EXAMINATIONS OF THE NATURE OF THE DEFICITS INDUCED BY N-METHYL-D-ASPARTIC ACID LESIONS OF THE RAT LATERAL HYPOTHALAMUS

Judith Clark

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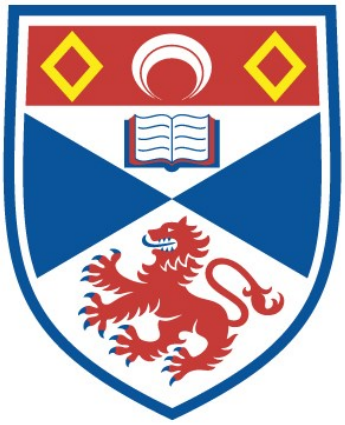

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A thesis submitted to the University of St. Andrews for the degree of Doctor of Philosophy.

by

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DECLARATION

a) I, Judith Mary Clark, hereby certify that this thesis has been composed by myself, that it is a record of my own work, and that it has not been accepted in partial or complete fulfilment of any other degree or professional qualification.

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b) I was admitted to the Faculty of Science of the University of St. Andrews under Ordinance General No. 12 in October 1986, and as a candidate for the degree of Ph.D. in October 1986.

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c) We hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate to the degree of Ph.D.

Signatures of Supervisors Date

12th April 1990

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ABSTRACT

The lateral hypothalamic syndrome of aphagia, adipsia akinesia and sensorimotor impairments induced by electrolytic lesions of the lateral hypothalamus (LH) has been suggested to be due to the destruction of two components of a single system controlling feeding and drinking behaviour. While the "motor" component has been attributed to disruption of dopaminergic fibres, it has been suggested that destruction of intrinsic LH neurones induces a "motivational" deficit. The nature of this motivational deficit was investigated using the excitotoxin N-methyl-d-aspartic acid (NMDA) to lesion cell bodies and leave fibres of passage intact. Such lesions induced temporary reductions in body weight, food and water intake and residual deficits in response to some physiological challenges. Most animals recovered food and water intake and body weight gain after a short period of time. It was shown that LH lesioned rats were able to perceive and respond to the palatability of food/fluid; they responded physiologically to intracellular dehydration caused by hypertonic saline injections, although they did not respond behaviourally; they responded as controls to a battery of long-term, "positive" physiological challenges, but not to short-term, "negative" ones; and they displayed increased rate of development of schedule-induced polydipsia and tail pinch-induced eating, demonstrating that they had no motor impairments and that they did not have an "activational"

deficit. These results indicate that the LH cannot be regarded as a feeding or drinking "centre" and that the motivational deficit following lesions of the LH is of a very complex nature. The implications of these data for the function of the LH are discussed in relation to electrophysiological and anatomical studies.

LIST OF ABBREVIATIONS

AII :- angiotensin II

ADH :- anti-diuretic hormone (vasopressin)

AMB :- ambiguus nuclei

ANOVA:- analysis of variance

AVP :- arginine vasopressin

DA :- dopamine

2-DG :- 2-deoxy-d-glucose

DMH :- dorsomedial hypothalamus

DMNX :- dorsal motor nucleus of the vagus

HPLC :- high performance liquid choromatography

HRP :- horseradish peroxidase

ICSS :- intracranial self-stimulation

IBO :- ibotenic acid

KAI :- kainic acid

LH :- lateral hypothalamus

MH :- medial hypothalamus

NA :- noradrenaline

NMA :- N-methyl-D,L,- aspartic acid

NMDA :- N-methyl-D-aspartic acid

6-OHDA :- 6-hydroxydopamine

PVN :- paraventricular nucleus of the hypothalamus

SFO :- subfornical organ

SIP :- schedule-induced polydipsia

5-TG :- 5-thioglucose

TP :- tail pinch

VMH :- ventromedial hypothalamus

CONTENTS

CHAPTER 1

INTRODUCTION

CLASSIC LATERAL HYPOTHALAMIC SYNDROME

The lateral hypothalamus (LH) is part of the hypothalamus, a diencephalic structure at the base of the brain, which, for many years, has been associated with the basic behaviours necessary for life, such as feeding, drinking and sexual behaviour. The LH has, in particular, been associated with feeding and drinking.

What became known as the lateral hypothalamic syndrome, as defined by **Anand and Brobeck** in 1951, is produced by bilateral electrolytic lesions of the LH, which result in a profound aphagia and adipsia. Further deficits included in the syndrome are sensorimotor impairments (**Marshall, Turner and Teitelbaum**, 1971; **Marshall and Teitelbaum**, 1974), akinesia (**Levitt and Teitelbaum**, 1975), finickiness (i.e. over consumption of palatable and undereating non-palatable foods) (**Teitelbaum and Epstein**, 1962), impairments in muricide (**Marshall and Teitelbaum**, 1974), EEG dysfunction and somnolence (**Danguir and Nicolaidis**,

1980; Levitt and Teitelbaum, 1975; Robinson and Whishaw, 1974).

The aphagia and adipsia are so profound that animals will die in the presence of food and water unless they are kept alive by intragastric feeding. Those which do survive this initial phase are found to recover in four clear stages which always follow the same sequence. These are:

Stage 1: Aphagia and Adipsia

All food and water is refused and animals must be kept alive by tube feeding.

Stage 2: Anorexia and Adipsia

Animals will eat wet and palatable foods but still refuse dry lab chow and water.

Stage 3: Adipsia with Secondary Dehydration-Aphagia

Animals regulate their intake of wet and palatable foods but still refuse water.

Stage 4: Recovery

Animals eat and maintain their weight in the presence of water as the only fluid.

These stages were first described by Teitelbaum and Epstein in 1962. Although lesioned animals appear to have recovered in stage 4 they still show regulatory deficits when tested

by a range of physiological challenges. For example, they do not drink in response to intracellular dehydration induced by injections of hypertonic saline (**Teitelbaum and Epstein**, 1962); and they do not eat in response to glucoprivation induced by 2-deoxy-d-glucose (2-DG) (**Wayner et al.**, 1971).

In addition to aphagia and adipsia, several authors have reported sensorimotor deficits following electrolytic LH lesions. **Marshall, Turner and Teitelbaum** (1971) observed that lesioned animals had deficits in orientation to visual, olfactory and somatosensory stimuli. Further experimentation revealed rigidity and impairments in limb use (**Marshall and Teitelbaum**, 1974).

Thus, the classic electrolytic lateral hypothalamic syndrome can be seen to include deficits in food and water intake and sensorimotor deficits. Through explanation of the causes of the syndrome, it was hoped that the functions of the LH could be determined.

THEORIES TO EXPLAIN THE ELECTROLYTIC SYNDROME

1) REGULATORY THEORIES

Observations on the profound deficits in feeding and drinking following lesions of the LH led several authors to

suggest that this area was involved in the control of food and water intake. In addition, among other behaviours such as gnawing (**Valenstein**, 1973), electrical stimulation of the LH elicited feeding (**Wyrwicka and Dobrzecka**, 1960). These results were then contrasted with data from similar experiments on the ventromedial area of the hypothalamus (VMH). Bilateral electrolytic lesion of the VMH caused animals to overeat and become obese (**Brobeck et al**, 1943) and electrical stimulation of the VMH suppressed feeding (**Larsson**, 1954).

Such observations led **Stellar** (1954) to propose the "dual centre hypothesis". He suggested that the hypothalamus contains a number of centres involved in the regulation of motivated behaviours and that, in the case of feeding, the LH is the "hunger centre" controlling active feeding and the VMH the "satiety centre" acting as a negative input to the LH. Lesion of the LH, therefore, removes the hunger centre and abolishes the motivation to eat, while lesion of the VMH removes the negative input from the satiety centre to the LH and, therefore, increases the motivation to eat.

The dual centre hypothesis, however, does not account for finickiness. If lesions of the VMH and LH have removed "satiety" and "hunger" centres respectively, then all foods should be overeaten or undereaten to the same extent. VMH lesioned animals and LH lesioned animals, however, will reject unpalatable food adulterated with quinine

(Teitelbaum, 1955; Teitelbaum and Epstein, 1962) and LH lesioned animals can only be encouraged to eat with palatable food. Neither does this hypothesis account for the sensorimotor deficits, akinesia and somnolence seen in the electrolytic LH syndrome.

Powley and Keesey in 1970 opposed the suggestion of "hunger" and "satiety" centres, and proposed that the hypothalamus functions as a body weight set-point regulator and that lesion of the LH lowers the set-point so the animal actively attempts to reduce weight, whereas VMH lesions raise the set point so the animal actively attempts to increase weight. They supported this theory by demonstrating that by reducing weight pre-lesion by starvation, the duration of aphagia and adipsia post-lesion was dramatically reduced and rats quickly reached the weight of ad-lib lesioned animals. Both groups then maintained weight gain at the same rate. Thus, whether the rat approached its post-lesion weight by gaining or losing weight initially, the same weight level plateau was achieved. This opposed the motivational "hunger centre" hypothesis which predicted loss of food intake concomitant with neural insult whatever the pre-lesion weight.

However, Powley and Keesey's hypothesis cannot account for the observation that feeding can be restored after electrolytic lesions by the presentation of palatable foods (Teitelbaum and Epstein, 1962). If LH lesioned rats are

actively attempting to reach a lowered body weight set point then surely they should cease eating all foods, however palatable, until the set point is reached. Also, this theory does not explain the sensorimotor deficits, akinesia and somnolence associated with the LH syndrome.

One further explanation for the aphagia and adipsia seen in LH syndrome arose from the observation that LH damage can produce gastric pathology (**Teitelbaum and Epstein**, 1962). Lesions of the LH have been reported to produce gastric ulcers and haemorrhages in stomach and caecum (**Grijalva et al**, 1980). **Lindholm et al** (1975) reported that gastric pathology was present within 24 hours of lesioning the LH. **Grijalva et al** (1980) attribute the formation of ulcers to an alteration in the permeability of the gastric mucosa, producing a significant increase in hydrogen ion loss and sodium ion gain from the gastric contents. Some authors (**Lindholm et al**, 1975) have suggested that the deficits in food and water intake arise as a result of the gastrointestinal pathology. However, this does not appear to be the case since not all aphagic and adipsic rats show this pathology (**Teitelbaum and Epstein**, 1962). Also, **Schallert et al** (1977) demonstrated that it was possible to dissociate, to some extent, between brain sites responsible for gastric pathology and aphagia. Lesions of LH produced gastric ulcers and haemorrhages and aphagia, whereas gastric pathology and no aphagia was produced by lesions of other hypothalamic areas.

These regulatory theories, therefore, could not account for all aspects of the LH syndrome. In opposition to the regulatory hypotheses, it was suggested that the feeding and drinking problems observed in the LH syndrome were actually secondary, and indeed a result of the sensorimotor deficits.

2) SENSORIMOTOR THEORIES

As already mentioned, electrolytic lesions of the LH do not only produce aphagia and adipsia, but also induce akinesia, somnolence and sensorimotor impairments. **Marshall, Turner and Teitelbaum** (1971) described the syndrome of sensory neglect associated with such lesions. They found that unilateral lesions of the LH produced severe deficits in the ability to orient to somatosensory, visual and olfactory stimuli in the contralateral field, while bilateral lesions impaired responsiveness to sensory stimuli on both sides. Responses to sensory stimuli presented to the ipsilateral field were normal. Deficits in limb use have also been observed (**Marshall and Teitelbaum**, 1974).

It was suggested that these sensorimotor deficits had profound consequences for feeding behaviour (**Marshall, Turner and Teitelbaum**, 1971) as sensory information

presented to the side contralateral to the lesion is ignored. Furthermore, recovery of feeding was seen to correlate with recovery of orientation to sensory stimuli. Progression from stage I (aphagia) to stage II (anorexia) took place on the same day as orientation to olfactory stimuli returned. This led **Marshall and Teitelbaum** (1974) to suggest that sensorimotor deficits may be causally related to at least the first stages of aphagia seen in the electrolytic LH syndrome.

It is difficult, however, to see how sensorimotor deficits might account for finickiness and failure to respond to regulatory challenges once recovery has taken place. Also, although initial recovery stages of sensorimotor and feeding abilities may appear in parallel, sensorimotor deficits generally disappear within the first 2 weeks post-operation, while feeding and drinking deficits may last for months (**Marshall and Teitelbaum**, 1974).

Thus, the above studies have not revealed the function of the LH through examination of the LH syndrome. It seems from these investigations that neither regulatory, nor sensorimotor deficits account for the syndrome individually. Matters were complicated still further when it was suggested that, in fact, the LH itself had little to do with the deficits seen following electrolytic lesion of the LH.

DESTRUCTION OF FIBRES OF PASSAGE

The LH is not a compact nucleus, but a relatively cell-poor area without clearly defined borders. It has been demonstrated that electrolytic lesions of the area do not simply destroy LH neurones, but produce damage in many other brain sites (**Mufson**, 1980). Some of this damage results directly from axonal degeneration from LH neuronal destruction. Such damage is found in the nucleus accumbens, the preoptic area, the lateral habenular nucleus, the VMH, the nucleus reuniens of the thalamus, the parafascicular nucleus, the posterior hypothalamus, the zona incerta, the central gray matter, the tegmentum, the substantia nigra pars compacta, the ventral tegmental area and the parabrachial area. In addition, electrolytic lesions damage fibres of passage which have no terminals in the LH. This results in axonal degeneration in the mediodorsal thalamus, the superior colliculus, the medial pontine gray, the raphe nuclei, the motor nucleus of V and the crus cerebri fibre system (**Mufson**, 1980). Such wide-spread damage has led several workers to suggest that, in fact, destruction of the LH itself is not crucial in producing aphagia and adipsia, but that damage to remote sites or fibres of passage produce these symptoms.

Morgane (1961) found that more severe feeding deficits were produced by damage to the far-lateral hypothalamus

than were seen after midlateral hypothalamic damage. As similar impairments were observed following lesions of the globus pallidus, Morgane proposed that electrolytic lesions of LH did not produce aphagia and adipsia by destruction of intrinsic LH neurones, but by the interruption of pallidofugal pathways which pass through this area.

Ungerstedt (1970; 1971) offered an alternative explanation. He agreed that feeding deficits were not attributable to LH cell loss, but suggested that the fibre pathway interrupted was the mesotelencephalic dopamine (DA) system, ascending in the medial forebrain bundle and coursing through the far-lateral hypothalamus. He found that injection of the neurotoxin 6-hydroxydopamine (6-OHDA) along the nigrostriatal bundle caused striatal DA and telencephalic noradrenaline (NA) depletions and produced severe feeding impairments; the LH was not damaged. He concluded that dopaminergic disruption was responsible for the aphagia since NA depletion alone did not produce the deficits.

Ungerstedt's proposal was supported by several studies. **Zigmond and Stricker** (1973) reported aphagia and adipsia in rats following intraventricular administration of 6-OHDA and pargyline. Recovery in these animals was found to follow the same stages as in animals electrolytically lesioned in the LH (**Teitelbaum and Epstein**, 1962). They also found that deficits could be reinstated in recovered electrolytic and 6-OHDA animals by

administration of an inhibitor of catecholamine synthesis, alpha-methyl-p-tyrosine. From this they concluded that the loss and recovery of feeding and drinking behaviours in LH syndrome involved catecholamine neurones.

Fibiger, Zis and McGeer (1973) found that 6-OHDA treated animals showed regulatory deficits similar to those seen in the electrolytic syndrome. They found deficits in daily food and water intake, prandial drinking and in response to intracellular dehydration . These animals also showed finickiness when given quinine water and reduced anorexia to amphetamine. Thus, the 6-OHDA and electrolytic syndromes were seen to be similar and aphagia and adipsia were attributed to loss of striatal DA, not to LH damage.

Further support for these suggestions came from observations of Parkinsonian patients. In Parkinson's disease, severe damage to the nigrostriatal DA system produces motor and sensorimotor deficits. DA replacement therapy can alleviate these symptoms and has been reported to induce appetite. **Sacks** (1973) describes the initial reaction to L-Dopa of one patient:

"Her capacity to chew and swallow were suddenly increased, and so too was her appetite: "Don't give me any of that slush!" she exclaimed, when presented at lunch-time with her usual thin soup. "I want a steak, well done!" The

steak, duly procured and grilled, was devoured with great relish"(pp 127-128).

Thus, DA replacement therapy has reinstated appetite in this patient. Taken together, all of this work would seem to indicate that the LH may have little to do with the regulation of food and water intake. Deficits following lesions of the LH were attributed to disruption in DA transmission caused by destruction of fibres passing through the area, rather than damage to the cells of the LH itself.

The hypothesis that DA loss was responsible for behavioural deficits seen following electrolytic lesions of the LH was questioned by **Willis and Smith** (1984) because, although behavioural recovery takes place in a large proportion of 6-OHDA treated animals, DA depletions in the striatum persist. This phenomenon has been claimed to be the result of DA receptor "supersensitivity" (**Zigmond and Stricker**, 1972, 1973), but Willis and Smith suggested that the aphagia and adipsia were not due to DA depletions, but to amine accumulation in the LH and that recovery reflected the activation of other catecholamine systems and the gradual reduction in accumulation. They supported this view by pointing out that severity of behavioural deficit did not seem to reflect extent of DA depletion, but was related to amount of amine accumulation (**Willis and Smith**, 1984). This hypothesis, however, cannot account for results which

indicate that serial bilateral 6-OHDA lesions separated by 8 months induce full aphagia and adipsia although amine accumulation has been reported to last no longer than 2 months (**Dunnett and Bjorklund**, 1984). These data suggest that the deficits in feeding and drinking arise when DA is depleted bilaterally and support the hypothesis that disruption of DA fibres produces the aphagia and adipsia in LH syndrome.

However, there are several problems with this interpretation of the data presented. First, the "LH" and "6-OHDA" syndromes do differ, in that the 6-OHDA animals show no finickiness (**Marshall, Richardson and Teitelbaum**, 1974), no somnolence and the sensorimotor impairments are greater and last longer. Secondly, the amount of DA loss needed to produce the syndrome is much greater (90-99% as compared to 50-60% loss in electrolytic LH lesions). Also, investigations of oral movement ability by **Whishaw and Kolb** (1983) showed that even after recovery of oral movements, electrolytically LH lesioned animals failed to maintain themselves on dry food and water. Thus, recovery of sensorimotor and motor abilities need not produce a recovery of feeding and drinking. This suggests a dissociation between the ability to make the appropriate movements and the motivation to eat or drink. Furthermore, while **Ljungberg and Ungerstedt** (1976) demonstrated that aphagia and adipsia following 6-OHDA lesions were due to disruption of DA transmisssion by reinstating feeding and

drinking with administration of DA agonist drugs, L-Dopa, apomorphine and ET-495, these DA agonists did not reinstate eating in LH lesioned animals.

These results suggest that the 6-OHDA and electrolytic LH syndromes do differ and that, while there is evidence that dopaminergic systems are involved in the regulation of food and water intake, disruption of DA fibres does not account for all symptoms of the electrolytic LH syndrome.

Electrophysiological recordings from single neurones in the substantia nigra during feeding and drinking suggest that the DA projection may be part of a system involved in the initiation and preparation of movement for feeding and drinking, rather than a component of a feeding and drinking motivational system. Activity of neurones in the substantia nigra was found to be related to movement of, for example, the mouth during feeding, rather than to the need for food (**Mora, Mogenson and Rolls**, 1977). It has been suggested from this that destruction of the nigrostriatal DA pathway produces aphagia and adipsia by interfering with the animal's ability to initiate feeding and drinking responses rather than by disturbing the motivational control of these behaviours (**Rolls and Rolls**, 1982). The full implications of this work will be discussed in Chapter 4.

SUMMARY

Electrolytic lesions of the LH produce a syndrome of aphagia, adipsia, finickiness and sensorimotor deficits. Following recovery, lesioned animals still show deficits in response to regulatory challenges. Some authors have suggested that the major components of the syndrome are the feeding and drinking disorders and have proposed that the LH functions as a feeding centre or weight set-point regulator. Others have proposed that the sensorimotor impairments are primary and that problems in feeding and drinking arise from these sensorimotor deficits. The latter hypothesis could gain some support from the studies which demonstrate that damage to DA fibres which pass through the LH also produces sensorimotor deficits and concommitant feeding disorders. This would suggest that the LH itself is not necessarily involved in the control of food and water intake.

What evidence is there that the LH is involved in the regulation of food and water intake? To answer this question, we must look at the anatomy of the LH, electrophysiological data and more recent lesion experiments using excitotoxins. These topics are covered in detail in the following Chapters.

CHAPTER 2

ANATOMY OF THE LATERAL HYPOTHLAMUS

The hypothalamus is regarded as the diencephalic representative of the limbic system. "Speaking in general terms the hypothalamus guards a part of the channels of entry and the majority of outflow of information to and from the internal environment of the mammalian body. It makes the first overall selection of certain inputs and a major selection of output to and from the "higher" integration centres of the limbic system" (**Luiten, ter Horst, and Steffens,** 1987). To be able to fulfill this function, the hypothalamus must have afferent and efferent connections with large numbers of structures. This is particularly true of the LH with its widespread pattern of connections. If one looks at the neuroanatomical connections of the LH, its relationship with feeding and metabolic regulation becomes apparent. The organisation of these connecting pathways is very complicated. To make things clearer, the description here has been split below into (i) afferents to and efferents from the LH to extra-hypothalamic brain regions; (ii) LH connections with the autonomic nervous system; (iii) intrahypothalamic connections. Before these connections are described, a

brief outline of the development of the LH will be presented as this may be of functional significance.

DEVELOPMENT OF THE LATERAL HYPOTHALAMUS

Evidence has been presented (**Altman and Bayer**, 1987) which indicates that the hypothalamus develops in three "waves" into the reticular, core and midline hypothalamic areas. The reticular hypothalamic area is formed in the "first wave" and includes the LH. The zona incerta and the entopeduncular nucleus are formed from the same anlage at the same time. The core hypothalamus is formed in the "second wave" and includes the preoptic area, the paraventricular, dorsomedial and ventromedial nuclei and the mammillary bodies. The midline hypothalamus produced in the "third wave" includes the suprachiasmatic and the arcuate nuclei and the periventricular area.

Produced as part of the reticular hypothalamus in the first wave, the LH is the first hypothalamic area to form and generation of its neurones stops on day 15 of embryonic development. It is not, however, formed from the classic hypothalamic anlage which produces the core and midline areas of hypothalamus, but arises from part of the third ventricle neuroepithelium which also produces the zona incerta and entopeduncular nucleus. Thus, the neurones of the LH are developmentally associated with the ventral thalamus, rather than the rest of the hypothalamus. It

develops before the rest of the hypothalamus and from different tissue. This may imply that the LH functions differently to, or has different functions from, the rest of the hypothalamus.

i) AFFERENTS AND EFFERENTS OF THE HYPOTHALAMUS

AFFERENTS FROM EXTRA-HYPOTHALAMIC BRAIN REGIONS

It is possible to visualise afferent connections of CNS structures by the method of retrograde transport of injected horseradish peroxidase (HRP). Most of the connections discussed here have been discovered using this method. HRP is injected into the LH and is transported back to the source of afferents to this region. As it is a difficult technique, some discrepencies arise between the findings of different groups, but the projections discussed here are, in general, accepted. Data is mainly obtained from studies of the rat brain, but also includes data from the cat.

Afferents from the prefrontal cortex to the LH have been demonstrated by several methods (**Kita and Oomura**, 1981; **Room et al**, 1987; **Luiten et al**, 1987). Cells in the medial prefrontal cortex, the sulcal prefrontal cortex and the

infralimbic cortex project predominantly to the posterior parts of the LH.

The nucleus accumbens provides a large input to the LH (**Kita and Oomura**, 1981) and a limited input comes from the globus pallidus. Afferents from the thalamus originate in the lateral habenular nucleus, the zona incerta and fields of Forel (**Kita and Oomura**, 1981). Projections from the septum, predominantly the lateral and medial septum, provide an indirect route for information from the hippocampus. Direct projections from the hippocampal formation to the medial portion of the LH arise in the subiculum (**Luiten et al**, 1987).

The amygdala sends projections directly and indirectly to the LH. The indirect information goes via the bed nucleus of the stria terminalis (**Krettek and Price**, 1978), which innervates most parts of the hypothalamus and preoptic area (**Swanson and Cowan**, 1979). Directly, the LH is innervated by the cortical, medial, central, basolateral, basomedial and lateral nuclei of the amygdala (**Ono et al**, 1985). The medial and central nuclei are the major sources of amygdaloid projections to the LH and the central and basolateral nuclei send most of their fibres to the LH. These connections are of particular functional interest as these amygdaloid structures receive sensory, gustatory, olfactory and visceral information from cortex, brainstem and other hypothalamic areas (**Ben-Ari**, 1981).

The LH is the hypothalamic area most heavily innervated from the lower brainstem. It receives input from the central gray substance, the midbrain raphe nuclei, the ventral tegmental area and the locus coerulus (**Ricardo**, 1983). The mesencephalic and pontine reticular formations also project to the LH (**Kita and Oomura**, 1982).

In summary, the LH receives input from prefrontal cortex, visual cortex, nucleus accumbens, amygdala, thalamus and brainstem. Some authors have suggested a complex feeding circuitry involving the prefrontal cortex, the visual cortex, the amygdala and the LH (**Rolls**, 1978; **Ono et al**, 1981). Behavioural experiments have shown that lesions of these areas effect food intake and food related learning tasks (**Kolb**, 1984; **Nachman and Ashe**, 1974; **Rolls and Rolls**, 1982) and neurophysiological single cell recordings have confirmed that prefrontal cortex participates in learned feeding behaviour (**Ono et al**, 1984) and that amygdaloid cells respond to food-related stimuli (**Fukuda et al**, 1987; **Ono et al**, 1980). The connections and functions of the structures which project to the LH are too complex to go into in detail here, but it is important to note that most have been reported to be involved in some way in feeding behaviour. Figures 1 and 2 give a visual outline of some important LH connections.

FIGURE 1

SOME AFFERENTS TO SPECIFIC LH REGIONS

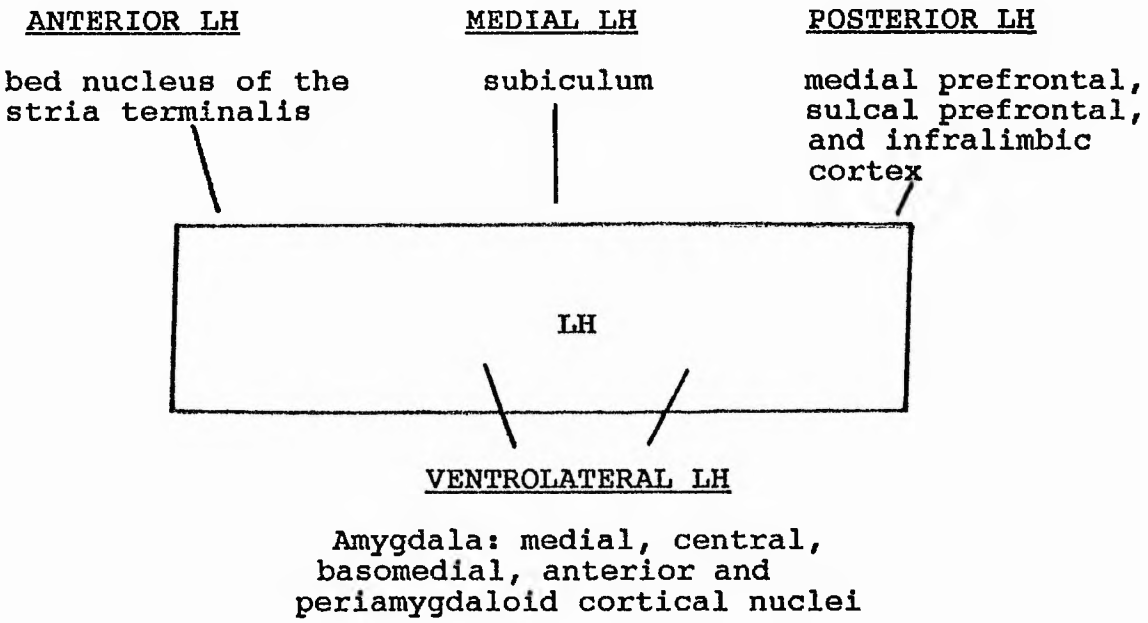


FIGURE 1: This figure depicts, in a very simplified form, some of the brain areas which project to specific regions in the LH. Regions of the LH are written in capital letters and underlined and brain areas which have afferents to specific sites in the LH are listed below the area to which they project.

FIGURE 2

SUMMARY OF LH CENTRAL CONNECTIONS

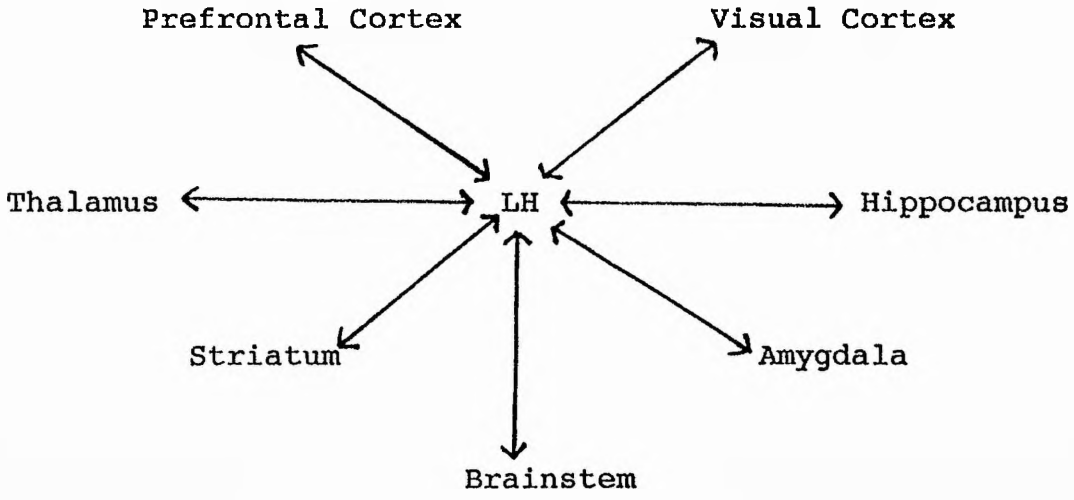


FIGURE 2: This figure depicts, in a very simplified form, a summary of the afferents to and efferents from the LH to other areas within the brain.

EFFERENTS TO EXTRA-HYPOTHALAMIC BRAIN SITES

Many of the connections described above are reciprocal. There are direct projections from LH to the neocortex and parallel pathways synaptically interrupted at the level of the claustrum (**LeVay and Sherk,** 1981). Efferents are also sent to the medial frontal cortex and the medial septal nucleus and scattered cells in the rostral and caudal thirds of the LH send direct projections to the hippocampus (**Wyss, Swanson and Cowan,** 1979).

The central nucleus of the amygdala appears to be the sole amygdaloid recipient of direct fibres from the LH (**Ottersen,** 1981) and indirect fibres via the ventral tegmental area, the substantia nigra pars compacta and the medial and dorsal raphe nuclei (**Ricardo,** 1983). It is interesting to note that the central nucleus of the amygdala projects in turn to the cerebral cortex via the basal nucleus of Meynert, the midbrain, pons and medulla oblongata and the ventromedial and dorsomedial hypothalamus (**Ben-Ari,** 1981).

Reciprocal connections with the thalamus also arise with efferents terminating in the thalamic paraventricular nucleus and the parataenial nucleus with anterior LH projections to the lateral habenula (**Veening et al,** 1987).

Projections to the limbic midbrain terminate in the central gray substance, the median and dorsal raphe nuclei, the ventral tegmental area and the pars compacta of the substantia nigra (**Saper et al, 1979; Hosoya and Matsushita,** 1981).

ii) LH CONNECTIONS WITH THE AUTONOMIC NERVOUS SYSTEM

The hypothalamus is believed to be part of the neural circuitry involved in the control of hormone release from the endocrine pancreas. This hormonal regulation plays an integral part in maintaining fuel substrate homeostasis. What anatomical evidence is there for LH involvement in this?

The connections between the hypothalamus and the endocrine pancreas have been studied in great detail by **Ter Horst, Luiten and Kuipers** (1984) and most of the findings given below are summarised from their work. It was found that the LH had one limited direct pathway and two more extensive indirect pathways to the parasympathetic motor nuclei of the lower medulla, the cell groups which innervate the pancreas. The direct pathway consists of rather diffuse LH projections to the dorsal motor nucleus of the vagus (DMNX) and the ambiguus nuclei (AMB). Efferents to the DMNX arise in the caudal parts of the LH, while projections to the AMB originate in the peripheral aspects of the entire

longitudinal aspects of the LH. The major indirect route for LH information lies in the widespread connections with the medullary reticular formation which arise throughout the LH. The second indirect route has been mentioned briefly above. The ventral and lateral aspects of the LH project to the ventral tegmental area and the mesencephalic central gray. These in turn project to the parvocellular reticular formation of the lower medulla. This provides entry to both sympathetic and parasympathetic autonomic cell groups. The diagram in Fig 3, modified from **Ter Horst et al** (1984), displays these connections. It might be implied from this that it would be possible for LH output to effect hormonal release from the endocrine pancreas. Such direct functional relationships have some support from studies described in Chapter 3 which show that stimulation of the LH, either electrically or chemically, has an effect on the release of insulin (**Steffens**, 1981) and glucagon (**Helman et al**, 1982).

The LH not only sends output to the periphery, but receives it via the hypothalamospinal nerve and the nucleus of the solitary tract (**Hosoya**, 1980; **Hosoya and Matsushita**; 1979).

FIGURE 3

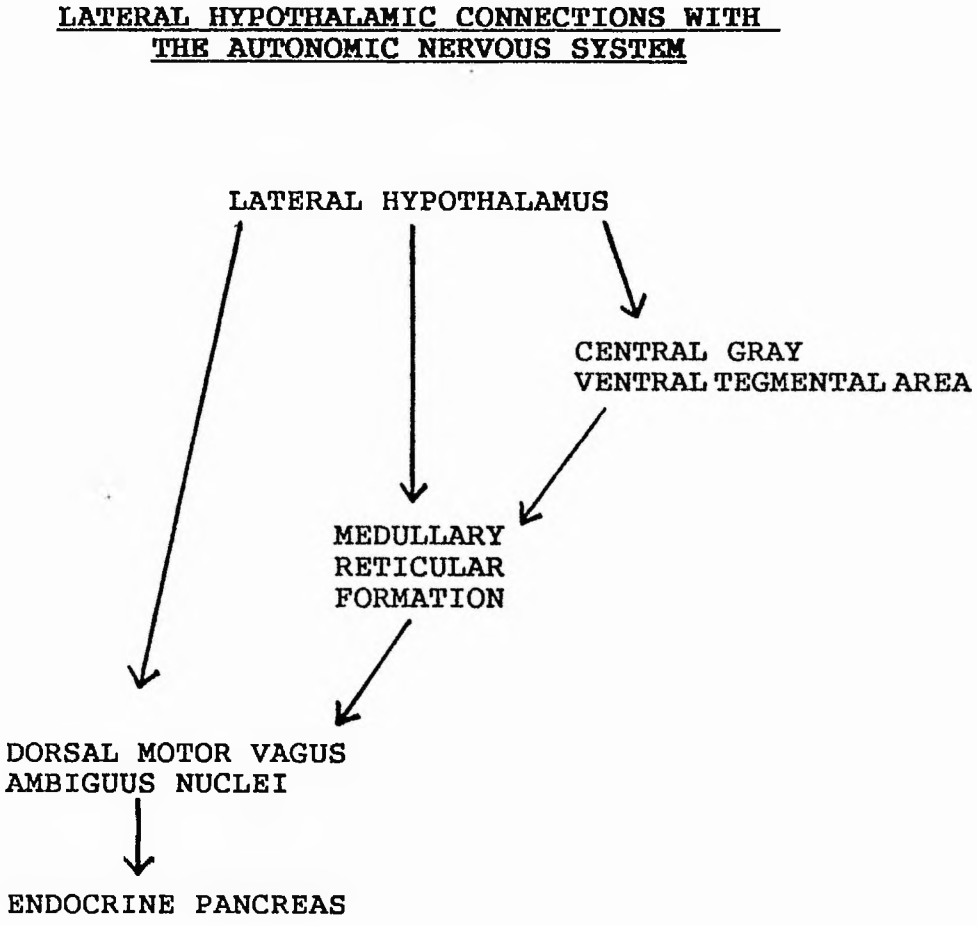


FIGURE 3: This diagram, modified from Ter Horst et al (1984), depicts the lateral hypothalamic connections with the autonomic nervous system. In addition to the direct connections with the dorsal motor nucleus of the vagus and ambiguus nuclei the LH has indirect access to sympathetic and parasympathetic autonomic cell groups via the ventral tegmental area, the central gray and the medullary reticular formation.

iii) INTRA-HYPOTHALAMIC CONNECTIONS

The evidence given above demonstrates hypothalamic connections with "higher order" cortical sites and with areas involved in the control of hormonal release and blood glucose homeostasis. The hypothalamus, however, cannot merely be regarded as a relay station. Some form of integration or information transfer must take place within it. Knowing which hypothalamic sites connect with the LH may help to elucidate its functions.

It has been reported (**Luiten et al,** 1987) that the major intra-hypothalamic output of the various LH cell groups is to other cell groups within the LH. This internal connectivity allows transfer of information since not all cell groups within the LH have the same afferents (see above). It suggests that some form of integration of peripheral and central information may take place within the LH itself.

The anterior hypothalamus does send some afferents to the LH, although not so many as the lateral and medial preoptic areas. The supraoptic and the suprachiasmatic nuclei also project to the LH (**Kita and Oomura,** 1982).

The LH sends efferents to and receives afferents from the VMH and the paraventricular nucleus of the hypothalamus

(PVN) (Luiten et al, 1987; Kita and Oomura, 1982). The LH projects to the nuclear core and the surrounding fibre shell of the VMH. The core and the lateral marginal regions of the VMH project to the ventromedial parts of the LH. The parvocellular cells of the PVN project to the medial and ventral peripheral zones of the LH. These connections are not substantial in either direction.

The hypothalamic area with which the LH has most connections is the dorsomedial hypothalamus (DMH). The LH receives a large number of afferents from the DMH which terminate in the medio-lateral peripheral zones of the LH and it projects to all anterior-posterior levels of the DMH (Saper et al, 1979), in particular to the lateral parts. The VMH also has strong connections with the DMH and it has been suggested (Luiten et al, 1987) that the DMH performs a bridge function between the LH and the VMH, allowing communication between the two structures. There are also reciprocal connections between the LH and the perifornical area, the posterior hypothalamic nucleus and the dorsal premammilary nucleus (Kita and Oomura, 1982).

This pattern of intra-hypothalamic connectivity suggests that, if the LH is performing any form of integrative function, it is not integration of information from various intrahypothalamic sites. Poor connections with the VMH make it difficult to believe that these structures are working together, but suggest that they may work in parallel. If

any hypothalamic structure integrates intra-hypothalamic information it is the DMH which has strong reciprocal connections with the LH, VMH and PVN. The DMH has also been implicated in the control of food and water intake, as lesions of the DMH induce hypodipsia, hypophagia and reduced body mass (**Bernadis and Bellinger**, 1987). These animals, however, are capable of complex intake regulation and such lesions produce a smaller but otherwise normal animal, leading to suggestions that the DMH is an integrator of hypothalamic and homeostatic information, rather than an essential "feeding" or "satiety" centre. The extensive intra-LH projections suggest that the function of the LH may have something to do with integration of the peripheral and central information converging on it.

SUMMARY

The neurones of the LH, the first hypothalamic cells to be generated, do not develop from the hypothalamic anlage but arise from the same tissue as the zona incerta and the entopeduncular nucleus. The LH receives input from and sends information to higher limbic areas, thought to be involved in behavioural selection, brainsites involved in perception of food or food related behaviour and peripheral areas involved in homeostasis. The information which arrives is communicated to all LH cell groups. The neuroanatomy of this area suggests that it may perform

some integrative function, perhaps in relation to consummatory behaviour.

CHAPTER 3

EVIDENCE FOR LATERAL HYPOTHALAMIC INVOLVEMENT IN THE REGULATION OF FLUID AND ENERGY BALANCE.

Anatomically, the LH would seem to be connected to many areas known to be involved in the regulation of food and water intake. It must be remembered that organisms do not simply eat and drink in response to physiological signals of deficit, but are influenced by sensory stimuli, previous experience and the parameters of the particular situation in which they find themselves. Thus, peripheral, central and external influences combine to determine which behaviour will be performed in the face of competing choices. When this is considered, it becomes obvious that terms such as "feeding centres" are not generally useful in investigations of how the brain controls feeding behaviour. In order to produce the behaviour, an integrated system comprising many different brain sites is more likely to be involved and it is, therefore, more useful to look at the LH as one component of a system rather than as a centre. In this way, it may be possible to see if the LH is involved at any stage of the regulation of food and water intake.

WATER INTAKE

Body water comprises 45-70% of total body volume and is the largest single component of the body. It is vitally important to maintain hydration as without water a healthy human being, rat or almost any other mammal will only survive for a few days. An appreciable volume of water is lost from the body each day as perspiration and urine, and this water must be replaced. Dehydration promotes thirst and, consequently, drinking and also activates mechanisms to conserve water. Is there any evidence that the LH is involved in any of these components of water regulation?

Total body water is divided into intra- and extra-cellular fluid. Thirst depends on monitoring these fluid levels inside and outside the cell, and either cellular or extracellular dehydration can initiate drinking, although it is thought that cellular dehydration is the more important (Rolls and Rolls, 1982).

Intracellular dehydration is monitored by cells called "osmoreceptors", which can be found both in the periphery and the brain. Peripheral osmoreceptors are found in the gut and stomach. They are also found in the hepatic portal system, which is the blood vascular system which carries absorbed substances from the gut to the liver. It appears

that the LH may play some role in the monitoring of peripheral cellular hydration through this system, as information about changes in the osmolality of the blood in the hepatic portal vein travels to the liver and causes changes in the discharge of the vagus nerve and firing in some LH neurones (**Schmitt**, 1973). The existence of central osmoreceptors was first observed when drinking was induced by altering the hydrational state of cells in the brain without affecting the periphery by injecting hypertonic saline into the carotid artery (**Wood et al.**, 1977). The sites of these central osmoreceptors are believed to be the lateral preoptic area, the paraventricular nucleus and the LH (**Rolls and Rolls**, 1982) as injection of hypertonic saline into these areas elicits drinking. Thus, the LH may be involved in monitoring peripheral and central cellular dehydration.

Extracellular dehydration is detected by receptors in the heart and kidney. In the heart, baroreceptors, or stretch receptors, respond to the volume of blood in the heart and, therefore, the volume of blood in the body as a whole. This information is sent to the brain which promotes either an increase or decrease in the probability of drinking and the release of vasopressin, a hormone involved in water conservation, as appropriate. The kidney detects a reduction in the volume of blood (hypovolemia) when renal blood flow drops. In response to this it releases the enzyme renin which acts on a substrate in the plasma to

produce angiotensin I. This is converted in the lungs to angiotensin II (AII) which has several effects. The first is to stimulate the adrenal cortex to produce aldosterone and thus to conserve sodium. The second is to stimulate "thirst" by action on particular brain sites. AII receptors have been reported in the supraoptic nucleus, the anteroventral third ventricle, the subfornical organ (SFO) (**Okuya et al**, 1987) and in the LH (**Tanaka et al**, 1986). **Tanaka et al** (1986) found that LH cells which were activated by electrical stimulation of the SFO, and some of those which were not, could be excited by microiontophoretically applied AII. **Swanson and Mogenson** (1981) reported that the medial preoptic area was found to be a AII sensitive zone and that this area projects to several hypothalamic nuclei, including the LH. They sugested that the LH and VMH may form part of a loop from the AII sensitive medial preoptic area to both the activating reticular formation and the vagus, thus initiating drinking and promoting peripheral water retention. The importance of AII in the stimulation of thirst under normal circumstances has been called into question (**Rolls and Phillips**, 1983), but if it is involved in the initiation of drinking, the LH may have a role to play.

Further evidence for the involvement of the LH in fluid regulation at some level comes from pharmacological studies. **Leibowitz** (1971) demonstrated that infusion of

noradrenaline (NA) into the LH inhibits drinking in water deprived rats and that infusion of the alpha-adrenergic blocker phentolamine into the LH induced drinking in satiated rats. **Mason et al** (1988) demonstrated that changes in extracellular catecholamine levels in the LH were linearly related to plasma osmolality, in that drinking increased catecholamine concentrations as it reduced plasma osmolality. They suggested that the effect was mainly due to changing levels of NA, although adrenaline and ascorbic acid may have been involved. Thus, increased NA concentrations in the LH appear to be related to fluid satiation.

Neurophysiological studies by E.T. Rolls and his colleagues (**Rolls, Burton and Mora**, 1980) have demonstrated that some neurones in the LH fire to the taste of water and some are activated by the sight of a stimulus which predicts fluid (i.e. the syringe from which they normally drink). **Vincent, Arnauld and Biolac** (1972) also showed that LH neurones which increased their firing rate following injections of hypertonic saline, decreased activity during and after drinking water. Thus, activity of these neurones would seem to be modulated by the hydrational state of the animal and activated by sensory stimuli related to fluid intake. An interaction between state of thirst and sensory information about the sight or taste of water may take place. This suggestion will find further support in the following discussion of LH involvement in food intake.

SUMMARY

There is substantial evidence to support the suggestion that the LH has some involvement in drinking. Not only have osmo-sensitive neurones been found in the LH itself, it also receives information from sites in the brain and the periphery known to host osmoreceptors. AII receptors have also been reported in the LH itself and in brain areas which innervate the LH. Catecholamine activity in the LH is seen to increase as plasma osmolality decreases and NA injection into the LH inhibits drinking. Single cell recordings demonstrate that LH neuronal activity is affected by the hydrational state of the animal and that specific LH neurones will fire to the sight or taste of water or water associated stimuli.

This evidence suggests that, while the LH may not be a "drinking centre", it receives and responds to information concerning hydrational state.

FOOD INTAKE

In order to function, the body requires energy. If there was a failure in the continual supply of fuel to the cells of the body, death would follow. This fuel, however, is not continually supplied from the external environment,

but is utilized from energy stores in the body. These stores gradually deplete and must be replenished by the intake of food. Energy balance involves not only the monitoring of energy stores and circulating levels of fuel, but also the initiation of feeding to restore falling energy levels. Is there any evidence to implicate LH involvement at any stage of the regulation of energy balance?

The principal source of energy for most tissue is glucose. Excess glucose is stored as fat or glycogen. Insulin facilitates the entry of glucose into cells and increases the conversion of glucose into glycogen or fat. Glucagon causes liver glycogen to be converted to glucose and promotes the breakdown of fats to free fatty acids for the use of body tissues as energy. Thus, during the absorptive phase of metabolism, high levels of insulin are used to promote energy storage and during the fasting phase, glucagon facilitates energy use. It has been postulated that the hypothalamus is involved in regulating circulating levels of fuel substrates by altering release of glucagon or insulin and by the initiation of feeding.

The autonomic hypothesis of the regulation of food intake (Bray, 1985) suggests that the VMH and LH control metabolism through the parasympathetic and sympathetic branches of the autonomic nervous system respectively. Bray (1985) reports that lesion of the VMH induces increased

activity in the parasympathetic nervous system and decreased activity in the sympathetic nervous system while lesions of the LH produce the opposite effects. LH lesions are reported to decrease insulin and increase glucagon secretion, while VMH lesions produce the opposite results. These data are interesting, but inconclusive without further evidence as the lesions described were electrolytic and, therefore, suffer from the criticisms laid out in Chapter 1; results could be due to disruption of catecholamine fibres or damage to other brain sites.

Further experiments have reported conflicting results. Electrical stimulation of the LH in anaesthetised rats has been observed to increase glucagon secretion without altering insulin levels (**Helman et al, 1982**). These changes were attributed to sympathetic alterations arising in the LH as section of the splanchnic nerve abolished the LH stimulation effects but vagotomy did not. Others have reported that electrical stimulation of the LH resulted in insulin secretion (**Steffens**, 1981) and that vagotomy abolishes electrically stimulated eating (**Ball**, 1974). The former data conflicts with the lesion results reported (**Bray**, 1982), but the latter data support it.

Chemical stimulation of the LH has also been investigated. Injection of NA into the LH has been reported to increase insulin secretion (**de Jong, Strubbe and Steffens**, 1977) and decrease plasma free fatty acids (**Steffens et al**,

1984) without altering glucose levels. Injection of adrenaline, on the other hand, has been reported to increase insulin and glucose levels without altering free fatty acids (Steffens et al, 1984). It has been suggested that these results indicate that the LH can arouse the parasympathetic nervous system. These results become confusing when it is noted that injection of adrenaline and noradrenaline both induce feeding in satiated rats (Grossman, 1960 and 1962).

It is difficult to draw conclusions from such conflicting data. It appears that the LH is involved in the regulation of circulating fuel substances, but what this involvement entails exactly is unclear. Stimulation and lesion of the LH effect insulin, glucagon and free fatty acid secretions but different authors report different effects. It is not surprising that stimulation or lesion of the LH should effect endocrine factors when the connections between the LH and the endocrine pancreas are considered (see Chapter 2). However, the exact role of the LH in the regulation of energy substrates remains unclear. The different results could be due to stimulation of different areas of the LH as commented by Oomura and Yoshimatsu (1985) who point out that stimulation of the ventral LH tends to facilitate vagal activity and stimulation of the dorsal LH inhibits it. As already mentioned, different areas of the LH have different inputs and outputs.

Electrophysiological studies have shown that the activity of some LH neurones is directly related to food and food intake. **Oomura and Yoshimatsu** (1984) have reported that 20-25% of LH neurones are glucose sensitive in that they decrease activity following the direct application of glucose to their surfaces. These neurones also increase activity following insulin administration. Behaviourally, insulin-induced eating can be abolished by glucose injection into the LH. Neurones in the VMH were found to be glucose responsive cells which increase activity after glucose administration. Thus, the glucose sensitive neurones of the LH and the glucose responsive cells of the VMH show opposing properties.

Two endogenous sugar acids, 2-DTA and 3-DPA have been shown to suppress and induce eating respectively in fasted rats (**Oomura**, 1986). They also decrease (2-DTA) and increase (3-DPA) the activity of LH glucose sensitive neurones and produce the opposite effects in the VMH. It was suggested by the authors that this indicates endogenous satiety and hunger substances mediating their effects through the LH and VMH. The glucose sensitive neurones of the LH appear to be involved in monitoring peripheral as well as central glucose levels, because glucose injection into the hepatic portal vein inhibits the activity of LH glucose sensitive neurones (**Shimizu et al**, 1983).

Direct application of 2-deoxy-D-glucose (2-DG) increased the activity of 38% of LH neurones immediately and decreased firing of 19% (**Katafuchi, Oomura and Yoshimatsu,** 1985). The decreased activity was attributed to a general decrease in neuronal metabolic rate caused by 2-DG. Following increased activity, 58% of the excited neurones were inhibited. This pattern of neuronal activity in the LH was shown to parallel the behavioural effects of 2-DG which induced an immediate increase but a long-term inhibition of food intake.

While these results show a clear indication of LH involvement in glucose monitoring, feeding in response to cerebral glucoprivation does not seem to be mediated solely by the hypothalamus. Intraventricular injections of the antimetabolic glucose analogue 5-thioglucose (5TG) induces hyperglycemia and feeding (**Ritter, Slusser and Stone,** 1981). The glucoreceptors which mediate these responses have traditionally been presumed to be in the hypothalamus, but experimental evidence suggests that they exist in the hindbrain (**Ritter, Slusser and Stone,** 1981). When the cerebral aqueduct was obstructed, infusion of 5TG into the lateral ventricle failed to induce feeding or hyperglycemia but infusion into the fourth ventricle still elicited these responses. Injection of AII into the lateral ventricle induced significant increases in drinking which were not diminished after cerebral aqueduct obstruction. This indicates that the failure to respond to 5TG was not

due to a general inability to respond to stimuli infused into the lateral ventricle after aqueduct obstrucion. These data indicate that brain glucoreceptors which mediate feeding in response to brain glucoprivation are located in the caudal hindbrain rather than the forebrain. This evidence may suggest that the LH, while in receipt of homeostatic data, is not essential for responding homeostatically to that data. This data may be processed for a different purpose in the LH.

In addition to these effects induced by manipulation of the chemical environment of the LH, much work has been performed to investigate single cell activity in the LH of the freely behaving animal. Early work (**Oomura et al.**, 1969) reported that neurones in the LH of the cat fired before and during eating and that neuronal activity was greatest in the hungry animal. More recent experiments in the monkey (**Rolls et al.**, 1980) have demonstrated that some LH neurones fire to the sight and taste of food while others have been reported to respond during the ingestion of food (**Fukuda, Ono and Nakamura**, 1987). The activity of these neurones, however, is not simply concerned with the perception of food stimuli. Two things have been found to modulate their activity; ingestion and hunger.

Kendrick and Baldwin (1986) have demonstrated that neurones of the sheep LH did not fire to a nonsense object or to the sight of a food that they would not eat, but that they did

fire to the sight or approach of a desired food. This response was shown to diminish and eventually extinguish when the sheep was not allowed to eat the food. The rate of extinction depended upon palatability, with the most preferred food eliciting responses for a longer time. Neuronal activity was found to be re-established very quickly if the animal was then allowed to ingest the food. Thus being able to eat the food had a direct effect on neuronal activity which could not be attributed solely to perception of the food stimuli. Neurones ceased to respond if food reward was not obtained, demonstrating that neuronal activity did not signal merely the visual perception of food, but the perception of available food.

These authors and others working with the monkey (**Burton et al.**, 1976) have shown that LH activity in relation to the sight or taste of food is modulated by the animal's state of hunger. Those neurones which were activated by the food related stimuli were found to decrease activity when the animal was satiated (**Kendrick and Baldwin**, 1986). Activity could be re-established if a novel food was introduced (**Burton et al.**, 1976). Thus, activity in these neurones did not signal hunger, but was dependent upon hunger; their responding was modulated by the internal state of the animal but was dependent upon external food related stimuli and the opportunity to eat. The activity of the LH neurones cannot be attributed to straightforward motor reponses made by the animals to get the food rewards

as latency of LH neuronal activity precedes muscle activation (reviewed in **Rolls**, 1980), although it may be related to preparation for movement.

These results may indicate that the LH is important in assessing the significance of stimuli by integrating information from the periphery about the state of energy balance with sensory information about the presence of food, its availability and palatability. Sensory and learnt information both appear to be used. Further evidence to support this idea has been provided by several authors. LH neurones have been shown to respond to the sight of a non-food object which is associated with food reinforcement (**Mora, Rolls and Burton**, 1976; **Kendrick and Baldwin**, 1986). Thus neurones were found to respond to the sight of a black bottle or syringe from which animals were fed, but not to the sight of a yellow bottle or white syringe unassociated with feeding. It has also been found that LH neurones may acquire a response to a tone associated with a glucose reward (**Ono et al**, 1985, 1986; **Nakamura and Ono**, 1986). Further, neurones have been shown to respond to the sight of food or non-food associated with a juice reward, but not to the sight of food or non-food associated with aversive saline (**Fukuda et al.**, 1986). Thus the significance of the stimulus seems to predict the activity of the LH neurone.

There is further evidence to support this idea. It has been found that animals actively will seek electrical stimulation of the brain to the extent that they will self-stimulate (Olds, 1977). This intracranial self-stimulation (ICSS) can be elicited from a large number of brain sites, including the LH, and is believed to be rewarding in that any behaviour which animals will actively seek to engage in and repeat must have rewarding properties (Redgrave and Dean, 1981). It has been suggested that ICSS in the LH mimics the naturally rewarding effects of food for a hungry animal as ICSS in the LH has been shown to increase with food deprivation and decrease with satiation (Hoebel and Teitelbaum, 1962; Margules and Olds, 1962). This ICSS has been shown to be dependent upon the intrinsic neurones of the LH, rather than upon stimulation of fibres of passage, as ibotenic acid lesions, removing intrinsic cells, attenuated ICSS; while 6-hydroxydopamine lesions, disrupting catecholamine transmission but leaving LH neurones intact, did not effect ICSS (Velley et al., 1988).

Single cell recordings in the LH also demonstrated that some neurones which were influenced by licking to obtain ICSS were also influenced by licking to obtain a glucose reward and that the neuronal response was usually similar to both rewards, i.e. excited or inhibited by both rewards (Ono et al., 1985,1986; Nakamura and Ono, 1986). Neurones in the LH have also been found to respond to aversive stimuli such as electric shock and those which responded

to reward and aversion were found to respond in the opposite direction, e.g. activation by reward and inhibition by aversion. Thus, the significance of the stimulus seems to predict the LH neuronal response.

Once the significance has been established, the LH seems to fire until the food reward has been obtained (Ono et al., 1980). Neurones which were found to respond only to the sight of food maintained responding throughout bar pressing to obtain food. Once food was placed in the mouth response diminished. This suggests that the LH is not just involved in the perception of food when the animal is hungry, but in the drive to obtain it.

Analyses of neuronal activity in higher brain sites which project to the LH have found that these area possess some of the components of the LH pattern of responding, but do not appear to be involved in the recognition of significance. In the inferior temporal visual cortex neurones repond to food and non-food related objects but their activity is not effected by hunger or satiation or by the significance of the stimuli i.e. rewarding glucose or aversive saline (reviewed in Rolls, 1981). The inferior temporal cortex projects to the amygdala, which in turn projects to the LH. In the amygdala, although the activity of many neurones can be attributed to arousal or to the physical characteristics of visual stimuli, some neurones have been found to respond specifically to food,

especially after a period of learning (Ono et al, 1980). In this study, the LH neurones were found to fire continually during bar pressing for food, whereas the neurones of the amygdala did not. In latency of responding, a clear progression was found from the inferior temporal cortex, through the amygdala to the LH and experiments in which reversible cooling was used to render brain sites inactive, LH food related neurones were affected when the amygdala was not functioning and amygdaloid food related neurones were affected when the inferior temporal cortex was not functioning (Fukuda, Ono and Nakamura, 1987). This indicates a system whereby food-related stimuli are processed at differing levels of complexity.

SUMMARY

There is evidence to suggest that the LH plays some role in the regulation of food intake. The anatomical connections between the LH and endocrine pancreas and sensory brain sites have been shown to have some functional significance. Not only are some LH neurones sensitive to glucose concentrations in the brain, they also respond to peripheral glucose levels. Stimulation and lesion experiments have shown the LH to have some involvement in regulation of insulin and glucagon levels. Thus the LH receives information about and responds to the animal's state of hunger. This information appears to be integrated

with sensory information about the sight or taste of food and food related objects and with learnt information about the consequences of ingestion.

DISCUSSION

The evidence discussed above indicates that the LH is not a feeding or drinking "centre". Its role in homeostatic regulation appears to be much more complex. The LH appears to receive information concerning hydrational state and glucoprivation but what it does with this information is not clear. Although LH neurones are influenced by homeostatic changes, the LH may not be essential for responding homeostatically to those changes. Sensory and physiological signals converge on the LH. Neuronal responding is not related to only sensory or only physiological information but an integration of both so that the significance of the stimuli for the animal may be assessed. That is, neurones of the LH do not respond simply to hunger or to all food related stimuli, but to the sight or taste of desired food when the animal is hungry until the food reward has been obtained. Thus, the LH may send information to higher cortical structures to assist in behavioural selection by emphasising the significance of food or fluid stimuli when the animal is hungry.

CHAPTER 4

LESION STUDIES USING EXCITOTOXIC AGENTS

Evidence has been provided from anatomical and electrophysiological studies which suggests LH involvement in the regulation of food and water intake. This involvement appears to be of a highly complex nature and does not support the hypothesis that the LH is a simple "feeding centre". However, neither does it support the suggestion that the consummatory deficits seen following electrolytic lesion of the LH are due simply to destruction of catecholamine-containing fibres of passage. The extent to which feeding and drinking deficits are dependent upon LH neuronal damage can be assessed by destruction of intrinsic LH neurones leaving passing fibres intact. Discovery of the excitotoxins has made this technique possible (e.g. **Winn, Tarbuck and Dunnett**, 1984).

KAINIC ACID

Kainic acid (KAI), a structural analogue of glutamate, is a neurotoxin used to destroy cell somata without disrupting fibres of passage. Infusion of KAI into the LH has been claimed to destroy cell bodies and leave DA fibres intact as catecholamine assays have shown no significant change in

DA levels in telencephalic areas which receive fibres which pass through the LH and are destroyed by electrolytic lesions (Peterson and Moore, 1980; Grossman et al., 1978). Histological examination using silver-staining also revealed no destruction of fibres.

Infusion of KAI into the LH has been reported to induce aphagia and adipsia (Grossman et al., 1978) and, following recovery post-surgery, residual deficits to physiological challenges have been found (Stricker, Swerdloff and Zigmond, 1978). Rats did not drink in response to intra- or extra-cellular dehydration or eat in response to glucoprivation. Thus, destruction of LH cell bodies as opposed to fibres of passage produced similar feeding and drinking deficits as electrolytic lesions, although behavioural deficits were not so long lasting (Wayner et al., 1981). This evidence would seem to indicate that consummatory deficits were attributable to LH neuronal destruction.

However, KAI was found to produce epileptic type seizures and neuronal death in sites distant from the site of injection which could not be explained by diffusion. Extra-hypothalamic damage was found in the ventral thalamus (which showed more complete cell loss than the LH), zona incerta, subthalamic nucleus, thalamic reticular nucleus and the dorsal thalamus (Peterson and Moore, 1980). In addition to non-specific tissue damage, some authors have

also reported that fibres of passage are damaged (**Wuerthele et al., 1978**). These results call into question the extent to which LH insult is responsible for the behavioural deficits seen.

IBOTENIC ACID

More recent investigations have used the neurotoxin ibotenic acid (IBO), which is also a structural analogue of glutamate. This amino acid neurotoxin was also believed to destroy cell bodies and leave fibres of passage intact and, in contrast to KAI, IBO had been reported not to induce seizures or damage areas remote from the site of injection (**Guldin and Markowitsch, 1981; Markowska et al., 1985**).

Infusion of IBO into the LH has been demonstrated to induce aphagia, adipsia and deficits in responding to physiological challenges (**Winn, Tarbuck and Dunnett, 1984**). This would indicate that the LH is involved in the regulation of food and water intake.

Detailed comparisons have been made between the syndrome produced by IBO and those produced by 6-OHDA or electrolytic lesions. Aphagia and adipsia have been found to be less severe in IBO lesioned animals in comparison to

electrolytic lesions (**Winn, Tarbuck and Dunnett,** 1984). It was also observed that IBO induced no akinesia (**Winn, Tarbuck and Dunnett,** 1984) and the sensorimotor deficits observed in 6-OHDA animals were not present in IBO treated rats (**Dunnett, Lane and Winn,** 1985). In their responses to physiological challenges, however, electrolytic and IBO lesioned rats were equally impaired (**Winn, Tarbuck and Dunnett,** 1984). These results have led to the suggestion that the classic electrolytic LH syndrome is formed from two components; "motor" and "motivational". **Winn, Tarbuck and Dunnett** (1984) suggest that damage to the ascending dopamine pathway produces sensorimotor deficits which interfere with the animal's ability to eat and drink and that LH damage produces impairments in the motivation or willingness to eat and drink, not related to motor dysfunctions. Thus, electrolytic lesions damage two elements involved in the regulation of food and water intake and following IBO lesions there appears to be a disconnection between motivational and motor components of the regulatory system (**Winn, Tarbuck and Dunnett,** 1984) as animals appear to be <u>able</u> to eat, yet show less willingness to do so than normal. A more detailed description of the concept of "motivation" can be found in a later section of this chapter.

This hypothesis finds some support from experiments by **Velley et al** (1988) who were examining two behaviours elicited by electrical stimulation of the LH; ICSS and

increased locomotor activity following non-contingent electrical stimulation. The experiments were designed to investigate whether these responses were elicited by stimulation of the same set of neurones or whether they could be dissociated anatomically. To investigate this, Velley et al examined the effects of 6-OHDA and IBO lesions on these behaviours. Rats were unilaterally injected in the LH with either 6-OHDA or IBO and implanted bilaterally with stimulation electrodes. In all rats, ICSS and locomotor activity were normal following stimulation of the LH contralateral to the lesion. It was found that, while lesions of the LH with IBO attenuated LH ICSS, they did not affect locomotor activity produced by non-contingent electrical stimulation; 6-OHDA lesions produced the opposite effects. Thus lesion of LH cell bodies abolished behaviour motivated by reward and lesion of DA fibres abolished locomotor responding. Although these behaviours are not directly food related, it has already been mentioned in Chapter 3 that ICSS is believed to resemble natural rewards in some ways and in the LH to resemble the reward of food to a hungry animal.

There are, however, some problems associated with the use of IBO as a neurotoxin in the LH. **Hastings, Winn and Dunnett** (1985) have reported that, in addition to LH destruction, infusion of IBO into the LH induces damage in the thalamus and amygdala. These unilateral lesions also produced a bilateral increase in DA levels in the

caudate-putamen (15% : non-significant) and nucleus accumbens (30%). Similar increases had been found following bilateral IBO lesions of the LH (21.8% increase in the caudate-putamen and 124.2% increase in the nucleus accumbens: **Winn, Tarbuck and Dunnett**, 1984). This increase was attributed to amygdaloid damage because other neurotoxins which did not damage the amygdala did not induce an increase in DA levels. **Hastings et al** (1985) suggested that destruction of the amygdala either interrupted the projection of this area to the A9/A10 dopaminergic cell bodies or disrupted a direct presynaptic influence of the amygdala upon the striatum. This increase in DA in the nucleus accumbens makes interpretation of feeding results difficult, as **Heffner, Hartman and Seiden** 1980 have demonstrated that DA metabolism increases in the nucleus accumbens during feeding after food deprivation and they suggest that feeding is facilitated by increased DA release from nigrostriatal neurones. This may confound feeding results obtained from rats lesioned in the LH with IBO. Also, IBO has been shown to induce demyelination in areas containing diffuse fibre systems (**Coffey et al.**, 1988) and in doing so to disrupt axonal transport. Thus, although more specific than KAI, because of extra-hypothalamic damage and increased DA levels, some doubt still remains as to whether homeostatic impairments of a motivational nature can be attributed to LH cell loss.

N-METHYL-D-ASPARTIC ACID

N-methyl-D-aspartic acid (NMDA) is a more recently introduced amino acid neurotoxin, which is again a glutamate analogue. The effects of NMA (N-methyl-D,L-aspartic acid) were compared to those of IBO after injection into the LH (**Hastings, Winn and Dunnett,** 1985). It was found that these neurotoxins produced different patterns of extra-hypothalamic damage as NMA tended not to induce any neuronal loss in the amygdala, although it did damage the thalamus to some extent. Lesions remote from the site of injection of NMA were not found. A further advantage of NMA over IBO was that it did not affect DA concentrations in the ventral striatum. Although NMA was found to be only approximately half as potent, on a molar basis, as IBO, it was predicted that NMDA would be equally potent as the L isomer in NMA is not biologically active. It has also been observed that NMDA does not induce demyelination in the LH (Coffey, pers. comm.).

With such advantages over other neurotoxins, experiments were undertaken to assess the histological and behavioural effects of NMDA administration to the LH (**Winn et al,** in preparation). Various doses of NMDA were injected in an attempt to produce the most effective LH lesion without causing extra-hypothalamic damage. From histological analysis, animals were split into two groups; those animals

which had sustained only LH damage (LH) and those which had also sustained substantial extra-hypothalamic damage (LH+). Behavioural analyses demonstrated that both groups showed an initial deficit in feeding and drinking, but that LH animals recovered much more quickly and showed normal weight gain after an initial loss. This pattern of initial weight loss followed, after a period of recovery, by normal weight gain, has been shown in normal control animals yoked to LH lesioned animals' food intake following surgery (Winn et al, in preparation). Thus, the decrease in weight appears to be related to reduced food intake following surgery, and diminishes the need to suggest alterations to body weight set-point "mechanisms". Control animals which had lost weight through reduced intake did not rebound when allowed free access to food, but gained weight at the same rate as unyoked control rats. The LH+ group never fully recovered food or water intake or body weight increase. Both groups, however, displayed deficits to physiological challenges.

Thus, destruction of LH cell bodies using NMDA with no or minimal extra-hypothalamic damage, or disruption of DA fibres of passage, was seen to induce temporary deficits in food and water intake and deficits in response to physiological challenges. The purpose of the following experiments was to investigate the nature of these deficits and in doing so to shed some light on LH involvement in the regulation of food and water intake.

WHAT IS THE NATURE OF THE MOTIVATIONAL IMPAIRMENT IN LH SYNDROME?

Evidence provided from anatomical, electrophysiological and lesion studies implies that the LH has a complex role to play in the regulation of food and water intake. Signals from the periphery concerning energy balance and hydrational state converge on several brain sites, including the LH and the rest of the hypothalamus. It seems unlikely that these areas should all be performing exactly the same function and while other hypothalamic sites may be directly involved in homeostatic regulation, single cell recordings imply that neuronal activity in the LH does not in itself signal hunger or thirst. Thus, the LH cannot be described as a hunger or thirst "centre". Rather, LH responding is modulated by hunger or thirst. It has been suggested that the LH is one part of a system controlling regulation which includes peripheral and central receptors which monitor energy and water levels, brain sites involved in the perception of food or water, motor areas controlling movements to obtain food/fluid and eat or drink and higher cortical sites involved in the selection of behaviour in the face of competing choices of activities (such as sexual behaviour, attack or defence, grooming etc.). The LH receives information from and sends information to all

parts of the system. What role is played by the LH in this system is as yet unclear, but electrophysiological evidence suggests that it may be involved in the assessment of the significance of food related stimuli and excitotoxic lesion studies suggest that it may be involved in the motivation to eat. **Dunnett, Lane and Winn** (1985) demonstrated that lesion of the LH did not produce sensorimotor or motor deficits. Thus, the animals would appear to be able to eat but show some lack of motivation to do so. The nature of this motivational deficit, however, remains unclear and the following experiments were designed to investigate this problem.

Let us consider at this stage the concept of motivation. The word "motivation" is used by many authors to mean many different things and it is important to define clearly what it denotes in this context. The concept of motivation and motivational systems is perhaps most clearly set out by **Toates** in his book "Motivational Systems" (1986). Here he draws heavily on the work of Bindra and refers to motivation as "the strength of the tendency to engage in behaviour when taking into account not only internal factors but also external factors" (p.6). He goes on to state that, "Motivational systems are a class that steers the animal in relation to primary incentive objects in its environment, to make or maintain contact with, escape or avoid such objects. Motivational states are caused by incentive objects (e.g. food) acting in collaboration with

internal states (e.g. energy depletion). Such systems are able to give behaviour a goal direction, since a given incentive object plays vital roles both in causing the motivational state and in defining its direction" (p.161). Thus, motivation arises as a function of both internal state and external factors. These external factors which may stimulate a motivational state are known as "incentives". As motivation arises as a function of internal and external factors, changes in either the internal state or in the incentive may change the motivational state.

If lesions of the LH induce a motivational deficit, what is the nature of the motivational impairment? Four possibilities were hypothesised;

a) INCENTIVE: Animals fail to recognise the rewarding qualities of food/fluid and this change in the perception of external factors or "incentive objects" has changed the motivational state.

b) STIMULUS: The physiological signals arising from changes in internal state which elicit feeding and drinking have been altered.

c) RECOGNITION: The physiological signals which elicit feeding and drinking are intact but are not recognised or understood.

d) RESPONSE: Animals understand intact signals but have a problem in generating the correct response.

These possibilities are described in detail and investigated in Chapters 7, 8, 9 and 10.

CHAPTER 5

GENERAL METHODOLOGY AND HISTOLOGY

A) GENERAL PROCEDURES

For each experiment the specific methodology used is described in detail with the relevant results. However, some procedures were always the same throughout the series of experiments and are therefore described in detail here instead of continually repeating the same methodology.

HOUSING

Male Lister-hooded rats were housed singly before and after surgery under a twelve hour dark-light cycle. The lights were switched on at 8.00am and off at 8.00pm. Wire bottom cages above spill trays were used and pieces of aluminium foil, measuring approximately 4 square inches, were placed under each food hopper to catch any food dropped. The weight of this spillage was then added to the weight of the food in the hopper each day to ensure accurate food intake

measurements (to the nearest 0.1g). In the experiments using wet mash the same procedure was used to collect and measure spillage. Unless otherwise stated, animals drank tap water given to them in plastic water bottles and ate dry lab chow in pellet form (S.D.S No.1 Maintenance Diet) with both food and water available ad libitum.

SURGERY

Surgery for all experiments followed the same general procedures. There were some variations in dose of NMDA or co-ordinates used, but it will be stated clearly in the method section of each experiment exactly which dose or co-ordinates were used for that particular experiment. The reasons for these variations will be discussed in detail in the histology section. LH co-ordinates are always given as anterior-posterior, mm from bregma; lateral, mm from midline; and vertical, mm from dura (**Pellegrino, Pellegrino and Cushman**, 1979).

Before surgery, 10ml/kg Avertin anaesthetic was administered i.p. The concentrate solution of Avertin was made from 100gms of 2,2,2,tri-bromo-ethanol crystals and 62mls of tertiaryamyl-alcohol liquid and was dissolved and stored in darkness. The anaesthetic solution was made from 1.25mls of the concentrate mixed overnight in a dark bottle with 62.5mls of 0.9% saline and 5.0mls of absolute alcohol. Any animal that did not fall unconscious within

approximately 5 minutes of Avertin administration was given more anaesthetic, 1ml at a time, until he did. The animals given extra Avertin did not show any behavioural differences post-surgery when compared to the other rats.

Solutions of NMDA (Sigma) and sterile phosphate buffer were adjusted to approximately pH7 by the addition of concentrated sodium hydroxide (NaOH). Exact pH readings and NMDA concentrations are given with each experiment.

Microinjections were made using a Harvard infusion pump. Injection needles were made by joining approximately 20mm of 30 guage stainless steel tubing to 30 guage polythene tubing with a small amount of araldite glue (Ciba-Geigy). The tubing was flushed through with saline before being attached to a 5 ul glass syringe (Scientific Glass Engineering PTY Ltd) filled with alcohol. A small air bubble was left to prevent the saline and alcohol mixing and to monitor flow. The injection apparatus was then back-filled by pushing the contents of the syringe into the tubing and , therefore, pushing saline out of the needle; drawing in an air bubble to prevent mixing of substances and to monitor flow; and then pulling the syringe plunger back while the needle was immersed in the substance to be injected. Two injection needles for bilateral infusions were attached firmly to the vertical arm of the stereotaxic apparatus by a metal cuff.

After anaesthesia, animals were placed in the stereotaxic frame and heads were held at the angle of de Groot, that is nose held 5mm above the ear bars. The dorsal surface of the skull was exposed and lateral co-ordinates taken from bregma. A dental drill was used to make holes in the skull and vertical co-ordinates were taken from dura, which was then pierced with a needle and the injection needles lowered into place. Infusions were of 1ul over 2 minutes and needles were then left in situ for a further 2 minutes to allow for diffusion away from the tip and to prevent toxin from travelling back up the injection track. Skull holes were filled with gelatin foam (Sterispon) and the wound closed using Michel clips.

NORMAL REGULATORY BEHAVIOUR

Body weight, food intake and water intake were measured daily before and after surgery. These data are provided at the beginning of each experiment so that state of recovery for these particular animals can be seen clearly. Body weight data are presented as percentages, taking day of surgery as 100%. Although body weights do not differ greatly across experimental groups, presenting this data as percentages means that weight loss following surgery is more readily comparable across experiments.

Before surgery, animals were divided into control and lesion groups. Allocation into groups was decided by weight

so that no difference existed pre-surgery between control and lesion groups' average weight or range of weights.

PHYSIOLOGICAL CHALLENGES

It was seen in the experiments carried out by **Winn et al.** (in preparation) that animals with NMDA lesions of LH recovered normal regulatory behaviour almost completely. However, these animals were unable to respond to a battery of physiological challenges. In the following experiments, intracellular dehydration is used as a standard physiological challenge given to all animals to ensure that there was a behavioural basis against which all animals could be measured in conjuction with histological material to assess lesion damage. The individual data will be presented with each experiment, but methodology is always the same and is described below.

Drinking in response to intracellular dehydration was measured by i.p. administration of 20ml/kg 5% (hypertonic) saline. Administration of 0.9% (physiological) saline served as a control and animals received injections of both substances with at least 48 hours between injections. Animals had ad libitum drinking water prior to injection at which point water was removed for 10 minutes and then returned. The amount drunk was measured at 1hr and 3hrs. The order of injections was randomised.

STATISTICS

In many experiments analysis of variance (ANOVA) is used to test for significant differences between groups or conditions. ANOVA was performed on a VAX mainframe computer using the ALICE statistical package available on St. Andrews University SAVA. Post-hoc testing was usually performed with the Tukey test.

B) HISTOLOGY

As the aim of these experiments was to investigate the nature of the deficits seen after lesion of the LH, close attention must be paid to the size and nature of the lesions produced. Conclusions about LH involvement in particular behaviours can only be drawn if comparisons between the nature of the lesions and the nature of the deficits can be made. It is important to look at the size and shape of the lesions and to assess whether fibres of passage have been disrupted and catecholamine levels altered. In the following pages general procedures used to examine the nature of the lesions and an overall picture of the results will be given. The specific histological data

for each group of animals will be described with the experimental results.

GENERAL METHODOLOGY

Animals were deeply anaesthetised with Euthatal (200mg/ml sodium pentobarbital; May and Baker) overdose and perfused intracardially with 0.9% saline followed by 10% phosphate buffered formalin. Brains were removed and stored in 10% formalin solution.

Brains were frozen with dry ice and 40um sections were cut using a bench microtome. Sections were mounted onto subbed glass slides and allowed to dry overnight or for several hours in formalin vapour. Slides were first immersed in xylene to defat sections and were then taken through decreasing concentrations of alcohol (100%, 60%, 30%) to water. Sections were then stained for 2 minutes in cresyl fast violet, followed by 5 minutes immersion in slowly running water. Slides were then taken back through the alcohol solutions to 100% alcohol and, once sections were clearly differentiated, immersed once more in xylene. Cover slips were affixed over sections with DPX mountant (B.D.H.).

Lesion volume was assessed using a drawing tube on a Leitz microscope and drawings from the stereotaxic atlas of the rat brain by **Paxinos and Watson** (1979). It was decided that

simply drawing round the lesion site and assessing lesion volume in mm3 would not account for differences in brain size, distortion during the histological procedure or brain shrinkage at the site of particularly gross lesions. This shrinkage was seen in several animals and simply drawing round lesion area could have given a false impression of damage as in these instances area did not signify cell loss. A standard was therefore used (i.e. pictures from the stereotaxic atlas) and cell loss up to and around the landmarks of the area surrounding the LH were used to describe volume lost. By this method, a series of pictures for each animal was obtained, showing how much and exactly where cell loss had been sustained.

This qualitative analysis was then used to make a quantitative analysis of lesion size by comparing area of damage with area of a standard complete LH as represented in the stereotaxic atlas (**Paxinos and Watson**, 1979). Using a SummaSketch II digitising tablet and Sigma-Scan version 3.0 (Jandel Corporation) on a Tandon AT computer, the size of a standard LH was calculated by drawing round the edges of the LH represented on 7 relevant pages of the stereotaxic atlas and calculating the sum of the area covered in square mm. The number generated is not given, as it represents the area of the pictorial LH, rather than the actual size of the LH in the brain. Lesions were calculated as percentages of the standard by drawing round the lesion

in the LH and calculating the area in square mm. Extra-hypothalamic damage is not included in the analysis.

Due to the nature of the experiments and the questions being asked, it was important to try to get as large a lesion of the LH as possible with as little extra-hypothalamic damage as possible. Because of this, after each experiment histological analyses were carried out and adjustments were made to the concentration or volume of NMDA used in the next experiment according to the histological results. Therefore, if lesions were too small or concentrated in one area, volume and strength of NMDA were increased; if they were too large, volume and strength were decreased in the next experiment. This accounts for the variations in the volume, concentration and placement of cannulae seen between experiments.

RESULTS

Due to the adjustments being made between experiments, lesion volume generally increased throughout the series of experiments. Lesions were found to be quite equal bilaterally and no animals were discarded because of unilateral damage. Animals which responded as controls to physiological challenges were found to have extremely small or misplaced lesions and were discarded from the results. Details of individual experimental groups are given with the behavioural data.

Typically, greatest cell loss occurred in the central region of the LH with greatest sparing at the anterior and posterior poles. Rather than loss of a small amount throughout the LH, most lesions removed the central region completely and did not damage one or both of the poles. This is not surprising due to the tubular shape of the LH. It would seem that it is virtually impossible to remove the whole area with one injection, without causing substantial extra-hypothalamic damage. In general, from the level of the paraventricular nucleus back to the level of the dorsomedial hypothalamus there was substantial cell loss extending medially to the fornix, laterally to the internal capsule, dorsally up to and including the zona incerta and ventrally to the optic tract. The extent to which damage extended beyond this area into the anterior and posterior poles varied between individuals and experimental groups and is reported with the behavioural data.

If the results from all experiments are summed, the average lesion size was 59.12% (Standard Error= 2.32). The lesion size varied from experiment to experiment and is described for individual groups with the behavioural data. Lesions ranged from 28.7% to 100%. This quantitative analysis of lesion size is intended to be used as a guide across experiments and to give the reader a general picture of NMDA damage, rather than as an exact measure of cell loss. Analysis of LH neuronal damage in a quantitative manner is

extremely difficult as the LH has no clear boundaries and is often called the "lateral hypothalamic area" because of this.

It is important to note here that, although 59.12% may not seem very large, cell loss in these animals was greater than in animals showing aphagia and adipsia following electrolytic lesions (e.g. **Powley and Keesey**, 1970). In such cases, the poles are nearly always spared and only the far lateral hypothalamus damaged.

Extra-hypothalamic damage varied from experiment to experiment, but there was consistent cell loss in the zona incerta and reticular nucleus of the thalamus. Other areas sometimes damaged included the globus pallidus, thalamus and subthalamic nucleus. Occasionally there was a little damage to the amygdala. Thalamic damage probably resulted from leakage back up the cannulae tracks.

Some examples of lesions are given in figures 4, 5, and 6. Figure 4 depicts an average or typical lesion, chosen as the closest representation of 59% cell loss, while figure 5 depicts the largest and figure 6 the smallest lesion accepted in any experiment. A picture of a "typical" lesion for each experimental group is presented with the hisological data for that particular group. The lesions presented were chosen as the closest to the mean lesion size of that group.

FIGURE 4: An average lesion is depicted on the following page. Shaded area represents lesion site. As can be seen, no cell loss was found at the posterior pole. (Rat J.38/88)

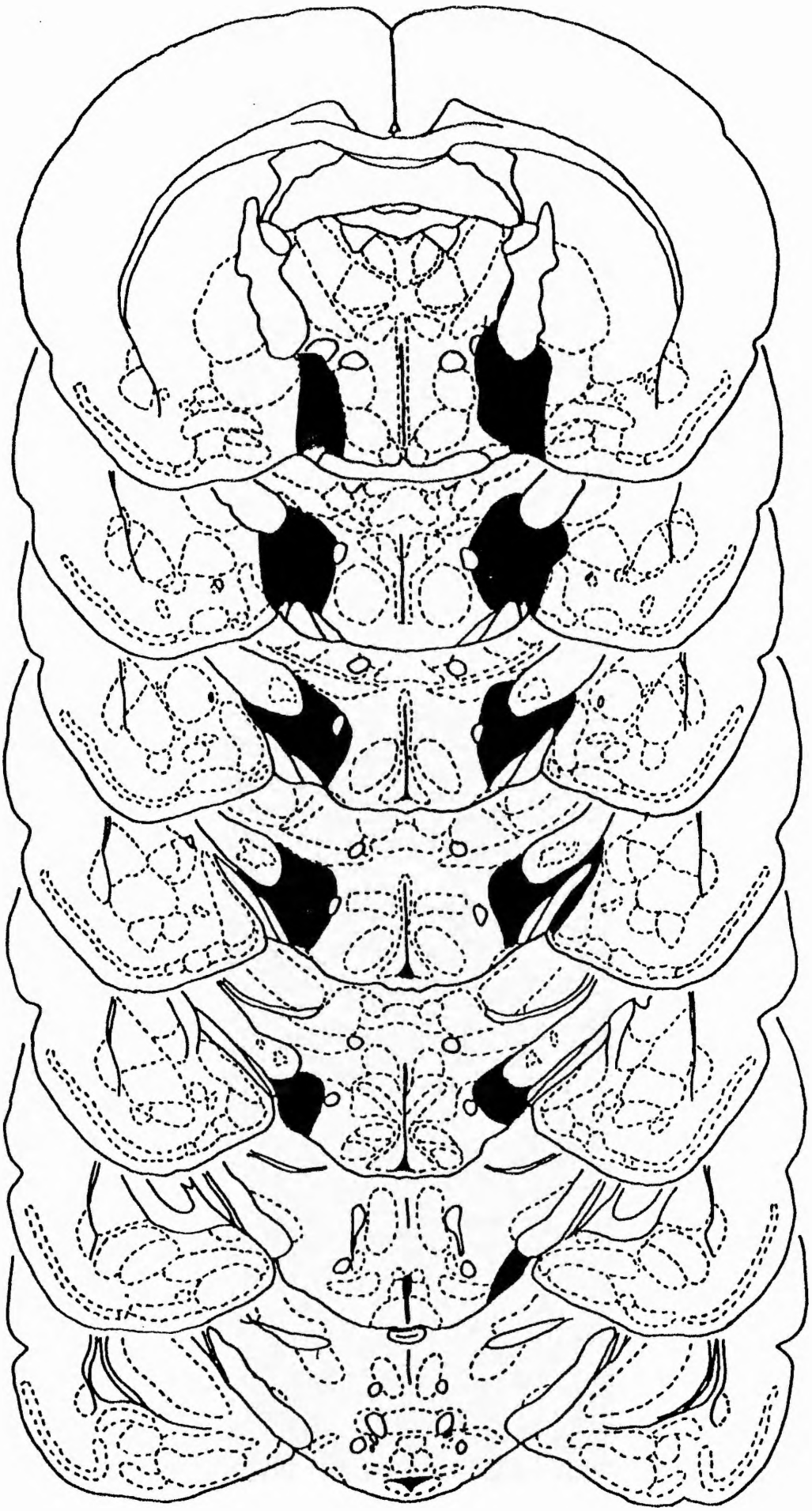


FIGURE 5: The lesion depicted in this figure was the largest found throughout experimentation. Shaded area represents the lesion site. The whole of the LH has been destroyed, but there is also substantial extra-hypothalamic damage. This animal was not used in a behavioural experiment. (Rat J.71/88)

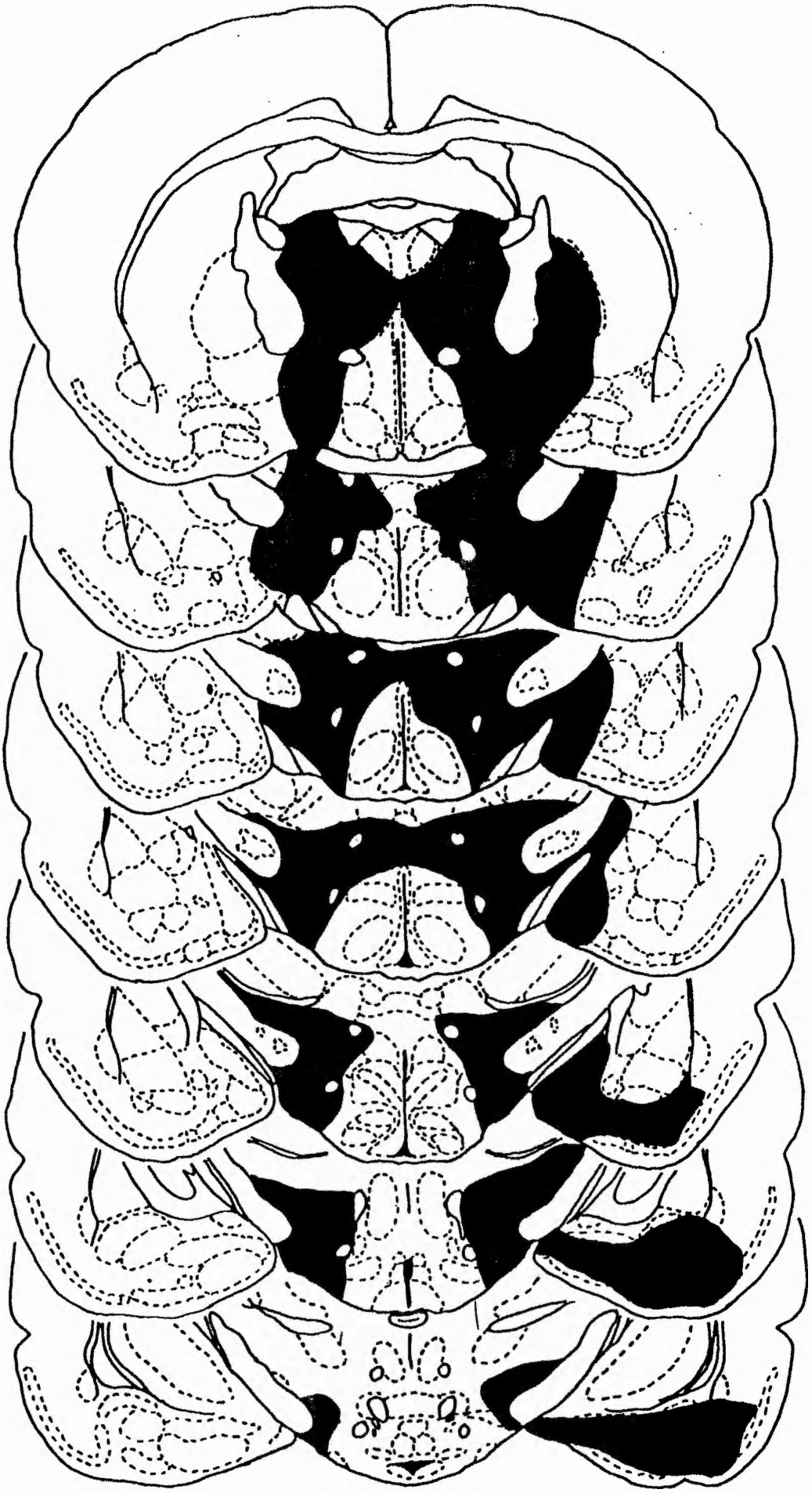
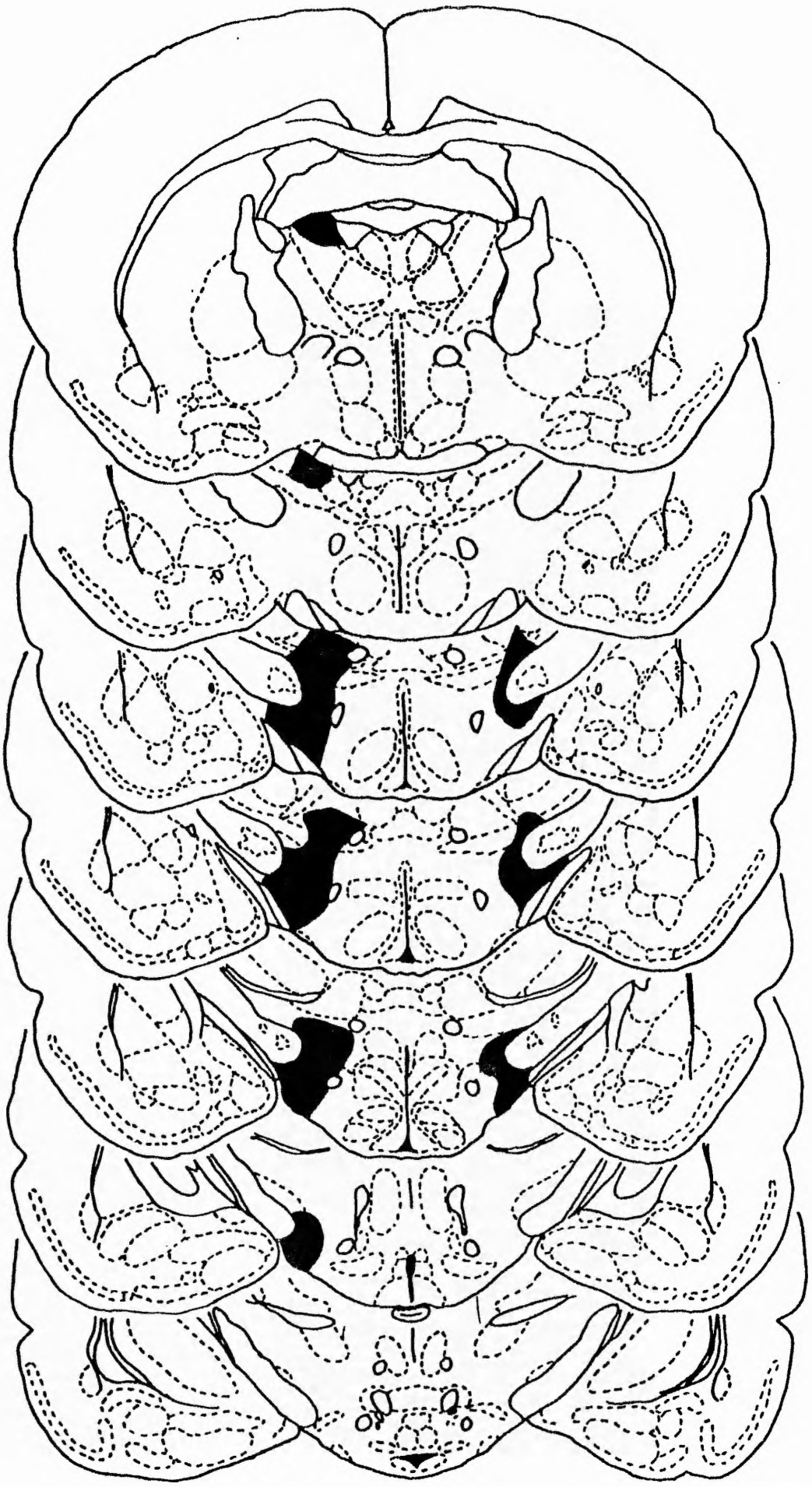


FIGURE 6: The lesion depicted in this figure was the smallest accepted in any experiment. Shaded area represents the lesion site. While substantial portions of the central core of the LH have been destroyed, the anterior and posterior poles have not been damaged. (Rat J.200/88)



CONCLUSIONS

Histological analysis of the NMDA LH lesions described in the following chapters indicates that cell loss was mainly from the central core of the LH, with some sparing at the anterior and posterior poles. Average lesion size was 59% loss of LH neurones and lesions ranged from 28.7% to 100%.

CHAPTER 6

DOPAMINE ASSAY

It was very important to assess the effects of NMDA lesions made in the LH on dopamine levels in the striatum. As mentioned in the introduction, feeding and drinking deficits seen following electrolytic LH lesions were attributed by some authors to the loss of striatal catecholamines, particularly DA, (**Ungerstedt**, 1971) rather than to the loss of intrinsic LH neurones. If NMDA lesions of the LH did not disrupt striatal DA, then consummatory deficits could not be attributed to striatal DA loss and could more readily be attributed to LH neuronal damage. Also, attempts to lesion the LH with the excitotoxin ibotenic acid (IBO) resulted in increased DA concentrations in the nucleus accumbens (**Winn, Tarbuck and Dunnett**, 1984). This DA increase may confound the results of IBO experiments as increases in DA metabolism in the nucleus accumbens have been associated with feeding following food deprivation (**Heffner, Hartman and Seiden**, 1980). Thus, if DA was not increased in the nucleus accumbens by NMDA lesions, then behavioural results would not be confounded in this way and NMDA could be suggested to be a more

suitable excitotoxin to use for making lesions of the LH than ibotenic acid and if DA was not reduced, then consummatory deficits could not be attributed to striatal DA loss.

Measurements of striatal DA following unilateral NMA lesions of the LH have already been performed (**Hastings, Winn and Dunnett**, 1985) and no significant DA alterations were found. However, bilateral lesions can sometimes produce much greater effects than unilateral lesions as can be seen when IBO is used. The bilateral IBO lesions reported in **Winn, Tarbuck and Dunnett** (1984) induced 124% DA increase in the nucleus accumbens, whereas unilateral lesions (**Hastings, Winn and Dunnett**, 1985) only induced a 30% DA increase. Thus bilateral NMDA lesions could produce differences in DA concentrations not seen following unilateral lesions. The differences in DA concentrations found in these studies could be attributed to different post-op survivial times, but all possibilities must be considered. Also, in the following experiments NMDA was used, as opposed to NMA used by **Hastings et al.** (1985) which is assumed to be only half as potent because it contains both the biologically active D isomer and the inactive L isomer. The more potent form of the neurotoxin may produce different results. Thus, analyses of levels of dopamine and its metabolites in the striatum were important to assess the specificity of the NMDA lesions as compared to other lesion techniques and to address the arguments put

forward suggesting that alterations in feeding following LH lesions are a result of DA alterations rather than destruction of the LH itself.

SURGERY

Ten male Lister-hooded rats were housed as described in the general procedures section under a twelve hour dark/light cycle. Using 10 ml/kg avertin as anaesthetic, bilateral LH lesions were made in 5 animals by microinjection of 1.0 ul of 0.12M NMDA (pH 6.8) at the following stereotaxic co-ordinates in the orientation of de Groot: 0mm from bregma; ±2.0mm lateral; -8.0mm vertical from dura (Pellegrino, Pellegrino and Cushman, 1979). NMDA was infused at 0.5 ul/min and injection cannulae were left in situ for 4 minutes. Control animals (n=5) were microinjected with phosphate buffer (pH 7.4) at the same co-ordinates. Average weight of all animals pre-surgery was 406.2 gms (SE=11.47; range 361.4gms-450.5gms).

NORMAL REGULATORY BEHAVIOUR

Body weight, food and water intake were measured daily. Aphagia, adipisia and body weight loss were severe in the LH lesioned animals and steps had to be taken to keep them alive. Post-surgery they were given 6.3% glucose solution to drink rather than tap water and were given Farex baby food to eat. Dry lab chow was still available, but was

mostly ignored by these rats. Some recovery took place and the glucose solution was replaced by water, but animals were still unable to maintain themselves on dry lab chow. Most of their daily water intake came from the Farex wet mash which was made up at 50% w/v with tap water. Normal body weight gain was never recovered (see Fig 7). Overall analysis of pre- and post-op percentage weights by ANOVA revealed a significant difference between the groups ($F=44.0361$; $df=1,8$; $p=0.0002$) and an interaction between groups and time ($F=34.2049$; $df=3,24$; $p<0.001$). One week pre-op there was no significant difference between groups (ANOVA: $F=0.1662$; $df=1,8$; $p=0.6942$), but weeks 1, 2 and 3 post-op revealed a significant groups effect (ANOVA: week 1, $F=56.7095$; $df=1,8$; $p<0.001$: week 2, $F=40.1968$; $df=1,8$; $p=0.0002$: week 3, $F=37.6386$; $df=1,8$; $p=0.0003$).

The regulatory deficits seen in these animals were greater than those observed in any subsequent experiments. Such severe lesions were thought to be less unfortunate in this context; if no alterations in catecholamine levels could be detected following such complete LH neuronal destruction, then no changes would be expected following smaller lesions. As no behavioural tests were being performed, extra-hypothalamic damage was not so important as complete LH destruction.

FIGURE 7: The percentage body weights of control and lesioned animals used for HPLC analysis of forebrain catecholamine levels are presented in this figure. One week pre- and three weeks post-operation are shown. Day of surgery is taken to be 100%. ANOVA revealed no significant differences between groups before surgery, but a permanent difference between groups post-surgery.

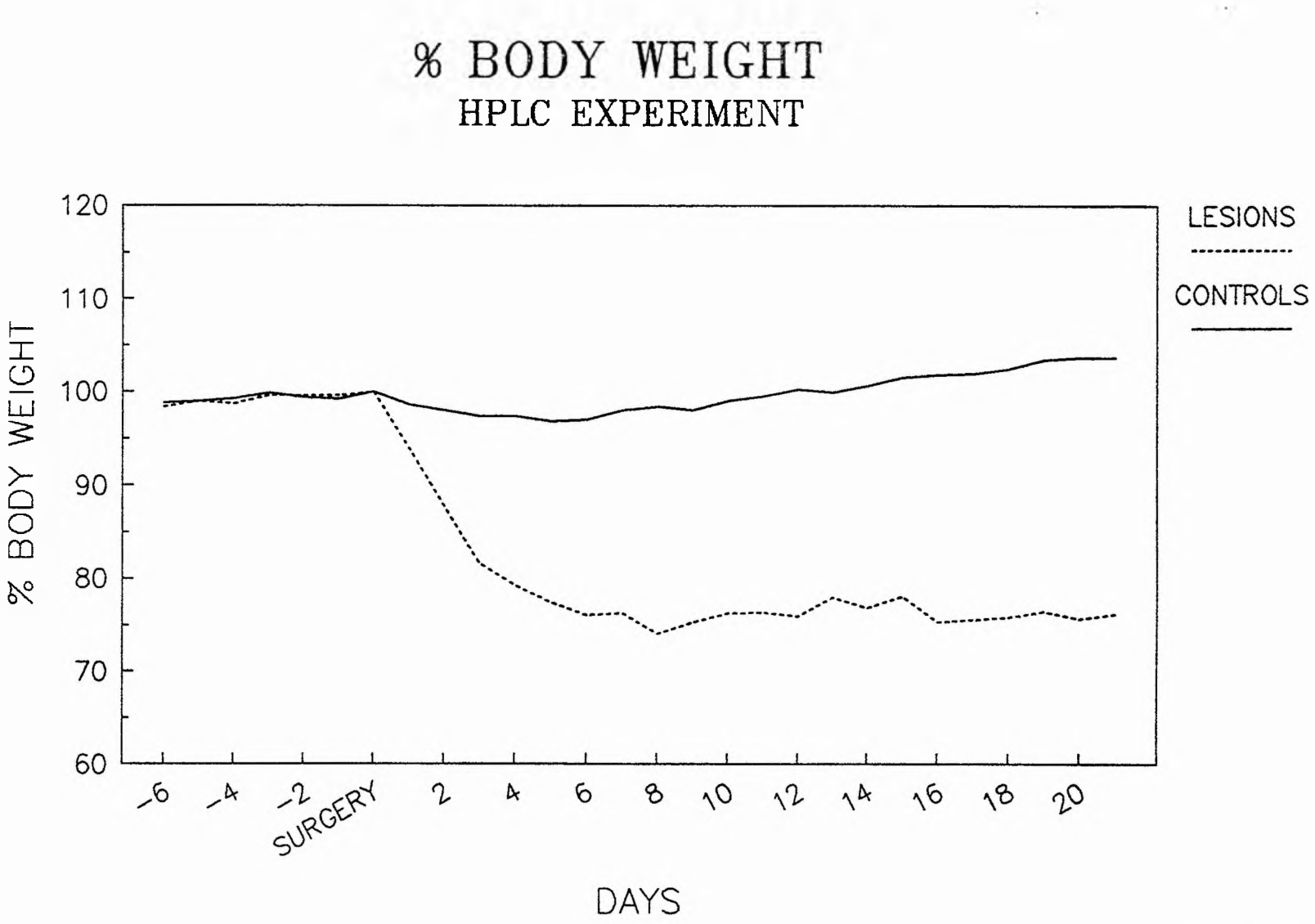

% BODY WEIGHT
HPLC EXPERIMENT
120
110
100
90
80
70
60
% BODY WEIGHT
LESIONS
CONTROLS
-6
-4
-2
SURGERY
2
4
6
8
10
12
14
16
18
20
DAYS

FIG 7

BIOCHEMICAL ANALYSIS

Animals were lightly anaesthetised with halothane (ICI) and decapitated. Brains were removed and two coronal sections were cut from which, first, anterior dorsal and anterior ventral striatum; and second, posterior dorsal and posterior ventral striatum were dissected freehand. These sections were frozen and stored at -20 degrees centigrade before assay. The remainder of the brain, including the LH, was stored in 10% formalin solution before histological analysis took place.

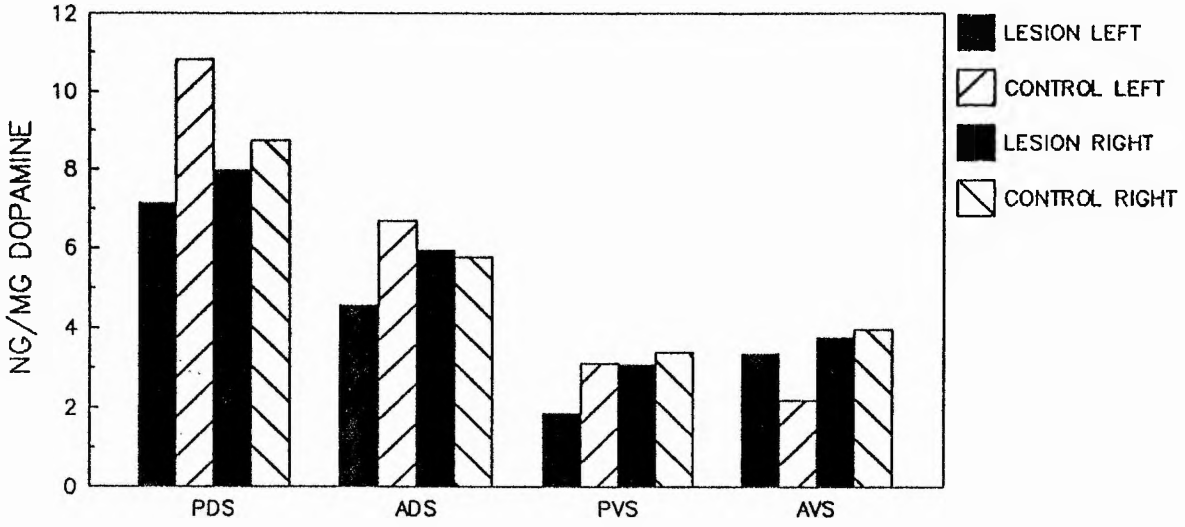
Levels of DA, DOPAC and HVA were assessed by HPLC according to the method of **Lin and Blank** (1983). Results can be seen in Fig 8. No significant differences were found between the lesioned or control animals on any measure.

HISTOLOGY

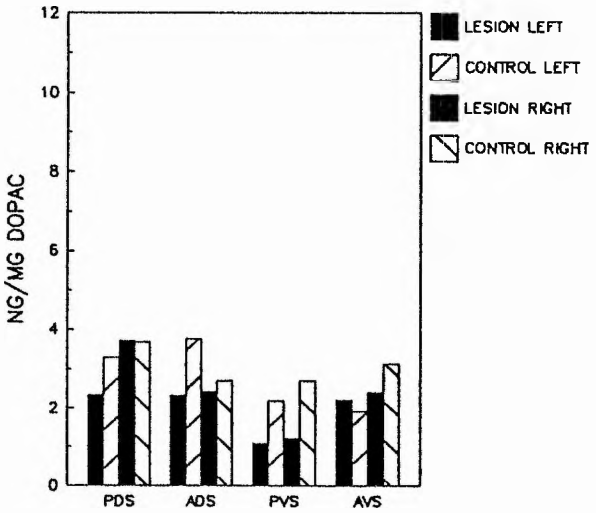
Histological data was gathered by the methods described above. Lesions in this group of animals were found to be particularly large, with an average size of 94.1% (SE=3.49) and a range of 86.3% to 100%. Damage was greatest from the anterior pole through the central core and past the level of the DMH. Cell loss was least at the posterior pole. There was extensive extra-hypothalamic damage, particularly

FIGURE 8: In this figure striatal levels of dopamine, DOPAC and HVA are presented. Levels in both the left and right hemisphere are presented for both lesioned and control animals. The clusters represent levels in the posterior dorsal striatum (PDS), the anterior dorsal striatum (ADS), the posterior ventral striatum (PVS) and the anterior ventral striatum (AVS). Standard errors were never greater than 2.14. Range and variance within groups was large and no differences were found between groups.

DOPAMINE IN THE STRIATUM



DOPAC IN THE STRIATUM



HVA IN THE STRIATUM

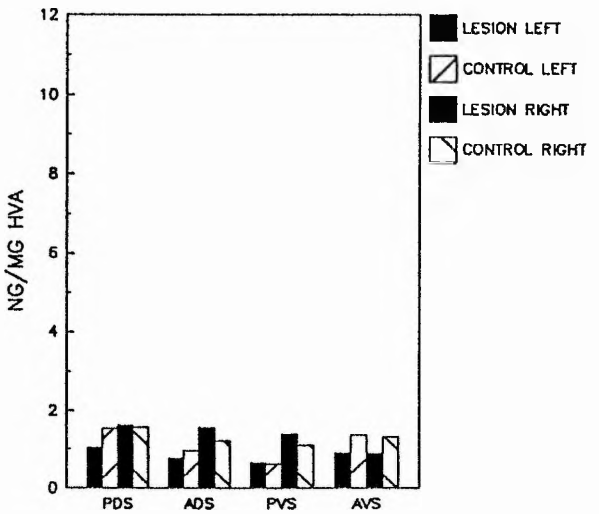
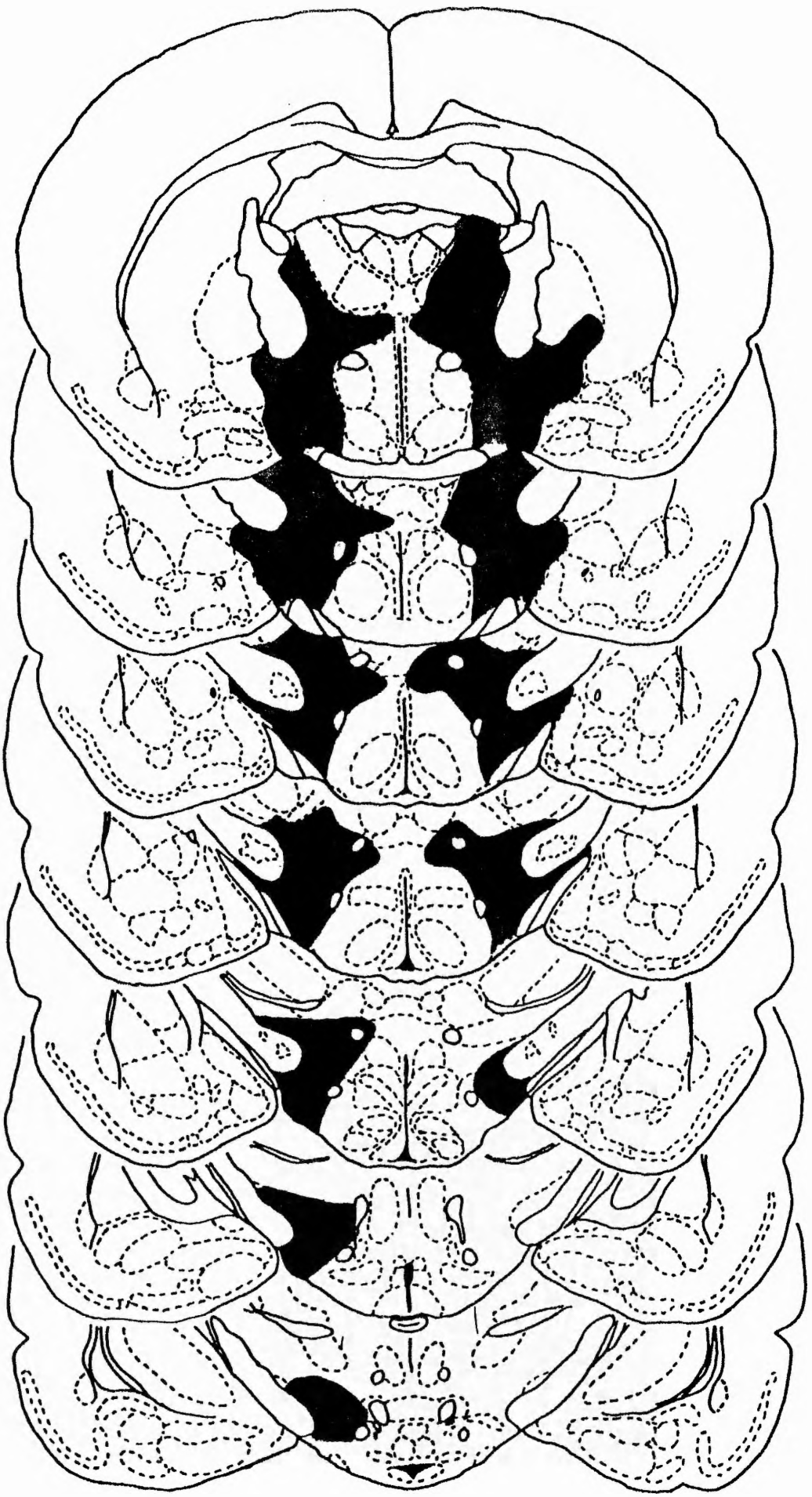


FIG 8

FIGURE 9: The following picture displays the size and pattern of a lesion typical for this experimental group. the shaded area represents the lesion site. Some sparing of the posterior pole was found and extra-hypothalamic damage was found in the thalamus. (Rat J.82.87)



in the thalamus, zona incerta and subthalamic nucleus. The pattern and size of a typical lesion can be seen in Fig 9.

DISCUSSION

The animals used for this experiment had received very large lesions, greater than any other group subsequently described, and showed marked impairments in regulating food and water intake. They did not show any significant differences in levels of striatal DA or metabolites in comparison to controls. Transmitter concentration and turnover (as indicated by metabolite levels) were found to be normal. This would indicate that, even in severe cases, lesion of the LH using NMDA does not disrupt or affect passing dopaminergic fibres such as to deplete DA. Although it is not possible to attribute the behavioural deficits reported to LH damage alone because of the extensive extra-hypothalamic damage, this would indicate that consummatory deficits seen in these animals could not be attributed to a malfunction of striatal DA. These results suggest that NMDA is a more effective excitotoxin for investigations of LH functioning than ibotenic or kainic acid.

CONCLUSIONS

The lesions in this experiment were very large, larger than in most subsequent experiments. As there was neither loss

of striatal DA nor metabolites in this study, it can be suggested that disruption of forebrain DA does not account for the behavioural changes which follow the lesions described in the following experiments, although replication with larger groups would be required to hold this position with confidence.

CHAPTER 7

INCENTIVE

The work of **Winn, Tarbuck and Dunnett** (1984), in which the cells of the LH were destroyed with IBO, demonstrated that electrolytic lesions of the LH damage two components of a single feeding system; a "motor" component and a "motivational" component. The motor component appears to be intact following IBO lesions, suggesting that destruction of intrinsic LH neurones induces a "motivational" deficit. The nature of this motivational deficit remains unclear.

In the definition given of motivation in Chapter 4, motivated behaviours, including feeding and drinking are seen to arise as a function of both internal state ("drive") and external factors ("incentive"). In simple terms, "drive" refers to the state of deprivation or dehydration, while "incentive" refers to external factors which are able to influence the motivational state. According to **Toates** (1986), stimuli are either hedonically positive, hedonically negative or neutral. Those stimuli which are hedonically positive or negative can be termed "incentive stimuli". The sensory qualities, the taste and texture, of food or fluid , are, therefore, "incentive stimuli".

As already mentioned, motivation arises as a function of "drive" and "incentive". This means that "drive" and "incentive" 'gate' each other so that food deprivation potentiates the incentive value of food and a highly palatable diet will be eaten beyond the amount needed to satisfy hunger (Sclafani and Springer, 1976). In other words, as drive falls, incentive could act to prolong feeding and as incentive falls, drive could act to prolong feeding. A food deprived rat is more likely to eat quinine adulterated food that a satiated one and satiated animals are more likely to eat palatable food than unpalatable or neutral food.

If LH lesioned animals display regulatory deficits because of a motivational deficit, this may be due to either a change in "drive" or "incentive" as a change in either would effect an overall change in motivational state. If LH lesioned animals lack the capacity to understand or recognise "incentive" in some sense, and eat only in response to "drive" arising from their internal state of deprivation, then they should not respond to purely sensory changes in their diet; that is they should not respond to adulteration of their food and water with agents which affect only the palatability of the diet, not the caloric density. They should be governed only by the state of dehydration or deprivation. This means that, whereas normal animals should overeat or overdrink a palatable diet and

undereat or underdrink an unpalatable diet, LH lesioned animals should not alter the amount consumed. Substances used in this experiment to alter the taste of the diet were quinine (negative) and saccharin (positive).

SURGERY

Average weight before surgery of the 30 male Lister-hooded rats used was 379gms (SE=6.84; range = 283.7gms - 441.5gms). Normal housing procedure was used as previously described.

Bilateral LH lesions were made in 18 rats by microinjection of 1.0ul of 0.06M NMDA (pH 6.98) [Sigma] and 12 control animals were microinjected with 1.0ul of sterile phosphate buffer (ph7.4) at the following stereotaxic co-ordinates: (in the orientation of de Groot): bregma +0; lateral $\pm$ 2.0mm; vertical $\pm$ 8.0mm.

One animal in the lesion group died within 24 hours of surgery and one sham-operated rat died after 2 weeks. Their data have not been included in the results.

GROUPS

Rats were split into 4 groups of approximately equal weight before surgery so that they could get used to the appropriate type of food delivery, and effects of novelty

in dietary texture post-op would not confound the results.

The groups were treated as follows:-

Number of Animals in group:		Treatment	Food
A	6	sham operation	dry lab chow
B	8	LH lesion	dry lab chow
C	5	sham operation	wet mash
D	9	LH lesion	wet mash

Wet mash was made up in a 1:1 w/v ratio from powdered lab chow (S.D.S. No.1 Maintainance Diet) and tap water. This made food adulteration easier, as powdered quinine or saccharin could be mixed in to the wet mash. There was no difference in calorific or nutrient value of the diet as the dry lab chow pellets given to groups A and B were ground up to make the powder for groups C and D.

NORMAL REGULATORY BEHAVIOUR

BODY WEIGHT: [Fig 10] Body weight was measured daily. For this analysis all lesioned animals are treated as one group and all control animals as one group. One week pre-op there was no significant difference between groups' percentage body weight (ANOVA: $F=0.8924$; $df=1,27$; $p=0.3532$) or rate of growth, indicated by the fact that there was no

FIGURE 10: The percentage body weight of control and lesioned animals in the experimental group given food or water adulteration are presented in this figure. One week pre- and three weeks post-operation are shown. Day of surgery was taken as 100%. ANOVA revealed a significant difference between the groups one week after surgery. This difference was not present at any other time.

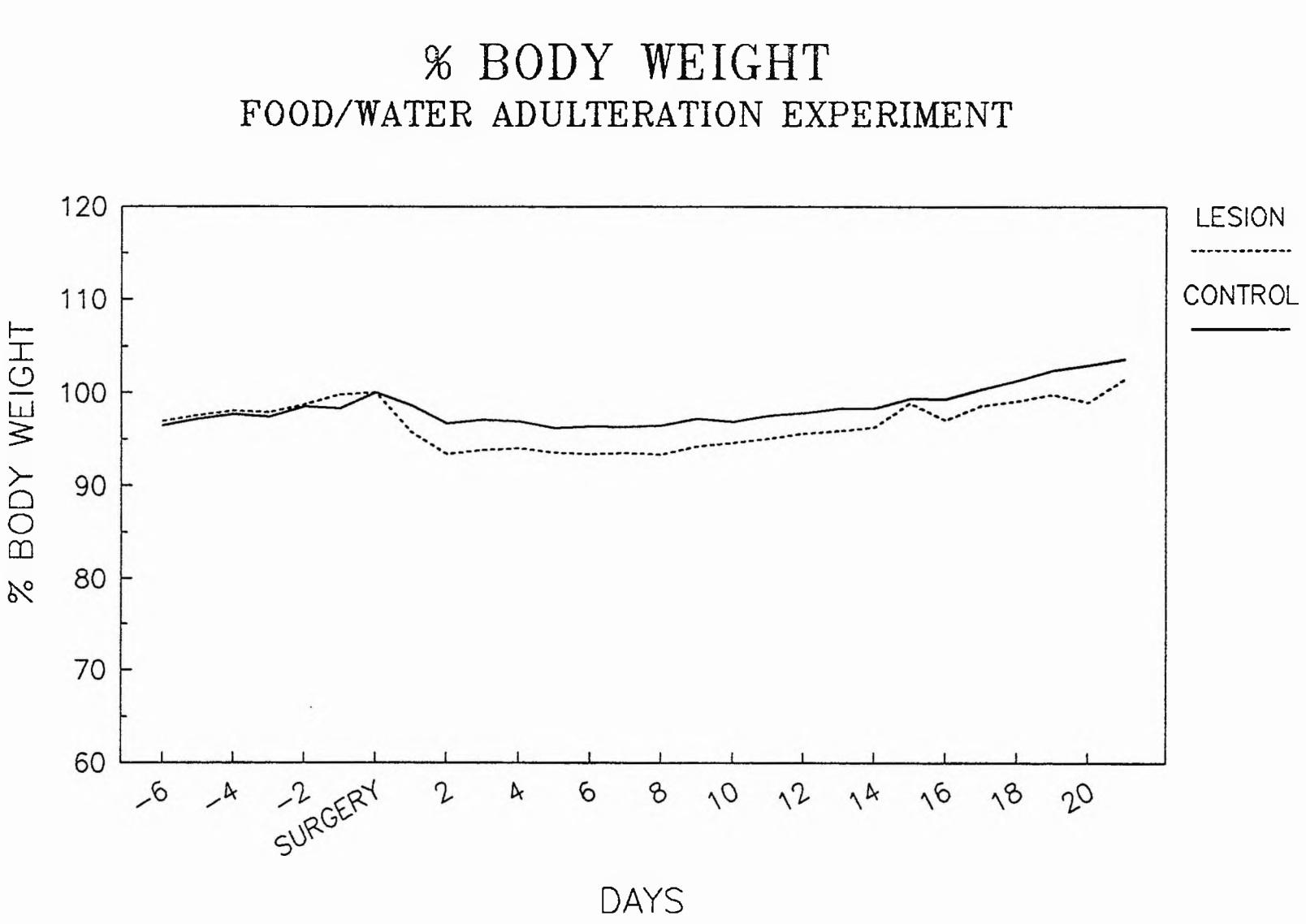

% BODY WEIGHT
FOOD/WATER ADULTERATION EXPERIMENT
120
110
100
90
80
70
60
% BODY WEIGHT
LESION
CONTROL
-6
-4
-2
SURGERY
2
4
6
8
10
12
14
16
18
20
DAYS

FIG 10

interaction between groups and time (ANOVA: F=0.4465; df=6,162; p=0.8467). One week post-op, ANOVA revealed a significant groups effect (F=6.7715; df=1,27; p=0.00149), but this difference between the groups was not found 2 weeks post-op (F=2.5256; df=1,27; p=0.1237) or 3 weeks post-op (F=2.1263; df=1,27; p=0.1563). No interaction between time and groups was found post-op (ANOVA: week 1, F=0.5783; df=6,162; p=0.7472: week 2, F=1.0316; df=6,162; p=0.4067: week 3, F=0.9959; df=6,162; p=0.43). Thus, after an initial drop in weight post-op, lesioned animals maintained their weight as controls.

FOOD INTAKE: [Figs 11 and 12] Food intake was measured daily pre- and post-op before adulteration. ANOVA revealed no significant differences in food intake between "lesioned/lab chow" animals and their controls either before (F=1.0836; df=1,12; p=0.3184) or after surgery (week 1, F=4.1737; df=1,12; p=0.0637: week 2, F=0.2618; df=1,12; p=0.6182: week 3, F=0.5186; df=1,12; p=0.4852) and no significant differences in food intake between "lesioned/wet mash" animals and their controls either before (F=0.1442; df=1,13; p=0.7103) or after surgery (week 1, F=0.3712; df=1,13; p=0.5529: week 2, F=0.0273; df=1,13; p=0.8714: week 3, F=0.0069; df=1,13; p=0.9350). Thus, lesioned animals maintained their food intake at the same level as control animals.

FIGURE 11: The food intake for "lesioned/lab chow" and "control/lab chow" groups for one week pre- and three weeks post-operation are presented in this figure. Both groups decreased intake following surgery and ANOVA revealed no significant differences between the groups at any stage.

FOOD INTAKE

LAB CHOW GROUPS

FOOD/WATER ADULTERATION EXPERIMENT

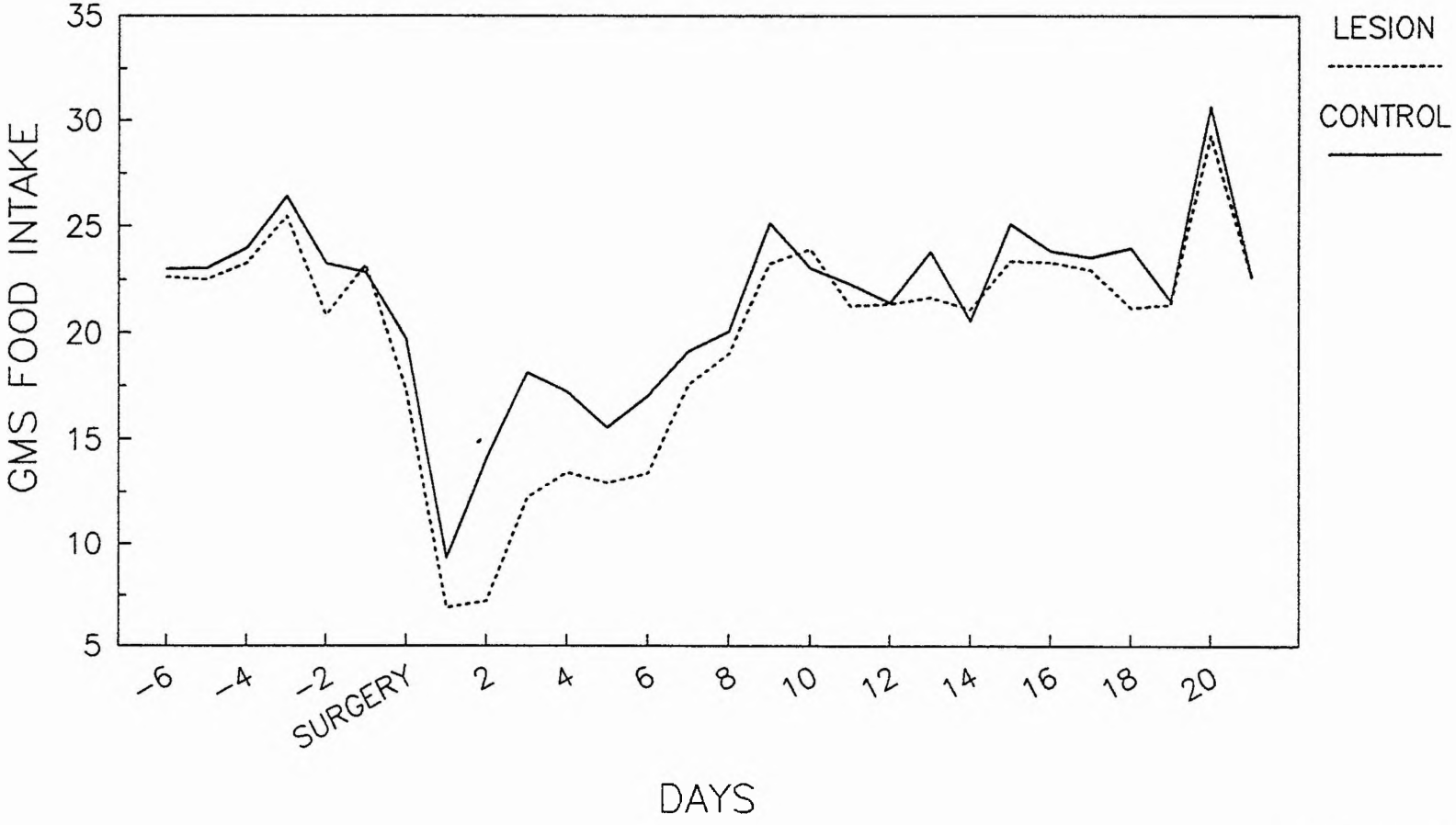


FIG 11

FIGURE 12: The food intake of "lesioned/wet mash" and "control/wet mash" groups for one week pre- and three weeks post-operation are presented in this figure. Gms food intake represents the amount of wet mash eaten and, therefore, includes some liquid intake. Both groups decreased intake following surgery and ANOVA revealed no significant differences between the groups at any stage.

FOOD INTAKE

WET MASH GROUPS

FOOD/WATER ADULTERATION EXPERIMENT

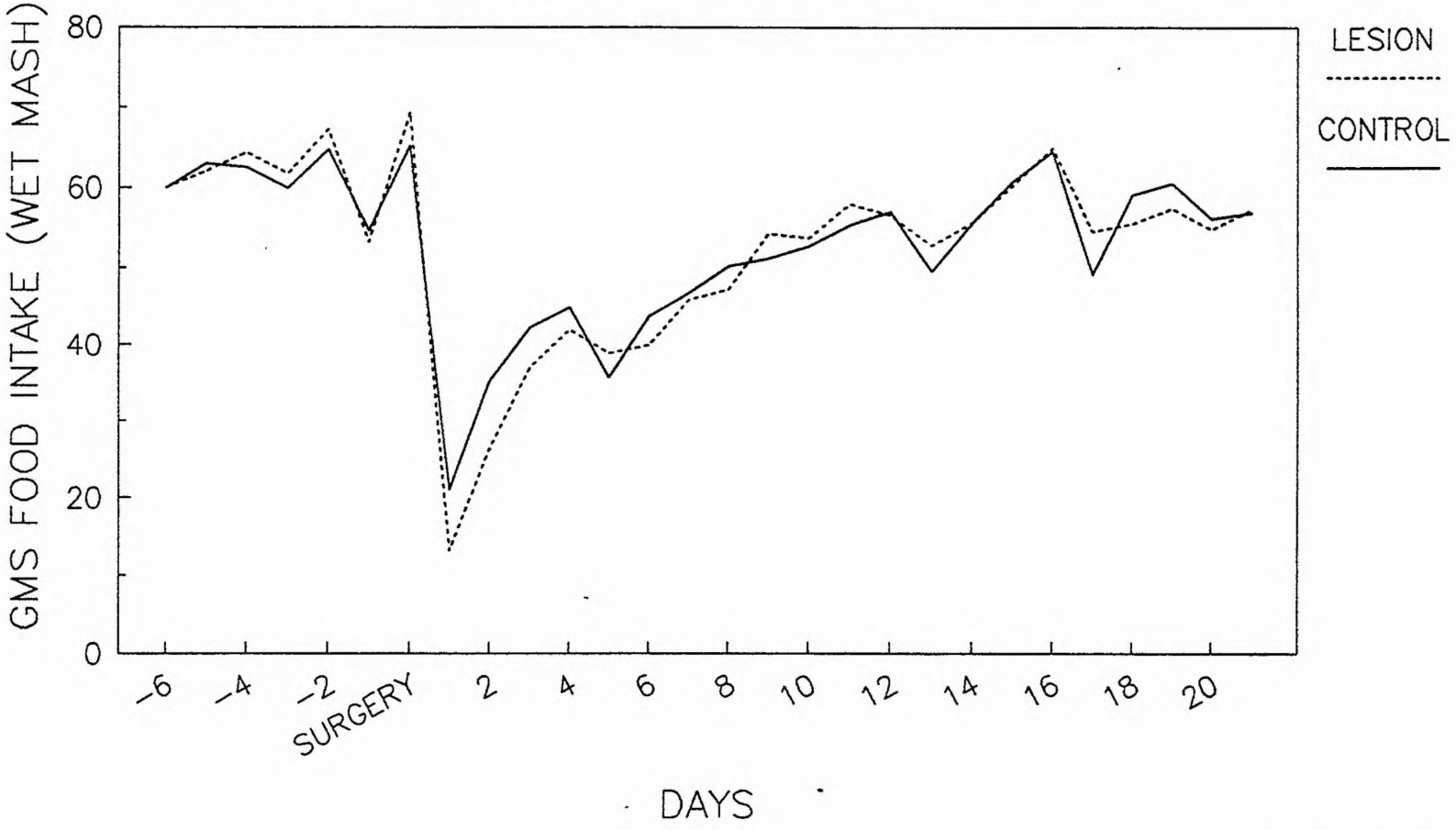


FIG 12

WATER INTAKE: [Figs 13 and 14] Water intake was also measured daily pre- and post-op before adulteration. "Lesioned/lab chow" animals did not differ from their controls either before (ANOVA: F=0.3115; df=1,12; p=0.587) or after surgery (ANOVA: week 1, F=4.1737; df=1,12; p=0.0637: week 2, F=0.2618; df=1,12; p=0.6182: week 3, F=0.5186; df=1,12; p=0.4852). "Lesioned/wet mash" animals did not differ significantly from their controls before surgery (ANOVA: F=0.5738; df=1,12; p=0.4622), but there was a significant difference between the groups 1 week post-op (ANOVA: F=13.613; df=1,13; p=0.0027). This difference was no longer present in week 2 (ANOVA: F=3.7566; df=1,13; p=0.0746) or week 3 (ANOVA: F=1.2427; df=1,13; p=0.2851). Thus, after an initial period of hypodipsia post-op, lesioned animals maintained their water intake at the same level as control animals.

PHYSIOLOGICAL CHALLENGES

(For the physiological challenges all lesioned animals are treated as one group and all sham animals as one group.)

Hypertonic saline was administered according to the method already discussed (p. 65). After 1hr, ANOVA revealed an interaction between groups and conditions (F=4.2075; df=1,26; p=0.05). Post-hoc testing with Tukey test showed that there was no significant difference between the

FIGURE 13: Water intake of "lesioned/lab chow" and "control/lab chow" groups for one week pre- and three weeks post-operation are presented in this figure. Both groups decreased intake following surgery and ANOVA revealed no significant differences between groups at any stage.

WATER INTAKE

LAB CHOW GROUPS

FOOD/WATER ADULTERATION EXPERIMENT

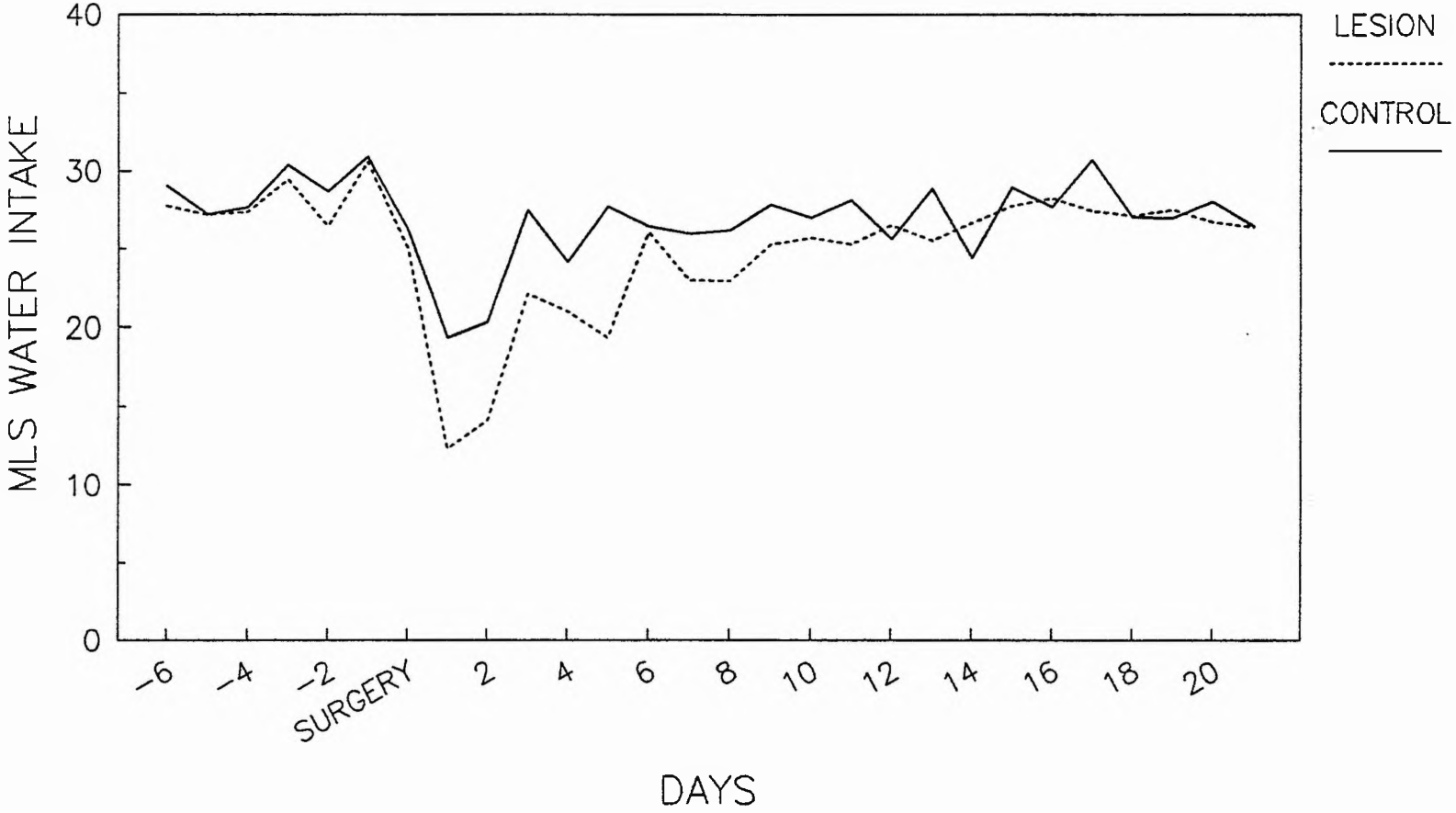


FIG 13

FIGURE 14: Water intake of "lesioned/wet mash" and "control/wet mash" groups for one week pre- and three weeks post-operation are presented in this figure. Lesioned animals reduced their intake for one week after surgery and ANOVA revealed a significant difference between the groups at this stage. No significant differences were found between groups at any other stage.

WATER INTAKE

WET MASH GROUPS

FOOD/WATER ADULTERATION EXPERIMENT

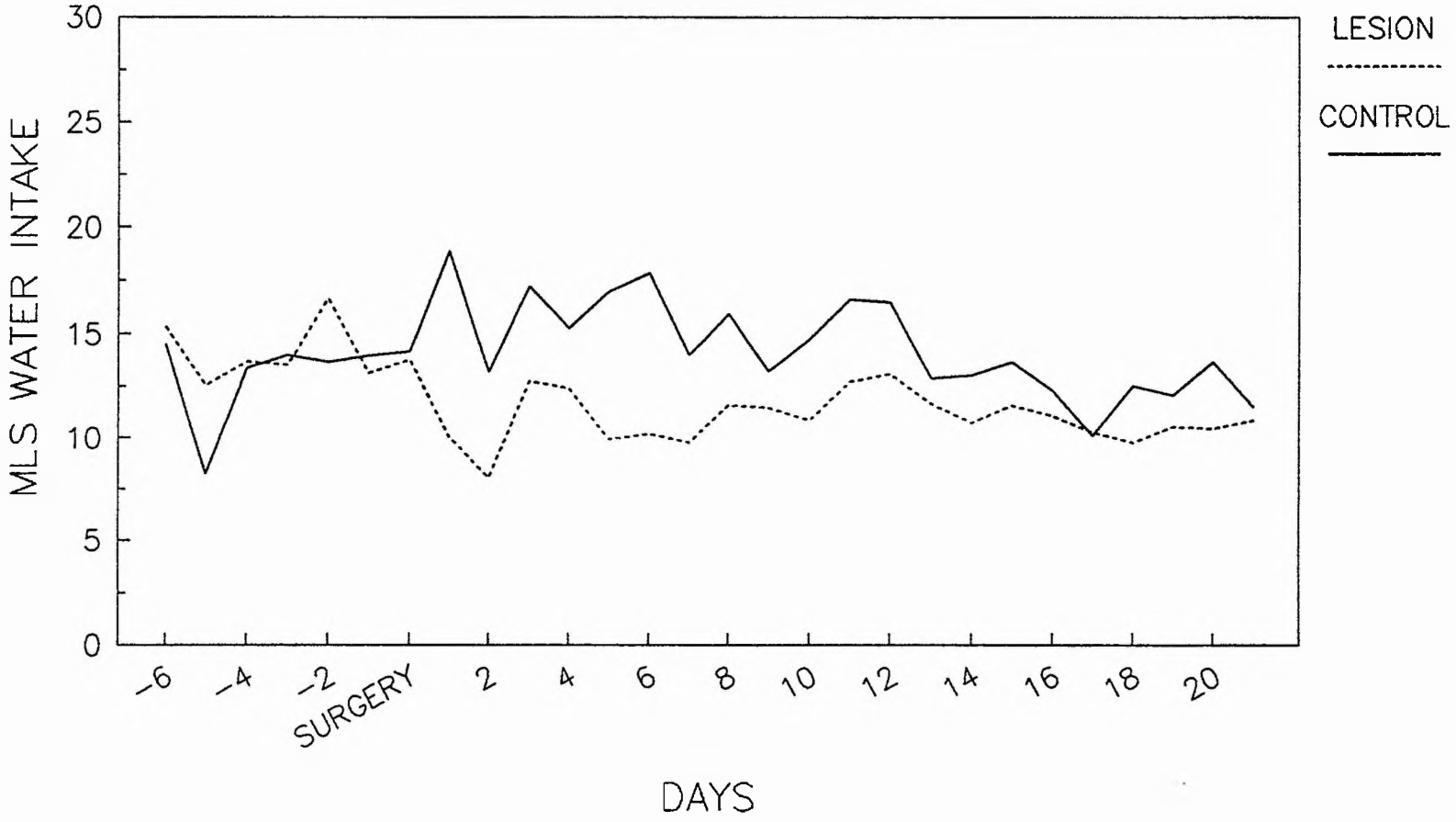
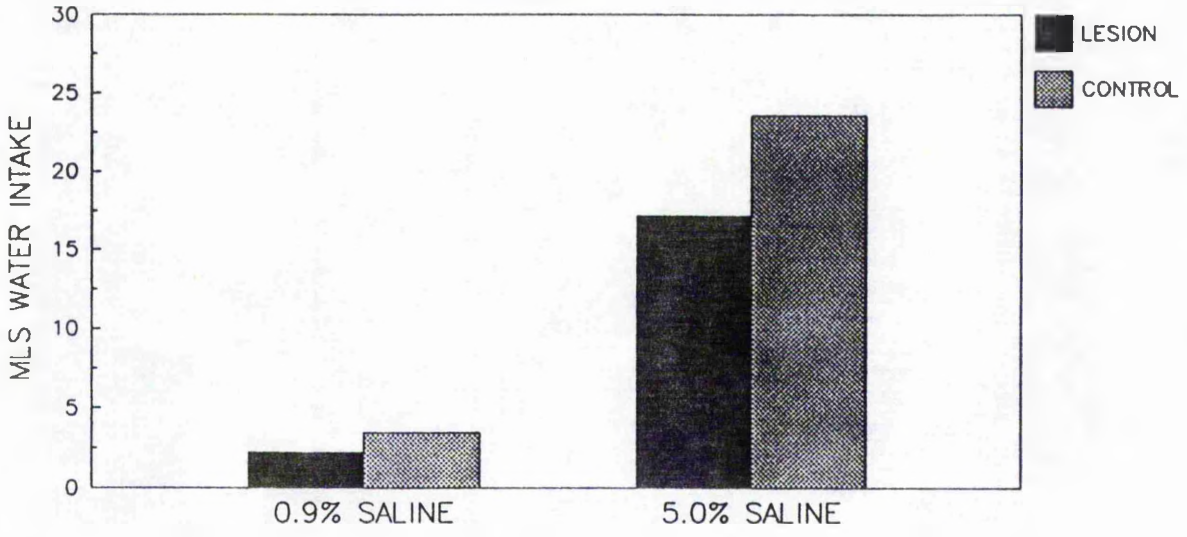


FIG 14

groups regarding amount drunk following 0.9% saline injection, but that there was a significant difference ($p<0.01$) between the groups after administration of hypertonic saline, even though both groups had increased their intake significantly ($p<0.01$) compared to isotonic saline administration. No interaction was found at 3hrs ($F=2.8033$; $df=1,26$; $p=0.1061$). These data are presented in Fig 15.

Eating in response to glucoprivation was measured by i.p. injection of 0.75g/kg 10% w/v 2-deoxy-D-glucose (2-DG) as compared to control injections of 0.9% saline. Methodology for 2-DG administration was similar to the procedure used for hypertonic saline. Injections were given to satiated rats and after 10 minutes they were given free access to lab chow, which was weighed again after 1hr, 3hrs and 24hrs. Animals acted as their own controls and injections were at least 48 hours apart, given in a random order. After 1hr ($F=0.0544$; $df=1,26$; $p=0.8173$) and 24hrs ($F=7.3074$; $df=1,26$; $p=0.3824$) there were no significant interactions. After 3hrs, ANOVA showed an interaction between groups and conditions ($F=7.3074$; $df=1,26$; $p=0.0119$) and Tukey post-hoc testing showed that, while there was no significant difference between the groups after 0.9% saline administration, there was a significant difference ($p<0.01$) after injection of 2-DG. These data are presented in Fig 16.

FIGURE 15: The results of the hypertonic saline physiological challenge are presented on the following page for the food or water adulteration experimental animals. Measurements taken 1 hour after the injection are presented at the top of the page and after 3 hours at the bottom. Standard error for lesion animals was never greater than 1.18 and for control animals was never greater than 1.6.


HYPERTONIC SALINE
1 HOUR
FOOD/WATER ADULTERATION EXPERIMENT
MLS WATER INTAKE
30
25
20
15
10
5
0
0.9% SALINE
5.0% SALINE
LESION
CONTROL

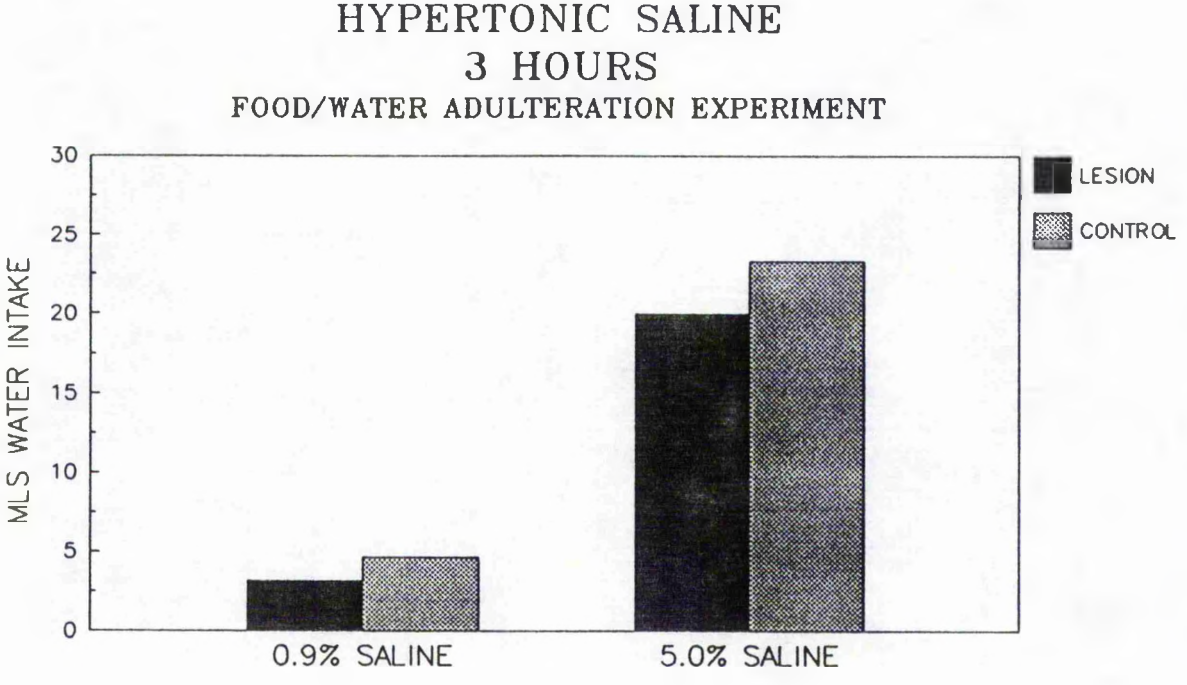

HYPERTONIC SALINE
3 HOURS
FOOD/WATER ADULTERATION EXPERIMENT
MLS WATER INTAKE
30
25
20
15
10
5
0
0.9% SALINE
5.0% SALINE
LESION
CONTROL

FIG 15

FIGURE 16: Food intake 3 hours after injections of either 0.9% saline or 2-DG are presented on the following page. ANOVA revealed a significant interaction between the groups and conditions as lesioned animals did not respond to the challenge. Standard errors were never greater than 0.34.

2-DG GLUCOPRIVATION
3 HOURS

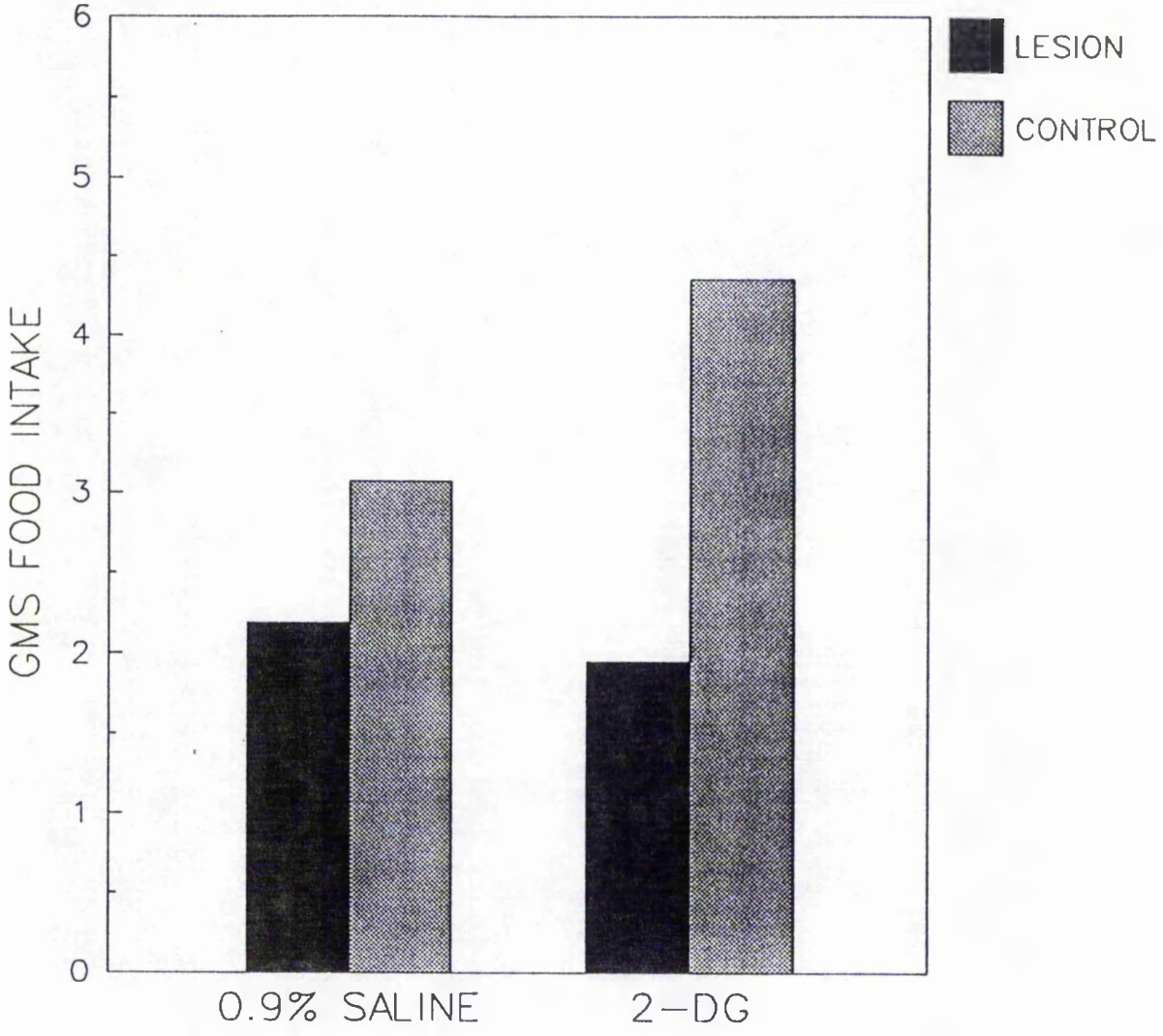


FIG 16

These results indicate that, while normal regulatory behaviour has recovered in the LH lesioned animals, they still show impairments in response to physiological challenges.

ADULTERATION OF DIET

Once it had been established that lesioned animals were not eating or drinking less than control groups, adulteration began, following the schedule outlined below:

TEST DAY	ADULTERATION	TEST DAY	ADULTERATION
1	0.1% quinine	2	No adulteration
3	0.1% saccharin	4	No adulteration
5	0.3% quinine	6	No adulteration
7	0.3% saccharin	8	No adulteration
9	0.5% quinine	10	No adulteration
11	0.5% saccharin	12	No adulteration
13	4% quinine	14	No adulteration
15	4% saccharin	16	No adulteration

This schedule was used for both food and water adulteration. Percentages are weight by volume. Groups A and B were fed normal lab chow but had adulterated tap water; groups C and D were given normal tap water and adulterated wet mash.

RESULTS

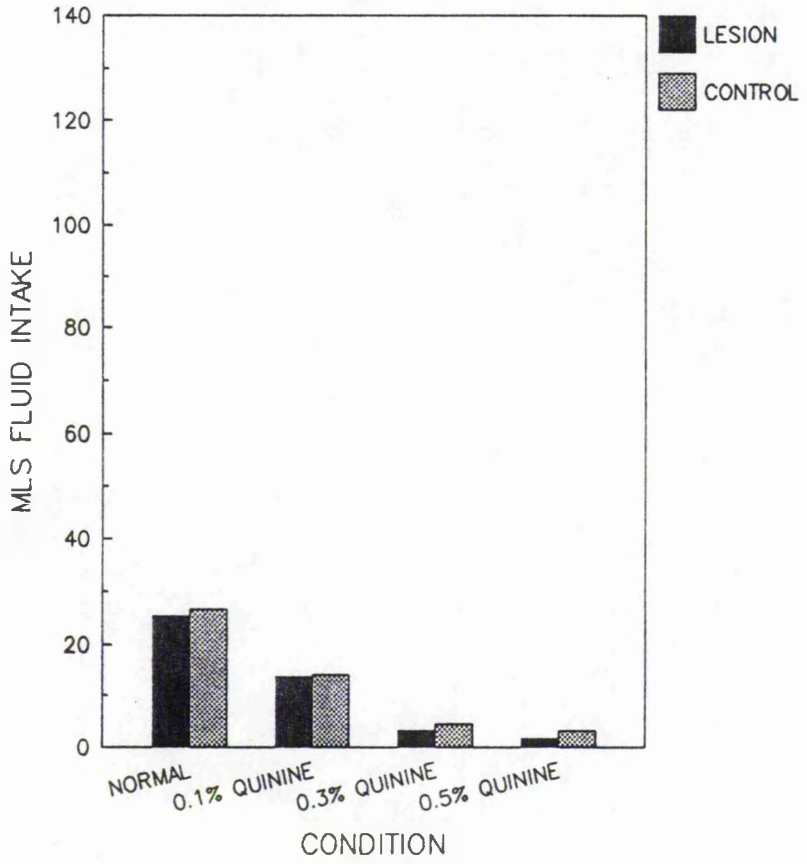
Water adulteration results are presented in Fig 17 and food adulteration results can be seen in Fig 18. It is clear that there was no difference between the groups and that all animals responded to the tastes in the same way. Rats given water adulteration clearly found quinine unpalatable and reduced their intake as a function of the amount of quinine given. They also found saccharin palatable and increased intake as the strength of solution increased. There was no significant difference between lesion or control groups. Adulteration of wet mash was not so successful, the wet mash itself probably having such a strong taste that adulterations were partially masked. However, as can be seen in Fig 18, intake was slightly reduced following the addition of quinine showing it to be less palatable at least to some degree and there was no difference between the groups' responses. The obvious results found after water adulteration do not lead one to expect to find differences between lesions and controls in reponse to food adulteration.

HISTOLOGY

In lesioned animals there was, in general, total destruction of the central core of the LH, extending

FIGURE 17: The effects of adulteration of drinking water with saccharin or quinine are presented on the following page. No differences were found between the groups. Standard errors were never greater than 12.81.

WATER ADULTERATION
QUININE



WATER ADULTERATION
SACCHARIN

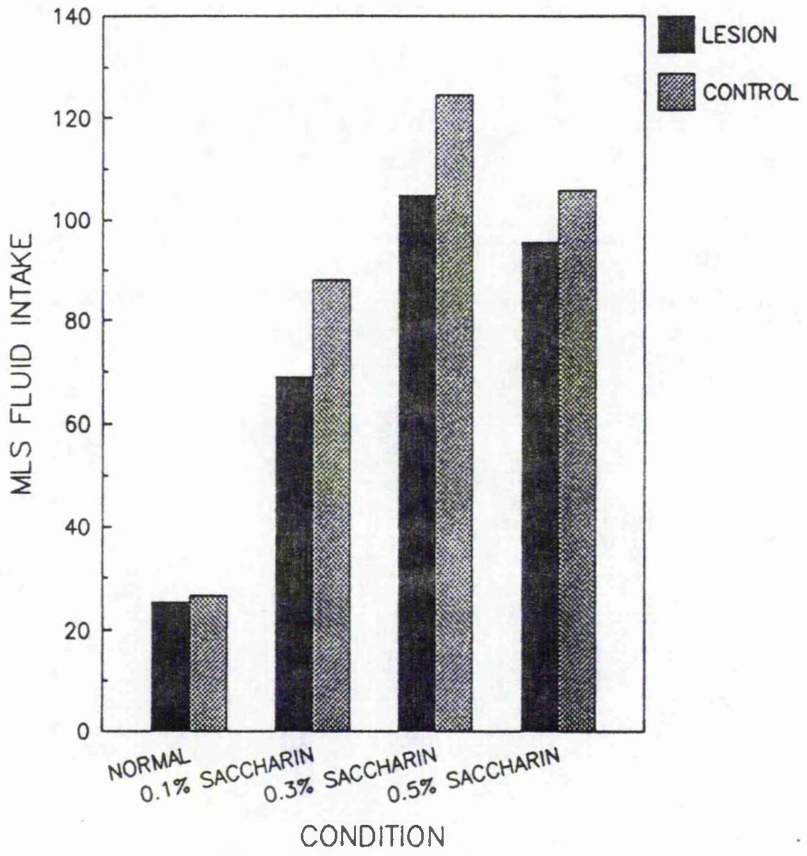
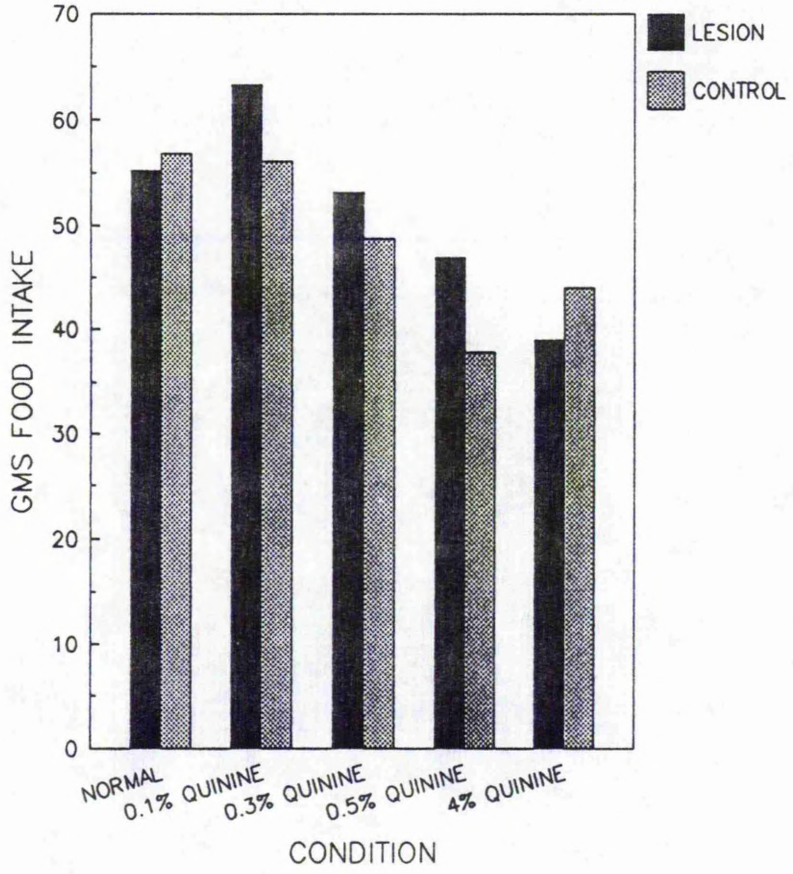


FIG 17

FIGURE 18: The effects of adulteration of food (wet mash) with saccharin or quinine are presented on the following page. No differences were found between the groups. Standard errors were never more than 8.43.

FOOD ADULTERATION
QUININE



FOOD ADULTERATION
SACCHARIN

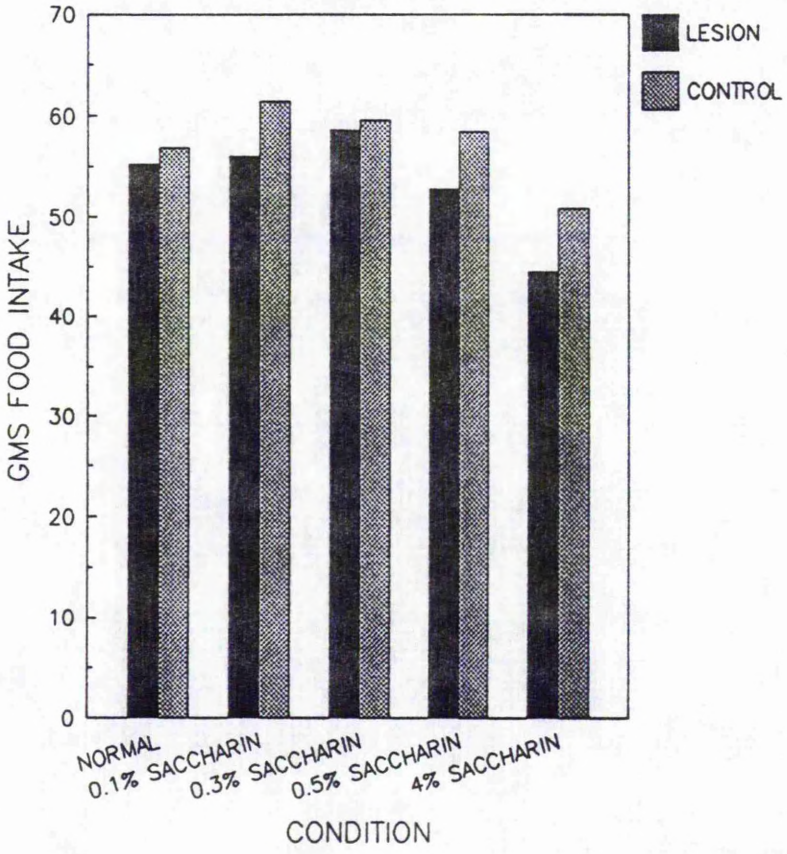


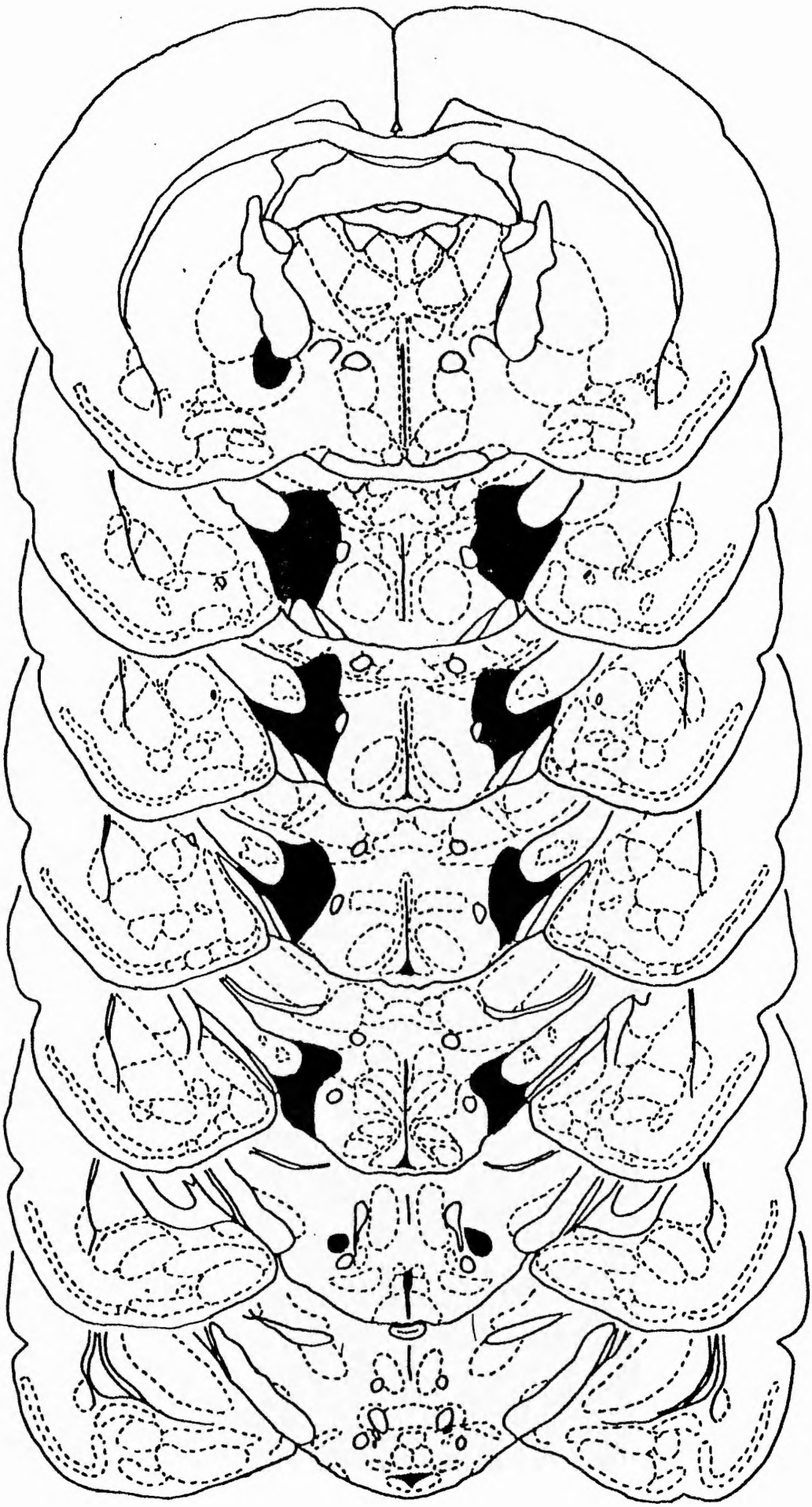
FIG 18

rostrally to the level of the paraventricular nucleus and caudally to the level of the dorsomedial hypothalamus, but sparing the poles, particularly the posterior pole. Average lesion size was 45.25% (SE=2.9) and lesions ranged from 29% to 76.9%. Extra-hypothalamic damage was very limited and most commonly found in the zona incerta and reticular nucleus of the thalamus. The pattern and size of a typical lesion can be seen in Fig 19.

DISCUSSION

The experimental results presented here suggest that the motivational deficit seen after LH lesions is not one of "incentive" in that animals can recognise and respond to the sensory qualities of food/fluid. They are therefore aware of the positive or negative rewarding properties of the stimuli. However, they are not over-responsive to these properties, or "finicky", as rats with electrolytic lesions of the LH have been described (**Teitelbaum and Epstein**, 1962). The results would indicate that NMDA LH lesioned rats can assess taste and respond to it as control animals. They should not, therefore, show any less motivation to eat for incentive reasons.

FIGURE 19: The pattern and size of a typical lesion in this experimental group is presented in this figure. Shaded area represents the lesion site. Lesions were restricted to the central core of the LH and extra-hypothalamic damage was found in the thalamus. (Rat J.20/87)



CHAPTER 8

STIMULUS

Since LH lesioned animals respond to the sensory qualities of their diet, the motivational deficit seen after such lesions cannot be said to be one simply of taste recognition. It is also unlikely to be a deficit in the recognition of "incentives" as external factors such as palatability still seem to influence the motivational state, as seen by changes in fluid intake. Assessing the sensory qualities of food, however, is a very "high order" process involving perception of taste, texture and smell. Perhaps the deficit is not due to failure to integrate information coming from other brain sites, but due to an alteration of the information coming from the periphery. This could involve either an alteration of the physiological signals which stimulate eating and drinking or failure to recognise intact signals once they reach the brain (or both). The first of these hypotheses will be dealt with in this section, with relation to drinking.

Dehydration promotes signals to the C.N.S. which work through "osmoreceptors" (see Chapter 3) to produce two effects; one is behavioural (drinking) and the other is physiological (the release of hormones which aid water

conservation). It has already been demonstrated that the behavioural response to dehydration is reduced following lesions of the LH, seen in the attenuated response to hypertonic saline. This may imply that the signals to the C.N.S. concerning dehydration have been altered. If this is the case, then the physiological response would also be expected to have been altered. Such an alteration in physiological responses to dehydration has already been demonstrated following ventral noradrenergic bundle lesion (**Lightman, Todd and Everitt**, 1983). If both behavioural and physiological responses to dehydration have been altered following lesions of the LH, this may imply that the signals which stimulate the responses to dehydration have been altered.

For this experiment, we chose to measure the levels of arginine vasopressin (AVP) in the blood. AVP is a marker of response to dehydration. As the body becomes dehydrated, AVP is secreted in the supraoptic and paraventricular nuclei of the hypothalamus and is released into the blood stream from the pituitary to aid water conservation. AVP promotes water reabsorption by increasing the permeability of the renal tubules and collecting ducts. Thus, water is reabsorbed from the urine into the interstitial fluid and the urine becomes more concentrated. The more AVP is secreted, the less water is excreted as urine. Therefore, levels of AVP rise as the body becomes dehydrated in an attempt to maintain fluid levels.

Measurement of AVP levels in the bloodstream should, therefore, give some indication of the hydrational state of the body. Administration of hypertonic saline causes intracellular dehydration which promotes drinking and increases AVP release, especially if access is not given to drinking water. It was hypothesised that, if physiological signals of dehydration have been altered after lesion of the LH, then release of AVP following injection of hypertonic saline would be altered in lesioned animals in comparison to controls. If AVP levels are not altered , then it seems likely that at least some physiological signals remain intact after LH lesions. This hypothesis is tested in the following experiments. In the first experiment baseline levels of AVP in non-deprived LH lesioned and control animals under normal conditions were compared. In the second experiment AVP levels were measured after administration of isotonic or hypertonic saline to see if dehydration induced increased AVP release.

BASELINE AVP

SURGERY

Twenty male Lister-hooded rats (mean body weight 325.2g, SE=6.84; range = 250.7gms - 367.7gms) were split into two groups of approximately equal weight on a random basis and were housed under a 12 hour dark/light cycle as described

in the general procedures section. Bilateral LH lesions were made in 12 rats by microinjection of 1ul of 0.06M NMDA (Sigma) (pH 7.0); the remaining 8 control animals were microinjected with phosphate buffer (pH 7.4). Injections were made at the following co-ordinates : (in the orientation of de Groot); 0mm AP from bregma; ± 1.9mm lateral; -8.0mm vertical from dura. NMDA and phosphate buffer were delivered bilaterally at 0.5ul/min and injection cannulae were left in situ for 2 mins.

One lesioned animal died within 24 hours of surgery and is therefore not included in the following results.

NORMAL REGULATORY BEHAVIOUR

BODY WEIGHT: [Fig 20] Body weights were measured daily pre- and post-op. Before surgery, there was no significant difference between lesion and control groups' percentage weights (ANOVA: $F=0.4558$; $df=1,17$; $p=0.5087$). One week after surgery, there was a significant difference between the groups (ANOVA: $F=24.5792$; $df=1,17$; $p=0.0001$) and an interaction between groups and days (ANOVA: $F=9.0371$; $df=6,102$; $p<0.001$). The significant difference between the groups was maintained in week 2 (ANOVA: $F=23.7398$; $df=1,17$; $p=0.0001$) and week 3 (ANOVA: $F=27.4721$; $df=1,17$; $p=0.0001$) but no interaction between groups and days was found (ANOVA: week 2, $F=1.6986$; $df=6,102$; $p=0.1289$: week 3, $F=1.389$; $df=6,102$; $p=0.2262$). This

FIGURE 20: Percentage body weights for animals used in baseline plasma AVP experiments are presented in this figure. No groups differences were found pre-surgery, but LH lesioned animals lost weight post-surgery. Two and three weeks post-op ANOVA revealed no groups by days interaction indicating that both groups were gaining weight at the same rate.

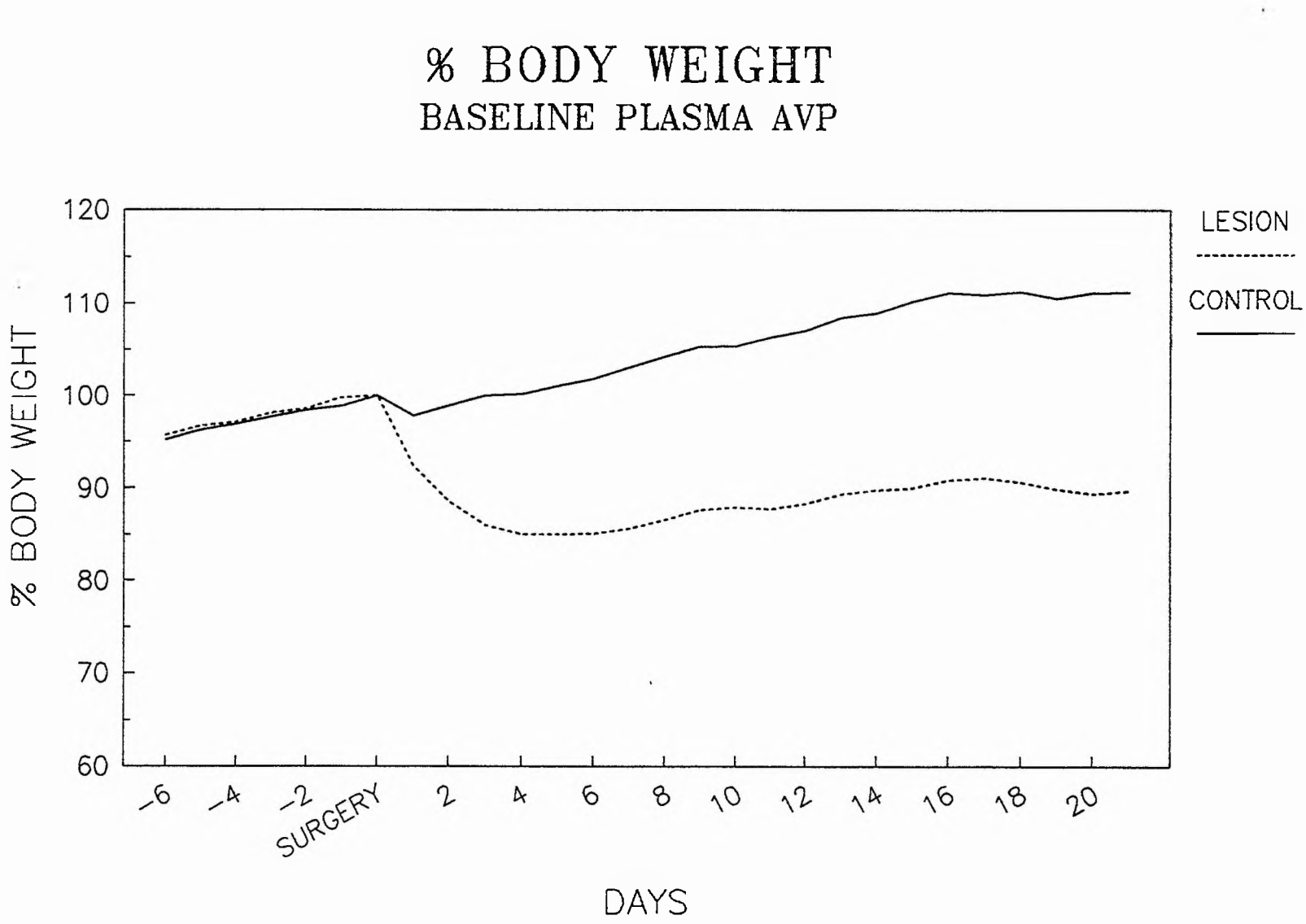

% BODY WEIGHT
BASELINE PLASMA AVP
120
110
100
90
80
70
60
% BODY WEIGHT
LESION
CONTROL
-6
-4
-2
SURGERY
2
4
6
8
10
12
14
16
18
20
DAYS

FIG 20

indicates that after an initial drop in weight, the lesioned animals gained weight at the same rate as controls.

FOOD INTAKE: [Fig 21] Intake of dry lab chow was measured daily pre- and post-op. Before surgery, there was no significant difference between groups (ANOVA: F=0.7356; df=1,17; p=0.403). After surgery, ANOVA revealed a significant difference between the groups which was maintained throughout the experiment (ANOVA: week 1, F=24.4387; df=1,17; p=0.0001: week 2, F=6.6901; df=1,17; p=0.0192: week 3, F=11.6408; df=1,17; p=0.0033). These measurements reflect intake of dry lab chow only. If an animal dropped below 80% body weight it was given wet mash (Farex). Five lesioned animals were given wet mash at some point and two of these animals received wet mash almost every day throughout the experiment. Wet mash was eaten in preference to lab chow so the dry lab chow intake does not include all food intake.

WATER INTAKE: [Fig 22] Water intake was measured daily pre- and post-op. Before surgery, there was no significant difference between the groups (ANOVA: F=0.0079; df=1,17; p=0.9304). After surgery, ANOVA revealed a significant difference between the groups which was maintained throughout the experiment (ANOVA: week 1, F=20.3066; df=1,17; p=0.0003: week 2, F=5.7902; df=1,17; p=0.0278: week 3, F=7.8333; df=1,17; p=0.0123). These measurements

FIGURE 21: Food intake for one week pre- and three weeks post-surgery are presented in this figure. No differences were found before surgery between the groups, but lesioned animals reduced intake post-op. The data presented only depicts intake of dry lab chow. Some animals were also receiving Farex wet mash, which they ate in preference to the lab chow.

FOOD INTAKE

BASELINE PLASMA AVP

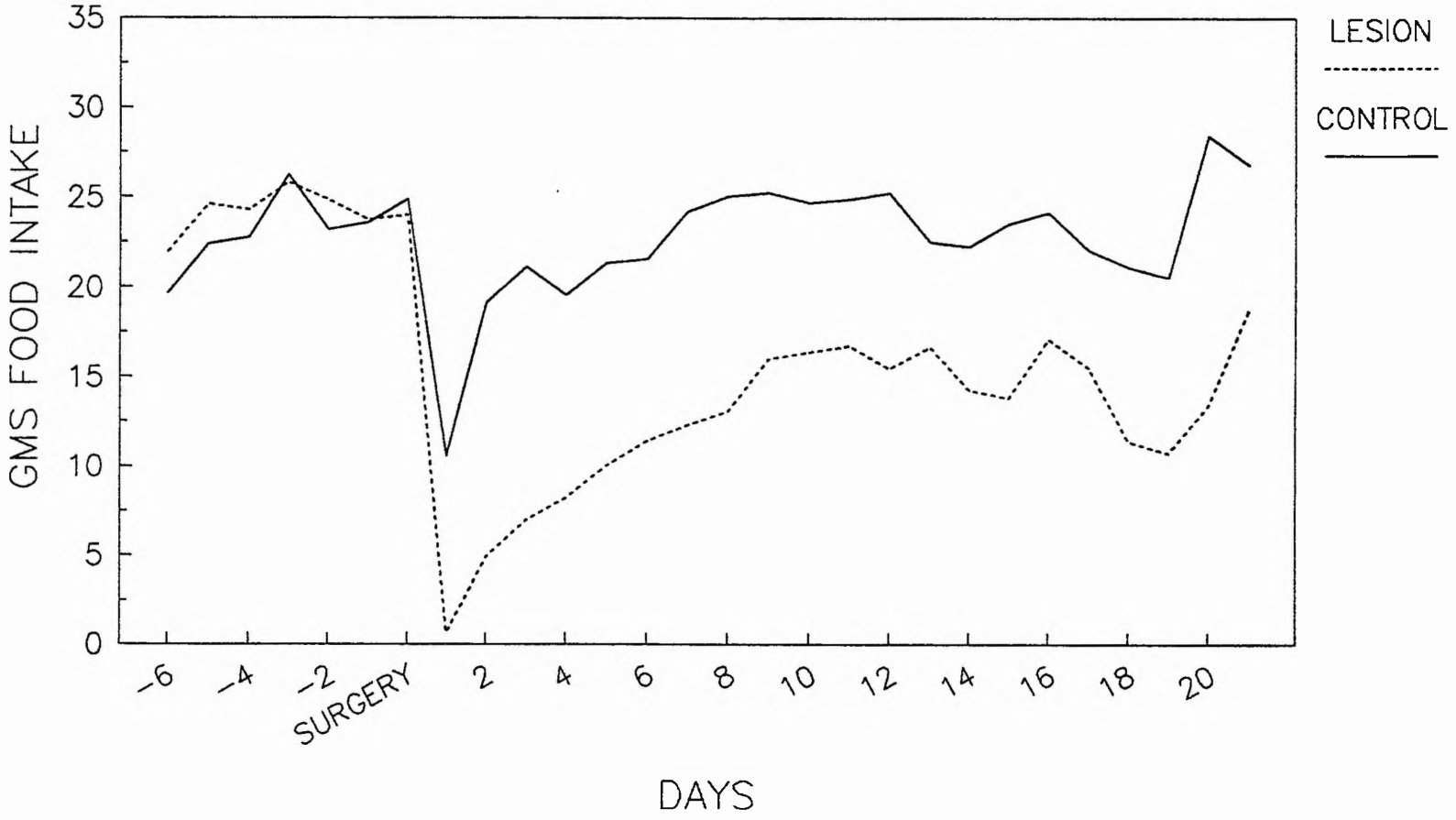


FIG 21

FIGURE 22: Water intake for one week pre- and three weeks post-surgery are presented in this figure. No differences were found between groups pre-surgery, but LH lesioned animals reduced intake post-surgery. The data presented represent intake of tap water from water bottles only. Some animals also consumed fluid when eating Farex wet mash.

WATER INTAKE
BASELINE PLASMA AVP

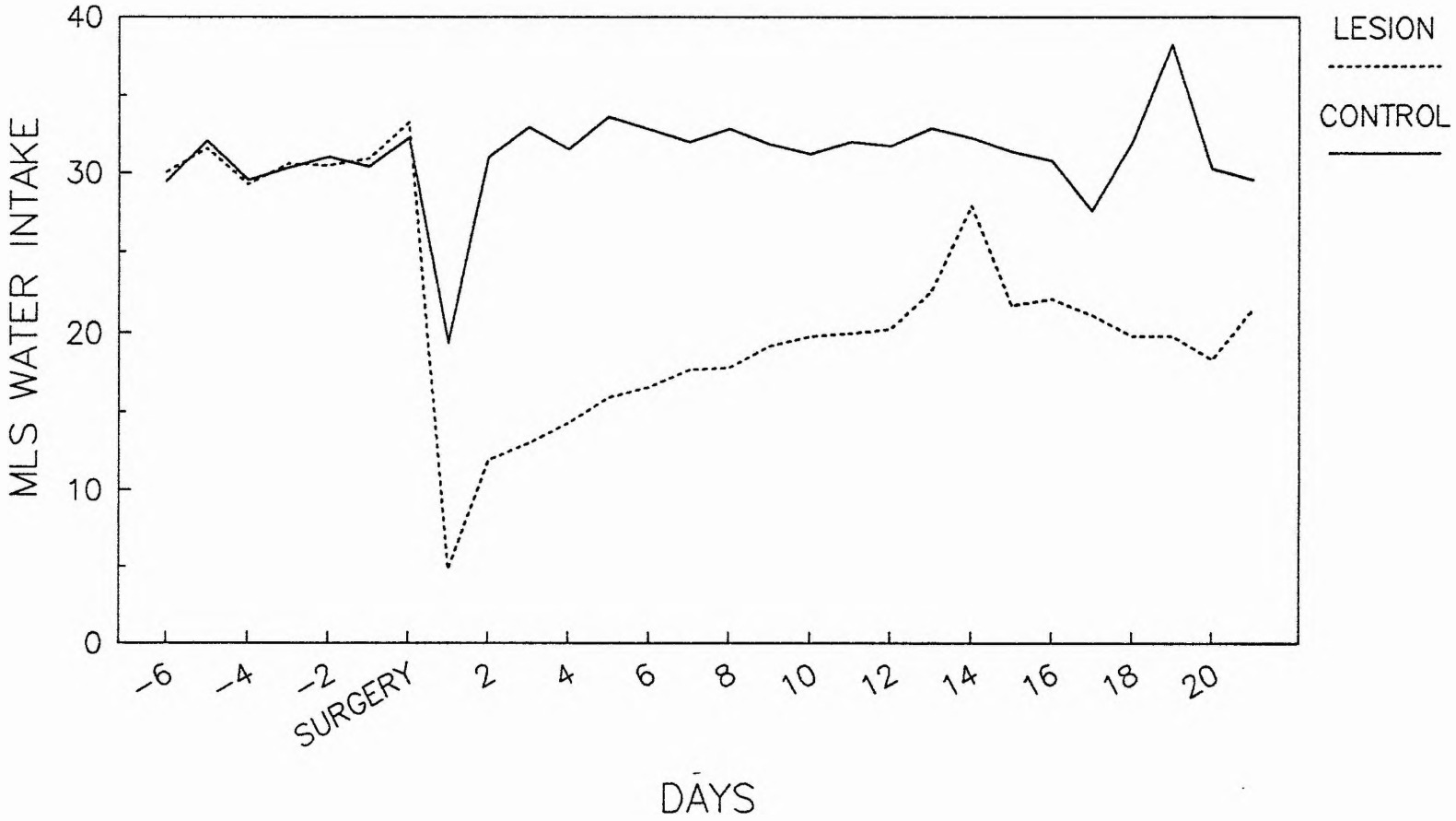


FIG 22

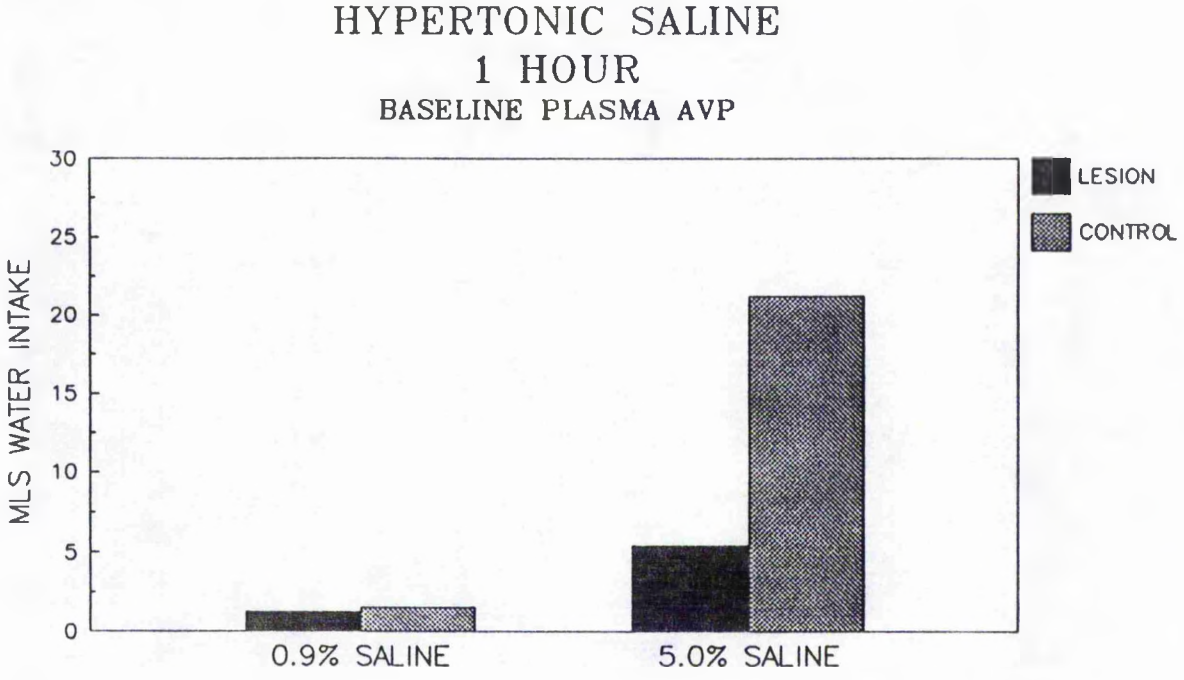
reflect intake of tap water from the water bottle only. Some animals as described above also consumed fluid when eating wet mash.

These lesioned animals reduced body weight, food intake and fluid intake in comparison to their controls. If given wet mash, however, they were capable of recovery and most were able to maintain themselves on dry food and water after approximately one week. Two animals were not capable of this, but could maintain themselves on wet mash.

HYPERTONIC SALINE

Hypertonic saline was given according to the standard procedure described in the general procedures section. Data can be seen in Fig 23. At 1 hr post-injection, ANOVA revealed an interaction between groups and conditions ($F=44.944$; $df=1,17$; $p<0.001$). Tukey post-hoc tests showed that, while control animals significantly increased their intake after hypertonic saline administration ($p<0.01$), lesioned rats did not and that there was no significant difference between the responses of lesioned and control animals to physiological saline administration. At 3 hrs post-injection, similar results were obtained. An interaction was found between groups and conditions (ANOVA: $F=34.0335$; $df=1,17$; $p=0.00$), again due to a significant increase in water intake by the control animals following hypertonic saline injection (Tukey test: $p<0.01$). The

FIGURE 23: The following figure presents the responses to hypertonic saline physiological challenge of the experimental groups used in baseline plasma AVP tests. Lesioned animals displayed a deficit in response to this challenge 1 hour and 3 hours following injections as revealed by ANOVA. Standard errors were never more than 1.91.


HYPERTONIC SALINE
1 HOUR
BASELINE PLASMA AVP
MLS WATER INTAKE
30
25
20
15
10
5
0
0.9% SALINE
5.0% SALINE
LESION
CONTROL

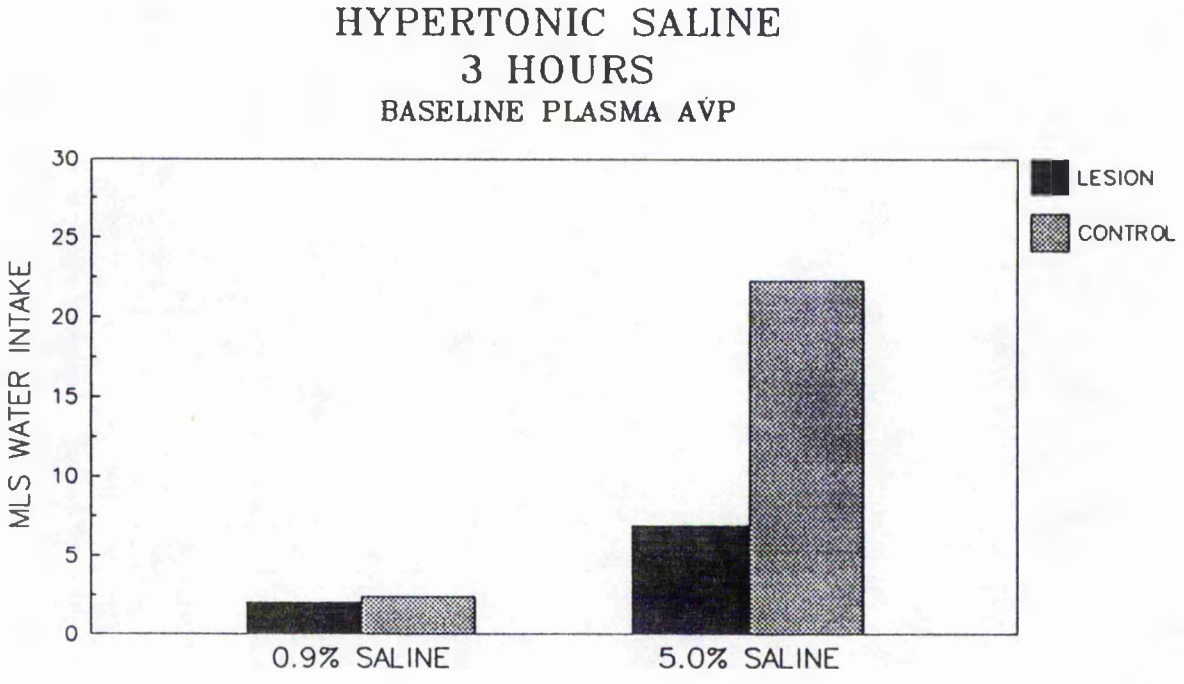

HYPERTONIC SALINE
3 HOURS
BASELINE PLASMA AVP
MLS WATER INTAKE
30
25
20
15
10
5
0
0.9% SALINE
5.0% SALINE
LESION
CONTROL

FIG 23

lesioned animals did not increase water intake significantly in response to hypertonic saline and there was no significant difference between the lesioned and control rats' responses to physiological saline administration. Thus, these LH lesioned rats did not respond to intracellular dehydration by drinking as control animals did.

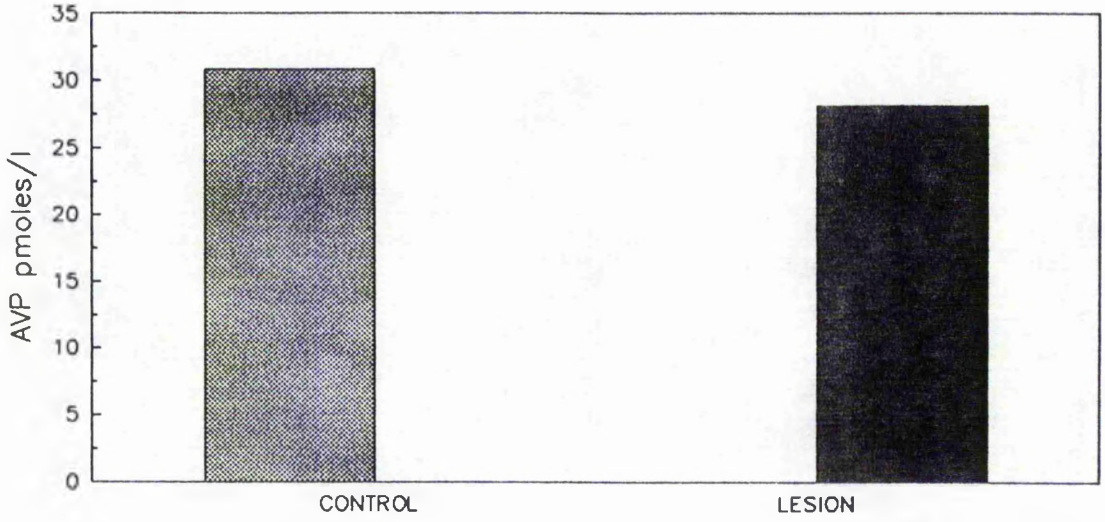
BLOOD SAMPLING

Animals were anaesthetised with halothane (ICI) and then decapitated using a guillotine. Brains were removed and placed in 20% formalin solution. Trunk blood was collected in a chilled heparinised tube and stored in the fridge until all samples had been collected. They were then placed in a cold centrifuge at between 0 and -5 degrees centigrade and spun at 3000rpm for 15 minutes to separate blood and allow plasma extraction. Plasma osmolality was measured using an osmometer; samples were stored at approximately -20 degrees centigrade prior to assay. AVP concentration was measured according to the method described by **Williams, Carter and Lightman** (1985).

Data is presented in Figure 24. Using Student's t-test, no significant differences were found between the osmolality readings of lesion and control groups (df=17, t=0.265, p=NS) or between the groups' AVP readings (df=17, t=0.298, p=NS). This indicates that the plasma of the animals in

FIGURE 24: The following figure presents baseline plasma AVP levels and baseline plasma osmolality. No differences were found between the groups. Standard errors never more than 9.45 in the osmolality test and 7.09 in the AVP test.

BASELINE PLASMA AVP



BASELINE PLASMA OSMOLALITY

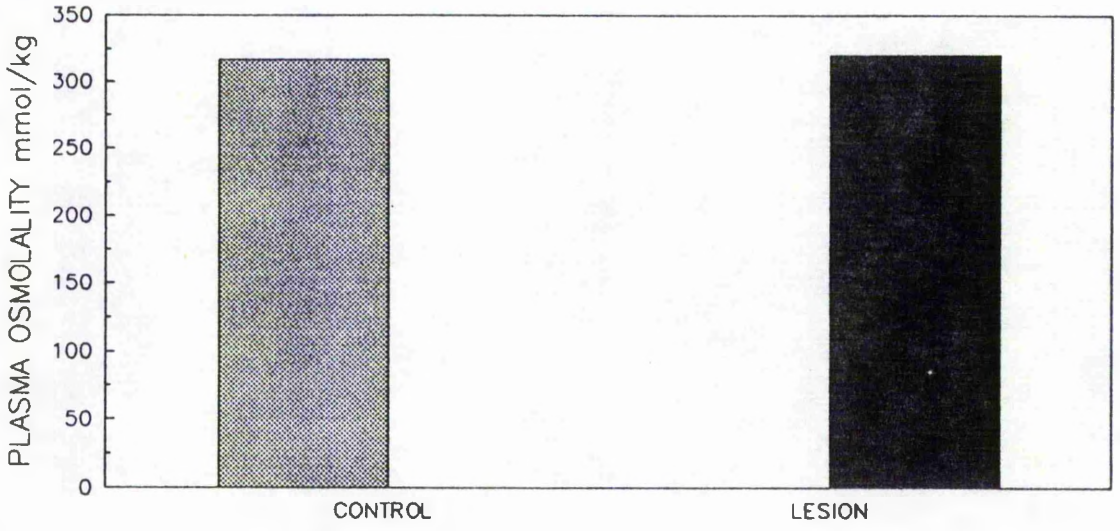


FIG 24

each group had the same water concentration and that baseline AVP levels were similar in LH lesioned and control animals.

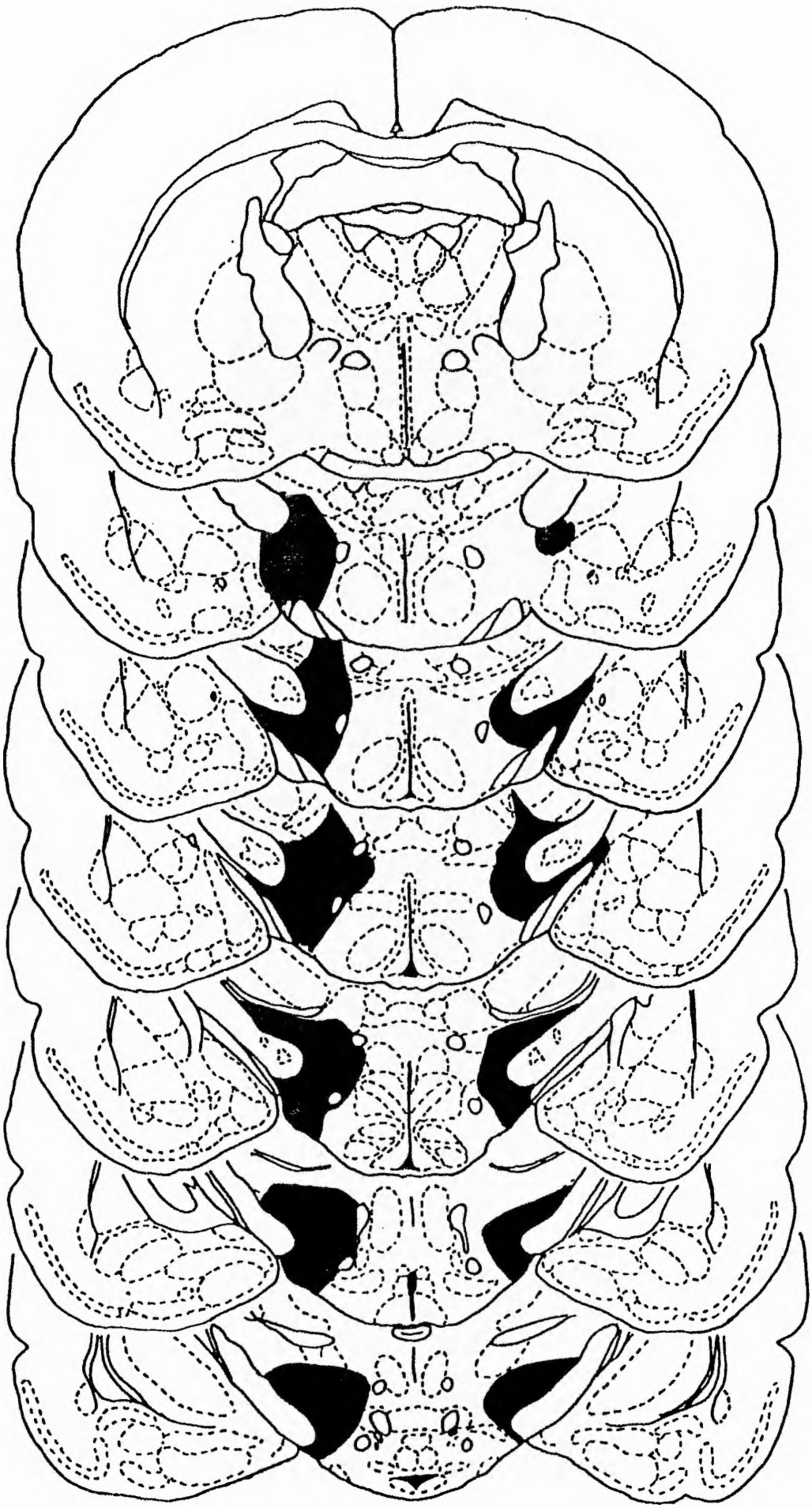
HISTOLOGY

Histological procedures are described in Chapter 5. In these lesioned animals greatest damage was found at the level of the VMH and cell loss in the central core of the LH was almost complete. Least damage was found at the posterior pole with only limited damage at the anterior pole. Average lesion size was 66.07% (SE=4.73) and lesions ranged from 44.9% to 95.8%. Extra-hypothlamic damage in this group was quite extensive. Cell loss was noted in the zona incerta, reticular nucleus of the thalamus and other ventral thalamic nuclei, subthalamic nucleus and globus pallidus. The pattern and size of a typical lesion can be seen in Figure 25.

SUMMARY

LH lesioned animals in this experiment showed impaired food and water intake, reduced body weight and deficits to physiological challenges. Lesioned animals gained weight at the same rate as controls from 1 week post-op. Lesions were found to be large with almost complete loss of the central core of the LH. Blood osmolality and baseline AVP levels, however, were found to be the same as controls. It must be

FIGURE 25: The size and pattern of a typical lesion for this experimental group is presented on the following page. Shaded area represents lesion site. Cell loss occurred mostly in the central core of the LH and the anterior pole was not damaged. Extra-hypothalamic damage occurred in the thalamus and zona incerta. (Rat J.55/88)



pointed out here that both control and lesioned animals showed elevated AVP levels in comparison to "normal" (eg see following experiment). This could be due to the use of anaesthetic or to the procedure used to give the anaesthetic (animals were placed in a bell jar and anaesthetised by halothane fumes). There is some debate as to whether AVP may also be released in response to stress (Williams, Carter and Lightman, 1985) which could account for the high levels found here. As a consequence, anaesthetic was not used in subsequent experiments. Despite these problems, the fact remains that the osmolality and the AVP levels were similar in both control and lesion groups. Even if levels were elevated by stress, lesioned and control animals responded in the same way. The results of the following experiment support the data presented on the preceding pages and alleviate concern that the results may have been confounded by the use of anaesthetic.

AVP RESPONSES TO ISOTONIC AND HYPERTONIC SALINE

SURGERY

Bilateral LH lesions were made in 14 male Lister-hooded rats by microinjection of 1ul 0.09M NMDA (pH 6.89) at 0.5ul/min the following co-ordinates: (in the orientation of de Groot) 0mm from bregma; $\pm$ 2.0mm lateral; - 8.0mm vertical. Control animals (n=18) were microinjected at the

same site with phosphate buffer (pH 7.4). Injection cannulae were left in situ for 2 minutes following the injection. Average weight before surgery was 334.04gms (SE=1.45; range = 208.2gms - 410.3gms).

NORMAL REGULATORY BEHAVIOUR

BODY WEIGHT: [Fig 26] Body weight was measured daily. One week pre-op there was no significant difference between the groups (ANOVA: $F=1.3842$; $df=1,30$; $p=0.2486$) or rate of growth, indicated by the fact that there was no interaction between groups and days (ANOVA: $F=1.295$; $df=6,180$; $p=3.47$). Following surgery, the lesioned animals lost weight, resulting in a significant difference between the groups, which lasted throughout the experiment (ANOVA: week 1, $F=161.2703$; $df=1,30$; $p\leq0.001$: week 2, $F=182.5132$; $df=1,30$; $p=0.00$: week 3, $F=183.667$; $df=1,30$; $p=0.00$). The lesioned animals' initial loss of weight produced an interaction between groups and days (ANOVA: week 1, $F= 37.8799$; $df=6,180$; $p\leq0.001$), but the significance of this interaction was reduced in week 2 (ANOVA: $F=2.3748$; $df=6,180$; $p=0.0312$) and had disappeared by week 3 (ANOVA: $F=1.1586$; $df=6,180$; $p=0.3306$). Thus, after an initial loss of weight, the lesioned animals gained weight at the same rate as control animals.

FOOD INTAKE: [Figs 27 and 28] Food intake was measured daily pre- and post-op. Before surgery, ANOVA revealed no

FIGURE 26: Percentage body weight for animals used in experiments testing for physiological responses to hypertonic saline injections are presented in this figure. No differences were found between the animals pre-surgery, but the LH lesioned animals lost weight post-surgery.

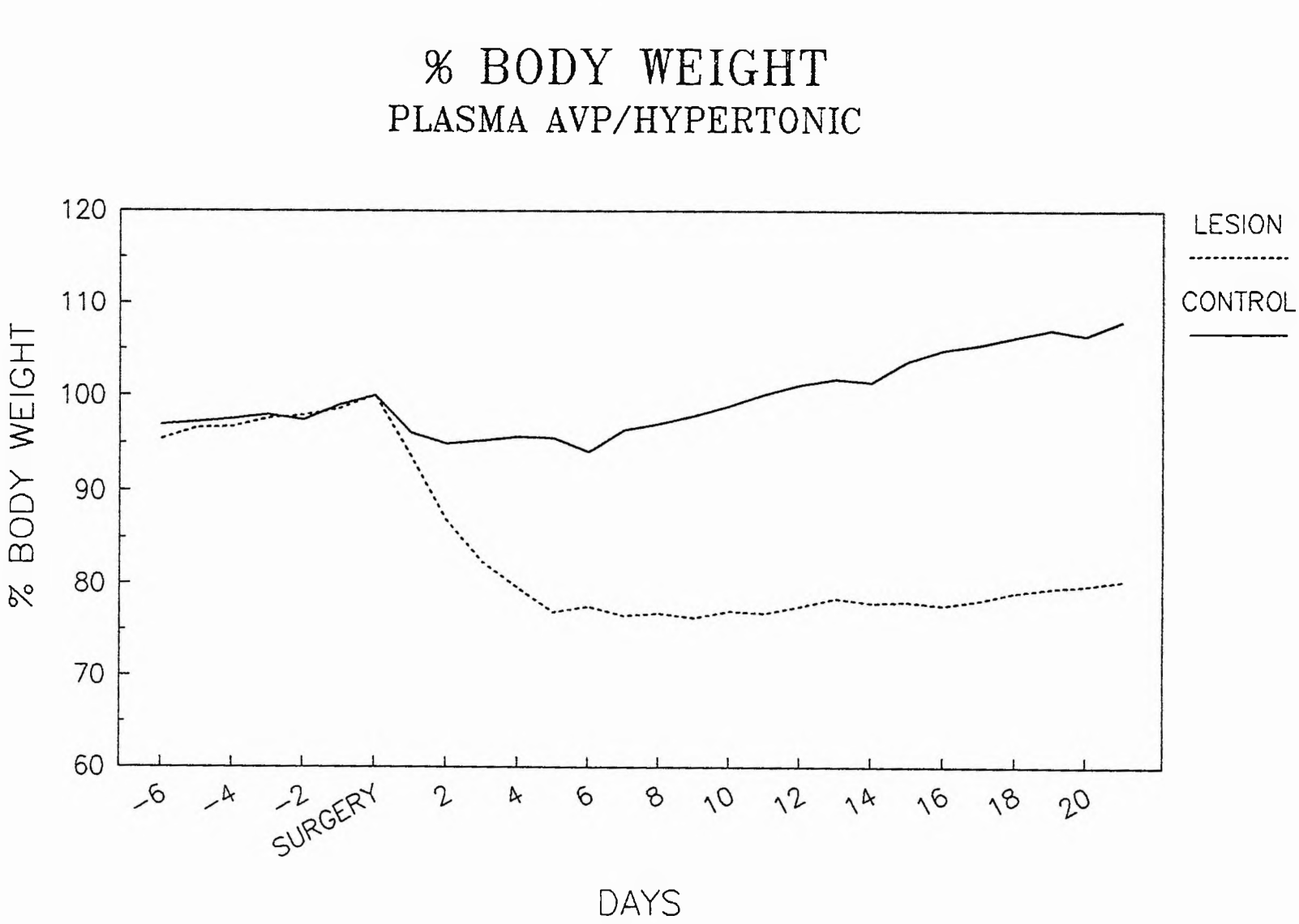

% BODY WEIGHT
PLASMA AVP/HYPERTONIC
120
110
100
90
80
70
60
% BODY WEIGHT
-6
-4
-2
SURGERY
2
4
6
8
10
12
14
16
18
20
DAYS
LESION
CONTROL

FIG 26

significant differences between the groups (F=2.1328; df=1,30; p=0.1546). After surgery, lesioned animals reduced intake of dry lab chow and several were given Farex wet mash (50% w/v) to eat in addition to the lab chow. Figure 27 shows intake of dry lab chow. On this measure there was a significant difference between lesioned and control groups post-op which lasted throughout the experiment (ANOVA: week 1, F=165.4074; df=1,30; p=0.00: week 2, F=70.2251; df=1,30; p=0.00; week 3, F=49.0201; df=1,30; p<0.001). As so many of these animals (N=7) were also being given Farex wet mash (which they ate in preference to the dry lab chow), Fig 28 shows lab chow intake of those animals maintaining themselves on dry lab chow only (N=7). It can be seen that, while intake is still reduced in comparison to the control group, more food is being consumed than Fig 27 seems to indicate. Those animals on Farex wet mash were able to maintain themselves on this diet, eating an average of 120gms daily.

WATER INTAKE: [Figs 29 and 30] Water intake was measured daily pre- and post-op. Before surgery, ANOVA revealed no significant differences between the groups (ANOVA: F=1.3775; df=1,30; p=0.2498). After surgery, lesioned animals reduced their intake, resulting in a significant difference between the groups which was maintained throughout the experiment (ANOVA: week 1, F=139.7573; df=1,30; p=0.00: week2, F=79.2226; df=1,30; p=0.00; week 3; F=28.3713; df=1,30; p=0.00). These results, presented in

FIGURE 27: Food intake for one week pre- and three weeks post-surgery are presented in this figure. Post-surgery, LH lesioned animals reduced intake and there was a significant difference between the groups using ANOVA. The data presented here represent intake of dry lab chow only. Half of the lesioned animals were also given Farex wet mash, which they ate in preference to the dry lab chow.

FOOD INTAKE

PLASMA AVP/HYPERTONIC

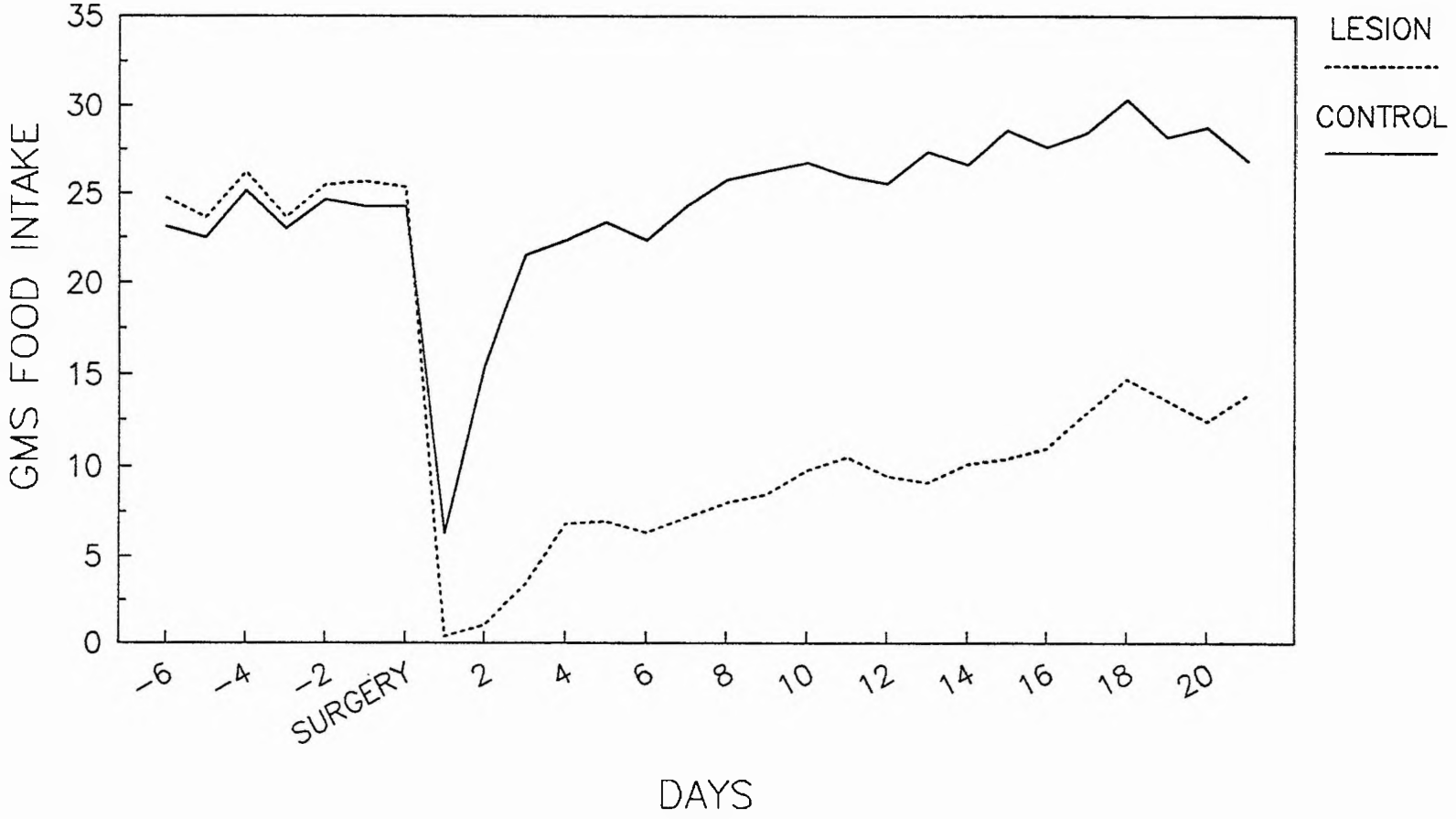


FIG 27

FIGURE 28: Food intake for one week pre- and three weeks post-surgery of those animals which maintained themselves throughout the experiment on dry lab chow only are presented on the following page. N=7 lesioned animals.

FOOD INTAKE

PLASMA AVP/HYPERTONIC

RECOVERED ANIMALS

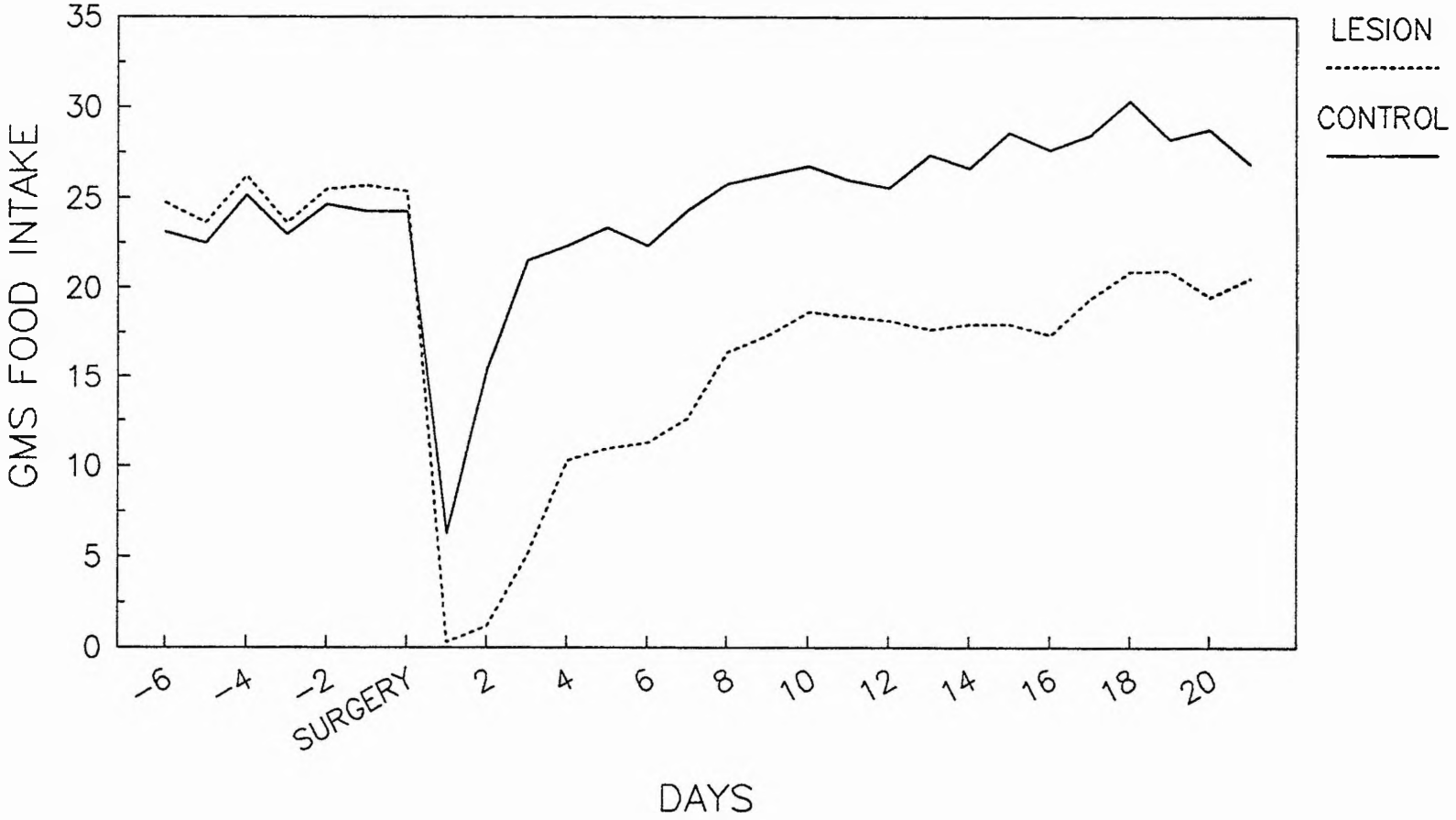


FIG 28

FIGURE 29: Water intake for one week pre- and three weeks post-surgery are presented in this figure. Post-surgery, lesioned animals reduced their intake and there was a significant difference between the groups. The data presented here represents intake of all animals including those given Farex wet mash, which also consumed liquid in their diet.

WATER INTAKE

PLASMA AVP/HYPERTONIC

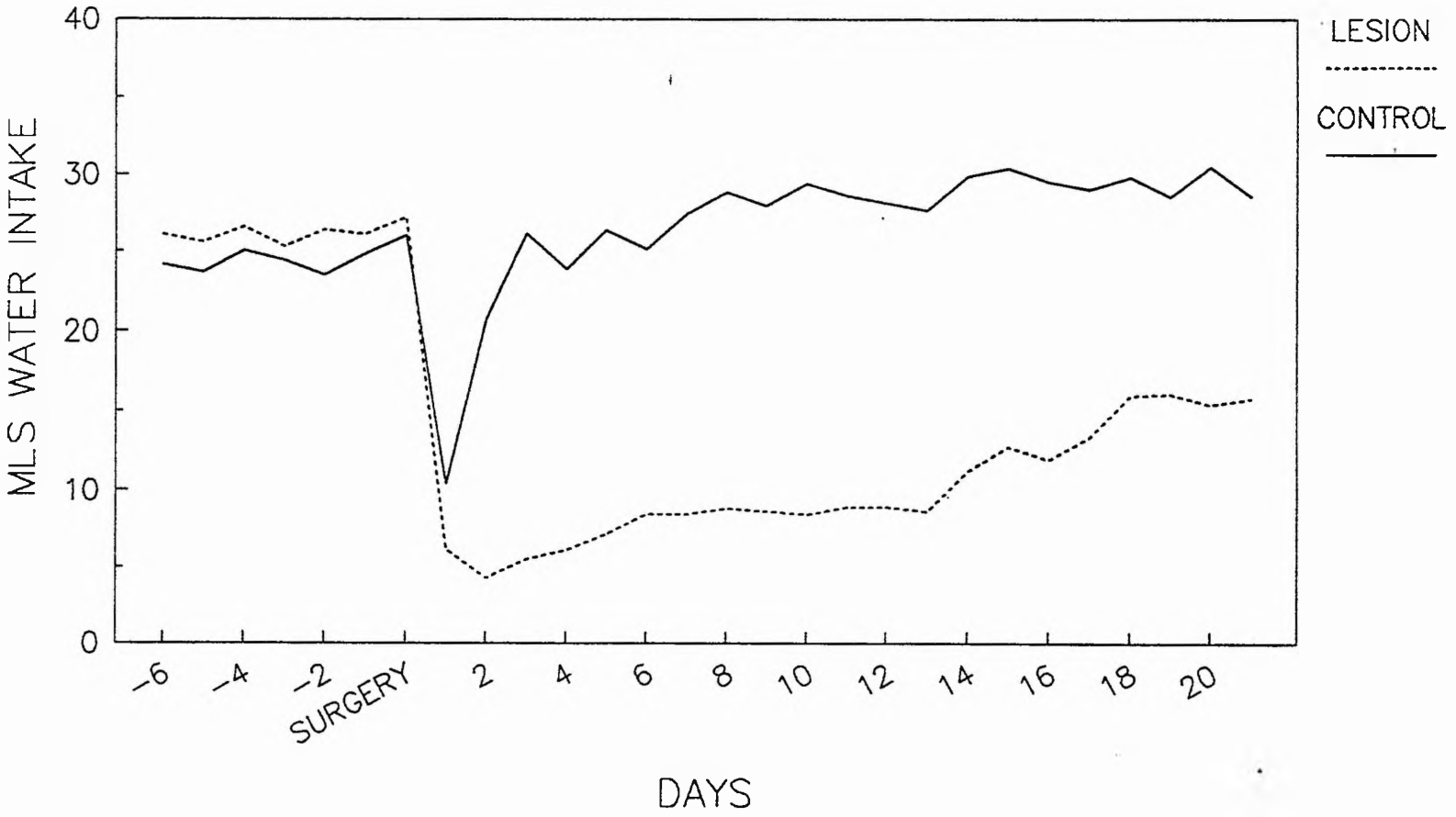


FIG 29

FIGURE 30: Water for one week pre- and three weeks post-surgery are presented for only those animals able to maintain themselves on dry lab chow throughout the experiment. N=7 lesioned animals.

WATER INTAKE

PLASMA AVP/HYPERTONIC

RECOVERED ANIMALS

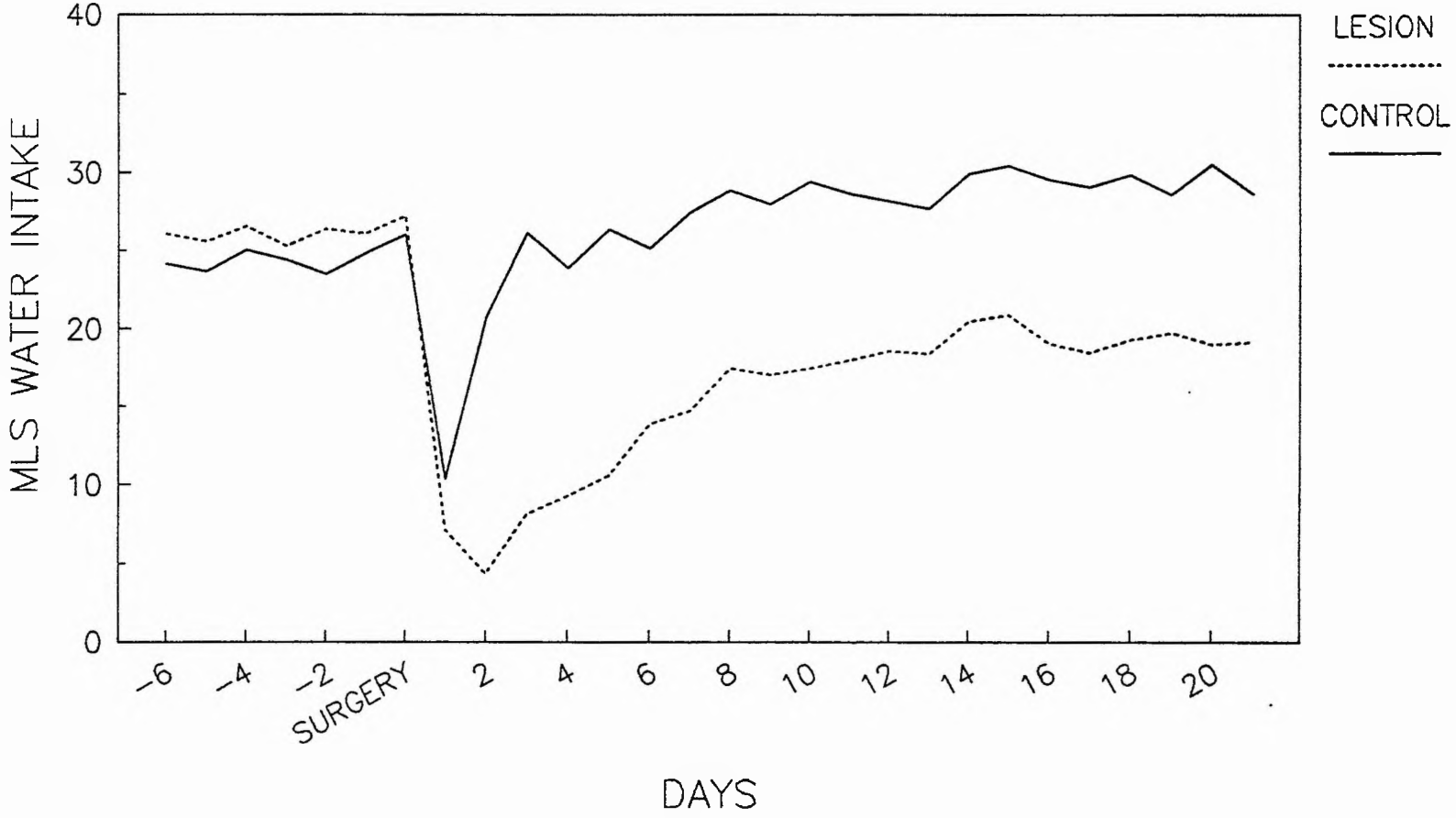


FIG 30

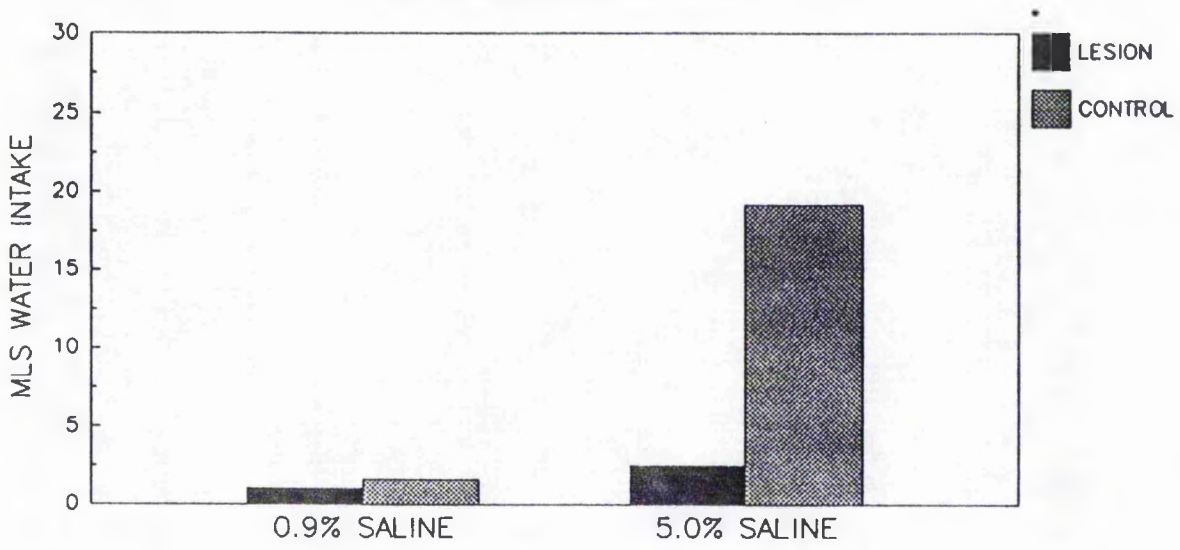
Figure 29, reflect tap water intake of all animals. As described above, several lesioned animals were given 50% w/v Farex wet mash to eat and therefore consumed liquid other than from their water bottles. Figure 30 shows water intake of lesioned animals maintaining themselves on dry lab chow only (N=7).

In summary, regulatory deficits following these lesions were severe. There was a marked loss of body weight and reduced food and water intake which remained throughout the experiment. Although some animals were able to maintain themselves on dry lab chow and tap water, others were given Farex wet mash to eat. These animals were able to maintain themselves on this diet with no other intervention.

HYPERTONIC SALINE

20ml/kg 5% hypertonic saline was administered to non-deprived rats following the method described in the general procedures section. ANOVA revealed an interaction between groups and conditions at 1 hr post-injection ($F=133.2188$; $df=1,30$; $p<0.001$) and at 3 hrs post-injection ($F=139.2886$; $df=1,30$; $p<0.001$). At both times, control animals significantly increased their water intake following hypertonic saline administration (Tukey; $p<0.01$) compared to administration of physiological saline (see Fig 31). Lesioned animals did not significantly increase intake in response to hypertonic saline and did not differ

FIGURE 31: Responses to hypertonic saline physiological challenge are presented for 1 hour and 3 hours post-injection. LH lesioned animals displayed a deficit in response to this challenge as revealed by ANOVA. Standard errors were never more than 1.16.


HYPERTONIC SALINE
1 HOUR
PLASMA AVP/HYPERTONIC
MLS WATER INTAKE
30
25
20
15
10
5
0
0.9% SALINE
5.0% SALINE
LESION
CONTROL

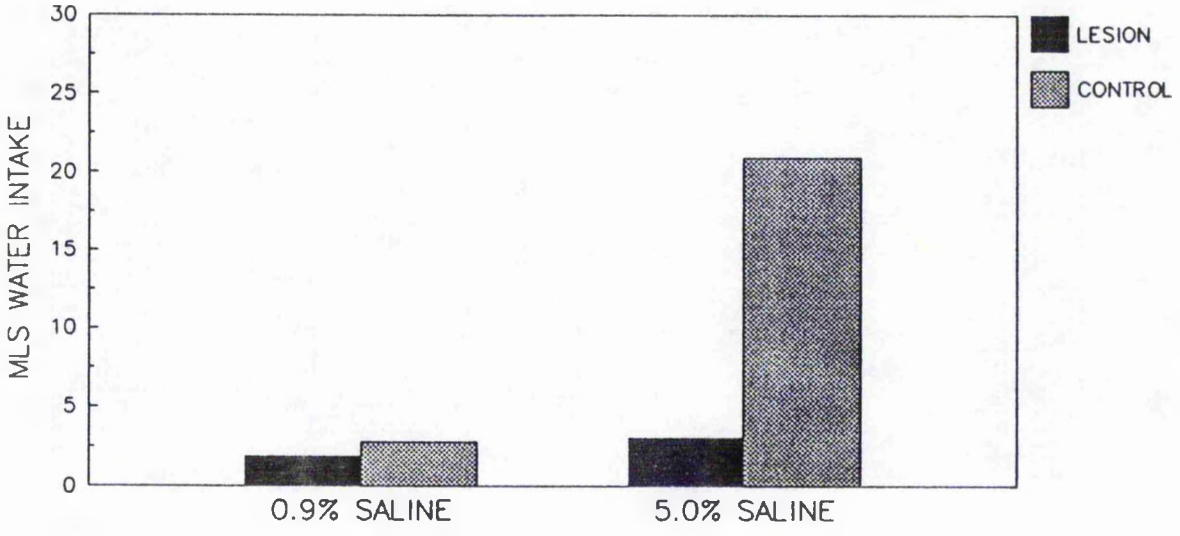

HYPERTONIC SALINE
3 HOURS
PLASMA AVP/HYPERTONIC
MLS WATER INTAKE
30
25
20
15
10
5
0
0.9% SALINE
5.0% SALINE
LESION
CONTROL

FIG 31

significantly from control rats in their response to physiological saline. Thus, these LH lesioned rats did not respond behaviourally to this physiological challenge.

BLOOD SAMPLING

In this experiment, injections of isotonic saline were used as a control for the injection procedure of hypertonic saline. Isotonic saline should not dehydrate the rats and should therefore have no effect on AVP levels. The lesioned animals were split into two equal groups (n=7), each group including some animals on wet mash; one group was injected with 20ml/kg 5% saline and the other with 20ml/kg 0.9% saline. The control animals were also split into two equal groups (n=9) which received injections as described above. All rats were non-deprived. After injection, drinking water was immediately removed. One hour later, rats were stunned by a sharp blow to the head and decapitated with a guillotine. Following this, the same procedure as described above was used for plasma collection, osmolality measurements and AVP analysis.

The effects of isotonic and hypertonic saline on plasma osmolality can be seen in Figure 32. ANOVA revealed that there was a conditions effect ($F= 23.0324$; $df=1,14$; $p=0.0003$), demonstrating that hypertonic and isotonic saline injections induce different plasma water concentrations. This effect was induced in both lesioned

FIGURE 32: Plasma osmolality measures for lesioned and control animals 1 hour injections of isotonic or hypertonic saline can be seen in this figure. ANOVA revealed a conditions effect, but no groups effect or interaction. Both groups had lower osmolality measures following isotonic saline administration. Standard errors were never more than 8.58.

OSMOLALITY
PLAMSA AVP/HYPERTONIC

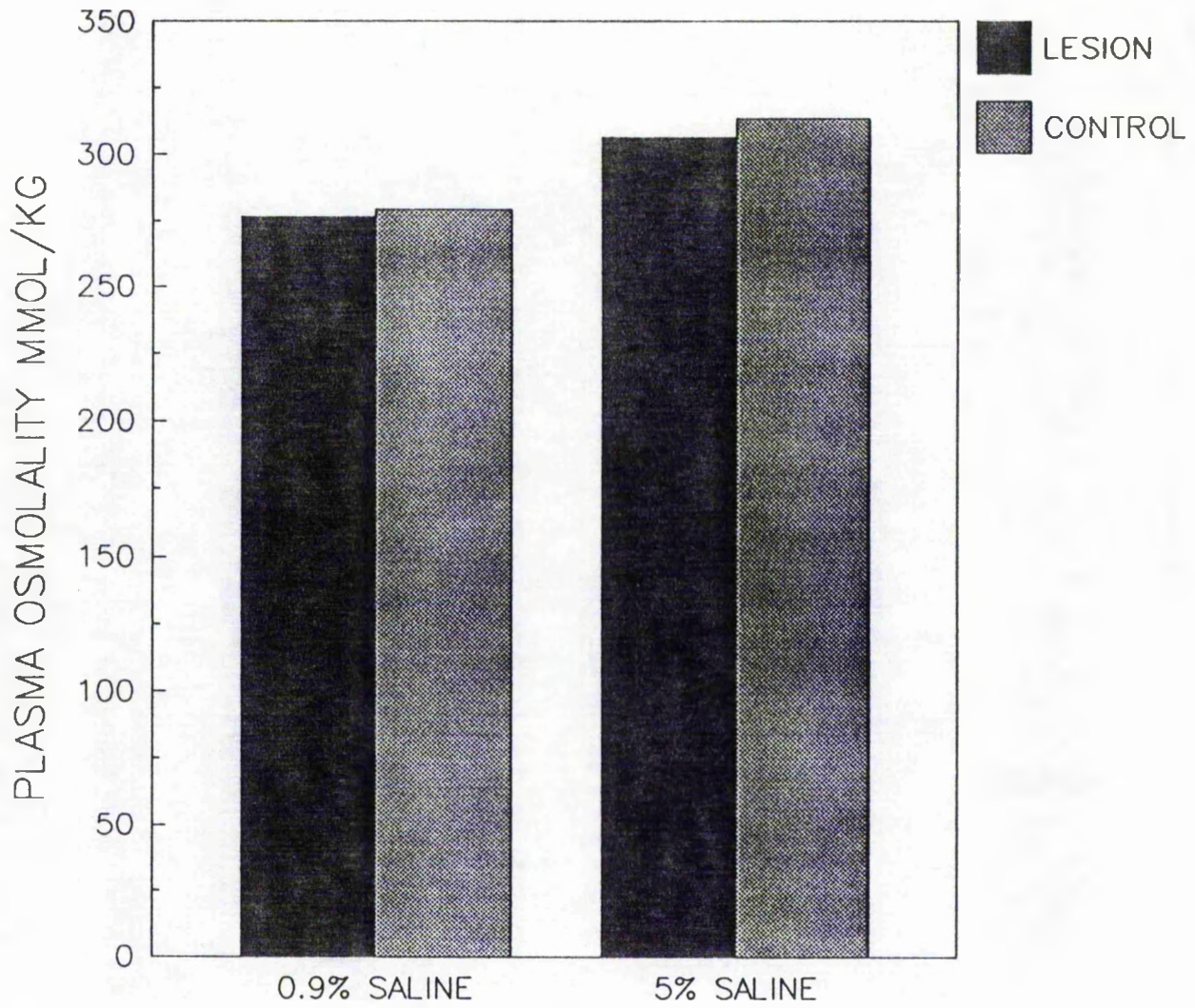


FIG 32

and control animals as there was no significant difference found between groups (ANOVA: F=0.2308; df=1,14; p=0.6383) and no interaction between groups and conditions (ANOVA: F= 0.0913; df=1,14; p=0.7669). By comparison with plasma osmolality reported in the last experiment when no injections had been given, it appears that injections of isotonic saline reduced plasma water concentrations. Lesioned and control animals were affected similarly.

Results from the AVP analysis can be seen in Fig 33. ANOVA showed that there was no interaction between groups and conditions (F=0.3344; df=1,14; p=0.5723) and no significant lesion effect on the groups (F=1.5537; df=1,14; p=0.233). However, both lesioned and control animals given hypertonic saline showed increased plasma AVP levels and ANOVA showed this to be a significant effect (ANOVA: F=19.7837; df=1,14; p=0.0006). This indicates that in this measure both lesioned and control groups responded in the same way to hypertonic saline and had increased AVP levels in response to dehydration. Thus, in contrast to the behavioural results reported above and presented in Figure 31, LH lesioned animals did <u>not</u> show a physiological deficit in response to hypertonic saline administration. That is, AVP levels in the plasma of both groups were significantly greater following hypertonic saline administration compared to administration of isotonic saline (ANOVA: F=16.073; df=1,31; p=0.001). Thus, both groups had increased AVP plasma levels in response to dehydration and, in contrast

FIGURE 33: Plasma AVP levels 1 hour after injections of isotonic or hypertonic saline can be seen in this figure. Both lesioned and control animals had increased plasma AVP following hypertonic saline administration and ANOVA showed a conditions effect but no groups effect or interaction. Standard errors were never more than 2.88.

PLASMA AVP LEVELS FOLLOWING 0.9% OR 5% SALINE

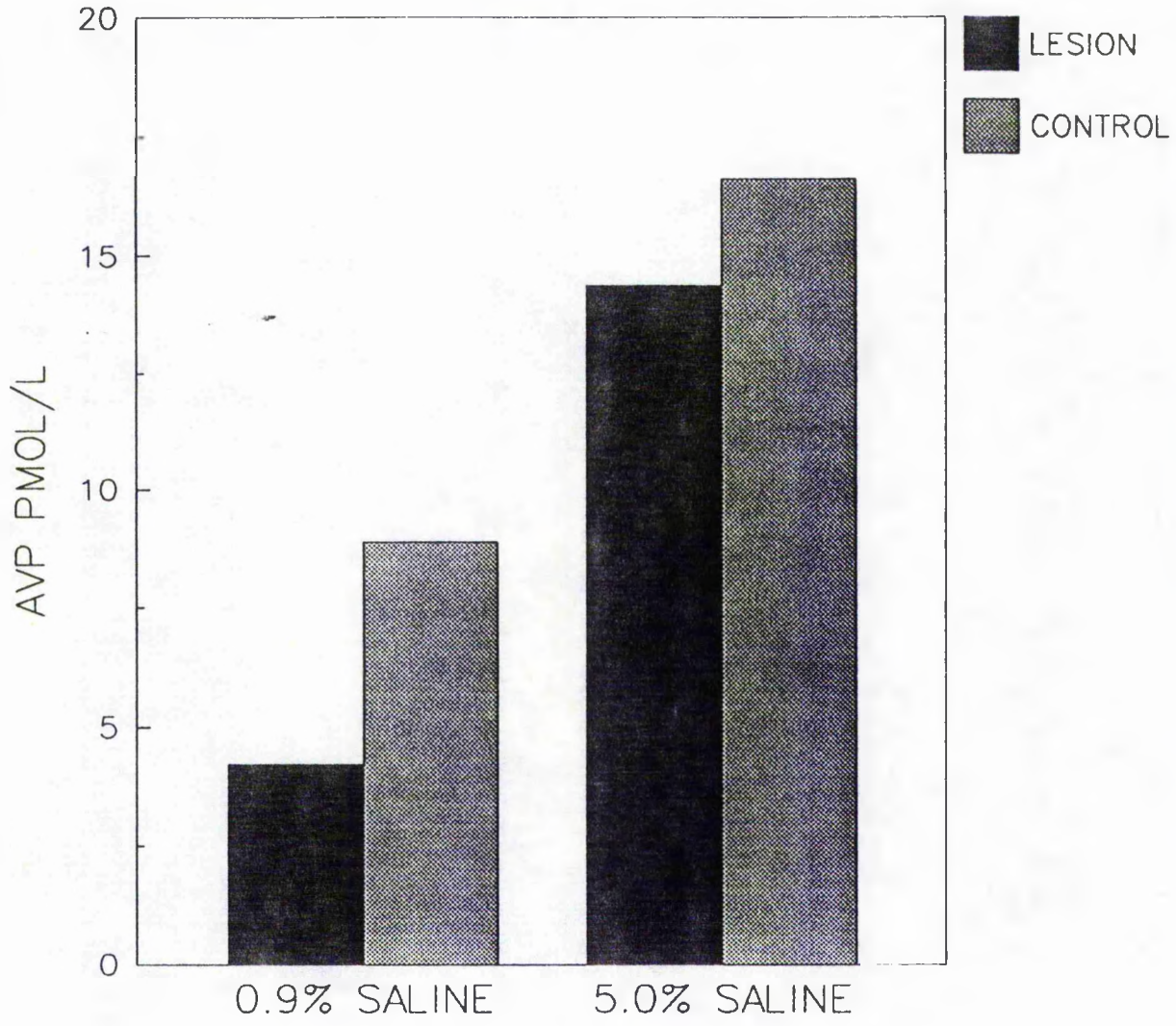


FIG 33

to the behavioural results reported above and shown in Figure 31, LH lesioned animals did not show a physiological deficit in response to hypertonic saline administration. Although lesioned animals did not respond behaviourally in response to intracellular dehydration by drinking, they did respond physiologically with increased levels of plasma AVP, which is used to conserve body water.

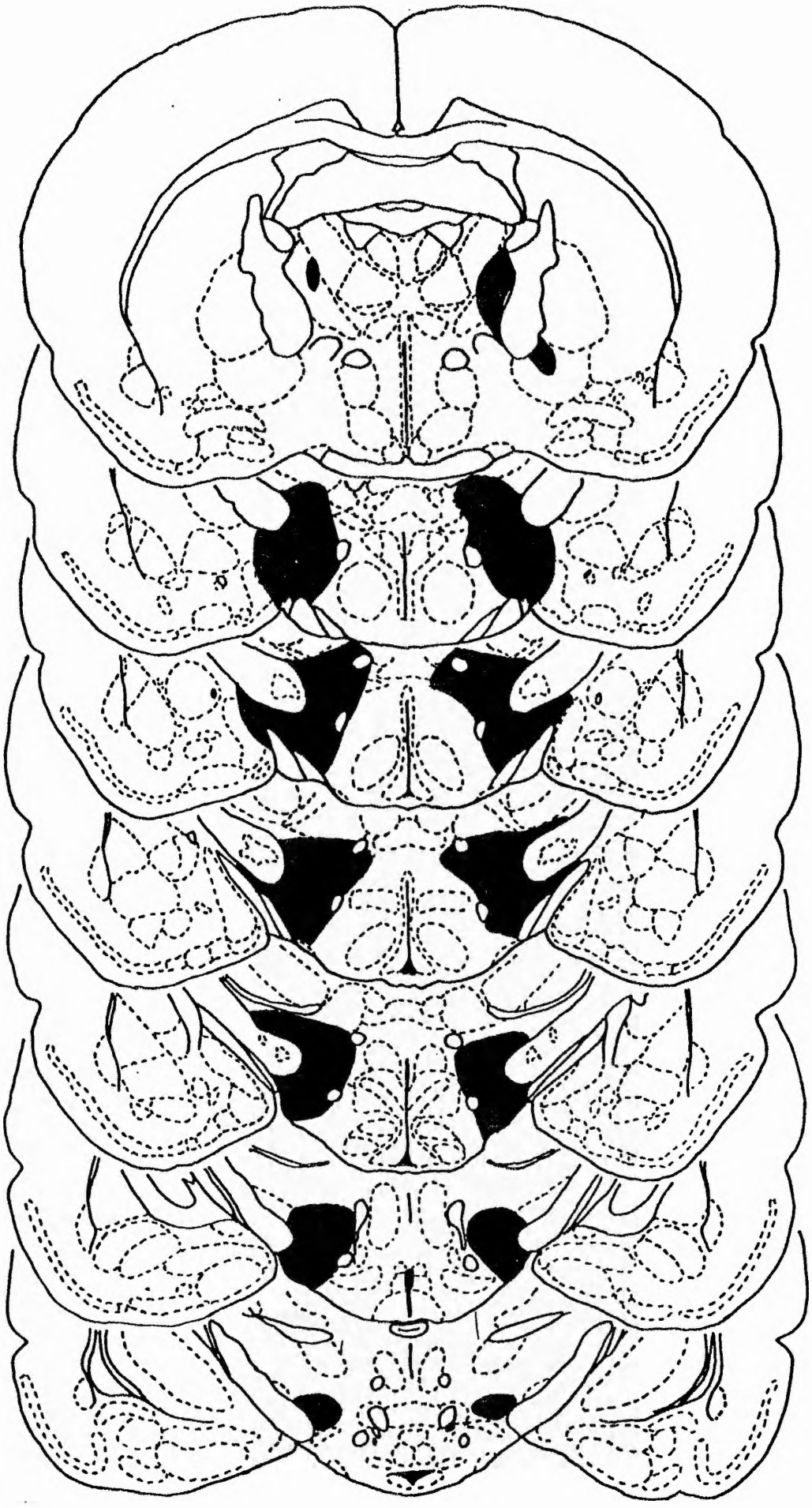
HISTOLOGY

Histological procedures are described in Chapter 5. As before, there was almost total destruction of the central core of the LH, especially between the levels of the PVN and the DMH. Damage to the poles, in particular the anterior pole, was minor. Average lesion size was 75.4% (SE=4.64) and lesions ranged from 34.6% to 92.7%. Extra-hypothalamic damage was found consistently in the zona incerta, reticular nucleus of the thalamus and subthalamic nucleus. Some damage was also found in the thalamus and amygdala of a few of the animals. The pattern and size of a typical lesion can be seen in Fig 34.

DISCUSSION

These experiments were designed to investigate whether or not there was an alteration in the physiological responses

FIGURE 34: The size and pattern of a typical lesion for this experimental group can be seen in this figure. Shaded area represents lesion site. Although most of the LH was removed, the anterior pole was not damaged. Extra-hypothalamic damage occurred in the thalamus, globus pallidus and zona incerta. (Rat J.149/88)



to dehydration. Such an alteration could imply that the physiological signals which stimulate the behavioural and physiological responses to dehydration have been changed following lesion of the LH and could account for the behavioural deficits seen in response to physiological challenges. As the most commonly observed deficit arises in response to hypertonic saline, measures of AVP, a hormone involved in the conservation of water, were taken.

The first experiment indicated that there was no difference between the LH lesioned and the control groups in the baseline levels of AVP in their bloodstream suggesting that, under normal conditions, at least one hormonal response to dehydration was normal in LH lesioned animals.

In the context of the hypothesis under examination in this chapter, the most important result is that LH lesioned animals respond as controls to the administration of hypertonic saline by an increase in release of AVP. These results are in stark contrast to the behavioural results; the increase in AVP (seen in Fig 33) following hypertonic saline administration is not accompanied by an increase in drinking (seen in Fig 31). This suggests that the deficit in responding to hypertonic saline is not due to a disruption of the physiological signals associated with dehydration as the physiological response is normal. If the signals had been altered, then the response would also be expected to be different. The LH lesioned animals do not

respond behaviourally to the dehydration produced by this challenge but they do respond physiologically by production of at least one hormone which promotes conservation of water, indicating that the dehydration stimulus must still be intact. The release of AVP is a response to a stimulus and its correctness after the lesion indicates that the dehydration stimuli are properly processed. Thus, although the appropriate physiological responses to dehydration are made, no behavioural measures are taken by these animals (i.e. drinking) in response. This suggests that the nature of the deficit following LH lesions may not be one of "stimulus" disruption. The contrast between behavioural and physiological data is quite startling.

The intact physiological response in these LH lesioned animals was not found in animals given lesions of the ventral noradrenergic bundle (**Lightman, Todd and Everitt,** 1983) which showed attenuated AVP release in response to hypertonic saline. The data presented in this chapter supports the hypothesis presented by those authors that a large proportion of the AVP response to hypertonic saline is dependent upon the integrity of the connections between the lateral tegmental area, the medulla and the hepatic portal system, rather than upon hypothalamic osmoreceptive mechanisms.

Vassopressin is, of course, only one physiological marker out of many. Perhaps future experiments should measure

levels of glucose, insulin, glycogen, glucagon or angiotensin.

The results of these experiments indicate that at least one physiological response to dehydration is not disrupted by lesions of the LH. This implies that the dehydration signals may be intact. The nature of the deficit following such lesions may not, therefore, be one of "stimulus" disruption.

CHAPTER 9

RECOGNITION

The previous experiments demonstrate to some extent that lesions of the LH do not alter animals' ability to perceive the sensory qualities of their diet and that at least one important physiological factor involved in the control of water balance appears to remain intact. These results suggest that the motivational impairment following lesions of the LH may not be due to either a problem of "incentive" or to disruption of a physiological process. Perhaps, therefore, intact signals are not recognised or understood and animals fail to respond to physiological signals because they fail to recognise the need to do so.

It may be suggested that this hypothesis cannot be true because some LH lesioned animals can recover normal daily food and water intake as seen in Chapter 7. If they can maintain homeostasis under normal circumstances then surely they must be able to recognise their internal state. However, it must be remembered that these are laboratory rats, housed singly in small cages and they may eat out of boredom (**Robbins and Fray**, 1980). They also live in an environment which follows a strict routine in terms of light/dark cycle and daily weighing. They may eat out of

habit or as a learned response. Some evidence has been provided that suggests that rats drink in anticipation of dehydration under normal circumstances, rather than in response to dehydration (Fitzsimons and Le Magnen, 1969). Rats appeared to learn to match water intake with water need and drank to prevent thirst, rather than in response to thirst. This was observed by changing the rats' carbohydrate diet to a protein diet. At first, rats drank after meals when the diet was changed in response to the greater need for water. They soon, however, began to drink the required amount before meals in anticipation of thirst. The animals used in the experiments described in this thesis were given the same diet throughout their lives and had probably learned how much fluid they needed. Thus, knowledge of hydrational state would only be necessary if the diet was changed or under extreme circumstances, such as in response to physiological challenges. Perhaps in an environment of free access it is not necessary to be able to recognise physiological signals and the necessity only arises under extreme conditions when it is vital to know what state your body is in; for example, following sudden dehydration or glucoprivation. Under such extreme conditions, the nature of the signal must also be extreme. To respond to physiological challenges it is necessary to understand physiological signals exactly.

It would seem from the data presented in this thesis that LH lesioned rats cannot understand peripheral signals

because they show an impairment in responding to the physiological challenges of intracellular dehydration by injection of hypertonic saline and of glucoprivation by injection of 2-DG. It could be suggested that their daily food and water intake may be learnt responses rather than direct consequences of their state of energy or fluid balance and that their lack of recognition of this state is revealed by the deficits shown to the physiological challenges mentioned above. However, there are two important things to consider about both of these challenges. The first is that both challenges are negative in nature; that is, they put the animals into a state of deprivation. The second is that both challenges are acute, in that they require an immediate response to a sudden change in internal state. It was reasoned that, to be able to claim that the motivational deficit was really one of "recognition", LH lesioned animals must be tested (a) in a positive and (b) in a chronic way; that is, animals must show a deficit in regulation when given physiological challenges which (a) involve a state of excess or overload as opposed to deprivation or (b) which involve long term responding as opposed to immediate responding. Without these tests, it is impossible to say whether the deficit shown by these animals lies in "recognition" or "response". The following experiments were devised to explore the hypothesis that the impairment seen in LH lesioned animals arises from a lack of recognition or understanding of intact physiological signals. Several groups of

experimental animals were used to investigate this hypothesis and the surgery and recovery data for each group will be given alongside the experiments in which they took part.

DEPRIVATION EXPERIMENTS

The most simple experiments performed in this investigation were ones of deprivation. An injection of hypertonic saline or of 2-DG places the animal into an almost immediate state of dehydration or glucoprivation. Perhaps it is the "immediacy" of the challenge which LH lesioned animals find difficult to respond to, either in the immediacy of the recognition or of the response needed. Food or water deprivation over 24 hours also places the animal in a state of dehydration or glucoprivation, but this state is reached over a much longer period of time. This, therefore, provides the same challenge in terms of physiological action, but alters the parameters of the challenge in terms of time course.

SURGERY AND NORMAL REGULATORY BEHAVIOUR

This experiment was performed on the animals described in Chapter 7 several weeks after finickiness tests. Surgery, body weight and recovery can be found on page 78. In summary, there were 17 lesioned and 11 control animals.

Recovery following lesions was good and animals did not show sustained body weight, food intake or water intake reductions.

METHOD

The procedure was similar for both food and water deprivation. Food or water was removed for 24 hours. On return to free access, amount consumed was measured after 1 hr, 3 hrs and 24 hrs. Animals deprived of food were given ad lib water and free access to food was given to animals deprived of water.

Results are presented in Figs 35 and 36. ANOVA revealed that there was no significant difference between the groups after water deprivation ($F=2.8386$; $df=2,26$; $p=0.104$) or after food deprivation ($F=3.2843$; $df=2,26$; $p=0.0815$) and that there was no interaction between groups and time after water deprivation ($F=0.0283$; $df=2,52$; $p=0.9721$) or after food deprivation ($F=0.0273$; $df=2,52$; $p=0.9731$). Thus, there was no significant difference in amount consumed by the groups or in their pattern of eating or drinking. The lesioned animals did not take longer than controls to respond as they do following hypertonic saline or 2-DG injections.

The state of dehydration or glucoprivation following either 24 hrs deprivation or the injections of hypertonic saline

FIGURE 35: Water intake 1 hour, 3 hours and 24 hours after free access was given to water following a period of 24 hours water deprivation are presented in this figure. No differences were found between the groups.

WATER DEPRIVATION

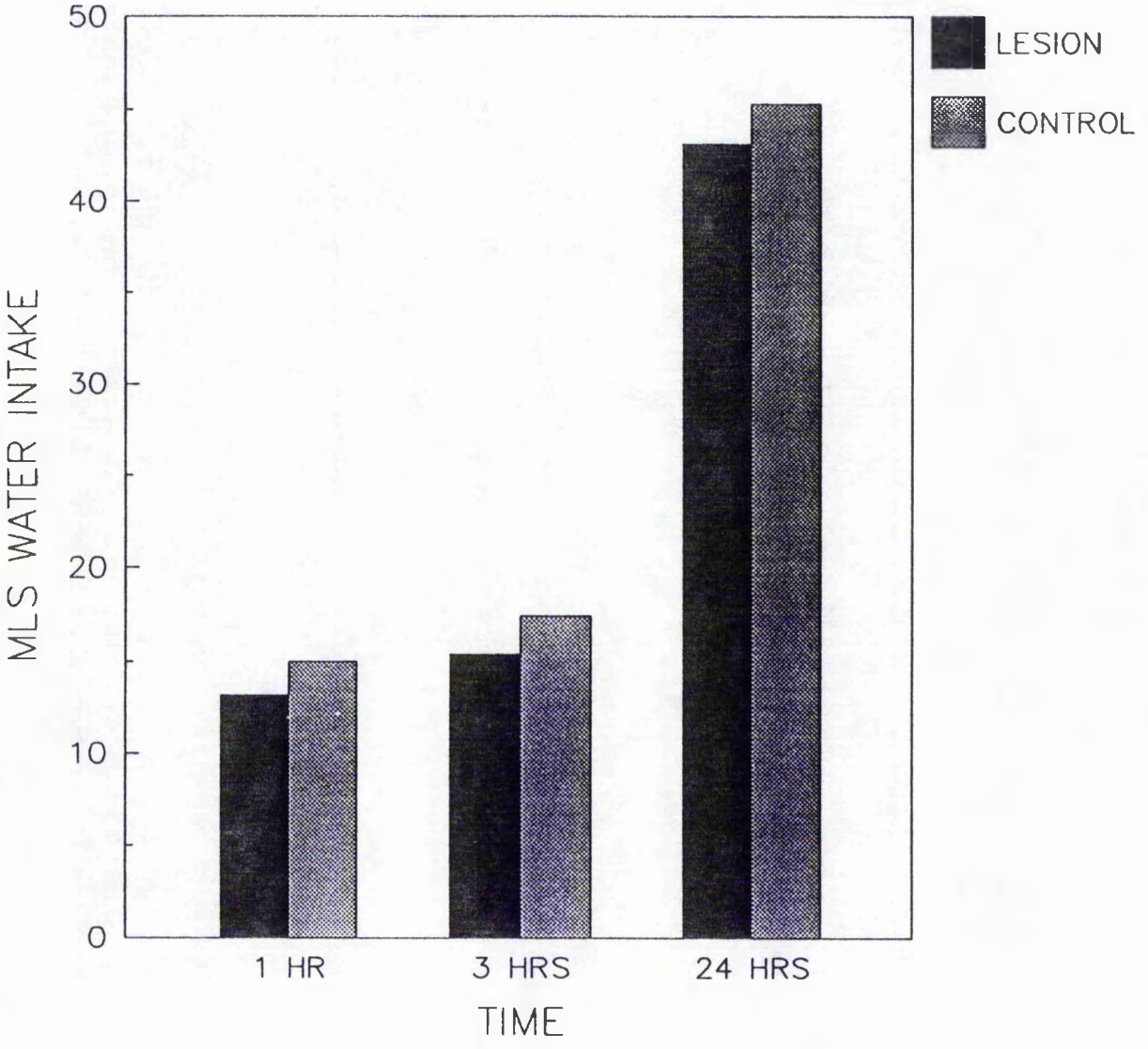


FIG 35

FIGURE 36: Food intake 1 hour, 3 hours and 24 hours after free access was given to food following a period of 24 hours food deprivation are presented in this figure. No differences were found between the groups.

FOOD DEPRIVATION

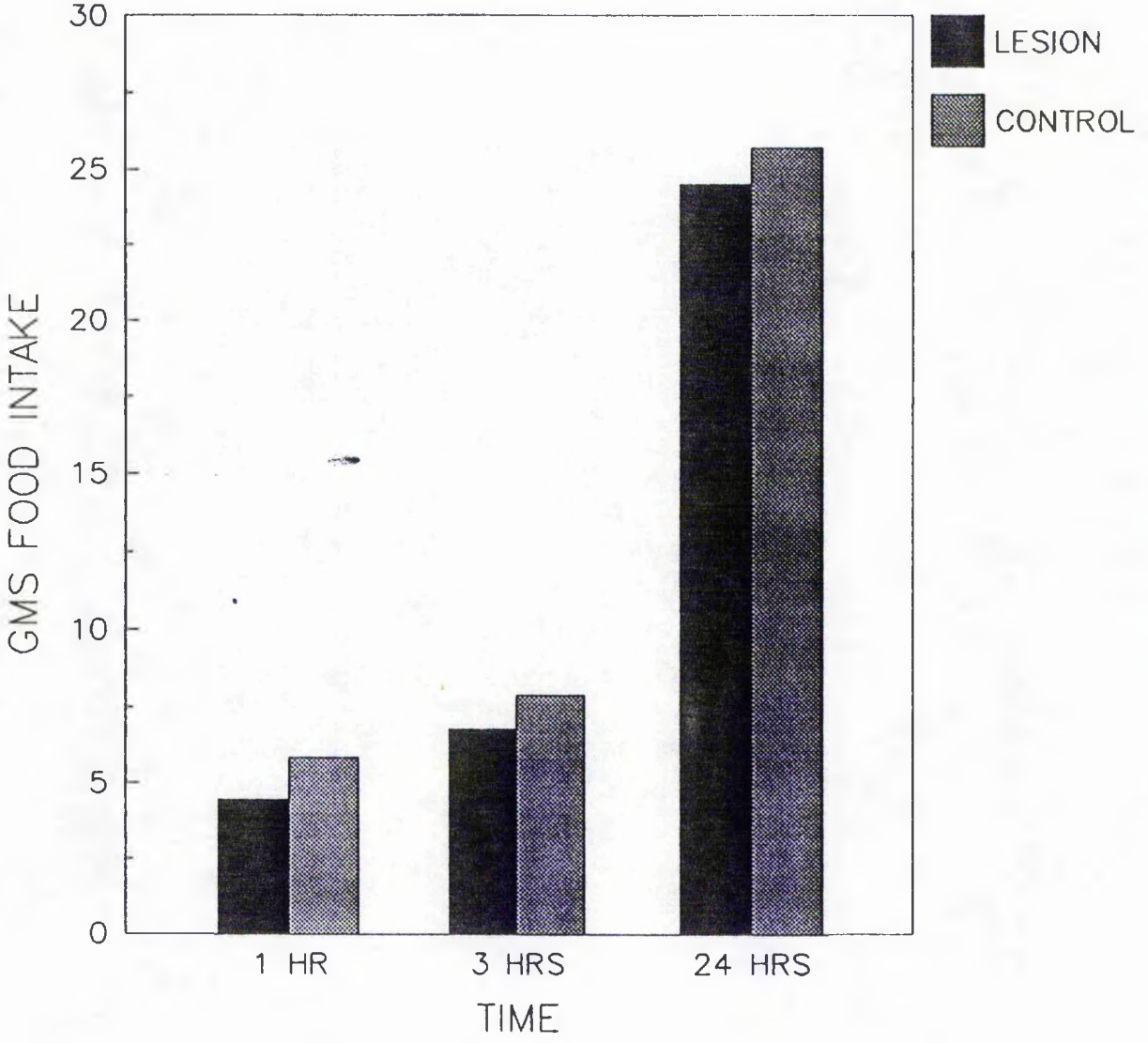


FIG 36

or 2-DG do not appear to be exactly the same since they produced slightly different responses from control animals. One hour after the return of water, control animals drank an average of 14.98mls (±0.57) following water deprivation in comparison with 21.33mls (±2.4) following hypertonic saline administration. Three hours after the return of food, injections of 2-DG induced an intake of 4.35gms (±0.42) of food (see Fig 16) in comparison with 7.89gms (±0.45) induced by 24 hrs food deprivation. Thus hypertonic saline administration would appear to be a more potent challenge than 24 hrs water deprivation and 24hrs food deprivation would appear to be a more potent challenge than injections of 2-DG. However, the fact remains that all of these challenges induce greater intake in control animals than is usual during the daytime (1hr normal water intake = 2.5mls ±0.37; 3hrs normal food intake = 3.07gms ±0.39) which suggests that all of these challenges induce a change in body energy and/or fluid balance to which the animals respond. Only the time course of the change to energy and fluid levels has been altered.

In the case of food deprivation and 2-DG, the LH lesioned rats response to the stronger stimulus (deprivation) is better than their response to the weaker stimulus (2-DG). This could lead to the suggestion that reduced responding to 2-DG arises because the challenge was not of sufficient strength and that the results indicate a shift to the right of the dose response curve, rather than an abolition of

responding. If this were the case, the LH lesioned animals' response to 2-DG should improve if a larger dose of 2-DG were given. However, in the case of water deprivation and hypertonic saline, the effects are reversed; the LH lesioned rats' response to the weaker stimulus (deprivation) is better than their response to the stronger stimulus (hypertonic saline). Thus the severity of the challenge does not seem to predict the LH lesioned animals' response, but there does seem to be an association between response and suddenness of onset of the challenge. These results appear to dissociate the suddenness of onset of the challenge from its severity and may imply that the lesioned animals are failing to respond to the "immediacy" rather than the strength of the challenge.

In summary, LH lesioned animals responded in the same way as their controls to food and water deprivation, suggesting that they were aware of their state of deprivation and able to respond appropriately. One might assume, therefore, that not only must the physiological signals be intact, but that the rats must be able to recognise them. In these experiments the "immediacy" of the physiological challenge had been removed and the fact that lesioned rats could respond appropriately in these circumstances suggests that it may be the "immediacy" of the hypertonic saline challenge which caused problems for LH lesioned animals. The results do not rule out the possibility that lesioned animals have problems in recognition, but suggest that

these problems must be complex ones which can be resolved if the animal has sufficient time to do so. It may not be recognition of the problem, but generation of the correct response which takes time. This hypothesis will be returned to later.

CALORIFIC INTAKE REGULATION

Glucose is necessary to meet the metabolic needs of the living cell. A constant glucose concentration is achieved by controlled release of hormones from the pancreatic islets of Langerhans. If the body is using sensitive mechanisms to maintain a relatively constant glucose concentration it seems sensible that this should also be translated into behaviour and that animals should regulate energy intake around an optimum level, or within an acceptable range. If this is the case, then rats should decrease the amount of dry lab chow eaten daily when given a palatable glucose solution to drink instead of tap water. Compensation should be made for the calorific intake in the water supply by decreasing calorific intake elsewhere. If, however, LH lesioned animals cannot understand their own physiological signals, then they should not realise that they are consuming excess calories and should not compensate behaviourally by reducing food intake. This could lead to the unexpected situation of finding an LH

lesioned animal with an increased calorific intake and possibly an increased weight.

SURGERY

Bilateral LH lesions were made in 10 male Lister-hooded rats by microinjection of 1.0ul of 0.12M NMDA (pH 7.0) at the following stereotaxic co-ordinates in the orientation of de Groot: bregma +0mm, lateral ± 2.0mm, vertical -8.0mm (Pellegrino, Pellegrino and Cushman, 1979). Control animals (n=10) were microinjected with phosphate buffer (pH 7.4). Average weight before surgery was 350.1 gms (SE=8.68; range = 268.7gms - 421.7gms). Five lesioned and 2 control animals died because of a skin infection unrelated to the surgical procedure. The data from these animals have not been included in the results.

NORMAL REGULATORY BEHAVIOUR

BODY WEIGHT: [Fig 37] Body weight was measured daily and calculated in terms of percentage weight from day of surgery. Both control groups are treated as one in the following calculations. Before surgery, no significant difference was found between groups (ANOVA: $F=1.9462$; $df=1,11$; $p=0.1905$). One week post-op, lesioned animals lost weight and there was a significant difference between the groups (ANOVA: $F=21.9854$; $df=1,11$; $p=0.0007$) and an

FIGURE 37: Percentage body weight for animals used in calorific intake regulation experiments are presented in this figure. Post-surgery there was a significant difference between the groups as lesioned animals lost weight. This difference was maintained, but lesioned animals gained weight at the same rate as controls after 1 week of recovery.

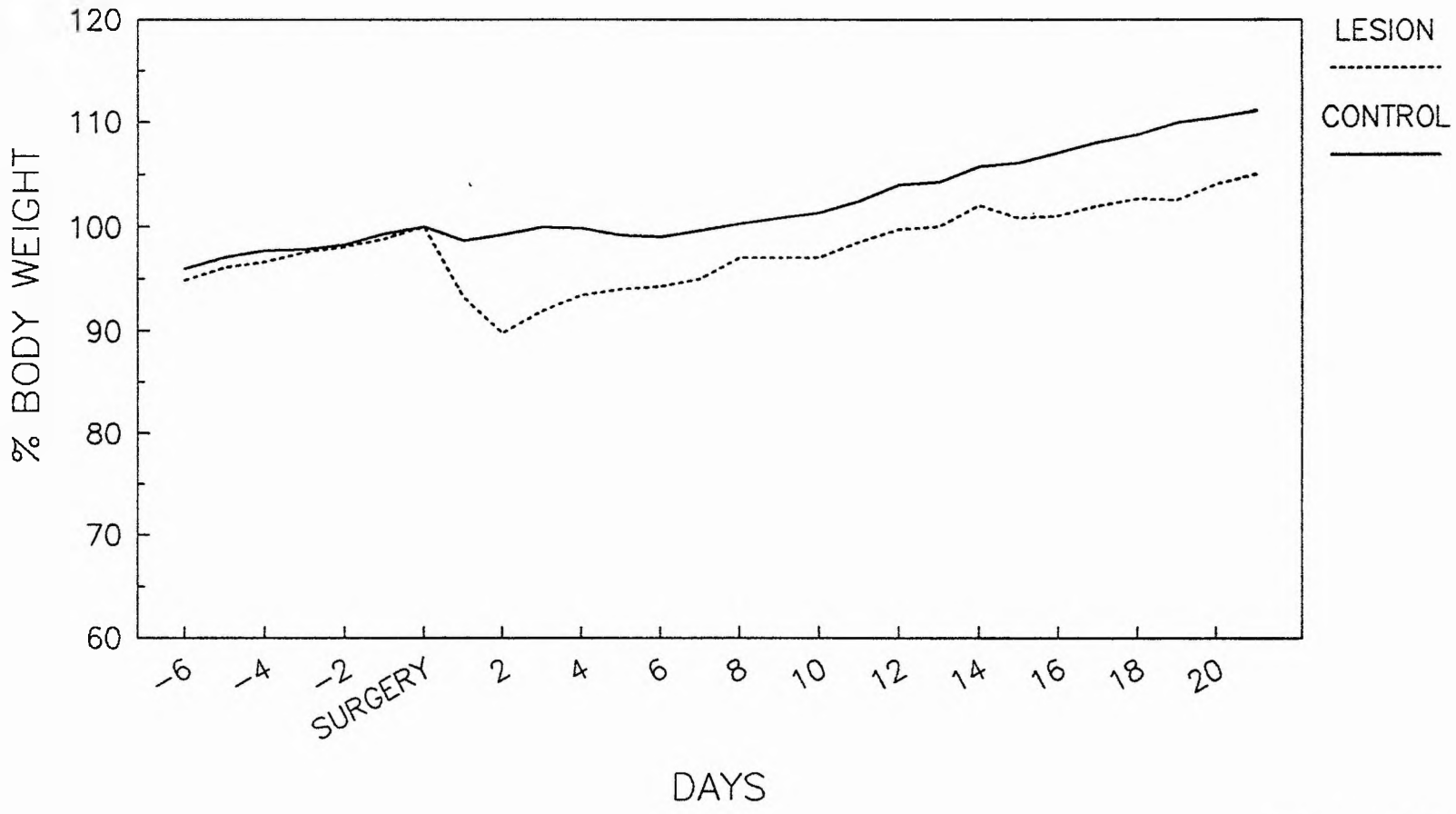

% BODY WEIGHT
CALORIFIC INTAKE REGULATION
120
110
100
90
80
70
60
% BODY WEIGHT
-6
-4
-2
SURGERY
2
4
6
8
10
12
14
16
18
20
DAYS
LESION
CONTROL

FIG 37

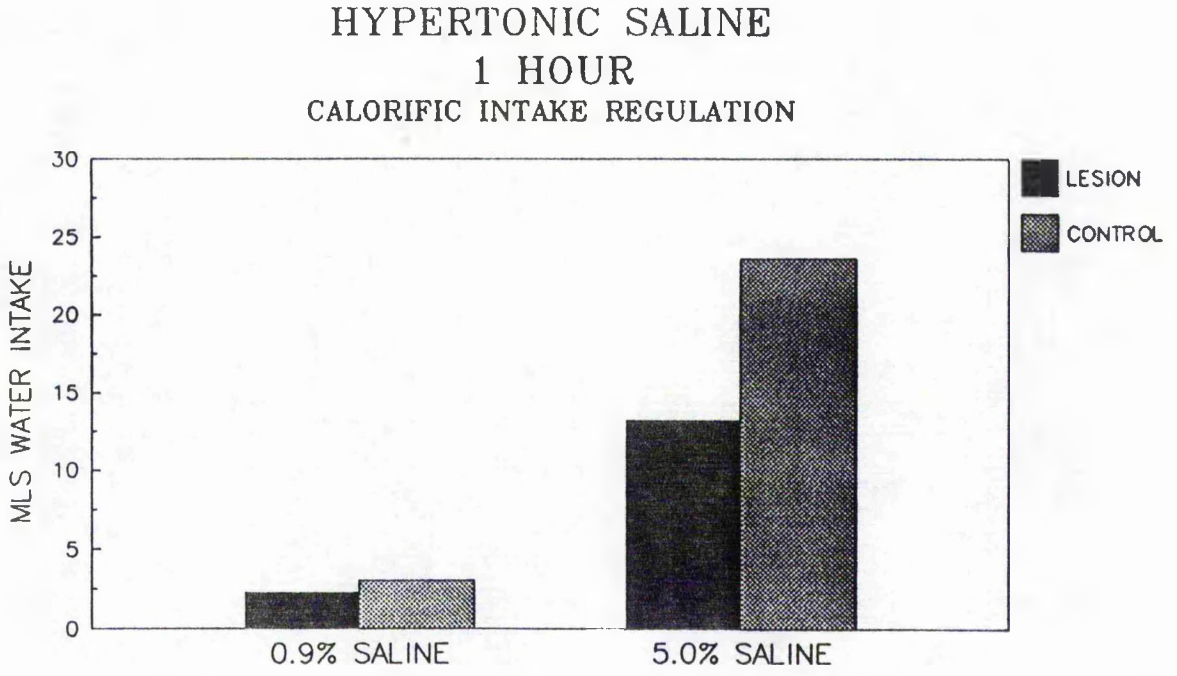
interaction between groups and days (ANOVA: F=6.6739; df=6,66; p<0.001). The difference between the groups remained in week 2 (ANOVA: F=6.5277; df=1,11; p=0.0268) and week 3 (ANOVA: F=8.9833; df=1,11; p=0.0121), but there was no longer an interaction between groups and days (ANOVA: week 2, F=0.5741; df=6,66; p=0.7496: week 3, F=1.4684; df=6,66; p=0.2027), indicating that the lesioned and control animals were now gaining weight at the same rate.

Due to the nature of this experiment, it is not appropriate to present normal food and water intake data at this stage. These will be incorporated with other results and presented later.

HYPERTONIC SALINE

Detailed methodology for the administration of hypertonic saline is outlined in the general procedures section. ANOVA revealed a significant interaction between groups and conditions after 1hr (F=8.9809; df=1,11; p=0.012) and 3 hrs (F=7.9776; df=1,11; p=0.016). This indicates that LH lesioned animals did not respond as control animals to the challenge and that, although they drank more in response to hypertonic saline than in response to isotonic saline, they still showed a deficit when compared to the control group [see Fig 38].

FIGURE 38: Responses to hypertonic saline physiological challenge at 1 hour and 3 hours post-injection are presented in this figure. Lesioned animals showed a deficit in response to this challenge as revealed by ANOVA. Standard errors were never more than 3.46.


HYPERTONIC SALINE
1 HOUR
CALORIFIC INTAKE REGULATION
MLS WATER INTAKE
30
25
20
15
10
5
0
0.9% SALINE
5.0% SALINE
LESION
CONTROL

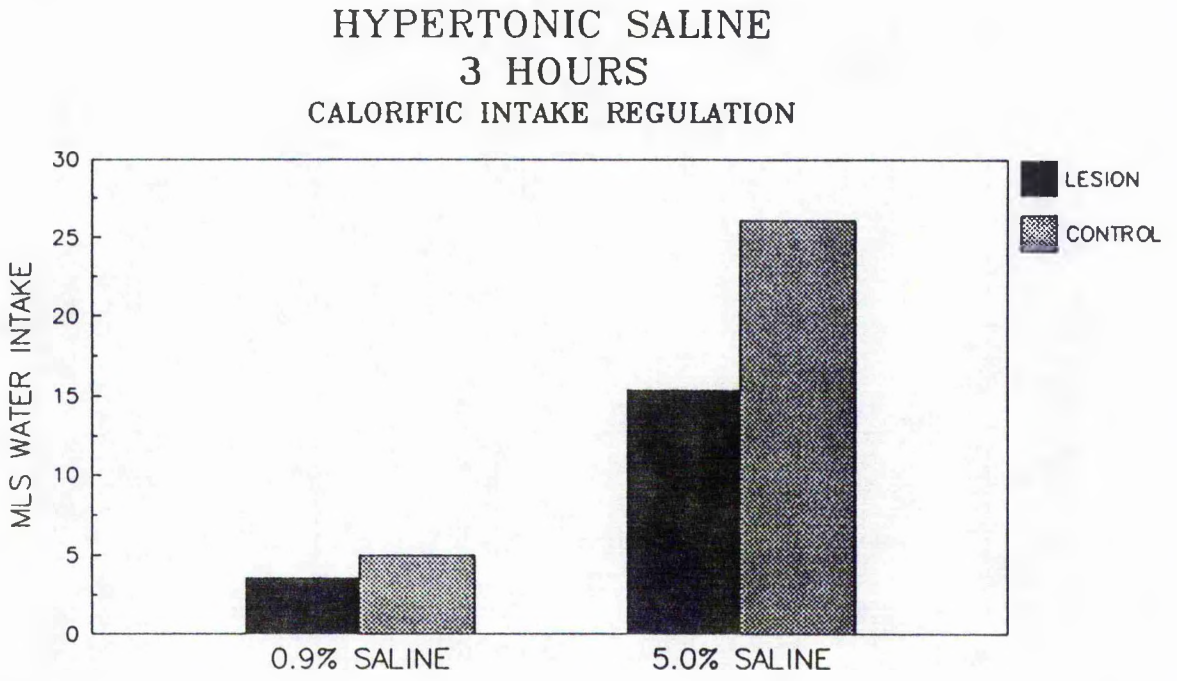

HYPERTONIC SALINE
3 HOURS
CALORIFIC INTAKE REGULATION
MLS WATER INTAKE
30
25
20
15
10
5
0
0.9% SALINE
5.0% SALINE
LESION
CONTROL

FIG 38

As the deprivation experiments had demonstrated the LH lesioned animals' ability to respond to a challenge over time, water intake was also measured 24hrs after hypertonic saline injections. The results can be seen in Figure 39. ANOVA revealed that there was a significant difference between the conditions (i.e. hypertonic or isotonic saline) (ANOVA: $F=69.3863$; $df=1,11$; $p<0.001$), but no groups effect (ANOVA: $F=1.5689$; $df=1,11$; $p=0.2363$) and no interaction between groups and conditions (ANOVA: $F=2.6815$; $df=1,11$; $p=0.1298$). Thus, both control and lesioned animals increased water intake over a 24hr period following an intracellular dehydration challenge.

These results lend further support to the idea that LH lesioned animals can respond to a challenge if allowed time to do so and that the "immediacy" of the challenge presents a problem either in recognition or response. The lesioned animals may need time to recognise or assess the meaning of internal signals or they may need time to generate the correct response.

PROCEDURE FOR CALORIFIC INTAKE REGULATION EXPERIMENTS

Half of the sham and all of the NMDA lesioned animals were given 6.3% glucose solution to drink after one week post-op; the other sham group were kept as a permanent control and given tap water to drink. All animals were

FIGURE 39: Responses to hypertonic or isotonic saline injections 24 hours after administration are presented in this figure. Both lesioned and control animals displayed increased drinking in response to hypertonic saline; there was a conditions effect, but not a groups effect or an interaction using ANOVA. Standard errors were never more than 4.98.

HYPERTONIC SALINE
24 HOURS
CALORIFIC INTAKE REGULATION

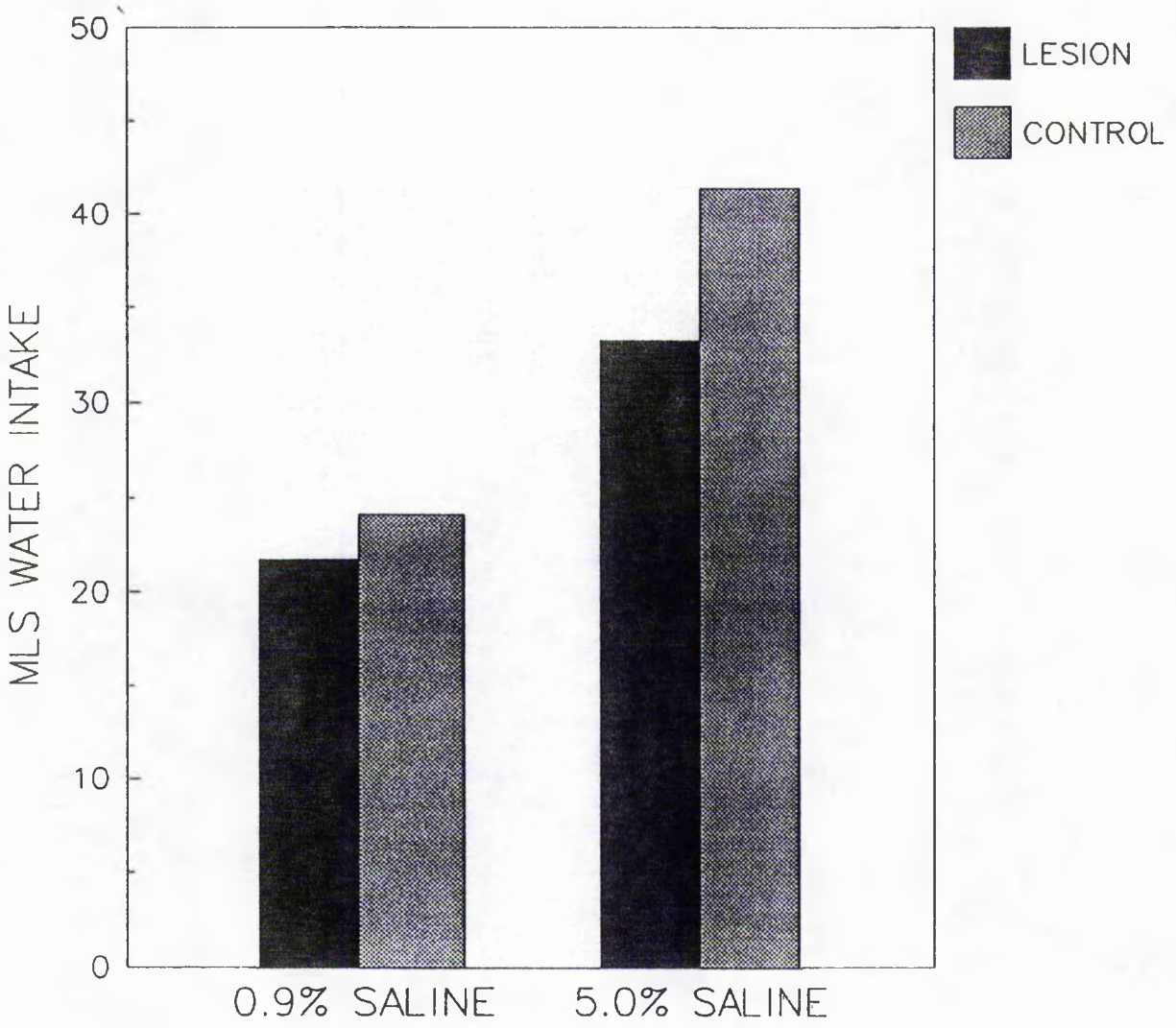


FIG 39

given dry lab chow to eat. 6.3% glucose solution was used because a previous pilot study by P. Winn and A. Clark had shown this concentration to be effective in reducing lab chow intake.

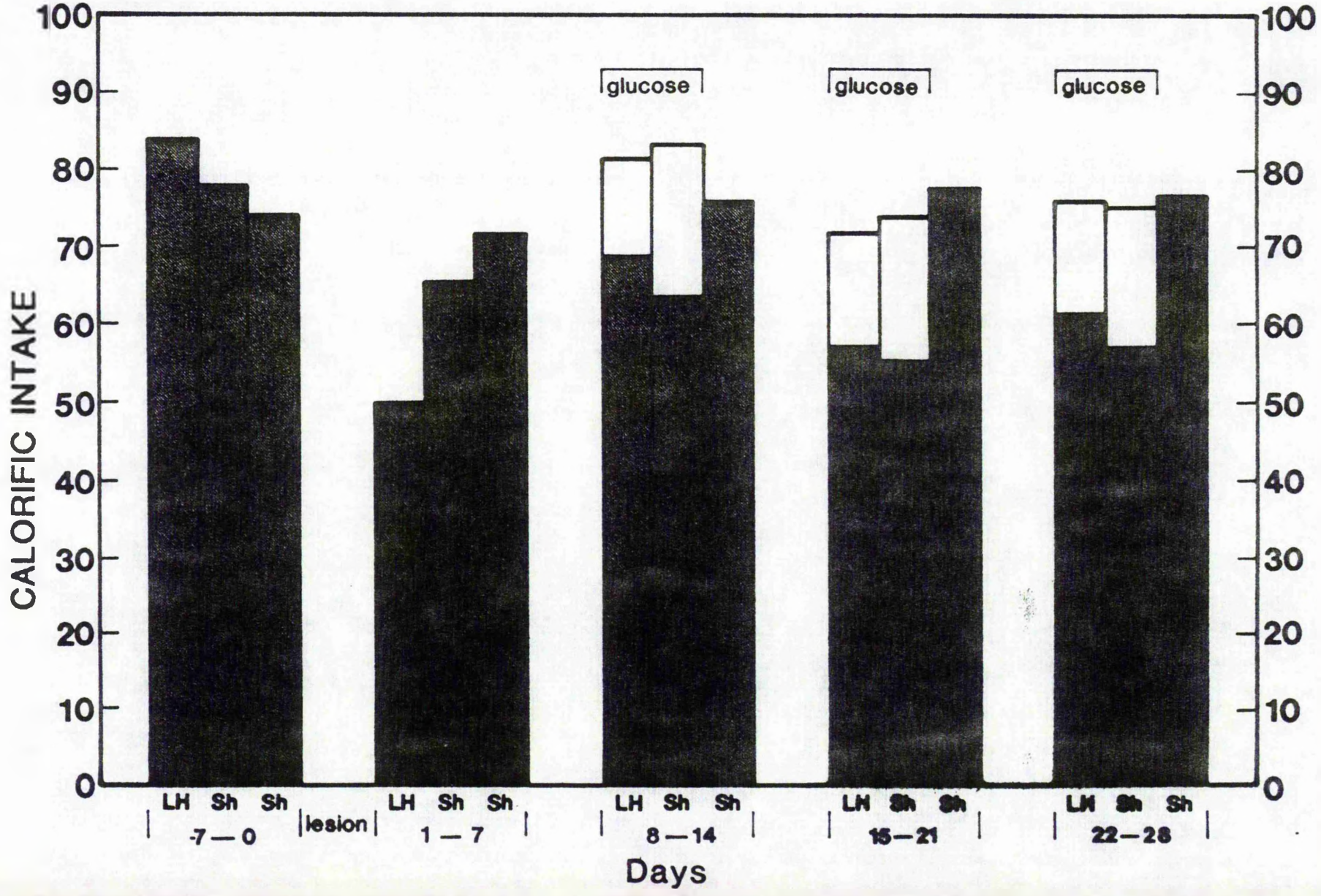
CALORIFIC INTAKE

Daily calorific intake was calculated by converting both lab chow and glucose into calories. The lab chow (S.D.S. No.1 Maintainance Diet) contained 3.04 Kcal/gm and the glucose (Sigma) contained 3.48 Kcal/gm, which meant that the 6.3% glucose solution contained 0.22 Kcal/ml.

As can be seen in Fig 40, after one week of reduced intake following surgery, there was no significant difference in calorific intake between the three groups (ANOVA: $F=0.2479$; $df=2,11$; $p=0.785$).

During the period of glucose administration, significant differences were found between the lab chow intake of the glucose and non-glucose groups (ANOVA: $F=7.1149$; $df=2,11$; $p=0.0104$). Tukey tests showed no significant difference between the two glucose groups (one lesioned and one control) in lab chow intake, but both ate significantly less lab chow than the control group ($p<0.01$). These results indicate proper energy intake regulation in both normal and LH lesioned animals. No significant differences

FIGURE 40: This relatively complicated figure depicts overall calorific intake of lesioned and control animals pre- and post-surgery. There were three groups: lesioned animals given glucose; control animals given glucose; and control animals not given glucose. The shaded area represents calorific intake from lab chow and the empty bars on top of shaded areas represent calorific intake from glucose. From this comparisons of overall calorific intake can be assessed in addition to comparisons of intake of lab chow and of glucose. Lesioned and control animals maintained a constant calorific intake by reducing lab chow intake when given glucose solution to drink.


GLUCOSE LOADING
CALORIFIC INTAKE
100
90
80
70
60
50
40
30
20
10
0
glucose
glucose
glucose
LH Sh Sh
-7 — 0
lesion
LH Sh Sh
1 — 7
LH Sh Sh
8 — 14
LH Sh Sh
15 — 21
LH Sh Sh
22 — 28
Days

were found between the glucose intake of lesioned or control animals (ANOVA: F=0.6057; df=1,8; p=0.4588).

It could be argued that animals given glucose reduced their food intake not because they were aware of energy intake, but simply because they were aware of stomach distention due to the large increase in fluid volume consumed. This hypothesis can probably be rejected: normal animals given a palatable saccharin solution to drink increase fluid intake without altering lab chow intake (Fig 41). The saccharin contains no calories and the animals do not need to compensate. The stomach distention due to the fluid overload does not affect food intake.

These results indicate that, given a long-term positive challenge, LH lesioned animals can regulate energy intake and are presumably, therefore, aware of homeostatic signals. It also means, by implication, that these signals are intact and lends support to the data described in Chapter 8.

WATER LOADING

It was hypothesised that, if animals were aware of their internal physiology, then injection with water after water deprivation should attenuate, if not prevent, post-deprivation drinking. Furthermore, injection with

FIGURE 41: The data presented in this figure are taken from a pilot study performed by P. Winn and A. Clark. The effects of saccharin solution to drink on fluid intake and food intake are presented; fluid intake was increased, but food intake was unaffected. Standard errors were never more than 9.46.

EFFECT OF SACCHARIN SOLUTION TO DRINK ON FOOD AND FLUID INTAKE

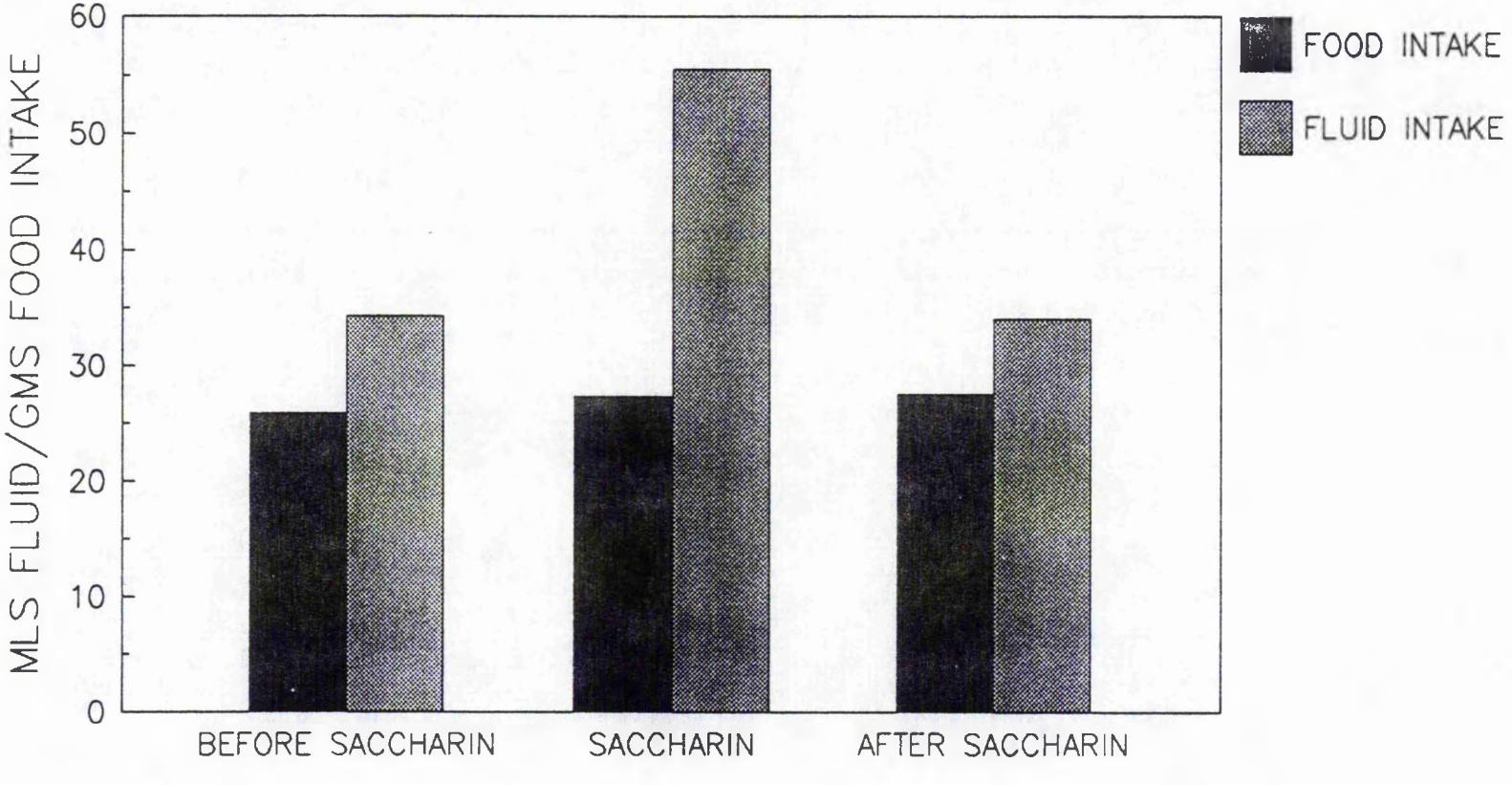


FIG 41

physiological saline after water deprivation should not affect rebound drinking to this extent, because the physiological saline would not immediately alter the intra-/extra-cellular balance in the way that water does. Animals which are unaware of or do not recognise their own internal physiological signals should respond similarly to both fluids and should not be affected by water deprivation.

PILOT STUDY

As this hypothesis was based on a relatively complicated assumption, a pilot study was performed to ensure that normal animals were indeed capable of reducing post-deprivation drinking in response to water injections and making the distinction between isotonic saline and water. This would indicate a sophisticated degree of regulation. Twelve male Lister-hooded rats (average weight 339gms, SE=4.32; range 280.2gms - 391.4gms) were water deprived for 24 hours. They were then split into 3 groups of 4 on a random basis. One group was injected i.p. with 15mls sterile water, one with 15mls 0.9% physiological saline and the third group had an injection needle inserted i.p., but had no fluid injected ('needle only' condition). 15mls of fluid were used because previous experiments (see above) had shown this to be the average volume drunk in the first hour of access to water after 24hrs water deprivation.

Water intake was measured after 1 and 3 hours. Fig 42 shows the results obtained. It can be seen that animals responded differently in the three conditions, reducing intake after water or 0.9% saline injections.

SURGERY AND NORMAL REGULATORY BEHAVIOUR

Surgery and normal regulatory behaviour for these experimental animals are described under "Calorific Intake Regulation" (See p 115). In summary, LH lesioned animals (n=5) lost weight immediately after surgery, but gained weight at the same rate as controls (n=8) after one week of recovery. Food and water intake were slightly impaired for one week post-op and were normal thereafter. These animals were tested for calorific intake regulation before water loading experiments took place. All of the lesioned animals and half of the control animals had been given glucose solution to drink instead of water. The animals were returned to water one week before water loading experiments began.

WATER LOADING

The same procedure was followed as described above for the pilot study, except that a repeated measures design was used and all animals were used in each condition. Control

FIGURE 42: This figure presents data from the pilot experiment used to assess the effects on post-deprivation drinking of water or 0.9% saline injections. Both injections reduced intake and the water injection was more effective than the saline injection. Standard errors were never more than 3.01.

WATER LOADING
PILOT STUDY

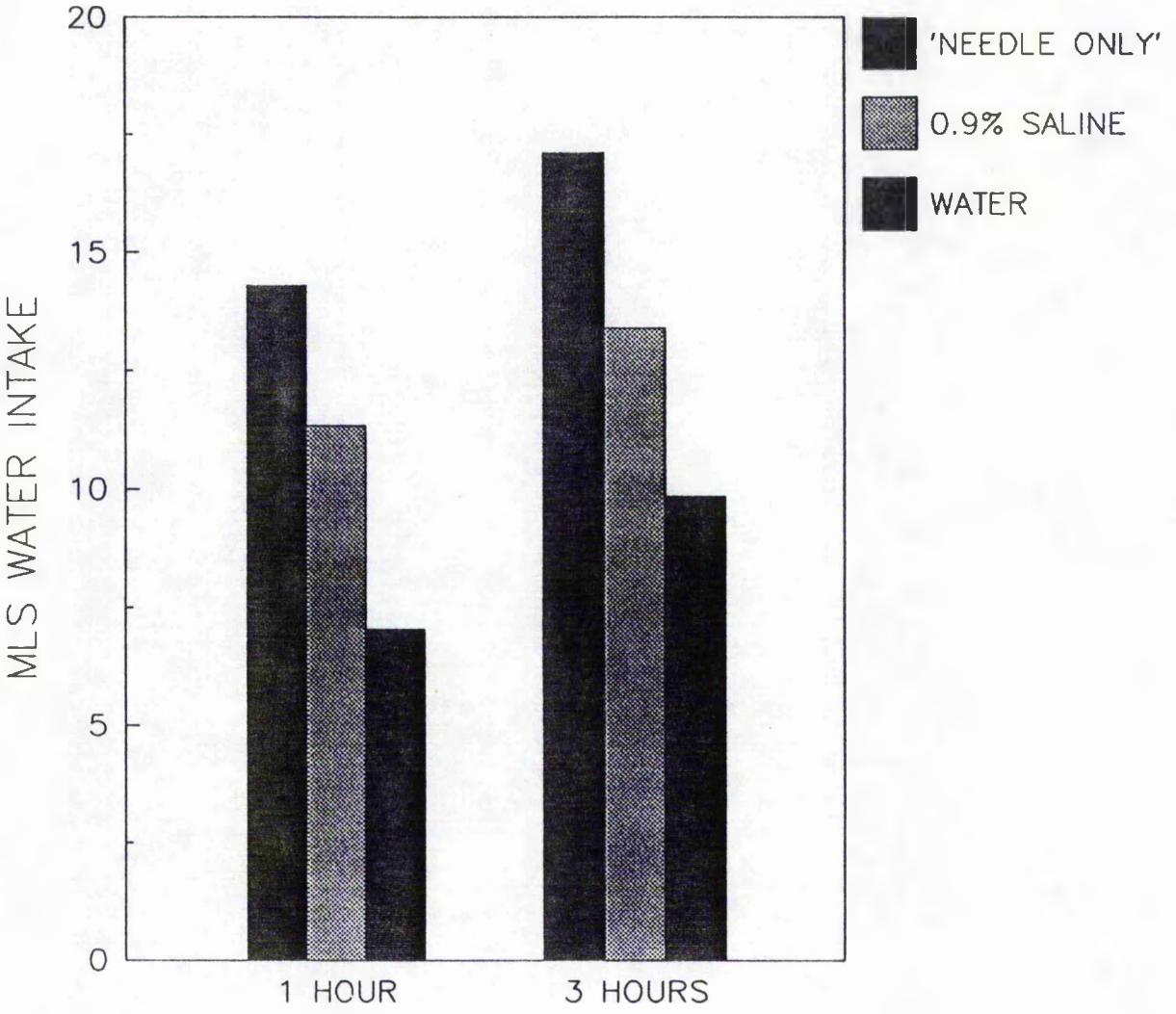


FIG 42

animals were treated as one group for this experiment. Following 24 hours water deprivation, animals were injected with 15mls sterile water, 15mls 0.9% saline or were given a 'needle only' injection. Rats were then allowed free access to tap water and intake was measured after 1hr, 3hrs and 24hrs. Injection conditions were given in a randomised order and at least 48hrs elapsed between each test.

Results can be seen in Figs 43, 44 and 45. Using ANOVA, no groups effect was found after 1 hour ($F=4.4069$; $df=1,14$; $p=0.0544$), 3 hours ($F=2.318$; $df=1,14$; $p=0.1501$) or after 24 hours ($F=3.3248$; $df=1,14$; $p=0.0897$) and no interaction between groups and conditions was found after 1 hour ($F=0.4673$; $df=2,28$; $p=0.6315$), 3 hours ($F=1.8019$; $df=2,28$; $p=0.1836$) or 24 hours ($F=.1569$; $df=2,28$; $p=0.8555$). Thus, the LH lesioned animals responded as controls in all conditions over the same period of time and showed no deficit to this challenge. Moreover, there was a significant difference between the conditions after 1 hour ($F=43.6181$; $df=2,28$; $p<0.001$) and after 3 hours ($F=55.500$; $df=2,28$; $p<0.001$). Post-hoc analysis with the Tukey test revealed that these significant effects arose because both lesioned and control rats drank significantly more after 'needle only' injection ($p<0.01$) or after injection of saline ($p<0.01$) than they did after water injection. No differences were found between saline injection and 'needle only' injection.

FIGURE 43: Water intake 1 hour following water, 0.9% saline or 'needle only' injections and the return of water following 24 hours water deprivation are presented in this figure. No differences were found between the groups as they both reduced intake following water injections. Standard errors were never more than 2.5.

WATER LOADING
1 HOUR

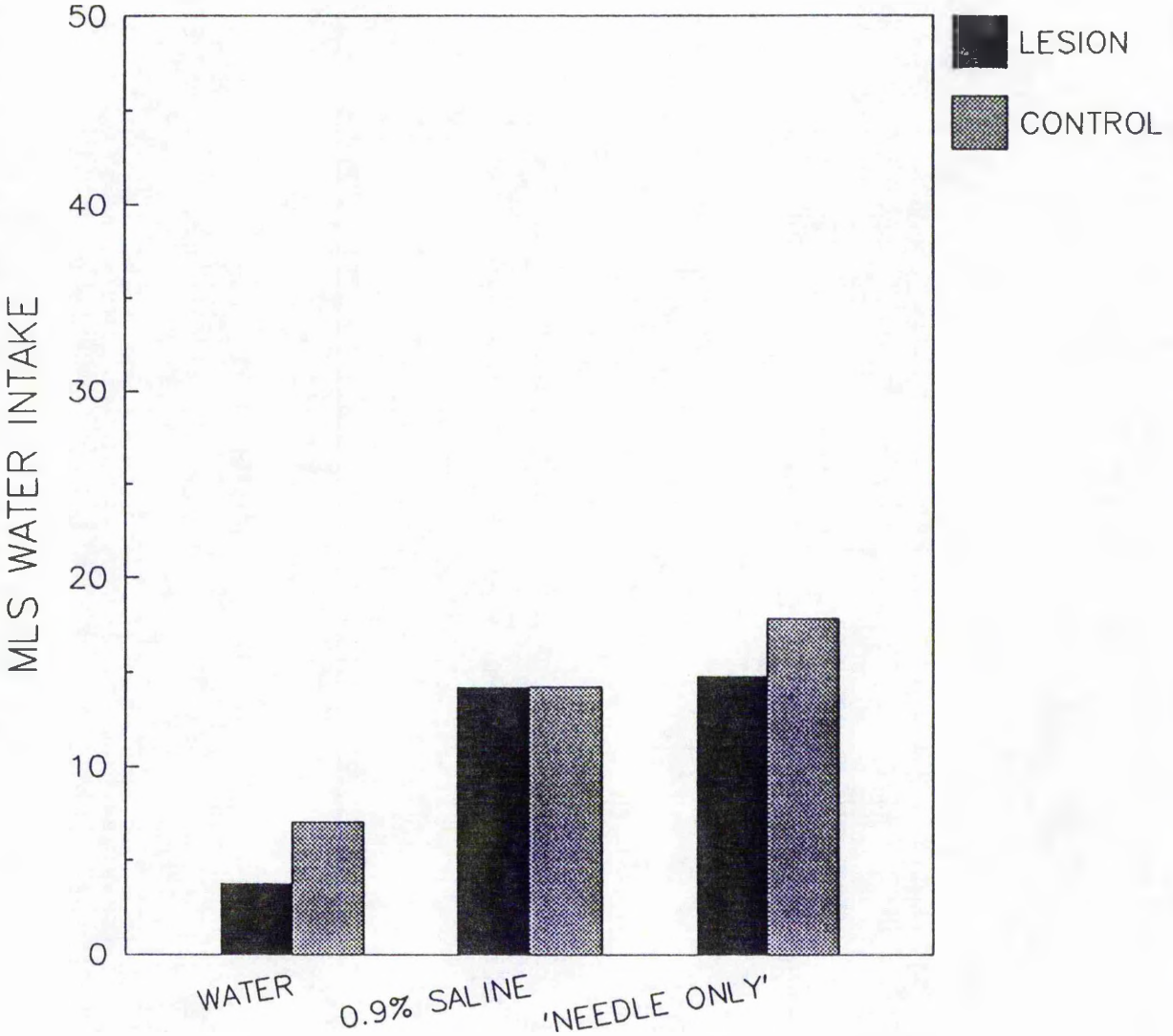


FIG 43

FIGURE 44: Water intake 3 hours following water, 0.9% saline or 'needle only' injections and the return of water following 24 hours water deprivation are presented in this figure. No differences were found between the groups as they both reduced intake following water injections. Standard errors were never more than 1.61.

WATER LOADING
3 HOURS

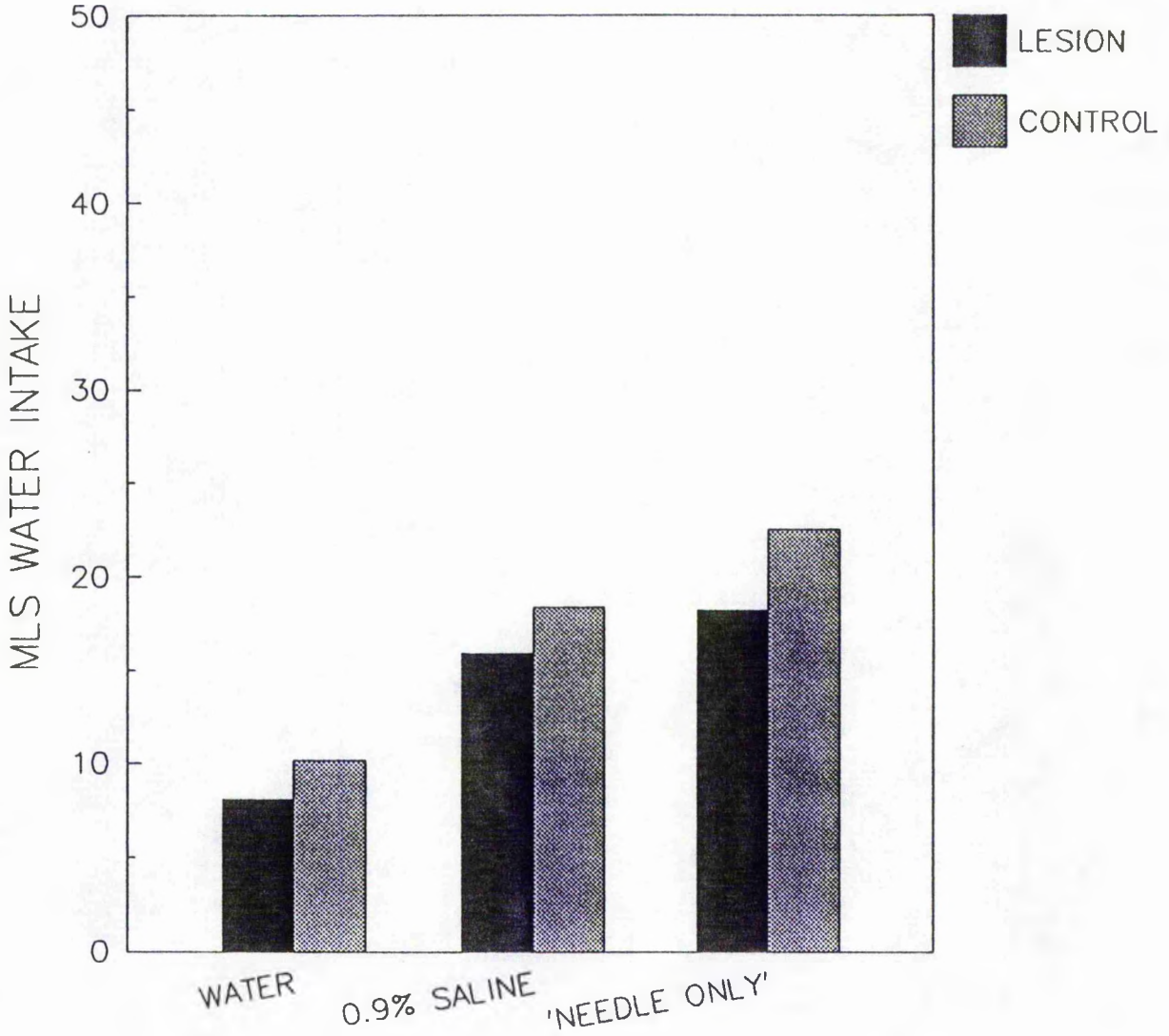


FIG 44

FIGURE 45: Water intake 24 hours following water, 0.9% saline or 'needle only' injections and the return of water following 24 hours water deprivation are presented in this figure. No differences were found between the groups. Satndard error was never more than 4.69.

WATER LOADING
24 HOURS

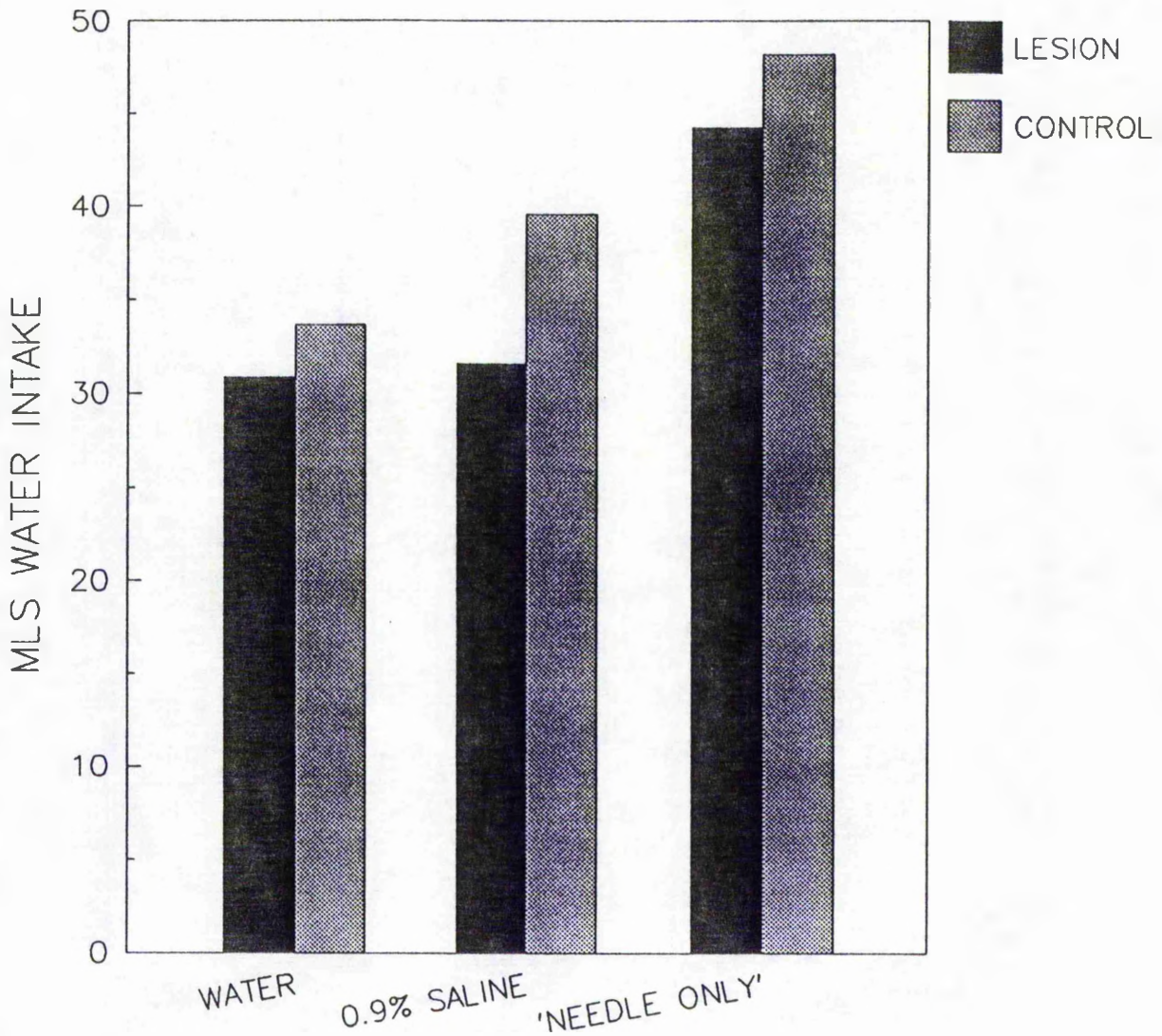


FIG 45

These results indicate three things; first, LH lesioned animals responded to water deprivation by drinking; secondly, this drinking was attenuated if rats were injected with the amount of water equivalent to that normally drunk after deprivation; and thirdly that they were aware of the physiological difference between water and 0.9% isotonic saline. These results are very important as they show that lesioned animals were able to recognise their hydrational state as quickly as control animals. In this test, recognition was demonstrated by a lack of response (ie attenuated post-deprivation drinking) and implies that deficits to physiological challenge may be due to a problem in generating the correct response.

HISTOLOGY

Histological procedures are presented in Chapter 5. Typically, the lesion was greatest at the level of the VMH and the DMH and decreased in size moving towards the poles. Most cells were lost from the central core of the LH, while most cells in the anterior and posterior poles were spared. Average lesion size was 48.04% (SE=8.05) and lesions ranged from 32.4% to 78.7%. Extra-hypothalamic damage occured most frequently in the zona incerta and reticular nucleus of the thalamus, but some cell death was found in the globus pallidus, ventral pallidum, substantia innominata, thalamus and subthalamic nucleus. Thalamic damage appeared

to result from leakage up the cannulae tracks. The pattern and size of a typical lesion can be seen in Fig 46.

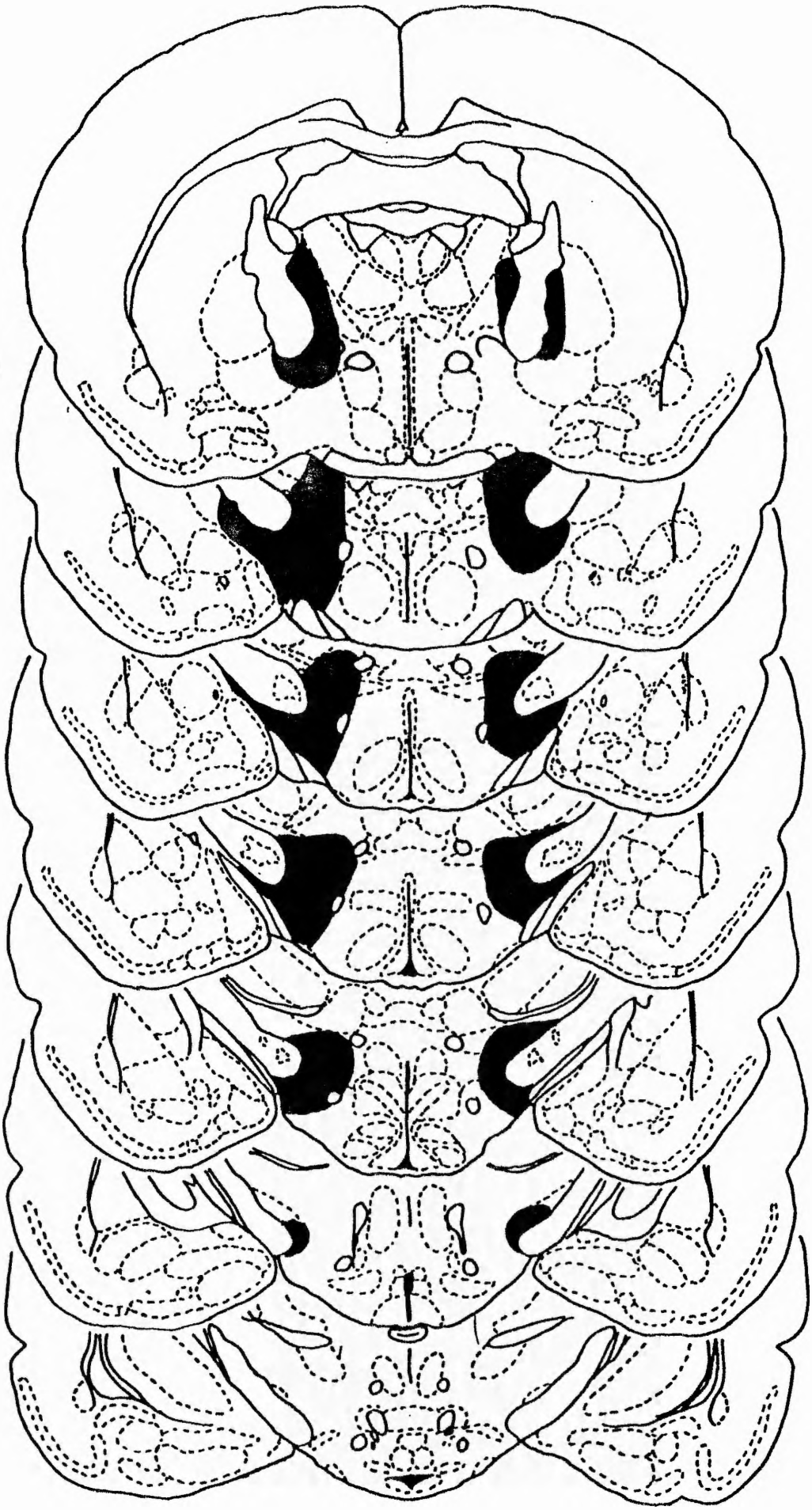
SALT ADULTERATION OF FOOD

If it is possible to make an animal reduce intake of lab chow by providing calories in its drinking water, then is it possible to make an animal increase fluid consumption by adding salt to the food? Salt added to the food should dehydrate the body by disturbing sodium balance and drawing water from the intracellular fluid. Animals which can recognise signals from the periphery indicating hydrational state should respond to this by drinking. If LH lesioned animals cannot recognise their internal state, then they should not alter water or food intake. This provides a long term non-invasive physiological challenge.

SURGERY

Three groups of male Lister-hooded rats were used for this experiment. Six animals were microinjected with 1ul of phosphate buffer (pH 6.98), six with 1ul 0.12M NMDA (pH 7) and five with 1ul 0.06M NMDA (pH 7). (There were two lesion groups as several animals had died following 0.12M NMDA administration and toxin concentration was therefore reduced.) Injections were made at 0.5ul/min at the

FIGURE 46: The size and pattern of a typical lesion for this experimental group is presented in this figure. Shaded area represents lesion site. The central core of the LH was removed, but the anterior and posterior poles were not damaged. Extra-hypothalamic damage occurred in the thalamus and zona incerta. (Rat J.51/88)



following co-ordinates (Pellegrino, Pellegrino and Cushman, 1979) in the orientation of de Groot: 0mm bregma; ± 1.8mm lateral; -8.0mm vertical from dura. Average weight before surgery was 410.3gms (SE=5.8; range = 370.4gms - 452.9gms).

NORMAL REGULATORY BEHAVIOUR

BODY WEIGHT: [Fig 47] Body weight was measured daily pre- and post-op and converted into percentage weight from the day of surgery. Before surgery, there was no significant difference between the groups (ANOVA: $F=1.8747$; $df=2,14$; $p=0.1899$). After surgery, lesioned animals lost weight and there was a significant difference between the groups which remained throughout the experiment (ANOVA: week 1, $F=10.7879$; $df=2,14$; $p=0.0015$: week 2, $F=12.4089$; $df=2,14$; $p=0.0008$: week 3, $F=14.8599$; $df=2,14$; $p=0.0003$). There was also an interaction between groups and days during the first and second weeks post-op (ANOVA: week 1, $F=4.1146$; $df=12,84$; $p<0.001$: week 2, $F=3.424$; $df=12,84$; $p=0.0004$), but this interaction was not present in week 3 post-op (ANOVA: $F=1.187$; $df=12,84$; $p=0.3058$) indicating that, following a two week recovery period, LH lesioned animals were beginning to gain weight at the same rate as controls.

FOOD INTAKE: [Fig 48] Food intake was measured daily pre- and post-op. Measurements represent amount of 50% w/v wet mash consumed made from powdered lab chow pellets (S.D.S) and tap water. Animals were fed wet mash to make salt

FIGURE 47: Percentage body weight for one week pre- and three weeks post-surgery of animals used in salt adulteration of food experiments are presented in this figure. Post-surgery, lesioned animals lost weight and there was a significant difference between the groups using ANOVA. Two weeks after surgery, there was no longer an interaction between groups and days indicating that lesioned animals were beginning to gain weight at the same rate as controls.

% BODY WEIGHT
SALT ADULTERATION OF FOOD

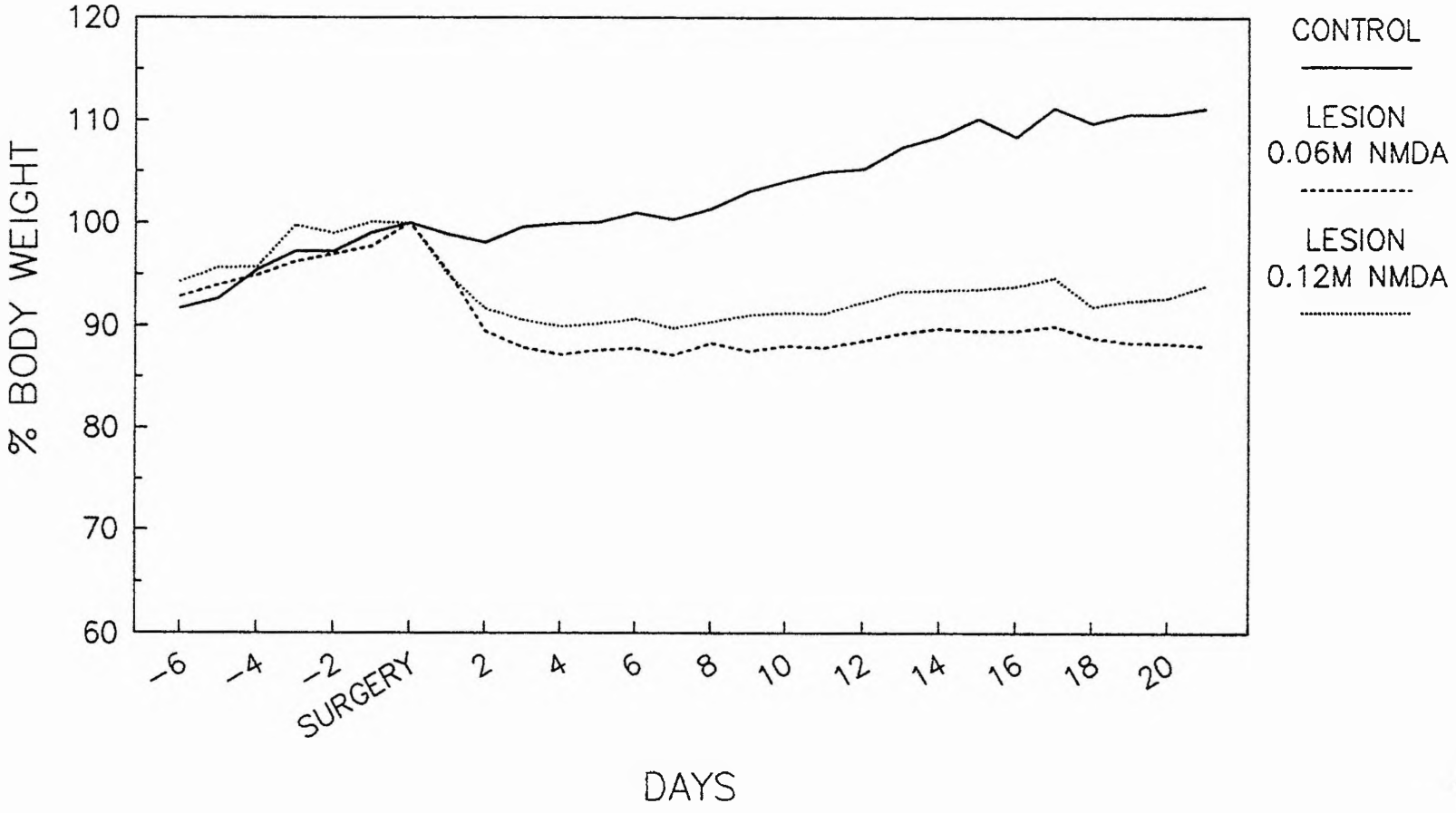


FIG 47

FIGURE 48: Food intake for one week pre- and three weeks post-surgery are presented in this figure. Food intake measurements represent amount of wet mash eaten. Lesioned animals reduced intake post-surgery and there was a significant difference between the groups.

FOOD INTAKE

SALT ADULTERATION OF FOOD

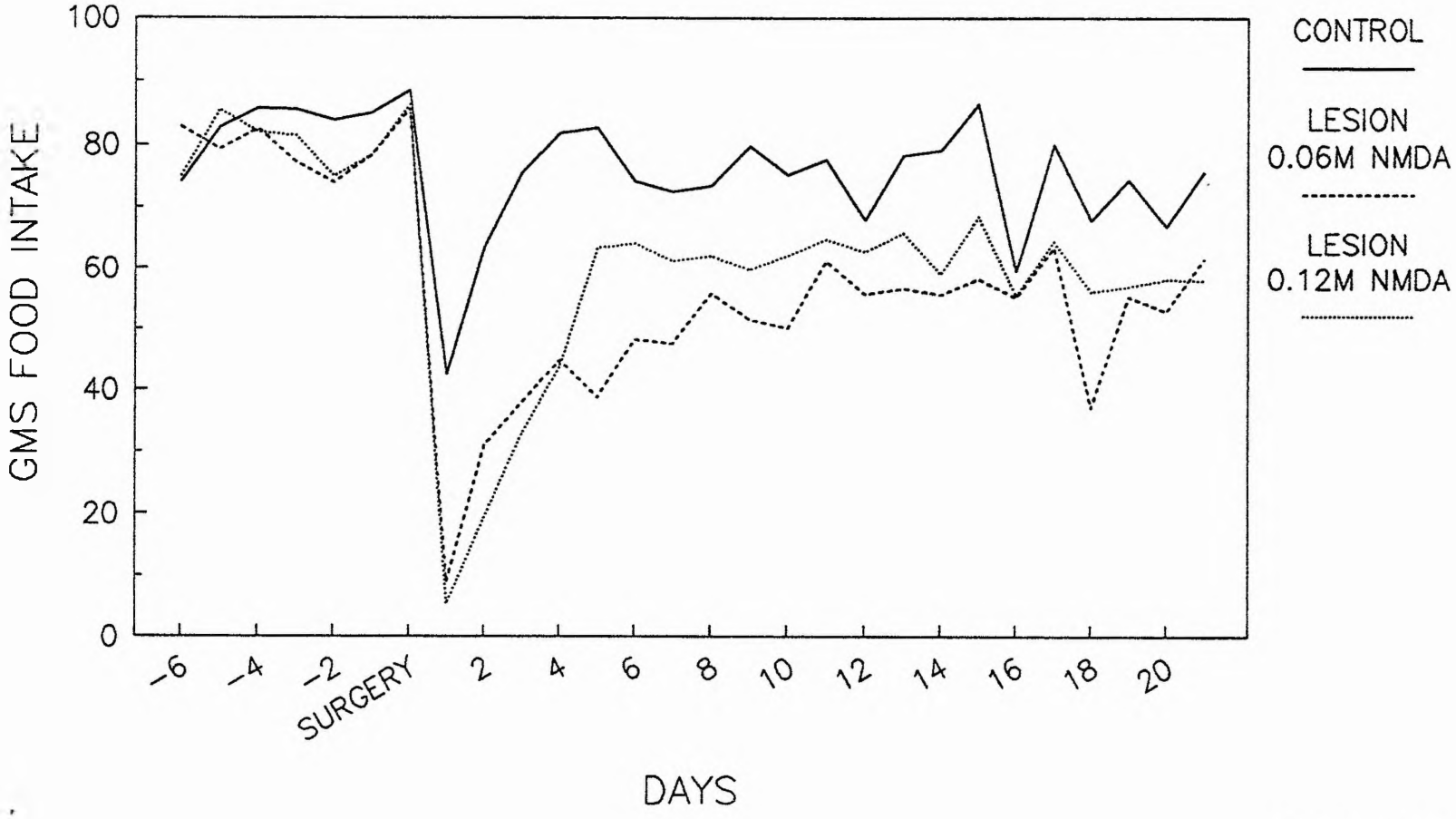


FIG 48

adulteration of food easier. Before surgery, there was no significant difference between the groups (ANOVA: $F=0.6353$; $df=2,14$; $p=0.5444$). After surgery LH lesioned animals reduced their food intake which resulted in a significant difference between the groups which lasted throughout the experiment (ANOVA: week 1, $F=15.0397$; $df=2,14$; $p=0.0003$: week 2, $F=7.5865$; $df=2,14$; $p=0.0059$: week 3, $F=5.3101$; $df=2,14$; $p=0.0192$). The lesioned animals showed a reduced intake, but they were able to maintain themselves on this diet and did not require any intervention to keep them alive.

WATER INTAKE: [Fig 49] Water intake was measured daily pre- and post-op. Before surgery, there was no significant difference between groups (ANOVA: $F=0.612$; $df=2,14$; $p=0.5562$). After surgery LH lesioned animals reduced intake and there was a significant difference between the groups which lasted throughout the experiment (ANOVA: week 1, $F=30.4884$; $df=2,14$; $p<0.001$: week 2, $F=17.3047$; $df=2,14$; $p=0.0002$: week 3, $F=19.8162$; $df=2,14$; $p=0.0001$).

In summary, LH lesioned animals did not recover body weight, food or water intake post surgery to the levels of the control animals, but they were able to maintain themselves on wet mash and tap water without intervention and they began to gain weight at the same rate as control rats after a 2 week recovery period. In the context of this experiment, lowered intake is not so important as a

FIGURE 49: Water intake for one week pre- and three weeks post-surgery are presented in this figure. Lesioned animals reduced intake post-surgery and there was a significant difference between the groups using ANOVA.

WATER INTAKE

SALT ADULTERATION OF FOOD

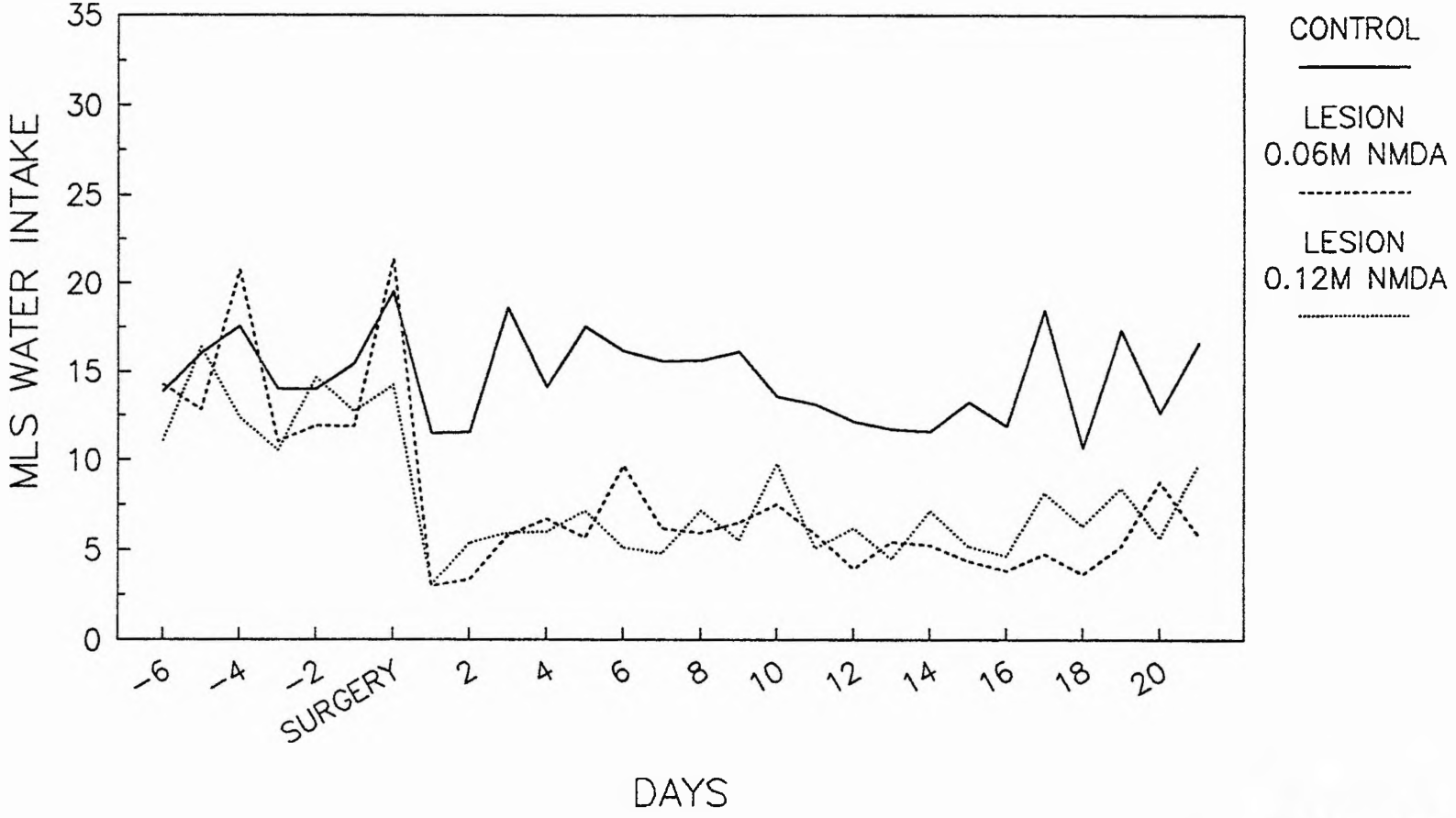


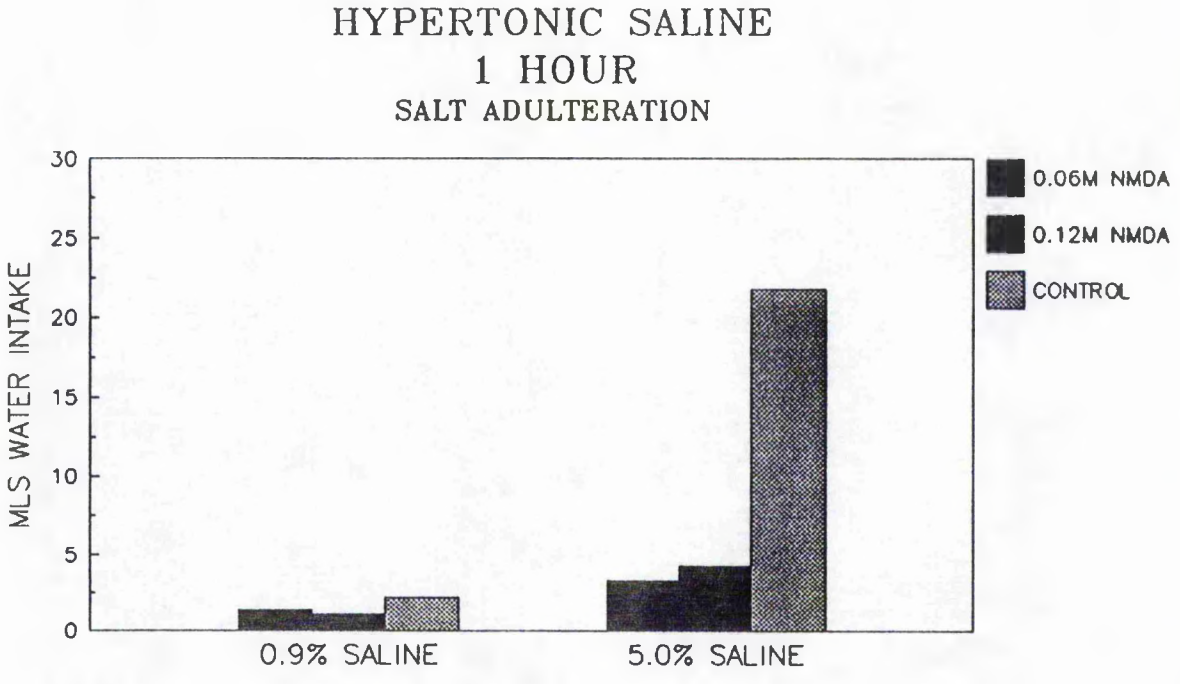
FIG 49

constant balanced intake so that the effects of adulteration can be monitored.

HYPERTONIC SALINE

Hypertonic saline was given according to the standard procedure described in the general procedures section. Data can be seen in Fig 50. At 1 hour post-injection, ANOVA showed an interaction between groups and conditions ($F=25.956$; $df=2,14$; $p<0.001$). Tukey tests showed that, while control animals significantly ($p<0.01$) increased their intake after hypertonic saline administration, there was no significant difference between the intake of the groups to 0.9% saline injection and there was no significant difference between the intake of the LH rats after physiological or hypertonic saline. The results at 3 hours were similar. There was an interaction between groups and conditions ($F=33.9423$; $df=2,14$; $p<0.001$) and post-hoc Tukey tests showed a significant difference between the intake of the control animals following injection of hypertonic as opposed to isotonic saline ($p<0.01$). No differences were found between the three groups' intake after physiological saline administration and no significant difference was found between lesions' response to physiological or hypertonic saline. Thus, these LH lesioned animals did not respond to intracellular dehydration caused by injection of hypertonic saline. They

FIGURE 50: Responses to hypertonic saline physiological challenge 1 hour and 3 hours post-injection are presented in this figure. Lesioned animals showed a deficit in response to this challenge as revealed by ANOVA. Standard errors were never more than 2.18.


HYPERTONIC SALINE
1 HOUR
SALT ADULTERATION
MLS WATER INTAKE
30
25
20
15
10
5
0
0.9% SALINE
5.0% SALINE
0.06M NMDA
0.12M NMDA
CONTROL

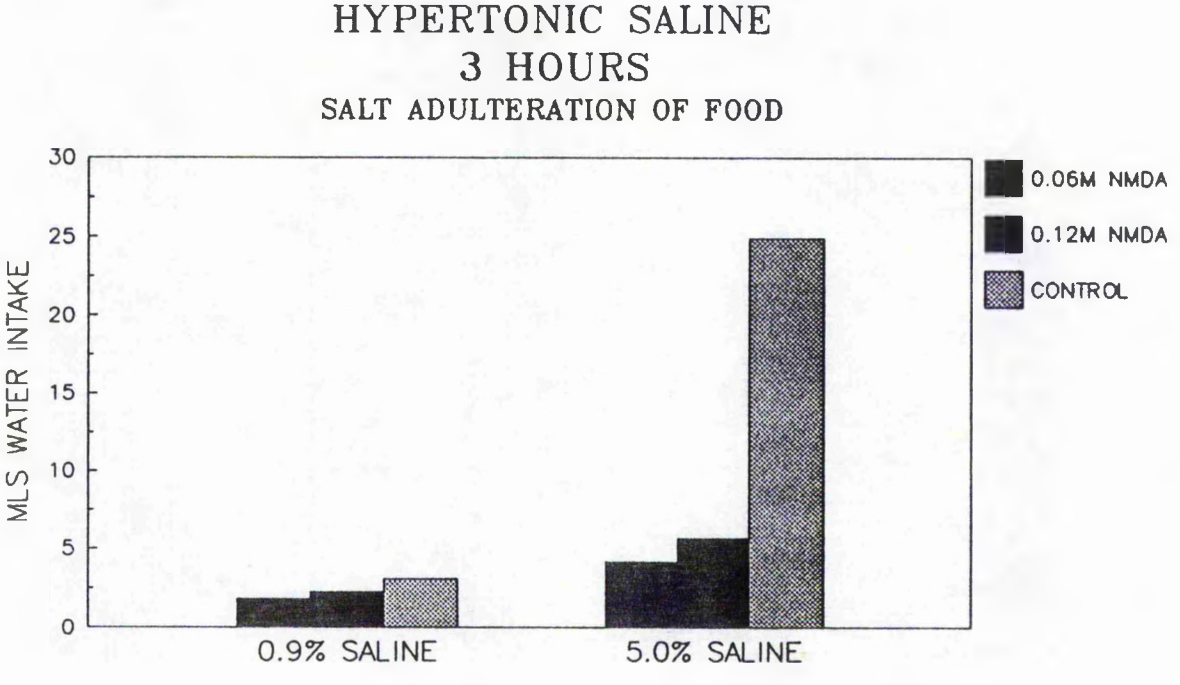

HYPERTONIC SALINE
3 HOURS
SALT ADULTERATION OF FOOD
MLS WATER INTAKE
30
25
20
15
10
5
0
0.9% SALINE
5.0% SALINE
0.06M NMDA
0.12M NMDA
CONTROL

FIG 50

were unable to respond correctly to this acute physiological challenge.

SALT ADULTERATION

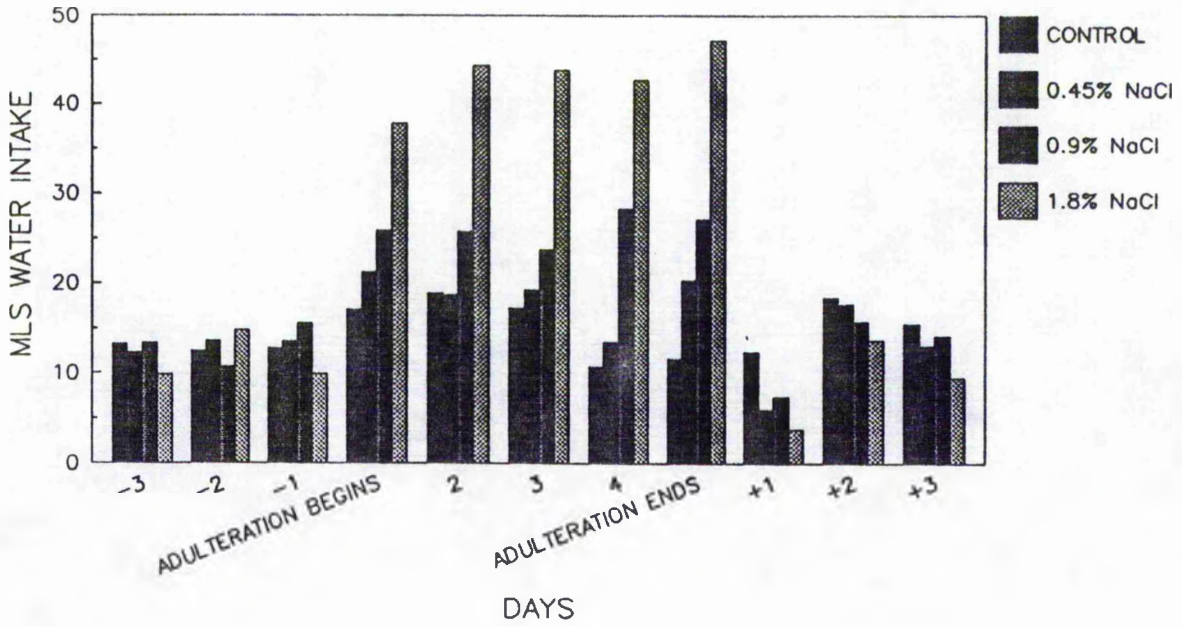
A pilot study was carried out to determine what concentration of NaCl could be mixed with food to increase fluid intake without affecting food intake. Twelve male Lister-hooded rats were fed 50% w\v wet mash made from ground lab chow pellets (S.D.S) and tap water, with ad lib water to drink. The animals were then split randomly into 4 groups of 3 and were given either tap water, 0.45%, 0.9% or 1.8% NaCl solution mixed with their ground lab chow. The results can be seen in Fig 51. Food intake was not affected, but fluid intake increased as a function of NaCl concentration with 1.8% NaCl solution producing the highest intake.

All experimental animals were fed wet mash, made to the above specifications, before and after surgery. Following a two week recovery period post-op, all animals were given wet mash adulterated with 1.8% NaCl solution to eat for a period of 5 days. Ordinary wet mash was then returned. Free access to tap water was available at all times.

In Fig 52, food intake is shown for 2 days before, 5 days during and 2 days after adulteration and demonstrates that

FIGURE 51: Data from a pilot study examining the effects of salt adulteration of food on food and water intake are presented on the following page. Four concentrations of salt solution were added to wet mash. No effects on food intake were found, but water intake increased as the concentration of the salt solution increased.

SALT ADULTERATION OF FOOD
PILOT STUDY
WATER INTAKE



SALT ADULTERATION OF FOOD
PILOT STUDY
FOOD INTAKE

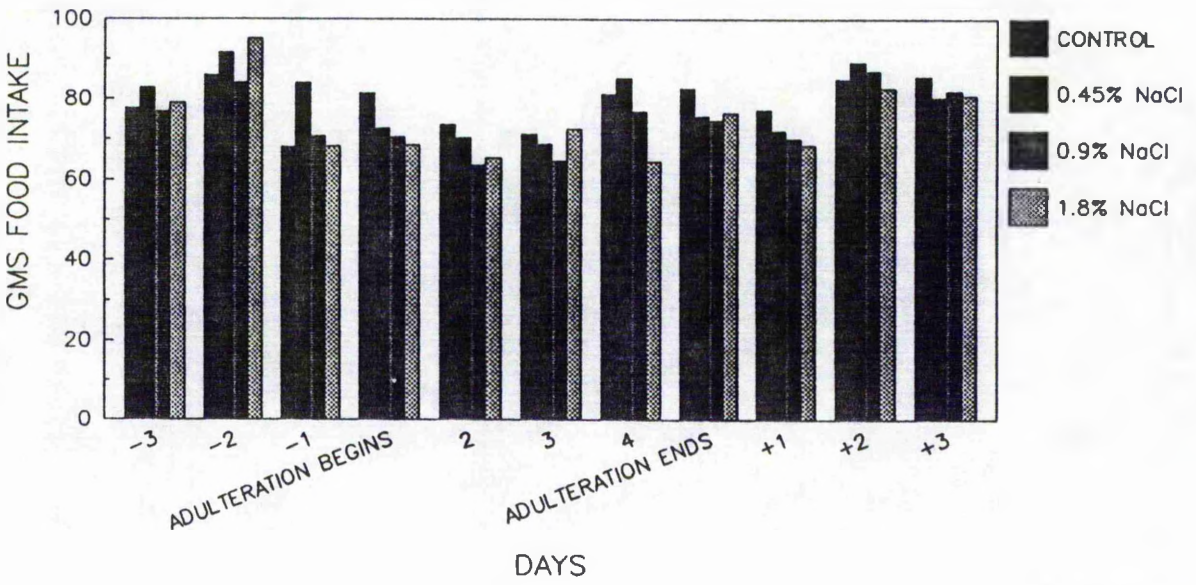


FIG 51

FIGURE 52: Food intake for 2 days pre- , 5 days during and 2 days post-adulteration of food with 1.8% salt solution are presented in this figure. No differences were found between the stages of adulteration and non-adulteration.

FOOD INTAKE DURING SALT ADULTERATION

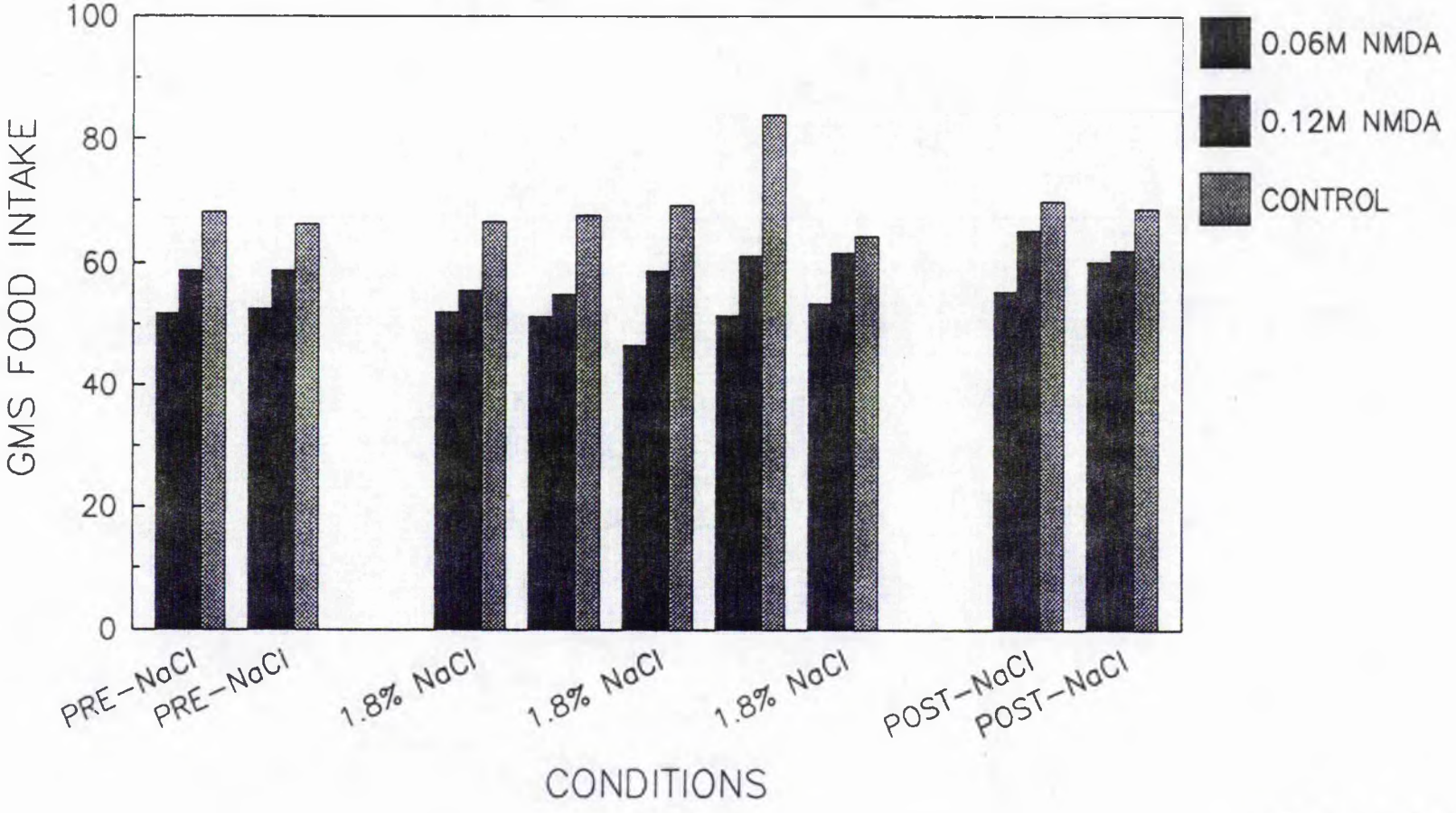


FIG 52

food intake was not altered during salt adulteration. Using ANOVA , there was a significant difference between the groups ($F=7.6668$; $df=2,14$; $p=0.005$), but no interaction between groups and days ($F=0.7361$; $df=4,28$; $p=0.575$). This indicates that, although lesioned animals had a lowered intake, the groups did not respond differently when salt was added to their food.

In Fig 53, water intake is shown for 2 days before, 5 days during and 2 days after adulteration of food. Again, lesioned animals had a lowered baseline, with groups showing significant differences in water intake (ANOVA: $F=14.66$; $df=2,14$; $p=0.0004$). However, adulteration of food affected the water intake of both groups (ANOVA: $F=93.7562$; $df=2,28$; $p<0.001$) as both groups significantly increased the amount of water consumed (TUKEY: $p<0.01$). Although the lesioned group did not increase intake quite so dramatically as control animals the interaction between groups and days did not reach significance (ANOVA: $F=2.6814$; $df=4,28$; $p=0.0521$). As lesioned animals were not eating as much food, and therefore not as much salt, the reduced effect is not surprising. The fact that both groups increased water intake significantly and no interaction was found indicates that lesioned animals were able to respond as controls to this challenge.

FIGURE 53: Water intake for 2 days pre-, 5 days during and 2 days post-adulteration of food with 1.8% salt solution are presented in this figure. Although lesioned animals had a lowered baseline intake, all groups increased water intake in response to the salt adulteration.

WATER INTAKE DURING SALT ADULTERATION

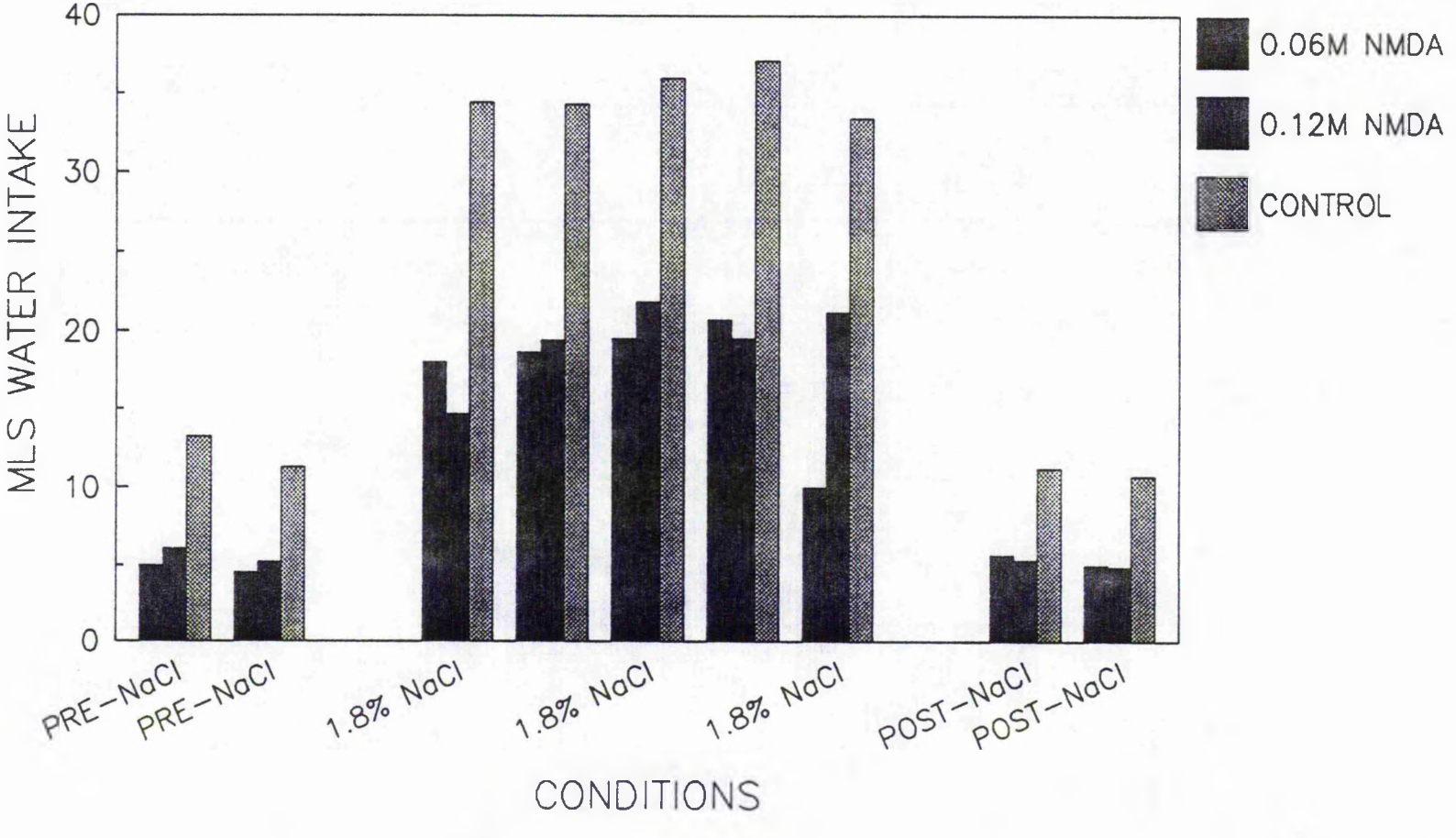


FIG 53

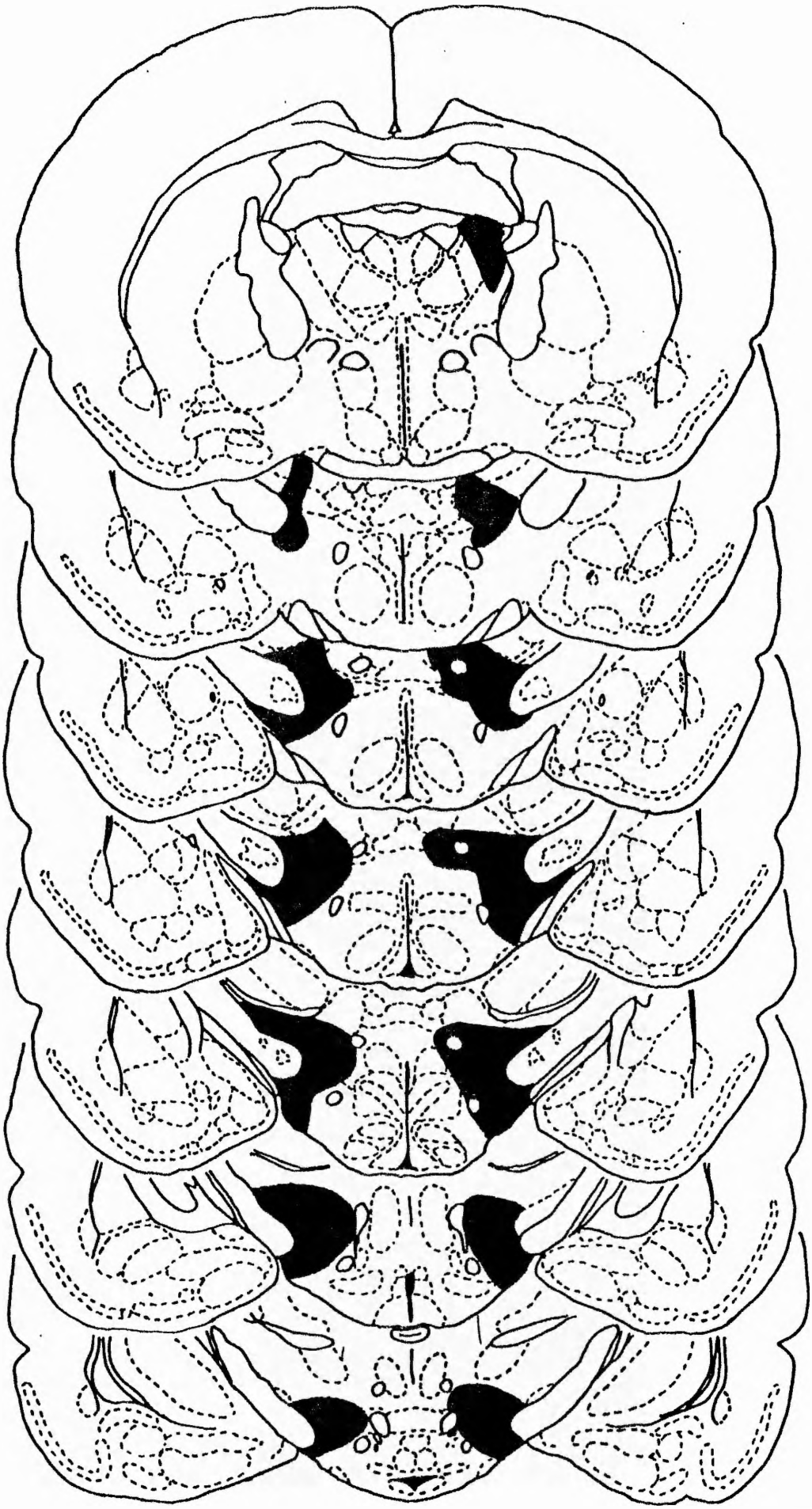
HISTOLOGY

Histological procedures are presented in Chapter 5. Typically, the anterior pole was virtually untouched, but the posterior pole received more damage than in previous experiments. As before, cell loss was greatest from the central core of the LH with almost complete loss of neurones at the level of the DMH. Average lesion size was 55.1% (SE=4.33) and lesions ranged from 31.7% to 77.3%. Extra-hypothalamic damage occured in the zona incerta and reticular nucleus of the thalamus with some loss in other thalamic nuclei and the subthalamic nucleus. The pattern and size of a typical lesion can be seen in Fig 54.

SUMMARY

These results demonstrate that, although LH lesioned animals had a lowered baseline, they responded as control animals to salt adulteration of their food by increasing water intake. To respond to NaCl loading in this way, LH lesioned animals presumably must have been able to understand and recognise their hydrational state.

FIGURE 54: The size and pattern of a typical lesion for this experimental group is presented in this figure. Shaded area represents the lesion site. Cell loss was greatest in the posterior regions of the LH and extra-hypothalamic damage occurred in the thalamus and zona incerta. (Rat J.48/88)



PHARMACOLOGICAL MANIPULATIONS

The above results have demonstrated that LH lesioned animals can respond to long term and positive, but not to acute negative, physiological challenges and that recognition of hydrational state can be done as effectively and as quickly by lesioned animals as by control animals. They have also shown that they can respond to the psychological aspects of food and fluid intake by reacting to the sensory qualities of their diet. Can they respond to pharmacological challenges? Certain pharmacological agents are known to reduce food intake but are not substances occurring naturally in the body. These agents may affect consumption by altering the operations of the regulatory system or by interfering with general processes of (e.g) "arousal" or motor skills (**Popplewell et al.**, 1986). The workings of these drugs are not yet fully understood but some authors have suggested that the anorectic effects result from disruption of regulatory processes mediated by the LH (**Liebowitz**, 1975; **Schwartz et al.**, 1987). Thus, lesions of the LH should abolish the anorectic actions of these substances. The drugs used for this experiment are amphetamine and fenfluramine which are known to induce anorexia (**Blundell and Latham**, 1978).

SURGERY AND NORMAL REGULATORY BEHAVIOUR

The animals used for this experiment have already been described in Chapter 7 and details of surgery and recovery food and water intake can be found there. It is sufficient to note here that there were 17 lesioned and 11 control animals and that recovery of body weight, food intake and water intake took place relatively quickly.

AMPHETAMINE AND FENFLURAMINE ANOREXIA

Animals were food deprived for 16 hours prior to injection. d-Amphetamine sulphate (Smith, Kline and French) and fenfluramine hydrochloride (Sigma) were injected i.p. at doses known to induce anorexia (1mg/kg and 3mg/kg respectively) (Blundell and Latham, 1978) and 0.9% saline was used as the control condition. After drug administration, rats were allowed free access to dry lab chow and amount consumed was measured after 3 hours. A repeated measures design was used, rats acting as their own controls, and 48 hours elapsed between each injection.

Using ANOVA, no interaction between groups and conditions was found ($F=0.907$; $df=3,81$; $p=0.4413$), indicating that the lesioned animals responded as controls to the drugs. There was a conditions effect (ANOVA: $F=75.7055$; $df=3,81$; $p<0.001$) as both drugs were shown to induce a significant

FIGURE 55: Food intake following injections of 0.9% saline, 1mg/kg amphetamine or 3mg/kg fenfluramine are presented on the following page. Both lesioned and control animals reduced intake following amphetamine and fenfluramine injections. Standard errors were never more than 0.67.

DRUG-INDUCED ANOREXIA

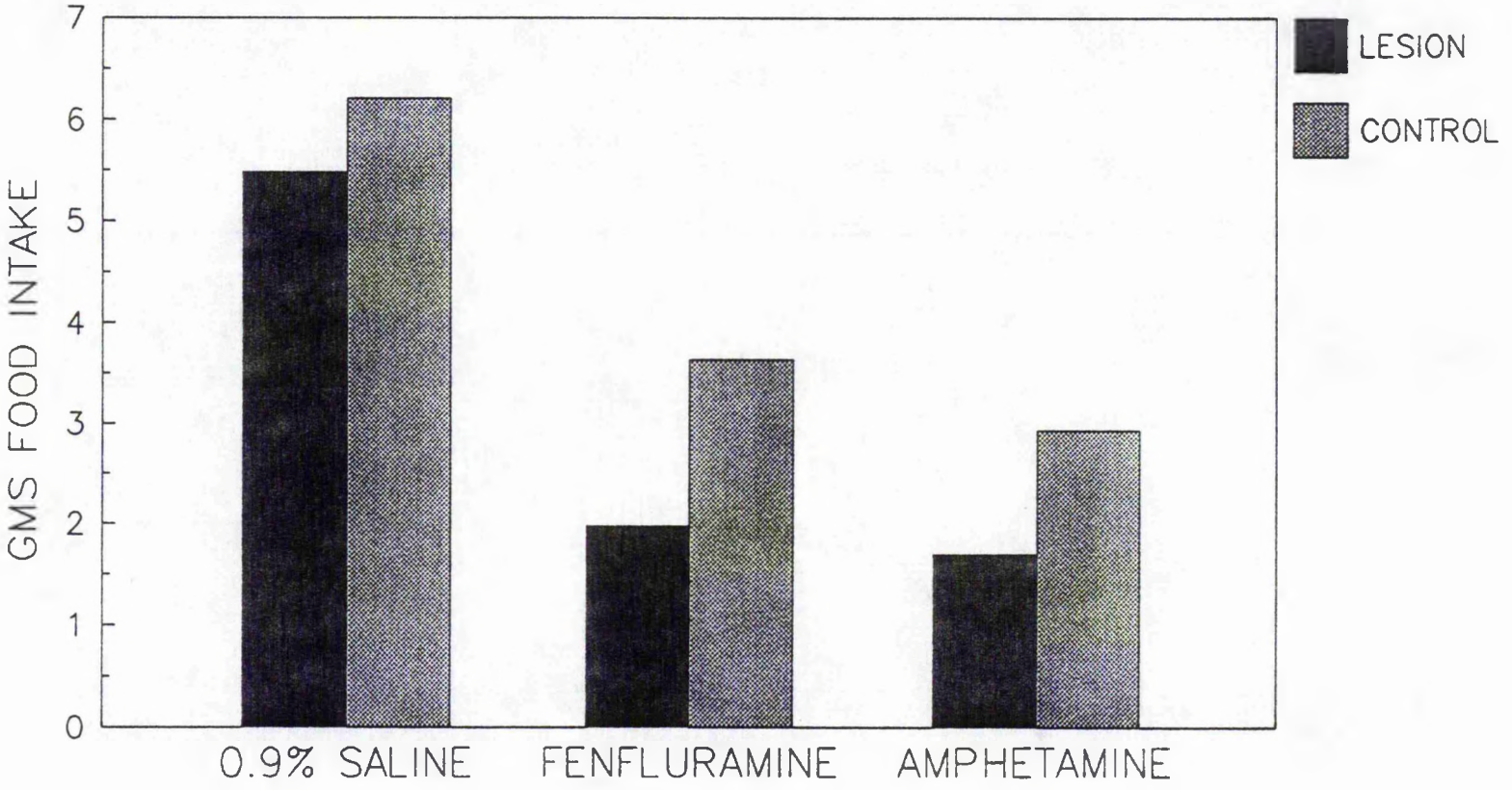


FIG 55

reduction in food intake (Tukey: $p<0.01$) in comparison to saline injections. Although there was no interaction, there was a groups effect (ANOVA: $F=9.5923$; $df=1,27$; $p=0.0045$) since lesioned animals had a lowered baseline [see Fig 55].

Thus, lesioned animals responded to the anorectic drugs by lowering their food intake, just as control animals did. This indicates that the site of action of these anorectic drugs is not the LH as had been suggested by some authors (**Schwartz et al, 1987**), and that lesions of the LH do not effect ability to respond to these pharmacological manipulations.

CONCLUSIONS

LH lesioned animals do not respond adequately to acute physiological challenges. Data presented above demonstrates that, initially, they do not respond as control animals do to injection of hypertonic saline, which causes intracellular dehydration. It was hypothesised that this deficit may be due to a disruption of the mechanisms involved in the recognition or interpretation of signals from the periphery. Perhaps LH lesioned animals are unaware of their bodies' state of glucose and fluid balance and, therefore, do not respond to a state of glucoprivation or dehydration behaviourally because they do not recognise that they are in such a state of privation.

This hypothesis can be rejected. If LH lesioned rats are given long term or positive physiological challenges, they can respond just as well as control animals. They "rebound eat or drink" after a period of deprivation, compensate for calorific or water overloading to maintain a constant energy and fluid balance and increase fluid consumption when challenged with salty food. They respond to hypertonic saline injections within 24hrs and are able to tell the difference between injections of water and 0.9% saline. These challenges require a sophisticated homeostatic control system and LH lesioned animals can cope adequately with them. This suggests that LH lesioned rats <u>can</u> understand physiological signals and the motivational deficit seen after such lesions, demonstrated by the behavioural response to hypertonic saline, is <u>not</u> a deficit in recognition.

CHAPTER 10

RESPONSE

The previous Chapters have demonstrated that animals with lesions of the LH are capable of responding normally to the sensory and calorific qualities of their diet and compensating for conditions of deprivation and overload if time is allowed for them to do so. We may suggest from this that the deficits seen after LH lesions do not arise from problems in "incentive", "stimulus" or "recognition". Why, therefore, are LH lesioned animals unable to repsond quickly to intracellular dehydration and glucoprivation? Why do they show an initial, and sometimes a sustained, reduction in food and water intake?

Hebb claims that, "in general terms...we can now distinguish two quite different effects of a sensory event. One is the 'cue function', guiding behaviour; the other, less obvious but no less important, is the 'arousal function'. Without a foundation of arousal, the cue function cannot exist"(Hebb, 1955, p.249). Since 1955 our definitions of "arousal" have become more complex and more sophisticated, but in this passage Hebb is using "arousal" to mean "a general drive state" which is "an energizer, but not a guide". Thus, in an integrated system, behaviour can only take place if a sufficient foundation of arousal is

present; and which behaviour will be performed depends upon the cues which direct and guide.

If lesions of the LH do induce a "motivational" deficit, then this deficit may arise because of a reduced "foundation of arousal". As Hebb points out, without this foundation of arousal the "cue function" which directs behaviour cannot exist. If the deficit lies in the "arousal function" then LH lesioned animals may have problems in performing the behaviour. There may, of course, be other explanations of data presented so far, but it seemed reasonable to investigate the hypothesis outlined above.

Due to the many arguments about the meaning of "arousal", it is important at this stage to set out a clear definition of what is meant by this and other terms in the following Chapters. A clear distinction has been made between "arousal" and "activation" by **Robbins and Everitt** (1981) and I shall follow their definitions of these terms. "Arousal" refers to "the processes resulting in altered EEG activity in the cerebral cortex" which exert "tonic influences on the processing of sensory information". Thus, <u>arousal</u> is represented by "changes in electrocortical activity". "Activation" refers to "the processes maintaining the capacity to move appropriately in response to inputs"; that is "response production" (**Robbins and Everitt**, 1981). Thus, <u>activation</u> is represented by the "rate and vigour of behavioural output". It can be seen

that Hebb's description of "arousal" as "energizing" encompasses both of the above definitions and is, therefore, too vague since there are occasions when arousal and activation are not correlated. In general, as arousal increases, so does activation until a critical level of arousal is reached at which point activation decreases. Thus, the level of arousal before an exam which induces locomotion in the form of pacing is surpassed in the animal freezing in response to an oncoming vehicle. There may be other definitions of "arousal" and "activation", but for the purposes of this thesis the above definitions will apply throughout.

Therefore, if lesions of the LH induce deficits in the "arousal function" of sensory events, this deficit could be one of either arousal or activation; that is, either tonic influences on the processing of sensory information could be altered or there may be a deficit in response production. An arousal or activational deficit could explain the previous experimental results as they should both lead to slowed responding to acute, negative physiological challenges such as administration of hypertonic saline. As demonstrated, a long-term deficit need not exist, but responding would take time when active responses were required.

The following experiments were designed to test the hypothesis that lesions of the LH induce a motivational deficit due to impairments in either arousal or activation.

SCHEDULE-INDUCED POLYDIPSIA

Schedule-induced polydipsia (SIP) is a phenomenon seen when hungry rats are exposed to the intermittent presentation of food in the presence of ad-lib water. If small food pellets are presented at 1 minute intervals to food-deprived animals, rats will drink excessive amounts in the period between presentations. Water intake far exceeds amounts normally consumed with meals or amounts needed to maintain body fluid levels. SIP is thought to be an adjunctive behaviour, which arises when attempts to perform a highly motivated behaviour, such as eating following food deprivation, are obstructed (**Robbins and Koob**, 1980).

It has been suggested that SIP may be a coping response (**Brett and Levine**, 1979), used to reduce the aversive aspects of the heightened arousal induced by the intermittent presentation of the food. In support of this theory, an inverse relationship between levels of plasma corticosterone, which is related to stress, and SIP has been found (**Mittleman, Jones and Robbins**, 1988); the higher the levels of SIP, the lower the levels of plasma corticosterone. This could suggest that SIP lowers stress

and corticosterone levels, helping the animal to cope with the aversive situation. However, evidence against the coping response hypothesis has also been reported, in that plasma corticosterone levels were significantly higher following SIP with water available than without water (**Mittleman, Jones and Robbins**, 1988). If the drinking helped the animal to cope with the situation, the corticosterone levels should have been lower when this option was available.

An alternative explanation proposed by **Robbins and Koob** (1980) is that SIP arises because the "motivational excitement accompanying delivery of a food pellet may outlast its consumption, and potentiate alternative activities evoked by available environmental stimuli". This suggests that SIP arises from excessive arousal or activation. If lesions of the LH produce deficits in responding because of a disruption of arousal or activation, then LH lesioned animals should not show, or be slower to demonstrate, SIP. This hypothesis was tested in the following experiments.

PILOT STUDY

A pilot study was carried out to establish the most effective methodology and to assess how much and over what time course SIP occurred in normal animals. Eight male

Lister-hooded rats were housed singly under a twelve hour light/dark cycle with ad-lib water. Average weight at the beginning of the experiment was 219.5gms (SE=4.12; range = 198.4gms - 247.3gms). Food intake was restricted to 12 gms a day to reduce weight. Once all animals were below 95% body weight (5 days) testing began. However, as some animals showed no SIP on the first trials, intake was restricted to 10gms a day until all animals were below 85%. At this stage, individual animals needed different amounts to maintain this reduced weight and daily intake ranged from 10gms to 15gms.

Animals were placed in Skinner boxes (Campden Instruments) at the same time every second day. House lights were permanently on, but room lights were put out just before testing began. Water bottles, with nozzle placed next to the food hopper, were weighed before the test began. During testing, one pellet (Campden Instruments) was dispensed every 60 seconds for one hour. Animals were returned to their home cages and water bottles were weighed again.

Results from this pilot experiment can be seen in Fig 56. A clear increase in water intake was seen over consecutive trials until a plateau was reached on trial 8 and intake stabilized out at approximately 16mls per trial. This was much greater than the 2.5mls drunk in the first trial and is large in comparison to the 18mls drunk on average over the whole of the non-trial days in the home cage.

FIGURE 56: Pilot data for schedule-induced polydipsia are presented in this figure. Animals were found to develop polydipsia over several trials. Trials on consecutive days reduced polydipsia while 24 hours food deprivation increased it. Presenting food pellets all at once reduced water intake.

SCHEDULE INDUCED POLYDIPSIA
PILOT STUDY

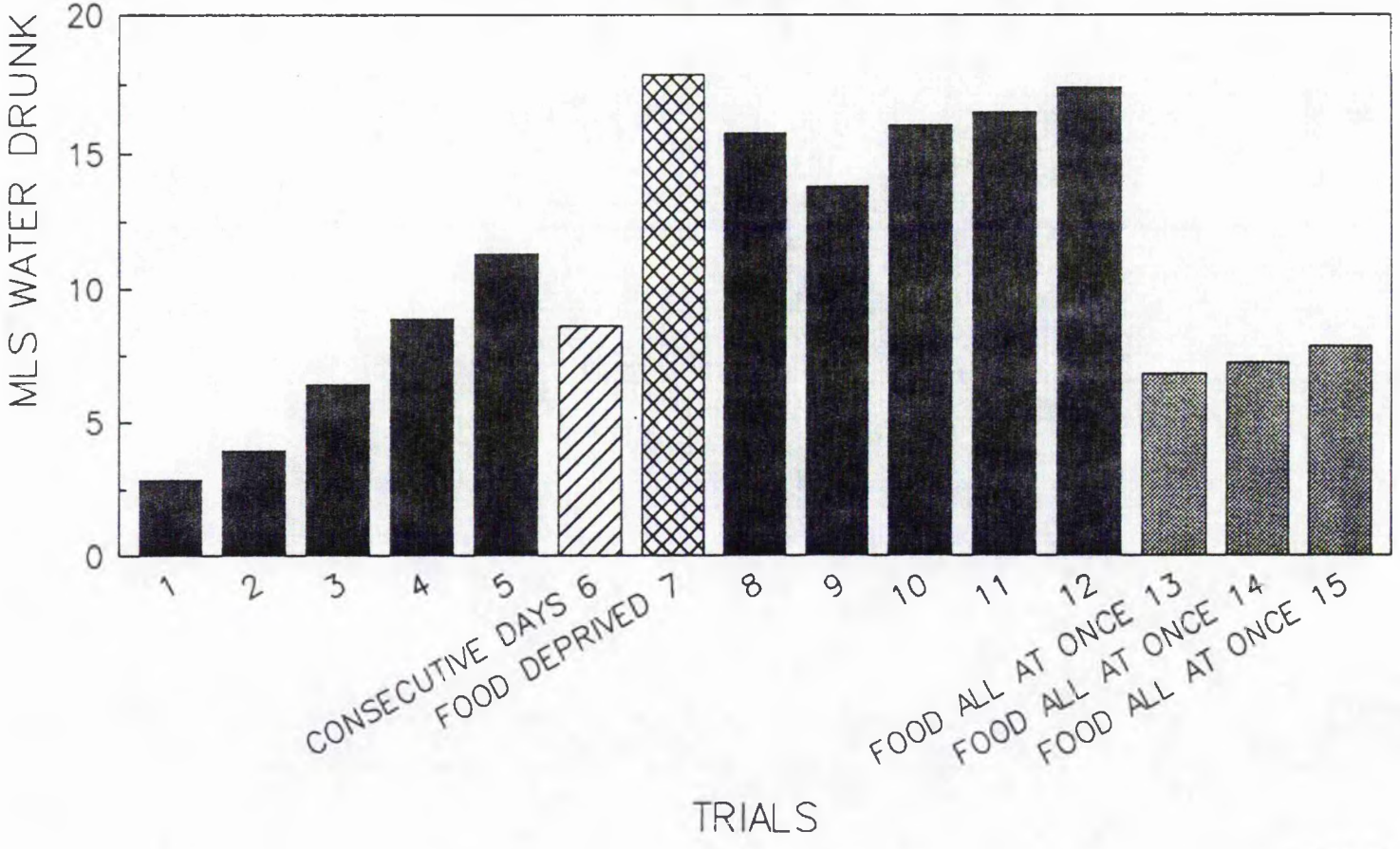


FIG 56

Several manipulations of the procedure were carried out. Trials 5 and 6 took place on consecutive days with no rest day in between. As can be seen in Fig 56, this reduced water intake on trial 6. Animals were food deprived for 24hrs before trial 7; this increased intake to some extent. On trials 13, 14 and 15, 60 pellets were placed in the food hoppers in the Skinner boxes all at once, rather than at 1 minute intervals. If water was consumed in quantities needed merely to compensate for dehydration caused by food intake, then this procedure should induce the same level of fluid intake as intermittent presentations. This was not the case, as can be seen in Fig 56. Water intake dropped by about 50% to approximately 7.5mls in 1hr. This indicates that presentation at 1 minute intervals effects a behavioural change in these animals.

SURGERY

Twenty male Lister-hooded rats, housed singly under a twelve hour dark-light cycle, were given ad-lib food and water. Average weight before surgery was 271.7 gms (SE=4.49; range = 248.6gms - 309.3gms). During surgery, 10ml/kg Avertin was used as anaesthetic. Bilateral LH lesions were made in 14 rats by microinjection of 1.0ul of 0.06M NMDA (pH 6.95) at the following stereotaxic co-ordinates in the orientation of de Groot: bregma +0mm, lateral $\pm$2.0mm, vertical -8.0mm (Pellegrino, Pellegrino and

FIGURE 57: Percentage body weights pre- and post-surgery for animals used in schedule-induced polydipsia are presented in this figure until food restictions began. Lesioned animals lost weight post-surgery, but began to gain weight at the same rate as controls after one week of recovery.

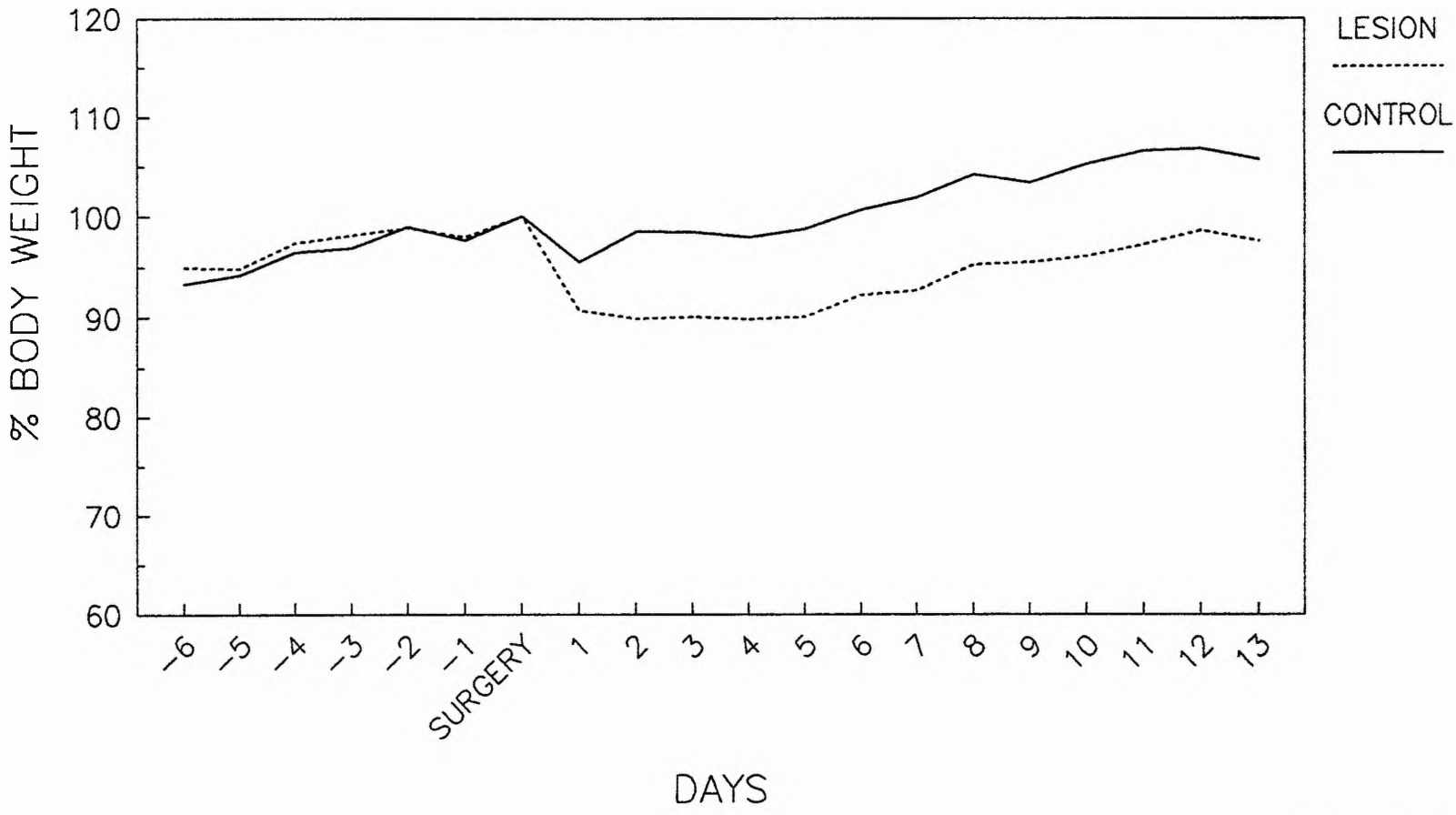

% BODY WEIGHT
SCHEDULE-INDUCED POLYDIPSIA
120
110
100
90
80
70
60
% BODY WEIGHT
LESION
CONTROL
-6
-5
-4
-3
-2
-1
SURGERY
1
2
3
4
5
6
7
8
9
10
11
12
13
DAYS

FIG 57

FIGURE 58: Food intake for one week pre- and two weeks post-surgery are presented in this figure. Lesioned animals reduced intake for one week post-surgery. Following this there were no significant differences between the groups.

FOOD INTAKE

SCHEDULE-INDUCED POLYDIPSIA

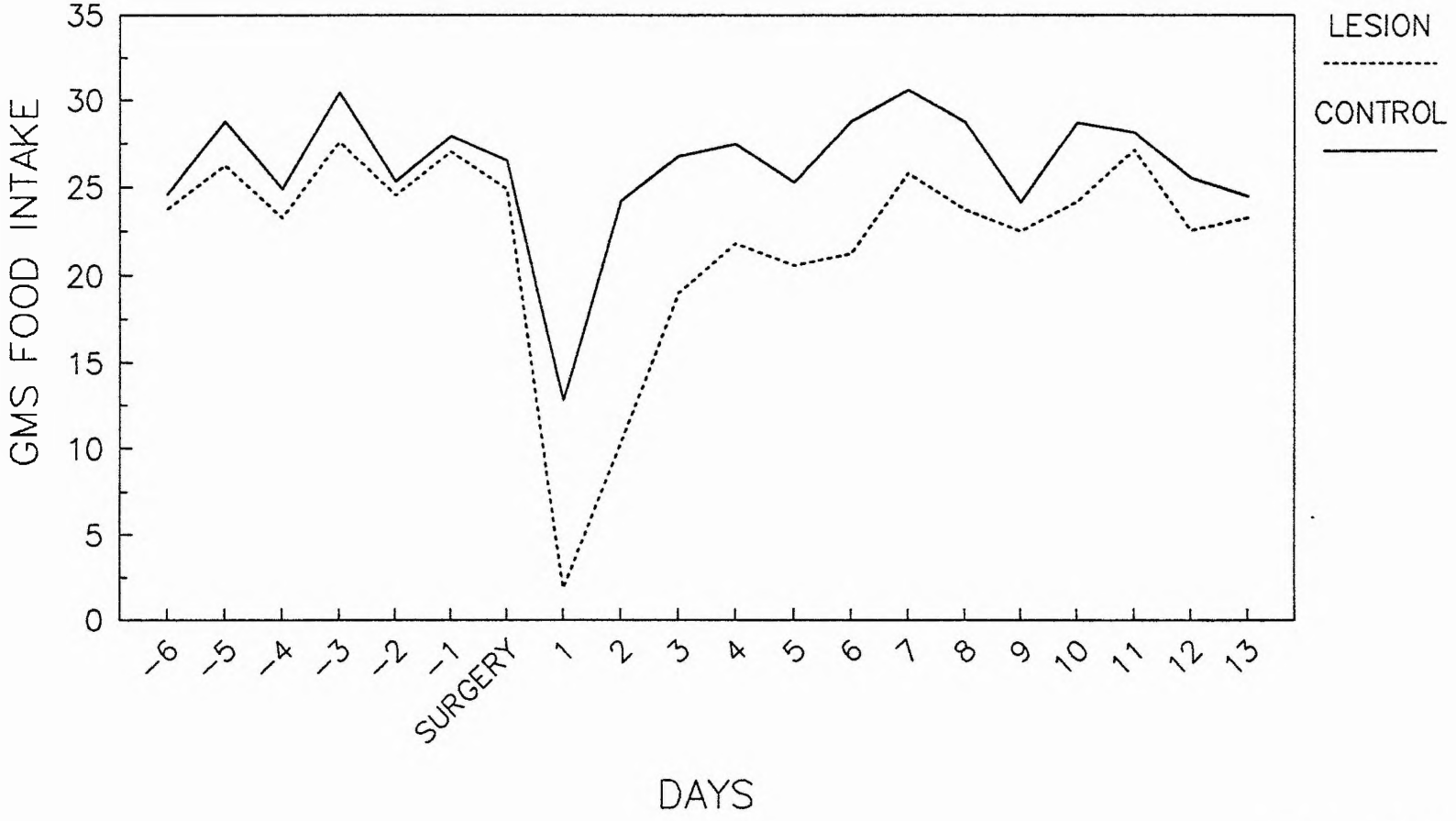


FIG 58

Cushman, 1979). Control animals (n=6) were microinjected with phosphate buffer (pH7) at the same co-ordinates.

Following histological examinations, three lesioned animals were found to have insubstantial lesions. It was also found that these animals responded as controls to hypertonic saline. These animals have, therefore, not been included in the results.

NORMAL REGULATORY BEHAVIOUR

BODY WEIGHT: [Fig 57] Body weight was measured every day pre- and post-surgery until food restrictions began 14 days post-op. Before surgery, there was no significant difference between the groups (ANOVA: $F=0.7956$; $df=1,15$; $p=0.3865$). One week following surgery, LH lesioned animals lost weight and there was a significant groups difference (ANOVA: $F=11.8935$; $df=1,15$; $p=0.0036$). This groups difference was maintained for the following 6 days leading up to restrictions (ANOVA: $F=6.9797$; $df=1,15$; $p=0.0185$), although there was no interaction between groups and days (ANOVA: $F=0.399$; $df=5,75$; $p=0.8481$) indicating that groups were gaining weight at the same rate.

FOOD INTAKE: [Fig 58] Food intake was measured daily pre- and post-op until food restrictions began. Before surgery, there was no significant difference between groups (ANOVA: $F=2.6310$; $df=1,15$; $p=0.1256$). One week following

surgery, LH lesioned animals reduced their food intake and there was a significant difference between the groups (ANOVA: F=12.2866; df=1,15; p=0.0032) but this difference was not found in the third week (ANOVA: F=4.0815; df=1,15; p=0.0616).

WATER INTAKE: [Fig 59] Water intake was measured daily pre- and post-op until food restrictions began. Before surgery, no significant difference was found between the groups (ANOVA: F=0.8106; df=1,15; p=0.3882). One week following surgery, LH animals reduced their water intake and there was a significant difference between the groups (ANOVA: F=14.1220; df=1,15; p=0.0019). In the third week this difference did not reach significance (ANOVA: F=4.2491; df=1,15; p=0.057).

In summary, LH lesioned animals lost weight and reduced their food and water intake for one week following surgery. After 2 weeks recovery, they were gaining weight and eating and drinking similar amounts to control animals.

HYPERTONIC SALINE

Hypertonic saline was administered according to the standard procedures described in the general procedures section (p. 60). At 1 hour post-injection, an interaction was found between groups and conditions (ANOVA: F=14.7914;

FIGURE 59: Water intake for one week pre- and two weeks post-surgery are presented in this figure. Lesioned animals reduced their water intake for one week post-surgery. Following this there were no significant differences between the groups.

WATER INTAKE
SCHEDULE-INDUCED POLYDIPSIA

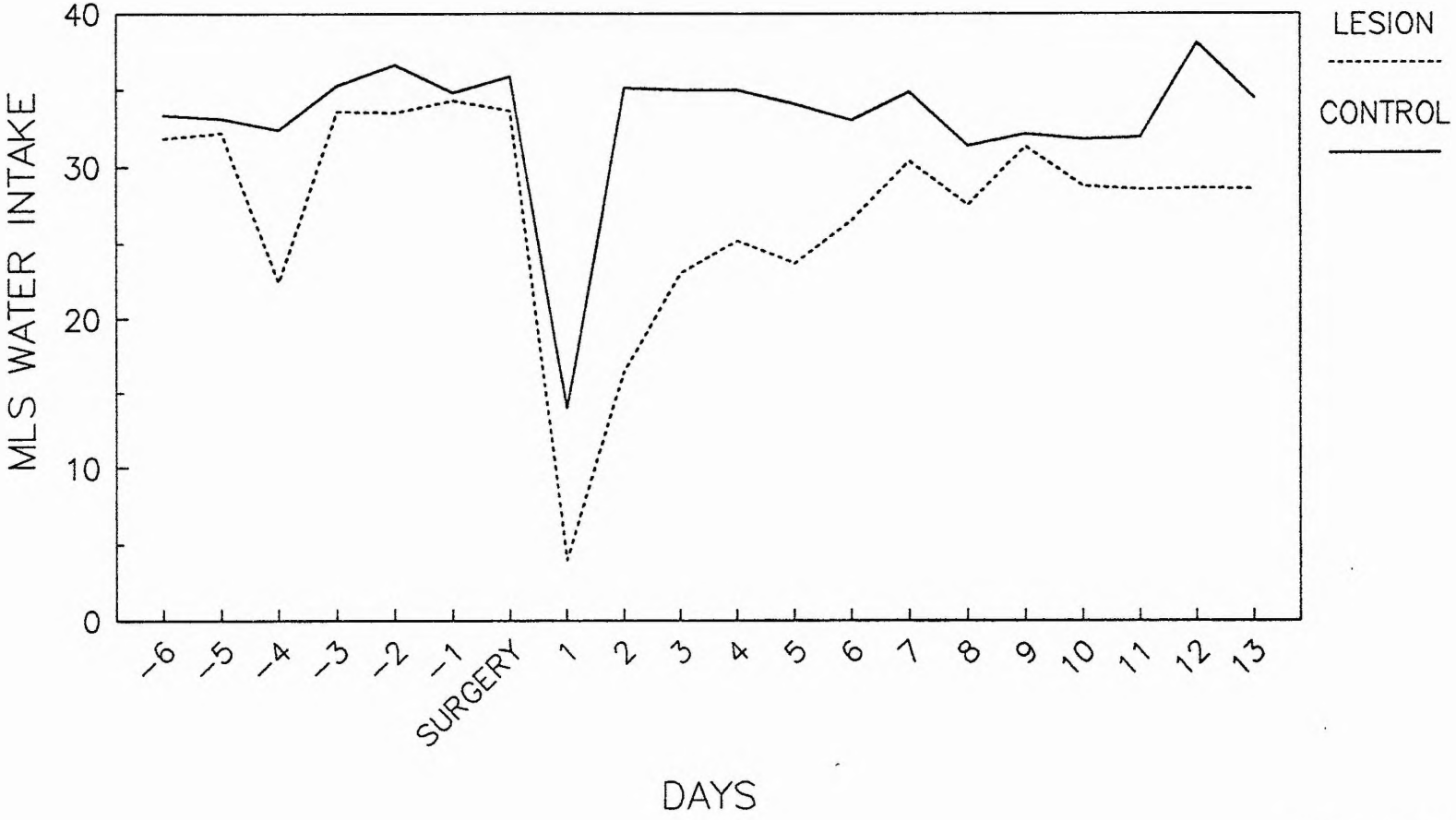


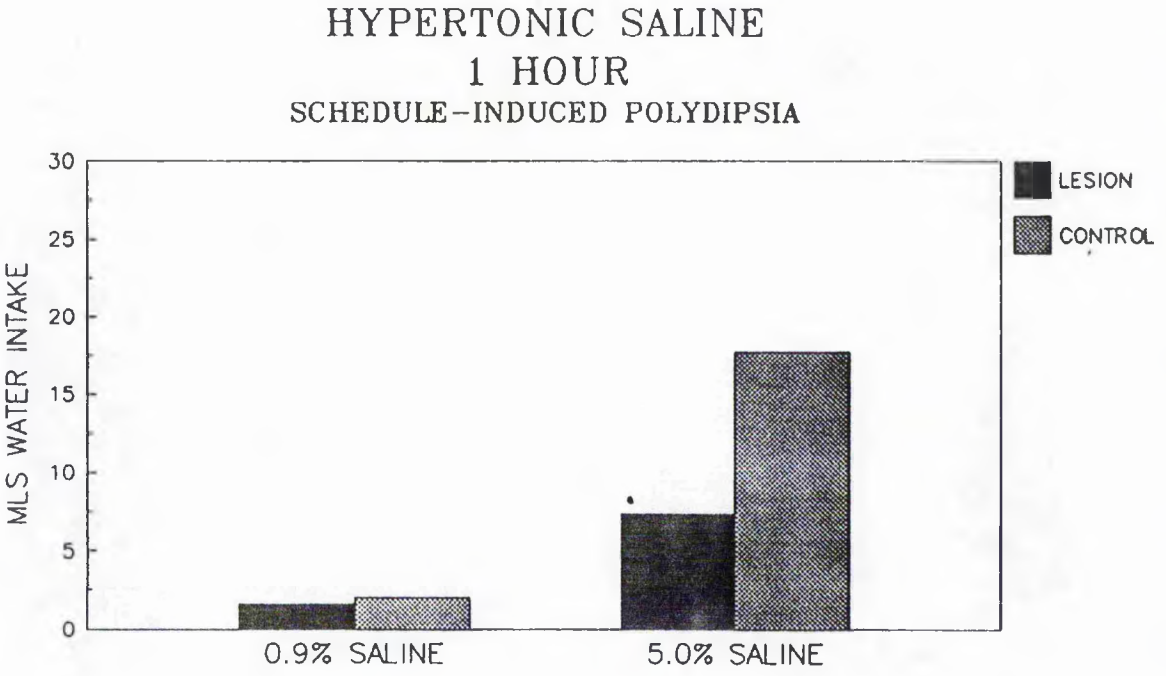
FIG 59

df=1,15; p=0.0016). Post-hoc testing (Tukey) showed a significant increase in water intake following hypertonic saline administration in control ($p<0.01$) and lesion ($p<0.05$) groups. Control animals drank significantly more ($p<0.01$) than lesioned animals in response to the challenge. At 3 hours post-injection, similar results were obtained. ANOVA showed an interaction between groups and conditions (F=12.3028; df=1,15; pn=0.0032). Both groups responded similarly to 0.9% saline injection, while controls drank significantly more (Tukey: $p<0.01$) than lesions in response to 5% saline administration [see Fig 60].

METHODS

Following a two week recovery period post-surgery, animals were placed on a restricted diet of 10gms of food per day until 85% of body weight was reached. Animals were then maintained at that weight, with daily intake ranging from 10gms to 15gms. On the fourth day of food restriction, testing began. At this stage, the average percentage weight of lesioned animals was 89.96% (SE=0.55) and of control animals was 91.23% (SE=1.09). Trials took place at the same time every second day with a rest day in between. Animals were placed in the Skinner boxes, room lights were switched off and testing began. Food pellets were dispensed every 60 seconds for 1 hour and then animals were returned to their

FIGURE 60: Responses to hypertonic saline physiological challenge 1 hour and 3 hours post-injection are presented in this figure. Lesioned animals showed a deficit in response to this challenge as revealed by ANOVA. Standard errors were never more than 2.58.


HYPERTONIC SALINE
1 HOUR
SCHEDULE-INDUCED POLYDIPSIA
MLS WATER INTAKE
30
25
20
15
10
5
0
0.9% SALINE
5.0% SALINE
LESION
CONTROL

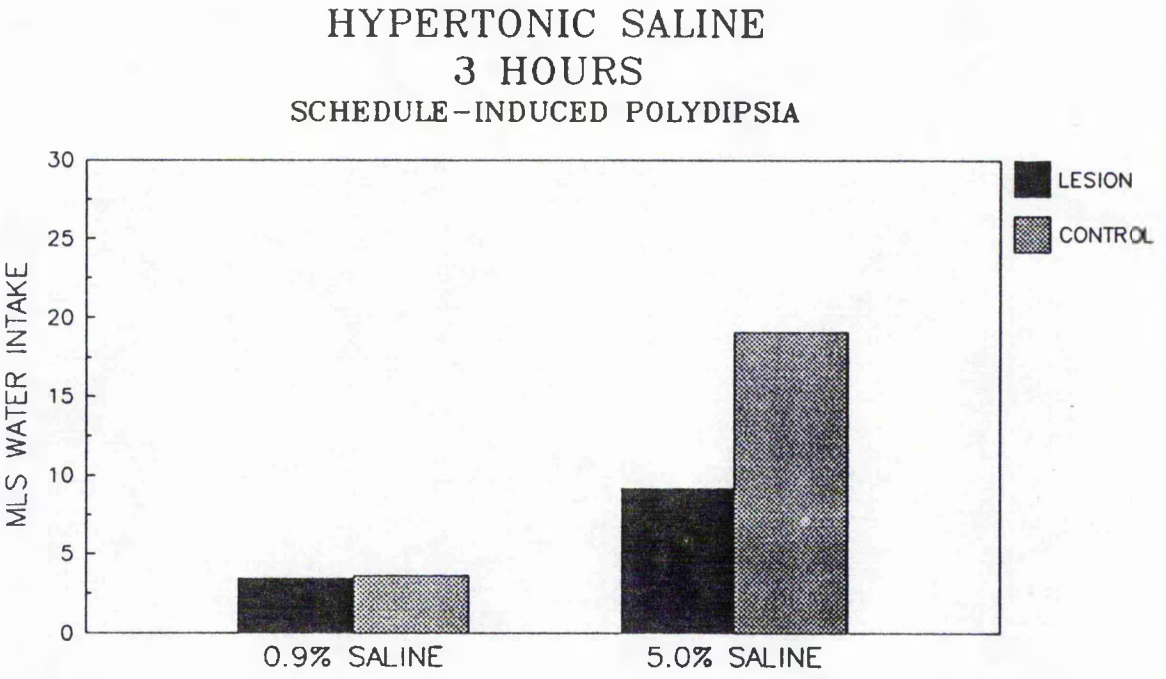

HYPERTONIC SALINE
3 HOURS
SCHEDULE-INDUCED POLYDIPSIA
MLS WATER INTAKE
30
25
20
15
10
5
0
0.9% SALINE
5.0% SALINE
LESION
CONTROL

FIG 60

home cages. Water bottles were weighed before and after each trial.

RESULTS

Results can be seen in Fig 61. On the first trial, both groups responded similarly. However, on subsequent trials lesioned rats drank more during the test than controls did until the last few trials. This represents a more rapid development of SIP in the lesioned animals and an ANOVA with trend analysis revealed that the shape of acquisition of SIP differed significantly in a quadratic manner between the groups (ANOVA: $F=7.777$; $df=1,135$; $p<0.01$), although the difference between groups was not found to be significant (ANOVA: $F=3.2916$; $df=1,15$; $p=0.0897$; interaction between groups and days $F=1.0263$; $df=9,135$; $p=0.4223$). Thus, lesioned rats developed SIP more quickly than control animals.

HISTOLOGY

Histological procedures are described in Chapter 5. Cell loss was greatest at the level of the VMH and the DMH with virtually no cell loss at either the anterior or posterior poles. Average lesion size was 48.6% (SE=4.16) and lesions ranged from 28.7% to 68.7%. There was very little extra-hypothalamic damage in this group. Some damage

FIGURE 61: Water intake during the intermittent presentation of food pellets is presented in this figure. Lesioned animals developed polydipsia quicker than control animals in that they drank more in earlier trials than control animals. This groups difference was found to be significant. Standard errors were never more than 2.5.

SCHEDULE-INDUCED POLYDIPSIA

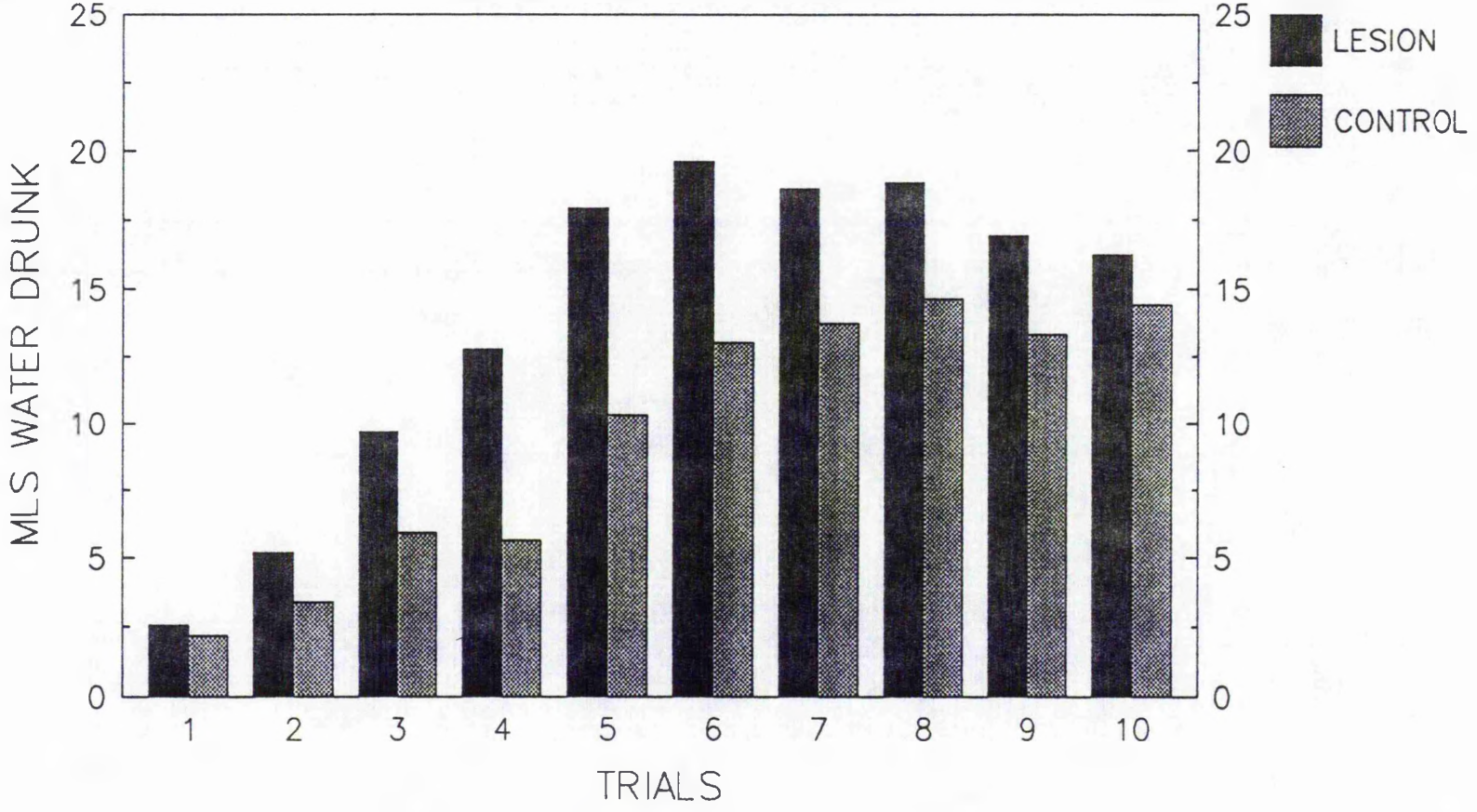


FIG 61

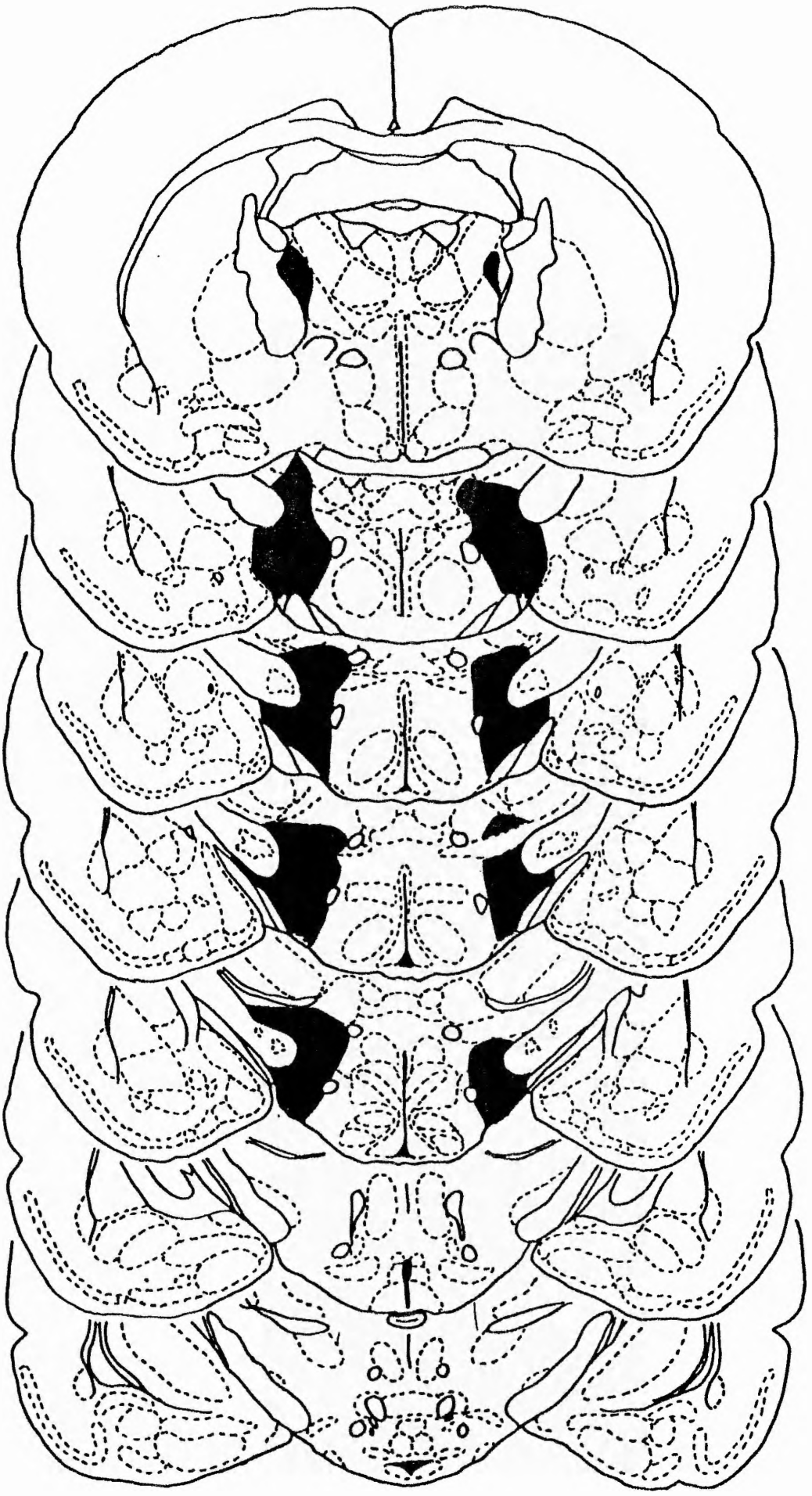
occurred in the zona incerta and reticular nucleus of the thalamus in most animals and in the ventral thalamus in some animals. The pattern and size of a typical lesion can be seen in Fig 62.

SUMMARY

The data presented indicate that LH lesioned animals developed SIP more quickly than control animals. If lesions of the LH induced deficits in arousal such that lesioned animals require greater stimulation to respond, or deficits in activation such that LH lesioned animals have difficulties in activating a response, then lower levels of SIP would be expected in these animals. However, LH lesioned rats did not show lower or even the same levels of SIP as controls, but HIGHER levels.

These results are in stark contrast to data obtained from hypertonic saline challenges. In comparison to controls, LH lesioned animals show significantly reduced drinking in response to intracellular dehydration caused by hypertonic saline administration, but increased drinking in response to the intermittent presentation of food. This clearly indicates that LH lesioned animals are capable of drinking and that the deficit to hypertonic saline does not arise because of a motor problem. It also indicates that LH lesioned animals do not appear to have either an arousal

FIGURE 62: The pattern and size of a typical lesion in this experimental group is presented in this figure. Shaded area represented lesion site. The central core of the LH was removed, but neither the anterior or posterior poles were damaged. Extra-hypothalamic damage occurred in the thalamus and zona incerta. (Rat J.191/88)



or activational deficit as polydipsia in such situations is believed to result from excessive excitement or activation (Robbins and Koob, 1980) and LH lesioned animals developed polydipsia before control animals.

TAIL-PINCH-INDUCED EATING

Eating in response to a mild but sustained pinch to the tail was first reported in 1975 by Antelman and Szechtman. They reported that eating, licking and gnawing could be induced by tail-pinch (TP) without producing any signs of distress. It does not seem likely that the eating observed following TP is a coping response used to lessen stress as anxiolytics (Robbins et al., 1977) and analgesics (Robbins and Fray, 1980) do not reduce the eating response to TP. It has been suggested that TP acts as an arousing stimulus which increases attention to external stimuli (Robbins and Fray, 1980). If food related cues are prominent animals will eat to TP; if wood chips are presented instead, animals will gnaw. The behaviour induced is determined by the stimuli presented during the pinch. The eating response does not appear on the first trial but develops over several trials, with animals initially showing high levels of activation to the pinch by increasing locomotion and rearing. The suggestion from this that the eating induced is a learned response (Robbins and Fray, 1980) is sometimes disputed, but the observation that TP involves arousal and

activation is widely accepted. If lesions of the LH induce deficits in arousal or activation, then eating in response to TP should not be observed in these animals or it should take longer or more pressure to induce eating in these animals.

SURGERY AND GENERAL PROCEDURES

The animals used in this experiment have already been described in Chapter 7. Details of surgical procedures, normal regulatory behaviour, responses to hypertonic saline and histological results can be found there. In summary, there were 17 lesioned and 11 control animals. Lesioned animals recovered well post-surgery in body weight and food and water intake, but showed a residual deficit to intracellular dehydration. Cell loss was found from the central core of the LH. As described in Chapter 7, rats were split into four groups: "lesioned/lab chow" (n=8); "lesioned/wet mash" (n=9); "control/lab chow" (n=6); "control/wet mash" (n=5). Wet mash consisted of powdered lab chow and water and was used as part of the experimental procedure, rather than the Farex wet mash presented to lesioned animals with reduced food and water intake described in previous Chapters. Type of food presented on a daily basis was used in the tail pinch trials; that is dry lab chow was presented to "lesioned/lab chow" and

"control/lab chow" groups and wet mash was presented to "lesioned/wet mash" and "control/wet mash" groups.

METHODS

After a five day recovery period, rats were tested individually every second day. The tail pinch arena consisted of a box 74cm square, with three metal sides and one of transparent plexiglas to allow observation. The floor of the arena was covered in food pellets for the dry lab chow groups, whereas the wet mash groups were given a ceramic bowl (as used for their everyday feeding) full of wet mash in each quadrant of the box. Rats were allowed to run around the arena freely for two minutes. Then a metal cuff, attached to a compressed air cylinder, was placed approximately two inches from the tip of the tail and pressure was applied. Initial pressure used was 10 psi and was maintained at this level if rats ate. For those animals which could not be induced to eat at this level, pressure was gradually increased (in 5psi steps) over a number of trials to a maximum level of 25 psi or until eating was observed. If the animal did not eat during the pinch, pressure was released after two minutes. If the animal ate for more than three seconds, the pressure was maintained for two minutes from the beginning of the eating bout. Latency to eat was recorded. If an animal did not eat after 10 trials, this was recorded as failure to respond to tail pinch.

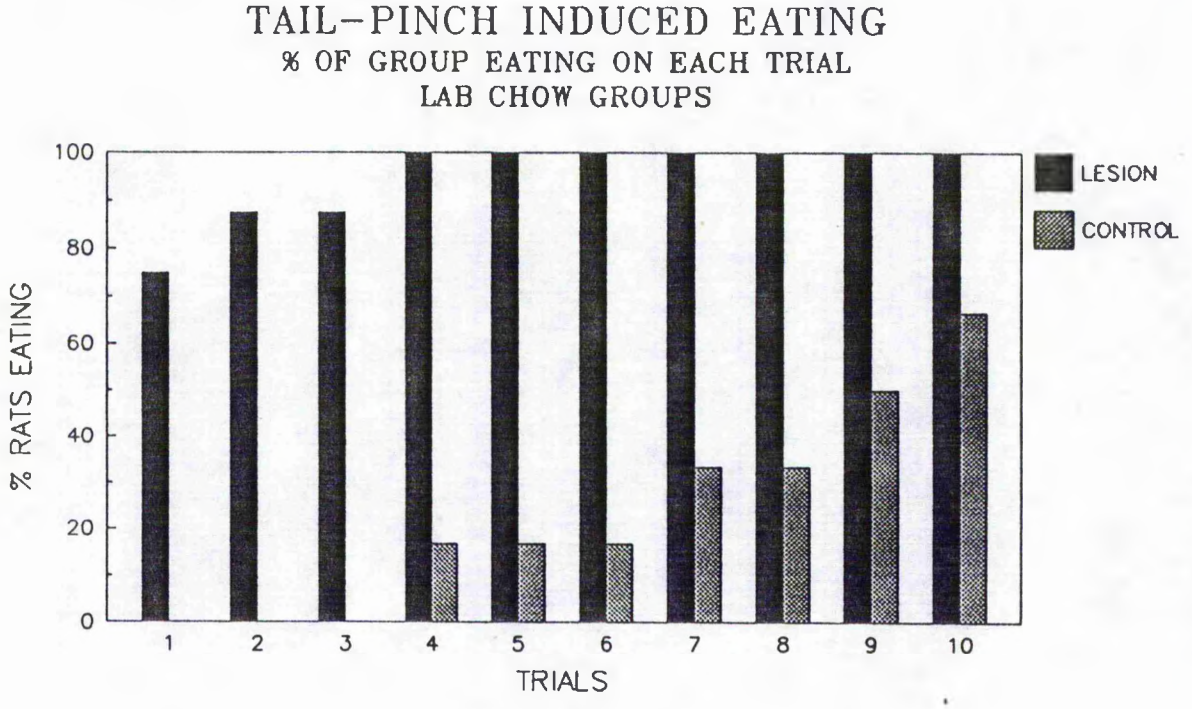
RESULTS

Data from this experiment can be seen in Fig 63. 75% of the "lesioned/lab chow" and 60% of the "lesioned/wet mash" animals ate on the first trial with latency to eat ranging from 4 secs to 116 secs. None of the control animals ate on the first or second trials. 40% of the "control/wet mash" group ate for the first time on trial 3, with latencies ranging from 29 secs to 115 secs, and 16.7% (one animal) of the "control/lab chow" group ate on the fourth trial, latency of 100 secs. By trial 4, 100% of the "lesioned/lab chow" were eating to TP and by trial 7, 100% of the "lesioned/wet mash" were responding. Neither of the control groups reached 100% response as by the end of trial 10 20% of the "control/wet mash" group and 33.3% of the "control/lab chow" group had never eaten in response to TP. Using chi-square analysis, there were significant differences between the lesioned and control groups (lab chow groups; $df=1$, $X^2=73.61$, $p<0.0001$: wet mash groups; $df=1$, $X^2=9.274$, $p<0.01$).

SUMMARY

As with the SIP results, the TP data are not compatible with the suggestion that lesion of the LH disrupts the "arousal function" of sensory events. If LH lesions produced a deficit in arousal or activation, then

FIGURE 63: The percentage number of each group of rats which ate in reponse to tail pinch on 10 trials is presented in this figure. An animals was included on the first trial in which it ate. 100% of lesioned animals developed tail pinch-induced eating and they did so on earlier trials than control animals, some of which never ate in response to tail pinch.


TAIL-PINCH INDUCED EATING
% OF GROUP EATING ON EACH TRIAL
LAB CHOW GROUPS
% RATS EATING
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
TRIALS
LESION
CONTROL

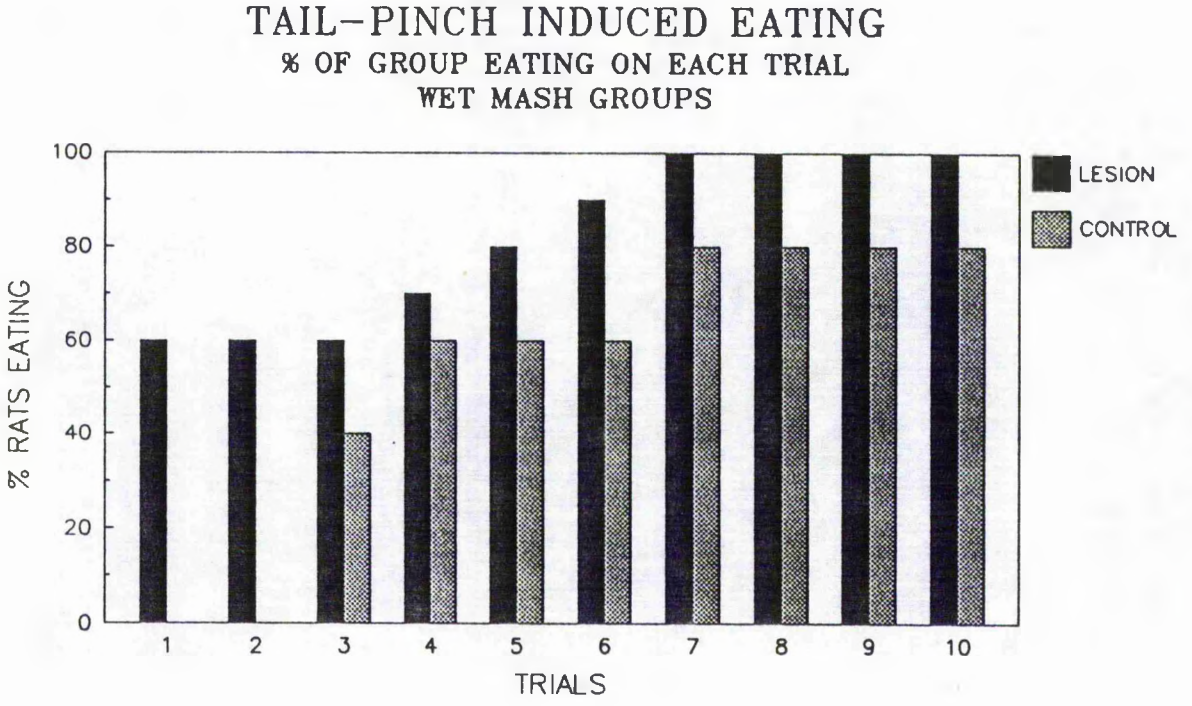

TAIL-PINCH INDUCED EATING
% OF GROUP EATING ON EACH TRIAL
WET MASH GROUPS
% RATS EATING
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
TRIALS
LESION
CONTROL

FIG 63

response to TP should be reduced, whereas the data indicates an increase in response. **Wolgin and Teitelbaum** (1978) reported that electrolytically lesioned animals which were aphagic could be made to eat by TP and interpreted this as loss of "endogenous activation", replaced by "exogenous activation". Even if this were the case, responses should be muted in comparison to controls who presumably have both "endogenous" and "exogenous activational" stimulation. However, the majority of LH lesioned animals responded on the first trial, whereas control animals did not respond until the third or fourth trial or, in some cases, not at all. This seems to indicate an increase in responsiveness rather than a decrease. It could be argued that "endogenous" and "exogenous activation" may be additive and reach a level in normal and control animals which becomes aversive and puts the animal in such a high state of arousal that it becomes immobile, thus dissociating arousal and activation and demonstrating that LH lesioned animals are, in fact, less aroused and therefore able to act. However, if this were so, control animals would be expected to eat to TP at a lower pressure, rather than having to increase the pressure above those used for lesioned animals before control animals would eat. This seems to indicate that the control animals needed higher levels of "exogenous activation" to induce the same response rather than that they were already more highly aroused and needed less "exogenous activation".

The fact that LH lesioned animals mostly responded on the first trial, some within 4 seconds of pressure being applied, also poses some problems for the theory that eating to TP is a learnt response which is acquired over several trials (Robbins and Fray, 1980).

The experiments described above do not seem to indicate that LH lesioned animals show a deficit in arousal or activation in that they display the behaviours expected in situations believed to induce activation and non-specific arousal. Furthermore, they actually respond more vigorously to these challenges than controls. Perhaps, then, removal of the LH induces an increase in activation or arousal rather than a decrease. This suggestion must be contemplated in the light of these results.

DO LH LESIONS INCREASE ACTIVATION?

It could be suggested that lesions of the LH increase arousal rather than decrease it. By the Yerkes-Dodson law, performance levels follow an inverted U shape in conjunction with arousal levels. That is, performance increases with arousal until a critical level is reached, beyond which performance decreases as arousal increases. As already mentioned, activation and arousal, in general, have the same relationship as performance and arousal, in that activation increases with arousal up to a critical level

and then decreases as arousal increases. Perhaps lesions of the LH increase arousal so that less external stimulation is needed to induce behaviours such as SIP or TP-induced eating. Injections of hypertonic saline are extremely stressful for the animal so perhaps deficits arise because the level of arousal is so great that activation has decreased; that is "rate and vigour of behavioural output" has decreased. This hypothesis could explain the data presented above, but could only account for the initial, sometimes permanent, decrease in daily food and water intake seen following surgery by proposing that animals are too active or aroused under normal conditions to eat or drink. These suggestions may not seem convincing, but in the light of the SIP and TP results it is important to look at baseline levels of arousal and activation in LH lesioned animals.

As already mentioned, up to a certain level arousal and activation are correlated. As the definition of activation given is "rate and vigour of behavioural output", increased locomotion indicates increased activation and may indicate increased arousal. If LH lesions have increased activation, then perhaps they show more locomotion and movement under normal conditions than controls. To test this hypothesis, the activity of lesioned and control animals was recorded in photocell cages.

SURGERY AND GENERAL PROCEDURES

Details of surgical procedures, normal regulatory behaviour, hypertonic saline responses and histological results for these animals have already been reported in Chapter 9 (p. 115). In summary, 5 LH lesioned and 8 control animals were used. Recovery post-operation was good but a residual deficit to physiological challenges was found. Lesions were found to lie in the central core of the LH. Following the experiment described below, these animals were given the long-term glucose challenge reported in Chapter 9 as "Calorific Intake Regulation".

PHOTOCELL RECORDINGS

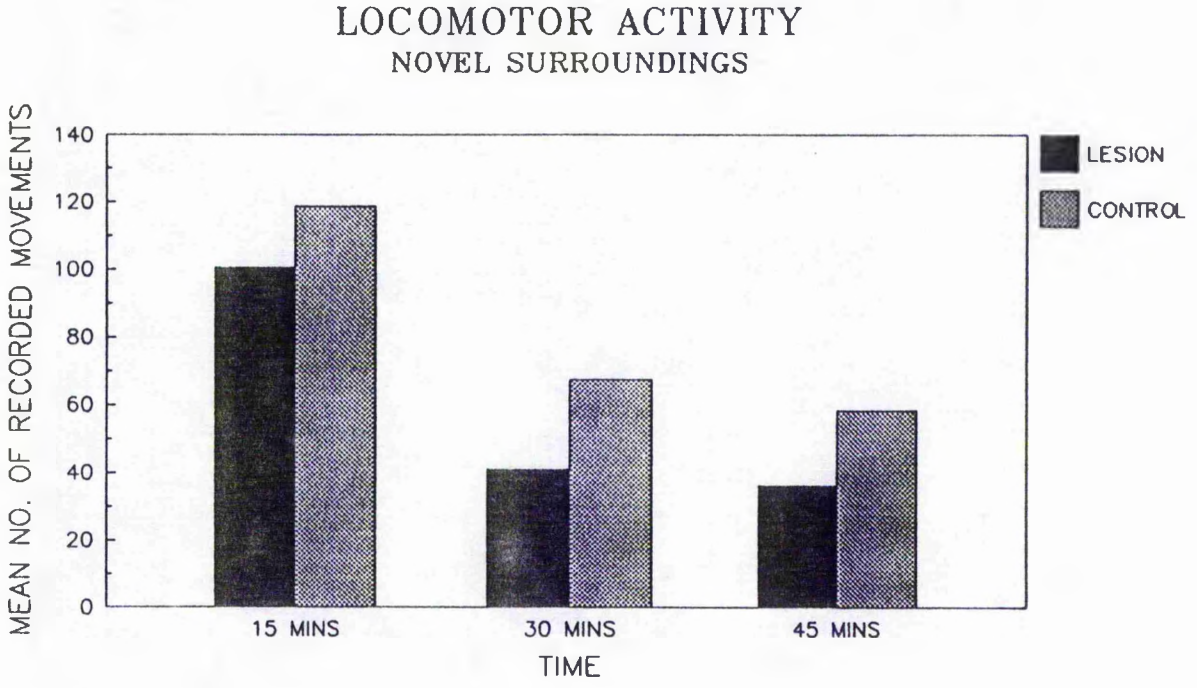
Following a three day recovery period post-surgery, animals were placed in photocell recording cages and recording of locomotion began immediately. The wire cages were of the same size as home cages and were run by a 'Spider' computer system (Paul Fray Ltd). Every time a beam of light was broken by the animal, the movement and time of movement were recorded. Each beam had to be broken in turn to register counts, rather than repeated breaking of one beam. In this way only genuine locomotion about the cage was recorded and measurement of grooming or stereotyped movements were avoided. Food and water were not available during testing. Measurements of activity during the first

45 minutes in the cages and then for 30 minutes following a two hour habituation period were taken. These measurements were used to assess differences in initial activation induced by novelty and speed to habituate, in addition to recording activity once familiarity had been established. Rats were then returned to home cages.

RESULTS

Data from this experiment can be seen in Fig 64. Activity is reported in 15 minute blocks. In Fig 64A it can be seen that both groups of animals responded similarly during the first 15 minutes in the photocell cages and were very active. This is important as animals with electrolytic lesions of the LH are reported to be akinetic (**Teitlelbaum and Epstein**, 1962). These recordings were taken only 3 days post-surgery and LH lesioned animals were as active as controls. During the next two 15 minute blocks, both groups reduced activity. ANOVA revealed an effect over time ($F=31.5536$; $df=2,22$; $p<0.001$) but no groups effect ($F=1.5082$; $df=1,11$; $p=0.245$) and no interaction between the groups over time ($F=0.1182$; $df=2,22$; $p=0.8891$). This would suggest that the LH group habituate to the novel surroundings in the same time as controls. As locomotion in a novel environment is likely to indicate level of arousal (at least to some extent), it would seem that LH lesioned animals and controls show the same level of arousal to this situation. Total activity over 45 minutes is greater in

FIGURE 64: Locomotor activity recorded in a photocell cage is presented in this figure. Activity in response to a novel environment is presented in the top graph and activity in response to a familiar environment is presented in the bottom graph. No differences in activity levels were found between the groups in either condition. Standard errors were never more than 21.14.


LOCOMOTOR ACTIVITY
NOVEL SURROUNDINGS
MEAN NO. OF RECORDED MOVEMENTS
140
120
100
80
60
40
20
0
15 MINS
30 MINS
45 MINS
TIME
LESION
CONTROL

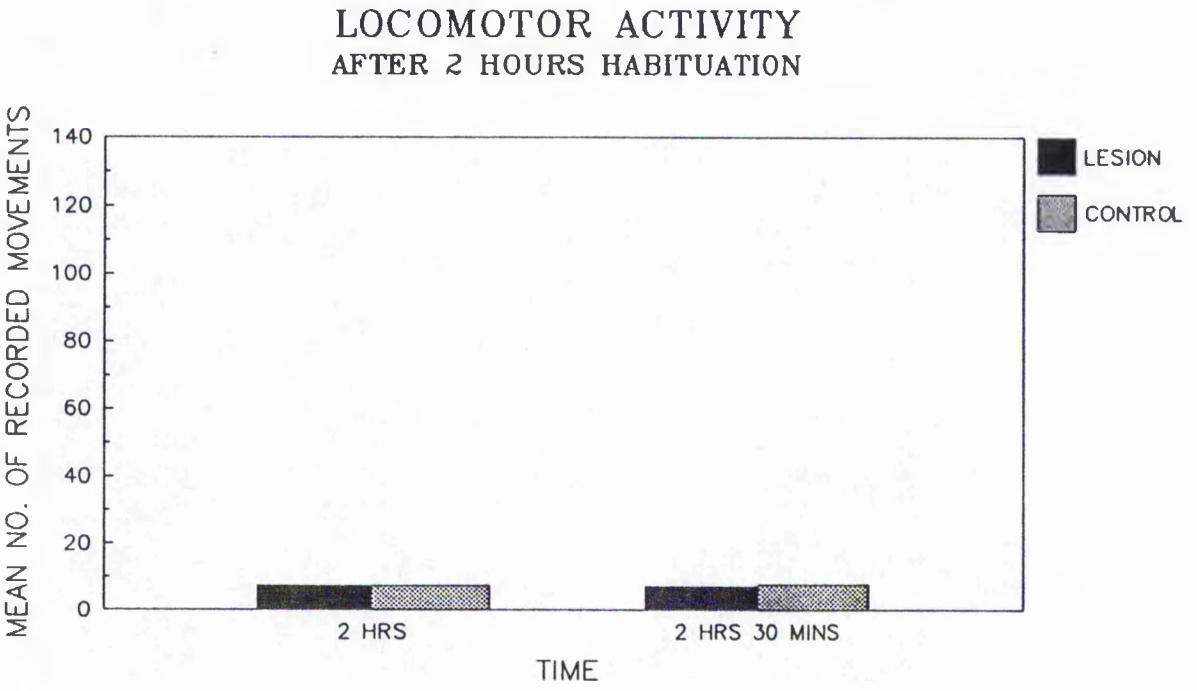

LOCOMOTOR ACTIVITY
AFTER 2 HOURS HABITUATION
MEAN NO. OF RECORDED MOVEMENTS
140
120
100
80
60
40
20
0
2 HRS
2 HRS 30 MINS
TIME
LESION
CONTROL

FIG 64

control rats. In Fig 64B, activity following a two hour habituation period is shown. No significant difference between lesioned and control animals was found (ANOVA: $F=0.001$; $df=1,11$; $p=0.9752$).

SUMMARY

Levels of activity in a familiar or a novel environment are not altered by LH lesions. These results do not support the hypothesis that LH lesions increase arousal or activation in general. The observation that these animals are obviously not akinetic supports the proposal put forward by **Winn et al** (1984) that the classic LH syndrome can be divided into two components; a motivational component mediated by the LH and a motor component mediated by the dopaminergic fibres of passage. NMDA lesions do not disrupt these fibres and do not affect locomotion. This data supports the observations that IBO lesioned LH rats show control levels of baseline activity and respond as controls to d-amphetamine and apomorphine injections by increasing activity; electrolytic lesions induce hypokinesia in general and in response to d-amphetamine, but hyperactivity in response to apomorphine (**Winn, Tarbuck and Dunnett,** 1984). This indicates that electrolytic lesioned animals have DA deafferentation and compensatory supersensitivity and that IBO animals do not. Due to the data presented in Chapter 6, similar results would be expected from NMDA lesioned animals given d-amphetamine or apomorphine as

reported for IBO lesioned rats. The NMDA results presented in the preceding pages also have implications for the hypothesis proposed by **Wolgin and Teitelbaum** (1978) outlined above. If LH lesioned animals had lost "endogenous activation" then the expected result would be a decrease in general activity levels. The result obtained, however, was no change in general activity levels.

DISCUSSION

The experiments described do not support the hypothesis that lesions of the LH induce a "motivational" deficit due to a disruption of the "arousal function" of sensory events as outlined by Hebb. Polydipsia during intermittent presentation of food is suggested to arise from nonspecific "motivational excitement or activation" (**Robbins and Koob**, 1980) and eating in respones to TP is believed to arise from "non-specific arousal" increasing attention to external stimuli (**Robbins and Fray**, 1980). Thus, the observable behaviours of drinking and eating can be used to measure internal levels of arousal and activation. LH lesioned animals respond more quickly and more vigorously than controls to SIP and TP indicating that they do not have decreased arousal or activation levels. Measurements of locomotion in familiar and novel environments do not indicate any differences between LH lesioned and control animals' general levels of arousal or activation. The

changes observed following these lesions do not, therefore, seem to be due to deficits in the "arousal function" of sensory events.

There are several important points raised by the results in this chapter. The first is that, as there were no differences found between lesioned and control animals' locomotor behaviour, the akinesia found in electrolytically lesioned animals does not arise because of damage to the intrinsic neurones of the LH. This finding supports the observations of **Winn, Tarbuck and Dunnett** (1984) and strengthens the argument that deficits seen following lesions of the LH are "motivational deficits".

This suggestion receives further support from the second important point; in the challenges described in this chapter the difference between lesioned and control animals is one of <u>excess</u> drinking or eating on the part of the lesioned animals. This result is in sharp contrast to the data collated from acute physiological challenges such as hypertonic saline or 2-DG administration which demonstrate <u>reduced</u> drinking or eating on the part of the lesioned animals. This emphasises the point that deficits are not due to sensorimotor problems or to a general feeling of malaise following lesions. These animals are obviously capable of eating and drinking.

So why do LH lesioned animals show reduced drinking to hypertonic saline challenge and increased development of drinking in a SIP paradigm? Why do they show decreased eating in response to 2-DG-induced glucoprivation and increased development of eating in response to TP? Where do the differences between these challenges lie?

The most obvious difference between these challenges lies in their specificity. Although the behavioural response induced in each situation may be the same, the specificity of the challenge is quite different. Injection of hypertonic saline induces intracellular dehydration and drinking behaviour is necessary to restore the body's fluid balance. Injection of 2-DG induces glucoprivation and feeding is necessary to restore the body's energy balance. Animals which display SIP are not dehydrated and eating in response to TP does not depend upon glucoprivation. Thus, these experiments demonstrate a dissociation between 'displacement' eating and drinking, arising in situations of non-specific motivational excitement, and 'deprivation' eating and drinking, arising in situations of genuine regulatory need.

This dissociation has been noticed before in the study of 6-OHDA lesions of DA terminals in the nucleus accumbens. In this study, deprivation drinking was unaltered while displacement drinking was greatly reduced following 6-OHDA lesions of the nucleus accumbens (**Robbins and Koob**, 1980).

These results were taken to indicate that such lesions do not impair regulatory behaviour, but do alter nonspecific motivational excitement. By Hebb's definition of the components of motivation, the "cue function" remains intact while the "arousal function" has been attenuated.

In the case of LH lesions, the "arousal function" appears to remain intact. Perhaps the "cue function" has been attenuated in some sense. Some support may be given to this hypothesis by the results presented, although the following explanations are put forward very tentatively as evidence is in many respects indirect.

The "cue function" is said to guide behaviour. How could this take place? How is one behaviour chosen as appropriate in the face of competing choices. In his discussion of motivation, Toates (1986) says, "..how does a motivational state select the appropriate response, so that the animal that is low in energy is directed to food and not to water? Bindra's answer is: by energy depletion accentuating the central representation of food." In Bindra's (1979) own words, "the central motive state is generated directly by organismic-state and incentive variables, and, once generated, 'feeds forward' to make certain particular environmental stimuli so potent that the animal must act in relation to them rather than in relation to any other stimuli." By this definition, behavioural selection is made by an integration of internal state and external cues which

may act to emphasise the relevant behaviour appropriate to the situation, for example, to eat when the animal is hungry and in the presence of food.

Lesions of the LH produce slowed responding to physiological challenges. Perhaps this is due to a disruption in the "cue function" which helps to guide behaviour. If the appropriate behaviour for that particular situation is not emphasised by the integration of internal and external cues, they may need longer to work out what the appropriate response is. Electrophysiological recordings from single cells in the LH found that LH neurones respond to the sight of food when the animal is hungry (Burton et al, 1976). The activity of these neurones does not signal hunger, but is dependent upon hunger and may help behavioural selection by firing when the animal is hungry and the option to eat is present. Thus, the appropriate behaviour in that situation is emphasised. In addition to responding to reward, LH neurones have been found to respond to aversive stimuli (Ono et al., 1985, 1986; Nakamura and Ono, 1986). Neurones which were activated by reward were inhibited by aversive stimuli. Thus, the significance of the stimulus to the animal predicts the LH neuronal response.

The concept of "emphasis" (either in a positive or a negative manner) is very important here. There is no suggestion that the LH processes or provides all the

information about energy or fluid balance. The rest of the hypothalamus, in particular the medial hypothalamus, appears to be much more involved in the actual processes of regulation. The LH has numerous connections with other central areas involved in the perception of food or food related behaviour and peripheral areas involved in homeostasis and it seems to be involved in the integration of this information rather than the transfer of direct information about the state of energy or fluid balance. Thus LH lesioned animals may not be deprived of information about regulation, as this is provided by other brainsites, but they may be deprived of the integrated information about internal state and external cues which infuences behavioural selection by emphasising the most appropriate behaviour to engage in at that particular time or inhibiting inappropriate behaviour.

Such a deficit could explain the opposite results obtained from hypertonic saline and SIP or 2-DG and TP. If the appropriate behaviour is not emphasised following hypertonic or 2-DG injections, it may take animals longer to respond to these challenges. In the case of SIP or TP the behavioural response induced is not necessarily appropriate to the situation. If activity of LH neurones aids behavioural selection by responding to the significance of stimuli, then control animals may be more aware in the Skinner box that they are hungry and that the appropriate behaviour is to eat, not to drink, and

therefore do not direct their attention to the water bottle so easily. Further experiments should be carried out to assess how much time animals spend at the food hopper between presentations. In the TP arena perhaps control animals are more aware that they are satiated and do not direct their attention to the food so easily. If the LH lesioned animals are unsure of the most appropriate response but prominent external cues are provided (such as food or water) there seems to be little advantage in hesitation and undirected activity. The inappropriate response may be inhibited to some extent in control animals during TP or SIP just as the appropriate response is emphasised by the integration of internal and external cues.

As already stated, these are merely suggestions as no direct support has been provided for this hypothesis. However, there is some support for this view in the anatomical and electrophysiological data cited in the introduction. This will be discussed in detail in the following chapter.

CHAPTER 11

DISCUSSION

Previous studies have suggested that the LH is involved in the regulation of food and water intake. The nature of this involvement, however, has been unclear and conclusions have been difficult to draw due to problems in the techniques used, particularly with electrolytic lesions which cause fibre damage and widespread disruption (Mufson, 1980). Lesion experiments using the neurotoxin ibotenic acid (Winn, Tarbuck and Dunnett, 1984) have suggested that classic electrolytic LH syndrome, which involves aphagia, adipsia and akinesia, induces such deficits by destruction of two systems involved in the control of food intake. Damage to the ascending dopamine fibre pathway has been shown to produce sensorimotor and motor deficits with direct consequences for feeding and drinking. Damage to the LH does not induce motor deficits and thus, the deficits in food and water intake have been attributed to a "motivational" deficit. The purpose of this thesis was to investigate the nature of this deficit using a more efficient excitotoxin, NMDA.

NMDA lesion of the LH induced reductions in food and water intake and loss of body weight. Consummatory deficits were sometimes permanent, but usually subsided after a period of

recovery. After an initial drop in body weight, LH lesioned animals generally began to gain weight at the same rate as control animals. This pattern of weight loss and gain has been demonstrated in control animals on a resticted diet and can, therefore, be attributed to reduced food intake, rather than to changes in a "body weight set-point" (**Powley and Keesey**, 1970; **Winn et al**, in preparation). Even after recovery of food and water intake, deficits were still found in response to intracellular dehydration caused by hypertonic saline injection. The behavioural effects found after NMDA LH lesions were therefore similar, but perhaps milder, than those reported following IBO lesions of LH (**Winn, Tarbuck and Dunnett**, 1984).

Histological examinations of the NMDA lesions revealed that cell loss was greatest from the central core of the LH with some sparing of the anterior and posterior poles. It appears to be almost impossible to remove 100% of LH neurones without causing extra-hypothalamic damage. This is probably due to the tubular shape of the LH in contrast to the spherical shape of NMDA lesions. Perhaps in future experiments two smaller injections separated by a small distance should be made in the LH, rather than one large one. Extra-hypothalamic damage was mostly found in the thalamus and zona incerta, but was not so great as to be prohibitive in assessments of the behavioural effects of LH damage. Although the LH was not generally removed completely and some cells were spared, lesions were much

greater than those reported to induce aphagia and adipsia in electrolytic lesion studies (Teitelbaum and Epstein, 1962). Fibres of passage did not seem to be damaged by NMDA as striatal catecholamine levels were found to be unaltered.

Thus, destruction of substantial portions of the LH with NMDA induced transient deficits in food and water intake and deficits in response to physiological challenges which could not be attributed to DA fibre disruption. This technique was therefore of use in investigations of the nature of the "motivational" deficits described by Winn et al. (1984).

It was suggested that the motivational deficit seen following destruction of the LH could take place at any one of four stages involved in food and water intake. The motivation to eat could be reduced if external factors no longer had any "incentive" value for the lesioned animals, because, for example, they no longer recognised the sensory qualities of food or fluid. The results from experiments designed to test this hypothesis suggest that animals are aware of the taste of food and fluid and that they respond to this sensory quality just as controls do. They demonstrated that external factors influenced their motivational state by drinking more of a palatable fluid and reducing their intake when presented with an unpalatable fluid. Thus, this component of the motivation

to eat or drink appeared to be intact in these NMDA LH lesioned animals.

In addition to sensory information from other brain sites, the LH receives input from the periphery and endocrine pancreas via the vagus, and has reciprocal efferents to these sites. Perhaps then, the "stimulus" which initiates feeding or drinking has been altered by destruction of a brain site (the LH) which may form part of an information loop with the endocrine system. Disruption of one link in this system may have altered the physiological signals which elicit feeding and drinking. There are many hormonal signals involved in the regulation of energy and fluid levels. The standard regulatory challenge used to test lesion deficits in all animals was intracellular dehydration so the hormone investigated was vasopressin, which is involved in the conservation of water and is released in response to dehydration. Measurements of baseline vasopressin in control and LH lesioned animals were found not to differ and levels in the blood of LH rats were found to increase in response to intracellular dehydration, even though the animals had been shown not to respond behaviourally to this challenge. This would indicate that this physiological signal remains intact and that the animals respond physiologically to dehydration although they do not respond behaviourally. Future experiments should be performed to analyse the

concentration of insulin, glucagon and AII in the blood of NMDA LH lesioned animals.

If the physiological signals associated with dehydration and glucoprivation remain intact, and yet the animal does not drink, perhaps there is a problem in "recognition" in that physiological signals are not understood or recognised. Thus changes in hormonal concentrations are no longer used to determine behaviour. To test this hypothesis, the nature of the physiological challenges was altered. When given long term challenges which were non-stressful, LH lesioned animals were found to be able to regulate calorific and water intake in the same manner as control animals. They reduced food intake when glucose, and therefore calories, were added to their water and increased water intake in response to salt adulteration of their food. Water deprived lesioned rats were also able to respond to the differences between injected physiological saline and water and could respond to intracellular dehydration over time. Thus, the ability to recognise energy and water levels in the body appears to be intact.

If physiological signals are intact and can be recognised by the LH lesioned animal, then why is there a deficit in response to administration of hypertonic saline? As this is an acute and immediate challenge, it is possible that animals have a problem in generating a "response", in that

"arousal" or "activation" have been altered by the lesion. Compensatory drinking in response to hypertonic saline does take place over 24 hours and the deficit is only seen 1 or 3 hours post-injection, which may suggest that the animals need time to respond. "Activation" and "arousal", however, do not seem to be impaired as LH rats showed similar general activity levels and actually increased responding in schedule-induced polydipsia and tail-pinch induced eating tests in which responding has been associated with arousal (Robbins and Koob, 1980).

Thus, NMDA LH lesioned rats can recognise the sensory and physiological properties of food and fluid and can respond to these. The deficits seen to physiological challenges cannot be attributed to a simple problem in activating responses, as some responses appear to be unimpaired. Thus, the nature of the deficit would not appear to be one of "incentive", "stimulus", "recognition" or "response" in the sense of activation.

The work presented may be criticised as merely a demonstration of what the LH does NOT do, rather than as a demonstration of what it DOES do. However, the data described has suggested several possibilities to explain the changes seen. Most of the results have been of a negative nature in that no differences could be found between lesion or control animals on many tests. This may cast doubt upon the efficacy of the lesion technique.

However, consistent deficits in response to hypertonic saline administration and the increased responding in schedule-induced polydipsia and tail-pinch experiments suggest that there was a difference between the two groups of animals. In fact, increased response indicates that the lesions did not just produce a general malaise which made animals slow to respond to hypertonic saline injections.

If anything these results indicate that our attempts to simplify brain function to "centres" is misguided. Without doubt the LH has some function in the regulation of food and water intake as indicated by electrophysiological studies, but although it receives homeostatic data, it may not be necessary to respond homeostatically to that data. The medial hypothalamus (MH) may be much more important for direct internal regulation. The LH does not develop from the same tissue as the rest of the hypothalamus and has very poor connections with the MH. Perhaps the LH and MH do not work together (as suggested by Stellar, 1954), but in parallel. While the MH is directly involved in regulatory processing, the function of the LH may be much more complex. Perhaps it is more useful to look at the brain as a series of filters connected into systems, rather than as a collection of "centres". Each area filters incoming information before sending it to connecting brain sites. By filtering information and only letting some pass through that stage, information is necessarily changed. Thus the LH may act as a filter for sensory, learnt and endocrine

signals. Some neurones only fire to the sight of food if the animal is hungry and the palatability and availability of the food modulate these neuronal responses (**Kendrick and Baldwin**, 1986). Thus converging information is integrated and passed on to higher cortical areas where behaviour is selected. Information about the sight or taste of food is not processed and passed through this filter in the system unless appropriate endocrine information is also available. When information passes through the filter, this aids behavioural selection.

It is useful here to look again at the concept of "motivation". As previously described, motivation arises as a <u>function</u> of internal state and external factors. Each of these components can effect the motivational state and it is the <u>interaction</u> of the two which determines the strength of the motivational state. Therefore, it is an interaction of present energy balance, palatability and availability of food stimuli, learned information and competing behavioural choices which determine whether an animal will eat or not and how much will be eaten. Thus, the motivational state arises from a complex integration of many inputs and does not arise from a simple state of glucoprivation. It is in the <u>integration</u> of internal and external factors that the LH seems to be involved and, as demonstrated, this information is fundamental to the motivational state which in turn is used in behavioural selection.

This analysis finds support from the anatomical data. Different sections of the LH receive different inputs. As a whole, the LH receives input from and sends information to brainsites involved in the perception of food or food related stimuli, brainsites implicated in learning and peripheral areas involved in homeostasis. Although these inputs may arrive at different sections of the LH, this structure has extensive intra-LH connections suggesting that it may perform an integrative function on the peripheral and central information converging on it. The LH also projects to higher limbic areas, believed to be involved in behavioural selection.

Electrophysiological data also supports this hypothesis. LH neuronal firing does not signal hunger, but is modulated by hunger; that is, LH neurones fire to the sight of food when the animal is hungry (**Burton et al**, 1976). This neuronal activity is also affected by the palatability of the food and whether the food is available for ingestion or not (**Kendrick and Baldwin**, 1986). The significance of the stimulus predicts the LH response. Thus, LH neuronal responding is not related to only physiological or only sensory information, but an integration of the two. This integration of internal physiological and external sensory stimuli (or "drive" and "incentive" in more theoretical terms; **Toates**, 1986) is fundamental to the motivational state and is important in "directing" that motivational state. **Toates** (1986) claims that "a motivational state

selects the appropriate response" by "energy depletion accentuating the central respresentation of food". Thus, behavioural selection is influenced by the integration of internal and external cues.

If this is the case, the different behavioural responses seen following LH lesions could be due to several things. If the LH does act as a filter which passes on information from the integration of internal and external cues about the significance of a stimulus in relation to physiological state then lesion of the LH should cause problems in behavioural selection. The results presented could be used to support this hypothesis, although they do not prove it. LH lesioned animals show a deficit in response to acute negative physiological challenges, but can respond over time. This may indicate that they have a behavioural selection problem in that it takes longer to work out the appropriate behaviour in that situation or that the appropriate behaviour does not "stand out" in the face of competing choices any more. During tail-pinch, they respond by eating more quickly than controls. External food cues are very prominent in such instances and perhaps LH lesioned animals are more easily distracted by prominent cues in a state of arousal or activation. It may be hypothesised that, in motivated behaviours such as feeding and drinking, the LH performs the "cue function" and focusses the rat on the most prominent 'need' by

emphasising the most appropriate behaviour in a situation of competing choices. Control rats may concentrate on the appropriate response, while LH lesioned rats are more easily distracted. This could account for the SIP results where LH lesioned rats display more adjunctive drinking rather than concentrating on the need for food; it could account for the TP results as LH lesioned rats may be more easily influenced by the prominent food cues than controls; it could account for the hypertonic saline results, which show that LH lesioned animals in their home cages, provided with no unusually prominent external cues, take time to make the appropriate response; and it may also account for the sometimes permanent reduction in daily food and water intake because, if the significance of stimuli in relation to physiological state has been reduced as one source of integrated internal and external information has been removed, although the animals maintain a sufficient intake to stay alive they would not be expected to engage in that behaviour as often or for as long as control rats as the emphasis may have been removed from engaging in that behaviour. As the motivational state arises from an integration of internal and external factors, without one source of that integration the motivational state is likely to be altered.

To test these hypotheses, animals could be put into a situation where immediate choices must be made. Would hungry LH lesioned animals be more easily distracted from

food by the presentation of a sexual partner than controls? If LH lesioned animals could be shown to have a problem in generating the appropriate behaviour in a situation of competing choices, then this might support the single cell recording results which implicate the LH in integrating information with regard to the significance of stimuli and thus aiding in behavioural selection.

As already stated, there may be other explanations of the results presented. One possibility is seen when the data presented here is compared to results obtained with anxiolytics. Rats given benzodiazepines show increased development of SIP (**Mittleman et al**, 1988) and increased eating to TP (**Robbins et al**, 1977). In these measures at least there appear to be direct parallels between changes in behaviour produced by LH lesions and by administration of benzodiazepines. Conclusions about such comparisons must be tentative because the same observable results do not necessarily arise from the same causes; 'displacement' and 'deprivation' drinking are obvious examples. Bearing this in mind, it is striking that the results from both groups should be so similar. This may imply that a "stress factor" has been removed by lesioning the LH. **Robbins et al** (1976) suggested that there are two components to TP-induced eating; an arousal and an aversive component. If the aversive component is removed by administration of chlordiazepoxide, then the arousal is enhanced and animals respond more vigorously. During SIP there may be a stress

component associated with the wait for food pellets. Removing the stress with diazepam may induce the animal to drink more. Administration of hypertonic saline is also stressful. Perhaps reduction in stress alleviates the immediate "drive" to drink. Thus, removal of the LH may be associated with removal of a stress component. This is not incompatible with the hypothesis given above, as a functioning LH may place pressure on behavioural selection and induce stress in extreme situations. To test this hypothesis, levels of corticosteroids in freely behaving, TP, SIP and hypertonic saline injected LH lesioned rats should be measured in addition to making further behavioural comparisons between LH lesioned and benzodiazepine treated rats.

These hypotheses are only tentative. The problems faced in interpreting the results raises the problem faced by many scientists using lesion techniques. As techniques improve, deficits become more difficult to find. This would seem to indicate the brain's amazing ability to compensate for insult and perhaps the complexity of the brain's organization which does not appear to include one site to perform one function, but multiple sites integrating and filtering information. It is not possible to remove tissue, observe the behavioural consequences and draw direct conclusions about the function of the tissue removed. Removing one link of the system may not tell you much about that link. If sequential processing is taking

place, then deficits may arise because information is not getting to an area further down the line and the behavioural disorder may arise as a result of malfunction in this area rather than in the site removed. If parallel processing is taking place, no deficit may be seen because other areas are working to compensate for the site lost. Lesion techniques are most useful, therefore, as one more source of information, rather than as THE source of information. Information from anatomy, neurochemistry, electrophysiology and physiology must be analysed with lesion and stimulation data if it an attempt is to be made to work out exactly how the brain works.

CONCLUSIONS

The results presented support the hypothesis that NMDA lesions of the LH do not induce regulatory deficits through motor or sensorimotor disruption and further support the idea that such deficits are of a motivational nature. This motivational deficit does not appear to be due to disorders of sensory perception, hormonal regulation or general arousal. The implication of this data is that the function of the LH is much more complex than terms such as "hunger centre" would imply and that future research must be detailed, thorough and multidisciplinary if the complex and subtle functions of the LH and similar brain areas are to be revealed.

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