentation showed that the initial rate for response prevented saline treated rats was significantly greater than for home cage saline controls ($p < 0.05$). This difference cannot be attributed to differences in reaction to the CS. However, there were components of the shuttle box attached to the transparent walls of the Skinner box for presenting the CS concurrently. Higher pre-CS response rates in response prevented groups suggest a change in the status of these components of the training environment.

Response prevention did not change suppression ratios relative to home cage saline controls during either Test 1 or 2. This contrasts with the result reported by Monti and Smith (1976) who found that response prevention significantly reduced suppression compared to non-prevented rats. In addition, Monti and Smith (1976) did not find that

response prevention increased the pre-CS response rate. Conflicting results from the present study and that of Monti and Smith (1976) may reflect procedural differences. In particular, Monti and Smith (1976) used only 15 prevention trials, tested suppression immediately afterwards and used a much longer inter-CS interval (four minutes).

The conflicting results from these experiments could be interpreted as differences in the interactions between the avoidance CS and operant responding resulting from schedule and treatment differences rather than as conflicting accounts of whether or not fear of the CS or apparatus cues are extinguished during prevention trials.

Extinction tests confirmed the results of previous experiments (see Experiments One and Three) that response prevention reduced avoidance responding in extinction. Failure to find differences in suppression ratios did not therefore reflect an ineffective response prevention procedure. Monti and Smith (1976) did not report the effects of their procedure on avoidance responding in extinction.

LVP injected after home cage retention did not alter pre-CS operant response rates. This suggests that although LVP and response prevention had similar effects on extinction responding when given separately (Experiment Three) they could be distinguished by their action on the operant baseline. This interpretation was supported by the finding that relative to saline LVP significantly increased period B responding and suppression ratios (p's < 0.05) in Test 1, trials 1-5, but did not affect period A responding during these trials. Failure to observe changes in the period A response rate between these groups suggests that the significant difference in suppression ratios must be attributed to the significant decrease in the period B response rate under LVP and not to differences in the baseline sensitivity of each group to the suppressive effects of the CS (Blackman 1968, 1974). As Test 1 proceeded into trials 6-10 home cage LVP treated rats retained their significantly lower period B response rates, although LVP also reduced period A responding during these trials (both p's < 0.05), thereby abolishing the effect of the peptide on suppression ratios observed in trials 1-5 of Test 1. Home cage LVP rats did not differ from home cage saline rats during Test 2; peptide effects on the operant rate therefore extinguished relatively rapidly. This rapid extinction coupled with additional CS exposure during the second test may account for the absence of any effect of the peptide during subsequent extinction testing.

Vasopressin induced changes in the operant rate and suppression ratios are novel and considered in isolation may be interpreted in the theoretical terms used by Kamin et al (1963) and Monti and Smith (1976)

to explain changes in the operant rate, ie that LVP increased conditioned fear of the CS. Indeed this would fit the many observations (see Chapter 2) that post learning injections of LVP significantly increased subsequent extinction responding. However, using identical training and treatment procedures it was found in Experiment Three that LVP reduced avoidance responding in extinction. This result is not compatible with an interpretation of LVP's effect on extinction responding in terms of increased conditioned fear, at least within the terms of two factor avoidance theory (Mowrer 1947; Miller 1948; Rescorla and Solomon 1967). Post training LVP injections clearly altered the status of the CS measured by operant response changes; however, the relationship between this and the peptide's effects on avoidance extinction remain to be clarified.

Relative to response prevented saline controls, the LVP treated rats did not differ in any aspect of their operant response rate during trials 1-5 of Test 1. During trials 6-10, LVP significantly increased period A responding, although this did not persist throughout later trials. LVP after prevention did, however, exert persistent effects during Test 2 by reducing period B response rates during trials 1-5 and 6-10 ($p's < 0.05$). During trials 1-5 this reduction was sufficient to significantly reduce ($p < 0.05$) suppression ratios. Insofar as LVP reduced both period B responding and suppression ratios after response prevention its effects are the same as those seen after retention in the home cage. However, these effects were not evident until Test 2 in response prevented rats whereas in home cage rats they were found only in Test 1. Thus, although response prevention reversed the effects of LVP on extinction of the avoidance response compared to home cage retention (Experiment Three) it delayed but did not reverse the peptide effect on the operant rate and suppression ratios. Once again this configuration of changes argues against any change in an inferred central state and in favour of schedule induced changes in the local interactions between the operant and avoidance schedules.

The complexity of interactions between response prevention and LVP are further illustrated by comparing data from home cage LVP rats and response prevented LVP rats. In Test 1, trials 1-5, response prevented LVP rats had higher period A and period B response rates and showed greater suppression than home cage LVP controls (all $p's < 0.05$). Similarly, during trials 6-10 response prevented LVP rats had greater period A and period B response rates ($p's < 0.05$) than home cage LVP treated rats. However, during Test 2, this relationship was reversed; response prevented LVP rats showed lower period B rates during trials 1-5

and lower period A and period B rates during trials 6-10 (all p's < 0.05). Reversal of this relationship from Test 1 to Test 2 suggests that when prevention and LVP are combined the outcome is not simply to reverse the effects of LVP upon some inferred psychological state, as may be suggested from considering the avoidance extinction data in isolation. Rather the data indicate that a complex interaction between the avoidance and the operant schedule is further complicated by altering the status of the CS using LVP. The reversal of the relationship between home cage LVP rats and response prevented LVP rats on operant rate between Tests 1 and 2 reflects a large increase in the period A response rate of home cage LVP rats between tests combined with a slightly decreasing rate for response prevented LVP rats. A similar pattern is evident for the period B data. Thus preceding LVP injections with a period of response prevention not only reverses the effect of LVP on avoidance extinction (Experiment Three) but also delayed recovery of period A and B response rates. In Experiment Three there is also evidence that the combination of response prevention and LVP is not the reversal of one simple effect. Whereas both LVP and response prevention, in isolation, reduced extinction responding throughout the three extinction tests, the combination of these treatments increased extinction responding but the increase was sustained only over two extinction tests and not over three.

The results may be summarised as follows. Response prevention increased the pre-CS response rate and also showed inconsistent effects on period A and period B response rates but did not affect suppression ratios as was reported by Monti and Smith (1976) and was predicted from deductive theories which suggest that changes in the suppression ratio indicate correspondent changes in conditioned fear (Kamin, Brimer and Black 1963; Rescorla and Solomon 1967; Monti and Smith 1976). If these deductive arguments are correct, it must be assumed that failure to confirm their predictions and the observations of Monti and Smith (1976) in the present experiment is due to a number of procedural factors which have been outlined. As an alternative, it has been argued that neither operant baseline changes or suppression changes reflect changes in inferred psychological states. Instead the results of the present experiment and those reported by Monti and Smith (1976) may be reconciled by theories which interpret operant rate changes and suppression changes in terms of interactions between the concurrent operant and avoidance schedules. The form of such interactions being dictated by schedule characteristics, the changes which have been observed may be attributed to changes in these schedule characteristics. This interpretation has been extended to classical-operant interactions by Henton and Iversen

(1978) and is closely allied to the competing response hypothesis of Brady and Hunt (1955).

When given to home cage retained rats LVP reduced period B responding and suppression ratios relative to saline treatment; there was no effect of the peptide during Test 2 or during subsequent extinction testing. From these data, and taking into account the observations of Experiment Three, it was argued that LVP altered the status of the CS. This effect could be detected with extinction testing (Experiment Three) or with the concurrent presentation of the CS during the operant schedule. Whereas the former test, in which performance of the response is possible, yielded long term effects of the peptide, the latter test, in which responding was not possible, rapidly extinguished the peptide effect. Again the data are not compatible with the theoretical position of deductive accounts but could be interpreted in terms of alterations in the local schedule interactions.

The interaction between response prevention and LVP were relatively straightforward during the extinction tests of Experiment Three (see also King and De Wied 1974). In the case of the operant rate tests, LVP had a similar effect after response prevention and after home cage retention. Period B responses and suppression rates were reduced, but in the case of response prevented rats this did not appear until the second test whereas in the home cage rats these effects were apparent in Test 1. The complexity of the interaction between these two treatments was apparent when home cage LVP and response prevented LVP rats were compared. Differential rates of change in period A and B response rates in these two groups contributed to a reversal in the magnitude of their respective response rates. The complexity and the direction of the operant rate and suppression ratio changes combined with the direction of the changes seen in the avoidance extinction data of the present experiment and Experiment Three do not fit an explanation in terms of changes in a single psychological construct such as conditioned fear or memory consolidation.

CHAPTER FIVE

THE EFFECTS OF VARYING POST TRAINING INJECTION INTERVALS, DOSES AND PEPTIDE STRUCTURE

5.0 Introduction

Five experiments are reported, the first showing that post training sensitivity to the response reducing effects of LVP (1 µg) is maximal one hour after training (Experiment Five). Tests with higher doses (Experiment Six) suggested that in the range 2-4 µg the dose response curve is negative when injected 30 minutes after training. A wider dose range was therefore examined and Experiment Seven, using a modified training and test procedure, showed that the dose response curve for 0.036-2.97 µg is an inverted U shape at this interval.

Opposite effects of 0.11 µg and 2.97 µg were still seen after training at a higher shock level (0.45 ma) (Experiment Nine). Furthermore, although 0.11 µg increased extinction responding when injected immediately 30 or 60 minutes after training, 2.97 µg was ineffective immediately after training, decreased responding when injected after 30 minutes and increased responding when injected after 60 minutes (Experiment Eight). The response reducing effects of various LVP doses do not appear to be mediated by classical endocrine effects of the peptide because none of the doses of DG-LVP which were tested increased subsequent extinction although several reduced it (Experiment Ten).

5.1 Experiment Five: The Effects of Varying the Interval between LVP Injections and Training or Response Prevention on Avoidance Extinction

Introduction

In Experiment Two it was shown that LVP (1 µg) increased avoidance responding in extinction when injected immediately after training in agreement with results reported in the literature (see Chapter Two). In Experiment Three rats were trained on the same schedule and injected after 30 minutes spent in the home cage. In this case LVP reduced extinction responding, a result which does not support the hypothesis that LVP enhances the consolidation of memory (see Chapter Two).

The conflicting results from Experiments Two and Three suggest that varying the interval between training and injection may alter the effect of LVP on extinction other than by a simple time dependent decrement as studies in Section 2.2 suggest. Therefore, in order to

re-examine this question LVP (1 µg) was injected at several intervals after training.

Response prevention reduced extinction responding when given alone (Experiment One) or when followed by a saline injection (Experiment Three). These results are in agreement with the effects of prevention trials which have been widely reported in the literature (see Chapter Three). Response prevention followed immediately by an LVP injection increased extinction responding relative to response prevention followed by saline (Experiment Three). This is in agreement with the results reported by King and De Wied (1974) who gave peptide before response prevention. Thus, comparing the results from King and De Wied (1974) with those of Experiment Three suggests that, relative to saline, LVP increases responding whether it precedes or follows a period of response prevention. In this respect, the LVP effect on response prevented rats resembles that for non prevented rats described in the literature (see Chapter Two). According to data from extinction testing (Experiment Three; King and De Wied 1974) LVP appears to counter the effect of response prevention, despite the evidence (Experiment Three) that given separately the effects of these treatments may be identical. However, the data from Experiment Four clearly distinguish LVP and response prevention by their differential effects on the operant response rate. Furthermore these data suggest that the interaction between LVP and response prevention cannot be interpreted as the summation of two effects on an inferred psychological state.

As LVP and response prevention may affect different aspects of behaviour and as the effect of LVP on extinction may be reversed when peptide treatment is preceded by prevention trials it was of interest to determine if this reversal varied as a function of the interval between prevention and injection and if the direction and magnitude of any changes were comparable to those seen in non prevented rats.

The basic design of Experiment Three was repeated in the present experiment. Rats were trained to a criterion of ten correct consecutive avoidances, half were randomly selected for response prevention (RP) and half for no behavioural treatment (home cage). Rats retained in the home cage were injected with either saline or LVP immediately (0 mins), 30 mins, 60 mins, 6 hours or 24 hours after the end of training. Response prevented rats were given 30 trials of response prevention followed by saline or LVP at one of the intervals mentioned previously. The interval was timed from the end of the behavioural procedure, whether or not this included response prevention.

Methods

Subjects

Adult male cfhb Wistar rats (250-350 g) were housed three or four to a cage and maintained on ad lib food and water. One hundred and sixty rats were used, 40 supplied by Anglia Laboratories Limited and the remainder taken from the Plymouth Polytechnic closed colony; 25 rats failed to reach the learning criterion and were dropped from the experiment.

Apparatus

The two way shuttle box and the schedule used have been described in detail in Experiment One.

Training

Each day rats were brought from the animal house to the laboratory at approximately 9.15-9.30 am. The experiment was run in a series of replications, each starting on a Monday. Animals were first weighed and then placed in the shuttle box for five minutes of adaptation to the environment. Training was according to the schedule described in Experiment One and continued for a maximum of 50 trials on each of two consecutive training days or until the animal had made ten consecutive correct responses. Subjects which did not attain the criterion within the hundred trials were dropped from the experiment.

Treatment

Having attained the criterion, half of the animals were randomly selected to receive 30 trials of response prevention, according to the method described in Experiment One. After response prevention animals were removed from the shuttle box and returned to the home cage. These animals were injected with either saline (0.5 ml physiological saline SC) or LVP (1 µg/0.5 ml physiological saline SC). The batch details, preparation details and method of administration have been described in Experiment Two. Rats were randomly allocated to receive injections immediately (0 mins), 30 mins, 60 mins, six hours or 24 hours after the end of response prevention. The remaining rats were returned to the home cage immediately after training injected with either saline or LVP after one of the intervals described.

Testing

Twenty-four hours after injection rats were returned to the shuttle box for 50 extinction trials (Test 1) and 24 hours later this was repeated (Test 2). The extinction test procedure has been described in detail in Experiment One.

Results

Acquisition performance

Groups were compared on five aspects of their performance during training, the number of trials taken to reach criterion, the number of avoidances, escapes and failures to respond whilst attaining the criterion and the number of shocks received. These data are presented in Table A36. Groups were compared on each of these measures using analysis of variance (Winer 1962) and these analyses are summarised in Table A37. There were no significant differences between groups on any of the measures used.

Extinction

The technique for analysing extinction data has been described in Experiment One. Briefly, responses for each subject were summed across five successive extinction trials. Group totals per trial block were computed by summing across subjects to obtain the total number of responses per group in each block of five trials. These data are presented in Table A38. The total number of responses in each trial block is the sum of the short avoidances (latency $\leq$ 10 secs) and the long avoidances (latency $\geq$ 10 secs). Groups were then compared statistically for differences in each class of response during extinction Tests 1 and 2 using Friedman's analysis of variance (Siegel 1956) followed by multiple comparisons between groups using the method outlined by Hollander and Wolfe (1973).

There were significant treatment effects in the total number of avoidances made during Test 1 ($p < 0.001$) and Test 2 ($p < 0.001$). Similarly, there were significant treatment effects in the number of short avoidances in Test 1 ($p < 0.001$) and in Test 2 ($p < 0.001$). There were no significant treatment effects on the number of long avoidance responses made in either test. The results of these tests are summarised in Table A .

Multiple comparisons were made between groups using the method of Hollander and Wolfe (1973) based on the differences between rank sums of groups (see Table A40) exceeding a critical difference (see Table A40) with α set experimentwise at 0.05. This method allows all possible comparisons to be made; however, as only selected comparisons are of interest, these are presented in Table A41.

Changes in the mean number of total responses and short avoidances during Test 1 as a function of the injection interval are shown in Figures 19 and 20 respectively.

The differences between home cage saline groups were evaluated in

Figure 19 Total number of avoidances in extinction test one.

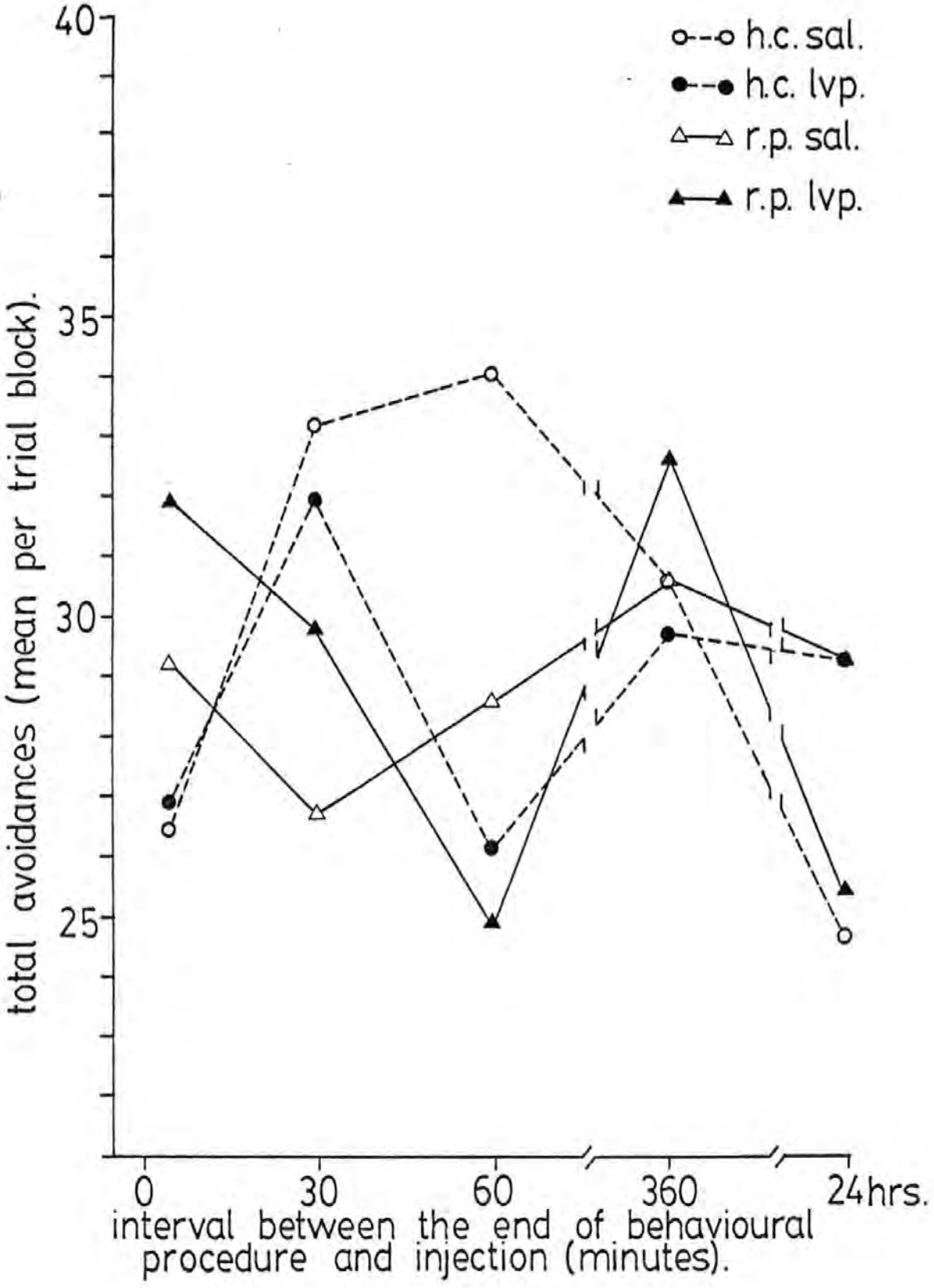
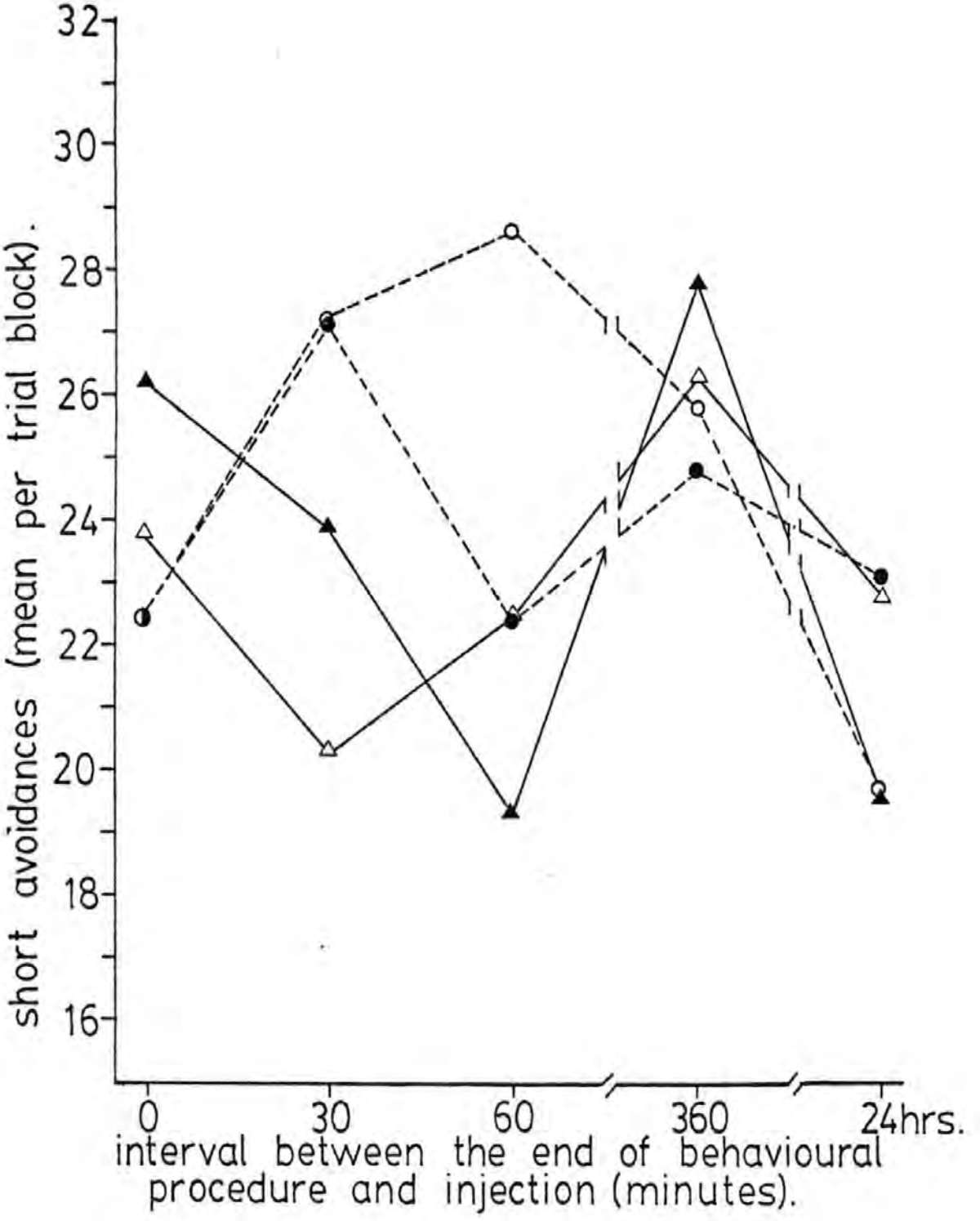


Figure 20 Mean number of short avoidances in extinction test one.



order to establish the effects of varying the time of saline injection after training. In Test 1 animals injected immediately after training (Hc sal 0) made significantly fewer total avoidances than animals injected after 30 minutes ($p < 0.05$) or 60 minutes ($p < 0.05$); there were no differences in the number of short avoidances. When animals were injected 24 hours after training they made significantly fewer total avoidances than those injected either 30 minutes ($p < 0.01$) or 60 minutes ($p < 0.05$) after training. Similarly rats injected 24 hours after training made significantly fewer short avoidances than those injected either 30 ($p < 0.025$) or 60 minutes ($p < 0.01$) after training. During Test 2 these differences had disappeared. Therefore during Test 1 animals injected immediately after training responded in extinction at a rate comparable to rats injected after 24 hours. In contrast, when injection was delayed for either 30 minutes or 60 minutes after injection responding was significantly increased. These effects can clearly be seen in Figures 19 and 20.

The differences between response prevented saline groups were evaluated in order to establish the effect of saline injections when a period of response prevention intervened between the end of training and the injection (see Table A41, Section 2). Figures 19 and 20 show that the rate of Test 1 extinction responding tended to decrease to a minimum at the 30 minute interval and thereafter to increase. There were no significant differences between response prevented saline groups in the total number of avoidances made during Extinction Test 1 but rats injected 30 minutes after response prevention made significantly fewer short avoidances ($p < 0.05$) than animals injected after six hours. This difference was greater during Test 2 as response prevented rats injected 30 minutes after prevention made significantly fewer total responses ($p < 0.025$) and short avoidances ($p < 0.01$) than animals injected after six hours. The data from response prevented saline injected rats therefore suggest that when a period of response prevention intervenes between training and injection this counters time dependent effects of the saline injection which were evident in home cage saline rats. Extinction responding tends to decrease and then significantly increase as a function of the increasing interval.

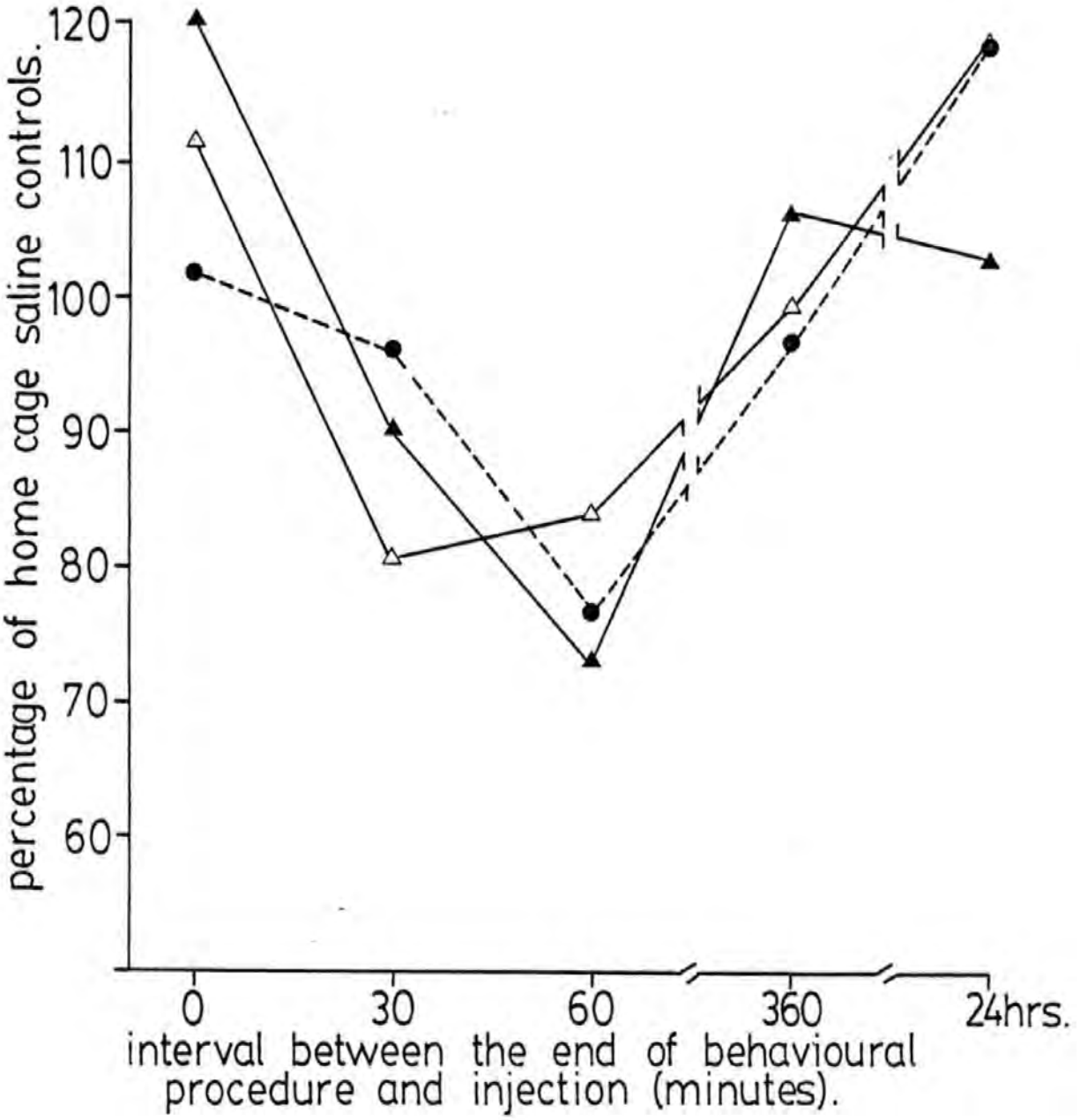
Comparing home cage saline groups with response prevented saline rats confirmed the response reducing effect of prevention trials (see Table A41, Section 5). During Test 1, response prevented rats injected after 30 minutes made significantly fewer total avoidances (both $p's < 0.05$) and short avoidances (both $p's < 0.01$) than home cage animals injected after 30 or 60 minutes. During Test 2 response prevented rats

injected with saline after 30 minutes maintained their low rate of responding and made significantly fewer short avoidances than home cage rats injected 60 minutes ($p < 0.01$), six hours ($p < 0.025$) or 24 hours ($p < 0.01$). Additional Freidman tests shows that response prevented rats injected with saline immediately after prevention made significantly fewer short avoidances ($p < 0.029$) than home cage saline rats injected 30 minutes after training, confirming the results of Experiment Three. However, when these data formed a subset of the overall experimental analysis (see Table A41, Section 5) the difference did not achieve significance, reflecting non-monotonicity in the Friedman test (Hollander and Wolfe 1973, p 118).

The significant differences in Test 1 extinction performance between home cage rats given saline injections at various intervals after training (see earlier discussion) were abolished by LVP (1 µg) (see Table A41, Section 3). During Test 2, however, animals injected with LVP immediately after training made significantly fewer total avoidances than rats injected 30 minutes ($p < 0.025$), 60 minutes ($p < 0.01$), six hours ($p < 0.01$) or 24 hours ($p < 0.01$) after training. Similarly, immediately injected rats made significantly fewer short avoidances than those injected 60 minutes ($p < 0.01$), six hours ($p < 0.01$) or 24 hours ($p < 0.05$) after training.

When response prevented rats were injected with saline the subsequent between group differences were mainly seen in the short avoidance data (see Table A41, Section 2). Furthermore, responding in both Tests 1 (see Figures 19 and 20) and 2 (see Table A38) first tended to decrease with the intermediate intervals (30 and 60 minutes) then increase with the six hour injection. When response prevention was followed by 1 µg of LVP this pattern was accentuated (see Figure 21). Statistical analysis (Table A41, Section 4) shows that in Test 1 rats injected 60 minutes after prevention made significantly fewer short avoidances than those injected immediately ($p < 0.025$) or six hours ($p < 0.01$) after injection. Rats injected immediately responded at a similar rate to those injected after six hours and both groups made significantly more short avoidances than those injected after 24 hours (p's < 0.025 and < 0.01 respectively). In Test 2 response prevented rats injected with LVP after 30 minutes made significantly fewer total avoidances ($p < 0.05$) and short avoidances ($p < 0.05$) than those injected after six hours. Therefore in Test 1 when response prevention was followed by LVP (1 µg) the rate of short avoidance responding varied in a U shaped function as the interval between prevention and injection increased. This time dependent function in the short avoidance data is opposite to that seen

Figure 21 Extinction test one: Total avoidances per group (percentage of home cage saline controls) as a function of the injection interval.



in the total avoidance data from the home cage saline controls (see Figures 19 and 20).

The effects of LVP were compared to the effects of saline in home cage animals (see Table A41, Section 7) and in no case did LVP increase the extinction response rate relative to saline. On the contrary, when significant differences did occur LVP reduced the response rate. In Test 1 rats injected with LVP immediately after training made significantly fewer total avoidances than rats injected with saline 60 minutes ($p = 0.05$) after training. Similarly, those rats injected with LVP after 60 minutes made significantly fewer total avoidances than those injected with saline either 30 minutes ($p < 0.01$) or 60 minutes ($p < 0.01$) after training. During Test 2, rats injected with LVP immediately after training made significantly fewer total avoidances than those injected with saline either 60 minutes ($p < 0.01$) or 24 hours ($p < 0.01$). Similarly, rats injected with LVP immediately after training made significantly fewer short avoidances than those injected with saline after 60 minutes ($p < 0.01$), six hours ($p < 0.01$) or 24 hours ($p < 0.01$).

During Test 1 there were no significant differences in the total avoidance responding made by response prevented saline and LVP treated rats. Differences in short avoidance responding between these two groups are summarised in Table A41 (Section 8).

Discussion

When the interval between the end of training and saline injection was increased, in rats which had been detained in the home cage, responding during Extinction Test 1 was lowest in groups which had been injected immediately or 24 hours after training. For groups with an intermediate interval the response rate was higher (see Figures 19 and 20). The statistical analysis of the data from Extinction Test 1 shows that response rates varied as an inverted U shaped function of the interval between the end of training and saline injection. This pattern was less clear in Test 2 although immediately injected rats still tended to make fewer responses than those injected after 60 minutes. The basis for this effect is unknown but may be related to the stress of handling and injection. Reports of endocrinological changes following handling stress in rats have recently been confirmed by van Dijk using a radioimmunoassay to measure plasma ACTH (van Dijk 1979, personal communication). In addition, Riffee et al (1979) have found that saline injections reduce locomotor activity and that both handling and pre injections with saline could alter behavioural arousal (composite locomotor activity) induced by apomorphine and dextroamphetamine. Therefore pituitary adrenal

activation or stress induced arousal changes may play a part in mediating the differences seen in extinction between saline injected animals, possibly by interacting with post training changes in the behavioural substrate. Kamin (1957) found that when rats were partially trained on an avoidance schedule and were returned for retraining at various intervals after the first session, then the level of avoidance responding during retraining varied as a function of the intervening interval. The intervals used by Kamin (1957) were the same as those in the present experiment; furthermore, responding in the Kamin (1957) study was minimal at the 60 minute interval, the interval showing maximum avoidance response rates in the present experiment and maximal sensitivity to the response inhibiting action of LVP (see Figure 21).

When LVP was injected there were no significant differences between home cage LVP groups during Test 1, although when injected immediately after training LVP significantly reduced total avoidance responses in Extinction Test 2 relative to all other LVP home cage groups. A similar pattern was seen in the short avoidance data. These data indicate that LVP disrupts the time dependent effects of saline injections. In Test 2 immediately injected rats made significantly fewer responses than rats injected with LVP after any other interval.

Comparing home cage saline groups with home cage LVP groups at different intervals confirmed that LVP significantly reduces responding in Extinction Test 1, as seen in Experiment Three. However, in the present experiment the response reducing effect of LVP was evident only when injections were given 60 minutes after training.

The increase in responding seen in Experiment Two when LVP was given immediately after training was not replicated. In the present experiment home cage LVP and home cage saline rats responded similarly in Test 1 at all injection intervals except 60 minutes. The sensitivity of the 60 minute injection interval to the response reducing effect of LVP can be seen from Figure 21 in which the Test 1 total avoidance data for each group is plotted as a percentage of the home cage saline control performance over all injection intervals.

Response prevention tended to reverse the inverted U shaped function in the extinction performance of home cage saline rats in Test 1, suggesting an interaction between response prevention and the saline injection procedure. Comparing response prevented saline rats with the home cage saline controls confirms the response reducing effect of prevention treatment (Experiments One, Three and Four). Lowest response rates were found when saline followed response prevention by 30 minutes; this was confirmed in Test 2.

Combining response prevention with LVP injections reversed the relationship between the injection interval and Test 1 avoiding seen in the home cage saline rats. In the response prevented LVP treated rats Test 1 responding declined to a minimum at the 60 minute interval, yielding a U shaped relationship between response rate and interval (see Figure 21). These data also suggest that at 60 minutes after training the animals are maximally sensitive to the response reducing effect of LVP. Response prevention is capable of reversing, only for a limited time, the response reducing effect of LVP (see Experiment Three).

Several aspects of the data point to sensitivity changes after training or response prevention. The 'placebo' effect of saline on Test 1 avoidance rates was greatest when the injection was given after 60 minutes; the response reducing effect of LVP was maximal 60 minutes after the end of either training or prevention and, although response prevention reversed the response reducing effect of LVP when injected immediately or after 30 minutes, after 60 minutes the response reducing effect of LVP was once more prominent. Kamin (1957) pointed out the response deficits evident in partially trained rats 60 minutes after original training and Anisman (1975) has suggested that this may be related to neurochemical changes after training. Performance changes in the present experiment may reflect interactions between time dependent neurochemical changes and the treatment variables of saline, response prevention and LVP. Subsequent experiments examined the interactions between various peptide doses and treatment intervals.

5.2 Experiment Six: The Effect of LVP (2,3,4 µg) on Extinction Responding when Injected 30 Minutes after Training or Immediately after 30 Response Prevention Trials

Introduction

Evidence discussed in Chapter Two suggested that increasing the dose of vasopressin injected after passive avoidance training increased subsequent passive retention latencies in a dose dependent manner. Experiments Three and Six found that a 1 µg of LVP injected either 30 minutes (Experiment Three) or 60 minutes (Experiment Six) after shuttle box training led to significant decreases in extinction responding. It was decided to examine whether higher doses injected 30 minutes after training would increase or decrease extinction responding. In the present experiment rats were injected with either 2, 3 or 4 µg of LVP after training or response prevention trials.

Methods

Subjects

Seventy adult male CFHB wistar rats (250-350 g) from the colony maintained at Plymouth Polytechnic were housed three or four to a cage with ad lib access to food and water. Twenty-two rats failed to attain the learning criterion and were therefore discarded from this experiment, but used in a subsequent experiment.

Apparatus and schedule

These have been described in Experiment One. Rats were trained to make ten consecutive avoidances during a maximum of two training sessions consisting of 50 trials each and run on two consecutive days. Rats which achieved this criterion were randomly allocated to one of two conditions, 30 minutes retention in the home cage or 30 response prevention trials as described in Experiment One.

Treatment

After 30 response prevention trials or 30 minutes in the home cage rats were randomly allocated to receive either a saline injection or one of three doses of LVP. Control animals were injected with 0.5 ml of physiological saline (0.9%). Experimental animals received either 2, 3 or 4 microgrammes of LVP in 0.5 ml of physiological saline and prepared from the batch described in Experiment Two. All injections were SC.

Testing

Approximately 24 hours after injection, animals were returned to the shuttle box for 50 extinction trials (Test 1) and this was repeated on the following day (Test 2).

Results

Acquisition

Performance during training was compared on five measures; avoidance responses, escape responses, failures to respond, trials to criterion and shocks received in training (see Table A42) and analysed using analyses of variance (see Table A43). There were no significant differences between groups during the acquisition phase of the experiment.

Extinction

Table A44 shows the number of short avoidance responses (< 10 seconds), long avoidances (> 10 seconds) and the total number of avoidances (short plus long) responses made during extinction Tests 1

and 2. Responses were summed across every block of five trials for each rat. Totals summed across subjects in each group are shown in Table A45 and formed the basis of comparisons using Freidman's analysis of variance (Seigel 1956). The outcome of these analyses is shown in Table A46. There were significant treatment effects in the total avoidance data of Test 1 ($p < 0.001$) and Test 2 ($p < 0.001$). Similarly there were significant treatment effects in the short avoidance data from Test 1 ($p < 0.001$) and Test 2 ($p < 0.01$). There were no significant treatment effects in the long avoidance data.

Multiple comparisons between groups were made, using the method described by Hollander and Wolfe (1973), in order to locate significant effects (Table A46). Within the home cage groups animals given 4 µg of LVP tended to make fewer total responses than those given 2 µg ($p < 0.1$), suggesting a negative dose response relationship between the dose of peptide and subsequent extinction response levels. Although response prevention (plus saline) did not reduce response levels compared to those of home cage saline controls, data from response prevented rats supported the suggestion that the dose response relationship is negative as differences in extinction as a function of dose were more apparent in the response prevented rats. During Test 1, response prevented rats given 4 µg LVP made significantly fewer total avoidances ($p < 0.01$) and short avoidances ($p < 0.01$) than those given 2 µg. These differences were maintained in Test 2; rats given 4 µg made fewer total ($p < 0.01$) and short ($p < 0.01$) avoidance responses than rats given 2 µg. In Test 2, response prevented rats given 4 µg tended to make fewer total responses than response prevented saline controls ($p < 0.06$). In Test 1, response prevented rats (3 µg) made significantly fewer short avoidances than rats given 2 µg LVP ($p < 0.01$). Similarly, in Test 2, rats treated with 3 µg made significantly fewer short avoidances than those given 2 µg ($p < 0.031$).

There were no significant differences between behavioural treatments (HC v RP) when comparisons were made within a single dose level, although there were a number of differences between home cage and response prevented rats across different dose levels, conforming to the negative dose response function with higher doses invariably producing lower response rates than low doses regardless of the behavioural treatment (see Table A46). Regression lines calculated for Test 1 short avoidance data using the method of least squares did not indicate any systematic effects in line slopes as a function of dose or behavioural treatment (see Table A47).

Discussion

Rats retained in the home cage for 30 minutes after training then injected with 4 µg of LVP tended to make fewer total avoidance responses in Test 1 than rats injected with 2 µg of LVP, suggesting a negative dose response relationship between the post training peptide dose and the subsequent extinction response rats, ie higher doses leading to lower response rates.

Response prevention per se did not affect extinction rates. Furthermore, this lack of effect does not appear to be due to rapid within test extinction rate differences, a factor which confounded the effects of extinction trials in Experiment Three. This lack of effect contrasts with the findings of Experiments One, Three, Four and Five. However, prevention trials rendered the rats more sensitive to the response reducing effects of high doses of LVP. Data from these rats suggest a negative dose response relationship as response prevented rats injected with 3 µg made significantly fewer short avoidance responses in Test 1 than those injected with either 2 µg or saline. Similarly, in Test 2, 3 µg produced fewer short avoidance responses than 2 µg. The effect of 4 µg was more pervasive. In Test 1, 4 µg reduced responding relative to 2 µg in both the total avoidance and short avoidance data. In Test 2, response prevented rats treated with 4 µg made fewer total responses than those treated with saline. In addition 4 µg produced fewer total and short avoidance responses than 2 µg. The absence of response prevention effects per se permits the comparison of doses across behavioural treatments. In all cases these differences conform to the principle that 3 or 4 µg yield lower response rates than either saline or 2 µg.

It may be concluded that, within the dose range tested, the relationship between the dose of a post training LVP injection and extinction responding is negative, higher doses leading to lower extinction responding. The results confirm the previous findings (Experiments Three and Five) that post training LVP may reduce subsequent extinction response levels.

5.3 Experiment Seven: The Effects of Five Doses of LVP Injected 30 Minutes after Shuttle Box Training

Introduction

Although Experiments Three, Five and Six showed that post training LVP injections reduced avoidance responding in extinction, they failed

to replicate the findings of Experiment Two and those in the literature (see Chapter Two) which show that vasopressin's increase extinction responding. As Experiment Six showed that higher doses than 2 µg tended to further reduce responding, the present experiment examined the effects of lower doses on a modified shuttle box task.

Methods

Subjects

Adult male Wistar rats of an inbred strain (cpb TNO, Zeist, Netherlands) were housed five to a cage with ad lib access to food and water under conditions of constant temperature (22°C) and regulated illumination; the animal house was in darkness between 1900 and 0500 hours. Animals weighed 200-220 g and were brought to the laboratory at least one hour before the experimental sessions which were run between 1300 and 1700 hours.

Apparatus

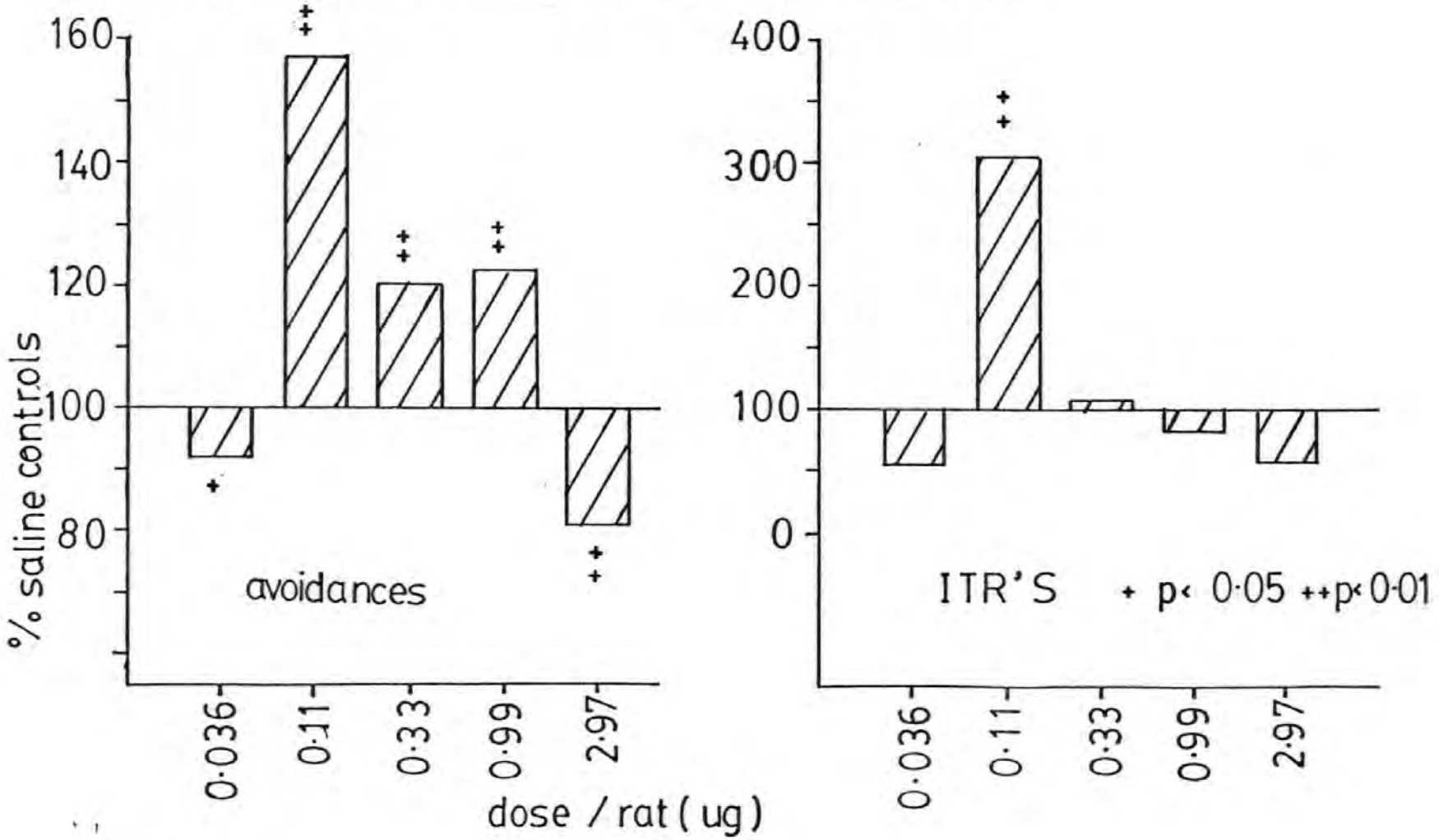
A manually controlled two-way shuttle box (internal dimensions 48 x 25 x 17 cm) with a centrally placed hurdle (height 4 cm) was housed in a sound attenuating chamber under reduced illumination. The shuttle box was lit by a single overhead houselight and a constant level of background noise was maintained by the foots hock scrambler. A loud buzzer was placed immediately behind the shuttle box to act as the conditioned stimulus (CS). Ten seconds of the CS alone were followed by ten seconds of the CS accompanied by scrambled foots hock as the unconditioned stimulus (UCS) set at 0.15 ma.

If the rat crossed the central hurdle after the onset of the CS but before the UCS, then impending shock was cancelled (avoidance); if the crossing occurred during the UCS, the shock was terminated (escape). In both cases the CS was also switched off. Training trials were not allowed to exceed 20 seconds in order to eliminate excessive exposure to shock. Each trial began with the onset of the CS every 60 seconds; therefore the inter trial interval varied as a function of response rapidity between the minimum of 40 seconds and a maximum approaching 60 seconds. A hurdle crossing in the absence of the CS was designated as an intertrial response (ITR).

Procedure

Five minutes of adaptation to the shuttle box preceded training. Learning then began and continued until each animal had made ten correct consecutive avoidance responses. Having reached the criterion, animals were removed from the shuttle box and returned to the home cage for

FIGURE 22 Post training LVP effects on extinction



treatment with the various doses described below. Approximately 24 hours later they were returned to the shuttle box and after two minutes of adaptation were tested with ten extinction trials. Shock was omitted during extinction but otherwise the schedule was the same as for training. A response within ten seconds of CS onset was an avoidance and responses in the absence of the CS were intertrial responses.

This experiment examined the effects of five doses of LVP injected 30 minutes after training. Animals were returned to the home cage immediately after training and were randomly allocated to receive either saline or LVP. The experiment was run in two consecutive, independent phases; in the first phase three doses were compared with saline, 0.11, 0.33 and 0.99 µg/rat. The second phase extended the dose range to 0.036 and 2.97 µg/rat.

Peptides

LVP was stored at 1-5°C as a dry powder and was freshly prepared before each session. A single drop of HCL (0.01 N) plus sufficient physiological saline were added to yield the required dose in a constant injection volume of 0.5 ml. Lysine vasopressin (LVP; pressor activity > 200 IU/mg) were supplied by Organon, Oss, Netherlands. All injections were subcutaneous (SC).

Data analysis

Acquisition performance was recorded using four measures, the number of trials to reach criterion and the number of avoidances, escapes and intertrial responses made in training. Independent t-tests and one way analysis of variance (Winer 1962) were used to determine significant differences between groups in acquisition on these measures. For the analysis of extinction data the number of avoidances or intertrial responses were summed across subjects within each group to obtain the total number of each response made by the group on every trial. The trial totals from each group, within each experiment, were then analysed using a two way analysis of variance (treatment x trials) with repeated measures on the trials factor (Winer 1962). Neuman-Keuls test (Winer 1962) was then used to determine significant differences between peptides and saline, between peptide doses and between trials. For all tests $p < 0.05$ (two tailed) was considered significant.

Results

The results are summarised in Table 2. Analysis of the acquisition data showed that there were no significant differences during training between treatment and control groups in either phase. Analysis

Table 2: Acquisition and extinction of shuttle box avoidance responding using 0.15 ma footshock in training and a 30 minute interval between the end of training and injecting various doses of lysine vasopressin

			ACQUISITION[1]			EXTINCTION[2]	
			Trials	Avoidances	Escapes	Avoidances	ITRs
Saline		(8)	18.63 ± 1.58	12.62 ± 0.42	5.87 ± 1.24	4.0 ± 0.36	2.9 ± 0.56
LVP	0.11 µg	(8)	20.87 ± 2.11	13.87 ± 1.53	6.37 ± 0.9	6.3 ± 0.26[c]	8.9 ± 0.84[c]
LVP	0.33 µg	(7)	21.28 ± 1.99	14.43 ± 1.04	6.0 ± 1.23	4.8 ± 0.44[c]	2.7 ± 0.49
LVP	0.99 µg	(8)	23.25 ± 2.87	13.25 ± 0.97	8.87 ± 2.31	4.9 ± 0.31[c]	2.4 ± 0.6
Saline		(8)	23.75 ± 2.51	15.0 ± 1.08	8.62 ± 1.74	5.5 ± 0.31	5.2 ± 0.64
LVP	0.036 µg	(8)	22.62 ± 2.25	13.75 ± 0.99	8.25 ± 1.37	5.1 ± 0.37[a]	2.9 ± 0.91
LVP	2.97 µg	(8)	23.25 ± 2.53	15.75 ± 1.38	7.12 ± 1.39	4.5 ± 0.31[c]	3.0 ± 0.69

[1] Mean ± SEM per subject

[2] Mean ± SEM per trial

( ) Number of subjects

[a] $p < 0.05$ (compared to saline controls)

[b] $p < 0.02$

[c] $p < 0.01$

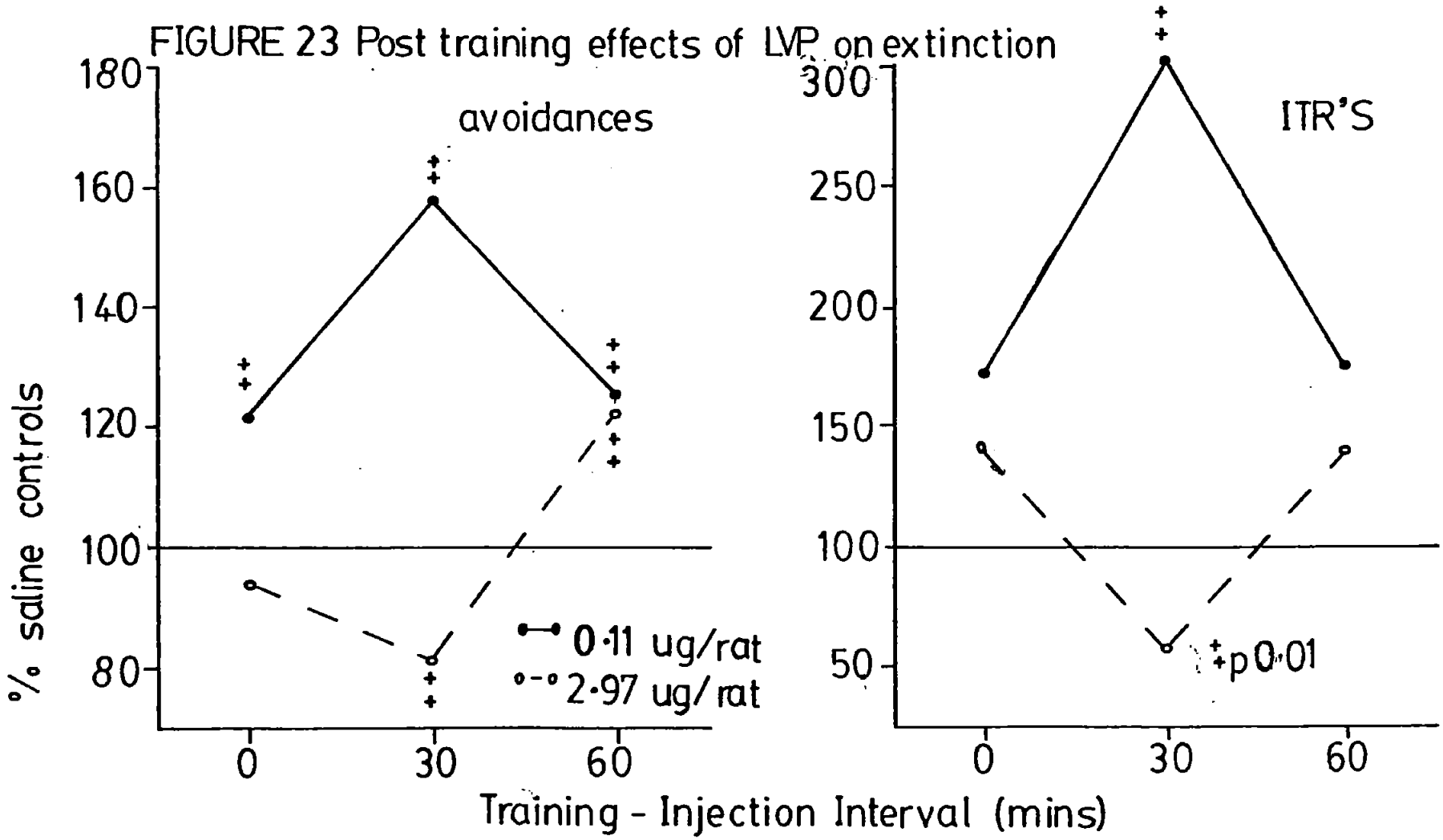
of the extinction data from phase one revealed significant effects of treatments on both avoidance ($F(3/27) = 16.646$, $p < 0.01$) and intertrial ($F(3/27) = 11.646$, $p < 0.01$) responding. In addition there were significant effects of trials in avoidance ($F(9/27) = 7.12$, $p < 0.01$) and intertrial responding ($F(9/27) = 15.33$, $p < 0.01$). Neuman-Keuls comparisons revealed that after 0.11, 0.33 and 0.99 µg avoidance responding was significantly greater than in saline controls (all p's < 0.01). Furthermore, 0.11 µg resulted in significantly more avoidances than either 0.33 or 0.99 µg (p's < 0.01). Neuman-Keuls comparisons on the trials effect in the avoidance data showed that responding was higher on trial one (p's < 0.05) and higher on trial two than on subsequent trials (p's < 0.05). Neuman-Keuls comparisons showed that 0.11 µg increased intertrial responding relative to saline ($p < 0.01$). Analysis of the trial effect in the intertrial response data showed that responding on trials 2 and 3 was significantly lower than on trials 5 to 10 (p's < 0.05), responding on trial 1 was lower than on both trials 6 and 8 ($p < 0.05$), and responding was maximal by trial 8 which had a significantly higher total than all other trials ($p < 0.05$).

Analysis of extinction data from phase two showed that there were significant effects of dose ($F(2,18) = 5.51$, $p < 0.05$) and trials ($F(9,18) = 5.23$, $p < 0.05$) on avoidance responding but no significant effects on intertrial responses. Neuman-Keuls tests showed that responding was significantly reduced by both 0.036 µg ($p < 0.05$) and 2.97 µg ($p < 0.01$) compared to saline controls. Furthermore the response level after 2.97 µg was significantly lower than after 0.036 µg ($p < 0.01$). Neuman-Keuls comparisons of trial effects in the avoidance data showed that the response level on trial 1 was significantly higher than on any subsequent trial (p's < 0.05); also levels on trials 2 and 10 were significantly greater than on trial 4 ($p < 0.05$).

Discussion

All five doses of vasopressin altered avoidance responding in extinction. However, the direction of change depended on the dose. This is clear from Figure 22 in which data from each dose, calculated as a percentage of saline controls, facilitate comparison between the two phases. Avoidance responding was reduced by the lowest (0.036 µg) and the highest dose (2.97 µg); in contrast the intermediate doses (0.11, 0.33 and 0.99 µg) increased avoidance responding. The most potent dose in this respect was 0.11 µg which yielded higher response levels than either 0.33 or 0.99 µg and was the only dose to significantly increase intertrial responding above the level of saline controls. The effects

FIGURE 23 Post training effects of LVP on extinction



of 0.11, 0.33 and 0.99 µg confirm previous reports that post training vasopressin injections increase resistance to extinction in intact rats (de Wied and Bohus 1966; de Wied 1971; Bohus et al 1972; King and de Wied 1974; Bohus et al 1978a,b; Krejci, Kupkova, Metys, Barth and Jost 1979; see also Experiment Two) whereas the effects with 0.036 and 2.97 µg confirm previous findings with high doses (Experiments Three, Five and Six). Taken together, the data suggest that the direction and magnitude of vasopressin's effect on extinction responding varies as an inverted U shaped function of the dose.

Analysis of the trials effects in the avoidance data from phases one and two showed that response levels were initially high and then declined rapidly. Phase two showed that the lowest response rate had been attained by trial 4 and thereafter gradually increased till the last trial although the final level was still significantly lower than on trial 1. In contrast, the pattern in intertrial data from phase one was for responding to increase from trials 1, 2 and 3 through to trial 8, suggesting that the trial dependent reduction in responding was due to a loss of stimulus control rather than a reduction in general activity.

5.4 Experiment Eight: The Effects of Oppositely Acting LVP Doses Injected Immediately or 60 Minutes after Training on Avoidance Extinction

Time dependent changes in the effectiveness of post training vasopressin injections have been a central aspect in the evidence relating the action of the peptides to processes concerned with memory consolidation (de Wied 1971; Bohus et al 1972; King and de Wied 1974; Bohus et al 1978a,b; van Wimersma Greidanus et al 1975). However, Experiments Three, Five, Six and Seven showed that LVP may also reduce extinction responding and that sensitivity to this effect increases rather than decreases 60 minutes after training (Experiment Five). It was therefore of interest to determine the pattern of time dependent changes for oppositely acting doses of LVP in this behavioural model. Experiment Seven established the relationship at the 30 minute interval. Therefore in the present experiment either 0.11 or 2.97 µg were injected immediately or 60 minutes after the end of training in the shuttle box.

Methods

All aspects of the methods and procedures were identical to those described for Experiment Seven. Rats were injected with saline, 0.11

or 2.97 µg LVP immediately or 60 minutes after the end of training; for the 60 minute groups the intervening period was spent in the home cage.

Results

During training there were no significant differences between groups according to the number of trials, avoidances, escapes or intertrial responses. Data from both the acquisition and extinction phases of the experiment are summarised in Table 3. Analysis of the extinction data revealed significant effects of dose ($F(2,18) = 9.948$, $p < 0.01$) and trials ($F(9,18) = 2.947$, $p < 0.05$) on avoidance responding but no significant effects on intertrial responding when animals were injected immediately after training. Neuman-Keuls tests showed that 0.11 µg resulted in significantly more avoidance responses than either saline or 2.97 µg (p's < 0.01), whereas 2.97 µg did not affect avoidance responding relative to saline. Neuman-Keuls comparisons of trial totals showed that responding on trial 1 was higher than on 5 ($p < 0.05$). When the injections were withheld for 60 minutes there were significant effects of dose on the avoidance data ($F(2,18) = 4.77$, $p < 0.025$) but no effects of trials. Neuman-Keuls tests showed that both 0.11 and 2.97 µg resulted in significantly more avoidances than saline (p's < 0.01). There were no significant effects in the intertrial response data.

Discussion

To facilitate comparison with data from Experiment Seven, the results from each group were calculated as a percentage of their saline controls and these data are shown in Figure 23. 0.11 µg LVP increased avoidance responding in extinction when injected either immediately or 60 minutes after training. Comparing the data from Experiment Seven shows that 0.11 µg enhanced responding when injected within one hour of training, thus confirming earlier indications on the most effective intervals for treatment (de Wied 1971; Bohus et al 1972; King and de Wied 1974). The low dose appeared to be equipotent at the 0 and 60 minute intervals but more active when injected 30 minutes after training. In contrast, the effect of the high dose (2.97 µg) varied in direction as a function of the intervening interval. When injected immediately after training, there was no effect; when injected after 30 minutes avoidance responding was reduced; and if the injection was delayed for 60 minutes avoidance responding was significantly increased.

The data suggest that there are time dependent changes in the dose response curve with particular sensitivity to both the low and high dose

Table 3 : Acquisition and extinction of a shuttle box avoidance response using 0.15 ma footshock in training followed by 0.11 µg or 2.97 µg LVP injected either immediately or 60 minutes after training

			Training injection interval	ACQUISITION[1] Trials	Avoidances	Escapes	ITRs	EXTINCTION[2] Avoidances	ITRs
Saline		(8)	0 minutes	19.5 ± 2.28	13.5 ± 1.24	5.75 ± 1.22	2.0 ± 0.65	5.5 ± 0.5	3.3 ± 0.68
LVP	0.11 µg	(8)	0 minutes	25.0 ± 2.85	15.62 ± 1.74	6.75 ± 1.13	4.75 ± 1.2	6.7 ± 0.15[c]	5.7 ± 0.77
LVP	2.97 µg	(8)	0 minutes	21.12 ± 1.74	13.87 ± 1.27	7.12 ± 1.26	5.75 ± 2.15	5.2 ± 0.2	4.7 ± 0.57
Saline		(8)	60 minutes	26.87 ± 3.82	16.37 ± 2.21	8.75 ± 1.21	5.0 ± 1.37	4.3 ± 0.26	2.8 ± 0.48
LVP	0.11 µg	(8)	60 minutes	24.5 ± 3.54	15.62 ± 2.32	8.0 ± 1.72	6.87 ± 2.11	5.4 ± 0.3[c]	4.9 ± 0.72
LVP	2.97 µg	(8)	60 minutes	24.12 ± 2.96	16.87 ± 1.87	6.5 ± 1.21	2.75 ± 0.92	5.3 ± 0.42[c]	3.9 ± 0.56

See Table 2 for key

effects 30 minutes after training.

5.5 Experiment Nine: The Effects of Oppositely Acting LVP Doses Injected 30 Minutes after Training with a Higher Shock Level (0.45 ma) on Avoidance Extinction

The results from Experiment Seven indicated that extinction avoidance responding varied as an inverted U shaped function of the vasopressin dose when injected 30 minutes after the training session. A similar dose response relationship has been reported for Adrenocorticotrophic hormone (Acth) when injected immediately after passive avoidance training (Gold and van Buskirk 1976a,b). These authors also found a strong interaction between dose and training shock level, thus a high dose facilitated retention after training with low shock but disrupted retention after an intermediate or high shock. Moreover a low dose facilitated retention after both low and intermediate training shock levels but disrupted retention after high shock (Gold and van Buskirk 1976b). This interaction was interpreted as support for the hypothesis that Acth modulated the normal hormonal response to training thereby mimicking the effects of higher footshock in training (Gold and van Buskirk 1976a,b; Gold and McGaugh 1977).

Previous research had shown that small increases in footshock intensity, or the use of overtraining procedures, in a passive avoidance task reduced the amnestic effects of protein synthesis inhibitors (Flood, Bennett, Rosenweig and Orme 1973; Flood et al 1974). Similarly the duration of amnestic treatment needed to be prolonged in order to be effective in mice which were overtrained in an active avoidance task (Flood, Bennett, Orme and Rosenweig 1975). Pharmacological manipulations of post training arousal using stimulant drugs also counteracted the amnestic effects of protein synthesis inhibitors (Flood, Jarvik, Bennett, Orme and Rosenweig 1977).

These data suggested that if either the hormonal consequences of training or post training arousal were affected in a dose dependent manner by LVP then the characteristics of the inverted U shaped dose response curve observed in Experiment Seven should be changed by increasing the footshock level in training. Two oppositely acting doses (0.11, 2.97 µg) were therefore selected and were injected 30 minutes after training in the shuttle box at a higher shock level.

Methods

All aspects of the procedure and methods were identical to those

described for Experiment Seven with the exception that the shock level in training was increased to 0.45 ma.

Results

The data from Experiment Nine are summarised in Table 4. During training there were no significant differences between groups in the avoidances, escapes, trials to criterion or intertrial responses. Analysis of the extinction data showed that there were significant effects of dose ($F(2,18) = 16.08$, $p < 0.01$) and trials ($F(9,18) = 14.575$, $p < 0.01$) on avoidance responding. Neuman-Keuls tests revealed that 0.11 µg resulted in significantly more avoidances than either saline ($p < 0.01$) or 2.97 µg ($p < 0.01$). Furthermore, 2.97 µg produced fewer avoidances than saline ($p < 0.01$). Neuman-Keuls comparisons between trial totals showed that avoidance responding on trial 1 was significantly higher than on all subsequent trials (p's < 0.05). Intertrial responding was almost totally suppressed during extinction and there were no significant effects of either trials or doses.

Discussion

The results from this experiment confirm those from Experiment Seven; 0.11 µg increased whereas 2.97 µg decreased subsequent avoidance responding in extinction. Unlike the effects of post training ACTH (Gold and van Buskirk 1976a), the effectiveness of the low and high dose of LVP remained essentially the same after training at the higher level of footshock; this tends to rule out an explanation in terms of LVP modulating the hormonal consequences of training. Furthermore it appears unlikely that LVP mediates its effects by altering post training arousal as has been found for other drugs which affect memory storage (Flood et al 1973, 1974, 1975, 1977).

Increased shock did not appear to affect the rate of response acquisition compared to Experiment Seven or later experiments. Intertrial responding appeared lower during training in the present experiment and in extinction was almost totally suppressed. During extinction the level of baseline avoidance responding was approximately 50% of that seen in the control groups of Experiments Seven and Eight yet despite the different baselines extinction also preceded very rapidly within the test. These baseline changes do not agree with previous suggestions of an inverse relationship between shock levels and acquisition rate in the shuttle box (Moyer and Korn 1964; Levine 1966; Theios, Lynch and Lowe 1966; McAllister and McAllister 1971) and in the passive avoidance task (Pearce 1978), but suggest that with relatively small increases in shock

Table 4: Acquisition and extinction of a shuttle box avoidance response using 0.45 ma of footshock in training followed by 0.11 or 2.97 µg of LVP injected 30 minutes after training

			ACQUISITION[1]				EXTINCTION[2]	
			Trials	Avoidances	Escapes	ITRs	Avoidances	ITRs
Saline		(8)	23.87 ± 3.32	14.87 ± 1.23	8.5 ± 2.44	2.0 ± 0.84	2.7 ± 0.42	0.3 ± 0.21
LVP	0.11 µg	(8)	22.12 ± 2.28	14.12 ± 1.12	8.25 ± 1.56	0.62 ± 0.37	3.2 ± 0.32[c]	0.2 ± 0.13
LVP	2.97 µg	(8)	22.5 ± 1.28	12.87 ± 0.76	9.5 ± 1.44	1.87 ± 1.06	2.0 ± 0.29[c]	0.3 ± 0.21

See Table 2 for key

decreased responding may be evident in extinction before the effects are seen in acquisition.

5.6 Experiment Ten: Dose Response Studies with DG-LVP

Structure activity studies using the pole jump active avoidance response have shown that the increased resistance to extinction seen after post training injections of LVP or AVP does not appear to be mediated by the peptides' effects on endocrine function (see Section 2.2). When the C terminal glycinamide was removed the resulting des-glycinamide analogs (DG-AVP, DG-LVP) retained approximately 50% of their behavioural activity but were almost devoid of classical endocrine pressor and anti-diuretic effects (Lande et al 1971; de Wied et al 1972) (see Section 2.2). In the present experiment a range of doses of DG-LVP were injected 30 minutes after the end of shuttle box training in order to determine if the entire vasopressin molecule was required for the inhibitory effects of low and high doses and whether or not this effect could be ascribed to the classical endocrine functions of the peptide.

Methods

The methods and procedures were identical to those described for Experiment Seven with the exception that des-glycinamide lysine vasopressin (DG-LVP (Organon, Oss, Netherlands) was used. The experiment was run in five independent phases in each of which saline was compared with a number of peptide doses ranging from 0.012 µg to 8.91 µg.

Results

The acquisition and extinction data from Experiment Ten are summarised in Table 5. Analysis of the acquisition data from phase one showed that there were no significant differences between groups during training. Analysis of the extinction data showed that 8.91 µg DG-LVP significantly reduced avoidance responding ($F(1,9) = 176.09$, $p < 0.01$). There was no significant effect of trials and no significant effects on intertrial responding.

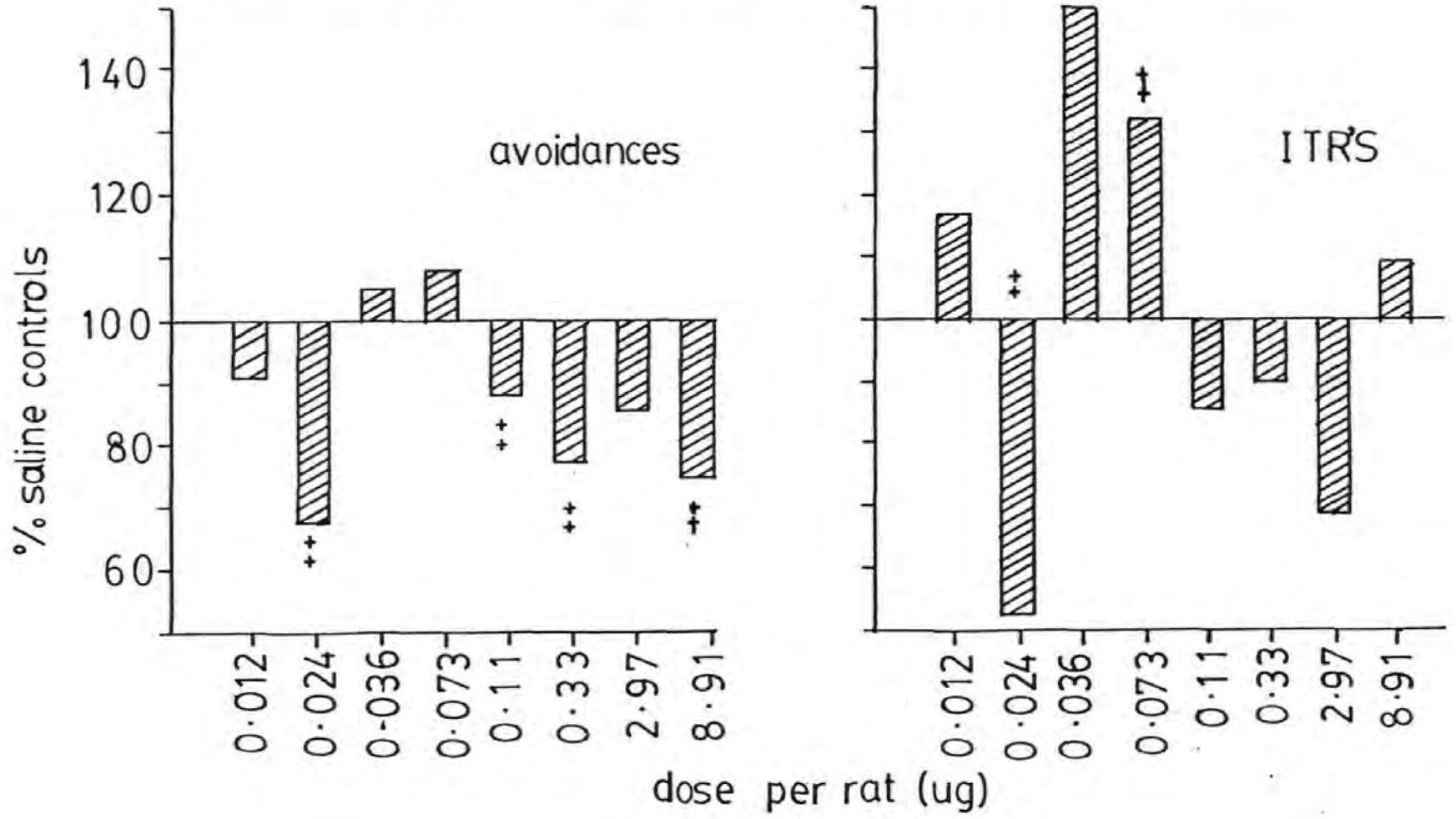
During phase two there were no significant differences between groups in training. Analysis of avoidances in extinction revealed a significant effect of doses ($F(2,18) = 5.72$, $p < 0.05$) but not trials. There were no significant effects on the intertrial response data. Neuman-Keuls comparisons showed that saline treated controls made significantly more avoidances than rats treated with either 0.11 or 0.33 µg (p's < 0.01). Furthermore the number of avoidances was higher after

Table 5: Acquisition and extinction of shuttle box avoidance responding using 0.15 ma footshock in training and a 30 minute interval between training and injection of various doses of DG-LVP

				ACQUISITION[1]				EXTINCTION[2]	
	Phase			Trials	Avoidances	Escapes	ITRs	Avoidances	ITRs
(1)	Saline		(9)	18.44 ± 1.29	13.0 ± 0.64	5.5 ± 1.01	3.11 ± 0.75	6.7 ± 0.36	5.2 ± 0.73
	DG-LVP	8.91 µg	(9)	18.66 ± 2.19	12.66 ± 1.4	6.0 ± 1.1	3.66 ± 0.91	5.0 ± 0.36[a]	5.7 ± 1.15
	Saline		(8)	17.5 ± 2.18	12.87 ± 1.14	4.25 ± 1.21	8.12 ± 2.63	7.1 ± 0.17	6.3 ± 0.45
(2)	DG-LVP	0.11 µg	(8)	17.75 ± 1.21	12.37 ± 0.82	4.62 ± 0.92	7.5 ± 1.96	6.3 ± 0.26[a]	5.4 ± 0.37
	DG-LVP	0.33 µg	(8)	19.75 ± 2.69	13.62 ± 1.37	5.37 ± 1.36	3.0 ± 0.86	5.5 ± 0.52[a]	5.7 ± 0.75
(3)	Saline		(10)	19.3 ± 1.82	13.3 ± 1.07	5.3 ± 1.03	4.4 ± 1.13	6.5 ± 0.65	6.4 ± 1.18
	DG-LVP	2.97 µg	(10)	20.5 ± 2.27	13.1 ± 1.2	6.3 ± 1.83	2.7 ± 0.93	5.6 ± 0.45	4.4 ± 0.62
	Saline		(8)	17.2 ± 1.0	12.62 ± 0.56	3.75 ± 0.62	6.75 ± 2.64	5.7 ± 0.36	3.0 ± 0.21
(4)	DG-LVP	0.012 µg	(8)	25.5 ± 3.14[a]	16.37 ± 2.09	8.12 ± 1.83	4.75 ± 2.47	5.2 ± 0.38	3.5 ± 0.68
	DG-LVP	0.036 µg	(8)	19.87 ± 2.17	13.12 ± 1.09	5.25 ± 1.22	5.37 ± 1.32	6.0 ± 0.45	4.5 ± 0.83
	Saline		(8)	22.25 ± 1.58	13.25 ± 0.84	7.87 ± 1.66	3.75 ± 1.53	6.0 ± 0.45	5.3 ± 0.56
(5)	DG-LVP	0.024 µg	(8)	22.12 ± 2.98	15.12 ± 1.53	6.5 ± 1.77	3.37 ± 1.32	4.1 ± 0.38[a]	2.8 ± 0.2[c]
	DG-LVP	0.073 µg	(8)	24.37 ± 3.49	15.62 ± 1.63	7.62 ± 1.97	2.12 ± 0.61	6.5 ± 0.27	7.0 ± 1.17[c]

See Table 2 for key

FIGURE 24 Post training (30 mins) DG LVP effects on Extinction.



0.11 µg than after 0.33 µg ($p < 0.01$).

Analysis of the acquisition data from phase three revealed no significant differences between groups. During extinction 2.97 µg of DG-LVP tended to reduce avoidance responding compared to saline ($F(1,9) = 4.314$, $p < 0.1$) and there was a significant effect of trials on the avoidance response ($F(9,9) = 5.734$, $p < 0.01$). Neuman-Keuls tests showed that responding was significantly higher on trial 1 than on all subsequent trials ($p's < 0.05$) with the exception of trial 2 and that responding on trial 2 was significantly greater than on trial 4 ($p < 0.05$). The lowest level of responding had been reached by trial 4.

During phase four the analysis of acquisition data showed a significant difference between groups in the number of trials to criterion ($F(2,21) = 3.5$, $p < 0.05$). Neuman-Keuls comparisons revealed that animals which were subsequently injected with 0.012 µg took more trials to attain the criterion than either saline controls or the 0.036 µg group ($p's < 0.05$). There were no other significant differences in acquisition. Analysis of the extinction data showed that there were no effects of dose on either avoidance or intertrial responding but there were significant trials effects in both ($F(9,18) = 2.504$, $p < 0.05$; $F(9,18) = 3.23$, $p < 0.05$ respectively). Neuman-Keuls comparisons of trial totals in the avoidance data showed that responding was significantly higher on trial 1 than on all subsequent trials with the exception of trial 10 ($p's < 0.05$). Neuman-Keuls comparisons of trial totals in the intertrial response data showed that responding on trial 8 was significantly greater than on trials 1, 2, 3, 4, 5 and 10 ($p's < 0.05$).

Finally, during phase five there were no significant differences between groups in acquisition. Analysis of the extinction data showed a significant effect of dose in both the avoidance ($F(2,18) = 15.518$, $p < 0.01$) and the intertrial response data ($F(2,18) = 8.66$, $p < 0.01$). There were no significant trial effects in either set. Neuman-Keuls comparisons showed that animals treated with 0.024 µg made fewer avoidance responses than either saline controls ($p < 0.05$) or animals treated with 0.073 µg ($p < 0.01$). Intertrial responding was significantly lower in the 0.024 µg group than in either the saline or 0.073 µg group ($p's < 0.01$). In addition 0.073 µg resulted in significantly more intertrial responses than saline ($p < 0.01$).

Discussion

The data from each treatment group in this experiment were calculated as a percentage of their saline controls and this form of the data is presented in Figure 24. None of the doses tested increased

avoidance responding in extinction although 0.073 µg significantly increased intertrial responding. On the contrary, significant reductions in avoidance responding and intertrial responding were seen with 0.024 µg and significant reductions in avoidance alone were seen with 0.11, 0.33 and 8.91 µg.

These data indicate that the C terminal glycinamide is not necessary in order to show response reductions when the peptide is injected 30 minutes after training. Therefore the effects observed in Experiments Three, Five, Six, Seven, Eight and Nine with various doses were probably not mediated by effects on classical endocrine targets (see Sections 1.8 and 1.9) (Lande et al 1971; de Wied et al 1972). Rather they suggest that the increased extinction responding seen with 0.11, 0.33 and 0.99 µg LVP in Experiments Two and Seven and in the literature (see Chapter Two) required the full molecular structure.

CHAPTER SIX

6.0 Introduction

Extensive evidence suggests that vasopressin injections alter catecholaminergic metabolism in discrete brain nuclei (Section 2.7) and that this may be the neurochemical mechanism which underlies their behavioural effects. Substantial evidence also implicates cholinergic neurons in memory mechanisms and therefore the first experiment of this chapter describes the effects of the cholinergic drugs scopolamine and physostigmine on the outcome of LVP injections and response prevention trials.

Tests with rats which failed to achieve learning criterion showed that the effects of LVP (1 µg) were opposite to those seen in rats which achieved criterion (Experiments Twelve and Thirteen). In addition, rats which failed to reach criterion showed a different interaction between cholinergic drugs and LVP injections (Experiment Fourteen).

6.1 Experiment Eleven: The Effects of LVP and Prevention Trials on Extinction Responding after Injections of Scopolamine or Physostigmine

Introduction

Cholinergic neurons have been implicated in processes related to storage and recall of learned responses. Deutsch et al (1966) found that the anticholinesterase diisopropyl fluorophosphate (0.01 mg) injected directly into rat hippocampi 30 minutes after training a Y maze escape response resulted in amnesia for the response for up to three days after the injection. Following intra-hippocampal injections of scopolamine hydrobromide (0.19 mg/6 µl), a cholinergic antagonist, Wiener et al (1973) showed good retention for three days after injection but performance was disrupted five, seven and ten days later. Localisation of the amnestic effect in the hippocampus could not be confirmed by Todd et al (1979) with subseizure doses of physostigmine (10 µg/µl) immediately after avoidance training. Earlier positive findings for this region (Deutsch et al 1966; Wiener et al 1973) could have been due to a spreading of effects as a result of relatively high doses and injection volumes. However, Todd et al (1979) did find an amnesic effect of physostigmine following post training injections into the

amygdala.

Retrieval processes are affected by physostigmine (0.4 mg/kg ip) injected seven days after passive avoidance training and 30 minutes prior to retention testing (Hanbury et al 1976). Signorelli (1976) confirmed that the retention effect could only be found when the drug was still pharmacologically active suggesting that apparent effects on retrieval may not be due to altering the substrate of memory.

Post training systemic injections of physostigmine may result in facilitation of subsequent retention. Alpern and Marriot (1973) trained rats on a reversal learning task in a T maze and found that a post training injection of scopolamine (2 mg/kg ip) disrupted responding 25 minutes later. In contrast, physostigmine (0.2 mg/kg ip) facilitated subsequent performance. The authors argued that the data indicated effects upon short term memory processes. However, this interpretation may be confounded by two factors: firstly, despite prolonged training, control animals reverted to responding at chance levels 25 minutes later; secondly, the injections were given immediately after training and 25 minutes before the retention test, allowing no clear temporal distinction between consolidation and retrieval stages. Stronger evidence for an amnesic effect of physostigmine was reported by Barrati et al (1979). These authors trained mice on a one trial step through passive avoidance task, injected physostigmine or oxotremorine, a cholinergic receptor agonist, immediately after training and during retention testing 24 hours later found that both drugs, in equimolar doses, produced dose dependent and time dependent increases in passive avoidance. A dose of 0.25 μ mol/kg of physostigmine enhanced passive avoidance 24 hours later when injected within ten minutes of the learning trial. These data suggest a role for cholinergic neurons in consolidation processes.

Extensive evidence that vasopressin plays a role in consolidation has been reviewed in Chapter Two (see also Wimersma Greidanus & Versteeg 1980). These findings have been confirmed in Experiments Two, Seven, Eight and Nine. However, it has also been shown that both high and low doses of LVP may reduce subsequent extinction responding (Experiments Three, Five, Six, Seven, Eight and Nine). Furthermore, the response reducing effects of both 1 μg (Experiment Five) and 2.97 μg (Experiments Seven and Eight) appear to first increase and then decrease during the post training period. The present experiment is designed to test the hypothesis that cholinergic neurons are involved in mediating the response reducing effect of LVP.

In an extensive study, Taub et al (1977) found that the only

effective pharmacological adjunct to response prevention was atropine in a dose of 3 or 6 mg/kg ip. One hundred and fifty ledge jump training trials were followed by an injection of the drug followed 30 minutes later by ten trials of response prevention. Extinction testing was carried out 72 hours after injection. The design did not allow a distinction to be made between possible consolidation type effects of the drugs, resulting from immediate post learning injections, and interactions with the prevention trials which followed the injection after 30 minutes. Non response prevented drug treated rats were not tested. However, in view of the possible interaction between the anticholinergic drug atropine and response prevention and considering that response prevention may, under certain conditions, reverse the effects of post training LVP (see Experiments Three and Six) it was of interest to determine the effect of scopolamine and physostigmine on extinction responding after prevention trials

Methods

Subjects

Seventy-two adult male wistar rats (350-450 gm) from the Plymouth Polytechnic colony were housed three or four to a cage with free access to food and water.

Procedure

The apparatus and schedule have been described in Experiment One. Rats were trained in the shuttle box to a criterion of ten correct consecutive avoidance responses within a maximum of two 50 trial training sessions, run on consecutive days. Rats which attained criterion were randomly assigned to be detained in the home cage for 30 minutes or to receive 30 trials of response prevention (see Experiment One). Following this each rat was then randomly assigned to receive one of six combinations of peptide and drug treatments. In the first of two injections each rat was given either saline or LVP (SC). The second injection followed immediately and was either saline, scopolamine or physostigmine (SC). The details of LVP preparation have been described in Experiment Two; the peptide was administered in a dose of 1 µg per rat in 0.5 ml of saline vehicle. Scopolamine hydrobromide crystals (Sigma Chemicals Limited, Lot Number 16c-0359) were dissolved in 0.9% saline (2 mg/ml), and injected in a dose of 0.5 mg/kg body weight calculated as the weight of the salt. Solutions were stored at 1-5^{o}C and injected at room temperature. Approximately 24 hours after injection rats were returned to the shuttle box for 50 extinction trials (Test 1) and repeated 24 hours later (Test 2).

Figure 25 Total avoidances for treatment groups in extinction test one (mean and s.e.m.)

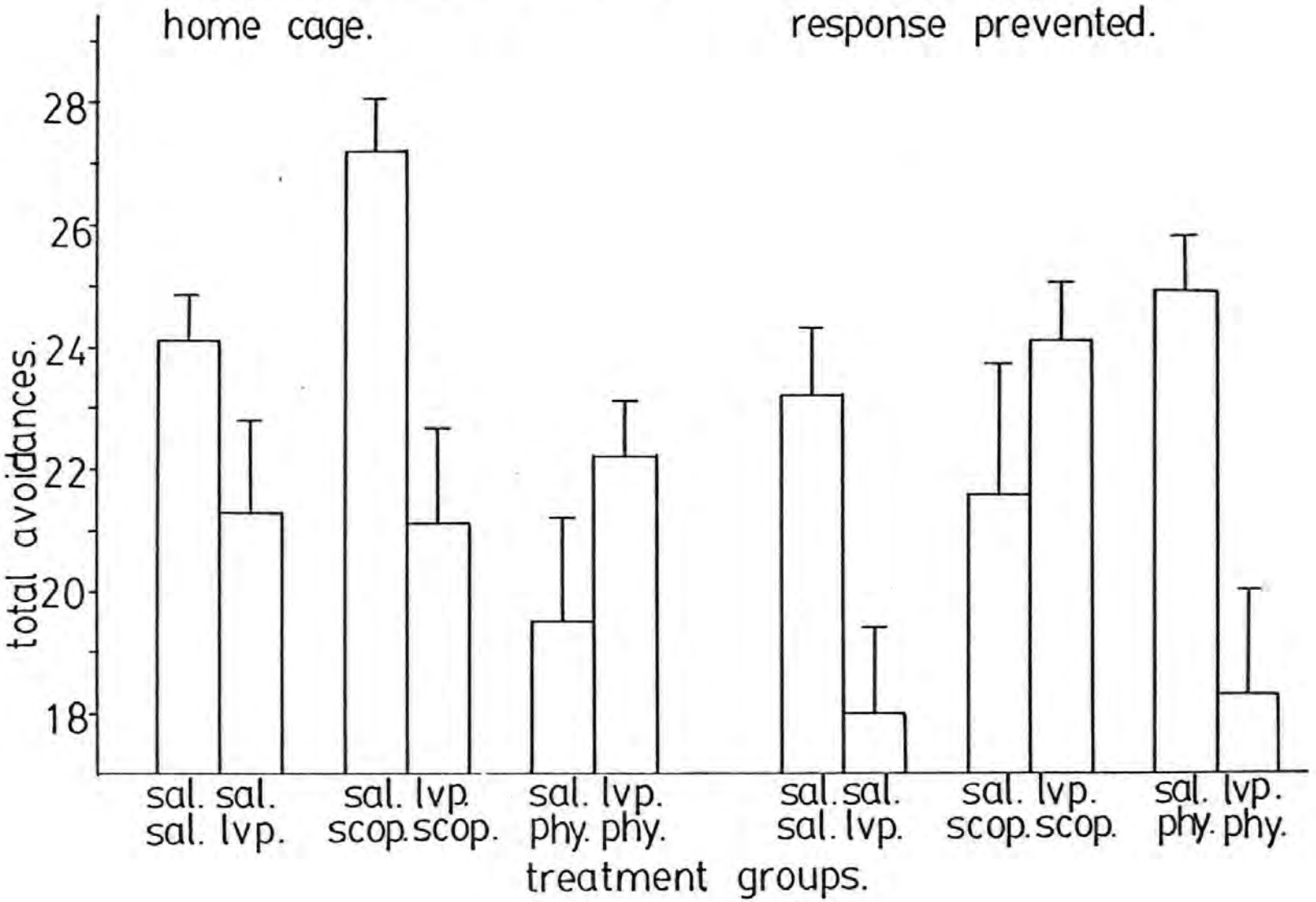
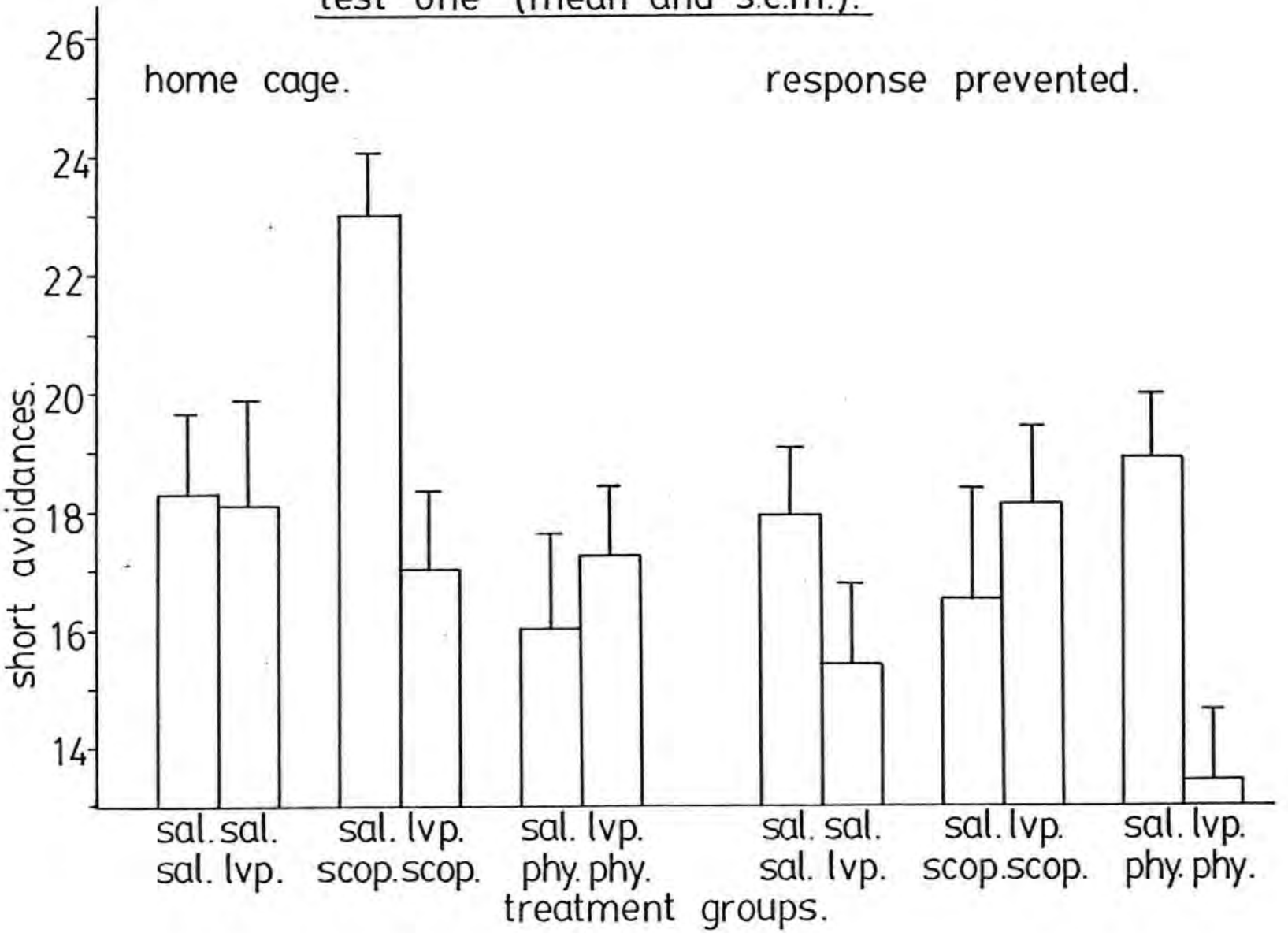


Figure 26 Short avoidances for treatment groups in extinction test one (mean and s.e.m.).



Results

Acquisition

Performance during training was recorded using five measures; the number of avoidance responses, escape responses, failures to respond, shocks received in training and trials to criterion (see Table A48) and analysed using analysis of variance (Winer 1962) (see Table A49). There were no significant differences between groups on any of these measures.

Extinction

The mean number of avoidance responses, short avoidance responses (< 10 seconds) and long avoidance responses (> 10 seconds) made in extinction is presented in Table A50. The number of responses made by each subject in each block of five extinction trials was summed across subjects to obtain the total number of each class of response per trial block (see Table A51). The performance of each group was compared using Freidman's two way non parametric analysis of variance (Seigel 1956); the outcomes of these tests are presented in Table A52. There were significant treatment effects in the total avoidance data in Test 1 ($p < 0.001$) and Test 2 ($p < 0.02$), in the short avoidance data in Test 1 ($p < 0.001$) and in the long avoidance data of Test 1 ($p < 0.02$) and Test 2 ($p < 0.02$). The method described by Hollander and Wolfe (1973) was used to locate significant differences between groups (see Table A52). The mean number of total avoidances and the mean number of short avoidances made by each group in Test 1 are shown in Figures 25 and 26.

The highest response rate was seen in rats injected with saline and scopolamine; these animals made more total responses than LVP saline rats ($p = 0.06$) and LVP scopolamine rats ($p < 0.03$). Saline scopolamine treated rats also made significantly more total responses ($p < 0.01$) and short avoidances ($p < 0.01$) than rats treated with saline and physostigmine. In addition saline scopolamine rats made more total avoidances ($p < 0.047$) and tended to make more short avoidances ($p < 0.1$) than rats treated with LVP and physostigmine.

The lowest number of total responses in Test 1 was found in response prevented rats injected with LVP and saline. These rats made fewer total avoidances than those treated with saline only ($p < 0.03$) or saline and physostigmine ($p < 0.01$) and tended to make fewer than rats injected with saline and scopolamine ($p = 0.06$), LVP and scopolamine ($p = 0.085$). Saline plus physostigmine treated rats made significantly more total avoidances ($p < 0.01$) and short avoidances ($p = 0.05$) than rats treated with LVP plus physostigmine.

Comparing results of drug and peptide treatments across the

behavioural conditions of home cage retention versus response prevention showed that home cage rats given saline and physostigmine made significantly fewer total responses than response prevented rats given saline and physostigmine ($p = 0.05$). Similarly home cage rats given saline and scopolamine made significantly more short avoidance responses than response prevented rats given saline and scopolamine ($p < 0.047$) (see also Table A52).

Discussion

LVP (1 µg) tended to reduce avoidance responses in Test 1 after home cage retention (see Figure 25). Failure to find a significant difference may be due to differential rates of change in the within session response levels (see also Experiment Three). Test 1 total avoidance data for saline control rats (see Table A53) yielded a slope coefficient of -0.31 compared to -1.32 for LVP treated rats indicating a higher within session rate of change in response levels.

Scopolamine injected 30 minutes after training tended to increase whilst physostigmine tended to decrease the level of Test 1 extinction responding relative to saline controls. Although the differences between drug treated rats and saline controls did not reach significance, scopolamine significantly increased Test 1 total avoidances and short avoidances relative to physostigmine. These drug induced changes in extinction support the hypothesis that cholinergic neurons are involved in mediating post training neurochemical processes and supports previous observations, using central injections, suggesting that physostigmine acts as an amnesic agent (Deutsch et al 1966; Todd et al 1979). The findings contrast with those from experiments which used systemic injections and found that physostigmine enhanced recall when injected immediately after learning (Barrati et al 1979; Hanbury et al 1976). These differences may reflect procedural differences, eg training task, injection route or dose.

LVP significantly reduced the total number of avoidance responses in Test 1 compared to saline when both of these treatments immediately preceded a scopolamine injection. LVP did not alter avoidance response levels compared to saline in physostigmine treated, home cage rats. Those rats which had been treated with either LVP or saline and physostigmine responded at similar levels in Tests 1 and 2 and both of these groups made significantly fewer total avoidance responses and short avoidance responses than rats treated with saline and scopolamine.

Assuming that the effects of the cholinergic drugs and LVP take comparable times to develop, the data suggest that the response reducing

effect of LVP seen in rats which have been detained in the home cage for 30 minutes is not dependent upon the level of activity in cholinergic neurons.

Response prevented rats injected with saline did not show significantly reduced extinction responding relative to home cage saline controls. However, as in the case of the LVP treated home cage rats, these animals had a greater within test regression slope (-1.11) than home cage saline controls (-0.31) indicating a greater rate of change in the probability of responding for the response prevented rats. When response prevention was followed by an injection of LVP there was a significantly lower number of total avoidance responses in Test 1 than response prevented rats injected with saline, contrasting with results from Experiment Three and King and de Wied (1974). When response prevented rats were injected with scopolamine they made significantly fewer short avoidance responses than home cage rats injected with scopolamine, supporting the findings of Taub et al (1977). Conversely, response prevented rats injected with physostigmine made significantly more total avoidances than home cage rats given physostigmine. Therefore the effects of scopolamine and physostigmine in home cage control rats were reversed when drug treatments were preceded by 30 response prevention trials. These results suggest that the response reducing effect of prevention trials widely reported in the literature (see Chapter Three) and confirmed in Experiments One and Three may be explained in terms of altered activity of cholinergic neurons. As the effects of prevention may also be found after 24 hours (Experiments One, Three Four and Six) this may indicate that prevention trials may activate a cholinergically controlled mechanism for the elimination or inhibition of irrelevant responding and not by fear extinction (see Section 3.1.2). This hypothesis is strongly supported by the consistent failure to find an 'anti anxiety' drug which acts as an effective adjunct to prevention trials (Kamano 1968, 1972; Baum 1973; Cooper et al 1974; Christy et al 1975, Taub et al 1977).

Response prevention trials reversed the effects of subsequent scopolamine and physostigmine injections found with home cage rats. Following prevention trials physostigmine increased the response rate relative to scopolamine. Despite this reversal of effect, the response reducing action of LVP prevailed and LVP significantly reduced responding relative to saline when given to physostigmine treated rats. Therefore the response reducing effect of LVP can be found regardless of the changes in response levels found after manipulating cholinergic activity. This supports the conclusion from the data with home cage rats in

suggesting that cholinergic neurons are not involved in mediating the response reducing effects of LVP.

6.2 Experiment Twelve: The Effect of LVP Conditioned Suppression and Avoidance Extinction in Rats which Failed to Reach Learning Criterion

Introduction

Experiment Four showed that LVP (1 µg) injected 30 minutes after training altered operant lever pressing rate changes during concurrent CS presentations 24 hours after injection. A number of rats, which were trained to lever press on the VI 60 second schedule, failed to attain the avoidance learning criterion of ten consecutive responses. Each of these rats received 100 training trials which should be sufficient to condition fear to the CS. If LVP increases conditioned fear, then suppression ratios should be significantly reduced relative to saline controls.

Methods

Methods and procedures were identical to those described in Experiment Four. The small number of animals available prohibited examining the effect of response prevention. Rats were injected with saline or LVP (1 µg) 30 minutes after the end of avoidance training trials.

Results

Acquisition

Performance during avoidance training is summarised in Table A54. Independent 't' tests (Winer 1962) showed that there were no significant differences between groups in the number of avoidances, escapes or failures to respond or in the number of shocks received in training (see Table A54).

Lever pressing data

The number of responses made by each animal in each period A (pre CS and inter CS) is presented in Table A55. Analysis has been described in Experiment Four. There were no significant differences between groups in the period A data from any of the trial blocks (see Table A55).

The number of responses made in each period B (during the CS) is also presented in Table A55. Analysis of variance revealed that during Test 1, trials 1-5, LVP treated rats ($\bar{x} = 4.25$) tended ($p < 0.1$) to make

more responses than saline controls ($\bar{x}$ = 2.15). There were no significant differences between groups on trials 6-10. In Test 2, trials 1-5, LVP treated rats ($\bar{x}$ = 8.35) made significantly ($p < 0.05$) more responses than saline controls ($\bar{x}$ = 6.55). In trials 6-10 of Test 2 there was a trend for LVP rats ($\bar{x}$ = 10.6) to make fewer responses ($p < 0.1$) than saline controls ($\bar{x}$ = 11.35).

Suppression ratios were calculated and analysed in the manner described for Experiment Four (see Table A55). In Test 1, trials 1-5, LVP treated rats ($\bar{x}$ = 0.2914) tended to show less suppression ($p < 0.1$) than saline controls ($\bar{x}$ = 0.1338). There were no significant differences between groups in any other trial block (see Table A56).

Extinction data

The number of responses made by each animal during extinction testing is presented in Table A57. Data from each group were compared using the Wilcoxon test (Seigel 1956) on responses summed across subjects on each block of five extinction trials. In Test 1 LVP treated rats made significantly more total responses ($p < 0.02$) than saline controls. This was due to a significantly greater number of short avoidances ($p < 0.01$) as there were no significant effects on long avoidance responses. During Test 2 LVP treated rats also made more total responses than saline controls ($p < 0.05$). However, in this test the difference was due to a greater number of long avoidance responses ($p < 0.01$) as there was no significant difference between groups on the number of short avoidances.

Discussion

LVP showed a strong trend ($p < 0.1$) to decrease suppression ratios compared to saline controls during the first five trials of Test 1. There were no differences between groups during any other trial blocks. LVP did not affect responding during period A but tended to increase period B responding during trials 1-5 of Test 1 and significantly increased period B responding in trials 1-5 of Test 2. This outcome contrasts with the results from Experiment Four in which LVP was found to decrease period B responding and consequently increase suppression ratios, and supports the conclusion of Experiment Four in which it was argued that increased fear of the CS cannot explain the effects of LVP on suppression ratios.

The pattern of operant response rate changes seen in the present experiment are the opposite of those seen in Experiment Four. Subjects in these two experiments differ primarily in their learning performance;

those in the present experiment failed to reach the learning criterion despite extended training trials. This suggests that the direction and magnitude of the LVP's effects on the operant response rate may be related to the level of response acquisition during avoidance learning. It is apparent from the present results that LVP may alter operant response rate changes induced by the CS even in rats which showed a very low probability of responding during training. Furthermore, LVP increased extinction responding in these animals; indeed, the response rate in extinction was only slightly lower than that seen in previous experiments in which rats had attained the learning criterion. This suggests that although attaining the criterion of ten correct consecutive avoidances is not essential to show considerable responding in extinction, the direction of the LVP effect seen in extinction may to some extent depend upon the level of responding during acquisition. Similarly the direction of operant response rate changes may depend upon this factor. Thus Experiments Three and Four showed that in rats which had acquired the response criterion LVP increased period B responding and suppression ratios and decreased extinction responding. In the present experiment, animals which failed to attain the criterion showed opposite effects on period B responding, suppression ratios and extinction responding.

6.3 Experiment Thirteen: The Effects of LVP (2, 3 or 4 µg) on Shuttle Box Extinction Responding in Rats which Failed to Reach Learning Criterion

Introduction

Experiment Twelve showed that 1 µg of LVP increased extinction responding when injected 30 minutes after training to rats which failed to reach the learning criterion of ten correct consecutive avoidances. This contrasts with the results from Experiments Three and Six in which this same dose given 30 or 60 minutes after training reduced subsequent extinction responding in rats which had attained the criterion. These findings may suggest that the outcome of LVP treatment may depend upon the level of response acquisition. Interpretation of the result from Experiment Thirteen is complicated by the interpolation of operant suppression tests. Therefore rats which failed to achieve criterion in Experiment Six were tested with various doses of LVP.

Methods

Subjects

Twenty-two adult male cfhb wistars from Experiment Six which failed to achieve the learning criterion were housed three or four to a cage with ad lib food and water.

Procedure

The apparatus and avoidance schedule have been described in Experiment One. Each of these rats received 50 training trials on each of two consecutive days. After the second acquisition session rats were returned to the home cage for 30 minutes and then injected with either 2, 3 or 4 µg of LVP or saline. Preparation and administration of the peptide has been described in Experiment Two. Approximately 24 hours after the end of training rats were returned to the shuttle box for 50 extinction trials (Test 1) and repeated 24 hours later (Test 2).

Results

Acquisition

The number of avoidance responses, escape responses, failures to respond and shocks received in training are summarised in Table A58. Performance was compared using the one way analysis of variance (Winer 1962) and the outcomes from these analyses are shown in Table A58. There were no significant differences between groups on any of these measures.

Extinction

Extinction performance is summarised in Table A59. Total number of each type of response, short avoidance (latency < 10 seconds), long avoidances (latency > 10 seconds) and total responses (short plus long avoidances) in each block of five trials was divided by n to obtain the mean number of responses per block of five trials for each group (see Table A59). Freidman's analysis of variance (Seigel 1956) was used to compare group performances in extinction (see Table A59). There were no significant effects of treatment in the total avoidance data of Test 1 or Test 2. There were significant treatment effects in the short avoidance data of Test 1 ($p < 0.02$) but not Test 2. There were no significant treatment effects in the long avoidance data from Test 1 or 2. In order to locate the significant treatment effects in the Test 1 short avoidance data multiple comparisons were made between groups using the procedure described by Hollander and Wolfe (1973). 3 µg of LVP tended to increase the number of short avoidances relative to saline ($p < 0.1$) and 2 µg significantly increased short avoidances relative to saline

($p < 0.046$). Furthermore, a 4 µg significantly increased short avoidances in Test 1 relative to 2 µg ($p < 0.05$).

Discussion

The results confirm the earlier indications (Experiment Twelve) which showed that LVP increased extinction responding when given to rats which failed to achieve the learning criterion. In the present study both 3 and 4 µg increased responding relative to saline and 2 µg. This contrasts with the results from Experiment Six in which the reverse relationship was found for increasing doses. Taken together with the results from Experiment Six, therefore, these results suggest that in this dose range the dose response relationship between LVP and extinction responding is positive in animals with very low levels of avoidance learning in training and negative in animals which had reached criterion.

6.4 Experiment Fourteen: The Effects of LVP, Scopolamine and Physostigmine on Extinction in Rats which Failed to Reach the Learning Criterion

Introduction

The results from Experiment Eleven showed that the response reducing effects of LVP given after 30 minutes of retention in the home cage did not involve cholinergic neurons. In Experiments Twelve and Thirteen LVP has been shown to have a different profile of effects when given to rats which failed to achieve the learning criterion. LVP (1 µg) given to criterion achievers 30 minutes after training decreased subsequent avoidance responding in extinction (Experiment Three) but increased responding in rats which failed to achieve the criterion (Experiment Twelve). This same dose increased suppression ratios in a conditioned suppression test when given to criterion achievers (Experiment Four) but tended to decrease suppression ratios when given to rats which failed to achieve the criterion (Experiment Twelve). Higher doses of the peptide yielded a negative dose response relationship in rats which had achieved the criterion (Experiment Six) but a positive dose response curve in those which had failed to achieve criterion (Experiment Thirteen). It was therefore of interest to examine the effect of LVP in rats which had failed to achieve the criterion and were also treated with either scopolamine or physostigmine.

Methods

Subjects

Thirty-four adult male rats which failed to attain the learning criterion in Experiment Eleven were used.

Procedure

The apparatus and training schedule have been described in Experiment One. During 50 training trials on each of two consecutive training days these rats failed to make ten correct consecutive avoidances. At the end of the second training session each animal was removed from the training cage and returned to the home cage for 30 minutes before injections.

Each animal was randomly allocated to receive one of six treatments: saline + saline, LVP + saline, saline + scopolamine, LVP + scopolamine, saline + physostigmine, LVP + physostigmine. The preparation, batch details and administration of these drugs has been described in Experiment Eleven.

Approximately 24 hours after treatment each rat was returned to the shuttle box for 50 trials of extinction testing (Test 1), repeated 24 hours later (Test 2).

Results

Acquisition

The performance of each animal during training was recorded using four measures: the number of avoidances, escapes, failures to respond and shocks received during training (see Table A60). The performance of each group was compared using analysis of variance (Winer 1962); outcomes are presented in Table A60. There were no significant differences between groups on any of the measures.

Extinction

The total number of avoidance responses, short avoidance responses (latency < 10 seconds) and long avoidance responses (latency > 10 seconds) made in extinction Tests 1 and 2 are summarised in Table A61. Data were summed to obtain the number of each type of response made by each animal in every block of five trials and then across rats to obtain the total number of each response type made by each group in every block of five trials. As there are unequal numbers in each group the total for each trial block was divided by n for each group (see Table A62). The total number of avoidances, the number of short avoidances and the number of long avoidances made by each group in each test was then compared using Freidman's two way analysis of variance (Seigel 1956) (see Table A62).

There were significant treatment effects in the total avoidances from Test 1 ($p < 0.001$) and Test 2 ($p < 0.001$), the short avoidance data from Test 1 ($p < 0.001$) and Test 2 ($p < 0.001$) and the long avoidance data from Test 1 ($p < 0.05$).

The significant differences between treatment groups in each test were located using the multiple comparison technique described by Hollander and Wolfe (1973). The outcome of these comparisons is shown in Table A63. LVP plus saline tended to increase Test 1 total avoidances and short avoidances relative to saline saline rats ($p < 0.1$); in Test 2 this difference achieved significance and LVP saline rats made significantly more total avoidances ($p < 0.009$) than saline saline controls. Rats injected with saline and scopolamine responded at similar rates to saline saline controls although in Test 1 there was a trend for scopolamine to increase the number of long avoidance responses ($p = 0.09$). Rats treated with saline and physostigmine made significantly more total avoidance responses ($p < 0.047$) and short avoidances ($p < 0.009$) than saline saline controls in Test 1. Similarly physostigmine increased both total avoidances ($p < 0.009$) and short avoidances ($p < 0.009$) in Test 2. Those rats which were injected with saline and physostigmine made significantly more total avoidance responses in Test 2 than those injected with saline and scopolamine ($p < 0.047$).

When LVP was injected in scopolamine treated rats the number of total avoidances was significantly increased ($p < 0.023$) and the number of long avoidances tended to increase ($p < 0.09$) relative to saline scopolamine treated rats in Test 1. These differences were not significant in Test 2. In addition, LVP scopolamine treated rats made significantly more total avoidances ($p < 0.023$) and short avoidances ($p < 0.009$) than LVP saline treated rats in Test 1 but not in Test 2. Although neither LVP nor scopolamine affected responding relative to saline saline controls in Test 1 when they were each given in combination with a saline injection when they were given together there were significant effects on extinction responding. Thus LVP scopolamine treated rats made significantly more total avoidances ($p < 0.009$) and short avoidances ($p < 0.009$) than saline saline controls in Test 1.

There were no significant differences between saline physostigmine treated rats and those treated with LVP and physostigmine. The significant increases seen when saline physostigmine rats were compared with saline saline controls were also found with LVP physostigmine rats. Thus, LVP physostigmine treated rats made significantly more total avoidance responses ($p < 0.023$) and short avoidances ($p < 0.009$) than saline saline controls in Test 1. Similarly, in Test 2 rats treated

with LVP and physostigmine made significantly more total avoidance responses ($p < 0.009$) and short avoidance responses ($p < 0.009$) than saline saline controls.

Discussion

When LVP was injected 30 minutes after the last training trial in rats which had failed to achieve the learning criterion the peptide increased avoidance responding in extinction Test 2 but not in Test 1. This increase is in agreement with the results of previous experiments (Experiments Twelve and Thirteen) and confirms that the effect of LVP at this dose in these rats is opposite to that seen in trained rats (Experiments Three and Six).

When scopolamine was injected with saline there were no significant differences in extinction with respect to saline saline controls. In contrast, when physostigmine was injected with saline, avoidance response rates in both Tests 1 and 2 were increased significantly with respect to saline saline controls. This contrasts with the effects of these drugs seen in Experiment Eleven when injected into rats which had attained the criterion. In that experiment neither treatment significantly altered response rates in extinction with respect to the saline saline controls although scopolamine treated rats made significantly more extinction responses than physostigmine treated animals. The differences between drug treatments were not significant in the present experiment although the trend in the relationship was the reverse of that seen in Experiment Eleven with scopolamine treated rats making fewer responses than physostigmine treated rats. The effect of these two drugs given in the same doses at the same time after training is different in rats which failed to learn the response than in those which learned the response.

There was evidence in Test 1 that scopolamine acted to facilitate the effects of LVP. The peptide alone did not significantly increase response levels in Test 1 and scopolamine did not affect response levels in either Test 1 or Test 2. However, when scopolamine and LVP were given together this combination increased Test 1 response levels relative to saline saline controls, LVP saline treated rats and saline scopolamine treated rats. LVP did not affect the response levels found in physostigmine treated rats. These data suggest the blockade of post training cholinergic activity with scopolamine facilitates the response increasing effect of LVP in rats which failed to achieve criterion.

CHAPTER SEVEN

EFFECTS OF LVP ON APPETITIVELY MOTIVATED RESPONDING

7.0 Introduction

The experimental literature on the behavioural effects of the vasopressins is largely dominated by experiments which have used aversively motivated responding. Pole jump avoidance, passive avoidance and shuttle box avoidance responding are very sensitive to these peptides. Appetitively motivated responding has received less attention. This may in part be attributed to negative results in earlier experiments; Garrud et al (1974) could find no effect of DG-LVP on an open field test or in the extinction of a straight runway task involving approach to food. However, more recent data from Hostetter et al (1977) have demonstrated that pitressin can affect responding in a T maze discrimination task. Food deprived rats were rewarded for making the correct choice between the black and the white arm of a maze. When pitressin (0.4 IU) was injected SC before each training session there was no effect on the number of trials required to reach the extinction criterion. However, when the same dose was injected before extinction testing the number of trials to reach the extinction criterion was increased although only in rats trained to enter the black arm of the maze but not in those trained to enter the white arm. Time required to execute the maze did not differentiate the groups confirming earlier negative results (Garrud et al 1974). Bohus (1977) was able to demonstrate an effect of vasopressin when male rats were rewarded with copulation following a correct choice in a T maze. These two studies demonstrate that the peptides are active under conditions of positive reinforcement when the measures used are sufficiently sensitive.

Garrud (1975) observed that 2 µg of LVP reduced responding on a variable interval appetitive baseline.

These studies suggest that appetitively motivated responses are sensitive to the action of the vasopressins. Experiments described in this chapter examine the effects of LVP on appetitively motivated responding maintained on Variable Interval (VI) and differential reinforcement of low rates (DRL) schedules.

7.1 Experiment Fifteen: The Effects of Five Doses of LVP on Lever Pressing Maintained on a Variable Interval 60 Second (VI 60) Schedule

Methods

Subjects

Six adult male rats (cfhb wistar) from the closed colony maintained at Plymouth Polytechnic were housed four to a cage with ad lib access to water. Rats weighed between 200 and 250 g at the start of the experiment.

Apparatus

The lever press apparatus has been described in detail in Experiment Four. Briefly, one lever was removed from a two lever rat operant chamber housed in a sound and light attenuating cabinet. Illumination was provided by a single house light located in the top of the operant chamber. Reinforcement was provided by 45 mg food pellets (Campden Instruments Company Limited) delivered automatically from a solenoid operated magazine.

Schedule

The variable interval schedule was programmed using the Grason Stadler 1201 solid state seriesof control modules. Intervals ranging from 2 to 120 seconds (Clarke 1958) were randomly arranged to yield a variable interval schedule with an arithmetic mean of 60 seconds. A limited hold of two seconds was incorporated in the programme. A further refinement was introduced in order to avoid confounding due to predictability in the interval sequence. The interval sequence was divided into two and these were randomly juxtaposed periodically. A response within two seconds of reinforcement becoming available automatically terminated the limited hold and the response availability to prevent multiple reinforcements. Inter response times were recorded, to the nearest tenth of a second, using automatic timers and a re-set printer.

Peptide treatment

The batch details, solution preparation and storage details have been described in Experiment Two. Five doses of LVP were used plus a saline control; 0.5 µg, 1 µg, 2 µg, 3 µg or 4 µg were injected SC in a constant volume of 0.5 ml 60 minutes before each experimental session.

Procedure

Rats were reduced to 80% of their free feeding body weight by reducing the weight of food available per day and restricting the time for which it remained available. In this way the subjects were adapted

to feeding at the same time each day; having reached their target weight the feeding schedule was stabilised for one week before starting to magazine train. Body weights were checked before each experimental session.

During magazine training the rats were placed in the operant chamber and a free food pellet delivered every 60 seconds for 40 minutes. This continued until each rat readily approached and ate from the hopper. Rats were then shaped to lever press; a food pellet was awarded for progressively closer approaches to the lever, then for touching and finally for depressing the lever. Once each animal had acquired the bar press response, it was introduced to and stabilised for two days on an automatically controlled schedule of continuous reinforcement (CRF). The variable interval schedule was programmed in such a way that the time base for the intervals could be reduced to milliseconds, effectively mimicking a CRF. The VI schedule was introduced by increasing the interval time base and reducing the limited hold until the required VI 60 seconds with two seconds limited hold had been reached. Rats were stabilised on the VI 60 second schedule for two weeks in 40 minute sessions at the same time of day. After each session additional standard lab diet was made available in the home cage in order to maintain each subject at 80% of its free feeding weight.

Experimental phase

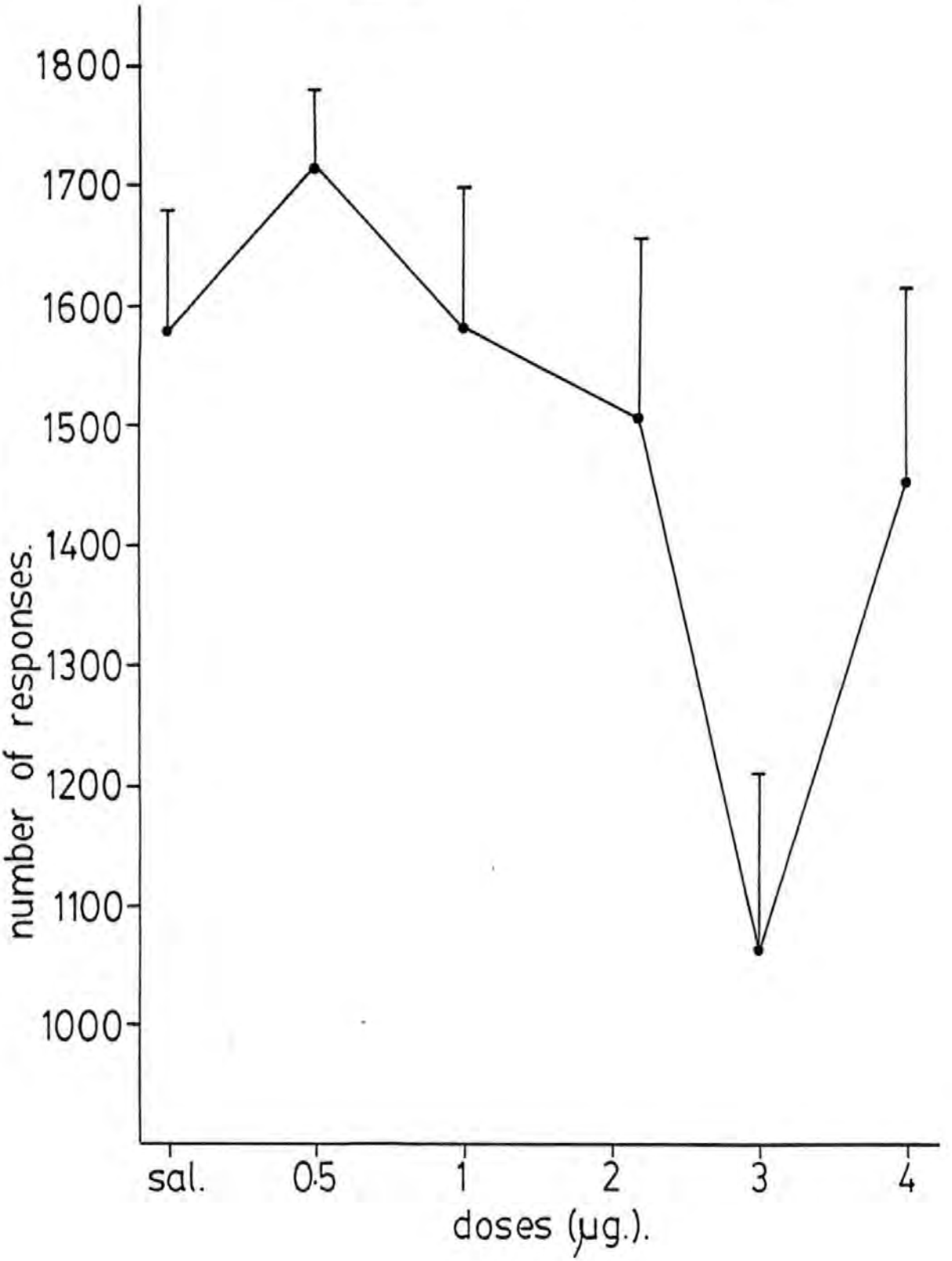
Each rat was given one of the six treatments on one of six experimental days. The order of treatments in this 6 x 6 x 6 design was specified by a latin square (Kirk 1968). The six treatment days were spaced over three calendar weeks. Two test days (Tuesday and Friday) were spaced by at least two rest days and each test day by at least one day when subjects responded on the schedule in the absence of any treatment (Monday and Thursday). Injections were made 60 minutes before the start of each test session, which lasted for 30 minutes and was preceded by ten minutes of warm up responding.

Results

Inter response times were checked and punched onto computer cards. Data from each of the 36 cells of the experiment were processed by a computer program which sorted the inter response times into 49 bins of 0.1 seconds. Bins 1-49 contained all the inter response times spanning from 0.1 second to 4.9 seconds. The 50th bin contained all inter response times in excess of 4.9 seconds.

After visual inspection, two aspects of the data were selected for statistical analysis: the total number of inter response intervals and

Figure 27 The number of responses per dose of lvp (mean and s.e.m.)



the 50% interquartile range. Table A64 shows the total number of response intervals in each cell. These values were divided by a constant (460) and analysed by analysis of variance for latin squares according to the method of Kirk (1968). The analysis determined whether or not the total number of responses intervals was affected significantly by one of the three factors of dose, treatment day or subjects. The outcome of this analysis is shown in Table A65. There was a significant difference in the number of inter response times as a function of the treatment dose ($F_{5,20} = 3.331$, $p < 0.05$). No other factors were significant. Newman-Keuls test (Kirk 1968) was used with the error rate set experimentwise at $\alpha = 0.05$ to locate the significant differences between doses. These are summarised in Table A65. 3 µg of LVP significantly reduced inter response intervals compared to saline, 0.5 µg, 1 µg, 2 µg and 4 µg of LVP ($p's < 0.05$). The change in the number of inter response intervals as a function of the dose is shown in Figure 27.

In order to locate changes which occurred in the response distribution independent of changes in the total number of inter response intervals the 50% interquartile range for each cell was calculated; this is the point in the range of bin values by which 50% of the total number of intervals had occurred. The 50% IQR did not coincide exactly with bin boundaries; therefore the value was estimated using a method of proportional allocation, eg suppose that an animal had 1786 inter response intervals, 50% of this value is 893. The cumulative total at bin 3 is 840 and at bin 4 is 947. Therefore 47.03% of the inter response intervals were less than 0.3 seconds and 53.02% of the inter response times were less 0.4 seconds. The 50% value lies in bin 4 and was estimated as the ratio of two differences:

(A) the difference between the 50% value (893) and the cumulative total at the first bin with value lower than this (bin 3 with a cumulative total of 840 inter response intervals);

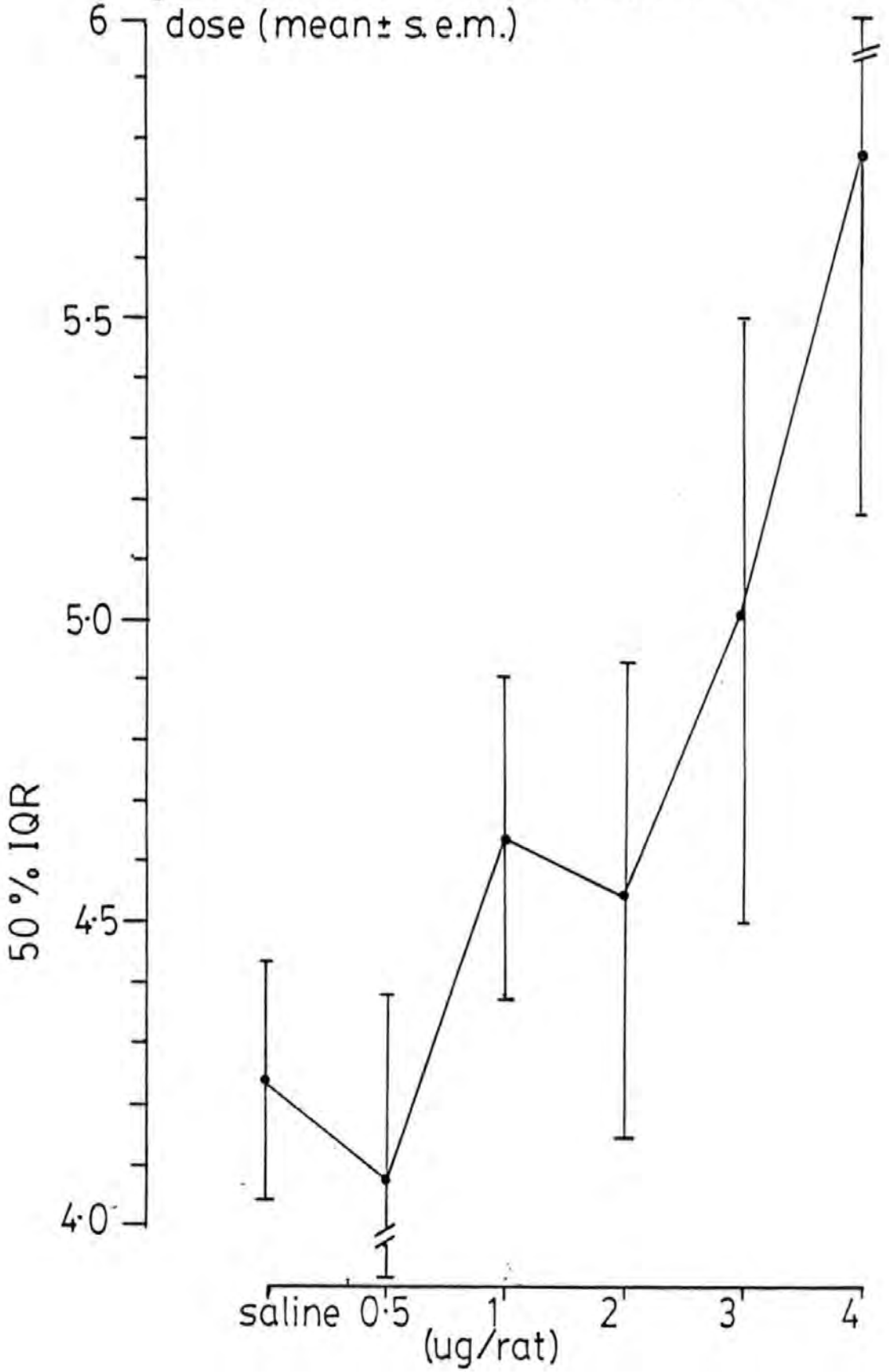
(B) the difference between the cumulative total at the first bin with a value lower than the 50% value (bin 3, 840) and the cumulative total at the first bin with a value higher than the 50% value (bin 4, 947).

Thus, for the example described,

$$\frac{893 - 840}{947 - 840} = 0.4953.$$

This value is added tothe number of the lower bin (bin 3) to yield 3.4953 as the estimated 50% IQR. According to this estimate 50% of the inter response times made by the rat in this cell were shorter than 0.34953 seconds and 50% were longer.

Figure28 50% IQR as a function of LVP dose (mean ± s.e.m.)



The 50% IQR was calculated for each of the 36 cells of the experiment and are shown in Table A66. Analysis of variance for this latin square design (Kirk 1968) was used to analyse the effects of LVP dose, treatment day and variation between subjects. There were significant effects of LVP dose ($F = 3.457$, df 5,20, $p < 0.05$) and treatment days ($F = 3.984$, df 5,20, $p < 0.05$) (see Table A67). Newman-Keuls tests (Kirk 1968), summarised in this table, revealed that 4 µg of LVP yielded a significantly greater 50% IQR value than saline, 0.5 µg, 1 µg and 2 µg (all p's < 0.05). The IQR decreased as a function of the treatment day. The mean on day one was significantly greater than on day four ($p < 0.01$) and on days five and six (p's < 0.05). The changes in IQR as a function of the LVP dose and the treatment day are shown in Figure 28.

Discussion

3 µg of LVP significantly reduced the total number of inter response intervals compared to saline and to all other LVP doses. This may have been due to very low responding from one rat at this dose. The IQR statistic allows an evaluation of changes in the response distribution independent of gross changes in the number of intervals. It is clear from Figure 28 that the IQR statistic increased as a function of dose of LVP. The highest dose used (4 µg) showed the highest mean IQR; this was significantly greater than the value found for saline, 0.05, 1 or 2 µg. Therefore 4 µg induced a significant shift in the distribution of the inter response intervals.

The IQR statistic decreased as a function of the treatment day and this shift occurred in the absence of any increase in the total number of inter response intervals as a function of treatment days. These two indices did not vary in a similar fashion as a function of treatment days. Progressive increases in motivation to respond could decrease the IQR but if this were the case then both the IQR and the total number of inter response intervals should vary in parallel or at least in a similar pattern, which they do not. Alternatively, the progressive decrease in the IQR as a function of the treatment day may reflect changes due to cumulative treatment effects, either as a result of repeated stressful injections per se or as a result of cumulative effects of LVP.

7.2 Experiment Sixteen: The Effects of Five Doses of LVP on Rats Responding on a Differential Reinforcement of Low Rates (DRL) 11.8 Second Schedule

Introduction

In Experiment Fifteen it was shown that LVP affected the total number of inter response intervals and the 50% interquartile range statistic of response interval distribution when rats were responding on a VI 60 second schedule. This schedule was characterised by rapid sustained responding which maximises reinforcement rate. In contrast, schedules using differential reinforcement of low response rates (DRL) require rats to withhold responses. Inter response intervals shorter than the pre determined interval do not produce reinforcement whilst those which are equal to or longer than the stipulated interval do produce a reinforcement. The distinction between responses which achieved reinforcement (hits) and those which did not (false alarm) permit an animal's response profile to be examined as a function of these two rates. A range of doses of LVP were tested on this schedule, to determine if there were effects on the total number of inter response intervals or if effects were restricted to the rate of false alarm or hit responding.

Methods

Subjects

Six adult male cfhb wistar rats were housed four to a cage, with other experimental animals, and ad lib access to water. They weighed 200-250 g at the time of the experiment.

Apparatus

The two lever Skinner box used in this experiment has been described previously (Experiments Four and Eleven). One lever was removed from the standard chamber; a single house light provided illumination and the cage was housed in a sound and light attenuating cabinet. Automatic schedule control and data recording were provided by the Grason Stadler 1201 series of solid state modules.

Schedule

The schedule was programmed so that a response could only be reinforced with a food pellet (45 mg Campden Instruments Limited) if 11.8 seconds or longer had elapsed from the previous response, whether or not the previous response had been reinforced. If the animal responded before 11.8 seconds had elapsed then the timers re-set the

interval and the 11.8 second period re-started. If the interval between responses was equal to or greater than 11.8 seconds the rat was reinforced with a single pellet and the interval re-set. There was no upper limit on the inter response times. Responding was monitored on a cumulative recorder and inter response times were recorded using a print out counter.

Procedure

Rats were reduced to 80% of their free feeding weight and stabilised at this weight for one week before being magazine trained. During magazine training rats were adapted to being fed at the same time each day; the procedure used to shape the bar press response has been described in Experiment Eleven. Having learned the response, each rat was shifted to the CRF schedule as described in Experiment Eleven. The interval for which rats were required to withhold responding was gradually increased to the full 11.8 seconds and responding with this interval was stabilised for three weeks before starting the experimental phase of the experiment.

The experiment design was a latin square design outlined by Kirk (1968) and identical to that used in Experiment Eleven. Six doses of LVP (0.5 µg, 1 µg, 2 µg, 3 µg, 4 µg) or saline were injected (SC) on one of six experimental days. The experimental phase extended over three weeks; two experimental sessions were run in each week and each experimental session was separated by at least two days with no treatment on at least one of which subjects were run on the DRL schedule.

Prior to each experimental session rats were taken from the home cage, weighed, injected and returned to the home cage for one hour. Then rats were placed in the Skinner box for the 40 minutes of bar pressing. At the end of each session the rats were removed from the cage and given free food sufficient to maintain their body weight at 80% of their free feeding levels. The 23 hour food deprivation state was maintained by running the animals in the same order and at the same time each day.

Data

Inter response times, to the nearest tenth of a second, were recorded for each session and these values were punched onto paper tape and processed by a PDP8 computer programmed to classify latencies into 30 bins of one second width. Bins 1-29 contained all inter response times up to 29 seconds. Thus bin 1 counted all response times up to one second long, bin 2 counted the number of inter response intervals which were between one and two seconds long, etc. Bin 30 counted inter

response times in excess of 29 seconds.

From histograms, obtained from each of the 36 cells of the experiment, the following indices were calculated:

(1) the total number of inter response intervals per cell;

(2) the total number of rapid responses (latency less than or equal to one second);

(3) the total number of long responses (latency greater than 29 seconds).

These indices refer to the total data in each cell; the following were calculated from an 11 second bandwidth, ie all inter response intervals occurring within 11 seconds of the target time of 11.8 seconds (bins 2-23):

(4) the number of false alarms (sum of intervals in bins 2-11 inclusive);

(5) the number of hits (sum of intervals in bins 12-23 inclusive).

From these values the conditional probability of a false alarm was calculated using the formula:

(6) $P(fa) = \frac{X}{Z - P}$, where X = sum of false alarms within the sample bandwidth
Z = sum of all inter response intervals
P = sum of all false alarms outside the sample bandwidth (rapid responses).

Similarly the conditional probability for a hit:

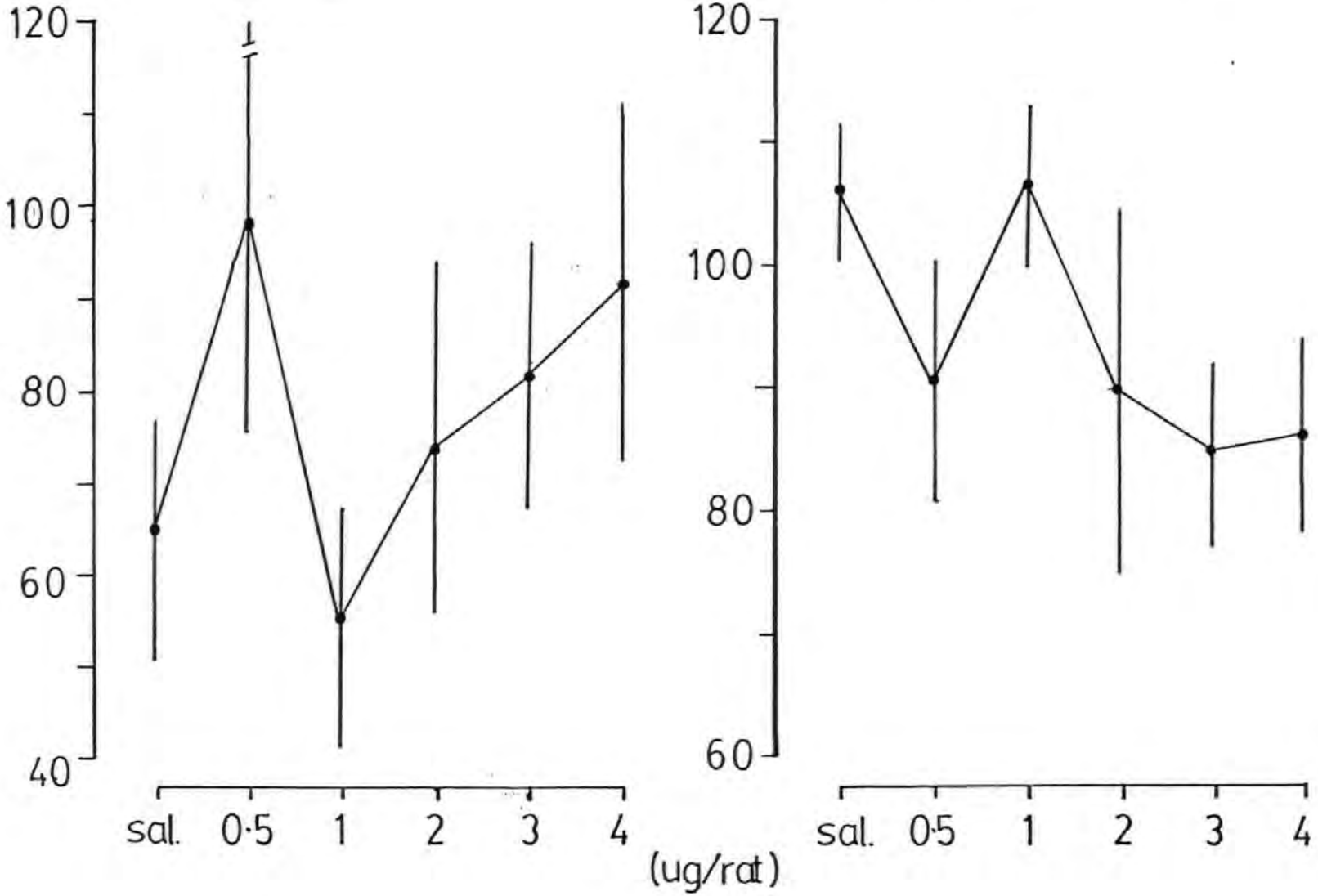
(7) $P(hit) = \frac{Y}{Z - P - X}$, where Z, X and P are defined above
Y = sum of hits within sample bandwidth.

A breakdown in the recording equipment resulted in loss of data from three of the 36 experimental cells.

Results

The data obtained under each peptide dose is presented in Table A68. Loss of data from three cells precluded a latin square analysis of variance on each of the indices. One tailed paired 't' tests were used to compare performance under each dose with performance under saline. The outcome of these tests is presented in Table A69. Total number of responses tended to increase with 0.5 µg ($p < 0.1$). There were no significant effects on either the number of rapid responses or the number of long latency responses. False alarms and the probability of a false alarm tended to increase after the highest dose, 4 µg ($p's < 0.1$). The total number of hit responses was significantly decreased after both 3 µg ($p < 0.05$) and 4 µg ($p < 0.01$), but the probability of a hit response was not significantly changed at any dose.

Figure 29. False alarms. Hits. (means ± s.e.m.)



The false alarm and hit data are shown in Figure 29. The variability between doses and within doses was higher in the false alarm than in the hit data; therefore significant effects were only seen in the hit data. Total number of responses did not vary as a function of the dose and therefore the mean number of false alarms was inversely proportional to the mean number of hit responses. The mean number of hit responses under each dose was negatively correlated with the mean number of false alarms ($r = 0.816$, $p < 0.05$ two tailed; slope = -0.4).

Discussion

LVP did not affect the total number of responses, the number of responses with latencies less than one second or greater than 29 seconds after any of the doses tested. The data show that rats remained under a constant state of motivation, and were not suffering from motor incapacities, therefore keeping the extremes of the response latency distribution constant.

Figure 29 shows that the number of false alarm responses tended to increase withthe dose of LVP as compared to saline control levels. These changes did not achieve significance due to the increased variability seen after each peptide treatment. However, the number of hit responses was significantly decreased by both 3 µg and 4 µg (see Figure 29). The data suggest that the significant decrease in the level of hit responding was parallelled by a non significant increase in false alarms. In the absence of shifts in the total number of responses this suggests that the higher doses affected a shift in the response latency distribution. The inverse relationship between false alarms and hits is also supported by the significant negative correlation seen between these measures.

In Experiment Eleven it was found that 4 µg LVP injected 60 minutes before responding on a VI 60 second schedule induced a significant shift in the response distribution, measured by the 50% IQR statistic, in the absence of significant changes in the total number of responses. This conclusion is supported by the results from the present experiment. Thus high doses of the peptide shifted the response latency distribution under two schedules which make very different demands in terms of response characteristics. A number of hypotheses may be excluded; motor inefficiency or incapacity is unlikely in view of the absence of changes in the total number of responses or in the number of very short and very long latency responses in the present experiment. Similarly, marked changes in motivation appear unlikely in view of the constancy in total responses in both experiments. Both experiments suggest that vasopressin

in some way alters the animals' capacity to guage the interval between responses; this may be due to disrupted 'timing' or to alterations in concommitant behaviour patterns.

CHAPTER EIGHT

CONCLUSIONS

It is well established that vasopressin, synthesised in the supra-optic and paraventricular nuclei of the anterior hypothalamus (Section 1.2.1) and secreted into circulating blood via the posterior lobe of the pituitary gland regulates water reabsorption at the kidney (Section 1.8.1) and pressor responses (Section 1.8.2). The neurochemical mechanism controlling this route of secretion involves complex interactions between catecholaminergic, cholinergic and histaminergic neurons (Section 1.4). Peripheral vasopressin levels are sensitive to changes in levels of hydration (Section 1.6.1), blood volume (Section 1.6.3), angiotensin and renin (Section 1.6.2), sexual stimulation (Section 1.6.5) and some stressors (Section 1.6.4). The identification of vasopressin as the CRF remains controversial (Section 1.9.3) although some evidence suggests that ACTH secretion is stimulated by vasopressin injections, particularly in high doses (Section 1.9.1). These findings implicate vasopressins in regulation of physiological functions and the maintenance of homeostasis through changing environmental and behavioural conditions.

In addition to its established endocrinological activity and its putative role as a CRF (Section 1.9.3) extensive evidence shows that vasopressins exert marked behavioural effects (see Chapter Two). Rats with depleted endogenous vasopressin levels as a result of surgical removal of the posterior lobe of the pituitary gland (Section 2.1), central injections of antivasopressin serum (Section 2.5.5) and in some instances as a result of genetic deficiencies in vasopressin synthesis (Sections 2.5.2, 2.5.3 and 2.5.4) show reduced active and passive avoidance extinction, which can be corrected with injections of pitressin, a posterior pituitary extract, vasopressin (Section 2.1) or its des-glycinamide analogues (Section 2.3). Conversely these substances have been universally reported to increase subsequent extinction responding after central or peripheral injections (see Chapter Two). These observations, coupled with the fact that the behavioural potency of vasopressin and centrally injected antivasopressin serum have been reported to decline as a function of the interval between injection and either the end of training or the first extinction test and exert long term influences on behaviour which far exceed the metabolic half life of the peptides (Section 2.2) have led to the widely accepted hypothesis that endogenous vasopressin plays an important physiological role in regulating the consolidation of associative or cognitive information

about the behavioural schedule or schedule changes from short to long term memory stores. Additional supportive evidence comes from studies showing that vasopressins antagonise the amnestic effects of puromycin (Section 2.4.1), anoxia (Section 2.4.2), electroconvulsive shock and pentylenetetrazol injections (Section 2.4.2).

The consolidation hypothesis appeared to account for much of the data described in Chapter Two although it was also necessary to propose additional involvement in "retrieval" mechanisms in order to explain effects of the peptide when injected 24 hours after training and one hour before extinction or passive avoidance retention testing (Sections 2.2 and 2.3). Further difficulties stemmed from the fact that much of the corroborative evidence from rats with a genetic incapacity to synthesise vasopressin is conflicting (Sections 2.5.2, 2.5.3 and 2.5.4) and that the physiological mechanisms which underlie the effects of experimental amnestic treatments and their relevance to normal memory processes are poorly understood. Furthermore, increasing evidence from human neuropsychological studies suggest that the consolidation hypothesis does not offer a satisfactory account of memory disturbances characteristic of clinical amnesia (Section 2.0).

A number of additional arguments, based on the experimental evidence described in Chapters Three to Six, may be advanced against the consolidation hypothesis and in favour of the contention that consolidation of short term memories are not invariably enhanced by vasopressin injections (King and de Wied 1974).

Vasopressin injections one hour before (King and de Wied 1974) and immediately after response prevention trials (Experiment Three) increased subsequent extinction responding despite extensive evidence that prevention trials alone (Sections 3.0 and 3.1) and when followed immediately by saline injections (Experiment Three) reduce subsequent extinction responding. The response reducing effects of prevention trials have been interpreted in terms of the additional "information" conveyed during confinement concerning the contingencies of the avoidance schedule whether this be conceived in terms of enhanced fear extinction, learning alternative responses or altered expectancies (Sections 3.1 and 3.2). The fact that response deficits are seen when tests are delayed for 24 hours after prevention trials (Experiments One and Three) argues against an explanation in terms of reduced mobility or perseveration of alternative responses. If the action of vasopressin was to enhance consolidation of "information" contained in short term stores then the peptides should have further reduced extinction responding when given in conjunction with extinction trials and this was not found. This shows

that the effects of vasopressin injections may be dissociated from the informational content of the behavioural procedure with which the injections were associated.

Vasopressin and prevention trials do not appear to affect the same mechanism. Cholinergic drugs did not substantially alter LVP's response reducing effects but did alter the outcome of response prevention trials (Experiment Eleven). It was shown in Experiment Four that the effects of LVP injections and prevention trials were clearly distinguishable on concurrent operant suppression during CS presentations. Prevention trials altered baseline response rates but neither response levels during CS presentations or suppression ratios whereas post training LVP injections increased suppression of the operant baseline during CS presentations but left baseline response rates undisturbed. Taken together with the results from Experiment Three, in which it was found that the effects of LVP injected after 30 minutes of home cage retention or 30 extinction trials were opposite to its effects after prevention trials, the data suggest that the effects of prevention trials and LVP injections cannot be explained in terms of consolidating the storage of information. Similarly, increased fear of the CS after post training LVP injections, which might be suggested by Experiment Four despite difficulties of interpreting operant suppression as a measure of conditioned fear (see Experiment Four) does not explain the data. According to two factor theory of avoidance greater suppression should be associated with more avoidance responding in extinction and the opposite was found, and according to the more recent cognitive explanation of avoidance responding variations in conditioned fear have no direct consequences for response rates (Seligman and Johnston 1973). As the peptide was invariably injected after training and approximately 24 hours before extinction testing this tends to rule out effects on arousal, attention or motor activity during training.

Furthermore, rats which were either retained in the home cage for 30 minutes or given 30 extinction trials before LVP (1 µg) injections showed reduced responding during subsequent extinction tests. These results stand in sharp contrast tothose discussed in Chapter Two in which vasopressin injections have been universally found to increase subsequent extinction responding. That this unusual result was not due to peculiarities in the avoidance training schedule, impurities in the vasopressin batch or faulty preparation, storage or injection procedures was shown by Experiment Two in which identical injections immediately after training increased subsequent extinction responding and by subsequent replications with manual shuttle box training (Experiments Seven,

Eight, Nine and Ten). Assuming that retention in the home cage per se has no bearing on the subsequent execution of a previously trained response and conveys no additional information the results show that LVP injections may reduce extinction responding in the absence of changes in the informational content of the training schedule and confirm conclusions from response prevented rats that vasopressin's effects on subsequent extinction responding are independent of the informational content of the behavioural procedures with which its injection is associated. This was further substantiated in Experiments Twelve and Thirteen which showed that in rats which failed to reach learning criterion LVP (1 µg) increased subsequent extinction responding.

Comparisons between Experiments Two and Three, which showed opposite effects with the same dose of LVP, suggested that the interval between the end of training and injection is an important variable in determining the direction of LVP's effects on subsequent extinction. Previous studies (Section 2.2) had shown interval to be an important determinant of potency but not direction. Studies using prolonged extinction tests (Experiment Five) confirmed the response reducing effects of LVP (1 µg) and showed maximal reductions when injections were delayed for 60 minutes after training but failed to confirm the response increasing effects of this dose injected immediately after training which were reported in Experiment Two. In addition at the 60 minute interval prevention trials acted as an effective adjunct to the response reducing effect of vasopressin injections, in contrast to the effects when injected immediately after prevention trials (Experiments Three and Five). Additional studies (Experiment Six) suggested that when injected 30 minutes after training in the range 2-4 µg LVP tended to decrease subsequent extinction responding in a dose dependent manner; this was more pronounced in response prevented rats. In rats which failed to reach learning criterion the dose response curve was positive (Experiment Thirteen) in this dose range.

Subsequent experiments examined more closely the interaction between dose and injection interval using a manually operated shuttle box and fewer extinction trials. With this procedure the response was learned rapidly and during extinction trials the probability of an avoidance response tended to diminish whereas that of an intertrial response tended to increase. Experiment Seven showed that in the range 0.036 to 2.97 µg the effects on avoidance responding in extinction varied as an inverted U shaped function of the dose. The lowest and highest doses reduced whilst intermediate doses increased subsequent responding. Maximal facilitation of the extinction response rate,

including elevated intertrial responding, was found after 0.11 µg. Although 0.99 µg also increased avoidance responding in extinction, the effect was smaller than for either 0.11 or 0.33 µg. Vasopressin injections affected within test rates of response change. Responding on initial test trials tended to be high regardless of dose, group differences depended on subsequent reductions or increases in response rate compared to saline controls. The dose response curve from 0.11 to 2.97 µg was negative, confirming indications from Experiment Six for a similar range and the weak effect seen after 0.99 µg may explain why the effects of 1 µg LVP reported in Experiment Two were not replicated in Experiment Five.

These results suggest a complex interaction between dose and injection interval. Experiment Eight showed that whereas 0.11 µg also increased responding when injected immediately or 60 minutes after training, the direction in which 2.97 µg influenced subsequent extinction responding was found to vary as a function of the interval between training and injection. In contrast to its response reducing effects when injected 30 minutes after training 2.97 µg was ineffective when injected immediately and increased responding when injected 60 minutes after training.

The mechanism by which LVP exerts its behavioural influence in these experiments is unknown. The opposite effects of 0.11 and 2.97 µg injected 30 minutes after training were confirmed using a higher shock level in training (Experiment Nine) and it was argued that, unlike a similar dose response curve reported for ACTH (Gold and van Buskirk 1976), the inverted U shaped dose response curve for vasopressin could not be attributed either to dose dependent changes in post training arousal or to modulation of the hormonal consequences of shock.

Data from Experiment Ten argue against a role for target organ related endocrine effects in mediating the response reducing effects of LVP. Endogenous vasopressin is known to affect endocrine target organs (Section 1.8) in addition to its behavioural effects. However, DG-LVP is thought to retain only behavioural activity, at least using acute treatments (Section 2.3). Therefore finding that DG-LVP injected in a wide range of doses 30 minutes after training reduced but never increased subsequent responding suggests that endocrine effects may in fact be required for the response increasing effects of intermediate doses of the full vasopressin molecule. Although these effects of vasopressin may reflect direct actions on the CNS accessed following peripheral injections via the CSF (Section 1.3.3) and possibly involving extra-hypothalamic vasopressinergic pathways (Section 1.3.4), the involvement

of corticosteroids and ACTH, particularly after high vasopressin doses (Section 1.9), cannot be ruled out.

It is interesting to note that others have reported similar anomalies using post training injection procedures. Gold, van Buskirk and Haycock (1977) reported an inverted U shaped dose response relationship between post training epinephrine injections and subsequent passive avoidance retention. Furthermore, a low dose of epinephrine (50 µg/kg) increased subsequent retention when injected immediately but not 10 or 30 minutes after training. In contrast, a ten times higher dose was ineffective when injected immediately or 30 minutes after training but improved retention when injected after 30 minutes. Recently Messing et al (1979) reported that intermediate doses of naloxone (1 mg/kg) increased subsequent retention when injected immediately or 30 minutes after passive avoidance training whereas a low dose (0.1 mg/kg) and a high dose (10 mg/kg) were ineffective. Furthermore, 0.5 mg/kg of naloxone was inactive when injected immediately after passive avoidance training but significantly reduced retention when injection was delayed for 30 minutes.

The observations of inverted U shaped dose response relationships and anomalous time related effects with such a wide range of drugs may suggest a complex interaction with post training neurochemical changes and a common mode of action. Recent work using experimentally induced "amnesias" suggests that these may have a common mechanism (Gold and Sternberg 1979). Pretreatment with the a-adrenergic blocker phenoxybenzamine blocked the development of several different types of amnesia. The extensive evidence outlined in Section 2.7 suggesting that vasopressin alters catecholamine metabolism in discrete brain nuclei may provide an explanation for the results of the experiments reported in terms of fluctuations in post training activity at catecholaminergic nerve terminals in the CNS. This is speculative but could be tested by examining the effects of altered post training CA activity on oppositely acting vasopressin doses. The complexity of dose response relationships and the effects of varying training - injection intervals coupled with the difficulties of explaining the data in terms of behavioural constructs such as consolidation, fear, anxiety or arousal suggests that, whilst experiments on the neurochemical and physiological bases for vasopressin's actions may prove fruitful, these must be accompanied by stringent analysis of behavioural variables in order to characterize more fully the behavioural importance of vasopressin's pharmacological effects. The use of global but imprecise psychological constructs such as memory is of doubtful significance in furthering our understanding of

these phenomena, more restricted but precise concepts are required. The data from Experiment Eleven argue against any significant involvement of cholinergic neurons in mediating the response reducing effects of LVP in well trained rats although this did not appear to be the case for poor responders (Experiment Fourteen). Poor shuttle box avoidance learners may also be distinguished from good performers by their lower disappearance rate for labelled catecholamines in the hippocampus, hypothalamus, brain stem and cortex (Hraschek, Paulik and Endroczi 1977). Investigations of the peptide's physiological basis of action may be facilitated by the use of appetitive response schedules. Experiments Fifteen and Sixteen show that although a variable interval schedule was sensitive to the effects of vasopressin a DRL schedule was not. This was thought to reflect different demands in the speed of responding for these schedules rather than different psychological processes involved.

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Table A1: Experiment One. Acquisition performance

	Home cage immediate	Home cage 24 hours	Extinction immediate	Extinction 24 hours	Response prevention immediate	Response prevention 24 hours
			Trials to criterion			
Sum	293	455	310	468	296	418
$\bar{x}$	36.625	56.875	38.75	58.5	37	52.25
SD	40.01	33.138	22.44	47.277	18.647	46.876
SE	14.146	11.716	7.93	16.715	6.593	16.573
			Avoidances to criterion			
Sum	165	201	189	246	161	211
$\bar{x}$	20.625	25.125	23.625	30.75	20.125	26.375
SD	16.071	11.407	10.809	20.886	5.592	15.061
SE	5.682	4.033	3.822	7.384	1.977	5.325
			Escapes to criterion			
Sum	99	102	87	119	45	102
$\bar{x}$	12.375	12.75	10.875	14.875	5.625	12.75
SD	18.913	9.823	11.051	10.973	6.301	16.49
SE	6.687	3.473	3.907	3.88	2.228	5.83
			Failures to criterion			
Sum	26	149	38	107	87	96
$\bar{x}$	3.25	18.625	4.75	13.375	10.875	12
SD	5.064	23.120	4.527	30.720	18.427	18.189
SE	1.79	8.174	1.60	10.861	6.515	6.431
			Shocks to criterion			
Sum	340	943	314	718	547	701
$\bar{x}$	42.5	117.875	39.25	89.75	68.375	87.625
SD	69.463	121.961	27.773	165.503	99.863	133.252
SE	24.559	43.12	9.819	58.514	35.307	47.112

Table A2: Experiment One. Analysis of acquisition data

	Source	SS	df	MS	F	P
Trials to criterion	Between groups	4258.917	5	851.78	0.64	NS
	Within groups	55879.75	42	1330.47		
	Total	60138.667	47			
Avoidances to criterion	Between groups	622.94	5	124.59	0.62	NS
	Within groups	8396.88	42	199.93		
	Total	9019.88	47			
Escapes to criterion	Between groups	410.42	5	80.28	0.48	NS
	Within groups	7058.5	42	168.06		
	Total	7459.92	47			
Failures to criterion	Between groups	1298.35	5	259.67	0.71	NS
	Within groups	15363.63	42	365.8		
	Total	16661.98	47			
Shocks to criterion	Between groups	36718.85	5	7343.77	0.58	NS
	Within groups	529135.63	42	12598.47		
	Total	565854.48	47			

Table A3: Experiment One. Extinction performance

	Home cage				Extinction				Response prevented			
	Immediate		24 hours		Immediate		24 hours		Immediate		24 hours	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
	Total responses in extinction											
S1	29	27	50	50	12	46	45	42	17	23	50	50
S2	49	50	49	49	31	35	50	46	46	50	43	48
S3	48	44	32	23	20	48	44	35	32	38	47	47
S4	38	43	47	40	46	18$^{\Delta}$	31	37	41	50	6	4
S5	24$^{\bullet}$	49	48	42	37	22	45	36	21	48	48	50
S6	50	48	48	44	2	17	12	28	2	27	50	46
S7	20	34	38	41	40	50	34	41^{+}	1	8	18	25
S8	50	50	43	46	42	45	42	37	35	50	8	41^{o}
$\bar{x}$	38.5	43.125	44.375	41.875	28.75	35.125	37.875	37.75	24.375	36.75	33.75	38.875
SD	12.581	8.425	6.346	8.442	15.791	14.126	12.159	5.392	17.02	15.881	19.543	16.305
SE	4.448	2.979	2.244	2.985	5.583	4.994	4.299	1.906	6.018	5.615	6.909	5.765

$^{\Delta}$ 25 trials lost

$^{\bullet}$ 20 trials lost

^{o} 8 trials lost

$^{+}$ 5 trials lost

Continued ...

Table A3 (continued)

	Home cage				Extinction				Response prevented			
	Immediate		24 hours		Immediate		24 hours		Immediate		24 hours	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
	Responses 0 - 10 seconds											
S1	21	12	50	43	10	43	36	31	14	14	49	46
S2	44	48	47	46	23	16	47	43	44	44	38	38
S3	46	32	11	17	19	36	34	25	26	34	37	37
S4	34	38	43	38	39	13$^{\Delta}$	26	30	27	50	4	2
S5	16$^{\bullet}$	48	42	30	34	17	41	33	18	41	47	50
S6	50	45	47	42	0	11	7	22	2	21	47	43
S7	15	29	27	35	35	48	28	31^{+}	1	5	11	20
S8	49	45	40	41	40	37	25	30	35	50	6	60^{o}
Sum	275	297	307	292	200	221	244	245	167	259	239	252
$\bar{x}$	34.375	37.125	38.375	36.5	25	27.625	30.5	30.625	20.875	32.375	29.875	31.5
SD	15.01	12.449	13.092	9.335	14.599	14.87	12.154	6.163	15.142	17.113	19.511	16.903
SE	5.308	4.401	4.629	3.3	5.162	5.257	4.297	2.179	5.353	6.050	6.898	5.976

$^{\Delta}$ 25 trials lost

$^{\bullet}$ 20 trials lost

^{o} 8 trials lost

$^{+}$ 5 trials lost

continued ...

Table A3 (continued)

	Home cage				Extinction				Response prevented			
	Immediate		24 hours		Immediate		24 hours		Immediate		24 hours	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
	Responses 10 - 20 seconds											
S1	8	15	0	7	2	3	9	11	3	9	1	4
S2	5	2	2	3	8	19	3	3	2	6	5	10
S3	2	12	21	6	1	12	10	10	6	4	10	11
S4	4	5	4	2	7	5$^{\Delta}$	5	7	14	0	2	2
S5	8$^{\bullet}$	1	6	12	3	5	4	3	3	7	1	0
S6	0	3	1	2	2	6	5	6	0	6	3	3
S7	5	5	11	6	5	2	6	10^{+}	0	3	7	5
S8	1	5	3	5	2	8	17	7	0	0	2	25^{o}
Sum	33	48	48	43	30	60	59	57	28	35	31	60
$\bar{x}$	4.125	6	6	5.375	3.75	7.5	7.375	7.125	3.5	4.375	3.875	7.5
SD	2.997	4.928	6.969	3.292	2.605	5.581	4.565	3.091	4.721	3.249	3.227	8.018
SE	1.06	1.742	2.464	1.164	0.921	1.973	1.614	1.093	1.669	1.149	1.141	2.835

$^{\Delta}$ 25 trials lost

$^{\bullet}$ 20 trials lost

^{o} 8 trials lost

$^{+}$ 5 trials lost

Table A4: Experiment One. Analysis of covariance on acquisition and extinction performance

Source	df	SS_x	SP	SS_y	df	$SS_{\hat{y}}$	$MS_{\hat{y}}$	F	
(1) x = number of trials to acquisition criterion; y = number of short avoidances in Extinction Test I									
Between groups	5	4258.92	1396.58	1581.17	5	1581.17	317.8	1.36	(F_x = 0.64;5;42 NS)
Within groups	42	55879.75	-847.25	9573.5	41	9573.5	233.19	NS	(F_y = 1.39;5;42 NS)
Total	47	60138.67	549.33	11154.67	46	11154.67			
(2) x = number of avoidances to acquisition criterion; y = number of short avoidances in Extinction Test I									
Between groups	5	622.94	283.25	1581.17	5	1560.2	312.04	1.34	(F_x = 0.62;5;42 NS)
Within groups	42	8396.88	197.25	9573.5	41	9568.87	233.39	NS	(F_y = 1.39;5;42 NS)
Total	47	9019.81	480.5	11154.67	46	11129.07			

Table A5: Experiment One. Total number of responses in every block of five trials for each group

Trial blocks	Home cage				Extinction				Response prevention			
	Immediate		24 hours		Immediate		24 hours		Immediate		24 hours	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
					Total responses							
1	31	29	37	40	29	35	31	38	20	39	28	36
2	32	35	37	39	34	34	34	36	24	31	30	33
3	35	39	38	37	32	29	32	27	20	33	32	35
4	35	33	39	38	28	29	34	31	21	32	29	36
5	37	34	37	36	18	31	34	36	19	35	27	34
6	37	35	37	36	21	28+	33	33	14	28	27	30
7	29+	33	35	31	27	23+	28	23	22	24	26	28
8	29+	38	34	31	20	29+	26	29	23	22	26	30
9	23+	37	29	25	12	26+	26	26	15	26	22	29
10	20+	32	32	22	9	17+	25	23	17	24	23	21+
					Short avoidances							
1	27	21	33	37	29	33	29	31	14	34	22	26
2	29	29	34	36	31	25	29	32	23	28	24	25
3	32	32	34	34	27	26	26	22	18	32	29	27
4	29	27	34	33	24	21	27	24	17	26	29	28
5	32	33	34	33	14	24	28	28	17	28	23	31
6	34	31	30	31	19	21+	27	27	13	23	25	25
7	27+	33	26	24	24	21+	24	15	19	23	25	24
8	27+	31	28	26	17	22+	20	27	20	22	23	27
9	19+	33	27	19	9	16+	19	20	11	23	20	22
10	19+	27	27	19	6	12+	15	19+	15	20	19	17+
Sum	275	297	307	292	200	221	244	245	167	259	239	252
					Long avoidances							
1	4	8	4	3	0	2	2	7	6	5	6	10
2	3	6	3	3	3	9	5	4	1	3	6	8
3	3	7	4	3	5	3	6	5	2	1	3	8
4	6	6	5	5	4	8	7	7	4	6	0	8
5	5	1	3	3	4	7	6	8	2	7	4	3
6	3	4	7	5	2	7+	6	6	1	5	2	5
7	2+	0	9	7	3	2+	4	8	3	1	1	4
8	2+	7	6	5	3	7+	6	2	3	0	3	3
9	4+	4	2	6	3	10+	7	6	4	3	2	7
10	1+	5	5	3	3	5+	10	4+	2	4	4	4+
Sum	33	48	48	43	30	60	59	57	28	35	31	60

+ 5 trials missing

Table A6: Experiment One. The outcomes of Friedman's non-parametric ANOVA applied to extinction data

	Immediate test		24 hour test	
	T1	T2	T1	T2
	Total responses			
ΣR_j^2	1326	1286	1382	1264.5
xr^2	12.6	8.6	18.2	6.45
p	< 0.001	0.012	< 0.001	0.05
	Short avoidances			
ΣR_j^2	1304	1287.5	1352	1219.5
xr^2	10.4	8.75	15.2	1.95
p	< 0.01	< 0.02	< 0.001	ns
	Long avoidances			
ΣR_j^2	1209.5	1269.5	1240.5	1246.5
xr^2	0.95	6.95	4.05	4.65
p	ns	< 0.05	ns	< 0.1

Table A7: Experiment One. Pairwise comparisons of group performance in extinction (Hollander and Wolfe 1973)

			Home Cage v Extinction	Home Cage v Response Prevented	Extinction v Response Prevented
Immediate test					
Total responses	T1	r	12	15	3
		p	< 0.05	< 0.01	NS
	T2	r	13	8	5
		p	< 0.01	NS	NS
Short avoidances	T1	r	10	14	4
		p	0.05	< 0.01	NS
	T2	r	12.5	10	2.5
		p	< 0.019	0.05	NS
Long avoidances	T1	r	0.5	4	3.5
		p	NS	NS	NS
	T2	r	8	3.5	11.5
		p	NS	NS	< 0.037
24 hour test					
Total responses	T1	r	11	19	8
		p	0.037	< 0.01	NS
	T2	r	9	10.5	1.5
		p	NS	0.05	NS
Short avoidances	T1	r	16	14	2
		p	< 0.01	< 0.01	NS
	T2	r	6	4.5	1.5
		p	NS	NS	NS
Long avoidances	T1	r	4.5	4.5	9
		p	NS	NS	0.1
	T2	r	7.5	9	1.5
		p	NS	0.1	NS

Critical differences for r (α,k,n) k = 3; n = 10 (from Hollander and Wolfe 1973); r = 11, p = 0.037; r = 12, p = 0.019; r = 13, p = 0.01.

Table A8: Experiment One. Trend line slope coefficients for extinction data

	Home cage		Extinction		Response prevented	
	Immediate	24 hours	Immediate	24 hours	Immediate	24 hours
			Test 1			
Total responses	-1.272	-0.806	-2.387	-0.963	-0.466	-0.848
Short avoidances	-1.036	-0.975	-2.46	-1.43	-0.381	-0.575
Long avoidances	-0.236	0.169	0.072	0.466	-0.084	-0.272
			Test 2			
Total responses	0.224	-1.884	-1.448	-1.345	-1.551	-1.309
Short avoidances	0.563	-2.12	-1.66	-1.18	-1.363	-0.727
Long avoidances	-0.339	0.236	0.218	-0.163	-0.187	-0.581

Table A9: Experiment One. Outcomes of Kruskall Wallis ANOVA on linear regression coefficients (Seigel 1956)

		Total responses	Short avoidances	Long avoidances
Test 1	H	2.571	4.57	2.512
	P	NS	0.067	NS
Test 2	H	0	0.2325	1.942
	P	NS	NS	NS

Table A10: Experiment Two: Performance in acquisition

Measures to criterion		Σx	$\bar{x}$	SD	SE
Avoidances	Sal	168	21.0	10.24	3.62
	LVP	149	16.55	2.24	0.74
Trials	Sal	316	39.5	23.53	8.31
	LVP	270	30.0	12.13	4.04
Escapes	Sal	73	9.12	12.26	4.33
	LVP	86	9.55	12.27	4.09
Failures	Sal	52	6.5	7.92	2.8
	LVP	21	2.33	4.87	1.62
Shocks	Sal	384	48.0	46.51	16.44
	LVP	227	25.22	28.54	9.51

Table A11: Experiment Two: Analysis of acquisition data
(two tailed 't' test, Winer 1962)

Measure	t	df	p
Avoidances	1.273	15	NS
Trials	1.065	15	NS
Escapes	0.07	15	NS
Failures	1.32	15	NS
Shocks	1.23	15	NS

Table A12: Experiment Two. Responses during extinction

Subject	Total avoidances		Short avoidances		Long avoidances	
	Sal	LVP	Sal	LVP	Sal	LVP
1	5	49	4	40	1	9
2	44	50	27	44	17	7
3	30	46	13	38	17	8
4	50	48	46	41	4	7
5	50	48	50	39	0	9
6	50	50	49	50	1	0
7	44	30	38	23	6	7
8	21	49	14	47	7	2
9		8		6		2
Σx	294	378	241	328	53	51
$\bar{x}$	36.75	42.0	30.125	36.44	6.625	5.66
SD	16.61	14.203	18.16	13.7	6.86	3.39
SE	5.87	4.734	6.42	4.56	2.42	1.13

Table A13: Experiment Two. Analysis of covariance on acquisition and extinction performance

(1) x = number of trials to acquisition criterion; y = number of short avoidances in Extinction Test 1

Source	df	SS_x	SP	SS_y	df	SS_y	MS_y	F	
Between	1	83.66	-118.95	169.14	1	94.6	94.6	0.35	f_x = 1.62 (1,15) NS
Within	15	774.22	-234.22	3813.1	14	3742.24	267.3	NS	f_y = 0.67 (1,15) NS
Total	16	857.88	-353.18	3982.24	15	3836.84			

(2) x = number of avoidances to criterion; y = number of short avoidances in Extinction Test 1

Between	1	382.24	-254.26	169.14	1	125.67	125.67	0.46	f_x = 1.13 (1,15) NS
Within	15	5054.0	-356.5	3813.1	14	3787.95	270.57	NS	f_y = 0.67 (1,15) NS
Total	16	5436.24	-610.76	3982.24	15	3913.62			

Table A14: Experiment Two. Responses made in extinction as a function of extinction trial block (saline n = 8; LVP n = 9)

Trial block	Total avoidances		Short avoidances		Long avoidances	
	Sal	LVP	Sal	LVP	Sal	LVP
			Test 1			
1	23	37	22	32	1	5
2	30	35	25	34	5	1
3	30	37	25	34	5	3
4	32	40	27	36	5	4
5	32	40	28	36	4	4
6	31	38	23	32	8	6
7	29	39	23	37	6	2
8	29	38	23	32	6	6
9	35	36	28	28	7	10
10	31	33	23	23	8	10
			Test 2			
1	34	40	24	38	10	2
2	29	38	20	32	9	6
3	37	41	30	39	7	2
4	29	42	21	40	8	2
5	29	39	23	35	6	4
6	30	35	21	28	9	7
7	28	39	20	17	8	12
8	23	38	17	18	6	10
9	21	29	18	22	3	7
10	20	28	12	21	8	7

Table A15: Experiment Two. Summary of Wilcoxon rank signed rank tests on extinction data (Seigel 1956) (one tailed tests)

	T	n	P
Test 1			
Total responses	5	10	0.0098
Short avoidances	4.5	10	< 0.009
Long avoidances	13	10	NS
Test 2			
Total responses	1	10	< 0.005
Short avoidances	6	10	0.0137
Long avoidances	13	10	NS

Table A16: Experiment Three. Acquisition performance

	Home cage		Extinction		Response prevented	
	Saline	LVP	Saline	LVP	Saline	LVP
	HCS	HCL	Ext S	Ext L	RPS	RPL
			Avoidances			
Σx	110	169	141	138	153	169
$\bar{x}$	13.75	21.125	17.625	17.25	19.125	21.125
SD	2.964	13.485	8.193	11.285	10.575	8.61
SE	1.048	4.768	2.897	3.99	3.739	3.044
			Trials			
Σx	307	486	355	331	377	434
$\bar{x}$	38.375	60.75	44.375	41.375	47.125	54.25
SD	28.137	42.04	27.428	20.021	22.242	26.092
SE	9.948	14.86	9.697	7.078	7.864	9.225
			Escapes			
Σx	39	68	39	73	95	121
$\bar{x}$	4.875	8.5	4.875	9.125	11.875	15.125
SD	6.221	11.326	7.24	9.862	15.142	17.78
SE	2.199	4.004	2.56	3.487	5.353	6.286
			Failures			
Σx	88	63	78	76	27	111
$\bar{x}$	11.0	7.875	9.75	9.5	3.375	13.875
SD	13.277	7.24	16.859	14.784	6.14	19.0
SE	4.694	2.56	5.96	5.227	2.171	6.717
			Shocks			
Σx	528	440	429	500	286	736
$\bar{x}$	66.0	55.0	53.525	62.5	35.75	92.0
SD	78.831	49.616	90.432	76.878	34.074	91.558
SE	27.871	17.542	31.973	27.181	12.047	32.371

Table A17: Experiment Three. Acquisition performance: outcomes from analyses of variance

	Source	SS	df	MS	F	P
Avoidances	Between groups	311.17	5	62.23	0.65	NS
	Within groups	3997.5	42	95.18		
	Total	4308.67	47			
Trials	Between groups	2812.42	5	562.48	0.69	NS
	Within groups	34213.5	42	814.61		
	Total	37025.92	47			
Escapes	Between groups	640.44	5	128.09	0.89	NS
	Within groups	6034.38	42	143.68		
	Total	6674.81	47			
Failures	Between groups	489.35	5	97.87	0.52	NS
	Within groups	7911.13	42	188.36		
	Total	8400.48	47			
Shocks	Between groups	13727.94	5	2745.59	0.51	NS
	Within groups	226157.38	42	5384.7		
	Total	239885.31	47			

Table A18: Experiment Three. Extinction performance

Total avoidance responses

		Σx	$\bar{x}$	SD	SE
Test 1	HC S	289	36.125	17.96	6.35
	HC L	213	26.625	20.61	7.29
	Ext S	345	43.125	5.27	1.86
	Ext L	285	35.625	14.04	4.96
	RP S	252	31.5	15.89	5.61
	RP L	321	40.125	12.12	4.28
Test 2	HC S	304	38.0	10.37	3.66
	HC L	216	27.0	18.97	6.7
	Ext S	284	35.5	14.16	5.0
	Ext L	248	31.0	17.82	6.3
	RP S	224	28.0	14.88	5.26
	RP L	261	32.62	13.49	4.76
Test 3	HC S	301	37.62	13.81	4.88
	HC L	179	29.83	18.01	7.35
	Ext S	257	32.125	14.8	5.23
	Ext L	217	27.125	20.71	7.32
	RP S	151	30.2	13.0	5.81
	RP L	214	30.57	25.06	5.69
Test 4	HC S	71	8.87	2.8	0.99
	HC L	46	5.75	4.16	1.47
	Ext S	67	8.37	3.27	1.16
	Ext L	56	7.0	3.74	1.32
	RP S	36	6.43	4.42	1.67
	RP L	71	8.87	1.35	0.47
Test 5	HC S	68	8.5	3.11	1.1
	HC L	66	8.25	2.96	1.04
	Ext S	60	7.5	3.34	1.18
	Ext L	64	8.0	3.16	1.11
	RP S	53	7.571	3.78	1.43
	RP L	70	8.75	1.91	0.67

Table A18 (continued)

Short avoidance responses

		Σx	$\bar{x}$	SD	SE
Test 1	HC S	260	32.5	19.17	6.78
	HC L	199	24.87	20.87	7.38
	Ext S	263	38.87	7.93	2.8
	Ext L	234	29.25	17.36	6.13
	RP S	213	26.62	15.87	5.61
	RP L	280	35.0	12.24	4.33
Test 2	HC S	261	32.62	12.18	4.3
	HC L	180	22.5	18.55	6.56
	Ext S	261	32.62	14.75	5.21
	Ext L	213	26.62	17.75	6.27
	RP S	182	22.75	14.63	5.17
	RP L	212	26.5	12.28	4.34
Test 3	HC S	262	32.75	13.54	4.78
	HC L	145	24.16	17.01	6.94
	Ext S	208	26.0	15.32	5.41
	Ext L	179	22.37	19.69	6.96
	RP S	133	26.6	14.79	6.61
	RP L	193	27.57	14.21	5.37
Test 4	HC S	68	8.5	2½72	0.96
	HC L	38	4.75	4.23	1.49
	Ext S	57	7.12	3.35	1.18
	Ext L	52	6.5	4.0	1.41
	RP S	37	5.28	3.98	1.5
	RP L	64	8.0	1.77	0.62
Test 5	HC S	60	7.5	3.11	1.1
	HC L	53	6.62	3.92	1.38
	Ext S	54	6.75	4.06	1.43
	Ext L	55	6.87	3.6	1.27
	RP S	44	6.28	3.98	1.5
	RP L	58	7.25	2.25	0.79

Table A18 (continued)

Long avoidance responses

		Σx	$\bar{x}$	SD	SE
Test 1	HC S	29	3.62	3.88	1.37
	HC L	14	1.75	1.98	0.7
	Ext S	34	4.25	3.61	1.27
	Ext L	51	6.37	5.73	2.02
	RP S	39	4.87	3.31	1.17
	RP L	41	5.12	3.35	1.18
Test 2	HC S	43	5.37	3.06	1.08
	HC L	36	4.5	2.82	1.0
	Ext S	23	2.87	2.69	0.95
	Ext L	35	4.37	4.95	1.75
	RP S	42	5.25	4.83	1.7
	RP L	49	6.12	4.22	1.49
Test 3	HC S	39	4.87	3.31	1.17
	HC L	34	5.66	3.32	1.35
	Ext S	49	6.12	5.05	1.78
	Ext L	38	4.75	4.95	1.75
	RP S	18	3.6	2.51	1.12
	RP L	21	3.0	2.76	1.04
Test 4	HC S	3	0.37	0.51	0.18
	HC L	8	1.0	1.19	0.42
	Ext S	10	1.25	1.28	0.45
	Ext L	4	0.5	0.92	0.32
	RP S	8	1.14	1.21	0.45
	RP L	7	0.87	1.35	0.48
Test 5	HC S	8	1.0	1.3	0.46
	HC L	13	1.62	1.76	0.62
	Ext S	5	0.75	1.16	0.41
	Ext L	9	1.12	1.64	0.58
	RP S	9	1.28	1.6	0.61
	RP L	12	1.5	1.19	0.42

Table A19: Experiment Three. Analysis of covariance on acquisition and extinction performance

Source	df	SS_x	SP	SS_y	df	$SS_{\hat{y}}$	$MS_{\hat{y}}$	F	
(1) x = number of trials to criterion; y = number of short avoidances in Extinction Test 1									
Between groups	5	311.17	-159.63	1118.19	5	1037.85	207.57	0.83	(F_x, df 5,42 = 0.65)
Within groups	42	3997.5	-1758.38	10989.13	41	10215.67	249.16	NS	(F_y, df 5,42 = 0.85)
Total	47	4308.67	-1918.0	12107.31		11253.52			
(2) x = number of avoidances to criterion; y = number of short avoidances in Extinction Test 1									
Between groups	5	2812.42	-642.63	1118.19	5	1117.87	223.57	0.83	(F_x, df 5,42 = 0.69)
Within groups	42	34213.5	306.25	10989.13	41	10986.38	267.96	NS	(F_y, df 5,42 = 0.85)
Total	47	37025.92	-336.38	12107.31	46	12104.26			

Table A20: Experiment Three

Mean number of short avoidances in every block of five trials

HC S	HC L	Ext S	Ext L	RP S	RP L
		Test 1			
3.125	3.0	4.25	3.0	3.75	3.875
3.25	3.0	3.875	3.0	2.625	4.125
3.125	3.25	4.25	3.375	3.875	4.0
3.875	3.25	4.75	3.125	3.0	4.25
3.8	3.125	3.625	2.875	2.125	3.5
3.125	2.125	4.125	2.875	2.75	4.125
3.125	1.625	4.375	2.875	2.25	3.625
3.125	1.875	3.625	2.875	2.42	2.857
2.875	2.0	3.0	2.875	2.85	3.0
3.25	1.625	3.0	2.375	1.625	2.714
		Test 2			
3.25	2.375	4.125	3.375	3.875	3.625
3.125	2.375	4.25	3.375	3.125	3.25
3.375	2.625	4.0	3.5	2.75	3.14
3.75	2.85	3.75	3.125	2.625	2.71
3.75	2.375	3.714	3.0	2.75	3.0
3.375	2.625	3.28	2.71	2.25	2.85
3.0	2.375	2.75	2.142	1.625	2.57
3.25	1.625	3.28	2.42	1.75	2.714
3.375	2.28	2.75	2.142	1.14	2.375
2.375	1.857	2.875	2.28	1.14	2.0
		Test 3			
3.375	3.5		2.5	3.6	4.57
3.75	3.83	3.125	2.75	3.6	4.0
4.125	2.66	3.0	2.25	3.8	2.85
4.25	2.66	3.25	2.875	4.2	3.43
3.375	3.0	3.5	2.375	2.6	2.28
3.5	1.833	3.0	2.375	2.8	2.0
2.625	2.3	2.142	1.625	1.6	2.28
3.0	1.66	2.375	1.5	1.6	2.42
2.625	0.833	2.25	2.0	1.6	2.28
2.125	1.833	2.0	2.125	1.2	1.428
		Test 4			
4.0	2.5	3.875	3.75	0.71	0.5
4.375	2.25	3.25	2.75	0.43	1.0
		Test 5			
4.125	3.25	3.5	3.75	0.57	0.5
3.375	3.375	3.125	3.125	0.86	0.625

Table A20 (continued)

Mean number of long avoidances in every block of five trials

HC S	HC L	Ext S	Ext L	RP S	RP L
		Test 1			
0.125	0.125	0.125	0.75	0.75	0.5
0.375	0.25	0.375	0.375	0.875	0.75
0.5	0.25	0.25	0.125	0.375	0.375
0	0.125	0.25	0.5	0.375	0.25
0.5	0.125	0	0.5	0.5	0.875
0.375	0	0.142	0.625	0.25	0.375
0.375	0.5	0.625	1.375	0.625	0.625
0.5	0.25	0.875	1.125	0.428	0.571
0.375	0	0.75	0.5	0.285	0.142
0.5	0.125	0.875	0.5	1.0	0.857
		Test 2			
0.25	0.25	0.125	0.25	0.25	0.625
0.5	1.0	0.25	0.5	0.25	0.75
0.5	0.75	0.25	0.25	0.75	0.714
0.25	0.428	0.5	1.0	0.625	1.0
0.625	0.5	0.285	0.5	0.625	0.571
0.875	0.375	0.428	0.428	0.625	0.285
0.75	0.125	0.5	0.857	0.75	0.428
0.625	0.5	0.142	0.714	0.25	0.571
0.375	0.25	0.375	1.0	0.571	1.0
0.625	0.375	0.25	0.571	0.428	0.625
		Test 3			
0.75	0.333	1.0	0.625	0	0.142
0.75	0.333	0.625	0.5	1.0	0.285
0.375	0.666	1.25	1.0	0	0.428
0.375	1.333	0.25	0.375	0.6	0.428
0.625	0.33	1.142	0.5	0.6	0.428
0.375	0.66	0.428	0.625	0.8	0.142
0.5	0.5	0.625	0.25	0	0.142
0.25	0.66	0.375	0.625	0	0.428
0.375	0.5	0.25	0.25	0	0.285
0.5	0.33	0.375	0	0.2	0.285
		Test 4			
0.375	0.625	0.5	0.25	0.714	0.25
0	0.375	0.75	0.25	0.428	0.625
		Test 5			
0.25	1.125	0.375	0.5	0.57	0.125
0.75	0.5	0.375	0.625	0.857	1.37

Table A20 (continued)

Mean number of total avoidances in every block of five trials

HC S	HC L	Ext S	Ext L	HC S	HC L
		Test 1			
3.25	3.125	4.375	3.75	4.5	4.375
3.625	3.25	4.25	3.375	3.5	4.875
3.625	3.5	4.5	3.5	4.25	4.375
3.875	3.375	5.0	3.625	3.375	4.5
4.3	3.5	3.625	3.375	2.625	4.375
3.5	2.125	4.267	3.5	3.0	4.5
3.5	2.125	5.0	4.25	2.875	4.25
3.625	2.125	4.5	4.0	2.848	3.428
3.25	2.0	3.75	3.375	3.135	3.142
3.75	1.75	3.875	2.875	2.625	3.571
		Test 2			
3.5	2.625	4.5	3.625	4.125	4.25
3.625	3.375	4.5	3.875	3.375	4.0
3.875	3.375	4.25	3.75	3.5	3.854
4.0	3.278	4.25	4.125	3.25	3.71
4.375	2.875	3.999	3.5	3.375	3.571
4.25	3.0	3.708	3.138	2.875	3.135
3.75	2.5	3.25	2.999	2.375	2.998
3.875	2.125	3.422	3.134	2.0	3.285
3.75	2.53	3.125	3.142	1.711	3.375
3.0	2.232	3.125	2.851	1.568	2.625
		Test 3			
4.125	3.833	4.125	3.125	3.6	4.712
4.5	4.163	3.625	3.25	4.6	4.285
4.5	3.326	4.5	3.25	3.8	3.278
4.625	3.993	3.75	3.25	4.8	3.858
4.0	3.33	4.142	2.875	3.2	2.708
3.875	2.493	2.57	3.0	3.6	2.142
3.125	2.8	3.0	1.875	1.6	2.422
3.25	2.32	2.625	2.125	1.6	2.848
3.0	1.333	2.25	2.25	1.6	2.565
2.625	2.163	2.375	2.125	1.4	1.713
		Test 4			
4.375	3.125	4.375	4.0	1.424	0.3
4.375	2.625	4.0	3.0	0.858	1.625
		Test 5			
4.375	4.375	3.875	4.25	1.14	0.625
4.125	3.875	3.5	3.75	1.717	1.995

Table A21: Experiment Three. The outcomes of Friedman's ANOVAs on extinction data

	Sum R_j^2	X_r^2	P	
		Test 1		
Short avoidances	8584.0	35.24	< 0.001	(for T1, T2 and T3 n = 10, k = 6)
Long avoidances	7895.5	15.585	< 0.02	
Total	8652.0	37.187	< 0.001	
		Test 2		
Short avoidances	8501.0	32.85	< 0.001	
Long avoidances	7784.0	12.388	< 0.05	
Total	8502.0	32.902	< 0.001	
		Test 3		
Short avoidances	7892.5	15.488	< 0.02	
Long avoidances	7598.5	7.089	ns	
Total	8086.0	21.017	< 0.001	
		Test 4		
Short avoidances	342.0	6.8376	ns	(for T4 and T5 n = 2, k = 6)
Long avoidances	334.5	5.766	ns	
Total	362.5	9.765	< 0.1	
		Test 5		
Short avoidances	323.5	4.1958	ns	
Long avoidances	310.0	2.268	ns	
Total	360.5	9.479	< 0.1	

Table A22: Experiment Three. Trend lines for short avoidances in extinction

	Home cage		Extinction		Response prevented	
	Saline	LVP	Saline	LVP	Saline	LVP
T1	-0.026	-0.194	-0.128	-0.059	-0.16	-0.153
T2	-0.056	-0.069	-0.174	-0.164	-0.284	-0.142
T3	-0.178	-0.261	-0.154	-0.097	-0.328	-0.279

Table A23: Experiment Three. The outcomes of Nemenyi's multiple comparisons in extinction data

		Short avoidances		Long avoidances		Total avoidances	
		r	p	r	p	r	p
HC S v HC L	T1	23.0	0.05	15.5	ns	22.0	0.08
	T2	30.0	< 0.009	11.5	ns	34.0	< 0.009
	T3	25.0	< 0.023	2.5	ns	27.0	< 0.023
HC S v Ext S	T1	15.5	ns	2.5	ns	24.5	< 0.047
	T2	7.5	ns	12.0	0.1	1.5	ns
	T3	16.5	ns	3.5	ns	14.0	ns
HC S v Ext L	T1	13.5	ns	14.0	ns	0	ns
	T2	13.5	ns	1.5	ns	17.5	ns
	T3	30.5	< 0.009	6.0	ns	34.0	< 0.009
HC S v RP S	T1	20.0	0.1	6.0	ns	7.0	ns
	T2	29.5	< 0.009	1.5	ns	20.0	0.1
	T3	21.5	0.1	11.0	ns	26.0	< 0.023
HC S v RP L	T1	8.0	ns	11.0	ns	13.5	ns
	T2	12.0	ns	2.5	ns	7.0	ns
	T3	17.5	ns	13.0	ns	25.0	< 0.047
Ext S v RP S	T1	35.5	< 0.009	3.5	ns	32.5	< 0.009
	T2	37.0	< 0.008	19.5	ns	31.5	< 0.009
	T3	5.0	ns	14.5	ns	12.0	ns

Table A23 (continued)

		Short avoidances		Long avoidances		Total avoidances	
		r	p	r	p	r	p
Ext L v RP L	T1	21.5	< 0.1	3.0	ns	13.5	ns
	T2	1.0	ns	1.0	ns	10.5	ns
	T3	13.0	ns	7.0	ns	9.0	ns
HC L v Ext L	T1	9.5	ns	29.5	< 0.009	22.0	< 0.1
	T2	16.5	ns	13.0	ns	16.5	ns
	T3	5.5	ns	8.5	ns	7.0	ns
HC L v RP S	T1	3.0	ns	21.5	< 0.1	15.0	ns
	T2	0.5	ns	10.5	ns	4.0	ns
	T3	3.5	ns	13.5	ns	1.0	ns
HC L v RP L	T1	31.0	< 0.009	26.5	< 0.023	35.5	< 0.009
	T2	17.5	ns	14.0	ns	27.0	< 0.023
	T3	7.5	ns	15.5	ns	2.0	ns
Ext S v Ext L	T1	29.0	< 0.009	11.5	ns	24.5	< 0.047
	T2	21.5	< 0.1	22.5	< 0.1	19.0	ns
	T3	14.0	ns	10.5	ns	20.0	0.1
RP S v RP L	T1	28.0	< 0.009	5.0	ns	20.5	0.1
	T2	17.0	ns	4.0	ns	23.0	0.05
	T3	4.0	ns	2.0	ns	1.0	ns

Table A24: Experiment Three. Responses made during 30 trials of extinction treatment

Subject	Extinction plus saline			Extinction plus LVP		
	Total responses	Short avoidances	Long avoidances	Total responses	Short avoidances	Long avoidances
1	30	30	0	22	17	5
2	30	30	0	28	24	4
3	30	30	0	30	29	1
4	30	28	2	29	28	1
5	30	30	0	28	27	1
6	17	12	5	30	30	0
7	30	30	0	14	14	0
8	26	26	0	29	27	2
Σx	223	216	7	210	196	14
$\bar{x}$	27.875	27	0.875	26.25	24.5	1.75
SD	4.611	6.23	1.807	5.574	5.879	1.832
SE	1.63	2.202	0.638	1.971	2.079	0.647

Table A25: Experiment Four. Acquisition performance

	Home cage		Response prevented	
	Saline	LVP	Saline	LVP
		Trials		
Σx	340	330	282	398
$\bar{x}$	42.5	41.25	40.285	44.222
SD	24.28	31.75	25.62	33.31
SE	8.58	11.22	9.68	11.10
		Avoidances		
Σx	165	171	127	187
$\bar{x}$	20.625	21.375	18.142	20.777
SD	7.05	8.77	6.44	9.4
SE	2.49	3.10	2.43	3.13
		Escapes		
Σx	83	91	100	154
$\bar{x}$	10.375	11.375	14.285	17.111
SD	8.72	13.08	22.32	18.46
SE	3.08	4.62	8.43	6.15
		Failures		
Σx	69	70	37	52
$\bar{x}$	8.625	8.75	5.285	5.777
SD	10.84	14.44	3.77	9.27
SE	3.83	5.10	1.42	3.09
		Shocks		
Σx	479	472	304	515
$\bar{x}$	59.875	59.0	43.428	57.222
SD	63.84	86.52	16.89	72.16
SE	22.57	30.59	6.38	24.05

Table A26: Experiment Four. Analyses of variance on acquisition data

	Source	SS	df	MS	F	P
	Between groups	70.39	3	23.46	0.03	NS
Avoidances	Within groups	23996.8	28	857.02		
	Total	24066.88	31			
	Between groups	44.71	3	14.9	0.23	NS
Trials	Within groups	1842.16	28	65.79		
	Total	1886.88	31			
	Between groups	235.43	3	78.48	0.3	NS
Escapes	Within groups	7446.07	28	265.93		
	Total	7681.5	31			
	Between groups	79.14	3	26.38	0.24	NS
Failures	Within groups	3054.36	28	109.08		
	Total	3133.5	31			
	Between groups	124302.14	3	432.24	0.1	NS
Shocks	Within groups	1296.73	28	4439.36		
	Total	125598.88	31			

Table A27: Experiment Four. Responses made by subjects in Periods A and B in Suppression Tests 1 and 2

	Period A										Period B									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Home cage saline: Test 1																				
Σx	42	39	35	35	42	46	72	84	68	62	15	14	15	24	28	38	46	59	54	60
$\bar{x}$	5.25	4.87	4.37	4.37	5.25	5.75	9.0	10.5	8.5	7.75	1.875	1.75	1.87	3.0	3.5	4.75	5.75	7.37	6.75	7.5
SD	4.26	3.56	5.09	5.7	6.86	5.28	7.59	5.68	6.56	5.97	1.72	1.98	1.72	5.01	2.51	4.77	4.43	5.55	4.94	4.66
SE	1.51	1.25	1.8	2.01	2.42	1.86	2.68	2.01	2.32	2.11	0.61	0.7	0.61	1.77	0.88	1.68	1.56	1.96	1.75	1.64
Home cage saline: Test 2																				
Σx	56	63	67	62	87	72	76	62	75	81	56	68	65	67	63	84	68	79	91	78
$\bar{x}$	7.0	7.87	8.37	7.75	10.8	9.0	9.5	7.75	9.37	10.12	7.0	8.5	8.12	.837	7.87	10.5	8.5	9.87	11.37	9.75
SD	6.0	6.1	4.83	6.27	6.97	6.59	6.39	4.02	4.89	5.51	5.34	7.38	6.97	6.45	4.76	5.95	4.5	5.59	6.16	4.71
SE	2.12	2.15	1.71	2.22	2.46	2.33	2.26	1.42	1.73	1.94	1.89	2.61	2.46	2.28	1.68	2.1	1.59	1.97	2.17	1.66
Home cage LVP: Test 1																				
Σx	41	16	15	19	24	21	17	28	27	26	12	6	8	14	16	22	16	26	32	42
$\bar{x}$	5.125	2.0	1.875	2.375	3.0	2.625	2.125	3.5	3.375	3.25	1.5	0.75	1.0	1.75	2.0	2.75	2.0	3.25	4.0	5.25
SD	4.02	2.67	3.56	3.85	5.01	5.04	4.02	5.42	4.69	4.53	1.77	1.39	2.14	2.49	3.42	4.50	3.21	3.69	5.90	5.70
SE	1.42	0.94	1.26	1.36	1.77	1.78	1.42	1.92	1.66	1.60	0.63	0.17	0.75	0.88	1.20	1.59	1.13	1.30	2.09	2.02
Home cage LVP: Test 2																				
Σx	87	64	64	59	84	77	80	76	88	72	46	50	55	70	93	87	80	83	76	72
$\bar{x}$	10.875	8.0	8.0	7.375	10.5	9.625	10.0	9.5	11.0	9.0	5.75	6.25	6.875	8.75	11.625	10.875	10.0	10.375	9.5	9.0
SD	5.67	6.52	6.74	4.98	2.67	6.32	6.65	9.55	6.80	5.50	3.37	4.53	4.55	4.98	7.39	6.38	5.40	7.17	7.56	7.78
SE	2.0	2.31	2.38	1.76	0.94	2.23	2.35	3.38	2.40	1.94	1.19	1.60	1.61	1.76	2.61	2.26	1.91	2.53	2.67	2.75

Table A27 (continued)

	Period A										Period B									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Response prevented saline: Test 1																				
Σx	76	15	50	44	40	35	41	49	33	58	17	16	33	34	44	30	39	38	49	60
$\bar{x}$	10.857	2.143	7.143	6.286	5.714	5.0	5.85	7.0	4.71	8.28	2.43	2.28	4.71	4.85	6.28	4.28	5.57	5.43	7.0	8.57
SD	5.18	3.24	7.49	5.56	3.98	4.93	5.84	7.09	6.29	6.44	2.51	2.42	4.07	3.76	4.27	4.68	4.86	4.24	6.11	4.46
SE	1.958	1.224	2.83	2.1	1.5	1.86	2.21	2.68	2.38	2.44	0.95	0.91	1.54	1.42	1.61	1.77	1.84	1.60	2.31	1.68
Response prevented saline: Test 2																				
Σx	50	43	57	53	71	44	69	57	53	52	35	48	48	63	56	64	58	47	57	67
$\bar{x}$	7.14	6.14	8.14	7.57	10.14	6.28	9.85	8.14	7.57	7.43	5.0	6.85	6.85	9.0	8.0	9.14	8.28	6.71	8.14	9.57
SD	5.39	4.87	6.03	6.18	8.07	4.71	7.9	6.52	6.85	7.11	3.46	4.09	5.64	6.27	6.29	5.95	6.1	5.64	7.33	7.65
SE	2.04	1.84	2.28	2.33	3.05	1.78	2.98	2.46	2.59	2.68	1.31	1.55	2.13	2.37	2.38	2.25	2.31	2.13	2.77	2.89
Response prevented LVP: Test 1																				
Σx	111	81	59	58	68	77	71	78	79	65	27	32	35	39	59	63	67	53	42	31
$\bar{x}$	12.33	9.0	6.55	6.44	7.55	8.55	7.88	8.66	8.77	7.22	3.0	3.55	3.88	4.33	6.55	7.0	7.44	5.88	4.66	3.44
SD	3.77	8.93	7.36	6.65	8.7	9.0	7.34	6.91	7.03	6.42	3.97	4.64	4.78	4.09	8.18	6.38	5.72	5.68	4.38	3.84
SE	1.26	2.97	2.45	2.22	2.9	3.0	2.44	2.3	2.34	2.14	1.32	1.54	1.59	1.36	2.73	2.13	1.91	1.89	1.46	1.28
Response prevented LVP: Test 2																				
Σx	65	60	68	53	52	78	65	55	52	62	33	35	34	44	54	57	59	57	76	51
$\bar{x}$	7.22	6.66	7.55	5.88	5.77	8.66	7.22	6.11	5.77	6.88	3.66	3.88	3.77	4.88	6.0	6.33	6.55	6.33	8.44	5.66
SD	5.74	6.55	8.23	6.31	6.87	8.27	8.13	5.68	6.16	7.22	5.47	5.39	4.23	6.33	6.57	6.06	6.08	5.14	6.26	4.79
SE	1.91	2.18	0.91	2.1	2.28	2.76	2.71	1.89	2.05	2.4	1.82	1.79	1.41	2.11	2.19	2.02	2.03	1.71	2.08	1.59

Table A28: Experiment Four. Suppression ratios in Suppression Test 1 (Blocks One and Two) and Test 2 (Blocks Three and Four)

	Block One					Block Two					Block Three					Block Four				
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Home cage saline: Test 1																				
Σx	2.715	1.397	3.716	2.508	4.254	3.027	3.41	2.719	3.658	4.04	3.081	3.373	3.045	3.221	3.103	4.148	4.063	3.947	3.807	3.48
$\bar{x}$	0.339	0.174	0.464	0.313	0.531	0.378	0.426	0.339	0.457	0.505	0.385	0.421	0.381	0.402	0.387	0.518	0.508	0.493	0.476	0.435
SD	0.319	0.226	0.373	0.351	0.357	0.204	0.265	0.182	0.255	0.279	0.278	0.204	0.22	0.257	0.241	0.279	0.101	0.208	0.218	0.181
SE	0.112	0.08	0.132	0.124	0.126	0.072	0.093	0.064	0.09	0.098	0.098	0.072	0.077	0.091	0.085	0.098	0.036	0.074	0.077	0.063
Home cage saline: Test 2																				
Σx	1.795	0.563	0.65	1.402	1.145	2.833	2.905	3.028	3.652	4.625	3.376	2.794	3.225	5.159	3.954	4.155	4.105	4.385	4.081	3.345
$\bar{x}$	0.224	0.070	0.081	0.175	0.143	0.354	0.363	0.378	0.456	0.578	0.422	0.349	0.403	0.644	0.494	0.519	0.513	0.548	0.510	0.418
SD	0.209	0.137	0.156	0.260	0.199	0.440	0.439	0.42	0.438	0.417	0.139	0.212	0.208	0.239	0.146	0.153	0.114	0.203	0.232	0.193
SE	0.073	0.048	0.055	0.091	0.07	0.155	0.155	0.148	0.155	0.147	0.049	0.074	0.073	0.084	0.052	0.054	0.04	0.072	0.082	0.068
Response prevented saline: Test 1																				
Σx	1.068	2.267	2.857	3.038	3.228	2.44	3.682	2.603	3.462	3.252	2.578	3.39	3.19	3.658	4.142	3.481	3.05	3.157	2.976	4.149
$\bar{x}$	0.153	0.324	0.408	0.434	0.461	0.349	0.526	0.372	0.495	0.465	0.368	0.484	0.456	0.523	0.592	0.497	0.436	0.451	0.425	0.593
SD	0.121	0.344	0.347	0.333	0.264	0.378	0.368	0.350	0.381	0.232	0.183	0.262	0.311	0.298	0.284	0.260	0.250	0.306	0.350	0.341
SE	0.046	0.126	0.131	0.126	0.099	0.143	0.139	0.132	0.144	0.088	0.069	0.099	0.118	0.113	0.107	0.098	0.094	0.116	0.132	0.129
Response prevented saline: Test 2																				
Σx	2.0	1.778	1.795	3.465	2.841	4.071	4.561	3.146	2.886	2.788	2.766	3.405	3.744	3.399	2.81	3.085	3.216	5.117	5.339	4.983
$\bar{x}$	0.222	0.198	0.199	0.385	0.316	0.452	0.507	0.349	0.321	0.310	0.307	0.378	0.416	0.378	0.312	0.343	0.357	0.569	0.593	0.554
SD	0.241	0.209	0.197	0.294	0.346	0.360	0.295	0.312	0.309	0.308	0.345	0.315	0.376	0.346	0.348	0.222	0.303	0.317	0.280	0.360
SE	0.080	0.070	0.066	0.098	0.115	0.120	0.098	0.104	0.103	0.103	0.115	0.105	0.125	0.115	0.116	0.074	0.101	0.106	0.093	0.120

Table A29: Experiment Four. Analysis of variance of the mean number of lever press responses during Period A (non CS presentation)

Source	SS	df	MS	F	P
	Suppression Test 1; trials 1-5				
Treatment	82.11	3	27.37	10.64	< 0.01
Trials	39.82	4	9.95	3.67	< 0.05
Error	30.88	12	2.57		
Total	152.8				
	Suppression Test 1; trials 6-10				
Treatment	93.46	3	31.15	23.15	< 0.01
Trials	7.85	4	1.96	1.46	NS
Error	16.15	12	1.34		
Total	117.45				
	Suppression Test 2; trials 1-5				
Treatment	14.78	3	4.92	3.34	(< 0.1)
Trials	12.43	4	3.11	2.11	NS
Error	17.7	12	1.47		
Total	44.92				
	Suppression Test 2; trials 6-10				
Treatment	25.25	3	8.42	7.24	< 0.01
Trials	3.29	4	0.82	0.71	NS
Error	13.94	12	1.16		
Total	42.48				

Table A30: Experiment Four. Analysis of variance of the mean number of lever press responses during Period B (under CS presentation)

Source	SS	df	MS	F	P
	Suppression Test 1; trials 1-5				
Treatment	28.69	3	9.563	19.15	< 0.01
Trials	16.9	4	4.225	8.46	< 0.01
Error	5.99	12	0.499		
Total	51.59				
	Suppression Test 1; trials 6-10				
Treatment	27.6	3	9.2	3.86	< 0.05
Trials	4.83	4	1.2075	0.51	NS
Error	28.63	12	2.358		
Total	61.06				
	Suppression Test 2; trials 1-5				
Treatment	40.8	3	13.6	11.55	< 0.01
Trials	23.22	4	5.805	4.93	< 0.01
Error	14.13	12	1.177		
Total	78.14				
	Suppression Test 2; trials 6-10				
Treatment	37.52	3	12.506	12.71	< 0.01
Trials	4.04	4	1.01	1.03	NS
Error	11.8	12	0.983		
Total	53.36				

Table A31: Experiment Four. Analysis of variance of mean suppression ratios

Source	SS	df	MS	F	P
	Suppression Test 1; trials 1-5				
Treatment	0.17	3	0.056	6.15	< 0.01
Trials	0.08	4	0.02	2.12	NS
Error	0.11	12	0.00916		
Total	0.35				
	Suppression Test 1; trials 6-10				
Treatment	0.01	3	0.003	0.42	NS
Trials	0.03	4	0.0075	1.38	NS
Error	0.07	12	0.0058		
Total	0.11				
	Suppression Test 2; trials 1-5				
Treatment	0.05	3	0.016	3.5	0.05
Trials	0.03	4	0.0075	1.56	NS
Error	0.06	12	0.005		
Total	0.14				
	Suppression Test 2; trials 6-10				
Treatment	0.0013	3	0.0004	0.07	NS
Trials	0.01	4	0.0025	0.38	NS
Error	0.08	12	0.0066		
Total	0.09				

Table A32: Outcome of Newman Keuls tests on differences between means (Winer 1962)

Period A responses: Test 1; trials 1-5

Treatment effects

Treatment totals	HC S	24.11		
	HC L	14.37	HC S < RP L	$p < 0.05$
	RP S	32.14	HC L < RP S	$p < 0.05$
	RP L	41.87	HC L < RP L	$p < 0.05$

Trial effects

Trial totals	1.	33.56	trial 1 > trial 2	$p < 0.05$
	2.	18.01	trial 1 > trial 3	$p < 0.05$
	3.	19.93	trial 1 > trial 4	$p < 0.05$
	4.	19.47	trial 1 > trial 5	$p < 0.05$
	5.	21.51		

Period A responses: Test 1; trials 6-10

Treatment effects

Treatment totals	HC S	41.5	HC S > HC L	$p < 0.05$
	HC L	14.88	HC S > RP S	$p < 0.05$
	RP S	30.84	RP S > HC L	$p < 0.05$
	RP L	41.0	RP L > HC L	$p < 0.05$
			RP L > RP S	$p < 0.05$

Period A responses: Test 2; trials 6-10

Treatment effects

Treatment totals	HC S	45.74	HC S > RP L	$p < 0.05$
	HC L	49.13	HC L > RP L	$p < 0.05$
	RP S	39.27	HC L > RP S	$p < 0.05$
	RP L	34.64		

Period B responses: Test 1; trials 1-5

Treatment effects

Treatment totals	HC S	12.0	HC S > HC L	$p < 0.05$
	HC L	7.0	RP S > HC S	$p < 0.05$
	RP S	20.55	RP S > HC L	$p < 0.05$
	RP L	21.31	RP L > HC L	$p < 0.05$
			RP L > HC S	

Table A32 (continued)

Period B responses: Test 1; trials 1-5

Trial effects

Trial totals	1.	8.81	trial 4 > trial 2	$p < 0.05$
	2.	8.33	trial 4 > trial 1	$p < 0.05$
	3.	11.46	trial 5 > trial 1	$p < 0.05$
	4.	13.93	trial 5 > trial 2	$p < 0.05$
	5.	18.33	trial 5 > trial 3	$p < 0.05$
			trial 5 > trial 4	$p < 0.05$

Period B responses: Test 1; trials 6-10

Treatment effects

Treatment totals	HC S	32.12	HC S > HC L	$p < 0.05$
	HC L	17.25	RP S > HC L	$p < 0.05$
	RP S	30.85	RP L > HC L	$p < 0.05$
	RP L	28.42		

Period B responses: Test 2; trials 1-5

Treatment effects

Treatment totals	HC S	39.86	HC S > RP L	$p < 0.05$
	HC L	39.25	HC L > RP L	$p < 0.05$
	RP S	35.7	RP S > RP L	$p < 0.05$
	RP L	22.19		

Trial effects

Trial totals	1.	21.41	trial 4 > trial 1	$p < 0.05$
	2.	25.48	trial 5 > trial 1	$p < 0.05$
	3.	25.62	trial 5 > trial 3	$p < 0.05$
	4.	31.0		
	5.	33.5		

Period B responses: Test 2; trials 6-10

Treatment effects

Treatment totals	HC S	49.99	HC S > RP S	$p < 0.05$
	HC L	49.75	HC S > RP L	$p < 0.05$
	RP S	41.84	HC L > RP S	$p < 0.05$
	RP L	33.31	HC L > RP L	$p < 0.05$
			RP S > RP L	$p < 0.05$

Table A32 (continued)

Mean suppression ratios: Test 1; trials 1-5

Treatment effects

Treatment totals	HC S	1.82	HC S > HC L	$p < 0.05$
	HC L	0.69	RP S > HC L	$p < 0.05$
	RP S	1.78	RP L > HC L	$p < 0.05$
	RP L	1.32		

Mean suppression ratios: Test 2; trials 1-5

Treatment effects

Treatment totals	HC S	1.98	RP S > RP L	$p < 0.05$
	HC L	2.31		
	RP S	2.42		
	RP L	1.79		

Table A33: Experiment Four. Extinction data

	Home cage		Response prevented	
	Saline	LVP	Saline	LVP
	Test 1: Total avoidances			
Σx	232	249	163	219
$\bar{x}$	29.0	31.125	23.28	24.33
SD	12.27	15.78	12.16	17.58
SE	4.338	5.58	4.597	5.86
	Test 1: Short avoidances			
Σx	173	192	116	157
$\bar{x}$	21.625	24.0	16.571	17.44
SD	11.134	15.39	11.83	14.52
SE	3.937	5.441	4.47	4.84
	Test 1: Long avoidances			
Σx	59	57	47	62
$\bar{x}$	7.375	7.125	6.714	6.888
SD	4.103	4.015	1.603	5.883
SE	1.451	1.419	0.606	1.961
	Test 2: Total avoidances			
Σx	229	233	161	175
$\bar{x}$	28.625	29.125	23.0	19.444
SD	10.446	9.417	6.831	18.31
SE	3.693	3.33	2.582	6.10
	Test 2: Short avoidances			
Σx	143	165	102	129
$\bar{x}$	17.875	20.625	14.57	14.33
SD	6.379	7.799	3.95	17.421
SE	2.255	2.758	1.493	5.807
	Test 2: Long avoidances			
Σx	86	68	59	46
$\bar{x}$	10.75	8.5	8.428	5.111
SD	5.8	2.725	4.894	5.464
SE	2.051	0.963	1.85	1.821

Table A34: Experiment Four. Extinction performance as a function of extinction trials

Trial	HC S	HC L	RP S	RP L
Test 1:	Mean number of total responses per trial			
1	4.125	3.75	2.142	3.443
2	3.25	2.875	3.142	2.443
3	3.0	3.5	2.428	2.888
4	3.125	3.25	2.0	2.666
5	2.625	2.875	2.5657	1.888
6	2.75	3.5	1.999	1.777
7	1.875	3.125	2.285	2.777
8	2.5	3.0	1.999	1.777
9	2.875	3.0	2.142	2.555
10	2.875	2.25	2.571	2.11
Test 1:	Mean number of short avoidances per trial			
1	3.375	3.375	1.714	2.777
2	2.375	2.5	2.0	1.666
3	1.875	2.625	1.571	2.0
4	1.875	2.25	1.0	1.666
5	2.625	2.25	2.28	1.222
6	2.0	2.625	1.571	1.222
7	1.375	2.25	1.285	2.111
8	2.0	2.375	1.428	1.555
9	2.25	2.25	1.857	2.0
10	1.875	1.5	1.857	1.222
Test 1:	Mean number of long avoidances per trial			
1	0.75	0.375	0.428	0.666
2	0.875	0.375	1.142	0.777
3	1.125	0.875	0.857	0.888
4	1.25	1.0	1.0	1.0
5	0	0.625	0.2857	0.666
6	0.75	0.875	0.428	0.555
7	0.5	0.875	1.0	0.666
8	0.5	0.625	0.5714	0.222
9	0.625	0.75	0.285	0.555
10	1.0	0.75	0.714	0.888

Table A34 (continued)

Trial	HC S	HC L	RP S	RP L
Test 2:	Mean number of total responses per trial			
1	3.75	3.625	3.571	2.333
2	2.625	3.125	2.999	1.888
3	2.25	2.875	2.142	1.999
4	2.875	2.625	1.571	1.777
5	3.0	3.25	1.999	1.885
6	2.5	2.75	2.142	1.666
7	2.625	3.625	2.4284	1.777
8	3.5	3.5	2.142	1.777
9	2.25	2.0	1.713	2.221
10	3.25	1.75	2.285	2.11
Test 2:	Mean number of short avoidances per trial			
1	3.125	2.625	2.714	2.0
2	1.75	2.125	1.857	1.555
3	1.375	1.75	1.142	1.111
4	2.0	1.625	1.0	1.555
5	1.375	2.25	1.142	1.33
6	1.5	2.375	1.142	1.111
7	1.0	2.25	1.5714	1.333
8	1.75	2.75	1.285	1.333
9	1.25	1.75	1.142	1.444
10	2.75	1.125	1.571	1.555
Test 2:	Mean number of long avoidances per trial			
1	0.625	1.0	0.857	0.333
2	0.875	1.0	1.142	0.333
3	0.875	1.125	1.0	0.888
4	0.875	1.0	0.571	0.222
5	1.625	1.0	0.857	0.555
6	1.0	0.375	1.0	0.555
7	1.625	1.375	0.857	0.444
8	1.75	0.75	0.857	0.444
9	1.0	0.25	0.571	0.777
10	0.5	0.625	0.714	0.555

Table A35: Experiment Four. Outcomes of Freidman's analysis of variance on extinction data (Seigel 1956)

	Total responses	Short avoidances	Long avoidances
		Test 1	
ΣR_j^2	2747.5	2695.5	2534.0
χ_r^2	14.85	11.73	2.04
P	< 0.01*	< 0.01	NS
		Test 2	
ΣR_j^2	2776.25	2658.0	2633.25
χ_r^2	16.57	9.48	7.995
P	< 0.001	< 0.05	< 0.05
	Multiple comparisons (Hollander and Wolfe 1973)		
		Test 1	
HC L v RP L	19.5	17.5	
P	< 0.029	< 0.01	
		Test 2	
HC L v RP L	20.0	17.0	17.0
P	< 0.01	< 0.029	< 0.029
		Test 1	
HC L v RP S	16.5		
P	< 0.029		
		Test 2	
HC S v RP L	19.5		
P	< 0.01		

* n = 10, k = 4

Table A36: Experiment Five. Acquisition performance

	Home cage saline					Home cage LVP					Response prevented saline					Response prevented LVP				
	Imm	30	60	6	24	Imm	30	60	6	24	Imm	30	60	6	24	Imm	30	60	6	24
Avoidances to criterion																				
Σx	136	180	141	154	182	135	144	202	127	145	177	135	186	120	167	192	157	114	165	138
$\bar{x}$	17.0	22.5	17.63	19.25	22.75	16.88	18.0	25.25	15.88	18.13	22.125	16.88	23.25	15.0	20.88	24.0	19.63	14.25	20.63	17.25
SD	2.39	6.48	2.72	7.09	12.74	3.76	2.2	18.54	3.83	8.84	8.67	8.63	14.9	3.02	9.19	4.38	4.78	3.92	6.7	4.86
Trials to criterion																				
Σx	266	359	292	265	298	259	260	362	306	232	304	238	349	235	256	374	279	206	389	257
$\bar{x}$	32.25	44.88	36.55	33.13	37.25	32.38	32.5	45.25	38.25	29.0	38.0	29.75	43.63	29.38	32.0	46.75	34.88	25.75	48.63	32.13
SD	10.9	25.65	24.45	13.66	21.65	25.71	10.85	25.81	25.69	16.04	16.0	15.26	25.91	14.83	13.55	19.72	16.22	18.21	28.11	13.68
Escapes to criterion																				
Σx	45	53	71	48	54	42	67	65	54	44	111	33	56	30	37	65	28	67	49	91
$\bar{x}$	5.63	6.63	8.87	6.0	6.75	5.25	8.37	8.125	6.75	5.5	13.87	4.12	7.0	3.75	4.63	8.125	3.5	8.37	6.125	11.37
SD	6.71	4.89	11.65	6.84	6.52	5.75	7.46	7.04	10.87	4.63	18.87	4.52	8.67	3.88	3.25	4.64	3.66	16.17	4.97	13.67
Failures to respond																				
Σx	56	108	32	23	37	32	48	47	67	39	26	34	35	15	36	99	42	24	153	29
$\bar{x}$	7.0	13.5	4.0	2.87	4.62	4.0	6.0	5.87	8.375	4.87	3.25	4.25	4.375	1.87	4.5	12.37	5.25	3.0	19.13	3.625
SD	9.57	22.4	6.25	5.76	7.33	8.52	9.68	5.97	12.14	4.99	3.5	5.59	6.3	2.1	6.54	18.68	5.12	7.31	22.28	5.09
Shocks in training																				
Σx	349	628	255	211	267	227	331	347	443	257	277	149	271	118	242	638	273	196	842	257
$\bar{x}$	43.63	78.5	31.87	26.4	33.4	28.37	41.37	43.4	53.4	32.13	34.63	18.63	33.8	14.75	30.25	79.75	34.12	24.5	105.25	32.12
SD	49.41	113.7	39.9	28.5	44.1	46.05	48.16	32.85	75.9	27.11	37.5	25.3	42.72	14.54	38.5	106.0	31.02	37.3	115.78	22.4

Table A37: Analysis of acquisition performance

	Source	SS	df	MS	F	P
	Between groups	1522.818	19	80.14	1.2199	NS
Avoidances	Within groups	9197.87	140	65.6991		
	Total	10720.0	159			
	Between groups	6901.275	19	363.225	0.9235	NS
Trials	Within groups	55066.5	140	393.33		
	Total	61967.0	159			
	Between groups	976.875	19	51.4145	0.6601	NS
Escapes	Within groups	10904.5	140	77.8893		
	Total	11881.375	159			
	Between groups	2752.725	19	144.8803	1.3319	NS
Failures	Within groups	15228.25	140	108.7732		
	Total	17980.97	159			
	Between groups	77146.7	19	4060.35	1.2527	NS
Shocks	Within groups	453767.25	140	3241.19		
	Total	530913.9	159			

Table A38: Experiment Five. Extinction data: Number of responses per block of five trials

	Home cage saline					Home cage LVP					Response prevented saline					Response prevented LVP				
	Imm	30	60	6	24	Imm	30	60	6	24	Imm	30	60	6	24	Imm	30	60	6	24
										Total responses: Test 1										
1	31	36	36	35	26	21	32	25	27	29	29	31	34	31	30	35	38	36	35	27
2	32	40	37	36	34	22	28	27	31	28	25	29	32	32	33	32	35	35	29	24
3	26	36	37	34	33	29	32	27	33	34	30	32	30	31	37	35	30	27	28	30
4	31	37	32	34	28	28	33	24	28	31	33	31	34	31	35	34	28	27	34	28
5	29	33	32	30	27	29	37	32	32	28	31	33	34	34	34	32	35	24	33	25
6	23	34	35	37	29	28	30	34	34	33	29	28	33	33	24	31	30	27	41	32
7	21	30	38	29	21	32	39	26	31	32	34	23	27	31	27	35	29	20	31	24
8	23	30	33	28	21	29	32	23	33	28	33	24	27	29	27	32	24	19	35	22
9	24	27	31	23	12	25	31	26	28	25	25	19	21	31	24	28	24	16	36	19
10	24	28	29	21	16	26	25	17	20	25	23	17	14	23	22	25	25	18	24	23
Σx	264	331	340	307	247	269	319	261	297	293	292	267	286	306	293	319	298	249	326	254
$\bar{x}$	26.4	33.1	34.0	30.7	24.7	26.9	31.9	26.1	29.7	29.3	29.2	26.7	28.6	30.6	29.3	31.9	29.8	24.9	32.6	25.4
SD	4.005	4.25	3.02	5.49	7.12	3.41	4.01	4.67	4.16	3.13	3.79	5.64	6.63	2.98	5.25	3.28	4.89	6.87	4.78	3.89
SE	1.26	1.34	0.95	1.74	2.25	1.07	1.27	1.48	1.32	0.98	1.198	1.78	2.09	0.94	1.66	1.04	1.55	1.89	1.51	1.23
										Total responses: Test 2										
1	34	36	38	37	34	26	36	32	33	35	33	32	33	31	37	40	33	33	38	31
2	28	37	38	35	35	18	35	36	32	32	37	36	33	35	32	34	31	34	34	35
3	28	34	32	34	31	25	36	39	33	36	36	29	31	33	30	32	28	35	34	28
4	30	31	33	31	39	23	31	31	39	33	31	31	34	33	35	39	25	38	36	25
5	30	33	38	30	37	20	32	32	34	36	33	29	33	34	34	33	35	32	36	27
6	27	30	32	27	35	24	30	31	32	29	32	20	29	32	28	31	27	26	37	28
7	29	24	28	22	24	18	29	31	33	30	23	16	20	34	26	31	23	17	29	29
8	23	21	29	21	28	18	27	23	27	23	19	10	20	33	24	30	24	19	24	26
9	21	17	28	24	28	14	17	27	23	30	33	13	17	26	22	20	27	20	23	19
10	19	17	20	20	23	14	13	22	24	31	19	14	17	17	16	26	19	19	32	17
Σx	269	280	316	281	314	200	286	304	310	315	296	230	267	308	284	316	272	273	323	265
$\bar{x}$	26.9	28.0	31.6	28.1	31.4	20.0	28.6	30.4	31.0	31.5	29.6	23.0	26.7	30.8	28.4	31.6	27.2	27.3	32.3	26.5
SD	4.58	7.65	5.69	6.19	5.48	4.35	7.82	5.25	4.89	3.92	6.72	9.39	7.26	5.45	6.53	5.79	4.82	7.97	5.31	5.29
SE	1.45	2.42	1.80	1.96	1.73	1.37	2.47	1.66	1.55	1.24	2.12	2.97	2.29	1.72	2.06	1.83	1.53	2.52	1.68	1.67

Table A38 (continued)

	Home cage saline					Home cage LVP					Response prevented saline					Response prevented LVP				
	Imm	30	60	6	24	Imm	30	60	6	24	Imm	30	60	6	24	Imm	30	60	6	24
Short avoidances: Test 1																				
1	28	35	33	31	25	20	29	25	24	24	27	24	28	29	26	29	31	25	31	25
2	26	35	35	31	29	20	27	25	26	27	20	24	24	28	28	28	29	31	26	19
3	24	30	33	29	25	24	31	26	31	25	25	24	20	25	31	27	26	20	25	20
4	28	32	28	28	23	23	27	23	23	27	27	25	27	26	25	28	27	21	30	22
5	25	27	26	25	21	27	33	26	26	19	26	24	28	29	26	28	28	18	26	18
6	17	26	30	32	19	20	27	31	31	26	23	23	24	26	21	26	23	20	32	25
7	15	24	28	22	19	26	29	22	25	25	30	17	24	27	23	24	23	13	26	20
8	17	21	25	22	15	24	24	16	27	19	23	18	21	26	19	27	17	15	29	18
9	21	22	25	20	8	21	23	17	21	20	20	12	18	27	14	25	15	14	30	15
10	23	20	23	18	13	19	21	13	14	19	17	12	11	20	15	20	20	16	23	14
Σx	224	272	286	258	197	224	271	224	248	231	238	203	225	263	228	262	239	193	278	196
$\bar{x}$	22.4	27.2	28.6	25.8	19.7	22.4	27.1	22.4	24.8	23.1	23.8	20.3	22.5	26.3	22.8	26.2	23.9	19.3	27.8	19.6
SD	4.72	5.59	4.03	5.07	6.32	2.79	3.66	5.5	4.94	3.45	3.96	5.14	5.25	2.58	5.53	2.66	5.28	5.49	2.97	3.68
SE	1.49	1.77	1.27	1.60	2.0	0.88	1.16	1.74	1.56	1.09	1.25	1.63	1.66	0.82	1.75	0.84	1.67	1.74	0.94	1.16
Short avoidances: Test 2																				
1	28	31	34	31	29	21	31	28	30	29	28	28	29	31	28	34	30	31	32	28
2	22	29	32	30	29	15	30	29	29	28	30	29	27	31	25	28	26	33	29	27
3	24	29	26	31	29	22	26	31	30	29	31	19	27	27	27	26	23	29	29	23
4	23	29	30	24	31	17	26	23	33	27	26	18	31	30	28	34	20	31	30	23
5	23	25	33	25	32	16	26	26	31	29	26	16	31	32	28	28	25	25	31	22
6	26	27	27	27	33	20	24	27	26	23	24	15	24	23	23	25	22	18	29	26
7	24	17	23	19	17	17	24	26	27	22	18	14	16	28	21	28	18	16	25	20
8	19	18	24	20	26	16	22	20	21	19	13	7	14	28	19	26	18	10	23	20
9	15	11	25	21	22	8	10	25	21	24	26	11	15	19	15	18	18	13	21	15
10	16	12	17	18	19	8	11	21	18	26	16	11	16	14	15	22	13	13	27	15
Σx	220	228	271	246	267	160	230	256	266	256	238	168	230	263	229	269	213	219	276	219
$\bar{x}$	22.0	22.8	27.1	24.6	26.7	16.0	23.0	25.6	26.6	25.6	23.8	16.8	23.0	26.3	22.9	26.9	21.3	21.9	27.6	21.9
SD	4.16	7.58	5.26	5.01	5.56	4.81	7.12	3.47	5.01	3.47	6.08	7.11	6.99	5.88	5.19	4.86	4.92	8.81	3.56	4.53
SE	1.32	2.39	1.66	1.59	1.76	1.52	2.25	1.09	1.59	1.09	1.92	2.25	2.21	1.86	1.64	1.54	1.56	2.78	1.13	1.43

Table A38 (continued)

	Home cage saline					Home cage LVP					Response prevented saline					Response prevented LVP				
	Imm	30	60	6	24	Imm	30	60	6	24	Imm	30	60	6	24	Imm	30	60	6	24
										Long avoidances: Test 1									
1	3	1	3	4	1	1	3	0	3	5	2	7	6	2	4	6	7	11	4	2
2	6	5	2	5	5	2	1	2	5	1	5	5	8	4	5	4	6	4	3	5
3	2	6	4	5	8	5	1	1	2	9	5	8	10	6	6	8	4	7	3	10
4	3	5	4	6	5	5	6	1	5	4	6	6	7	5	10	6	1	6	4	6
5	4	6	6	5	6	2	4	6	6	9	5	9	6	5	8	4	7	6	7	7
6	6	8	5	5	10	8	3	3	3	7	6	5	9	7	3	5	7	7	9	7
7	6	6	10	7	2	6	10	4	6	7	4	6	3	4	4	11	6	7	5	4
8	6	9	8	6	6	5	8	7	6	9	10	6	6	3	8	5	7	4	6	4
9	3	5	6	3	4	4	8	9	7	5	5	7	3	4	10	3	9	2	6	4
10	1	8	6	3	3	7	4	4	6	6	6	5	3	3	7	5	5	2	1	9
Σx	40	59	54	49	50	45	48	37	49	62	54	64	61	43	65	57	59	56	48	58
$\bar{x}$	4.0	5.9	5.4	4.9	5.0	4.5	4.8	3.7	4.9	6.2	5.4	6.4	6.1	4.3	6.5	5.7	5.9	5.6	4.8	5.8
SD	1.88	2.23	2.36	1.28	2.71	2.27	3.08	2.91	1.66	2.57	2.01	1.35	2.51	1.49	2.5	2.31	2.18	2.72	2.29	2.48
SE	0.59	0.71	0.75	0.41	0.86	0.72	0.97	0.92	0.53	0.81	0.63	0.43	0.79	0.47	0.79	0.731	0.69	0.86	0.73	0.78
										Long avoidances: Test 2										
1	6	5	4	6	5	5	5	4	3	6	5	4	4	0	9	6	3	2	6	3
2	6	8	6	5	6	3	5	7	3	4	7	7	6	4	7	6	5	1	5	8
3	4	5	6	3	2	3	10	8	3	7	5	10	4	6	3	6	5	6	5	5
4	7	2	3	7	8	6	5	8	6	6	5	13	3	3	7	5	5	7	6	2
5	7	8	5	5	5	4	6	6	3	7	7	13	2	2	6	5	10	7	5	5
6	1	3	5	0	2	4	6	4	6	6	8	5	5	9	5	6	5	8	8	2
7	5	7	5	3	7	1	5	5	6	8	5	2	4	6	5	3	5	1	4	9
8	4	3	5	1	2	2	5	3	6	4	6	3	6	5	5	4	6	9	1	6
9	6	6	3	3	6	6	7	2	2	6	7	2	2	7	7	2	9	7	2	4
10	3	5	3	2	4	6	2	1	6	5	3	3	1	3	1	4	6	6	5	2
Σx	49	52	45	35	47	40	56	48	44	59	58	62	37	45	55	47	59	54	47	46
$\bar{x}$	4.9	5.2	4.5	3.5	4.7	4.0	5.6	4.8	4.4	5.9	5.8	6.2	3.7	4.5	5.5	4.7	5.9	5.4	4.7	4.6
SD	1.91	2.09	1.18	2.22	2.16	1.76	2.01	2.44	1.71	1.28	1.47	4.34	1.7	2.63	2.27	1.42	2.08	2.95	2.0	2.5
SE	0.6	0.66	0.37	0.70	0.68	0.56	0.63	0.77	0.54	0.41	0.46	1.373	0.54	0.83	0.72	0.45	0.66	0.93	0.63	0.79

Table A39: Experiment Five. Outcomes of Freidman's Analysis of variance on extinction data (Siegel 1956)

		ΣR_j^2	χ_r^2	P
Total avoidances	Test 1	248094.5	78.83	0.001
	Test 2	252439.0	91.24	0.001
Short avoidances	Test 1	252199.5	90.559	0.001
	Test 2	251365.59	87.973	0.001
Long avoidances	Test 1	228965.5	24.177	NS
	Test 2	227254.25	19.28	NS

Table A40: Experiment Five. Extinction Analysis: Sums of Freidman ranks per group

		Imm	30	60	6	24
		Test 1				
Total avoidances	HC sal	135.0	41.0	39.5	74.0	156.0
	HC LVP	132.5	84.5	161.0	95.5	107.5
	RP sal	118.0	138.5	114.5	83.0	107.5
	RP LVP	66.5	89.0	139.5	56.0	161.0
Short avoidances	HC sal	87.0	153.5	171.0	130.5	51.5
	HC LVP	84.5	155.0	77.5	111.0	93.5
	RP sal	109.0	45.5	86.5	143.0	90.5
	RP LVP	147.5	114.0	47.0	157.5	45.5
		Test 2				
Total avoidances	HC sal	145.5	105.5	53.5	133.0	68.0
	HC LVP	194.0	94.0	78.0	80.5	65.0
	RP sal	95.0	169.5	141.5	65.5	121.0
	RP LVP	64.5	144.5	96.5	50.5	134.5
Short avoidances	HC sal	75.5	94.5	157.5	129.5	145.5
	HC LVP	21.5	99.5	131.0	148.5	117.5
	RP sal	108.5	29.5	91.5	140.0	83.0
	RP LVP	144.0	62.5	89.5	158.5	72.5

Reject $H_0(R_u = R_v)$ if $R_u - R_v \geq q(\alpha,k,\infty)[\frac{n(k).(k+1)}{12}]^{1/2}$

$R_{u(v)}$ = Σ ranks for groups u/v

Critical differences for rank sums

α (experimentwise)	d (critical difference)
0.1	87.816
0.05	93.76
0.025	99.134
0.01	105.607

Table A41: Experiment Five. Selected comparisons between groups in extinction data

	Index	Groups	d	P
(1) Comparison between home cage saline groups				
Test 1	Total avoidances	HC sal 30 > HC sal 0	94.0	< 0.05
		HC sal 60 > HC sal 0	95.5	< 0.05
		HC sal 30 > HC sal 24	115.0	< 0.01
		HC sal 60 > HC sal 24	116.5	< 0.01
Test 1	Short avoidances	HC sal 30 > HC sal 24	102.0	< 0.025
		HC sal 60 > HC sal 24	119.5	< 0.01
Test 2	Total avoidances	HC sal 60 > HC sal 0	91.5	[< 0.1]
Test 2	Short avoidances			NS
(2) Comparison between response prevented saline groups				
Test 1	Total avoidances			NS
Test 1	Short avoidances	RP sal 30 < RP sal 6	97.5	< 0.05
Test 2	Total avoidances	RP sal 30 < RP sal 6	104.0	< 0.025
Test 2	Short avoidances	RP sal 30 < RP sal 6	110.5	< 0.01
(3) Comparison between home cage LVP groups				
Test 1				NS
Test 2	Total avoidances	HC LVP 0 < HC LVP 30	100.0	< 0.025
		HC LVP 0 < HC LVP 60	116.0	< 0.01
		HC LVP 0 < HC LVP 6	113.6	< 0.01
		HC LVP 0 < HC LVP 24	129.0	< 0.01
Test 2	Short avoidances	HC LVP 0 < HC LVP 60	109.5	< 0.01
		HC LVP 0 < HC LVP 6	127.0	< 0.01
		HC LVP 0 < HC LVP 24	96.0	< 0.05
(4) Comparison between response prevented LVP groups				
Test 1	Total avoidances	RP LVP 0 > RP LVP 24	94.5	< 0.05
		RP LVP 6 > RP LVP 24	105.0	< 0.01
Test 1	Short avoidances	RP LVP 0 > RP LVP 60	100.5	< 0.025
		RP LVP 0 > RP LVP 24	102.0	< 0.025
		RP LVP 60 < RP LVP 6	110.5	< 0.01
		RP LVP 6 > RP LVP 24	112.0	< 0.01

Table A41 (continued)

	Index	Groups	d	P
Test 2	Total avoidances	RP LVP 30 < RP LVP 6	94.0	< 0.05
Test 2	Short avoidances	RP LVP 30 < RP LVP 6	96.0	< 0.05
(5) Comparison between response prevented saline and home cage saline groups				
Test 1	Total avoidances	HC sal 30 > RP sal 30	97.5	< 0.05
		HC sal 60 > RP sal 30	99.0	<<0.05
Test 1	Short avoidances	HC sal 30 > RP sal 30	108.0	< 0.01
		HC sal 60 > RP sal 30	125.5	< 0.01
		HC sal 60 > RP sal 60	84.0	[0.1]
		HC sal 24 < RP sal 6	91.5	0.06
Test 2	Total avoidances			NS
Test 2	Short avoidances	HC sal 60 > RP sal 30	127.5	< 0.01
		HC sal 6 > RP sal 30	100.0	< 0.025
		HC sal 24 > RP sal 30	116.0	< 0.01
(6) Comparison between response prevented LVP and home cage LVP groups				
Test 1	Total avoidances	HC LVP 60 < RP LVP 6	105.0	< 0.025
Test 1	Short avoidances	HC LVP 30 > RP LVP 60	108.0	< 0.01
		HC LVP 30 > RP LVP 24	109.5	< 0.01
Test 2	Total avoidances	HC LVP 0 < RP LVP 0	129.5	< 0.01
		HC LVP 0 < RP LVP 6	144.0	< 0.01
Test 2	Short avoidances	HC LVP 0 < RP LVP 0	122.5	< 0.01
		HC LVP 0 < RP LVP 6	137.0	< 0.01
(7) Comparison between home cage saline and home cage LVP groups				
Test 1	Total avoidances	HC sal 30 > HC LVP 0	91.5	0.06
		HC sal 30 > HC LVP 60	120.0	< 0.01
		HC sal 60 > HC LVP 0	93.0	0.05
		HC sal 60 > HC LVP 60	121.5	< 0.01
		HC sal 6 > HC LVP 60	87.0	[< 0.1]
Test 1	Short avoidances	HC sal 60 > HC LVP 60	93.5	0.05
		HC sal 24 < HC LVP 30	103.5	< 0.025

Table A41 (continued)

	Index	Groups	d	P
Test 2	Total avoidances	HC sal 30 > HC LVP 0	88.5	[< 0.1]
		HC sal 60 > HC LVP 0	141.0	< 0.01
		HC sal 24 > HC LVP 0	126.0	< 0.01
Test 2	Short avoidances	HC sal 60 > HC LVP 0	136.0	< 0.01
		HC sal 6 > HC LVP 0	108.0	< 0.01
		HC sal 24 > HC LVP 0	124.0	< 0.01
(8)	Comparison between response prevented saline and response prevented LVP groups			
Test 1	Total avoidances			NS
Test 1	Short avoidances	RP sal 30 < RP LVP 0	102.0	< 0.025
		RP sal 30 < RP LVP 6	112.0	< 0.01
		RP sal 6 > RP LVP 60	96.0	< 0.05
		RP sal 6 > RP LVP 24	97.5	< 0.05
Test 2	Total avoidances	RP sal 30 < RP LVP 0	105.0	0.01
		RP sal 30 < RP LVP 6	109.0	< 0.01
		RP sal 60 < RP LVP 6	91.0	0.06
Test 2	Short avoidances	RP sal 30 < RP LVP 0	114.5	< 0.01
		RP sal 30 < RP LVP 6	129.0	< 0.01
(9)	Comparison between home cage saline and response prevented LVP groups			
Test 1	Total avoidances	HC sal 30 > RP LVP 60	98.5	< 0.05
		HC sal 30 > RP LVP 24	120.0	< 0.01
		HC sal 60 > RP LVP 60	100.0	< 0.025
		HC sal 60 > RP LVP 24	121.5	< 0.01
		HC sal 24 < RP LVP 0	89.5	[< 0.1]
		HC sal 24 < RP LVP 6	100.0	< 0.025
Test 1	Short avoidances	HC sal 30 > RP LVP 6	106.5	< 0.01
		HC sal 30 > RP LVP 24	108.0	< 0.01
		HC sal 60 > RP LVP 60	124.0	< 0.01
		HC sal 60 > RP LVP 24	125.5	< 0.01
		HC sal 24 < RP LVP 0	96.0	< 0.05
		HC sal 24 < RP LVP 6	106.0	< 0.01

Table A41 (continued)

	Index	Groups		d	P
Test 2	Total avoidances	HC sal 0	RP LVP 6	95.0	< 0.05
		HC sal 60	RP LVP 60	91.0	0.05
Test 2	Short avoidances	HC sal 60	RP LVP 30	95.0	< 0.05
(10) Comparison between response prevented saline and home cage LVP groups					
Test 1	Total avoidances				NS
Test 1	Short avoidances	HC LVP 30 >	RP sal 30	109.5	< 0.01
Test 2	Total avoidances	HC LVP 0 <	RP sal 0	99.0	0.025
		HC LVP 0 <	RP sal 6	128.5	< 0.01
		HC LVP 60 >	RP sal 30	91.5	0.05
		HC LVP 6 >	RP sal 30	89.0	0.06
Test 2	Short avoidances	HC LVP 0 <	RP sal 0	87.0	[0.1]
		HC LVP 0 <	RP sal 6	118.5	< 0.01
		HC LVP 60 >	RP sal 30	101.5	< 0.025
		HC LVP 6 >	RP sal 30	119.0	< 0.01
		HC LVP 24 >	RP sal 30	88.0	[< 0.1]

Table A42: Experiment Six. Number of avoidances, escapes, trials, failures to respond and shocks received during acquisition training

	Home cage				Response prevented			
	Saline	2 µg	3 µg	4 µg	Saline	2 µg	3 µg	4 µg
				Avoidances				
Σx	87	143	128	148	103	144	129	159
$\bar{x}$	14.5	23.83	21.33	24.66	17.16	24.0	21.5	26.5
SD	3.88	8.06	8.66	10.63	5.23	7.79	7.96	9.64
SE	1.58	3.29	3.53	4.34	2.136	3.18	3.25	3.94
				Trials				
Σx	211	286	311	245	184	320	278	250
$\bar{x}$	35.16	47.66	51.83	40.83	30.66	53.33	46.33	41.66
SD	22.81	15.32	18.01	25.4	10.46	22.6	17.95	16.74
SE	9.316	6.25	7.35	10.37	4.27	9.23	7.33	6.84
				Escapes				
Σx	30	88	58	41	27	60	87	33
$\bar{x}$	5.0	14.66	9.66	6.83	4.5	10.0	14.5	5.5
SD	2.75	12.13	9.11	8.08	3.98	7.13	8.43	4.97
SE	1.12	4.95	3.72	3.3	1.63	2.91	3.44	2.03
				Failures				
Σx	80	47	98	48	40	116	45	47
$\bar{x}$	13.33	7.83	16.33	8.0	6.66	19.33	7.5	7.83
SD	18.47	8.25	16.22	5.47	7.36	24.45	7.96	6.25
SE	7.54	3.37	6.62	2.23	3.0	9.98	3.25	2.55
				Shocks				
Σx	465	345	596	304	244	689	343	290
$\bar{x}$	77.5	57.5	99.33	50.66	40.66	114.83	57.16	48.33
SD	98.93	37.55	82.21	34.43	30.45	117.1	36.87	34.59
SE	40.39	15.33	33.57	14.06	12.43	47.82	15.06	14.125

Table A43: Experiment Six. Analysis of variance on acquisition data

	Source	SS	df	MS	F	P
	Between groups	685.479	7	97.92	1.527	NS
Avoidances	Within groups	2564.83	40	64.12		
	Total	3250.3125	47			
	Between groups	2616.64	7	373.8	1.0143	NS
Trials	Within groups	14741.16	40	368.52		
	Total	17357.81	47			
	Between groups	700.66	7	100.09	1.7198	NS
Escapes	Within groups	2328.0	40	58.2		
	Total	3028.66	47			
	Between groups	979.48	7	139.9	0.7681	NS
Failures	Within groups	7826.5	40	182.16		
	Total	8265.97	47			
	Between groups	29561.0	7	4223.0	0.9297	NS
Shocks	Within groups	181694.0	40	4542.35		
	Total	211255.0	47			

Table A44: Experiment Six. Extinction data

	Home cage				Response prevented			
	Saline	2 µg	3 µg	4 µg	Saline	2 µg	3 µg	4 µg
				Total avoidances: Test 1				
Σx	232	235	221	206	241	247	210	188
$\bar{x}$	38.66	39.16	36.83	34.33	40.16	41.16	35.0	31.33
SD	11.893	5.56	15.75	12.71	11.78	10.72	8.34	7.5
SE	4.856	2.27	6.43	5.18	4.81	4.38	3.4	3.062
				Total avoidances: Test 2				
Σx	224	223	186	189	221	244	190	187
$\bar{x}$	37.33	37.16	31.0	31.5	36.83	40.66	31.66	31.16
SD	7.58	4.62	15.96	12.11	10.51	9.58	6.71	13.54
SE	3.09	1.88	6.52	4.94	4.29	3.91	2.74	5.53
				Short avoidances: Test 1				
Σx	185	189	188	163	190	204	145	146
$\bar{x}$	30.83	31.5	31.33	27.16	31.66	34.0	24.16	24.33
SD	12.86	8.96	15.48	9.174	11.91	11.55	11.48	7.53
SE	5.25	3.66	6.32	3.746	4.86	4.72	4.68	3.07
				Short avoidances: Test 2				
Σx	168	176	137	151	170	200	137	130
$\bar{x}$	28.0	29.33	22.83	25.16	28.33	33.33	22.83	21.66
SD	9.96	5.95	12.7	13.79	8.24	10.76	8.7	10.91
SE	4.06	2.43	5.18	5.63	3.36	4.39	3.55	4.45
				Long avoidances: Test 1				
Σx	47	46	33	43	51	43	67	42
$\bar{x}$	7.83	7.66	5.5	7.16	8.5	7.16	11.16	7.0
SD	4.12	4.46	3.51	6.55	2.58	3.06	4.07	3.28
SE	1.68	1.82	1.43	2.67	1.056	1.25	1.66	1.34
				Long avoidances: Test 2				
Σx	56	47	49	38	51	44	53	57
$\bar{x}$	9.33	7.83	8.16	6.33	8.5	7.33	8.83	9.5
SD	2.65	2.86	5.03	4.32	3.78	4.32	3.92	4.72
SE	1.08	1.16	2.05	1.764	1.54	1.76	1.6	1.928

Table A45: Experiment Six. Extinction responding as a function of the trial block

	Test 1								Test 2							
	Home cage				Response prevented				Home cage				Response prevented			
	Sal	2 µg	3 µg	4 µg	Sal	2 µg	3 µg	4 µg	Sal	2 µg	3 µg	4 µg	Sal	2 µg	3 µg	4 µg
	Total avoidances															
1	21	17	20	21	22	23	15	20	21	30	22	26	22	28	22	21
2	22	24	22	22	23	28	17	21	25	25	17	23	20	25	19	25
3	22	26	21	25	23	28	20	23	22	23	15	25	26	23	24	22
4	23	26	21	19	22	28	23	20	23	26	17	23	23	24	22	21
5	22	28	22	20	26	28	23	21	26	28	18	25	20	24	22	21
6	26	26	24	25	26	25	23	20	20	27	23	19	27	25	23	20
7	28	25	24	26	27	23	26	19	26	22	21	14	22	25	21	13
8	25	24	22	20	25	22	23	17	23	21	22	12	22	26	14	16
9	23	20	26	16	29	23	22	16	21	10	20	13	20	23	13	12
10	20	19	19	12	18	19	18	11	17	9	15	9	19	21	10	16
Σx	232	235	221	206	241	247	210	188	224	221	190	189	221	244	190	187
$\bar{x}$	23.2	23.5	22.1	20.6	24.1	24.7	21.0	18.8	22.4	22.1	19.0	18.9	22.1	24.4	19.0	18.7
SD	2.44	3.59	2.08	4.32	3.14	3.19	3.39	3.39	2.84	7.18	2.98	6.35	2.64	1.89	4.87	4.22
SE	0.77	1.14	0.657	1.36	0.99	1.01	1.07	1.073	0.89	2.27	0.94	2.0	0.84	0.6	1.54	1.33

Table A45 (continued)

	Test 1								Test 2							
	Home cage				Response prevented				Home cage				Response prevented			
	Sal	2 µg	3 µg	4 µg	Sal	2 µg	3 µg	4 µg	Sal	2 µg	3 µg	4 µg	Sal	2 µg	3 µg	4 µg
	Short avoidances															
1	16	15	16	20	18	19	11	17	18	26	18	23	19	21	15	19
2	12	21	19	15	22	22	10	13	17	21	12	21	14	21	12	19
3	18	25	19	21	20	24	17	18	11	19	8	19	23	20	17	12
4	21	20	20	16	12	20	15	16	16	19	14	22	15	17	19	15
5	17	23	15	14	21	22	18	17	16	22	13	15	16	20	18	13
6	23	24	19	21	19	22	14	17	15	21	13	14	24	21	18	15
7	23	16	19	19	23	20	18	18	20	16	17	10	15	23	12	9
8	21	18	19	17	23	19	17	13	23	16	19	9	16	20	9	8
9	19	12	24	11	20	21	11	10	18	8	14	9	16	20	9	7
10	15	15	18	9	12	15	14	7	14	8	9	9	12	17	8	13
Σx	185	189	188	163	190	204	145	146	168	176	137	151	170	200	137	130
$\bar{x}$	18.5	18.9	18.8	16.3	19.0	20.4	14.5	14.6	16.8	17.6	13.7	15.1	17.0	20.0	13.7	13.0
SD	3.59	4.38	2.39	4.137	4.03	2.45	3.03	3.74	3.29	5.83	3.59	5.76	3.86	1.82	4.22	4.18
SE	1.14	1.38	0.76	1.31	1.27	0.77	0.96	1.18	1.04	1.84	1.13	1.82	1.22	0.57	1.33	1.32
	Long avoidances															
1	5	2	4	1	4	4	4	3	3	4	4	3	3	7	7	2
2	10	3	3	7	1	6	7	8	8	4	5	2	6	4	7	6
3	4	1	2	4	3	4	3	5	11	4	7	6	3	3	7	10
4	2	6	1	3	10	8	8	4	7	7	3	1	8	7	3	6
5	5	5	7	6	5	6	5	4	10	6	5	10	4	4	4	8
6	3	2	5	4	7	3	9	3	5	6	6	5	3	4	5	5
7	5	9	5	7	4	3	8	1	6	6	4	4	7	2	9	4
8	4	6	3	3	2	3	6	4	0	5	3	3	6	6	5	8
9	4	8	2	5	9	2	11	6	3	2	6	4	4	3	4	5
10	5	4	1	3	6	4	4	4	3	1	6	0	7	4	2	3
Σx	47	46	33	43	51	43	65	42	56	45	49	38	51	44	53	57
$\bar{x}$	4.7	4.6	3.3	4.3	5.1	4.3	6.5	4.2	5.6	4.5	4.9	3.8	5.1	4.4	5.3	5.7
SD	2.11	2.67	1.94	1.94	2.92	1.83	2.55	1.87	3.47	1.9	1.37	2.82	1.91	1.71	2.16	2.45
SE	0.66	0.84	0.61	0.61	0.92	0.57	0.81	0.59	1.09	0.6	0.43	0.89	0.6	0.54	0.68	0.77

Table A46: Experiment Six. Analysis of extinction data using Freidman's analysis of variance (Seigel 1956)

		ΣR_j^2	χ_r^2	P*
Total avoidances	Test 1	18064.5	31.135	< 0.001
	Test 2	17757.75	26.021	< 0.001
Short avoidances	Test 1	17752.5	25.93	< 0.001
	Test 2	17559.0	22.708	< 0.01
Long avoidances	Test 1	16586.75	6.50112	NS
	Test 2	16509.5	5.21337	NS

Multiple comparisons (Hollander and Wolfe 1973)

	Groups	d	P+
Test 1: Total avoidances	HC LVP 2 > HC LVP 4	32.0	[< 0.1]
	RP LVP 2 > RP LVP 4	47.0	< 0.01
	HC Sal > RP LVP 4	37.0	< 0.031
	HC LVP 2 > RP LVP 4	49.0	< 0.01
Test 1: Short avoidances	RP Sal > RP LVP 3	31.0	[0.1]
	RP LVP 2 > RP LVP 3	42.5	< 0.01
	RP LVP 2 > RP LVP 4	40.0	< 0.01
	HC LVP 2 > RP LVP 3	30.5	[0.1]
Test 2: Total avoidances	RP Sal > RP LVP 4	32.5	[0.06]
	RP LVP 2 > RP LVP 4	42.5	< 0.01
	HC LVP 3 < RP LVP 2	38.5	< 0.01
	HC LVP 4 < RP LVP 2	35.0	0.031
Test 2: Short avoidances	RP LVP 2 > RP LVP 3	35.0	0.031
	RP LVP 2 > RP LVP 4	40.5	< 0.01
	HC LVP 3 < RP LVP 2	40.5	< 0.01

* Reject H_0 when $\chi_r^2 \geq \chi_r^2\alpha$; for df k - 1 (k = 8)

+ Critical differences in sums of ranks (for N = 10; k = 8)

d	P
34	0.04
35	0.031
38	0.01

Table A47: Experiment Six. Extinction data

Test 1: Total avoidances

	Home cage				Response prevented			
	Sal	2 µg	3 µg	4 µg	Sal	2 µg	3 µg	4 µg
Correlation coefficient (r)	+0.341	-0.456	+0.4138	-0.581	-0.1	-0.507	+0.236	-0.636
Slope	+0.406	-0.66	+0.327	-0.794	-0.13	-0.412	+0.236	-0.787
Y' intercept	12.2	22.5	17.0	20.66	19.73	22.66	13.2	18.93
Y = 10 predicted	16.26	15.92	20.27	12.72	18.4	18.54	15.56	11.05

Table A48: Experiment Eleven. Acquisition data

	Home cage						Response prevented					
	Sal Sal	LVP Sal	Sal Scop	LVP Scop	Sal Phy	LVP Phy	Sal Sal	LVP Sal	Sal Scop	LVP Scop	Sal Phy	LVP Phy
Trials												
Σx	211	290	276	388	289	372	210	211	226	280	345	270
$\bar{x}$	35.16	48.33	46.0	64.66	48.166	62.0	35.0	35.16	37.66	46.66	57.5	45.0
SD	15.66	23.38	28.29	28.91	34.22	31.85	32.57	8.84	15.0	28.02	27.46	30.29
SE	6.39	9.54	11.54	11.803	13.97	13.0	13.29	3.61	6.12	11.44	11.21	12.36
Avoidances												
Σx	108	132	173	214	165	166	144	126	127	128	181	151
$\bar{x}$	18.0	22.0	28.83	35.66	27.5	27.66	24.0	21.0	21.16	21.33	30.16	25.16
SD	8.44	9.05	18.77	14.36	17.7	12.59	23.29	5.83	7.27	8.214	12.44	15.727
SE	3.44	3.69	7.66	5.86	7.22	5.14	9.51	2.38	2.97	3.35	5.08	6.42
Escapes												
Σx	81	32	75	92	49	130	31	52	66	109	103	46
$\bar{x}$	13.5	5.33	12.5	15.33	8.16	21.66	5.16	8.66	11.0	18.16	17.16	7.66
SD	12.09	4.41	9.42	18.99	8.612	12.22	6.88	4.88	5.14	20.4	17.05	5.5
SE	4.93	1.8	3.84	7.75	3.516	4.99	2.81	1.99	2.09	8.32	6.96	2.24
Failures												
Σx	13	117	32	66	60	82	22	14	27	23	63	74
$\bar{x}$	2.16	19.5	5.33	11.0	10.0	13.66	3.66	2.33	4.5	3.83	10.5	12.33
SD	3.92	17.23	6.05	9.51	22.05	16.657	4.96	4.08	7.064	3.816	16.07	23.51
SE	1.6	7.03	2.47	3.88	9.0	6.8	2.03	1.66	2.88	1.56	6.56	9.59
Shocks												
Σx	180	650	275	449	378	607	151	153	250	295	452	445
$\bar{x}$	30.0	108.33	45.83	74.83	63.0	101.16	25.16	25.5	41.66	49.16	75.33	74.16
SD	17.37	85.02	33.03	53.39	111.31	88.35	34.67	18.66	38.85	35.66	82.26	114.15
SE	7.09	34.71	13.48	21.79	45.44	36.06	14.15	7.619	15.86	14.55	33.58	46.61

Table A49: Experiment Eleven. Analysis of acquisition data

	Source	SS	df	MS	F	P
	Between groups	6997.11	11	636.101	0.9040	NS
Avoidances	Within groups	42175.3	60	702.92		
	Total	49172.44	71			
	Between groups	1627.0417	11	147.9129	0.7772	NS
Trials	Within groups	11418.8333	60	190.3139		
	Total	13045.875	71			
	Between groups	1850.9444	11	168.2677	1.2064	NS
Escapes	Within groups	8369.0	60	139.4833		
	Total	10219.9444	71			
	Between groups	1940.1528	11	176.3775	0.9983	NS
Failures	Within groups	10600.8333	60	176.6806		
	Total	12540.9861	71			
	Between groups	51903.4861	11	4718.4987	1.0147	NS
Shocks	Within groups	279006.5	60	4650.1083		
	Total	330909.9861	71			

Table A50: Experiment Eleven. Extinction data

	Home cage						Response prevented					
	Sal Sal	LVP Sal	Sal Scop	LVP Scop	Sal Phy	LVP Phy	Sal Sal	LVP Sal	Sal Scop	LVP Scop	Sal Phy	LVP Phy
Test 1: Short avoidances												
Σx	183	181	230	170	160	172	179	154	165	181	189	134
$\bar{x}$	30.5	30.16	38.33	28.33	26.66	18.66	19.83	25.66	27.5	30.16	31.5	22.33
SD	12.91	15.38	8.64	10.96	12.72	14.66	12.49	15.08	11.39	9.02	7.5	13.03
SE	5.27	6.28	3.53	4.47	5.19	5.98	5.1	6.16	4.65	3.68	3.06	5.22
Test 2: Short avoidances												
Σx	177	180	182	166	160	164	151	145	181	152	169	168
$\bar{x}$	29.5	30.0	30.33	27.66	26.66	27.33	25.16	24.16	30.16	25.33	28.16	28.0
SD	9.89	10.54	9.54	9.31	9.81	9.91	17.209	12.76	13.21	7.08	9.78	11.24
SE	4.04	4.3	3.89	3.8	4.0	4.05	7.02	5.21	5.39	2.89	3.99	4.56
Test 1: Long avoidances												
Σx	58	32	42	41	35	50	53	26	51	58	60	49
$\bar{x}$	9.66	5.33	7.0	6.83	5.83	8.33	8.83	4.33	8.5	9.66	10.0	8.16
SD	5.92	3.2	4.81	5.07	3.06	4.8	3.18	2.5	4.41	3.72	3.4	3.76
SE	2.42	1.31	1.96	2.07	1.25	1.96	1.3	1.02	1.8	1.52	1.39	1.54
Test 2: Long avoidances												
Σx	32	40	51	68	54	53	38	40	37	61	41	29
$\bar{x}$	5.33	6.66	8.5	11.33	9.0	8.83	6.33	6.66	6.16	10.16	6.83	4.83
SD	4.13	3.72	3.14	4.54	4.86	2.93	3.33	2.34	1.94	3.37	4.99	2.86
SE	1.68	1.52	1.28	1.85	1.98	1.19	1.36	0.95	0.79	1.37	2.04	1.16
Test 1: Total avoidances												
$\bar{x}$	40.16	35.5	45.33	35.16	34.16	37.0	38.66	30.0	36.0	39.83	41.5	30.5
SD	8.82	17.78	4.76	10.03	15.26	16.07	10.87	16.97	10.12	7.41	5.17	14.85
SE	3.6	7.26	1.94	4.09	6.23	6.56	4.44	6.93	4.13	3.03	2.11	6.06
Test 2: Total avoidances												
$\bar{x}$	34.83	36.66	38.83	39.0	35.66	36.16	33.16	30.83	36.0	35.5	35.0	32.83
SD	9.66	10.31	8.11	7.59	5.64	9.74	13.26	13.66	11.78	10.07	12.89	12.64
SE	3.94	4.21	3.31	3.09	2.30	3.97	5.41	5.57	4.81	4.11	5.26	5.16

Table A51: Experiment Eleven. Extinction data: Sum of responses per block of five trials

	Home cage						Response prevented					
	Sal Sal	LVP Sal	Sal Scop	LVP Scop	Sal Phy	LVP Phy	Sal Sal	LVP Sal	Sal Scop	LVP Scop	Sal Phy	LVP Phy
						Test 1: Total avoidances						
1	23	25	24	20	21	21	28	20	24	23	25	22
2	26	25	28	20	25	24	27	24	28	25	28	23
3	24	25	30	26	24	24	26	22	25	23	24	17
4	24	22	30	27	24	23	26	20	30	22	26	20
5	28	24	29	28	25	26	25	19	27	27	24	23
6	27	22	28	24	20	23	21	20	22	28	25	22
7	21	24	28	19	16	23	20	19	19	25	29	23
8	25	19	27	18	18	23	21	15	14	27	23	13
9	22	18	27	16	9	19	19	10	15	23	26	8
10	21	9	21	13	13	16	19	11	12	18	19	12
$\bar{x}$	24.1	21.3	27.2	21.1	19.5	22.2	23.2	18.0	21.6	24.1	24.9	18.3
SD	2.42	4.99	2.78	4.98	5.48	2.86	3.52	4.57	6.31	2.96	2.77	5.5
SE	0.77	1.58	0.88	1.57	1.73	0.9	1.11	1.45	2.0	0.94	0.87	1.74
						Test 2: Total avoidances						
1	25	30	28	26	24	24	23	25	33	27	27	28
2	24	26	26	28	26	22	24	27	25	27	24	25
3	29	22	29	26	27	27	19	26	25	23	24	25
4	25	24	27	25	23	27	21	25	25	26	25	24
5	21	28	27	24	24	24	20	19	29	22	26	25
6	19	23	28	28	27	17	23	21	21	20	25	20
7	22	16	27	25	20	23	20	16	16	24	15	19
8	15	16	22	22	17	18	15	9	17	16	15	9
9	17	17	11	18	13	17	14	8	15	15	13	11
10	12	18	8	12	13	18	10	6	12	13	16	11
$\bar{x}$	20.9	22.0	23.3	23.4	21.4	21.7	18.9	18.2	21.8	21.3	21.0	19.7
SD	5.2	5.1	7.54	4.97	5.4	3.95	4.53	8.04	6.73	5.12	5.5	6.98
SE	1.64	1.61	2.39	1.57	1.71	1.25	1.43	2.54	2.13	1.62	1.74	2.21

Table A51 (continued)

	Home cage						Response prevented					
	Sal Sal	LVP Sal	Sal Scop	LVP Scop	Sal Phy	LVP Phy	Sal Sal	LVP Sal	Sal Scop	LVP Scop	Sal Phy	LVP Phy
						Test 1: Short avoidances						
1	19	22	20	20	16	19	21	17	21	21	19	18
2	23	25	25	17	23	19	21	19	18	21	24	16
3	20	23	28	19	17	22	24	22	18	17	17	15
4	19	19	25	21	24	18	20	17	26	13	21	13
5	23	21	26	23	20	19	19	16	21	20	21	16
6	22	20	23	19	15	19	17	18	20	24	13	14
7	11	18	23	14	13	20	14	15	11	19	18	17
8	18	16	23	15	12	14	16	12	10	19	17	9
9	17	9	20	12	9	11	13	10	11	17	22	6
10	11	8	17	10	11	11	14	8	9	10	17	10
$\bar{x}$	18.3	18.1	23.0	17.0	16.0	17.2	17.9	15.4	16.5	18.1	18.9	13.4
SD	4.35	5.67	3.27	4.16	5.06	3.82	3.67	4.27	5.84	4.09	3.18	3.89
SE	1.37	1.79	1.03	1.32	1.6	1.21	1.16	1.35	1.85	1.29	1.0	1.23
						Test 2: Short avoidances						
1	19	20	23	20	21	19	17	21	24	25	18	28
2	22	21	19	23	17	17	16	21	23	18	21	23
3	24	17	17	21	21	22	15	15	20	18	16	24
4	22	20	24	19	19	21	17	23	21	20	23	19
5	17	22	23	18	20	22	16	16	25	17	22	17
6	18	23	23	17	19	11	18	17	17	12	21	17
7	19	14	25	16	11	13	15	9	16	17	10	17
8	11	13	15	9	11	12	14	9	14	9	13	6
9	14	13	8	13	9	12	13	6	12	7	12	8
10	11	17	5	10	12	15	10	5	9	9	13	9
$\bar{x}$	17.7	18.0	18.2	16.6	16.0	16.4	15.1	14.2	18.1	15.2	16.9	16.8
SD	4.52	3.74	6.99	4.65	4.71	4.38	2.33	6.56	5.38	5.73	4.72	7.27
SE	1.43	1.18	2.21	1.47	1.49	1.38	0.74	2.08	1.7	1.81	1.49	2.3

Table A51 (continued)

	Home cage						Response prevented					
	Sal Sal	LVP Sal	Sal Scop	LVF Scop	Sal Phy	LVP Phy	Sal Sal	LVP Sal	Sal Scop	LVP Scop	Sal Phy	LVP Phy
						Test 1: Long avoidances						
1	4	3	4	0	5	2	7	3	3	2	6	4
2	3	0	3	3	2	5	6	5	10	4	4	7
3	4	2	2	7	7	2	2	0	7	4	7	2
4	5	3	5	6	0	5	6	3	4	9	5	7
5	5	3	3	5	5	7	6	3	6	7	3	7
6	5	2	5	5	5	4	4	2	2	4	12	8
7	10	6	5	5	3	3	6	4	8	6	11	6
8	7	3	4	3	6	9	5	3	4	8	6	4
9	5	9	7	4	0	8	6	0	4	6	4	2
10	10	1	4	3	2	5	5	3	3	8	2	2
$\bar{x}$	5.8	3.2	4.2	4.1	3.5	5.0	5.3	2.6	5.1	5.8	6.0	4.9
SD	2.44	2.57	1.4	1.97	2.46	2.4	1.42	1.58	2.56	2.25	3.27	2.38
SE	0.77	0.81	0.44	0.62	0.78	0.76	0.45	0.5	0.81	0.71	1.03	0.75
						Test 2: Long avoidances						
1	6	10	5	6	3	5	6	4	9	2	9	0
2	2	5	7	5	9	5	8	6	2	9	3	2
3	5	5	12	5	6	5	4	11	5	5	8	1
4	3	4	3	6	4	6	4	2	4	6	2	5
5	4	6	4	6	4	2	4	3	4	5	4	8
6	1	0	5	11	8	6	5	4	4	8	4	3
7	3	2	2	9	9	10	5	7	0	7	5	2
8	4	3	7	13	6	6	1	0	3	7	2	3
9	3	4	3	5	4	5	1	2	3	8	1	3
10	1	1	3	2	1	3	0	1	3	4	3	2
$\bar{x}$	3.2	4.0	5.1	6.8	5.4	5.3	3.8	4.0	3.7	6.1	4.1	2.9
SD	1.62	2.83	2.96	3.26	2.67	2.11	2.49	3.27	2.31	2.13	2.6	2.23
SE	0.51	0.89	0.94	1.03	0.85	0.67	0.79	1.03	0.73	0.67	0.82	0.71

Table A52: Experiment Eleven. Analysis of extinction data

		ΣR_j^2	χ_r^2	P*
Total avoidances	Test 1	58020.5	56.3111	< 0.001
	Test 2	53645.5	22.6572	< 0.02
Short avoidances	Test 1	56022.0	40.938	< 0.001
	Test 2	52847.5	16.518	NS
Long avoidances	Test 1	53666.5	22.818	< 0.02
	Test 2	53841.0	24.16112	< 0.02

Multiple comparisons (Hollander and Wolfe 1973)

Groups	d	P
Test 1: Total avoidances		
HC Sal Scop > HC LVP Sal	52.0	0.06
HC Sal Scop > HC LVP Scop	56.5	< 0.03
HC Sal Scop > HC Sal Phy	73.0	< 0.01
HC Sal Scop > HC LVP Phy	54.0	< 0.047
RP Sal Sal > RP LVP Sal	56.5	< 0.03
RP Sal Scop > RP LVP Sal	52.0	0.06
RP LVP Scop > RP LVP Sal	50.5	0.085
RP Sal Phy > RP LVP Sal	68.0	< 0.01
RP Sal Phy > RP LVP Phy	61.0	< 0.01
HC Sal Sal > RP LVP Sal	57.0	< 0.03
HC Sal Sal > RP LVP Phy	50.0	[0.1]
HC Sal Scop > RP LVP Sal	88.5	< 0.01
HC Sal Scop > RP LVP Phy	81.5	< 0.01
HC Sal Phy < RP Sal Phy	52.5	0.05
Test 1: Short avoidances		
HC Sal Scop > HC Sal Phy	64.0	< 0.01
HC Sal Scop > HC LVP Phy	50.0	[0.1]
RP LVP Scop > RP LVP Phy	50.0	[0.1]
RP Sal Phy > RP LVP Phy	52.5	0.05
HC Sal Sal > RP LVP Phy	51.5	0.07
HC LVP Sal > RP LVP Phy	51.5	0.07
HC Sal Scop > RP LVP Sal	72.0	< 0.01
HC Sal Scop > RP Sal Scop	53.0	< 0.047
HC Sal Scop > RP LVP Phy	87.5	< 0.01

Table A52 (continued)

Groups	d	P
Test 1: Long avoidances		
RP LVP Sal < RP LVP Scop	49.0	0.1
Test 2: Total avoidances		
HC Sal Scop > RP Sal Sal	53.0	0.047
HC Sal Scop > RP LVP Sal	49.0	0.1
HC LVP Scop > RP Sal Sal	49.0	0.1
Test 2: Long avoidances		
HC LVP Scop > HC Sal Sal	45.0	0.1
RP LVP Scop > HC Sal Sal	45.0	0.1

* df = 11

Table A53: Experiment Eleven. Extinction data: Trend lines for total avoidances in Test 1

	Home cage						Response prevented					
	Sal Sal	Sal LVP	Sal Scop	LVP Scop	Sal Phy	LVP Phy	Sal Sal	Sal LVP	Sal Scop	LVP Scop	Sal Phy	LVP Phy
Correlation coefficient (r)	-0.39	-0.8	-0.37	-0.58	-0.81	-0.56	-0.96	-0.87	-0.85	-0.17	-0.42	-0.69
Slope (s)	-0.31	-1.32	-0.34	-0.96	-1.47	-0.53	-1.11	-1.31	-1.77	-0.17	-0.38	-1.25
Y' (x = 1)	25.8	28.6	29.0	26.4	27.6	25.13	29.33	25.2	31.33	25.06	27.0	25.2
Y' (x = 10)	22.71	15.33	25.6	16.76	12.87	19.8	18.18	12.12	13.63	23.3	23.18	12.65

Y' = predicted number of responses on trial blocks (x) 1 and 10

Table A54: Experiment Twelve. Analysis of acquisition data

		Avoidances	Escapes	Failures	Shocks
Saline	Σx	16	44	318	1661
	$\bar{x}$	4.0	11.0	79.5	415.25
	SD	2.449	16.186	28.5	118.7
	SE	1.224	8.093	14.25	59.35
LVP	Σx	37	90	258	1745
	$\bar{x}$	9.25	22.5	64.5	436.25
	SD	7.088	35.123	36.05	59.264
	SE	3.54	17.56	18.02	29.63
	df	6	6	6	6
	t*	1.4	0.595	0.653	0.316
	P	NS	NS	NS	NS

* t test for independent samples (Winer 1962)

Table A55: Experiment Twelve. Lever press responses made by subjects during Periods A and B of Suppression Tests 1 and 2

	Period A										Period B									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Test 1: Saline group																				
Σx	33	18	10	12	11	9	23	30	30	35	3	10	12	7	11	12	25	35	33	20
$\bar{x}$	8.25	4.5	2.5	3.0	2.75	2.25	5.75	7.5	7.5	8.75	0.75	2.5	3.0	1.75	2.75	3.0	6.25	8.75	9.25	5.0
SD	5.73	5.44	5.0	3.56	3.2	3.86	3.77	1.0	1.29	0.5	1.5	5.0	6.0	3.5	3.77	2.45	3.68	4.03	4.35	2.16
SE	2.86	2.72	2.5	1.78	1.6	1.93	1.88	0.5	0.64	0.25	0.75	2.5	3.0	1.75	1.88	1.22	1.84	2.01	2.17	1.08
Test 2: Saline group																				
Σx	22	21	36	35	32	27	37	41	31	29	12	22	25	38	34	48	38	47	49	45
$\bar{x}$	5.5	5.25	9.0	8.75	8.0	6.75	9.25	10.25	7.75	7.25	3.0	5.5	6.25	9.5	8.5	12.0	9.5	11.75	12.25	11.25
SD	3.78	4.5	4.89	4.57	0.81	3.3	3.59	3.59	4.57	3.3	4.24	5.4	1.89	1.0	2.08	3.16	4.79	3.3	1.25	3.86
SE	1.89	2.25	2.45	2.28	0.41	1.65	1.79	1.79	2.28	1.65	2.12	2.7	0.94	0.5	1.04	1.58	2.39	1.65	0.63	1.93
Test 1: LVP group																				
Σx	37	14	15	29	33	30	29	25	25	27	5	12	15	22	31	29	32	35	28	25
$\bar{x}$	9.25	3.5	3.75	7.25	8.25	7.5	7.25	6.25	6.25	6.75	1.25	3.0	3.75	5.5	7.75	7.25	8.0	8.75	7.0	6.25
SD	5.62	5.06	4.5	5.18	5.73	5.97	4.85	4.19	4.19	4.57	2.5	4.69	4.78	4.51	6.39	5.12	5.41	6.18	4.69	4.19
SE	2.81	2.53	2.25	2.59	2.86	2.98	2.43	2.09	2.09	2.28	1.25	2.34	2.39	2.25	3.19	2.56	2.71	3.09	2.34	2.09
Test 2: LVP group																				
Σx	34	26	33	38	29	33	27	28	39	30	22	28	29	41	47	34	35	47	47	49
$\bar{x}$	8.5	6.5	8.25	9.5	7.25	8.25	6.75	7.0	9.75	7.5	5.5	7.0	7.25	10.25	11.75	8.5	8.75	11.75	11.75	12.25
SD	4.12	4.36	5.5	6.55	4.99	5.9	4.71	3.46	2.06	5.44	2.08	4.96	5.25	5.37	4.03	5.56	6.34	2.06	2.06	4.35
SE	2.06	2.18	2.75	3.27	2.49	2.95	2.35	1.73	1.03	2.72	1.04	2.48	2.62	2.68	2.01	2.78	3.17	1.03	1.03	2.17

Table A55 (continued): Experiment Twelve. Suppression ratios during Tests 1 and 2

	Trials									
	1	2	3	4	5	6	7	8	9	10
Test 1: Saline group										
Σx	0.214	0.476	0.545	0.5	0.946	2.357	2.052	2.044	1.948	1.47
$\bar{x}$	0.053	0.119	0.136	0.125	0.236	0.589	0.513	0.511	0.487	0.367
SD	0.107	0.238	0.272	0.25	0.284	0.401	0.147	0.145	0.177	0.119
SE	0.053	0.119	0.136	0.125	0.142	0.201	0.073	0.072	0.088	0.059
Test 2: Saline group										
Σx	0.802	2.439	1.757	2.221	2.04	2.582	2.016	2.161	2.558	2.473
$\bar{x}$	0.2	0.609	0.439	0.555	0.51	0.645	0.504	0.54	0.639	0.618
SD	0.254	0.336	0.125	0.179	0.049	0.149	0.131	0.044	0.149	0.036
SE	0.127	0.168	0.063	0.089	0.024	0.074	0.065	0.022	0.075	0.018
Test 1: LVP group										
Σx	0.25	1.976	0.982	1.27	1.355	1.505	1.571	1.736	1.585	3.333
$\bar{x}$	0.063	0.494	0.245	0.317	0.338	0.376	0.392	0.434	0.396	0.833
SD	0.125	0.41	0.303	0.22	0.246	0.28	0.262	0.292	0.265	0.786
SE	0.063	0.204	0.152	0.11	0.123	0.14	0.131	0.146	0.132	0.393
Test 2: LVP group										
Σx	1.644	1.542	1.387	2.46	2.755	2.493	3.687	2.555	2.186	2.717
$\bar{x}$	0.411	0.385	0.346	0.615	0.688	0.623	0.921	0.638	0.546	0.679
SD	0.088	0.263	0.237	0.273	0.207	0.263	0.719	0.091	0.094	0.241
SE	0.044	0.131	0.118	0.137	0.104	0.131	0.359	0.045	0.047	0.12

Table A56: Analysis of variance for Periods A and B and suppression ratios

Source	SS	df	MS	F	P
Period A: Test 1; trials 1-5					
Between groups	12.1	1	12.1	3.5	NS
Trials	36.79	4	9.19	2.66	NS
Error	13.84	4	3.46		
Total	62.73				
Period A: Test 1; trials 6-10					
Between groups	0.51	1	0.51	0.11	NS
Trials	8.91	4	2.227	0.5	NS
Error	17.96	4	4.49		
Total	27.38				
Period A: Test 2; trials 1-5					
Between groups	1.23	1	1.23	1.0	NS
Trials	13.4	4	3.35	2.73	NS
Error	4.9	4	1.225		
Total	19.53				
Period A: Test 2; trials 6-10					
Between groups	0.4	1	0.4	0.14	NS
Trials	3.16	4	0.79	0.28	NS
Error	11.16	4	2.79		
Total	14.73				
Period B: Test 1; trials 1-5					
Between groups	11.03	1	11.03	4.88	< 0.1
Trials	18.91	4	4.727	2.09	NS
Error	9.04	4	2.26		
Total	38.98				
Period B: Test 1; trials 6-10					
Between groups	3.6	1	3.6	1.69	NS
Trials	17.47	4	4.367	2.05	NS
Error	8.5	4	2.125		
Total	29.58				

Table A56 (continued)

Source	SS	df	MS	F	P
Period B: Test 2; trials 1-5					
Between groups	8.1	1	8.1	14.64	< 0.05
Trials	50.41	4	12.6	22.79	< 0.01
Error	2.21	4	0.552		
Total	60.73				
Period B: Test 2; trials 6-10					
Between groups	1.41	1	1.41	1.0	NS
Trials	12.4	4	3.1	2.2	NS
Error	5.63	4	1.407		
Total	19.43				
Suppression Ratios: Test 1; trials 1-5					
Between groups	0.06	1	0.06	6.56	< 0.1
Trials	0.08	4	0.02	2.05	NS
Error	0.04	4	0.01		
Total	0.18				
Suppression Ratios: Test 1; trials 6-10					
Between groups	0.000129	1	0.000129	0.00356	NS
Trials	0.03	4	0.075	0.22	NS
Error	0.15	4	0.03		
Total	0.18				
Suppression Ratios: Test 2; trials 1-5					
Between groups	0.00174	1	0.00174	0.1	NS
Trials	0.13	4	0.0325	1.88	NS
Error	0.07	4	0.0175		
Total	0.2				
Suppression Ratios: Test 2; trials 6-10					
Between groups	0.02	1	0.02	1.11	NS
Trials	0.02	4	0.005	0.26	NS
Error	0.08	4	0.02		
Total	0.12				

Table A57: Experiment Twelve. Extinction performance for each subject

	Saline			LVP		
	Total responses	Short avoidances	Long avoidances	Total responses	Short avoidances	Long avoidances
			Test 1*			
$\bar{x}$	20.0	12.75	7.25	27.0	20.75	6.25
SD	16.431	7.141	5.678	23.36	20.139	4.193
SE	8.215	3.57	2.839	11.68	10.069	2.096
			Test 2*			
$\bar{x}$	17.5	12.75	4.75	26.0	14.5	11.5
SD	20.24	19.05	6.601	12.987	10.63	5.446
SE	10.12	9.525	3.3	6.493	5.31	2.723

* n = 4

Table A57 (continued): Experiment Twelve. Extinction performance as a function of extinction trials

	Total responses		Short avoidances		Long avoidances					
	Sal	LVP	Sal	LVP	Sal	LVP				
			Test 1					T**	N	P*
1	6	9	5	7	1	2				
2	4	11	2	9	2	2				
3	6	12	4	8	2	4	Total responses	4.5	9	< 0.02
4	7	11	6	9	1	2				
5	6	10	4	8	2	2	Short avoidances	1.0	9	< 0.01
6	11	11	7	9	4	2				
7	9	11	4	8	5	3	Long avoidances	19.0	7	NS
8	6	12	2	9	4	3				
9	12	11	9	8	3	3				
10	13	10	8	8	5	2				
			Test 2							
1	6	14	5	8	1	6				
2	10	12	6	8	4	5				
3	7	9	6	4	1	5	Total responses	6.5	10	< 0.05
4	6	15	4	9	2	6				
5	6	9	5	5	1	4	Short avoidances	17.5	9	NS
6	8	9	5	7	3	2				
7	7	11	6	5	1	6	Long avoidances	1.5	9	< 0.01
8	8	10	6	8	2	2				
9	8	5	6	1	2	4				
10	7	9	5	3	2	6				

** Wilcoxon signed ranks test (Seigel 1956)

* P = two tailed

Table A58: Experiment Thirteen. Acquisition data

	Sal	2 µg	3 µg	4 µg
		Avoidances		
Σx	42	82	54	46
$\bar{x}$	7.0	16.4	10.8	7.66
SD	2.828	21.836	8.93	7.06
SE	1.1547	9.765	3.99	2.88
		Escapes		
Σx	94	56	22	109
$\bar{x}$	15.66	11.2	4.4	18.16
SD	12.42	6.79	4.39	21.36
SE	5.07	3.04	1.96	8.72
		Failures		
Σx	461	363	423	430
$\bar{x}$	76.83	72.6	84.6	71.66
SD	12.73	21.915	13.01	31.66
SE	5.198	9.801	5.818	12.92
		Shocks		
Σx	2451	1889	2170	2420
$\bar{x}$	408.5	377.8	434.0	403.33
SD	47.89	106.95	56.414	106.18
SE	19.55	47.83	25.23	43.35

Table A58 (continued): Experiment Thirteen. Analysis of variance on acquisition data

	Source	SS	df	MS	F	P
	Between groups	293.93	3	97.9798	0.7012	NS
Avoidances	Within groups	2515.33	18	139.7407		
	Total	2809.27	21			
	Between groups	587.697	3	195.899	1.064	NS
Escapes	Within groups	3314.1667	18	184.1204		
	Total	3901.8636	21			
	Between groups	543.297	3	181.099	0.3871	NS
Failures	Within groups	8420.5667	18	467.8093		
	Total	8963.86	21			
	Between groups	7976.1849	3	2658.7283	0.3788	NS
Shocks	Within groups	126331.633	18	7018.4241		
	Total	134307.0	21			

Table A59: Experiment Thirteen. Extinction data

	Total avoidances				Short avoidances				Long avoidances			
	Sal	2 µg	3 µg	4 µg	Sal	2 µg	3 µg	4 µg	Sal	2 µg	3 µg	4 µg
						Test 1						
S1	50	22	41	46	33	13	31	39	18	9	10	7
S2	30	38	19	15	16	30	15	7	14	8	4	8
S3	24	20	49	40	15	13	44	31	9	7	5	9
S4	27	35	27	30	16	25	21	23	11	10	6	7
S5	32	15	30	23	21	10	13	15	11	5	17	10
S6	25			35	13			29	12			6
Σx	188	130	166	189	114	91	124	144				
$\bar{x}$	31.33	26.0	33.2	31.5	19.0	18.2	24.8	24.0	12.5	7.8	8.4	7.83
SD	9.626	9.975	11.84	11.327	7.348	8.757	12.814	11.576	3.146	1.923	5.319	1.472
SE	3.929	4.461	5.29	4.624	3.0	3.916	5.73	4.725	1.284	0.86	2.379	0.6
						Test 2						
S1	35	36	39	45	27	16	29	40	8	20	10	5
S2	37	32	8	6	25	21	7	1	12	11	1	5
S3	7	20	18	37	3	16	11	27	4	4	7	10
S4	32	44	24	30	23	34	17	13	9	10	7	17
S5	21	33	20	17	9	23	12	18	12	10	8	5
S6	38				25				13			
Σx	170	165	195	135	112	110	76	99	58	55	33	42
$\bar{x}$	28.33	33.0	21.8	27.0	18.66	22.0	15.2	19.8	9.66	11.0	6.6	8.4
SD	12.127	8.66	11.278	15.6	10.07	7.38	8.497	14.686	3.386	5.74	3.36	5.272
SE	4.95	3.87	5.044	6.978	4.112	3.3	3.8	6.568	1.382	2.56	1.503	2.358

Table A59 (continued): Experiment Thirteen. Extinction data: Mean number of responses/subject on each trial block

	Total avoidances				Short avoidances				Long avoidances			
	Sal	2 µg	3 µg	4 µg	Sal	2 µg	3 µg	4 µg	Sal	2 µg	3 µg	4 µg
						Test 1						
1	0.999	1.0	2.6	1.830	0.666	0.6	2.4	1.5	0.333	0.4	0.2	0.33
2	2.333	1.8	3.6	2.49	1.5	1.0	2.6	2.16	0.833	0.8	1.0	0.33
3	3.666	3.0	3.8	2.66	1.5	2.2	2.8	1.16	2.166	0.8	1.0	1.5
4	2.99	3.0	3.0	3.0	1.66	1.8	2.8	2.0	1.33	1.2	0.2	1.0
5	3.666	2.8	3.6	3.17	2.5	2.2	2.6	2.5	1.166	0.6	1.0	0.666
6	3.166	2.6	3.8	3.166	1.5	1.8	2.6	2.166	1.666	0.8	1.2	1.0
7	3.33	3.2	2.4	4.0	2.5	2.2	1.2	2.833	0.83	1.0	1.2	1.166
8	3.5	2.2	4.0	4.0	2.5	2.0	3.0	3.0	1.0	0.2	1.0	1.0
9	3.49	2.8	3.0	4.17	2.83	1.8	2.6	3.833	0.66	1.0	0.4	0.333
10	2.666	3.6	3.4	3.333	1.833	2.6	2.2	2.833	0.833	1.0	1.2	0.5
$\bar{x}$	2.98	2.6	3.32	3.182	1.899	1.82	2.48	2.398	1.0817	0.78	0.84	0.782
SD	0.82	0.754	0.543	0.741	0.667	0.599	0.5	0.776	0.528	0.304	0.408	0.409
SE	0.26	0.238	0.172	0.234	0.211	0.189	0.158	0.245	0.167	0.096	0.129	0.129
						Test 2						
1	3.166	3.0	4.0	3.6	1.666	2.0	2.8	2.8	1.5	1.0	1.2	0.8
2	3.326	3.2	2.8	3.6	2.666	1.4	2.2	2.2	0.66	1.8	0.6	1.4
3	2.833	3.6	3.8	3.2	1.333	2.6	2.6	2.0	1.5	1.0	1.2	1.2
4	2.993	3.0	2.0	2.8	1.833	1.6	1.0	1.8	1.16	1.4	1.0	1.0
5	3.0	4.8	1.75	3.2	2.0	3.8	1.5	2.2	1.0	1.0	0.25	1.0
6	3.16	3.4	2.0	3.2	2.0	2.6	1.5	1.8	1.16	0.8	0.5	1.4
7	3.16	3.2	1.0	2.8	1.83	2.0	0.75	1.8	1.33	1.2	0.25	1.0
8	2.5	4.4	1.8	2.2	2.0	3.6	1.4	2.0	0.5	0.8	0.4	0.2
9	2.5	2.0	2.2	2.0	2.0	1.6	1.4	1.8	0.5	0.4	0.8	0.2
10	1.66	2.8	1.8	1.6	1.33	1.2	1.0	1.4	0.33	1.6	0.8	0.2
$\bar{x}$	2.829	3.34	2.315	2.82	1.866	2.24	1.615	1.98	0.964	1.1	0.7	0.84
SD	0.496	0.794	0.946	0.683	0.384	0.898	0.694	0.37	0.436	0.4137	0.358	0.4789
SE	0.157	0.251	0.299	0.215	0.121	0.284	0.219	0.117	0.138	0.131	0.113	0.1514

Table A59 (continued): Experiment Thirteen. Outcomes of Freidman's analyses of variance on extinction data

		ΣR_j^2	χ_r^2	P
Total avoidances	Test 1	2558.5	3.51	NS
	Test 2	2546.5	2.79	NS
Short avoidances	Test 1	2686.5	11.19	< 0.02
	Test 2	2597.0	5.82	NS
Long avoidances	Test 1	2563.5	3.81	NS
	Test 2	2602.0	6.12	NS

Table A60: Experiment Fourteen. Acquisition performance

	Sal Sal	LVP Sal	Sal Scop	LVP Scop	Sal Phy	LVP Phy
			Avoidances			
$\bar{x}$	8.66	15.66	15.5	19.33	29.75	3.33
SD	11.64	12.801	16.28	26.23	17.29	3.44
SE	4.75	5.23	6.647	10.71	8.64	1.41
			Escapes			
$\bar{x}$	14.33	28.0	17.16	9.5	28.0	4.5
SD	13.31	32.93	18.84	9.61	17.34	4.59
SE	5.43	13.44	7.69	3.92	8.67	1.87
			Failures			
$\bar{x}$	77.66	56.5	67.5	67.66	41.5	82.0
SD	21.31	36.2	25.28	38.18	22.52	20.57
SE	8.29	14.78	10.32	15.58	11.26	8.39
			Shocks			
$\bar{x}$	407.83	330.16	365.33	355.66	256.25	470.33
SD	82.35	139.63	108.73	178.91	98.73	18.24
SE	33.62	57.0	44.39	73.04	49.36	7.45

Table A60 (continued): Acquisition data: Analysis of extinction performance

	Source	SS	df	MS	F	P
	Between groups	2036.89	5	407.38	1.58	NS
Avoidances	Within groups	7219.58	28	257.84		
	Total	9256.47	33			
	Between groups	2509.45	5	501.89	1.47	NS
Escapes	Within groups	9551.17	28	341.11		
	Total	12060.62	33			
	Between groups	5298.86	5	1059.77	1.3	NS
Failures	Within groups	22740.67	28	812.17		
	Total	28039.53	33			
	Between groups	131615.61	5	26323.12	1.93	NS
Shocks	Within groups	381466.42	28	13623.8		
	Total	513082.03	33			

Table A61: Experiment Fourteen. Extinction data: Responses in extinction

	Test 1						Test 2					
	Sal Sal	LVP Sal	Sal Scop	LVP Scop	Sal Phy	LVP Phy	Sal Sal	LVP Sal	Sal Scop	LVP Scop	Sal Phy	LVP Phy
Total avoidances												
$\bar{x}$	20.0	24.33	24.0	36.83	30.5	30.83	25.5	33.83	30.0	36.5	38.0	36.83
SD	8.55	7.03	13.87	11.46	11.15	10.34	9.31	13.01	11.45	12.69	8.48	7.65
SE	3.49	2.87	5.66	4.67	5.57	4.22	3.8	5.31	4.67	5.18	4.24	3.12
Short avoidances												
$\bar{x}$	10.33	14.0	18.83	26.66	22.0	22.0	15.16	25.66	22.0	28.33	30.25	27.66
SD	6.62	5.73	13.04	10.27	7.35	8.15	5.77	11.34	12.13	10.29	10.24	6.89
SE	2.7	2.34	5.32	4.19	3.67	3.33	2.36	4.63	4.95	4.2	5.12	2.81
Long avoidances												
$\bar{x}$	9.66	10.33	5.16	10.16	8.5	8.83	10.33	8.16	8.0	8.16	7.75	9.16
SD	3.56	3.01	2.64	3.12	4.12	3.12	5.04	3.66	4.29	3.97	4.11	4.02
SE	1.45	1.23	1.07	1.27	2.06	1.27	2.06	1.49	1.75	1.62	2.06	1.64

Table A62: Experiment Fourteen. Extinction data: Responses per block of five trials

	Test 1						Test 2					
	Sal Sal	LVP Sal	Sal Scop	LVP Scop	Sal Phy	LVP Phy	Sal Sal	LVP Sal	Sal Scop	LVP Scop	Sal Phy	LVP Phy
						Total avoidances						
1	0.833	1.999	2.5	2.999	3.0	1.166	2.832	3.832	3.666	4.666	5.25	3.666
2	2.466	3.333	3.666	3.996	3.0	3.333	3.666	3.5	4.166	4.166	4.25	4.666
3	1.499	2.993	2.333	3.999	3.5	2.666	2.499	4.166	4.332	4.332	4.0	3.666
4	2.5	2.833	3.666	4.333	3.0	3.666	4.166	3.666	3.8	4.332	4.5	4.833
5	2.3	1.666	3.166	4.333	3.5	2.999	2.832	3.166	3.499	4.499	4.5	3.832
6	2.332	2.332	2.499	3.833	3.5	3.666	3.333	3.933	2.832	3.833	5.0	3.666
7	2.166	2.999	1.666	3.833	2.5	4.166	2.2	3.332	2.833	3.666	2.5	3.499
8	2.666	2.499	1.666	3.833	2.75	3.499	1.0	2.999	1.666	1.999	2.5	3.999
9	2.166	2.333	1.332	3.332	3.0	3.0	1.996	2.833	2.0	2.833	2.5	2.832
10	1.0	1.332	1.5	2.333	2.75	2.666	1.499	2.499	1.833	2.166	3.0	2.666
$\bar{x}$	1.993	2.432	2.399	3.682	3.05	3.083	2.602	3.383	3.063	3.649	3.8	3.732
SD	0.648	0.634	0.872	0.626	0.349	0.825	0.974	0.515	0.979	0.976	1.079	0.681
SE	0.205	0.2	0.276	0.178	0.111	0.261	0.308	0.163	0.309	0.309	0.341	0.215
						Short avoidances						
1	0.5	0.833	2.0	1.833	2.25	1.0	1.666	2.666	2.833	3.833	4.25	3.0
2	1.3	1.833	2.5	2.166	1.75	2.5	1.666	3.0	2.666	3.333	3.5	3.0
3	0.666	2.16	1.833	2.333	3.0	2.0	1.833	3.0	3.166	3.166	3.5	2.555
4	1.0	1.833	3.0	3.5	1.75	2.833	2.5	2.666	2.2	3.666	4.25	4.0
5	1.3	0.666	2.333	3.833	2.25	1.666	1.666	2.833	2.166	3.166	3.75	3.166
6	1.166	1.166	1.833	3.0	2.75	2.5	2.333	2.5	2.666	3.0	4.5	2.666
7	1.0	1.666	1.666	3.5	1.75	2.666	1.0	2.166	2.5	2.666	2.5	2.666
8	1.666	1.166	1.5	2.5	2.25	2.833	0.6	2.666	1.166	1.666	0.75	2.833
9	1.166	1.5	1.166	2.166	2.5	2.0	1.33	1.833	1.5	2.0	1.5	2.166
10	0.5	1.166	1.0	1.833	1.75	2.0	0.833	2.333	1.5	1.833	1.75	1.5
$\bar{x}$	1.026	1.399	1.883	2.666	2.2	2.199	1.543	2.566	2.236	2.833	3.025	2.766
SD	0.377	0.478	0.609	0.737	0.453	0.581	0.615	0.37	0.658	0.766	1.315	0.649
SE	0.119	0.151	0.193	0.233	0.143	0.184	0.194	0.117	0.208	0.242	0.416	0.205
						Long avoidances						
1	0.333	1.166	0.5	1.166	0.75	0.166	1.166	1.166	0.833	0.833	1.0	0.666
2	1.166	1.5	1.166	1.83	2.15	0.833	2.0	0.5	1.5	0.833	0.75	1.666
3	0.833	0.833	0.5	1.666	0.5	0.666	0.666	1.166	1.166	1.166	0.5	1.0
4	1.5	1.0	0.666	0.833	1.25	0.833	1.666	1.0	1.6	0.666	0.25	0.833
5	1.0	1.0	0.833	0.5	1.25	1.333	1.166	0.333	1.333	1.333	0.75	0.666
6	1.166	1.166	0.666	0.833	0.75	1.166	1.0	1.333	0.166	0.833	0.5	1.0
7	1.166	1.333	0	0.333	0.75	1.5	1.2	1.166	0.333	1.0	0	0.833
8	1.0	1.333	0.166	1.333	0.5	0.666	0.4	0.333	0.5	0.333	1.75	1.166
9	1.0	0.833	0.166	1.166	0.5	1.0	0.666	1.0	0.5	0.833	1.0	0.666
10	0.5	0.166	0.5	0.5	1.0	0.666	0.666	0.166	0.333	0.333	1.25	1.166
$\bar{x}$	0.966	1.033	0.516	1.016	0.85	0.883	1.059	0.816	0.826	0.816	0.775	0.966
SD	0.341	0.375	0.346	0.505	0.316	0.385	0.494	0.433	0.533	0.319	0.506	0.312
SE	0.107	0.119	0.109	0.159	0.1	0.122	0.156	0.137	0.168	0.1	0.16	0.988

Table A62 (continued): Experiment Fourteen. Analysis of extinction data: Outcomes of Freidman's two way analyses of variance

		ΣR_j^2	χ_r^2	P
Total avoidances	Test 1	8330.0	27.988	< 0.001
	Test 2	8255.5	25.859	< 0.001
Short avoidances	Test 1	8339.5	28.252	< 0.001
	Test 2	8346.25	28.452	< 0.001
Long avoidances	Test 1	7757.5	11.631	< 0.05
	Test 2	7432.0	2.332	NS

Table A63: Experiment Fourteen. Multiple comparisons tests on extinction data (Hollander and Wolfe 1973)

	Groups	d	P
	Test 1		
Total avoidances	Sal Sal < LVP Scop	41.5	< 0.009
	Sal Sal < Sal Phy	24.5	< 0.047
	Sal Sal < LVP Phy	27.0	< 0.023
	LVP Sal < LVP Scop	26.5	< 0.023
	Sal Scop < LVP Scop	26.5	< 0.023
Short avoidances	Sal Sal < LVP Scop	38.0	< 0.009
	Sal Sal < Sal Phy	29.0	< 0.009
	Sal Sal < LVP Phy	29.5	< 0.009
	LVP Sal < LVP Scop	28.0	0.009
Long avoidances	Sal Sal > Sal Scop	22.0	[0.09]
	LVP Sal > Sal Scop	21.5	[0.1]
	LVP Scop > Sal Scop	22.0	[0.09]
	LVP Phy > Sal Scop	23.0	[0.06]
	Test 2		
Total avoidances	Sal Sal < LVP Sal	29.5	< 0.009
	Sal Sal < Sal Phy	34.0	< 0.009
	Sal Sal < LVP Phy	30.5	< 0.009
	Sal Scop < Sal Phy	24.5	< 0.047
Short avoidances	Sal Sal < LVP Sal	22.5	[0.075]
	Sal Sal < LVP Scop	36.0	< 0.009
	Sal Sal < Sal Phy	37.0	< 0.009
	Sal Sal < LVP Phy	31.5	< 0.009

Reject H_0 where $d \geq r(\alpha,k,n)$, $k = 6$, $n = 10$ (Table A17, Hollander and Wolfe 1973)

Critical values of r; 24, $\alpha = 0.047$; 26, $\alpha = 0.023$; 28, $\alpha = 0.009$

Table A64: Experiment Fifteen. Lever press data: Total number of responses per cell

Dose (µg per rat)	Days 1	2	3	4	5	6	Σx	$\bar{x}$	SE
	S1	S5	S4	S2	S3	S6			
Sal	1120	1540	1640	1620	1840	1720	9480.0	1580.0	100.94
	S2	S1	S3	S4	S6	S5			
0.05 µg	1480	1840	1640	1740	1700	1920	10320.0	1720.0	63.05
	S3	S2	S1	S6	S5	S4			
1 µg	1600	1540	1100	1700	1600	1960	9500.0	1583.3	114.26
	S4	S6	S5	S1	S2	S3			
2 µg	960	1380	1380	1520	1800	2000	9040.0	1506.6	148.4
	S5	S4	S6	S3	S1	S2			
3 µg	1000	1360	1380	940	1260	460	6400.0	1066.6	142.6
	S6	S3	S2	S5	S4	S1			
4 µg	1080	1780	1500	1400	2000	960	8720.0	1453.3	162.6
Σx	7239.9	9440.0	8640.0	8920.0	10200.0	9020.0			
$\bar{x}$	1206.66	1573.33	1440.0	1486.66	1700.0	1503.33			
SE	109.0	81.45	82.96	120.43	103.79	262.0			

Table A65: Experiment Fifteen. Summary of analysis of variance on the number of lever responses per cell (Kirk 1968)

Source	SS	df	MS	F	PP
Dose	7.086	5	1.417	3.311	< 0.05
Days	3.786	5	0.7572	1.769	NS
Subjects	2.236	5	0.4472	1.045	NS
Residual	8.558	20	0.4279		
Total	21.666	35			

Newman Keuls pairwise comparisons to locate significant differences between doses (Kirk 1968)

Ordered means (mean number of responses per dose)

	Dose (µg)					
	3	4	2	Sal	0.5	1
$\bar{x}$	2.315	3.153	3.273	3.43	3.436	3.735
	Differences (D)					
3	-	0.838*	0.928*	1.115*	1.121*	1.42*
4		-	0.12	0.277	0.283	0.582
2			-	0.157	0.163	0.462
Sal				-	0.006	0.305
0.05					-	0.299
1						-

Critical values of w

$w_6 = 1.188$; $w_5 = 1.129$; $w_4 = 1.057$; $w_3 = 0.956$; $w_2 = 0.787$

When $D \geq W_R$ reject H_0 at α (0.05)

* $p \leq 0.05$

Table A66: Experiment Fifteen. Lever press data : 50% interquartile range per cell (50% IQR)

Dose (µg/0.5 ml)	Days 1	2	3	4	5	6	Σx	$\bar{x}$	SE
	S1	S5	S4	S2	S3	S6			
Sal	4.775	4.918	4.266	4.234	3.745	3.517	25.455	4.242	0.225
	S2	S1	S3	S4	S6	S5			
0.05 µg	5.353	3.871	4.11	3.832	3.128	4.135	24.43	4.071	0.296
	S3	S2	S1	S6	S5	S4			
1 µg	5.73	4.845	4.75	3.776	4.617	4.077	27.79	4.632	0.277
	S4	S6	S5	S1	S2	S3			
2 µg	4.758	4.86	6.322	3.949	3.48	3.927	27.29	4.549	0.415
	S5	S4	S6	S3	S1	S2			
3 µg	6.923	5.166	4.838	3.336	6.025	3.773	30.06	5.01	0.55
	S6	S3	S2	S5	S4	S1			
4 µg	7.652	4.52	5.641	5.102	4.205	7.531	34.65	5.775	0.608
Σx	35.191	28.18	29.927	24.229	25.2	26.96			
$\bar{x}$	5.865	4.696	4.987	4.038	4.2	4.49			
SE	0.483	0.185	0.345	0.243	0.423	0.614			

Table A67: Experiment Fifteen. Summary of analysis of variance on 50% interquartile range data (50% IQR) (Kirk 1968)

Source	SS	df	MS	F	P
Dose	11.298	5	2.2596	3.457	< 0.05
Days	13.021	5	2.6042	3.984	< 0.05
Subjects	5.588	5	1.1176	1.709	NS
Residual	13.073	20	0.6536		
Total	42.98	35			

Newman Keuls pairwise comparisons to locate significant differences between doses and days (Kirk 1968)

		Doses (ordered means)					
		0.5	Sal	2	1	3	4
		4.0715	4.2425	4.5493	4.6325	5.0101	5.7751
		Differences (D)					
Doses	0.5	-	0.171	0.4778	0.561	0.9386	1.7036*
	Sal		-	0.3068	0.39	0.7676	1.5326*
	2			-	0.083	0.4608	1.2258*
	1				-	0.3776	1.1426*
	3					-	0.765
	4						-

Critical values of w ($\alpha = 0.05$)

$w_6 = 1.468$; $w_5 = 1.396$; $w_4 = 1.306$; $w_3 = 1.181$; $w_2 = 0.973$

		Days (ordered means)					
		4	5	6	2	3	1
		4.038	4.2	4.493	4.697	4.987	5.865
		Differences (D)					
Days	4	-	0.162	0.455	0.659	0.949	1.827**
	5		-	0.293	0.497	0.787	1.665*
	6			-	0.204	0.494	1.372*
	2				-	0.29	1.168
	3					-	0.878
	1						-

Critical values of w ($\alpha = 0.01$)

$w_6 = 1.818$; $w_5 = 1.745$; $w_4 = 1.656$; $w_3 = 1.531$; $w_2 = 1.326$

Table A68: Experiment Sixteen. Summary of lever press response data for DRL schedule

	Dose								
	Sal	0.5	1	2	3	4	$\bar{x}$	SD	SE
(1) Total responses									
S1	196	242	-	149	180	192	191.8	33.57	15.01
S2	189	162	-	151	173	243	183.6	36.05	16.12
S3	167	245	149	181	199	170	185.16	33.63	13.73
S4	201	210	164	-	157	143	175.0	29.02	12.98
S5	215	163	179	229	193	250	204.83	32.51	13.27
S6	168	202	181	185	222	163	186.83	22.03	8.99
$\bar{x}$	189.3	204.0	168.25	179.0	187.3	193.5			
SD	18.93	36.36	14.9	32.49	22.6	43.99			
SE	7.73	14.84	7.45	14.53	9.22	17.96			
(2) Rapid responses (latency ≤ 1 sec)									
S1	15	13	-	2	3	1	6.8	6.64	2.97
S2	17	7	-	9	25	15	14.6	7.12	3.18
S3	3	12	3	6	12	4	6.66	4.27	1.74
S4	17	15	3	-	3	4	8.4	6.98	3.12
S5	31	7	5	25	37	33	23.0	13.74	5.6
S6	0	4	2	3	7	1	2.83	2.48	1.01
$\bar{x}$	13.83	9.66	3.25	9.0	14.5	9.66			
SD	11.17	4.27	1.26	9.35	13.73	12.54			
SE	4.56	1.74	0.63	4.18	5.61	5.12			
(3) Long responses (latency > 29 secs)									
S1	3	9	-	15	2	1	6.0	5.91	2.64
S2	6	3	-	1	3	9	4.4	3.13	1.4
S3	0	3	1	0	3	3	1.66	1.5	0.61
S4	5	4	0	-	2	0	2.2	2.28	1.02
S5	4	0	3	2	3	1	2.16	1.47	0.6
S6	1	0	1	5	4	5	2.66	2.25	0.91
$\bar{x}$	3.16	3.16	1.25	4.6	2.83	3.16			
SD	2.31	3.31	1.258	6.1	0.75	3.37			
SE	0.94	1.35	0.63	2.73	0.31	1.37			
(4) Total false alarms (latency 2 ≥ s < 11.8)									
S1	83	172	-	85	78	114	106.4	39.3	17.56
S2	75	40	-	18	71	159	72.6	53.65	23.99
S3	46	145	33	49	65	66	67.3	40.0	16.33
S4	67	83	42	-	58	25	55.0	22.39	10.01
S5	70	31	53	132	63	133	80.33	42.5	17.35
S6	41	118	91	85	154	52	90.16	41.83	17.07
$\bar{x}$	63.66	98.16	54.75	73.8	81.5	91.5	78.63		
SD	16.6	56.85	25.51	42.9	36.18	51.8	18.05		
SE	6.78	23.21	12.75	19.19	14.77	21.14	7.37		

Table A68 (continued)

	Dose								
	Sal	0.5	1	2	3	4	$\bar{x}$	SD	SE
(5) Probability of a false alarm									
S1	0.458	0.75	-	0.578	0.441	0.596	0.564	0.124	0.055
S2	0.436	0.258	-	0.127	0.527	0.697	0.409	0.233	0.1
S3	0.28	0.622	0.228	0.28	0.347	0.397	0.358	0.142	0.058
S4	0.364	0.425	0.26	-	0.376	0.179	0.321	0.099	0.044
S5	0.38	0.198	0.304	0.647	0.404	0.613	0.424	0.175	0.071
S6	0.244	0.598	0.508	0.467	0.716	0.322	0.475	0.173	0.071
$\bar{x}$	0.36	0.475	0.324	0.419	0.468	0.467			
SD	0.084	0.218	0.126	0.214	0.136	0.199			
SE	0.345	0.089	0.063	0.096	0.055	0.081			
(6) Total hits (latency > 12 secs)									
S1	95	48	-	44	92	76	71.0	23.97	10.72
S2	87	105	-	115	69	54	86.0	25.07	11.21
S3	118	83	111	125	114	94	107.5	15.83	6.46
S4	104	107	114	-	86	106	103.4	10.43	4.66
S5	107	120	117	70	90	82	97.66	20.14	8.22
S6	125	79	85	94	58	104	90.83	22.81	9.31
$\bar{x}$	106.0	90.33	106.75	89.6	84.83	86.0	92.64		
SD	14.08	25.87	14.7	33.09	19.49	19.63	13.26		
SE	5.75	10.56	7.35	14.8	7.96	8.01	5.4156		
(7) Probability of a hit									
S1	0.969	0.842	-	0.709	0.911	0.987	0.884	0.112	0.05
S2	0.897	0.800	-	0.927	0.896	0.783	0.86	0.064	0.028
S3	1.0	0.943	0.982	0.99	0.934	0.94	0.964	0.029	0.011
S4	0.888	0.955	0.957	-	0.895	0.929	0.924	0.032	0.014
S5	0.928	0.96	0.966	0.972	0.967	0.976	0.963	0.013	0.005
S6	0.984	0.987	0.966	0.969	0.951	0.045	0.968	0.014	0.006
$\bar{x}$	0.946	0.914	0.967	0.913	0.926	0.926			
SD	0.046	0.075	0.01	0.116	0.029	0.073			
SE	0.018	0.03	0.005	0.052	0.012	0.03			

Table A69: Experiment Sixteen. Outcome of paired "t" tests on lever press data (one tailed p)

		Sal v 0.5	Sal v 1	Sal v 2	Sal v 3	Sal v 4
Total responses	t	0.747	1.67	0.565	0.132	0.264
	df	5	3	4	5	5
	p	NS	< 0.1	NS	NS	NS
Rapid responses	t	0.877	1.45	1.332	0.153	1.387
	df	5	3	4	5	5
	p	NS	NS	NS	NS	NS
Long latency responses	t	0	0.951	0.612	0.299	0
	df	5	3	4	5	5
	p	NS	NS	NS	NS	NS
False alarms	t	1.353	0.072	0.525	0.915	1.554
	df	5	3	4	5	5
	p	NS	NS	NS	NS	< 0.1
Probability of false alarm	t	1.117	0.087	0.584	1.453	1.696
	df	5	3	4	5	5
	p	NS	NS	NS	NS	< 0.1
Total hits	t	1.268	0.573	1.139	2.206	4.152
	df	5	3	4	5	5
	p	NS	NS	NS	< 0.05	< 0.01
Probability of hit	t	1.034	0.728	0.806	1.311	0.762
	df	5	3	4	5	5
	p	NS	NS	NS	NS	NS