

THE EFFECT OF PEDUNCULOPONTINE TEGMENTAL
NUCLEUS AND LATERAL HYPOTHALAMIC
EXCITOTOXIC LESIONS ON MOTIVATIONAL
STRENGTH AS MEASURED BY THE PROGRESSIVE
RATIO PARADIGM

Alison Helen Robertson

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**THE EFFECT OF PEDUNCULOPONTINE TEGMENTAL NUCLEUS AND LATERAL
HYPOTHALAMIC EXCITOTOXIC LESIONS ON MOTIVATIONAL STRENGTH AS
MEASURED BY THE PROGRESSIVE RATIO PARADIGM**

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

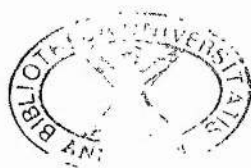
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September 1994



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(ii) I was admitted as a research student under Ordinance No. 12 in October, 1993 and as a candidate for the degree of Master of Philosophy in October, 1993; the higher study for which this is a record was carried out in the University of St. Andrews between 1993 and 1994.

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ABSTRACT

Motivated behaviours are associated with the nucleus accumbens (NAS). For example, microinjection of *d*-amphetamine into the NAS results in increased motivated behaviours, and 6-hydroxydopamine lesions attenuate responding for a rewarding stimulus. Two efferent targets of the NAS are the pedunculopontine tegmental nucleus (PPTg) and lateral hypothalamus (LH). Excitotoxic lesions of the PPTg result in deficits in certain incentive-driven behaviours such as conditioned reinforcement. The LH, on the other hand, is involved in more subtle behaviours. LH excitotoxic lesions result in an apparent inability to suppress inappropriate behaviours with regard to food and water intake regulation. LH-lesioned rats do not respond to certain physiological challenges. Thus, both structures are apparently involved in different aspects of motivated behaviours. Motivational strength has not yet been examined in the PPTg and LH. Progressive Ratio (PR) schedules involve a systematic increase in the number of presses on a conditioned lever following each reinforcer delivery. The stage at which responding ceases is called the breaking point (BP) and it is an indicator of motivational strength. This study involved lesioning the PPTg and LH by excitotoxins and testing them on a PR5 schedule. Ibotenate PPTg lesioned rats produced a clear deficit in responding indicated by significantly lower BPs than quinolinate or sham lesioned rats, indicating the possibility that PPTg neurones mediated motivational strength. Ibotenate lesioned rats pressed the non-contingent lever and the panel significantly more often than the other groups. It was hypothesised that these rats were unable to dissociate between the two levers or the panel, and the reward. NMDA lesions of the LH had no effect on motivational strength. They did, however, continually fail to respond appropriately to hypertonic saline. Thus, it seems that by their connections to the NAS, the PPTg and the LH are implicated in motivated behaviours, with only the PPTg being involved in the expression of motivational strength.

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MOTIVATION

Motivation is an "extremely important but definitionally elusive term" (Reber, 1985). The major primary drives of hunger, thirst, copulation and thermoregulation satisfy the fundamental needs of all organisms and under normal circumstances elicit behaviours in order to maintain the actively appropriate level for survival. Within the field of motivation there are two constructs that are widely used - drive and incentive. Drive was initially used by learning theorists in the 1940's and 1950's. It was thought to be induced by deprivation, such that hunger drives arose when an organism was food deprived. The term, however, is not water-tight. For example, it is easy to thwart drive by not eating when on a diet, or by doing another behaviour, and it is not an appropriate term for sexual behaviours since deprivation does not induce copulation. Although a new, more flexible construct "organismic state" was coined, the term drive survives because it is neat. More relevant to this thesis is the term incentive. This relates to the fact that objects and events in the environment can be hedonically loaded - that is, carry meaning for the animal - and the stimuli can be appetitive or aversive. The frequently used procedure of operant conditioning is brought about by an organism responding to a stimulus which is hedonically positive, and thus repeating the act.

One brain site particularly associated with the production of incentive-related behaviour is the nucleus accumbens (NAS), situated in the striatum. This is thought to be mediated by the neurotransmitter dopamine (DA) and it is believed that DA is critical for the operation of a neural system which mediates the rewarding properties of food, drink, drugs of abuse and electrical brain stimulation. The acquisition of and responding for conditioned reinforcement is a well known and widely used paradigm for investigating rewarding behaviour. Classical Pavlovian conditioning results in a previously neutral stimulus, such as lights or tones, gaining motivational significance through repeated pairings with an unconditioned stimulus (primary reinforcer), such as food or water. Such stimuli are known as conditioned reinforcers (CR). In the past few

years, a great deal of interest has been shown in the relationship between accumbens DA and the reinforcing effects of psychostimulant drugs (for example, Koob and Bloom, 1988). Taylor and Robbins (1984) found a dose-dependent selective increase in responding on the lever that produced a CR with intra-accumbens *d*-amphetamine infusions, suggesting that the NAS played an important role in *d*-amphetamine's enhanced control over behaviour exerted by CRs. To add more credence to this statement, Kelley and Delfs (1991a) microinjected *d*-amphetamine into various striatal subregions and found a dose-dependent, selective increase in responding after accumbens injections. They also found increases after injections into two regions surrounding the NAS, the anterior dorsal and the ventromedial striatum, although the increases were significantly less than intra-accumbens. Injections into ventrolateral striatal regions produced nonselective and changeable results. Thus, the NAS was the most potent site for mediating responses to a previously learned reward-related stimulus. McCullough, Cousins and Salamone (1993) were one of many experimental groups to show that NAS DA had a role to play in responding on a continuous reinforcement operant schedule. Rats were trained to press a lever for a food reward on a CR schedule and were implanted with dialysis probes in the NAS. Pressing the lever resulted in a significant increase in both DA and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) relative to controls. NAS lesions were then made by 6-hydroxydopamine (6-OHDA), and results showed that DA depletion produced a slowing of the initial lever pressing response rate.

As well as these experiments, there are other ways to measure incentive-related behaviour. Carr and White (1983) investigated the conditioned place preference (CPP) paradigm. Amphetamine injections into the NAS or caudate putamen (CP) were paired with a distinctive environment and control treatments paired with another. On given a free choice, rats showed a significant preference for the environment paired with amphetamine only when the injection had been aimed at the NAS. Hoebel et al. (1983) showed that rats would self-administer amphetamine into the NAS, and selective lesions of the DA mesolimbic neurones abolished amphetamine self-administration

(Lyness et al., 1979). Thus it seems clear from the above selected studies that NAS DA is an important mechanism in the production of stimulus-reward associations. In fact, more direct evidence has come from Ljungberg, Apicella and Schultz (1992) who recorded the responses of monkey DA neurones in the ventral tegmental area (VTA) during learning of behavioural reactions. The VTA is the site of the mesolimbic DA system cell bodies which project forward principally to the NAS. In the first task, animals learned to reach a small box for a morsel of apple when it opened visibly and audibly. The second task employed the operant conditioning of a reaction time situation in which animals reached from a resting key toward a lever when a small light was illuminated. During task acquisition, a drop of liquid reward was delivered for reinforcing the reaching movement. Each DA neurone was found either to respond to a particular stimulus during each learning phase or to lack responses to any stimuli. Effective stimuli included (i) novel, unexpected stimuli inducing orienting reactions; (ii) primary reward, when delivered as a reinforcer during conditioning, and (iii) conditioned incentive stimuli, which predicted reward and had the capacity to elicit behavioural reactions. Ljungberg et al. (1992) concluded that during the acquisition of simple behavioural tasks, mesolimbic DA neurones respond to unconditioned and conditioned stimuli that attract the animal's attention, elicit a behaviour and are associated with reward.

Despite the widespread support for the role of NAS DA in reward-related processes over the past decade or so, there is a growing body of evidence claiming that the relationships may not be quite as clear cut. Initial findings claimed that DA antagonists produced effects similar to extinction, that is, a decrease in the learned response resulting from withholding the reinforcer. It is now known, however, that DA antagonists and extinction do not produce similar effects (for example, Ettenberg and Carlisle, 1985). Berridge et al. (1989) found that rats with 6-OHDA lesions of DA pathways had intact appetitive taste reactivity to sweet solution, thus showing that despite a lack of DA, rats were able to perceive rewarding stimuli and produce a behaviour in response. Kelley and Delfs (1991b) investigated the effect of

microinjecting various neuropeptides into the VTA, site of the A10 mesolimbic dopaminergic cell bodies projecting to the NAS. They found small increases in DA turnover, but this was not enough to initiate responding for conditioned reward. They concluded that the important requirement for potentiation of CR was excessive release of DA in the NAS. On the other hand, Parker (1993) found that rats increased responding for CR after microinjection of ACh into the VTA which resulted in an increase in NAS DA efflux. Thus it seems that the rewarding effects mediated by DA are more specific and complicated than previously thought.

The basic reward paradigms may prove to be too global and hide more detailed aspects of motivation abolished by DA depletion which could be dissociated if a more specific task was used. This idea has been taken up by several authors in recent years. Salamone, Steinpreis, McCullough, Smith, Grebel and Mahan (1991) tested rats in an operant chamber in which they had a choice between pressing a lever for a preferred food or free feeding on the less preferred food. Administration of the DA antagonist, haloperidol, made rats eat more less preferred food and decrease the number of lever presses. This was true of either systemic administration or direct intra-accumbens microinjection of haloperidol. Thus, DA antagonists dissociated aspects of motivated behaviour impairing some and leaving others intact. DA depletion did not impair the behaviours oriented towards reinforcing stimuli because some aspects of the process were left intact. They suggested that the role of NAS DA was to facilitate the ability of conditioned stimuli (CS) to elicit and maintain vigorous instrumental responses. Hence the instrumental response of lever pressing which was elicited by the CS was disrupted by haloperidol. Another experiment carried out by Whishaw and Kornelson (1993) found two types of motivation that were dissociated by ibotenate lesions of the NAS. Both control and NAS lesioned food-deprived rats carried food to eat it. On the other hand, only sated control rats carried food to leave, whereas NAS sated rats did not. This showed that food carrying could be dissociated into two separate actions and controlled by two different neural mechanisms. Kelley and Stinus (1985) found exactly the same results when they lesioned NAS DA fibres using 6-

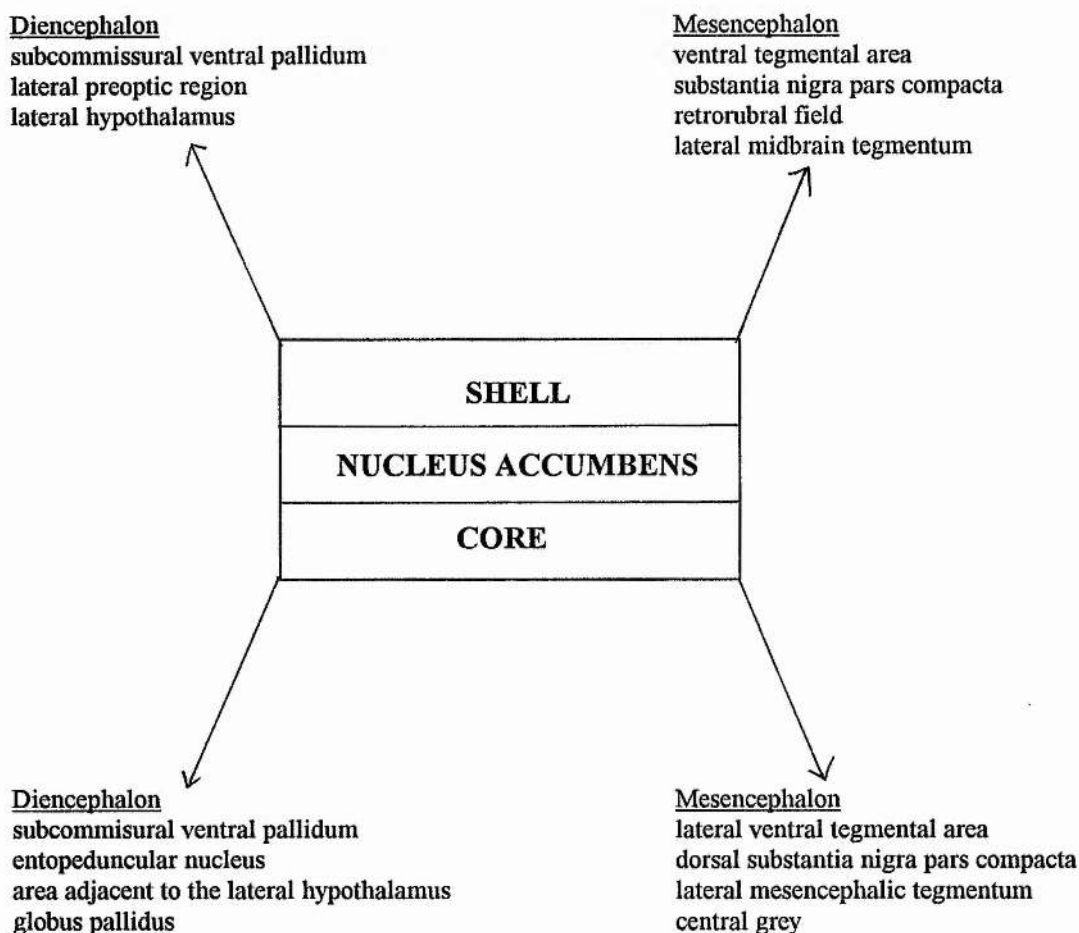
OHDA. Wishaw and Kornelson (1993) explained this dissociation in terms of an incentive-motivation hypothesis. They suggested that NAS DA was involved in the mediation of behaviour directed towards secondary features of food. That is, control and NAS rats were unaffected in the motivation that directed behaviour to primary food cues, whereas NAS rats were unable to direct their behaviour to food's secondary features. When rats were sated, only controls saw food as still of value to them. The dichotomy between motivation of primary and secondary drives may be the feature that is crucial in determining the function of NAS DA.

There is not substantial evidence that impairment of DA systems produces a fundamental or general interruption in rewarding processes. Taken from the above evidence, rats with a DA depletion are still able to perform consummatory behaviours and carry food with the intention of consuming it at its destination. Several recent theories have been put forward to account for the results of previous studies. Blackburn et al. (1987, 1989) proposed that mesolimbic DA was important for preparatory behaviours as they found that DA depleted rats were impaired in the latency and frequency of entry into a feeding compartment but unimpaired in actual food consumption. Salamone (1988) proposed that DA depletion interrupted activational aspects of motivated behaviour such as response rate, vigour and persistence. Salamone (1992) integrated these hypotheses in a review paper which stated that: "...the behaviours most easily disrupted by DA antagonists are highly activated and complex learned instrumental responses that are elicited or supported by mild conditioned stimuli, and maintained for considerable periods of time." This accounts for the findings of Wishaw and Kornelson (1993) since carrying food to hoard is a more complex goal-directed behaviour than to food consumption. It is also in agreement with Salamone et al. (1991) who showed that DA depleted rats failed to press levers for a preferred food, but consumed food that was freely available.

With the NAS being involved in certain specific reward-related behaviours, it is of interest to discover whether the structure has any influence of this kind on any of its efferent target structures. The two target areas of interest to this thesis are the

pedunclopontine tegmental nucleus (PPTg) and the lateral hypothalamus (LH). The NAS sends projections containing the inhibitory transmitter γ -aminobutyric acid (GABA) to the ventral pallidal complex (including the substantia innominata and the lateral preoptic area) and then descending GABA projections to the PPTg, located in the brainstem. The LH also receives a high number of projections from the medial part of the rostral pole of the NAS. Projections to both the ventral pallidum and the LH come from the "shell" part of the NAS (Zahm and Heimer, 1993). The following chapters will discuss the anatomy of the PPTg and LH, and put forward evidence relating these structures to incentive-motivated behaviours.

Figure 1: Efferent projections from the nucleus accumbens.



MORPHOLOGY OF THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS

The pedunculopontine tegmental nucleus (PPTg), which contains the Ch5 cholinergic neurones and non-cholinergic neurones, is located in the brainstem, and forms part of the pontomesencephalo tegmental (PMT) complex. It is intricately associated with other brainstem structures via ascending and descending fibre pathways. The PPTg is between 500-800 μ m long (Rugg et al., 1992), and is in close contact with the ascending limb of the cerebellar peduncle. It extends from the caudal pole of the substantia nigra to the rostral parabrachial nucleus, lies medial and dorsal to the fibres of the lateral lemniscus, and ventral to the cuneiform nucleus (Rye et al., 1987).

The PPTg can be distinguished into two different areas according to the cell density although this distinction is no longer widely used. The densest portion is the pars compacta which contains large, darkly staining cholinergic neurones, and the pars dissipatus which is a collection of less dense, smaller, non-cholinergic neurones. In general, the percentage of cholinergic cells among the total population of identifiable neurones increases linearly proceeding from the medial to the lateral extremes of the PPTg, and there are approximately 1700 cholinergic neurones per hemisphere (Rugg et al., 1992). These neurones have ascending projections to areas such as the thalamus, substantia nigra pars compacta (SNc), superior colliculus, lateral hypothalamus (LH), globus pallidus and subthalamic nucleus (Woolf and Butcher, 1986; Rye et al., 1987; Hallanger and Wainer, 1988; Lee et al., 1988; Gould et al., 1989). There are also descending ACh projections to cranial nerve nuclei, raphe nuclei, the medulla and the pons (Woolf and Butcher, 1989). (These connections will be discussed in more detail below). Using immunohistochemical histological techniques, investigators have been able to show the exact nature of the PPTg cholinergic neurones. The most widely used indicator is monoclonal antibodies raised to choline acetyltransferase (ChAT), the enzyme which facilitates synthesis of ACh from its precursors, acetate and choline. Cells labelled with this technique are called ChAT positive cells, and PPTg cholinergic

cells identified in this way have been found to be typically between 15-25 μ m in size, range from fusiform to triangular in shape, and tend to cluster in groups of three or four (Woolf and Butcher, 1986).

Several authors (Rye et al., 1987; Lee et al., 1988; Steininger et al., 1992) have distinguished between the PPTg and what they called the midbrain extrapyramidal area (MEA). Using this distinction, *only* cholinergic neurones are found in the PPTg-proper and the non-cholinergic neurones constitute a completely separate area - the MEA, located medially adjacent to the PPTg-pars compacta. On the basis of size and connectivity, many MEA non-cholinergic neurones are found mixed in with the cholinergic PPTg neurones (Spann and Grofova, 1992), a number often underestimated (Rye et al., 1987).

Using both ChAT and tyrosine hydroxylase (TOH; a catecholamine marker) immunohistochemistry, Rye et al. (1987) found that the PPTg was interposed between the A8 and A9 dopaminergic cell groups (located in the retrorubral nucleus and the substantia nigra respectively). The rostral third of the PPTg remains separated dorsally from the A8 cell group by the small celled ventral portion of the central tegmental field (CTF). The medial aspect of the PPTg is embedded within a diffuse collection of TOH positive axons, thought to comprise a portion of the central tegmental tract. Semba and Fibiger (1992) carried out a more detailed investigation by examining the relationship between 5-hydroxytryptamine (5-HT) and TOH-containing fibres and cholinergic neurones in the PPTg and the laterodorsal tegmental nucleus (LDTg), by combining nicotinamide diphosphate (NADPH diaphorase) histochemistry with 5-HT and TOH immunohistochemistry. They found not only TOH positive fibres mixed with the NADPH diaphorase positive cholinergic neurones, but also found dense varicose 5-HT fibres. These fibres were either thick or thin in diameter. The labelled fibres were seen to have bouton-like swellings when they were closely associated with NADPH positive cholinergic neurones in both the PPTg and the LDTg.

AFFERENTS TO THE PPTg

The study of afferents to the PPTg is important in that they may show the source of activation of neurones and thus allow a better understanding of their role in behavioural state regulation. Semba and Fibiger (1992) carried out a detailed histological analysis of the afferents to only the cholinergic mesopontine tegmentum, the area consisting of both the PPTg and the LDTg. They were able to identify the neurotransmitters used in these afferents by retrograde axonal transport using wheatgerm agglutinin conjugated horseradish peroxidase (WGA-HRP), by immunofluorescence using Fluorogold (FG) and by anterograde tracing using *Phaseolus vulgaris* leucoagglutinin (PHA-L). NADPH diaphorase histochemistry and immunohistochemistry were used to specifically mark cholinergic neurones. A similar method was employed by Steininger, Rye and Wainer (1992), but they distinguished between the cholinergic PPTg and the non-cholinergic MEA. The following description is taken from results found by both Steininger et al. (1992) and Semba and Fibiger (1992) unless stated. A small number of afferent projections originated in the prefrontal cortex and terminated in the cholinergic cell bodies of the PPTg. Of the diencephalon, the hypothalamus appeared to be the most densely retrogradely labelled, especially in the tuberal and posterior lateral hypothalamic area. A moderate density of labelling was found in the paraventricular hypothalamus, the zona incerta and the lateral and medial preoptic area. A few retrogradely labelled neurones were observed in the ventral lateral geniculate nucleus and the reticular nucleus. Small numbers of neurones were retrogradely labelled in the entopeduncular nucleus (medial globus pallidus in primates), the subthalamus (part of the basal ganglia) and the substantia innominata.

The majority of retrograde labelling was found in the central tegmental field (CTF), the periaqueductal gray (PAG; especially the lateral quadrant) and the dorsal raphe nucleus (DR). Light labelling was found in the anterior pretectal area, interstitial nucleus of the medial longitudinal fasciculus, substantia nigra pars reticulata (SNR),

and SNC. These substantia nigra terminals were found in the non-cholinergic neurones of the PPTg, the area Rye et al. (1987) originally called the MEA. In fact, all basal ganglia afferents terminated in the non-cholinergic PPTg neurones. Others, however, have suggested that the projections from the SNR to the PPTg are very dense (Spann and Grofova, 1991). Moderately dense retrograde labelling was found in the deep and intermediate layers of the superior colliculus. Retrograde PPTg injections resulted in numerous labelled neurones in the pontine reticular formation including both the oral and caudal parts of the pontine reticular nucleus. Labelled also was the dorsal raphe magnus, parabrachial region, median raphe nucleus, nucleus raphe pontis, locus coeruleus, nucleus of the lateral lemniscus, inferior colliculus, superior olive and the ventral cochlear nucleus. Following PPTg retrograde injections, labelling was found in the contralateral PPTg and bilaterally in the LDTg with ipsilateral dominance.

After injections into the PPTg, heavy retrograde labelling was seen in the medullary reticular formation - the lateral paragigantocellular nucleus and the gigantocellular reticular nucleus. Occasionally, labelled cells were seen in the vestibular nuclei, nucleus of the solitary tract, cuneate nuclei and the prepositus hypoglossal nucleus. A high number of labelled cells were seen in the spinal trigeminal nuclei. Retrogradely labelled neurones were also found in the cerebellum and spinal cord. These were located in the medial, interposed and lateral cerebellar nuclei, and also the dorsal horn, dorsolateral funiculus of the spinal cord at the cervical, thoracic and lumbar levels.

In order to identify the neurotransmitters present in these neurones, Semba and Fibiger (1992) injected FG in combination with immunohistochemical methods using secondary antibodies conjugated to the fluorochrome Texas Red. Primary antibodies immunoreactive to ChAT, TOH and 5-HT were used. The majority of retrogradely labelled neurones from the PPTg were ChAT immunoreactive, and were seen both contra- and ipsilateral to the injection site. FG labelled neurones which were 5-HT immunoreactive were occasionally found in the raphe nuclei. FG labelled cells in the

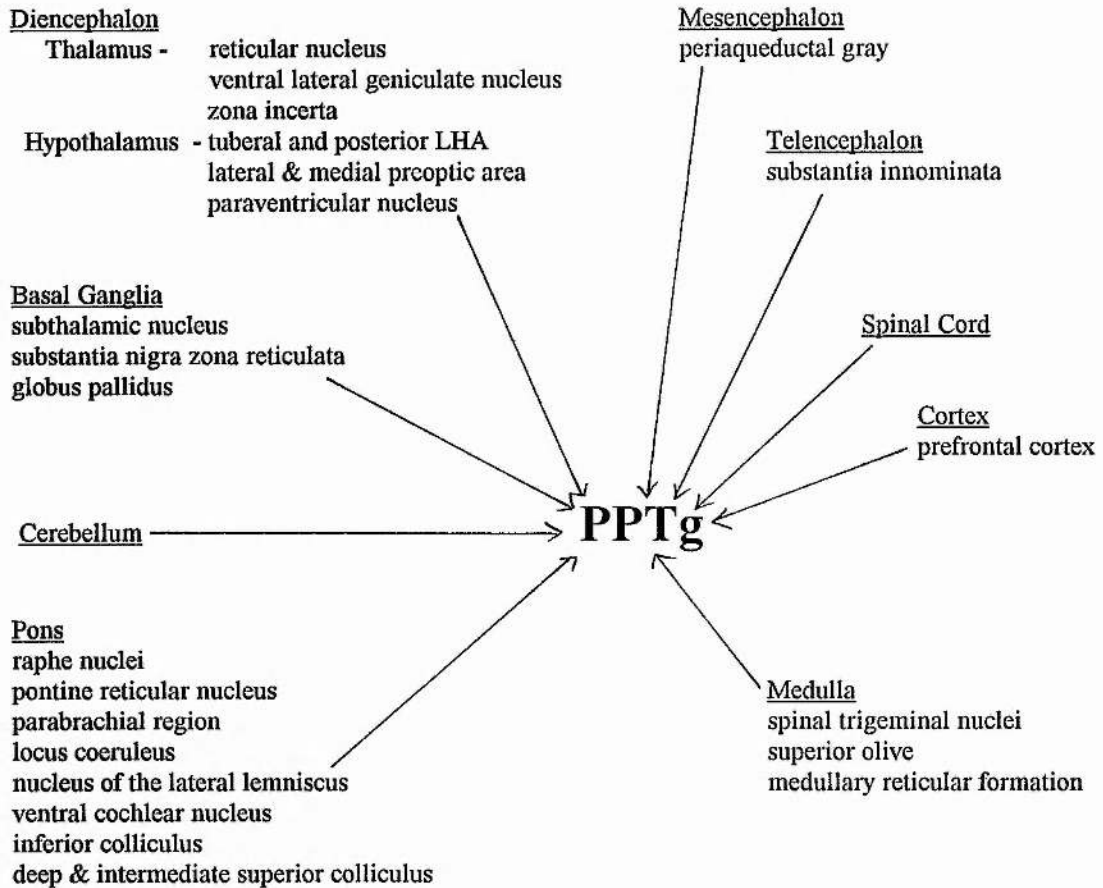
locus coeruleus were TOH immunoreactive. Closely packed 5-HT fibres were seen to be mixed with NADPH-diaphorase positive cholinergic neurones in the PPTg.

Two of the heaviest labelled afferents of the PPTg were from limbic structures, namely the LH and the midbrain central gray. These major inputs point to a role in sensorimotor and autonomic feedback control for the entire PMT complex, as the limbic system is critically involved in emotional behaviour, motivation and learning (Carlson, 1986). It is also possible that the limbic inputs have an excitatory effect on PMT cholinergic neurones which could bring about further facilitation of thalamocortical sensorimotor processing, since the thalamus receives the majority of its cholinergic innervation from the PMT complex.

Basal forebrain neurones projecting to the PPTg are not cholinergic, and have relatively slow conduction velocities. It is thought that gamma-aminobutyric acid (GABA), an inhibitory transmitter substance, may be the main neurotransmitter because many GABAergic neurones are found together with cholinergic neurones in the basal forebrain, and some have been found projecting to the LH. Also, stimulation of the substantia innominata produces both excitatory and inhibitory responses in the PPTg (Swanson et al., 1984).

Steininger et al. (1992), having distinguished between the PPTg-pars compacta and the MEA, found differences between their afferents. This shows that it is important to distinguish the two different areas. The major afferent to the MEA was found to be the basal ganglia. Steininger et al. (1992) propose that the MEA may have a role in the "modulation of motor systems", but this has not been studied in any more depth as yet. By studying the widespread distribution of the PPTg afferents, it becomes clear that this structure must have a role to play in behavioural state regulation. It also seems plausible to assume that cholinergic neurones of the entire PMT complex form part of the ascending reticular activating system which innervates the thalamus to a great extent, and which then continues on to innervate the cerebral cortex.

Figure 2: Major afferents to the PPTg



EFFERENTS FROM THE PPTg

Efferents from the PPTg form ascending and descending anatomical projections. By far the greatest number of neuronal projections are part of the ascending pathway which has been studied in great detail over the past ten years (Hallanger and Wainer, 1988; Lee, Rye, Hallanger, Levey and Wainer, 1988; Gould, Woolf and Butcher, 1989; Jourdain, Semba and Fibiger, 1989; Semba, Reiner and Fibiger, 1990). Woolf and Butcher (1986) and Woolf et al. (1990) have carried out detailed immunohistochemical studies in order to investigate the destination of the rat and feline pontomesencephalic ascending projections respectively. Woolf and Butcher (1986) used fluorescent tracer histology in combination with ChAT immunohistochemistry and AChE pharmacohistochemistry. Retrograde fluorescent

tracers into all thalamic areas resulted in a high density of cholinergic neurones marked in the PPTg. The cholinergic projections to the superior colliculus, especially the deep and intermediate layers, came from the PPTg. Retrograde tracers also identified the parafascicular nuclear area, subthalamic nucleus, globus pallidus, entopeduncular nucleus and the LH as major target areas for PPTg cholinergic innervation. Woolf et al. (1990) followed up the work on the rat by studying the ascending anatomical projections of the feline pontomesencephalon. In addition to histological techniques, lesions of the pontomesencephalon were made, and measures of ChAT fibre density were taken. Woolf et al. (1990) distinguished two major cholinergic pathways originating in the PMT complex. The lateral pathway originated in the PPTg. ChAT fibres were observed projecting to the inferior colliculus and SNC. Both ChAT fibres and terminal fields were observed in all the thalamic nuclei except the ventral nuclei. As well as the thalamus, widespread ChAT projections from the PPTg were identified terminating in the lateral geniculate nucleus, central tegmental field, zona incerta, globus pallidus and substantia innominata. As well as using ChAT immunohistochemistry, the results of radio frequency lesions of the PMT complex were also studied. In cats with lesions involving the PPTg and LDTg, the overall intensity of ChAT positive fibres was reduced throughout the diencephalon, midbrain and basal telencephalon, whereas there was no observable change in density of ChAT positive fibres in the midectosylvian gyrus, basolateral amygdala or the caudate nucleus. This suggests that a proportion of the fibres in these structures is derived from cholinergic cell bodies in the PPTg and LDTg. Woolf et al. (1990) also observed that ChAT fibres cut in cross section were thickest near the pontomesencephalon and were finest at rostral sites, that is, the anterior reticular thalamus, substantia innominata and lateral septal nucleus, suggesting that axon collaterals could have divided from original fibres to innervate other thalamic and hypothalamic sites. They also observed that ascending cholinergic fibres from the pontomesencephalon appeared to travel in larger tracts which contained many non-cholinergic fibres themselves projecting to basal ganglia and basal forebrain nuclei.

A controversy in this area of investigation has been the long standing disagreement as to whether the PPTg innervates the SNC via cholinergic neurones. Lee et al. (1988) proposed that the main source of SNC innervation was the non-cholinergic neurones of the MEA. Using combined ChAT immunohistochemistry and WGA-HRP retrograde tracing, they found that in all five cases, large numbers of retrogradely labelled neurones were found in the MEA. On the other hand, Woolf and Butcher (1986) used a combination of fluorescent tracer histology with ChAT immunohistochemistry and AChE pharmacohistochemistry, and found a distribution of cholinergic and non-cholinergic cells projecting to or through the SNC from the PPTg. Hence two different technique combinations have resulted in different conclusions. Lee et al. (1988) explained the differences as possibly due to spread of the injection site and/or uptake by damaged axons of passage are the result of use of fluorescent retrograde tracers. This argument seemed impossible to settle, but there have been several recent findings that reinforce the conclusions of Woolf and Butcher (1986).

Gould, Woolf and Butcher (1989) used highly retrograde and anterograde tract-tracing methods in combination with ChAT immunohistochemistry. The fluorescent tracers True Blue, propidium iodide or FG were injected into either the pars compacta or reticulata. ChAT and fluorescent tracer positive cells were found in the PPTg. Approximately 2-3 times more cholinergic cells were labelled in the PPTg when the tracer injections were centred in the compact area. The anterograde marker PHA-L was injected into the PMT complex and resulted in uptake and transport of that label to both nigral subnuclei. This supports the view that the PMT cholinergic complex does innervate the substantia nigra. Blaha and Winn (1993) microinjected cholinergic agonists into the SNC and measured DA efflux in the anterior dorsomedial striatum. As a result, they found an increase in DA following either nicotine or carbachol administration. Microinjection of the cholinesterase inhibitor neostigmine also resulted in an increase in striatal DA. Quinolinate lesions of the PPTg lead to a reversal of the stimulatory effects of intranigral neostigmine but also lead to an increase in DA efflux following nicotine or carbachol microinjection. This shows that the PPTg

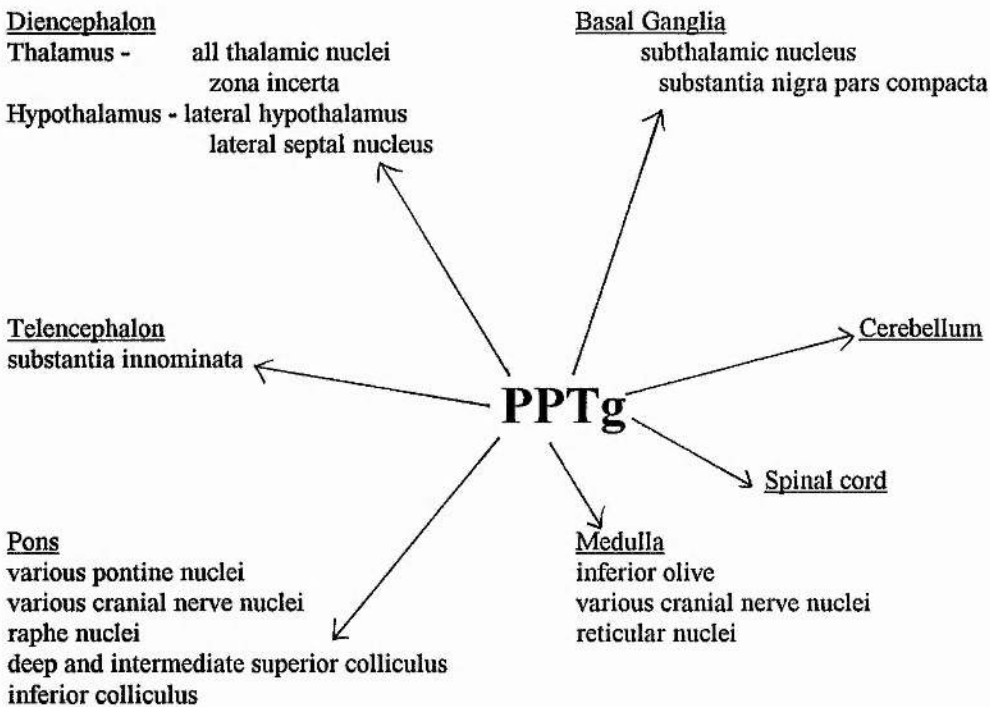
has a direct effect on the SNC which in turn regulates striatal DA. Bolam, Francis and Henderson (1991) discovered ChAT immunoreactive axons and axonal boutons throughout the substantia nigra, especially in the pars compacta, clearly showing evidence for a cholinergic input. The findings of Gould, Woolf, and Butcher (1989), Blaha and Winn (1993) and Bolam et al. (1991) together add favour to the argument proposing a SN input from the PPTg. There is no other known cholinergic input to the SN, and it has a high density of nicotinic and muscarinic receptors.

The PPTg not only sends out ascending efferents. A significant but generally fewer numbers have been found to be descending fibre efferents. These fibres are not all cholinergic, but numerous muscarinic and nicotinic receptor types have been found in various brainstem nuclei - several cranial nerve nuclei, the reticular formation, the cerebellum, the inferior olive and various pontine nuclei. Woolf and Butcher (1989) studied these projections by the use of FG and True Blue in combination with ChAT immunohistochemistry or AChE pharmacohistochemistry. ChAT and AChE positive neurones in the PPTg were labelled following infusions into the motor nuclei of cranial nerves 5, 7 and 12. Following tracer infusions into the dorsal raphe, median raphe or raphe magnus, ChAT immunopositive cells were found in the predominantly contralateral PPTg. ChAT positive neurones were also found in the PPTg following infusions into the oral pontine, caudal pontine and medullary reticular nuclei. The following associated cerebellar nuclei were labelled ChAT and AChE positive: pontine reticulotegmental nucleus, ventral tegmental nucleus, pons, paramedian and lateral reticular nucleus, perihypoglossal, dorsal column nuclei, inferior olive, deep cerebellar nuclei, pontine nuclei and the spinal cord.

It is obvious that both the ascending and descending cholinergic projections must play an important role in behaviour regulation because of their dense network of projecting fibres to diverse regions of the diencephalon, telencephalon and reticular formation. For example, (i) the cholinergic PMT complex mostly lies within the area termed the brainstem reticular formation, (ii) iontophoretic administration and local infusion or implantation of ACh and cholinergic agonists in the lateral geniculate,

caudate nucleus, hypothalamus, thalamus and other PMT targets produce significant excitatory or inhibitory effects on the workings of that structure, (iii) although the PMT cholinergic complex does not innervate cortical regions directly, its control of thalamic activity gives it defacto control over cortical processing.

Figure 3: Efferents from the PPTg



PPTg BEHAVIOURAL STUDIES

As the PPTg neurones project to a wide variety of target structures throughout the brain, it is not surprising that the PPTg has been implicated in many aspects of behaviour. Jones and Webster (1988) studied the effects of PPTg lesions upon cholinergic innervation of various brain sites, as it is commonly believed that these neurones constituted the ascending cholinergic reticular activating system (Shute and Lewis, 1967). They found that kainic acid lesions of the PMT complex of cats resulted in a significant decrease in both AChE staining and ChAT immunoreactivity in the

dorsal lateral geniculate, lateral posterior, pulvinar, intralaminar, mediodorsal and reticular nuclei of the thalamus, and the superior colliculus and reticular formation. Jones and Webster (1988) concluded that the PMT cholinergic neurones influenced the forebrain by sending both direct and indirect projections to the thalamus thus being a part of the ascending reticular activating system (ARAS).

Excitotoxins are one of the most common and effective ways of lesioning the PPTg. These lesions are useful in that they selectively kill cell bodies and leave fibres of passage intact. Rugg et al. (1992) investigated the following excitotoxins and their selectivity on cholinergic and non-cholinergic neurone loss in the PPTg: α -amino-3-hydroxy-5-methyl-4-isoxazdepropionic acid (AMPA), folate, ibotenate, kainate, N-methyl-D-aspartate (NMDA), quinolinate and quisqualate. Previous studies had been unable to specify the exact nature of the various excitotoxins used in lesioning experiments. The largest lesions were made by kainate and AMPA, and the smallest by quisqualate and quinolinate. Quinolinate produced the greatest ratio of cholinergic cell loss to general neuronal loss, making the most selective lesions. Using these various excitotoxins, authors have been able to investigate a wide variety of behaviours without confounding their results with possible damage to fibre pathways.

Many authors have associated the PPTg with locomotor activity. The PPTg is thought to be part of a neuronal circuit which mediates the expression of locomotion. Anatomically, it is located surrounding an area known as the mesencephalic locomotor region (MLR), a region which was discovered by electrically stimulating a decerebrate cat to stimulate walking (Shik, Severin and Orlovsky, 1966). Garcia-Rill, Houser, Skinner, Smith and Woodward (1987) carried out a study on rats to show the exact brain location of stimulation-induced locomotion. They performed a precollicular transection and fitted the rats to a treadmill whereupon they applied low amplitude current pulses to various brain sites. Stepping was seen at the lowest threshold ($<50\mu$ A) after stimulation of the caudal PPTg and sites in the middle portions of the PPTg. It took slightly higher thresholds to induce any sign of locomotion in areas located outside the PPTg. Histological techniques identified the locomotor-inducing sites as

populated by cholinergic cell bodies. Thus, the study showed further evidence linking the PPTg and the MLR.

Gordon Mogenson and his colleagues have been the most prolific exponents of the idea that the PPTg is critically involved in locomotion. Brudzynski, Wu and Mogenson (1988) described the PPTg as a major part of the MLR, receiving afferents from a variety of structures connected with locomotor activity, and sending projections to the spinal cord to generate limb movements. They investigated the effect of the cholinergic system on locomotion by microinjecting the muscarinic cholinergic agonist, carbachol, into the PPTg and studying the effects on freely moving rats. Results showed a decrease in spontaneous locomotor activity whereas microinjection into areas surrounding the PPTg elicited an increase in spontaneous locomotor activity. Pretreatment with the muscarinic antagonist, atropine, resulted in the absence of any locomotor changes. The increase in locomotion after administration of amphetamine into the NAS was attenuated by injection of carbachol into the PPTg. These results showed that the muscarinic cholinergic system was playing a role in adjusting locomotor activity by influencing the PPTg neurones concerned with relaying this information to associated structures. It seems to play an inhibitory role in locomotor activity. Milner and Mogenson (1988) compared the effects of electrical stimulation of two sites whose afferents originated in the MLR: the cuneiform nucleus (CnF) and the PPTg. Similar increases in locomotor activity were found in both areas. They also injected inhibitory neurotransmitter GABA antagonists into the PPTg, which resulted in an increase in locomotor activity. These results made the authors conclude that the PPTg was an integral part of the MLR and that it had an important role in locomotor activity.

In order to identify this apparent locomotor neuronal circuit, Mogenson, Wu and Tsai (1989) made lesions of possible target sites of the accumbens-subpallidal neural circuit, that is, the mediodorsal thalamus (MD) or the MLR (including the PPTg). The NAS is closely associated with locomotor activity since microinjection of *d*-amphetamine into the NAS elicits locomotion (for example, Pijnenburg, Honig, van

der Heyden and van der Rossum, 1976). A main target site for accumbens efferents is the subpallidal area which in turn projects to both the MD and the MLR. The local anaesthetic procaine was bilaterally microinjected via cannulae into the MD or PPTg and rats were tested in open-field apparatus containing novel objects or wooden panels. Novelty-elicited spontaneous activity decreased significantly only after procaine was injected into the PPTg. These results backed up the previous studies reported, which proposed that the main circuit implicated in locomotor activity was via the NAS, subpallidum and PPTg (Mogenson, Wu and Tsai, 1989).

In opposition to this is the idea that the PPTg has no role to play in locomotor activity. Swerdlow and Koob (1987) lesioned several of the VP efferent terminal regions, namely the DMT, PPTg and the medial prefrontal cortex (MPC). The NAS was denervated by 6-OHDA making the DA cell bodies supersensitive, and apomorphine was administered. Rats were tested in locomotor cages which measured locomotor activity using infrared photocell beams. Lesions of the DMT, but not the MPC or PPTg reduced the locomotor response to apomorphine. This is contrary to the findings of Mogenson, Wu and Tsai (1989) who claimed that it was the PPTg terminal region that formed an intricate part of the locomotor activation neural circuit. Swerdlow and Koob (1987), on the other hand, claim that the circuit critical in the mesolimbic DA controlled locomotor activity is accumbens-pallidum-thalamus. This important difference in findings can be accounted for in several ways. Mogenson, Wu and Tsai (1987) and an earlier paper by Brudzynski and Mogenson (1985) found locomotion attenuated after procaine administration into the PPTg. Close scrutiny of the injection sites used in both studies show that the injections are, in fact, aimed at the cuneiform nucleus, which is a structure strongly implicated in locomotion (for example, Garcia-Rill, 1986). Hence it is not surprising that they found effects of procaine on locomotion when they injected into their so-called "PPTg". There are also differences between the methods used to reduce PPTg neural activity, that is, lesions and procaine administration. Procaine prevents the flow of neural impulses through the structure's membranes, thus blocking the activity of fibres of passage as well as the local

neurones. On the other hand, excitotoxic lesions leave fibres of passage intact. Thus, the difference in findings could be due to disruption of fibres of passage rather than the PPTg itself. Therefore, it seems that the discrepancy between the findings could be due to several factors. Further evidence to support the view that the PPTg is not associated with the initiation of locomotor activity comes from a study by Inglis, Allen, Whitelaw, Latimer, Brace and Winn (1994a). They made bilateral ibotenate lesions of in either the PPTg or the deep mesencephalic nucleus (DpMe) and tested rats for spontaneous locomotion after subcutaneous administration of *d*-amphetamine or apomorphine. Spontaneous locomotion was not affected in any way by injection of *d*-amphetamine or apomorphine into either the PPTg or the DpMe. This shows that locomotion is not dependent on an intact and fully functioning PPTg. In a similar experiment, Inglis, Dunbar and Winn (1994b) measured spontaneous locomotion in both sham and PPTg lesioned rats after direct microinjection of *d*-amphetamine into the NAS. They found that both control and lesioned rats were more active following any dose of *d*-amphetamine compared to saline, but there was no difference in locomotor counts between the control and lesioned rats. These two studies compliment the findings by Swerdlow and Koob (1987), demonstrating that NAS induced locomotion is not affected by PPTg lesions.

It seems to be the case that the PPTg is not directly involved in locomotor activation, but there is evidence that points to a role in certain aspects or components of motor activity. Kelland and Asdourian (1989) investigated the role of the PPTg in muscle activity. They proposed that one role of the PPTg non-cholinergic neurones was to receive and influence striatal efferents concerning muscle activity inhibition, and to direct the afferents to a descending reticulospinal system. In order to investigate this, they electronically recorded the responses of selected neck and shoulder muscles during electrical stimulation of either the PPTg or an inhibitory area in the reticulospinal system, namely the nucleus reticularis gigantocellularis and ventralis (nrGi-V). Stimulation of 0.1Hz in the PPTg elicited bilateral muscular excitation and any increase resulted in a decrease in excitation. Anodal DC current inactivation of the

nrGi-V at the same time as 10Hz stimulation of the PPTg resulted in an increase in neck and shoulder muscle activity. As well as this, PPTg lesions caused an increase in baseline muscular activity. Taken together, these findings showed that the PPTg was a major source of inhibitory control over muscular activity, as destruction resulted in an attenuation of this inhibition. Dunbar, Hitchcock, Latimer, Rugg, Ward and Winn (1992) examined the effects of excitotoxic lesions of the PPTg on various activities using either ibotenate or quinolinate. They found that ibotenate and quinolinate destroyed PPTg cholinergic neurones equally well, whereas ibotenate produced lesions of non-cholinergic neurones which were twice as widespread as quinolinate lesions. These different lesions produced functional differences in behaviour. When turning in response to amphetamine and apomorphine was measured, quinolinate rats turned ipsilaterally and ibotenate contralaterally, showing that the different forms of rotation may be elicited from different neuronal cell populations in the PPTg. A dissociation was also found in a staircase task, a test which required a rat to reach steps of different levels using different paws in order to collect food pellets. Quinolinate lesioned rats showed normal grasping but impaired reaching abilities, whereas ibotenate lesioned rats were unimpaired. It was possible that these rats could have been motivationally impaired, but this was deemed unlikely since they all ate and drank normally in their home cages. Thus it seems that the PPTg has a role to play in motor control, but as yet, the exact nature of this role is to be discovered.

The PPTg has been associated with many more aspects of behaviour other than motor activity. Ever since the classical studies of Moruzzi and Magoun (1949), the pontomesencephalic tegmentum has been known to contain neurones responsible for the generation and maintenance of sleep-wake states. Cholinergic neurones in this location are part of the ARAS and lesions here result in severe deficits in behavioural arousal - an indicator of wakefulness (Lindsley, Schreiner, Knowles and Magoun, 1949). Webster and Jones (1988) injected kainic acid into the dorsolateral pontomesencephalic tegmentum, including the PPTg, of cats to study the effects of cholinergic cell destruction on sleep-wake states. The most marked effect was on rapid

eye movement (REM) sleep, in the first month after lesioning: the PGO spike rate decreased and the EMG amplitude increased marking a loss of neck muscle tone. The larger the lesion, the fewer the number of REM sleep bouts. The PGO spike rate and percentage REM sleep bouts were significantly correlated with the remaining number of ChAT immunoreactive cholinergic neurones, thus showing that it was the PMT complex cholinergic neurones mediating REM sleep. In addition to this study, there have been numerous further studies published describing the intimate association of the PPTg with sleep-wake states showing that the cholinergic neurones are critical for the generation of REM sleep (for example, Gnadt and Pegram, 1985; Shiromani and McGinty, 1986; and Harrison, Woolf and Buchwald, 1990).

An intact PPTg has also been shown to be important in certain learning mechanisms. Dellu, Mayo, Cherkaoui, Le Moal and Simon (1991) evaluated the behavioural consequences of excitotoxic PPTg lesions in the rat. Locomotor and memory processes were tested using a locomotor cage, a cross maze task (four arms round a box designated north, south, east and west, with a start box in the middle and one arm with a food pellet and visual cues present), a place navigation task (a swimming pool filled with milky water and a platform in the middle) and a radial maze task (eight maze arms projecting from a central platform with food pellets at the end of each arm and no visual cues). Control and lesioned rats did not differ in locomotor activity, and both groups reduced their number of errors over the duration of the experiment in the cross maze task. Lesioned rats, however, were slower to find the platform in the place navigation task, and made more errors in the radial maze task compared to controls. The differences in performance of lesioned rats in the two tasks designed to test reference memory (cross and water maze) showed that memory itself was not affected, rather the critical factor was task difficulty. Dellu et al. (1991) suggested that the intact PPTg was involved in sustained attention required to correctly perform in the water and radial maze tasks. No matter how these results are finally interpreted, it is clear that the PPTg is involved in more aspects of motor

activity. Cognitive deficits can be explained anatomically as the PPTg is connected to cortical structures via the thalamic relay nuclei.

In order for rats to perform any of the tasks previously described, they must be suitably motivated to do so. The PPTg has been implicated in aspects of motivation to the extent that it is possibly becoming the most studied feature of the structure. The PPTg is ideally situated to play a role in motivation due to both its cholinergic and non-cholinergic connections with numerous other brain regions. The PPTg has reciprocal cholinergic connections with the LH, a structure known to be associated with motivation (see later section), and it also sends cholinergic projections to the substantia nigra, known to elicit motivated behaviours upon stimulation (for example, Winn and Redgrave, 1979, 1981; and Winn, 1991). Non-cholinergic neurones from the VP and the ventral tegmental area (VTA) project to the PPTg. These two structures are also highly regarded as important structures in the display of motivated behaviours and are target sites of the NAS. Dunbar et al. (1992) investigated the effects of ibotenate and quinolinate PPTg lesions on the basic motivated behaviours of eating and drinking. They found no differences between the control and lesioned groups showing that the PPTg must be involved in another more complex aspect of motivated behaviour.

Bechara and van der Kooy (1989, 1992a, 1992b) and Bechara, Harrington, Nader and van der Kooy (1992c) carried out a series of elegant experiments studying the relationship between the PPTg and reward mechanisms. Rats with PPTg ibotenate lesions were given either amphetamine or morphine and tested in a conditioned place preference (CPP) paradigm. These rats had the reinforcing effects blocked but were, however, still able to show a CPP to an environment paired with the peripheral opiate antagonist methylnaltrexone. Rats that had acquired a morphine CPP prior to the PPTg lesion retained the CPP afterwards. Taken together, these results showed that the PPTg lesions were producing deficits in motivation rather than learning or memory (Bechara and van der Kooy, 1989). In order to study this in more depth, Bechara and van der Kooy (1992a) tested drug naive and drug dependent rats in the CPP paradigm.

Drug naive and morphine dependent rats both preferred places paired with morphine, but only morphine dependent rats showed withdrawal by avoiding places paired with a lack of morphine. They also found that food deprived, but not sated rats avoided places paired with the lack of food. PPTg lesions blocked the morphine and food CPP in drug naive and food sated rats but did not block CPP in drug dependent and food deprived rats, and morphine withdrawal conditioned and hunger conditioned place aversions. They concluded that separate neural mechanisms were the basis of deprivation and non deprivation induced motivation. To address the issue of tolerance to the rewarding properties of opiates, Bechara and van der Kooy (1992b) investigated the effects of withdrawal alleviating doses of morphine on rats in the CPP paradigm. PPTg lesions blocked CPP in high and low doses of morphine in drug naive rats, but only the high dose of morphine in drug dependent rats. When drug dependent rats received withdrawal alleviating doses of morphine 3.5 hours before pairing, they showed CPP which were abolished by PPTg lesions. These findings suggested that chronic exposure to morphine did not alter the neural tissue mechanisms underlying the immediate rewarding properties of morphine. The final paper in the series by Bechara et al. (1992c) investigated the two motivational mechanisms underlying drug naive and drug dependent rats. It had already been established that the PPTg was a crucial substrate in the neural system mediating the rewarding effects of opiates in drug naive rats. It is also commonly thought that DA is important in reward mechanisms produced by various opiates (for example, Wise and Bozarth, 1987). In contrast to this, however, it is strongly believed that opiate reward is observed only when DA is blocked by, for example, neuroleptics (for example, Koob and Bloom, 1988). The final paper in the series aimed to discover the effects of neuroleptics on drug naive and drug dependent rats. They found that neuroleptics blocked CPP in drug dependent but not drug naive rats. Thus, taking all the results together, it seem that there is a double dissociation in motivational systems concerning the rewarding effects of opiates on drug naive and drug dependent rats, and it is only the motivational system which represents the drug dependent state that relies on the utilisation of DA.

In addition to these experiments providing evidence for a role in rewarding mechanisms for the PPTg, Inglis et al. (1994b) investigated the effects of PPTg lesions on the acquisition of responding for CR stimulated by *d*-amphetamine administered into the NAS. This incentive-related paradigm has been shown to be an accurate method of measuring rewarding stimuli. Inglis et al. (1994b) found that there were significant differences between control and PPTg lesioned rats in the CR paradigm. Lesioned rats directed almost all their attention to the levers, leaving the food panel alone. They did not, however, discriminate accurately between the reinforced and non-reinforced lever, whereas controls on the whole pressed the reinforced lever and ignored the non-reinforced lever and the food panel. This showed quite clearly that by lesioning the PPTg, rats were unable to direct incentive-driven behaviour. In 1988, Koob and Bloom stated that "positive-reinforcing" effects of psychoactive drugs occurred via neural excitation of the ventral tegmental - NAS - ventral pallidal circuit, mediated by the mesolimbic DA system, commonly known to be associated with the expression of reward-related behaviour (for example, Carr and White, 1983). The PPTg receives descending non-cholinergic projections from the VP, which enhances the probability that the PPTg is concerned with the behavioural expression of reinforcement brought about by the ventral tegmental - NAS - ventral pallidal circuit. Inglis et al. (1994b) propose that the PPTg is involved in the expression of appropriate and inhibition of inappropriate behaviour. They claim that its "task is to form simultaneous associations between more than one novel stimulus and one incentive, in order to direct incentive-driven behaviours". This explains the fact that PPTg lesioned rats in their study were able to make the lever/press reward association, but failed to discriminate between the reinforced and non-reinforced lever.

Despite numerous authors claiming that they have pinpointed the exact role that the PPTg plays in motivated behaviour, it is clear from all the studies mentioned in this chapter that the PPTg is concerned with many different, subtle aspects of motivated behaviour, and that the exact nature of this role is yet to be defined. There have been no studies to date addressing the issue of the role the PPTg has to play in

expression of motivational strength. The following experiments aim to show whether an intact PPTg is necessary for rats to work to their full potential in a rewarding situation.

MORPHOLOGY OF THE LATERAL HYPOTHALAMUS

The hypothalamus is a tiny structure which is located directly below the thalamus in the diencephalon. It lies in the walls of the third ventricle below the hypothalamic sulci, and externally it is bounded rostrally by the optic chiasm, laterally by the optic tracts, and posteriorly by the mammillary bodies, forming a diamond shaped area containing several small protuberances. Despite being a relatively small structure, it is an extremely important one. It is crucial to homeostatic regulation and is a principal site for regulating the behaviour essential to the survival of the individual and species - often called the four Fs: fighting, feeding, fleeing and mating. The hypothalamus is ideally located to be in control of the regulation of the internal environment by correlating endocrine and neural functions. This is partly achieved by the zone forming the floor of the third ventricle which is called the median eminence. It represents the final point of convergence of pathways from the central nervous system on the peripheral nervous system. The median eminence is the crucial link between the brain and the anterior pituitary, a gland which secretes hormones into the blood in response to the hypothalamic hormones. The hypothalamic-hypophyseal portal system interconnects the hypothalamus and the anterior pituitary gland (adenohypophysis). Arterioles of the hypothalamus branch into capillaries that drain into small veins, which travel to the anterior pituitary, where they branch into another set of capillaries, signalling a release of hormones into the main blood stream.

The LH is connected to a wide variety of brain structures and is bounded medially by the mammillothalamic tract, and the anterior column of the fornix; the lateral boundary is the medial margin of the internal capsule and the subthalamic region. It is continuous with the lateral preoptic nucleus rostrally, and caudally it

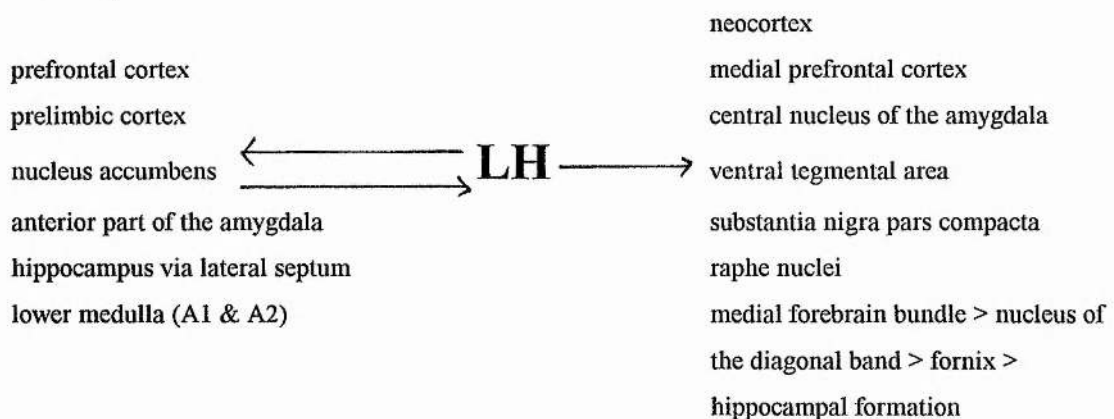
merges with the midbrain tegmentum. Throughout the LH, there are groups of large, dark staining cells which make up the LH nucleus, and surrounding these are small, pale multipolar cells encircled by a delicate fibre capsule known as the tuberal nuclei. In depth investigation of the LH connections reveals an intricate and complex network of afferents and efferents which unsurprisingly point to a role in the regulation of the internal environment, which is known to be its main function. Luiten, Ter Horst and Steffens (1987) carried out a detailed tract-tracer study of the intrinsic connections and outflow pathways of the endocrine system in relation to the control of feeding and metabolism in the hypothalamus. Most of the anatomical connections summarised below are taken from their work. They used the tracer horseradish peroxidase (HRP), HRP conjugated with wheat-germ agglutinin (WGA-HRP), ^3H -leucine, and the lectin *Phaseolus vulgaris* leuco-agglutinin (PHA-L). The tracer was injected using iontophoretic delivery methods in order to produce the smallest possible injection sites and minimise tissue damage. Due to the complexity of the LH connections, the description will be divided up into the following sections: (i) afferents to and efferents from the LH to extra hypothalamic brain regions; (ii) intrahypothalamic connections; and finally (iii) LH connections with the autonomic and neuroendocrine nervous systems.

(i) AFFERENTS TO AND EFFERENTS FROM THE LH CONNECTED TO EXTRA HYPOTHALAMIC BRAIN REGIONS

The retrograde tracer HRP injected into the LH resulted in the labelling of a large number of somata in the medial and orbital regions of the prefrontal cortex and especially the prelimbic cortex. The anterograde tracer PHA-L labelled both terminal and en-passant presynaptic boutons predominantly in the ventral part of the posterior LH. More importantly, a high number of cells were labelled in the NAS. The anterior part of the amygdala has limited afferents to the LH although these have been shown to play an important part in food intake (for example, Ono, Tamura, Nishijo, Nakamura

and Tabuchi, 1989). Hippocampal information enters the LH, in the majority, only via the lateral septal pathway. The hippocampal formation, by way of output from the ventral subiculum, may reach the LH via the lateral septum. Noradrenergic fibres originating in the lower medulla (A1 and A2), via the ventral noradrenergic bundle, have a moderate projection to the LH (Sawchenko and Swanson, 1981). This is important as Liebowitz (1978) has shown that noradrenergic stimulation of the paraventricular nucleus of the hypothalamus (PVN) elicits feeding. The vast majority of sites sending afferents to the LH receive efferents from the LH. Different areas of the cortex receive many efferents from the LH. Direct projections are found terminating in the neocortex and the medial prefrontal cortex, an area known to be involved in reinforcement. Routtenberg and Sloan (1972) discovered that electrical stimulation of the medial prefrontal cortex increased lever pressing for food reinforcement. The LH sends direct fibres to the central nucleus of the amygdala (Otterson, 1980) and indirect connections to the amygdaloid complex via the VTA and SNC, both areas in the tegmentum, and the medial and dorsal raphe nuclei located in the reticular formation (Ricardo, 1983). The medial forebrain bundle contains fibres from the LH which project rostrally to the nucleus of the diagonal band and to the medial septal nuclei, which in turn send fibres to the hippocampal formation via the fornix.

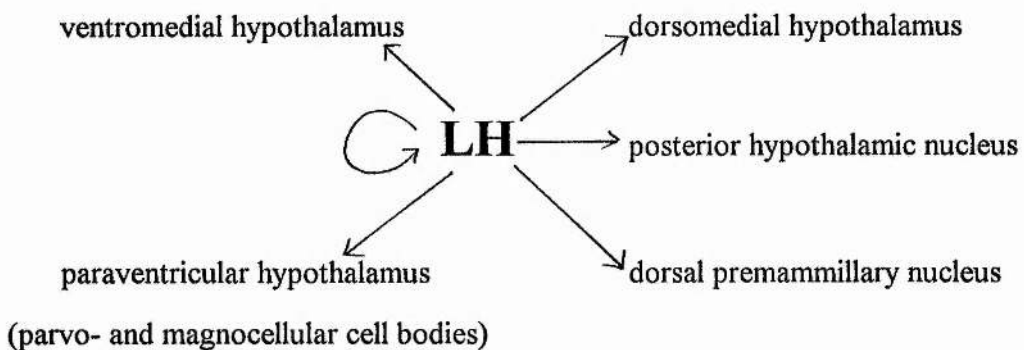
Figure 4: Major afferents to and efferents from the LH connected to extrahypothalamic brain regions.



(ii) INTRAHYPOTHALAMIC CONNECTIONS

The hypothalamus is involved in a wide variety of complex functions, and this is reflected by the neural organisation within the structure itself. Most of the intrahypothalamic output of the LH is aimed at other parts of the LH itself. There is a large information exchange between the cells that make up the LH. There are minor projections to the ventromedial hypothalamus (VMH) and the PVN from the medial and ventromedial aspects of the LH, whereas projections to the dorsomedial nucleus of the hypothalamus (DMH), posterior hypothalamic nucleus and dorsal preammillary nucleus are more extensive. In general, the connections of the LH indicate that this area is more of an output station to the autonomic nervous system (ANS) rather than specialising in intrahypothalamic information relay.

Figure 5: Major intrahypothalamic connections

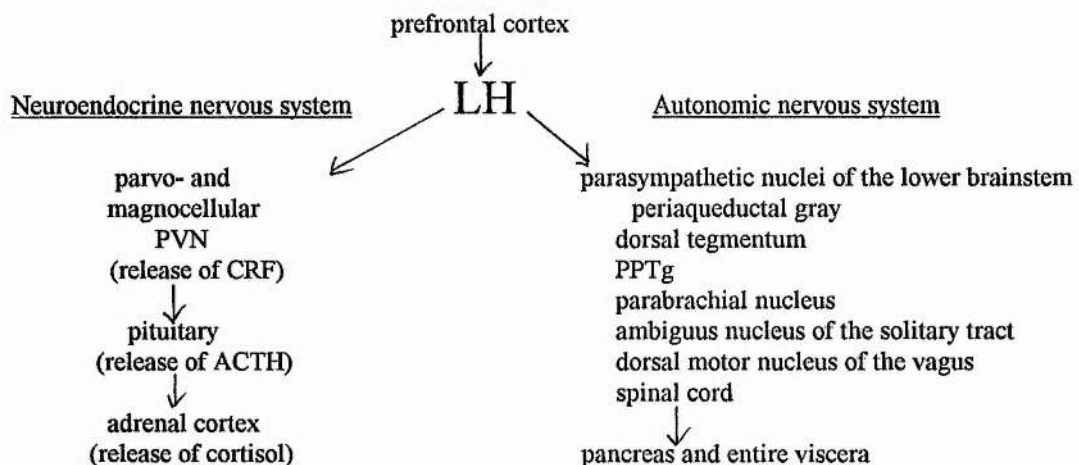


(iii) LH CONNECTIONS WITH THE AUTONOMIC AND NEUROENDOCRINE NERVOUS SYSTEMS

Corticotropin releasing factor (CRF) is produced by cells in the parvocellular PVN. It reaches the anterior pituitary via the hypophyseal portal vein, whereupon it stimulates the release of, amongst others, adrenocorticotrophic hormone (ACTH). This

stimulates the adrenal cortex to secrete cortisol, a hormone which regulates various aspects of organic metabolism. Thus, the hypothalamus plays an intricate role in the expression of motivational drives. The LH projects to both parvocellular and magnocellular PVN, and therefore provides a critical link between prefrontal cortex and the pituitary-adrenal complex. As well as output to the neuroendocrine system via the release of hormones into the blood supply, the hypothalamus can also influence endocrine target organs via the ANS. By far the most important target organ is the pancreas as it influences digestion and maintains and regulates fuel substrate homeostasis. Descending autonomic connections of the LH have direct projections to the parasympathetic nuclei of the lower brainstem. The main target sites are the periaqueductal gray and the adjacent dorsal tegmentum and more caudal, the PPTg and parabrachial nucleus. To a lesser extent, there are terminations in the ambiguous nucleus of the solitary tract. There is a fairly dense pattern of projections to the dorsal motor nucleus of the vagus, with fewer fibres continuing to the spinal cord. The dorsal motor vagus and ambiguous nuclei contain parasympathetic nerve cells that project to the pancreas and the entire viscera. The LH-lower brainstem connection is the main hypothalamic parasympathetic outflow pathway. The LH is, however, not exclusively parasympathetic. Connections with the PAG allows the LH to be in contact with the main sympathetic circuit via the main dorso- and ventromedial circuit.

Figure 6: LH connections with the autonomic and neuroendocrine nervous systems.



LH BEHAVIOURAL STUDIES

The control of food and water intake is both a complicated and delicately balanced bodily mechanism because so many features of the interstitial fluid must be regulated, for example, water concentration, levels of solutes and amounts of nutrients available. All these fluctuating levels trigger control mechanisms involving detection by receptors throughout the body, secretion of hormones, changes in neural activity, all resulting in changes in order to maintain homeostasis. In 1951, Anand and Brobeck discovered what is commonly known as the lateral hypothalamic syndrome. Rats given bilateral electrolytic lesions of the LH became aphagic, adipsic and lost body weight. These rats died unless they were maintained on a food supplement or fed via a tube into their stomachs. Teitelbaum and Epstein (1962) identified four stages of recovery of rats with lateral hypothalamic syndrome:

1. **Aphagia and Adipsia:** rat is motionless and will not drink water or eat even palatable food.
2. **Adipsia and Anorexia:** signs of recovery because rat begins to eat wet mash food, that is, baby food.
3. **Adipsia and Dehydration Aphagia:** rat starts to gain weight. Will eat dry food only if fill the rat full of water first.
4. **Recovery:** rats eat dry lab chow and drinks water as before. Food and water intake is low compared to controls. Rate of body weight gain is good.

Teitelbaum and Epstein (1962) carried out a variety of physiological challenges on recovered LH rats. (i) Rats were injected with hypertonic saline promoting intracellular dehydration. Control rats drank over 15ml of water in one hour in response to the injection, whereas LH-lesioned rats drank nothing in response. (ii) Polyethylene glycol was injected into the rats to promote extracellular dehydration. LH-lesioned rats did not drink in response, whereas controls increased their intake accordingly. (iii) The antimetabolic analogue of glucose, 2-deoxy-d-glucose, blocks conversion of glucose to simpler substrates. Normal rats injected with this were

stimulated to eat because they were in an apparent state of glucoprivation. LH-lesioned rats did not respond by eating.

There was a single shift in time course for responding to these challenges. Rats with electrolytic lesions failed to respond to the challenges immediately, but responded to some extent after 24 hours. Another feature that Teitelbaum and Epstein (1962) observed was also paradoxical. LH-lesioned rats showed finnickiness, that is, they rejected mildly quinine-adulterated food and water, and over consumed palatable foods.

In addition to feeding and drinking deficits, Marshall and Teitelbaum (1974) observed several sensorimotor deficits. Unilateral damage to the LH caused deficits only on the side contralateral to the lesion. Bilateral lesions caused bilateral sensorimotor impairments and also problems in killing mice. Orientation to olfactory, visual, tactile and proprioceptive stimuli were deficient. These deficits were seen to be partly responsible for the initial deficits in motivated behaviours, that is, feeding and mice killing. Marshall, Richardson and Teitelbaum (1974) also found akinesia, rigidity and limb impairments which contributed to the feeding deficits.

All in all, the classic lateral hypothalamic syndrome consists of feeding, drinking and sensorimotor impairments. Several explanations for the syndrome have been put forward, and these are discussed in the next section.

EXPLANATIONS FOR THE CLASSIC LH SYNDROME

Stellar (1954) introduced the concept of a physiological mechanism believed to be in control of motivated behaviour. His basic assumption was that "the amount of motivated behaviour is a direct function of the amount of activity in certain excitatory centres of the hypothalamus". He proposed that the LH was the hunger centre, and bilateral electrolytic lesions destroyed this so that the rats could no longer perceive hunger. The satiety centre located in the VMH would then be uninhibited. This concept was discounted because the rats do not behave as if satiated. For example, they over

consume palatable foods. The brain is not organised into discrete centres that control specific functions, rather, individual functions are performed by neural circuits distributed among several structures of the brain.

Another theory that has come up against opposition is the idea that there are body weight set point mechanisms (Powley and Keeseey, 1970). Lesioning the LH alters this set point to a lower level and animals must adjust their food intake accordingly. It could, however, be that the animal had a new food intake level and their body weight fell to fit it. This theory again cannot explain finnickiness or the sensorimotor deficits.

Marshall and Teitelbaum (1974) proposed that many of the sensorimotor dysfunctions they discovered after electrolytic LH lesions had a role to play in the feeding disorders. They could not explain how the sensorimotor loss accounted for the finnickiness, so they put forward the proposal that the deficits were probably only a major cause of stage 1 (aphagia and adipsia), and the mice killing deficits.

The above theories fail to explain all the deficits found in the classic lateral hypothalamic syndrome and the exact function of the LH in relation to motivated behaviours. In fact, the entire classic LH syndrome was challenged in 1971 by Ungerstedt. He claimed that destruction of the nigrostriatal DA pathway, which passes through the far lateral hypothalamus, by 6-OHDA produced similar aphagia, adipsia, loss of body weight and an inability to initiate movement, that is, essentially the LH syndrome. This pathway originates in the SNC and terminates in the CP and it is this fibre bundle that degenerates in Parkinson's disease. He claimed that the electrolytic lesions of the LH also destroyed these fibres of passage, the real site responsible for the LH syndrome. Teitelbaum and Epstein (1962) showed that animals with lesioned nigrostriatal pathways recovered by the same stages as rats with electrolytic LH lesions, thus supporting Ungerstedt's claim that it was the destruction of passing DA fibres rather than the LH lesion itself that caused the classic LH syndrome. Marshall, Richardson and Teitelbaum (1974), however, carried out an experiment showing that there were subtle differences between the LH-lesioned and nigrostriatal pathway

lesioned rats. Despite striking similarities, only rats with fibre pathway 6-OHDA lesions ate small quantities of food when sufficiently activated, they were also less finicky and showed greater sensorimotor impairments. Therefore, the two syndromes were not the same, as Ungerstedt had proposed. Rolls et al. (1976) and Ono et al. (1989) found cells in the hypothalamus that responded to food and food-associated stimuli showing that the LH did indeed have a role to play in perception of food. It has also been shown that the classic LH syndrome cannot be accounted for completely by loss of DA neurones from the nigrostriatal bundle. Levels of forebrain DA were measured by *in vivo* sampling techniques (Robbins and Everitt, 1987). Electrolytic LH lesions produced around 60% DA depletion in forebrain sites, whereas a depletion of around 95% was found to be necessary to produce the effects of DA fibre selective lesions, which left the LH unharmed. These studies showed that contrary to what Ungerstedt claimed, lesions of the nigrostriatal DA pathway do not produce the same syndrome as electrolytic lesions of the LH.

In order to lesion only the cell bodies of the LH while keeping the passing fibre pathways intact, excitotoxins were introduced in the late 1970's. These promote uncontrolled influx of calcium into cells, which in turn stimulates enzymes to destroy the cells. Dunnett, Lane and Winn (1985) injected the excitotoxin ibotenate into the LH and found initial hypophagia, hypodipsia, loss of body weight, failure to respond to physiological challenges but no finickiness. Unilateral lesions of the LH and nigrostriatal bundle were compared. Rats with unilateral 6-OHDA nigrostriatal pathway lesions failed to respond to a contralateral stimulus, showing a motor deficit. On the other hand, ibotenate lesioned LH rats did respond to a contralateral stimulus, therefore showing that they had no motor problems, but they had a motivational deficit. This clearly showed a dissociation in the LH syndrome. Why do rats with LH lesions recover food and water intake but fail to respond to regulatory challenges? Winn, Clark, Hastings, Clark, Latimer, Rugg and Brownlee (1990) gave both controls and LH-lesioned rats hypertonic saline. After one week of recovery, the LH rats drank 1ml water in response to the dehydration whereas controls drank 18ml. When given

water with 0.2% saccharin, all rats drank the same amount which demonstrated that the rats' perception of taste of the incentive quality of its diet was unimpaired. There were no motor deficits to prevent the LH-lesioned rats from drinking. The LH-lesioned rats had normal neuroendocrine responses so the deficit was not a physiological problem. Both control and LH-lesioned rats were able to decrease their intake of dry lab chow when given glucose, showing they could detect their own physiological state. LH-lesioned rats were also able to react appropriately to salt adulteration, and discriminate between water and saline. Thus it seems that the LH-lesioned rats have a motivational deficit which is not a product of their physiology.

A recent theory to account for deficits after LH lesions has been proposed by Clark et al. (1991a&b). The LH contains neurones that project indirectly to the cerebral cortex (Saper, 1985), and it is thought that it is disruption of these that explains the behavioural deficits resulting from LH lesions. LH neurones normally have an inhibitory function which suppresses inappropriate and aids selection of appropriate behaviour. Clark et al. (1992) found that the acquisition of eating in response to tail pinch was greatly enhanced in LH-lesioned rats, that is, they ate on earlier test sessions than controls, and did not need as much tail stimulation as controls. This seems to agree with the theory. Tail pinch eating has no function for the rat, and hence the LH neurones usually inhibit the action. Further evidence has come from an experiment by Winn et al. (1992). Schedule-induced polydipsia (SIP) occurs when hungry rats are given food at intervals with water continually available. Between these intervals, rats will drink in excess of normal amounts necessary to maintain homeostasis. LH-lesioned rats acquired SIP significantly more quickly than controls only in the first six sessions. Again, SIP is of no functional use to the rat, and the LH neurones which usually inhibit this action, have been lesioned so the inappropriate behaviours are left intact. The inability of LH-lesioned rats to respond to hypertonic saline could also fit into this theory. Administration of hypertonic saline induces drinking in normal rats, so absence of drinking is an inappropriate behaviour. As the intact LH neurones suppress

inappropriate behaviours, that is, not drinking in response to hypertonic saline, then by lesioning the LH, the suppression is abolished.

As yet, nothing has been discussed concerning the LH and its effect on motivational strength. It is clear that the LH has an important role to play in motivation as lesions produce certain deficits which are not a product of physiological impairments or motor dysfunction. The following experiments aim to address the question of motivational strength by using the progressive ratio paradigm. Lesions of the LH may produce deficits in the amount of work a rat will do to receive reward. As well as this, food and water intake and body weight will be studied, together with two physiological challenges: injection of hypertonic saline and prandial drinking. The results of these experiments will help to clarify the role the LH has to play in behaviour.

PROGRESSIVE RATIO

Hodos (1961) was the first author to use the progressive ratio (PR) paradigm as a measure of reward strength, initiating an area of research that is continuing from strength to strength to the present day. A typical PR schedule involves "a systematic increase in the value of the fixed-ratio (FR) requirement following each reinforcer delivery, requiring the subject to exert progressively more effort (for example, increased lever presses) to obtain successive reinforcers" (Skjoldager, Pierre and Mittleman, 1993). At some point in the PR schedule, the subject decreases the frequency of its behaviour, eventually leading to a complete cessation. At this time, the subject's energy expenditure outweighs the value of the reinforcement. Hodos (1961) termed this point the "Breaking Point" (BP), and it was thought to reflect the measure of reinforcer effectiveness, that is, the maximum amount of energy a subject was prepared to expend in order to receive a reinforcer. Hodos (1961) found that on a PR schedule with an increment of two (PR2), rats working for condensed milk pressed

the lever up to 70 times. As the concentration of condensed milk decreased, so did the number of presses on the lever during the final ratio.

This pioneering work was soon followed by a study by Hodos and Kalman (1963) investigating the effect of increment size and reinforcer volume on PR performance. They found that the number of responses in the final ratio increased as a function of the size of the ratio increment. Both studies showed that the number of responses in the final ratio was sensitive to manipulation of a number of motivational variables, including the degree of food deprivation, concentration of the reinforcer or the volume of the reinforcer. Baron, Mikorski and Schlund (1992) investigated the effect of reinforcement magnitude on post reinforcement pausing on a PR schedule. They put forward two hypotheses: (i) that pausing was controlled by excitatory properties of the reinforcer to come, and so a larger reinforcer should produce a shorter pause, (ii) that pausing may reflect inhibitory aftereffects of the previous reinforcer and so an increase in its size would increase pause duration. They found that pausing was longer following a large reinforcer, increased as the ratio increment increased and were reduced by a stimulus signalling a large up-coming reinforcer. Thus, they concluded that pausing was under control of aspects of both the inhibitory properties of the past reinforcer and excitatory properties of stimuli correlated with the up-coming reinforcer. One of the most recent and in-depth experiments carried out on progressive ratio schedules was by Skjoldager, Pierre and Mittleman (1993). They discovered that reinforcer magnitude did not affect lever press acquisition, but rats given three pellets, as opposed to one pellet had higher BPs and shorter response durations. Overall, rats receiving three pellets had a more efficient response style. They also investigated whether rats given larger reinforcers were more resistant to alterations in controlling conditions. Increasing the lever height reduced BPs and disrupted the temporal pattern of responding, that is, lengthened post reinforcement pauses, response durations and time taken to collect the food. Rats receiving one pellet reinforcement were affected more as shown by lower BPs and time to collect the food. After continual exposure to the lever height manipulation, the three pellet rats were

less influenced by the effort changes and engaged a more efficient response strategy. Finally, to further assess the effects of reinforcer magnitude on resistance to change, they tested rats on the effects of extinction. They found that extinction reduced BPs in both groups, however, the three pellet group had a greater decrease. They concluded from the series of experiments that larger magnitudes of reinforcement increased the resistance to effort, but not to extinction, and they enhanced the development of a more efficient method of responding on a lever. These papers investigating the effect on responding for natural rewards show that PR performance is a useful method of measuring rewarding parameters, such as the effect on time to respond, the rate of responding and the extent to which a subject will respond, that is, motivational strength.

Since PR performance correlates well with reward parameters, it is not surprising that the PR paradigm has been used to evaluate the relative rewarding properties of drugs of abuse. Roberts, Loh and Vickers (1989) investigated intravenous cocaine self-administration behaviour using a PR schedule. They found that BPs were relatively stable over all the sessions, with higher doses of cocaine producing higher BPs. On administration of the DA antagonist haloperidol, the authors found an increase in injection rate and a decrease in BP. They concluded that the rats were able to compensate for the DA depletion by increasing their drug intake, but this was lost at higher ratios. Thus, the PR schedule provided essential information about the strength of responding for drug reinforcement. Robledo and Koob (1993) investigated ibotenate lesions of the subcommisural ventral pallidum (SVP) and the sublenticular region of the extended amygdala (SEA) on responding for cocaine on a FR and PR schedule. Rats with both SVP and SEA lesions decreased responding on a FR5 schedule for intravenous cocaine. When tested on the PR schedule, differences in performances by the lesioned rats were found. Lesions of the SVP did not produce significant changes in the BPs of rats compared to controls. On the other hand, rats with SEA lesions displayed a significant decrease in BP. These results showed that by carrying out a PR schedule, differences in performances by the lesioned rats were

found which were not identified using the FR paradigm. PR can also be used to investigate the effects of aversive stimuli. Shaham and Stewart (1994) looked into the effect of mild footshock on intravenous heroin self-administration. Rats were exposed to ten minutes of intermittent footshock before daily sessions of heroin self-administration. No group differences were found in heroin-reinforced behaviour acquisition. When rats were put on a PR schedule, those previously given footshock had higher rates of lever pressing and higher BPs. They concluded that exposure to mild stress enhanced the reinforcing results of heroin.

Winger and Woods (1985) carried out a study comparing FR and PR schedules on monkey self-administration of the drugs cocaine and nomifensine. Monkeys maintained self-injection of both drugs on a FR30 schedule, with nomifensine appearing slightly more potent overall. With the PR schedule, both drugs maintained high rates of responding and maximum BPs at a dose of 0.32mg/kg per injection. On the FR schedule, the highest doses of both drugs produced less rapid rates of responding compared to the lower doses. On the PR schedule, the BPs did not rise as rapidly with increasing doses as did the FR rates, and the decrease in rates at the highest dose was not as marked. The authors concluded that the two schedules produced similar effects and were possibly controlled by similar processes. The apparent differences found were seen to be due to temporal discrepancies in the schedules themselves caused by, for example, different drug half lives. The difference found between the schedules by Robledo and Koob (1993) could have been task specific, as the general consensus is that FR and PR schedules provide similar information about the relative reinforcing efficacy of differing drug doses.

As already mentioned in the previous chapters, there are several brain areas that are heavily implicated in rewarding mechanisms, the most cited being the NAS. The two efferent target structures important to this thesis are the PPTg and the LH. Both structures have clear motivational roles, and yet they seem to display different aspects of these motivated behaviours. It is important to discover whether these structures have a role to play in motivational strength when a rat is working for a reward, as this

aspect of motivated behaviour has not yet been investigated. The strength of motivation will be measured using the PR paradigm, as the BP is a relatively straightforward construct to interpret.

SUMMARY

1. The PPTg is a possible outflow station for NAS mediated behaviour, a structure clearly involved in incentive-driven behaviours.
2. Lesions of the PPTg affect certain specific aspects of motivated behaviours.
3. The LH is intimately connected to the neuroendocrine system and the autonomic nervous system via numerous neural pathways.
4. The LH receives heavy input from the NAS shell.
5. Lesions of the LH affect very specific aspects of motivated behaviours.
6. By lesioning the PPTg and LH, motivational strength will be investigated to discover whether these structures are involved in its expression since they both seem to be involved in certain aspects of motivated behaviour.

METHODS

EXPERIMENT 1 - THE EFFECT OF PPTg LESIONS ON RESPONDING TO A PROGRESSIVE RATIO SCHEDULE

Animals

Twenty male hooded Listar rats were used. Prior to surgery, all rats had been trained for one month to respond in an operant chamber on both fixed and progressive ratio schedules. Animals were housed individually in cages in a room kept at 20-22°C, with lights on 7am-7pm. Two weeks after surgery, rats were food deprived by allowing them only 18g of food per day - an amount found to maintain the animal above its 85% pre-operative body weight and allow it to gain weight with a small but steady increase. Tap water was available *ad libitum*. Mean body weight at the time of surgery was 412.2g (range 368.0 to 460.7g).

Surgery

The rats were divided into 3 groups: PPTg quinolinate lesions (n=7), PPTg ibotenate lesions (n=7) and PPTg phosphate buffer controls (n=6). Rats undergoing ibotenate lesions were anaesthetised with 1ml/kg "Sagatal" (May and Baker; sodium pentobarbitone, 60mg/ml), whereas rats undergoing quinolinate excitotoxic lesions were anaesthetised with 10ml/kg Avertin (10g tribromoethanol/5g tertiary amyl-alcohol; 10ml of this concentrate then dissolved in 40ml ethanol and 450ml 0.9% saline/0.01M phosphate buffer; pH 7.2-7.4). The majority of these rats needed a top up of approximately 0.4ml Avertin to ensure deep anaesthesia. Once completely anaesthetised, rats were placed in the stereotaxic frame.

Infusions of ibotenate (Cambridge Research Biochemicals, Cambridge, UK, made up as 0.12M solution in sterile phosphate buffer (pH 7.4); final pH adjusted to pH 7.00 using 2M NaOH) were delivered in a volume of 0.2µl (24nmol) to each site in 0.02µl steps at 10 second intervals (100 seconds). Infusions of quinolinate (Sigma, St Louis, MO, made up as 0.12M solution in sterile phosphate buffer (pH 7.4); final pH

adjusted to pH 7.4 using 2M NaOH) were delivered in a volume of 0.2 μ l (24 nmol) to each site in 0.02 μ l steps at 10 second intervals (100 seconds). Needles were left *in situ* for 300 seconds to allow for diffusion. Control rats received the same volume of phosphate buffer delivered at the same rate as the excitotoxins. All injections were made using a 1 μ l syringe mounted on a stereotaxic frame and care was taken to ensure that the needle bevel was pointing forwards. Two injections were made in each hemisphere at the following stereotaxic co-ordinates:- 0.8mm anterior to the interaural line, \pm 1.6mm from midline and 7.0mm below skull surface (posterior PPTg); and 1.5mm anterior to the interaural line, \pm 1.7mm from midline and 7.8mm below skull surface (anterior PPTg): all with level skull - incisor bar set at -3.3mm. For bilateral lesions, rats had 2 separate unilateral operations separated by 24 hours, as previous experience has shown that bilateral ibotenate infusions in the same operation proved fatal. On removal from the stereotaxic frame at the end of each operation, all rats were immediately injected i.p. with 5ml 6% glucose/saline in order to reduce the gastrointestinal irritant effects of the anaesthetic (this is necessary with Avertin, but was used in all cases regardless). Two rats failed to respond to the Sagatal on the first day of surgery, so were given bilateral phosphate buffer injections on the second day. Rats were carefully observed during the post operative period as barrel rolling and respiratory difficulties are side effects of excitotoxins. One ibotenate rat died after the second day of surgery.

Rats were allowed 20 days after surgery before training was resumed. Wet mash was available for those rats whose body weights fell below 85% of their pre-operative measures - 2 ibotenate lesioned rats. All rats, however, recovered sufficiently well and began training as planned.

Apparatus

Four operant chambers (Campden Instruments Ltd.) measuring 25cm across, 26cm high, and 23cm deep were used. Three walls and the roof were made from steel and the floor was a grid with a tray of sawdust below. One wall was made from hinged

plexiglass and opened to allow rat entry. On the left hand wall of the chamber, 2 levers were located 10.5cm apart and 6cm from the grid floor. Each lever was 4cm wide and extended 2cm from the chamber wall. The reinforced lever was the lever positioned to the left of the panel. The food hopper was located in the centre of the 2 levers, separated from each by 2.5cm. The panel allowing entry to the food hopper was made of plexiglass hinged at the top, and was 5.5cm wide by 6cm high. Whenever the reinforced lever was pressed, a light located in the centre of the roof was switched off, and a light in the food hopper was illuminated to indicate reinforcement. A pellet dispenser delivered 45mg dustless precision sucrose pellets (rodent grain-base formula, Campden Instruments Ltd.) into the food hopper upon reinforced lever pressing. Each experimental operant chamber was enclosed with a sound proof box. An extractor fan was also used to give some background noise and provide continuous airflow. Data were collected on a Spider real - time processor (Paul Fray Ltd.).

Procedure

Rats were randomly allocated into 4 groups and tested on alternate days. Three groups were run on one day and one group the next.

Pre surgery training: Over a period of 30 days before surgery, all 20 rats were trained to respond in an operant chamber to various reinforcement schedules. On the first 3 days of training, rats were initially rewarded on a schedule of continuous reinforcement (CR). Thereafter, the rats responded to a progressive ratio schedule of 1 (PR1) - the number of lever presses to obtain a reward was increased by 1 after every pellet delivery - 1, 2, 3, 4 and so on. Over the next 10 training days, the initial CR schedule was gradually decreased from 20 trials to 1 trial, with a PR schedule of 1 always following each CR session. The final 2 days of training pre-surgery were both on a CR schedule of 1 followed by a PR schedule of 2 on the first day and a PR schedule of 3 on the final day.

Post surgery training: Food deprivation was started 2 weeks after surgery, and training was initiated 6 days later. On the first 2 days of training, rats were put on CR

for 30 trials, followed by a PR1 schedule. On the 3rd day of training, rats were put on a CR for 10 trials and thereafter PR1. Day 4 to 8, a single reinforced trial was followed by a PR1 schedule.

Testing: Rats were tested on alternate days for a total of 6 days. One CR trial was followed by a PR5 schedule. The breaking point was established and the test session terminated when the rat did not press the lever for 5 min. If rats had not finished responding within 4 hr they were returned to their home cages, and their breaking point noted as the number of lever presses they had reached in the allocated time. After 2 days of testing on PR5, one quinolinate lesioned rat was dropped because of continual failure to respond in the operant chamber.

The following data were collected for every reinforced lever press: reward - number of pellets earned so far in session; lever 1 - number of presses on reinforced lever; lever 1+ - number of presses on reinforced lever after pellet dispersed; lever 2 - number of non-reinforced lever presses; panel - number of panel pushes; latency 1 - time to collect pellet (1/10 sec); latency 2 - time to resume pressing the reinforced lever after pellet collected (1/10 sec).

Statistical Analysis

Data were analysed by analysis of variance (ANOVA) using the SPSS for Windows statistical package and graphs were produced using the Sigma Plot for Windows graphics package.

Histological Analysis

At the end of behavioural testing, rats were humanely sacrificed by an overdose of barbiturate (Euthatal, May and Baker; sodium pentobarbitone, 200mg/ml) and perfused transcardially with phosphate buffered saline followed by 4% paraformaldehyde in 0.1M phosphate buffer. Brains were removed and stored in paraformaldehyde prior to histological analysis. Sections of 40µm were cut on a freezing microtome and processed for immunohistochemistry - nicotinamide adenine

dinucleotide phosphate (NADPH) diaphorase. Cresyl Violet sections were also made in order to map out lesion volumes and assess damage. Individual sections were examined using a "Leitz" Diaplan microscope fitted with a Sony DXC-3000P video camera for visualisation of sections on a high resolution colour monitor.

RESULTS

EXPERIMENT 1 - THE EFFECT OF PPTg LESIONS ON RESPONDING TO A PROGRESSIVE RATIO SCHEDULE

Histological Analysis

Following recovery from the anaesthetic, rats which had been given either ibotenate or quinolinate, were observed barrel rolling at a high frequency and had a high respiration rate. These behavioural signs were seen as a normal reaction to administration of an excitotoxin. Post mortem assessment of lesion volumes showed all rats to have their lesion in the correct place. Table 1 shows the average lesion volume and the modal damage scores in structures adjacent to the PPTg after either ibotenate or quinolinate lesions. The overall average lesion volume value disguises an important point: there is a large difference in average lesion size between the two excitotoxins used. Ibotenate produced an average lesion volume of 3.43mm^3 , whereas quinolinate produced an average lesion volume of 2.36mm^3 . Figure 7 shows the largest and smallest lesions, the largest being an ibotenate and the smallest being a quinolinate lesion. All lesions showed moderate damage in the microcellular tegmental nucleus, retrorubral field, retrorubral nucleus and the paratrochlear glial substance. The larger lesions caused extensive damage to the superior cerebellar peduncle, rubrospinal tract and the cuneiform nucleus. The areas most damaged were the PPTg (60-90%) and the various parabrachial nuclei (30-60%). Damage to the PPTg was greatest from the central core through to the posterior pole to the level of the motor trigeminal nuclei. Cell loss was least at the anterior pole.

The number of healthy cholinergic neurones remaining after lesions were investigated using ANOVA. A significant difference was revealed between the three lesioned groups ($F[1,33] = 22.97, p=0$). Tukey post-hoc testing showed this difference to be between the sham and quinolinate lesioned groups at the 0.05 level of significance, and between sham and ibotenate groups at the 0.01 level of significance. Ibotenate lesioned rats had the least number of diaphorase positive cells, and the sham

lesioned group had the most. A significant difference was also found between the groups in the nissl cell counts, representing remaining healthy neurones after the lesioning procedure ($F[1,33] = 22.97, p=0$). Tukey post-hoc testing revealed differences between all the groups at the 0.01 level of significance, with the ibotenate group having the least number of nissl cells and the sham group having the most.

Table 1. Summary of damage and lesion volumes

Damage assessment:

(modal scores)

Cuneiform nucleus	ND
Deep mesencephalic nucleus	ND
Laterodorsal tegmental nucleus	ND
Mesencephalic trigeminal nuclei	ND
Microcellular tegmental nucleus	X
Motor trigeminal nuclei	ND
Parabrachial nuclei	XX
Pedunculopontine tegmental nucleus	XXX
Paracochlear glial substance	XX
Retrorubral field	X
Retrorubral nuclei	XX
Rubrospinal tract	X
Subpeduncular tegmental nucleus	ND
Substantia nigra pars compacta	ND
Substantia nigra zona reticulata	ND
Superior cerebellar peduncle	XX
Supratrigeminal nucleus	ND
Lesion volume (mm ³):	3.01
(mean ± S.E. value)	+0.22

A summary of damage and average lesion volumes computed from the Cresyl Violet sections for each group. Damage was identified by glial infiltration and degeneration of neuronal somata, and a summary of affected structures for every animal estimated. The modal score in the group was then taken for each structure. The key is as follows: ND, no damage; X, < 30%; XX, 30-60%; XXX, 60-90%; XXXX, > 90%.

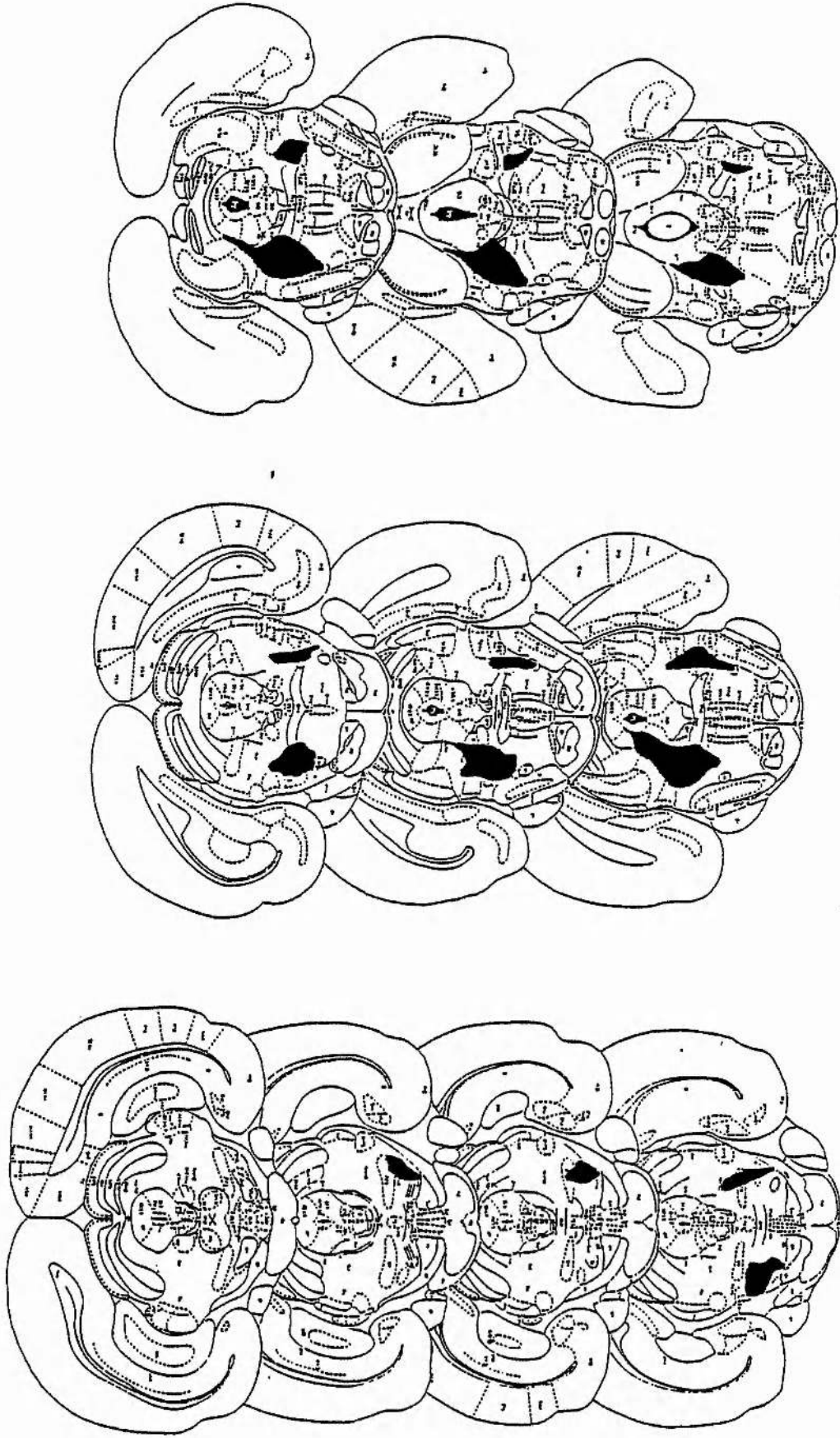


Figure 7. Representative sections re-drawn from the atlas of Paxinos and Watson illustrating the largest and smallest PPTg lesion sizes.

Progressive ratio

Figure 8 shows the variance of breaking points for each rat over the 6 test days. The median values and standard deviations are also shown. ANOVA revealed a significant difference between the groups ($F[2,17] = 5.27, p < 0.02$) and Newman-Keuls post-hoc testing showed this difference to be between the ibotenate and the quinolinate lesioned groups at the 0.05 significance level. Figure 9 shows the mean breaking points for each group (ibotenate=38.3, quinolinate=81.8, sham=59.6) and clearly indicates a significant difference between the ibotenate and quinolinate lesioned groups.

For the remaining data analysis, one ibotenate rat was dropped because it only had data up to breaking point 10. All analysis was carried out on data up to breaking point 35. The length of time taken to resume pressing the reinforced lever after collecting a reward was analysed using ANOVA. No significant group effect ($F[2,14] = 0.98$) or group x PRI interaction ($F[12,84] = 0.50$) was found, but there was a highly significant PRI effect revealed ($F[6,84] = 4.91, p = 0$). Thus, as shown in figure 10, as the PRI increases, so does the latency to resume pressing the reinforced lever. It is also interesting to note that the differences in latencies *between* the groups also increases as the PRI increases. The latency to collect pellets from the hopper was analysed using ANOVA. No group effect ($F[2,14] = 0.64$), PRI effect ($F[6,84] = 0.59$) or group x PRI interaction were found, and figure 11 shows the lack of any relationship. ANOVA of the number of presses on the non-contingent lever revealed no group effect ($F[2,14] = 3.38$). There was, however, a highly significant PRI effect ($F[6,84] = 6.34, p = 0$) and a significant group x PRI interaction ($F[12,84] = 1.98, p < 0.04$). This significant interaction indicates that as the PRI increases, the groups also differ significantly in the number of non-contingent lever presses. By looking at figure 12, there is virtually no difference between the 3 groups at PRI of 5 and 10. From PRI 15 onwards, the ibotenate lesioned group press the non-contingent lever increasingly more often than both the quinolinate and sham lesioned groups. By PRI 35, the ibotenate lesioned group are pressing, on average, over 8 times, whereas the quinolinate and sham lesioned groups are pressing less than 2 times between rewards. ANOVA carried out

on pushes on the food magazine panel revealed a significant group effect ($F[2,14] = 7.28, p < 0.008$) and figure 13 shows that the ibotenate lesioned rats pushed the panel more times than the quinolinate and sham lesioned groups. It is a very similar pattern to that of the non-contingent lever presses shown in figure 12. There was, however, no group \times PRI interaction ($F[12,84] = 1.73$) but a highly significant PRI effect ($F[6,84] = 7.39, p = 0$) showing that as the PRI increased, so did the number of panel pushes.

Figure 8: Graph of PPTg lesioned and control rats' breaking points (indicating variance, median and standard deviation).

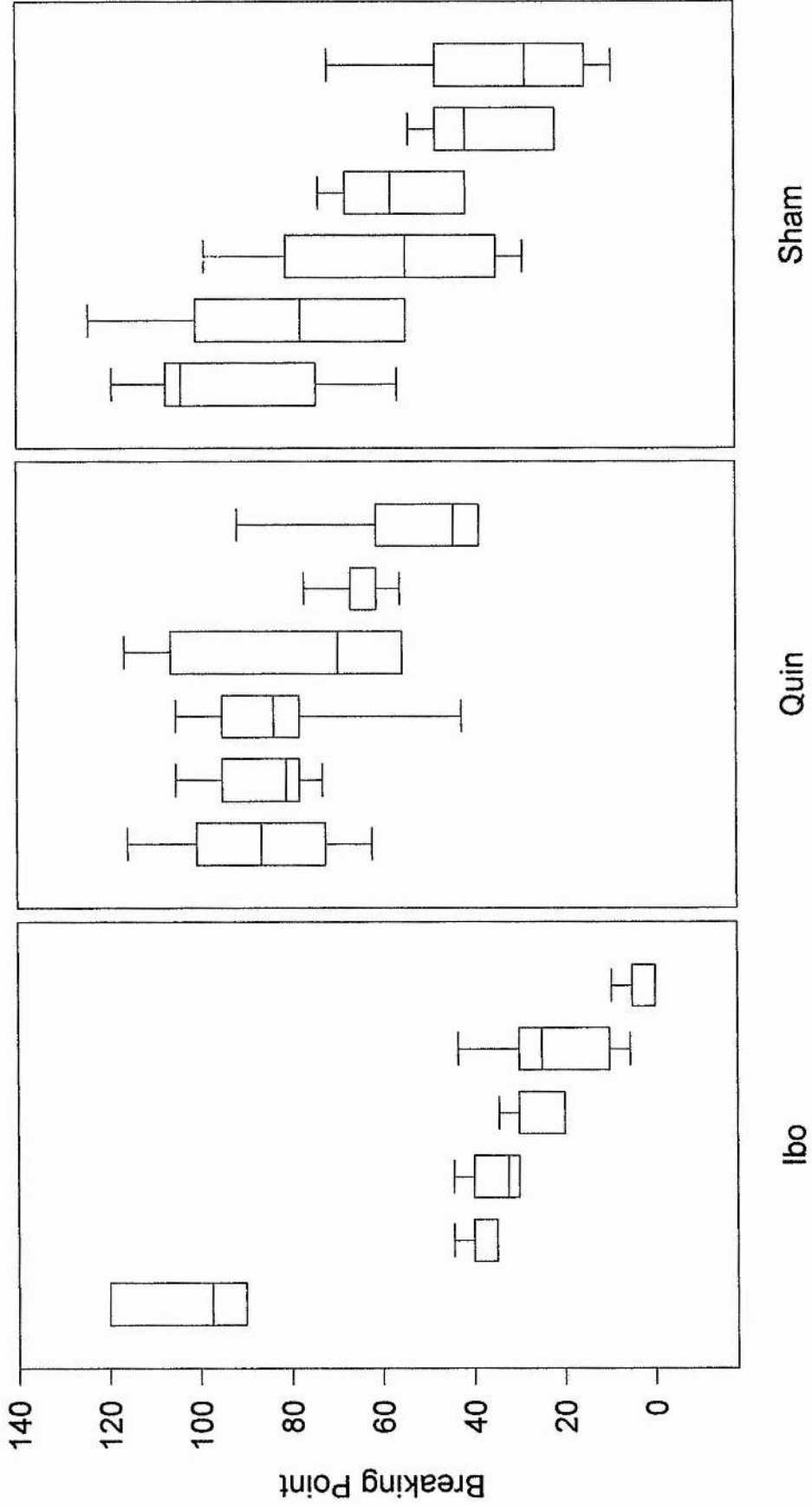


Figure 9: Graph of mean breaking points for each group.

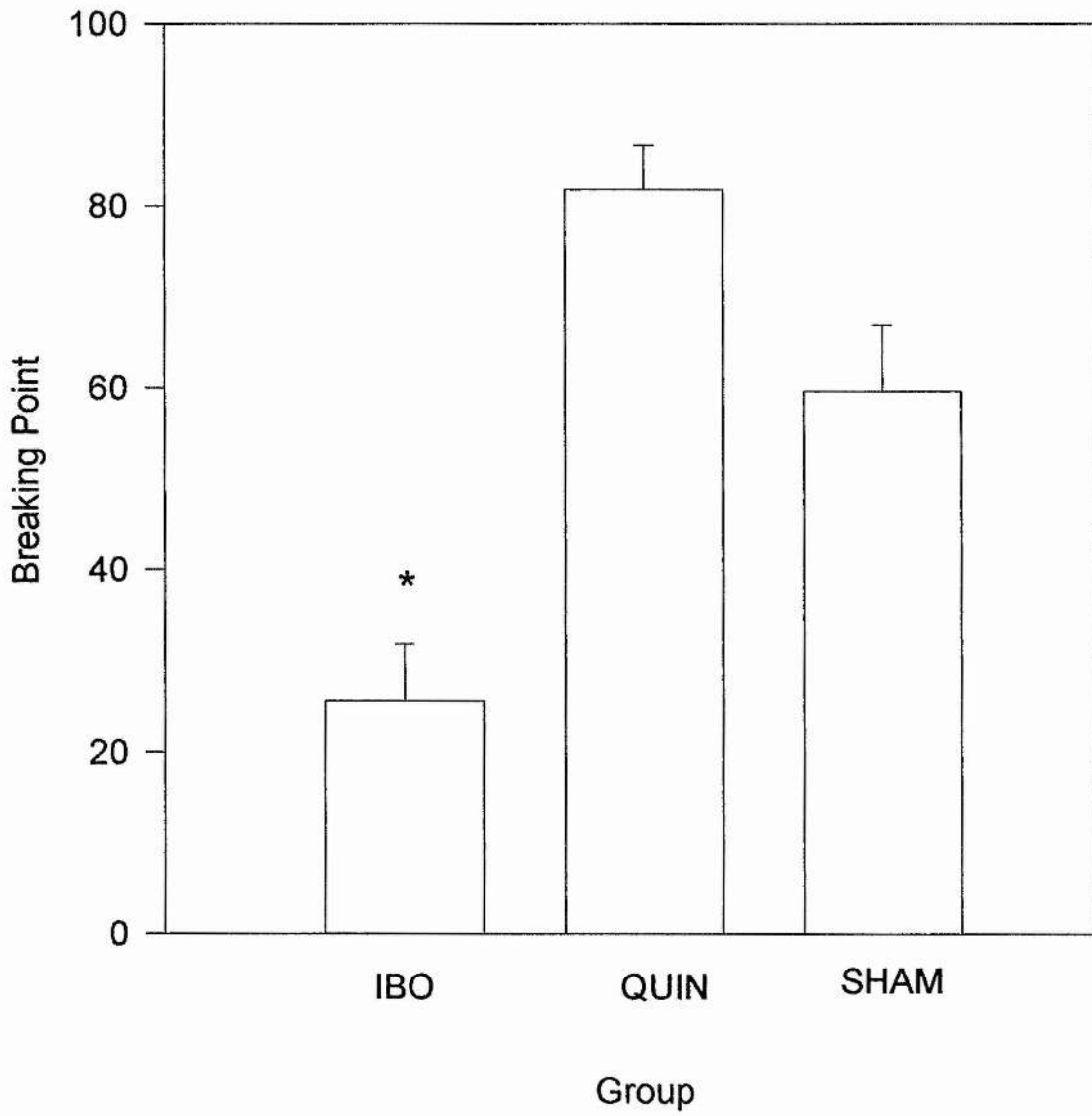


Figure 10: Graph of PPTg lesioned and control rats' latencies to resume pressing the non-contingent lever.

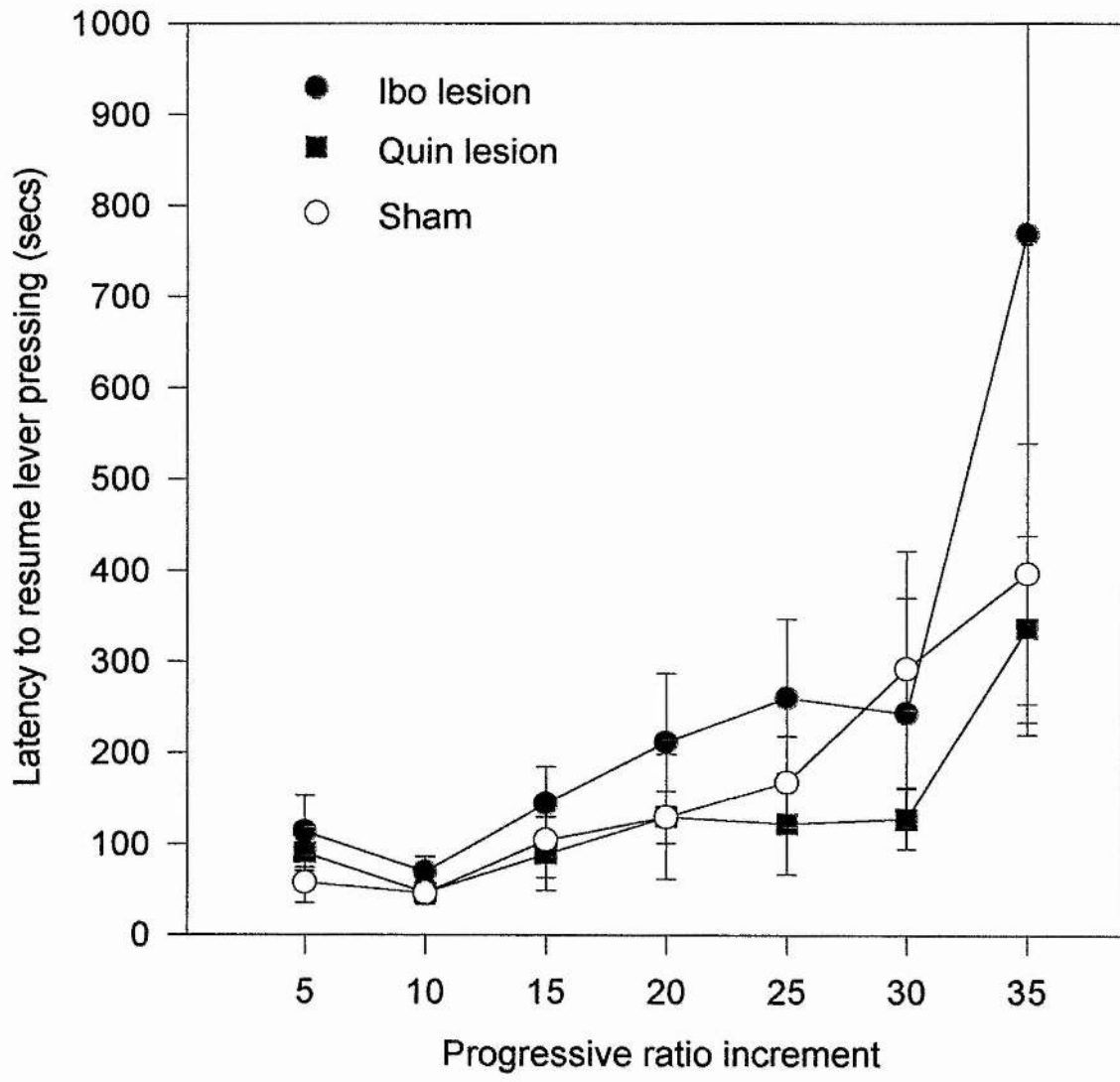


Figure 11: Graph of PPTg lesioned and control rats' latency to collect pellets.

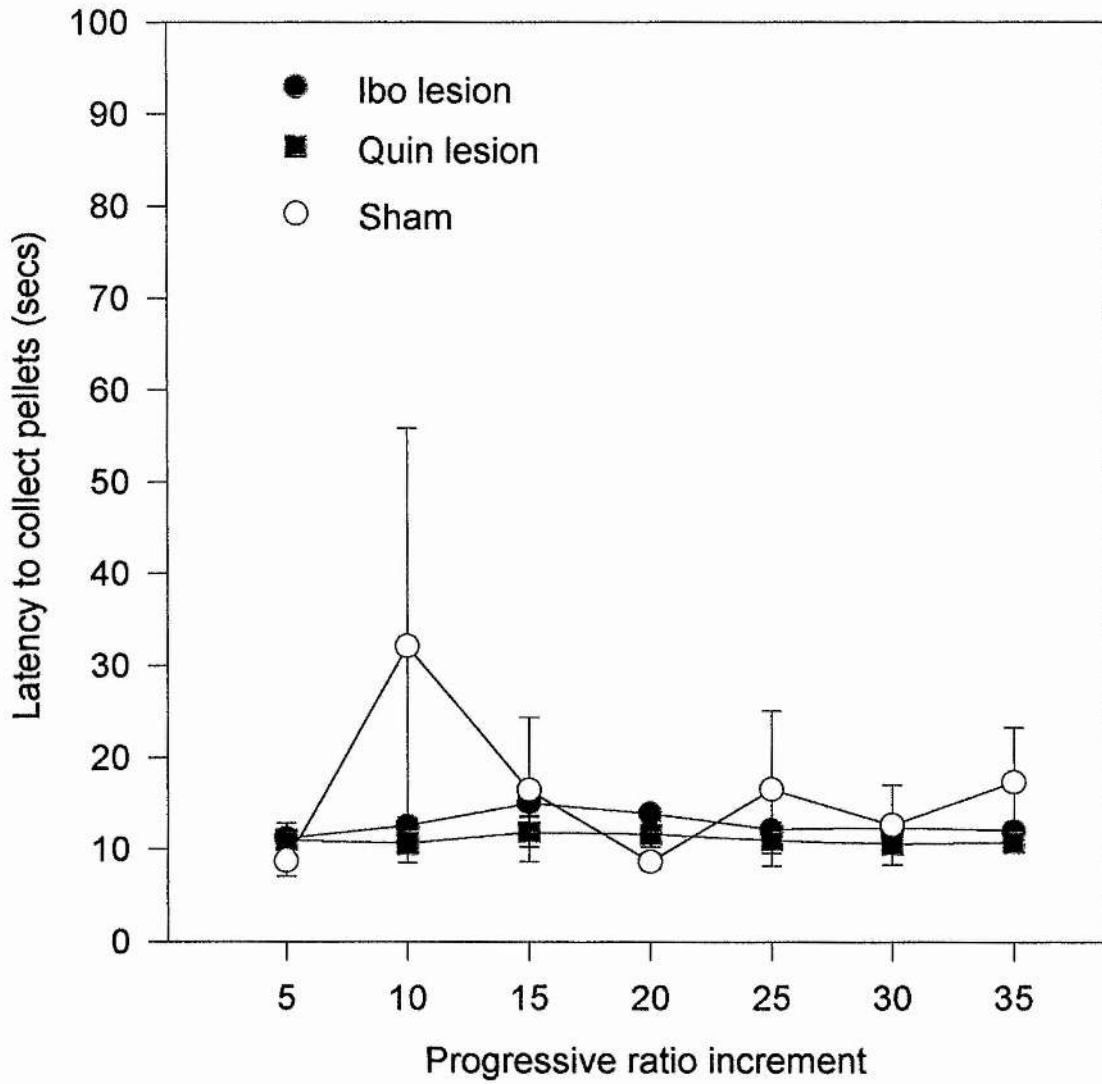


Figure 12: Graph of number of non-contingent lever presses for both PPTg lesioned and control rats.

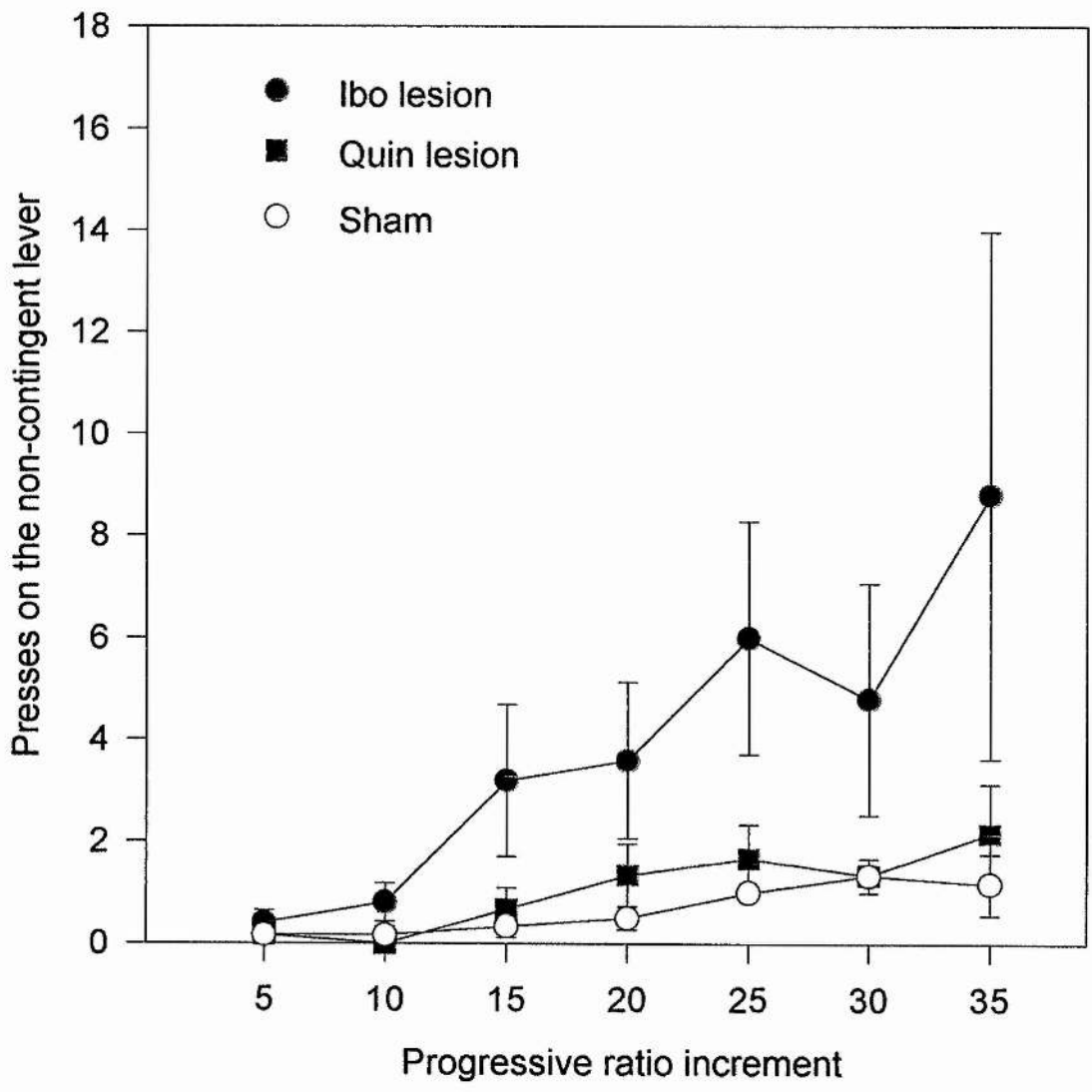
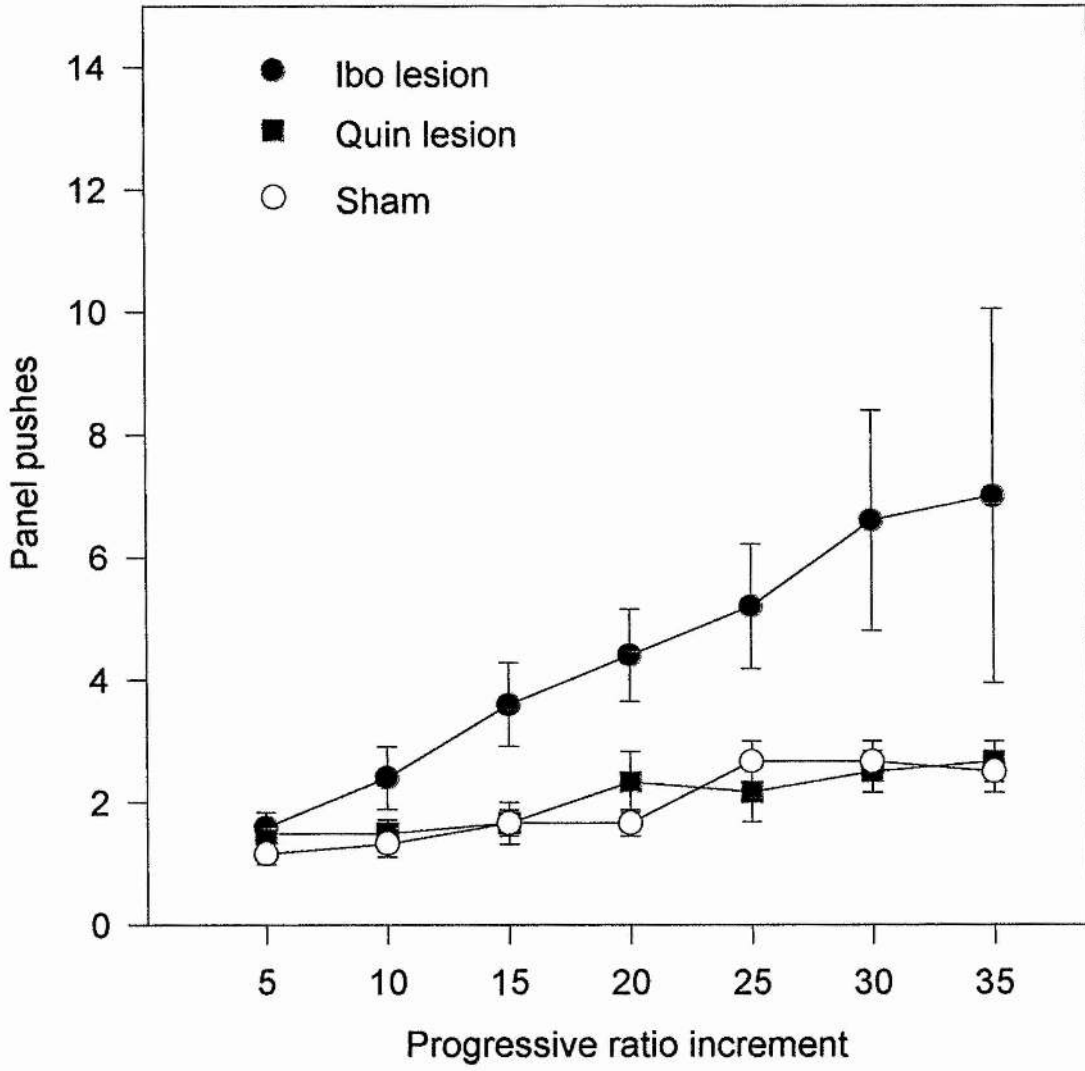


Figure 13: Graph of PPTg lesioned and control rats' number of panel pushes.



DISCUSSION

EXPERIMENT 1

These data showed that ibotenate lesions of the PPTg had a detrimental effect on the amount of work a rat was prepared to do to receive a reward. BPs of ibotenate lesioned rats were significantly lower than rats with quinolinate lesions. Within the ibotenate lesioned group, there was a large variance of BPs across the 6 rats, with one definite outlier which was confirmed having a substantial lesion in the PPTg. Rats with quinolinate or sham lesions did not differ from each other in BPs, but it was clear that the sham lesioned rats had more variable scores. Ibotenate lesioned rats were seen to press the non-contingent lever increasingly more often as the PRI increased, and also pressed the food hopper panel significantly more than the quinolinate and sham lesioned groups. No differences were found between the groups in the time taken to resume pressing the lever after the collection of the reward, nor was there any difference found in collecting the reward upon delivery.

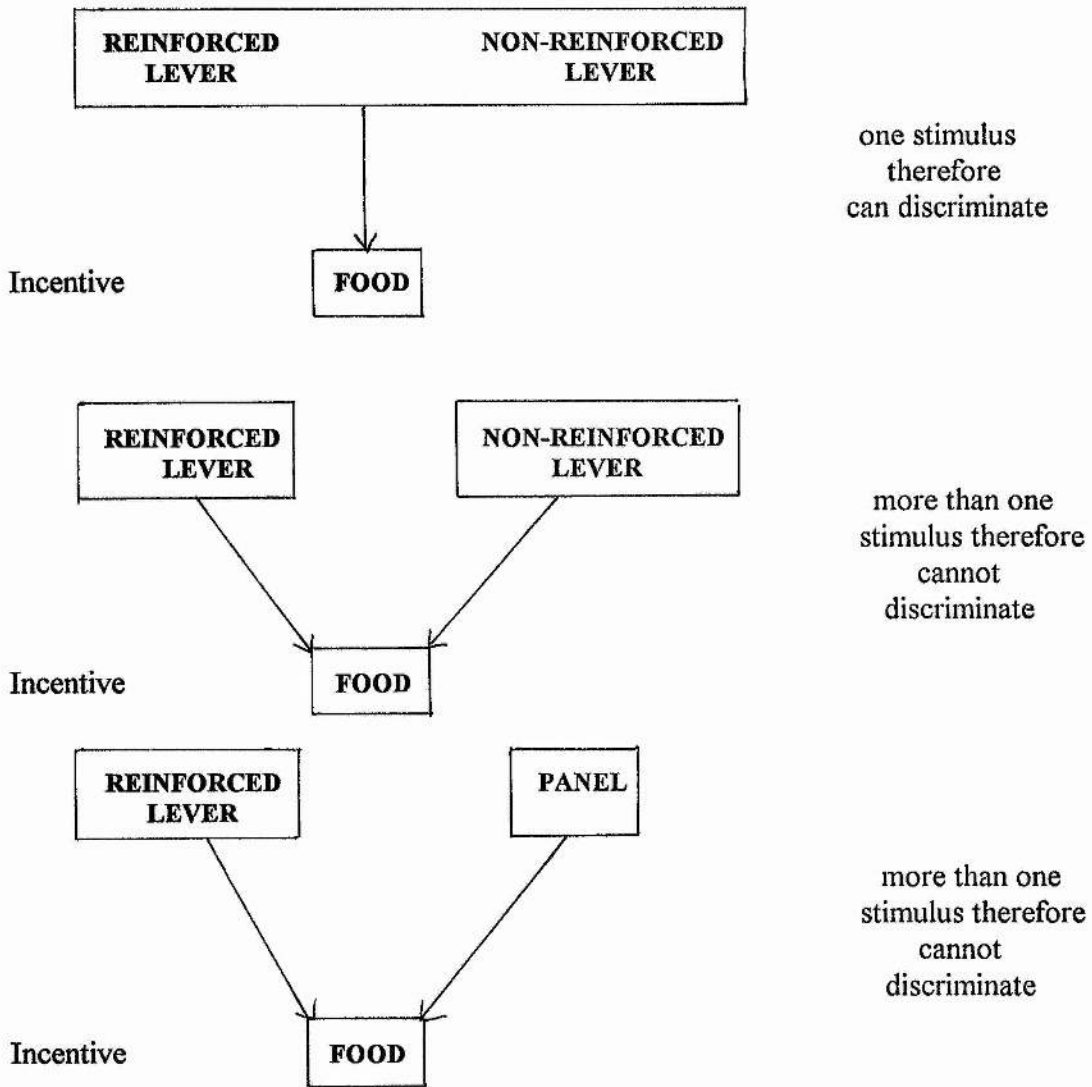
Analysis of the lesions revealed an interesting pattern of results. Rats given ibotenate lesions lost significantly more cholinergic neurones, marked by NADPH diaphorase, when compared to either quinolinate or sham lesioned rats. Rugg et al. (1992) compared the effect of various excitotoxins on the PPTg with special attention paid to the loss of cholinergic neurones identified using immunohistochemistry. Two of the excitotoxins tested were quinolinate and ibotenate. They found that quinolinate was the only excitotoxin which showed any selectivity for cholinergic neurones. Microinjection of 24 nmol produced a 40% loss of ChAT labelled neurones while sparing the majority of non-cholinergic neurones. Ibotenate was found to destroy both cholinergic and non-cholinergic neurones to the same extent. This was not what had happened in the present experiment. Ibotenate produced the most complete lesion of both cholinergic and non-cholinergic neurones. Quinolinate produced a smaller lesion but there was no evidence of selectivity for cholinergic neurones in the data. The area of the PPTg was lesioned to the same extent in both the ibotenate and quinolinate

lesioned rats. It was the areas surrounding the PPTg that differed in damage after the different excitotoxins. It has previously been noted that choice of anaesthesia is important in the action of ibotenate and quinolinate. Inglis, Dunbar and Winn (1993) investigated the two anaesthetics, Avertin and sodium pentobarbitone, on the effectiveness of both ibotenate and quinolinate excitotoxins. They found that under sodium pentobarbitone, but not Avertin, quinolinate lesions lost cholinergic selectivity and reduced its size, whereas ibotenate lesions were unaffected by anaesthetic type. This result was taken into account in the present experiment where quinolinate lesioned rats were anaesthetised by Avertin, and ibotenate rats by sodium pentobarbitone (Sagatal) in order to maximise apparent selectivity as previously reported by Inglis et al. (1993). Despite carrying out this exact procedure, ibotenate and quinolinate were not found to differ other than in terms of the magnitude of the lesion. The ibotenate lesioned rats had the highest degree of overall cell loss as shown by the highly significant difference among the three lesion groups in nissl counts, with ibotenate lesions having the least number of healthy neurones. Further experiments will be required to differentiate the effects of lesions selective for either cholinergic or non-cholinergic neurones in the PPTg. At present, reliable lesion technology to do this does not appear to be available. In the basal forebrain, 192 IgG-saporin (a protein synthesis inhibitor and potent immunotoxin) has been used to destroy selectively cholinergic neurones, sparing non-cholinergic ones. This lesion is based on the unique occurrence of nerve growth factor receptors on these cholinergic neurones. This demonstrates that selective neurochemical lesions *can* be made, but regrettably this procedure will not work in the PPTg, as there are no nerve growth factors present (Torres, Perry, Blokland, Wilkinson, Wiley, Lappi and Dunnett, in press).

Thus it seem clear from the results of the PR schedule experiment that PPTg neurones are critical for the behavioural expression of motivational strength and also to distinguish between the relevant aspects of the PR chamber. Rats with the highest level of neuronal loss, that is after ibotenate lesions, increasingly pressed the non-contingent lever more often as the PRI increased, whereas the quinolinate and sham lesioned

groups pressed it at a low rate throughout. In comparison to the CR study by Inglis et al. (1994b), ibotenate lesioned rats in the present experiment pressed the food panel significantly more than quinolinate and sham lesioned rats, whereas the rats in the Inglis et al. (1994b) study all pressed the food panel to the same extent. Both studies resulted in ibotenate lesioned rats pressing the non-contingent lever more often than the other groups. Inglis et al. (1994b) suggested that the PPTg was involved in the expression of appropriate and inhibition and inappropriate behaviours. Pressing the non-contingent lever is a behaviour which has never had any rewarding consequences for the rat, so an increase in pressing it is an inappropriate behaviour. An intact, fully functioning PPTg would normally inhibit this action, and so in accordance with the theory, the ibotenate lesioned rats cannot inhibit the inappropriate behaviour, resulting in an increase in non-contingent lever pressing. Unfortunately, this theory does not fully account for the increase in panel pushes found after ibotenate lesions in the present study. This was not found in the Inglis et al. (1994b) study, so the theory was satisfactory for only that particular set of results. Pressing the food panel does have rewarding consequences if done at the correct time. Hence increasing the number of panel pushes is only an inappropriate behaviour at specific times in the schedule. They also claimed, however, that the task of the PPTg was "...to form simultaneous associations between more than one novel stimulus (lever/press) and one incentive (food reward), in order to direct incentive-driven behaviours". This claim accounted for the ability of lesioned rats to make the lever/press reward association, and the failure of the reinforced and non-reinforced lever discrimination, and also for the panel push increases found in the present experiment after ibotenate lesions. These rats were unable to discriminate between the levers and the food panel because, as according to the theory of Inglis et al. (1994b), the panel and the lever constituted more than one novel stimulus. Hence without an intact PPTg, these rats were unable to discriminate between the panel and the lever, resulting in a higher number of presses on both compared to quinolinate and sham lesioned rats.

Figure 27: the behavioural relationship between stimuli and incentive (food) according to the theory of Inglis et al. (1994b).



The differences found between the present study and that of Inglis et al. (1994b) with respect to the number of panel pushes, could be a result of the different experimental procedures used. In the actual test phase of the Inglis et al. (1994b) study, responding on the CR lever resulted in the presentation of the compound stimulus without food, that is, food was only available in the training sessions. In the present study, however, food was the incentive that the rats were working for. Thus, the differences in the panel pushes could be due to the fact that rats in the present

study knew that food was going to be presented at some point in the schedule and so pressed the food panel more often, whereas rats in the Inglis et al. (1994b) study learned to associate the light stimulus as rewarding rather than food itself in the food hopper.

Thus it seems clear from the results that PPTg neurones, which are destroyed by ibotenate, reduce the amount of work a rat is prepared to do in order to receive a reward. The BPs were significantly lower than both the quinolinate and sham lesioned groups, showing that ibotenate rats stopped pressing the reinforced lever after fewer rewards indicating a reduction in motivational strength. Relating back to the anatomy of the PPTg and its numerous cholinergic efferents and afferents, it is not surprising to discover that cholinergic lesions of the PPTg affect motivational strength. Projections to structures heavily implicated with motivated behaviours, such as the LH, SNC, VP and indirectly the NAS, are numerous.

METHODS

EXPERIMENT 2(i) - THE EFFECT OF LATERAL HYPOTHALAMIC LESIONS ON FOOD AND WATER INTAKE, DRINKING IN RESPONSE TO HYPERTONIC SALINE AND PRANDIAL DRINKING.

Animals

Twenty experimentally naive, male hooded Listar rats were used, with a mean weight of 374.5g (range 325.5 to 409.5g) on the day of surgery. Subjects were housed individually in cages in a room kept at 20-22°C, lights on 7am-7pm. Daily measurements of food and water intake and spillage were made before and after surgery.

Surgery

Rats were anaesthetised with 10ml/kg Avertin (25g tribromoethanol/15.5ml tertiary amyl alcohol; of this, 2.5ml/125ml 0.9% saline + 10ml absolute alcohol) and placed in a stereotaxic frame. Simultaneous bilateral infusions into the LH were made at the following co-ordinates: 0mm from bregma; 2.0mm from midline; and 8.0mm below dura, with the incisor bar set 5.0mm above the interaural line. Ten rats received bilateral lesions of the LH made by 1.0µl 0.06M (60nmol) NMDA (Sigma) dissolved in 0.1M phosphate buffer (pH7.4) and pH adjusted to 7.00 with 2M NaOH. Ten rats received bilateral infusions of 1.0µl phosphate buffer. Infusion rate was 0.5µl/min and the cannulae were left *in situ* for a further 2 min post-infusion to allow for diffusion. Infusions were made via 30ga stainless steel tubing connected by PE10 polyethylene tubing to a 10µl SGE syringe mounted in an infusion pump. One lesioned rat failed to recover after surgery. Two further rats, one lesion and one sham, failed to make a sufficient recovery after surgery and despite being given wet mash, were killed by an overdose of barbiturate (Euthatal, May and Baker; sodium pentobarbitone 200mg/ml).

Procedure

For 9 days before and 30 days after surgery, body weight, water and food intake and spillage were measured to the nearest 0.1g. Rats were kept in wire floored cages allowing the food spillage to fall on to a sheet of aluminium foil below. One sham rat persistently pulled the foil through the cage floor and ate it; all data from this animal were discounted. All measurements were taken at the same time of day throughout testing.

Drinking in response to hypertonic saline

Twelve days after surgery, rats were given either an i.p. injection of 20ml/kg hypertonic saline (5%) or an equal amount of 0.9% isotonic saline. Two days later, the rats received the opposite injection to that which they had received on the previous test day. On these two days of testing, food and water intake, spillage and body weight were recorded as before. Water bottles were then filled with fresh water. Rats were given i.p. injections and drinking water given to them immediately after, noting the exact start time. Water intake was then measured 1 hr and 3 hr after the injections.

Prandial Drinking

On post operative day 16, all water bottles were removed. Exactly 23 hr later, after normal body weight, spillage and food intake were measured, the food was removed from the cage hoppers, and all rats were given access to water for exactly 1 hr. Water intake was calculated for the hour's access and food intake over 23 hr at the same time every day for 5 consecutive days.

Statistical Analysis

Data were analysed by analysis of variance (ANOVA) using the SPSS/PC+ Statistical Package for IBM PC. Graphs were produced using the Harvard Graphics package for Viglen PC.

METHODS

EXPERIMENT 2(ii) - RESPONDING ON A PROGRESSIVE RATIO SCHEDULE OF REINFORCEMENT

Animals

Eight sham and 8 lesioned rats were initially included in this part of the experiment. The rats were moved from the wire floored cages to solid floored cages with sawdust, but were otherwise kept under the same condition. Rats were given 18g of food per day - an amount previously discovered to keep the animal above its 85% pre-operative body weight, and allow it to gain weight at a small but steady increase.

Apparatus

Four operant chambers (Campden Instruments Ltd) measuring 25cm across, 26cm high, and 23cm deep were used. Three walls and the roof were made from steel and the floor was a grid with a tray of sawdust below. One wall was made from hinged plexiglass and opened to allow rat entry. On the left hand wall of the chamber, 2 levers were located 10.5cm apart and 6cm from the grid floor. Each lever was 4cm wide and extended 2cm from the chamber wall. The reinforced lever was the lever positioned to the left of the panel. The food hopper was located in the centre of the 2 levers, separated from each by 2.5cm. The panel allowing entry to the food hopper was made of plexiglass hinged at the top, and was 5.5cm wide by 6cm high. Whenever the reinforced lever was pressed, a light located in the centre of the roof was switched off, and a light in the food hopper was illuminated to indicate reinforcement. A pellet dispenser delivered 45mg dustless precision sucrose pellets (rodent grain-base formula, Campden Instruments Ltd) into the food hopper upon reinforced lever pressing. Each experimental operant chamber was enclosed with a sound proof box. An extractor fan was also used to give some background noise and provide continuous airflow. Data were collected on a Spider real-time processor (Paul Fray Ltd.).

Procedure

Three days following the start of food deprivation, the 16 rats were divided into 4 groups, each group containing 2 control and 2 lesioned rats. In order to accustomise the rats to the operant chamber and to teach them the association between the food and the panel, 20 food pellets were placed behind the propped-open food hopper panel. Rats were then left for 30 min. On the second day of training, the rats were left in the boxes for 60 min, and 25 food pellets were put in the open food hopper. All rats ate their pellets within the time limit. This was repeated on the third day. On the fourth day of training, a panel or lever press was rewarded with a food pellet for the duration of 30 minutes. For the next 4 training days, only reinforced lever presses were rewarded during the 30 minute sessions. At this point, one sham and one lesioned rat were dropped from the study because they failed to push the panel to collect a food reward after pressing the reinforced lever, despite remedial training. On the ninth and tenth day of training, rats were put on a schedule of continuous reinforcement (CR) for 30 trials, followed by a progressive ratio schedule of 5 (PR5). That is, for the first 30 lever presses, the rat received a food pellet after every press, thereafter the rat only received a pellet every 5 presses - 5, 10, 15, 20 and so on. On the eleventh and twelfth day of training, rats completed 10 CR trials before beginning the PR5 schedule.

Rats were tested on alternate days for a total of 7 days. A CR schedule for 1 trial was followed by a PR5 schedule. A maximum time of 4 hr was allowed, and if the rat was still responding at this time, they were returned to their home cages. The breaking point was established and the session terminated when the rat did not press the lever for 5 min.

The following data were collected for every reinforced lever press: reward - number of pellets earned so far in session; lever 1 - number of presses on reinforced lever; lever 1+ - number of presses on reinforced lever after pellet dispersed; lever 2 - number of non-reinforced lever presses; panel - number of panel pushes; latency 1 -

time to collect pellet (1/10 sec); latency 2 - time to resume pressing the reinforced lever after pellet collected (1/10 sec).

Statistical Analysis

Data were analysed by analysis of variance (ANOVA) using the SPSS for Windows statistical package and graphs were produced using the Sigma Plot for Windows graphics package.

Histological Analysis

At the end of all behavioural testing, rats were humanely sacrificed by a barbiturate overdose (Euthatal, May and Baker; sodium pentobarbitone, 200mg/ml) and perfused transcardially with phosphate buffered saline followed by 4% paraformaldehyde in 0.1M phosphate buffer. Brains were removed and stored in paraformaldehyde prior to histological analysis. Sections of 40µm were cut and stained with cresyl violet for examination of lesion volume and placement. Individual sections were examined using a Leitz "Diaplan" microscope and lesion silhouettes were drawn.

RESULTS

EXPERIMENT 2(i) - THE EFFECT OF LATERAL HYPOTHALAMIC LESIONS ON FOOD AND WATER INTAKE, DRINKING IN RESPONSE TO HYPERTONIC SALINE AND PRANDIAL DRINKING.

Histological Analysis

Immediately following surgery, rats which had been given NMDA convulsed for several hours, but this was seen as a normal result of excitotoxin administration. Post mortem assessment of lesion placements from Cresyl Violet sections identified 2 rats with lesions of insufficient size. One had an overall small lesion, and the second had a unilateral lesion on the left side. Both rats were excluded from the following behavioural data analyses. Table 2 shows the average lesion volume and the modal damage scores in structures adjacent to the LH after NMDA lesions. All lesions showed a moderate amount of damage in the subthalamus, perifornical nucleus, medial forebrain bundle and fornix. The larger lesions also showed damage extending into the entopeduncular nucleus, subincertal nucleus, zona incerta and to a lesser extent, the optic tract and supraoptic nucleus. The areas with the highest lesion damage were the magnocellular nucleus of the LH (over 90% damage), the paraventricular hypothalamic nucleus, posterior (60-90% damage), and the LH itself (30-60% damage). Damage in the LH was greatest from the anterior pole through the central core and past the level of the ventromedial hypothalamus. Cell loss was least at the posterior pole (see figure 14).

Table 2. Summary of damage and lesion volumes

Damage assessment:

(modal scores)

Anterior hypothalamic area	ND
Bed nucleus of the stria terminalis, medial division, posterolateral part	X
Entopeduncular nucleus	X
Fornix	X
Internal capsule	X
Lateral hypothalamus	XX
Lateroanterior hypothalamic nuclei	ND
Magnocellular nucleus of the lateral hypothalamus	XXXX
Medial forebrain bundle	XX
Medial tuberal nucleus	X
Nucleus of the stria medullaris	XX
Optic tract	ND
Paraventricular hypothalamic nucleus (posterior)	XXX
Perifornical nucleus	XX
Reticular thalamic nucleus	ND
Stria medullaris of the thalamus	X
Subincertal nucleus	XX
Substantia nigra pars compacta	ND
Substantia nigra zona reticulata	ND
Subthalamic nucleus	X
Supraoptic decussation	X
Supraoptic nucleus	ND
Zona incerta	X
Lesion volume (mm ³):	3.99
(mean ±S.E. value)	+0.38

A summary of damage and average lesion volumes computed from the the Cresyl Violet sections for each group. Damage was identified by glial infiltration and degeneration of neuronal somata, and a summary of affected structures for every animal estimated. The modal score in the group was then taken for each structure. The key is as follows: ND, no damage; X, < 30%; XX, 30-60%; XXX, 60-90%; XXXX, > 90%.

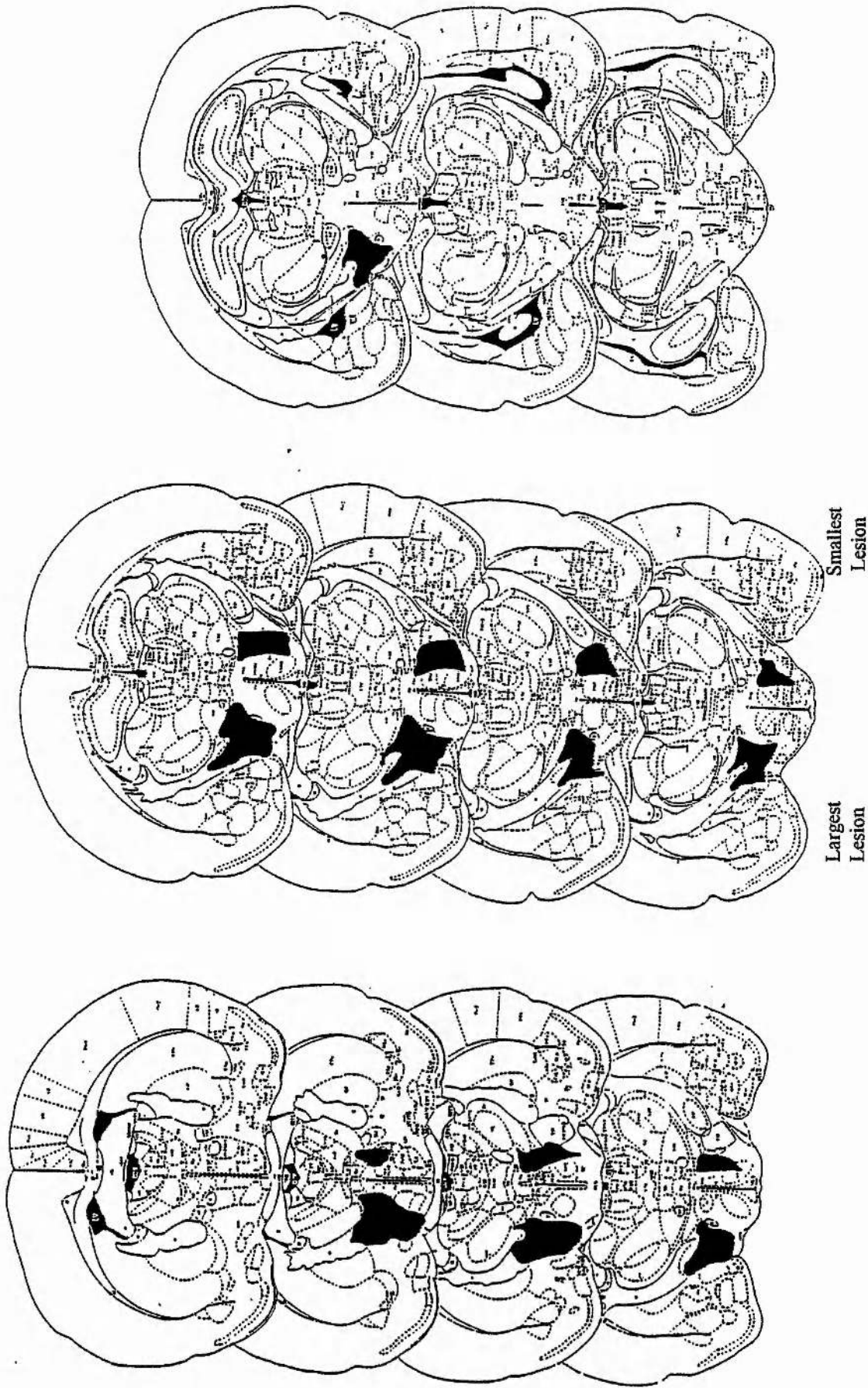


Figure 14. Representative sections re-drawn from the atlas of Paxinos and Watson illustrating the largest and smallest LH lesion sizes.

Body weight, food and water intake

In the week before surgery, there was no significant difference between the lesioned and control groups' body weights ($F[1,12] = 0.00$), and there was no group \times day interaction ($F[8,96] = 0.50$). In the first 16 days after surgery, the difference between control and lesioned groups' body weights was very close to significance ($F[1,12] = 4.70$) and there was a highly significant group \times day interaction ($F[15,180] = 2.95, p < 0.001$). It can be seen from figure 15 that after the initial body weight drop in the lesioned group after surgery, there was a small but steady weight gain in both groups for the remaining test days up until the physiological challenges were initiated after day 11. The lesioned group always weighed approximately 30g less than the control group. There was no significant difference in food intake before surgery ($F[1,12] = 0.01$). In the 11 days after surgery, before any physiological challenges had been given, there was a significant difference between the control and lesioned groups ($F[1,12] = 14.84, p < 0.005$) and also a highly significant group \times day interaction ($F[10,120] = 7.35, p < 0.001$). Post-hoc testing showed that there was a significant difference between the groups on the first 6 days after surgery ($p < 0.05$ on the first day and $p < 0.01$ for the following 5 days post-surgery), and no significant difference between groups on days 7 to 11. Therefore, rats with LH lesions recovered their food intake to the same level as controls 6 days after surgery. The pattern for water intake shows a very similar pattern to food intake as can be seen from figures 16 and 17. There was no difference in water intake between groups before surgery ($F[1,12] = 1.99$) and there was no group \times day interaction ($F[7,84] = 1.76$). In the 11 days after surgery before any physiological challenges, there was a significant difference between the groups ($F[1,12] = 11.30, p < 0.01$). As can be seen from the figure 17, the lesioned rats drank less water compared to the control rats. There was also a highly significant group \times day interaction ($F[10,120] = 7.95, p < 0.001$). Post-hoc testing showed that there was a highly significant difference in water intake between groups on the first 6 days post-surgery ($p < 0.01$). Thus after an initial significant drop in water intake, the lesioned rats had recovered to the same level as control rats. Before surgery, as

expected, there was no significant difference in the amount of food spilled between groups ($F[1,12] = 0.45$) nor was there any significant group \times day interaction ($F[7,84] = 1.81$). In the 11 days following surgery, the control and lesioned groups did not differ significantly from one another ($F[1,12] = 3.12$). There was, however, a group \times day interaction ($F[10,120] = 4.08, p < 0.001$). Post-hoc testing showed that in the first 6 days after surgery, there was no significant difference in food spillage between lesioned and control groups, whereas there was a significant difference ($p < 0.01$) on days 7 to 11. LH lesioned rats spilled more food than controls after the initial post-surgery recovery phase (figure 18).

Fig15: Post Op Body Weight

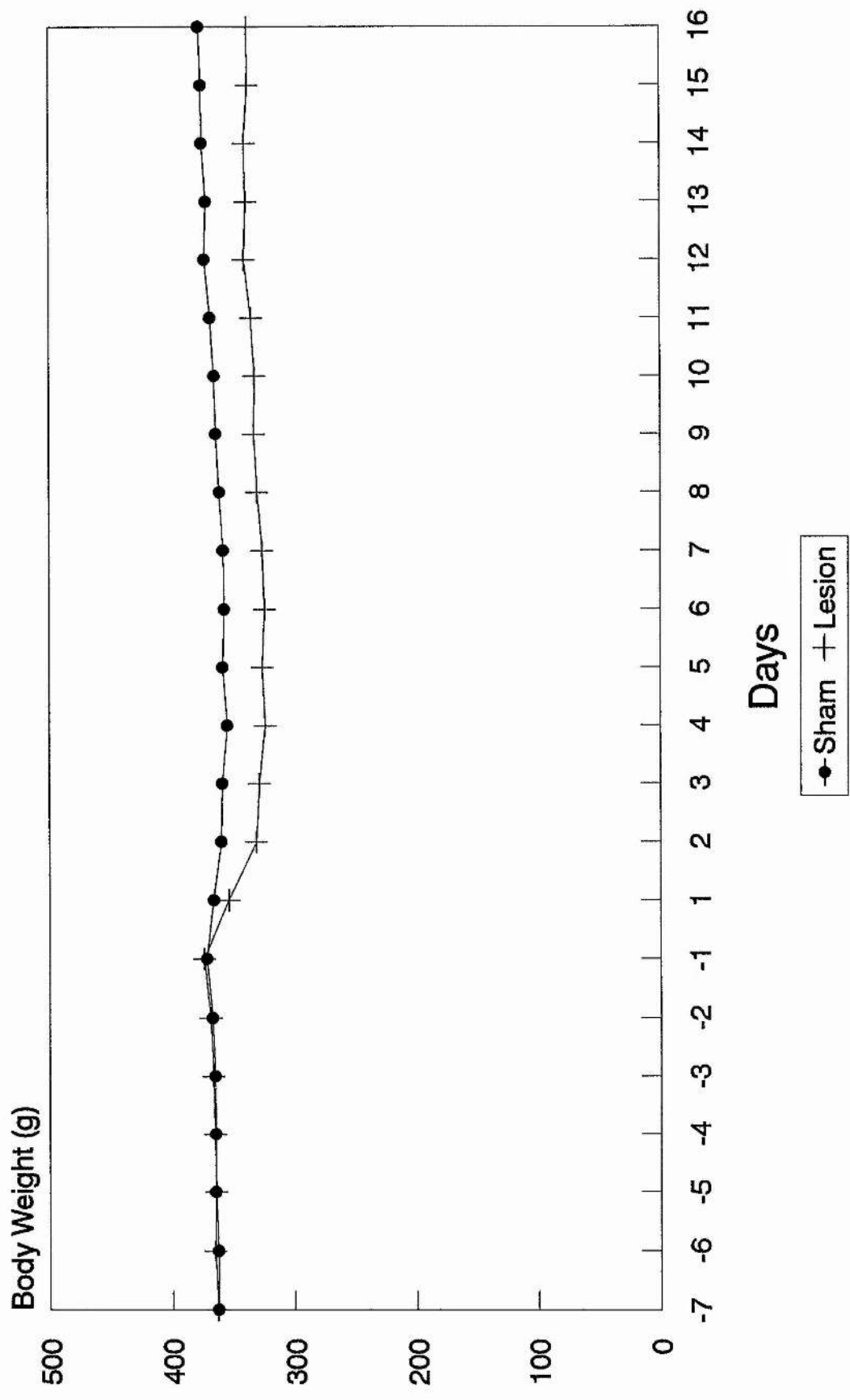


Fig16: Post Op Food Intake

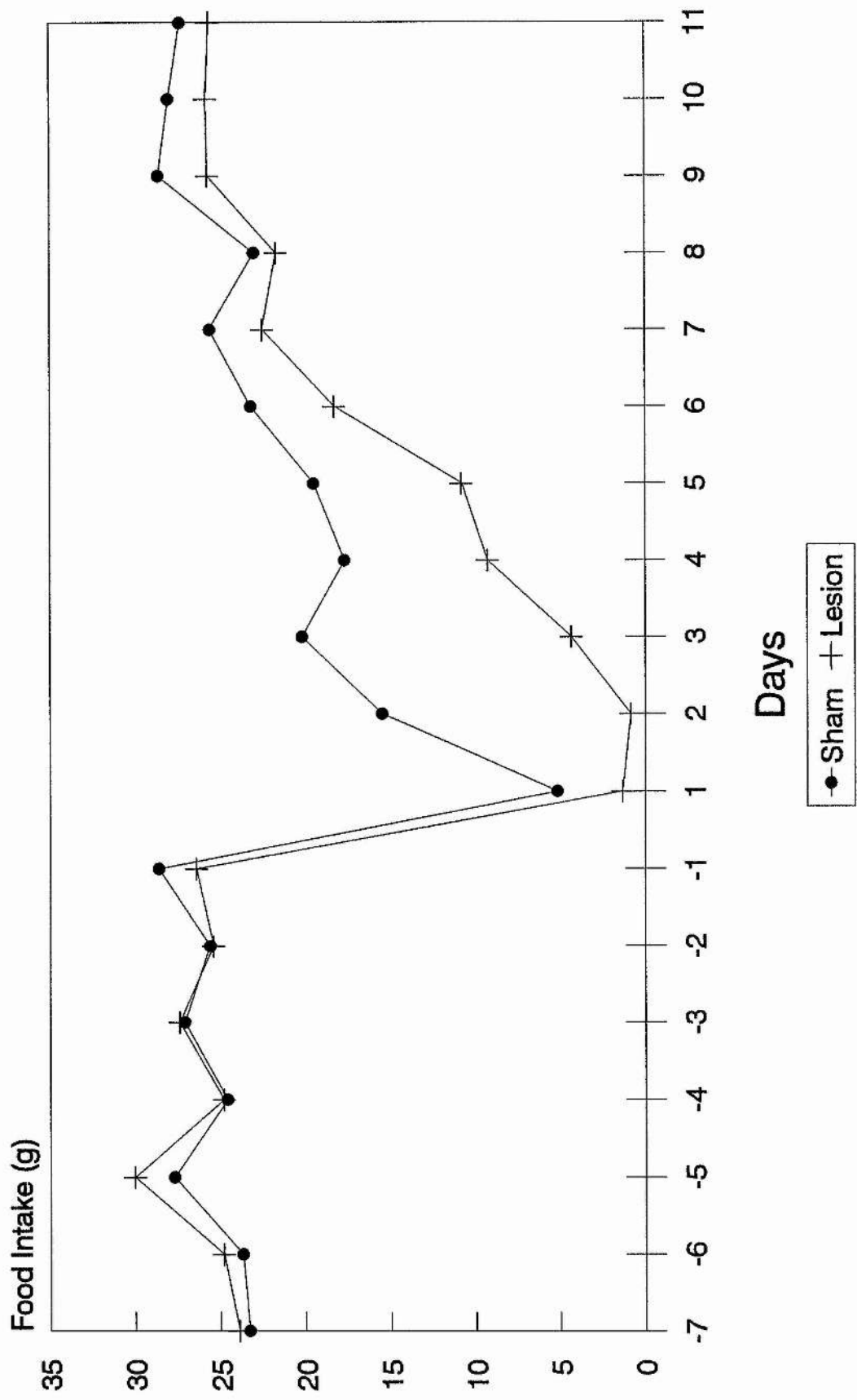


Fig17: Post Op Water Intake

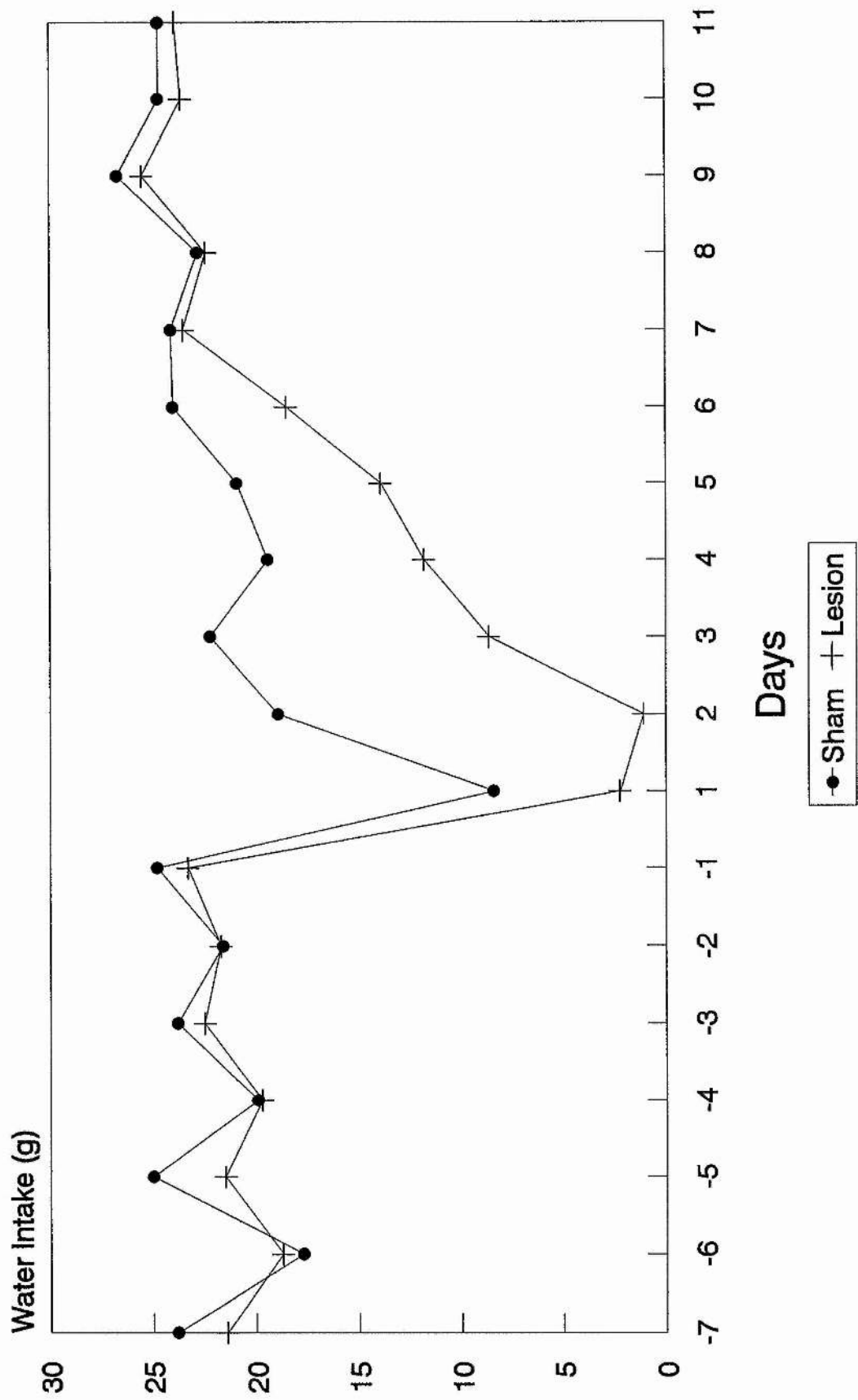
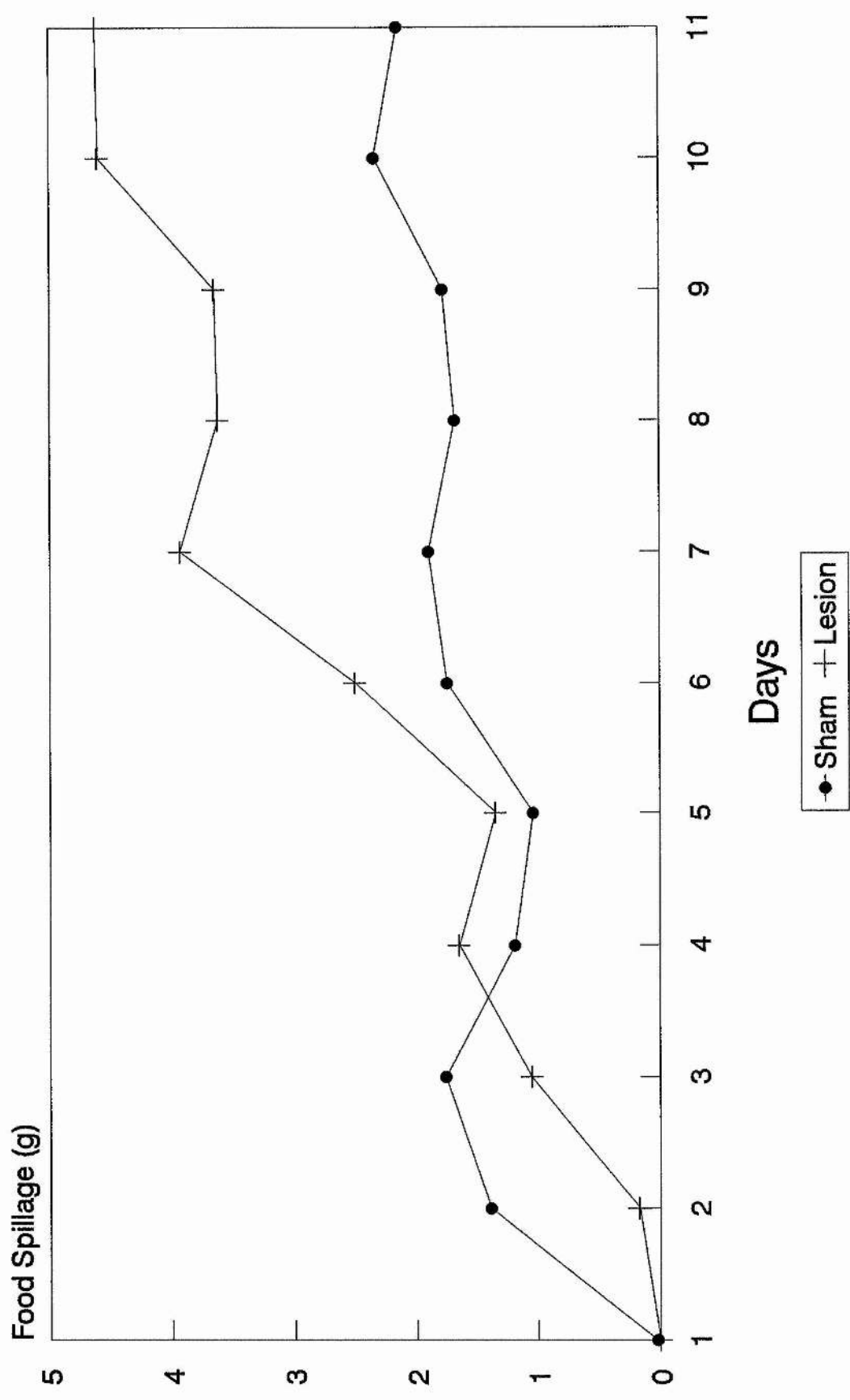


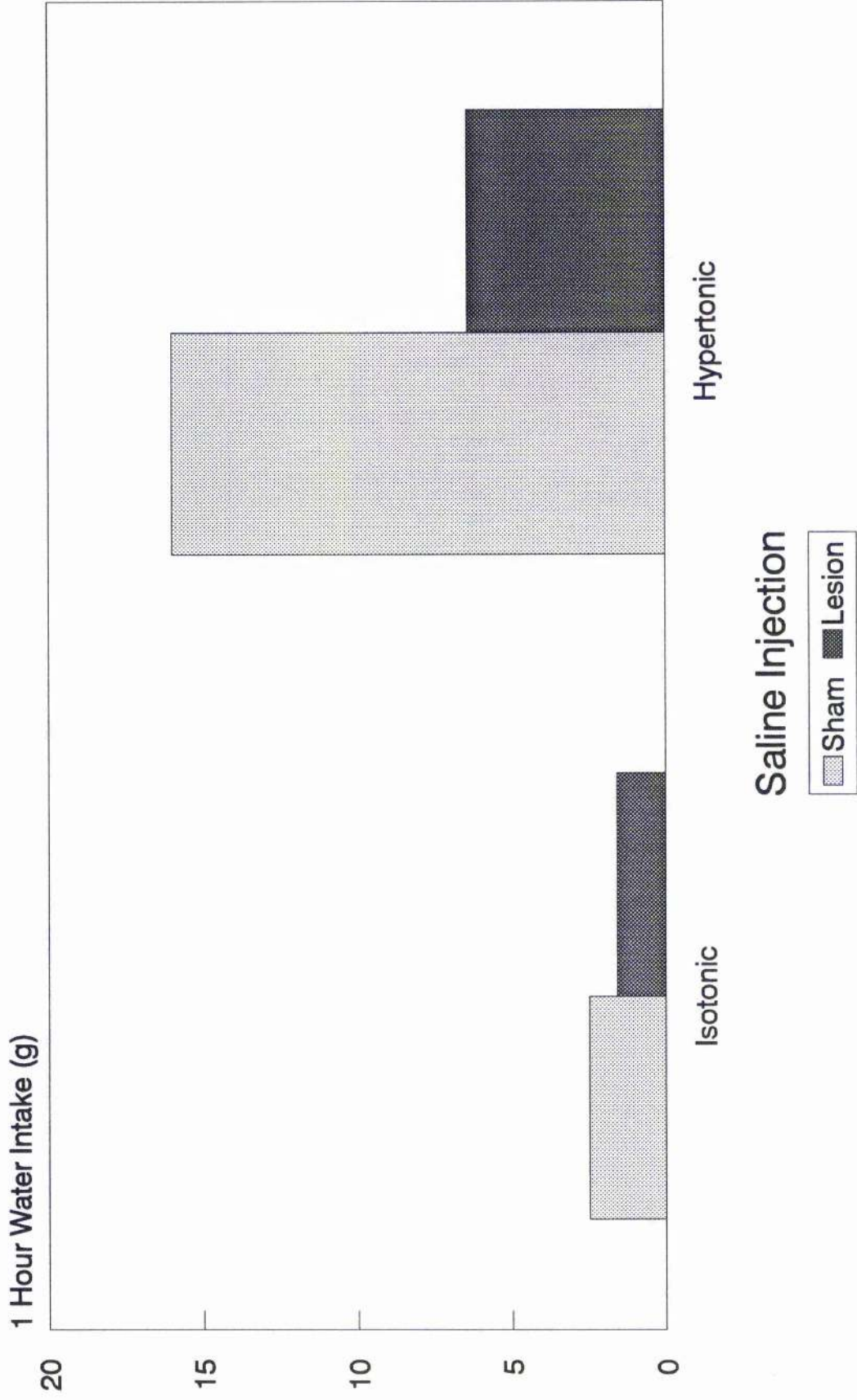
Fig18: Post Op Spillage



Hypertonic/Isotonic Saline Challenge

By comparing the isotonic and hypertonic saline conditions together, ANOVA revealed a significant group effect ($F[1,12] = 8.61, p < 0.005$) and also a significant group x condition interaction ($F[1,12] = 6.92, p < 0.05$). Post-hoc testing showed that there was no significant difference between the groups regarding amount drunk following 0.9% saline injection, but that there was a significant difference ($p < 0.01$) after injection of hypertonic saline (figure 19).

Fig19: Physiological Challenges



Prandial Drinking and Feeding

Analysis was carried out on the 2 days before prandial drinking was measured, the 5 test days of prandial drinking, and the 2 days following measurement - a total of 9 days. No significant difference between the groups was found ($F[1,12] = 1.08$) showing that both control and lesioned rats drank similar amounts of water after dehydration had been induced by 23 hr water deprivation. The 2 groups also drank similar amounts both before and after the prandial testing. No group x day interaction was found ($F[8,96] = 1.70$). There was, however, a highly significant days effect ($F[8,96] = 58.53, p < 0.001$) (figure 20). Food intake over this period revealed a very similar effect when compared to prandial drinking (figure 21). ANOVA, however, revealed a significant group effect ($F[1,12] = 12.25, p < 0.005$) but no group x day interaction ($F[8,96] = 0.53$). Lesioned rats ate significantly less than control rats during the 2 days pre and post testing and throughout the prandial testing phase.

Fig20: Pre & Post Prandial Drinking

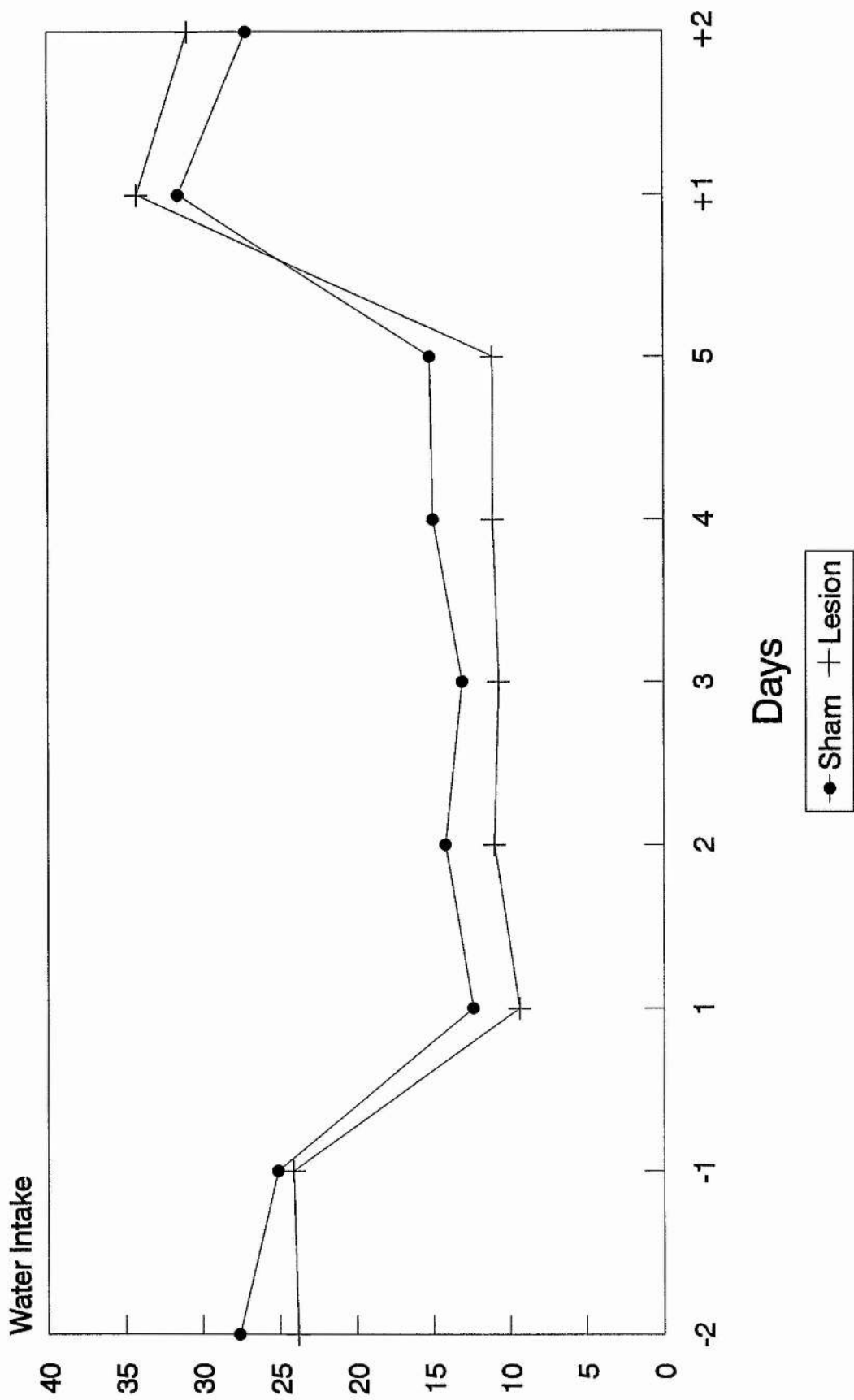
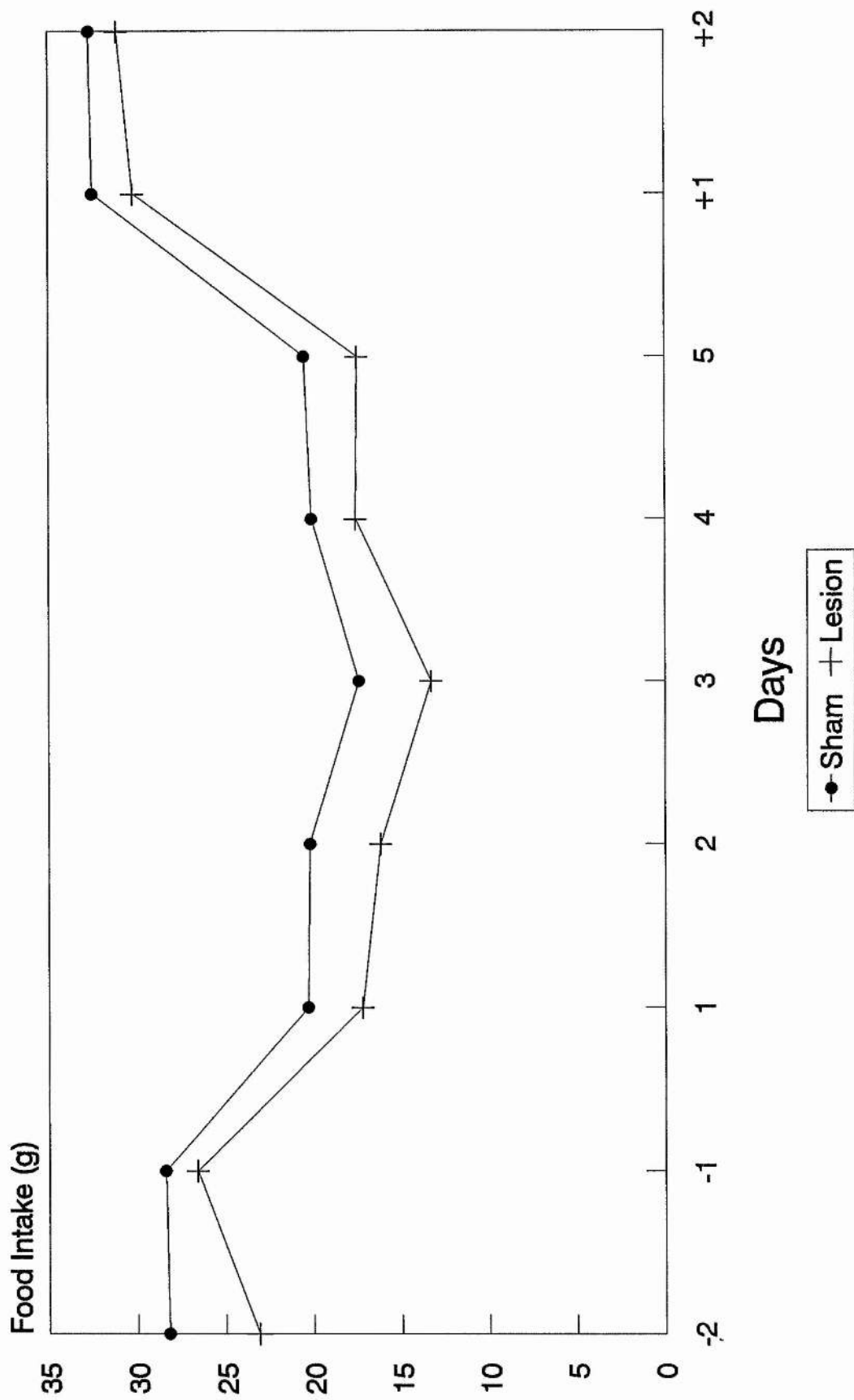


Fig21: Pre & Post Prandial Eating



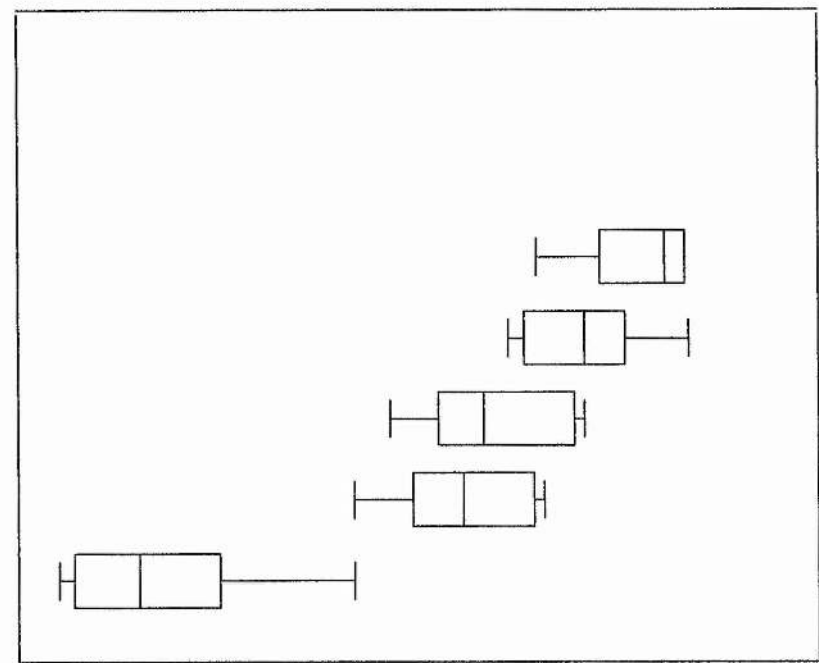
EXPERIMENT 2(ii) - RESPONDING ON A PROGRESSIVE RATIO SCHEDULE OF REINFORCEMENT.

The lesion group in this experiment was reduced to 5 rats after 1 was excluded during testing and a further 2 were dropped after histological analysis. Figure 22 shows the breaking points of both the control and lesioned rats over all 7 test days. It is apparent from this figure that there was a relatively high variance within individual rats' performance across test days. ANOVA revealed no difference in breaking points between the 2 groups ($F[1,10] = 0.39$), showing that rats with LH lesions exerted similar amounts of energy as control rats to gain a reinforcer. There was also no group x day interaction ($F[6,60] = 1.30$). It was interesting to note that in the majority of rats, the 5 min period of no responding necessary to end the session took place immediately after a reinforcer rather than during the next attempted ratio. Thus, rats tended to break off instantaneously at a certain point rather than trail off responding midway through an attempted ratio.

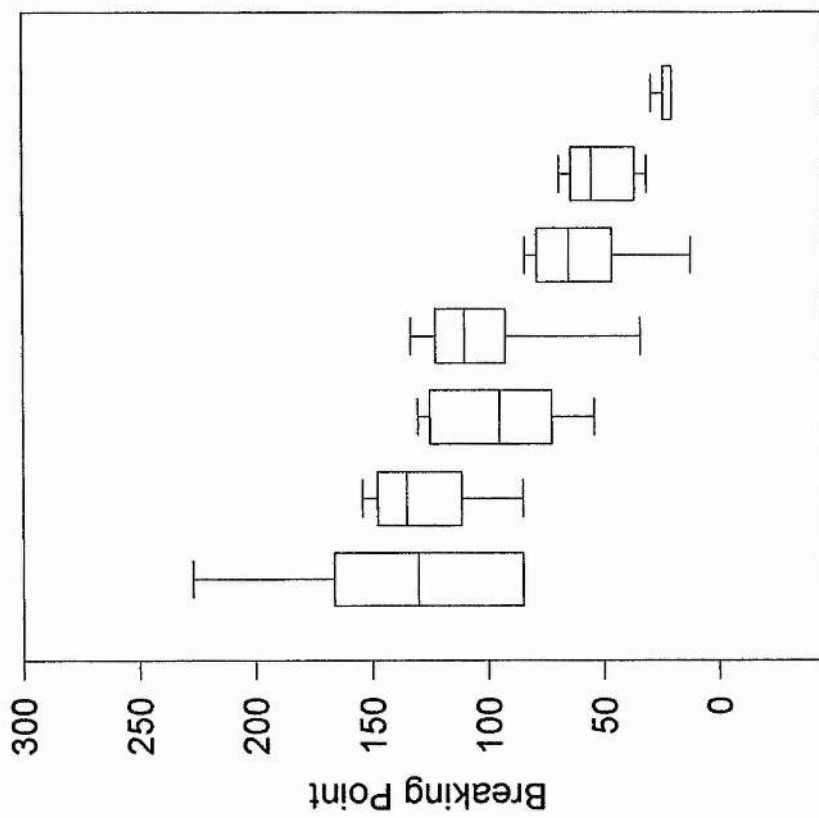
For the remaining data analysis, one sham rat was dropped because it only had data up to a breaking point of PR 30. All analysis was carried out on data up to breaking point 55 - a total of 11 rats. The length of time taken to collect a pellet from the food hopper was analysed using ANOVA. No groups effect was found ($F[1,9] = 2.72$) nor any group x PR increment interaction ($F[10,90] = 0.87$). Thus, there was no difference in the time taken by sham and lesioned rats to collect the pellet. Figure 23 shows that each group showed little variance across PR increment, and on the whole, the sham group took longer to collect. It was found that once the pellet had been collected, there was no difference between the sham and the lesioned groups in the latency to resume pressing the reinforced lever ($F[1,9] = 0.03$). There was also no group x PR increment interaction ($F[10,90] = 0.55$). By looking at figure 24, it is clear that there was a high degree of variance within groups, and also that as the PR increment increased, so did the latency to resume pressing - this is shown by a highly

significant PR increment effect ($F[10,90] = 4.53, p < 0.001$). Figure 25 shows the number of non-contingent lever presses as a function of PR increment. ANOVA revealed that there was a significant lever effect, that is, pressing on the non-contingent lever increased as the PR increment increases ($F[10,90] = 2.42, p < 0.05$). There was, however, no group effect indicating that both the control and lesioned groups responded in a similar overall manner. There does seem to be a pattern in the figure in that after position 30 on the schedule, the lesioned group pressed increasingly more often whereas the control group had a similar number of non-contingent lever presses throughout, which is reflected by the group \times PR increment interaction nearing significance ($F[10,90] = 1.74$). The number of panel pushes did not show any significant difference between the lesioned and control groups ($F[1,9] = 0.44$), and the group \times PR increment neared significance ($F[10,90] = 1.72$). There was, however, a significant PR increment effect ($F[10,90] = 3.77, p < 0.001$) indicating that, in general, as the PR increment increased the number of panel pushes also increased (figure 26).

Figure 22: Graph of LH lesioned and control rats' breaking points (indicating variance, median and standard deviation).



NMDA Lesion



Sham

Figure 23: Graph of LH lesioned and control rats' latencies to collect pellets.

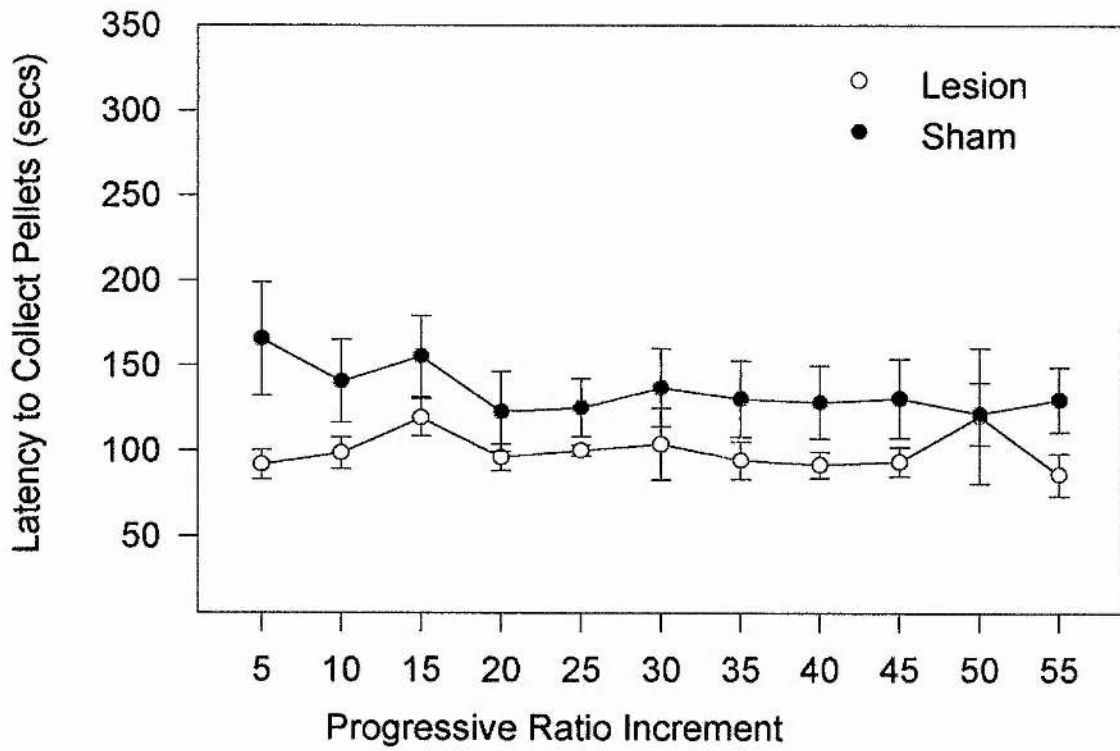


Figure 24: Graph of LH lesioned and control rats' latencies to resume pressing the reinforced lever.

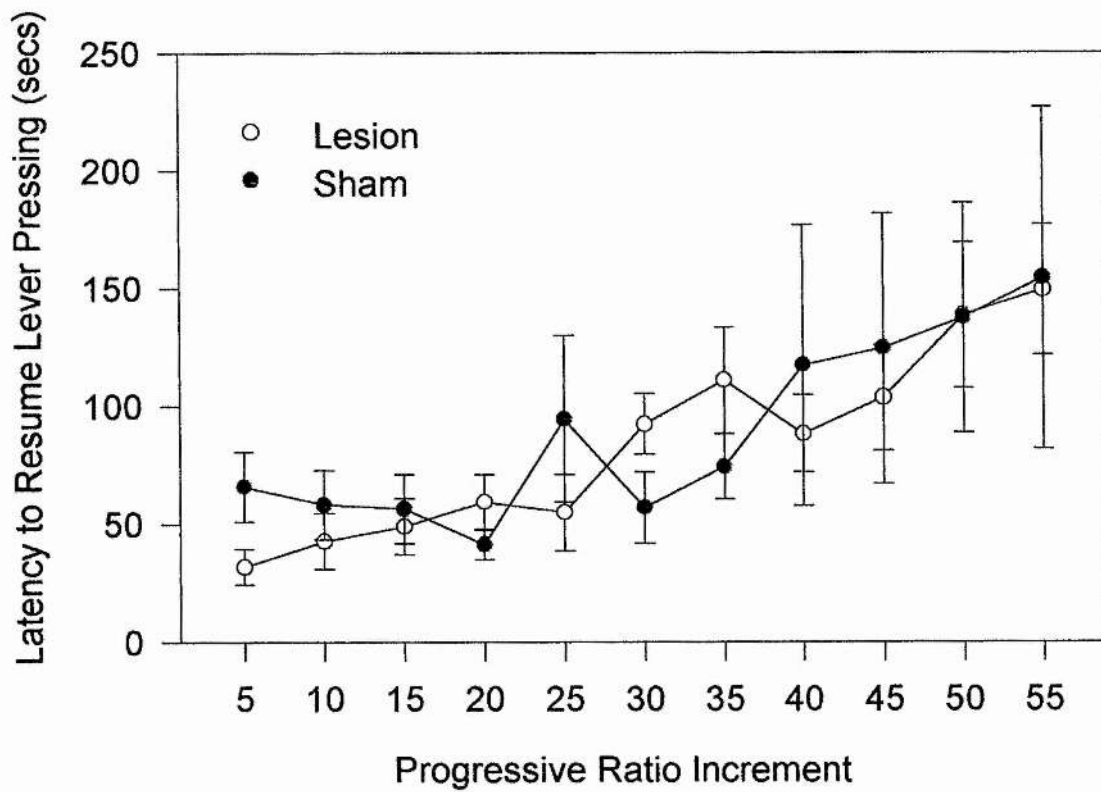


Figure 25: Graph of LH lesioned and control rats' number of non-contingent lever presses.

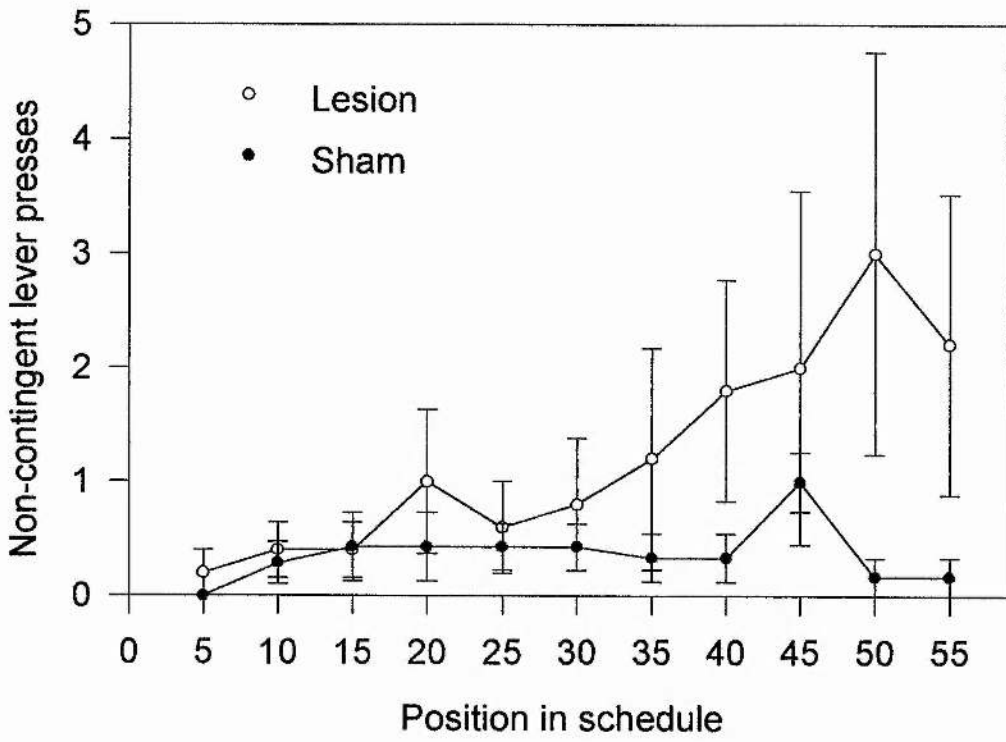
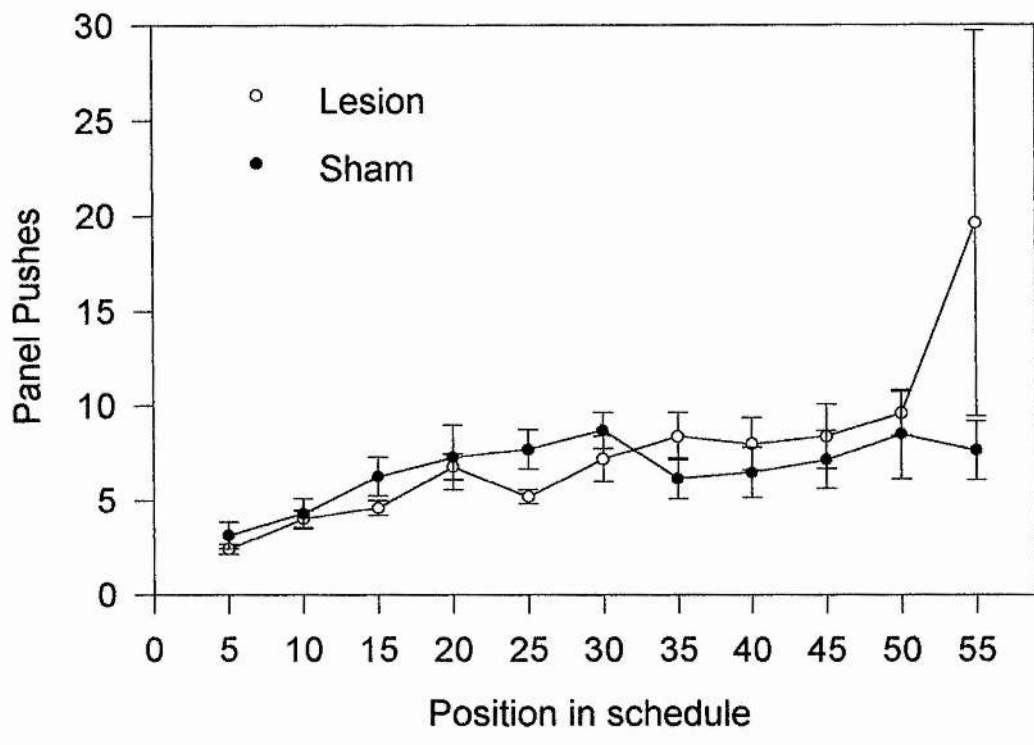


Figure 26: Graph of LH lesioned and control rats' number of panel pushes.



DISCUSSION

EXPERIMENT 2

After the initial post operative period of 6 days, lesioned and control rats had similar food and water intake measurements. This was reflected in their body weights which showed that lesioned rats only had a small overall decrease after surgery. Lesioned rats were found to spill significantly more than control rats, but this difference was expected as a consequence of the excitotoxin NMDA. Clearly, by using excitotoxic lesions, there were no long lasting food or water intake deficits as previously thought when using electrolytic lesions. The motivational deficits seemed to be more specific and subtle. This could be seen by looking at the results of the physiological challenges given to the rats. Injection of hypertonic saline resulted in lesioned rats drinking significantly less than control rats, whereas both groups drank the same volume of water after injection of isotonic saline. Rats with LH lesions drank similar volumes of water as control rats during the prandial testing. These two physiological challenges induced states of dehydration but clearly over a different time course. Hypertonic saline induced dehydration immediately whereas deprivation of water for 23 hours induced a slow and gradual onset of dehydration. It was clear that the rats were able to recognise their own internal states, since they responded appropriately to the 23 hour water deprivation. To support this finding, Clark, Clark, Warne, Rugg, Lightman and Winn (1990) injected rats with hypertonic saline and measured blood vasopressin levels. Within 1 hour, rats had made a normal vasopressin response, implying that they could detect signals of dehydration induced by the hypertonic saline. Taken together, these results seem to show that it is the immediacy of the challenge rather than the power which is the critical factor in whether the LH-lesioned rats can respond or not. Thus, the deficits after LH lesions are not solely regulatory, but are more complex and specific with the time factor playing an important role in normal LH control over feeding and drinking.

The PR results showed that there was only one difference between the LH-lesioned and control rats in the parameters measured. Both sham and LH-lesioned rats had a high variance of BPs across the groups. The only significant difference to appear was in responding on the non-contingent lever. Lesioned rats increased the number of presses as the PRI increased, especially from PRI30 onwards. Thus it seemed that NMDA LH-lesioned rats were not deficient in motivational strength, that is, they worked just as hard and to the same extent as control rats. BPs were very similar for both groups, and in fact, the LH-lesioned group had the highest and lowest mean BP for the 7 test days. The LH does not seem to be a critical structure in the expression of motivational strength.

The hypothesis that was put forward in the introduction by Clark et al. (1991a&b) was that the LH is involved in normal inhibitory function which suppresses inappropriate and helps select appropriate behaviour. The hypothesis was supported by the fact that LH-lesioned rats increased eating in response to tail pinch (Clark et al., 1992) and they acquired SIP more quickly than controls (Winn et al., 1992). Both of these behaviours were classified as an inappropriate behaviour since there were no rewarding behavioural consequences. LH-lesioned rats pressed the non-contingent lever more often as the PRI increased. This fits in with the Clark et al. (1991a&b) hypothesis. The destruction of the LH neurones removes its ability to suppress the non-contingent lever pressing, thus increasing the frequency. The hypothesis comes up against a problem when food and water intake data are examined. It is obvious that rats not responding to hypertonic saline are producing an inappropriate behaviour, since the normal appropriate response is to drink to relieve the state of dehydration. Although the hypothesis seems to be able to account for the failure to respond to hypertonic saline after LH lesions, it cannot explain the ability of rats to drink after 23 hour water deprivation. Rats seem to be focussing their behaviour on responding to only long term dehydration. Why should the hypothesis account for the behaviour resulting from one method of inducing dehydration and not another? It seems to be only valuable for explaining certain results found in the present experiment.

From the present results, it can be seen that lesioning the LH does not affect the amount of work a rat will do to obtain reward, that is, it does not affect motivational strength as measured by the PR paradigm. LH lesions do not affect food and water intake, but do impair the rats' responses to certain physiological challenges. As already mentioned, there seems to be an importance in the time factor of the onset of dehydration. LH-lesioned rats are unable to respond immediately to dehydration, but can after a time lapse. They are able to make the necessary vasopressin response which regulates the amount of water excreted by the kidneys by monitoring blood pressure. It seems as if LH-lesioned rats are able to produce the correct responses to dehydration but are unable to respond to them immediately, as shown by the inability to respond to the immediacy of hypertonic saline. The prandial drinking physiological challenge allows the LH-lesioned rats time to respond to the dehydration signals, and so the response is intact. They are deficient in their immediate motivated behaviour in response to dehydration.

GENERAL DISCUSSION

To say that the PPTg and the LH are involved in motivated behaviours is a gross over generalisation. It is clear from the previous experiments reported that each structure plays a complex role in only certain motivated behaviours. The PPTg has been shown to be important in the expression of the rewarding effects of psychoactive drugs (Bechara and van der Kooy, 1989, 1992a, 1992b; Bechara, Harrington, Nader and van der Kooy, 1992c) and now the present study shows that it is implicated in motivational strength. On the other hand, the LH is important in the expression of motivated behaviours concerned with subtle aspects of food and water intake regulation.

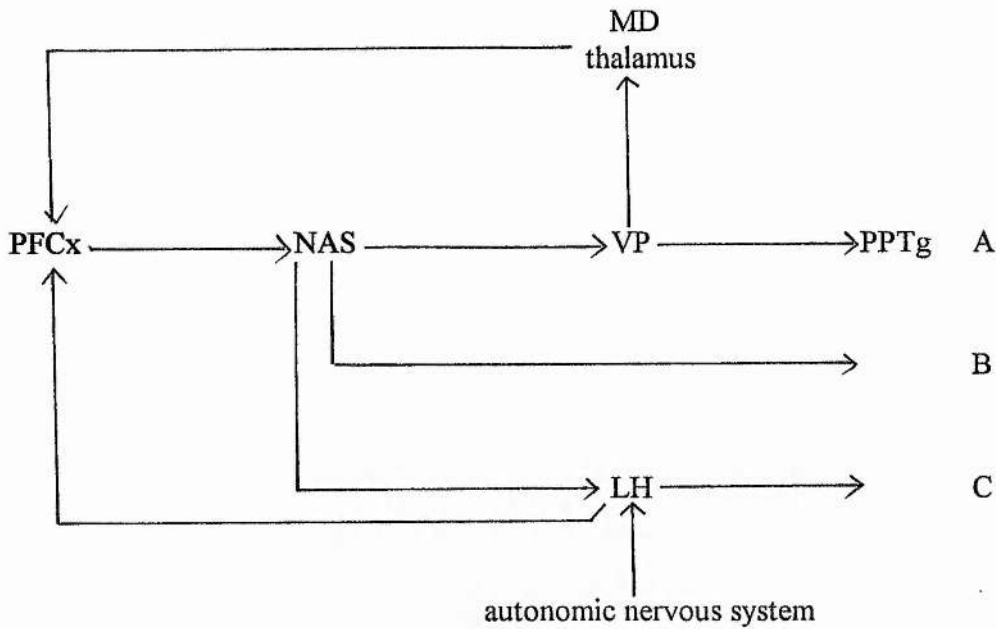
Both the PPTg and the LH are connected to the NAS, a site known to be important in rewarding mechanisms. Thus it does not seem surprising that both structures have a certain role to play in motivation. It does, however, seem that the PPTg is a more likely output station for the NAS-mediated incentive behaviour. Salamone, Mahan and Rogers (1993) found that NAS DA depletion did not impair food intake, feeding rate or time spent feeding in 30 minute feeding tests. Dunbar et al. (1992) found the same results after lesioning the PPTg with excitotoxins. LH-lesioned rats, however, appeared hypophagic and hypodipsic for the first week or so after surgery. Further evidence to support the idea that the PPTg is an output station for the NAS is from a study by Salamone, Kurth, McCullough, Sokolwski and Cousins (1993). They investigated the involvement of DA in the local rate of responding on a FR5 instrumental lever pressing schedule. Administration of the DA receptor antagonist, haloperidol, produced a dose-related impairment in lever pressing on the FR5 schedule. These results showed that DA was involved in instrumental lever pressing responses. This was deemed especially true for instigating responding early, since DA depletion decreased responding in the early portions of the test sessions. Thus, NAS DA is important for incentive-driven behaviours. The present study shows that only lesions of the PPTg affected lever pressing responses whereas LH-lesioned

rats responded in the same manner and to the same extent as control rats. It is possible to compare FR and PR lever pressing tasks since studies have shown them to produce comparable results (for example, Winger and Woods, 1985). From the above studies, it is possible to hypothesise that it is the PPTg which mediates NAS output, since the two structures seem to be involved in similar aspects of motivated behaviour. The VP is the intervening structure between the NAS and the PPTg, and has been implicated in reward-related behaviours. For example, ibotenate lesions of the VP disrupted opiate and cocaine CPP, with the rostral VP lesions producing a significant attenuation, whereas caudal VP lesions produced only a weak disruption (McAlonan, Robbins and Everitt, 1993). These studies, together with anatomical evidence showing a major projection from the NAS to the VP (Zahm and Heimer, 1993) add strength to the evidence that the VP is a part of the pathway involved in reward. Semba and Fibiger (1992) showed that the substantia innominata, part of the ventral pallidal complex, was retrogradely labelled by WGA-HRP injections into the PPTg. Thus the PPTg is connected to the NAS via the ventral pallidal complex.

Zahm and Heimer (1993) showed that the LH, as well as the VP, is a major efferent target of the shell part of the NAS, but instrumental responding for a rewarding stimulus seems to be governed by the NAS-VP-PPTg pathway rather than the NAS-LH pathway. Microinjections of *d*-amphetamine into the NAS results in a dose-dependent selective increase on the lever that produced a CR (Taylor and Robbins, 1984). Hubner and Koob (1990) found that VP lesions produced a detrimental performance in both FR and PR schedules of reinforcement, and Inglis et al. (1994b) showed that excitotoxic lesions of the PPTg disrupted responding on a CR schedule of reinforcement. Thus it is clear that these 3 structures are implicated in instrumental responding. The present study shows that lesioning the LH apparently does not produce any detriment to responding on levers. CR, FR and PR schedules, however, are different tasks, therefore it would be more appropriate to test LH-lesioned rats on CR and FR schedules. The hypothesis would be that they would

respond on a similar level as control rats, since they were able to respond equally well on the PR schedule - all tests involving lever pressing instrumental responses.

Figure 28: Diagram of possible outflow pathways from the NAS mediating incentive-driven behaviours.



The above diagram represents the possible outflow pathways from the NAS which are involved in motivated behaviours. From the present study and previous experiments, it seems that the NAS-VP-PPTg pathway (A on figure 28) is involved in expression of motivational strength. There may be other outflow pathways mediating the same behaviour that have yet to be discovered and these are represented by output to B. It is not surprising that the LH is associated with motivated behaviours towards the regulation of food and water intake, due to its direct connection with the neuroendocrine and autonomic nervous systems. Additional evidence comes from studying the remaining connections of the NAS. Efferents are found projecting to several other areas associated with different motivated behaviours, such as the SNC and the VTA (Winn, 1991 and Parker, 1993, respectively). The LH is also connected

to the PPTg via heavy cholinergic fibres, but as the PPTg has been shown to be involved in motivational strength, and the LH has not, the sites associated with this aspect of motivated behaviour must be relatively site specific. The most influential input to the LH comes from the autonomic nervous system and information is relayed from there to the prefrontal cortex, on to the NAS, finally projecting back to the LH itself. The NAS may only be a relay station for feedback from the prefrontal cortex to the LH. Despite numerous studies suggesting that the NAS-VP-MD is the important pathway in the expression of motivated behaviours, all the experiments carried out in this laboratory to date have found the PPTg to be a critical site in the process.

Future experiments could determine whether lesioning the NAS affects responding on a PR schedule of reinforcement. By looking at the effect of NAS lesions on normal CR responding, it is likely that a detrimental performance would be the result. Robledo and Koob (1993) found that rats with lesions of the VP did not differ significantly from controls when tested on a PR schedule. Their lesions were, however, of the subcommissural VP, and as already discussed, it is the rostral VP that is critical in the expression of NAS mediated reward-related behaviour. This specific area needs to be lesioned and the rats tested on the PR schedule to determine the effect on motivational strength. Lesioning the NAS and the rostral VP of rats and then testing them on a PR schedule of reinforcement would clarify the issue.

CONCLUSIONS

1. The LH and the PPTg are involved in different aspects of motivated behaviours.
2. Both structures receive projections from the shell of the NAS.
3. The PPTg is involved in the expression of motivational strength as measured by the PR paradigm.
4. Evidence indicates that the PPTg is an outflow station for NAS mediated incentive-driven behaviours, via the rostral VP.
5. The LH plays an important role in the regulation of food and water intake. Lesions displayed subtle deficits in the responses to physiological challenges.
6. The LH does not seem to be involved in the expression of motivational strength.

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