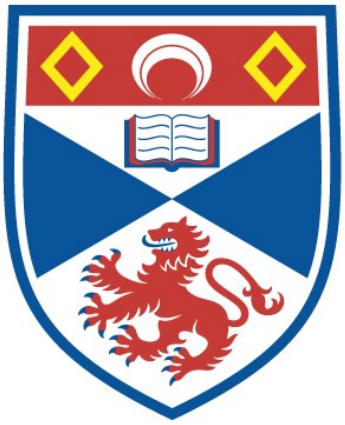
THE FUNCTION OF THE LATERAL HYPOTHALAMUS WITH REGARD TO GUSTATORY AND REWARD RELATED PROCESSES

Jennifer Margaret Scollon

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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

By

Jennifer Margaret Scollon

School of Psychology
University of St. Andrews
September 1998

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Declaration

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Abstract

The lateral hypothalamus (LH) has been shown to be involved in consummatory behaviour by a number of different experimental techniques including behavioural and electrophysiological methods. Lesion studies indicate that loss of the LH does not significantly alter normal feeding and drinking in the home cage, responding to food and water deprivation or responding to glucose or salt adulteration of the diet. However, when injected with dehydrating, dipsogenic or glucoprivic agents, the so called needle challenges, LH lesioned rats failed to respond as sham lesioned rats. This is despite the fact that the injections described induced the same deficits in homeostasis as food and water deprivation. Both sets of challenges are cued by internal visceral signals but only deprivation has additional environmental cues; animals are aware that their food or water are missing and may even anticipate its return. These different types of cues may be conveyed by different neural pathways and it has been proposed that lesioning the LH removes a pathway whereby visceral signals reach higher neural structures thus accounting for why LH lesioned rats responded appropriately to deprivation but not needle challenges. The present study examined the hypothesis that the LH acts as a gateway for signals concerning internal state to reach structures involved in behavioural planning and action. This was tested by the use of tests known to be susceptible to damage or change in the paraventricular system, responsible for monitoring the internal milieu, and frontostriatal systems responsible for behavioural planning and execution. The functions known to be dependent on the paraventricular system which were tested were conditioned taste aversion, benzodiazepine induced hyperphagia and taste perception but no deficits were found in responding in any of these procedures as a result of lesioning the LH. The functions known to be dependent on frontostriatal systems that were examined with LH lesioned rats were conditioned reinforcement and conditioned place preference but again few deficits were found. Hence, the present study failed to provide evidence to substantiate the hypothesis that the LH stands as an interface between the paraventricular system and frontostriatal systems. However, it did provide evidence that lesioning the LH induces deficits in consummatory responses dependent on the circumstances of the tests.

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Abbreviations

AMPA	(RS)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	analysis of variance
D-AP5	D(-)-2-Amino-5-phosphonopentanoic acid
CS	conditioned stimulus
CR	conditioned reinforcer
CRH	corticotrophin releasing hormone
D1	dopamine receptor type 1
D2	dopamine receptor type 2
$GABA_A$	γ-Aminobutyric acid receptor type A
HCl	hydrochloride
6-OHDA	6-hydroxydopamine
LH	lateral hypothalamus
LiCl	lithium chloride
MFB	medial forebrain bundle
mRNA	messenger ribonucleic acid
NaCl	sodium chloride
NaOH	sodium hydroxide
NCR	non-conditioned reinforcer
NT/NMN	neurotensin/neuromedin N
NTS	nucleus of the solitary tract
NMDA	N-Methyl-D-aspartic acid
PBN	parabrachial nucleus
PPTg	pedunculopontine tegmental nucleus
TTX	tetrodotoxin
US	unconditioned stimulus
VMH	ventromedial hypothalamus
VPMpc	ventral posteromedial thalamic nucleus
VTA	ventral tegmental area

General Introduction

The lateral hypothalamus was thought to be part of a dual control mechanism responsible for the regulation of feeding. Electrolytic lesions of the ventromedial hypothalamus (VMH) caused obesity while in complete contrast, electrolytic lesions of the lateral hypothalamus (LH) rendered rats aphagic and adipsic to such an extent that they died if they did not have intragastric feeding (Anand & Brobeck 1951). This lead to the belief that feeding was controlled by two opposing centres, the VMH and LH which were thought to be satiety and feeding centres respectively. Electrical stimulation studies supported this theory inasmuch as stimulation of the LH induced feeding whereas stimulation of the VMH inhibited feeding (Wyrwicka & Dobrzecka 1960).

A number of studies followed, further characterising the LH syndrome described by Anand & Brobeck (1951). Teitlebaum & Epstein (1962) reported that LH lesioned rats recovered from surgery but through a series of defined stages characterised as aphagia and adipsia; anorexia and adipsia; adipsia with secondary dehydration-aphagia, followed finally by recovery. However, even when at a stage where sufficient dry food and water were ingested to sustain life, deficits were found in regulatory and consummatory responses. The lesioned rats failed to drink in response to i.p. injection of hypertonic saline, a procedure that induced drinking in control rats, and they exhibited prandial drinking and therefore only drank water while eating. Furthermore, it was found that the inhibitory effect of adulterating food or water with quinine hydrochloride was greatly enhanced by lesioning the LH, and thus the lesioned rats were said to be finicky.

Despite reports of differences in responding to physiological challenges such as dehydration, it was suggested that changes in feeding patterns were secondary to body weight regulation. Powley & Keesey (1970) reported that the post-lesion period of aphagia and anorexia could be shortened or even reversed to an immediate post-lesion hyperphagia by lowering body weight prior to surgery. They hypothesised that lesioning the LH lowered a set-point for body weight and the disruption in feeding was simply a means to reduce body weight to this new level. In contrast, Mufson & Wampler (1972) proposed that the decrease in body weight was because the LH lesioned rats were more finicky than the control rats.

They found that LH lesioned rats actually maintained a greater weight than control rats if sustained on palatable foods. This indicated that rather than controlling a body weight set-point, the LH was in fact involved in the regulation of feeding behaviour.

Role of Dopamine Fibres

The role of the LH in the lateral hypothalamic syndrome itself was thrown into doubt by the development of a more selective lesion technique. Electrolytic lesions damaged tissue in a non-specific manner destroying neuronal cell bodies and axons and therefore, effects of the lesion could be attributed to the structure in question or to the fibres traversing it. It became clear that the nigrostriatal dopamine pathway ascended through the LH (Ungerstedt 1971a) and hence any effects produced by electrolytic lesions of the LH could potentially have been caused by destruction of cells intrinsic to the LH or disruption of the nigrostriatal pathway. The development of selective lesions of catecholamine pathways using 6-hydroxydopamine (6-OHDA) permitted examination of the extent to which catecholamine pathways contributed to the LH syndrome.

Ungerstedt (1971b) reported that complete bilateral degeneration of the nigrostriatal dopamine system induced by injection of 6-OHDA rendered rats aphagic, adipsic and akinetic. Thus, a group of rats appeared to have the main attributes of the LH syndrome without suffering any loss of neurones intrinsic to the LH. It was found that rats with 6-OHDA lesions recovered from surgery through the same defined stages as described in the LH syndrome and moreover, even after recovery, the same persistent deficits were found in response to a number of the regulatory challenges that had been found with LH lesioned rats (Marshall *et al.* 1974; Fibiger *et al.* 1973). It was demonstrated that rats bearing electrolytic lesions of the LH and rats bearing 6-OHDA lesions of the substantia nigra failed to drink in response to hypertonic saline or polyethylene glycol, failed to eat in response to 2-deoxy-D-glucose and they both exhibited prandial drinking (Marshall *et al.* 1974; Fibiger *et al.* 1973). This indicated that the LH syndrome could in fact be attributed to damage to this fibre system.

In addition to the similarities in ingestive behaviour, it was found that both LH lesioned rats and 6-OHDA nigrostriatal lesioned rats exhibited sensorimotor deficits. Several measures were taken which were indicative of such a deficit including responsiveness to olfactory, visual and somatosensory stimuli and ability to move limbs reflexively and in tasks requiring their precise placement in space (Marshall *et al.* 1971; Zigmond & Stricker, 1973; Marshall *et al.* 1974; Marshall & Teitlebaum, 1974). Rats with unilateral electrolytic or unilateral 6-OHDA lesions failed to orient to contralateral stimuli whereas they responded promptly to the same stimuli when presented ipsilaterally. After bilateral lesions, responsitivity to sensory stimuli on either side was impaired. It was suggested that deficits in regulatory behaviour could simply have been caused by these sensorimotor deficits, as recovery from the early stages of aphagia induced by bilateral lesions was correlated with recovery of orientation to olfactory and somatosensory stimuli, including stimuli concerned with food (Marshall & Teitlebaum 1974; Marshall *et al.* 1971). Thus, it appeared that not only was the LH syndrome not caused by damage to the LH but the primary effect of the lesions was not impaired feeding and drinking.

However, differences were found between rats bearing electrolytic LH lesions and 6-OHDA lesions. Unlike LH lesions, nigrostriatal bundle damage did not consistently result in an increase in finickiness but, it did result in a greater delay in recovery of orientation to sensory stimulation compared to LH lesions (Fibiger *et al.* 1973; Marshall *et al.* 1974). Furthermore, a difference was found in the degree of striatal dopamine loss required to induce aphagia. Greater than 90% loss of striatal dopamine was associated with aphagia produced by 6-OHDA lesions, whereas aphagia produced by electrolytic LH lesions was associated with only 50-60% striatal dopamine loss (Zigmond & Stricker 1973). This indicated that loss of another neural substrate in addition to the nigrostriatal dopamine system was at least partly responsible for inducing the behavioural syndrome seen after electrolytic lesions of the LH.

Contribution of LH Neurones

Despite evidence that the LH syndrome could be induced without causing any damage to cells intrinsic to the LH, electrophysiological data indicated that the

LH did have a role in ingestive behaviour. It was found that LH neurones of the monkey were responsive to the sight and taste of food in a manner that was dependent on the monkey being hungry (Burton *et al.* 1976). As the monkey was fed to satiety, the responsiveness of the LH neurones declined until when satiety was reached (as measured by rejection of food), the firing rate of the LH neurones had returned to basal levels. In a later study, Rolls *et al.* (1986) demonstrated that the responsiveness of LH neurones to the sight and taste of food was dependent not only on the deprivation state of the animal, but also on the sensory quality of the food supplied. While responsiveness to a substance declined as it was fed to satiety, responsiveness to other foods that had not been fed to satiety was relatively unchanged. Hence, the LH neurones responded to the sight and taste of food in a pattern termed sensory specific satiety.

Although electrophysiological data was indicative of a role for the LH in feeding behaviour, it was not until the development of excitotoxins that the contribution of LH neurones to the syndrome induced by electrolytic lesions of the LH could be determined. Excitotoxins are analogues of the neurotransmitter glutamate which, when infused in the central nervous system at sufficient concentration, result in cell death by inducing powerful excitation at glutamate receptors (Winn 1991). However, they act only at the cell bodies leaving fibres of passage intact thereby permitting the selective lesioning of the intrinsic cells of the LH.

Deficits Induced by Ibotenic Acid Lesions of the LH

Winn *et al.* (1984) used the excitotoxin ibotenic acid to lesion the LH in a comparative study of rats bearing electrolytic and excitotoxic lesions of the LH. It was found that like electrolytic lesions of the LH, ibotenic acid lesions induced aphagia and adipsia, albeit to a lesser degree. The excitotoxic lesioned rats were also impaired in responding to glucoprivation induced by 2-deoxy-D-glucose and dehydration induced by hypertonic saline or polyethylene glycol which were all regulatory challenges electrolytic LH lesioned rats had previously failed to respond to appropriately (Teitlebaum & Epstein 1962; Marshall *et al.* 1974). Nevertheless, it was demonstrated that rats bearing excitotoxic lesions of the LH exhibited neither prandial drinking or finickiness (Winn *et al.* 1984; Robertson 1994) which was in contrast to previous findings with rats bearing electrolytic

lesions (Teitlebaum & Epstein 1962; Marshall *et al.* 1974). This implied that the syndromes induced by excitotoxic and electrolytic lesion methods were not identical and more definitive group differences were found in measures of locomotor activity. Unlike electrolytic lesions, ibotenic acid lesions caused no deficits in spontaneous locomotion or locomotion induced by *d*-amphetamine or apomorphine. Since deficits in locomotor activity were a product of selective nigrostriatal damage, this indicated that these fibres remained functional after ibotenic acid lesions of the LH and histological and biochemical analysis supported this. Therefore since deficits in consummatory behaviour but not locomotion were found as a result of destruction of LH neurones alone, it appeared that LH neurones and the nigrostriatal dopamine system mediated different components of the classic LH syndrome. Destruction of LH neurones contributed to consummatory and regulatory behaviour deficits whereas disruption to the nigrostriatal pathway mediated the altered locomotor activity.

One aspect of the consummatory deficits included in the classic LH syndrome that was not found with excitotoxic lesioned rats and not consistently found with nigrostriatal damage was finickiness, indicating that loss of neither intrinsic cells of the LH or nigrostriatal fibres was the substrate for this effect. However, a study by Sahakian *et al.* (1983) indicated that finickiness was induced by damage to the ventral noradrenergic bundle and hence non-selective damage to this fibre system could have been the cause of finickiness found in electrolytic LH lesioned rats since like the nigrostriatal pathway, it has been shown to course in the medial forebrain bundle (MFB) which traverses the LH (Ungerstedt 1971a).

It had been suggested that the consummatory deficits found after electrolytic LH lesions were secondary to sensorimotor deficits. However, ibotenic acid lesions of the LH induced no sensorimotor deficits, further differentiating the effects of LH lesions and lesions of the fibres traversing the LH (Dunnett *et al.* 1985). Moreover, it indicated that the impairment in consummatory behaviour was not simply due to impaired orientation to food stimuli. Nevertheless, there were problems with ibotenic acid lesions that complicated interpretation of the results found. Dopamine levels in the nucleus accumbens were greatly enhanced as a result of ibotenic acid lesions of the LH (Winn *et al.* 1984; Dunnett *et al.* 1985)

and since the nucleus accumbens was known to be involved in feeding behaviour (Heffner *et al.* 1980), a more selective excitotoxin was sought. Hastings *et al.* (1985) compared the effects of injections of the neurotoxins ibotenic acid, NMDA and quisqualate in the LH by histological and biochemical means. In contrast to ibotenic acid, it was found that lesioning the LH using NMDA or quisqualate produced no change in dopamine levels in the accumbens. Since it was found that the majority of neurones within the LH were sensitive to all 3 neurotoxins, the increase in dopamine was attributed to the different pattern of extra-hypothalamic damage produced by ibotenic acid. It was shown that neurones of the medial amygdala were susceptible to damage by ibotenic acid but not NMDA or quisqualate and hence it was inferred that the increase in dopamine levels found in the nucleus accumbens as a result of ibotenic acid lesions of the LH was due to damage to the medial amygdala.

Deficits Induced by NMDA Lesions of the LH

It was found that rats bearing NMDA lesions of the LH closely resembled ibotenic acid lesioned rats in all consummatory and regulatory responses tested (Winn *et al.* 1990; Clark *et al.* 1990). The NMDA LH lesioned rats recovered rapidly from surgery resuming ingestion of dry chow and water within two post-operative weeks. Residual long-term deficits in body weight were found but when unoperated rats had food intake yoked to that of lesioned rats they showed identical long-term changes in body weight indicative that the changes in body weight were secondary to changes in ingestive behaviour. This provided further evidence to dispute the theory of Powley & Keesey (1970) that lesioning the LH simply altered a body weight set-point mechanism. Like ibotenic acid LH lesioned rats, NMDA LH lesioned rats exhibited impairments in responding to dehydrating, dipsogenic and glucoprivic challenges. Moreover, neither ibotenic acid nor NMDA LH lesions induced finickiness indicating that taste perception was not altered by either neurotoxin. However, despite the fact that NMDA induced the same regulatory and consummatory responses as ibotenic acid, biochemical analysis revealed that NMDA lesions of the LH did not affect the concentration of dopamine in the dorsal or ventral striatum, replicating the findings of Hastings *et al.* (1985). Hence, it appeared that NMDA was preferential to ibotenic acid in the production of lesions of the LH and was

subsequently used in several studies that further characterised the impairments induced by LH lesions (Clark *et al.* 1990; Clark *et al.* 1991; Clark *et al.* 1991; Robertson 1994).

It was found that despite the failure to respond to glucoprivic, dipsogenic and dehydrating challenges, LH lesioned rats responded appropriately to 24h food and water deprivation. A number of tests were conducted to investigate why LH lesioned rats could respond to glucoprivation and dehydration when induced by water and food deprivation but not by i.p. injection of 2-deoxy-D-glucose or hypertonic saline. It was shown that LH lesioned rats could maintain a constant energy intake when glucose was added to their water supply and they increased water intake appropriately when salt was added to their water supply indicating that they were aware of the physiological consequences of the substances they had ingested and were able to respond accordingly (Clark *et al.* 1991). In agreement, it was also shown that drinking in response to water deprivation was inhibited by i.p. injection of water but not isotonic saline when administered immediately before water was returned (Clark *et al.* 1991). The degree of inhibition of water intake did not differ from that seen with control rats illustrating that LH lesioned rats could respond to rapid changes in internal state. It appeared that lesioning the LH only disrupted regulation of energy and water balance that under particular circumstances.

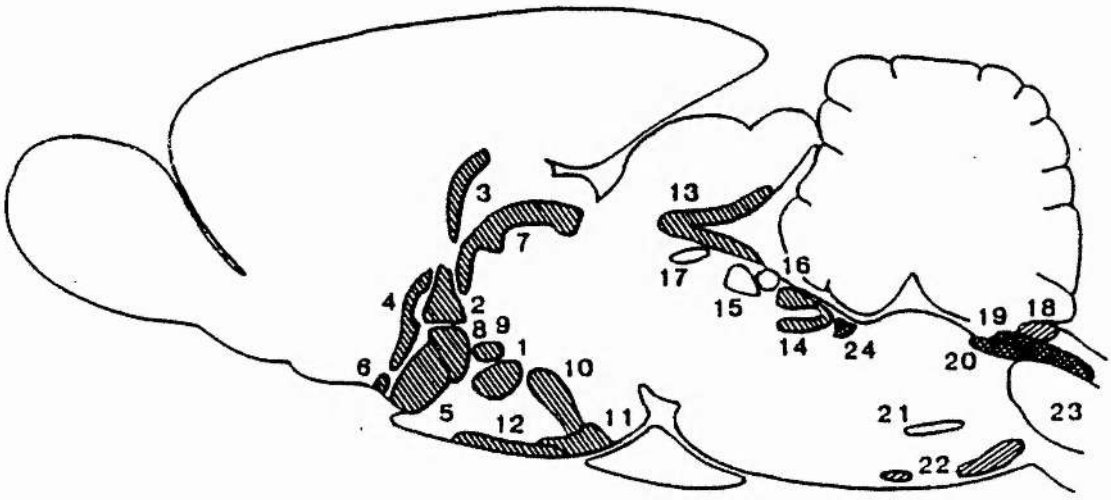
Perturbations in energy and water balance are addressed by co-ordinated behavioural, autonomic and endocrine responses. For instance, a decrease in the volume of extracellular fluid can be rectified not only by the behavioural response of drinking but also by the autonomic response of vasoconstriction, and by the release of the hormone arginine-vasopressin that increases water retention by the kidney. An impairment in responding to an insult to water or energy balance could reflect disruption to just one or a combination of these response mechanisms. In the case of intracellular dehydration induced by injection of hypertonic saline or water-deprivation, increased drinking and increased arginine-vasopressin secretion are exhibited to address the resultant increase in plasma osmolality. Clark *et al.* (1991) found that despite the lack of drinking in response to i.p. hypertonic saline, there was nevertheless an appropriate increase

in the plasma concentration of arginine-vasopressin. Hence, the behavioural and endocrine components of the response had been dissociated and it appeared that the nature of the deficits induced by lesioning the LH could be behavioural rather than physiological. Despite this, Robertson *et al.* (1994) has demonstrated that the deficits in responding were unlikely to be due to a decrease in the motivation to eat. Using the progressive ratio paradigm, she found that the number of lever presses rats were willing to make in order to receive food was not altered by lesioning the LH, indicating that motivational strength was normal in these rats.

Winn (1995) proposed that the key to why LH lesioned rats failed to respond to dehydration and glucoprivation, induced by injections, lay in the cues signalling the homeostatic deficit. Changes in water and energy balance are detected internally and cues concerning the internal environment can induce ingestive behaviour. However, internal cues are not the only driver of such responses. Animals can learn from past experiences of feeding with regard to location and availability of food for instance. This can shape anticipation and expectation and animals can use all this information to adapt to their environment. It was suggested that the importance of this was the implication that several neural systems were likely to be involved in the control of ingestion. If lesioning the LH only disrupted one of several neural systems responsible for driving ingestion this would account for why drinking was seen in response to dehydration induced by deprivation but not hypertonic saline. In addition to signals concerning loss of body water, water deprived rats may also have been aware that water bottles were missing and even anticipated their return. It was predicted that lesioning the LH resulted in loss of a pathway whereby signals concerning visceral state reached neural structures involved in behavioural responding.

It is the contention of Winn (1995) that the internal milieu is monitored by the paraventricular system. This system, illustrated in Figure 1, incorporates a series of structures, formulated by Lawes on the basis of the anatomical connections of the component structures to the area postrema (Lawes 1994). Not only had the structures to be within two extrinsic synapses of the area postrema, but they had to be connected to at least two other established members of the paraventricular system. Secondary to the anatomical connections, it was found that despite being

distributed throughout the brain, most of the structures were subependymal or subpial, and thus lay adjacent to the cerebrospinal fluid. It was proposed that the paraventricular system was responsible for all antinoxious behaviour including avoidance and expulsion of noxious stimuli, visceral and somatic functions related to them and in addition, other homeostatic functions which may have evolved from antinoxious behaviour.



Telencephalon
1. Central nucleus of the amygdala; 2. Bed nucleus of the stria terminalis; 3. Subfornical organ; 4. Median preoptic nucleus; 6. Vascular organ of lamina terminalis.

Diencephalon
7. Paraventricular nucleus of the thalamus; 8. Periventricular nucleus of the hypothalamus; 9. Paraventricular nucleus of the hypothalamus; 10. Dorsomedial nucleus of the hypothalamus; 11. Arcuate nucleus; 12. Median eminence.

Midbrain
13. Periaqueductal gray matter; 14. Parabrachial nucleus; 15. Dorsal raphe; 16. Dorsal tegmental nucleus; 17. Edinger-Westphal nucleus.

Hindbrain
18. Area postrema; 19. Solitary nucleus; 20. Dorsal vagal nucleus; 21.Nucleus ambiguus; 22. A1, A5; 23. Spinal nucleus of V; 24. Locus ceruleus.

Figure 1. Summary of the component structures of the paraventricular system and their anatomical position in a parasaggital outline of the rat brain reproduced from Lawes (1994) (pg 81).

Afferent Connections of the LH

Although the LH is not included in the paraventricular system as it fails to meet the criterion set by Lawes, it does have anatomical connections with many of its component structures. By means of iontophoretic injections of horseradish peroxidase into the LH, it was found that the central amygdaloid nucleus, the bed nucleus of the stria terminalis, dorsomedial hypothalamus, ventromedial hypothalamus, parabrachial nucleus (PBN), raphe nuclei and locus coeruleus all projected to the LH (Hosoya & Matsushita 1980; Kita & Oomura 1982; Luiten *et al* 1987; Fulwiler & Saper 1984). Thus, since the LH receives extensive input from the paraventricular system it can be inferred that it is in receipt of information regarding the internal milieu. This would include for instance, visceral and gustatory information via the PBN. Further to illustrating anatomical connections between the LH and structures of the paraventricular system, it is important to show that the function of these connections is relevant to the hypothesis proposed by Winn (1995). In this context, it is noteworthy that Kelly & Watts (1998) have indicated that a functional connection between the LH and PBN may exist with regard to water balance. It was found that LH neurones which expressed increased cellular levels of mRNAs encoding the precursor peptides for corticotrophin releasing hormone in response to intracellular dehydration projected to both the medial and lateral subnuclei of the PBN. Although this indicates a functional projection from the PBN to the LH the connections between the PBN and LH have been shown to be reciprocal (Moga *et al.* 1990a; Fulwiler & Saper 1984; Saper & Loewy 1980).

In addition to afferents from the paraventricular system, the studies previously described revealed that the LH received projections from infralimbic cortex, medial prefrontal cortex, lateral and dorsal septal nuclei including the nucleus of diagonal band, nucleus accumbens, olfactory tubercle, medial and lateral preoptic areas, preoptic lateral and medial amygdaloid nuclei, lateral habenular nucleus, peripendicular nucleus, ventral tegmental area, mesencephalic and pontine central gray and ventral nucleus of the lateral lemniscus. In addition using the anterogradely transported lectin *Phaseolus vulgaris leucoagglutin* it was found that the zona incerta projected to the LH (Wagner *et al.* 1995). Of importance is the fact that the LH receives afferents from the limbic system directly from the

infralimbic cortex and amygdala and it also receives afferents from the nucleus accumbens which in turn has projections from the amygdala and hippocampus. It would therefore appear that in addition to signals concerning internal state, motivationally significant signals may reach the LH. Thus in addition to acting as a gateway for signals concerning internal state to reach structures involved in behavioural planning and execution, it may be a locus for making computations of sensory quality of stimuli according to internal state. In support of this is the evidence described previously from Rolls and his colleagues which indicated that LH neurones responded to the sight and taste of food in a manner that was dependent on the deprivation state of the animal and the sensory quality of the food presented (Burton *et al.* 1976; Rolls *et al.* 1986).

In order to act as a gateway for signals concerning internal state the LH would have to project to frontal cortex, the structure generally believed to be responsible for behavioural planning and execution. The general term given to the tasks that the frontal cortex has been shown to be involved in is "executive functions". This describes a number of functions that require complex computations to be made between internal state, environmental stimuli and previous experience, including newly acquired information. One particular function that the prefrontal cortex has been shown to be involved in is spatially based foraging behaviour on a radial arm maze (Seamans *et al.* 1995; Floresco *et al.*1997). The nature of the role of prefrontal cortex in this response has been shown to involve functional connections between hippocampus, prefrontal cortex and nucleus accumbens. In order to complete these tasks efficiently it was necessary to remember which arms of the radial arm maze had been baited previously using spatial cues. However, implicit to this task is the need to recognise the deprivation state under which the rats were tested and the association between the testing apparatus and the provision of food. Elecrophysiological data suggests that the LH is important for the association of food and neutral stimuli (Nakamura & Ono 1986) and examination of the efferent connections of the LH indicate that it is possible that frontostriatal systems gain access to such computations made in the LH.

Efferent Connections of the LH

The LH has ascending and descending connections throughout the brain and medial connections with the hypothalamus including connections between different areas of the LH itself.

Ascending Projections

Saper *et al.* (1979) and Berk & Finkelstein (1982) have traced the efferent connections of the LH using autoradiographic techniques. Included in the ascending projections of the LH were efferents to the medial cortex and cingulate bundle. Saper (1985) replicated these findings and further reported that anterograde tracing using autoradiographic methods revealed two efferent pathways to the cerebral cortex. A medial pathway was demonstrated that ran through the MFB traversed the diagonal band and medial septal nuclei and entered the fornix and cingulate bundle from which it distributed to the hippocampal formation and the medial cortical fields respectively. In addition, a lateral pathway was found that ran through the lateral part of the MFB then turned laterally through the substantia innominata to enter the external capsule from which it distributed to the lateral cortical fields. The LH was also shown to have rostral projections which projected through the MFB into the lateral preoptic area to the medial septal-diagonal band complex and through the stria medullaris to parataenial and paraventricular thalamic nuclei and the lateral habenular nucleus (Saper *et al.* 1979; Berk & Finkelstein 1982). In addition, projections were noted from the LH via the ventral amygdalafugal pathway and via the stria medullaris to the ventral and medial nuclei of the amygdala (Berk & Finkelstein 1982).

Intrahypothalamic Projections

In addition to projecting to other areas within the LH itself, LH neurones project medially terminating in the paraventricular hypothalamic nuclei, posterior hypothalamic area, supramammillary nucleus, dorsomedial hypothalamic nucleus and ventral and dorsal premammillary nuclei (Saper *et al.* 1979; Berk & Finkelstein 1982).

Descending Projections

Berk & Finkelstein (1982) described three descending pathways from the LH to the brainstem.

1. Projections via the periventricular system to the ventral and lateral parts of the midbrain central gray, dorsal raphe nucleus and laterodorsal tegmental nucleus of the pons.
2. Dorsal projections via the central tegmental tract to central tegmental fields and lateral and medial parabrachial nuclei. Some fibres were reported to extend to the ventrolateral pontine and medullary reticular formation to the nucleus of the solitary tract (NTS), dorsal motor nucleus of the vagus and the nucleus commissuralis.
3. From the MFB fibres were found which descended into the ventral tegmental area the median raphe and raphe magnus nuclei.

Thus it appears that the LH has reciprocal connections with cortical areas and there is also evidence that the nucleus accumbens has reciprocal connections with the LH (Zahm & Brog 1992). There is evidence that at least some of the connections between LH and frontostriatal systems are functional. Maldonado-Irizarry *et al.* (1995) demonstrated that blockade of glutamate receptors in the nucleus accumbens induced a pronounced feeding response which could be blocked by concurrent infusion of the $GABA_A$ agonist muscimol into the LH indicating that a functional link may exist between the nucleus accumbens and LH with regard to feeding behaviour.

Hence together this provides evidence that the LH is favourably placed anatomically to act as an interface between the paraventricular system responsible for the monitoring of internal state and frontal cortex responsible for behavioural planning and execution. Furthermore, tests have already been carried out which suggest a functional relationship between the LH and at least some of these areas. The aim of this thesis was to investigate the hypothesis that the LH acts as interface between the paraventricular system and frontostriatal systems and this was investigated by using tests known to be susceptible to damage or change in either system independently.

Chapter 1. The Function of the LH in Relation to Gustatory Processing

One structure that may project information concerning internal state to the cortex via the LH is the PBN. It is located within the dorsolateral pons flanking the brachium conjuctivum and surrounding the superior cerebellar peduncle. The PBN receives afferents concerning both gustatory and visceral signals and it is generally acknowledged that they terminate in a topographical manner with gustatory afferents terminating in the medial PBN and visceral afferents terminating in the lateral PBN (Norgren 1978; Ricardo & Koh 1978; Norgren and Leonard 1973). The PBN has been implicated in the regulation of a diverse array of autonomic processes: for instance, the medial PBN has been implicated in feeding behaviour (Spector *et al.* 1992; Flynn *et al.* 1991a; Flynn *et al.* 1991b; Spector *et al.* 1993) and the lateral PBN has been implicated in fluid balance (Ohman & Johnson 1986; Edwards & Johnson 1991).

Anatomical Evidence of a Projection Between PBN and LH

Moga *et al.* (1990a) reported that the PBN has three primary sources of forebrain input: the cerebral cortex, the hypothalamus and the basal forebrain. Included in the projection of the hypothalamus were afferents originating from two areas of the lateral hypothalamus:

1. a group of cells dorsal and lateral to the fornix which projected to the rostral PBN including the central lateral, superior lateral, extreme lateral and external lateral subnuclei of the lateral PBN, and
2. a group of cells adjacent to the subthalamic nucleus and internal capsule, which in addition to overlapping the projection to the subnuclei aforementioned also projected to the inner zone of the external lateral subnucleus and the medial PBN.

Anterograde autoradiographic studies have shown that both the lateral PBN and medial PBN project to the LH indicating that the projections might be reciprocal. (Fulwiler & Saper 1984; Saper & Loewy 1980). Thus since both medial and lateral PBN have been shown to project to the LH, this could potentially be a relay for both visceral and gustatory information. Furthermore, since the LH has been shown to project to both the medial and lateral PBN, these descending inputs could modulate synapses between the PBN and other structures.

Involvement of the PBN in Feeding Behaviour

In rodents, the medial PBN is an important relay in the central gustatory system (Figure 2). The anatomical connections of the central gustatory system have received much attention and have been described extensively in the literature concerning feeding behaviour (Reilly *et al.* 1993; Flynn *et al.* 1991a; Spector *et al.* 1992). Gustatory afferent information is projected via the facial, glossopharyngeal, and vagus nerves (Hamilton & Norgren 1984) to the NTS and from the NTS axons ascend to the PBN (Norgren & Leonard 1971). The PBN projects to the parvicellular division of the ventral posteromedial thalamic nucleus (VPMpc) (Norgren & Leonard 1973; Nomura *et al.* 1979) an area shown to be involved in gustatory function (Flynn *et al.* 1991b) which in turn projects to the gustatory cortex (Kosar *et al.* 1986). In addition, the PBN also sends axons to other forebrain areas (Saper & Loewy 1980; Fulwiler & Saper) known to be involved in regulation of feeding and drinking including the amygdala (Hatfield & Gallagher 1995), bed nucleus of the stria terminalis (Zardetto-Smith *et al.* 1994) and LH (Clark *et al.* 1991a).

Structures of the central gustatory system also receive afferents concerning visceral state; there are direct projections from the NTS and the lateral PBN to forebrain structures including the bed nucleus of the stria terminalis and the amygdala (Ricardo & Koh 1978; Saper & Loewy 1980). This indicates a potential for gustatory information to be altered according to the internal milieu at various stages of the central gustatory system and not simply at the level of the brainstem.

In theory, signals may be relayed from forebrain structures other than the VPMpc to the cortex. In this way, the LH could modify gustatory signals from the PBN before they reached the cortex. Alternatively, due to the reciprocal connections with the PBN, the LH may modulate synapses between the PBN and other structures of the gustatory system. One of the objectives of this thesis is to study any modulatory role the LH has on behavioural responses mediated by the PBN. The behavioural responses studied were conditioned taste aversion, benzodiazepine induced hyperphagia and taste perception.

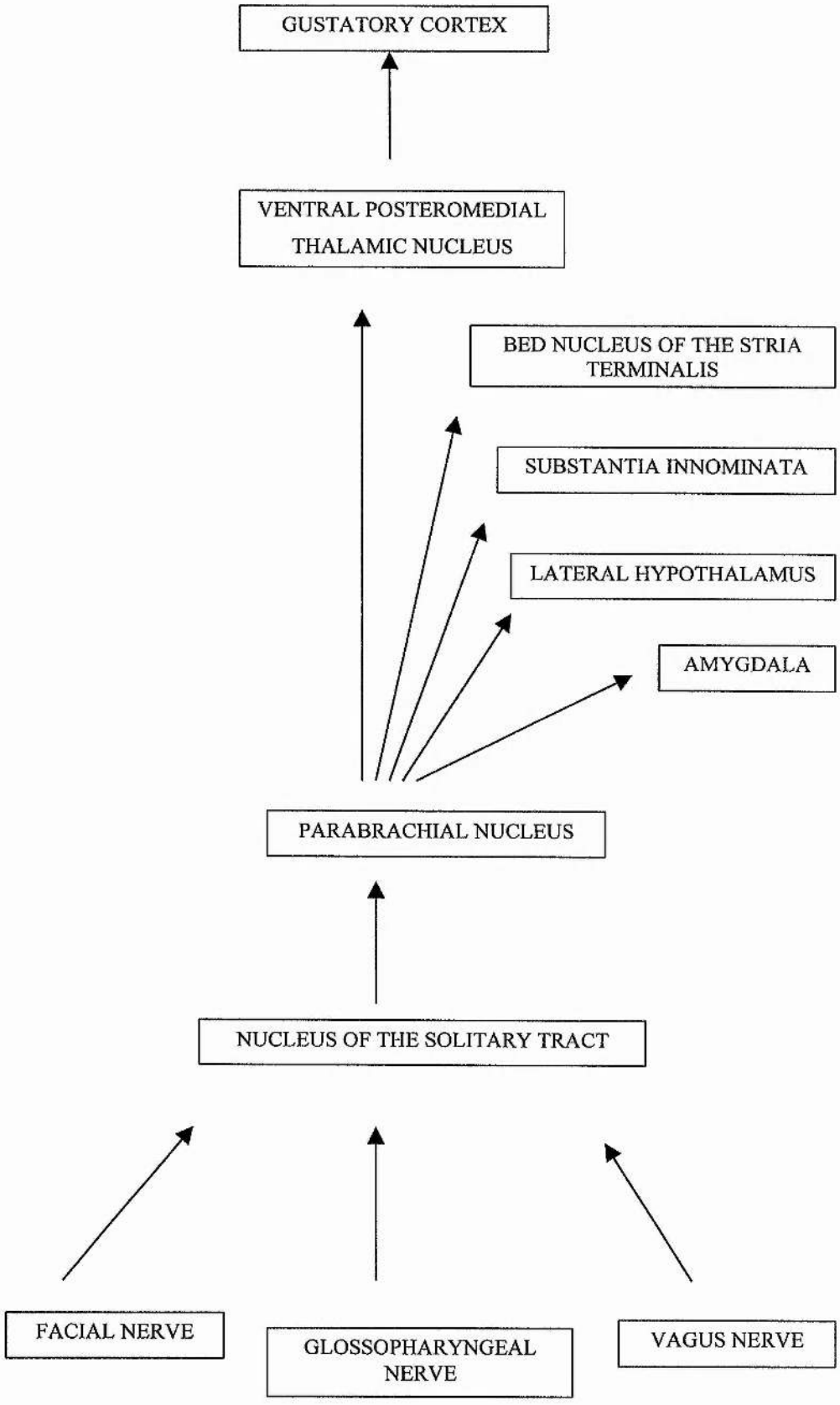


Figure 2. Summary of the central gustatory system in the rat.

CONDITIONED TASTE AVERSION

If ingestion of a substance is followed by internal malaise (including gastrointestinal distress and nausea) an animal will make an association between the taste of the substance and the feeling of malaise, causing it to avoid the substance if later presented with it. This type of behaviour, known as conditioned taste aversion or taste aversion learning, is robust and easily induced under laboratory conditions. Lithium chloride (LiCl) induces malaise when either drunk or injected and is the most common unconditioned stimulus (US) used experimentally to induce conditioned taste aversions. Following exposure to a conditioned stimulus (CS) such as sucrose solution, the animal is injected with the US, LiCl, and on subsequent exposures the animal fails to ingest the CS. In addition to detecting both the taste of the ingested substance and the internal malaise, an association must be made between these which is then used to alter behaviour on further exposures to the CS.

Role of the PBN in Conditioned Taste Aversion Learning

Given the anatomical evidence that the PBN receives afferents concerned with both visceral and gustatory signals it may not be surprising that this region has been implicated in the acquisition of conditioned taste aversions. Many lesion studies have reported the PBN as crucial for the formation of a conditioned taste aversion (Flynn *et al.* 1991a; Reilly *et al.* 1993; Scalera *et al.* 1995). In rats with electrolytic lesions of the PBN, Flynn *et al.* (1991a) showed that when an oral infusion of a CS was paired with an injection of LiCl these animals failed to reject the CS on further exposures to it. Additionally, Bielavska & Bures (1994) implicated the PBN in the mediation of conditioned taste aversion by inactivation studies using tetrodotoxin (TTX). When injection of TTX into the PBN was made after ingestion of the CS and before administration of the US rats failed to acquire a conditioned taste aversion when a variety of US were used (LiCl, carbachol, amphetamine) indicating that the PBN was not simply important for taste aversion learning involving LiCl. However, interpretation of these results is complicated by the non-specific nature of these lesions; electrolytic lesions destroy fibres of passage in addition to intrinsic fibres and TTX, a non-specific sodium channel blocker, blocks transduction of signals in fibres of passage.

Nevertheless, later studies using the excitotoxin ibotenic acid confirmed that these deficits were due to destruction of intrinsic neurones of the PBN. Scalera *et al.* (1995) reported that rats with excitotoxic lesions of the PBN failed to acquire a conditioned taste aversion; after pairing the CS with LiCl, the rats with lesions of the PBN failed to suppress free intake of the CS.

Reilly *et al.* (1993) reported that this deficit was not due to a general inability to process either gustatory or visceral afferent information *per se* by illustrating that rats with PBN lesions could form a conditioned flavour preference and a conditioned place aversion. The ability of rats with lesions of the PBN to use taste cues to form a conditioned flavour preference illustrated that they were not ageusic (i.e. sense of taste was not lost) and the ability to form a conditioned place aversion illustrated that the visceral signals conveying the internal malaise were processed adequately to guide at least some behaviour. Like the conditioned taste aversion procedure, in these tests it was necessary to associate at least two different cues in order to alter behaviour: conditioned flavour preference required association of the conditioned taste cue with either post-ingestional factors or the taste of the unconditioned solution it was paired with and conditioned place aversion required association of the visceral malaise with a spatial cue. Since PBN lesioned rats failed to acquire a conditioned taste aversion but acquired a conditioned flavour preference and a conditioned place aversion it would appear that the association of different external and internal cues have different specific loci. Hence, the deficit seen with PBN lesioned rats appears not only to be an associative deficit as opposed to a sensory deficit but the associative deficit appears to be exclusive for the acquisition of a conditioned taste aversion i.e. the association of the gustatory signal with the visceral malaise.

Evidence gained from two different methods suggests that the PBN may be the site of association of the CS and US. Hermann & Rogers (1985) provided anatomical evidence that the PBN could be an important site for the integration of visceral and gustatory input by illustrating that despite the general topographic separation of gustatory and visceral input to the PBN, both hepatic and gustatory divisions of the NTS sent efferents to a confined region of the PBN at a posterior

site suggesting convergence of hepatic-vagal and gustatory afferents. Yamamoto *et al.* (1994) used a different approach, studying the neural activity in the PBN during the different stages of the conditioned taste aversion procedure by means of c-Fos immunohistochemistry. Activation of neurones results in increases in the activity of the c-Fos gene with a concomitant increase in the protein product c-Fos. Because levels of this protein are normally low, its presence can be used as an indicator of neural activity. Yamamoto *et al.* (1994) illustrated that specific subnuclei in the PBN were concerned with palatable and aversive tastes and that the recipient zone for a taste in the PBN was altered by taste aversion learning. Although this alteration in hedonic value occurred even after large ibotenic lesions of the gustatory cortex, VPMpc and amygdala, it was found that aversive and avoidance behaviour could not be expressed without these higher systems in the CNS. This indicates that despite the apparently crucial role the PBN has in the acquisition of a conditioned taste aversion it may not be sufficient for behavioural expression.

Role of the Forebrain Conditioned Taste Aversion Learning

The involvement of the ventral forebrain in the acquisition of conditioned taste aversions has been investigated by various researchers using lesion studies but the results are equivocal. The VPMpc and amygdala have been reported to be crucial in acquisition of conditioned taste aversion by some authors and unnecessary by others. Within these studies are many variations in experimental protocol including method of producing lesions, size of lesions and the way in which acquisition of a taste aversion was tested. Scalera *et al.* (1997) and Dunn & Everitt, (1988) have addressed this disparity in results.

Dunn & Everitt (1988) reported that while electrolytic lesions of the amygdala did disrupt learning of a conditioned taste aversion, ibotenic acid lesions of the amygdala did not. After illustrating that ibotenic acid lesions of the insular cortex disrupted conditioned taste aversion learning, it was concluded that the deficit seen with electrolytic lesions of the amygdala was due to damage to axons traversing the amygdala. It has been demonstrated that projections from the PBN, known to be crucial for the acquisition of conditioned taste aversions, to insular cortex traverse the amygdala (Saper & Loewy 1989) and thus it is

possible that disruption to such fibres would have disrupted taste aversion learning.

Scalera *et al.* (1997) studied the disparity in results of lesion studies of the VPMpc which had shown that large but not small electrolytic lesions of the VPMpc disrupted taste aversion learning by producing extensive ibotenic acid lesions of this structure. These rats were shown to learn a taste aversion in a single trial and concluded that the critical difference between rats with small as opposed to large electrolytic lesions of the VPMpc with regard to acquisition of a conditioned taste aversion was the incomplete destruction of axons of passage rather than incomplete damage of intrinsic cells of the VPMpc.

Thus the use of more specific lesion methods lead to the conclusion that the amygdala and VPMpc were not involved in taste aversion learning and that electrolytic lesions of either area destroyed fibres projecting from PBN to the forebrain. Yamamoto (1993) has reported deficits in conditioned taste aversion learning after ibotenic lesions of VPMpc and amygdala but without more detailed accounts of methods or histology these are difficult to interpret.

Role of Cortex in Conditioned Taste Aversion Learning.

Other experimental procedures in addition to the lesion study by Dunn & Everitt, (1988) have implicated gustatory cortex in conditioned taste aversion learning. Microinjections into the cortex which result in the transient disruption of cortical function have been shown to block conditioned taste aversion learning implicating both cholinergic and glutamate transmission in the processing of a taste aversion:

1. The muscarinic antagonist scopolamine blocked conditioned taste aversion learning when injected into insular cortex before exposure to the novel CS (Naor & Dudai 1996)
2. Injection of the NMDA receptor antagonist AP5 into insular cortex during conditioned taste aversion training blocked acquisition of a conditioned taste aversion (Rosenblum *et al.* 1997).

Chronic hemi-decerebrate rats which had a unilateral brain transection at the level of the superior colliculus were reported to acquire a conditioned taste aversion indicating that unilateral connections between the forebrain and brainstem were sufficient to support acquisition and expression of a conditioned taste aversion (Schafe *et al.* 1995). This technique also allowed the comparison of neural activity within the brainstem with and without connections to the forebrain. It was demonstrated that after a CS was paired with LiCl, the presentation of the CS induced a different pattern of c-Fos to that induced by the unpaired CS or even an innately aversive stimuli but which was similar to that induced by LiCl (Swank & Bernstein 1994; Swank *et al.* 1995). Examination of the NTS of the hemi-decerebrate rat revealed that the conditioned pattern of c-Fos expression to the CS was evident only on the side of the brain which retained neural connections with the forebrain (Schafe *et al.* 1995). This evidence supports the theory that the forebrain is essential for the acquisition and expression of a conditioned taste aversion.

Thus, the mechanism and the structures involved in acquisition of a conditioned taste aversion remain unclear. Nonetheless, it is evident that the PBN has a fundamental role in this behavioural paradigm and that input from higher centres is required. Given the anatomical connections between the PBN and the LH it is possible that the LH operates in the some way to facilitate conditioned taste aversion learning. Previous studies have produced conflicting evidence as to whether or not the LH is involved; microinjections of TTX and the specific D2 dopaminergic antagonist SCH 23390 into the LH have blocked acquisition of a conditioned taste aversion (Caulliez *et al.*1996) indicating that transient disruption of the LH is sufficient to block acquisition of a taste aversion, while ibotenic acid lesions of LH have been shown to have no effect on taste aversion learning (Yamamoto, 1993). Problems arise in interpreting this lesion study. It has been reported that ibotenic acid injected into the LH resulted in damage of the amygdala (Winn *et al.* 1984; Hastings *et al.* 1985), a region known to be involved in taste guided behaviour (Zardetto-Smith *et al.* 1994). Without a more detailed report on the formation of the lesions and histological analysis of them, it is difficult to determine the implications of them.

The present experiment was conducted in an endeavour to clarify the involvement of the LH in the acquisition of a conditioned taste aversion by using the more specific toxin NMDA to lesion the LH. If lesioning the LH inhibited behaviour mediated by the PBN then this would provide evidence that the LH does at least in part function as a gateway for information to reach the cortex.

Experiment 1.1: The Role of the LH in Conditioned Taste Aversion Learning

METHODS

Animals

Forty-eight male Lister Hooded rats (Charles River) were individually housed in grid bottomed cages under a 12h light/dark cycle. Animals had *ad lib* food (SDS maintenance diet no. 1 chow pellets) and tap water in their home cages except when indicated. Daily measurements were taken of body weight, water remaining, food remaining in the food hopper, and spillage (collected on foil sheets beneath the food hoppers). Twenty-four animals with an average pre-surgery weight of 366.1g (SD 16.42) were assigned to the sham lesioned group and 24 animals with an average pre-surgery weight of 365.8g (SD 16.32) were assigned to the LH lesioned group

Surgery

Animals were anaesthetised by i.p. injection of 10ml/kg Avertin (10g tri-bromo-ethanol, 5g tertiary amyl-alcohol, 500ml 0.01M phosphate buffer, 4.5g sodium chloride). Since avertin can cause the intestines to adhere to the abdominal muscle wall, thereby preventing passage of faecal matter (producing a syndrome known as "bloat"), the injection of avertin was followed by an i.p. injection of 6.3% glucose saline solution (10ml/kg), a procedure which has previously been thought to have some preventative power against this. The rats were placed in a stereotaxic frame with the incisor bar 5.0mm above the interaural line. Animals received a simultaneous bilateral infusion of either 1µl 0.06M NMDA or 1µl phosphate buffer. NMDA (Sigma) was dissolved in 0.1M phosphate buffer (pH 7.4) and pH adjusted to pH 7.4 with 0.2M sodium hydroxide NaOH. The infusion rate was 0.5µl/min with a further 2min period allowed for diffusion. The co-ordinates for cannulae were anterior-posterior 0mm from bregma; medial-lateral ±2.0mm from midline; ventral 8.0mm from dura.

Post-Operative Care

One LH lesioned rat died shortly after surgery. Body weight, food intake and water intake for the remaining rats were measured for 28 days after surgery. If body weight fell below 85% pre-surgery weight, these individual rats were given wet mash (Farley's baby food and glucose) which was changed daily and removed if the animals body weight exceeded 85% of its pre-operative weight. Eighteen of the LH lesioned rats required wet mash with 8 only requiring it for a maximum of 2 days and 9 requiring it for a maximum of 12 days. Four rats were found to be capable of maintaining their reduced body weight without further dietary supplements resulting in wet mash being removed before body weight returned to 85% of pre-surgery levels. One LH lesioned rat was found to be unable to maintain its weight without dietary supplements and was terminated.

Hypertonic Saline Challenge

Eighty-eight days after surgery all rats received i.p. injections of hypertonic saline (5%, 20ml/kg) and isotonic saline (0.9%, 20ml/kg), with 48h between injections. Injections were given in a counterbalanced order and tap water consumed in the home cage 1h and 3h after injection was measured.

Conditioned Taste Aversion

An experimental and control group were matched for body weight and food and water intake (Table 1) for both the LH lesioned rats and the sham lesioned rats 70 days after surgery.

	LH lesion		Sham lesion	
	Experimental	Control	Experimental	Control
Body weight (g)	365.8 (±13.7)	363.7 (±13.14)	500.1 (±13.92)	492.2 (±7.25)
Water intake (ml)	28. 20 (±3.64)	29.0 (±3.50)	27.1 (±0.82)	27.9 (±0.68)
Food intake (g)	23.50 (±0.95)	23.3 (±1.12)	30.2 (±0.71)	30.1 (±0.42)
N	n = 11	n = 10	n = 12	n = 12

Table 1. Mean (± SE) body weight and food and water intake for sham lesioned and LH lesioned experimental and control groups 70 days after surgery. Experimental groups are defined as rats treated with LiCl and control groups are defined as rats treated with saline, irrespective of lesion type.

The rats were put on a 19h water deprivation schedule 3 days before the conditioning day with water available in the home cage daily during the same 5h period within the light phase. On the conditioning day the rats received a novel 0.15% saccharin solution (Sweetex ® sodium saccharin tablets) for 30min in a novel test cage in a novel room. The amount of fluid drunk was measured to the nearest 0.1ml. No other fluid was available on the conditioning day and the animals remained on the 19h water deprivation schedule. Immediately after the 30min drinking period the experimental rats received an i.p. injection of 0.15M LiCl (20ml/kg) and the control rats received an equal quantity of the vehicle alone (isotonic saline). Injections were administered in a third room (not the home cage or CTA room) and the order in which rats received injections was counterbalanced. The animal's behaviour was then rated once a minute for 25min in locomotor cages. The behaviours rated were still, locomotion, rear, sniff, fore-paw groom and other groom using the methods and criteria of Fray *et al.* (1980). In addition "lying on belly" and "doggy scratch" were rated (defined as lying prostrate on belly with no detectable movement and scratching of the body or head with hind limbs respectively); each had to be present for more than 3sec to be scored.

Three days after conditioning the rats were given a 2-bottle test (one of saccharin solution, one of tap water) to test for saccharin preference. Both saccharin and water were introduced separately and, after both fluids had been sampled, rats were given access to both for 30min. Half the rats received saccharin on the side they received it on the conditioning day and half on the opposite side and the amount of water and saccharin consumed was measured at the end of the test period.

Histological Analysis

At the end of this study the rats were used to study the role of the LH in benzodiazepine induced hyperphagia prior to processing tissue for histological analysis; procedures used to analyse the lesioned tissue and the results of the histological analysis are described in Experiment 1.2; "Benzodiazepine induced hyperphagia".

Statistical Analysis

The results were examined statistically using analysis of variance (ANOVA) with, when appropriate Tukey's post-hoc tests. Due to the substantial group (sham lesion/LH lesion) differences with regard to body weight, all measures of fluid consumed during the conditioned taste aversion procedure were expressed as volume drunk with respect to body weight [(ml/g bw)*100]. The body weight values used for this calculation with respect to the conditioning day and the 2-bottle test were those measured on the conditioning day. Although the 2-bottle test was performed 3 days after the conditioning day, repeated measures ANOVA of body weight with condition (LiCl/NaCl) and group (sham lesion/LH lesion) as between subjects factors and days (4 days before, until 4 days after the conditioning day) as the within subjects factors revealed no condition × group interaction ($F(2,82) = 23.37$) and no group × days interaction ($F(2,82) = 0.29$) indicating that any change in body weight that occurred was equivalent for all the groups.

RESULTS

Histology

Analysis of the histology revealed that all the rats in the LH lesioned group had complete lesions of the LH and thus were all included in the further analysis. The extent of the lesioned tissue is described fully in Experiment 1.2; "Benzodiazepine induced hyperphagia" (pg 72).

Regulatory Behaviour

Body weight and food and water intake before and after surgery are shown in Figures 3, 4, and 5. In the week preceding surgery there was no difference between the LH lesioned and sham lesioned groups in respect of body weight ($F(1,45) = 0.00$), food intake ($F(1,45) = 0.00$) or water intake ($F(1,45) = 0.31$). Since the rats were still increasing in size there was of course a significant main effect of days for body weight, ($F(7,315) = 523.10$, $p<0.001$). In addition food and water intake were not constant before surgery [main effects of days: food intake ($F(7,315) = 3.06$, $p<0.004$); water intake ($F(7,315) = 2.38$, $p<0.022$)] but nevertheless, there were no significant interactions between groups and days for body weight ($F(7,315) = 1.87$), food intake ($F(7,315) = 0.81$) or water intake ($F(7,315) = 0.97$) indicating that changes in each factor were comparable between the sham lesioned and LH lesioned groups.

Following surgery, the LH lesioned rats lost weight and their body weight remained lower than the sham lesioned rats [main effect of group ($F(1,45) = 88.41$ $p<0.001$)] for the duration of the study. Food and water intake for the LH lesioned rats were also reduced compared to the sham lesioned rats [main effect of group: food intake ($F(1,45) = 74.89$, $p<0.001$); water intake ($F(1,45) = 72.75$, $p<0.001$)]. There were main effects of days with respect to body weight ($F(27,1215) = 131.63$ $p<0.001$), water intake ($F(27,1215) = 23.68$ $p<0.001$) and food intake ($F(27,1215) = 50.86$ $p<0.001$) as both groups recovered from surgery but statistically significant interactions were also found in respect of each factor [body weight ($F(20,900) = 46.79$ $p<0.001$); food intake ($F(20,900) = 6.75$ $p<0.001$); water intake ($F(20,900) = 7.70$ $p<0.001$)] indicating that the groups followed a different pattern of recovery. It is clear from Figures 3, 4 and 5 that

the sham lesioned group made a more rapid and complete recovery than the LH lesioned group.

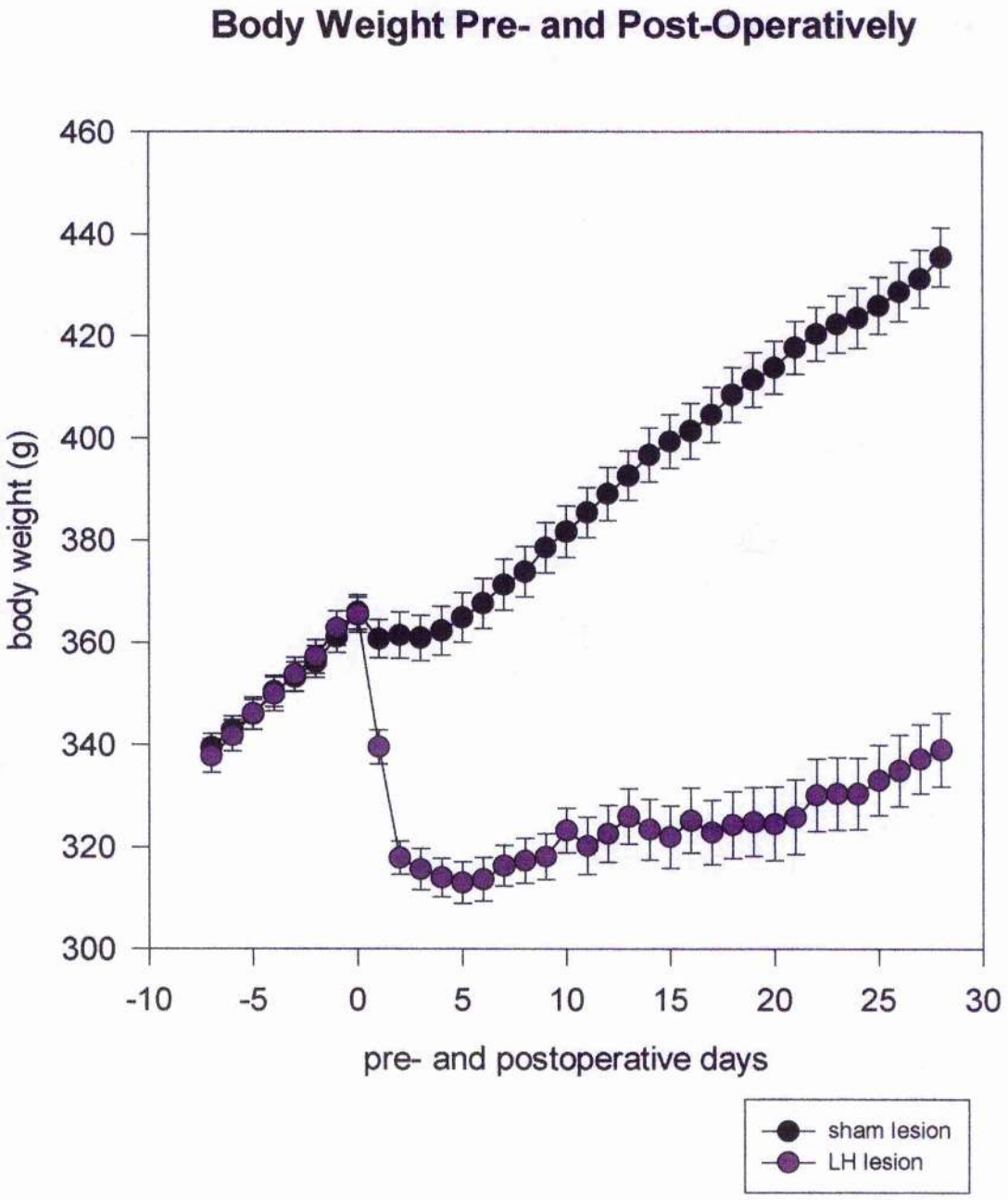


Figure 3. Mean (± SE) body weight of the sham lesioned group (n=24) and LH lesioned group (n=23) 7days before and 28 days after surgery.

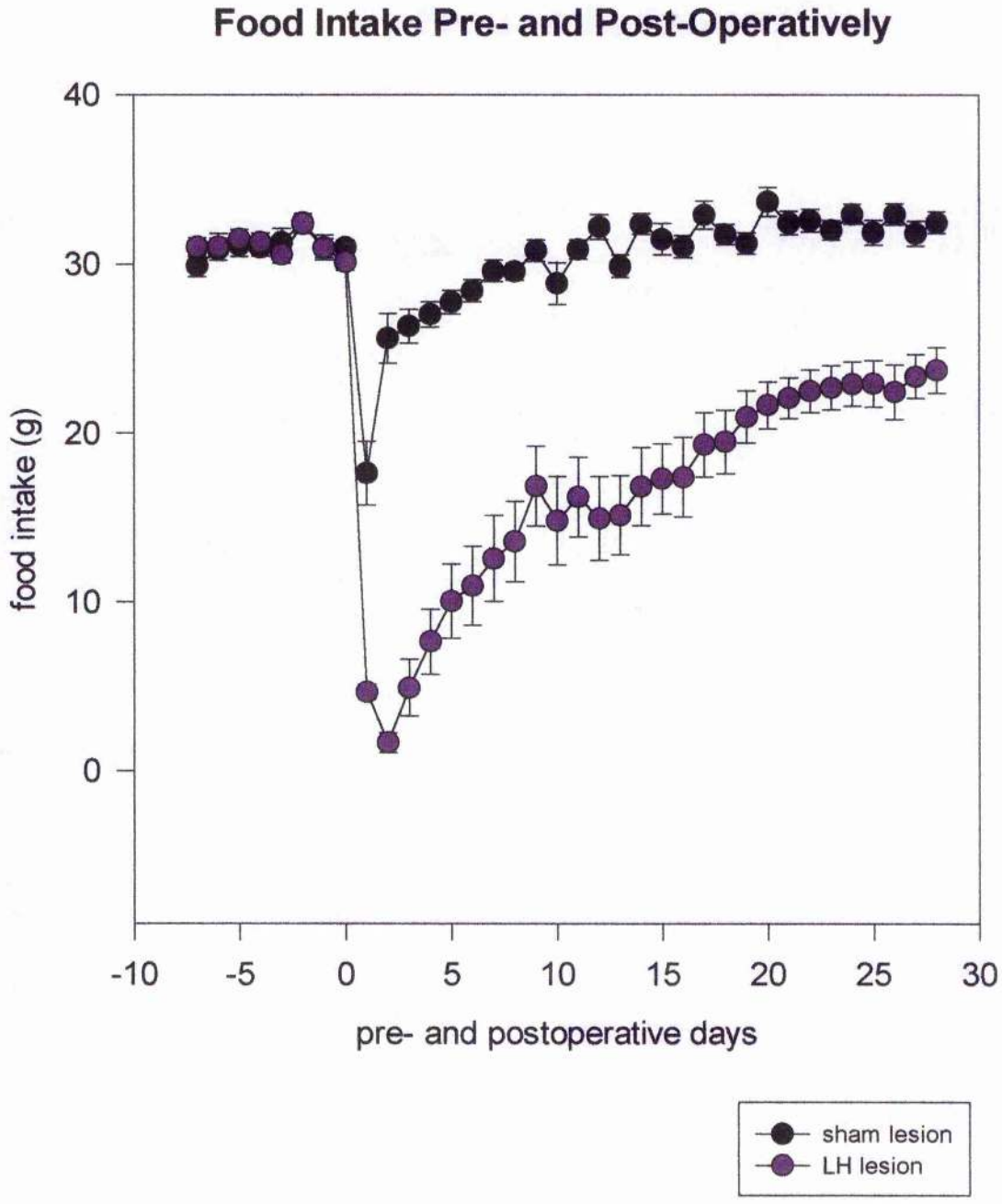


Figure 4. Mean (± SE) food intake for the sham lesioned group (n=24) and LH lesioned group (n=23) 7 days before and 28 days after surgery.

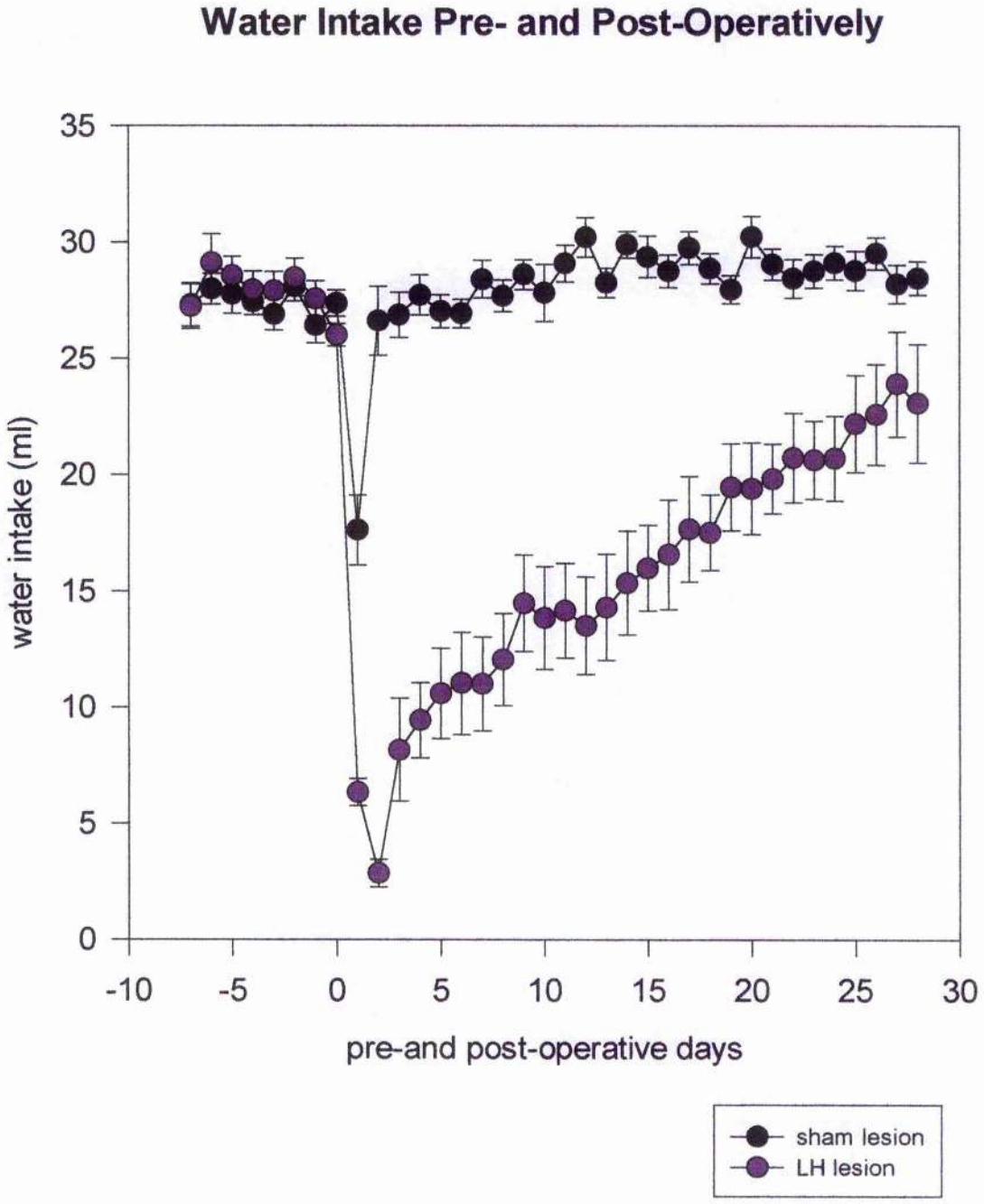


Figure 5. Mean (± SE) water intake for the sham lesioned group (n=24) and LH lesioned group (n=23) 7 days before and 28 days after surgery.

Figures 6 and 7 present food intake and water intake plotted as a function of body weight, and give a clearer indication of the recovery made by the LH lesioned rats. ANOVA of the data before surgery showed no main effect of group for food intake ($F(1,45) = 0.02$) or water intake ($F(1,45) = 0.14$) and no group × days interaction for food intake ($F(7,315) = 0.70$) or water intake ($F(7,315) = 0.93$). After surgery however there were significant main effects of group for both food intake ($F(1,45) = 43.17$ $p<0.001$) and water intake ($F(1,45) = 23.47$ $p<0.001$) and a significant group × days interaction for each factor [food intake ($F(27,1215) = 17.52$ $p<0.001$); water intake ($F(27,1215) = 13.95$ $p<0.001$)]. It is clear from Figures 6 and 7 that after an initial deficit which was greater than that seen with the sham lesioned rats, the LH lesioned rats recovered a normal relationship between body weight and food intake and water intake over the first three weeks after surgery. Tukey's post-hoc tests showed that there were significant differences between the groups with respect to food intake ($p<0.003$ at least) until post-surgery day 18 and water intake ($p<0.05$ at least) until post-surgery day 22, after which the groups no longer differed in respect to food or water intake as a function of body weight.

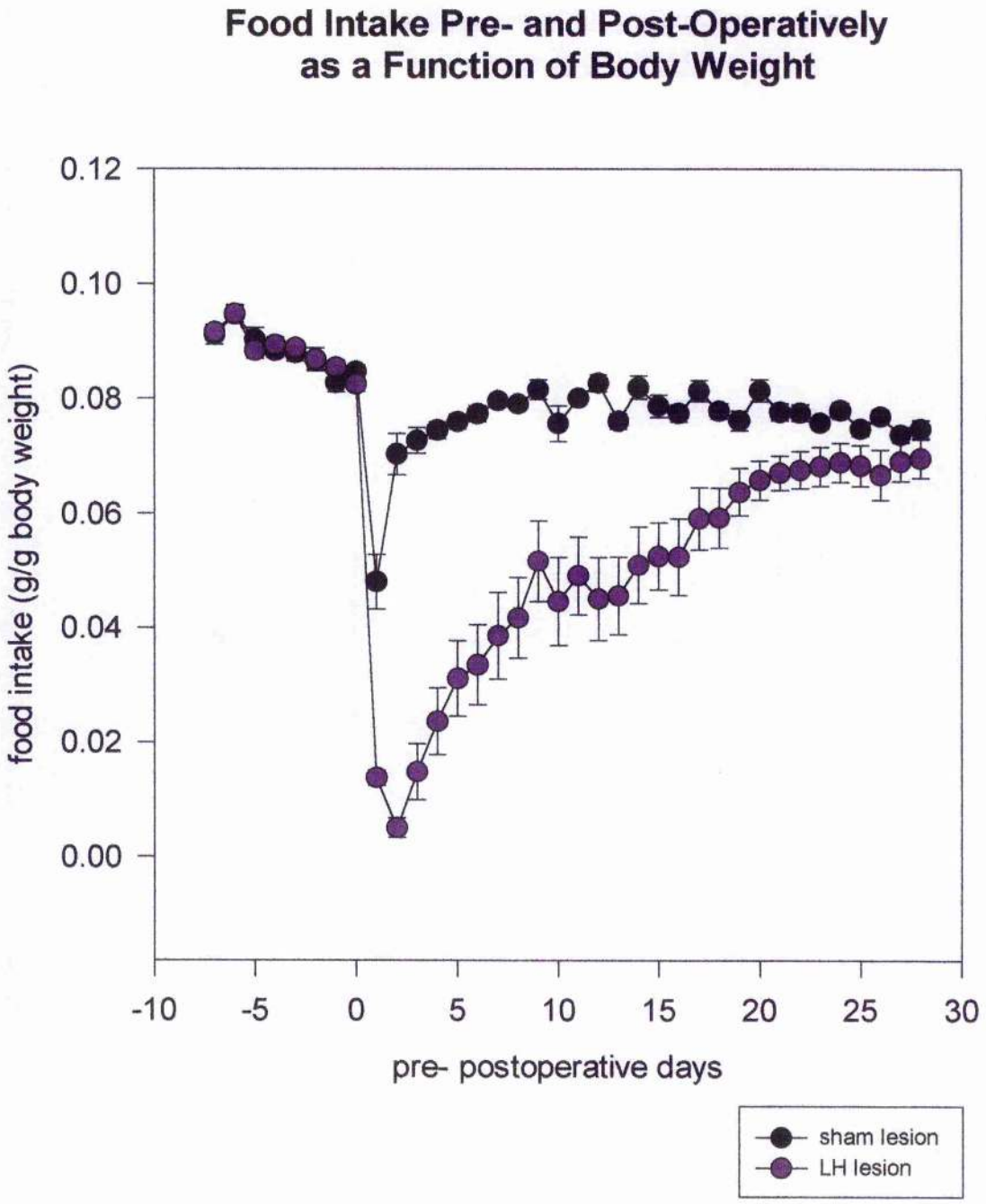


Figure 6. Mean (± SE) food intake as a function of body weight for the sham lesioned group (n=24) and LH lesioned group (n=23) 7 days before and 28 days after surgery.

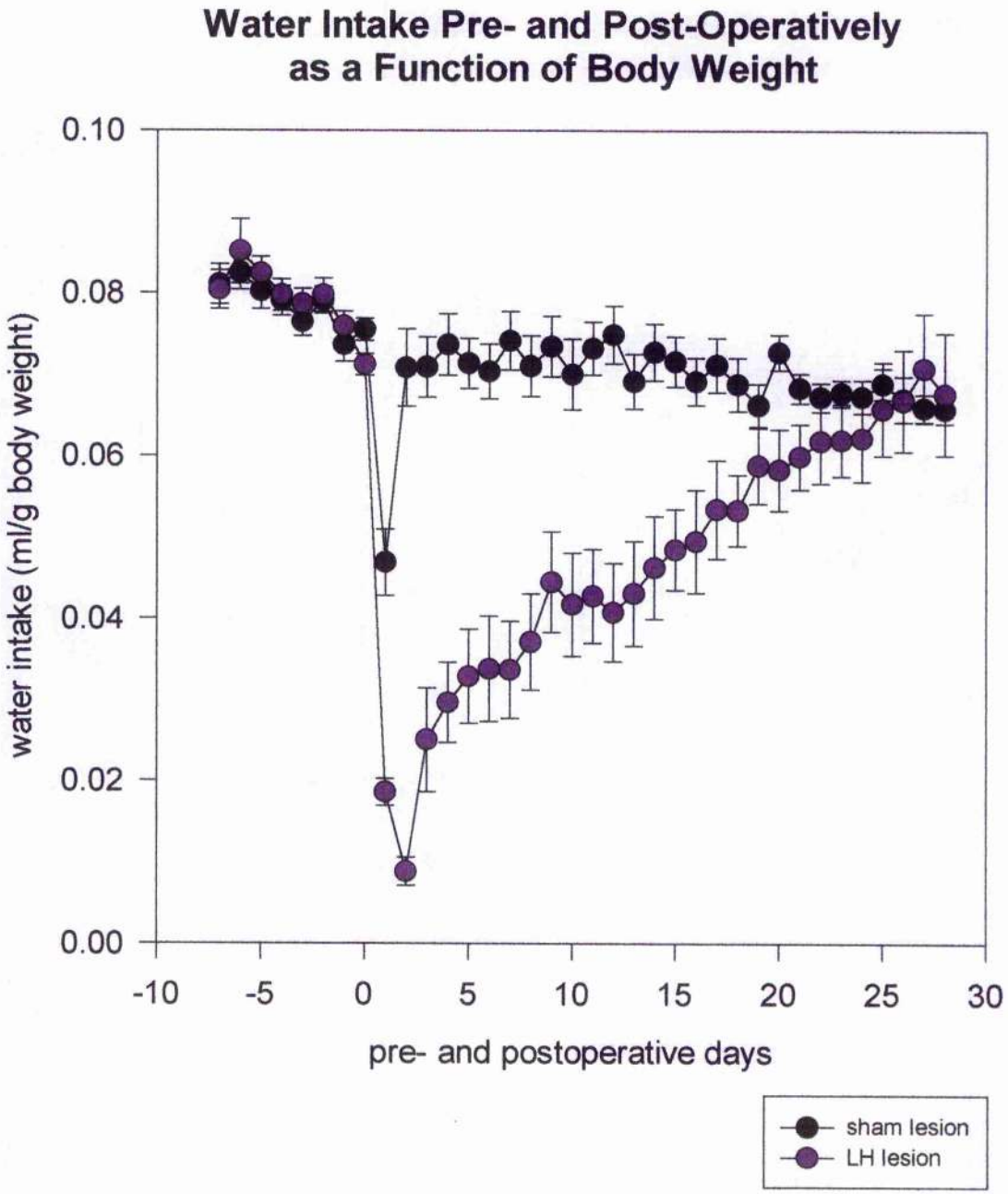


Figure 7. Mean (± SE) volume of water ingested as a function of body weight by the sham lesioned group (n=24) and LH lesioned group (n=23) 7 days before and 28 days after surgery.

Hypertonic Saline Challenge

The volumes of water drunk in 3h after i.p. injection of 20ml/kg hypertonic saline (5%) and isotonic saline (0.9%) are shown in Figure 8. ANOVA of these data showed a significant main effect of injection ($F(1,43) = 88.71$, $p<0.001$) indicating that hypertonic saline induced a greater degree of drinking than isotonic saline. However a main effect of group ($F(1,43) = 44.58$ $p<0.001$) and a significant group × injection interaction ($F(1,43) = 34.43$, $p<0.001$) suggest that the increase in drinking induced was not the same for the sham lesioned and LH lesioned groups. Tukey's post-hoc tests revealed that there were no significant differences in the amount drunk by LH lesioned and sham lesioned groups after isotonic saline. Although both the sham lesioned and LH lesioned groups did drink more after hypertonic compared to isotonic saline (sham lesioned, $p<0.001$; LH lesioned, $p<0.002$), the volume drunk by the sham lesioned group was significantly greater than that drunk by the LH lesioned group ($p<0.001$). These data indicate that, although the LH lesioned group did show an increase in drinking after hypertonic saline injection, this response was very significantly blunted in comparison to the sham lesioned group.

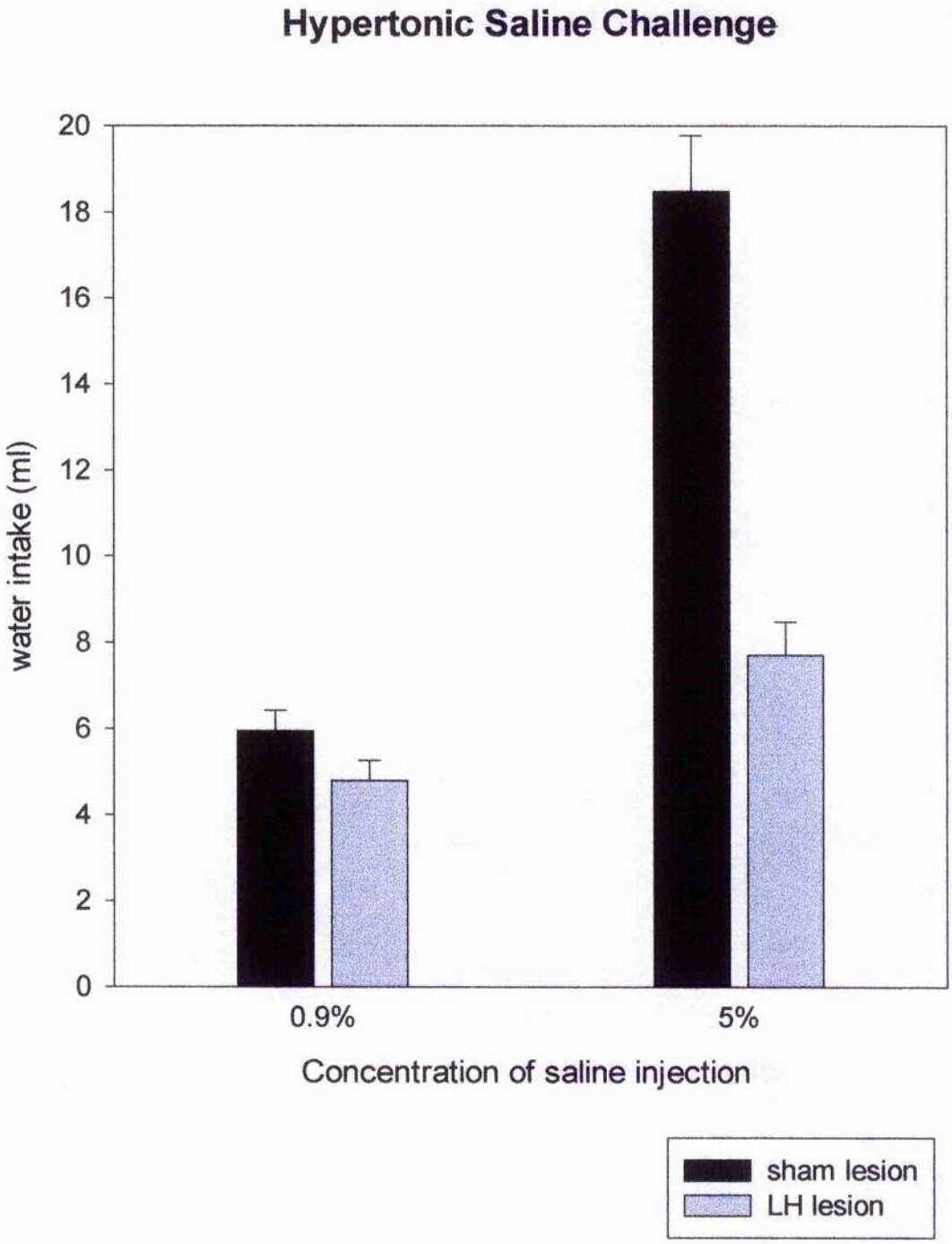


Figure 8. Mean (± SE) volume of water consumed in 3hr following i.p. injections of hypertonic (5%) and isotonic (0.9%) saline for the sham lesioned group (n=24) and the LH lesioned group (n=21).

Conditioned Taste Aversion

Behavioural responses to LiCl injection

	Experimental groups (LiCl)		Control groups (NaCl)	
	Sham lesion	LH lesion	Sham lesion	LH lesion
Still	4.25 (±0.84)	2.80 (±0.84)	3.58 (±0.69)	1.3 (±0.47)
Locomotion	3.08 (±0.67)	3.36 (±1.05)	8.08 (±0.60)	8.4 (±0.90)
Rear	2.50 (±0.67)	3.45 (±1.50)	5.00 (±0.60)	8.6 (±1.59)
Sniff	7.67 (±1.53)	4.55 (±1.58)	10.83 (±1.04)	13.5 (±1.56)
Lying on belly	9.92 (±2.62)	13.00 (±3.30)	0.08 (±0.08)	0.1 (±0.10)
Doggy-scratch	0.25 (±0.13)	1.00 (±0.49)	1.75 (±0.49)	2.1 (±0.71)
Fore-paw groom	1.83 (±0.63)	2.18 (±0.69)	3.58 (±0.62)	3.7 (±0.75)
Other groom	0.92 (±0.31)	0.64 (±0.31)	1.83 (±0.46)	1.3 (±0.47)
	n =12	n = 11	n = 12	n = 10

Table 2. Mean (± SE) ratings of behaviour elicited by LH lesioned and sham lesioned rats after injection of either LiCl or NaCl. Behaviour was rated once per minute for 25min. Experimental groups are defined as sham lesioned or LH lesioned rats treated with i.p. LiCl during the conditoned taste aversion procedure and control groups are defined as sham lesioned or LH lesioned rats treated with i.p. NaCl during the conditioned taste aversion procedure.

The frequency of the behaviours shown after injection of either LiCl or NaCl are illustrated in Table 2 expressed as the average number of times the behaviour was rated in the test period. These were analysed using multivariate ANOVA with conditioning (LiCl/NaCl) and group (sham/LH lesion) as independent factors and rated behaviours as dependent factors; details of the analysis are included in the appendix (pg 264). Analysis revealed no group × condition interactions indicating that any differences seen between LiCl and NaCl conditioned rats was similar for the sham lesioned and LH lesioned groups. The only behaviours showing significant main effects of group were still and rear. There were however significant main effects of conditioning for all behaviours rated except still. The important thing to note is that "lying on belly" was the only behaviour rated significantly more for the LiCl treated rats than the control rats. All other behaviours except still were rated more for the control groups

than the experimental groups. Thus the predominate response in every case for the LiCl treated groups was for the rat to lie flat on its belly, with other behaviours effectively suppressed. In contrast lying on belly was only rated twice for the control rats. These results indicate that lesioning the LH does not prevent the feeling of malaise induced by i.p. LiCl.

There is disagreement over what this particular data is classed as, whether it is parametric data or frequency counts. If they were frequency counts then ANOVA would not be the relevant analysis to use. However in this particular case, the important point to be gained is from one particular behaviour rated, "lying on belly". In this case, a group difference is evident from the mean values.

Preference Tests

	Experimental groups		Control groups	
	Sham lesion	LH lesion	Sham lesion	LH lesion
Pre-conditioning				
Saccharin	2.14 (±0.16)	2.50 (±0.26)	1.90 (±0.20)	2.81 (±0.32)
Post-conditioning				
Saccharin	0.20 (±0.03)	0.46 (±0.14)	2.71 (±0.26)	3.45 (±0.33)
Water	1.92 (±0.10)	1.84 (±0.22)	0.76 (±0.13)	0.47 (±0.06)
n	n=12	n=11	n=12	n=10

Table 3. Mean (± SE) volume of saccharin consumed before conditioning and the volume of water and saccharin consumed in the two-bottle test after conditioning [(ml/g body weight)×100]. Experimental groups are defined as sham lesioned or LH lesioned rats treated with i.p. LiCl during the conditoned taste aversion procedure and control groups are defined as sham lesioned or LH lesioned rats treated with i.p. NaCl during the conditioned taste aversion procedure.

The amounts of saccharin solution and water consumed in the post-conditioning 2-bottle test and the amount of saccharin solution consumed before conditioning were expressed as a function of body weight (×100) for purposes of analysis and are shown in Table 3. Figure 9 illustrates the volumes of saccharin consumed before and after conditioning with the US.

The amount of saccharin drunk before conditioning with the US was analysed using general ANOVA with group (sham lesion/LH lesion) and condition (LiCl/NaCl) as between subjects factors and volume of saccharin drunk as the within subjects factor. There was a main effect of group ($F(1,41) = 7.25$ $p<0.010$) but no main effect of condition ($F(1,41) = 0.02$) and no condition × group interaction ($F(1,41) = 1.33$). Thus, on first exposure to saccharin the LH lesioned rats expressed a significantly higher preference for saccharin with respect to volume drunk (but the rats within each group (sham lesion/LH lesion) destined to different US did not differ from each other).

The 2-bottle test was analysed using multivariate ANOVA with saccharin and water as dependent factors and group (sham/LH lesion) and condition

(LiCl/NaCl) as independent factors. Analysis showed that the LH lesioned group consumed more saccharin than the sham lesioned group ($F(1,41) = 5.44$ $p<0.025$) but there was no difference between the groups in respect of water consumed ($F(1,41) = 1.75$). There were main effects of condition for saccharin ($F(1,41) = 1.64$ $p<0.001$) and water ($F(1,41) = 80.68$ $p<0.001$) indicating that conditioning with LiCl did inhibit ingestion of saccharin. Although the LH lesioned group drank more saccharin than the sham lesioned group, analysis revealed no significant group × condition interaction for either saccharin ($F(1,41) = 1.25$) or water ($F(1,41) = 0.56$) indicating that the inhibition of saccharin consumption was comparable between the sham lesioned and LH lesioned groups. Figure 9, although not accounting for the volume of water consumed during the 2-bottle test, compares saccharin consumed before and after conditioning. Despite the elevated saccharin consumption shown by the LH lesioned rats, the changes in intake after conditioning appear to mirror those of the sham lesioned rats.

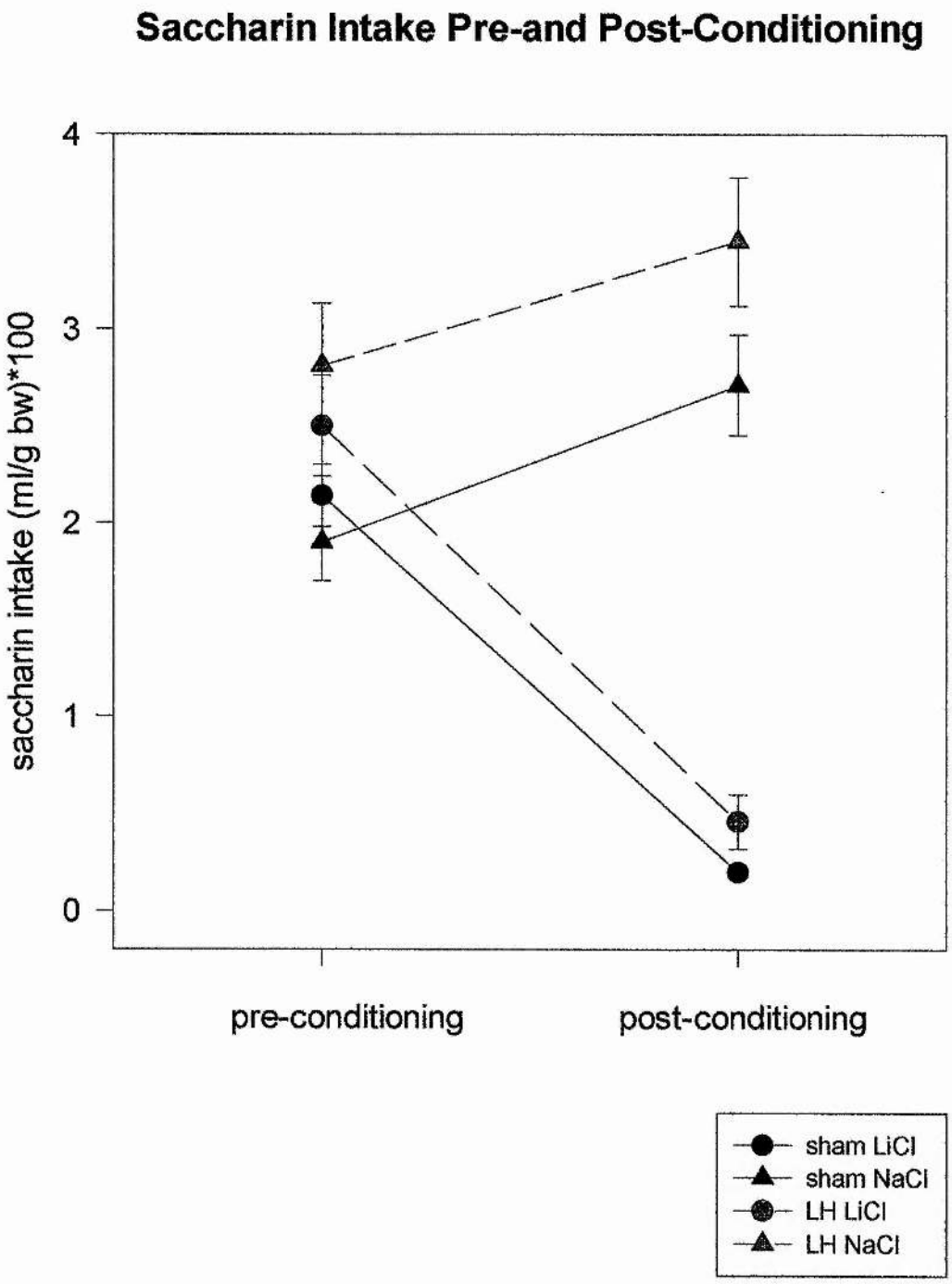


Figure 9. Mean (± SE) volume of saccharin drunk before and after conditioning for sham lesioned rats conditioned with either LiCl (n=12) or NaCl (n=12) and LH lesioned rats conditioned with either LiCl (n=11) or NaCl (n=10).

Saccharin Preference Scores

Figure 10 represents saccharin preference scores calculated as (saccharin consumed/saccharin + water consumed) where a preference of 1 represents the highest preference for saccharin possible and 0 represents complete aversion for saccharin. ANOVA of these data showed significant main effects of group (LH lesioned/sham lesioned) ($F(1,41) = 6.49$ $p<0.015$) and treatment (LiCl/NaCl) ($F(1,41) = 236.05$ $p<0.001$) but there was no significant group × treatment interaction ($F(1,41) = 0.04$). This analysis shows that LiCl effectively suppressed the preference for saccharin in all rats, but also that LH lesioned rats had significantly greater preferences for saccharin than sham lesioned rats.

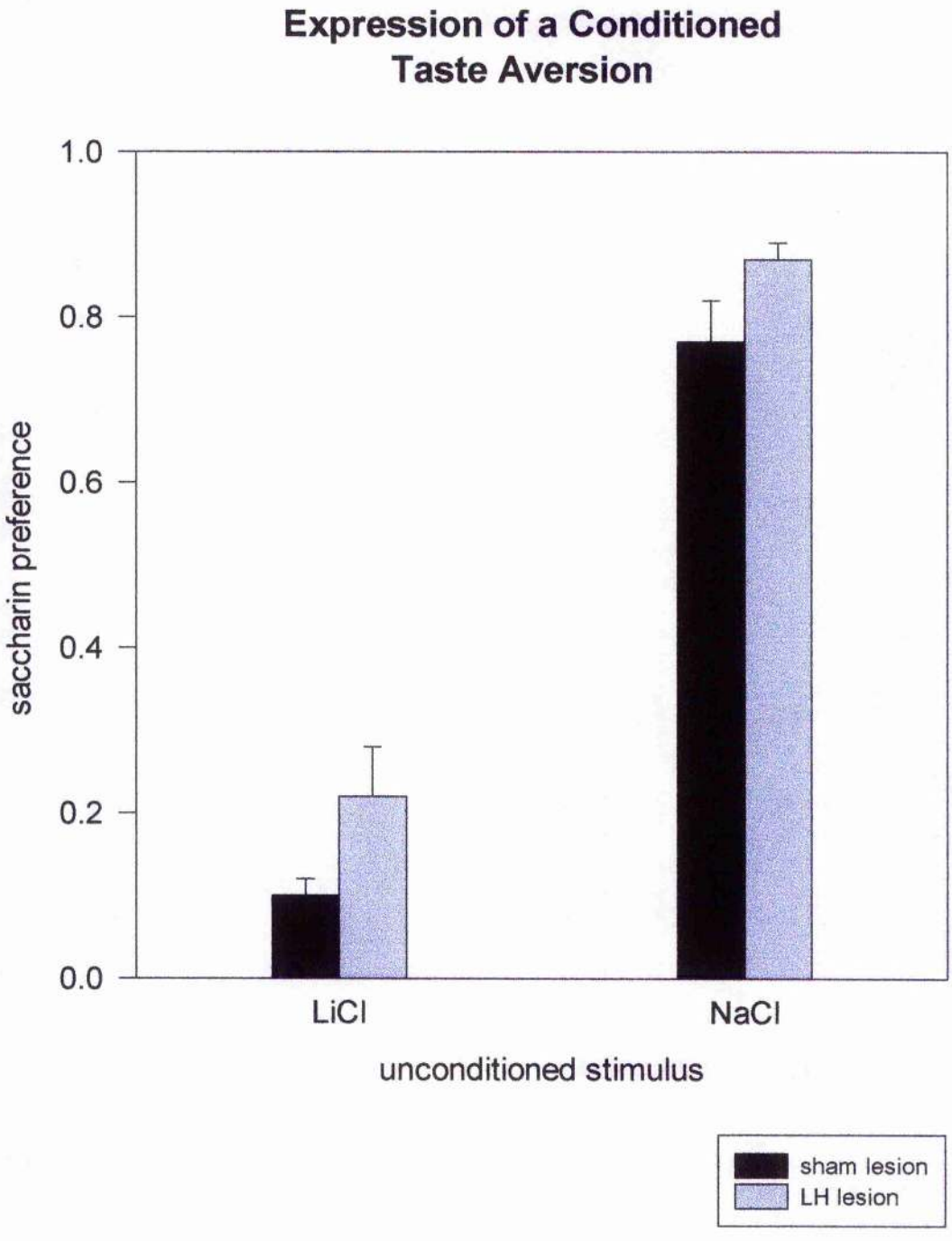


Figure 10. Mean (± SE) saccharin preference scores for sham lesioned rats after conditioning with LiCl (n=12) or NaCl (n=12) and LH lesioned rats after conditioning with LiCl (n=11) or NaCl (n=10). Preference score was calculated as saccharin intake/saccharin intake + water intake.

DISCUSSION

The present results show that lesioning the LH did not prevent the acquisition and expression of a conditioned taste aversion to saccharin but did elevate saccharin consumption *per se*. Home cage behaviour recorded and the response to the hypertonic saline challenge indicated that the area and volume of tissue lesioned was comparable to previous studies and histological analysis which is reported in Experiment 1.2 confirmed this. These data clearly indicate that the LH is not essential for the acquisition of a conditioned taste aversion. Since the PBN is clearly involved in conditioned taste aversion, it can be inferred that PBN connections with the LH are not critical for conditioned taste aversion function.

The results of previous studies, which investigated the involvement of the LH in the acquisition of a conditioned taste aversion, are contradictory. In agreement with the present results, Yamamoto (1993) demonstrated that rats with ibotenic acid lesions of the LH could acquire a CTA. It is notable however, that these results are difficult to interpret due to the meagre reporting of lesioning methods and histology. In addition, the toxin they used to produce LH lesions, ibotenic acid, has been shown to be less specific in lesioning the LH than NMDA since ibotenic acid diffuses into the amygdala (Winn *et al.* 1984).

In contrast, Caulliez *et al.* (1996) using a different experimental technique implicated the involvement of the LH in taste aversion learning. They showed that infusion of TTX and a specific D1 receptor antagonist, SCH 23390, into the lateral hypothalamic area blocked the acquisition of a conditioned taste aversion while microinjection of a D2 receptor antagonist, sulpiride, did not. Nevertheless, it is well documented that damage to fibres of passage in the LH produces deficits not seen with damage to intrinsic neurones of the LH. It does not seem prudent therefore, to infer that the LH is involved in a particular type of behaviour by showing that microinjection of TTX into this area inhibits it due to the nature of TTX, a sodium ion channel blocker which would block transmission in both intrinsic neurones and fibres of passage. Moreover, they confirmed that TTX did in fact block transmission in fibres of passage, reporting that microinjection of TTX rendered the animal immobile, aphagic and adipsic

for 24-48h, a state likened to that induced by electrolytic but not excitotoxic lesions of the LH. Although they also studied specific blockade of dopaminergic transmission, they stated that the highest density of dopamine receptors in the LH are D2 receptors whereas the highest density of D1 receptors are in areas around the LH including the zona incerta and the entopenducular nucleus. Since assessment revealed that these areas were within the extent of diffusion of the drugs and the fact that it was the D1 receptor antagonist and not the D2 receptor antagonist which blocked the acquisition of a taste aversion it appears that the effect can not specifically be attributed to blockade of transmission within the LH.

Unlike Caulliez *et al.* (1996), Roldan & Bures (1994) report that non-specific blockade of neuronal transmission in the LH with TTX did not disrupt acquisition of a conditioned taste aversion. While the deprivation schedule was similar in both studies, water availability being 15min or 30min a day, Caulliez *et al.* (1996) tested for a taste aversion using a one bottle test and Roldan & Bures (1994) in similarity with the present study used a two bottle test. Caulliez *et al.* (1996) state the theory that a one-bottle test is more sensitive than a two bottle test. However, it may be the case that it could occlude an aversion to some extent if animals are on a deprivation schedule of the severity of 15min water access a day and then presented with an aversive stimulus without any other access to fluid.

Electrophysiological studies indicate that signals concerning a taste cue are actually diverted from the LH after it has been paired with internal malaise. Aleksanyan *et al.* (1976) recorded unit responses to mouth perfusion of saccharin and found that the number of responses recorded in anaesthetised rats that had saccharin paired with LiCl was half that found with naïve animals. This could indicate that the alteration in hedonic value of the solution resulted in signals being conveyed in at least part by a different pathway. However, it is also important to note that it is not only a change in the hedonic value of the saccharin solution that occurs after pairing it with LiCl. Internal malaise is associated with many endocrine responsed in addition to behavioural responses and the change in the signals recorded from the LH could signify such responses.

It would therefore appear that the LH might not be essential in the acquisition and expression of a conditioned taste aversion. Despite this, previous studies indicate that it may be necessary to study many different techniques in order to elucidate the involvement of an area in a particular behavioural paradigm. Lesions of the NTS, a relay in the central gustatory system, were found not to attenuate the acquisition of a conditioned taste aversion, yet when neural activity in the NTS was studied during the different stages of the conditioned taste aversion procedure, it appeared that the NTS did have a role to play in this behaviour (Swank & Bernstein 1994). Administration of the US LiCl induced c-Fos staining in the NTS with the densest staining seen in the intermediate zone of the nucleus. In comparing the pattern of c-Fos induced by exposure to saccharin that had previously been paired with LiCl or saline, only saccharin paired with LiCl induced c-Fos in regions of the NTS activated by LiCl (Swank & Bernstein 1994). Furthermore this alteration in staining pattern was also seen when amphetamine was used as the US (Swank *et al.* 1995), a drug which only induced light c-Fos staining in the NTS, implying that it is a general response to taste aversion learning as opposed to a specific response to LiCl.

A series of papers have been published comparing the involvement of the NTS and PBN in taste guided behaviours (Shimura *et al.* 1997; Grigson *et al.* 1997a; Grigson *et al.* 1997b). In agreement with previous literature, they showed that while lesioning the PBN did disrupt acquisition of a conditioned taste aversion, lesioning the NTS did not. However, they also showed that lesioning the NTS rendered rats incapable of responding appropriately to changes in concentration of a variety of appetitive and aversive stimuli including sucrose and salt. The experimental protocol used was almost identical to that used by Spector *et al.* (1993) to investigate concentration-dependent licking of salt and sucrose in rats with lesions of the PBN. Although the response to increasing concentrations of sucrose by PBN lesioned rats was attenuated, the extent of the attenuation was far less than that seen with NTS lesioned rats when tested over the same range of concentrations. Furthermore when tested over the same range of salt concentrations, the response of the PBN rats did not differ from that of the control rats whereas the NTS lesioned rats failed to modify their responses in

relation to the changes in concentration. Grigson *et al.* (1997b) hypothesise that the NTS may be involved in sensory processing while the PBN may be involved in associative and regulatory tasks.

The volumes of fluid drunk during the first presentation of CS before pairing with the US gives some indication of whether or not taste processing has in some way been altered by lesions of discrete areas of the brain. Although comparison of absolute volumes of the CS drunk are made difficult by the transformations made on raw data before presentation, such as calculations of preference score (volume of CS drunk/volume of CS and water drunk), there are a number of studies which have included the actual volumes of fluid consumed during preference tests. Rats with either electrolytic or excitotoxic lesions of the PBN have been shown to drink significantly more 0.3M alanine than control rats (Scalera *et al.* 1995) whereas a study using a similar experimental procedure revealed that rats with electrolytic lesions of the NTS did not exhibit an elevated intake of 0.3M alanine (Grigson *et al.* 1997b). In similarity with this study of the PBN, we have presently shown that LH lesioned rats exhibit an elevated consumption of saccharin. This may be due to reduced neophobia, the phenomenon whereby rats only consume a small amount of a novel substance. However, this elevated consumption of the CS was seen not only on the first exposure to saccharin on the conditioning day but also on the test day in the present study. Furthermore, PBN lesioned rats were shown to drink more of the CS alanine than unoperated controls treated with saline as opposed to LiCl on 3 consecutive exposures to alanine. It therefore seems unlikely that the elevated intake was simply due to an attenuation of neophobia indicating that both the PBN and LH may play some role in taste perception.

Ferssiwi *et al.* (1987) revealed that ibotenic acid lesions of the LH shifted the preference curve for saccharin to the right, i.e. it was necessary to use higher concentrations of saccharin to obtain equivalent preference scores in LH lesioned rats compared to control rats. Moufid-Bellancourt & Velley (1994) reported that this shift in taste preference was reversed by injection of the opiate morphine into the PBN and subsequently hypothesised that the palatability of the saccharin

solutions used was modulated by input from the LH to the gustatory part of the PBN.

Thus, although the LH may not be an essential part of the relay forming conditioned taste aversions, it may have a functional link with the PBN in the perception of taste. However, in order to elucidate why electrophysiological evidence suggests that the involvement of the LH in gustatory processing is altered by conditioned taste aversion learning it would be useful to study the neural activity within the LH during the different stages of the conditioned taste aversion learning procedure. Further testing is also required to reveal the role of the LH in taste processing. Although ibotenic acid lesions of the LH have been shown to alter taste processing (Ferssiwi *et al.* 1987), Winn *et al.* (1984) reported that when lesions were made in this area using ibotenic acid, the toxin diffused laterally producing damage in the amygdala and furthermore, the lesions were associated with an increase in dopamine levels in the caudate putamen and the nucleus accumbens. This complicates the interpretation of the results since manipulations of both the amygdala and nucleus accumbens affect ingestive behaviour (Zardetto-Smith *et al.* 1994; Maldonado-Irizarry *et al.* 1995).

In order to form a conditioned taste aversion it was necessary to process visceral signals concerning the gastric malaise and associate these signals with the substance previously ingested. Behavioural observation indicated that lesioning the LH did not abolish the feeling of malaise. However, studies of the PBN have shown that the while lesioning the PBN prevents the feeling of malaise being used to form a conditioned taste aversion it does not prevent it from being used to form a conditioned place aversion suggesting that visceral signals concerning malaise must be processed by more than one pathway (Reilly *et al.* 1993). Hence, different structures may be concerned with the association of malaise with a gustatory cue as opposed to a spatial cue.

Previous studies have shown that LH lesioned rats failed to respond to physiological challenges involving changes in internal state induced by i.p. injections (Winn *et al.* 1984; Winn *et al.* 1990; Clark *et al.* 1990). By illustrating that LH lesioned rats formed a conditioned taste aversion we have shown that

although lesioning the LH appears to render the animal incapable of responding to visceral signals alone, it is still possible to use at least particular signals in conjunction with gustatory signals to alter behaviour. In similarity, Clark *et al.* (1991a) showed that LH lesioned rats responded appropriately when diet was adulterated with salt; here the rats had cues of a changing internal state from both taste and viscera. Since many studies have shown that the PBN is essential for both conditioned taste aversion and sodium appetite it would appear that a functional link between the LH and the PBN was not essential for either of these functions. If the LH were involved in CTA, one would expect lesions to attenuate CTA, but other techniques could show some more subtle involvement. Since the PBN does send gustatory information to the LH, what does the LH do with it?

BENZODIAZEPINE INDUCED HYPERPHAGIA

Drugs which act at the benzodiazepine receptor have a modulatory effect on ingestive behaviour which may be bi-directional. While benzodiazepine receptor agonists have been reported to have a hyperphagic effect, increasing feeding (Cooper *et al.* 1985) and drinking (Cooper & Francis 1979b), inverse agonists have been reported to have an anorectic effect decreasing feeding (Cooper *et al.* 1985) and drinking (Higgs & Cooper 1996a). The significant increase in feeding induced by benzodiazepine receptor agonists has been characterised as a reduction in latency to feed and a prolonging of the duration of feeding (Cooper & Francis 1979a). Observed in both deprived and non-deprived animals (Cole 1983) this effect has been induced by a wide range of benzodiazepine receptor agonists such as diazepam (Wise & Dawson 1974), midazolam (Cooper *et al.* 1985), clonazepam (Cooper & Gilbert 1985) and chlordiazepoxide (Cooper & Francis 1979a; Cole 1983) and has been blocked by benzodiazepine antagonists such as flumazenil, (Cooper & Yerbury 1986; Cooper & Gilbert 1985) indicating that it was mediated via the benzodiazepine receptor complex. Wise & Dawson (1974) reported a dose dependent increase in lever pressing for food after administration of diazepam indicating that the increase in food intake was also well motivated.

Mode of Action of Benzodiazepines on Feeding Behaviour

Although the exact mode of action of benzodiazepine receptor agonists on feeding behaviour is still under investigation, the opposing theories that it may be via a direct or indirect effect have been reviewed thoroughly and thus will only be discussed briefly (Cooper 1980a; Berridge & Pecina 1995; Cooper & Estall 1985). Drugs that act at the benzodiazepine receptor have a number of properties, which, it may be argued, induce feeding as a secondary indirect effect. Cooper & Estall (1985) have proposed that the increase in food consumption cannot be due to either the sedatory effects of the drugs or stereospecific gnawing induced by the drugs since while benzodiazepine treated animals rapidly develop tolerance to both these effects they do not develop tolerance to the benzodiazepine induced hyperphagia indicating that they are mediated by independent processes.

It is well known that benzodiazepines also have anxiolytic properties which give rise to the possibility that the increase in food consumption induced by benzodiazepines is merely due to a reduction in the "stressfulness" of the novel test environment or the novel test food. On initial contact with a novel food rats exhibit neophobia, that is they restrict their intake regardless of the palatability of the food item. Although this may be a contributing factor under novel circumstances, benzodiazepine receptor agonists have been shown to induce increased feeding even under conditions of familiarity (Cooper and Yerbury 1986; Wise & Dawson 1974). Wise and Dawson (1974) reported no significant difference in the degree of "vigorous eating" that diazepam produced with familiar food in either test area or home cage strongly indicating that at least part of the effect may be due to a direct action on feeding behaviour.

Cooper & Crummy (1978) replicated a study devised by Rolls & Rolls (1973) to further investigate the relationship between the action of benzodiazepine receptor agonists and the expression of neophobia. Rolls & Rolls (1973) had illustrated that when food deprived rats were exposed to a selection of foods composed of one familiar food (chow) and 5 other novel foods the predominate response was to consume the familiar chow despite the fact that all the food substances had been sampled. After a number of repetitions of this procedure, as the novelty of the different food substances declined, the composition of the diet changed with a smaller proportion gained from the familiar chow and more from more palatable substances such as cookies. This suggested that under conditions of food deprivation, familiarity had a stronger influence over food choice than palatability alone. Utilising this protocol allowed Cooper & Crummy (1978) to distinguish between a simple enhancement of appetite and a reduction in the influence of novelty. The increase in feeding time induced by chlordiazepoxide administration was found to be selective for familiar chow rather than generally increasing consumption of all foods which would have been expected if chlordiazepoxide acted by means of reducing neophobia. Cooper & McClelland (1980) found that prior familiarisation with the test food substances abolished this effect with the result that consumption of the more palatable food substances increased excluding an increase in the consumption of chow. This provided

evidence that the selective increase in consumption of chow was not simply because of an innate preference for it over the other food substances used. Further investigation using a range of concentrations revealed dual effects of chlordiazepoxide (Cooper & McClelland 1980). At low doses, (5, 10mg/kg) chlordiazepoxide promoted eating of familiar food substances whereas a higher dose (15mg/ml) promoted eating of palatable food regardless of its novelty. It was postulated that these dual effects of benzodiazepines were mediated by different mechanisms of action (Cooper & McClelland 1980).

The development of more selective benzodiazepine receptor agonists and partial agonists such as the pyrazoloquinoline compounds, indicated that the various properties of benzodiazepines could well be mediated by different mechanisms of action. In order to consider hyperphagia as secondary to anxiolysis, hyperphagia would have to be induced concomitantly with anxiolysis. However, it was demonstrated that compounds acting at the benzodiazepine receptor had potent anxiolytic activity but failed to stimulate feeding and in fact dose-dependently antagonised clonazepam induced hyperphagia (reviewed Cooper *et al.* 1987; Cooper *et al.* 1987). This indicated that these two different actions were mediated via different mechanisms.

Together this evidence indicates that benzodiazepine receptor agonists induce feeding by an action, which at least in part is mediated directly as opposed to a secondary indirect effect.

Neural Substrate for Benzodiazepine Modulation of Ingestive Behaviour

Higgs & Cooper (1996b) reported that administration of midazolam into the IVth ventricle in rats caused a significant increase in the consumption of a palatable wet mash suggesting that the neural substrate for benzodiazepine induced hyperphagia was in the brainstem. Moreover, this increase in consumption of palatable mash was blocked by the benzodiazepine antagonist flumazenil indicating that it was in fact due to an action mediated by a specific benzodiazepine receptor.

Higgs & Cooper (1996c) have investigated the locus of the benzodiazepine induced hyperphagia more specifically by studying the role of the PBN, a structure positioned adjacent to the IVth ventricle. They reported that direct administration of midazolam into the PBN significantly increased the consumption of a wet mash and a 3% sucrose solution. In this series of experiments, they also addressed the problem of sedation induced by benzodiazepine receptor agonists. By illustrating that doses of midazolam which induced significant increases in consumption of wet mash failed to alter locomotor activity they have provided evidence to suggest that benzodiazepine receptors in the PBN may be specific for feeding behaviour as opposed to sedation.

Although this evidence suggests that the receptors mediating benzodiazepine-induced hyperphagia are located within the PBN it does not mean that this is the only area or neurotransmitter system involved. Opioids have a modulatory effect on ingestive behaviour that is similar to the effect of drugs active at the benzodiazepine receptor. Like benzodiazepine receptor agonists, opioid receptor agonists administered systemically have been reported to induce ingestive behaviour. For instance, low doses of the opioid receptor agonist morphine increased feeding and drinking in non-deprived rats (Cooper 1981; Sanger & McCarthy 1980). However, unlike benzodiazepine antagonists which do not appear to affect ingestion when administered alone, the opioid receptor antagonist naloxone has been shown to decrease food and water intake in non-deprived rats (Cooper 1980b).

It is possible that rather than modulating ingestive behaviour via separate mechanisms, benzodiazepine and opioid receptor agonists may influence the same relay. Higgs & Cooper (1997) have examined the relationship between opioid receptor and benzodiazepine receptor mediated events with respect to enhanced feeding behaviour. Midazolam was administrated to non-deprived rats that had been trained to consume a palatable fat emulsion, Intralipid, from a lickometer. They illustrated that the increase in total number of licks (expressed as an increase in the mean bout duration) induced by midazolam was blocked not only by the benzodiazepine receptor antagonist but also by the opioid antagonist

naloxone. Since both flumazenil and naloxone were ineffective when administered alone, this indicated that neither of these drugs acted simply by producing an action opposite to that of midazolam, but actually antagonised the effect of midazolam.

Anatomical Location of Opioid Positive Cells

Using the combined retrograde fluorescence-immunofluorescence method, a population of cells located within the LH which project to the PBN have been found to be immunoreactive for the endogenous opioid neuropeptide dynorphin (Moga *et al.* 1990b; Zardetto-Smith *et al.* 1988). There is evidence suggesting that opioid receptors located in the LH are important in the control of food intake; naloxone injected into the LH reliably depressed food intake in food–deprived rats (Thornhill & Saunders 1984). Hence, it is possible that the LH exerts a controlling influence over the benzodiazepine receptor mediated hyperphagia. This hypothesis was tested presently by measuring any hyperphagia induced by systemically administered midazolam to rats with excitotoxic lesions of the LH.

Experiment 1.2: The Role of the LH in Benzodiazepine Induced Hyperphagia

METHODS

Subjects

Forty-four male Lister Hooded rats (Charles River) previously used in a study of conditioned taste aversion (Experiment1.1 "Conditioned Taste Aversion") were individually housed under a 12h light/dark cycle with ad lib food (SDS maintenance diet no. 1 chow pellets) and tap water available. Twenty-four of the rats had received sham lesions and twenty had received excitotoxic LH lesions using NMDA (details included in Experiment 1.1, pg 38).

Procedure

Ninety-seven days after surgery body weight, and food and water intake were measured for 6 days in order to gain baseline measurements. The rats were then given a daily 30min exposure to a palatable mash, consisting of 400ml ground lab chow (SDS maintenance diet 1), 200ml of water and 100ml of Nestlé condensed milk, until a steady daily intake level was reached. When measuring the amount of mash left after the 30min session, the test cage was checked for any food spillage and this was included in the measurement of remaining food. After 10 days exposure to mash, injections of 1ml/kg isotonic saline and midazolam (0.3mg/ml, 1mg/ml and 3mg/ml) were administered in a counterbalanced order, prior to exposure to the mash, with 48h between injections. The amount of mash consumed in the 30min period immediately after the injection was measured. Body weight and food and water intake were then measured for a further 10 days. All measures of intake for this experiment (both in the test cage and home cage) were converted to KJ: 1g mash = 10KJ, 1g lab chow = 14.8KJ to enable a direct comparison of intake from lab chow and palatable mash.

Histological Analysis

At the end of the experiments all the rats were given an overdose of 200mg/ml pentobarbitone (Euthatal UAB ®) and were subsequently perfused transcardially

with physiological saline followed by 10% formalin. The brains were then removed and stored in 10% formalin until they were sectioned on a freezing microtome. 25μm slices were cut at 200μm intervals and stained with cresyl violet stain for nissl substance and Gallyas stain for myelin (Gallyas 1970). Lesions were identified by areas of cell loss and reactive gliosis. In order to ascertain lesion volume, histological sections were scanned and then silhouettes of the areas of the lesion were drawn onto the scanned sections. The areas of the lesioned tissue was determined using a microcomputer and lesion volumes calculated.

Statistical Analysis

Due to the substantial difference in body weight between the sham lesioned and LH lesioned groups all measures of intake were expressed as a function of body weight for the purpose of statistical analysis and graphical presentation. The results were examined statistically using ANOVA with, when appropriate Tukey's post hoc tests. The behavioural data was also examined to ensure the previous study did not interact with the present results.

RESULTS

Histological Analysis

Figure 11 illustrates the biggest and smallest LH lesions produced. The average size of the lesions was 1.74mm^3 (SE 0.16) on the left side and 1.87mm^3 (SE 0.14) on the right side with the greatest damage level with the paraventricular nucleus decreasing towards the anterior and posterior poles. There was little extra-hypothalamic damage: two-thirds of the animals had damage (30%-80%) in the zona incerta, reticular nucleus of the thalamus, subincertal nucleus and the ventromedial thalamic nucleus. In a minority of animals there was also slight damage (<30%) in the substantia innominata, subthalamic nucleus and antero-medial and antero-ventral thalamic nuclei. In 3 cases the toxin diffused along the border of the internal capsule causing minimal damage to the ansa lenticularis. Analysis of the behavioural results showed no difference between the rats with differing amounts of extra-hypothalamic damage.

Analysis of the Gallyas stained sections showed that myelin was present. However although myelin was shown to be present in the LH, the staining was lighter and a less regular pattern than that found in the sham lesioned rats. Moreover, in a number of animals there were small areas unstained within the LH. In addition to a change in the pattern of staining in the LH there was also a change in the density and staining pattern in the fornix in 6 animals suggesting an alteration to the myelination in these individuals. One rat was terminated 30 days after surgery and analysis of the LH revealed that in this individual remyelination had not occurred. These observations are consistent with those we and others have presented previously: that NMDA in the LH demyelinates fibres, but that remyelination occurs over approximately 3 months (Brace *et al.* 1997).

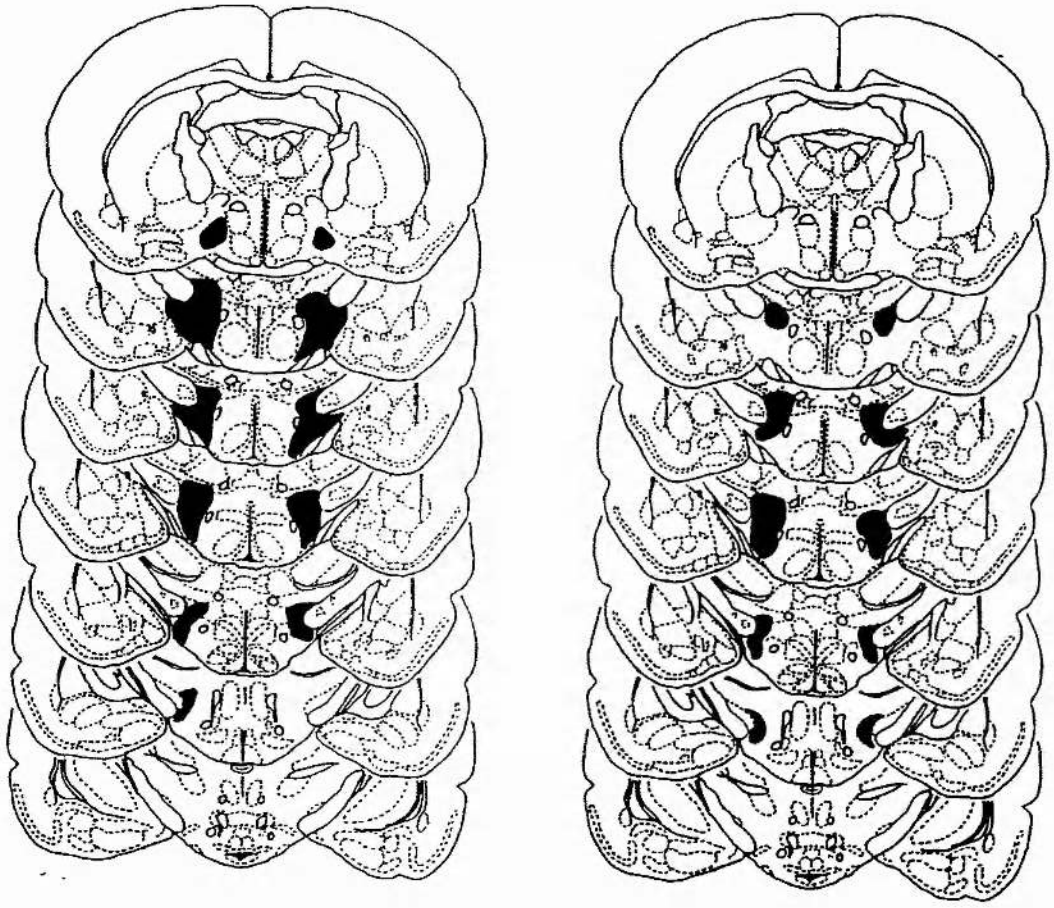


Figure 11. Representative sections redrawn from the atlas of Paxinos & Watson (1982) illustrating the extent of the biggest and smallest lesions produced by injection of NMDA into the LH. The shaded regions represent areas of neuronal loss.

Diet Adulteration

Total Energy Intake

With respect to diet adulteration the study can be split into 3 stages: first, intake of home cage lab chow alone prior to exposure to mash, second, intake from lab chow plus palatable mash supplements and third, intake from lab chow alone after exposure to palatable mash. In order to compare total energy intake in these three stages an average daily energy intake was determined for each; illustrated in Figure 12. These data were analysed using repeated measures ANOVA with group (sham lesion/LH lesion) as the between subjects factor and diet (lab chow alone both before and after exposure to palatable mash and lab chow plus mash) as the within subjects factor. ANOVA showed a main effect of group ($F(1,41) = 6.99$ $p<0.012$) indicating that the LH lesioned group did not regulate energy intake in the same manner as the sham lesioned group. Total energy intake differed when diet was supplemented [main effect of diet ($F(2,82) = 117.89$ $p<0.001$)] and a significant group × condition interaction ($F(2,82) = 4.39$ $p<0.015$) indicated that this change in energy intake was not comparable between the groups. Tukey's post-hoc tests showed that energy intake was significantly greater for the LH lesioned group compared to the sham lesioned group when lab chow alone was available (pre-mash $p<0.001$; post-mash $p<0.001$) but when diet was supplemented with palatable mash there was no difference between sham lesioned and LH lesioned groups. The introduction of palatable mash into rats' daily routine did not change the average energy intake of sham lesioned rats, but in fact *reduced* the average energy intake of the LH lesioned rats ($p<0.001$).

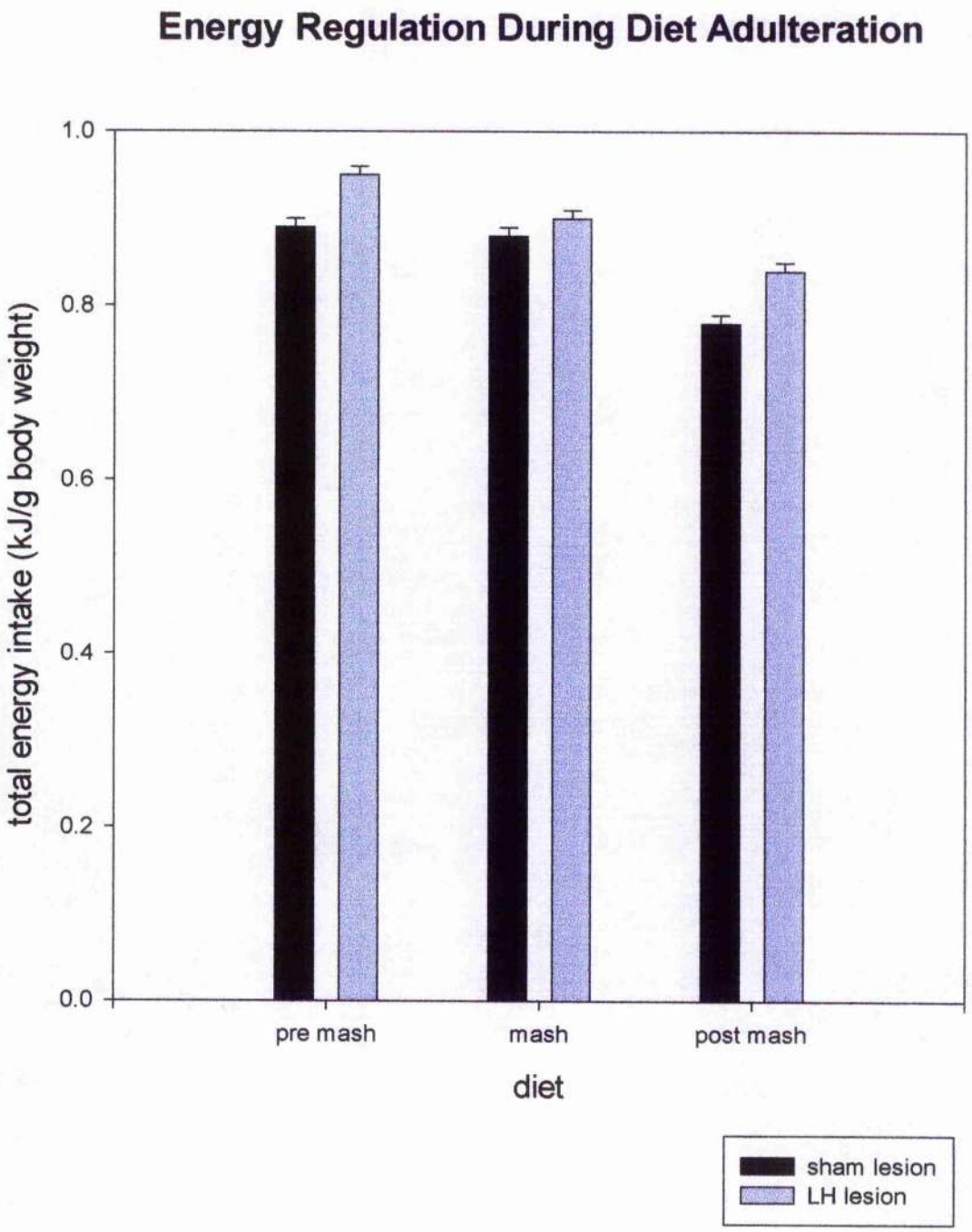


Figure 12. Mean (± SE) total energy intake as a function of body weight for the sham lesioned group (n=24) and LH lesioned group (n=20) with respect to body weight before, during and after introduction of palatable mash.

For further analysis of energy intake in this study, separate analyses were made of the energy intake from lab chow and mash using repeated measures ANOVA.

Energy Intake From Lab Chow

Figure 13 presents daily energy intake from lab chow. ANOVA showed no main effect of group ($F(1,41) = 0.00$) but a significant effect of day ($F(33,1353) = 284.47$ $p<0.001$) and a significant group × day interaction ($F(33,1353) = 8.78$ $p<0.001$). Clearly, LH lesioned rats were obtaining more energy from lab chow than sham lesioned rats when mash was not available, but less during the period when mash was presented.

Energy Intake From Palatable Mash

Figure 14 shows energy intake from mash; ANOVA revealed significant main effects of group ($F(1,42) = 12.85$ $p<0.001$) and day ($F(17,714) = 59.88$ $p<0.001$) and a significant group × day interaction ($F(17,714) = 2.45$ $p<0.001$). Palatable mash was available for 18 days from day 7-24. Tukey's post-hoc tests showed significant differences ($p<0.05$ at least) between the two groups on all days except 8, 9, 10, 17, 20, 21 and 23. Thus, the LH lesioned group consumed significantly more mash than the sham lesioned group on eleven of the eighteen days where mash was available. It is important to consider that for the first 10 of these days, rats were receiving no drug treatment. Midazolam was given on days 17, 19, 21 and 23.

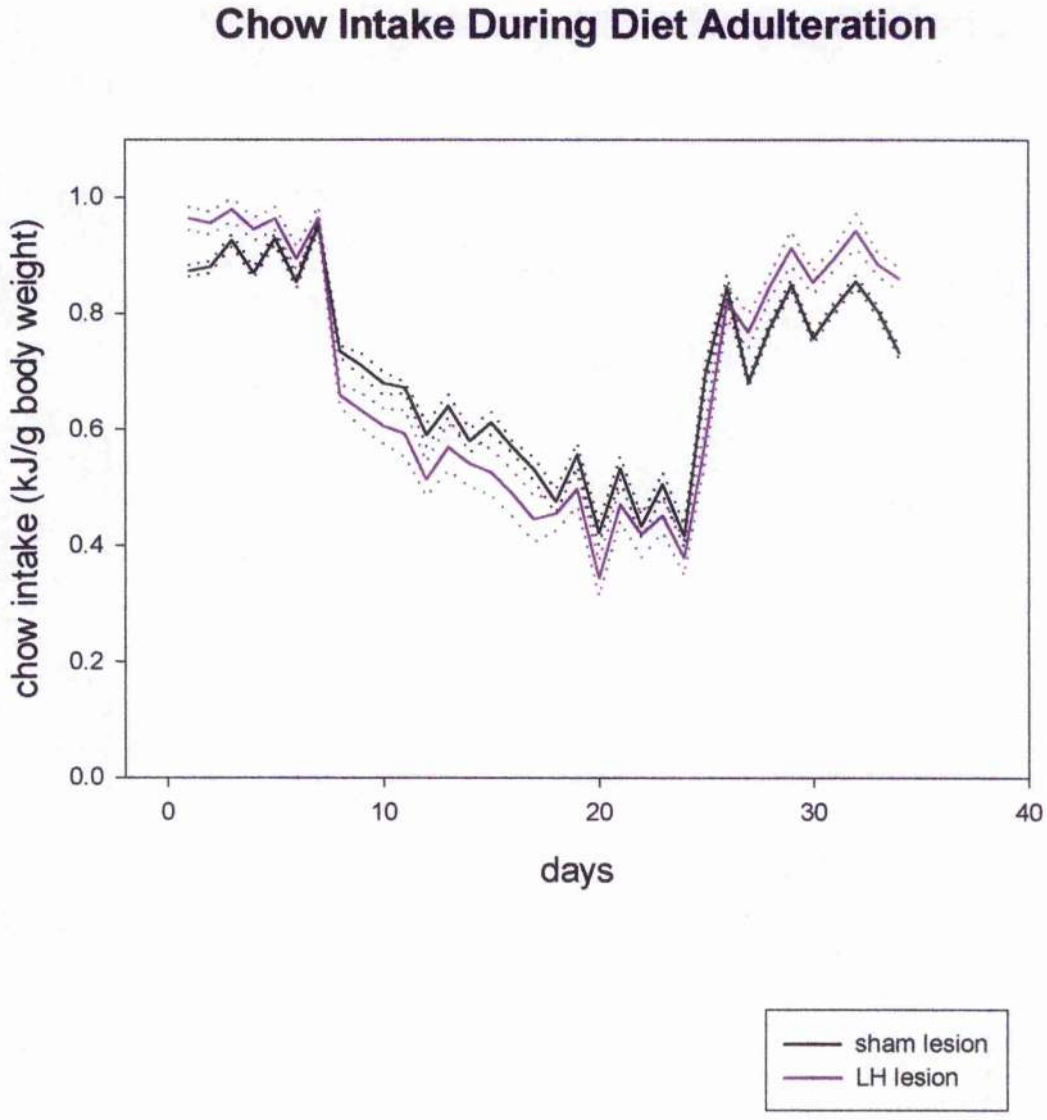


Figure 13. Mean (± SE) energy intake as a function of body weight from lab chow before (days 1-6), during (days 7-24) and after (days 19-34) availability of palatable mash for the sham lesioned group (n=24) and LH lesioned group (n=20).

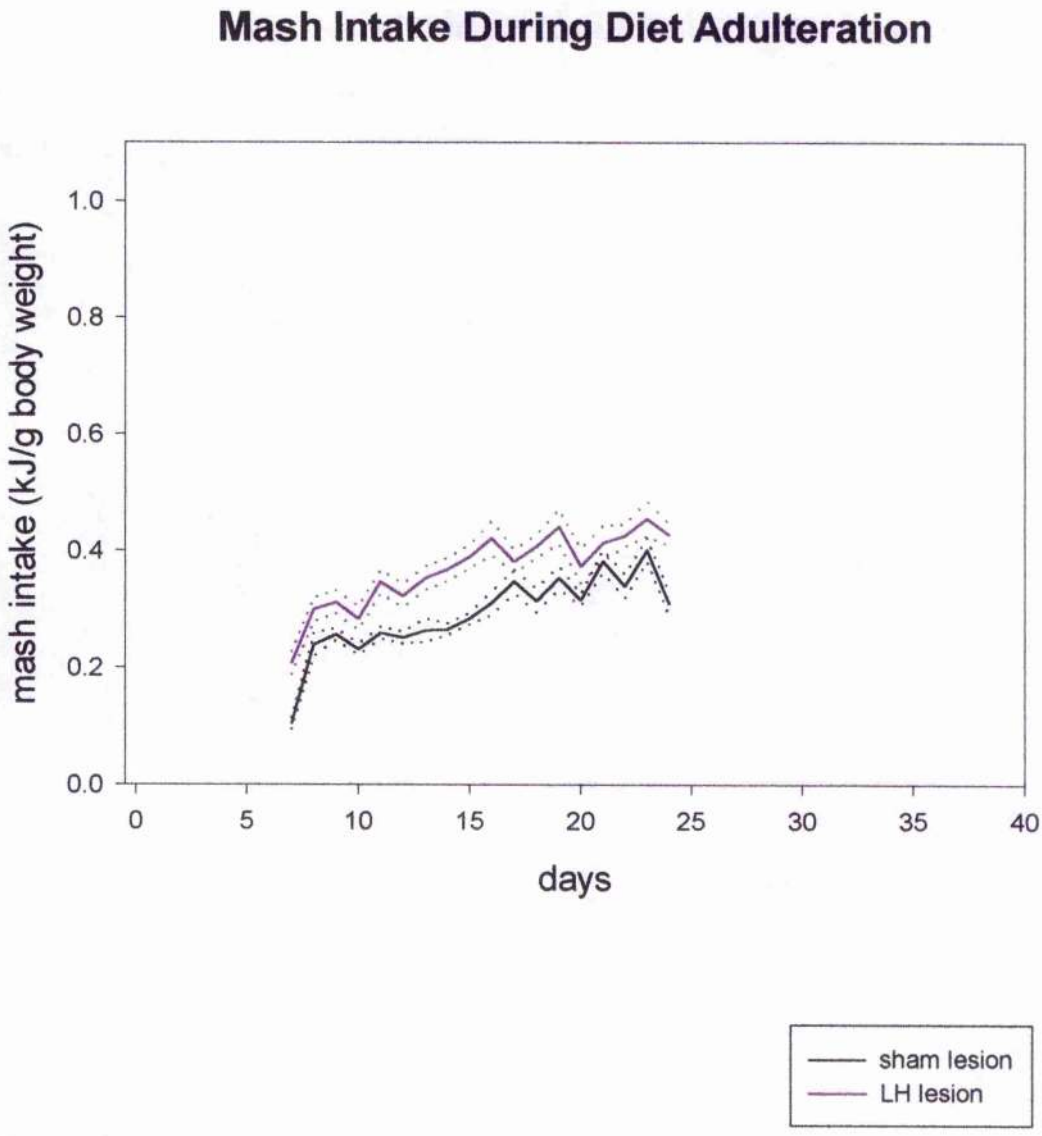


Figure 14. Mean (± SE) energy intake as a function of body weight from palatable mash for the sham lesioned group (n=24) and LH lesioned group (n=20).

Midazolam Induced Hyperphagia

From day 11 after the introduction of the palatable mash all rats were given i.p. injections of midazolam (0.3, 1, 3 mg/ml) and isotonic saline. Figure 15 presents the mean amounts of mash consumed/g body weight after each injection. ANOVA showed no significant group × drug interaction ($F(3,126) = 0.47$). There was however a significant main effect of drug ($F(3,126) = 3.05$ $p<0.031$) indicating that midazolam did induce hyperphagia. The main effect of group did not quite achieve statistical significance ($F(1,42) = 3.74$ $p<0.06$). If anything however, it was the LH lesioned group which was over-responding to the drug in comparison to the sham lesioned group. Post hoc analysis indicated that 1.0 mg/kg midazolam stimulated greater intake than saline ($p<0.039$). These results suggest that lesioning the LH does not prevent hyperphagia induced by midazolam.

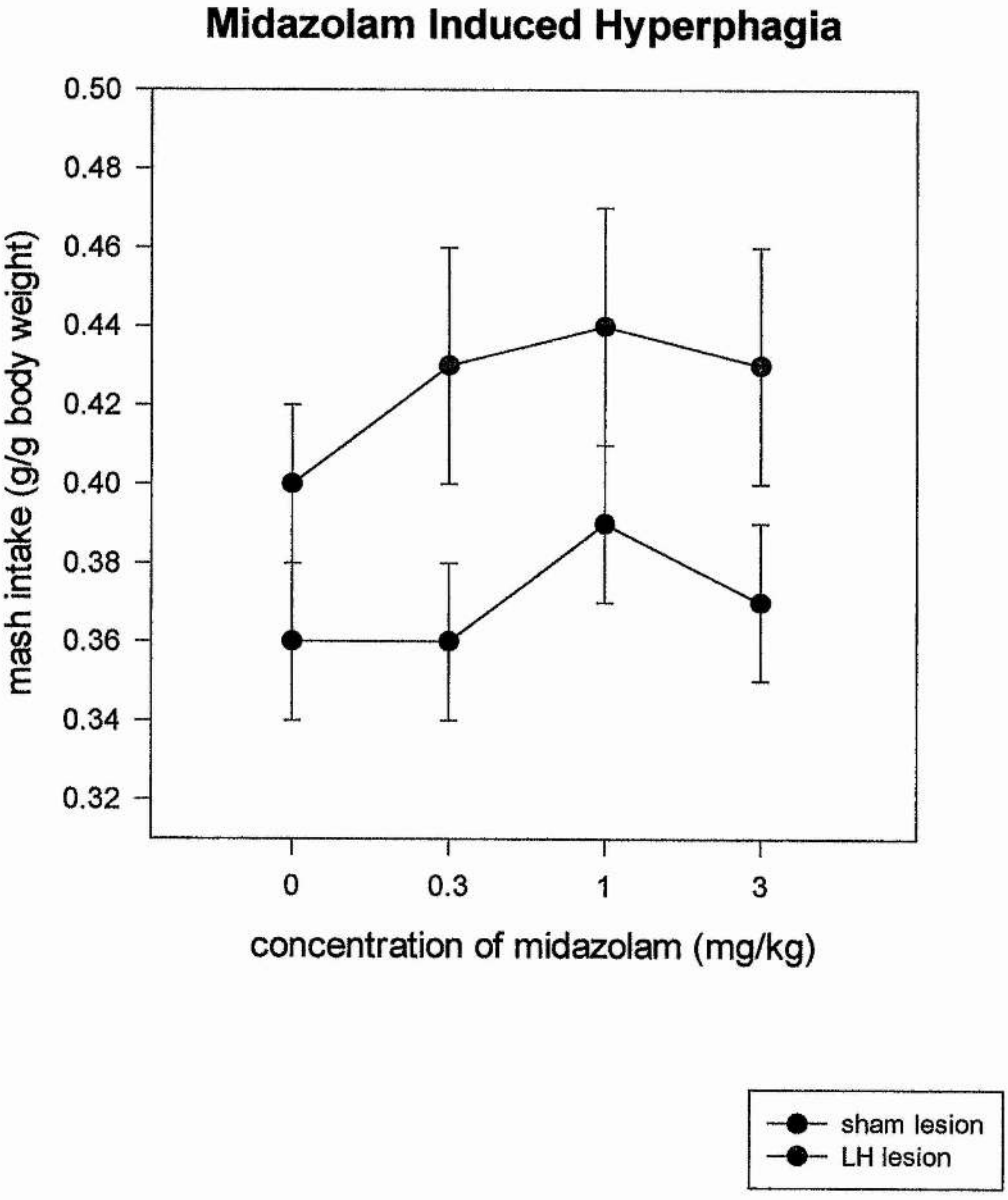


Figure 15. Mean (± SE) intake of palatable mash for the sham lesioned group (n=24) and LH lesioned group (n=20) after i.p. injection of isotonic saline and 0.3, 1, 3mg/ml midazolam.

DISCUSSION

The present results show that lesioning the LH did not disrupt the hyperphagia induced by midazolam while in contrast, examination of diet during the test period suggests that lesioning the LH did disrupt energy regulation.

Evidence strongly suggests that the PBN is the locus for benzodiazepine induced hyperphagia (Higgs & Cooper 1996c) but it was proposed that the PBN might not be the only area involved. Thus while the PBN appears to be crucial, other areas may have a lesser but still influential role in this benzodiazepine mediated effect. The action of drugs active at opioid receptors has been reported to be comparable to those acting at benzodiazepine receptors with respect to feeding behaviour (Cooper 1981; Sanger & McCarthy 1980). Moreover, these have even been shown to interact suggesting the involvement of more than one neurotransmitter in this particular mode of feeding behaviour (Higgs & Cooper 1997). Since a projection from the LH to the PBN has been shown to be immunoreactive for opioids (Moga *et al.* 1990b; Zardetto-Smith *et al.* 1988), it was hypothesised that the LH may influence the benzodiazepine induced hyperphagia. However, the present results show that the degree of overeating induced by the benzodiazepine receptor agonist midazolam was equivalent for the LH lesioned and sham lesioned rats. Thus, it appears that a functional connection between the LH and the PBN is not essential for the mediation of midazolam induced hyperphagia. The LH is not the only region to be implicated in morphine induced feeding. For instance, morphine injected into the nucleus accumbens also induced hyperphagia (Evans & Vaccarino 1990). Therefore it may be this as opposed to the LH which interacts with the PBN to control this particular type of feeding behaviour while the LH may be involved in some other more subtle form of control which wasn't revealed by this test.

While these results show that a connection between the LH and PBN was not essential for the mediation of this midazolam induced hyperphagia, group differences were seen in the degree of feeding induced. Although this failed to reach significance, there was a tendency for the LH lesioned group to overeat in comparison to the sham lesioned group suggesting an impairment in performance

that may not have been detected by this particular measurement. Examination of the intake of mash for the whole test period provided evidence of a more apparent impairment in the behaviour of the LH lesioned group. While mash consumption induced by administration of midazolam was not significantly different between groups, analysis of mash consumption for the whole period mash was available (i.e. the habituation period, the alternate drug and drug free days of the test period) revealed that the LH lesioned group did in fact eat significantly more mash than the sham lesioned group. There are a number of possible explanations that could account for this. Since the early studies of the "LH syndrome" which involved electrolytic lesions of the LH, different groups have disputed the cause of the loss of body weight after LH lesions. Two opposing theories were proposed:

1. Lesioning the LH resulted in an alteration in body weight set-point which was reached by active weight loss (Powley and Keesey 1970)
2. Lesioning the LH altered feeding behaviour which resulted in a secondary fall in body weight (Winn *et al.* 1984).

Regulation of a Body Weight Set-Point

A new body weight set-point could be reached by a change in metabolism or a change in energy intake. Thus, the mild difference in the degree of hyperphagia induced by midazolam and the significant overall group difference in palatable mash consumption may be a secondary indirect result of a change in energy regulation. Due to the measurements taken before the actual test period it is possible to address the issue of body weight regulation.

Three weeks after surgery, energy intake as a function of body weight for the LH lesioned rats was not significantly different from that of the controls rats. However, when this measure was repeated 4 months after surgery, energy intake as a function of body weight was significantly greater for the LH lesioned group compared to the control group. A simple explanation for this discrepancy can be found in the substantial difference in body weight between the groups; smaller animals have higher metabolic rates. However, when their diet was adulterated by introducing a palatable mash in addition to *ad lib* chow, the LH lesioned rats failed to maintain a constant energy intake unlike the control rats. According to

the theory that lesions of the LH altered a "body weight set-point" a change in energy intake or metabolic rate would simply be a means to maintain a lowered set-point but surely to maintain a decrease in body weight it would be necessary to decrease energy intake rather than increasing it as was found presently. Moreover, even if lesioning the LH did somehow result in a changed body weight set-point, this new set-point would presumably be defended when offered a choice of diet, which did not appear to occur. This would therefore seem to be too simple an explanation for the deficits incurred by lesioning the LH.

Feeding Behaviour

Many internal and external cues control feeding behaviour. For instance, food eaten can depend on the taste of the food substances available, previous experience of these substances and internal state. When a range of familiar foods is presented, the more palatable options are eaten preferentially (Cooper & McClelland 1980). In the present study, the two food substances available differed not only in taste but also calorific density. The palatable mash was a sweetened mixture based on chow but it is important to note that it had a lower calorific density. Thus, a greater volume of palatable mash had to be consumed to obtain the same energy intake as that gained from eating chow. Examination of the dietary components when given limited daily access to the palatable mash in addition to *ad lib* chow revealed that the sham lesioned group consumed the palatable mash and decreased consumption of chow. Despite this choice of dietary components, they maintained an energy intake comparable to that seen during control conditions. The pattern of behaviour exhibited by the LH lesioned group was similar but somewhat exaggerated in comparison to the sham lesioned group. The fall in consumption of chow was significantly greater for the LH lesioned group who also ate significantly more mash than the sham lesioned group. Thus, overall the palatable mash made up a larger proportion of the LH lesioned group's diet, the consequence of which was that unlike the sham lesioned group, they failed to maintain a constant energy intake. This in contrast to the findings of Clark *et al.* (1991a) who reported that LH lesioned rats displayed appropriate energy regulation when access was given to a 6.3% glucose solution in addition to the normal diet of chow; they drank normal volumes of glucose solution, decreased their chow intake appropriately and had a

calorific intake no different from that of the sham lesioned group. Furthermore, following salt adulteration of food, the LH lesioned group increased their intake of water to the same degree as the sham lesioned group again indicating appropriate behaviour in order to maintain homeostasis.

There are a number of points that distinguish the present study from that of Clark *et al.* (1991a), which may account in at least part for the differences in results between studies. Firstly the home cage measurements of body weight and food intake indicate that the lesions produced presently may cover a greater volume of the LH; recovery for the present LH lesioned group was slower despite the fact that the post-operative care regime was the same in both studies. Secondly, there are also methodological differences between the two studies with respect to access to the additional food source and the duration between surgery and testing. In the present study, rats were given access to the palatable mash during a 30min session in a testing room whereas Clark *et al.* (1991a) provided *ad lib* 6.3% glucose solution in the home cage. The time course however may be a more crucial factor considering that the group relationship for the parameter under scrutiny, energy intake, did not remain constant for the duration of the present study even under control conditions where chow was the only food source. Although there were no group differences in energy intake at 21 days post-surgery, a time period equivalent to the test period of Clark *et al.* (1991a), this relationship had changed 97 days after surgery when the present LH lesioned group were tested. Thus while Clark *et al.* (1991a) reported no group differences in daily energy intake either before or during diet adulteration, in the present study the LH lesioned rats were overeating before diet was supplemented with palatable mash in addition to overeating this dietary supplement.

In order to elucidate why the LH lesioned animals were overeating, it is helpful to examine hyperphagia induced in neurological intact animals. Sclafani *et al.* (1996) have investigated the cues essential to palatability induced hyperphagia by pairing two solutions of different palatability (one bitter, one sweet) with intragastric infusions of polycose. A choice test at the end of the experiment showed that a preference had developed for the bitter taste. However, despite this and the fact that the solutions had the same postingestive consequence, the

fluid with the more palatable sweet flavour was drunk in greater volumes and as result, the animals exposed to this had a higher energy intake than the animals which had access to the bitter fluid. This indicated that the palatability of the available food substances was important in determining the amount of food eaten. However, the food substances in the present study differed not only in palatability but also in calorific density and thus would have had different postingestive consequences. Rigaud *et al.* (1994) has shown that increasing the duodenal energy load decreased sham meal size in a dose dependent manner implicating the postingestive properties of food substances in the determination of meal size. By combining sham meals of variable palatability with different duodenal energy loads, Rigaud *et al.* (1994) also studied the interaction between palatability and postingestive consequence. From the finding that the absolute decrease in feeding was greatest for the more palatable meals it was inferred that duodenal load had a more pronounced effect on the intake of highly palatable diets though it should be noted that the percentage decrease for each meal was approximately the same. Whether or not there is an interaction between palatability and postingestive properties, it appears that both have a role in determining meal size. Warwick & Weingarten (1994) demonstrated that a preference for an associated flavour can be established by the palatability or the calorific value of a fluid and thus provided evidence that both taste and postingestive consequence may be important factors in choice of food intake in addition to volume of food intake.

Although it is perhaps a simplistic view of the control of feeding it appears that in at least part, the components of a meal are determined by a balance between palatability and postingestive cues. While food that has a high calorific density is more likely to be eaten than that with a low calorific density, it is likely to be eaten in smaller quantities due to postingestive feedback. Rigaud *et al.* (1994) has also suggested that while increased palatability can lead to increased eating this is over-ridden after a certain postingestive consequence is reached. In this way, the integration of palatability cues and postingestive cues could maintain feeding within reasonable limits.

It is possible that lesioning the LH disturbed the balance between these cues. As previously stated, lesioning the LH resulted in a decrease in consumption of the food with a higher calorific density but lower palatability while elevating consumption of the food with a lower calorific density but higher palatability. Presuming palatability and postingestive factors form a type of inhibitory feedback loop, it would appear that palatability has a stronger influence on feeding behaviour than postingestive consequence as a result of lesioning the LH. This could be due to a number of possibilities including:

1. Altered taste perception
2. Deficit in monitoring of postingestive consequences
3. Failure to change behaviour appropriately to perceived cues.

It has been illustrated that changes in positive hedonic responses are not necessarily accompanied by changes in aversive taste responses (Berridge & Grill 1983). It is possible that lesioning the LH may have altered taste perception in a manner which increased positive hedonics and therefore selectively increased the palatability of the palatable mash. According to the work of Warwick & Weingarten (1994), this would have increased the likelihood of the palatable mash being the chosen food. The effect of lesioning the LH on taste perception has been examined but with disparity in the results seen. While Clark *et al.* (1990a) found no difference in the preference-aversion curves for saccharin or quinine when the LH was lesioned, Ferssiwi *et al.* (1987) found that lesioning the LH shifted the preference curve for saccharin to the right suggesting a down regulation of the perceived taste of saccharin. The results of Ferssiwi *et al.* (1987) are compromised though by the fact that ibotenic acid was used to produce the LH lesions; Hastings *et al.* (1985) reported that ibotenic acid lesions were less selective than NMDA lesions, being associated with damage to the amygdala and increased dopamine levels in the nucleus accumbens and the caudate-putamen. Clark *et al.* (1990), like the present study, used NMDA to produce lesions of the LH but these animals nevertheless appeared less impaired than the LH lesioned group examined presently. A study of the involvement of the LH in taste perception which employed lesions comparable to those seen in the present study would be necessary to give a clearer indication of whether a deficit in taste perception was the cause of the present differences in responding.

Ingestion of the different foods would have produced different degrees of change in internal state factors such as blood sugar levels. If there was a failure to distinguish the different calorific densities of the two different food substances, this could have lead to enhanced consumption of the more palatable food. Lesioning the LH has been shown to reduce the capacity to respond appropriately to changes in internal state induced by i.p. injections (Clark *et al.* 1990). It is well known that the response to injection or ingestion of the same substance is not always equivalent, for instance, the response to glucose injected intravenously is blunted in comparison to ingestion of the same glucose load. Nevertheless, these studies may give some indication of the capacity to respond to changes in internal state. It has been illustrated that lesioning the LH attenuated responding to 2-deoxy-D-glucose, a glucose mimetic which induces hunger (Clark *et al.* 1990). Furthermore, as was illustrated presently, LH lesioned rats fail to drink appropriately in response to injections of hypertonic saline that induce intracellular dehydration. This is in spite of appropriate arginine-vasopressin responses, which under normal circumstances induce thirst (Clark *et al.* 1991b). Although this would provide some indication of failure to respond to changes in internal state, LH lesioned rats have been shown to respond appropriately to 24hr food and water deprivation (Clark *et al.* 1990). Winn (1995) has suggested that this difference in responding to dehydration and glucoprivation induced by deprivation or i.p. injections is due to the different cues available. When thirst and hunger are induced by injections the only cues available are those from the viscera whereas in food and water deprivation, the animals may also be aware that their source of food and water are missing. It might be the case that while lesioning the LH does not impair the ability to monitor the postingestive consequences of food, it may impair the behavioural responses to them.

Alternatively, the hyperphagia may be due to disruption of an another aspect of feeding behaviour which, the LH has already been implicated in, sensory specific satiety (Rolls *et al.* 1986). Sensory specific satiety limits the degree to which a particular substance is eaten. It was found that the firing rate of LH neurones in the monkey corresponded to behavioural responses to satiety, measured as

rejection of the food item (Rolls *et al.* 1986). Thus, during the course of testing, the responsiveness of LH neurones to a particular food substance declined with the transition from hunger to satiety until a point was reached where firing rate had returned to baseline levels and the food substance was rejected. However, just as the monkey accepted different food substances which had not been fed to satiety, LH neurones responded to the sight or taste of such food. Hence it appeared that the LH may have a role in limiting ingestion in a manner that was dependent on the sensory qualities of the food presented. Disruption of such a phenomenon could potentially alter dietary composition and thus could account for the results found presently. Although satiety for palatable mash may well have been reached in the exposure period for mash in the testing paradigm used presently, this would not necessarily have excluded the consumption of chow on returning to the home cage. However, if this sensory specific satiety effect was disrupted by lesioning the LH, this could have lead to such a high level of consumption of the mash that less of the alternative food supply could be eaten.

Summary

It appears that neither acquisition of a conditioned taste aversion or benzodiazepine induced hyperphagia are dependent on an intact LH. However, examination of the results revealed perturbations in energy regulation and taste processing. A long-term study of energy regulation would provide information on the changes in energy intake after surgery. Presently, energy intake was only monitored for 28 days after surgery at the end of which the LH lesioned group had recovered energy intake to levels no different from the sham lesioned group and then resumed 70 days later when it was found that energy intake was greater for the LH lesioned group. It may be the case that energy intake increased until a plateau was reached and remained steady there after, or it may be the case that it there was a constant steady increase. Analysing any concomitant changes in body weight would give an indication of whether or not this change in energy intake was sufficient to alter body weight.

Although the present experiments also indicate a shift in taste perception, it would be necessary to examine this systematically in order to elucidate the role of the LH in taste processing. In addition to examining taste perception, it would

be interesting to study behaviour when given a choice of palatable substances simultaneously. It may be the case that lesioning the LH impairs the ability to disengage from behaviours once they are initiated and thus would not switch responding from one substance to another to the same degree as control subjects. If true, this could explain the overeating of the palatable mash found presently; it may simply be that they were persevering in feeding despite its inappropriateness.

TASTE PERCEPTION

Taste cues are important in guiding ingestive behaviour. Not only do they enable simple discrimination between different food substances, but by association with other cues, for instance environmental cues and internal cues they can guide ingestive behaviour in relation to previous experience and internal physiological state. Both positive and negative conditioning of taste has been shown experimentally (Reilly *et al.* 1993), phenomena which may be important for selection of substances of nutritional importance and avoidance of poisonous substances under more natural circumstances. Under experimental conditions, if ingestion of a substance was followed by illness this resulted in avoidance of it on further exposures thereby preventing further intake of the potentially malaise-inducing substance (Scalera *et al.* 1997). In the opposite direction the palatability of a tastant solution can be enhanced or even changed from aversive to preferred if the animal is depleted of a nutrient it contains. For instance, concentrations of salt solution which were rated aversive while sodium replete were preferred under conditions of sodium depletion (Berridge *et al.* 1984) and similarly, preference for a solution containing the amino acid lysine increased when rats were changed from a lysine containing to a lysine free diet (Tabuchi *et al.* 1991). Thus although taste is a constant, the palatability of tastes can be altered either positively or negatively. The result of a change in palatability can change not only behavioural responses but endocrine responses too. In addition to oral responses, placement of food into the mouth has endocrine consequences such as an increase in blood insulin. Berridge *et al.* (1981) illustrated that by pairing an insulin inducing substance with LiCl, not only was the behavioural response changed but the increase in insulin release was also abolished.

Not surprisingly, many studies of taste guided behaviour have concentrated on the PBN, the second gustatory relay in the rat. Although a number of studies have illustrated that rats with PBN lesions were not ageusic, under particular conditions responsitivity to sapid stimuli was blunted. Flynn *et al.* (1991b) measured taste preference using a one bottle 15min intake test with 18hr water-deprived rats and taste reactivity with non-deprived rats. Results of the intake test showed little change in total volume of the tastant solutions drunk as a result

of lesioning the PBN, but the preference aversion curves for all the tastants deviated from those expressed by control rats. When taste reactivity was measured for the same tastant solutions in the same group of rats, only two tastant solutions induced ingestive responses in PPN lesioned rats which were different from those induced in control rats; responses to salt and HCl solutions were unaffected by lesioning the PBN while sensitivity to sucrose and quinine HCl was reduced. The two different tests appeared to produce differing results although this may be accounted for by the different deprivation states at the time of testing. Spector *et al.* (1993) reported changes in concentration dependent licking as a result of lesions of the PBN but only under specific conditions. Responsiveness to salt was decreased only when rats were tested in a water-deprived state but was unchanged when tested in a non-deprived state whereas the reverse was true for sucrose. Responsiveness to sucrose was reduced by lesioning the PBN when rats were tested in a non-deprived state and was unchanged when tested in a deprived state. Caution was advised in the interpretation of these results due to the possibility of ceiling and floor effects. Salt intake even by control rats was very low in the non-deprived state leaving little scope to see a reduction caused by PBN lesions and consumption of sucrose when deprived was so high that it is possible that the control rats may not have been physically able to increase licking to a greater degree than that induced in PBN lesioned rats. Spector (1995) provided further evidence that lesioning the PBN does not render rats ageusic by illustrating that although taste-guided quinine responsiveness was attenuated in water-deprived PBN lesioned rats it was not abolished.

Although it appears that at least under particular conditions lesioning the PBN does not result in an inability to taste, it has been shown to consistently abolish some associative taste guided behaviours indicating a deficit in associative processing or the production of a behavioural response to it. Lesioning the PBN has repeatedly been shown to prevent acquisition of a conditioned taste aversion whether the lesions were produced by electrolytic means or using excitotoxins indicating that the deficit was due to loss of cell bodies intrinsic to the PBN (Flynn *et al.* 1991a; Scalera *et al.* 1995). Expression of a sodium appetite when depleted of sodium has also been shown to be abolished by lesioning the PBN

when sodium appetite was measured by either taste reactivity or intake tests (Flynn *et al.* 1991b; Scalera *et al.* 1995). These tests both required a behavioural adjustment based in part on integration of taste and visceral cues. It is important to note however, that this deficit may be specific to certain behavioural paradigms as opposed to a general failure to integrate behaviourally significant cues. Rats with lesions of the PBN have been shown to form a conditioned flavour preference and a conditioned place aversion to LiCl, the US used in the CTA paradigm (Reilly *et al.* 1993). Together these provide evidence that despite failing to acquire a conditioned taste aversion, both the unconditioned stimulus (LiCl) and conditioned stimulus (tastant solution) used in this paradigm could be used to guide behaviour in alternative behavioural tests which also required the association of different cues. Further evidence of an ability to respond to changes in internal state is provided by the findings that rats with lesions of the PBN increased consumption in response to injections of 2-deoxy-D-glucose or insulin and suppressed consumption after nutritive stomach loads (Flynn *et al.* 1991a).

Thus, impairment induced by lesions of the PBN cannot be classed as a general disorder in associating behaviourally significant cues. Neither does the ability to change behaviour in relation to these associations appear to depend on whether they induce or inhibit behaviour: conditioned taste aversion causes a decrease in the palatability of the stimulus whereas salt appetite increases the palatability of the stimulus, both paradigms which the PBN produces deficits in. Moreover, it is not possible to correlate deficits in one behavioural paradigm with deficits in another. Spector *et al.* (1995) tested a group of PBN lesioned rats which had all previously failed to acquire a CTA in a detectabililty task. Only a third had elevated thresholds compared to controls while one third had marginal impairments and the remaining third were no different from controls. This suggests that the neural circuitry subserving different taste guided behaviours may to some extent be independent.

The contribution of the first gustatory relay in rats to taste related behaviour has also been considered. In contrast to PBN, the greater deficits produced by lesions of the nucleus of the solitary tract (NTS) appear to lie in the area of

discrimination of tastes as opposed to association of cues. Shimura *et al.* (1997) extensively tested the involvement of the NTS in taste reactivity using the same method and apparatus that Spector *et al.* (1993) had used to test PBN lesioned rats. Although Shimura *et al.* (1997) extended the range of tastant solutions studied, comparison of the solutions which overlap between the studies provides evidence of a difference in the extent of the deficits induced by these two lesions of the gustatory system. While lesions of the PBN did not significantly alter responsiveness to sucrose solutions and merely attenuated responding to salt solutions when rats were tested in a water deprived state, lesions of the NTS abolished responding to increasing concentrations of both sucrose and salt. In further contrast to PBN lesioned rats, these same NTS lesioned rats expressed a significant salt appetite and acquired a conditioned taste aversion.

In interpreting lesion studies of both the NTS and PBN it is important to consider that by removing either of these nuclei, the gustatory information reaching the forebrain is also compromised. Thus, the results may be due to either loss of these structures or loss of higher processing or even both. Decerebrate rat studies demonstrated that even when the PBN and NTS were intact there were deficits in taste guided behaviour as a result of loss of forebrain connections. Grill *et al.* (1986) demonstrated that although physiological responses to changes in internal sodium state (measured as the level of sodium excretion) were comparable between decerebrate and control rats, the decerebrate rats expressed no behavioural responses to changes in internal sodium state. In contrast, the control rats altered the volume of salt solutions drunk and the number of taste-reactivity responses to salt solutions according to internal sodium state. This indicated that the behavioural responses to changes in internal sodium state were dependent on forebrain mechanisms.

The contribution of the forebrain to taste guided behaviour has been analysed. For instance, the involvement of the VPMpc in the paradigms already described has been studied revealing that electrolytic lesions of this region decreased sucrose intake in a 15min intake test, impaired behavioural expression of a salt appetite but did not prevent increased food intake after injections of insulin and 2-deoxy-D-glucose (Flynn *et al.* 1991a; Flynn *et al.* 1991b). Nevertheless, much

of this work is tainted by the fact that electrolytic lesions were employed making interpretation of the results difficult. Dunn & Everitt (1988) have attributed deficits first thought to be due to loss of the amygdala to the insular cortex. They illustrated that although electrolytic lesions of the amygdala disrupted acquisition of a conditioned taste aversion, selective lesioning of cells intrinsic to the amygdala did not. They reported that it was destruction of areas which received afferents which traversed the amygdala, rather than the amygdala itself that disrupted it. In similarity Scalera *et al.* (1997) dispute the involvement of the VPMpc in conditioned taste aversion showing that while electrolytic lesions disrupted acquisition of a conditioned taste aversion, excitotoxic lesions did not.

The involvement of the LH has been studied with regard to taste perception but with equivocal results. Ferssiwi *et al.* (1987) reported a downward shift in taste preference induced by lesioning the LH while Clark *et al.* (1990) found no difference in taste processing. The use of different excitotoxins to produce the lesions in the two studies described may account for the disparity in results. Ferssiwi *et al.* (1987) used ibotenic acid to lesion the LH introducing difficulties in interpretation since Winn *et al.* (1984) showed that ibotenic acid injected into the LH also caused damage in the amygdala and was associated with increased levels of dopamine in the nucleus accumbens and the caudate-putamen. Neither of these effects were reported with LH lesions induced by NMDA (Hastings *et al.* 1985), the excitotoxin used by Clark *et al.* (1990). Since Clark *et al.* (1990) showed no deficit in taste perception by rats with NMDA lesions of the LH, it may be inferred that more specific lesions of the LH did not affect taste perception. However, in a study of conditioned taste aversion NMDA LH lesioned rats overconsumed a saccharin solution on first exposure to it (Experiment 1.1 "Conditioned Taste Aversion"), a characteristic which has also been noted in similar experiments with PBN lesioned rats (Scalera *et al.* 1995). Moreover, this same group of LH lesioned rats were shown to overconsume a palatable mash indicating that taste processing may in fact have been compromised in some way. In order to elucidate the role of the LH in taste processing, LH lesioned rats were presently tested in two different intake tests for the tastant solutions saccharin and salt. In addition, home cage behaviour was monitored to provide information on body weight and food and water intake

after surgery since previous experiments indicated that it could be a change in energy regulation which was responsible for the impairments found in intake tests.

Pilot Taste Preference

A pilot study of taste preference for salt and saccharin was run in order to gain initial information on taste preferences in naive rats. Twelve naive male Lister Hooded rats (Charles River) were individually housed under a 12h light/dark cycle with ad lib food (SDS maintenance diet no. 1 chow pellets) and tap water in their home cages except when indicated. Preferences for 6 concentrations of salt (0.025M, 0.05M, 0.1M, 0.2M, 0.4M, 0.8M) and saccharin (0.05%, 0.1%, 0.2%, 0.4%, 0.8%,1.6%) were tested in the home cage. During the test period, the test solution was the only fluid the rat had access to. The rats were exposed to each concentration for 24h with 24h between exposures. The test solutions were made fresh daily by series dilution and the order in which the rats were exposed to them was counterbalanced. Half the rats were exposed to salt followed by saccharin and half the rats were exposed to saccharin followed by salt. The results of the pilot taste preference tests were used to select concentrations of salt and saccharin solutions to be used in further tests of taste preference both in the home cage and in drinking boxes described below

Pilot Choice Taste Preference

Eight rats were used to test 2 identical drinking boxes (described in Experiment 2.1: The Role of the LH in Taste Perception, pg). Access was given to a range of salt or saccharin solutions simultaneously and the volume consumed of each was measured. In addition to volumes consumed, behavioural measures of number of initiations to drink and duration of drinking were measured using "Observer"[1]. The results of the pilot choice taste preference study indicated that the drinking boxes were suitable for studying drinking behaviour in rats. In addition, it appeared prudent to fill the drinking burettes with a palatable solution during the habituation phase in order to train the rats to drink from them.

[1] "Observer" is a Noldus Information Technology software package which allows the allocation of timer switches and counts specific key presses enabling the user to record the frequency and duration of various behaviours simultaneously.

Experiment 2.1: The Role of the LH in Taste Perception

METHODS

Animals

Twenty male Lister Hooded rats (Charles River) were individually housed under a 12h light/dark cycle with ad lib food (SDS maintenance diet no. 1 chow pellets) and tap water in their home cages except when indicated. Daily measurements were taken of body weight, food remaining in the food hopper, water remaining and food spillage (collected on foil sheets). On the basis of this information 10 of the rats were assigned to the sham lesioned group and 10 were assigned to the LH lesioned group whose average weights were 344.61g (SD 10.46) and 344.04g (SD 17.27) respectively.

Surgery

Animals were anaesthetised by i.p. injection of 20ml/kg avertin (10g tri-bromo-ethanol, 5g tertiary amyl-alcohol, 500ml 0.01M phosphate buffer, 4.5g sodium chloride). Since avertin can cause the intestines to adhere to the abdominal wall thereby preventing the passage of faecal matter (producing a syndrome known as "bloat") the injection of avertin was followed by an i.p. injection of 6.3% glucose solution (20ml/kg), a procedure which has been found previously to have some preventative power against this. The rats were placed in a stereotaxic frame with the incisor bar 5.0mm above the interaural line. Animals received a simultaneous bilateral infusion of either 1μl 0.06M NMDA or 1μl phosphate buffer. NMDA (Sigma) was dissolved in 0.1M phosphate buffer (pH 7.4) and pH adjusted to pH 7.4 with 0.2M NaOH. The infusion rate was 0.5μl/min with a further 2min period allowed for diffusion. The co-ordinates for cannulae were anterior-posterior 0mm from bregma; medial-lateral ±2.0mm from midline; ventral 8.0mm from dura.

Post-Operative Care

Two LH lesioned rats died shortly after surgery. Body weight, food intake and water intake for the remaining rats was measured for 50 days after surgery and, if

body weight fell below 85% pre-surgery weight, these individual rats were given wet mash (Farley's dry baby food and glucose) which was made up fresh daily. All 8 of the LH lesioned rats received wet mash for a variable number of days ranging from 2-5 with an average of 2.75. Consumption of lab chow and tap water was monitored during this period and if the rat resumed normal eating and drinking wet mash was removed. This resulted in wet mash being removed before the rat's body weight returned to 85% of pre-surgery body weight but they were found to be capable of maintaining their lower body weight without any further dietary supplements.

Hypertonic Saline Challenge

The rats were given a hypertonic saline challenge 54 days after surgery. All rats received an i.p. injection of both hypertonic saline (5%, 20ml/kg) and isotonic saline (0.9%, 20ml/kg), with 48h between injections. Injections were given in a counterbalanced order and tap water consumed 1h and 3h after injection was measured.

Taste Preference

The rats were tested for salt and saccharin taste preferences 95 days post-surgery using concentrations based on the findings of the pilot study. The concentrations of saccharin solution used were: 0.01%, 0.06%, 0.36%, 2.16% and the concentrations of salt solution used were: 0.02M, 0.06M, 0.2M, 0.6M. Water bottles in the home cage were replaced with a bottle of solution and 24h consumption was measured. There was a 24h washout period between exposures where *ad lib* water was available. Exposure to the different solutions was in a random order; animals were given salt then saccharin on alternate test days in a random order of concentration and half were first exposed to salt then saccharin and half were exposed to saccharin then salt.

In order to gain a similar range of preferences for saccharin as that seen for salt, it was necessary to use a range of concentrations seven times greater for saccharin than salt. At the end of the test period it was decided to use a further 3 concentrations of saccharin which were 0.03%, 0.18% and 1.08% to gain further information on this wider curve. These were given in a counterbalanced order.

Choice Taste Preference

Salt and saccharin taste preferences when given a choice of 4 different concentrations simultaneously was examined 131 days after surgery using 2 identical drinking boxes. These consisted of metal boxes lit by a house light (2.5W, 24V). They had a mesh floor under which was a tray of sawdust. On one wall there were 4 holes, each with a drinking spout connected to a burette immediately behind the hole. These burettes were filled with a range of salt or saccharin solutions and the volume consumed of each was measured. The concentrations of salt used were: 0.02M, 0.06M, 0.2M, 0.6M and the concentrations of saccharin used were: 0.01%, 0.06%, 0.18%, 2.16%.

Sessions in the test boxes lasted 30min and were at 48h intervals. Animals were first habituated to the boxes where all 4 drinking tubes were filled with water and later trained to drink from the tubes by filling all the tubes with 0.18% saccharin, a concentration found to be optimally preferred in the previous tests. The rats were then tested for saccharin and salt preference with half tested for salt preference and half tested for saccharin preference on day 1.

The order in which the 4 concentrations were presented at the 4 tubes was counterbalanced. Analysis of the habituation and training sessions revealed that there was no preferred tube to drink from across the groups and as a result the order in which the solutions were presented was counterbalanced across the whole group rather than individually. In box 1 the solutions were presented in an incremented order with the lowest concentration in tube 1 and the highest concentration in tube 4 whereas in box 2 the solutions were presented as: tube 1 - concentration 2; tube 2 - concentration 1; tube 3 - concentration 4; tube 4 - concentration 1. The box each rat was tested in for salt and saccharin preference was counterbalanced across the groups with half of each lesioned group tested for salt and saccharin preference in box 1 and half tested in box 2.

Deprivation Choice Taste Preference

24h after completion of testing the rats in a non-deprived state, the rats were put on a 22h water deprivation schedule. The choice taste preference test was then repeated with the rats in a water deprived state.

Histological Analysis

At the end of the study all the experimental rats were given an overdose of 200mg/ml pentobarbitone (Euthatal) and were subsequently perfused transcardially with physiological saline followed by 10% formalin. The brains were then removed and stored in 10% formalin until they were sectioned on a freezing microtome. 25μm sections were cut at 200μm intervals and stained with cresyl violet stain for nissl substance. Lesions were identified by areas of cell loss and reactive gliosis. In order to ascertain lesion volume, histological sections were scanned and then silhouettes of the areas of the lesion were drawn onto the scanned sections. The areas of the lesioned tissue was determined using a microcomputer and lesion volumes calculated.

Statistical analysis

The results were examined statistically using repeated measures analysis of variance (ANOVA) with, when appropriate, Tukey's post hoc tests.

RESULTS

Histological Analysis

One rat was found to have a unilateral lesion and was excluded from all analysis. Figure 16 illustrates the size and pattern of the biggest and smallest lesions produced. The average size of the lesions was 2.12mm^3 (SE 0.20) on the left side and 2.13mm^3 (SE 0.29) on the right side with the greatest damage level with the paraventricular nucleus and decreasing towards the anterior and posterior poles. The majority of lesions started level with the CA3 region of the hippocampal formation and ended level with the subthalamic nucleus. There was little extra-hypothalamic damage: all 7 LH lesioned rats showed slight bilateral damage to the zona incerta, reticular thalamic nucleus and subincertal nucleus. In addition, 6 of the rats showed slight unilateral damage to the ventromedial thalamic nucleus and minimal bilateral damage to the subthalamic nucleus. Unilateral damage was found in the ventrolateral thalamic nucleus of 4 rats. In 2 animals unilateral tract damage was found in the anteroventral thalamic nucleus. More substantial unilateral damage was found in the anteroventral thalamic nucleus of 1 rat in addition to unilateral damage to the border of the inter anterodorsal and posterolateral thalamic nuclei and the nucleus stria medullaris. In 2 rats damage travelled unilaterally to the substantia innominata and in one instance damage occurred as dorsal as the globus pallidus unilaterally. Analysis of the behavioural results showed no difference between the rats with differing amounts of extra-hypothalamic damage

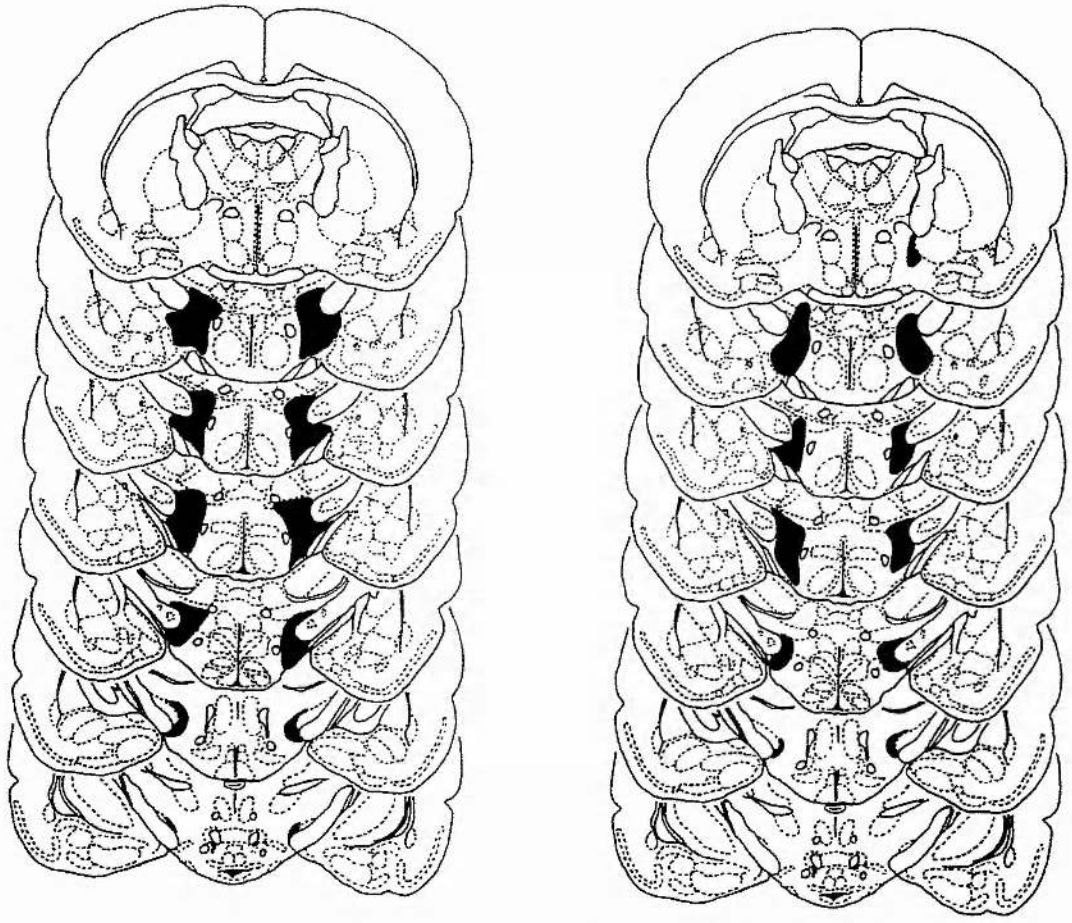


Figure 16. Representative sections redrawn from the atlas of Paxinos & Watson (1982) illustrating the extent of the biggest and smallest lesions produced by injection of NMDA into the LH. The shaded regions represent areas of neuronal loss.

Home Cage Regulatory Behaviour

Pre-Surgery Measures

Body weight and food and water intake before and after surgery are shown in Figures 17, 18 and 20. It can be seen that in the week preceding surgery there were no differences between the LH lesioned and sham lesioned groups in respect of body weight ($F(1,17) = 0.01$), food intake ($F(1,17) = 0.23$) or water intake ($F(1,17) = 1.21$). Both the sham lesioned and LH lesioned groups were gaining weight [main effect of days ($F(7,119) = 287.81$, $p<0.001$)] but ANOVA showed no significant group × days interaction ($F(7,119) = 0.45$) indicating that they were gaining weight at similar rates. There were main effects of days for food intake ($F(7,119) = 7.88$ $p<0.001$) and water intake ($F(7,119) = 3.64$ $p<0.001$) but ANOVA of these data revealed no significant interaction between groups and days for food intake ($F(7,119) = 0.65$) or water intake ($F(7,119) = 0.95$) suggesting that daily changes in either factor were seen for both groups.

Post-Surgery Measures

Statistical analysis of post-surgery home cage behaviour only included the first 50 days after surgery thus analysing the effect of surgery before further experimental manipulations were executed.

Body Weight

Following surgery, the LH lesioned rats lost weight and their body weight remained lower than the sham lesioned rats [main effect of group ($F(1,15) = 76.21$ $p<0.001$)] for the duration of the study as seen in Figure 17. There was a significant main effect of days ($F(49,735) = 59.47$ $p<0.001$) as the rats recovered from surgery and a significant group × days interaction ($F(49,735) = 30.01$ $p<0.001$) suggesting that the degree to which the groups recovered differed across days. In addition to the greater deficit in body weight seen with the LH lesioned group immediately after surgery, the difference in body weight between the groups continued to increase for the duration of the study.

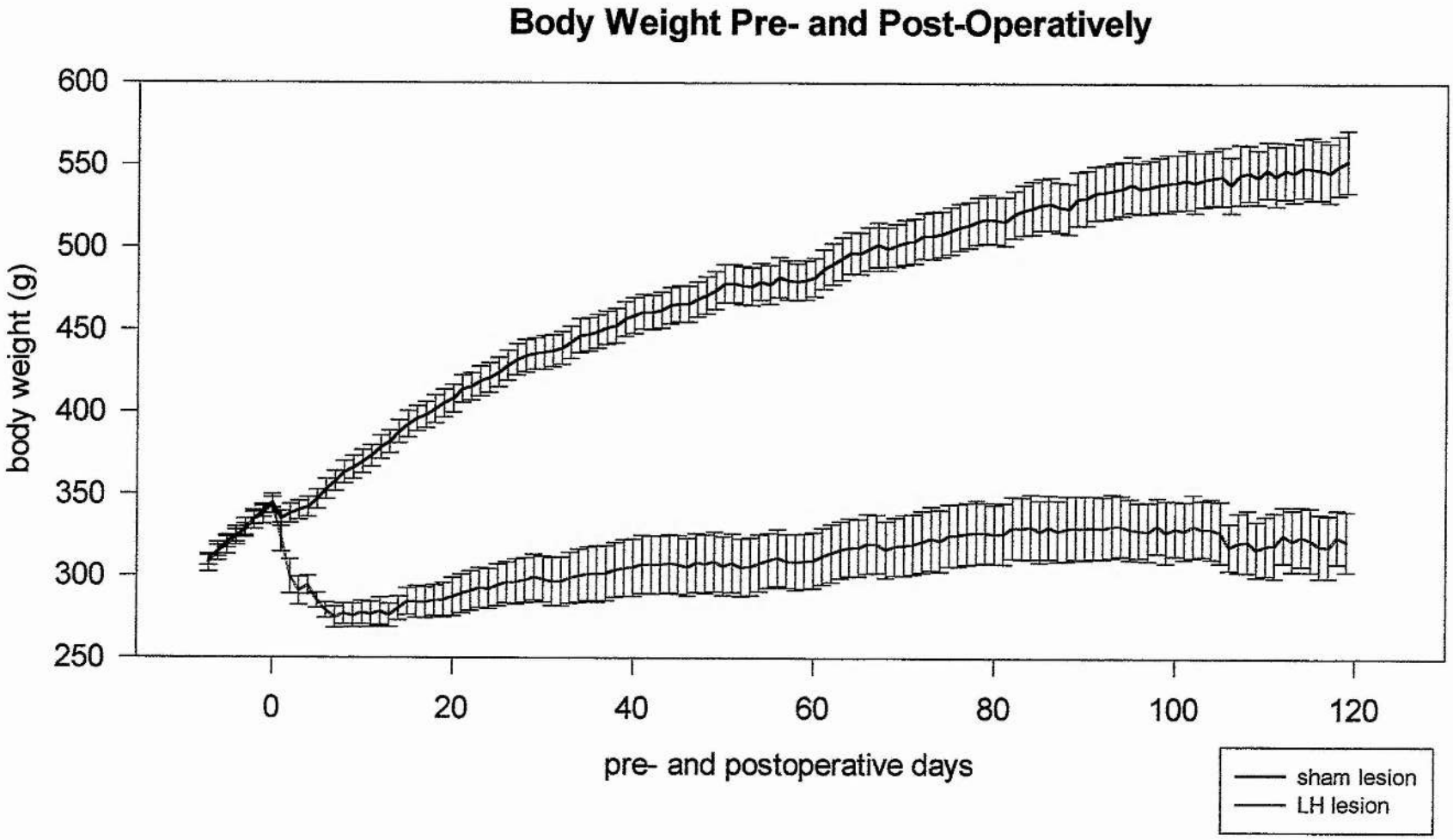


Figure 17. Mean (± SE) body weight for the sham lesioned group (n=10) and LH lesioned group (n=7) 7 days before and 119 days after surgery.

Food Intake

Figure 18 illustrates that food intake was reduced to a greater degree for the LH lesioned group than the sham lesioned group after surgery [main effect of group ($F(1,15) = 67.96$ $p<0.001$)]. ANOVA showed a statistically significant group × days interaction ($F(49,735) = 10.36$ $p<0.001$) and a significant main effect of days ($F(49,735) = 31.59$ $p<0.001$) indicating that the sham lesioned group made a more full and rapid recovery after surgery in comparison with the LH lesioned group. Nevertheless, Figure 18 illustrates that after the initial recovery period the LH lesioned group maintained a steady level of food intake for the duration of the study.

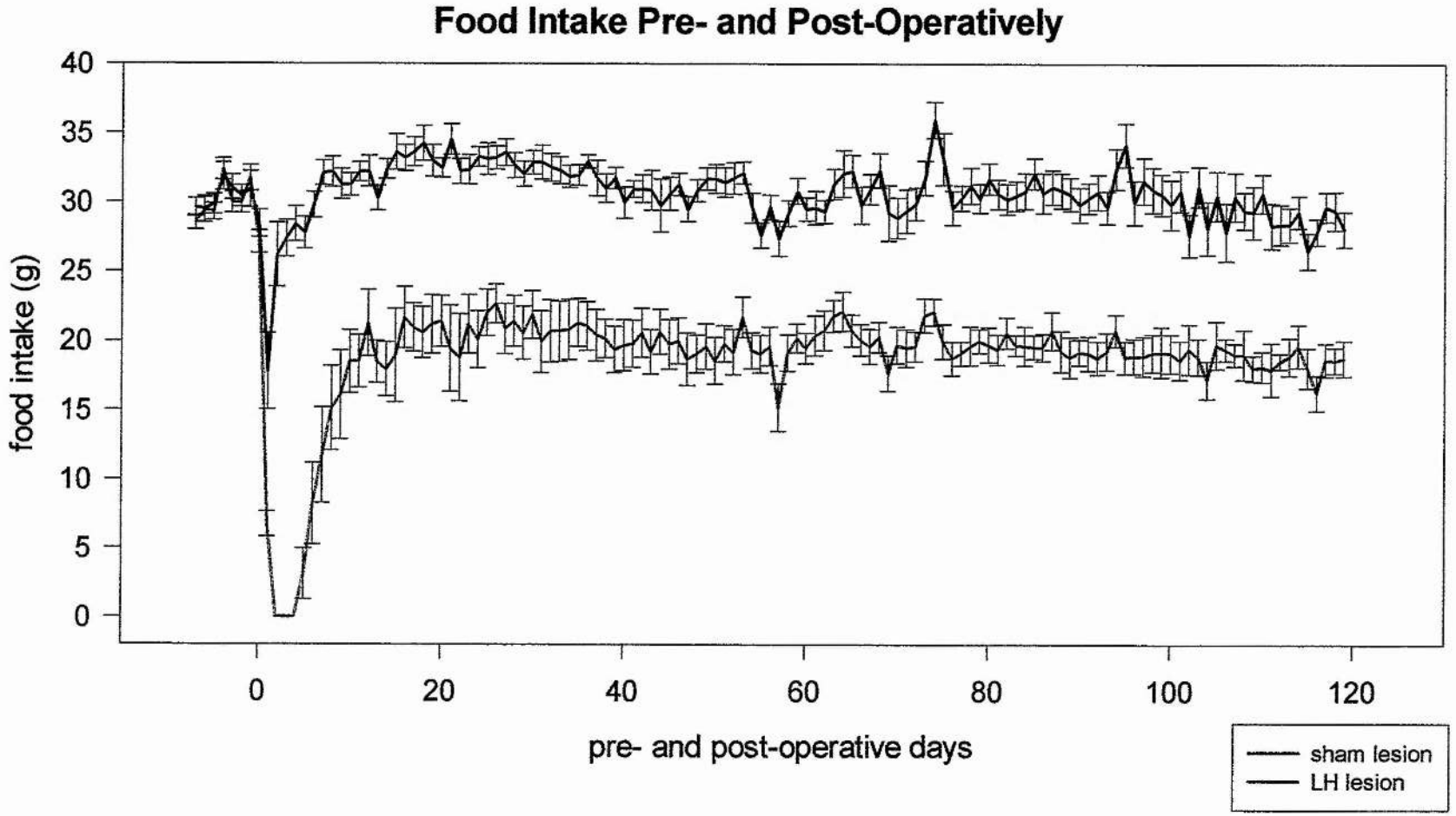


Figure 18. Mean (± SE) food intake for the sham lesioned group (n=10) and the LH lesioned group (n=7) 7 days before and 119 days after surgery.

Food Intake as a Function of Body Weight

Despite the apparent poor recovery with respect to food intake, as illustrated in Figure 19, when the difference in body weight is considered the LH lesioned rats appear to make a more complete recovery after surgery. Like food intake, ANOVA of food intake as a function of body weight before surgery showed no main effect of group ($F(1,17) = 0.27$), no group × days interaction ($F(7,119) = 0.79$) but a main effect of days ($F(7,119) = 14.11$ $p<0.001$). After surgery, ANOVA showed a main effect of group ($F(1,15) = 24.75$ $p<0.001$) a main effect of days ($F(49, 735) = 26.27$ $p<0.001$) and a significant group × days interaction ($F(49,735) = 27.83$ $p<0.001$). Although this indicates that food intake as a function of body weight for the LH lesioned rats decreased to a greater degree than the sham lesioned rats after surgery, Tukey's post-hoc tests showed that there were no significant differences between the groups 15 days after surgery and this relationship remained stable for the duration of the experiment. For the first 9 days after surgery, there were significant differences between the groups ($p<0.001$) after which was an intermediate period where these differences varied between reaching significance ($p<0.05$) and not reaching significance. This is related to the extent to which the LH lesioned group was dependent on wet mash. The initial 9 days after surgery correspond with the period where the majority of LH lesioned rats were receiving wet mash, whereas the number of rats receiving wet mash between days 9-14 was only 2. The last day any rat was given wet mash was day 14 which was also the last day a significant difference was found between the groups. Hence, after an initial recovery period where the LH lesioned rats received food supplements they resumed normal food intake at a level which was not significantly different from that of the sham lesioned rats when their reduced body weight was considered and this remained stable for the duration of the study.

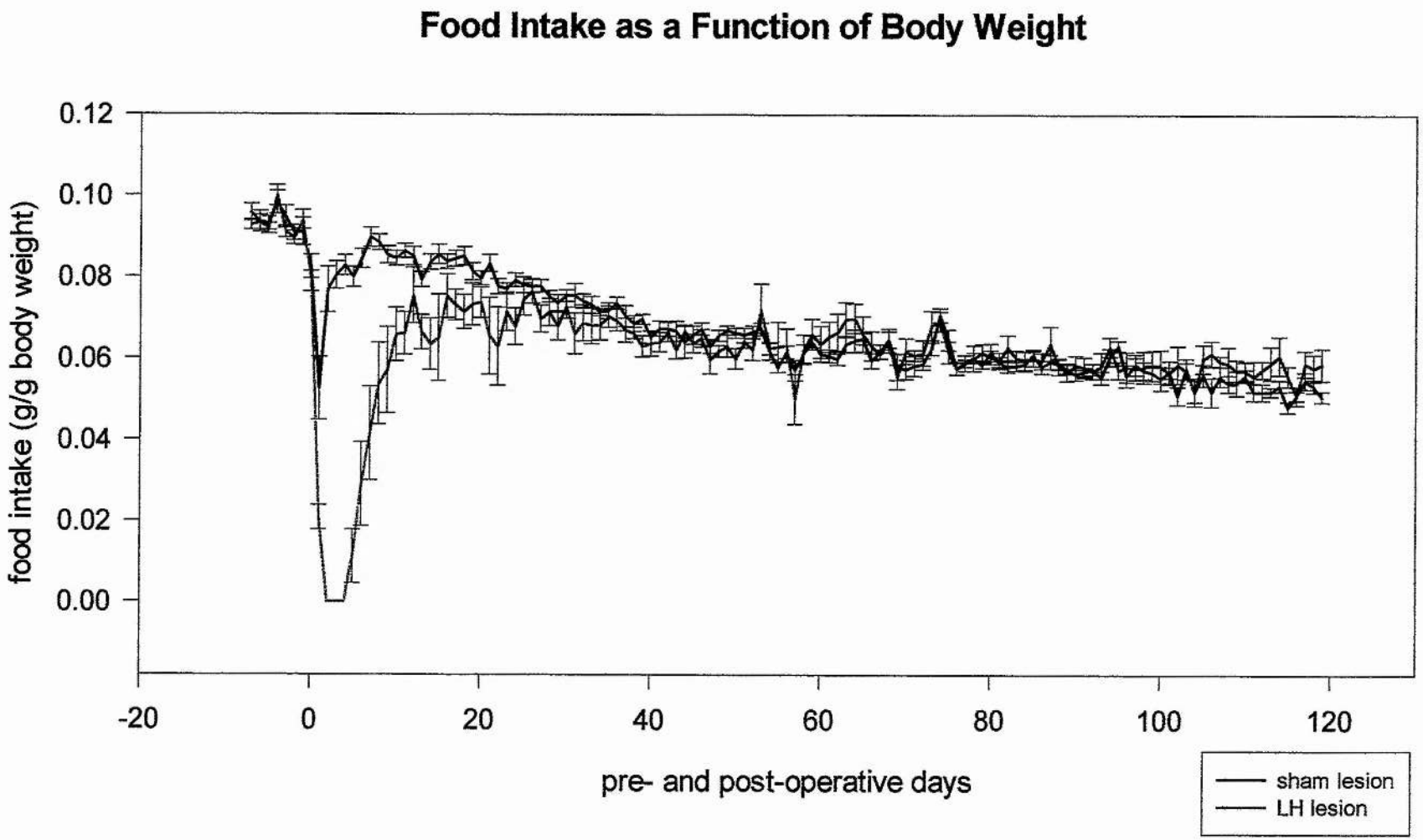


Figure 19. Mean (± SE) food intake as a function of body weight for the sham lesioned group (n=10) and the LH lesioned group (n=7) 7 days before and 119 days after surgery.

Water Intake

The effect of surgery on water intake was similar to that of food intake; water intake for the LH lesioned group was reduced to a greater extent than that of the sham lesioned group [main effect of group ($F(1,15) = 12.03$, $p<0.003$)]. There was a main effect of days ($F(49,735) = 6.31$ $p<0.001$), and a significant group × days interaction, ($F(49,735) = 4.41$ $p<0.001$) suggesting the sham lesioned and LH lesioned groups recovered to different extents. Figure 20 illustrates that water intake for the LH lesioned rats fell to a greater degree than that of the sham lesioned rats following surgery and then recovered to a lower level than the sham lesioned rats which remained fairly stable for the remainder of the study.

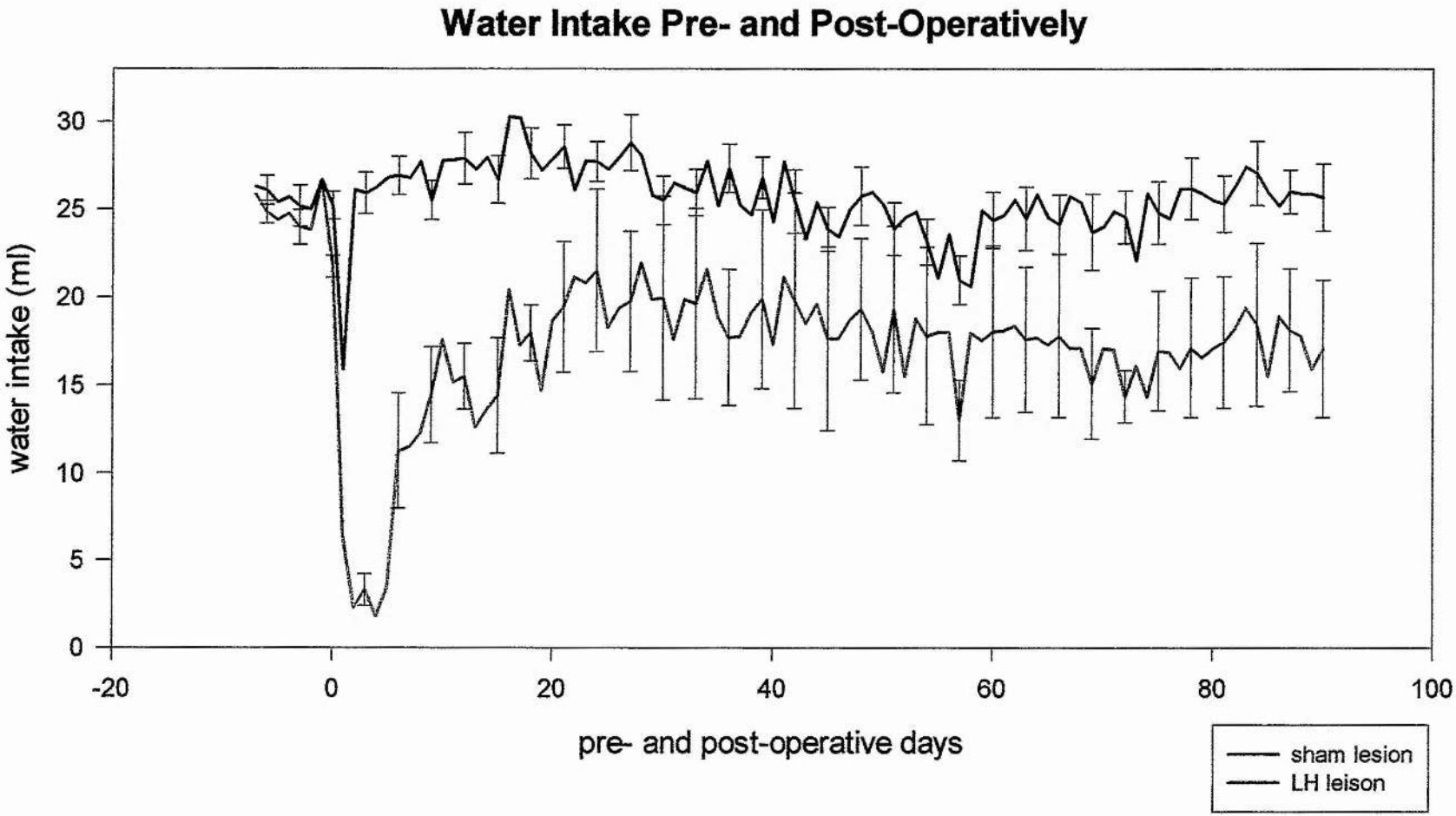


Figure 20. Mean (± SE) water intake for the sham lesioned group (n=10) and the LH lesioned group (n=7) 7 days before and 90 days after surgery.

Water Intake as a Function of Body Weight

Figure 21 illustrates water intake plotted as a function of body weight and suggests a greater degree of recovery by the LH lesioned rats. After surgery, there was no main effect of group in respect of water intake as a function of body weight ($F(1,15) = 2.27$) despite the fact that there appears to be a substantial difference between the groups until day 10. There was a main effect of days ($F(49,735) = 5.87$ $p<0.001$) and a significant group × days interaction ($F(49, 735) = 10.27$ $p<0.001$) indicating that water intake as a function of body weight was not constant over time and the relationship between the 2 groups did not remain stable for the duration of the study. In the period immediately after surgery, intake for the LH lesioned group was lower than that of the sham lesioned group, whereas approximately 20 days after surgery this relationship was reversed; on the majority of days the lesioned group drank more than the sham lesioned group. However, this may not be an accurate representation of the LH lesioned group. Water intake as a function of body weight for each rat is plotted in Figure 22. The range of data for each day is much smaller for the sham lesioned group in comparison to the LH lesioned group. The large range for the LH lesioned group seen from day 1-15 can be accounted for by the fact that a varying number of LH lesioned rats were receiving wet mash during this period which would have had a direct effect on the volume of water consumed. Nevertheless, even after the LH lesioned group recovered normal food intake with respect to the sham lesioned group, the range of values for water intake was still up to ×3 greater than that of the sham lesioned group. From Figure 22 it can be seen that this is mainly due to one rat who, after day 20, consumed double the maximum level of intake seen with any of the sham lesioned rats. Analysis of the histology revealed no apparent differences in lesion volume or placement between this rat and the other LH lesioned rats.

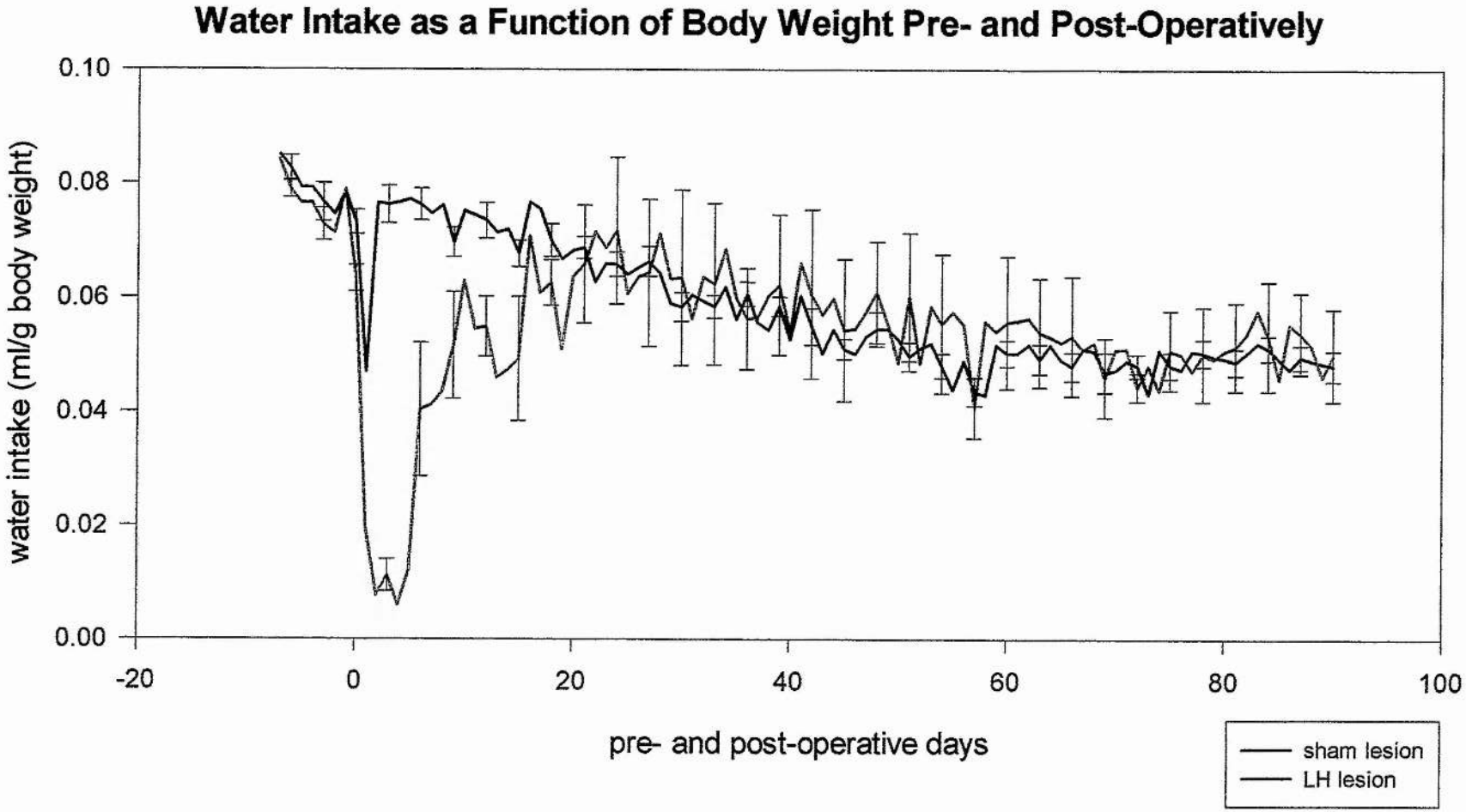


Figure 21. Mean (± SE) water intake as a function of body weight for the sham lesioned group (n=10) and the LH lesioned group (n=7) 7 days before and 90 days after surgery.

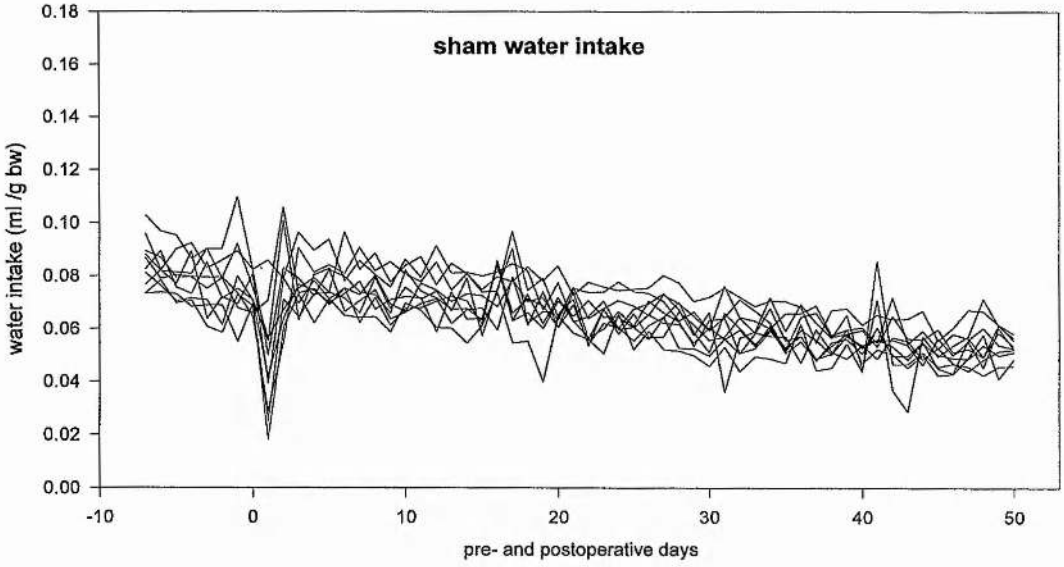


Figure 22 a. Water intake as a function of body weight plotted separately for each sham lesioned rat 7 days before and 50 days after surgery.
Sham lesioned group: n = 10.

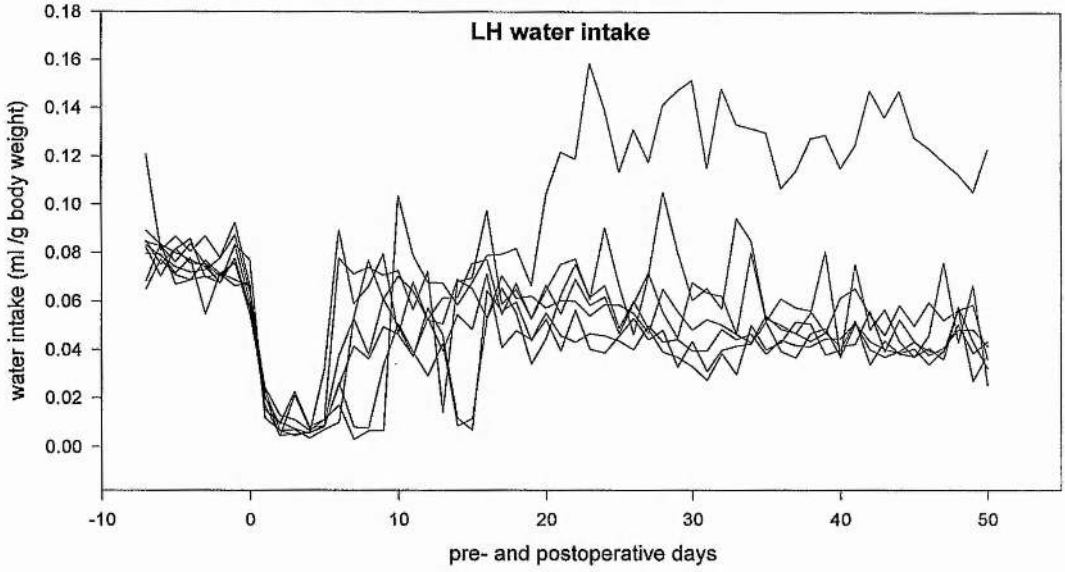


Figure 22 b. Water intake as a function of body weight plotted separately for each LH lesioned rat 7 days before and 50 days after surgery.
LH lesioned group: n = 7.

Hypertonic Saline Challenge

The volumes of water drunk in 3h after i.p. injection of 20ml/kg hypertonic saline (5%) and isotonic saline (0.9%) are shown in Figure 23. ANOVA of these data showed significant main effects of group ($F(1,15) = 30.43$ $p<0.001$) and injection ($F(1,15) = 54.31$, $p<0.001$) and a significant group × injection interaction ($F(1,15) = 23.52$, $p<0.001$). Figure 23 illustrates that hypertonic saline induced more drinking in both the sham lesioned and LH lesioned groups than isotonic saline. However, Tukey's post-hoc tests revealed that although the increase in drinking induced by hypertonic saline was significant for the sham lesioned group ($p<0.001$), the volume consumed by the LH lesioned group after treatment with hypertonic saline did not significantly differ from the volume of drinking induced by isotonic saline in either the sham lesioned group or LH lesioned group. Therefore lesioning the LH abolished the drinking response induced by i.p. hypertonic saline.

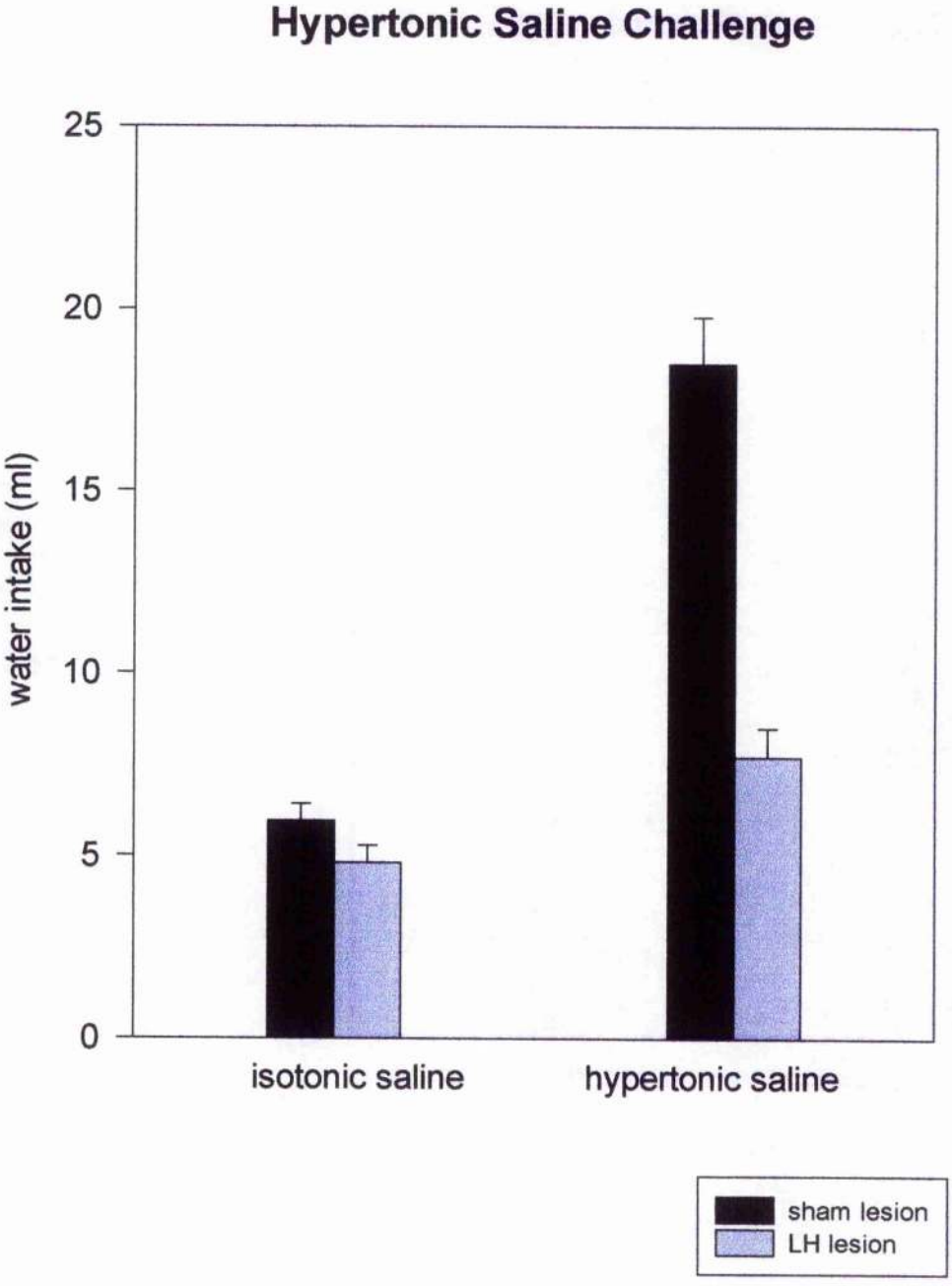


Figure 23. Mean (± SE) volume of water consumed in 3hr following i.p. injections of hypertonic (5%) and isotonic (0.9%) saline for the sham lesioned group (n=10) and LH lesioned group (n=7).

Taste Preference

Due to the substantial difference in body weight between the sham lesioned and LH lesioned groups, fluid consumption in all the preference tests has been expressed as volume per g body weight for the purpose of statistical analysis and graphical presentation. Analysis of the raw data is included in the appendix (pg 266) for direct comparison with other studies.

Saccharin Preference

The saccharin taste preference curve is presented in Figure 24. Repeated measures ANOVA revealed a significant main effect of concentration ($F(6,84) = 24.18$ $p<0.001$) indicating that preference differed over the range of concentrations used. Tukey's post-hoc analysis revealed that the most preferred concentration of was 0.18% saccharin ($p<0.001$). There was no main effect of group ($F(1,14) = 2.18$) and no group × concentration interaction ($F(6,86) = 1.99$) and thus when the difference in body weight between the groups was considered, there appeared to be no difference in perception of taste for saccharin between the sham lesioned and LH lesioned groups.

Salt Preference

Figure 25 clearly shows little difference in salt taste preference between the sham lesioned and LH lesioned groups ($F(1,14) = 0.06$) and no group × concentration interaction ($F(3,42) = 0.71$). ANOVA revealed a main effect of concentration ($F(3,42) = 31.92$ $p<0.001$) and this was further analysed using Tukey's post-hoc analysis which revealed that 0.2M salt was the most preferred salt solution ($p<0.001$). Thus it appears that lesioning the LH does not alter taste perception of salt.

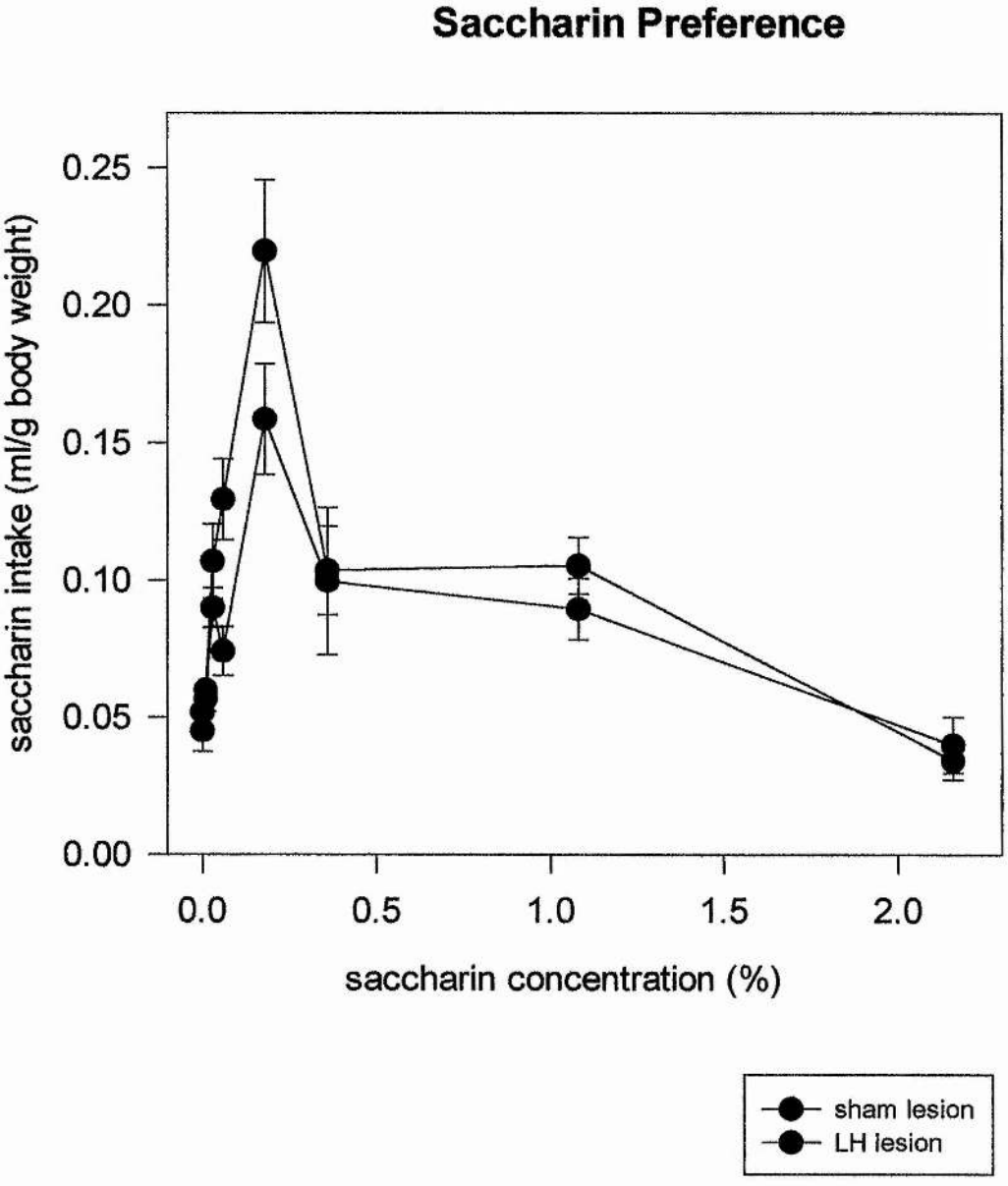


Figure 24. Mean (± SE) volume of water and saccharin (0.01%, 0.03%, 0.06%, 0.18%, 0.36%, 1.08%, 2.16%) drunk as a function of body weight for the sham lesioned group (n=10) and the LH lesioned group (n=7).

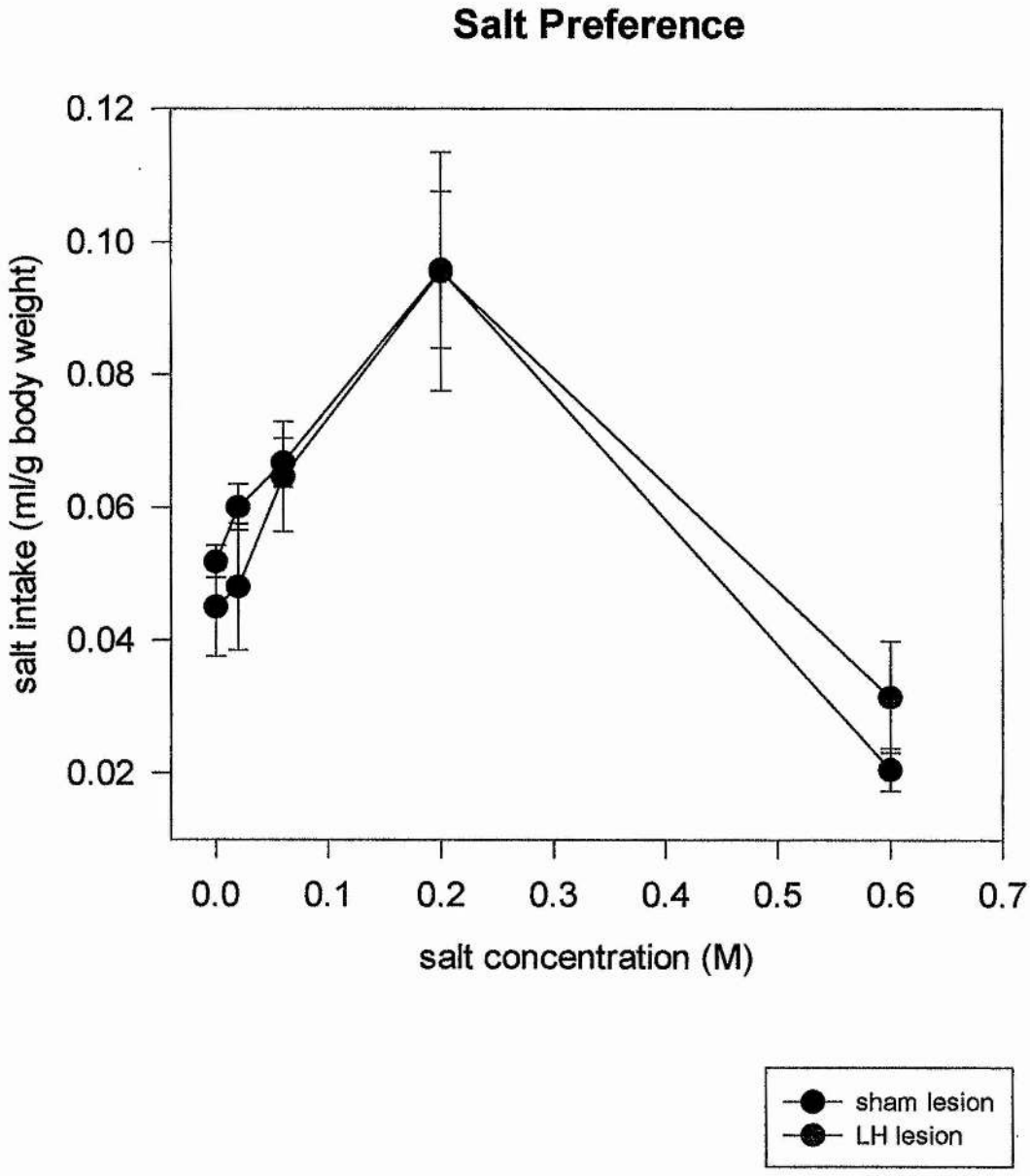


Figure 25. Mean (± SE) volume of water and salt (0.02M, 0.06M, 0.2M, 0.6M) consumed as a function of body weight for the sham lesioned group (n=10) and the LH lesioned group (n=7).

Choice Taste Preference

Choice taste preference with respect to volume drunk, duration of drinking and initiations to drink was analysed using repeated measures ANOVA. Separate analyses were made for each factor with group (sham lesion/LH lesion) as the between subjects factor and concentration and deprivation state (non-deprived/22h deprived) as within subjects factors.

Saccharin Preference

Figures 26, 28 and 30 illustrate taste preference and the pattern of drinking saccharin in a non-deprived state and in a 22h water deprived state.

Volume of Saccharin Solutions Consumed

ANOVA revealed a main effect of concentration ($F(3,36) = 26.68$ $p<0.001$) indicating that taste preference differed over the range of concentrations used. There was no main effect of group ($F(1,12) = 2.71$) and no group × concentration interaction ($F(3,36) = 2.25$) suggesting that lesioning the LH did not alter saccharin taste preference when presented with various concentrations of saccharin simultaneously.

There was a main effect of deprivation state ($F(1,12) = 41.62$ $p<0.001$) but no group × deprivation state interaction ($F(1,12) = 1.48$) indicating that both the sham lesioned and the LH lesioned groups increased drinking in response to 22h water deprivation to similar extents.

ANOVA revealed a significant concentration × deprivation state interaction ($F(3,36) = 10.52$ $p<0.001$). It is clear from Figure 27 that despite the general increase in consumption for all concentrations this was greatest for the optimally preferred concentration.

There was no group × concentration × deprivation state interaction ($F(3,36) = 2.49$).

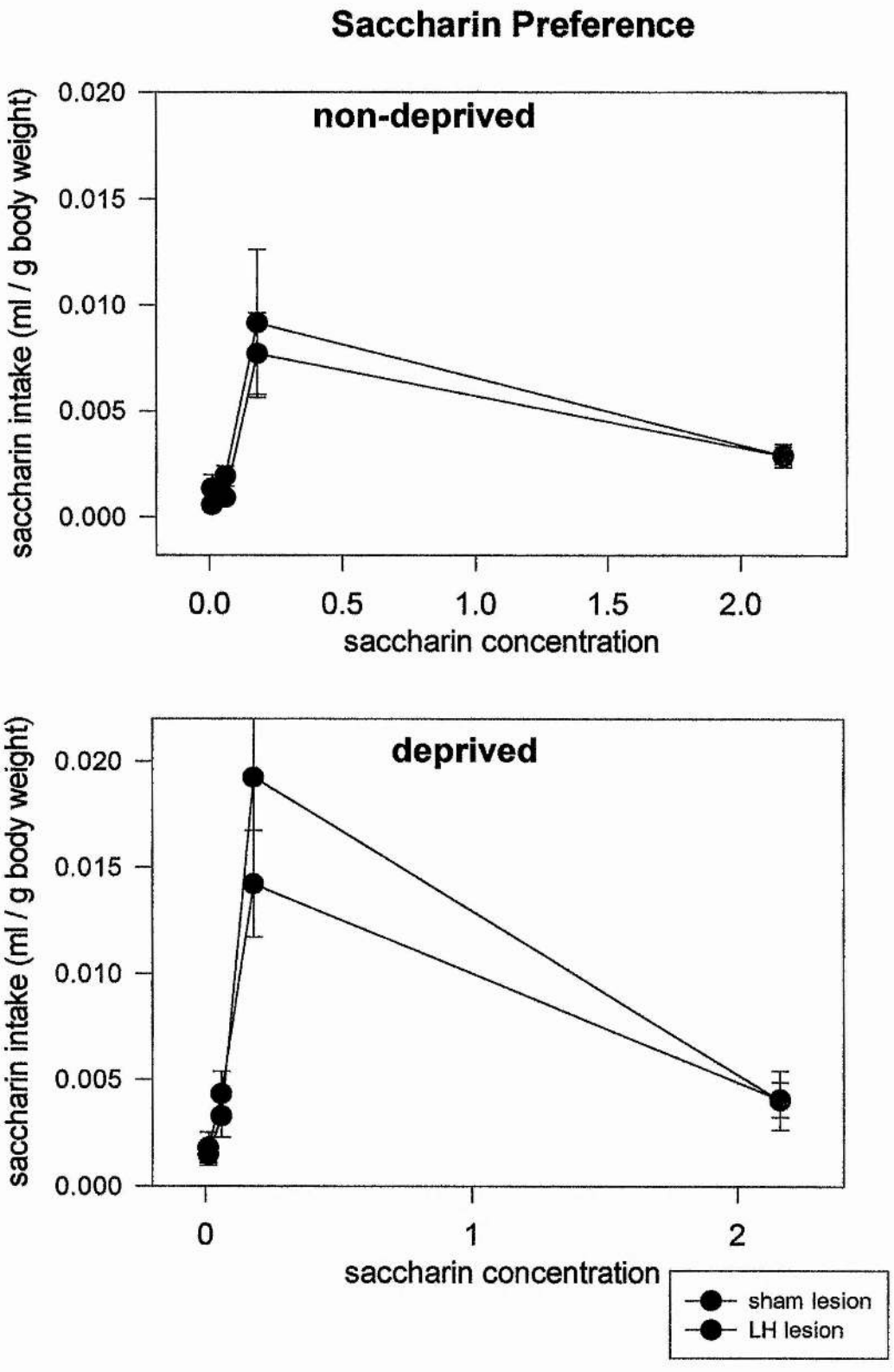


Figure 26. Saccharin taste preference when 22h water deprived and when non-deprived. Mean (± SE) volume of saccharin (0.01%, 0.06%, 0.18%, 2.16%) drunk for the sham lesioned (n=10) and the LH lesioned group (n=6).

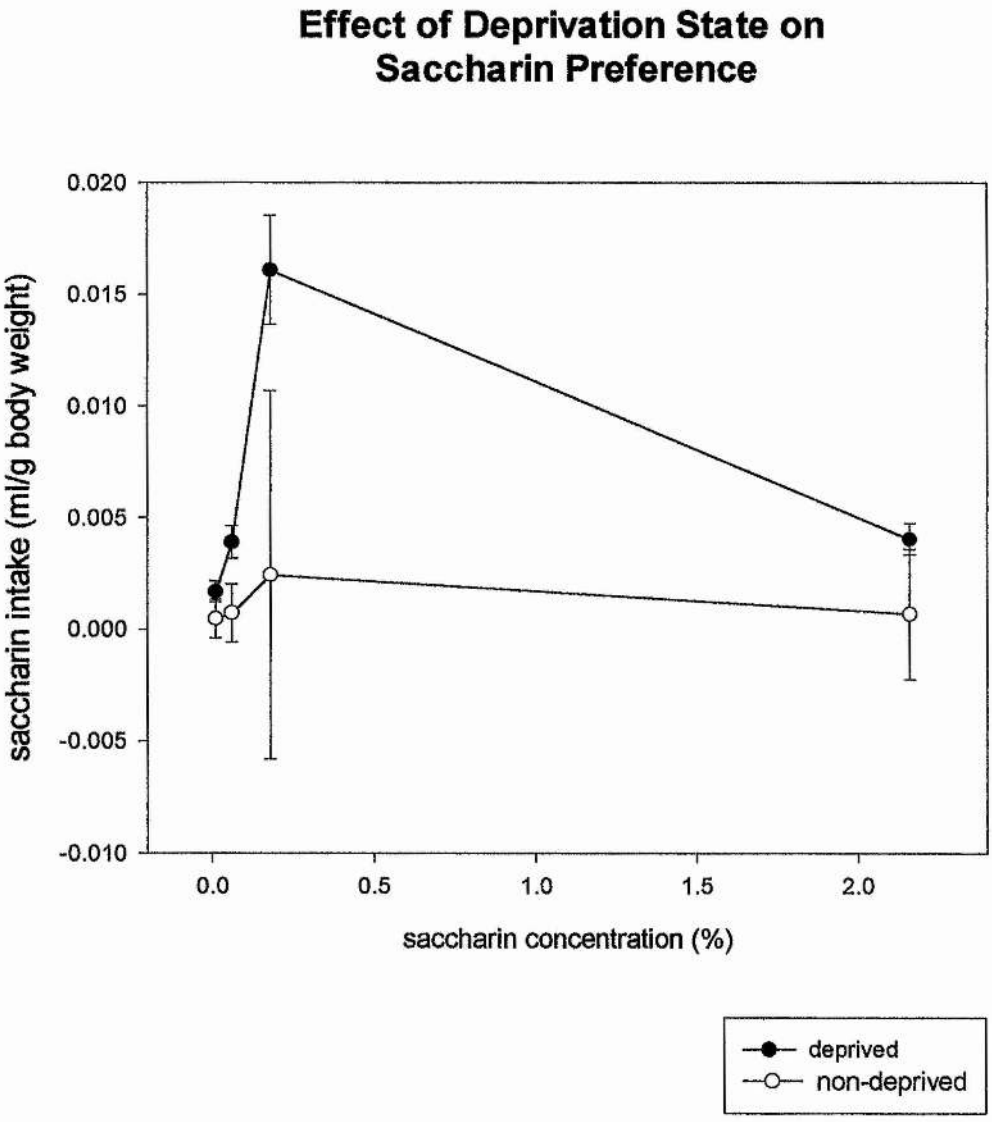


Figure 27. Group (sham lesion\LH lesion) collapsed to illustrate concentration x deprivation state interaction; mean volume of saccharin drunk when non-deprived (n=16) and 22h water deprived (n=16).

Duration of Drinking Saccharin Solutions

ANOVA showed a main effect of concentration ($F(3,42) = 25.04$ $p<0.001$), but no group × concentration interaction ($F(3,42) = 0.63$) and no main effect of group ($F(1,14) = 1.15$) for the duration of saccharin drinking. This suggests that lesioning the LH did not alter time spent drinking over this range of saccharin concentrations.

There was a main effect of deprivation state ($F(1,14) = 20.96$ $p<0.001$) but no group × deprivation state interaction ($F(1,14) = 0.02$) indicating that the sham lesioned and LH lesioned groups increased duration of drinking to the same extent in a deprived versus non-deprived state.

Figure 29 illustrates the concentration × deprivation state interaction ($F(3,42) = 7.63$ $p<0.001$) which reflects the greater increase in duration of drinking for the optimally preferred concentration.

There was no group × concentration ×deprivation state interaction ($F(3,42) = 0.92$).

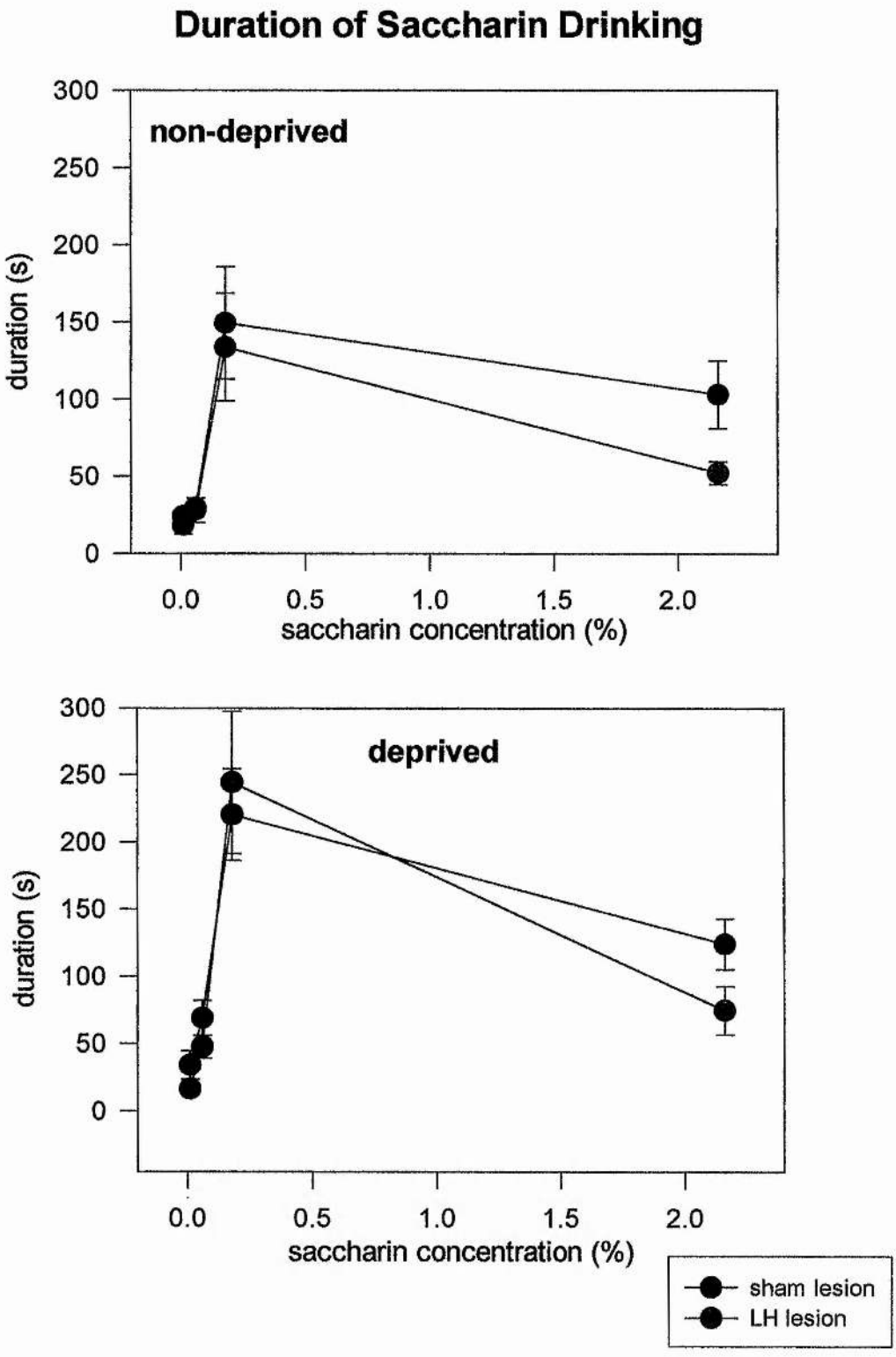


Figure 28. Mean (± SE) duration of time spent drinking saccharin solutions (0.01%, 0.06%, 0.18% 2.16%) by the sham lesioned group (n=10) and the LH lesioned group (n=6) in non-deprived and 22h water deprived states.

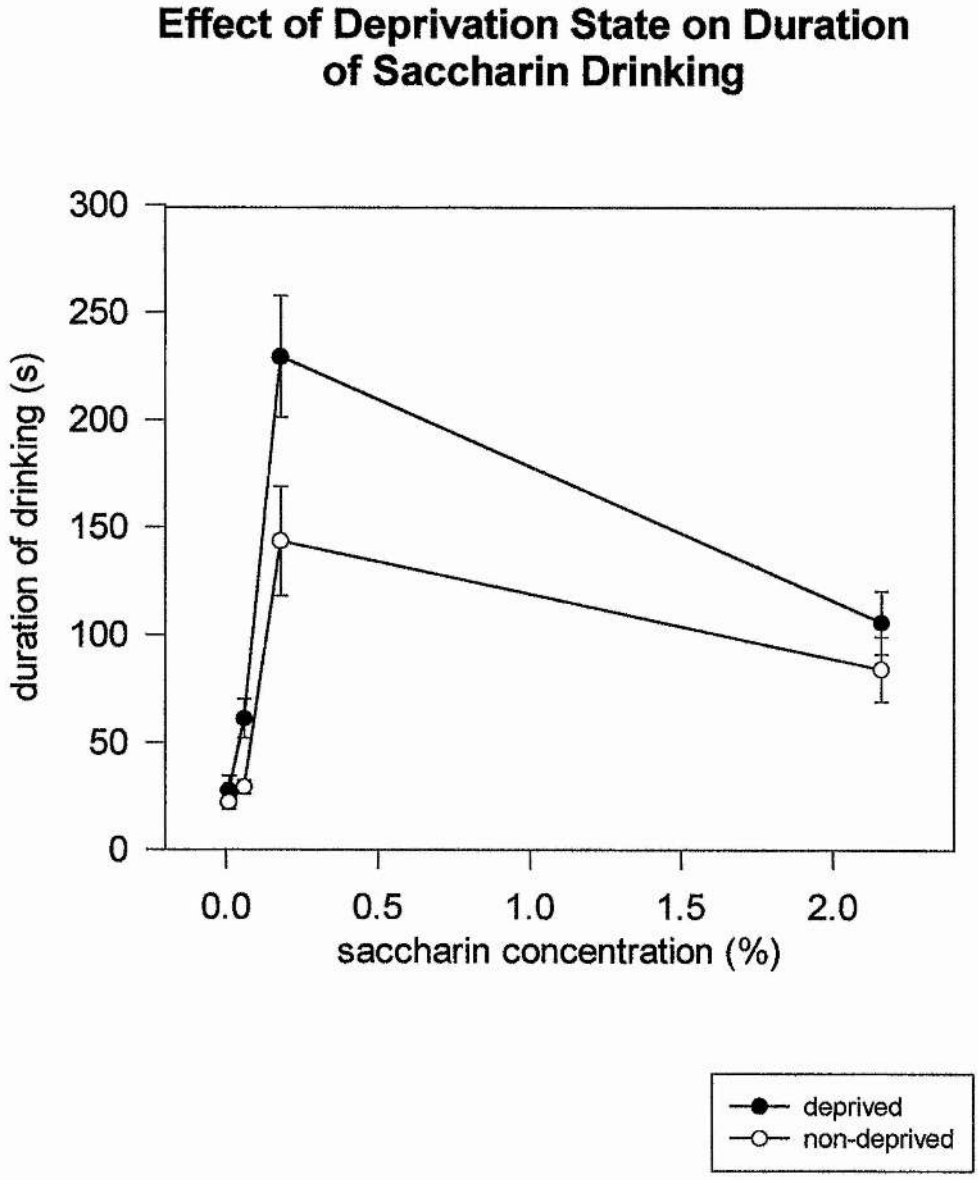


Figure 29. Group (sham lesion\LH lesion) collapsed to illustrate concentration x deprivation state interaction; mean duration of drinking saccharin when non-deprived (n=16) and 22h water deprived (n=16).

Frequency of Drinking Saccharin Solutions

ANOVA revealed a main effect of concentration ($F(3,42) = 13.88$ $p<0.001$) but no main effect of group ($F(1,14) = 1.01$) and no group × concentration interaction ($F(3,42) = 1.26$) indicating that frequency of drinking differs to the same extent for the sham lesioned and LH lesioned group over the range of saccharin concentrations.

There was a main effect of deprivation state ($F(1,14) = 5.17$ $p<0.039$) and a group × deprivation state interaction ($F(1,14) = 4.94$ $p<0.043$). In Figure 31, concentration has been collapsed to show the deprivation state × group interaction. It is clear that the sham lesioned group increased frequency of drinking when deprived whereas the LH lesioned rats did not.

There was a concentration × deprivation state interaction ($F(3,42) = 3.42$ $p<0.026$). From Figure 32 it appears that whereas deprivation state did not affect the number of initiations to drink the least preferred solutions, initiations to drink the most preferred solutions did increase when in a deprived state.

There was no group × concentration × deprivation state interaction ($F(3,42) = 0.84$).

In summary, it appears that volume of drinking, frequency of drinking and duration of drinking were all dependent on the concentration of saccharin but lesioning the LH did not alter taste preference for saccharin with respect to any of these three factors. Lesioning the LH had no significant effect the on the increase in drinking and duration of time spent drinking when deprived versus non-deprived but it did appear to abolish the increase in number of initiations to drink. The concentration drunk was dependent on deprivation state; when deprived, the volume drunk, time spent drinking and initiations to drink increased disproportionately in favour of the most preferred solution.

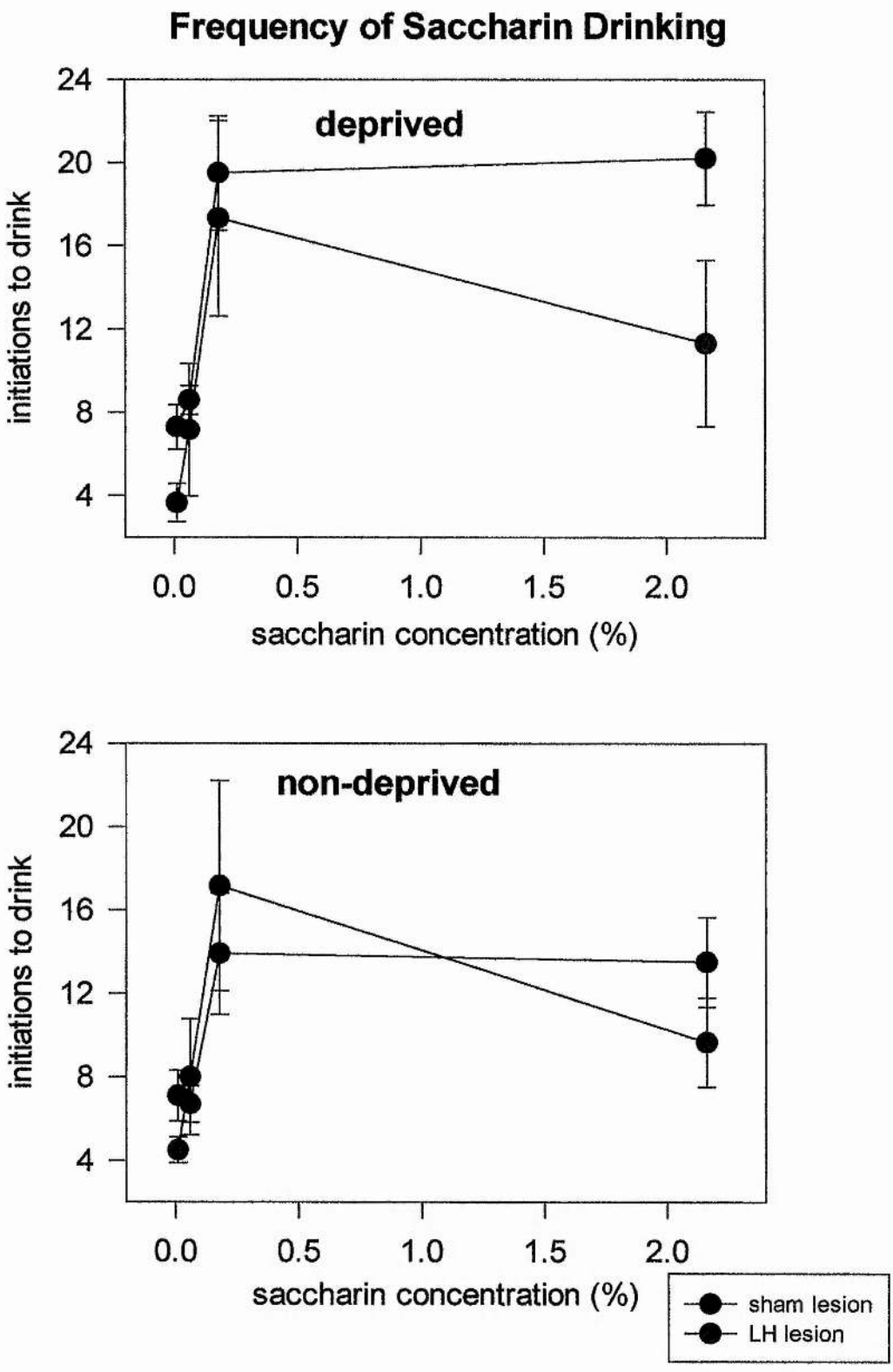


Figure 30. Mean (± SE) frequency of initiations to drink saccharin solutions (0.01%, 0.06%, 0.18%, 2.16%) by the sham lesioned group (n=10) and the LH lesioned group (n=6) in non-deprived and 22h water deprived states.

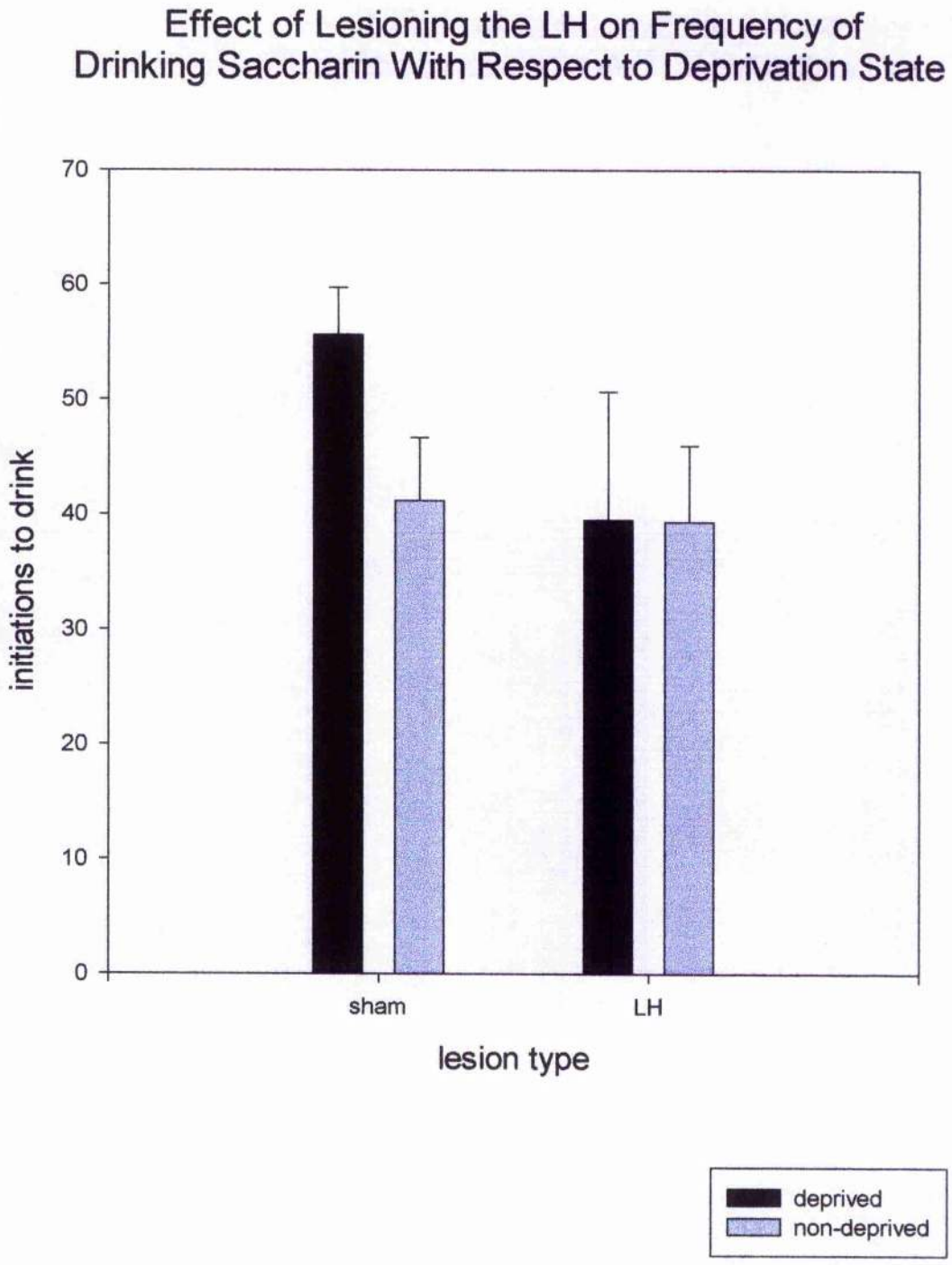


Figure 31. Saccharin concentration collapsed to illustrate group x deprivation state interaction for frequency of drinking saccharin solutions. Sham lesioned group: n=10; LH lesioned group: n=6

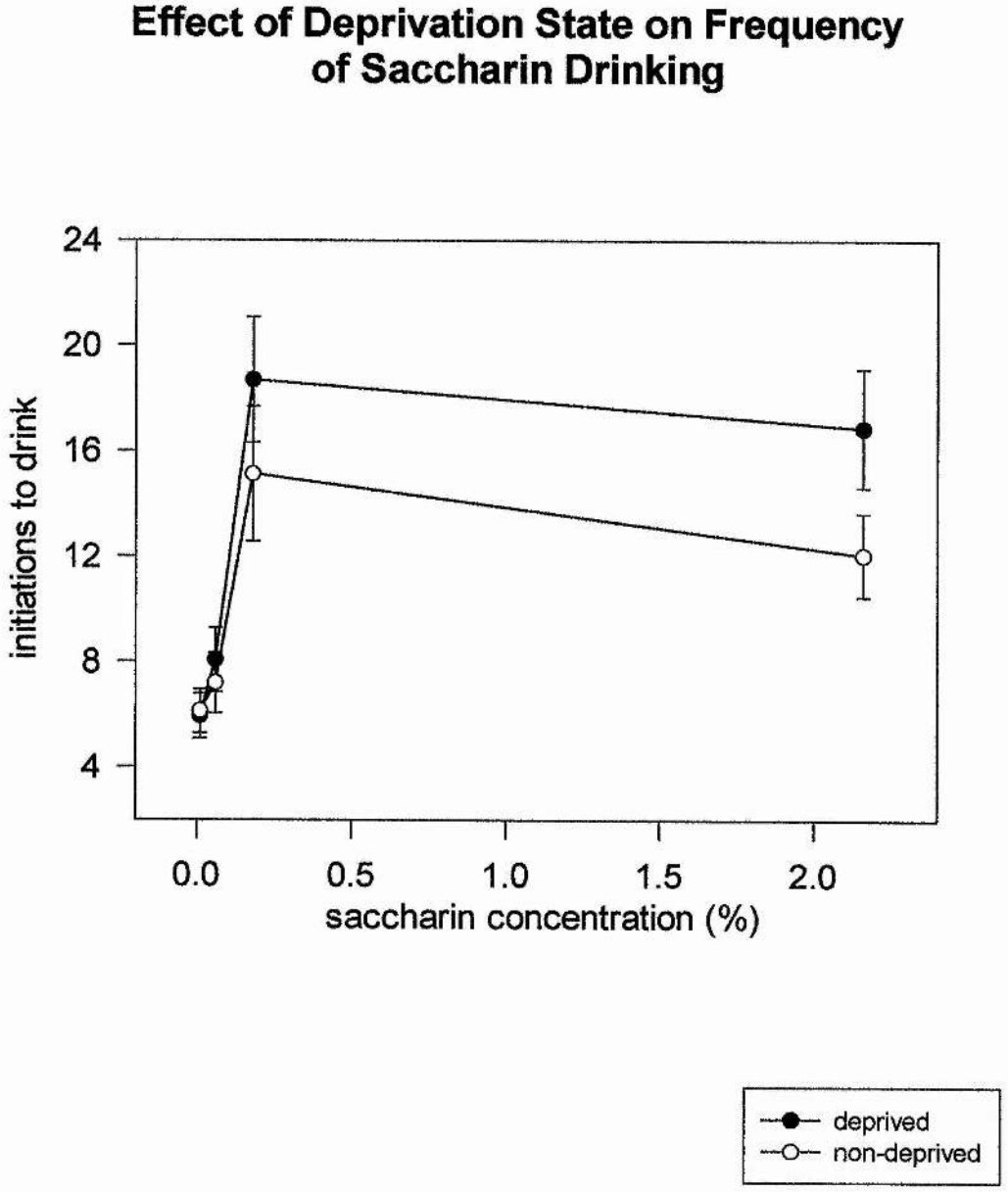


Figure 32. Group (sham lesion\LH lesion) collapsed to illustrate concentration x deprivation state interaction for frequency of drinking saccharin solutions. Deprived group: n=16; non-deprived group: n=16.

Salt Preference

Figures 33, 36 and 38 illustrate salt taste preference and the pattern of drinking salt in a non-deprived state and in a 22h water deprived state.

Volume of Salt Solutions Consumed

Salt taste preference curves in a deprived and non-deprived state are illustrated in Figure 33. ANOVA revealed a main effect of concentration ($F(3,39) = 6.50$ $p<0.001$) but no group × concentration interaction ($F(3,39) = 2.57$) and no main effect of group ($F(1,13) = 0.05$) suggesting that lesioning the LH did not alter taste preference for salt.

There was a main effect of deprivation state ($F(1,13) = 35.96$ $p<0.001$) indicating that the volume of drinking increased when deprived versus non-deprived. Figure 34 illustrates that this increase was greater for the sham lesioned group compared to the LH lesioned group [significant group × deprivation state interaction ($F(1,13) = 5.50$ $p<0.036$)].

There was a significant concentration × deprivation state interaction ($F(3,39) = 4.30$ $p<0.010$). Figure 35 shows that when deprived the lowest concentration was the most preferred whereas when non-deprived, preference increased as concentration increased until an optimally preferred concentration was reached.

There was no group × concentration × deprivation state interaction ($F(3,39) = 1.47$).

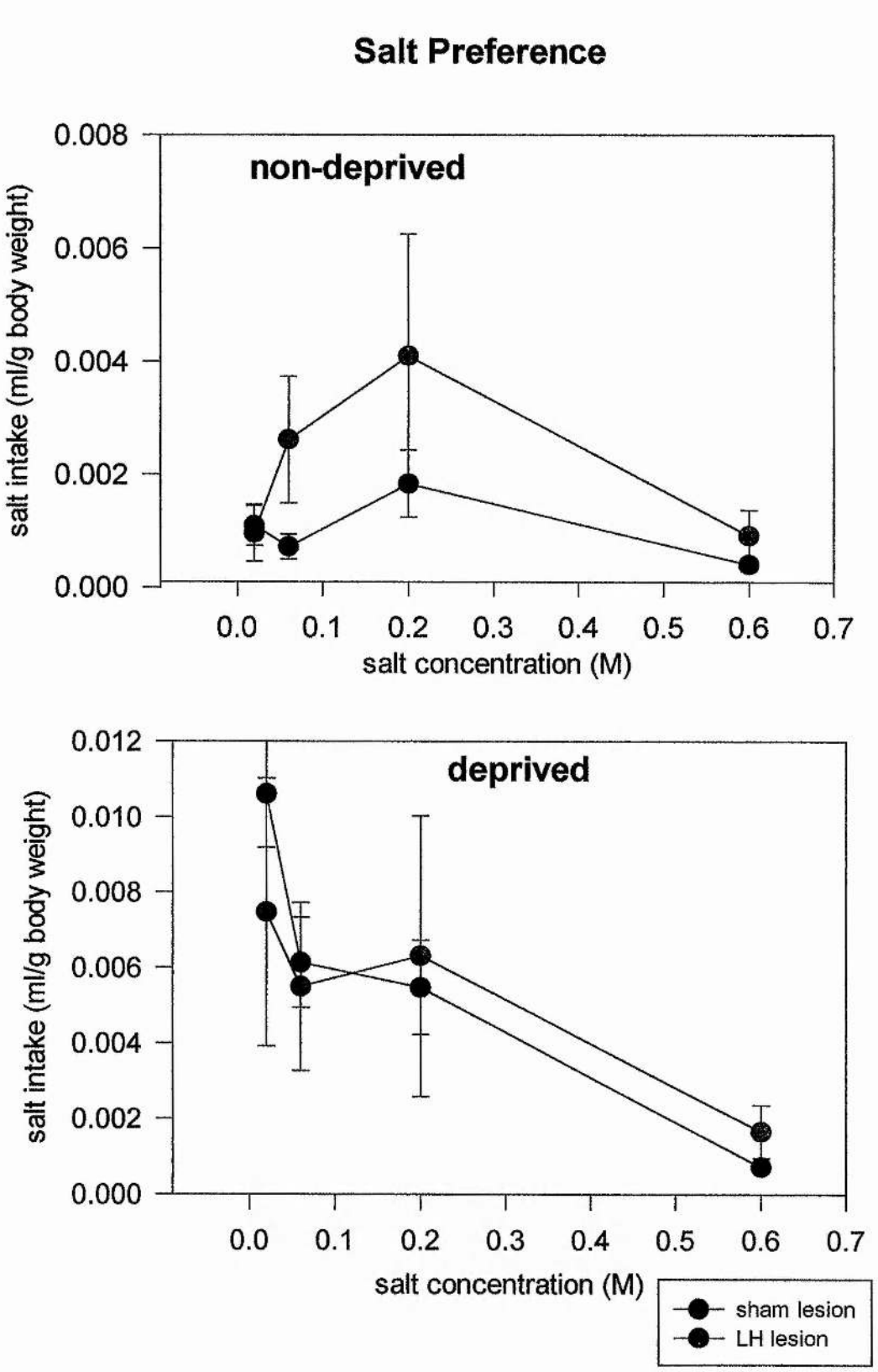


Figure 33. Salt taste preference when 22h water deprived and when non-deprived. Mean (± SE) volume of salt solution (0.02M, 0.06M, 0.2M, 0.6M) drunk as a function of body weight for the sham lesioned group (n=10) and the LH lesioned group (n=6).

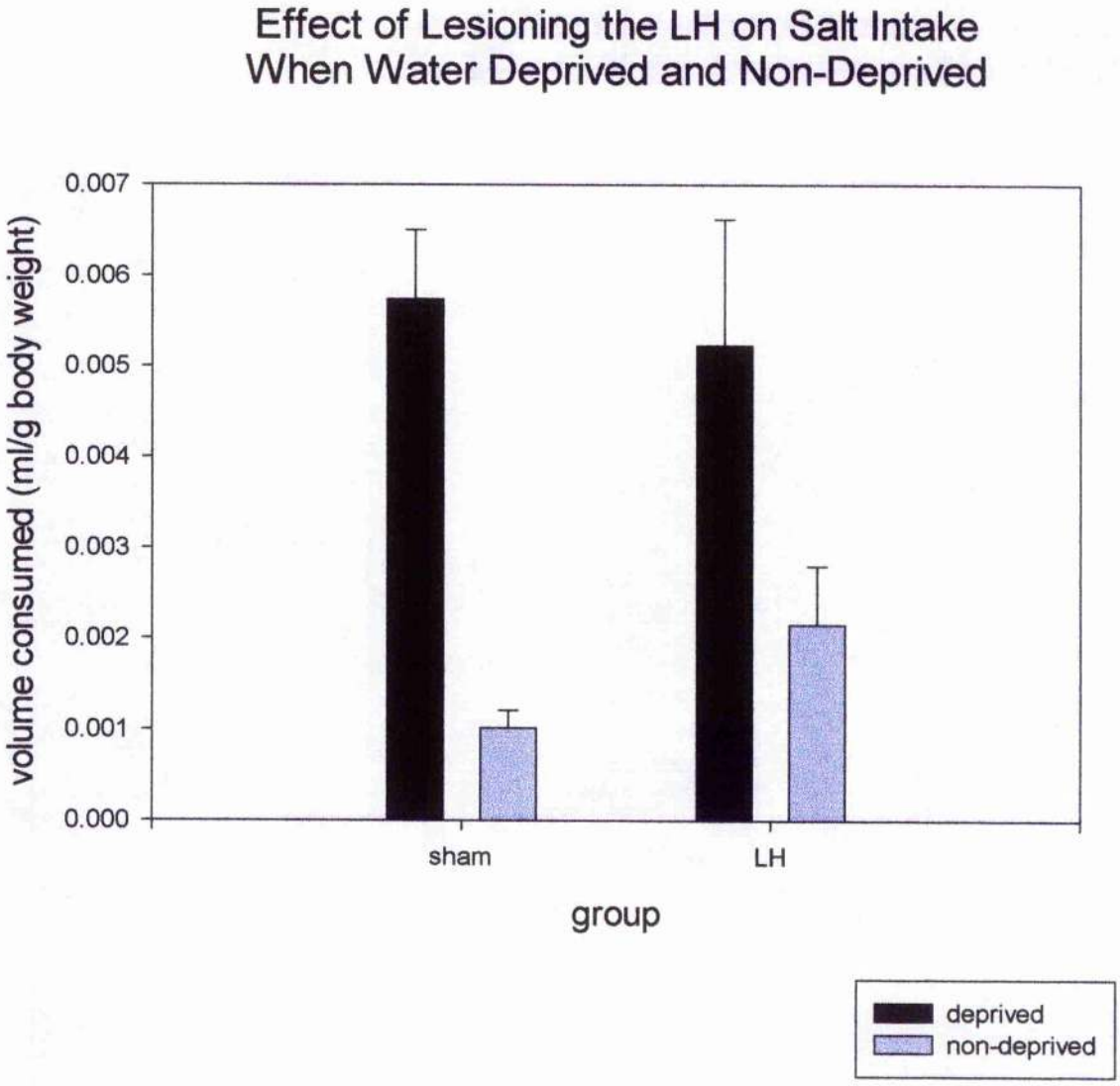


Figure 34. Concentration collapsed to illustrate group x deprivation state interaction for volume of drinking salt.
Sham lesioned group: n=10; LH lesioned group; n=6

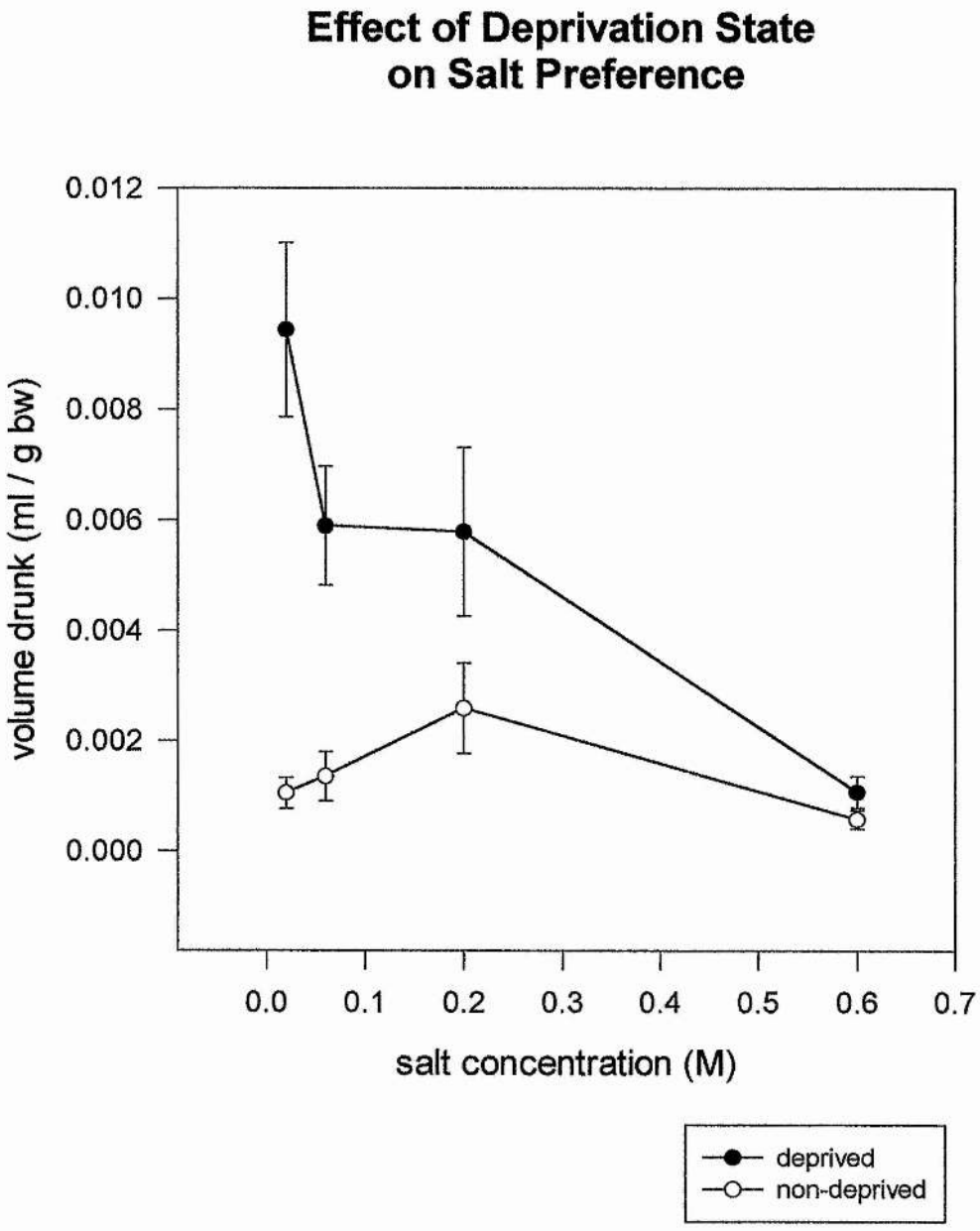


Figure 35. Group (sham lesion\LH lesion) collapsed to illustrate concentration deprivation state interaction for drinking salt. Deprived: n=16; non-deprived: n=16.

Duration of Drinking Salt Solutions

It is clear from Figure 36 that lesioning the LH did not alter the duration of time spent drinking salt solutions. ANOVA revealed a main effect of concentration ($F(3,42) = 4.75.p<0.006$) but no main effect of group ($F(1,14) = 0.00$) and no group × concentration interaction ($F(3,42) = 0.09$).

There was a main effect of deprivation state ($F(1,14) = 30.29$ $p<0.001$) but no group × deprivation state interaction ($F(1,14) = 0.36$) indicating that the LH lesioned group responded appropriately to the change in deprivation state with respect to time spent drinking.

As with volume drunk, there was a significant concentration × deprivation state interaction ($F(3,42) = 6.13$ $p<0.001$) reflecting the different preferences when deprived versus non-deprived. Figure 37 illustrates that in the non-deprived state the majority of time was spent drinking a high concentration of salt whereas in a deprived state a larger proportion of time was spent drinking the low concentrations of salt.

There was no group × concentration × deprivation state interaction ($F(3,42) = 0.09$).

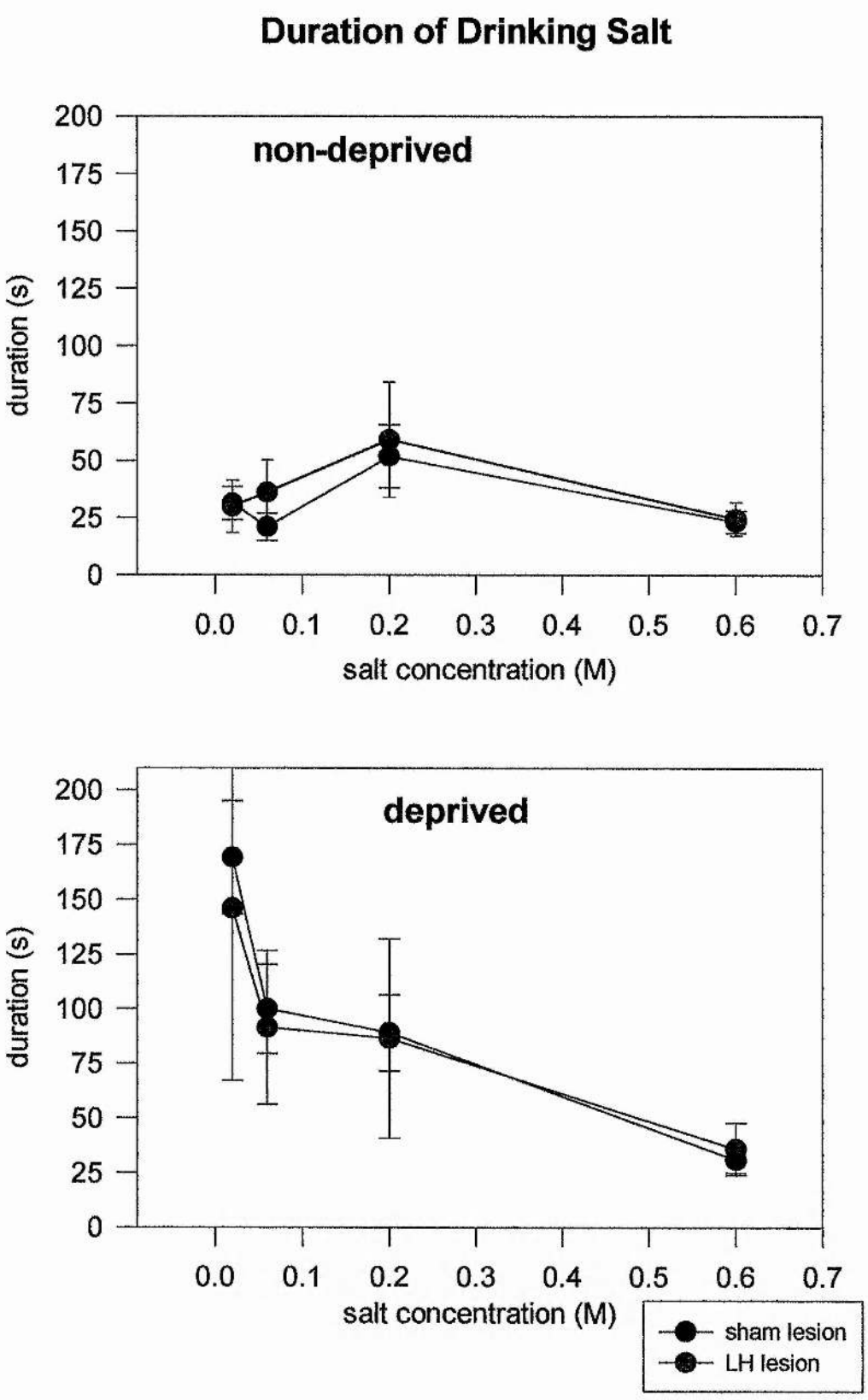


Figure 36. Mean (± SE) duration of drinking salt solutions (0.02M, 0.06M, 0.2M, 0.6M) by the sham lesioned group (n=10) and the LH lesioned group (n=6) in non-deprived and 22h water deprived states.

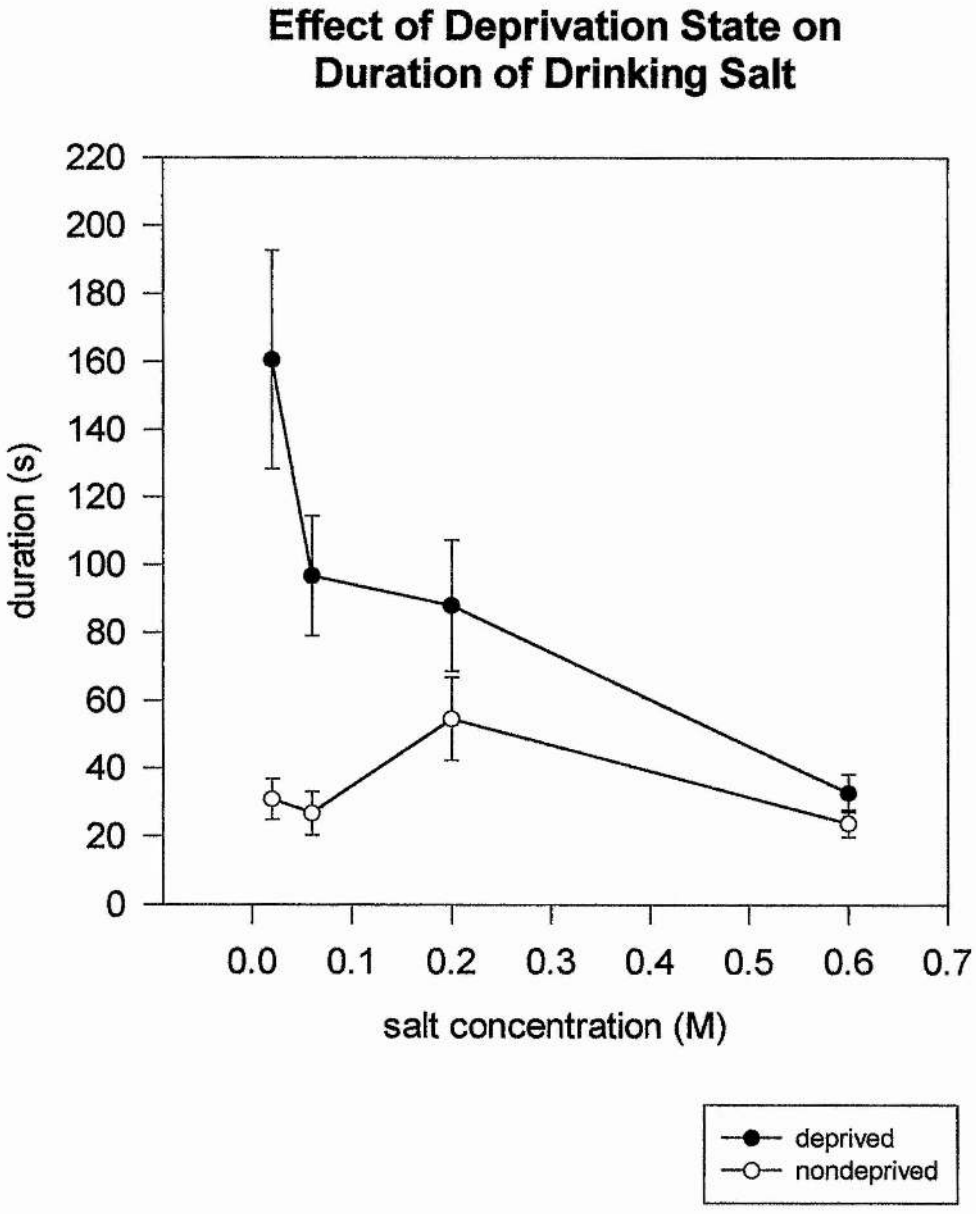


Figure 37. Group collapsed to illustrate concentration x deprivation state interaction for duration of drinking salt.
Deprived: n=16; nondeprived: n=16.

Frequency of Drinking Salt Solutions

The frequency of drinking salt solutions in a deprived and non-deprived state is illustrated in Figure 38. There was no main effect of group ($F(1,14) = 0.03$) or concentration ($F(3,42) = 0.62$) suggesting that the number of initiations to drink salt solutions was independent of concentration and was not affected by lesioning the LH. There was a significant increase in the number of initiations to drink when deprived versus non-deprived [main effect of deprivation state ($F(1,14) = 9.06$ $p<0.009$)] which was comparable between the sham lesioned and LH lesioned groups [no group × deprivation state interaction ($F(1,14) = 0.02$)]. In addition there was no concentration × deprivation state interaction ($F(3,42) = 0.44$) suggesting that unlike the volume consumed and the duration of drinking, deprivation state does not alter the preference for salt solutions with respect to initiations to drink.

There was no group × concentration × deprivation state interaction ($F(3,42) = 0.42$).

In summary, the volume of salt solutions drunk and the time spent drinking salt solutions were dependent on concentration whereas frequency of drinking salt solutions appears to be independent of concentration. Lesioning the LH did not alter taste preference when the difference in body weight was considered but did attenuate the increased drinking response to deprivation. The sham lesioned and LH lesioned groups increased duration of drinking and frequency of drinking to the same extent when deprived versus non-deprived but the pattern of preference was different between deprivations states. When non-deprived preference increased as concentration increased until an optimally preferred concentration was reached whereas when deprived the lowest concentrations of salt solution was optimally preferred with preference decreasing as concentration increased.

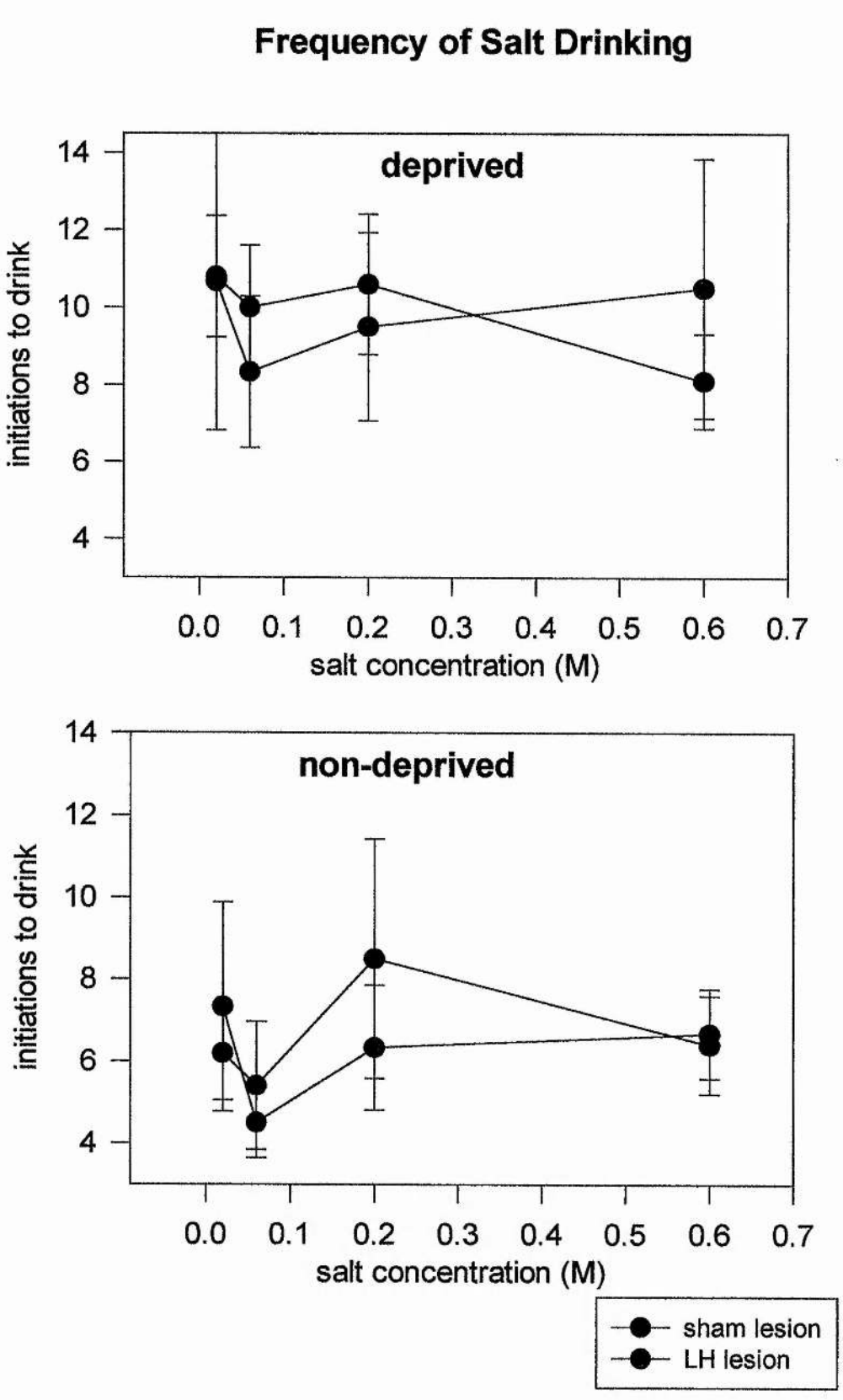


Figure 38. Mean (± SE) frequency of drinking salt solutions (0.02M, 0.06M, 0.2M, 0.6M) by the sham lesioned group (n=10) and the LH lesioned group (n=6) in non-deprived and 22h water deprived states.

DISCUSSION

The current results revealed that lesioning the LH produced little deficit in taste preference for salt or saccharin solutions when examined using intake tests. In addition, evaluation of home cage regulatory behaviour over a prolonged time period has extended knowledge of this area which, previously was only noted for the first 3 weeks after surgery. It is illustrated that the deficit seen in body weight as a result of lesioning the LH remained stable for at least 4 months post-surgery and furthermore, after a short recovery period, there was no deficit in feeding or drinking as a result of lesioning the LH.

When taste preference was tested in the home cage, there was no effect of lesion for salt or saccharin preference indicating that the LH was not involved in at least this aspect of taste perception. The concentrations of the tastant solutions used in the drinking boxes consisted of a more limited number but covered the same range as that tested in the home cage. The preference-aversion curves for salt and saccharin found when rats were tested in the drinking boxes were similar to that found when tested in the home cage. This is in spite of the substantial difference in exposure time to the tastant solutions, 30min versus 24h and the difference in presentation; simultaneous exposure to all tastants versus single exposure to each tastant. This is an interesting point to note when comparing related studies; much of the variation between studies has been attributed to differences in the duration of the test period or the type of test employed. Thus using two differing tests, it has been illustrated that the LH was not essential for the processing of taste information relating to salt and saccharin when rats were tested in a non-deprived state. This is in general agreement with Clark *et al.* (1990) who reported no differences in either saccharin preference or quinine aversion as a result of lesioning the LH.

The possible interaction between deprivation state and taste preference was examined using the drinking boxes by testing preference for both saccharin and salt solutions in a non-deprived and a water-deprived state. Although drinking was increased when testing for saccharin preference in a water-deprived state, little difference was found in the preference-aversion curve, aside from a

disproportionate increase in preference for the concentration optimally preferred when tested in a non-deprived state. Moreover, the LH lesioned group responded appropriately indicating that the LH was not essential for perception of saccharin taste when water–deprived. This is in contrast to Ferssiwi *et al.* (1987) who found that lesioning the LH produced a rightward shift of the preference-aversion curve for saccharin when rats were tested in a water-deprived state.

A number of methodological differences could have contributed to the variation between these contrasting studies. Different excitotoxins were used to lesion the LH: Ferssiwi *et al.* (1987) used ibotenic acid whereas presently, NMDA was used. Ibotenic acid and NMDA have been associated with different extrahypothalamic damage. Ibotenic acid has been reported to produce damage in the amygdala and increase nucleus accumbens and caudate-putamen levels of dopamine when injected into the LH whereas neither effect was associated with NMDA LH lesions (Hastings *et al.* 1985). Comparison of home cage regulatory behaviour implies that the lesions produced in the two studies may not be comparable. While the LH lesioned group in the study of Ferssiwi *et al.* (1987) regained weight 2 days post-surgery and began eating dry food from day 3, presently the LH lesioned group continued to lose weight for 7 days post surgery and it was 14 days after surgery before all the LH lesioned rats commenced eating of dry food. In addition, different testing protocols were used: Ferssiwi *et al.* (1987) presented the solutions in ascending order, while presently the solutions were tested in a random order. Comparison of the preferences for particular concentrations of saccharin revealed an unexpected feature. Although Ferssiwi *et al.* (1987) reported a group difference in saccharin preference, the particular concentration most preferred by the LH lesioned group actually corresponds to the concentration preferred by both the sham lesioned and LH lesioned groups in the present study.

In contrast to the present findings for saccharin preference, there was a substantial difference in the pattern of preference for salt solutions when tested deprived versus non-deprived. When non-deprived, the volume of salt solution ingested increased until an optimal concentration was reached after which the volume ingested declined whereas when water-deprived, preference decreased as

the concentration of salt increased. This divergence in results between tests for saccharin and salt preference would be the predicted outcome considering salt is osmotically active and thus ingestion of it would result in intracellular dehydration. When in a water-deprived state there is a physiological need to consume fluid in order to regain normal fluid balance. However, if the available salt solutions were hypertonic it is possible that a larger volume would be required to excrete the ingested salts than the volume ingested thereby exacerbating the dehydration instead of attenuating it. Thus, the rational response physiologically would be to avoid salt solutions of a high concentration and ingest solutions that are more dilute instead. This present result therefore provides evidence that the LH lesioned rats were not only aware of their water deprived state but were also able to integrate this with the different taste cues of salt and saccharin and modify their behaviour accordingly. This is in agreement with Clark *et al.* (1991a) who showed that LH lesioned rats responded as control rats to salt adulteration of the diet, increasing water intake appropriately. In both the present study and that of Clark *et al.* (1991a) these suitable responses were made despite the fact that the LH lesioned rats failed to drink in response to hypertonic saline, a treatment which causes dehydration. It should be noted that although the LH lesioned rats made an appropriate choice of solution they did however show a deficit in the increase in the volume of salt solutions ingested when deprived.

A previous study (Experiment 1.1 "Conditioned Taste Aversion") indicated that when water-deprived rats were given access to saccharin, the volume ingested was increased by lesioning the LH. It was proposed that this could be due to altered taste perception or a deficit in the ability to disengage from inappropriate behaviour. The current results indicate that it is unlikely that altered taste perception caused the increase in responding.

By testing taste preference in taste boxes that provided simultaneous access to four different concentrations of a tastant, the pattern of drinking was also studied. Lesioning the LH did not alter the duration of time spent drinking saccharin but did produce a deficit in the increase in the number of initiations to drink when water deprived. Since the volume of drinking was the same for the sham

lesioned and LH lesioned groups, presumably the rate of drinking must have increased. No deficit in duration of drinking or number initiations to drink salt was induced by lesioning the LH. In general it appears that the pattern of drinking was not altered by lesioning the LH. The present results would indicate that the increase in saccharin consumed as a result of lesioning the LH Experiment 1.1 "Conditioned Taste Aversion" was not due to failure to terminate behaviour that the animal was engaged in.

Rather than a failure to terminate behaviours or a deficit in taste perception, the enhanced consumption of the palatable mash and saccharin solution might have been due to a difference in energy regulation. Previously, 97 days after surgery, LH lesioned rats had a higher energy intake with respect to body weight than the sham lesioned group. However, the present findings do not consolidate this theory. Presently, food intake was seen to remain constant up to 120 days after surgery at similar levels for both groups. One aspect in which these studies differed was that the measurements were taken daily for the present experiment but were only measured for the first 28 days after surgery and then restarted 97 days after surgery for the previous study. It may be that the act of daily weighing affected food intake. It should be remembered though, that while the previous group were weighed for a shorter period they were part of another study and were handled during the break in home cage measurements.

In conclusion, it appears that a functional connection between the PBN and LH is not required for acquisition of conditioned taste aversion, benzodiazepine-induced hyperphagia or taste perception. This does not disqualify the possibility that a functional relay exists between the PBN and the LH. Both the PBN and LH have been shown to be involved in at least some aspects of water balance (Edwards & Johnson 1991; Winn *et al.* 1990). It would be interesting to compare the ability of PBN lesioned rats to drink in response to hypertonic saline with the deficits induced in this test by lesioning the LH. Rather than modulating aspects of feeding behaviour shown to be related to the PBN, the LH may be involved in processing the rewarding properties of food. Maldonado-Irizarry *et al.* (1995) have shown that there may be an important link between the LH and

the nucleus accumbens, an area important for reinforcement processes and appetitive behaviour.

Chapter 2: The Function of the LH in Relation to the Limbic System

The limbic system is composed of a number of structures originally thought to be involved in motivation and emotion and now also considered to be involved in learning and memory. It is generally thought to include the limbic cortex, amygdala, hippocampus and hypothalamus though both the anatomy and function of the limbic system have been disputed. Recently, it has been suggested that it is in fact a redundant term (Blessing 1997) but nevertheless, Herbert (1997) noted in response that there are anatomical grounds for defining the limbic system because it does describe a number of areas with similar function unique to those areas of the brain. Herbert (1997) has indicated that the function of the limbic system is "adaptation". It enables appropriate responses to be made to concurrent surrounding and internal stimuli thereby permitting the interaction of individuals with their environment.

The affective value attached to a particular stimulus can depend on internal state and previous experience of it and thus the most appropriate response to any one stimulus is dependent on the circumstances in which it is encountered. In this way, memories of biologically significant cues can be used to optimise behavioural choices. The ability of an animal to interact with its environment can be tested using specific experimental techniques, which have indicated that the hippocampus and amygdala are involved in different aspects of learning and memory.

The amygdala is important for emotional learning where a neutral stimulus is associated with an incentive stimulus of biological significance (reviewed by Ono *et al.* 1995). One particular task employed extensively to study emotional learning is the fear-potentiated startle paradigm. A startle response is induced in the presence of a cue paired with foot-shock producing an enhancement of this simple reflex. It has been found that lesions of the amygdala impaired the fear-potentiated startle response, while electrical stimulation of the amygdala induced an enhancement of it providing convergent evidence that it has a role in the expression of conditioned fear (reviewed Davis 1986; Davis 1992; LeDoux 1992). Evidence from studies of c-Fos induction substantiates this, implicating the amygdala in conditioned and unconditioned fear (Campeau *et al.* 1991).

In contrast, the hippocampus is important for the learning of relationships between stimuli. It was illustrated that ablation of the hippocampus produced deficits in tests dependent on spatial discrimination, such as the Morris water maze and specific experimental designs using both the radial arm maze and T-maze (Morris *et al.* 1982; Bouffard & Jarrard 1988; Aggleton *et al.* 1986). Aggleton *et al.* (1986) indicated that the memory deficit due to lesions of the hippocampus might be specific to spatial memory. It was illustrated that while lesions of the hippocampus disrupted performance in a spatial memory task, in the same group of rats, performance in a non-spatial memory task was unimpaired.

Studies have been devised which directly addressed the dissociation between the roles of the hippocampus and amygdala in learning and memory. Selden *et al.* (1991) studied the contributions of the hippocampus and amygdala in fear conditioning to explicit and contextual cues by pairing footshock with an auditory cue in a distinctive environment. Rats with amygdaloid lesions expressed conditioned fear in the presence of the distinctive environment in which shock was administered (measured as avoidance of that environment) but were impaired in expressing conditioned fear to the auditory stimulus which was paired with shock (measured as suppression of drinking in the presence of the auditory cue). The reverse was true for rats with hippocampal lesions who expressed conditioned fear towards the auditory stimulus but not the distinctive environment. Thus, lesioning the hippocampus and the amygdala had contrasting effects with lesions of the amygdala impairing conditioned fear to an explicit cue and lesions of the hippocampus impairing conditioned fear to contextual cues.

McDonald & White (1993) provided further evidence that different aspects of learning could be mediated by multiple memory systems. They illustrated clearly, the roles of not only the amygdala and hippocampus but also the dorsal striatum. Three experimental paradigms were used in which information about the relationships between cues, association of a neutral cue with a natural reward or a reinforced stimulus-response association was required for accurate performance.

Each lesioned group was only impaired on one task:

- lesions of the hippocampus disrupted learning of relationships between stimuli
- lesions of the amygdala disrupted the association of neutral cues with natural reward
- lesions of the dorsal striatum disrupted reinforced stimulus-response associations.

In addition to behavioural paradigms, *in vivo* electrophysiology has been used to investigate the neural networks responsible for learning and memory. Considering the evidence implicating the amygdala in learning previously described, it may not be surprising that electrophysiological techniques have implicated this region in associative learning (Uwano *et al.* 1995; Muramoto *et al.* 1993; Romanski *et al.* 1993). Uwano *et al.* (1995) have provided evidence that sensory modalities converged in the amygdala and moreover, the number of neurones responsive to rewarding or aversive cues increased when they were associated with sensory cues indicating that they may be important in associative learning. However, it is important to note that other regions such as the LH have been implicated in the association of natural rewards with neutral cues. It was demonstrated that LH neurones that responded to glucose drinking also responded to a cue tone stimulus associated with glucose drinking (Nakamura & Ono 1986).

While evidence implicating the LH in associative learning has been provided by *in vivo* electrophysiology, little is known about the contribution of LH to behavioural paradigms requiring learning and memory. In order to investigate this further, two experiments are reported here. The role of the LH in conditioned reinforcement and conditioned place preference was examined, procedures which both study the formation of a stimulus-reward association and the subsequent control over behaviour by conditioned stimuli.

CONDITIONED REINFORCEMENT

The likelihood of a behavioural response being repeated is dependent on the outcome of that particular behaviour. Thus, if the result is favourable the behaviour will be positively reinforced increasing the likelihood that the behaviour will be repeated, whereas if the result is aversive it is unlikely that the behaviour will be repeated – that is, it will be negatively reinforced. Remembering these consequences enables an animal to shape its actions based on previous experience. This phenomenon has been tested extensively using operant chambers where, if lever pressing produced an appropriate reward, for instance provision of intracranial self-stimulation (ICSS) or food (Crow 1972; Hodos 1961), an animal will continued to press the lever.

The mesolimbic dopamine pathway has an important role in the mediation of reinforcement (Crow 1972; Fibiger *et al.* 1987). This pathway projects from the ventral tegmental area (VTA) through the MFB to structures including the olfactory tubercle, amygdala, the lateral septum, the bed nucleus of the stria terminalis, the hippocampus and the nucleus accumbens (reviewed by Nieuwenhuys *et al.* 1982). In particular, the projection from the VTA to the nucleus accumbens has been implicated in the mediation of reinforcement by a number of different experimental techniques.

- Disruption of dopamine transmission by 6-OHDA lesions of the VTA or nucleus accumbens or infusion of dopamine antagonists into the nucleus accumbens disrupted the reinforcing effects of brain stimulation (Fibiger *et al.* 1987; Phillips *et al.* 1994).
- Enhancement of dopamine transmission in the nucleus accumbens is in itself a reinforcer; rats lever pressed for administration of *d*-amphetamine directly into the nucleus accumbens (Phillips *et al.* 1994).
- Examination of the neurotransmitters present in the nucleus accumbens using *in vivo* microdialysis demonstrated that electrical stimulation of the VTA and MFB capable of supporting ICSS evoked a significant increase in the concentration of dopamine in the nucleus accumbens that was later shown to

be positively correlated with rate/intensity functions for ICSS (Fibiger *et al.* 1987; Nakahara *et al.* 1989; Fiorino *et al.* 1993).

Thus in addition to chemical manipulation, monitoring of extracellular concentrations of neurotransmitters has implicated a dopaminergic projection from the VTA to the nucleus accumbens in reinforcement.

Reinforcement can be induced by not only primary reinforcers but also secondary reinforcers. By repeatedly pairing a neutral stimulus with a primary reinforcer, this previously neutral stimulus acquires, by simple Pavlovian conditioning, the ability to serve as a reinforcer and thus is termed a conditioned reinforcer. To test the acquired affective value of a stimulus, it is used to maintain a different behaviour. After a primary reinforcer such as food or water is associated with a neutral compound stimulus (light and noise), two levers are introduced, one of which when depressed, delivers the compound stimulus (now conditioned reinforcer). At this time, the animal presses the lever to obtain the conditioned reinforcer only as the primary reinforcer is removed at this stage of testing. By introducing a second lever that has no consequence – non-conditioned reinforcer, it can be concluded that lever pressing is specifically to gain the conditioned reinforcer as opposed to a non-specific motor effect.

Like reinforcement induced by primary rewards, the nucleus accumbens has been shown to have an important role in conditioned reinforcement. Taylor and Robbins (1984) illustrated that *d*-amphetamine in the nucleus accumbens increased the control of behaviour by conditioned reinforcers and hence, selectively increased responding on the lever providing conditioned reinforcement. This effect has been shown to be dependent on the dopaminergic innervation of the nucleus accumbens. Thus, it was antagonised by dopamine depletion of the ventral but not dorsal striatum by 6-OHDA (Taylor & Robbins 1986) and was mimicked by intra-accumbens infusions of dopamine but not noradrenaline (Cador *et al.* 1991). Wolterink *et al.* (1993) further characterised it by revealing that both D_1 and D_2 receptors were involved in mediating the effects of dopamine in potentiating the control over behaviour by conditioned reinforcers.

Further studies of the anatomical substrate of conditioned reinforcement implicated components of the limbic system in this behavioural response. Excitotoxic lesions of the ventral subiculum blocked the potentiative effect of intra-accumbens *d*-amphetamine on responding with conditioned reinforcement (Burns *et al.* 1993). In addition, excitotoxic lesions of the amygdala disrupted responding for conditioned reinforcement but the nature of the impairment was dependent on the particular area lesioned (Burns *et al.* 1993; Robledo *et al.* 1996; Cador *et al.* 1989). Although the basolateral amygdala decreased selective responding for the conditioned reinforcer, intra-accumbens *d*-amphetamine potentiated responding for it albeit at a reduced level (Cador *et al.* 1989). In contrast, lesions of the central nucleus of the amygdala profoundly impaired the intra-accumbens *d*-amphetamine-induced potentiation of responding with conditioned reinforcement without affecting the control over behaviour by the conditioned reinforcer in the absence of the drug (Robledo *et al.* 1996). It appeared that ventral subiculum-accumbens and amygdala-accumbens interactions could be important in the mediation of reward related processes.

In order to influence behavioural responses, limbic structures must be able to influence motor output stations. The nucleus accumbens is a major target for the efferent projections of forebrain limbic structures such as the basolateral amygdala and hippocampal formation (Kelley *et al.* 1982; Kelley & Domesick 1982) and it in turn projects to basal ganglia circuitry directly and indirectly via connections with the ventral pallidum (Deniau *et al.* 1994; Heimer *et al.* 1991; Haber *et al.* 1985). Evidence indicating that limbic structures gained access to motor output regions via the nucleus accumbens lead to this area being termed "the limbic motor interface" (reviewed, Mogenson *et al.* 1980). However, this may not be the sole relay whereby limbic information is translated into action. Winn *et al.* (1997) proposed that rather than simply being one structure, the limbic-motor interface may in fact be a distributed system. Based on anatomical and behavioural evidence, it was proposed that, the pedunculopontine tegmental nucleus (PPTg) has a critical role in the translation of motivation to behavioural output.

Anatomical studies indicate that the LH is favourably situated to interact with the systems described. The amygdala projects to the LH (Kita & Oomura 1982; Hosoya & Matsushita 1980) indicating that signals from at least this structure of the limbic system may access the LH directly. In addition, the LH receives innervation from structures of the proposed limbic-motor interface, the nucleus accumbens (Kita & Oomura 1982; Hosoya & Matsushita 1980) and the PPTg (Ford *et al.* 1995) from which it may be inferred that signals from both the hippocampus and amygdala access the LH indirectly. Kirouc & Ganguly (1995) have provided evidence that there is an overlap in the nucleus accumbens between the terminal fields of neurones originating in the amygdala and neurones which project to the LH. Furthermore, they reported apparent neuronal contacts suggestive that neurones of the basolateral amygdala made synaptic contact with neurones of the nucleus accumbens that projected to the LH. Not only do the afferent connections of the LH position it favourably to have a role in reward related processes but likewise, the efferent connections of the LH which include the amygdala, VTA and components of the ventral pallidal complex (lateral preoptic area and substannia innominata) also predict this (Berk & Finkelstein 1982).

It appears that in addition to the electrophysiological evidence implicating the LH in incentive learning, the LH is suitably located to influence relays shown to be critical to this function. The aim of the present study was to examine the role of the LH in incentive learning and in doing so, investigate the possibility of the existence of a functional amygdala-accumbens-LH interaction. In order to attain this, the ability of LH lesioned rats to respond for conditioned reinforcement was tested since it has been suggested that amygdala-accumbal interactions play an important role in the mediation of this behaviour (Cador *et al.* 1989).

Experiment 2.1: The Role of the LH in Conditioned Reinforcement

METHODS

Animals

Thirty-four male Lister Hooded rats (Charles River) were pair housed under a 12hr light/dark cycle with *ad lib* water available throughout the study. During the training and test phases food intake was specifically restricted and body weight was monitored to ensure 85% of free feeding body weight was maintained. Fourteen rats were assigned to the sham lesioned group and 20 rats were assigned to the LH lesioned group according to body weight and performance in the last pre-surgery training schedule. Mean body weights at the time of surgery were 341.69g (SD 18.98) and 334.33g (SD 11.80) for the sham lesioned and LH lesioned groups respectively and the mean number of consecutive trials with scores of at least 80% on the last training schedule were 7.93 (SD 3.85) and 6.95 (SD 2.39) for the sham lesioned and LH lesioned groups respectively.

Surgery

Animals were anaesthetised by i.p. injection of Sagatal (60mg/ml; 1ml/kg) with the injection volume doubled using sterile water. Eight of the sham lesioned rats were left to recover from anaesthesia in the post-operative care area without incurring any further procedures. The remaining rats were placed in a stereotaxic frame with the incisor bar 5.0mm above the interaural line. LH lesioned animals received a simultaneous bilateral infusion of 1µl 0.06M NMDA and eight of the sham lesioned rats received a simultaneous bilateral infusion of 1µl phosphate buffer. NMDA (Sigma) was dissolved in phosphate buffer (pH 7.4) and pH adjusted to 7.4 with 0.2M NaOH. The infusion rate was 0.5µl/min with a further 2min period allowed for diffusion. The co-ordinates for cannulae were anterior-posterior 0mm from bregma; medial-lateral ±2.0mm from midline; ventral 8.0mm from dura.

Post-Operative Care

After surgery rats were singly housed in grid bottomed cages with *ad lib* food and water available. Previously post-operative care consisted of monitoring body weight and if it fell below 85% pre-surgery weight these individual rats were given wet mash (Farley's dry baby food and glucose). However, it has been found that waiting until body weight had already fallen to this extent before intervening resulted in 90% of LH lesioned rats requiring wet mash. Moreover, the increase in body weight as a result of consumption of wet mash was not stable with body weight falling below 85% pre-surgery weight when wet mash was removed. Since it has also been found that LH lesioned rats ate lab chow from wet mash bowls but not from food hoppers after surgery, approximately 10g of lab chow was placed on the cage bottom of all rats after surgery. Body weight was monitored and if this fell to approximately 85% pre-surgery weight in addition to the lab chow on the cage floor either not being eaten or only a minimal amount being eaten, these individual rats were given dilute wet mash in their water bottles. After lab chow consumption had resumed the rats returned to receiving lab chow from the food hoppers and tap water. Two LH lesioned rats received a bottle of dilute wet mash: 1 received it for 2 days the other 6 days and in addition 6 rats were given access to lab chow on the cage floor for an average of 5 days.

Apparatus

The apparatus used to train and test the groups consisted of 8 operant chambers (Campden Instruments Ltd.) measuring 25cm across, 26cm high and 23cm wide. In each chamber there were 2 levers on either side of a food hopper which was accessed via a plexiglas panel hinged at the top. An automated dispenser delivered food pellets (Noyes Precision Food Pellets, Formula P) to the food hopper and a photocell located on the side of the hopper registered nose-pokes (head entries into the hopper). There was a house light situated in the centre top of the box and a hopper light above the food hopper. In order to reduce interference noise from adjacent boxes and the surrounding environment the chambers were in sound proof boxes and a fan was positioned next to each of the pellet dispensers. Chambers were interfaced to a Eurobeeb microcomputer

system (Paul Fray Ltd.) which operated the chambers and recorded presses on the panel covering the food hopper and lever presses.

Pre-Surgery Training

Prior to training the rats were habituated to the operant chambers in a 30min session where the house light was off and the hopper light was on. Twenty pellets were placed in the hopper and 10 were used to prop the panel door open. In this way, the door gradually closed as the pellets were eaten and the door subsequently had to be pushed open to reach the remaining pellets. One rat failed to eat the pellets and was habituated in a second 20min session in which the pellets were consumed. Animals were trained on a progressive training program which is summarised in Figure 39. The arrival of a food pellet was associated with a compound stimulus: the switching off of the house light, switching on of the hopper light and a tone. The house light remained off and the hopper light remained on until either the panel was pushed open or else 5s elapsed without a panel push after which the hopper light went off and the house light came on. Subsequent trials followed the panel push after a variable delay for a total of 100 trials per training session. Training began with a variable delay between delivery of pellets of 3-10s. If the rat pushed the panel prematurely this reset the variable delay ensuring that the delivery of food was associated with the compound stimulus. The minimum delay was set at 3s to allow ample time to collect the pellet before the next trial thus ensuring premature panel responses were not made to collect pellets. Dependent variables recorded were the total number of panel pushes, the percentage of panel pushes which were "correct" (defined as a panel push in response to the compound stimulus) and the delay to push the panel in response to the compound stimulus. If the rat failed to make a panel response within 5s of the compound stimulus this was regarded as a missed trial and was also recorded. The rats progressed to the subsequent reinforcement schedules (variable delay 3-20s, variable delay 3-30s) when 80% of their panel pushes were correct. Thus they progressed when 80% of the responses that were made at the panel were in response to the conditioned stimulus rather than during the variable delay period that occurred between the occurrence of the conditoned stimuli. Animals were trained until they reached at least 5 consecutive trials with 80% of panel pushes correct on the 3-30s variable delay schedule. The number

of training sessions to reach this ranged from 14-83 for all the rats trained. The average number of training sessions to reach a performance of 80% of panel pushes correct on the 3-30s variable delay schedule was 45.14 [± 24.64 (SD)] for the rats assigned to the sham lesioned group and 47.14 [± 17.32 (SD)] for the rats assigned to the LH lesioned group. Animals were trained 1-3 times a day. During pre-surgery training, rats were fed a specific amount in the home cage to produce a combined daily intake of lab chow and reward food pellets of 18g and feeding in the home cage only commenced after training was complete.

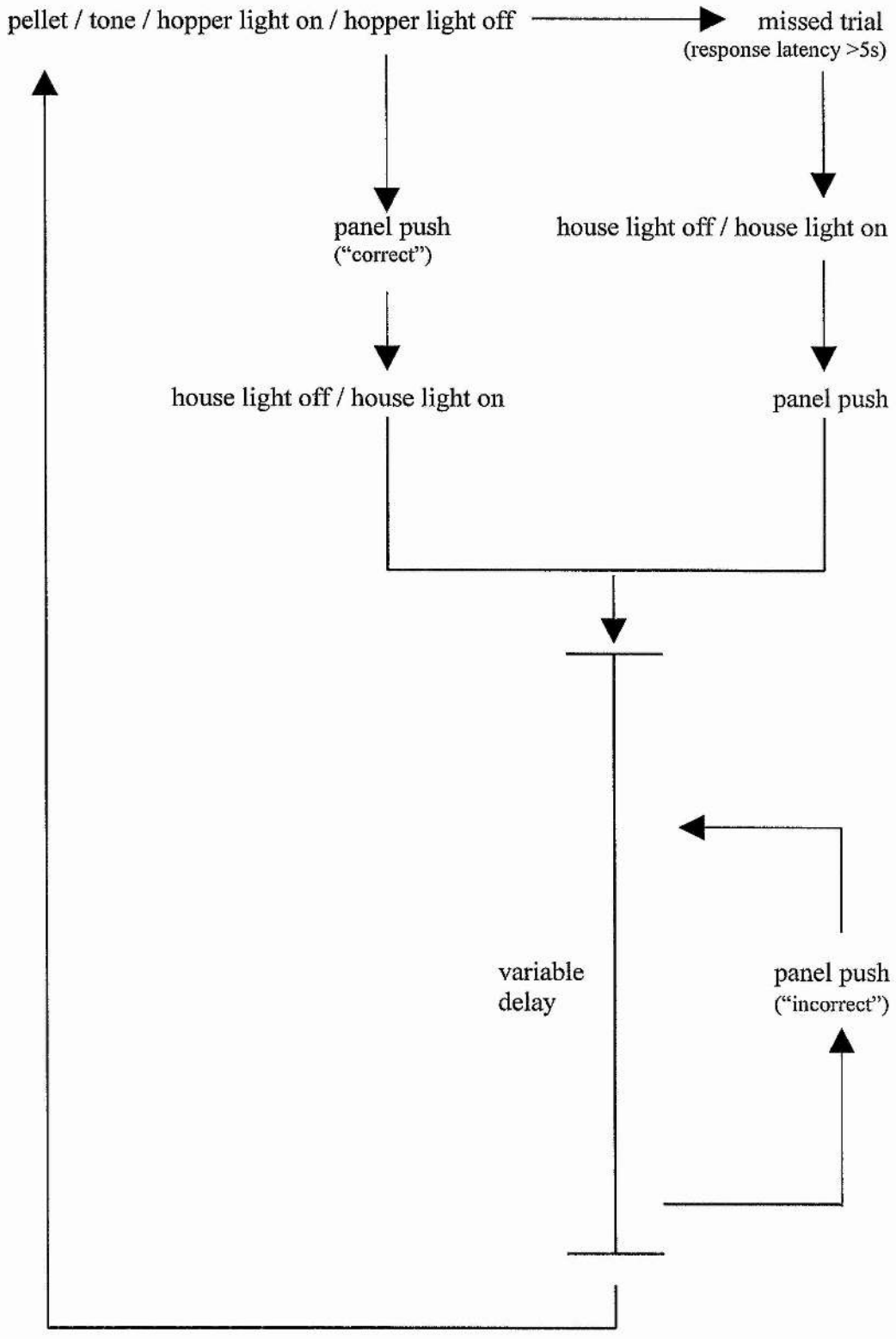


Figure 39. Summary of training programme used to associate food reward with compound stimulus. Correct panel push defined as a panel push in response to the compound stimulus.

Post-Surgery Training

Thirty-three days after surgery the rats were put on a food restriction schedule and training started 2 days later. Since the LH lesioned rats had a significantly lower intake than the sham lesioned rats it was considered inappropriate to use a standard food restriction regime of 18g per day. Instead an average intake was calculated for each rat for the 5 days previous to food restriction and they were fed 60% of this average value. The rats were trained on the 3-30s variable delay schedule for 8 sessions.

Testing

The day following the last training session the rats began the test phase. During the test phase the 2 levers either side of the food hopper were designated as the conditioned reinforcer (CR) lever and the non-conditioned reinforcer (NCR) lever with the lever assigned to the CR counter balanced. Here depression of the CR lever resulted in the compound stimulus, now the conditioned reinforcer, but no pellets were dispensed. Depression of the NCR lever and panel pushes had no consequence but were recorded. In a preliminary session the rats were placed in the chambers until 10 responses on the CR lever had been made. Immediately following the preliminary session the rats were placed in the chamber for 30min and the responses on the CR lever, NCR lever and panel were recorded. After completion of the test phase, the rats were returned to the home cage with *ad lib* food provided.

Hypertonic Saline Challenge

Forty-five days after surgery and at least 2 days after the completion of conditioned reinforcement testing the rats were given a hypertonic saline challenge. All rats received an i.p. injection of both 5% hypertonic saline (20ml/kg) and isotonic (0.9%) saline (20ml/kg), with 48h between injections. Injections were given in a counterbalanced order and tap water consumed 1h and 3h after injection was measured.

Histological Analysis

At the end of the study all the experimental rats were given an overdose of 200mg/ml pentobarbitone (Euthatal) and were subsequently perfused

transcardially with physiological saline followed by 10% formalin. The brains were then removed and stored in 10% formalin until they were sectioned on a freezing microtome. 25μm slices were cut at 200 μm intervals and stained with cresyl violet stain for nissl substance. Lesions were identified by areas of cell loss and reactive gliosis. In order to ascertain lesion volume, histological sections were scanned and then silhouettes of the areas of the lesion were drawn onto the scanned sections. The areas of the lesioned tissue was determined using a microcomputer and lesion volumes calculated.

Statistical Analysis

The results were examined statistically using ANOVA with, when appropriate Tukey's post hoc tests. The control group in the present study consisted of two conditions; rats which had received LH infusions of phosphate buffer or no infusion. ANOVA with group as the between subjects factor (phosphate buffer infusion/no infusion) revealed no main effect of group with regard to body weight before or after surgery ($F(1,5) = 1.90$) or drinking induced by hypertonic saline ($F(1,5) = 2.68$). Furthermore there was no group × day interaction for body weight ($F(45,225) = 0.87$) and no group × treatment interaction for drinking induced by hypertonic saline ($F(1,5) = 2.02$). It appeared that there was no difference between the sham conditions with regards to either body weight after surgery or drinking induced by i.p. hypertonic saline, variables consistently impaired by NMDA lesions of the LH. Therefore, sham conditions were collapsed for the purpose of statistical analysis and graphical presentation.

RESULTS

Histology

Analysis of the histology revealed that 14 rats had incomplete lesions of the LH and 1 sham lesioned rat had thalamic damage and all were excluded from further analysis. The average size of the LH lesions for the 7 LH lesioned rats remaining was 2.14mm^3 (SE 0.21) on the left side and 1.88mm^3 (SE 0.20) on the right side with the greatest damage level with the paraventricular nucleus decreasing towards the anterior and posterior poles. Figure 40 illustrates the biggest and smallest lesions found within the LH lesioned rats remaining.

Damage anterior to the level of the paraventricular nucleus was limited to the thalamus with reticular thalamic damage ranging from less than 30%-80% found bilaterally in 4 rats and unilaterally in 2 rats although reticular thalamic damage greater than 30% was only found bilaterally in 1 rat. In addition, 1 rat suffered bilateral damage and 2 rats suffered unilateral damage in the anteroventral thalamic nucleus where damage ranged from less than 30%-50% and 1 animal suffered minor damage (<30%) unilaterally in the anteromedial thalamic nucleus.

The greatest extra-hypothalamic damage level with the paraventricular nucleus was found in the zona incerta where 30%-75% of tissue was lesioned bilaterally in all rats. Lesioned tissue found bilaterally in 4 rats and unilaterally in 3 rats in the reticular thalamic nucleus did not exceed 30% except in 2 rats unilaterally where 50%-70% of the tissue was lesioned. Damage greater than 30% was only seen in 1 rat unilaterally in the ventrolateral thalamic nucleus where damage extended to 70%. More extensive thalamic damage ranging from less than 30%-80% was seen in the ventromedial thalamic nucleus bilaterally in 3 rats and unilaterally in 3 rats where the greatest bilateral damage was approximately 75%.

Posterior to the paraventricular nucleus lesioned tissue was only found more dorsal than the mammilliary thalamic tract in 2 rats unilaterally where 75% of the ventromedial thalamic nucleus was lesioned. The extent of the damage at this level was 75% of the zona incerta and subincertal nucleus lesioned bilaterally in all rats, 30%-90% of the subthalamic nucleus lesioned bilaterally in 6 rats and

minor (<20%) bilateral damage in the ventromedial thalamic nucleus of all rats.

Due to the substantial number of LH lesioned animals lost from the study, the sham lesioned and LH lesioned groups were no longer balanced with respect to pre-surgery body weight and performance in pre-surgery training. In order to compare pre- and post-surgery variables between and within groups more accurately it was decided to include in the analysis, only those 7 sham lesioned rats which most closely resembled the LH lesioned rats with respect to pre-surgery measures, thus producing two groups of equal numbers which did not significantly differ before surgery. Body weight for the sham lesioned and LH lesioned groups before surgery was 346.40g (SD 16.31) and 341.34g (SD 13.50) respectively and the number of consecutive trials with scores above 80% was 6.43 (SD 1.90) and 7.29 (SD 3.15) for the sham lesioned and LH lesioned groups respectively.

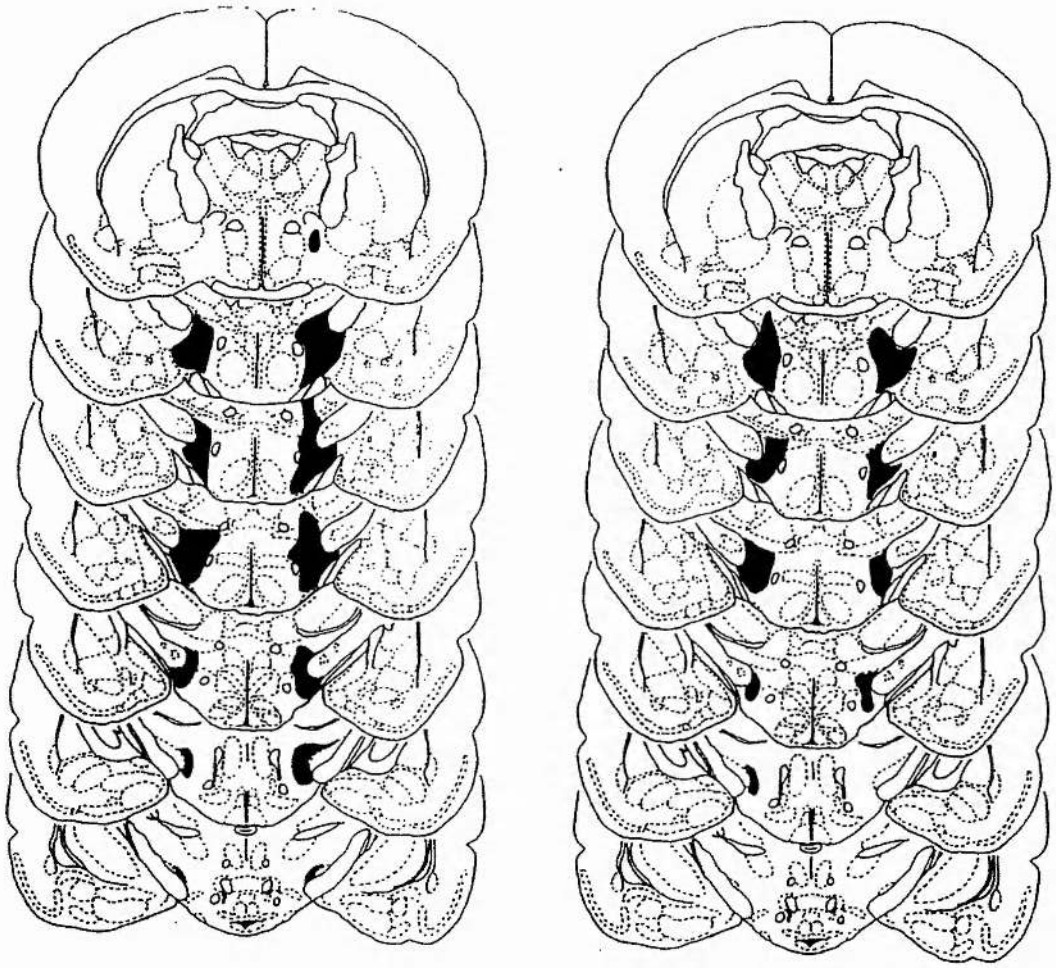


Figure 40. Representative sections redrawn from the atlas of Paxinos & Watson (1982) illustrating the extent of the biggest and smallest lesions produced by injection of NMDA into the LH. The shaded regions represent areas of neuronal loss.

Body Weight

Body weight 7 days before and 32 days after surgery are illustrated in Figure 41. ANOVA of body weight before surgery revealed no main effect of group ($F(1,12) = 1.82$) indicating that there was no significant difference between the groups with respect to body weight. Rats were given ad lib access to food in the home cage 1-2 days before surgery and as can be seen in Figure 41 body weight increased at this time [main effect of day ($F(7,84) = 22.0$ $p<0.001$)]. However, there was no significant group × day interaction ($F(7,84) = 1.02$) indicating that the increase in body weight was similar for both the sham lesioned and LH lesioned groups.

Figure 41 illustrates that unlike the sham lesioned group who did not incur any weight loss due to surgery, body weight for the LH lesioned group fell below pre-surgery levels [main effect of group ($F(1,12) = 54.85$ $p<0.001$)]. Although body weight of both the sham lesioned and LH lesioned groups increased after surgery [main effect of day ($F(29,348) = 89.66$ $p<0.001$)], this was clearly more substantial for the sham lesioned group [significant group × day interaction ($F(29,348) = 6.27$ $p<0.001$)].

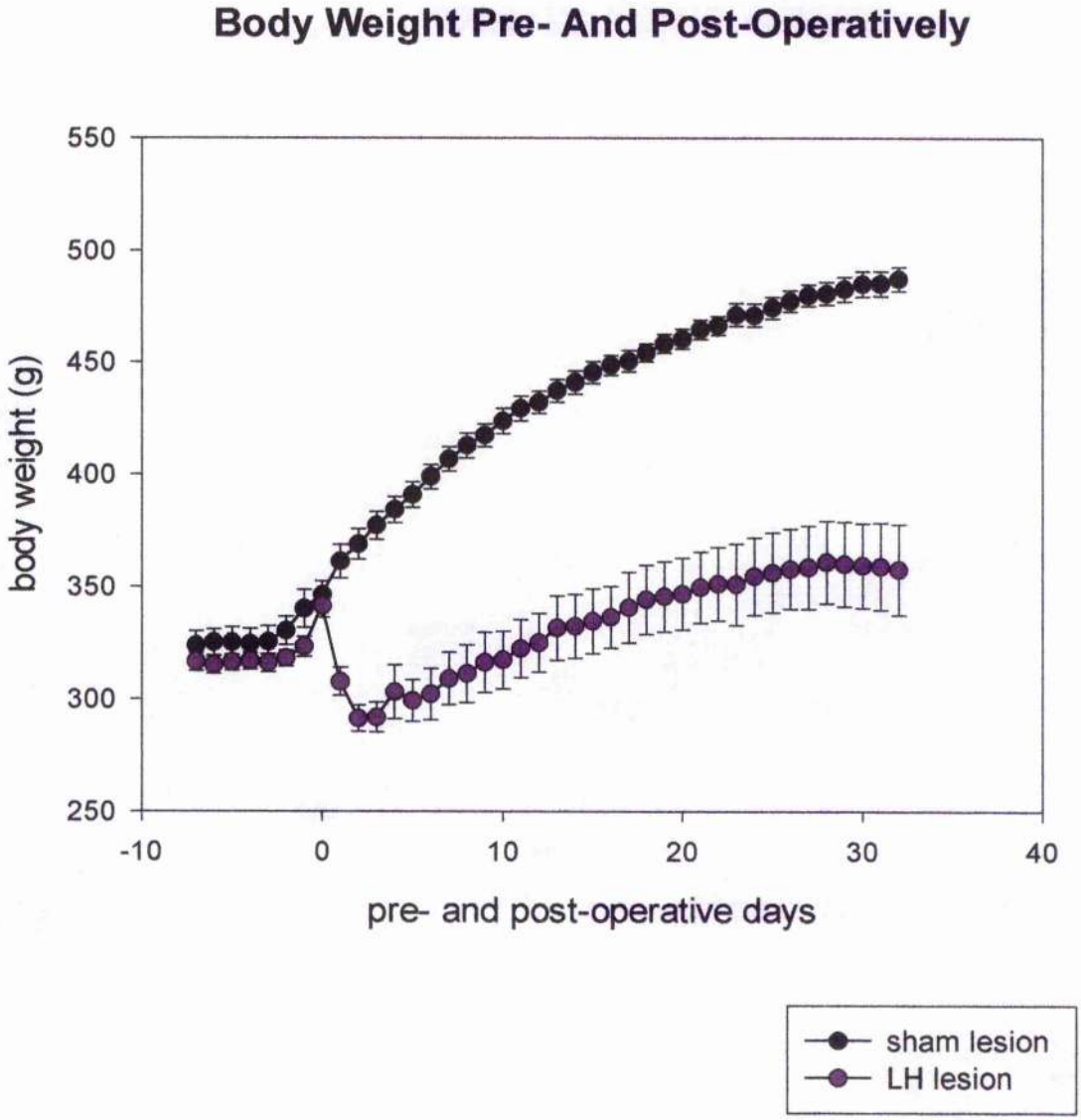


Figure 41. Mean (± SE) body weight 7 days before and 32 days after surgery for the sham lesioned group (n=7) and the LH lesioned group (n=7).

Pre-Surgery Training

The discriminative properties of the compound stimulus were evaluated by calculating the proportion of total panel pushes that were made in response to the compound stimulus (as opposed to during the variable delay period). The mean scores for discriminated approach to the compound stimulus for the last 5 pre-surgery training sessions and the mean latency to respond to the compound stimulus during these sessions are presented in Table 4 and 5 respectively. Before surgery there was no difference between the sham lesioned or LH lesioned groups with respect to discriminated approach ($F(1,12) = 0.60$) or latency to respond ($F(1,12) = 0.15$). Furthermore, there was no main effect of training session for discriminative approach ($F(4,48) = 0.911$) or latency ($F(4,48) = 0.131$) and no significant group × training session interaction for either discriminative approach ($F(4,48) = 0.495$) or latency ($F(4,48) = 0.91$). This suggests that a maximal plateau which was comparable between the sham lesioned and LH lesioned groups had been reached for performance in the pre-surgery training sessions.

Only a minimal number of trials were missed during the last 5 pre-surgery training sessions; 12 trials were missed by the sham lesioned group and 10 by the LH lesioned group out of a total of 500 trials for each rat.

	Training Session				
	1	2	3	4	5
Sham lesion	87.57 ±2.40	89.43 ±1.31	87.14 ±1.14	86.86 ±1.28	88.14 ±1.74
LH lesion	86.71 ±0.97	86.57 ±1.36	88.71 ±1.77	87.43 ±1.43	85.57 ±1.29

Table 4. Mean (± SEM) score for discriminative properties of the compound stimulus for the last 5 pre-surgery training sessions for the sham lesioned and LH lesioned groups (groups determined by histological analysis). Score calculated as the number of "correct" panel pushes as a percentage of the total number of panel pushes where a correct panel push is defined as a panel push in response to the compound stimulus.

Sham lesioned group: n = 7; LH lesioned group: n = 7.

	Training Session				
	1	2	3	4	5
Sham lesion	0.93 ±0.27	0.68 ±0.07	0.75 ±0.09	0.77 ±0.09	0.87 ±0.19
LH lesion	1.57 ±0.73	0.72 ±0.08	0.80 ±0.14	0.63 ±0.03	0.70 ±0.06

Table 5. Mean (± SEM) latency (s) to push the panel in response to the compound stimulus for the last 5 pre-surgery training sessions for the sham lesioned and LH lesioned groups (groups determined by histological analysis).

Sham lesioned group: n = 7; LH lesioned group: n = 7.

Post-Surgery Training

Discriminated Approach Response to the Compound Stimulus

Figure 42 illustrates that unlike the sham lesioned group, performance in the post-surgery training sessions was impaired for the LH lesioned group [main effect of group ($F(1,10) = 15.44$ $p<0.003$)]. It is clear however that discriminated approach for the LH lesioned group did improve during the training sessions [main effect of training session ($F(7,70) = 13.64$ $p<0.001$); significant group × session interaction ($F(7,70) = 6.47$ $p<0.001$)]. Tukey's post-hoc analysis showed that there was no change in performance from training session 1-8 for the sham lesioned group who almost reached optimal scores of approximately 80% from session 1. Although the LH lesioned group was impaired in comparison to the sham lesioned group for the initial 5 training sessions (at least $p<0.05$), Tukey's post hoc tests showed that there was no significant difference between the sham lesioned and LH lesioned groups for the remaining training sessions. This implies that lesioning the LH may have impaired memory for this task but the sharp increase in score indicated that it did not prevent the relearning of it. A comparison has been made in performance in the first training sessions and the post-surgery training sessions for the LH lesioned group in the appendix (pg 267). Since training began with the variable delay schedule of 3-10s and post-surgery training was with the variable-delay schedule of 3-30s it is not a true comparison but it does suggest that the behaviour of the LH lesioned group was more like that of rats naive to the training schedule than that of the sham lesioned group.

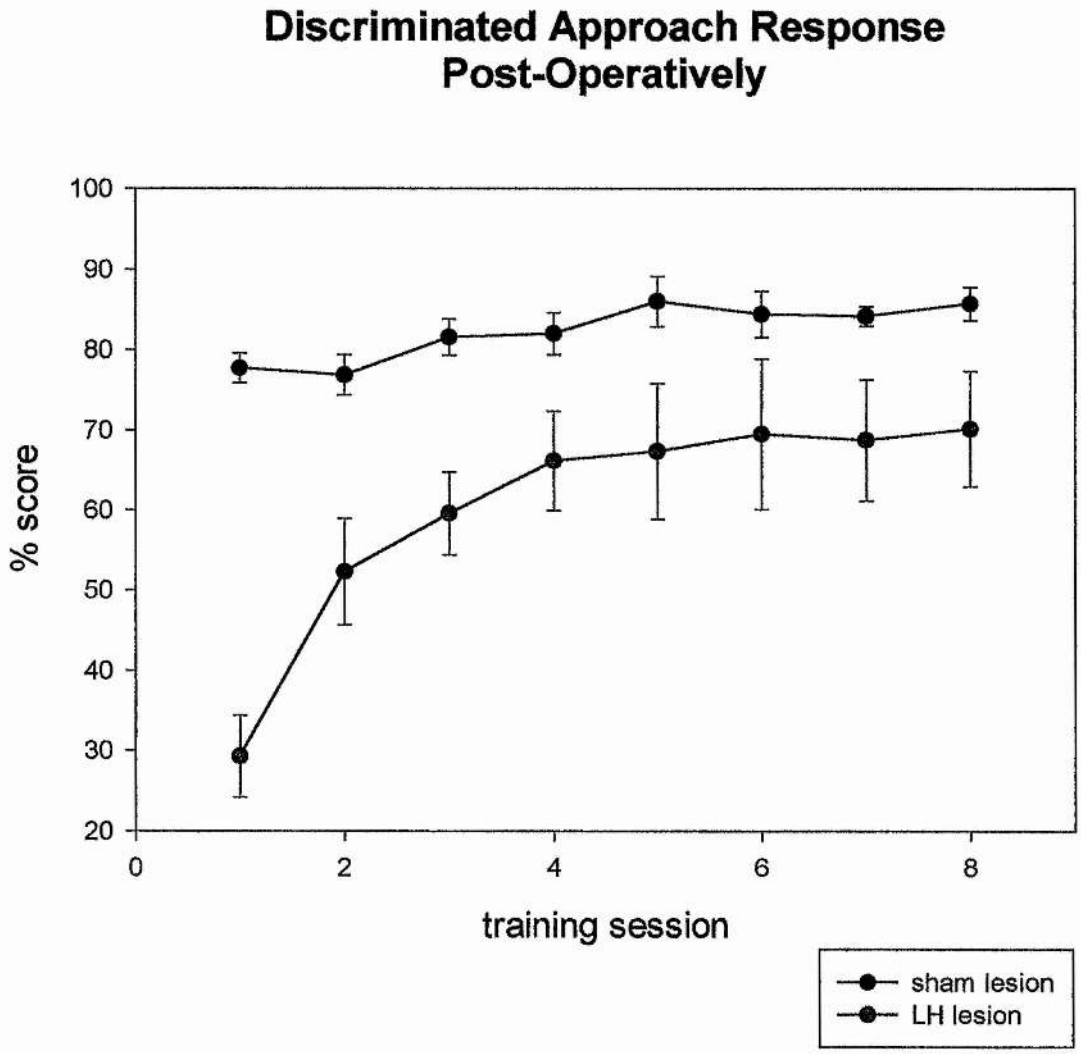


Figure 42. Mean (±SE) scores for discrimated approach response for the sham lesioned group (n=7) and the LH lesioned group (n=7) during the 8 training sesssions after surgery. Score was calculated as percentage correct panel pushes where a correct panel push was defined as a panel push in response to the compound stimulus.

Latency to Respond

The latency to respond to the compound stimulus during post-surgery training is presented in Table 6. ANOVA revealed no group effect ($F(1,10) = 0.42$), a main effect of training session ($F(7,70) = 4.03$ $p<0.001$) and no significant group × training session interaction ($F(7,70) = 1.89$) indicating that the latency to respond decreased with subsequent training sessions at a similar rate for both the sham lesioned and LH lesioned groups.

Missed Trials

The number of trials missed during post-surgery training are presented in Table 6. Like ANOVA of latency to respond, ANOVA of the number of missed trials revealed no main effect of group ($F(1,9) = 0.81$) and a main effect of session ($F(7,63) = 4.05$ $p<0.001$) but there was a significant group × session interaction ($F(7,63) = 3.27$ $p<0.005$) suggesting that the number of trials missed was not comparable between the groups for all the training sessions. Tukey's post hoc analysis showed that the only session where the LH lesioned group missed a significantly greater number of trials than the sham lesioned group ($p<0.001$) was session one.

Training	Latency		Missed Trial	
Session	Sham Lesion	LH Lesion	Sham Lesion	LH Lesion
1	2.30 ±0.33	4.73 ±1.97	1.57 ±0.43	6.29 ±2.32
2	2.48 ±0.98	1.59 ±0.37	1.43 ±0.65	2.29 ±1.48
3	1.45 ±0.24	1.86 ±0.56	1.00 ±0.44	3.29 ±2.30
4	1.17 ±0.16	1.34 ±0.31	0.86 ±0.26	1.14 ±0.70
5	1.29 ±0.19	1.33 ±0.25	1.17 ±0.40	1.33 ±0.61
6	1.69 ±0.50	1.51 ±0.35	1.29 ±0.36	0.83 ±0.54
7	1.08 ±0.16	2.27 ±0.83	1.14 ±0.51	1.57 ±0.78
8	1.33 ±0.32	1.33 ±0.27	1.14 ±0.46	1.14 ±0.83

Table 6. Mean (± SEM) latency to push the panel in response to the compound stimulus during post-surgery training and the mean (± SEM) number of trials with a response latency greater than 5s for the sham lesioned and LH lesioned groups.

Sham lesioned group: n = 7; LH lesioned group: n = 7.

Acquisition of a New Response with Conditioned Reinforcement

From Figure 43 it appears that both the sham lesioned and LH lesioned groups made more responses on the CR lever than the NCR lever or panel during the 30min test. Furthermore, it appears that responding on both the CR and NCR levers was elevated for the LH lesioned group. Despite this, ANOVA of all responses revealed no main effect of group ($F(1,12) = 0.76$) or panel/lever choice ($F(2,24) = 1.68$) and no group × panel/lever choice interaction ($F(2,24) = 0.60$). This disparity may be explained by the substantial difference in the variability of lever pressing between the sham lesioned and LH lesioned groups clearly illustrated in Figure 43.

Since the range of responding on both CR and NCR levers was elevated for the LH lesioned group the percentage of the total lever presses made on the CR lever was calculated and illustrated in Figure 44. General factorial ANOVA with group as the independent factor and percentage score as the dependent factor revealed no main effect of group ($F(1,12) = 0.10$) suggesting that lesioning the LH did not alter the selective responding for conditioned reinforcement. However, Figure 45 clearly illustrates that the range of scores for the sham lesioned group was substantially less than the LH lesioned group (60%-83% and 8%-90% respectively). Scores for 6 of the LH lesioned rats lay out with 1 standard deviation of the mean score for the sham lesioned group. Two of the LH lesioned rats had scores which were at least 4 standard deviations less and 2 had scores which were at least 2 standard deviations higher than the sham lesioned group mean. Analysis of the histology revealed no difference in lesion volume or lesion placement between these rats indicating that the LH lesioned rats may simply be over responding on the levers non-specifically as opposed to selectively responding for conditioned reinforcement.

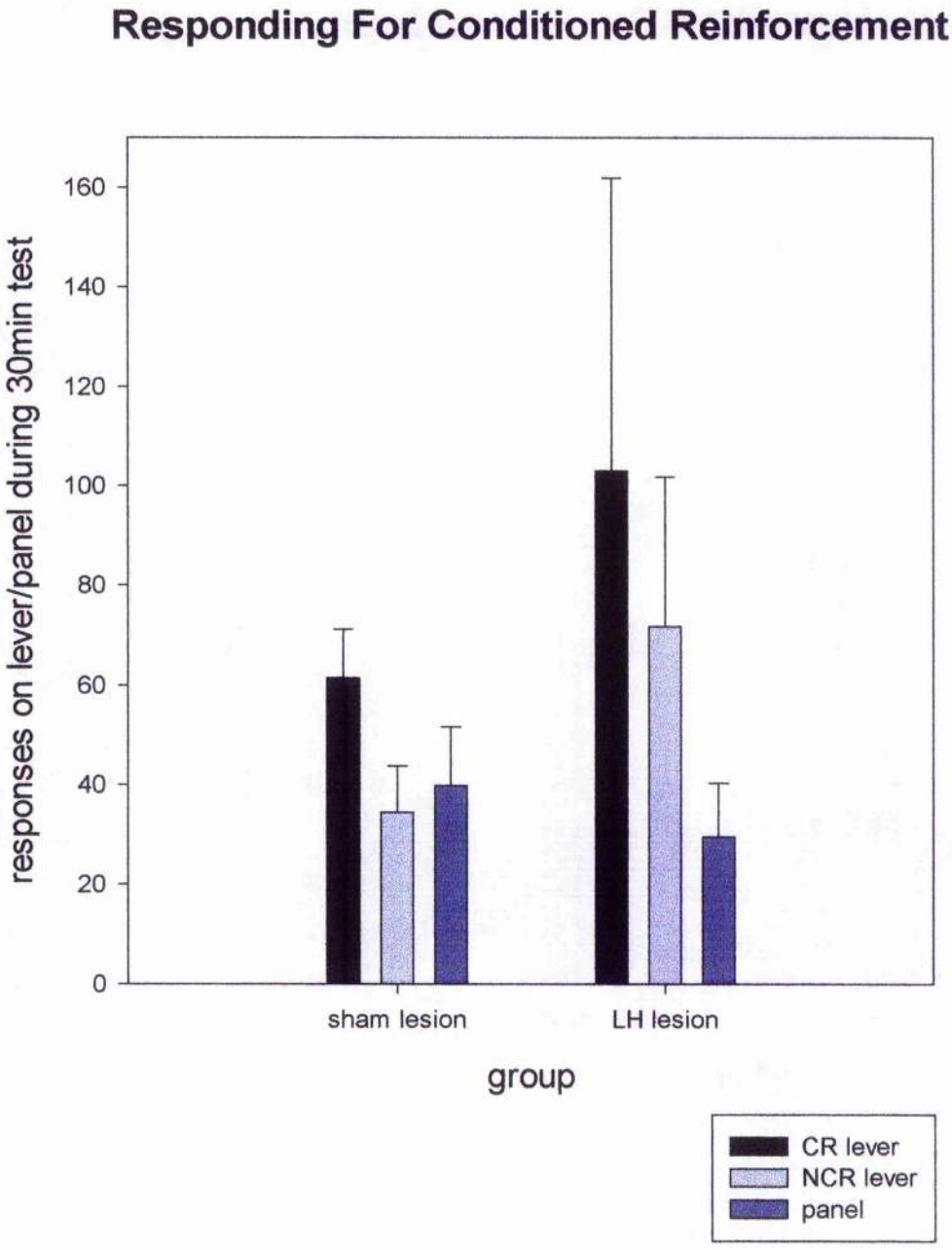


Figure 43. Mean (± SE) responses on CR lever, NCR lever and panel during the conditioned reinforcement test for the sham lesioned group (n=7) and the LH lesioned group (n=7).

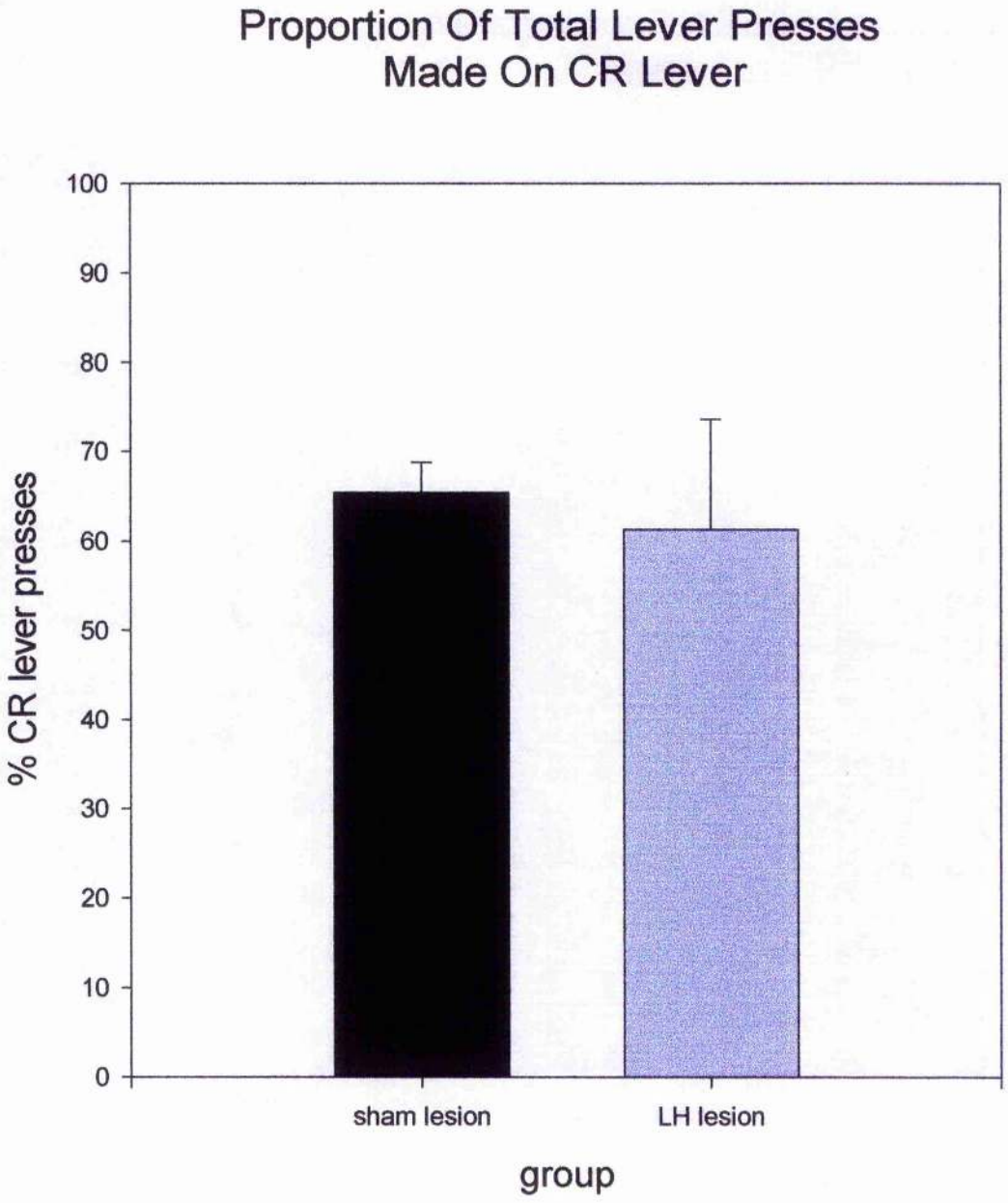


Figure 44. Mean (± SE) responses on CR lever as a percentage of total lever pressing during the conditioned reinforcement test for the sham lesioned group (n=7) and the LH lesioned group (n=7).

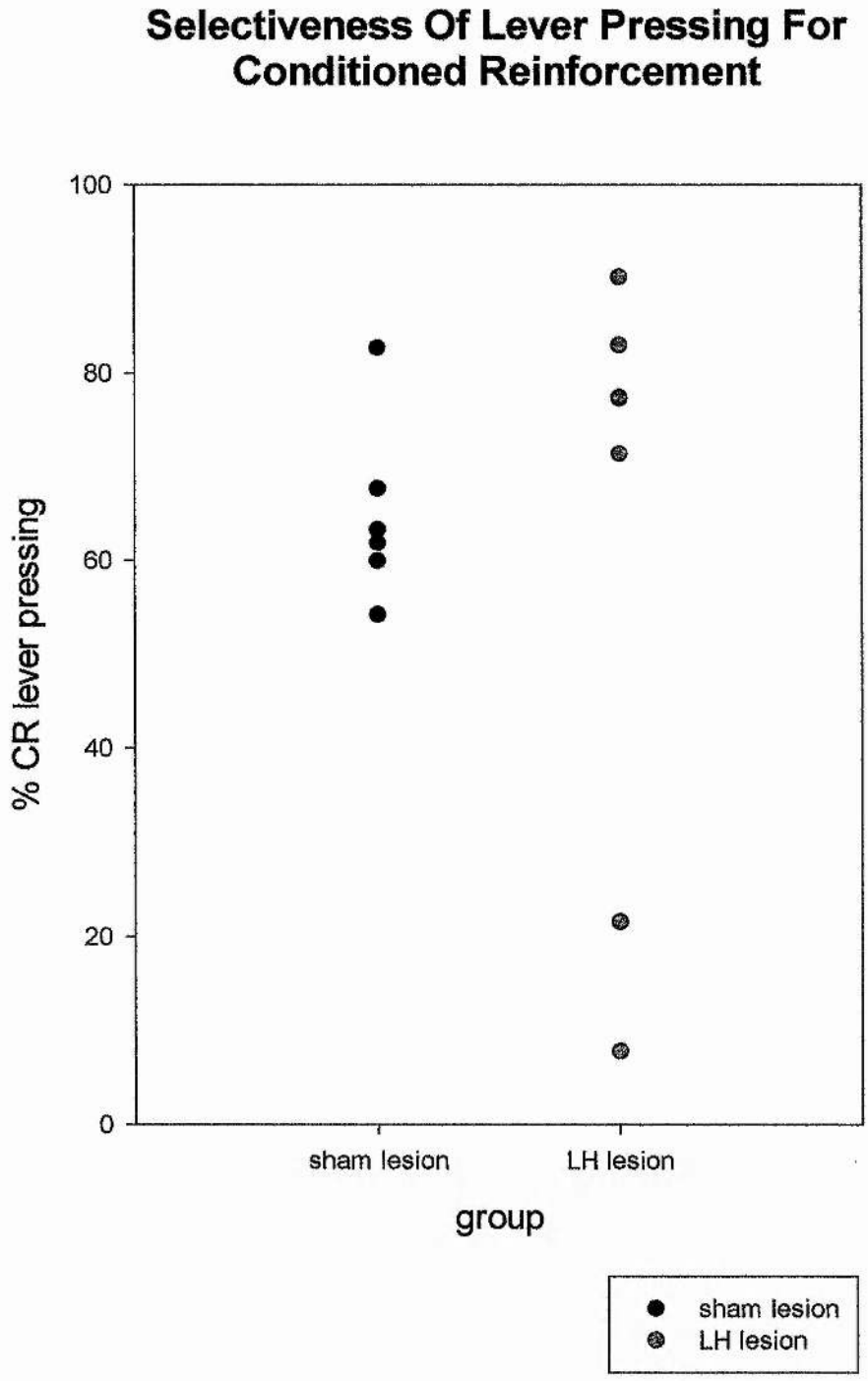


Figure 45. Mean number of lever presses on the CR lever as a percentage of total lever pressing for individual sham lesioned rats (n=7) and LH lesioned rats (n=7) during the test of acquisition of responding for conditioned reinforcement.

The responses made on the CR and NCR levers and panel made in 3min intervals during the test session are illustrated in Figures 46, 47 and 48. The results were analysed using repeated measures ANOVA with lever/panel choice and trial number as within subject factors and lesion group as the between subjects factor. There was no main effect of group ($F(1,12) = 0.76$) or lever/panel choice ($F(2,24) = 1.68$) and no group × choice interaction ($F(2,24) = 0.60$) indicating that neither group pressed the CR lever significantly more than the NCR lever during any of the 3min intervals.

From Figure 46 it appears that while the sham lesioned group only showed a mild decrease in responding on the CR lever, the LH lesioned group expressed sporadic increases in responding during the 30min test session. In addition while Figure 47 illustrates a fall in responding on the NCR lever after trial 5 for the sham lesioned group, the LH lesioned group appeared to increase responding on the NCR lever after trial 6. Nevertheless, it is clear from Figures 46 and 47 that the variability of responding on the CR and NCR levers by the LH lesioned group was substantially greater than that of the sham lesioned group. ANOVA revealed no main effect of trial number ($F(9,108) = 1.14$) and no group × trial interaction ($F(9,108) = 1.04$) suggesting that total responding remained constant throughout the test period for both groups.

There was no trial × lever/panel choice interaction ($F(18,216) = 1.45$) indicating that the ratio of the responses on the CR and NCR levers and the panel remained constant between the trials. ANOVA revealed no three way interaction ($F(18,216) = 0.83$).

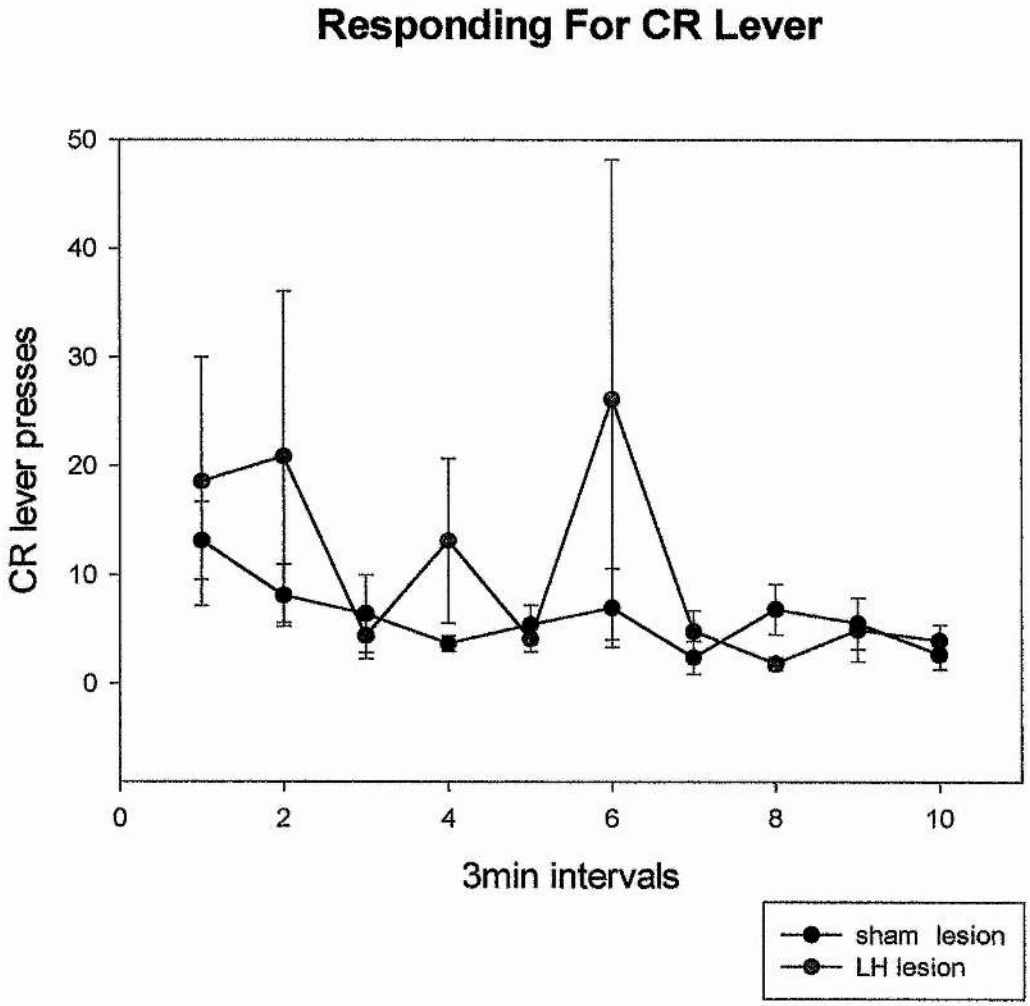


Figure 46. Mean (± SE) responses on CR lever measured at 3min intervals during the conditioned reinforcement test for the sham lesioned group (n=7) and the LH lesioned group (n=7).

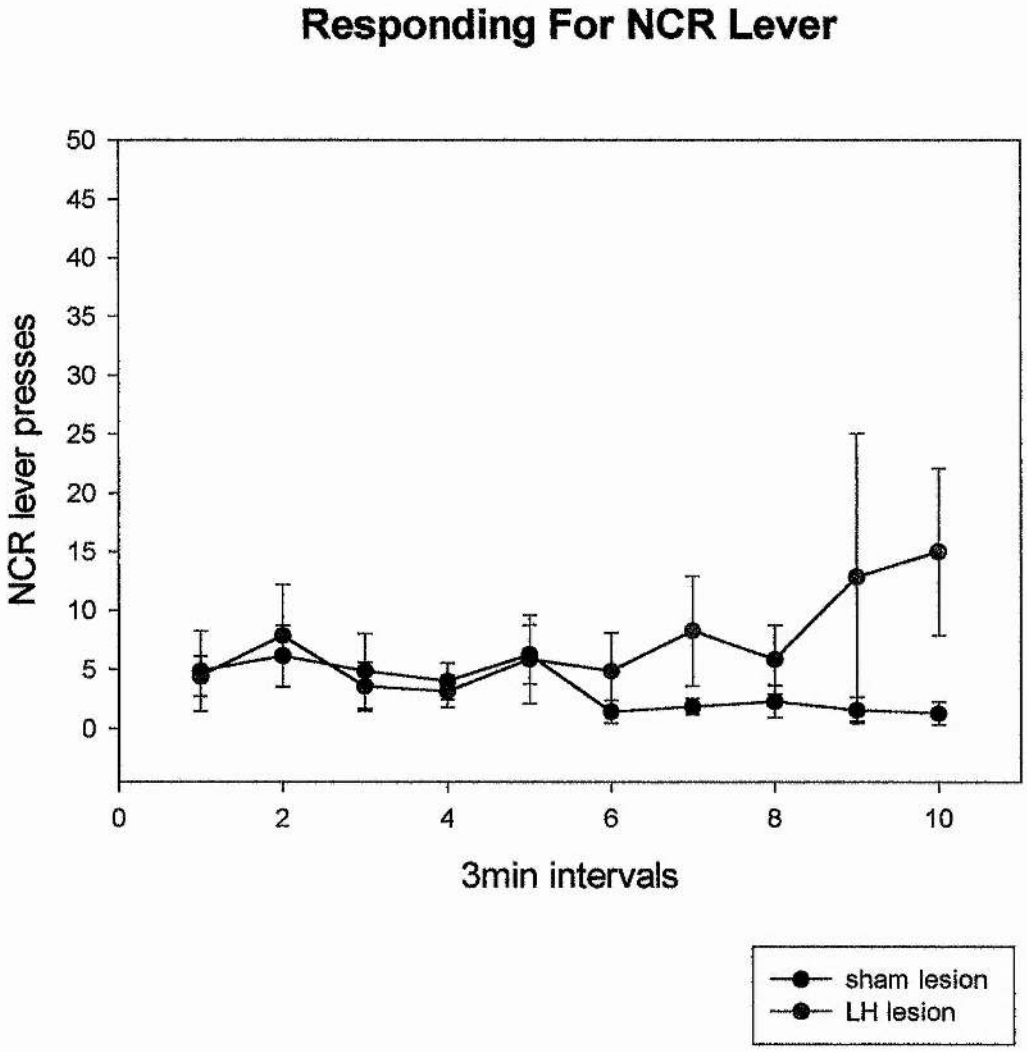


Figure 47. Mean (± SE) responses on NCR lever measured at 3min intervals during the conditioned reinforcement test for the sham lesioned group (n=7) and the LH lesioned group (n=7).

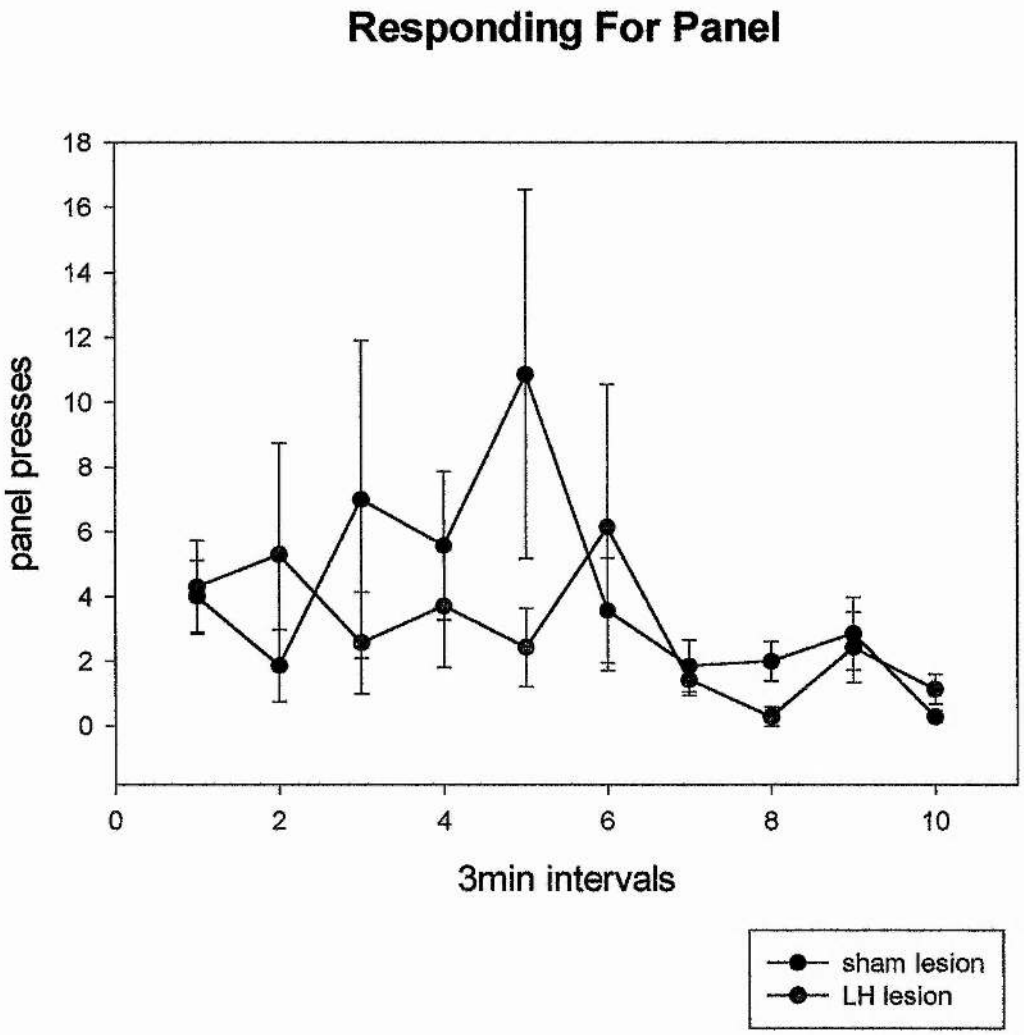


Figure 48. Mean (± SE) responses on panel measured at 3min intervals during the conditioned reinforcement test for the sham lesioned group (n=7) and the LH lesioned group (n=7).

In summary, ANOVA suggests that lesioning the LH did not alter responding for conditioned reinforcement. However, detailed study of the results indicates that the LH lesioned group was not responding as the sham lesioned group. It is clear that the range of behavioural responses varied to a greater degree for the LH lesioned group versus the sham lesioned group. Whereas the maximum number of responses made on the panel within any three minute interval was more or less comparable between the groups: 42 for the sham lesioned group, 32 for the LH lesioned group, the maximum number of responses on both the CR and NCR levers differed substantially between the groups. The maximum number of CR lever presses in one 3min interval made by any of the sham lesioned group was 28 compared to 158 for the LH lesioned rats and the maximum number of NCR lever presses made in any 3min interval by the sham lesioned rats was 25 compared to 86 by the LH lesioned rats. Thus rather than responding for conditioned reinforcement the LH lesioned rats may be over responding non-specifically with increased bouts of responding as opposed to a general increase in responding over the whole test period.

Hypertonic Saline Challenge

It is clear from Figure 49 that the LH lesioned group did not respond as the sham lesioned group [main effect of group ($F(1,12) = 50.26$ $p<0.001$)] in the hypertonic saline challenge. ANOVA revealed a main effect of condition ($F(1,12) = 96.08$ $p<0.001$) indicating that hypertonic saline induced more drinking than isotonic saline and a significant group × condition interaction ($F(1,12) = 71.59$ $p<0.001$) indicating that the increase in drinking induced by hypertonic saline was not comparable between the groups. Tukey's post-hoc tests indicated that although hypertonic saline induced significantly more drinking than isotonic saline in the sham lesioned group ($p<0.001$), the amount of drinking induced by hypertonic saline in the LH lesioned group did not significantly differ from the amount of drinking induced by isotonic saline in the sham lesioned or LH lesioned groups. This indicates that lesioning the LH abolished the drinking response to hypertonic saline.

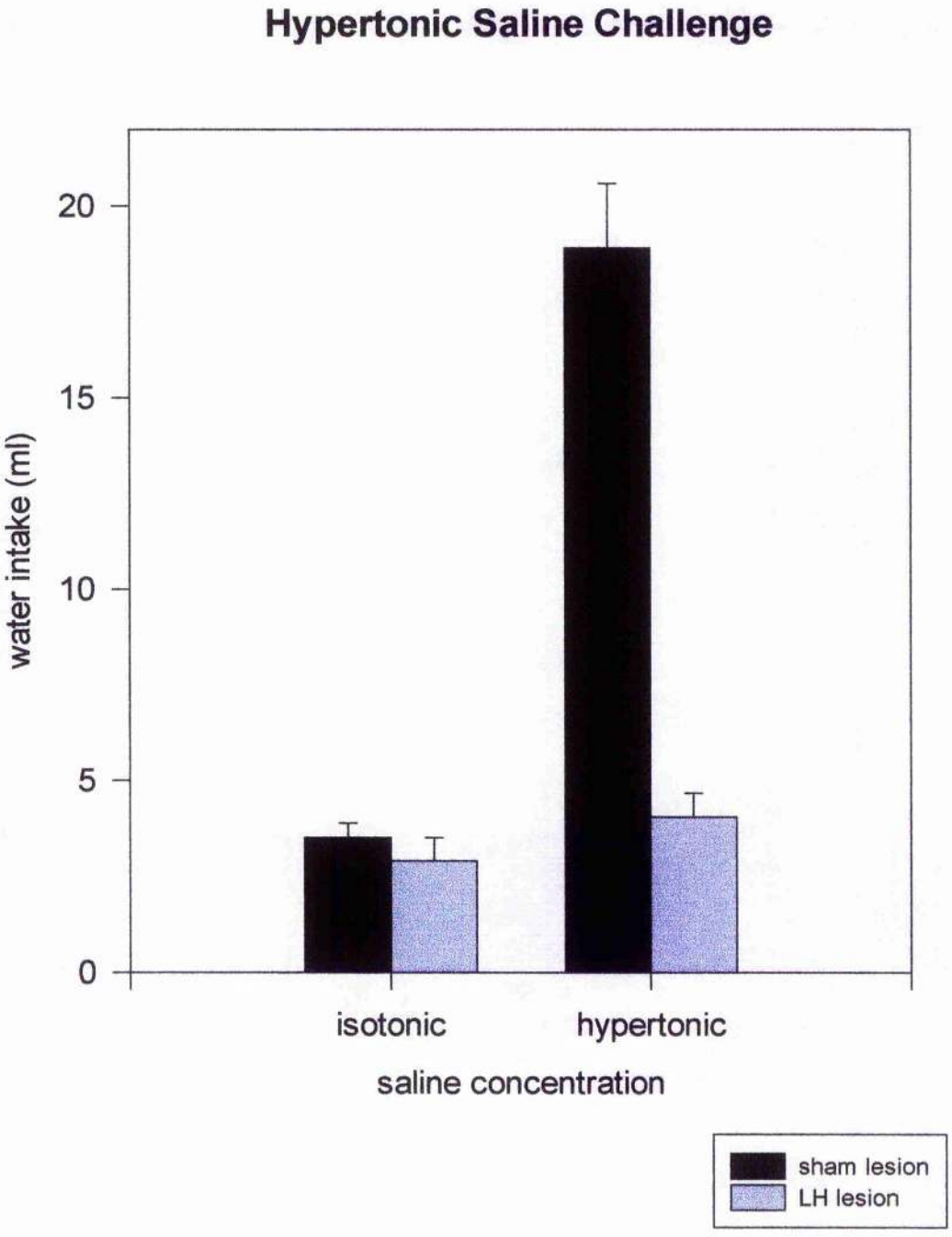


Figure 49. Mean (± SE) volume of water drunk by the sham lesioned group (n=7) and the LH lesioned group (n=7) 3h after i.p. injection of hypertonic (5%) and isotonic (0.9%) saline.

DISSCUSSION

The present data demonstrate that rats with NMDA lesions of the LH respond differently to controls in particular aspects of a reward-related paradigm. Differences were observed in the post-operative training phase of the conditioned reinforcement procedure suggestive that lesioning the LH disrupted retention of the stimulus-reward association. However, a deficit was only seen in the initial stages of post-operative training indicating that even if knowledge of the stimulus-response association was lost as a result of lesioning the LH, it was rapidly regained. Despite the fact that analysis revealed no significant effects of lesioning the LH in the test phase of the conditioned reinforcement procedure, it is clear that the variability of responding was greatly increased by lesioning the LH.

Post-Operative Training

The reinforcement schedule employed in the pre- and post-operative training phases consisted of the association of a compound stimulus with a reward. Training the rats in this reinforcement schedule prior to surgery allowed the role of the LH in the retention of stimulus-reward associations to be examined. For the purpose of analysing acquisition of the stimulus-reward association, the discriminative properties of the conditioned stimulus were determined by calculating the proportion of approaches to the food hopper made in response to the conditioned stimulus. Since there were no group differences in the discriminated approach response induced by the conditioned stimulus before surgery, any differences seen after surgery could be attributed to lesioning the LH.

Lesioning the LH significantly diminished the discriminative properties of the conditioned stimulus with the greatest deficit seen in the initial stages of post-operative training. In conjunction, the number of trials classed as missed where the latency to respond to the conditioned stimulus was greater than 5s was significantly greater for the LH lesioned group compared to the sham lesioned group during the first post-operative training trial. One explanation for this could be that the LH lesioned rats were less motivated to gain food rewards. However,

two pieces of evidence argue against this hypothesis. If lack of motivation was the reason for the deficit, it would be expected that this would have been a constant finding for the duration of the post-operative training period whereas, in fact, from trial 6 no difference in the discriminated approach response was seen between the groups. Furthermore, previous tests that specifically addressed motivational state suggested that NMDA lesions of the LH did not produce impairments in motivation. When operant responding was tested on a progressive ratio, breaking points of LH lesioned rats were no different from that of control rats indicating that lesioning the LH did not decrease motivation to gain food (Robertson, 1994).

An alternative explanation is that lesioning the LH impaired the stimulus-reward association learned prior to surgery. If true however, it would appear that this impairment was specific in relation to the compound stimulus. The reinforcement schedule required the rats to attend to the compound stimulus in order to gain food in an efficient manner. In addition to this stimulus-reward association, an association may have been made between the location of the panel and the delivery of food. Since the LH lesioned rats continued to respond at the panel, albeit inappropriately, it may be the case that this association was retained. A similar finding was reported for the effects of basolateral amygdala lesions in a behavioural study that indicated that the basolateral amygdala was important in the association of aversive cues with explicit but not contextual cues. Rats with lesions of the basolateral amygdala were able to avoid a distinctive environment that had been paired with footshock but failed to express fear in the presence of an auditory cue which had also been associated with the same footshock (Selden *et al.* 1991).

Studies have been completed concerning the role of the basolateral nucleus of the amygdala in discriminative approach induced by a conditioned stimulus permitting a more direct comparison of the function of the basolateral amygdala and LH. Like the effect of lesions of the LH seen presently, lesions of the basolateral amygdala impaired discriminative approach and moreover, it was found that this deficit was greater during the initial stages of post-operative training (Burns *et al.* 1993).

While to a certain extent the effects of lesioning the LH appear to mirror the effects of lesioning the basolateral amygdala, this is not true for other regions implicated in discriminated approach to a conditioned stimulus. Lesions of the central nucleus of the amygdala failed to disrupt latency to respond to the conditioned stimulus or discriminated approach (Robledo *et al.* 1996) and rats with ventral subiculum lesions made prior to discriminative approach training only showed a small albeit significant deficit in the discriminated approach response (Burns *et al.* 1993). In contrast, lesioning the PPTg had a more permanent effect on discriminative approach behaviour (Inglis *et al.* 1994b). Whereas lesioning the LH only produced an increase in the latency to respond to the conditioned stimulus during the first half of post-surgery training, lesions of PPTg increased latency to respond for the entire duration of the post-operative training phase.

Acquisition of a New Response with Conditioned Reinforcement

In order to reinforce a new behaviour, the compound stimulus must gain affective properties. Although both groups responded more on the CR lever than NCR lever indicating that this had occurred, it should be noted that the difference in responding between the levers failed to reach significance. The reason for this may be due to subtle differences in the training procedure used presently, compared to previous studies that have shown significant levels of selective responding. Unlike previous studies the conditioned reinforcer and non-conditioned reinforcer levers were present during the training session where they had no consequence and in addition, there was no minimum delay between rats being returned to the home cage after training trials and being fed the remainder of their daily food allowance. Furthermore, in previous studies where a significant difference was found in lever choice, the nucleus accumbens was infused with at least vehicle (saline) prior to testing. It is possible that some non-specific mechanical effect of the microinfusions potentiated lever choice (Inglis et al. 1994b; Robledo *et al.* 1996). Nevertheless, 60% of responding on the levers was for the CR lever only suggesting that the conditioned stimulus had gained affective properties even if only to a mild degree.

The proportion of selective responding for the CR lever was not significantly different between the groups indicating that lesioning the LH did not disrupt the process whereby the compound stimulus gained such affective properties. Nevertheless, variability of responding was much higher for the LH lesioned group, suggesting some sort of deficit in the processing of such information. Although overall the proportion of total lever pressing which was made on the CR lever was the same for both groups, 4 out of 7 LH lesioned rats lay either above or below the range of responding for the sham lesioned group. This indicates that responding may not actually have been specific for the CR lever. However, since approximately half the lesioned group responded as the control rats and half responded differently, with no correlation between these different responses and the placement of the lesions, it is difficult to predict a definitive hypothesis on the role of the LH in this behavioural paradigm without further testing.

A comparison similar to that made between rats with basolateral amygdala lesions and LH lesions on discriminated approach response cannot be made for responding for conditioned reinforcement. Lesions of the basolateral amygdala, unlike lesions of the LH, reduced selective responding on the CR lever (Cador *et al.*1989; Burns *et al.* 1993). Hence, while the results of the post-operative training phase were suggestive that the proposed relay between the LH and basolateral amygdala may be functional, it would appear that the LH and the basolateral amygdala may not be so closely linked with regard to responding for conditioned reinforcement.

Summary

The attenuation of discriminated approach behaviour seen in the initial stages of post-surgery training implies that lesioning the LH produced a deficit in the stimulus-response association formed prior to the lesion. However, this deficit was only seen until the sixth post-surgery training trial suggesting that although the LH lesion may have disrupted the retention of the stimulus-reward association, it did not preclude the relearning of it. The effect of lesioning the LH on responding for conditioned reinforcement is less clear. Overall responding for conditioned reinforcement was not altered by lesioning the LH

but the variability of responding within the LH lesioned group was greater than within the sham lesioned group. This difference in the degree of deficits found with the discriminated approach response and responding for conditioned reinforcement may reflect the idea of McDonald & White (1993) who suggested that subtle differences in the tests used to study memory systems produced great differences in the results obtained. In order to examine the role of the LH in memory systems further the ability of LH lesioned rats to form other stimulus-reward associations is required.

CONDITIONED PLACE PREFERENCE

Carr *et al.* (1989) stated that two aspects of reward can be utilised in the study of reward related processes. In addition to reinforcing behaviour, which can be monitored by procedures such as conditioned reinforcement, rewarding stimuli also elicit approach responses and maintain contact with them. This latter facet of reward is utilised in the conditioned place preference procedure. A rewarding stimulus is paired with a distinctive neutral environment thereby enlisting the environment as a secondary reinforcer. In a later test, if the animal approaches and maintains contact with this distinctive environment it is assumed that the stimuli of the environment did in fact gain reinforcing properties via association with the primary reward.

This procedure has been utilised in parallel to conditioned reinforcement to elucidate the involvement of limbic striatal interactions in incentive learning. Like conditioned reinforcement, evidence from studies of conditioned place preference suggested that release of dopamine in the nucleus accumbens could increase the incentive value of the neutral stimuli with which it was paired. For instance, microinjections of *d*-amphetamine into the nucleus accumbens but not the dorsolateral caudate nucleus induced a conditioned place preference (Carr & White 1983).

Furthermore, Everitt *et al.* (1991) neatly illustrated an amygdala-ventral striatal interaction in reward-related processes using the conditioned place preference procedure, a finding also reflected by studies of conditioned reinforcement (Cador *et al.* 1989). Not only did bilateral lesions of the basolateral amygdala or bilateral lesions of the ventral striatum attenuate conditioned place preference learning, but combining a unilateral lesion of the basolateral amygdala with a contralateral lesion of the ventral striatum also attenuated it. While sole destruction of each area was indicative of a role in the conditioned place preference procedure, the disconnection of the amygdala and ventral striatum by asymmetric unilateral lesions strongly supported the hypothesis that the amygdala and ventral striatum interacted in the formation of a conditioned place preference. Thus, using two different experimental procedures the basolateral

amygdala and the ventral striatum were shown to be important in the association of neutral stimuli with reward in a manner which could involve a relay between these two regions.

It has been suggested that the limbic system affects motivational processes via the nucleus accumbens, a component of the proposed limbic-motor interface (Mogenson 1980). Using the conditioned place preference procedure, the efferent connections of the nucleus accumbens involved in reward-related processes have been examined. Lesions of the anterior or posterior domains of the ventral pallidum which receives projections from the ventral striatum, or the dorsomedial thalamic nucleus which receives projections from the ventral pallidum disrupted the formation of a conditioned place preference indicating that this process may depend on ventral striatopallidal outflow to the dorsomedial thalamus (McAlonan *et al.* 1993).

Therefore the use of conditioned reinforcement and conditioned place preference has indicted that amygdala-striatal interactions may be important in determining approach to and the reinforcing properties of reward-related stimuli and the association of rewarding properties with neutral stimuli. In turn, motor processes necessary for the expression of these behavioural responses may be accessed by projections from the striatum to the ventral pallidum, which projects, via the dorsomedial thalamic nucleus to cortical areas involved in motor output.

Everitt *et al.* (1991) suggested that the procedures of conditioned place preference and conditioned reinforcement monitor related aspects of reward-related processes. However, McDonald & White (1993) in a study which utilised three different tests using the same piece of equipment, the radial arm maze, has illustrated that there are different types of learning responsible for reward-related processes which may be mediated by independent memory systems. It was proposed that procedures which required a stimulus-reward association in the absence of a response may be mediated by different neural substrates from procedures which required a stimulus-response association.

In the case of the conditioned reinforcement procedure, both stimulus-reward and stimulus-response associations may be required. In the training phase, a stimulus-reward association is formed by pairing the delivery of a reward such as food with a compound stimulus in a Pavlovian manner. The control over behaviour by this stimulus is then tested by a stimulus-response association; depression of a lever is required to gain this secondary reward. Thus, the hypothesis proposed by McDonald & White (1993) would suggest that the different stages of the conditioned reinforcement procedure would require different neural substrates. In consideration of this, it is noteworthy that lesions of the basolateral amygdala impaired performance on the discriminated approach to a primary reinforcer and reduced the control over behaviour by a conditioned reinforcer but did not further impair the potentiation of control over behaviour by a secondary reinforcer (Burns *et al.* 1993). Furthermore, lesions of the central nucleus of the amygdala did not impair discriminated approach to a primary reinforcer, or the control over behaviour by a conditioned reinforcer but profoundly impaired the potentiation of responding with conditioned reinforcement by intra-accumbens *d*-amphetamine (Robledo *et al.* 1996). Thus with either lesion, any change reported in the training phase was reflected in the test phase but this appeared to bear no relation to the potentiation of the control over behaviour by the secondary reinforcer in the test phase.

Acquisition of a conditioned place preference requires the formation of a stimulus-reward association in order that the environment with which the reward was paired may stimulate approach and maintain contact with that environment. However, it is possible that more than one mechanism may contribute to the expression of a conditioned place preference. In order to obtain the secondary reward, the animal must actively respond by moving to the paired environment indicating that a stimulus-response association may be required. However, unlike conditioned reinforcement where lever pressing must be repeated to gain further secondary rewards, no other response is required to maintain contact with the secondary reward unless the animal exits the paired environment. Hence, it is possible that a stimulus-reward association is necessary for the maintenance of contact with it.

It would therefore appear prudent to study various reward related procedures when examining the role of neural structures in learning and memory not only to verify the relevance of such structures but also to clarify more specifically the aspect of reward learning to which they contribute. The LH has been implicated in the conditioned reinforcement procedure (Experiment 2.1: The Role of the LH in Conditioned Reinforcement) but few other behavioural studies exist which examined the contribution of the LH to reward-related processes. Hence, the aim of the present study was to examine the role of the LH in such processes by investigating the effect of NMDA lesions of the LH on acquisition and expression of a conditioned place preference.

Experiment 2.2: Role of the LH in Conditioned Place Preference Learning

METHODS

Animals

Thirty-two naïve male Lister Hooded rats (Charles River) were individually housed under a 12h light/dark cycle with ad lib food (SDS maintenance diet no. 1 chow pellets) and tap water in their home cages except when indicated. Daily measurements were taken of body weight and on the basis of this information 16 of the rats were assigned to the sham lesioned group and 16 were assigned to the LH lesioned group whose average weights were 409.00g (SD 22.94) and 408.30g (SD 20.42) respectively. Body weight and food and water intake were monitored throughout the study. Although food spillage was not accounted for, previous studies have shown that lesioning the LH failed to disrupt the amount of food spilled (Winn *et al.* 1990) and thus it was considered that a valid comparison of food eaten across the groups could still be made.

Surgery

Animals were anaesthetised by i.p. injection of Sagatal (60mg/ml; 1ml/kg) with the injection volume doubled using sterile water. Eight of the sham lesioned rats were left to recover from anaesthesia in the post-operative care area without incurring any further procedures. The remaining rats were placed in a stereotaxic frame with the incisor bar 5.0mm above the interaural line. LH lesioned animals received a simultaneous bilateral infusion of 1µl 0.06M NMDA, and 8 of the sham lesioned rats received a simultaneous bilateral infusion of 1µl phosphate buffer. NMDA was dissolved in phosphate buffer (pH 7.4) and pH adjusted to 7.4 with 0.2M NaOH. The infusion rate was 0.5µl/min with a further 2min allowed for diffusion. The co-ordinates for cannulae were anterior-posterior 0mm from bregma; medial-lateral ±2.0mm from midline; ventral 8.0mm from dura.

Post-Operative Care

Body weight was monitored for 28 days after surgery and if it fell below 85% pre-surgery weight these individual rats were given wet mash (Farley's © dry baby food and glucose) which was made up fresh daily. Seven of the LH lesioned rats received wet mash for a variable number of days that ranged from 1-6 with an average of 2.4. Since in previous studies it has been found that body weight gain caused by consumption of wet mash was not stable, wet mash was removed when body weight started to increase as opposed to waiting for body weight to return to 85% of pre-surgery levels. In order to supplement diet without precluding the consumption of lab chow, the rats were given dilute wet mash in their water bottles until their body weight returned to 85% of pre-surgery levels. Five of the LH lesioned rats received dilute wet mash in their water bottles for a range of 2-6 days with an average of 5.

Hypertonic Saline Challenge

The rats were given a hypertonic saline challenge 54 days after surgery. All rats received an i.p. injection of both 5% hypertonic saline (20ml/kg) and isotonic (0.9%) saline (20ml/kg), with 48h between injections. Injections were given in a counterbalanced order and tap water consumed 1h and 3h after injection was measured.

Conditioned Place Preference

The method used for testing conditioned place preference was based on that used by Carr & White (1985).

Equipment

The equipment used consisted of two pairing boxes measuring 80cm across, 60cm high and 30cm wide separated by a neutral box measuring 20cm across, 60cm high and 30cm wide. All three boxes were made of wood with Plexiglas fronts which, apart from a 3cm strip at the bottom, were occluded with an appropriate colour and each box had an appropriately coloured lid made from perforated wooden board. The pairing boxes were differentiated with regard to three sensory cues:

- visual cues; one pairing box was painted black the other white,
- tactile cues; the flooring consisted of steel bars in the white box and perforated zinc painted black in the black box,
- olfactory cues; petri dishes were placed in spill trays beneath the flooring containing 2% acetic acid in the white box and floral scented fluid in the black box.

Markers on the outside of the pairing boxes divided each into 3 equal sized areas allowing a quantitative analysis of activity within them by counting the number of times the animals moved from one area to another. Each of the pairing boxes had a hole drilled in the end wall through which a drinking nozzle attached to a burette was placed. The neutral box, painted grey with a grey painted 1cm grid floor, gave access to both the pairing boxes through entrances that could be closed by removable metal guillotine doors. In order to minimise distraction from the environment surrounding the apparatus the room was darkened and the boxes were illuminated by lamps positioned above the lids of the pairing boxes.

Procedure

For the duration of the conditioned place preference procedure, the rats were on a 22h food deprivation schedule and body weight, food intake and water intake were monitored. During the procedure, the rats were fed in the home cage for 2h commencing 30min after they were removed from the place preference apparatus. The behavioural procedure consisted of three phases: a habituation phase, a conditioning phase and a testing phase.

Habituation Phase

In addition to familiarising the rats with the taste of 20% sucrose and the environment of the place preference apparatus, the habituation phase provided a measure of spontaneous preference for the pairing boxes. A second water bottle filled with 20% sucrose was introduced to the home cage and consumption over 48h was monitored. Subsequently, the rats were habituated to the conditioned place preference apparatus over 2 days. Throughout the habituation session the drinking nozzles were present in each of the pairing boxes but the drinking tubes remained empty. On day 1 the rats were placed in the neutral box for 1min after

which the guillotine doors were removed giving access to all areas for a further 10min. On day 2 this procedure was repeated with the rats remaining in the boxes for 15min. During this period the presence of a spontaneous preference was examined. The dependent measures recorded using "Observer[2]" were time spent in the three boxes and while in the pairing boxes, the number of crosses made from one area to another. Based on this information the side which was subsequently paired with sucrose was counter-balanced with half of each lesion group receiving sucrose in their preferred side, half in their non-preferred side and half of each lesion group receiving sucrose in the black side and half in the white side.

Conditioning Phase

On days 3-14 sucrose was associated with the paired side by confining the rats to one pairing box for 30min pairing sessions. In the paired side the drinking tube was filled with 20% sucrose and consumption monitored while in the unpaired side although the drinking nozzle was present the drinking tube was empty. The box in which the rat was confined alternated from day to day resulting in a total of 6 pairings in each box. On the first pairing day half of each lesion group were confined to their paired side and half were confined to their unpaired side. Between pairing sessions the boxes were wiped down with 20% alcohol to remove any odours left by the rats.

Testing Phase

24h after the last pairing session the rats were tested for a conditioned place preference using the same method as that used for measuring spontaneous place preference. The drinking tubes for both the paired and unpaired sides were empty but the drinking nozzles were not removed. The rats were placed in the neutral box for 1min after which the guillotine doors were removed allowing access to the whole apparatus and time spent in each box and the number of crosses into the different areas of the pairing boxes were measured using "Observer".

[2] "Observer" is a software package produced by Noldus Information Technology which allows the allocation of timer switches and counters to specific key presses enabling the user to record the frequency and duration of various observed behaviours simultaneously.

Sucrose Preference

Seventy-two days after surgery the groups were tested for sucrose preference in the home cage where ad lib food and water were available throughout testing. The concentrations of sucrose used were 1%, 2%, 4%, 12%, 20% and 40%. A water bottle filled with sucrose solution was placed in the home cage with the rats exposed to each concentration for 24h with 24h between exposures.

Histological Analysis

At the end of the study all the experimental rats were given an overdose of 200mg/ml pentobarbitone (Euthatal) and were subsequently perfused transcardially with physiological saline followed by 10% formalin. The brains were then removed and stored in 10% formalin until they were sectioned on a freezing microtome. 25μm sections were cut at 200μm intervals and stained with cresyl violet stain for nissl substance. Lesions were identified by areas of cell loss and reactive gliosis. In order to ascertain lesion volume, histological sections were scanned and then silhouettes of the areas of the lesion were drawn onto the scanned sections. The areas of the lesioned tissue was determined using a microcomputer and lesion volumes calculated.

Statistical Analysis

Despite the vast literature spanning over 35 years describing the conditioned place preference paradigm (reviewed by Schechter & Calcagnetti 1993), there still does not appear to be a standard method of analysing the results of these experiments. The data resulting from a study of conditioned place preference consists of three measurements of time: duration of total time spent in the paired, unpaired and neutral areas. These measurements are mutually exclusive since the animal can only be present in one area at a time. Thus the values found for each variable are not independent, as the duration of time spent in one area increases, the duration of time spent in the other areas must decrease. Although a score is made for each variable for each rat, analysis of the results by repeated measures ANOVA is flawed since in addition to the variables not being independent the total score for each rat will always be equal, being the total

duration of the test session. Therefore repeated measures ANOVA will fail to show any group differences that may be present. While many different analyses have been employed to overcome these problems including Wilcoxon's non-parametric test (Cervo & Samanin 1995) and one-tailed t-test for correlated designs (Carr & White 1983) none of these solutions are ideal. For instance, non-parametric tests fail to show interactions. Although repeated measures ANOVA fails to show a main effect of group, it is possible to find a main effect of side and an analysis of hypothetical data included in the appendix, pg 268, illustrates that the inability to find a main effect of group does not preclude the chance of finding a significant group × side interaction. This method has been employed previously (White & Carr 1985) and is the method used to analyse the results obtained presently.

The remaining results were examined statistically using repeated measure ANOVA with, when appropriate Tukey's post hoc tests. Previous studies revealed no differences between control rats that had been anaesthetised only and control rats that had received phosphate buffer infusions of the LH (Experiment2.1: "The Role of the LH in Conditioned Reinforcement) and so for all statistical analysis the two different classes of control rats were collapsed as one group. Since the body weights of the LH lesioned rats were significantly smaller than the sham lesioned rats for the duration of the study all measurements of intake were expressed as a function of body weight both for the purpose of statistical analysis and graphing the data.

RESULTS

Histology

Analysis of the histology revealed that 4 animals did not have LH lesions and were excluded from any further analysis. The pattern and size of the biggest and smallest lesions of the LH produced are illustrated in Figure 50. The average volume of the lesions for the LH lesioned group was 1.78mm^3 (SE 0.16) on the left side and 2.25mm^3 (SE 0.21) on the right side with the greatest damage level with the paraventricular nucleus decreasing towards the anterior and posterior poles. Damage out with the LH was limited with more extensive damage only present unilaterally. Seven animals suffered unilateral damage anterior to the paraventricular nucleus situated in the reticular thalamic nucleus. In addition, unilateral damage was found in the anteroventral and ventromedial thalamic nuclei of 3 rats, in the anteromedial thalamic nucleus of 1 rat and the stria medullaris of the thalamus of 2 rats. The extent of the damage to thalamic nuclei at this level ranged from 30%-80%.

Level with the paraventricular nucleus, lesions extended to the thalamus resulting in 50%-90% damage of the zona incerta bilaterally in all rats. Bilateral reticular thalamic damage was seen in 10 rats and unilateral damage was seen in 2 rats. However, extensive damage ranging from 30%-75% was only seen unilaterally. At this level there was also damage which may have been caused by reflux of the toxin found bilaterally in 4 rats and unilaterally in 6 rats. Damaged ranged from less than 30%-90% of the ventromedial thalamic nucleus and less than 30%-50% of the ventrolateral thalamic nucleus. Damage more extensive than 50% was not found in any thalamic structures bilaterally.

Posterior to the paraventricular nucleus damage was limited dorsally to the mammilliary thalamic tract except in 3 rats unilaterally where 60-75% of the ventromedial thalamic nucleus was lesioned. 30%-75% damage was found bilaterally in the zona incerta and subincertal nucleus in all rats and bilaterally in 4 rats and unilaterally in 1 rat in the subthalamic nucleus. Minor damage (<30%) was seen bilaterally in 6 rats and unilaterally in 1 rat in the ventral border of the ventromedial thalamic nucleus.

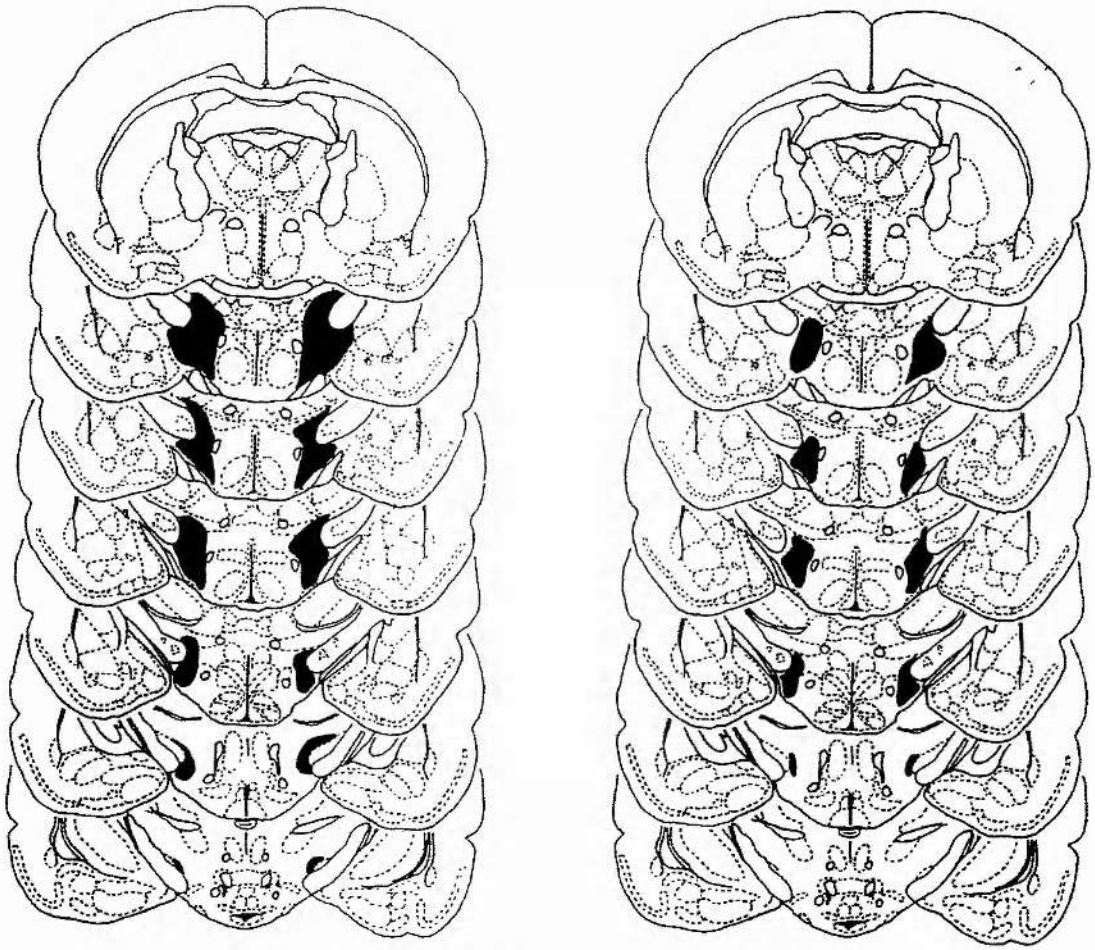


Figure 50. Representative sections redrawn from the atlas of Paxinos & Watson (1982) illustrating the extent of the biggest and smallest lesions produced by injection of NMDA into the LH. The shaded regions represent areas of neuronal loss.

Body Weight

Body weight before and after surgery is illustrated in Figure 51. Repeated measures ANOVA showed no main effect of group before surgery ($F(1,26) = 0.10$), a main effect of days ($F(6,156) = 58.52$ $p<0.001$) and no significant group × days interaction ($F(6,156) = 1.50$) indicating that although body weight was increasing before surgery, the rate at which body weight was increasing across days was comparable between the groups.

After surgery, body weight fell further for the LH lesioned group compared to the sham lesioned group [main effect of group ($F(1,26) = 26.70$ $p<0.001$)]. ANOVA revealed a main effect of days ($F(27,702) = 113.64$ $p<0.001$) and a significant group × day interaction ($F(27,702) = 21.53$ $p<0.001$) indicating that in addition to the greater initial deficit in body weight seen with the LH lesioned groups, the pattern of recovery significantly differed between groups. It is clear from Figure 51 that the sham lesioned group made a more rapid and complete recovery than the LH lesioned group with respect to body weight.

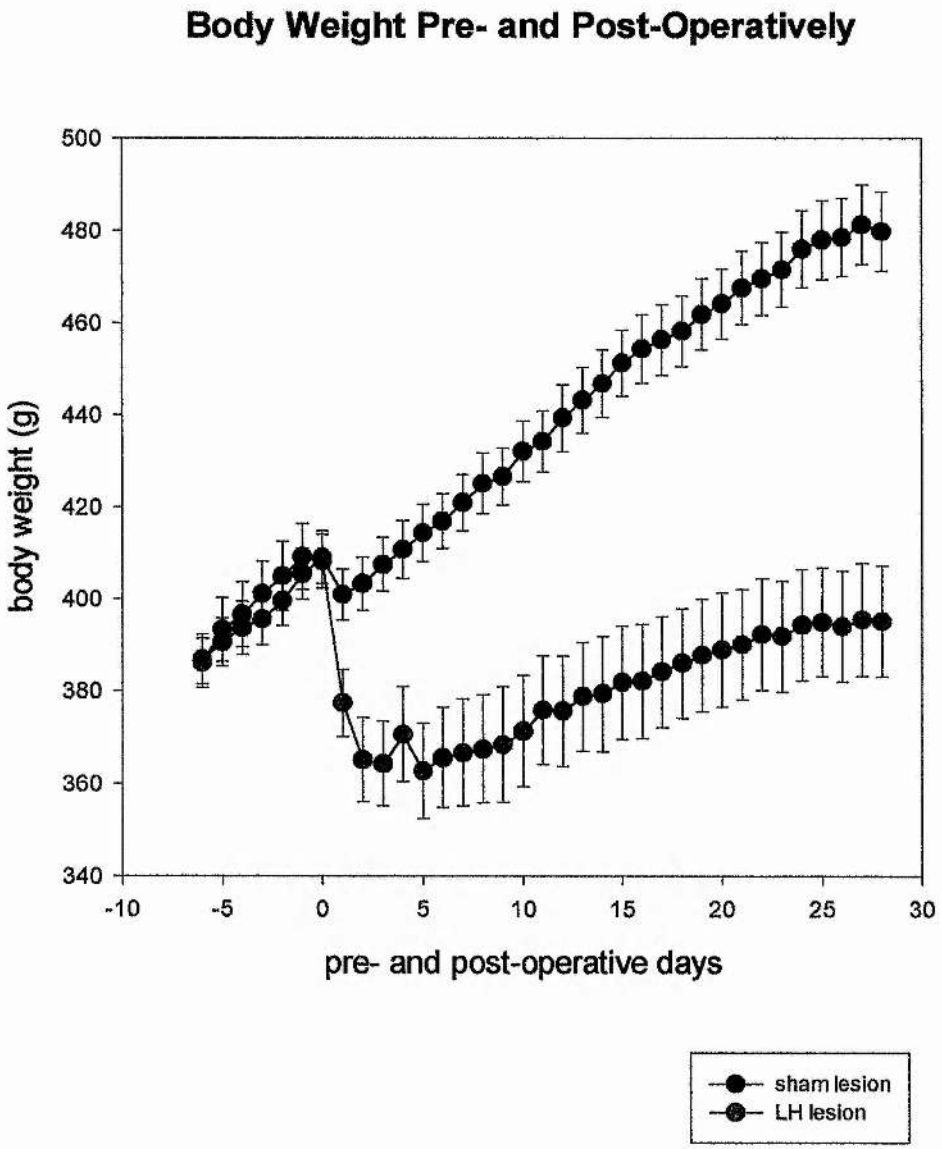


Figure 51. Mean body weight (± SE) for the sham lesioned group (n=16) and the LH lesioned group (n=12) 6 days before and 28 days after surgery.

Hypertonic Saline Challenge

Figure 52 illustrates that hypertonic saline induced a greater degree of drinking than isotonic saline in sham lesioned and LH lesioned groups [main effect of treatment ($F(1,26) = 68.54$ $p<0.001$)]. ANOVA revealed a main effect of group ($F(1,26) = 52.60$ $p<0.001$) and a significant group × treatment interaction ($F(1,26) = 33.35$ $p<0.001$) indicating that the volume of drinking induced by saline injections was not equal across the groups. Tukey's post-hoc tests showed that hypertonic saline induced a significantly greater degree of drinking than isotonic saline in the sham lesioned group ($p<0.001$). Although the increase in drinking for the LH lesioned group did reach significance ($p<0.007$), it was significantly smaller than that seen with the sham lesioned group ($p<0.001$) indicating that lesioning the LH attenuated the volume of drinking induced by hypertonic saline.

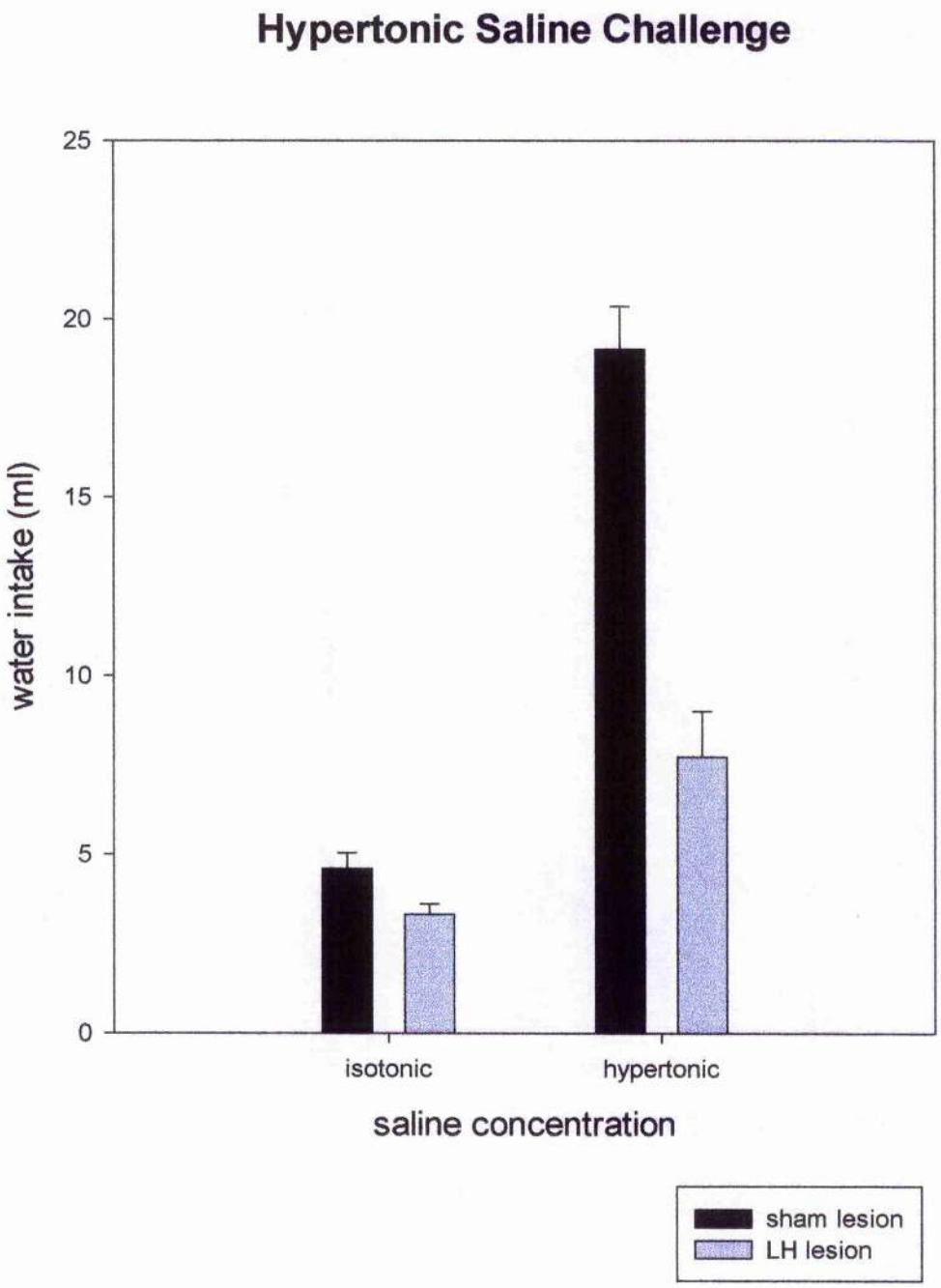


Figure 52. Mean (± SE) volume of water consumed by the sham lesioned group (n=16) and the LH lesioned group (n=12) in 3h after i.p. injections of hypertonic saline (5%) and isotonic saline (0.9%).

Conditioned Place Preference Test

Time Spent in Compartments

The amount of time spent in the paired, unpaired and neutral compartments is illustrated in Figure 53. ANOVA revealed a main effect of compartment ($F(2,50) = 21.11$ $p<0.001$) but no group × compartment interaction ($F(2,50) = 1.39$) indicating that lesioning the LH did not prevent the expression of a conditioned place preference.

Crossing in Paired and Unpaired Compartments

Figure 54 shows that the number of crosses made in the paired compartment was greater than in the unpaired compartment [main effect of compartment ($F(1,25) = 14.29$ $p<0.001$)]. ANOVA revealed no main effect of group ($F(1,25) = 0.79$) and no group × compartment interaction ($F(1,25) = 0.96$) thus indicating that lesioning the LH did not alter activity levels and that the rats were more active in the paired versus non-paired compartment.

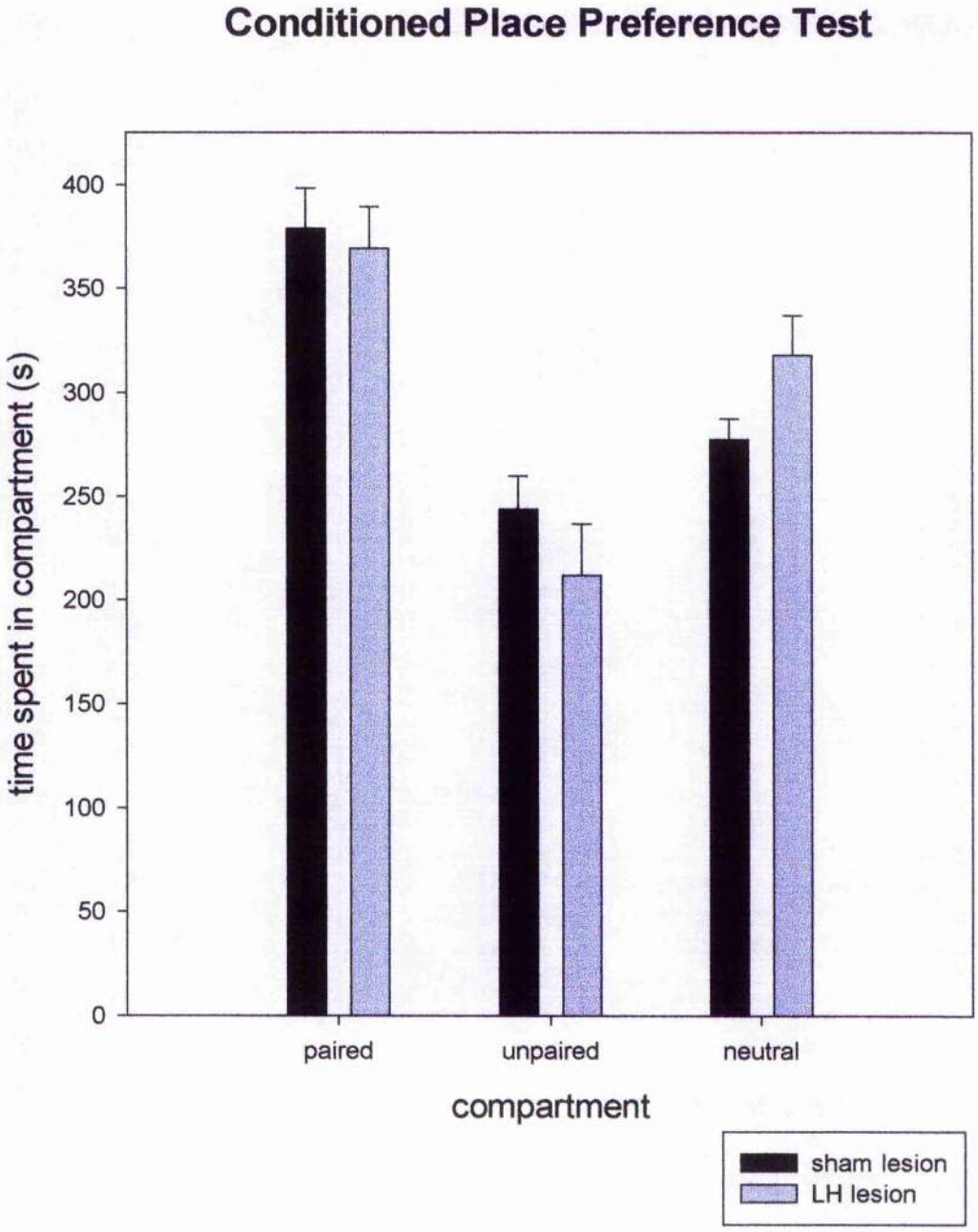


Figure 53. Mean (± SE) time spent in paired, unpaired and neutral compartments by the sham lesioned group (n=16) and the LH lesioned group (n=11) during the 15min test period.

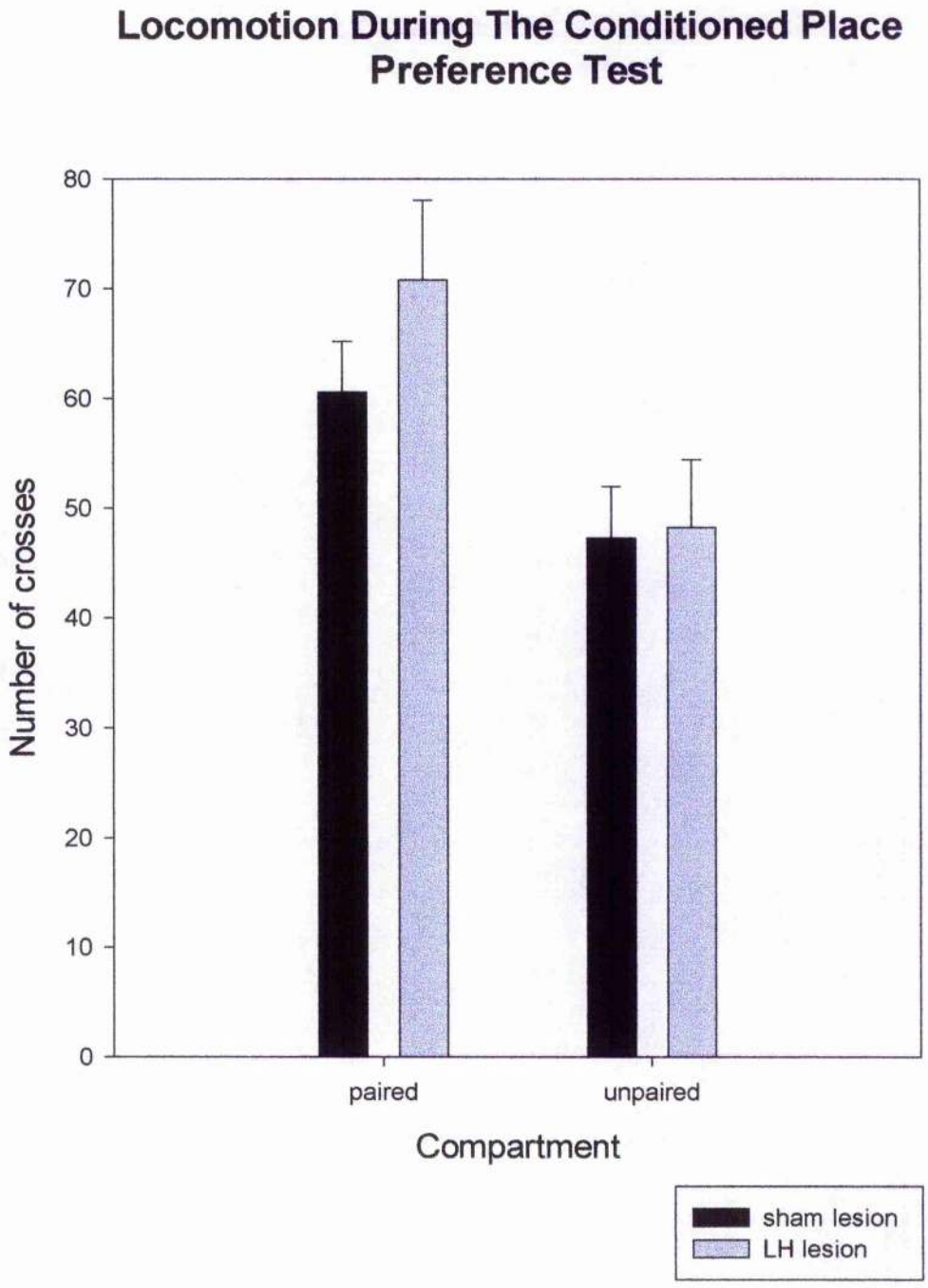


Figure 54. Mean (± SE) number of crosses made in the paired and unpaired compartments by the sham lesioned group (n=16) and the LH lesioned group (n=11) during the conditioned place preference test.

Sucrose Consumption on Exposure to the Paired Compartment

From Figure 55 it appears that the amount of sucrose drunk during pairings sessions in the paired compartment was elevated by damage in the LH [main effect of group ($F(1,25) = 36.48$ $p<0.001$)]. The volume of sucrose drunk increased from the first pairing session [main effect of pairing session ($F(5,125) = 10.44$ $p<0.00$)] but the group difference in sucrose drinking was constant for all pairing sessions [no significant group × pairing session interaction ($F(5,125) = 1.16$)].

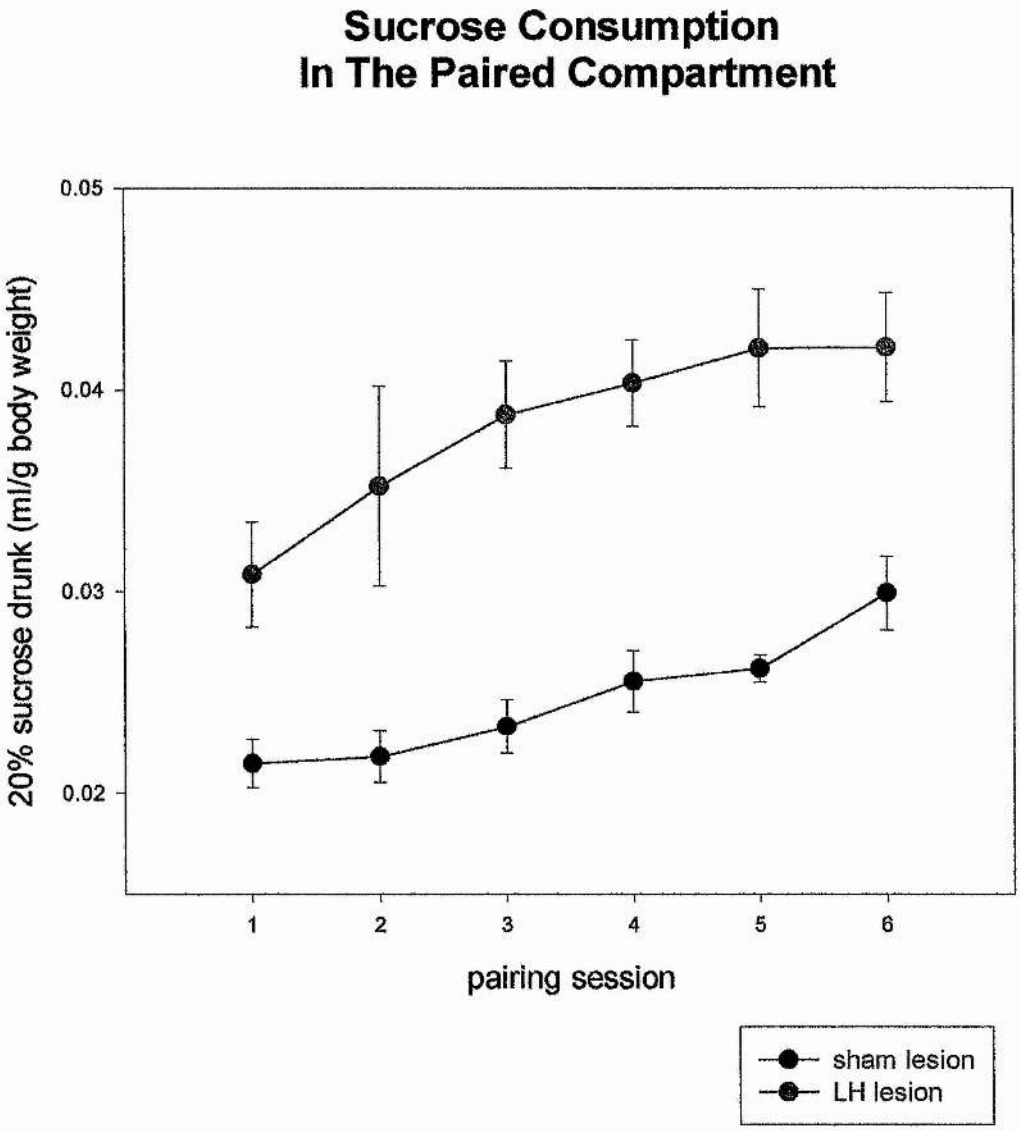


Figure 55. Mean (± SE) volume of sucrose drunk on exposure to the paired compartment for the sham lesioned group (n=16) and the LH lesioned group (n=11).

Regulatory Behaviour During The Conditioned Place Preference Procedure

The effect of the place preference procedure on home cage regulatory behaviour is illustrated in Figures 56, 57 and 58. Body weight, food intake and water intake were monitored for 3 days before the place preference procedure began in order to obtain baseline values for these variables. The habituation phase corresponds with days 4–7 with the first pairing session occurring on day 8.

Body Weight

Analysis of body weight during the conditioned place preference procedure revealed a main effect of group ($F(1,25) = 38.08$ $p<0.001$), a main effect of day ($F(20,500) = 87.13$ $p<0.001$) and a significant group × day interaction ($F(20,500) = 3.43$ $p<0.001$). Figure 56 illustrates that while on the 22h food deprivation schedule, damage in the LH resulted in a mild elevation of the rate of decrease in body weight.

Food Intake

Repeated measures ANOVA of food intake during the conditioned place preference procedure revealed no main effect of group ($F(1,25) = 4.61$), a main effect of day ($F(19,475) = 161.55$ $p<0.001$) and a significant group × day interaction ($F(19,475) = 2.11$ $p<0.004$). Overall food intake was no different for the sham lesioned and LH lesioned groups when body weight was considered but the relationship between the groups was not constant for the duration of the test period.

Water Intake

Repeated measures ANOVA of water intake during the conditioned place preference procedure revealed a main effect of group ($F(1,25) = 10.74$ $p<0.003$), a main effect of day ($F(19,475) = 21.31$ $p<0.001$) and a significant group × day interaction ($F(19,475) = 3.10$ $p<0.001$). Tukey's post-hoc analysis indicated that there was no difference in water intake for the sham lesioned and LH lesioned groups from day 1-6, corresponding with the period of baseline measurements and 48hr access to sucrose in the home cage. Figure 58 illustrates that water intake for LH lesioned group was lower than that of the sham lesioned group

after day 7 and Tukey's post-hoc analysis revealed that the difference in water intake between the groups was significant (at least $p<0.018$) on days 7, 9, 11, 13, 15 and 17.

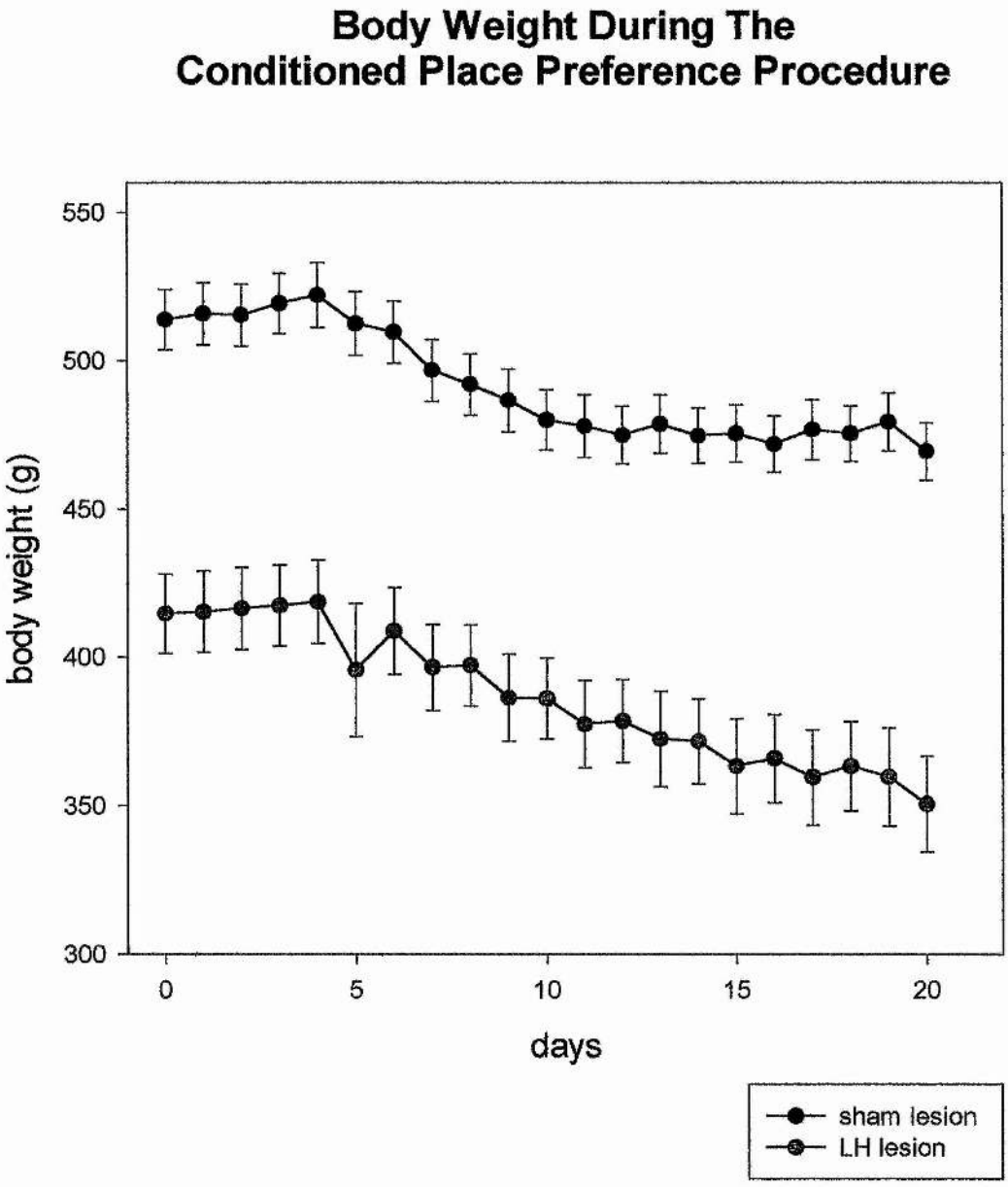


Figure 56. Mean (± SE) body weight for the sham lesioned group (n=16) and the LH lesioned group (n=11) during the conditioned place preference procedure.

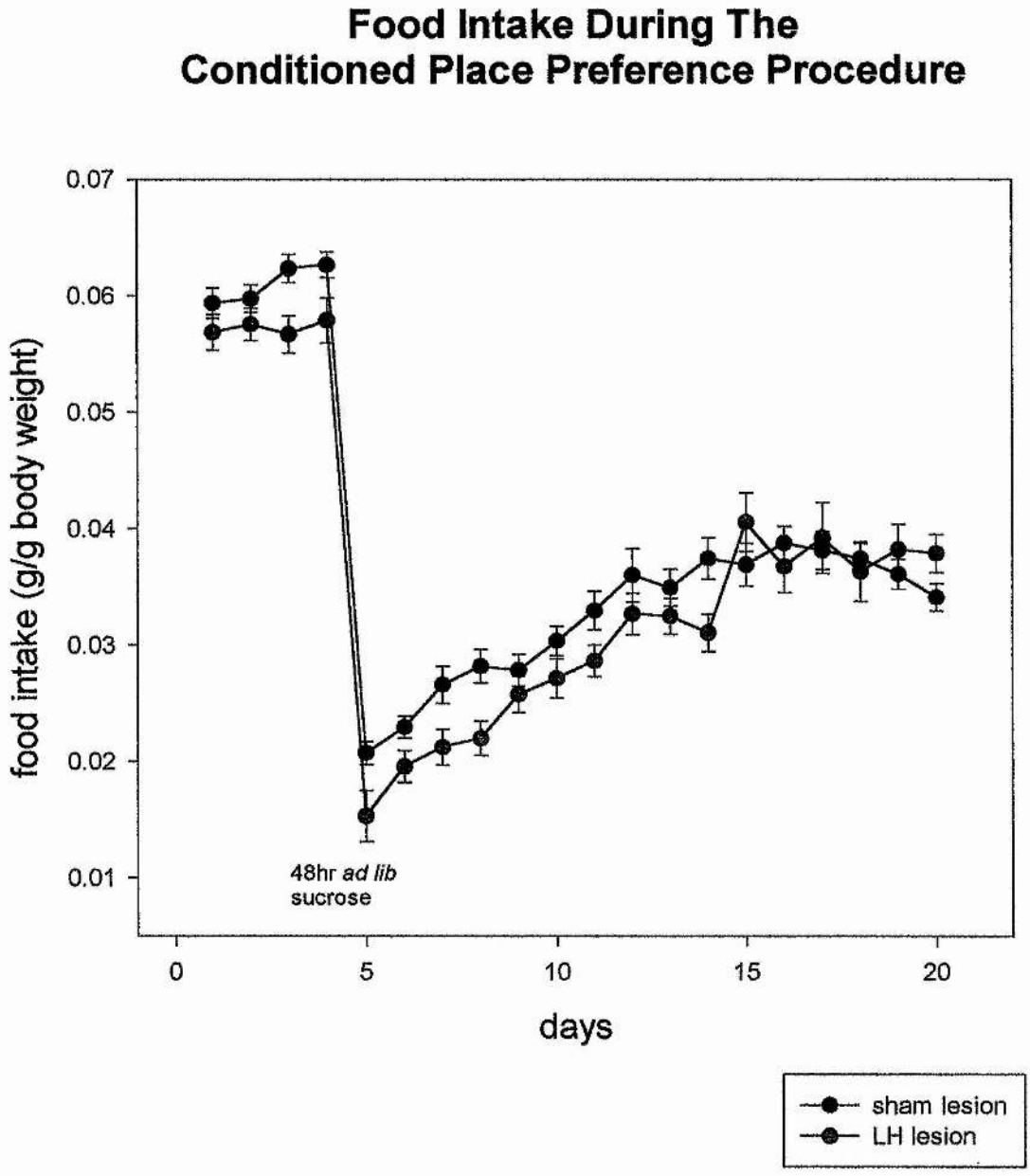


Figure 57. Mean (± SE) food intake as a function of body weight for the sham lesioned group (n=16) and LH lesioned group (n=11) during the conditioned place preference procedure.

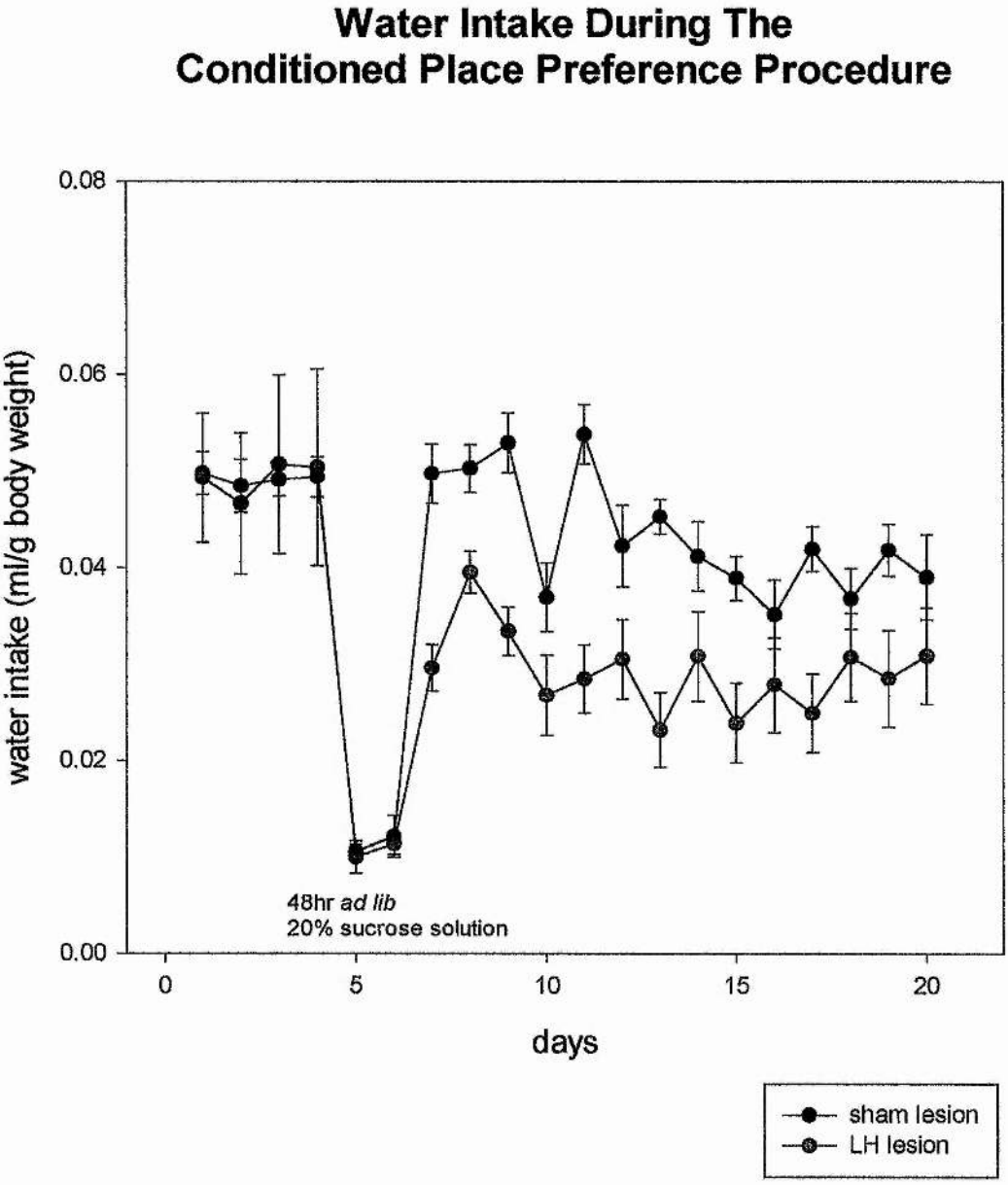


Figure 58. Mean (± SE) water intake as a function of body weight for the sham lesioned group (n=16) and the LH lesioned group (n=11) during the conditioned place preference procedure.

Sucrose Preference

Sucrose preference is illustrated in Figure 59. The volume of sucrose consumed was analysed using repeated measures ANOVA with lesion type as the between subjects factor and concentration of sucrose as the within subjects factor. In addition to the volume of the 6 concentrations of sucrose drunk, the volume of water drunk the day before testing was included in the analysis in order to compare preference for sucrose versus water. ANOVA revealed a main effect of concentration ($F(6,132) = 40.59$ $p<0.001$) indicating that sucrose preference differed for the concentrations used but no main effect of group ($F(1,22) = 0.89$) suggesting that overall sucrose consumption was not effected by lesioning the LH. However the relationship between the groups was not constant for all the concentrations used [significant group × concentration interaction ($F(6,132) = 4.03$ $p<0.001$)]. The range of concentrations most preferred was extended by lesioning the LH. Tukey's post hoc analysis revealed that sucrose preference for the sham lesioned group peaked at 4% sucrose ($p<0.001$) and began to fall at 20% sucrose ($p<0.025$). In contrast, there was no difference in preference for 4%, 12% or 20% sucrose for the LH lesioned group. It is noteworthy that preference for 20% sucrose, the concentration used in the place preference procedure was not altered by lesioning the LH.

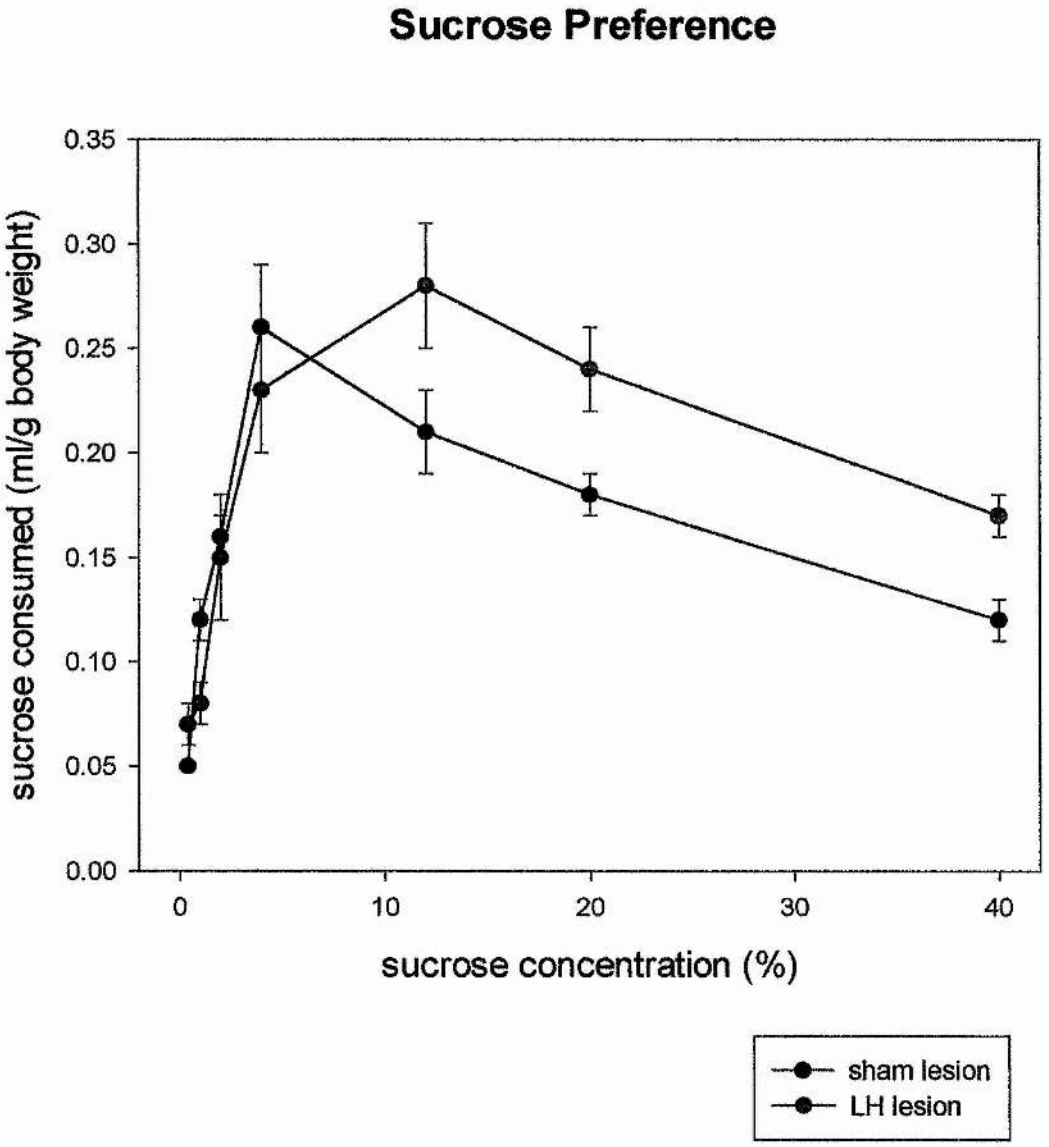


Figure 59. Sucrose preference curve for the sham lesioned group (n=16) and the LH lesioned group (11). Mean (± SE) volume of water, 1%, 2%, 4%, 12%, 20% and 40% sucrose drunk in 24h.

Physiological Regulation During The Sucrose Preference Test

Body weight and water intake during the sucrose preference test are illustrated in Figure 60 and 61. Sucrose was given on days 2, 4, 6, 8, 10, and 12.

Body Weight

ANOVA revealed a main effect of group ($F(1,25) = 50.19$ $p<0.001$), a main effect of day ($F12,300) = 113.19$ $p<0.001$) and a significant group × day interaction ($F(12,300) = 3.30$ $p<0.001$). Thus in addition to the overall difference between the groups the relationship between the groups with respect to body weight was not constant throughout the sucrose preference test.

Water Intake

ANOVA revealed no main effect of group ($F(1,25) = 0.21$), a main effect of day ($F11,275) = 108.42$ $p<0.001$) and a significant group × day interaction ($F(11,275) = 3.32$ $p<0.001$). The rats were exposed to sucrose on alternate days and from Figure 61 it can be seen that water intake fluctuated with peaks when sucrose was not available and troughs when sucrose was available as would be expected.

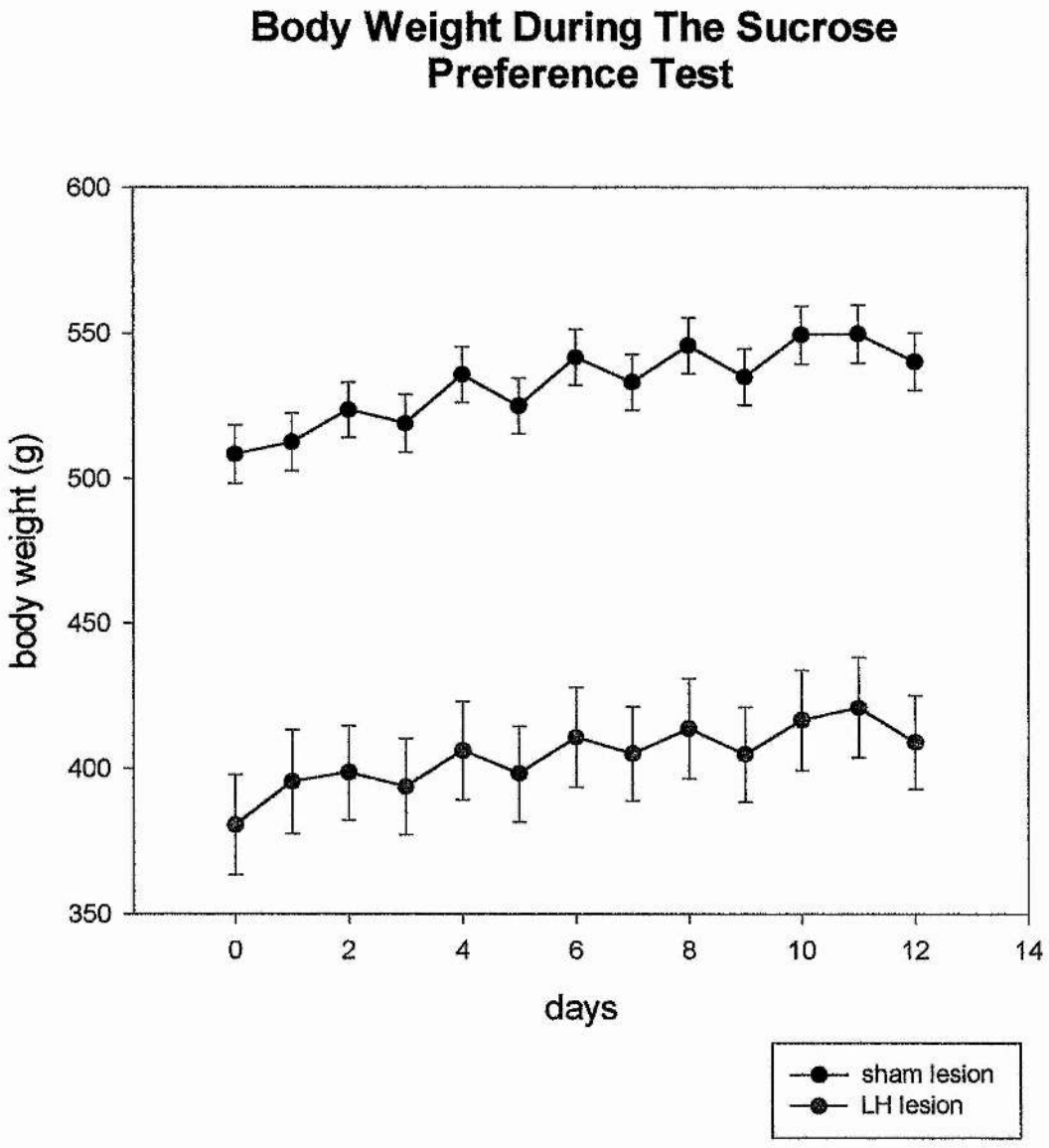


Figure 60. Mean (± SE) body weight for the sham lesioned group (n=16) and the LH lesioned group (n=11) during the sucrose preference test.

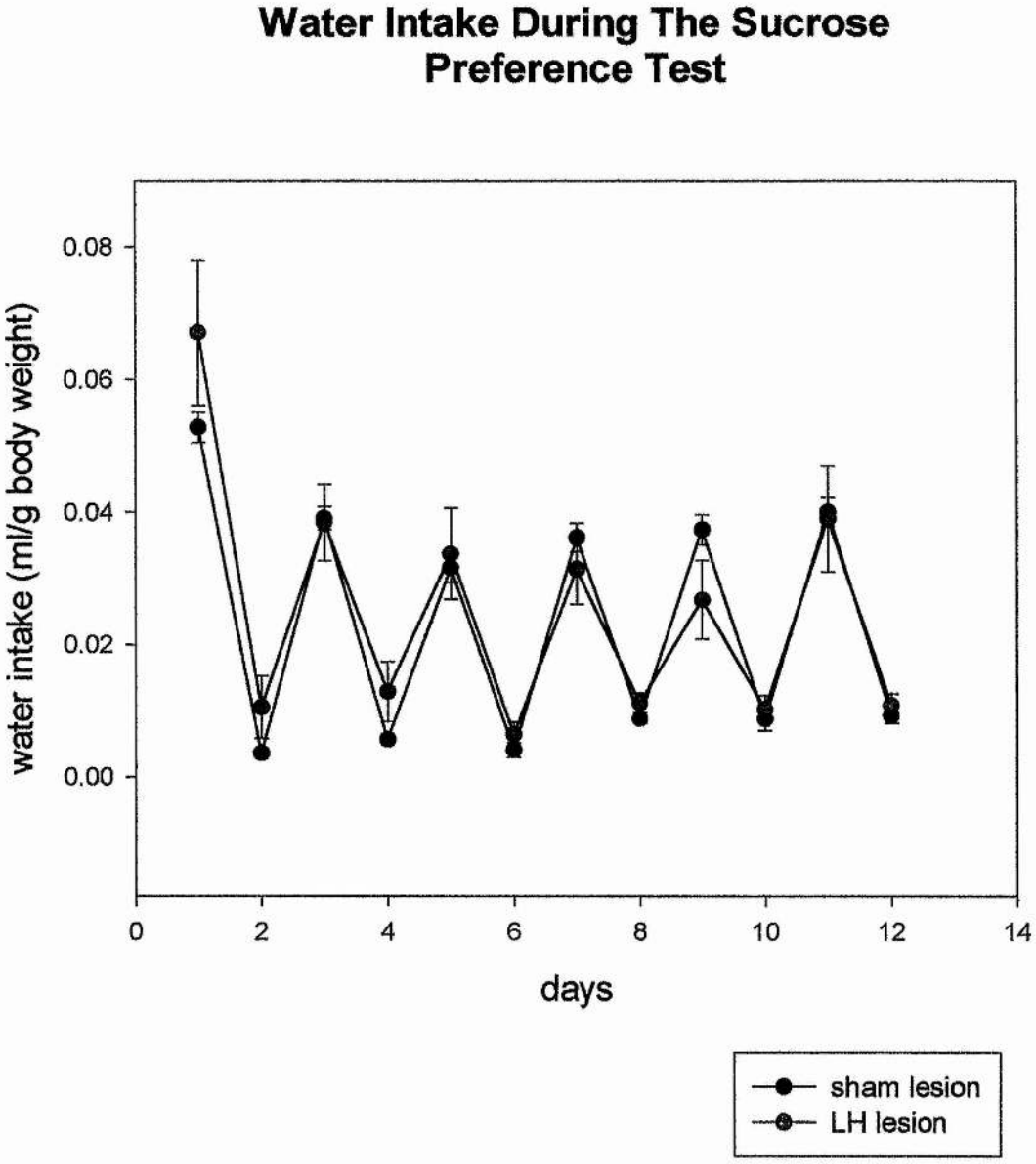


Figure 61. Mean (± SE) water intake as a function of body weight for the sham lesioned group (n=16) and the LH lesioned group (n=11) during the sucrose preference test.

Energy Intake

Since sucrose is an energy source it may be expected that food intake would have fluctuated during the sucrose preference test in relation to the volume of sucrose consumed. For the purpose of analysis, all intake values were expressed as energy intake as a function of body weight (1g lab chow = 14.8KJ, 1g sucrose = 17KJ). Gross daily energy intake and intake from sucrose and lab chow are illustrated in Figures 62, 63 and 64. Total intake and the intake from sucrose and lab chow were analysed separately by repeated measures ANOVA.

Figure 62 illustrates that daily total energy intake was comparable for the LH lesioned and sham lesioned groups during the sucrose preference test period [no main effect of group ($F(1,22) = 1.08$)]. The rats had free access to sucrose solutions on days 2, 4, 6, 8, 10 and 12, and as would be expected, this elevated energy intake was reflected in the main effect of day ($F(11,242) = 13.05$ $p<0.001$). No group × day interaction ($F(11,242) = 1.34$) was found indicating that the fluctuations seen in energy intake were not effected by lesioning the LH.

Although total energy intake was undisturbed by lesioning the LH, Figure 63 and Figure 64 suggest that the relative contributions of sucrose and chow to diet may have been altered. Although there was no group difference for the energy intake from lab chow ($F(1,25) = 0.85$), there was a main effect of day ($F(11,275) = 16.21$ $p<0.001$) and a significant group × day interaction ($F(11,275) = 2.11$ $p<0.020$). Energy intake from lab chow for both the sham lesioned and LH lesioned groups fluctuated daily with intake falling when sucrose was present. However, intake for the LH lesioned group appeared to fluctuate to a greater degree than for the sham lesioned group.

Figure 64 illustrates that the LH lesioned group had a greater energy intake from sucrose than the sham lesioned group [main effect of group ($F(1,22) = 7.20$ $p<0.014$)]. Intake did not differ across days ($F(5,110) = 0.91$) and there was no group × days interaction ($F(5,110) = 0.35$) indicating that the LH lesioned group consistently drank more sucrose than the sham lesioned group on all exposures to it.

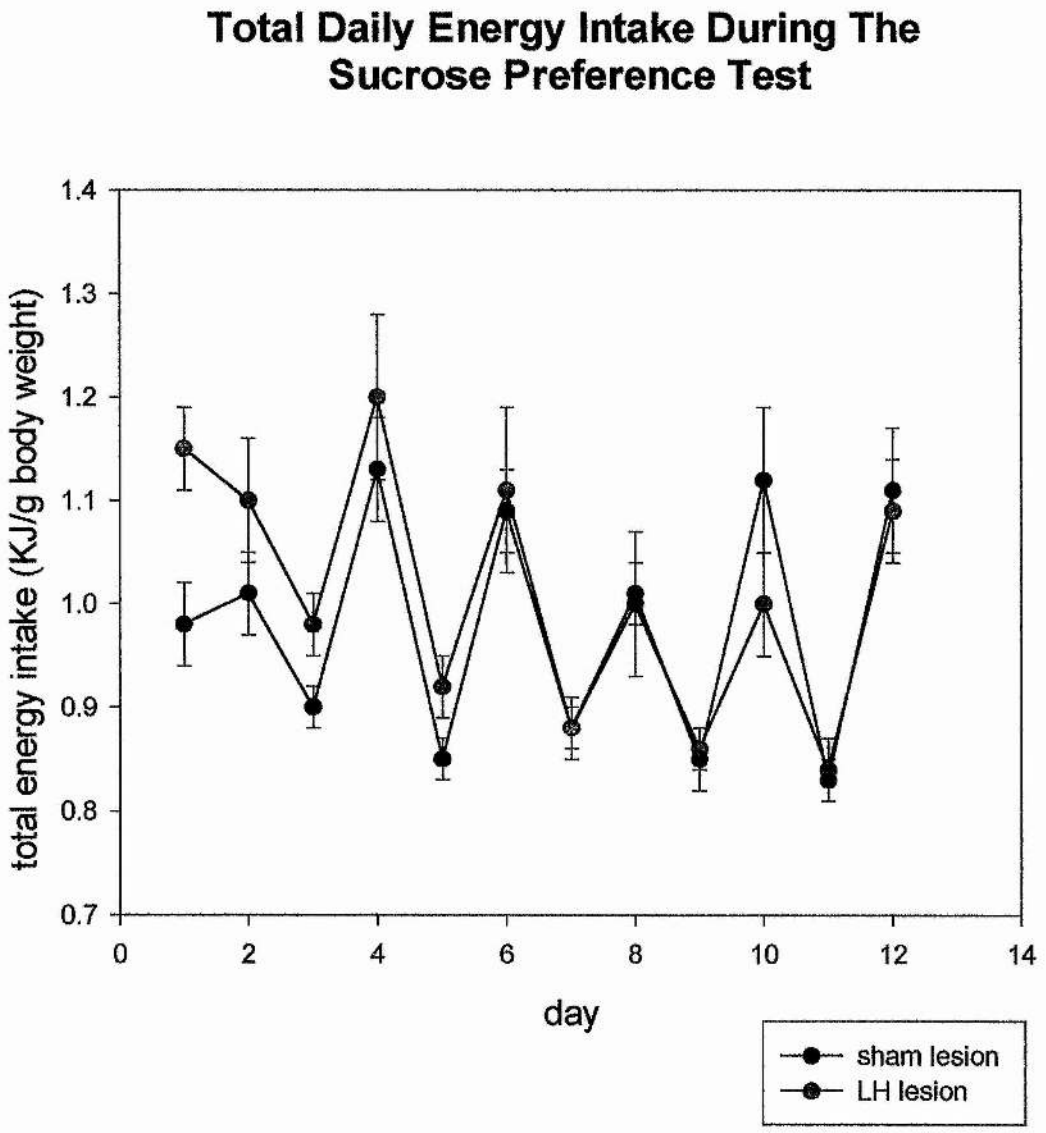


Figure 62. Mean (± SE) daily energy intake from chow and sucrose for the sham lesioned group (n=16) and the LH lesioned group (n=11) during the sucrose preference test.

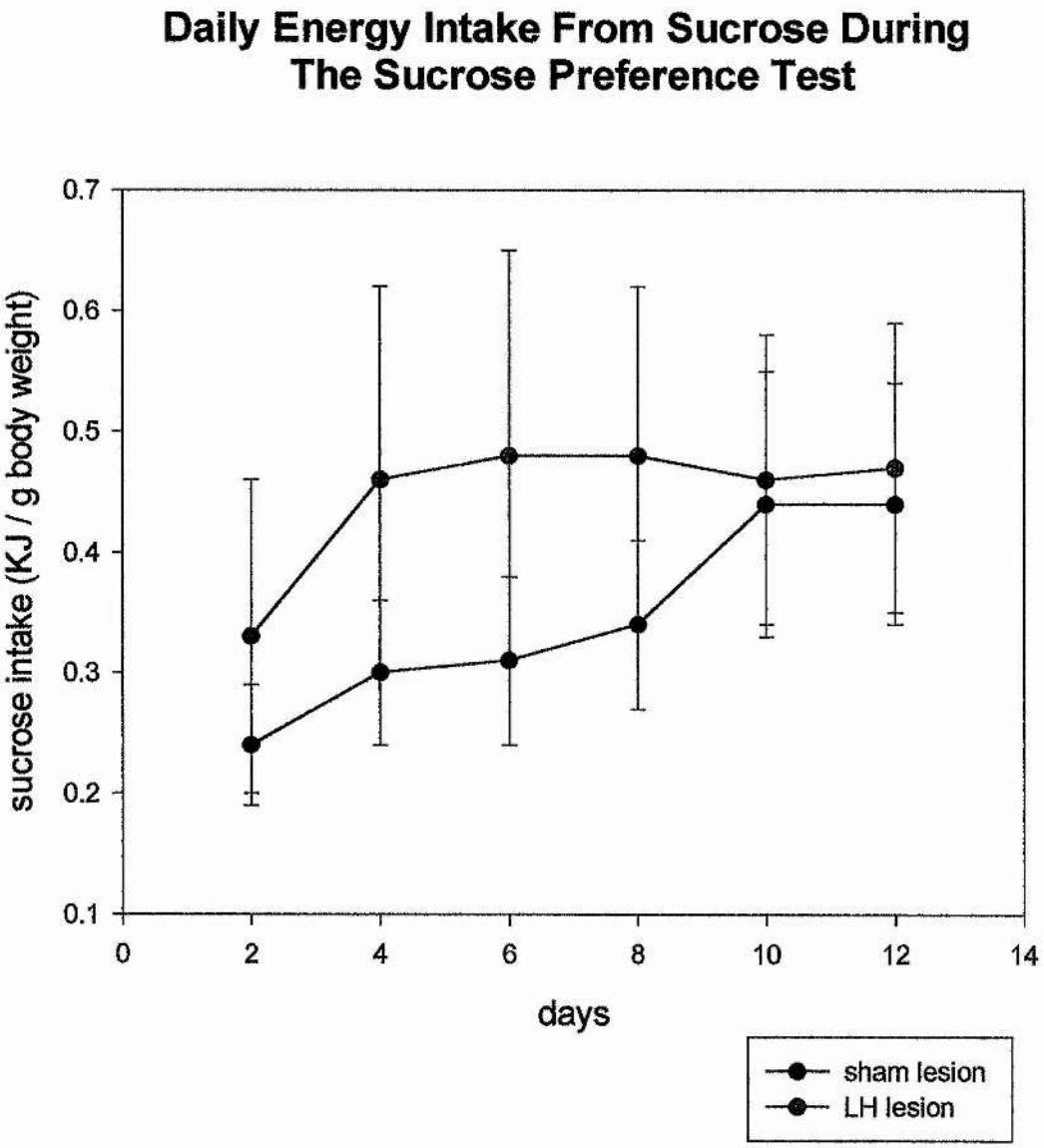


Figure 63. Mean (± SE) daily energy intake from sucrose during the sucrose preference test for the sham lesioned group (n=16) and the LH lesioned group (n=11).

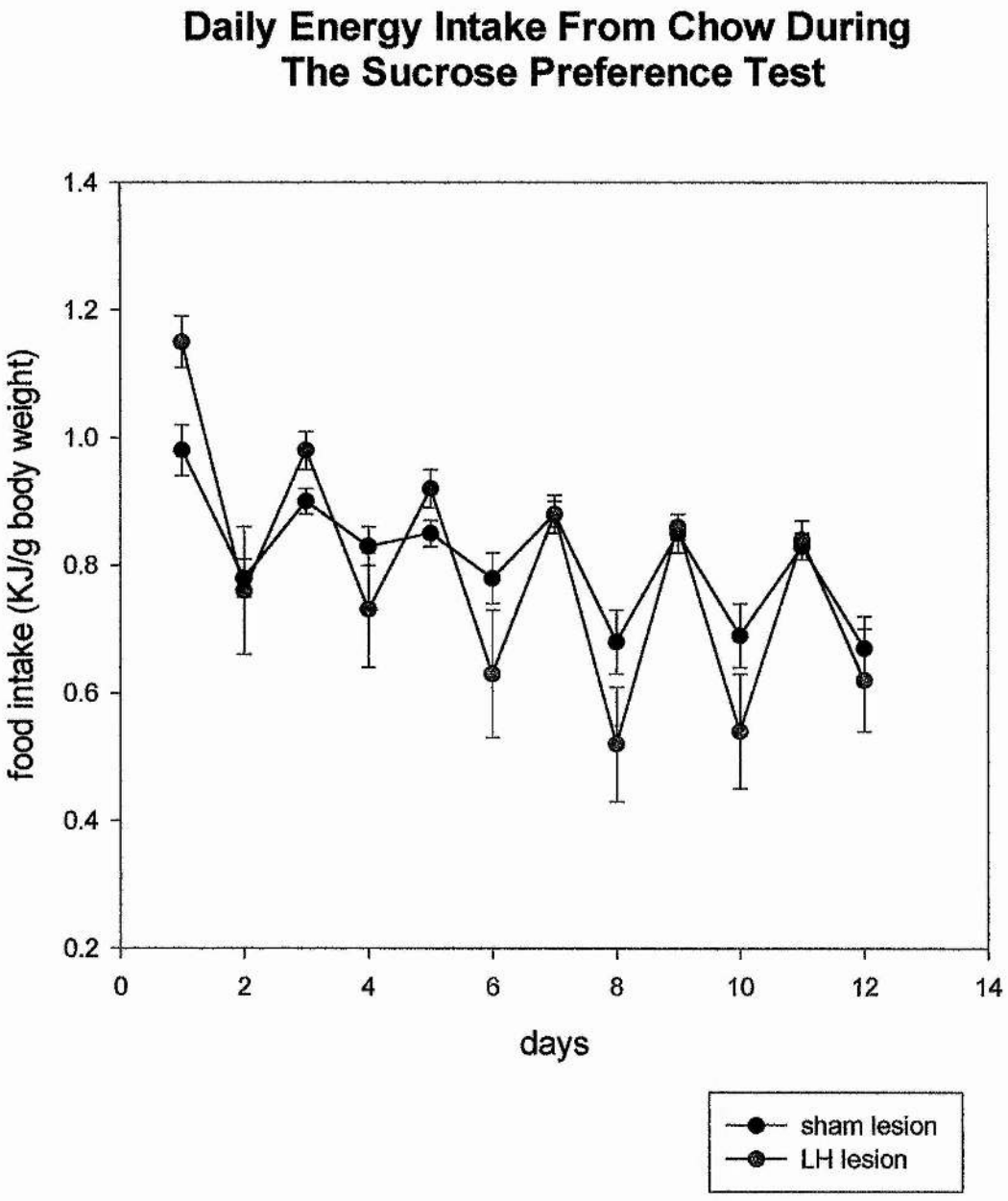


Figure 64. Mean (± SE) daily energy intake from lab chow during the sucrose preference test for the sham lesioned group (n=16) and the LH lesioned group (n=11).

In summary, it appears that the although energy regulation was not altered by lesioning the LH, there was a tendency to show a greater fall in chow consumption on exposure to sucrose solutions and a greater consumption of the sucrose solutions than the sham lesioned group. However, it would be necessary to have a measurement of total energy intake before the test period to investigate the effects of introducing sucrose solutions to energy intake regulation.

DISCUSSION

The results of the present study displayed for the first time, a failure of NMDA lesions of the LH to impair the acquisition or expression of a conditioned place preference to sucrose. In addition, no change was seen in exploratory behaviour with respect to duration or location. In contrast, lesioning the LH appeared to enhance sucrose ingestion but in a manner dependent on the circumstances in which it was supplied. While sucrose ingestion during the pairing sessions of the conditioned place preference procedure was increased by lesioning the LH, when sucrose preference was tested in the home cage no difference was found in the volume consumed. It did appear nevertheless that sucrose preference may have been shifted by lesioning the LH.

The formation of a conditioned place preference requires that neutral environmental cues gain incentive/rewarding properties by association with a primary reward. It was found that lesioning the LH did not prevent the acquisition and expression of a conditioned place preference suggesting that the LH is not essential for the perception of gustatory cues as rewarding, or the association of such gustatory cues with neutral environmental cues. This would indicate that the LH is not of fundamental importance to the guiding of behaviour by stimulus-reward association learning. Since a projection from the amygdala to the nucleus accumbens has been strongly implicated in this behavioural procedure, it can be inferred that connections between this relay and the LH are not critical for the formation of a conditioned place preference.

It may be predicted that lesioning a particular structure would have the opposite outcome to stimulating it. This however, has not been found to be true in the case of the LH. Ettenberg & Duvauchelle (1988) reported the establishment of a conditioned place preference by the rewarding properties of lateral hypothalamic stimulation. This was shown to be blocked by i.p. haloperidol indicating that it may have been mediated at least in part by dopamine. Although it is possible that at least some of the stimulated cells responsible for the formation of the conditioned place preference reported by Ettenberg & Duvauchelle (1988) were intrinsic to the LH, it is unlikely that the present results were due to incomplete

lesioning of the LH. The same group of LH lesioned rats failed to drink in response to hypertonic saline, a challenge that has consistently been disrupted by lesions of the LH.

In studies of electrical stimulation however, it is rudimentary to consider the fibres that traverse the target structure as candidates for the mediation of the results obtained. This is of considerable importance in the study of the LH in reward as the mesolimbic dopamine pathway, so critically involved in the mediation of reward-related processes, traverses the LH. In addition to the ascending projection from the VTA to the nucleus accumbens shown to be involved in reward, there is evidence implicating descending fibres originating in the forebrain in the rewarding effects of stimulation of the mesolimbic dopamine pathway (Bielajew & Shizgal 1986). Furthermore, included in the descending projection are LH efferents to the VTA (Berk & Finkelstein 1982). Although this anatomical evidence would suggest that intrinsic neurones of the LH are favourably placed to mediate the effects of stimulation of the mesolimbic dopamine pathway, it is difficult to accurately ascribe effects of electrical stimulation due to its non-specific nature.

While the formation of a conditioned place preference appeared to be completely unaffected by lesioning the LH, the same was not true for the consumption of the sucrose solution used as the primary reward in this procedure. Sucrose consumption in the paired compartment during the conditioning phase of the conditioned place preference procedure was consistently elevated for the LH lesioned group. It may be that lesioning the LH modified the perceived affective value of the sucrose solution although it seems unlikely since the place preference conditioned by this solution was unaffected by the lesion. A test of sucrose preference conducted after the completion of the conditioned place preference procedure revealed a mild rightward shift in sucrose preference. Although this would suggest that the perception of sucrose may have been altered by lesioning the LH, it is unlikely that this would have significantly influenced the volume of sucrose drunk in the pairing sessions since lesioning the LH did not effect the overall volume of sucrose consumed in the preference tests and moreover, there was no significant difference in the preference for the

concentration of sucrose used as the primary reward in the conditioned place preference procedure. In a similar experiment carried out in this lab, the role of the PPTg in conditioned place preference was examined. As previously noted, the PPTg projects to the LH and thus comparison of deficits produced by excitotoxic lesions of either area could potentially reveal the function of this anatomical connection. In similarity to the present findings with LH lesioned rats, it was demonstrated that lesions of the PPTg enhanced drinking of sucrose during the pairing sessions, but failed to produce a deficit in the formation of a conditioned place preference. However, while lesioning the LH produced inconsistent results for drinking sucrose in the home cage and the preference boxes, lesioning the PPTg resulted in elevated consumption of sucrose both during the conditioned place preference procedure and during sucrose preference tests in the home cage. Hence it would appear that although the LH and PPTg are both involved in the regulation of sucrose intake, the nature of these are not comparable.

There are a number of factors which could have contributed to the anomaly found in the present results with regard to sucrose intake including deprivation state and location of drinking. During behavioural testing the rats were maintained on a restricted food schedule that may have interacted with the perceived palatability of the sucrose solution especially considering that in addition to being palatable, it is an energy source. The food restriction schedule consisted of 30min free feeding with food delivered in containers on the bottom of the home cage. This meant that at the end of the free feeding period, not only had the container to be removed but the cage bottom was searched for pieces of food. During this procedure, it was noted that the rats hoarded whole food pellets by concealing them in their mouths and only proceeded to eat them after they were replaced in their cages. Being an unexpected finding and the fact that it required examining the interior of a food restricted rats mouth, this was not systematically examined, but it did appear to occur more frequently and had a more rapid onset in the LH lesioned group. A few instances of food hoarding were noted by sham lesioned rats but unlike the LH lesioned rats it was not observed until the latter stages of the conditioning phase and even then it was not every rat in the group. Food hoarding has been used previously to show that rats

are aware of their deprivation state (Herberg & Blundell 1982; Winn & Herberg 1985). This was tested by providing rats with an area in which food could be concealed. Food hoarding was observed when animals were on food restriction schedules but not when they were permitted to free feed. In the present experiment it was found that the LH lesioned rats lost weight more rapidly that the sham lesioned rats when food was restricted and hence the enhanced sucrose consumption and apparent food hoarding may reflect an awareness of this. In order to test this a systematic study of food hoarding by LH lesioning rats in a range of deprivation states would be required.

The duration of sucrose availability also differed between behavioural testing and the sucrose preference test. The pairing sessions lasted 30min whereas sucrose solutions were available in the home cage for 24hr. The difference may simply lie in the pattern of drinking exhibited by the LH lesioned rats. If in the initial exposure period to sucrose the LH lesioned rats exhibited a rate of drinking greater than the sham lesioned rats, this would produce a difference in the volume of fluid consumed in brief tests of consumption but would disappear as the duration of the test was extended. This is unlikely to have occurred however, since in a previous test no difference was found in the duration or frequency of drinking bouts for LH lesioned rats compared to controls (Experiment 1.3: " Role of the LH in Taste Perception").

Alternatively, the increased sucrose ingestion may be due to the location where the sucrose was ingested. Despite the habituation phase used, the conditioned place preference apparatus would still have been a novel environment in relation to the home cage. It is possible that transfer to the different environment produced an enhanced state of arousal that could have affected drinking. In agreement with this, it has been shown that lesioning the LH enhanced tail pinch-induced eating (Clark *et al.* 1992), a response which may also be due to increased arousal. Clark *et al.* (1992) suggest that the disinhibition of tail pinch-induced eating reflects a role of the LH in the suppression of inappropriate behaviour.

Summary

With regard to reward related processes, the role of the LH remains unclear. Lesions of the LH failed to produce a deficit in the acquisition of a conditioned place preference but increased the variability of responding for conditioned reinforcement. The role of the LH in the disinhibition of inappropriate behaviour proposed by Clark *et al.* (1992) may explain why lever pressing by the LH lesioned rats was less selective. However, if the LH does inhibit inappropriate behaviour it could be inferred that lesions of the LH should have disrupted the formation of a conditioned place preference, a result not found presently. An explanation for the apparent failure to disrupt one test of incentive learning but not another may be found in the proposal by McDonald and White (1993) that different procedures may require different memory systems. The LH has long been thought to have a role in feeding behaviour but the exact nature of this deficit has not been characterised. While LH lesioned rats show little deficit in day to day feeding or deprivation tests, they fail to respond appropriately in ingestion tests induced by i.p. injections. Furthermore, the present findings suggest that the results of ingestion tests are dependent on the nature of the test environment. Rather than simply regulating feeding *per se*, it may be the rewarding aspects of feeding which the LH is involved in and thus it may interact with a number of other structures in the control feeding. It was illustrated recently that feeding induced by microinfusions into the nucleus accumbens, an area strongly implicated in reward, could be blocked by inhibiting synaptic transmission in the LH. In order to elucidate the function of the LH it may be appropriate to compare the involvement of the LH in responses attributed to both gustatory and limbic processing taking careful consideration of the nature of the tests conducted.

GENERAL DISCUSSION

The function of the LH is difficult to define precisely. It is clear that the LH is not simply a feeding centre. Rats with excitotoxic lesions of the LH have consistently been shown to suffer only mild impairments in home cage food and water intake and appropriate responses have been made in response to food and water deprivation (Clark *et al.* 1990;Winn *et al.* 1990; Clark *et al.* 1991a). In agreement with previous studies, all the LH lesioned groups tested presently recovered normal food and water intake in relation to body weight within 3 weeks of surgery. In fact, the present results would indicate that the severity of the deficits found in body weight and food and water intake were dependent on the type of post-operative care granted. By placing food pellets on the floor of the home cage after surgery, the post-surgery deficits in body weight and food and water intake were attenuated.

So what exactly does the LH do if it is not a feeding centre? One challenge LH lesioned rats consistently failed in past studies, and has been replicated here, was drinking in response to i.p. injection of hypertonic saline (Clark *et al.* 1990; Clark *et al.* 1991a). Hence, an anomaly was found in the results of two experimental manipulations that had the same outcome. LH lesioned rats responded to changes in fluid homeostasis induced by water deprivation but not by injection of hypertonic saline. This indicated that the LH was involved in the maintenance of homeostasis but only under particular circumstances. Both of these procedures disrupted fluid balance by intracellular water loss but only one induced a compensatory drinking response. Thus, when examining the results of such physiological challenges it appears to be crucial to consider the manner in which the homeostatic insult was induced in addition to the nature of the insult. Winn (1995) proposed that LH lesioned rats responded to water deprivation but not i.p. hypertonic saline because of the additional environmental cues signalling water deprivation; animals were aware that their water bottles had been missing. This indicated that LH lesioned rats could not act on visceral signals alone, from which it was inferred that lesioning the LH had removed a pathway whereby signals concerning the viscera could reach neural structures responsible for behavioural responding. It was proposed that the LH acts as an interface between the paraventricular system responsible for monitoring internal state and frontostriatal systems responsible for planning and executing behaviour. The

present hypothesis was that destruction of the LH would disrupt behavioural responses dependent on either the paraventricular system or frontostriatal systems but the present findings would suggest that, for at least the behavioural responses tested, this is not true.

Paraventricular System

The candidate structure chosen from the paraventricular system was the PBN. It is known to be crucial for a number of aspects of feeding behaviour (Flynn *et al.* 1991a; Scalera *et al.* 1995) and it has reciprocal connections with the LH (Moga *et al.* 1990a; Fulwiler & Saper 1984). The particular paradigms chosen were conditioned taste aversion and benzodiazepine induced feeding. Lesioning the LH failed to produce a deficit in either procedure implying that a functional connection between the LH and the PBN was not required for at least the procedures tested. However, this does not preclude the possibility of a functional connection between the PBN and LH.

From the work of Kelly & Watts (1998) it appears that a functional connection between the LH and PBN does exist at least with regard to water balance; feeding regulation was not investigated by Kelly & Watts (1998). As previously mentioned, behavioural studies have indicated a role for the LH in water balance but only under particular circumstances, namely drinking in response to intracellular dehydration induced by i.p. hypertonic saline. Rather than employing behavioural techniques, Watts (1992) studied the neuropeptide response to intracellular dehydration within the LH. It was found that within a discrete subpopulation of neurones of the LH, cellular dehydration induced an increase in the cellular levels of mRNAs encoding the precursor peptides for corticotrophin releasing hormone (CRH) and neurotensin/neuromedin N (NT/NMN).

Further to examining the neuropeptide response of the LH, Kelly & Watts (1996) described the afferent and efferent connections of this subpopulation of LH neurones they labelled LHA-crh. Using a combination of knife cuts placed between the rostral forebrain and the LH, retrograde staining and *in situ* hybridisation for c-Fos mRNA, it was found that the LHA-crh neurones had

direct functional connections with the median preoptic nucleus, the subfornical organ and the fusiform nucleus which, in part, mediated the increases in CRH and NT/NMN mRNA seen after intracellular dehydration. It was a study of the efferent connections of the LHA-crh however, that revealed connections between the PBN and the LH (Kelly & Watts 1998). Using both anterograde and retrograde tracing techniques, it was found that significant numbers of LH neurones that expressed CRH mRNA in response to intracellular dehydration, projected to both the medial and lateral subnuclei of the PBN. Thus, the nature of the connection between the LH and the PBN differed in two ways from the present hypothesis: the function of the connection appeared to be water balance as opposed to feeding regulation and it was the LH which projected to the PBN rather than the PBN projecting to the LH. It should be noted however, that anatomical studies revealed reciprocal connections between the PBN and LH (Moga *et al.* 1990a; Fulwiler & Saper 1984).

Winn (1995) proposed that the lack of response to intracellular dehydration induced by i.p. hypertonic saline by LH lesioned rats was due to the loss of a route whereby visceral signals reached neural structures responsible for planning and execution of behaviour. Since the afferent connections of the LHA-crh previously described, are known to have a role in fluid balance it may be that the LH does act as a bridge for such signals to reach higher neural structures. With regard to elucidating the neural network responsible for monitoring fluid balance, Kelly & Watts (1998) focused on the efferent connections of the LH to the PBN since neurones of this structure also expressed neuropeptides associated with intracellular dehydration. Kelly & Watts (1998) proposed that osmoregulatory signals conveyed from the LH to the PBN could be integrated with other inputs within the PBN and then used to modify behavioural and autonomic function. In support of this theory, previous studies of conditioned taste aversion indicated that the PBN has an associative function in relation to visceral and gustatory signals (Reilly *et al.* 1993). In order to clarify the contribution of the PBN to water balance, it would be interesting to examine any deficits in responding to manipulations of water balance induced by lesioning the PBN. Ohman & Johnson (1986) studied the ability of rats bearing electrolytic lesions of the ventrolateral region of the lateral PBN to respond to a number of thirst

challenges examined previously in rats with LH lesions. Unlike LH lesioned rats who failed to drink in response to angiotensin II, rats with PBN lesions expressed enhanced drinking to this stimulus. Again in contrast to LH lesioned rats, rats with PBN lesions responded appropriately to hypertonic saline. Therefore lesions of the LH and PBN did not induce similar deficits in thirst challenges and moreover the difference in responding by LH lesioned and PBN lesioned rats was not constant across different challenges. This is difficult to reconcile with the idea that the LH simply projects visceral signals to the PBN. It should be noted however that the LH neurones responsive to intracellular dehydration projected to not only the lateral but also the medial PBN. Therefore, it would be more appropriate to examine the contribution of both the lateral and medial PBN to thirst challenges of that kind.

Frontostriatal Systems

The two functions of frontostriatal systems studied were conditioned place preference and conditioned reinforcement. With regard to conditioned place preference, lesioning the LH did not produce a deficit. Since a connection between the nucleus accumbens and the amygdala is crucial for this response, it can be inferred that a further serial connection with the LH is not essential. Responding for conditioned reinforcement, like conditioned place preference, is known to be affected by manipulations of the amygdala and nucleus accumbens but the present results of the conditioned reinforcement procedure are less clear. On average responding for conditioned reinforcement by the LH lesioned group was no different from that by the sham lesioned group but variability within the group was increased. The significance of these results will be discussed fully later but it would appear that the LH was not essential for at least the functions of frontostriatal systems studied.

In contrast, a study by Maldonado-Irizarry *et al.* (1995) reported a functional link between the nucleus accumbens and the LH with regard to feeding. It was found that concurrent infusion the $GABA_A$ receptor agonist muscimol into the LH completely blocked a robust, long lasting feeding response induced by infusions of AMPA and kainate receptor antagonists into the medial nucleus accumbens. This feeding response was also modulated by systemic administration of D1 and

D2 receptor antagonists but the decrease found in this case was less profound than that caused by muscimol infusion in the LH. Further evidence implicating the nucleus accumbens in feeding behaviour has been provided by *in vivo* microdialysis studies of neurotransmitter effluxes which indicated that deprivation induced feeding was associated with an increase in release of dopamine in the nucleus accumbens (Taber & Fibiger 1997). Parada *et al.* (1995) found that an increase in extracellular dopamine in the nucleus accumbens was also induced by infusion of the dopamine receptor antagonist sulpiride into the lateral hypothalamus. Together these data provide evidence that the function of the anatomical connections illustrated between the nucleus accumbens and LH may be concerned with feeding regulation. Since several studies have reported that the increase in extracellular dopamine in the nucleus accumbens is more closely associated with the anticipatory aspects of obtaining a reward as opposed to feeding *per se*, it is possible that the LH is involved in the gating of sensory signals in order that they may be used to alter behaviour (Salamone *et al.* 1994; Richardson & Gratton 1996).

Despite the existence of studies indicating that the LH does have functional connections with structures of the paraventricular system and frontostriatal systems, taken together, the results of the present experiments fail to provide evidence to verify the hypothesis that the LH acts as an interface between the paraventricular system and frontostriatal systems. However, the results do provide further insight into the function of the LH with regard to feeding behaviour and learning and memory.

Feeding Behaviour

Although lesioning the LH failed to disrupt the feeding responses dependent on the PBN, there was a specific enhancement in ingestive behaviour common to several of the experiments conducted. Conditioned taste aversion, benzodiazepine induced hyperphagia and conditioned place preference all entailed the consumption of a palatable substance (saccharin solution, palatable mash and sucrose solution respectively) in a specific test area other than the home cage and consumption of each was significantly increased by lesioning the LH. It was proposed that the LH could be important in the perception of taste

and in order to investigate this further, preference for a range of sucrose, saccharin and salt solutions was systematically tested in the home cage and preference for salt and saccharin solutions was tested in drinking boxes.

It was found that taste preference tested in the home cage was unaltered by lesioning the LH and it can therefore be inferred that the enhanced ingestion of palatable substances during the behavioural procedures was unlikely to be due to an alteration in taste perception. Although the duration of the home cage preference tests differed from the behavioural procedures, it is equally unlikely that this was the reason the LH lesions were without effect in the saccharin preference tests considering that taste preference for saccharin remained unaffected by lesioning the LH even when tested in the drinking boxes using a test duration equal to that of the behavioural tests.

A third factor that was not constant in the behavioural procedures and the home cage preference tests was the deprivation state of the rats at the time of testing. During the conditioned taste aversion procedure the rats were water deprived whereas in the benzodiazepine-induced hyperphagia procedure and the home cage preference tests the rats were non-deprived with respect to food and water intake. The simple fact that enhanced consumption was seen during both the conditioned taste aversion procedure and the benzodiazepine induced hyperphagia procedure indicated that deprivation state was not the crucial determining factor since the rats were in different deprivation states during each test. However, taste preference for saccharin in the drinking boxes provided a more formal examination of a possible role of deprivation state in the differing results found with the behavioural procedures and the home cage preference tests. In this case, the only factor that was varied was deprivation state and thus any difference in preference would have been attributed to it. However, no interaction between group and deprivation state was found for saccharin preference providing strong evidence that deprivation state was not the cause of any aberration in the results between the behavioural procedures and the home cage preference tests.

Why then did the LH lesioned rats express enhanced saccharin consumption in the conditioned taste aversion procedure but not in the preference tests conducted in the drinking boxes? In two different test procedures that were both conducted in test areas other than the home cage and where saccharin was consumed by rats in comparable deprivations states, conflicting results were found. Although the LH lesions produced in each of these studies appeared to be comparable, it is possible that some subtle difference between the lesion groups accounted for the differences in consumption found in the behavioural procedures and the taste preference tests. Conditioned taste aversion and benzodiazepine induced hyperphagia were tested in the same group of rats and enhanced consumption of the palatable substances used was reported in both instances. Taste preference for salt and saccharin were tested in the same group of rats and preference for both substances was consistently reported to be unaltered by the LH lesioned group. The same cannot be said, however, for the consumption of sucrose. Sucrose taste preference was tested in the same group of rats that had been used to examine the expression of a conditioned place preference with sucrose. Interestingly, the same group of rats that expressed enhanced consumption of sucrose in the conditioned place preference boxes expressed normal sucrose consumption in the home cage. Although it has been noted that deprivation state is unlikely to be the cause of any difference in preference in the home cage and other testing areas, it is noteworthy in this case that during the conditioned place preference procedure the rats were food deprived whereas during the taste preference test they were non-deprived.

The results of all the experiments conducted presently which involved ingestion of palatable substances are summarised in Table 7 with regard to the substance ingested, deprivation state at the time of testing and location of testing. The only constant that can clearly be found is that lesioning the LH never enhanced consumption of palatable substances in the home cage. Could it be that this strange aberration in feeding regulation reflects some form of context dependent disinhibition? Studies of tail pinch induced feeding and schedule induced polydipsia have lead to the belief that the LH is important for the selection of appropriate responses and inhibition of inappropriate responses in conditions of motivational excitement, because rats with LH lesions expressed enhanced

Procedure	Palatable Substance	Test Area	Deprivation State	Effect of lesioining LH on Intake	n for lesion groups
conditioned taste aversion	saccharin solution	test apparatus	water deprived	significant increase ($p<0.025$)	sham (24) LH (21)
midazolam induced hyperphagia	palatable mash	test apparatus	non-deprived	significant increase ($p<0.000$)	sham (24) LH (20)
conditioned place preference	sucrose solution	test apparatus	food-deprived	significant increase ($p<0.001$)	sham (16) LH (11)
taste preference	saccharin solutions	home cage	non-deprived	no effect	sham (10) LH (6)
	saccharin solutions	test area	non-deprived	no effect	sham (10) LH (6)
	saccharin solutions	test area	water-deprived	no effect	sham (10) LH (6)
	salt solutions	home cage	non-deprived	no effect	sham (10) LH (6)
	sat solutions	test area	non-deprived	no effect	sham (10) LH (6)
	salt solutions	test area	water deprived	no effect	sham (16) LH (11)
	sucrose solutions	home cage	non-deprived	no effect	sham (16) LH (11)

Table 7. Summary of the effects of lesioning the LH on intake of palatable food substances with regard to the substance ingested, the location of ingestion and the deprivation state of the rats at the time of ingestion.

acquisition of both (Clark *et al.* 1992; Winn *et al.* 1992). It is possible that placement in the test areas increased arousal and in this case destruction of the LH disinhibited the consumption of the palatable substances. The importance of location of testing has previously been shown to be crucial. It was found that lesioning the amygdala increased consumption of a novel saccharin solution only when it was presented in a novel environment and not a familiar environment (Dunn & Everitt, 1998). However, this was dependent on the novelty of the taste solution as on the second exposure to it, the amygdala lesioned rats responded appropriately.

Yet again the present data demonstrates that this is too simple an explanation. When taste preference was tested in drinking boxes no overall change was found in taste preference dispelling the theory that location was the determinant factor in the degree of consumption of palatable substances. It would appear that no one factor that was varied in the procedures conducted was singularly essential for this enhancement of ingestion reported. For every variation which could explain the differences in results between tests, be it deprivation state, test duration or test location, there is evidence from the present findings that suggest otherwise. Instead, the ability to show a deficit in feeding regulation with LH lesioned rats may be dependent on the complexity of the task employed.

Analysis of the volume of drinking during the preference tests in the drinking boxes did reveal one significant interaction involving lesion group. While the LH lesioned rats did not deviate from the controls in responding for saccharin solutions in the drinking boxes, there was an interaction between lesion group and deprivation state when responding for salt solutions. Salt differs from saccharin in that it affects fluid homeostasis. When presented with saccharin in a non-deprived state a bell-shaped preference-aversion curve emerged. This pattern of preference was simply exaggerated when tested in a water-deprived state with consumption of the most preferred concentration increasing disproportionately compared to the rest. Since saccharin is not osmotically active, ingesting this solute does not pose a further problem to fluid balance and thus, the concentration consumed is not crucial when addressing the problem of

reduced body water. This, in addition to the fact that it is not an energy source, made it an ideal solution for testing taste related behaviour only.

Consumption of salt on the other hand is a more complex matter and the pattern of preference changed dramatically when in a non-deprived versus water deprived state. When water deprived, the behavioural response to return fluid homeostasis to normal is obviously fluid ingestion. But in addition to maintaining the volume of body water, the concentration of osmotically active solutes such as salt is also closely regulated. The concentration of salt which can be excreted is limited and, as a result, if a salt solution is ingested that is of a higher concentration than that which can be excreted, intracellular fluid will be sacrificed in order to return the osmolality of the extracellular fluid compartment to within set limits. Consequently, if when water deprived the fluid ingested contains a high concentration of salt, the state of intracellular water loss will be exacerbated as opposed to diminished. In view of this, it would appear that presentation of a range of salt solutions to a water-deprived rat may well be a more complex task than presentation of a range of saccharin solutions. Not only does the rat have to ingest fluid but an appropriate choice of salt concentration has to be made in order that the fluid ingestion may address the homeostatic insult. The general shape of the preference curve was unaltered by lesioning the LH but the increase in the volume of drinking in response to water deprivation was attenuated. The LH lesioned group drank a greater volume than the sham lesioned group when non-deprived and a lesser volume when water-deprived resulting in the significant interaction between lesion group and deprivation state.

The behavioural procedures examined also involved a combination of determining factors. In the case of conditioned taste aversion, recognition that the taste of saccharin had previously been associated with visceral distress was required in a specific testing environment while in a water deprived state. In the case of conditioned place preference, sucrose solution was presented in a novel testing environment to food deprived animals. Thus, in this instance hungry animals were being presented with an alternative energy source. Could it be that while LH lesioned rats respond appropriately to food and water deprivation in the home cage, when placed in an environment other than the home cage and/or

having to make a choice of responses, not all of which would address the homeostatic deficit, responding is altered? This could be tested using a similar method to the taste preference test for salt in the drinking boxes. If lesioning the LH attenuated the ability to make appropriate choices to address homeostatic deficits it could be predicted that deficits in responding would be found if a food deprived rat were presented with a range of sucrose solutions simultaneously.

As with the previous arguments, there is a flaw in this latter conjecture. It still does not account for the fact that LH lesioned rats ate more palatable mash than sham lesioned rats when non-deprived and drank more saccharin solution on first exposure to it during the conditioned taste aversion procedure and yet drank appropriate quantities of saccharin during all the preference tests. One factor which differentiates the conditioned taste aversion procedure from all the other tests conducted was that the rats were not habituated to the taste of the palatable substance prior to behavioural testing simply due to the nature of the test. It may be that the effect of novelty was reduced by lesioning the LH which would explain the initial enhancement in saccharin consumption. This could also account for the initial elevation of mash consumption but not the elevation seen in the latter stages of testing. However, it has been proposed that even limited daily access to palatable mash induced a self-induced deprivation state with rats reducing intake of lab chow in the face of more palatable substances (Chen *et al.* 1995). If true then the latter stages of the benzodiazepine induced hyperphagia could have been similar in nature to the conditioned place preference procedure in that food deprived rats were exposed to an alternative food source in a distinctive environment. In favour of this theory, it was found that all rats decreased their intake of chow after the introduction of palatable mash and, in fact, the LH lesioned rats expressed a greater decrease than the sham lesioned rats.

Although it would not account for any changes found in consumption of saccharin, the concept that lesioning the LH altered energy regulation may account in part for the changes seen in consumption of alternative food sources which do have calorific content. Anecdotal notes were made that the LH lesioned rats began food hoarding when food access was limited, a function

which is known to be correlated with loss of body weight (Winn & Herberg 1985; Herberg & Blundell 1970). The increase in sucrose intake during the conditioned place preference procedure may simply reflect an awareness of the LH lesioned rats of being underweight. Examination of body weight during the conditioned place preference procedure revealed that the LH lesioned rats were losing weight at a mildly enhanced rate. Thus, it could be this extra drop in body weight that prompted the hoarding responses rather than the constant deficit in body weight in comparison to the sham lesioned rats seen throughout the study.

This leads back to the old argument of whether or not lesioning the LH changes some hypothetical body weight "set-point" to a new level which is defended. Examination of the original data is complicated by the fact that electrolytic lesions were employed but nevertheless, Powley & Keesey (1970) indicated that post-surgery disruption in feeding was simply a means to reach a new body weight set-point.. In the present study, surgery was followed by a brief disruption in feeding and a persistent deficit in body weight. However, even after the LH lesioned rats had recovered from surgery with regard to feeding and drinking, when a palatable mash was introduced into the diet, the LH lesioned rats failed to maintain a constant energy intake, unlike the sham lesioned rats. Presumably, a fall in body weight would be concomitant with the fall in energy intake and so if lesioning the LH did produce a new body weight set point why was it not defended. It is not only lesions of the LH which have been found to induce deficits in body weight. Although the deficits seen with other lesions such as the pedunculo pontine tegmental nucleus were not quite so great, body weight for the lesioned groups and sham lesioned groups increased in parallel after surgery, with the sham lesioned groups remaining heavier than the lesioned groups (Inglis *et al.* 1994a). It may be that the fall in body weight as a result of lesioning the LH is due to a non-specific effect of surgery, and the reason this decrease is greater with LH lesioned rats compared to rat bearing lesions of other neural structures is because of the longer recovery period to commence normal consummatory behaviour. It has been shown that non-lesioned rats yoked to the intake of LH lesioned rats had identical changes in body weight, implying that it was the loss of intake that could lead to loss of body weight rather than vice versa (Winn *et al.* 1990).

And so to another contradiction. If we presume that the LH lesioned rats increased sucrose intake in the face of falling body weight why did another group of lesioned rats not maintain a constant energy intake when given free access to lab chow and limited access to a palatable mash? The present evidence failed in its attempt to elucidate the function of the LH in feeding regulation. The measures collected clearly indicate that the LH lesioned rats are impaired in some way. Furthermore, simple observation indicated that these animals were not behaving normally. In addition to the anecdotal notes that the LH lesioned rats hoarded food it was noticed that the deficits in both feeding and drinking immediately after surgery may not simply have been due to a lack of desire to eat or drink. While the rats failed to eat or drink from the food hopper and water bottle to the extent that substantial losses in body weight were encountered, if food and water were placed on the cage floor, normal feeding was resumed. This could not even be accounted for by an inability to feed and drink from either the water bottle or food hopper since the rats could be enticed back to feeding from the normal locations by placing minimal quantities of palatable wet mash at the water bottle and food hopper. This was most frequently found with rats that had been given wet mash after surgery for a prolonged period. It was thought that it may reflect a perseverance in the location of feeding but this would surely have lead to a stronger conditioned place preference which was not found when LH lesioned rats were tested in this procedure.

On reflection, it appears that less severe techniques than actually lesioning the LH may be useful in elucidating the role of the LH in feeding behaviour. Stanley *et al.* (1996) has shown that feeding can be induced by infusing non-toxic doses of glutamate receptor agonists such as kainate, AMPA and NMDA into the LH. This is a more specific technique than electrically stimulating the LH since any effects seen can be attributed to cells intrinsic to the LH as opposed to stimulation of fibres of passage. Although there is always the problem with microinfusions that the effects seen could be due to diffusion of the agonists to structures adjacent to the one actually being infused, Stanley *et al.* (1993) addressed this problem by demonstrating that infusions into areas adjacent to the LH failed to induce a feeding response. The specificity of the feeding response

to NMDA was indicated by the fact that it was blocked by prior infusion of the NMDA receptor antagonist AP5 into the LH. Moreover, it was shown that infusion of AP5 alone into the LH attenuated the feeding response to food deprivation and nocturnal feeding indicating that NMDA receptors may be involved in the physiological regulation of feeding behaviour under normal circumstances.

Learning and memory

The results of the present experiments also provide insight into the role of the LH with regard to learning and memory. McDonald & White (1993) illustrated that there are different types of learning which may be mediated by independent neural systems. While the present study did not include one type of learning described by McDonald & White (1993), the learning of relationships among stimuli and events, several types of associations were examined.

- Conditioned taste aversion: association of neutral gustatory cue and visceral state.
- Conditioned place preference: association of neutral environmental cues and reward.
- Conditioned reinforcement: association of stimulus and response.

Association of Neutral Gustatory Cues and Visceral State

In order to acquire and express a conditioned taste aversion it was necessary to remember the taste of the substance ingested prior to the visceral distress. An association had to be made between this taste and the visceral distress which, then had to be remembered when later presented with the taste. Hence, the ability of LH lesioned rats to express a conditioned taste aversion illustrated that the LH was not essential in the association of signals concerning visceral state and gustatory cues. There are two reasons why this deviates from the predicted result. LH lesioned rats have consistently been shown to express deficits in "needle challenges" whether they were in relation to food or water deprivation. An explanation for this deficit was that lesioning the LH rendered the rat unaware that homeostasis had been challenged. However, the ability to acquire

and express a conditioned taste aversion illustrated that the LH lesioned rats were able not only to monitor their internal environment, but they were able to use information from it to modify behaviour. Data from Rolls *et al.* (1986) concerning sensory specific satiety also predicted that lesioning the LH would produce deficits in the acquisition of a conditioned taste aversion. It was found that the responsiveness of LH neurones depended on both internal state and the sensory qualities of the food being given. When rats were presented with a palatable food substance LH neurones fired. However, after that particular food was eaten to satiety, presentation of it no longer induced firing of LH neurones and it was then necessary to present a different palatable food substance in order to induced such firing. This indicated that the LH was involved in the association of visceral signals and gustatory cues since firing depended on the animal's immediate past experience of the food in addition to its sensory qualities.

Stimulus-Reward Associations

The procedures conducted also permitted the study of stimulus-reward associations. In order to form a conditioned place preference, a reward must be associated with one of two distinct environments such that when exposed to both environments in the absence of the reward, the conditioned environment acts as a conditioned stimulus attracting the rat and maintaining contact with it. The ability to form a conditioned place preference revealed that the LH was not essential for at least the formation of that type of stimulus-reward association. Furthermore, due to the nature of the conditioned reinforcement schedule, it was also possible to study the effect of lesioning the LH on a stimulus-reward association learned prior to the lesion. When the ability to perform a discriminated approach response learned prior to surgery was tested, a deficit was found as a result of lesioning the LH. While the control group performed at almost optimal levels from the first post-surgery training session, performance of the LH lesioned group was more comparable to that of naïve untrained animals. This was comparable to the findings in a similar study studying the role of the basolateral amygdala in responding for conditioned reinforcement (Burns *et al.* 1993). Nevertheless, like lesions of the basolateral amygdala, lesioning the LH did not preclude the relearning of the discriminated approach response and

hence, it would appear that the LH was not essential for the association of reward with the compound light/tone stimulus.

The finding that the LH was not essential for the association of neutral cues and reward is difficult to reconcile with electrophysiological data. Nakamura & Ono (1986) reported that neurones of the LH which were responsive to rewarding stimuli, were also responsive to conditioned stimuli which predicted their presentation.

Stimulus-Response Associations

In order to test the acquired affective value of the compound stimulus used for the discriminative approach response in the conditioned reinforcement procedure, it was used to drive a different behavioural response i.e. lever pressing. When specificity of lever pressing in order to gain this compound stimulus was tested, although the LH lesioned group on average responded as the sham lesioned group the variability of responding was much greater. Intensive lever pressing was found with both the CR and NCR levers and furthermore, bursts of pressing on the CR lever were found throughout the test period rather than the steady decline in lever pressing found in the sham lesioned group.

Overall it is difficult to define precisely the deficit produced in responding for conditioned reinforcement induced by lesioning the LH. This increased variability in responding may reflect disinhibition of inappropriate responding. When lever pressing of individual animals was examined it was found that there was an increase in the range of lever pressing on both the CR and the NCR levers. It is unlikely that this reflects a general increase in activation as responses on the panel were no different from control rats. It is likely that an association was made between the levers and the presentation of the conditioned reward but then there was some form of deficit in focusing on the CR lever only. This may reflect the inappropriate responding when presented with the palatable food substances in the conditioned taste aversion, benzodiazepine induced hyperphagia and conditioned place preference procedures.

Perhaps the most convincing evidence from the present findings for a role for the LH in incentive learning is found in the results of the conditioned reinforcement procedure. Like lesions of the basolateral amygdala (Burns *et al.* 1993), lesioning the LH produced an initial deficit in the discriminated approach to the conditioned stimulus. Although lesions of the amygdala produced a significant deficit in responding for conditioned reinforcement and LH lesions did not, the variability of responding was greatly increased by lesioning the LH.

When considering the lack of convergence between electrophysiological data concerning the LH and the behavioural data presented at this time, it is important to remember that even with the most precise lesion methods, the lesions produced are not perfect. In the present study the entire LH was never completely destroyed and so it may be that the lesions did not block functions attributed to the LH by electrophysiological means because the remaining neurones were capable of mediating such functions. This can be considered an unlikely possibility however, since all the LH lesioned groups studied exhibited deficits in drinking in response to hypertonic saline, a response consistently failed by LH lesioned rats. Although excitotoxins are selective in destroying cell bodies, demyelination of fibres does occur, a process that would radically alter conduction velocity. Brace *et al.* (1997) illustrated that remyelination does occur but the pattern of myelin staining found presently in the LH of lesioned rats differed from that found in control rats. Despite this altered pattern of staining, it has been noted that destruction of the fibres of passage traversing the LH did not induce the same deficits found after cell body lesions of the LH providing further indication that disturbance of fibres of passage was not responsible for the effects found presently. A further problem in attributing effects of lesions is that damage outside the structure in question is always found. This complicates the interpretation of the results found, even when the damage to other structures is limited as was found in all the LH lesioned groups tested presently. For instance, some degree of damage was found in the zona incerta of the majority of the lesioned rats. This is important when considering drinking behaviour since the zona incerta has been shown to be involved in this (Rowland *et al.* 1979).

Conclusion

The aim of this thesis was to examine the possibility that the LH acts as an interface between the paraventricular system, responsible for monitoring internal milieu and frontostriatal systems responsible for behavioural planning and execution. Although the present study failed to provide evidence that the LH has such properties this does not represent a complete failure to produce a result. The present study covers a wide range of studies which, when taken together, indicate that the LH should be eliminated from the list of possible structures that could act in such a manner.

Simply observing the behaviour of LH lesioned rats is suggestive that that there is something profoundly wrong with their behaviour. However, finding a test that can quantitatively describe this deficit has remained elusive. The present findings do not entirely conflict with previous reports that the LH is involved in ingestive behaviour and learning and memory but they fell short of characterising the exact nature of this role.

Future Experiments

As described previously, at least some of the results found with tests of gustatory processing could be accounted for in terms of attenuated neophobia and an inability to chose appropriately from a range of food sources in order to address a specific homeostatic deficit. In order to verify the validity of these arguments they would have to be tested using lesion studies. Neophobia in LH lesioned rats could be tested using a method similar to that used by Rolls & Rolls (1973) in which hungry rats were presented with a range of novel palatable substances in addition to familiar lab chow. However, since after an initial recovery period deficits in consummatory behaviour were only found in novel test areas and not the home cage, it would be important to examine the expression of neophobia in novel and familiar environments.

In order to examine the ability of LH lesioned rats to make appropriate choices to address homeostatic deficits it would be necessary to present LH lesioned rats with a variety of tastant solutions when food or water deprived or even deprived of particular dietary components such as lysine. Since LH lesioned rats were shown to sacrifice energy intake in the face of food which was more palatable but less dense in calories, it would be interesting to examine whether LH lesioned rats would choose appropriately from a range of saccharin and sucrose solutions when in a food deprived state. Anecdotal observations of food hoarding would suggest that the LH lesioned rats were aware of their reduced body weight from but a more formal analysis would be required to confirm this. In addition to studying the behavioural consequences of lesioning the LH on energy regulation, it would also be interesting to examine the effects of infusing the protein leptin in the LH. Leptin has been implicated in the maintenance of energy balance (White & Martin 1997; Zhang *et al.* 1994) and recent observations from immunohistochemical localization of leptin receptors in the rat brain demonstrated that leptin receptors are found in the lateral hypothalamus (Shioda *et al.* 1998).

Microinfusions of the LH could also be used as an alternative method of studying the involvement of the LH in the behavioural tests examined presently. It has been shown that inhibition of LH neurones modulated dopamine levels in the

nucleus accumbens (Parada *et al.* 1995). Since manipulations of dopamine are known to effect reward related processes, it may be that microinfusions of dopamine receptor antagonists or even glutamate receptor antagonists in the LH prior to tests such as responding for conditioned reinforcement would result in clearer results that those reported here.

Stanley *et al.* (1996) demonstrated that chronic infusion of AP5 into the LH produced a fall in body weight that was similar to the initial decrease in body weight found with LH lesions. In addition to the initial fall in body weight after surgery, LH lesioned rats have been characterised by the failure to respond to "needle challenges" such as i.p. hypertonic saline and thus using infusions of antagonists in the LH could potentially block responding to the needle challenges which would reveal the neurotransmitters involved. By studying the patterns of c-Fos induced in response to the "needle challenges" other neural structures involved in such responses could be revealed. Furthermore, this technique could be used to reveal the reason why LH lesioned rats respond to food and water deprivation but not dehydration or glucoprivation induced by the "needle challenges" by comparing the pattern of c-Fos induced by both sets of challenges in neurally intact and LH lesioned rats.

References

AGGLETON, J. P., HUNT, P. R. & RAWLINS, J. N. P. (1986). The effects of hippocampal lesions upon spatial and non-spatial tests of working memory. *Behavioural Brain Research* **19**, 133-146.

ALEKSANYAN, Z. A., BURESOVA, O. & BURES, J. (1976). Modification of unit responses to gustatory stimuli by conditioned taste aversion in rats. *Physiology and Behavior* **17**, 173-179.

ANAND, B. K. & BROBECK, J. R. (1951). Hypothalamic control of food intake in rats and cats. *Yale Journal of Biology and Medicine* **24**, 123-140.

BERK, M. L. & FINKELSTEIN, J. A. (1982). Efferent connections of the lateral hypothalamic area of the rat: an autoradiographic investigation. *Brain Research Bulletin* **8**, 511-526.

BERRIDGE, K. C., FLYNN, F. W., SCHULKIN, J. & GRILL, H. J. (1984). Sodium depletion enhances salt palatability in rats. *Behavioral Neuroscience* **98 No.4**, 652-660.

BERRIDGE, K. C. & GRILL, H. J. (1983). Alternating ingestive and aversive consummatory responses suggest a two-dimensional analysis of palatability in rats. *Behavioral Neuroscience* **97, No.4**, 563-573.

BERRIDGE, K. C., GRILL, H. J. & NORGREN, R. (1981). Relation of consummatory responses and preabsorptive insulin release to palatability and learned taste aversions. *Journal of Comparative and Physiological Psychology* **95, No.3**, 363-382.

BERRIDGE, K. C. & PECINA, S. (1995). Benzodiazepines, appetite and taste palatability. *Neuroscience and Biobehavioral Reviews* **19, No.1**, 121-131.

BIELAJEW, C. & SHIZGAL, P. (1986). Evidence implicating descending fibers in self-stimulation of the medial forebrain bundle. *The Journal of Neuroscience* **6, No.4**, 919-929.

BIELAVSKA, E. & BURES, J. (1994). Universality of parabrachial mediation of conditioned taste aversion. *Behavioural Brain Research* **60**, 35-42.

BLESSING, W. W. (1997). Inadequate frameworks for understanding bodily homeostasis. *Trends in Neuroscience* **20, No.6**, 235-239.

BOUFFARD, J. -P. & JARRARD, L. E. (1988). Acquisition of a complex place task in rats with selective ibotenate lesions of hippocampal formation: combined lesions of subiculum and entorhinal cortex versus hippocampus. *Behavioral Neuroscience* **102, No.6**, 828-834.

BRACE, H., LATIMER, M. & WINN, P. (1997). Neurotoxicity, blood-brain barrier breakdown, demyelination and remyelination associated with NMDA-induced lesions of the rat lateral hypothalamus. *Brain Research Bulletin* **43, No.5**, 447-455.

BURNS, L. H., ROBBINS, T. W. & EVERITT, B. J. (1993). Differential effects of excitotoxic lesions of the basolateral amygdala, ventral subiculum and medial prefrontal cortex on responding with conditioned reinforcement and locomotor activity potentiated by intra-accumbens infusions of D-amphetamine. *Behavioural Brain Research* **55**, 167-183.

BURTON, M. J., ROLLS, E. T., MORA, F. (1976). Effects of hunger on the responses of neurons in the lateral hypothalamus to the sight and taste of food. *Experimental Neurology* **51**, 668-677.

CADOR, M., ROBBINS, T. W. & EVERITT, B. J. (1989). Involvement of the amygdala in stimulus-reward associations: interaction with the ventral striatum. *Neuroscience* **30, No.1**, 77-86.

CADOR, M., TAYLOR, J. R. & ROBBINS, T. W. (1991). Potentiation of the effects of reward-related stimuli by dopaminergic-dependent mechanisms in the nucleus accumbens. *Psychopharmacology* **104**, 377-385.

CAMPEAU, S., HAYWARD, M. D., HOPE, B. T., ROSEN, J. B., NESTLER, E. J. & DAVIS, M. (1991). Induction of the c-*fos* proto-oncogene in rat amygdala during unconditioned and conditioned fear. *Brain Research* **565**, 349-352.

CARR, G. D., FIBIGER, H. C. & PHILLIPS, A. G. (1989). Conditioned place preference as a measure of drug reward. In *The Neuropharmacological Basis of Reward*, eds. LIEBMAN, J. M. & COOPER, S. J., pp. 265-319. New York: Oxford University Press.

CARR, G. D. & WHITE, N. M. (1983). Conditioned place preference from intra-accumbens but not intra-caudate amphetamine injections. *Life Sciences* **33**, 2551-2557.

CAULLIEZ, R., MEILE, M. -J. & NICOLAIDIS, S. (1996). A lateral hypothalamic D1 dopaminergic mechanism in conditioned taste aversion. *Brain Research* **729**, 234-245.

CERVO, L. & SAMANIN, R. (1995). Effects of dopaminergic and glutamatergic receptor antagonists on the acquisition and expression of cocaine conditioning place preference. *Brain Research* **673**, 242-250.

CHEN, S. -W., DAVIES, M. F. & LOEW, G. H. (1995). Food palatability and hunger modulated effects of CGS 9896 and CGS 8216 on food intake. *Pharmacology Biochemistry and Behavior* **51, Nos.2/3**, 499-503.

CLARK, A. J. M., CLARK, J. M. & WINN, P. (1990). NMDA lesions of rat lateral hypothalamus: effects of dietary and physiological challenges. *Neuroreport* **1**, 263-266.

CLARK, J. M., CLARK, A. J. M., BARTLE, A. & WINN, P. (1991a). The

regulation of feeding and drinking in rats with lesions of the lateral hypothalamus made by N-methyl-D-aspartate. *Neuroscience* **45, No.3**, 631-640.

CLARK, J. M., CLARK, A. J. M., WARNE, D., RUGG, E. L., LIGHTMAN, S. L. & WINN, P. (1991b). Neuroendocrine and behavioural responses to hyperosmolality in rats with lesions of the lateral hypothalamus made by N-methyl-D-aspartate. *Neuroscience* **45, No.3**, 625-629.

CLARK, J. M., CLARK, A. J. M. & WINN, P. (1992). N-methyl-D-aspartate lesions of the lateral hypothalamus do not reduce amphetamine or fenfluramine anorexia but enhance the acquisition of eating in response to tail pinch in the rat. *Psychopharmacology* **109**, 331-337.

COLE, S. O. (1983). Combined effects of chlordiazepoxide treatment and food deprivation on concurrent measures of feeding and activity. *Pharmacology Biochemistry and Behavior* **18**, 369-372.

COOPER, S. J. (1980a). Benzodiazepines as appetite-enhancing compounds. *Appetite* **1**, 7-19.

COOPER, S. J. (1980b). Naloxone: effects of food and water consumption in the non-deprived and deprived rat. *Psychopharmacology* **71**, 1-6.

COOPER, S. J. (1981). Behaviourally-specific hyperdipsia in the non-deprived rat following acute morphine treatment. *Neuropharmacology* **20**, 469-472.

COOPER, S. J., BARBER, D. J., GILBERT, D. B. & MOORES, W. R. (1985). Benzodiazepine receptor ligands and the consumption of a highly palatable diet in non-deprived male rats. *Psychopharmacology* **86**, 348-355.

COOPER, S. J. & CRUMMY, Y. M. T. (1978). Enhanced choice of familiar food in a food preference test after chlordiazepoxide administration. *Psychopharmacology* **59**, 51-56.

COOPER, S. J. & ESTALL, L. B. (1985). Behavioural pharmacology of food, water and salt intake in relation to drug actions at benzodiazepine receptors. *Neuroscience and Biobehavioral Reviews* **9**, 5-19.

COOPER, S. J. & FRANCIS, R. L. (1979a). Feeding parameters with two food textures: interaction of chlordiazepoxide administration, alone or in combination with *d*-amphetamine or fenfluramine. *Psychopharmacology* **62**, 253-259.

COOPER, S. J. & FRANCIS, R. L. (1979b). Water intake and time course of drinking after single or repeated chlordiazepoxide injections. *Psychopharmacology* **65**, 191-195.

COOPER, S. J. & GILBERT, D. B. (1985). Clonazepam-induced hyperphagia in nondeprived rats: tests of pharmacological specificity with Ro5-4864, Ro5-3663, Ro15-1788 and CGS 9896. *Pharmacology Biochemistry and Behavior* **22**, 753-760.

COOPER, S. J., KIRKHAM, T. C. & ESTALL, L. B. (1987a). Pyrazoloquinolines: second generation benzodiazepine receptor ligands have heterogenous effects. *Trends in Pharmacological Sciences* **8**, 180-184.

COOPER, S. J. & McCLELLAND, A. (1980). Effects of chlordiazepoxide, food familiarization, and prior shock experience on food choice in rats. *Pharmacology Biochemistry and Behavior* **12, No.1**, 1-6

COOPER, S. J. & YERBURY, R. E. (1986). Benzodiazepine-induced hyperphagia: stereospecificity and antagonism by pyrazoloquinolines, CGS 9895 and CGS 9896. *Psychopharmacology* **89**, 462-466.

COOPER, S. J., YERBURY, R. E., NEILL, J. C. & DESA, A. (1987b). Partial agonists acting at benzodiazepine receptors can be differentiated in tests of ingestional behaviour. *Physiology and Behavior* **41,No3**, 247-255.

CROW, T. J. (1972). A map of the rat mesencephalon for electrical self-stimulation. *Brain Research* **36**, 265-273.

DAVIS, M. (1986). Pharmacological and anatomical analysis of fear conditioning using the fear-potentiated startle paradigm. *Behavioral Neuroscience* **100, No.6**, 814-824.

DAVIS, M. (1992). The role of the amygdala in fear-potentiated startle: implications for animal models of anxiety. *Trends in Pharmacological Sciences* **13**, 35-41.

DENIAU, J. M., MENETREY, A. & THIERRY, A. M. (1994). Indirect nucleus accumbens input to the prefrontal cortex via the substantia nigra pars reticulata: a combined anatomical and electrophysiological study in the rat. *Neuroscience* **61, No.3**, 533-545.

DUNN, L. T. & EVERITT, B. J. (1988). Double dissociations of the effects of amygdala and insular cortex lesions on conditioned taste aversion, passive avoidance, and neophobia in the rat using the excitotoxin ibotenic acid. *Behavioral Neuroscience* **102, No.1**, 3-23.

DUNNETT, S. B., LANE, D. M. & WINN, P. (1985). Ibotenic acid lesions of the lateral hypothalamus: comparison with 6-hydroxydopamine-induced sensorimotor deficits. *Neuroscience* **14, No.2**, 509-518.

EDWARDS, G. L. & JOHNSON, A. K. (1991). Enhanced drinking after excitotoxic lesions of the parabrachial nucleus in the rat. *American Journal of Physiology* **261**, R1039-R1044.

ETTENBERG, A. & DUVAUCHELLE, C. L. (1988). Haloperidol blocks the conditioned place preferences induced by rewarding brain stimulation. *Behavioral Neuroscience* **102, No.5**, 687-691.

EVANS, K. R. & VACCARINO, F. J. (1990). Amphetamine- and morphine-induced feeding: evidence for involvement of reward mechanisms. *Neuroscience and Biobehavioral Reviews* **14, No.1**, 9-22.

EVERITT, B. J., MORRIS, K. A., O'BRIEN, A. & ROBBINS, T. W. (1991). The basolateral amygdala-ventral striatal system and conditioned place preference: further evidence of limbic-striatal interactions underlying reward-related processes. *Neuroscience* **42, No.1**, 1-18.

FERSSIWI, A., CARDO, B. & VELLEY, L. (1987). Gustatory preference-aversion thresholds are increased by ibotenic acid lesion of the lateral hypothalamus in the rat. *Brain Research* **437**, 142-150.

FIBIGER, H. C., LePIANE, F. G., JAKUBOVIC, A. & PHILLIPS, A. G. (1987). The role of dopamine in intracranial self-stimulation of the ventral tegmental area. *The Journal of Neuroscience* **7, No.12**, 3888-3896.

FIBIGER, H. C., ZIS, A. P. & McGEER, E. G. (1973). Feeding and drinking deficits after 6-hydroxydopamine administration in the rat: similarities to the lateral hypothalamic syndrome. *Brain Research* **55**, 135-148.

FIORINO, D. F., COURY, A., FIBIGER, H. C. & PHILLIPS, A. G. (1993). Electrical stimulation of reward sites in the ventral tegmental area increases dopamine transmission in the nucleus accumbens of the rat. *Behavioural Brain Research* **55**, 131-141.

FLORESCO, S. B., SEAMANS, J. K. & PHILLIPS, A. G. (1997). Selective roles for hippocampal, prefrontal cortical, and ventral striatal circuits in radial-arm maze tasks with or without a delay. *The Journal of Neuroscience* **17, No.5**, 1880-1890.

FLYNN, F. W., GRILL, H. J., SCHULKIN, J. & NORGREN, R. (1991a). Central gustatory lesions: II. Effects on sodium appetite, taste aversion learning, and feeding behaviours. *Behavioral Neuroscience* **105, No.6**, 944-954.

FLYNN, F. W., GRILL, H. J., SCHWARTZ, G. J. & NORGREN, R. (1991b). Central gustatory lesions: I. Preference and taste reactivity tests. *Behavioral Neuroscience* **105, No.6**, 933-943.

FORD, B., HOLMES, C. J., MAINVILLE, L., JONES, B. E. (1995). GABAergic neurons in the rat pontomesencephalic tegmentum: codistribution with cholinergic and other tegmental neurons projecting to the posterior lateral hypothalamus. *Journal of Comparative Neurology* **363**, 177-196.

FRAY, P. J., SAHAKIAN, B. J., ROBBINS, T. W., KOOB, G. F. & IVERSEN, S. D. (1980). An observational method for quantifying the behavioural effects of dopamine agonists: contrasting effects of *d*-amphetamine and apomorphine. *Psychopharmacology* **69**, 253-259.

FULWILER, C. E. & SAPER, C. B. (1984). Subnuclear organisation of the

efferent connections of the parabrachial nucleus in the rat. *Brain Research Reviews* **7**, 229-259.

GALLYAS, F. (1970). Silver staining of fibrous neuroglia by means of physical development. *Acta Neuropathol. (Berl.)* **16,** 39-40.

GRIGSON, P. S., SHIMURA, T. & NORGREN, R. (1997a). Brainstem lesions and gustatory function: III. The role of the nucleus of the solitary tract and the parabrachial nucleus in retention of a conditioned taste aversion in rats. *Behavioral Neuroscience* **111, No.1**, 180-187.

GRIGSON, P. S., SHIMURA, T. & NORGREN, R. (1997b). Brainstem lesions and gustatory function: II. The role of the nucleus of the solitary tract in Na^+ appetite, conditioned taste aversion, and conditioned odor aversion in rats. *Behavioral Neuroscience* **111, No.1**, 169-179.

GRILL, H. J., SCHULKIN, J. & FLYNN, F. W. (1986). Sodium homeostasis in chronic decerebrate rats. *Behavioral Neuroscience* **100, No.4**, 536-543.

HABER, S. N., GROENEWEGEN, H. J., GROVE, E. A. & NAUTA, W. J. H. (1985). Efferent connections of the ventral pallidum: evidence of a dual striato pallidofugal pathway. *The Journal of Comparative Neurology* **235**, 322-335.

HAMILTON, R. B. & NORGREN, R. (1984). Central projections of gustatory nerves in the rat. *The Journal of Comparative Neurology* **222**, 560-577.

HASTINGS, M. H., WINN, P. & DUNNETT, S. B. (1985). Neurotoxic amino acid lesions of the lateral hypothalamus: a parametric comparison of the effects of ibotenate, N-methyl-D,L-Aspartate and quisqualate in the rat. *Brain Research* **360**, 248-256.

HATFIELD, T. & GALLAGHER, M. (1995). Taste-potentiated odor conditioning: impairment produced by infusion of an N-Methyl-D-aspartate antagonist into basolateral amygdala. *Behavioral Neuroscience* **109, No.4**, 663-668.

HEFFNER, T. G., HARTMAN, J. A. & SEIDEN, L. S. (1980). Feeding increases dopamine metabolism in the rat. *Science* **208**, 1168-1170.

HEIMER, L., ZAHM, D. S., CHURCHILL, L., KALIVAS, P. W. & WOHLTMANN, C. (1991). Specificity in the projection patterns of accumbal core and shell in the rat. *Neuroscience* **41, No.1**, 89-125.

HERBERG, L. J. & BLUNDELL, J. E. (1970). Non-interaction of ventromedial and lateral hypothalamic mechanisms in the regulation of feeding and hoarding behaviour in the rat. *The Quarterly Journal of Experimental Psychology* **22**, 133-141.

HERBERT, J. (1997). Do we need a limbic system? *Trends in Neuroscience* **20, No.11**, 508-509.

HERMANN, G. E. & ROGERS, R. C. (1985). Convergence of vagal and gustatory afferent input within the parabrachial nucleus of the rat. *Journal of the Autonomic Nervous System* **13**, 1-17.

HIGGS, S. & COOPER, S. J. (1996a). Effects of the benzodiazepine receptor inverse agonist Ro 15-4513 on the ingestion of sucrose and sodium saccharin solutions: a microstructural analysis of licking behavior. *Behavioral Neuroscience* **110, No.3**, 559-566.

HIGGS, S. & COOPER, S. J. (1996b). Increased food intake following injection of the benzodiazepine receptor agonist midazolam into the IVth ventricle. *Pharmacology Biochemistry and Behavior* **55, No.1**, 81-86.

HIGGS, S. & COOPER, S. J. (1996c). Hyperphagia induced by direct administration of midazolam into the parabrachial nucleus of the rat. *European Journal of Pharmacology* **313**, 1-9.

HIGGS, S. & COOPER, S. J. (1997). Midazolam-induced rapid changes in licking behaviour: evidence for involvement of endogenous opioid peptides. *Psychopharmacology* **131**, 278-286.

HODOS, W. (1961). Progressive ratio as a measure of reward strength. *Science* **134**, 943-944.

HOSOYA, Y. & MATSUSHITA, M. (1980). Cells of origin of the descending afferents to the lateral hypothalamic area in the rat, studied with the horseradish peroxidase method. *Neuroscience Letters* **18**, 231-236.

INGLIS, W. L., ALLEN, L. F., WHITELAW, R,B., LATIMER, M. P., BRACE, H. M. & WINN, P. (1994a). An investigation into the role of the pedunculopontine tegmental nucleus in the mediation of locomotion and orofacial stereotypy induced by *d*-amphetamine and apomorphine in the rat. *Neuroscience* **58, No.4**, 817-833.

INGLIS, W. L., DUNBAR, J. S. & WINN, P. (1994b). Outflow from the nucleus accumbens to the pedunculopontine tegmental nucleus: a dissociation between locomotor activity and the acquisition of responding for conditioned reinforcement stimulated by *d*-amphetamine. *Neuroscience* **62, No.1**, 51-64.

KELLEY, A. E. & DOMESICK, V. B. (1982). The distribution of the projection from the hippocampal formation to the nucleus accumbens in the rat: an anterograde- and retrograde-horseradish peroxidase study. *Neuroscience* **7, No.10**, 2321-2335.

KELLEY, A. E., DOMESICK, V. B. & NAUTA, W. J. H. (1982). The amygdalostriatal projection in the rat - an anatomical study by anterograde and retrograde tracing methods. *Neuroscience* **7, No.3**, 615-630.

KELLY, A. B. & WATTS, A. G. (1996). Mediation of dehydration-induced

peptidergic gene expression in the rat lateral hypothalamic area by forebrain afferent projections. *The Journal of Comparative Neurology* **370**, 231-246.

KELLY, A. B. & WATTS, A. G. (1998). The region of the pontine parabrachial nucleus is a major target of dehydration-sensitive CRH neurons in the rat lateral hypothalamic area. *The Journal of Comparative Neurology* **394**, 48-63.

KIROUAC, G. J. & GANGULY, P. K. (1995). Topographical organization in the nucleus accumbens of afferents from the basolateral amygdala and efferents to the lateral hypothalamus. *Neuroscience* **67, No.3**, 625-630.

KITA, H. & OOMURA, Y. (1982). An HRP study of the afferent connections to rat lateral hypothalamic region. *Brain Research Bulletin* **8**, 63-71.

KOSAR, E., GRILL, H. J. & NORGREN, R. (1986). Gustatory cortex in the rat. II. Thalamocortical projections. *Brain Research* **379** 342-352.

LAWES, I. N. C. (1994). The central connections of area postrema define the paraventricular system involved in antinoxious behaviors. In *Nausea and Vomiting: Recent Research and Clinical Advances*, eds. KUCHARCZYK, J., STEWART, D. J. & MILLAR, A. D., pp. 77-101. CRC Press.

LeDOUX, J. E. (1992). Brain mechanisms of emotion and emotional learning. *Current Opinion in Neurobiology* **2**, 191-197.

LUITEN, P. G. M., TER HORST, G. J. & STEFFENS, A. B. (1987). The hypothalamus, intrinsic connections and outflow pathways to the endocrine system in relation to the control of feeding and metabolism. *Progress in Neurobiology* **28**, 1-54.

MALDONADO-IRIZARRY, C. S., SWANSON, C. J. & KELLEY, A. E. (1995). Glutamate receptors in the nucleus accumbens shell control feeding behavior via the lateral hypothalamus. *The Journal of Neuroscience* **15 (10)**, 6779-6788.

MARSHALL, J. F., RICHARDSON, J. S. & TEITELBAUM, P. (1974). Nigrostriatal bundle damage and the lateral hypothalamic syndrome. *Journal of Comparative and Physiological Psychology* **87, No.5**, 808-830.

MARSHALL, J. F. & TEITELBAUM, P. (1974). Further analysis of sensory information following lateral hypothalamic damage in rats. *Journal of Comparative and Physiological Psychology* **86**, 375-395.

MARSHALL, J. F., TURNER, B. H. & TEITELBAUM, P. (1971). Sensory neglect produced by lateral hypothalamic damage. *Science* **174**, 523-525.

McALONAN, G. M., ROBBINS, T. W. & EVERITT, B. J. (1993). Effects of medial dorsal thalamic and ventral pallidal lesions on the acquisition of a conditioned place preference: the ventral striatopallidal system in reward-related processes. *Neuroscience* **52, No.3**, 605-620.

McDONALD, R. J. & WHITE, N. M. (1993). A triple dissociation of memory systems: hippocampus, amygdala, and dorsal striatum. *Behavioral Neuroscience* **107, No.1**, 3-22.

MOGA, M. M., HERBERT, H., HURLEY, K. M., YASUI, Y., GRAY, T. S. & SAPER, C. B. (1990a). Organisation of cortical, basal forebrain, and hypothalamic afferents to the parabrachial nucleus in the rat. *The Journal of Comparative Neurology* **295**, 624-661.

MOGA, M. M., SAPER, C. B. & GRAY, T. S. (1990b). Neuropeptide organization of the hypothalamic projection to the parabrachial nucleus in the rat. *The Journal of Comparative Neurology* **295**, 662-682.

MOGENSON, G. J., JONES, D. L. & YIM, C. Y. (1980). From motivation to action: functional interface between the limbic system and the motor system. *Progress in Neurobiology* **14**, 69-97.

MORRIS, R. G. M., GARRUD, P., RAWLINS, J. N. P. & O'KEEFE, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature* **297**, 681-683.

MOUFID-BELLANCOURT, S. & VELLEY, L. (1994). Effects of morphine injection into the parabrachial area on saccharin preference: modulation by lateral hypothalamic neurons. *Pharmacology Biochemistry and Behavior* **48, No.1**, 127-133.

MUFSON, E. J. & WAMPLER, R. S. (1972). Weight regulation with palatable food and liquids in rats with lateral hypothalamic lesions. *Journal of Comparative and Physiological Psychology* **80, No.3**, 382-392.

MURAMOTO, K., ONO, T., NISHIJO, H. & FUKUDA, M. (1993). Rat amygdaloid neuron responses during auditory discrimination. *Neuroscience* **52, No.3**, 621-636.

NAKAHARA, D., OZAKI, N., MIURA, Y., MIURA, H. & NAGATSU, T. (1989). Increased dopamine and serotonin metabolism in rat nucleus accumbens by intracranial self-stimulation as measured by in vivo microdialysis. *Brain Research* **495**, 178-181.

NAKAMURA, K. & ONO, T. (1986). Lateral hypothalamus neuron involvement in integration of natural and artificial rewards and cue signals. *Journal of Neurophysiology* **55, No.1**, 163-181.

NAOR, C. & DUDAI, Y. (1996). Transient impairment of cholinergic function in the rat insular cortex disrupts the encoding of taste in conditioned taste aversion. *Behavioural Brain Research* **79**, 61-67.

NIEUWENHUYS, R., GEERAEDTS, L. M. G. & VEENING, J. G. (1982). The medial forebrain bundle of the rat. I. General Introduction. *The Journal of*

Comparative Neurology **206**, 49-81.

NOMURA, S., MIZUNO, N., ITOH, K., MATSUDA, K., SUGIMOTO, T. & NAKAMURA, Y. (1979). Localization of parabrachial nucleus neurons projecting to the thalamus or the amygdala in the cat using horseradish peroxidase. *Experimental Neurology* **64**, 375-385.

NORGREN, R. (1978). Projections from the nucleus of the solitary tract in the rat. *Neuroscience* **3**, 207-218.

NORGREN, R. & LEONARD, C. M. (1971). Taste pathways in rat brainstem. *Science* **173**, 1136-1139.

NORGREN, R. & LEONARD, C. M. (1973). Ascending central gustatory pathways. *The Journal of Comparative Neurology* **150**, 217-238.

OHMAN, L. E. & JOHNSON, A. K. (1986). Lesions of the lateral parabrachial nucleus enhance drinking to angiotensin II and isoproterenol. *American Journal of Physiology* **251**, R504-R509.

ONO, T., NISHIJO, H. & UWANO, T. (1995). Amygdala role in conditioned associative learning. *Progress in Neurobiology* **46**, 401-422.

PARADA, M. A., PUIG DE PARADA, M., HOEBEL,B.G. (1995). Rats self-inject a dopamine antagonist in the lateral hypothalamus where it acts to increase extracellular dopamine in the nucleus accumbens. *Pharmacology Biochemisty and Behavior* **52, No.1**, 179-187.

PAXINOS, G., WATSON, C. (1982). The brain in stereotaxic co-ordinates. New York: Academic Press.

PHILLIPS, G. D., ROBBINS, T. W. & EVERITT, B. J. (1994). Bilateral intra-accumbens self-administration of *d*-amphetamine: antagonism with intra-accumbens SCH-23390 and sulpiride. *Psychopharmacology* **114**, 477-485.

POWLEY, T. L. & KEESEY, R. E. (19700). Relationship of body weight to lateral hypothalamic feeding syndrome. *Journal of Comparative and Physiological Psychology* **70, No.1**, 25-36.

REILLY, S., GRIGSON, P. S. & NORGREN, R. (1993). Parabrachial nucleus lesions and conditioned taste aversion: evidence supporting an associative deficit. *Behavioral Neuroscience* **107, No.6**, 1005-1017.

RICARDO, J. A. & KOH, E. T. (1978). Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat. *Brain Research* **153**, 1-26.

RICHARDSON, N. R. & GRATTON, A. (1996). Behavior-relevant changes in nucleus accumbens dopamine transmission elicited by food reinforcement: an electrochemical study in the rat. *The Journal of Neuroscience* **16, No.24**, 8160-

8169.

RIGAUD, D., BETOULLE, D., CHAUVEL, A., ALBERTO, L. A. & APFELBAUM, M. (1994). Effect of postgastric loads on intake of sham meals of different palatability in rats. *American Journal of Physiology* **267**, R150-R155.

ROBERTSON, A. H. (1994). The effect of pedunculopontine tegmental and lateral hypothalamic excitotoxic lesions on motivational strength as measured by the progressive ratio paradigm. *PhD Thesis, University of St. Andrews.*

ROBLEDO, P., ROBBINS, T. W. & EVERITT, B. J. (1996). Effects of excitotoxic lesions of the central amygdaloid nucleus on the potentiation of reward-related stimuli by intra-accumbens amphetamine. *Behavioral Neuroscience* **110, No.5**, 981-990.

ROLDAN, G. & BURES, J. (1994). Tetrodotoxin blockade of amygdala overlapping with poisoning impairs acquisition of conditioned taste aversion in rats. *Behavioural Brain Research* **65**, 213-219.

ROLLS, E. T., MURZI, E., YAXLEY, S., THORPE, S. J. & SIMPSON, S. J. (1986). Sensory-specific satiety: food-specific reduction in responsiveness of ventral forebrain neurons after feeding in the monkey. *Brain Research* **368**, 79-86.

ROLLS, E. T. & ROLLS, B. J. (1973). Altered food preference after lesions in the basolateral amygdala in the region of the amygdala in the rat. *Journal of Comparative and Physiological Psychology* **83, No.2**, 248-259.

ROMANSKI, L. M., CLUGNET, M. -C., BORDI, F. & LeDOUX, J. E. (1993). Somatosensory and auditory convergence in the lateral nucleus of the amygdala. *Behavioral Neuroscience* **107, No.3**, 444-450.

ROSENBLUM, K., BERMAN, D. E., HAZVI, S., LAMPRECHT, R. & DUDAI, Y. (1997). NMDA receptor and the tyrosine phosphorylation of its 2B subunit in taste learning in the rat insular cortex. *The Journal of Neuroscience* **17, No.13**, 5129-5135.

ROWLAND, N., GROSSMAN, S,P. & GROSSMAN, L. (1979). Zona incerta lesions: regulatory drinking deficits to intravenous NaCl, angiotensin, but not to salt in the food. *Physiology and Behavior* **23**, 745-750.

SAHAKIAN, B. J., WINN, P., ROBBINS, T. W., DEELEY, R. J., EVERITT, B. J., DUNN, L. T., WALLACE, M. & JAMES, W. P. T. (1983). Changes in body weight and food-related behaviour induced by destruction of the ventral or dorsal noradrenergic bundle in the rat. *Neuroscience* **10, No.4**, 1405-1420.

SALAMONE, J. D., COUSINS, M. S., MCCULLOUGH, L. D., CARRIERO, D. L. & BERKOWITZ, R. J. (1994). Nucleus accumbens dopamine release increases during instrumental lever pressing but not free food consumption. *Pharmacology Biochemistry and Behavior* **49, No1**, 25-31.

SANGER, D. J. & MCCARTHY, P. S. (1980). Differential effects of morphine on food and water intake in food deprived and freely-feeding rats. *Psychopharmacology* **72**, 103-106.

SAPER, C. B. (1985). Organization of cerebral cortical afferent systems in the rat. II. Hypothalamocortical projections. *The Journal of Comparative Neurology* **237**, 21-46.

SAPER, C. B. & LOEWY, A. D. (1980). Efferent connections of the parabrachial nucleus in the rat. *Brain Research* **197**, 291-317.

SAPER, C. B., SWANSON, L. W. & COWAN, W. M. (1979). An autoradiographic study of the efferent connections of the lateral hypothalamic area in the rat. *The Journal of Comparative Neurology* **183**, 689-706.

SCALERA, G., GRIGSON, P. S. & NORGREN, R. (1997). Gustatory functions, sodium appetite, and conditioned taste aversion survive excitotoxic lesions of the thalamic taste area. *Behavioral Neuroscience* **111, No.3**, 633-645.

SCALERA, G., SPECTOR, A. C. & NORGREN, R. (1995). Excitotoxic lesions of the parabrachial nuclei prevent conditioned taste aversion and sodium appetite in rats. *Behavioral Neuroscience* **109, No.5**, 997-1008.

SCHAFE, G. E., SEELEY, R. J. & BERNSTEIN, I. L. (1995). Forebrain contribution to the induction of a cellular correlate of conditioned taste aversion in the nucleus of the solitary tract. *The Journal of Neuroscience* **15(10)**, 6789-6796.

SCHECHTER, M. D. & CALCAGNETTI, D. J. (1993). Trends in place preference conditioning with a cross-indexed bibliography; 1957-1991. *Neuroscience and Biobehavioral Reviews* **17**, 21-41.

SCLAFANI, A., LUCAS, F. & ACKROFF, K. (1996). The importance of taste and palatability in carbohydrate-induced overeating in rats. *American Journal of Physiology* **270**, R1197-R1202.

SEAMANS, J. K., FLORESCO, S. B. & PHILLIPS, A. G. (1995). Functional differences between the prelimbic and anterior cingulate regions of the rat prefrontal cortex. *Behavioral Neuroscience* **109, No.6**, 1063-1079.

SELDEN, N. R. W., EVERITT, B. J., JARRARD, L. E. & ROBBINS, T. W. (1991). Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues. *Neuroscience* **42, No.2**, 335-350.

SHIMURA, T., GRIGSON, P. S. & NORGREN, R. (1997). Brainstem lesions and gustatory function: I. The role of the nucleus of the solitary tract during a brief intake test in rats. *Behavioral Neuroscience* **111, No.1**, 155-168.

SHIODA, S., FUNAHASHI, H., NAKAJO, S., YADA, T., MARUTA, O.,

NAKAI, Y. (1998). Immunohistochemical localization of leptin receptor in the rat brain. *Neuroscience Letters* **243**, 41-44.

SPECTOR, A. C. (1995). Gustatory parabrachial lesions disrupt taste-guided quinine responsiveness in rats. *Behavioral Neuroscience* **109, No.1**, 79-90.

SPECTOR, A. C., GRILL, H. J. & NORGREN, R. (1993). Concentration-dependent licking of sucrose and sodium chloride in rats with parabrachial gustatory lesions. *Physiology and Behavior* **53**, 277-283.

SPECTOR, A. C., NORGREN, R. & GRILL, H. J. (1992). Parabrachial gustatory lesions impair taste aversion learning in rats. *Behavioral Neuroscience* **106, No.1**, 147-161.

STANLEY, B. G., WILLETT III, V. L., DONIAS, H. W., HA, L. H. & SPEAS, L. C. (1993). The lateral hypothalamus: a primary site mediating excitatory amino-acid elicited eating. *Brain Research* **630**, 41-49.

STANLEY, B. G., WILLETT III, V. L., DONIAS, H. W., DEE II, M. G., DUVA, M. A. (1996). Lateral hypothalamic NMDA receptors and glutamate as physiological mediators of eating and weight control. *American Journal of Physiology* **270**, R443-R449

SWANK, M. W. & BERNSTEIN, I. L. (1994). c-Fos induction in response to a conditioned stimulus after single trial taste aversion learning. *Brain Research* **636**, 202-208.

SWANK, M. W., SCHAFE, G. E. & BERNSTEIN, I. L. (1995). c-Fos induction in response to taste stimuli previously paired with amphetamine or LiCl during taste aversion learning. *Brain Research* **673, No.2**, 251-261.

TABER, M. T. & FIBIGER, H. C. (1997). Feeding-evoked dopamine release in the nucleus accumbens: regulation by glutamatergic mechanisms. *Neuroscience* **76, No.4**, 1105-1112.

TABUCHI, E., ONO, T., NISHIJO, H. & TORII, K. (1991). Amino acid and NaCl appetite, and LHA neuron responses of lysine-deficient rat. *Physiology and Behavior* **49, No.5**, 951-964.

TAYLOR, J. R. & ROBBINS, T. W. (1984). Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. *Psychopharmacology* **84**, 405-412.

TAYLOR, J. R. & ROBBINS, T. W. (1986). 6-Hydroxydopamine lesions of the nucleus accumbens, but not of the caudate nucleus, attenuate enhanced responding with reward-related stimuli produced by intra-accumbens *d*-amphetamine. *Psychopharmacology* **90**, 390-397.

TEITELBAUM, P. & EPSTEIN, A. N. (1962). The lateral hypothalamic syndrome: recovery of feeding and drinking after lateral hypothalamic lesions.

Psychological Review **69, No.2**, 74-90.

THORNHILL, J. A. & SAUNDERS, W. (1984). Ventromedial and lateral hypothalamic injections of naloxone or naltrexone suppress the acute food intake of food-deprived rats. *Appetite* **5**, 25-30.

UNGERSTEDT, U. (1971a). Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta Physiologica Scandinavia* **Supplementum 367**, 1-48.

UNGERSTEDT, U. (1971b). Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigrostriatal dopamine system. *Acta Physiologica Scandinavia* **Supplementum 367**, 95-122.

UWANO, T., NISHIJO, H., ONO, T. & TAMURA, R. (1995). Neuronal responsiveness to various sensory stimuli, and associative learning in the rat amygdala. *Neuroscience* **68, No.2**, 339-361.

WAGNER, C. K., EATON, M. J., MOORE, K. E. & LOOKINGLAND, K. J. (1995). Efferent projections from the region of the medial zone incerta containing A13 dopaminergic neurons: a PHA-L anterograde tract-tracing study in the rat. *Brain Research* **677**, 229-237.

WARWICK, Z. S. & WEINGARTEN, H. P. (1994). Dissociation of palatability and calorie effects in learned flavor preferences. *Physiology and Behavior* **55, No.3**, 501-504.

WATTS, A. G. (1992). Osmotic stimulation differentially affects cellular levels of corticotrophin-releasing hormone and neurotensin/neuromedin N mRNAs in the lateral hypothalamic area and central nucleus of the amygdala. *Brain Research* **581**, 208-216.

WHITE, N. M. & CARR, G. D. (1985). The conditioned place preference is affected by two independent reinforcement processes. *Pharmacology Biochemistry and Behavior* **23**, 37-42.

WHITE, B. D., MARTIN, R. J. (1997). Evidence for a central mechanism of obesity in the Zucker rat: role of neuropeptide Y and leptin. *Proceedings of the Society for Experimental Biological and Medicine* **214**, 222-232.

WINN, P. (1995). The lateral hypothalamus and motivated behavior: an old syndrome reassessed and a new perspective gained. *Current Directions in Psychological Science* **4, No.6**, 182-187.

WINN, P. (1991). Excitotoxins as tools for producing brain lesions. In *Methods in Neurosciences*, ed. CONN, P. M., pp. 16-27. San Diego: Academic Press, INC.

WINN, P., BROWN, V. J. & INGLIS, W. L. (1997). On the relationships between the striatum and the pedunculopontine tegmental nucleus. *Critical Reviews in Neurobiology* **11, No.4**, 241-261.

WINN, P., CLARK, A. J. M., HASTINGS, M., CLARK, J. M., LATIMER, M., RUGG, E. L. & BROWNLEE, B. (1990). Excitotoxic lesions of the lateral hypothalamus made by N-methyl-d-aspartate in the rat: behavioural, histological and biochemical analyses. *Experimental Brain Research* **82**, 628-636.

WINN, P., CLARK, J. M., CLARK, A. J. M. & PARKER, G. C. (1992). NMDA lesions of lateral hypothalamus enhance the acquisition of schedule-induced polydipsia. *Physiology and Behavior* **52**, 1069-1075.

WINN, P. & HERBERG, L. J. (1985). Changes in actual versus defended body weight elicited by a varied, palatable ("supermarket") diet in rats. *Physiology and Behavior* **35**, 683-687.

WINN, P., TARBUCK, A. & DUNNETT, S. B. (1984). Ibotenic acid lesions of the lateral hypothalamus: comparison with the electrolytic lesion syndrome. *Neuroscience* **12, No.1**, 225-240.

WISE, R. A. & DAWSON, V. (1974). Diazepam-induced eating and lever pressing for food in sated rats. *Journal of Comparative and Physiological Psychology* **86, No.5**, 930-931.

WOLTERINK, G., PHILLIPS, G., CADOR, M., DONSELAAR-WOLTERINK, I., ROBBINS, T. W. & EVERITT, B. J. (1993). Relative roles of ventral striatal D_1 and D_2 dopamine receptors in responding with conditioned reinforcement. *Psychopharmacology* **110**, 355-364.

WYRWICKA, W. & DOBRZECKA, C. (1960). Relationship between feeding and satiation centers of the hypothalamus. *Science* **132**, 805-806.

YAMAMOTO, T. (1993). Neural mechanisms of taste aversion learning. *Neuroscience Research* **16**, 181-185.

YAMAMOTO, T., SHIMURA, T., SAKO, N., YASOSHIMA, Y. & SAKAI, N. (1994). Neural substrates for conditioned taste aversion in the rat. *Behavioural Brain Research* **6, No.2**, 123-127.

ZAHM, D. S. & BROG, J. S. (1992). Commentary on the significance of subterritories in the "accumbens" part of the rat ventral striatum. *Neuroscience* **50, No.4**, 751-767.

ZARDETTO-SMITH, A. M., BELTZ, T. G. & JOHNSON, A. K. (1994). Role of the central nucleus of the amygdala and bed nucleus of the stria terminalis in experimentally-induced salt appetite. *Brain Research* **645**, 123-134.

ZAR, J. H. (1986). *Biostatistical analysis.* 3rd edition.Prentice Hall International (UK) Limited, London p81-85.

ZARDETTO-SMITH, A. M., MOGA, M. M., MAGNUSON, D. J. & GRAY, T. S. (1988). Lateral hypothalamic dynorphinergic efferents to the amygdala and brainstem in the rat. *Peptides* **9, No.5**, 1121-1127.

ZHANG, Y., PROENCA, R., MAFFEI, M., BARONE, M., LEOPOLD,L., FRIEDMAN, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature* **372,** 425-432.

ZIGMOND, M. J. & STRICKER, E. M. (1973). Recovery of feeding and drinking by rats after intraventricular 6-hydroxydopamine or lateral hypothalamic lesions. *Science* **182**, 717-720.

APPENDIX

Multivariate analysis of behaviour after i.p. injection of LiCl or NaCl. Data from Experiment 1.1, "Conditioned Taste Aversion".

Behaviour	Degrees of Freedom.	F ratio	p value
Still	1,41	7.66	0.008*
Locomotion	1,41	0.14	0.713
Rear	1,41	4.10	0.050*
Sniff	1,41	0.03	0.875
Lying on Belly	1,41	0.52	0.474
Doggy Scratch	1,41	1.32	0.258
Fore-paw groom	1,41	0.12	0.729
Other groom	1,41	1.07	0.308

Main effect of Group (sham lesion/LH lesion)

Behaviour	Degrees of Freedom.	F ratio	p value
Still	1,41	1.76	0.192
Locomotion	1,41	38.65	0.000*
Rear	1,41	11.51	0.002*
Sniff	1,41	17.81	0.000*
Lying on Belly	1,41	28.12	0.000*
Doggy Scratch	1,41	7.35	0.010*
Fore-paw groom	1,41	5.00	0.019*
Other groom	1,41	4.02	0.052

Main effect of condition (LiCl/NaCl).

Behaviour	Degrees of Freedom.	F ratio	p value
Still	1,41	0.16	0.690
Locomotion	1,41	0.0005	0.982
Rear	1,41	1.38	0.247
Sniff	1,41	4.06	0.050*
Lying on Belly	1,41	0.51	0.478
Doggy Scratch	1,41	0.17	0.679
Fore-paw groom	1,41	0.30	0.863
Other groom	1,41	0.10	0.750

Group × condition interaction

ANOVA of intake of saccharin and salt solutions during taste preference tests, volume expressed as ml. Data from Experiment 1.3, "Taste Perception".

saccharin preference

significant group × concentration interaction: $F(7,98) = 5.32$ $p<0.001$

main effect of concentration: $F(7,98) = 22.36$ $p<0.001$

main effect of group: $F(1,14) = 19.89$ $p<0.001$

Salt preference

group × concentration interaction: $F(4,56) = 2.84$ $p<0.032$

main effect of concentration: $F(4,56) = 24.81$ $p<0.001$

main effect of group: $F(1,14) = 6.81$ $p<0.021$

Choice taste preference

Volume of saccharin solution consumed (ml)

no group × concentration interaction: $F(3,36) = 0.03$

main effect of concentration: $F(3,36) = 24.02$ $p<0.001$

no main effect of group: $F(1,12) = 1.58$

main effect of deprivation state: $F(1,12) = 37.22$ $p<0.001$

no group × deprivation state interaction: $F(1,12) = 0.40$

significant concentration × deprivation state interaction: $F(3,36) = 5.82$ $p<0.002$

no group × concentration × deprivation state interaction: $F(3,36) = 0.61$

Volume of salt solution consumed (ml)

main effect of group: $F(1,13) = 7.28$ $p<0.018$

main effect of concentration: $F(3,39) = 7.36$ $p<0.001$

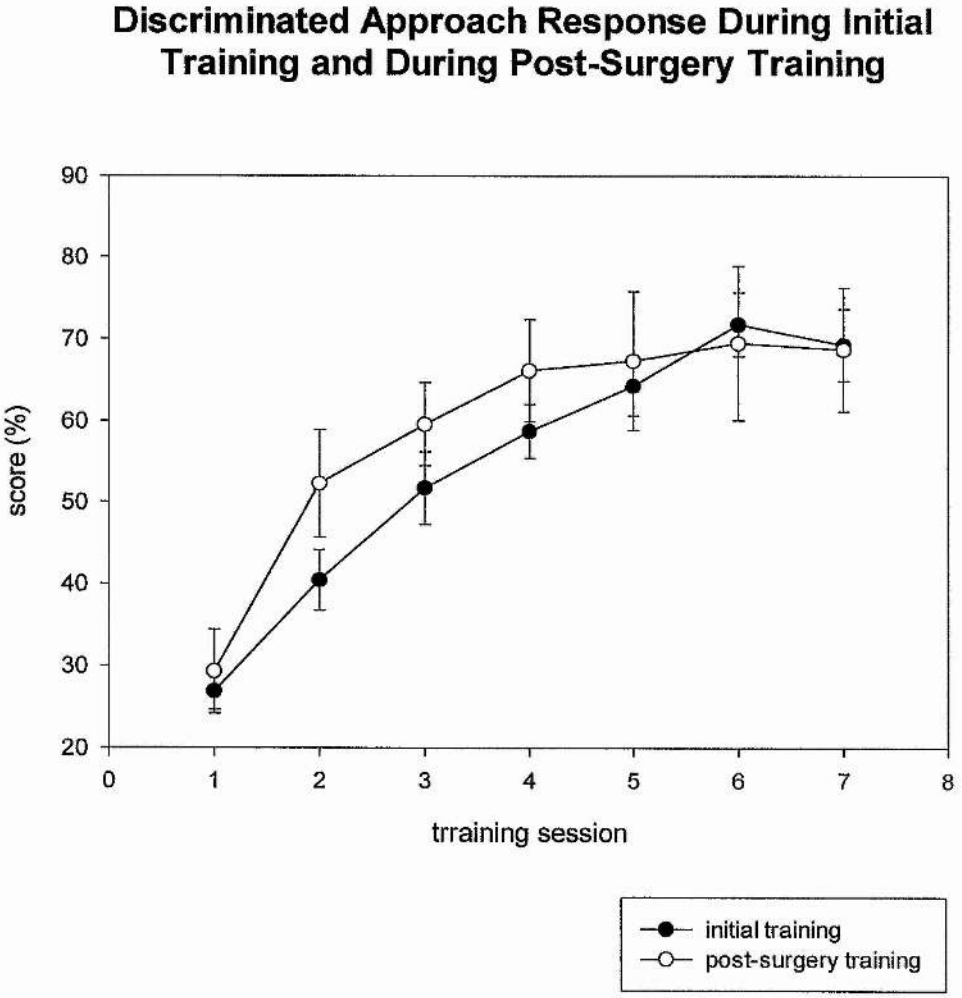
significant group × concentration interaction: $F(3,39) = 3.45$ $p<0.026$

main effect of deprivation state: $F(1,13) = 50.72$ $p<0.001$

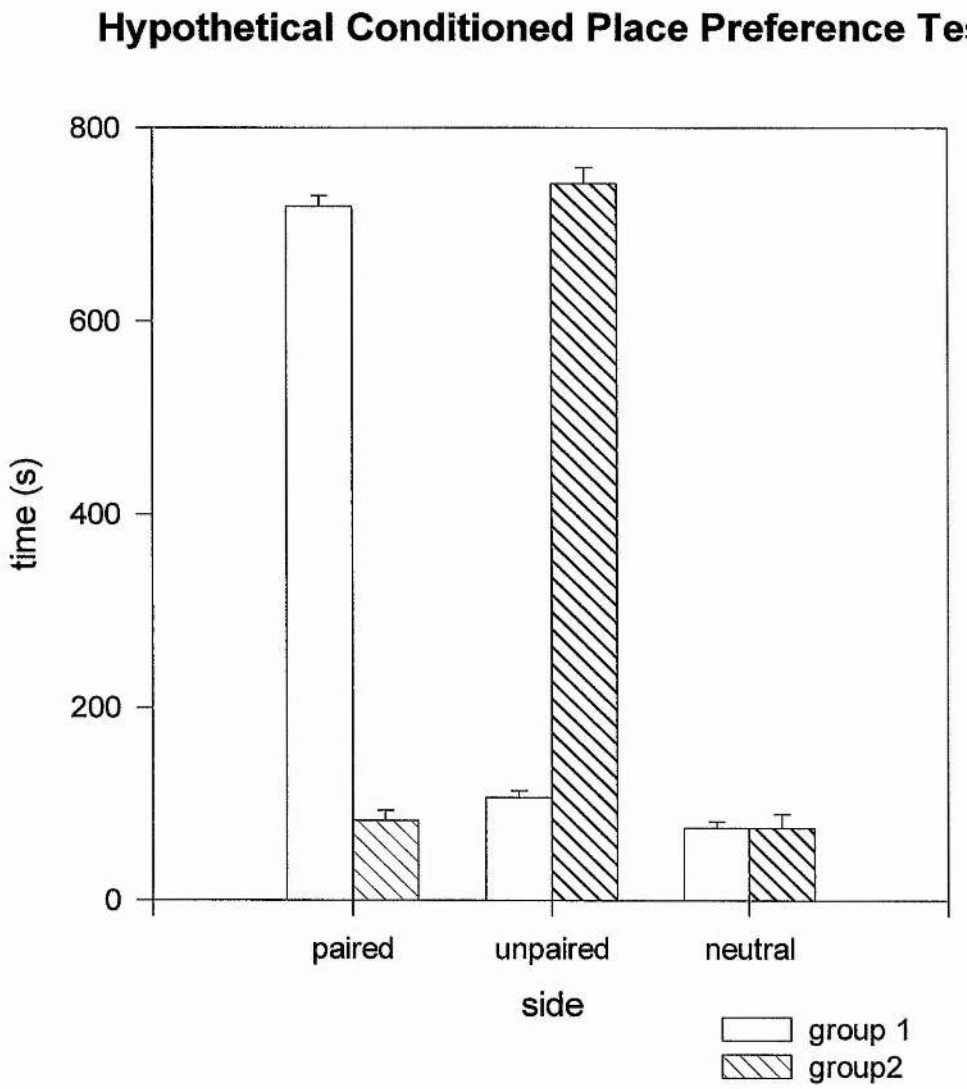
significant group × deprivation state interaction: $F(1,13) = 18.76$ $p<0.001$

significant concentration × deprivation state interaction: $F(3,39) = 4.73$ $p<0.007$

no significant group × concentration × deprivation state interaction: $F(3,39) = 2.5$



Comparison of the discriminated approach response before and after lesioning the LH; data from Experiment 2.1 "Conditioned Reinforcement". Mean (± SE) pre-surgery training scores for the discriminated approach response for the 7 initial training sessions for the group destined to be the LH lesioned group and mean (± SE) scores for the discriminated approach response for the first 7 post-surgery training sessions for the LH lesioned group.



Graph of hypothetical data from a conditioned place preference test illustrating mean (± SE) time spent in the paired, unpaired and neutral compartments.

Repeated measures ANOVA of the sample data graphed above revealed a main effect of side ($F(2,36) = 309.17$ $p<0.000$) and a significant group × side interaction ($F(2,36) = 821.03$ $p<0.000$) illustrating that the inability to reveal a main effect of group does not preclude the chance of revealing a significant group × side interaction.

Potential Problems

There are a number of methodological problems within this study. All the experiments carried out examined the behaviour of LH lesioned rats but several factors that were changed between studies make it difficult to compare data between them. Firstly, the anaesthetic used during surgery was not constant throughout the study. During the examination of gustatory processes i.e. in Experiments 1.1, 1.2 and 1.3, the rats were anaesthetised using avertin whereas in Experiments 2.1 and 2.2 which examined reward related processes, the rats were anaesthetised with Sagatal. The type of anaesthetic used was changed due to the unstable nature of avertin in comparison to Sagatal and due to the possibility of inducing gastrointestinal problems with avertin. It is possible that the type of anaesthetic used could determine the nature of the lesions induced to some extent. However, a comparison of the lesions produced with avertin and Sagatal did not reveal any clear differences in extra-hypothalamic damage produced.

Another factor that was changed between experiments was the post-operative care regime used. In experiment 1.1 body weight was monitored after surgery and if this fell below 85% of pre-surgery weight wet mash was given. Fresh wet mash was then given on a daily basis until body weight returned to 85% of pre-surgery weight. However, it was found that this was inadequate in enabling recovery from lesions of the LH. Access to wet mash resulted in the increase in body weight required but, when wet mash was removed, body weight returned to a level below 85% pre-surgery weight as the rats were not eating lab chow or drinking water. This resulted in a situation where the LH lesioned rats were given wet mash on alternate days for a prolonged period. In further studies it was found that simply placing pellets of lab chow on the floor of the home cage and supplementing the normal water supply with glucose was sufficient to produce rapid recovery from lesions of the LH in terms of food and water intake. The alterations made in the post-operative care regime resulted in a more rapid return to eating and drinking by the LH lesioned rats. However, this also resulted in a decrease in the long-term deficit in body weight seen with LH lesioned rats in comparison to the sham lesioned rats. It is possible that this could have affected the results found.

The procedure for inducing sham lesions was also modified between experiments in the present study. In experiments 1.1, 1.2, and 1.3 all sham lesioned rats received bilateral injections of phosphate buffer into the LH. However, in experiments 2.1 and 2.2 only half the sham lesioned rats received bilateral infusions of phosphate buffer while the other half were simply allowed to recover from anaesthesia without any further invasive procedures being conducted. When examining the behaviour of lesioned rats it is vital to have an appropriate control group with which to compare them. It is possible that the physical insertion of the cannula and/or the infusion of the vehicle solution (solution in which the toxin was dissolved) could have damaged neural tissue in some way leading to alterations in behaviour. However, sham lesions of the LH have been shown repeatedly to be without effect on every test examined. Although it is possible that simply leaving one group of rats to recover from anaesthesia and administering bilateral LH infusions of phosphate buffer to another produced distinct groups, no significant difference was found between the behaviour of these different sham lesioned groups. Therefor it was concluded that it was appropriate to collapse the different types of sham lesioned groups.

Statistical Problems

In addition to the procedural problems found in this study there are also a number of weaknesses in the statistical tests used to analyse the data collected. In order to analyse a set of data by ANOVA the data must meet a number of requirements:

- the data must have been measured on an interval scale
- the data must be normally distributed
- there must be homogenity of variance.

In Experiment 1.1 behaviour was rated after administration of either LiCl or saline. A problem arises in analysing this set of data using ANOVA because it violates the assumption of homogenity of variance. Because the LiCl treated rats expressed only "LOB" behaviour for practically the whole of the test period, excluding the expression of all other behaviours rated, while the control group expressed all behaviours rated except for "LOB", the variance found across

conditions varied greatly. This condition also introduces floor and ceiling effects: maximal scores were rated for some variables while other variables were not rated at all in particular cases.

Throughout the present study, a number of preference tests for sapid solutions were carried out. In each of these tests preference for a range of concentrations of was tested. Concentration can be classed as an interval scale, since there is a constant size between adjacent units on the concentration range whether it is measured as molarity or percentage concentration. However, in the present experiment inappropriate concentrations were used to examine taste preference since there were unequal intervals between the concentrations used. Hence, in these studies the assumptions of ANOVA were violated.

Statistical analysis of a group of subjects is used to infer characteristics about the population to which that group of subjects belongs. However, to be able to do this the subjects examined must be picked by random sampling. In experiment 3.1, this was violated in an attempt to balance two different lesion groups with respect to body weight. The appropriate statistical test in this case would have been ANCOVA. In testing body weight as a co-variant, this would have allowed the examination of the extent to which the difference in body weight contributed to the variance found in the data examined while maintaining random sampling.

There is also a problem in the way the data in experiment 2.2 was examined. This study examined conditioned place preference which, as it was stated in experiment 2.2 violates the assumptions of ANOVA because the variables measured are not independent. Although this a widely used technique, there does not appear to be a standard method of analysing the resultant data from it. However, one test which could be used to test it which was not mentioned in experiment 2.2 is the randomisation test.

In the present study a number of behavioural tests were carried out as it was predicted that lesioning the LH would have impaired performance in these tests. However, statistical analysis of the data from each of these tests failed to show a difference between the sham lesioned and LH lesioned group. Thus if our null hypothesis was that lesioning the LH would have no difference on the parameters measured we would have accepted the null hypothesis. It is possible for a null hypothesis to be wrongly accepted when there is in fact a difference between the experimental groups. When this occurs it is known as a Type II error. It is possible to calculate the probability that a statistical test will correctly reject the null hypothesis when it is false and this value is termed the statistical power. Statistical power is increased by increasing the number of subjects tested. Before carrying out an experiment it is possible to estimate the minimum number of subjects per level required to achieve a specified power, given the significance level and detectable difference desired among means. It is also possible to calculate the minimum detectable difference.