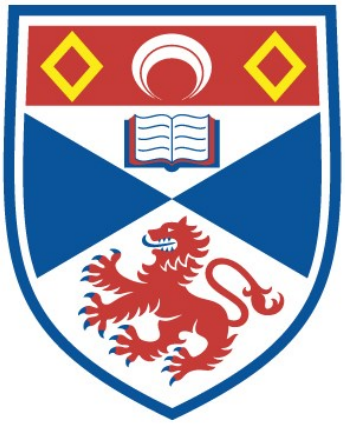
EXAMINATION OF THE ROLE OF THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS IN THE CONTROL OF BEHAVIOURAL PROCESSES

Glenda Louise Keating

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



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Examination of the role of the pedunculopontine tegmental nucleus in the control of behavioural processes

A thesis submitted to the University of St. Andrews

for the degree of Doctor of Philosophy

by

Glenda Louise Keating

School of Psychology

University of St. Andrews

September 1998

DECLARATION FOR THE DEGREE OF Ph.D.

I, Glenda Louise Keating, hereby certify that this thesis, which is approximately 50 000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

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Abstract

The role of the pedunculopontine tegmental nucleus (PPTg) in the control of behavioural processes was investigated in this thesis. This was achieved through examination of:

(1) Conditioned place preference formation: PPTg lesioned rats were not impaired in forming an appropriate place preference, regardless of their deprivation state.

(2) Reward-related responding: both food deprived and non-deprived lesioned rats displayed disinhibited intake across a gradient of sucrose rewards, the degree of disinhibition increasing as the reward became stronger. This disinhibited responding was disassociated from simple approach behaviour as shown by similar runway completion times across control and lesioned rats.

(3) Radial arm maze performance: PPTg lesioned rats were impaired in their ability to retrospectively plan and forage in a random foraging task. This impairment was seen in both acquisition and retention tasks. PPTg lesioned rats were also impaired in the acquisition of a spatial working memory task in which they had to prospectively plan and execute responses.

(4) These behavioural tasks are related to striatal output. To complement them anatomical experiments examining altered striatal outflow on neurotransmitter expression in the PPTg were conducted. Neither dopamine receptor blockade nor 6-hydroxydopamine (6-OHDA) lesions of striatal dopamine produced changes in nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase expression in the

PPTg. This work did, however, lay the foundation for future experimentation to address this question.

The combination of these findings extends current literature to outline a role for the PPTg in the control of complex behaviours that have been previously associated with sites higher up the neuraxis. This thesis demonstrates that removal of the PPTg results in behaviours that are inappropriate and disinhibited. In conclusion the PPTg is important for both accurate response selection and execution of goal directed behaviours, elements crucial for effective behavioural responding.

Abbreviations

2-DG	2-deoxyglucose
5-HT	5-hydroxytryptamine; serotonin
6-OHDA	6-hydroxydopamine
8-OH-DPAT	8-hydroxy-2-(di-*n*-propylamino) tetralin
ACh	acetylcholine
AChE	acetylcholinesterase
ADS	antibody diluting solution
ANOVA	analysis of variance
ARAS	ascending reticular activating system
cAMP	adenosine-3', 5'-monophosphate
CCK	cholecystokinin
Ch5	cholinergic area 5; cholinergic cells of PPTg and SPTg
ChAT	choline acetyltransferase
CP	caudate-putamen
CPP	conditioned place preference
DA	dopamine
DAB	diaminobenzidine
DAMGO	Try-D-Ala-Gly-MePhe-Gly(ol)
DSWS	delayed spatial win shift task
EEG	electroencephalograph
FR-1/FR-2	fixed ratio 1 or fixed ration 2
GABA	γ- amino butyric acid
IgG	immunoglobin
i.p.	intraperitoneal
LC	locus coeruleus
LDTg	laterodorsal tegmental nucleus
L-NAME	N^G-Nitro-L-arginine methylester hydrochloride
LTS	low threshold inward calcium spike
MAO	monoamine oxidase
MEA	midbrain extrapyramidal area

mPRF	medial pontine reticular formation
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NAcc	nucleus accumbens
NADPH	nicotinamide adenine dinucleotide phosphate
NaOH	sodium hydroxide
NBM	nucleus basalis magnocellularis
NLA	N^G-nitro-L-arginine
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NOS	nitric oxide synthase
PBS	phosphate buffered saline
PGO	ponto-geniculo-occipital
PPI	pre-pulse inhibition
PPN/PPTg	pedunculopontine tegmental nucleus
PR	progressive ratio
PTX	pertussis toxin
RBD	REM sleep behaviour disorder
REM	rapid eye movement
RF	random foraging task
s.c.	subcutaneous
SN	substantia nigra
SNAP	S-Nitroso-N-acetyl-penicillamine
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
SPTg	subpeduncular tegmental nucleus
TOH	tyrosine hydroxylase
VLCP	ventolateral caudate-putamen
VTA	ventral tegmental area

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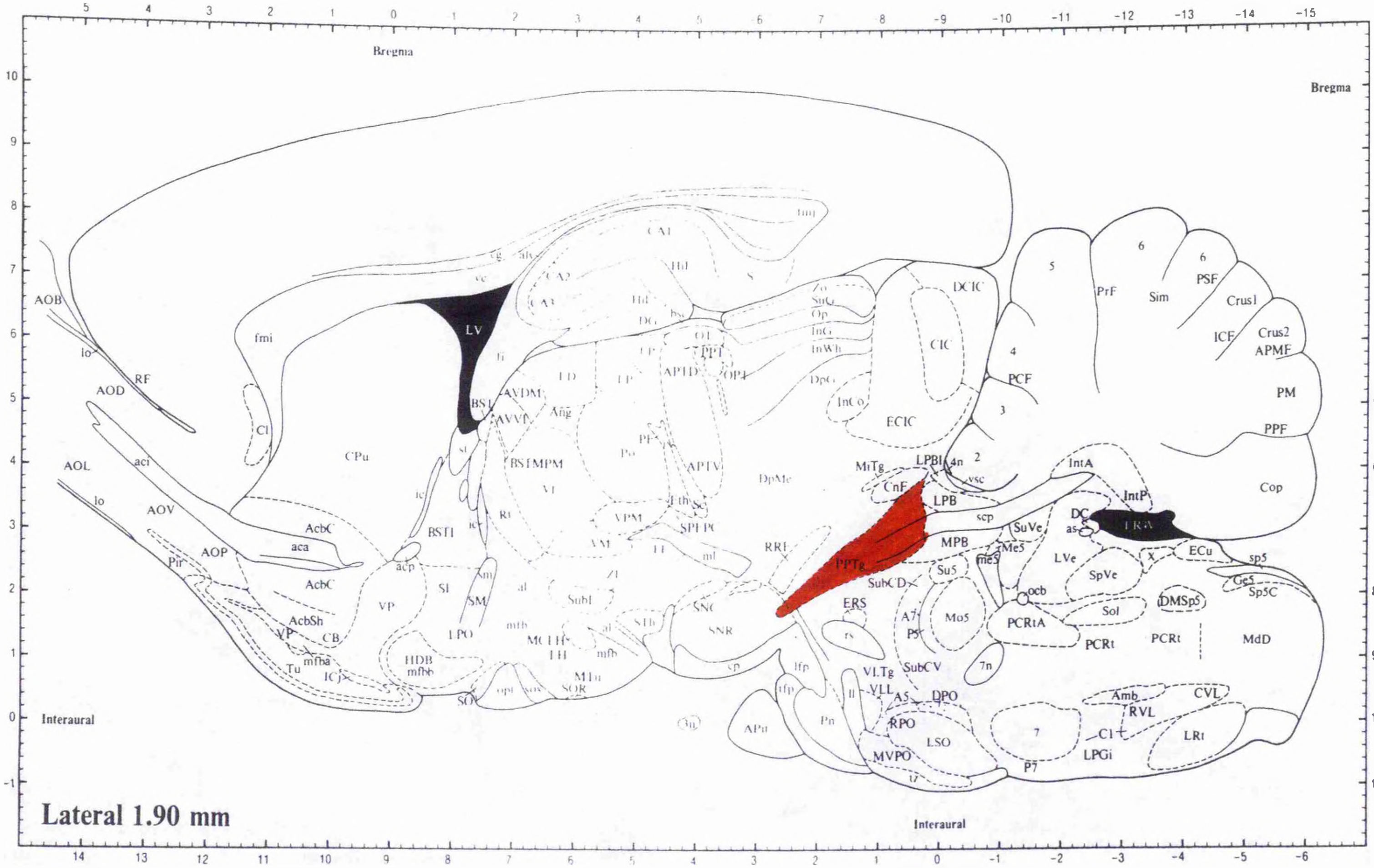
Chapter 1. The pedunculopontine tegmental nucleus

1.0 Introduction

Understanding the function of the pedunculopontine tegmental nucleus (PPTg) is a task in its infancy. Though the area has been anatomically recognized and described for decades, relating this anatomy to function has been a comparatively recent venture. The anatomy itself is controversial and still very much debated. In the rodent brain, and hence the definition used by this thesis, the PPTg is a heterogeneous collection of cholinergic and non-cholinergic neurons that extends from the caudal pole of the substantia nigra to the rostral edge of the parabrachial nucleus. It lies in close association with the ascending limb of the superior cerebellar peduncle (see Figure 1.0.1). It is bordered laterally by the lemniscal fibers and dorsally by the cuneiform and deep mesencephalic nuclei. There are approximately 1600-1700 cholinergic neurons per hemisphere in the rodent PPTg (Rye et al., 1987) and along with the subpeduncular (SPTg) and laterodorsal tegmental (LDTg) nuclei form a continuous column of brainstem cholinergic nuclei. Over the years this area has been defined using various terminology, but the above delineation comes from the work of Paxinos and Watson (1986) and their development of a stereotaxic atlas for the rat brain. Wainer and colleagues, (Rye et al., 1987) and subsequent work (Hallanger et al., 1987; Hallanger and Wainer, 1988; Steininger et al., 1992), for example, described this area based on the cytoarchitecture and cytochemistry of neurons. Their description includes a cholinergic PPTg area and the 'midbrain term extrapyramidal area' (MEA), which refers to the non-cholinergic neuronal population that is interdigitated through the

Figure 1.0.1

Sagittal section of the rat brain demonstrating the anatomical position of the pedunculopontine tegmental nucleus (PPTg; outlined in red). This figure was taken from the stereotaxic atlas of the rat brain by Paxinos and Watson (1986, Figure 81).



Lateral 1.90 mm

larger cholinergic neurons. Though both cholinergic and non-cholinergic neurons are mixed through the nucleus, Rye and his colleagues refer only to the cholinergic neurons as the PPTg proper. The presence of the MEA has been debated and work in the squirrel monkey has led Lavoie and Parent (1994a; p. 205) to report *'A region equivalent to the rodent midbrain extrapyramidal area (MEA) could not be identified in the squirrel monkey'*.

Mesulam and colleagues (1983) classified all cholinergic neurons of the central nervous system. They refer to PPTg cholinergic neurons as Ch5, or cholinergic area 5. In this classification, however, they included the cholinergic neurons of the subpeduncular tegmental nucleus, SPTg. Along with Woolf and Butcher (1989), they regard the SPTg as an extension of the PPTg. In contrast, Paxinos and Watson (1986) consider the SPTg as a separate nucleus. Debate continues over the appropriate classification. This may be resolved in the future as continued work examines the connectivity of this area, but for the purpose of this thesis the delineation of Paxinos and Watson (1986) will serve as reference to the PPTg. Though it is acknowledged that there are separate neuronal populations incorporated in this definition, it is their interdigitation and presumed functional interaction that has prompted inclusion of them both in the terminology of this thesis. The work of this lab has argued that the substantia nigra (SN) can serve as a model for the PPTg (Inglis and Winn, 1995; Winn et al., 1997). The substantia nigra may contain two populations of neurons, dopaminergic in the SN pars compacta (SNc) and non-dopaminergic in the SN pars reticulata (SNr), which are distinct in

cytoarchitecture, connectivity and electrophysiological properties, but are still considered one structure because of their functional interaction.

Phylogenetically, the PPTg is a very old structure. Cholinergic PPTg nuclei have been demonstrated in the amphibian (*Rana perezi*) brain as evidenced using choline acetyltransferase (ChAT) staining [1] (Marin et al., 1997). Similarly an obvious PPTg structure has been observed and is evident in other anurans (Marin et al., 1997), urodeles (newts; Marin et al., 1997) and lizards (Luebke et al., 1992; Medina et al., 1993). A series of studies have confirmed the presence of PPTg cholinergic collections in a teleost fish, (the plainfin midshipman; Brantley and Bass, 1988), reptiles (crocodiles; Brauth et al., 1985), birds (pigeon; Medina and Reiner, 1994), rats (Tago et al., 1989; Woolf, 1991), cats (Vincent and Reiner, 1987), ungulates (particularly horses; Gillilan, 1943), primates (Noback, 1959; Nauta and Mehler, 1966; Lavoie and Parent, 1994a,b,c) and man (Olszewski and Baxter, 1954; Rye et al., 1996). Homology of these neurons across species is supported by several facts. In each species the location of the cell groups are in similar areas of the brain and contain large cholinergic neurons with long dendrites that extend into adjacent areas. Across species the projection sites of these neurons are similar and in many species these homologue cholinergic neurons all express NADPH-diaphorase [2], though co-localization experiments for non-mammalian species is not complete. The

[1] ChAT identifies the essential catalytic enzyme for the formation of acetylcholine (ACh); this technique is regarded as the most reliable marker of cholinergic neurons

[2] NADPH-diaphorase is a nitric oxide (NO) synthase responsible for the formation of citrulline and NO from arginine (Vincent et al., 1983), and approximately 90% of cholinergic neurons in the mesopontine tegmentum stain for diaphorase (Vincent and Hope, 1992); thus neurons in the PPTg or LDTg that are reactive for NADPH-diaphorase are presumed to be cholinergic.

degree of homology of this area across species seems to speak volumes for the functional importance of this nucleus. As will be outlined further in this chapter and in the following chapters of this thesis, the PPTg plays a role in a number of functions including locomotor activity, modulation of behavioural state and plays a role in goal directed behaviour and response selection. All of these functions are crucial for basic survival such as capturing prey and avoiding being captured by predators. Evidence of such a structure as far back evolutionary as fish and lizards, and the fact that it has survived and existed through the evolution of so many species, outlines a fundamental role for this structure. It also outlines the importance of study into understanding such a structure.

A further justification for studying the PPTg is that it has been associated with the neuropathology of Parkinson's disease, progressive supranuclear palsy, Alzheimer's disease, and schizophrenia (Hirsch et al., 1987; Jellinger, 1988; Zweig et al., 1989; Garcia-Rill et al., 1995). Cholinergic neurons of the PPTg have been found to be significantly reduced in number and atrophied in Parkinson's disease, progressive supranuclear palsy and Alzheimer's disease (Hirsch et al., 1987; Jellinger, 1988; Zweig et al., 1989). It is uncertain whether loss of cholinergic neurons is primary in any of these disease processes, or is secondary to other degeneration (of the nigrostriatal pathway in Parkinson's disease, for example). However, in some cases association of PPTg pathology with particular symptoms has been made. Loss of PPTg neurons in Parkinson's disease, for instance, has been linked to movement dysfunction (hypokinesia, gait and postural abnormalities) and sleep disturbances seen in this disease.

Garcia-Rill and his colleagues (1995) have implicated the mesopontine tegmentum, particularly the PPTg, in the clinical disorder of schizophrenia in their reported finding of an increased number of cholinergic neurons in the PPTg. Schizophrenia is marked by slow wave sleep disturbances with reductions in stage 3 and 4 of slow wave sleep and reduction in REM latency (Tandon and Greden, 1989). The PPTg has a substantial role in modulation of sleep-wake cycles and deficits in sleep would seem to implicate the PPTg in the pathogenesis of schizophrenic signs and symptoms. Deficits in sensory gating are another hallmark of schizophrenia and sensory gating deficits are accompanied by motor and postural anomalies as well as impaired smooth pursuit eye movements (Reese et al., 1995). In the mesopontine tegmentum, both the PPTg and LDTg have substantial connections with motor regions within the medulla and spinal cord (Grofova and Keane, 1991), as well as the superior colliculus (Beninato and Spencer, 1986; Hallanger and Wainer, 1988) and it could be suggested that disturbances of the PPTg may be involved aspects of schizophrenia. Conclusions drawn from the study of Garcia-Rill and colleagues (1995) include the suggestion that an increase in cholinergic neuronal number in the PPTg could cause a hyperactivity in this area of the system, potentially accounting for some of the signs and symptoms of schizophrenia and will be discussed further in Chapter 8 of this thesis.

1.1 Neuronal identity

A summary of the electrophysiological properties of identified PPTg neurons is outlined in Table 1.1.1. The electrophysiological properties of PPTg and LDTg neurons have been documented using recording techniques from mesopontine slices

Table 1.1.1. A summary of the electrophysiological properties of identified PPTg neurons.

Leonard and Llinás (1990)

Type	Membrane properties	Classification	Identity
Type I	low threshold inward calcium spikes (LTS)	burst firing	small; not reactive for NADPH-diaphorase staining; presumed non-cholinergic
Type II	prominent transient outward potassium current (A-current)	Non-bursting	larger; reactive for diaphorase; presumed cholinergic
Type III	displayed both a LTS and A-current	Tonic and/or phasic	large; reactive for diaphorase; presumed cholinergic

Kang and Kitai (1990)

Type	Membrane properties	Classification	Identity
Type I	LTS	burst firing	small; immuno-negative for ChAT
Type II (A)	58 of 69 neurons: A-current	Non-bursting	both Type II types: larger; 50% ChAT positive
Type II (B)	11 of 69 neurons: LTS and A-current	Tonic and/or phasic	
Type III	neither and A-current nor an LTS	?	larger; immuno-negative for ChAT

in the rat and guinea pig and from this work several classes of neurons have been identified. Using intracellular recording in the guinea pig, Leonard and Llinás (1990) identified three types of neurons. Type I neurons were characterised by displaying low threshold inward calcium spikes (LTS) and classified as burst firing; Type II comprised the majority of the population sampled and exhibited a prominent transient outward potassium current (A-current) and were non-burst firing; and Type III neurons were similar to both Type I and Type II in displaying both a LTS and an A-current whose firing could be classified as either tonic and/or phasic. Display of the LTS and A-current in Type III neurons was voltage dependent suggesting they tend to cancel each others action and thus may act as a mediator mechanism of neuronal firing. Combined retrograde tracing of identified neurons using Lucifer Yellow and rhodamine labelled microspheres injected into the dorsolateral thalamus determined that both Type I and Type II were thalamic projecting. Further work identified neurons with Lucifer Yellow dye and used NADPH-diaphorase histochemistry to identify specific morphology and to determine if any of these neurons were classified as cholinergic. This process determined that Type I neurons were small, with ovoid to fusiform somas that gave rise to 3-4 narrow primary dendrites and were not reactive for diaphorase staining (indicative that they were probably non-cholinergic). Type II and Type III neurons were larger and had 4-9 primary dendrites and both were reactive for diaphorase and thus determined to be cholinergic. These cholinergic neurons have been furthered described as giving rise to long dendrites which can be traced for up to 300 μm from the cell body (Jones, 1990), invading nearby non-cholinergic neuronal populations and interacting with them.

Identification of neuronal types in the PPTg following stimulation from the SNr in the rat brain slice produced somewhat similar results (Kang and Kitai, 1990; Takakusaki and Kitai, 1997). In this classification Type I and Type II neurons were the same as those outlined above for the guinea pig, but Type II neurons were inclusive of those that displayed an A-current only as well as those (11 of 69 identified neurons; Takakusaki and Kitai, 1997) that displayed an A-current followed by an LTS. The latter neuronal type is the same as that classified as Type III by Leonard and Llinás (1990). The addition in the work by Kang and Kitai (1990) and Takakusaki and Kitai (1997), and what they classified as the Type III neurons, were neurons that displayed neither an A-current nor an LTS and thus effectively make up the fourth type of electrophysiologically identified neuronal type of PPTg neurons. Morphological identification of these neurons was similar to the results expressed by Leonard and Llinás (1990), but with the Type III neuron of Kang and Kitai (1990) being small to medium in size, polygonal or triangular in shape with 4-6 primary dendrites. Kang and Kitai (1990) then used ChAT immunocytochemistry to identify cholinergic neurons and found that approximately 50% of their Type II neurons were immuno-positive for ChAT (Type II and III in Leonard and Llinás), while their Type I and Type III neurons were negative and presumed non-cholinergic. Again these results are similar to those found in the work of Leonard and Llinás (1990) with the addition that neurons identified as expressing neither an A-current nor an LTS were non-cholinergic. In these studies the identity of the neurotransmitter type of the non-cholinergic neurons, however, has not been forthcoming.

Although the non-cholinergic neurons of the PPTg have not been characterised with a particular neurotransmitter type(s), glutamate immunoreactivity has been detected in the PPTg in both the rat (Clements and Grant, 1990) and in the squirrel monkey (Lavoie and Parent, 1994a) indicating that at least some of the non-cholinergic neurons are glutamatergic. Glutamate receptor subunits have been found co-localized with NADPH-diaphorase positive neurons with the GluR1 receptor subunit having the highest double labelling of the subunits identified in the PPTg (Inglis and Semba, 1996). There are also γ-aminobutyric acid (GABA) terminals in the PPTg (Moriizumi and Hattori, 1992) as well as serotonergic (Honda and Semba, 1995) and catecholaminergic (Krosaka et al., 1988) input to neurons in the PPTg. Finally several peptides, including substance P, gastrin-releasing peptide, corticotropin-releasing factor and the atrial natriuretic peptide, atriopeptin, have been found co-localised in cholinergic and non-cholinergic neurons (Vincent et al., 1986; Standaert et al., 1986; Austin et al., 1995).

1.2 Connectivity

The PPTg, as defined above, has extensive afferent and efferent connections. Detailed and comprehensive descriptions of the connectivity of the PPTg have been reported on several occasions (Rye et al., 1987; Hallanger and Wainer, 1988; Semba and Fibiger, 1992; Steininger et al., 1992; Inglis and Winn, 1995), and thus this thesis makes no attempt to provide an exhaustive review, but a thorough outline of the major connectivity.

1.2.1 Afferents

The major afferent inputs to the PPTg are outlined in Table 1.2.1. The outline of this table comes from the work of Inglis and Winn (1995) with various revisions and updates. While the majority of the identification of these connections were conducted using rodents, a proportion of work has been carried out using cats or primates and these are specifically identified in Table 1.2.1. The functional significance of all of these will not be discussed here, other than an overview of the most extensively researched. It is also noted that alternate nomenclature was used in several of the studies outlined here, as in the case from studies of Wainer, Rye and their colleagues and their use of the PPN/MEA distinction (Hallanger et al., 1987; Rye et al., 1987; Hallanger and Wainer, 1988; Rye et al., 1988; Steininger et al., 1992; Rye et al., 1996; Steininger et al., 1997a; Steininger et al., 1997b), and from other work in which cats were used in which the area in question is referred to as the 'peribrachial area of the pedunculopontine nucleus' (Smith et al., 1988; Steriade et al., 1988). To maintain contiguity, the anatomical area in question is referred to as the PPTg in this thesis. The PPTg receives vast input ranging from the cortex to downstream sites of the ascending reticular activating system reflecting the functional diversity of this structure. The face, arm, leg and trunk areas of Brodmann's area 4 of the motor cortex projects to the PPTg and thus provides a source of corticomotor input there (Hartmann-von Monakow et al., 1979). This motor input is thought to converge with basal ganglia input from structures such as the internal segment of the globus pallidus (Nauta and Mehler, 1966; Rye et al., 1996; Shink et al., 1997; or the rat homologue, the entopeduncular nucleus, Jackson and Crossman, 1981a; Steininger et al., 1992) and the substantia nigra (Nakamura et

Table 1.2.1 A summary of the efferent and afferent connections of the pedunculopontine tegmental nucleus (PPTg). See text and Appendix 1 for references. The majority of studies were conducted using rodents; * indicates those that used primates and † indicates those that used cats.

EFFERENT CONNECTIONS

Cortex

medial frontal cortex (including prelimbic and anterior cingulate)[23,41]
motor cortex[28]
sulcal frontal cortex[41]
area 17 of the visual cortex[18]

Basal Ganglia and Related Structures

caudate-putamen[23,26*,28,33*,41]
nucleus accumbens[41]
internal segment of the globus pallidus/ entopeduncular nucleus[6*,7,8,21,26*,28,38,41]
globus pallidus (external)[6*,21,22,23,28,41]
substantia nigra (SNc and r)[2,7,9,21,25*,26*,27*,28,35,38,41]
subthalamic nucleus[4,8,21,23,26*,28,38,41]
ventral tegmental area[6*,21,26*,35]
superior colliculus[1,13†,24]

Thalamus

all principal nuclei, including the reticular nucleus[14,23,26*,28,29,41,46†,47,48,51*†]
intralaminar nucleus[21,48]
parafascicular[21,48]

Limbic system

magnocellular preoptic area[22,23,29,42]
lateral hypothalamus[15,21,23,41]
suprachiasmatic nucleus[5]
amygdala[15]
medial and lateral septal area[15,23]
horizontal limb of the diagonal band [2,29,42]
substantia innominata[22,23,29]
zona incerta[23]

Pons, Medulla and Spinal cord

raphe nuclei (magnus, dorsal and median)[53]
locus coeruleus and subcoeruleal region[39,53]
deep cerebellar nuclei[37,53]
pontine reticular formation[21,44]
pontomedullary reticular nuclei[11,37,53,54]
gigantocellular tegmental field[11,30†]
spinal cord (as far as lumbosacral)[21,39]

AFFERENT CONNECTIONS

Cortex

precentral motor cortex (Brodmann area 4)[16*]
medial prefrontal cortex[36,43]

Basal Ganglia and Related Structures

ventrolateral caudate-putamen[3]
globus pallidus (medial)[19,31,38,49]
internal segment of the globus pallidus/ entopeduncular nucleus[19,34*,38,40*,43,45*,49]
subthalamic nucleus[19,20,43]
substantia nigra (SNc and r)[12,19,32,38,43,49]
ventral tegmental area[19,43,49]
superior colliculus[43,49]

Ventral striatum and related structures

nucleus accumbens (shell and rostral pole)[3,55]
substantia innominata[10,43,49,52]
bed nucleus of the stria terminalis[19,43,49,52]

Limbic system

central nucleus of the amygdala[19,43,49]
lateral hypothalamus[19,43,49]
zona incerta[19,43,49]
amygdala[19,43,49]
lateral habenula[43,49]

Ascending reticular activating system (ARAS) and medulla/spinal cord

locus coeruleus[23,43,49]
dorsal and medial raphé nuclei[43,49,50]
laterodorsal tegmental nucleus[43,49]
contralateral PPTg[43,49]
pontine reticular formation[43,49]
gigantocellular tegmental field[23,49]
deep cerebellar nuclei[17*,49]
spinal cord (as far as lumbar levels)[43,49]

al., 1989) which would then be further directed to motor portions of the pons, medulla and spinal cord to affect motor activity. These descending projections include sites in the pontine reticular formation (Mitani et al., 1988), medullary reticular formation (Nakamura et al., 1989; Woolf and Butcher, 1989; Grofova and Keane, 1991), gigantocellular medulla (Mitani et al., 1988; Grofova and Keane, 1991) and cervical and lumbar portions of the spinal cord (Jackson and Crossman, 1983; Rye et al., 1988; Semba and Fibiger, 1992; Steininger et al., 1992), thus establishing a potential pathway by which the PPTg can affect motor processes. It is thought that excessive inhibition from the globus pallidus to the PPTg is the source of hypokinesis in Parkinson's disease, a theory which may be partially supported by the finding of increased globus pallidus and PPTg activity in metabolic 2-deoxyglucose (2-DG) uptake measures in cynomolgus monkeys made hemiparkinsonian (Mitchell et al., 1989). Indeed release of this excessive inhibition is thought to restore movement function and thus pallidotomy is used as a source of treatment for Parkinsonian patients. Post pallidotomy anatomy reveals degenerating pallidotegmental axons in the dorsolateral PPTg (Rye et al., 1996) further confirming this connectivity. The PPTg in fact receives a vast amount of basal ganglia output from structures such as the striatum (Berendse et al., 1992; Zahm and Heimer, 1993), the subthalamic nucleus (Jackson and Crossman, 1981a, b; Semba and Fibiger, 1992) and the substantia nigra pars reticulata (Jackson and Crossman, 1981a, Semba and Fibiger, 1992; Steininger et al., 1992; Grofova and Keane, 1991; Grofova and Zhou, 1998). It is uncertain how much of this output is directed to the cholinergic versus non-cholinergic neurons of the PPTg, or if in fact both portions are in receipt. Some work has suggested that it is the non-cholinergic neurons that are in receipt of this

basal ganglia output (Rye et al., 1996; Grofova and Zhou, 1998), though at the same time it is acknowledged that terminal fields of these output fibers partially overlap the area occupied by the cholinergic neurons in the PPTg, emphasising that such segregation is not as distinct and easy to define (Grofova and Zhou, 1998).

The PPTg also receives input from downstream sites of the ascending reticular activating system. This includes noradrenergic input from the locus coeruleus (Jones and Yang, 1985; Steininger et al., 1992), serotonergic and other input for the dorsal and median raphe nuclei (Steininger et al., 1992; 1997b) and input from cholinergic LDTg neurons and contralateral PPTg neurons (Steininger et al., 1992). These inputs are believed to contact the cholinergic elements of the PPTg, but may, as in the case of the dorsal raphe input, contact both cholinergic and non-cholinergic elements with equal proportion. Recent work by Steininger and colleagues (1997b), using the sensitive biotinylated dextran anterograde labelling technique, found that 12% of dorsal raphe terminals synapsed on ChAT immunoreactive dendrites and that many contacted non-ChAT labelled dendrites, thus indicating dual distribution to both cholinergic and non-cholinergic elements from the dorsomedial region of the dorsal raphe. Input to the PPTg from these ascending reticular activating sites is thought to play a role in behavioural state in control of modulation of rapid eye movement (REM) sleep phenomenology and modulation of PPTg neuronal discharge patterns (Steininger et al., 1997b).

Though mentioned briefly above, the PPTg is in receipt of both dorsal and ventral striatal outflow, including input from the ventrolateral caudate-putamen and

the shell and rostral pole portions of the nucleus accumbens (Berendse et al., 1992). Processing in the nucleus accumbens has been particularly attributed as limbic or emotive and in the past the nucleus accumbens has been referred to as a limbic motor interface (Mogenson et al., 1980). Since then further research has outlined a role for the PPTg with limbic processing and in receipt of limbic outflow (Inglis et al., 1994b; Robertson et al., 1994). It has been suggested that the PPTg can be regarded as a limbic motor interface as well, integrating information from striatal limbic sites and other basal ganglia motor sites and in turn affecting downstream motor output and related activity (Winn et al., 1997). The limbic-motor interface is a catch all phrase and applying it to either the nucleus accumbens or the PPTg is far too simplistic. The processing of limbic information and then relaying output to affect motor sites is carried out in a number of additional sites including the hippocampus and amygdala and thus referring to one structure or another as the site of such activity is inaccurate and effectively incorrect. The nucleus accumbens and the PPTg may play a role in these processes of limbic and motor integration, but that is not to say that either is *the* limbic-motor interface, but rather *a* structure amongst a network that carries out these roles.

1.2.2 Efferents

The major efferent projections of the PPTg are outlined in Table 1.2.1. Again the outline of this table was taken from the work of Inglis and Winn (1995) with various revisions and updates. It is also again noted that while most of the anatomical work in identifying these connections has been carried out using rodents, several studies have used either cats or primates and these are specifically identified

in Table 1.2.1. One of the most detailed foci of the PPTg connections has been that of the widespread cholinergic innervation of the thalamus. Initial studies demonstrated PPTg connections to specific thalamic nuclei including the ventromedial, ventroanterior and ventrolateral (Saper and Loewy, 1982) as well as the anteroventral, rostral intralaminar and reticular nuclei (Sofroniew et al., 1985). Then, work by Hallanger and her colleagues (1987), using small injections of retrograde tracer localized to discrete thalamic subnuclei found that the PPTg cholinergic cell group labelled after every injection. These connections were soon verified in the cat and monkey (Steriade et al., 1988), confirming that all sensory, motor, associational and limbic thalamic nuclei were innervated by the PPTg. Double labelling of identified retrogradely labelled neurons revealed that 70-85% of these neurons were positive for ChAT immunohistochemistry. One role of this extensive thalamic, and predominantly cholinergic, innervation is thought to be involved in regulating sleep and wakefulness, as stimulation of the PPTg directly excites thalamocortical neurons, increases metabolic activity of the thalamic nuclei and causes desynchronization of the cortical electroencephalograph (EEG) and behavioural arousal (Steriade and Llinás, 1988). It has also been implicated in REM sleep-related events such as the production of ponto-geniculo-occipital (PGO) waves. Kainate lesion studies have found that extensive loss centered on the mesopontine cholinergic area, including both the PPTg and nearby LDTg, as well as the locus coeruleus (LC) was associated with loss of REM sleep and correlated with a decreased rate of PGO spiking (Webster and Jones, 1988). These projections may play a role in the switching of thalamic modes from slow wave sleep and burst firing to single spiking and the waking state, and thus act to regulate states of sleep and

wakefulness. Further examination of the role of the PPTg in behavioural state control will be outlined in the following chapter.

Related to cortical activation, arousal and behavioural state, the PPTg sends a small proportion of output to basal forebrain structures including the substantia innominata, magnocellular preoptic area, nucleus basalis magnocellularis, nucleus of the horizontal limb of the diagonal band and the medial septum (Jones and Yang, 1985; Semba and Fibiger, 1992; Losier and Semba, 1993). The work of Losier and Semba (1993) found that a population of neurons projecting to the basal forebrain were immunoreactive for ChAT and a subpopulation of these neurons had dual projections that innervated both the basal forebrain and the thalamus, lending support to the notion that these neurons may be related to behavioural state activation and maintenance. Here the PPTg can play a role in modulating cortical activation by modulating the activity of cortically projecting structures and have activational affects on alertness and vigilance.

Another important source of PPTg output that has been widely examined has been its connectivity to structures of the basal ganglia. These have included the substantia nigra (Saper and Loewy, 1982; Jackson and Crossman, 1983; Rye et al., 1987; Lee et al., 1988; Beninato and Spencer, 1987; Gould et al., 1989; Lavoie and Parent, 1994b,c; Oakman et al., 1995; Charara et al., 1996), the subthalamic nucleus (Saper and Loewy, 1982; Jackson and Crossman, 1983; Jones and Yang, 1985; Rye et al., 1987; Lee et al., 1988; Lavoie and Parent, 1994b; Bevan and Bolam, 1995; Clarke et al., 1997), the external segment of the globus pallidus (Saper and Loewy,

1982; Jackson and Crossman, 1983; Jones and Yang, 1985; Lee et al., 1988; Charara and Parent, 1994), the entopeduncular nucleus/internal segment of the globus pallidus (Saper and Loewy, 1982; Jackson and Crossman, 1983; Jones and Yang, 1985; Rye et al., 1987; Lee et al., 1988; Lavoie and Parent, 1994b; Charara and Parent, 1994; Clarke et al., 1996; Clarke et al., 1997) and the caudate-putamen (Saper and Loewy, 1982; Jones and Yang, 1985; Lee et al., 1988; Nakano et al., 1990; Lavoie and Parent, 1994b). Afferent input from these structures has been noted above, but reciprocal connectivity to these basal ganglia sites has also been explored. Input to these sites is believed to be related to the extrapyramidal role of these basal ganglia structures and this site provides a point from which output feedback can be relayed back into the system. A proportion of the input back into these structures, particularly the substantia nigra, subthalamic nucleus and entopeduncular nucleus is believed to be cholinergic and glutamatergic as identified by double labelling studies involving retrograde tracer identification from these structures to the PPTg and immunohistochemistry for acetylcholine and glutamate (Bolam et al., 1991; Lavoie and Parent, 1994c; Bevan and Bolam, 1995; Clarke et al., 1996; Clarke et al., 1997). In the squirrel monkey, double labelling for retrograde tracer and ChAT immunoreactivity found approximately 25% of the PPTg projection to the substantia nigra was ChAT positive (Lavoie and Parent, 1994c), while approximately 39% of the input to the internal segment of the globus pallidus was ChAT immunoreactive (Charara and Parent, 1994). Input to both the substantia nigra and globus pallidus is then thought to project to the striatum. The indirect role of the PPTg in modulation of the striatum via the substantia nigra has been identified using *in vivo* chronoamperometry in the rat. Blaha and Winn (1993) examined the

effect of substantia nigra manipulation on dorsomedial striatal dopamine efflux and found increases in dopamine efflux following intranigral injections of the cholinesterase inhibitor, neostigmine, which were attenuated in rats with quinolinic acid lesions of the PPTg. Similarly, unilateral excitation of the PPTg using scopolamine resulted in dose dependent increases in dopamine efflux in the dorsal striatum, effects which were thought to have acted via a cholinergic PPTg input to substantia nigra dopamine neurons (Chapman et al., 1997). While some studies suggest a proportion of cholinergic input to the basal ganglia sites outlined above, this is not the only source of PPTg efferent connectivity to these sites. Identification of additional neurotransmitter sources which further make up these PPTg efferent influences are under exploration and are yet to be fully outlined.

Finally, further downstream sites of PPTg innervation include the pontine reticular formation (Jackson and Crossman, 1983; Semba, 1993), the gigantocellular field of the medulla (Rye et al., 1988) and output to points as far away as the cervical and lumbar portions of the spinal cord (Jackson and Crossman, 1983; Rye et al., 1988). These latter innervation sites are thought to relate to the proposed role of the PPTg in locomotion as output from extrapyramidal sites of the basal ganglia is thought to be relayed through the PPTg to these medullary and spinal cord points to effect locomotor output and responding. Further details outlining the proposed role of the PPTg in locomotion will be addressed in Chapter 2. Downstream innervation to the pontine reticular formation, also referred to as the REM sleep induction zone (Semba, 1993), is thought to be part of the cascade of events that relates to the role of the PPTg in behavioural state control and the switch from the waking state to REM

sleep. Approximately 30% of the input from the PPTg was found to be positive for ChAT immunoreactivity, relating the partial role of cholinergic innervation to this process. Innervation to the pontine reticular formation and its role in behavioural state will be discussed further in the following chapter.

Overall then, the cholinergic and non-cholinergic neurons of the PPTg are ideally connected to affect the input and output of key brain areas crucial for behavioural processing. Input to basal forebrain and thalamic nuclei affect behavioural arousal and output from the striatum and basal forebrain are crucial for goal directed behaviour and response selection, mediated with input from extrapyramidal sites of the basal ganglia and output from the superior colliculus to affect movement and visually guided orientation (or visuomotor co-ordination). The PPTg can then relay feedback to these extrapyramidal sites to update response processing whose information can then make its way back to cortical sites. Alternatively, the PPTg can send output to downstream sites in the medulla and spinal cord to carry out the appropriate behavioural response that has been processed in the PPTg. The role of the PPTg in behaviour and the type of response processing will be further outlined in the following chapter and experimentally examined in subsequent chapters of this thesis.

Chapter 2: Behavioural processes and the PPTg

2.0 Introduction

As outlined briefly in the previous chapter, understanding of the functional role of the PPTg is by no means complete. Over the years various theories have been proposed and the PPTg has been implicated in a range of functions including behavioural state control, locomotion (resulting in the term 'mesencephalic locomotor region' applied to this area), and a range of behaviours related to striatal outflow that can be classified, but not limited to, goal directed or motivated behaviours. Experimentation related to each of these fields is currently prominent in research facilities around the world, indicating the interest that exists in the function of the PPTg, but at the same time providing indication that definitive answers to the function(s) of this structure are yet to be elucidated. The following sections of this chapter provide an overview of the existing research relating to theories of the function of the PPTg and ends with the experimental outline that is the basis of this thesis.

2.1 Behavioural state control

The role of the PPTg in behavioural state control has long been examined. Cholinergic neurons of the PPTg send dense innervation to the thalamus, including the medial, intralaminar and lateral thalamic nuclei, which results in prolonged depolarisation switching these thalamocortical neurons from burst firing to single-spiking mode and thus a switch from cortical electroencephalograph (EEG)

synchrony to an EEG desynchrony (Steriade et al., 1990a; Reiner, 1995; Rye, 1997). The desynchronisation of the cortical EEG is a hallmark sign of both waking and of REM sleep. PPTg neurons increase their firing rates and their synaptic excitability during waking as well as REM as compared with EEG synchronised or slow wave sleep (Steriade, 1992), and extracellular acetylcholine in the thalamus, presumed to come partially at least from the PPTg, is also high during wake and REM sleep and significantly lower during slow wave sleep (Williams et al., 1994). This increase in PPTg firing during REM sleep is believed to result from a release of inhibition from serotonergic dorsal raphe neurons (Jones, 1991; Steriade, 1992; Leonard and Llinás, 1994; Rye, 1997). These serotonin neurons increase firing during slow wave sleep and then cease firing prior to the onset of REM and remain silent throughout REM sleep (Wainer et al., 1993).

Recording from PPTg neurons during behavioural states have identified several categories of PPTg neurons which include wake/REM-on neurons, REM-on only neurons and REM-off neurons. Wake/REM-on neurons increase their firing rates during both the waking and REM states, whereas REM-on only neurons show preferential discharge during REM sleep and not in waking (Steriade et al., 1990b; Thakkar et al., 1998). This difference is thought to be related to the differential effect of monoaminergic inhibition on these neurons as REM-on only neurons are almost completely suppressed by microdialysis perfusion of the selective serotonin (5-HT) 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), while this agonist has little or no effect on wake/REM-on neurons (Thakkar et al., 1998). Wake/REM-on neurons appear not to be inhibited by serotonin thus

explaining their continued firing during waking compared to the REM-on only neurons. REM-off neurons display progressively lower firing rates as the animal moves from the quiet awake state to slow-wave sleep and to paradoxical sleep (Steriade et al., 1990b; Leonard and Llinás, 1990). Finally, some PPTg neurons fire in advance of the EEG desynchronisation, sometimes up to 1 minute in advance (Steriade et al., 1990b). Firing in the PPTg is also associated with PGO waves, spiky field potentials which heralds REM sleep by approximately 30-90 s and carries on throughout REM sleep (Paré et al., 1990; Steriade, 1992). Stimulation in the PPTg induces a sharp PGO wave in the lateral geniculate nucleus of the thalamus and kainic acid lesions of the mesopontine tegmental area, including the PPTg, reduce or abolish PGO waves (Webster and Jones, 1988). These lesions also reduce the amount of REM sleep but it must be noted that the lesions were not focused solely on the PPTg, but included lesion of the LDTg and LC (Webster and Jones, 1988). Disruptions to REM have also been found with bilateral electrolytic or radio frequency lesions of the PPTg (Shouse and Siegel, 1992). Here PGO spikes were reduced as were the number of REM cycles and the percent of total time in REM. The percent of active REM was correlated with lesion size and ChAT positive areas damaged by PPTg lesions. Pharmacological manipulation of cholinergic PPTg neurons produce the opposite effects. REM sleep can be induced by local injection of neostigmine, an inhibitor of the acetylcholine degradive enzyme acetylcholinesterase (AChE; Jones, 1991), and microinjection of L-glutamate into the cholinergic compartment of the PPTg decreased the latency to REM onset and increased the mean duration of REM sleep (Datta and Siwek, 1997). These effects on behavioural state by cholinergic PPTg neurons are believed to be related to nitric oxide

synthesised there. Reduction of endogenous nitric oxide by microinjection of N^G-Nitro-L-arginine methylester hydrochloride (L-NAME), a competitive inhibitor of nitric oxide synthase enzyme, into the PPTg reduced REM sleep by 62.05% and increasing concentrations of exogenous nitric oxide by microinjection of the nitric oxide donor, S-Nitroso-N-acetyl-penicillamine (SNAP), increased REM by 72.10% (Datta et al., 1997).

A further feature of REM sleep is muscle paralysis or atonia. This is not mediated in the PPTg directly. Bilateral electrolytic or radio frequency lesions did not prevent complete atonia during REM (Shouse and Siegel, 1992), but atonia is hypothesised to be mediated by descending projections from the PPTg and LDTg to the subcoeruleus and then to the ventral medullary gigantocellular field (Wainer et al., 1993; Kohyama et al., 1994). It is disruption of this pathway as it carries outflow from the internal segment of the globus pallidus that is believed to underlie the pathophysiological basis of REM sleep behaviour disorder (RBD). RBD is a clinical disorder hallmarked by interference with the maintenance of REM sleep atonia manifested in the form of nocturnal REM sleep twitches and leg movements (Rye, 1997). Reported to occur in 15% of all Parkinson's disease patients it is hypothesised to result from heightened discharge from the internal segment of the globus pallidus which would excessively inhibit the PPTg and thereby result in limb movement, including leg twitches, that overrides REM atonia (Rye, 1997).

The PPTg is further implicated in behavioural state control through its projection to the cholinoceptive REM sleep-generating structure, the medial pontine

reticular formation (mPRF; Baghdoyan et al., 1993). Microinjection of carbachol into the mPRF increased the number of REM sleep periods and reduced the latency to REM sleep (Gnadt and Pegram, 1986) and increased the total amount of REM sleep from 5.3 ± 6.4 % following saline injection, to 55.8 ± 16.4 % following 4 μg/0.25 μl carbachol (Baghdoyan et al., 1993). This carbachol induced REM sleep has been shown to produce an increase in fos-immunoreactive labelled neurons in the PPTg, LDTg and contralateral mPRF (Shiromani et al., 1995) indicating an increase in neuronal activation in these sites as a result of this carbachol induced REM sleep activation. [3] This increase in fos activity was specific to REM sleep induction as application of a lower dose of carbachol into the mPRF that didn't induce REM did not show an increase in fos immunoreactivity. Further work demonstrated that of the neurons in the PPTg and LDTg that displayed fos immunoreactivity, 11.2% of them were cholinergic as determined by the co-localization of fos and ChAT immunoreactivity in these neurons (Shiromani et al, 1996). Acetylcholine release in the mPRF is increased during REM sleep (Rye, 1997), and this release can be further increased through the dialysis of the muscarinic antagonist scopolamine into the mPRF (Roth et al., 1996). From these findings the authors concluded that acetylcholine release in the mPRF is, in part, regulated by muscarinic autoreceptors presumed to reside on PPTg/LDTg terminals that exist there. Again the role of nitric oxide in acetylcholine release is suspected as microdialysis delivery of the nitric oxide synthase inhibitor, N^G-nitro-L-arginine

[3] FOS is the DNA binding protein product resulting from activation of c-*fos*, an immediate early gene, whose activation is indicative of cellular stimulation and presumed increased neuronal activity. Fos expression has a low basal level in the central nervous system but can be induced to higher levels by physiological stimuli and is transiently expressed in neurons after synaptic stimulation (Hunt et al., 1987; Sagar et al., 1988).

(NLA), resulted in reduced acetylcholine release in the PPTg and mPRF and microinjection of NLA into the mPRF decreased the duration of REM sleep (Leonard and Lydic, 1997).

2.2 Behavioural processes in relation to basal ganglia, particularly striatal, outflow

The PPTg receives basal ganglia outflow particularly from the striatum (caudate-putamen and nucleus accumbens), internal and external segments of the globus pallidus, substantia nigra and subthalamic nucleus as outlined in the previous chapter (Rye et al., 1987; Berendse et al., 1992; Semba and Fibiger, 1992; Steininger et al., 1992; von Krosigk, 1992; Groenewegen et al., 1993; Zahm and Heimer, 1993; also see Table 1.2.1). Activation in the PPTg following dopaminergic loss from these structures has been outlined in a 2-DG uptake study conducted by Mitchell and colleagues (1989). In this study dopamine depletion was brought about by administration of 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP) in the cynomolgus monkey (*Macaca fascicularis*) and accumulation of 2-DG, a marker of glucose metabolism which is indicative of neuronal activation, was examined. Accumulation of 2-DG was evident in several structures but most notably in the PPTg. The authors suggested that increased activity of the globus pallidus in this hemiparkinsonian cynomolgus monkey produced such increased PPTg activity. Miwa and colleagues (1996) support this view in suggesting that the PPTg is under GABAergic control of pallidal output and in Parkinson's disease GABA disinhibition results in rigidity. In their study, haloperidol induced catalepsy was

reduced following injection of the GABA antagonist picrotoxin into the PPTg. Rigidity and flexed posture of upper and lower limbs contralateral to the lesions side was also seen in monkeys following unilateral kainic acid lesion of the PPTg (Kojima et al., 1997), further implicating the role of disrupted basal ganglia outflow to the PPTg.

2.2.1 Oral Stereotypy

Further functional attribution of basal ganglia outflow and relationship with PPTg behaviour comes in the form of orofacial stereotypies. Microinjection of *d*-amphetamine, the dopamine D_1 receptor agonist SKF 38393, or the D_2 receptor agonist, quinpirole, into the rat ventrolateral caudate-putamen (VLCP; the rodent homologue of the primate putamen) produced oral stereotypies such as self-biting, licking, and/or gnawing (Delfs and Kelley, 1990). As the VLCP provides output to the PPTg (Berendse et al., 1992), the role of the PPTg in such behaviour has been examined. Systemic injection of 3.0 or 5.0 mg/kg *d*-amphetamine result in biting and licking stereotypies following ibotenic acid lesions of the PPTg (Inglis et al., 1994a) and similar results were found following microinjection of *d*-amphetamine directly into VLCP in rats bearing bilateral ibotenic acid lesions of the PPTg (Allen and Winn, 1995). In the latter study, as well as producing oral stereotypies such as biting and licking, lesions of the PPTg increased both the number as well as the duration of time engaged in displaying an orofacial stereotypy following *d*-amphetamine into the VLCP. PPTg lesions, then, seem to produce a disinhibition of this striatal stimulated stereotyped behaviour and recent work has identified

polysynaptic connectivity between the PPTg and the masticatory, orofacial and lingual muscles that would mediate this activation (Fay and Norgren, 1997a,b,c).

2.2.2 Locomotion

Early work outlined a role for the nucleus accumbens as a neural substrate important for locomotion. Pijnenburg and colleagues (1973) found that low doses of ergometrine (0.5 or 1.0 μg bilaterally) microinjected into the nucleus accumbens acted on dopamine receptors there and stimulated locomotion which could be blocked by pre-injection of the dopamine receptor antagonist haloperidol (0.1 mg/kg). Work by Kelly and colleagues (1975) found that hyperactive locomotion in response to systemic *d*-amphetamine (1.5 mg/kg) was reduced 14 days after 6-hydroxydopamine (6-OHDA) [4] lesions of the nucleus accumbens. Work by Winn and Robbins (1985) supported this finding in their demonstration that while 6-OHDA lesions of the nucleus accumbens did not block exploratory behaviour in an exploratory choice box, it did decrease the locomotor response to intraperitoneal (i.p.) injection of 1.5 mg/kg *d*-amphetamine. Finally, Jones and colleagues (1981) demonstrated that locomotor activity could be activated by microinjection of dopamine into the nucleus accumbens. Outflow from the nucleus accumbens was then implicated in locomotor mediation when increased locomotor activity stimulated by microinjection of *d*-amphetamine into the nucleus accumbens was reduced by injection of the local anaesthetic procaine in to the PPTg (Brudzynski and Mogenson, 1985). From here, theories of limbic-motor integration involving the nucleus accumbens and outflow to the PPTg were borne (Brudzynski and Mogenson,

[4] 6-OHDA is a neurotoxin specific for destruction of catecholaminergic neurons including dopamine.

1985; Mogenson, 1987). From these early days the PPTg has long been implicated as having a role in locomotion. Studies in which micro-stimulation around the pars compacta of the PPTg resulted in treadmill stepping in the pre-collicular, post-mammillary decerebrate rat and cat preparations (Skinner and Garcia-Rill, 1984; Garcia-Rill, 1991; Lai and Siegel, 1990) combined with existing theories. This type of generated locomotion, however, requires continuous high frequency trains of electrical pulses for several seconds before movement becomes evident, as single electrical impulses never induce stepping. Electrical stimulation of the PPTg in the freely moving rat has also been shown to increase locomotion, rising from 22 ± 5 responses/10 min in the non-stimulation period to 104 ± 27 responses/10 min in the stimulation period (Milner and Mogenson, 1988). Earlier pharmacological manipulation studies also provided evidence for a role for the PPTg in locomotion. Mogenson and Wu (1988) administered picrotoxin into the substantia innominata to elicit locomotion and then found that unilateral injection of procaine into the PPTg reduced this elicited locomotion by 50%. Later work by Mogenson and colleagues (1989) demonstrated that increases in open field locomotor activity to the introduction of novel partitions could be decreased with an injection of 20% procaine directly into the PPTg. The problem with using procaine however, is that it is a local anaesthetic that acts to prevent electrical impulse conduction through all neural membranes and disrupts fibers of passage as well through the region of injection. Procaine's effect on the PPTg then, could have acted at either the neuronal level or through its fibers of passage and makes results less conclusive. Other work by Brudzynski and colleagues (1988) demonstrated that an injection of carbachol could decrease spontaneous locomotor activity measured in a simple plexiglas cage and

could also reduce intra-accumbens amphetamine induced increases in locomotion back to control levels. Here the authors suggest that carbachol modulates the activity of neurons involved in locomotion and does this through its influence on muscarinic receptors in the PPTg which are associated with synaptic transmission. Infusion of opioid receptor agonist into the PPTg has also been shown to effect locomotion. The μ agonist Try-D-Ala-Gly-MePhe-Gly(ol) (DAMGO) administered at 0.1 nmol/side, increased locomotor activity, an effect which was blocked by pre-treatment with i.p. administration of naloxone or haloperidol, both dopamine receptor antagonists (Klitenick and Kalivas, 1994). Finally, work by Bechara and van der Kooy (1992a) examined the effects of PPTg lesions on conditioned locomotion. In this study rats received 2 days of either systemic morphine or *d*-amphetamine administration. On the third day, a 2 minute pre-drug injection session of activity level was used as a measure of the conditioned locomotion to that drug. Control rats showed significant increases in locomotor activity scores to both morphine and amphetamine while bilateral lesions of the PPTg blocked this conditioned hyperactivity. While these studies would suggest a role for the PPTg in both spontaneous and conditioned locomotion, several excitotoxic lesion studies since then have found no such effect. Bilateral N-methyl-D-aspartate (NMDA) lesions have been found to have no effect on spontaneous locomotion or that stimulated by s.c. injections of 0.5, 1.5 or 3.0 mg/kg of *d*-amphetamine (Olmstead and Franklin, 1994). Similarly, bilateral ibotenic acid lesions did not affect locomotion stimulation by i.p. administration of 1.5, 3.0 or 5.0 mg/kg *d*-amphetamine (Inglis et al., 1994a), nor did ibotenic lesions disrupt supersensitive 0.1 mg/kg apomorphine stimulated locomotion following 6-OHDA lesions of the nucleus accumbens that was disrupted by electrolytic lesion of

the dorsomedial nucleus of the thalamus (Swerdlow and Koob, 1987). Bilateral ibotenic acid removal of the PPTg does not affect general home cage locomotor activity as revealed following examination of 24 h home cage locomotion (Winn, 1998 in press), so while a role for the PPTg in locomotion may not be entirely ruled out, these observations do reveal that such locomotor ability is not reliant on structural integrity of the PPTg and is mediated elsewhere.

2.2.3 Startle Reflex

The startle reflex is a stereotyped behaviour elicited by loud acoustic stimuli resulting in muscle contractions of the face and body. The response can be attenuated by pre-presentation of a weak warning stimulus (pre-pulse inhibition or PPI). Work by Swerdlow and colleagues (1990) have shown that PPI is disrupted when there is an increase in dopaminergic activity in the nucleus accumbens or when the GABA antagonist picrotoxin is injected into the ventral pallidum. The accumbens-ventral pallidum pathway provides output to the PPTg, and the PPTg, in turn, has been implicated in mediation of this sensorimotor startle reflex. Recordings of evoked potentials in the PPTg have shown waveforms whenever a startle response was elicited, but no such waveforms were seen if the stimulus failed to produce a motor response (Ebert and Ostwald, 1991). Electrolytic lesions of the PPTg have been found to reduce the PPI (Swerdlow and Geyer, 1993) and more selective quinolinic acid lesions have also shown a significantly reduced PPI without producing an effect on the startle response in the absence of pre-pulse stimuli (Koch et al., 1993). These data reveal that the PPTg may play a role in stimulus-response

association as, when lesioned, the rats are unable to associate the pre-stimulus with the subsequent louder stimulus to inhibit their resulting startle response.

2.2.4 Response selection: motivated and goal directed behaviour

While the PPTg may not play a crucial role in general locomotor ability, it has been implicated as important in various behavioural paradigms related to motivation and goal direction. The ventral striatum has been attributed as playing a role in these motivated and goal directed behaviours, but more recent work has outlined a role for the PPTg in mediation of appropriate responding and inhibition of inappropriate response selection. Consummatory processes do not appear to be affected as excitotoxic lesions of the PPTg do not affect eating and drinking (Dunbar et al., 1992) thus demonstrating no loss in the motivational significance of food and water. The role of the PPTg in motivated behavioural paradigms seems to be more related to response selection, stimulus association and inhibition of responding. Work by Fujimoto and colleagues (1989, 1992) has demonstrated a role for the PPTg in acquisition of passive and active avoidance. In the passive avoidance task the rat is placed in one chamber and then a guillotine door opens to provide access to an adjacent darkened chamber. Once the rat moves across to this new chamber it receives a foot shock. Several days later the rat is re-tested and the time to move into the darkened, previous shock giving, chamber is scored. If the negative association with the darkened environment has been formed then the rats should show increased latencies to now re-enter this chamber. Control rats show exactly this pattern, in that latency to enter the chamber before receiving shock was 17 ± 7 s, but on the post shock trial the latency to enter the room is 241 ± 104 s. PPTg lesioned rats,

however, while having a similar response latency on the pre-shock trial (17 ± 9 s), show a shorter latency than controls on the post-shock trial (105 ± 107 s). While these lesioned rats show a degree of association formation in that their response latencies are much longer than the initial trial, they are much quicker than control rats to re-enter the 'shock' chamber, revealing an impairment in their acquisition and re-use of appropriate negative conditioning cues. A similar acquisition impairment is evident in the active avoidance task. In this task the animal receives a light-tone conditioned stimulus which alerts them of an upcoming foot shock (the unconditioned stimulus). Once the conditioned stimulus comes on, the rat has 5 s to move across to an adjacent safety chamber and avoid the shock. Control rats acquire this association and improve from a avoidance response of 20% in initial trials to over 75% with subsequent trials. PPTg lesioned rats, however, fail to acquire this association and maintained avoidance responding at less than 25% across all trials. The PPTg lesions did not alter the perception of foot shock pain as once shocked PPTg lesioned rats moved to escape and when shock was directly applied to the foot the jump thresholds and vocalizations were equal in both the sham and PPTg lesioned rats (Fujimoto et al., 1989). The problem PPTg lesioned rats had was that they were not able to associate the conditioned stimulus cues with the upcoming foot shock to move in advance to avoid the shock and thus the lesion of the PPTg resulted in inappropriate response selection and impaired task performance.

The PPTg has also been indicated in several operant tasks including responding for self-administration of lateral hypothalamic electrical brain stimulation. Yeomans and colleagues (1993) examined the role of muscarinic drugs

unilaterally injected into the PPTg on bar pressing for such stimulation and found that application of carbachol, a muscarinic agonist, raised frequency thresholds to elicit bar pressing by as much as 400%. Application of the antagonist scopolamine, however, reduced frequency thresholds for bar pressing by 20-80%. Here activity of muscarinic receptors on PPTg neurons either inhibits or excites reward stimulation as shown by the increased or decreased stimulation frequencies required to sustain bar pressing following carbachol and scopolamine, respectively. This revealed that activation of PPTg neurons is essential for response acquisition of hypothalamic rewarding brain stimulation. This result is confirmed by work of Lepore and Franklin (1996) who demonstrated that bilateral NMDA lesions of the PPTg block the acquisition of lever pressing for such lateral hypothalamic brain stimulation reinforcement. Here lesions of the PPTg blocked the ability to acquire appropriate operant responding related to receipt of reinforcement.

Another operant responding task found to involve the PPTg is responding for conditioned reinforcement. Here the animal must associate a reinforcer with neutral environmental stimuli (such as a light and/or tone combination) such that the stimuli then serves as an indicator when a reinforcer is available. The animal learns to press a lever to receive the reinforcer and at the same time learns that the other available lever does not provide reinforcement. In such a conditioned reinforcement task, rats with lesions of the PPTg receiving *d*-amphetamine stimulation of the nucleus accumbens were found to have impaired performance (Inglis et al., 1994b). While lesioned rats responded on the reinforced lever equally as controls, they also press disproportionately on the non-reinforced lever and spent little time on the food-

hopper panel. This inappropriate response selection occurred at all doses of *d*-amphetamine used (10, 20 or 30 μg). Here the lesioned rats demonstrated that they had not distinguished between the reinforced and non-reinforced levers and persisted with unrewarded responding. Lesions of the PPTg then, resulted in impaired appropriate responding and impaired inhibition or suppression of inappropriate response selection.

Responding on schedules of reinforcement for food or for intravenous drug reinforcers has produced varying results following lesions of the PPTg. Work by Robertson and colleagues (1994) revealed that PPTg lesioned rats were able to learn to respond on a fixed-ratio 1 schedule of responding (FR-1; rats need to press the reinforced lever only once to receive the reinforcer) for food reinforcement, but when these rats were moved onto a progressive ratio (PR) schedule test they were significantly impaired. In this task the number of bar presses to receive the next reinforcer increases and the point at which the animal will no longer bar press to receive the reinforcer is termed the breaking point. On a PR schedule of reinforcement for food, food deprived PPTg lesioned rats responded less and had significantly lower breaking points than controls. Here PPTg lesioned rats stopped responding and refused to work as hard as controls for food reinforcement. A similar result was found in recent work by Olmstead and colleagues (1998) examining acquisition of heroin intravenous self-administration. In this study the group found that acquisition of FR-1 responding was blocked in more than half of the rats that had received bilateral NMDA lesions of the PPTg. Those that did acquire the responding were found to have reduced levels of self administration on the FR-1 task

compared to controls, that is received fewer infusions of the 0.1 mg/kg heroin reinforcer. These same rats were then found to have reduced breaking points when moved onto a progressive ratio responding task. Again PPTg lesioned rats ceased responding to the reinforcer at breaking points significantly lower than controls. These results are in contrast to work by Keating and colleagues (1997) using *d*-amphetamine as the self-administered reinforcer. In this study rats with bilateral ibotenic lesions of the PPTg responded eagerly on a FR-2 schedule of reinforcement, taking more infusions of the 0.1 mg/kg *d*-amphetamine and receiving most of these infusions in the first hour of the three hour testing session. These rats then went on to surpass control rats on the progressive ratio task, having significantly higher breaking points than controls and thus receiving more *d*-amphetamine infusions. Here lesions of the PPTg seem to have affected response selection in that the rats' responding on the reinforced lever appeared perseverative or disinhibited. In all of these studies the effect that PPTg lesions had on perception of reward may also need to be considered. In each case the effect on responding could reflect either an increased or decreased perception of the reinforcer. With food and heroin the decreased responding could reflect that the lesioned rats found the reinforcer less rewarding than the controls and so they were not prepared to bar press as much for said reinforcer. Alternatively, the reinforcer could have been more rewarding and the lesioned rats responding was reduced as they were satiated compared to controls and did not want any more reinforcer. The opposite effects could provide an explanation for the *d*-amphetamine responding. Here the lesioned rats may have responded more as they found the reinforcer more rewarding and couldn't get enough or they found the reinforcer less rewarding than controls and thus needed to

take more infusions of the *d*-amphetamine to get the same level of reward effect as controls. Answers are not forthcoming at this time and require more experimentation. Replication of the existing findings is required, along with examinations of the effects of other reinforcers on such responding. Cocaine and morphine self-administration needs to be examined, as well as varying the degree of heroin, *d*-amphetamine and food reinforcers already used to explore responding to different levels of the same reinforcer on lever responding. These studies will also begin to address the nature of responding to natural (food) versus artificial (drug) rewards and the nature of drugs that have different modes of action (stimulants versus depressants).

Spatially mediated behavioural tasks have been found to be influenced by lesions of the PPTg. An earlier place memory task study by Kessler and colleagues (1986) found delay dependent impaired performance following ibotenic acid lesions of the PPTg. In this task rats had access to three cue specific compartments, one of which contained food reward. The object of the task was that the rats had to remember in which compartment the food was held following delays of 0, 1, 15 or 120 min and return there. PPTg lesioned rats were not impaired during the test phase following no delay, but made significantly more errors than controls in trials following all time delays and in fact made an increasing number of errors with increasing delays. Here as the task demands increased, lesions of the PPTg caused significant impairments in response selection and resulted in disrupted performance. Further work on the role of the PPTg in spatially mediated behaviour came in a study by Dellu and colleagues (1991). In these experiments Dellu and colleagues

examined the role of the PPTg in performance in cross maze, water maze and radial arm maze tasks, all of which require use of environment specific spatial cues to mediate appropriate responding and accurate task performance. Performance in the simple cross maze was unimpaired following bilateral quisqualic acid lesions of the PPTg, while performance in both the water maze and radial arm tasks was disrupted. PPTg lesioned rats took longer to find a hidden underwater platform in the water maze task compared to controls but took a similar amount of time as controls to find the visible platform. Finally, in the radial arm maze lesioned rats made more errors than controls in their first 8 choices and more errors than controls to find all the food pellets. These results would suggest that as spatial task demands increase, lesions of the PPTg impair the rats ability to make accurate and appropriate response selections, resulting in disrupted performance. (Further details of spatially mediated radial arm maze performance following lesions of the PPTg are examined in Chapter 6 and 7 of this thesis.)

Finally, the PPTg has been implicated in tasks involving approach response behaviour and associative learning. Work by Ikemoto and Panksepp (1996) examined the effect of intra-PPTg infusion of the cholinergic antagonist atropine and GABA on reward approach behaviour (judged by run speed) and reward consumption. In this study they found that 100 μg of GABA into the PPTg attenuated run speed and 50 μg of atropine reduced the 20% sucrose reward intake and reduced run speed. This latter result seems contrary to the finding of decreased locomotion following injection of the cholinergic agonist carbachol into the PPTg (Brudzynski et al., 1988) and thus warrants further examination. (Exploration of the

role of the PPTg in reward intake and reward approach behaviour is examined in Chapter 5 of this thesis.)

The role of the PPTg in associative learning approach behaviour has been examined through the use of the conditioned place preference (CPP) paradigm. In this task the basic principle of associative learning are used in that the animal makes paired associations with environment specific cues and reward availability and displays the acquisition of this association through prolonged approach behaviour, in the absence of reward presence, to the reward paired environment. Rats that acquire this association are said to have displayed a positive place preference. Most of the work examining the role of the PPTg in formation of a positive place preference has come from the van der Kooy research group. In their studies they have found varying and sometimes theoretically inconsistent results. The crux of their explanation is that the PPTg plays a role in CPP formation when the animal is non-deprived or naïve (for food and drug respectively), but CPP formation is mediated elsewhere when the animal is in a state of deprivation or dependency. This theory was borne out of studies examining formation of place preferences following morphine, *d*-amphetamine or food availability (Bechara and van der Kooy, 1989; 1992b). In each of these cases PPTg lesions blocked CPP formation in drug naïve and food satiated rats, but normal place preferences were formed in rats bearing PPTg lesions when the rat is drug dependent or food deprived. In fact, the results of the drug naïve morphine place preference experiment have been replicated by an independent group (Olmstead and Franklin, 1993). This dissociative role of the PPTg in CPP formation becomes less consistent, however, with further

experimentation. Saccharin CPP, for example, is blocked in both water deprived and water satiated rats bearing ibotenic lesions of the PPTg (Stefurak and van der Kooy, 1994). With heroin, formation of a CPP following PPTg lesions is blocked at low doses (0.05 mg/kg) but not at high doses (0.5 mg/kg) and finally, cocaine CPP is not blocked in either naïve or dependent PPTg lesioned rats (Parker and van der Kooy, 1995). These combined results suggest that for some rewards the PPTg plays a role in the ability to acquire associative learning cues and later display them in the form of environment specific approach behaviour. It also suggests that there is no definitive role for the PPTg in CPP formation; further examination of this is presented in Chapter 4 of this thesis.

In summary then, these data outline a role for the PPTg in mediation of striatal outflow in behavioural responding. Lesions of the PPTg produce disruption in response selection, appropriate responding and associative learning, all of which have previously been examined in relation to striatal function. There are, however, many questions yet to be addressed in outlining a role for the PPTg in mediation of responding to incentive or motivated stimuli. The role of the PPTg in integrating external stimuli with internal drive needs to be explored further as does the nature of the PPTg in task demand response selection warrant examination. Some of these issues have been discussed in this thesis.

2.3 Outline of current research

As outlined above, the PPTg has been identified in behavioural processing that has been specifically related to the striatum. Placing the control of these

behaviours at a site further downstream than has been imagined is both novel and intriguing. The PPTg is situated at a point that allows it to receive and process basal ganglia and striatal outflow and either to provide feedback from processing this information to thalamic, forebrain and basal ganglia sites or to affect motor control sites downstream in the medulla and spinal cord. The main aim of this thesis then, was to examine further the role of the PPTg in behavioural processes including response selection and goal directed behaviour in relationship with the processing of striatal outflow. This was conducted through a series of experiments focusing on different elements of response selection including motivated and appropriate responding and the planning and execution of appropriate responding. This was concluded by an examination of the effect of striatal output on the neuronal structure and composition of the PPTg to understand the potential role this influence may play in subsequent modulation of behavioural processing.

The first task was to re-assess the proposed role of state dependence in associative learning performance by PPTg lesioned rats as outlined above (Bechara and van der Kooy, 1989; 1992b). The inconsistency of their theory across reward types and the incongruent findings with other studies that have found deficits from deprived PPTg lesioned rats (Fujimoto et al., 1992; Inglis et al., 1994b; Olmstead et al., 1998) warrant a re-examination and is further addressed in Chapter 4 of this thesis. The work of this chapter is then followed by an examination of the role of the PPTg in elements of motivated responding and appropriate responding to reward and its influence, and subsequent effect on responding, related to internal state. The next step was to examine the role of the PPTg in appropriate responding when task performance demanded the planning and execution of accurate responding and when

these task demands increased. Finally, this thesis finishes with an examination of the role of effect of direct and indirect striatal influence on the neurotransmitter expression of the PPTg. This aim addressed the question of neuronal correlates which would cause subsequent modulation of the behavioural processing of the PPTg.

Chapter 3. General Methods

3.1 Animals

All rats were adult male Lister hooded purchased from Charles River (London, UK). They were maintained on a controlled 12 hr light/dark cycle with lights on at 0700 hr and were initially pair housed. In some cases rats were later individually housed. This was carried out for experiments in which food, water and sucrose home cage intake was being monitored as individual measures of intake were required. It was also done in some cases for rats on food deprivation where one animal of the pair was losing more weight than the other due to this animal eating most of the food pellets. All rats were given *ad libitum* access to food (SDS maintenance diet no.1 chow pellets) and tap water except where otherwise indicated. In experiments where food deprivation was employed, the rats' body weight was monitored and maintained at no less than 85% of free feeding weight.

3.2 Anaesthesia

All rats received i.p. injections of 60 mg/kg 'Sagatal' (sodium pentobarbitone; Rhône Mérieux, Essex) before being placed in a David Kopf stereotaxic frame. Appropriate depth of anaesthesia was assessed by testing the rats' eye-blink reflex and paw withdrawal reflex and procedures did not proceed until both were negative. The level of anaesthesia was monitored throughout surgical procedures and maintained through administration of additional Sagatal if necessary. Sodium pentobarbitone is a barbiturate anaesthetic and its effectiveness can be time-of-day and temperature dependent, and length of time until full recovery can be

affected by the specific surgical lesion performed. In regard to the latter, full recovery from surgery involving excitotoxic lesions of the PPTg can range from 3-6 hr. During this time rats can experience respiratory problems as well as thermoregulatory control problems and therefore, were carefully monitored while recovering from anaesthesia.

3.3 Surgical Procedures

3.3.1 Toxins

6-hydroxydopamine (6-OHDA; Sigma Chemicals) was prepared as the freebase in 0.1 mg/ml ascorbic acid and kept on ice throughout surgery. Injections of 6-OHDA (for caudate-putamen [CP], nucleus accumbens [Nacc], and medial forebrain bundle lesions) were made using a stereotaxically mounted 30 ga stainless steel cannula connected via polyethylene tubing to a 5 μl SGE syringe driven by a Harvard Pump 22.

Ibotenate (Genosys) was prepared as a 0.12 M solution in sterile phosphate buffer; the final pH of the ibotenate solution was adjusted with 2 M sodium hydroxide (NaOH) to 7.2-7.4. Injections of ibotenate were made using a 0.5 μl SGE syringe mounted on a stereotaxic frame. Except for the medial forebrain bundle, all lesions were made with the skull level (incisor bar set at approximately -3.3 mm below the interaural line). Medial forebrain bundle lesions were performed with the skull in the orientation of De Groot (incisor bar 5 mm above the interaural line).

3.3.2 Lesions

For the caudate-putamen, a unilateral injection was made in either the left or right hemisphere (randomised across rats) at the following stereotaxic co-ordinates: 2 mm anterior-posterior from bregma, ± 3.0 mm from midline and 6.5 mm below the skull surface. 6-OHDA or phosphate buffer was delivered in a volume of 2 µl/ 4 min with the cannula left *in situ* for 4 min to allow for the diffusion of solution from the cannula tip.

For the nucleus accumbens, a unilateral injection was made in either the left or right hemisphere (randomised across rats) at the following stereotaxic co-ordinates: 3.4 mm anterior-posterior from bregma, ± 1.5 mm from midline and 7.8 mm below the skull surface. 6-OHDA or phosphate buffer was delivered in a volume of 2 µl/ 4 min with the cannula left *in situ* for 4 min to allow for the diffusion of solution from the cannula tip.

For the medial forebrain bundle, a unilateral injection was made in either the left or right hemisphere (randomised across rats) at the following stereotaxic co-ordinates: 1.2 mm anterior to the interaural line, ± 2.0 mm from the midline and 8.0 mm below the skull surface. 6-OHDA or phosphate buffer was delivered in a volume of 3.0 µl/ 6 min with the cannula left *in situ* for 4 min to allow for diffusion of solution from the cannula tip.

For the PPTg, 2 injections were made in each hemisphere at the following stereotaxic co-ordinates: 0.8 mm anterior to the interaural line, ± 1.6 mm from midline and 7.0 mm below skull surface (posterior PPTg); and 1.5 mm anterior to the interaural line, ± 1.7 mm from midline and 7.8 mm below skull surface (anterior PPTg). Ibotenate or phosphate buffer was delivered in a volume of 0.2 µl to each

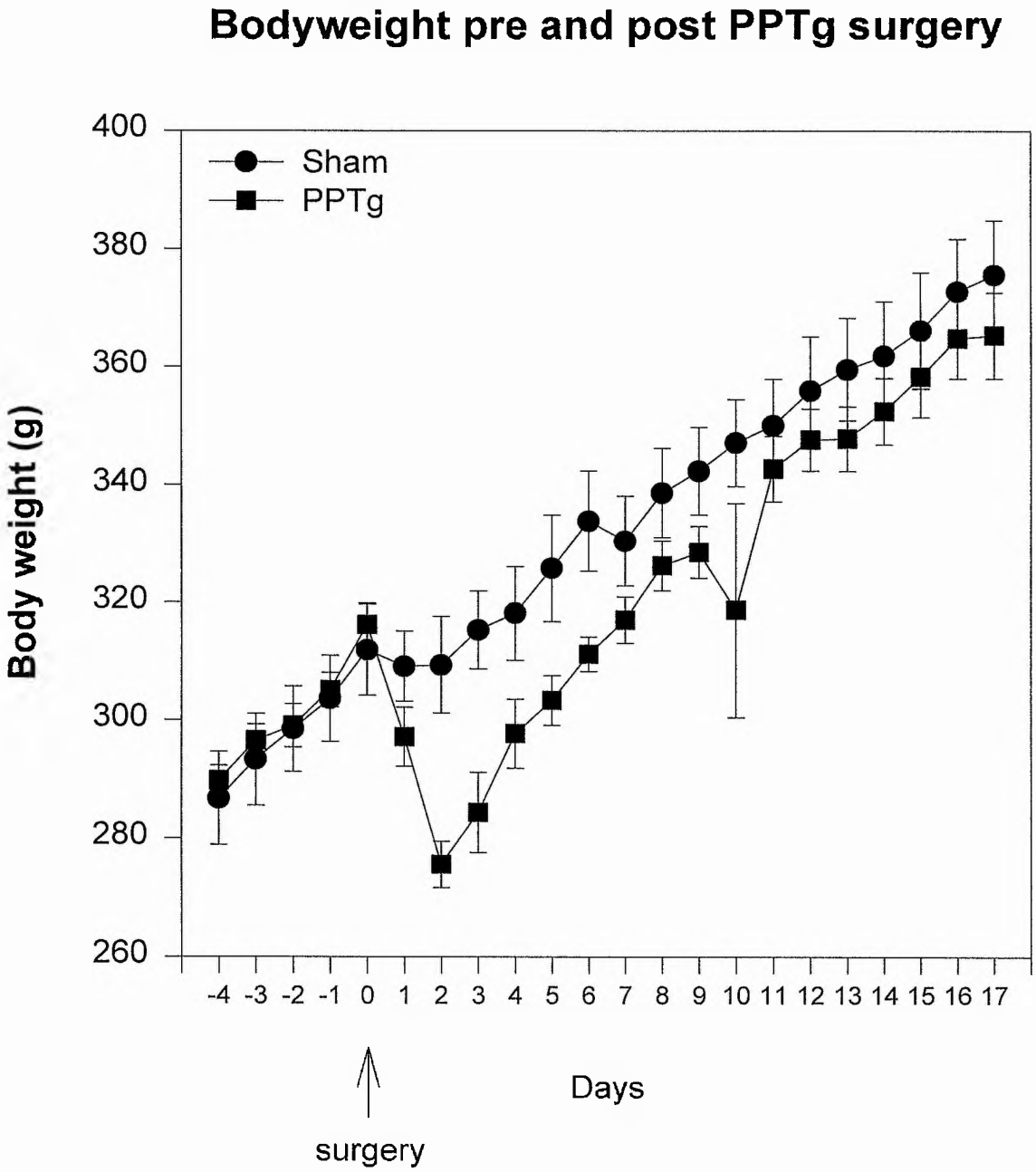
site with the needle left *in situ* for 5 min to allow diffusion of solution from the needle tip. Bilateral lesions were made over 2 days, 48-72 hours apart. Previous experience in this laboratory indicates that ibotenate infused bilaterally into these regions during one surgical procedure is fatal.

3.3.3 Post-operative care

Immediate post-operative care, particularly for the PPTg lesioned rats, was in the form of body temperature monitoring and in some cases mechanical stimulation of the chest to maintain heartbeat and breathing. PPTg lesioned rats lose immediate homeostatic body temperature control and post-surgery need to be placed under a heat lamp. As the rat re-acquires normal body temperature it must be carefully monitored as it can quickly over-heat, which can prove fatal. Once the rats are completely recovered from the anaesthesia, PPTg lesioned rats then often require post-operative attention of their food and water intake to ensure survival. Rats' body weight was monitored as an indicator of food intake and water bottle levels monitored for fluid intake. The first 3-7 days post-surgery rats received a strawberry yoghurt baby food mixture and a wet mash mixture of regular food pellets dissolved in water, in addition to their *ad libitum* access to food pellets and water. These mixtures were given when rats would not consume their regular food pellets and when their weight was decreasing. Rats were maintained on one or both of these mixtures until signs of steady weight gain were evident . Rats were then weaned off these mixtures and maintained on their regular food pellet diet until behavioural testing began. Figure 3.3.1 represents a typical body weight measure of rats pre and post PPTg surgery.

Figure 3.3.1

Representative sample of average daily body weight pre and post surgery for both sham and ibotenic acid PPTg lesioned animals (N=14; Data from Experiment 1, Chapter 7). Negative numbers indicate the days pre-operatively, with zero marking the first day of surgery. Repeated measures analysis of variance revealed no main effect of group ($F_{1,12} = 1.55$), but a main effect of days ($F_{21,252} = 98.57$, $p < 0.001$), and a two-way interaction of group x days ($F_{21,252} = 4.27$, $p < 0.001$). These latter results reflect the increase in weight gain with time, and the interaction reflects the immediate post-surgery decrease in body weight for PPTg ibotenate lesioned rats, which recovers with time.


Bodyweight pre and post PPTg surgery
Sham
PPTg
Body weight (g)
400
380
360
340
320
300
280
260
-4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Days
surgery

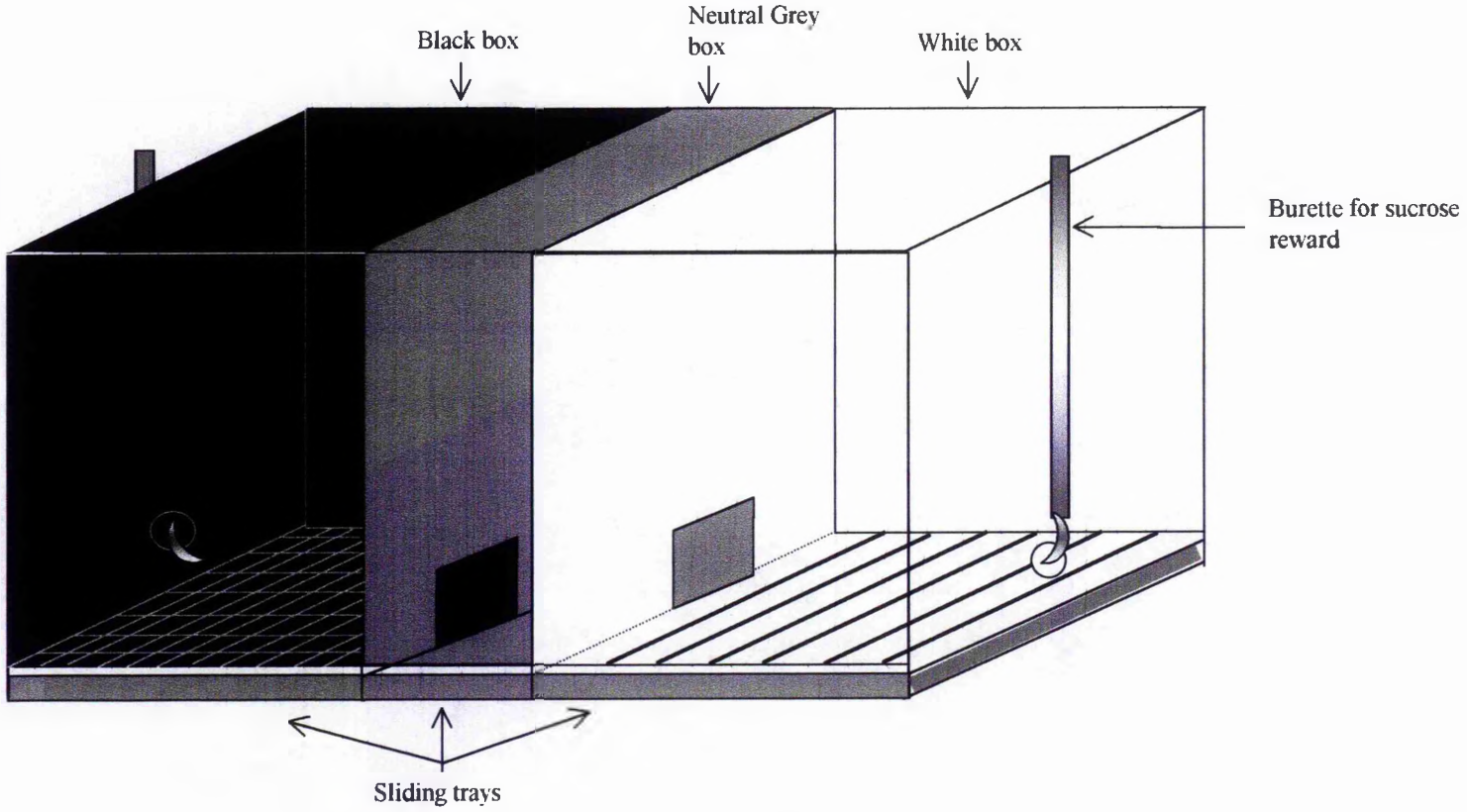
3.4 Behavioural Testing

3.4.1 Conditioned Place Preference

3.4.1.1 Procedure 1 (partially adopted from Mucha et al., 1982): Schematic representation of the place preference apparatus is illustrated in Figure 3.4.1. After 2-3 weeks post-operative recovery, rats were initially acclimatised in a rectangular wooden box (60 h x 100 l x 30 w cm) subdivided into 3 compartments. Two measured 60 h x 80 l x 30 w cm, and one 60 h x 20 l x 30 w cm, with the 2 large compartments separated by the smallest of the three. This middle compartment was considered the neutral zone and consisted of walls painted grey and a grey wire grid floor with a clear perspex front, neutral odour and covered with a grey metal lid. The two large end compartments were distinguished by colour, texture and smell. The walls of the boxes were painted black or white and at the far end of each box there was a hole centrally located approximately 20 cm from the bottom. The black box had a uniform zinc mesh floor painted black, a clear perspex front, metal lid and a petri dish filled with 40 ml of 2% acetic acid was placed underneath the floor prior to the rats being placed in the compartment. The white box had a floor of evenly spaced stainless steel bars, a clear perspex front, metal lid and a petri dish filled with 40 ml of a liquid cleaning soap ('Stag') solution was placed underneath the floor prior to the rats being placed in the compartment. All compartments were wiped with 20% alcohol between trials to remove individual rats' odour. In each of the larger compartments the floor area was divided into three equal parts by transition lines that were perpendicular to the perspex front. These two large end boxes were separated from the small grey area by appropriately coloured metal guillotine doors

Figure 3.4.1

Schematic representation of the conditioned place preference apparatus, shown without lids. The floor texture of the three boxes is not evident but consisted of steel cylindrical rods for the white box, metal grey painted grid flooring for the neutral grey box and zinc perforated mesh flooring, painted black for the black box. The burettes indicated on the sides of the boxes were only in place for the relevant experiment and were not a permanent feature of the apparatus.


Black box
Neutral Grey box
White box
Burette for sucrose reward
Sliding trays

(12 x 12 cm). Behavioural training was carried out in 3 stages: acclimatisation (3 days), conditioning (8 days), and testing (1 day).

Acclimatisation

On each of the 3 days the animal was placed in the neutral grey compartment for 1 min after which the guillotine doors were removed and on days 1 and 2 the rat was allowed access to all 3 compartments for 30 min. On the third day individual preference for the black or white compartment was determined by recording the time spent in each of the two larger compartments over a 15 min period. Behaviour was recorded through the use of the behavioural analysis computer package Observer 2.0 (Noldus Information Technology). This package allows individual computer keys to be assigned to represent separate behavioural actions (e.g. the letter 'B' key can be assigned to indicate when pressed when the animal is in the black compartment). Along with time spent in each compartment, the number of partition crosses made within the larger compartments, as well as the number of rearing responses, were recorded. A cross was recorded when at least 3 of the rat's paws had crossed a transition line. A rear was recorded when the animal moved from having all 4 paws on the floor to a position where the body was elevated more than 45° above the floor. Based on this initial preference rats were assigned to 2 counterbalanced groups (food deprived and non- food deprived) for conditioning. Rats assigned to the food deprived group were taken off *ad libitum* food access and did not have access to food for at least 1 day before place conditioning.

Conditioning stage

During this stage conditioning was based on compartment specific availability of food (approximately 50 g of food pellets; standard lab chow, SDS

maintenance no. 1 diet). The compartment in which rats had access to food pellets was referred to as the 'paired' side while the absence of food availability was the 'unpaired' side. Conditioning then involved placing each rat 4 times in the paired side of the apparatus and 4 times in the unpaired side, carried out on alternate days (i.e. days 1, 3, 5 etc.: paired side; days 2, 4, 6 etc.: unpaired side); each pairing lasted for 30 min. Rats in the food deprived group were given access to food beginning 30 min after a conditioning trial had ended. This access was either 30 min when placed in the 'paired' compartment box and then 30 min in the home cage, or 1 hr only in the home cage after a conditioning trial with lack of food (when placed in the 'unpaired' side). At the end of conditioning training all rats were given free access to food for at least 24 hr before the testing stage.

Testing

In this stage a post conditioning preference was assessed in a similar manner as that described in the acclimatisation stage. Rats were placed in the neutral, grey compartment for 1 min and were then given a 15 min period of access to the entire apparatus. Time spent in each of the compartments was scored as were number of crosses and rears.

3.4.1.2 Procedure 2 (partially adopted from Mucha et al., 1982 with apparatus modification): This place preference procedure is identical to procedure 1 but in this experiment the pairing boxes described above in the 'apparatus' were modified. After CPP experiment 1 (using procedure 1) was completed, the perspex fronts on all 3 compartments were covered with appropriate (black, white or grey) card leaving a gap of 40 cm at the bottom through which the rat's feet and lower part of the body

were visible. As the view of the rats' body was obscured time spent in each pairing box was scored as were the number of crosses made, but scoring of the number of rears was omitted.

3.4.1.3 Procedure 3: (taken from the CPP procedure methodology of White and Carr, 1985). The basic methodology was similar to that described in Procedure 1. The apparatus used was the modified CPP boxes outlined in Procedure 2. In this paradigm the rats received a 20% sucrose solution as their rewarding stimulus in contrast to the food reward used in Procedures 1 and 2. To acclimatise the rats to the taste of this reward stimuli the rats received 20% sucrose solution in their home cages for 3 days before acclimatisation to the CPP apparatus began. A group of food deprived and non-food deprived rats were used, similar to that as described in Procedure 1. In this procedure, however, rats in the food deprived group were given 2 hr access to food beginning 30 min after a conditioning trial had ended (to coincide with the methodology used in White and Carr, 1985). The other methodological difference in this procedure was the number of conditioning trials the rats received. In this procedure 14 conditioning days was used, 7 conditioning trials for the reward paired environment and 7 conditioning trials for the non-reward paired environment. In this procedure the sucrose reward was available through an externally located drinking tube, the spout of which protruded through the central hole on the far wall of the conditioning boxes (described in the apparatus of Procedure 1) and the amount of sucrose consumed over each 30 min pairing session was recorded. The box that served as the unpaired reward box had a similar drinking tube but no reward was

available from this spout. Time spent in each compartment was scored, as was the number of crosses, omitting rears as per Procedure 2.

3.4.2 Radial arm maze

Apparatus

A standard eight arm radial maze was used for experiments. A photographic image of the exact radial arm maze used in this lab is provided in Figure 3.4.2. Arms measured 80 cm x 10 cm with a cylindrical food cup (4 cm diameter) at the end of each arm. Arms were equally spaced and radiated out from an octagonal centre platform measuring 50 cm in diameter. Access to arms could be blocked with plastic guillotine doors (10 cm x 13 cm) covered with adhesive labels to make them opaque. The maze was elevated 37 cm from the floor as it rested on a breaking wheel mechanism from a model 'E' Ford. The wheel allowed the maze arms to be moved to different positions about the room. The maze was surrounded by several extra maze cues such as posters, cupboards, wall plugs, a door, and so on. The maze was situated in a 4.4 m x 4.9 m x 2.6 m room which was illuminated with 2 overhead fluorescent strip lights. Two tasks were employed on the maze; the delayed spatial win shift task and the random foraging task.

3.4.2.1 Delayed Spatial Win Shift Task

This task was adapted from Seamans and Phillips, 1994; Seamans et al., 1995; Floresco et al., 1997; and Floresco, 1998 (personal communications). A schematic representation of this task is provided in Figure 3.4.3 (A). For the first 2 days of training the rats were habituated to the maze. Rats were given 10 minute

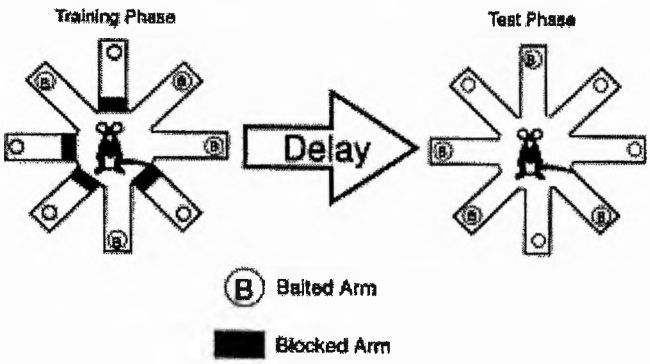
Figure 3.4.2

Photographic representation of the 8 arm radial maze used in this laboratory. In this picture the guillotine doors to the arms are open and note the various number of external cues (plugs, posters, door) available to the rat to aid foraging.

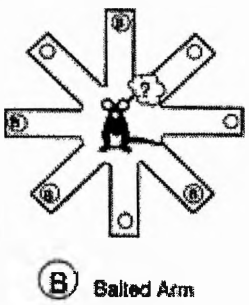
Figure 3.4.3

Schematic diagram of the delayed spatial win-shift (SWSh) and the random foraging (RF) eight arm radial maze tasks. A: The delayed spatial win shift task. This task consists of a training phase and a testing phase. During the training phase 4 of the 8 arms are randomly baited with food pellets and the remaining 4 are blocked. Once the rat has retrieved these pellets, it is removed from the maze for a 5 minute delay. After the delay, the rat is placed back onto the maze and now all the maze arms are open. The arms that were blocked previously are now open and baited and the arms that were previously baited are empty. The rat must remember which arms were previously blocked and enter them to retrieve food pellets. B: The non-delayed RF task. In this task all arms are always open and 4 of the 8 arms are randomly baited each day. To retrieve pellets in an optimal fashion the rat must remember which arms it's chosen and not return there, but unlike the test phase of the delayed SWSh task, the animal has no previous knowledge of the location of the food at the beginning of the non-delayed RF trial. Schematic taken from Floresco et al., 1997.

A The Delayed Spatial Win-Shift (SWSh) Paradigm



B The Non-Delayed Random Foraging (RF) Paradigm



access trials where no food was available on the maze. Once returned to their home cage rats received 5 x 45 mg Noyes food pellets (Sandown Scientific, Surrey). Subsequent to this, daily trials were run which consisted of a training phase and a test phase separated by a 5 min delay. In the training phase rats had access to a set of 4 arms, each baited with one 45 mg Noyes pellet. The other 4 arms were blocked by the plastic doors. The set of arms to be baited/blocked were chosen from a randomly generated list of numbers chosen such that no more than two adjacent arms were blocked on any given trial. The rats were allowed a maximum of 10 min to retrieve the 4 pellets before being placed back in their home cage for the 5 min delay period. Subsequent to the delay (the test phase) all arms of the maze were open, but now those that were previously blocked were now baited while those that were previously baited were now empty. The rats had a maximum of 10 min in this test phase to retrieve the 4 pellets. Rats were given trials once daily until a criterion performance was reached. The criterion was set such that rats had to retrieve the 4 pellets in 5 or fewer choices over 2 consecutive days. For each trial the performance of the animal was recorded using the 'Observer' 2.0 behavioural testing program. For each rat the number and order of arms entered were recorded. An arm entry was scored when the animal had entered the arm and reached the food cup at the end of the arm. In the training phase, errors were scored when the animal revisited the baited arms having already retrieved the pellet. In the test phase errors were divided into 2 error types- across phase and within phase errors. An across phase error was defined as an entry and re-entry to an arm that had been baited during the training phase but was unbaited during the test phase. Within phase errors were defined as re-entry to arms that were baited during the test phase (that is the arms that were blocked in the

training phase that were now open during the test phase). The latency to initiate the session was recorded as well as errors. The latency was the time it took the animal to make its first choice and reach the food cup of the first arm visited. The time to complete the phase was also recorded. From these measures the average time to make choices, subsequent to the first choice, could be calculated through the formula:

$$\frac{\text{Time to complete phase - time to initiate phase}}{\text{number of choices made during the phase}}$$

3.4.2.2 Random Foraging

This task was adapted from Seamans and Phillips, 1994; Seamans et al., 1995, Floresco et al., 1997 and Floresco, 1998 (personal communications). A schematic representation of this task is provided in Figure 3.4.3 (B). The first two days of training were habituation days, exactly as described above for the delayed spatial win shift task. Subsequent to habituation, daily training trials began. In each trial the animal was required to retrieve four pellets placed randomly on 4 of the 8 arms of the maze. The assignment of baited arms came from a list of randomly generated numbers. Rats were trained to a criterion of one re-entry error or less per daily trial over three consecutive days. Re-entry errors were scored as re-entry to baited arms (arms baited at the start of the trial) and re-entry to non-baited arms (arms not baited at the start of the trial). As with the delayed spatial win shift task, measures of the latency to make the first choice, time to complete the trial and the average time to make subsequent arm choices were recorded and used for data analysis.

3.4.3 Runway Task

Apparatus

This task employed a simple wooden runway measuring 3 m long x 30 cm wide x 30 cm high. The runway was painted grey and at one end there was a centrally located hole approximately 10 cm from the bottom through which the spout of an externally placed drinking tube protruded.

Task

This task was partially adapted from the maze experiments of Tolman (Tolman and Honzik, 1930a; Tolman and Honzik, 1930b; Tolman, Honzik and Robinson, 1930) and the experiments of Butter and Campbell (1960) and Ikemoto and Panksepp (1996) that looked at run speed (as a measure of motivation) and reward intake to comment on the role of reward influenced approach behaviours. Following at least 2 weeks of post-operative recovery rats were initially acclimatised to the runway in 2 x 10 min habituation trials (Trials 1 and 2). In these trials rats were given free access to the alleyway and access to the drinking tube spout, but no reward was available from the spout. Following this rats were given a series of trials to establish that sucrose was available from the drinking tube at the far end of the runway. These trials ran as follows:

Trial 3: The rat was placed in the alleyway approximately one half its body length from the drinking tube, facing the drinking tube. The time to contact the drinking tube was recorded (using a stopwatch) and then the animal was given access to the sucrose solution for 2 min. Half the rats received 4% sucrose solution in the

drinking tube while the other half received 20% sucrose solution (randomised across rats).

Trial 4: same as Trial 3.

Trial 5: similar to trial 3 but when the rat contacted the drinking tube the animal was lifted (but still able to maintain visual contact with the drinking tube) and placed at a distance of 1.5 m from drinking tube (half the entire length of the alleyway). The time until the rats contacted the tube from this distance was measured and then the animal was given free access to their assigned sucrose solution for 2 min.

Trial 6: Rats were placed 1.5 m from drinking tube. The time to contact tube was measured and the animal was then given 2 min access to sucrose reward.

Trials 7-13: Rats were placed at end of alleyway (3 m from drinking tube): Rats were placed facing away from the drinking tube and timing was started from the point when the animal turned itself around to face the drinking tube. The time to contact the drinking tube from this point was measured. Rats were then given 30 min access to their assigned sucrose solution and the total amount of solution consumed was recorded.

3.5 Histological Analysis

On completion of experimental testing rats were deeply anaesthetised with an i.p. injection of 0.7 ml 'Euthanal' (sodium pentobarbitone, 200 mg/ml) and were then perfused transcardially with 0.1 M phosphate buffered saline (PBS) followed by at least 300 ml fixative (4% paraformaldehyde in 0.1M phosphate buffer) at a rate of 20 ml/min. The brains were removed and post fixed in 4% paraformaldehyde for 30 min at room temperature and then stored in 20% sucrose for at least 30 min.

3.5.1 NADPH-diaphorase Expression: Enzyme histochemistry

NADPH-diaphorase is a NO synthase responsible for the formation of citrulline and NO from arginine (Vincent et al., 1983), and approximately 90% of cholinergic neurons in the mesopontine tegmentum stain for diaphorase (Vincent and Hope, 1992); thus neurons in the PPTg or LDTg that are reactive for NADPH-diaphorase are presumed to be cholinergic.

For analysis of NADPH-diaphorase expression brains were cut into 50 μm coronal sections, 200 μm apart, on a freezing microtome from the anterior portion of the cerebellum through to the anterior substantia nigra.

Following cutting, sections were sorted into a fine net bottom container and washed in 20% sucrose for 60 min followed by 5 x 4 min in 0.1 M PBS. Sections were then transferred to a 24 well tissue culture plate (0.3-0.5 ml volume/well; up to 6 sections/well) and incubated at 37 °C for 30- 60 min in a solution containing 0.1 mg/ml nitro blue tetrazolium (Sigma) and 1 mg/ml β-NADPH (tetra sodium salt type 1; Sigma) in 0.3% triton X-100/ 0.1 M phosphate buffer. Sections were then rinsed 3 x 5 min in 0.1 M phosphate buffer and mounted on chrome-alum-gelatin subbed slides and air dried overnight. As this stain is unstable in xylene, coverslips were applied with gelatin.

3.5.2 Cresyl Fast Violet Stain

Analysis of lesion extent was determined through the use of staining tissue for Nissl bodies using a fast cresyl violet staining procedure. Simply, brains were cut using a freezing microtome and 50 μm coronal sections cut 200 μm apart, from the anterior portion of the cerebellum through to the anterior substantia nigra, were

collected for processing. Sections were immediately mounted on chrome-alum-gelatin subbed slides and allowed to air dry overnight. Sections were then treated in xylene for 2 min followed by rehydration through a graded alcohol series: 100%-50% for 2 min each and then placed in tap water for 2 min. Sections were then placed in a fast cresyl violet solution for 2 min and then back into tap water for 5 min. Sections were then differentiated in graded alcohol solutions, 50%-100% and then placed into xylene to clear. Slides were then coverslipped from xylene using the xylene based mountant, DPX.

3.5.3 Immunohistochemistry: Analysis of CP, NAcc and medial forebrain bundle lesions

50 μm sections 200 μm apart were taken from the anterior pole of the nucleus accumbens to the tail of the caudate-putamen for tyrosine hydroxylase (TOH) staining. Sections were initially washed with 20% sucrose for 1 hr and then washed 5 x 4 min in PBS.

Incubations with antibodies and other reagents were carried out in 24 well tissue culture plates (as above). For washing, sections were transferred into a fine net bottom container and placed on a flat-bed shaker. All incubations were carried out at room temperature on a flat bed shaker, with the exception of the primary antibodies for TOH which were at 4 °C.

Sections were placed in blocking solution (20% normal goat serum, 0.1% triton X-100 in PBS) for 60 min and then washed 5 x 4 min with PBS. They were incubated with anti-TOH (from mouse-mouse hybridomas, Boehringer) 1:50 in antibody diluting solution (ADS) for approximately 15 hr followed by 5 x 4 min

washes with PBS. The ADS used was 0.1% normal goat serum and 0.1% triton X-100 in PBS.

The sections were then incubated with anti-mouse IgG (1:30 in ADS) (sheep, Sera-lab) for 1.5 hr and washed 5 x 4 min in PBS. Following the wash sections were incubated in monoclonal mouse peroxidase anti-peroxidase (1:100 (Sigma) in ADS) for 50 min and washed 5 x 4 min in PBS. The IgG and peroxidase anti-peroxidase incubations were repeated in the same order for the same length of time with PBS washes between each step. Finally sections were incubated with diaminobenzadine (DAB) for 7-10 min. The sections were washed 5 x 4 min in PBS, mounted on chrome-alum-gelatin coated slides and air dried. Coverslips were applied with DPX.

3.5.4 Analysis of PPTg excitotoxic lesions

For analysis of lesions, 2 sets of parallel 50 μm coronal sections were cut 200 μm apart from the anterior portion of the cerebellum through to the anterior substantia nigra. One set of sections was stained with cresyl violet according to normal histological procedures (as described above). The other set of sections were stained for NADPH-diaphorase as described above.

3.5.5 Overall Assessment of Lesions

All sections were inspected using a Leitz "Diaplan" microscope fitted with a Sony DXC-3000P video camera for visualisation of sections on a high resolution colour monitor. NADPH-diaphorase expressing cells were directly evaluated by comparing NADPH-diaphorase cell counts in lesioned and control brains. Lesions were identified in TOH stained sections by the overall presence of cell loss and

degenerated neuronal somata. Lesions were identified in cresyl violet stained sections by the presence of gliosis and degenerating neuronal somata.

3.6 Statistical Analysis

All data were analysed using the statistics packages SPSS and Statistica. Following histological assessment, only rats with appropriately placed lesions were included for analysis. Data were analysed parametrically using analysis of variance (ANOVA) and where appropriate was followed by post-hoc tests using the Tukey-HSD post-hoc test (p set at 0.05).

Chapter 4. The role of the PPTg in formation of a conditioned place preference.

4.0 Introduction

The conditioned place preference (CPP) paradigm relies on the basic principles of associative learning theory. Rewarding stimuli, whether drug reward or access to natural rewards such as food or water, are paired in association with the neutral stimulus of a particular environment such as a testing box or a room. The neutral environmental stimuli typically include the smell and/or the texture and/or the view of the specific environment. This association is then repeated sufficiently often such that the neutral stimulus, in the absence of reward, acquire the ability to serve as the reward itself and elicit approach behaviours (Carr et al., 1989). For example, a drug treatment that serves as a reward is paired with the neutral stimuli of one specific environment while the neutral stimuli of another environment is paired with the lack of such reward, or some other less rewarding stimuli. The animal is given an equal number of pairings to both environments and is then tested. In this test session the animal has equal access to both environments, but now in the absence of any of the previous rewards, and the amount of time the animal spends in both environments is measured. If the animal spends more time in the environment with which it received pairings with the drug reward, then it is assumed that the drug treatment was rewarding.

To date the CPP paradigm has been used to test a range of stimuli for reward properties including, but not limited to, availability of sex, food, water, morphine, heroin, amphetamine, cocaine and saccharin (Schetchter and Calcagnetti, 1993;

Guyon et al., 1993; Olmstead and Franklin, 1993; Hiroi and White, 1991; Parker and van der Kooy, 1995; White and Carr, 1985). The strength of these rewards can be assessed through varying the salience of such stimuli by, for instance, using a range of drug doses, or by altering the internal drive of the animal such as testing food reward in an animal that has been food deprived versus food satiated. Often in combination with this, the CPP paradigm has been used to determine the involvement of a range of anatomical structures in such reward related approach behaviours. This is usually assessed through the absence of such structures following a specific lesion or by pharmacological inactivation, such as microinjection of the D_2 receptor antagonist sulpiride into the nucleus accumbens (Hiroi and White, 1991). The ability to then form, or not form, a positive place preference can identify areas necessary for such a process to occur.

Specifically addressing this point, previous experiments by van der Kooy and colleagues have indicated an involvement of the PPTg in CPP performance using a variety of rewarding stimuli. They have shown that the formation of a CPP, when morphine, amphetamine or food is used as a rewarding stimuli, is blocked in rats following lesions of the PPTg in conditions when the rat is in a state of non-deprivation or drug naiveté, whereas when it is deprived or drug dependent, it is able to form a normal place preference to the morphine, amphetamine or food paired environment (Bechara and van der Kooy, 1989; Bechara et al., 1992; Bechara and van der Kooy, 1992b; Nader and van der Kooy, 1994). van der Kooy and his colleagues have concluded that the role of the PPTg in formation of a CPP is state dependent and the ability of an animal to form a place preference in the deprived

state is not mediated by the PPTg, but elsewhere. Data using other reward stimuli for CPP formation following PPTg lesions however, has been inconsistent. For example, when van der Kooy's group looked at formation of a CPP using saccharin in both a deprived and non-deprived condition, they found that lesions of the PPTg blocked the ability to form a place preference in both conditions (Stefurak and van der Kooy, 1994). These data then seem to imply that the role of the PPTg in CPP formation processes is not state dependent, as was suggested from the morphine data. Further variance in the role of the PPTg in mediating CPP reward came when the group used cocaine as a rewarding stimuli. In this case they found that lesions of the PPTg did not block formation of a CPP (Parker and van der Kooy, 1995). Here the motivating effects of cocaine do not seem to rely on the integrity of the PPTg but are mediated by a different substrate. Finally, in using heroin as the CPP reward stimuli in PPTg lesioned rats Nader and colleagues (1994) found that CPP was blocked with a 0.05 mg/kg dose of heroin but not with a higher dose of 0.5 mg/kg. Here, the results imply that the role of integrity of the PPTg in heroin place preference relies on the intensity of reward stimuli used. Lower doses rely on an intact PPTg while the formation of a CPP to a higher heroin dose is mediated elsewhere. These varying results make it difficult to provide a summary explanation of function of the PPTg in CPP performance. It is also difficult considering the common influence these stimuli have on mesolimbic sites known to be important for mediating reward and reinforcement and that project to the PPTg. Opiates, psychomotor stimulants and ingestive stimuli have been shown to act on a shared final pathway in that they all increase extracellular dopamine concentrations in the mesolimbic dopamine system, specifically the ventral tegmental area (VTA) and nucleus accumbens (Pontieri et al.,

1995; Yoshida et al., 1992; Bardo, 1998). This extracellular dopamine (DA) increase is under influence from increased impulse flow from VTA and accumbens neurons (Matthews and German, 1984; Gonon and Buda, 1985). Though these reinforcers act at different receptors, both mu and delta opiate receptors and D_2-like receptors activate a similar mechanism at the postreceptor, signal transduction level (Self et al., 1994; Self and Nestler, 1995). At the postreceptor level, these reinforcers inhibit adenylate cyclase activity through the guanine nucleotide binding proteins G_i and G_o. This results in inhibition of second messenger synthesis, particularly adenosine-3',5'-monophosphate (cAMP) which in turn activates potassium channels and regulates voltage sensitive calcium channels to effect neuronal activation. Specific inactivation of these proteins with pertussis toxin (PTX) reduces the reinforcing effect of drugs such as cocaine and heroin in self administration tasks (Self et al., 1994), implicating this signal transduction system as a common reinforcement pathway.

The findings of the van der Kooy group and their conclusion that PPTg lesions are most effective when the animal is non-deprived, or in a motivationally neutral state, may also be considered incomplete in that they do not agree with previous studies which have found disturbances in performance when rats were in a state of deprivation. Food restricted PPTg ibotenic acid lesioned rats used by Kessler and colleagues (1986) were found to have impaired performance on a positively reinforced memory place learning task. This result was similar to the finding of Dellu and colleagues (1991) who reported that food deprived PPTg lesioned rats were impaired in acquisition performance on both water and radial arm

maze place learning tasks. Finally, work by Inglis and colleagues (1994b) demonstrated disrupted performance on a conditioned reinforcement task from PPTg lesioned rats that were food deprived and maintained at approximately 85% of their free-feeding weight. Collectively these studies indicate that task performances following lesions of the PPTg are impaired when the animal is in a state of deprivation, and not only non-deprivation or neutral state, as the conclusions of van der Kooy would suggest.

The following experiments then, sought to re-evaluate the role of the PPTg in formation of a food CPP. Previous work has found inconsistent results across reward types for the role of the PPTg under deprived and non-deprived conditions (as outlined above; Bechara and van der Kooy, 1992b; Nader and van der Kooy, 1994; Stefurak and van der Kooy, 1994; Parker and van der Kooy, 1995) and thus warrants a re-examination of the role of the PPTg in this association paradigm. As well, the activity performance of the rats during the CPP formation was of interest. Previous examination of the role of the PPTg in CPP formation has only provided information in regard to time spent in the various parts of the apparatus and not details as to what the rats were doing during this time. Are they running about more than controls and are they exploring, or are they simply sitting or sleeping? The pattern of activities have not been reported in previous examinations of the role of the PPTg in formation of CPP and this omission was rectified in the following experiments.

4.1 Experiment 1: Effects of excitotoxic lesions of the pedunculopontine tegmental nucleus on the formation of a food conditioned place preference in food deprived and non-deprived animals.

Methods

Animals

28 adult male rats (Charles River) were used. Mean weight at surgery time was 330.49 ± 62.44 (SD).

Surgery

Rats were anaesthetised with sodium pentobarbitone (Sagatal, Rhône Mérieux; 60 mg/kg) before being placed in the stereotaxic frame. 12 rats were given bilateral lesions of the PPTg while 16 were given phosphate buffer control lesions (see General Methods). Of these rats 6 ibotenate lesioned rats and 8 control lesioned rats were in the food deprived condition, while 6 ibotenate lesioned rats and 8 control lesioned rats were in the non-deprived condition. Rats were given at least two weeks post-operative recovery before training began.

Behavioural testing

Rats were trained under the conditioned place preference procedure 1 as outlined in General Methods. When running the CPP paradigm care must be taken to control for a number of things including equal number of pairings to both environments, making sure that both environments serve as the reward paired environment for an equal proportion of rats as inequalities could result in false positive place preferences (Carr et al., 1989). In this experiment rats underwent CPP training with lab chow food pellets as their rewarding stimulus and were given a set of 4 food pairings and 4 non-

food pairings in their experimental paradigm with an equal number of rats having the white box as their reward environment and the black box as their reward environment.

Results

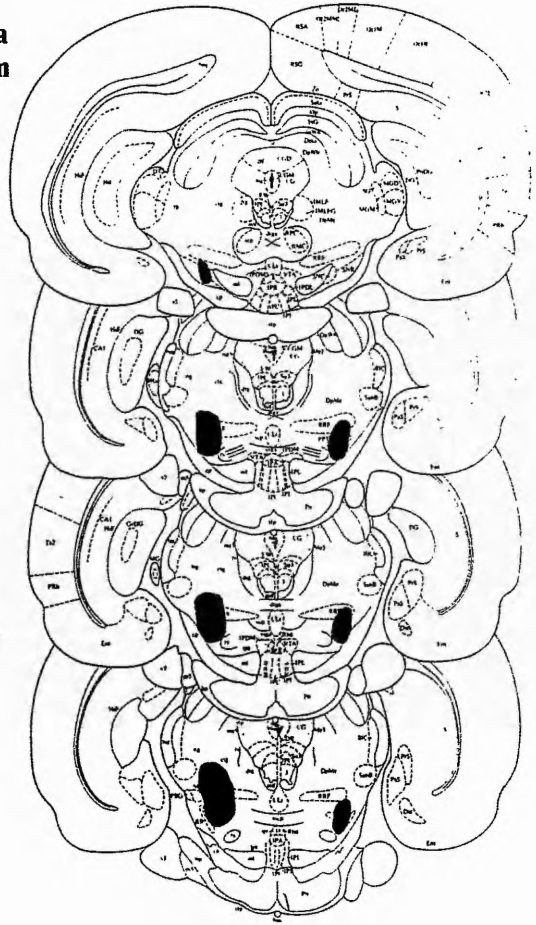
Figure 4.1.1 represents the largest and smallest lesion sizes for the PPTg ibotenate lesioned rats of this experiment. Damage to nearby structures was negligible and inconsistent across rats. 2 rats had damage to the deep mesencephalic nucleus. 6 rats had damage to the adjoining retrorubral field and 2 rats had damage to the parabrachial nucleus. All rats had minimal damage to the superior cerebellar peduncle. The inconsistent damage to these structures did not produce a change in behaviour across the PPTg lesioned rats. This was examined through direct comparison of damage sites and behavioural outcome. No consistent relationship between damage to a structure other than the PPTg and behaviour were found.

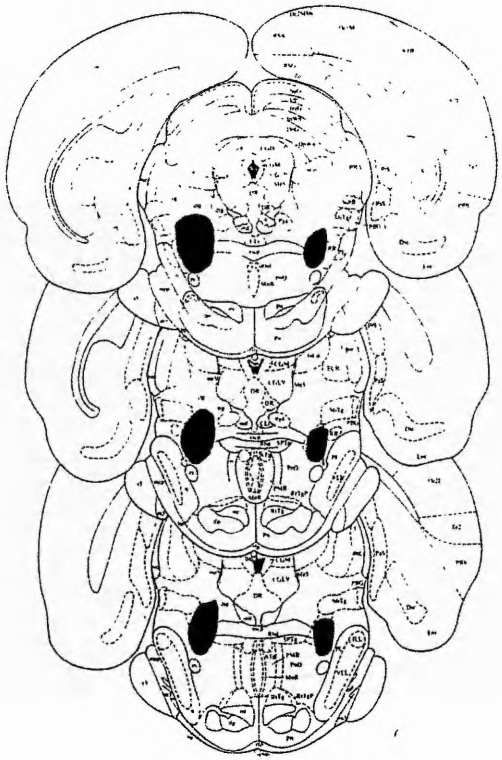
Measurement of time spent in the reward paired environment and unpaired environment were represented as proportion of the total test time. Mean proportion of time for each group examined are included in Table 4.1.1. Data were analysed using a repeated measures analysis of variance with lesion group (sham vs. ibotenate) and deprivation state (deprived vs. non-deprived) as the between subjects factors and side or compartment (paired, unpaired and neutral) as the within subjects factor. This type of repeated measures analysis, with all compartments as the within subjects variable, is a common analysis used in the conditioned place preference literature and was thus chosen for this experiment. Analysis of variance revealed no main effect of group ($F_{1,24} = 1.16$), side ($F_{2,48} = 0.47$) or state (deprived vs. non-

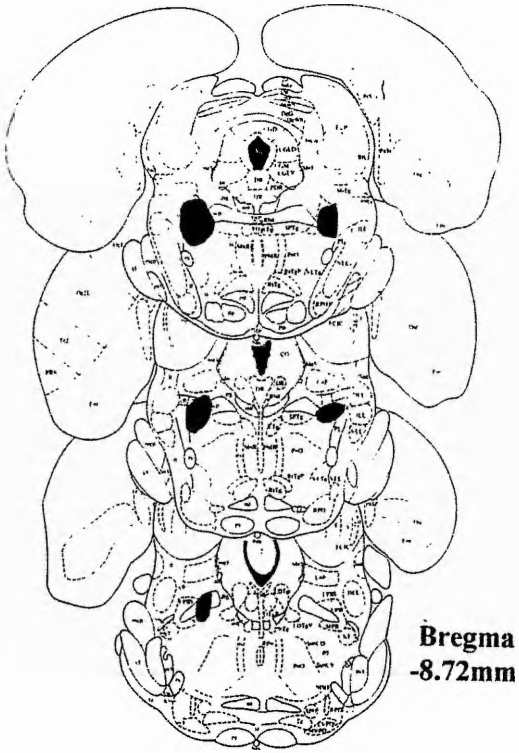
Figure 4.1.1

Representative sections from Bregma -6.30 to -8.72 mm redrawn from the atlas of Paxinos and Watson (1986) which illustrate the largest (left) and smallest (right) lesions for PPTg ibotenic acid lesioned rats in this experiment.

Bregma
-6.30mm







Bregma
-8.72mm

Table 4.1.1

Conditioned Place Preference: Procedure 1: The mean proportion of time spent in the paired, unpaired and neutral environments for deprived and non-deprived animals as well as measures of locomotion (CP Loco - conditioned probability of locomotion = # crosses in compartment / time spent in compartment; see p.75) and rearing (CP Rear - conditioned probability of rearing; as CP Loco) in the paired and unpaired sides and a measure of rearing for the neutral environment.

Deprived

	Paired	Unpaired	Neutral
SHAM			
Time (proportion; ± SEM)	32.35 (2.35)	34.15 (2.79)	33.50 (3.43)
CP Loco (± SEM)	0.21 (0.02)	0.21 (0.02)	
CP Rear (± SEM)	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)
PPTg			
Time (proportion; ± SEM)	31.68 (1.84)	38.90 (2.50)	29.42 (1.50)
CP Loco (± SEM)	0.15 (0.01)	0.14 (0.01)	
CP Rear (± SEM)	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)

Non-Deprived

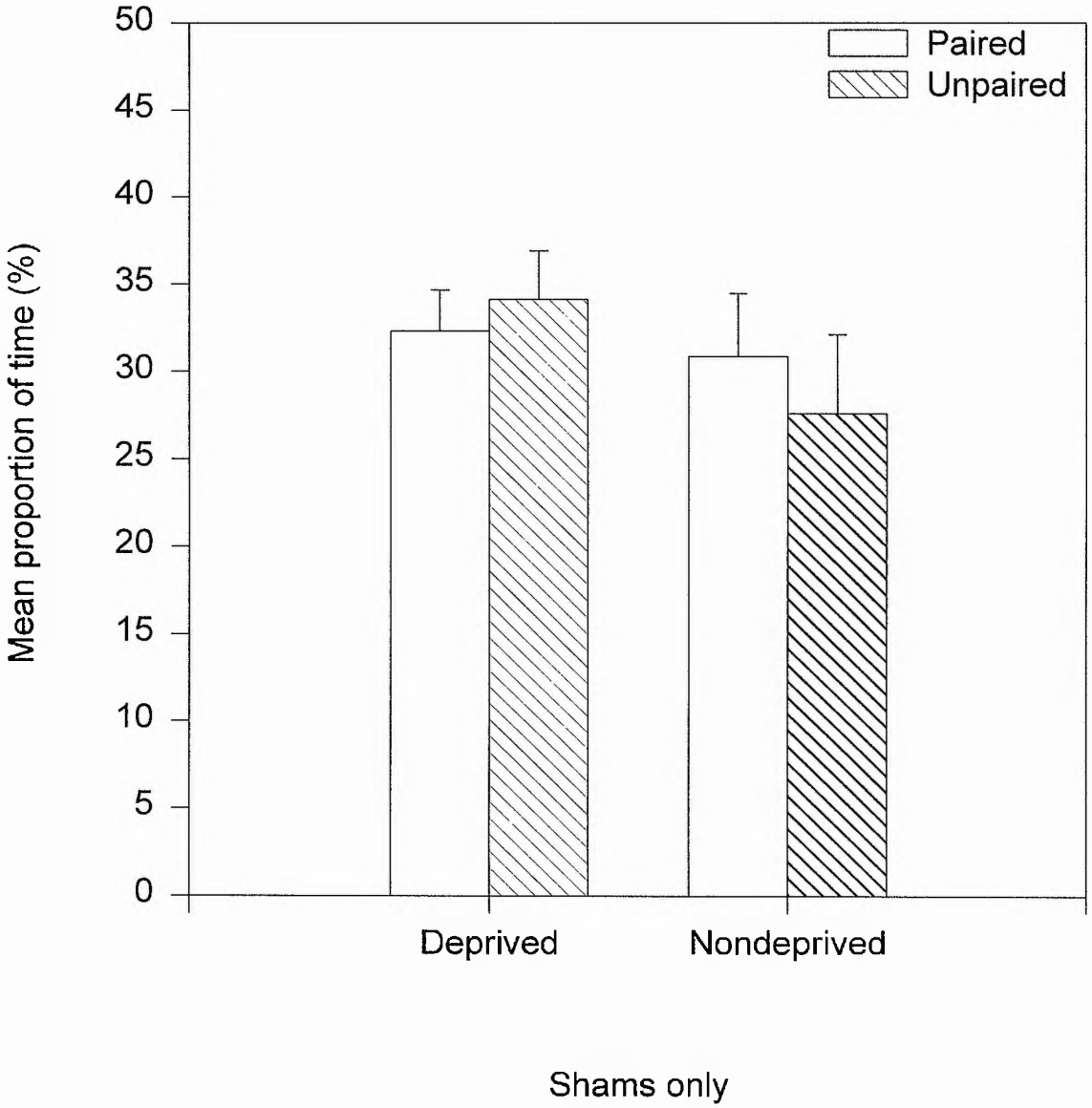
	Paired	Unpaired	Neutral
SHAM			
Time (proportion; ± SEM)	30.90 (3.60)	27.60 (4.52)	41.50 (2.60)
CP Loco (± SEM)	0.16 (0.02)	0.15 (0.02)	
CP Rear (± SEM)	0.03 (0.01)	0.03 (0.00)	0.03 (0.00)
PPTg			
Time (proportion; ± SEM)	34.87 (2.57)	30.47 (1.84)	34.66 (1.22)
CP Loco (± SEM)	0.16 (0.03)	0.16 (0.02)	
CP Rear (± SEM)	0.04 (0.01)	0.03 (0.00)	0.04 (0.01)

deprived) ($F_{1,24} = 0.29$). There were also no significant two-way interaction of group x side ($F_{2,48} = 1.74$), group x state ($F_{1,24} = 0.29$), or a three way interaction of group x side x state ($F_{2,48} = 0.30$). However, there was a significant effect of the two-way interaction of side x state ($F_{2,48} = 3.71$, $p = 0.032$). Simply, the proportion of time spent in the unpaired side by non-deprived rats was less than that spent in the neutral environment by non-deprived rats (Tukey-HSD post-hoc test, p=0.05). In essence, both sham and PPTg lesioned rats, in either a deprived or non-deprived state, spent an equal proportion of time on the paired and unpaired side on the test day. The lack of preference for either environment over the other indicates the animal did not form a CPP to the reward paired environment nor a conditioned aversion to the non-reward paired environment. The specific lack of preference formed by the sham rats in either the food deprived or non-deprived state is shown in Figure 4.1.2. This lack of preference displayed by the sham rats was worrying and indicates something wrong with the paradigm used. In this case it may indicate something wrong with either the apparatus used or the reward stimuli used (see below).

Measures of generalised arousal in the number of quadrant crossings the rats made in either the paired or unpaired environment, as well as the number of rears made in all three environments, were analysed as well. Both were calculated as a proportion of the time spent in each environment, for example, the total number of crosses or rears in the reward-paired environment / time spent in reward environment over the 15 min. test period. The resulting score is referred to as conditional probability of locomotion (crosses) and conditional probability of rears. Means for both of these for the 4 groups are presented in Table 4.1.1. Repeated measures

Figure 4.1.2

Mean proportion of time spent in the paired and unpaired boxes of CPP procedure 1 following sham lesion of the PPTg. This graph illustrates data for both food deprived and non-deprived animals. As evident from this figure there was no difference in the amount of time spent on either the paired or unpaired side for these animals. (See text for statistical details.)


50
45
40
35
30
25
20
15
10
5
0
Mean proportion of time (%)
Paired
Unpaired
Deprived
Nondeprived
Shams only

analysis of variance, again with side as the within subjects factor and deprivation state and lesion group as the between subjects factors, for the number of crosses revealed no main effect of group ($F_{1,24}$ = 2.71), no main effect of side (here only referring to either paired or unpaired side as there was no room for crosses in the neutral environment; $F_{1,24}$ = 0.23), and no main effect of deprivation state ($F_{1,24}$ = 1.19). There were no significant two way interaction of group x side ($F_{1,24}$ = 0.05), group x state ($F_{1,24}$ = 2.98), or side x state ($F_{1,24}$ < 0.001) or a three-way interaction of group x side x state ($F_{1,24}$ = 0.39), but there was a significant effect of the interaction of group x state ($F_{1,48}$ = 4.68, p = 0.036).

Repeated measures analysis of variance for the number of rears revealed no main effect of group ($F_{1,24}$ = 0.91), side ($F_{2,48}$ = 1.17) or state ($F_{1,24}$ = 1.33). There were no significant two way interaction of group x side ($F_{2,48}$ = 1.03), group x state ($F_{1,24}$ = 1.47), or side x state ($F_{2,48}$ = 0.39), or a three way interaction of group x side x state ($F_{2,48}$ = 0.01).

Discussion

While it is clear from the results that the PPTg lesioned rats were unable to form a positive place preference using food as a rewarding stimulus, it is also clear that rats with control phosphate buffer lesions were unable to form a preference either. This finding negates any conclusions to be made on the role of the PPTg in mediating the motivational effects of food reward stimuli in the CPP paradigm as adequate controls have not been established. This result was unexpected considering previous results of food reward CPP (Bechara and van der Kooy, 1992b).

Consideration of the apparatus, however, indicates that the preference boxes used in this experiment were larger than those used in many other place preference experiments. On the surface this does not seem to be a problem, but the front of these boxes are made from clear perspex which allows the rats to view a larger proportion of the room environment the boxes are placed in. The animal therefore has both box specific cues as well as room specific cues to which it can pay attention and use for association purposes with the food, and lack of food, pairings. This itself would provide confusion as the importance of box contextual cues versus room global cues would be in direct competition. In addition, however, as the room view is considerably expansive from both the pairing box and the non-pairing box, similar cues would be used for associations in both situations. As similar cues are being used for the reward and non-reward associations then a consolidation of an environmental incentive association is not established. With this step in the associative learning not established, the animal later displays this disruption in the form of equal approach behaviours to both environments, and thus the lack of a positive place preference.

In an attempt to overcome this problem, Procedure 2 (see General Methods) was employed. This modification simply involved the altering of the preference boxes such that the view from the perpex front was eliminated. Appropriately coloured card was attached to the front of the 3 experimental compartments, leaving a gap of 4 cm at the bottom through which the rats' feet and lower body were visible. This allowed the observer accurately to score when the rat was in each specific compartment but removed the global room cues the animal could focus on. This limited the association cues to the specific environment contextual cues of the

pairing and non-pairing boxes. It was hoped this would provide a focus for the rats' attention and subsequent ability to establish appropriate environment incentive associations leading to the formation of a positive place preference.

4.2 Experiment 2: Effects of excitotoxic lesions of the pedunculopontine tegmental nucleus on the formation of a food conditioned place preference in food deprived and non-food deprived animals.: Procedure 2.

Methods

Animals

23 rats were used (Charles River). Mean weight at time of surgery was 274.86 ± 11.94 (SD).

Surgery

Rats were anaesthetised with sodium pentobarbitone (Sagatal, Rhône Mérieux; 60 mg/kg) before being placed in the stereotaxic frame. 7 rats were given a lesion of the PPTg while 8 were given a phosphate buffer control lesion (see General Methods). Of these rats 3 ibotenate lesioned rats and 4 control lesioned rats were in the food deprived condition, while 4 ibotenate lesioned rats and 4 control lesioned rats were in the non-deprived condition. *Note*: In this experiment, 8 unoperated rats served as a control for potential surgical effects on formation of a place preference. Of these rats 4 were place in the food deprived condition and 4 were placed in the non-deprived condition. Rats were given at least 2 weeks postoperative care before behavioural testing began.

Behavioural Testing

Rats were trained under the conditioned place preference Procedure 2 as outlined in the General Methods.

Results

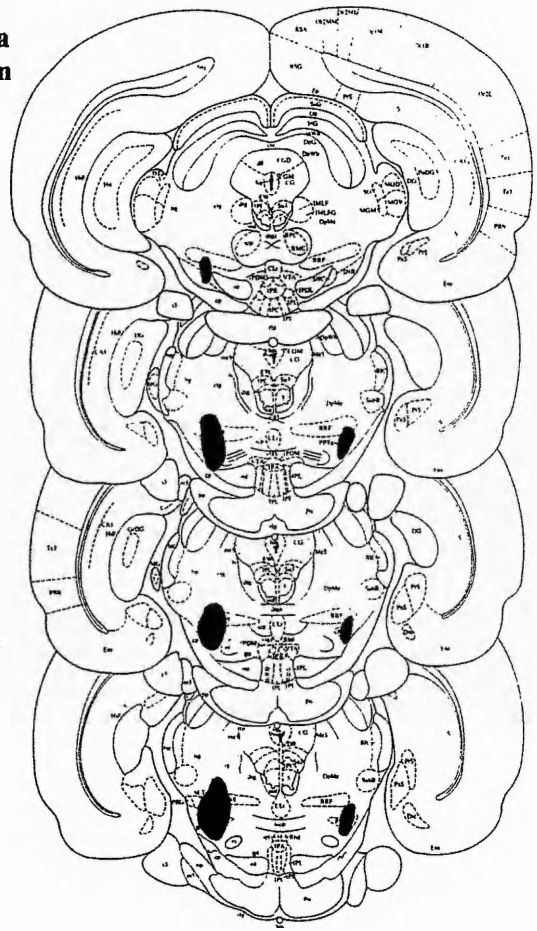
Figure 4.2.1 represents the largest and smallest lesion sizes for the PPTg ibotenate lesioned rats of this experiment. Damage to nearby structures was negligible and inconsistent across rats. 4 rats had damage to the adjoining retrorubral field, 2 rats had damage to the deep mesencephalic nucleus and 1 rats had unilateral damage to the cuneiform nucleus. 5 of the rats had minimal damage to the superior cerebellar peduncle. The inconsistent damage to these structures did not produce a change in behaviour across the PPTg lesioned rats. This was examined through direct comparison of damage sites and behavioural outcome. No consistent relationship between damage to a structure other than the PPTg and behaviour were found.

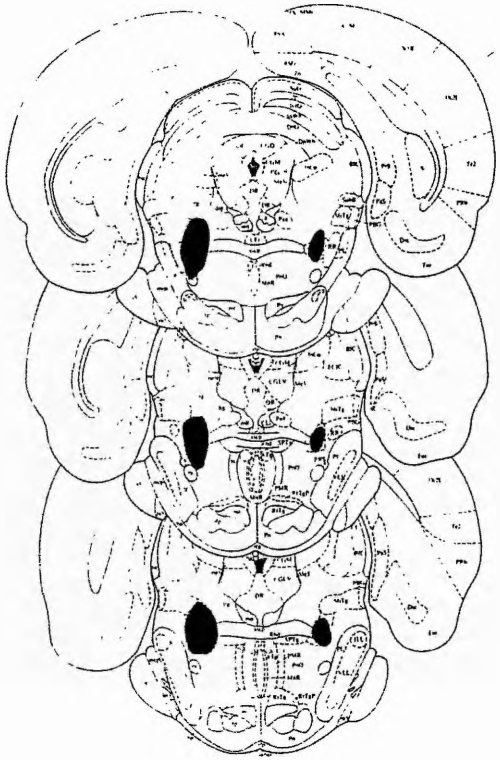
Measurement of time spent in the reward paired environment and unpaired environment were represented as proportion of time. The mean proportion of time spent in all environments for the deprived and non-deprived rats are outlined in Table 4.2.2. Data were analysed using a repeated measures analysis of variance with lesion group (sham vs. ibotenate) and deprivation state (deprived vs. non-deprived) as between subjects factors and side or compartment (paired, unpaired and neutral) as the within subjects factor. Again it is noted that this type of repeated measures analysis, with all compartments as the within subjects variable, is a common analysis used in the conditioned place preference literature and was thus chosen for this experiment. First, comparison of the two types of sham groups (operated vs. un-operated) is shown in Table 4.2.1. Analysis of these data revealed no main effect of group ($F_{1,12} = 1.00$), deprivation state ($F_{1,12} = 1.00$) or side ($F_{2,24} = 0.48$) and no

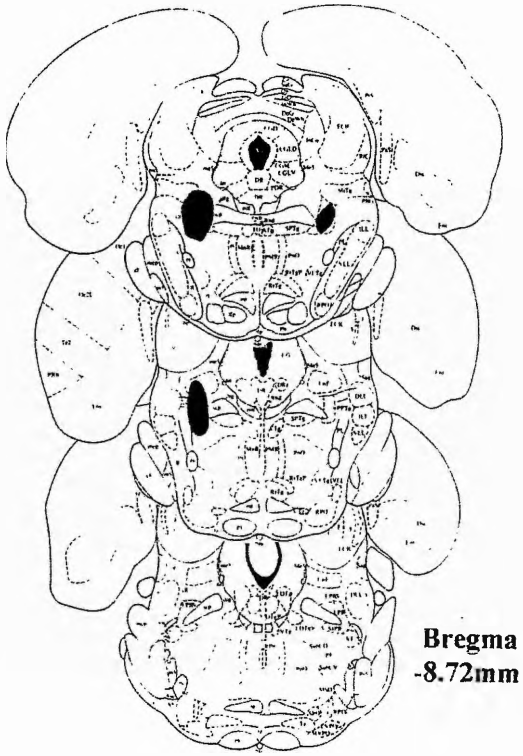
Figure 4.2.1

Representative sections from Bregma -6.30 to -8.72 mm redrawn from the atlas of Paxinos and Watson (1986) which illustrate the largest (left) and smallest (right) lesions for PPTg ibotenic acid lesioned rats in this experiment.

Bregma
-6.30mm







Bregma
-8.72mm

Table 4.2.1

Conditioned Place Preference: Procedure 2: The mean proportion of time spent in the paired, unpaired and neutral environments for deprived and non-deprived sham animals only. Analysis reveals no main effect of group as is evident from below. See text for statistical details.

Deprived

	Paired	Unpaired	Neutral
SHAM: Operated			
Time (proportion; ± SEM)	33.88 (5.85)	29.35 (1.82)	36.78 (5.64)
SHAM: Un-operated			
Time (proportion; ± SEM)	36.70 (1.71)	32.23 (5.06)	31.20 (3.67)

Non-Deprived

	Paired	Unpaired	Neutral
SHAM: Operated			
Time (proportion; ± SEM)	39.03 (5.37)	33.83 (2.23)	27.15 (3.41)
SHAM: Un-operated			
Time (proportion; ± SEM)	31.13 (3.19)	35.67 (2.52)	33.20 (1.59)

Table 4.2.2

Conditioned Place Preference: Procedure 2: The mean proportion of time spent in the paired, unpaired and neutral environments for deprived and non-deprived animals as well a measure of locomotion in the paired and unpaired environments.

Deprived

	Paired	Unpaired	Neutral
SHAM			
Time (proportion; ± SEM)	35.29 (2.87)	30.79 (2.55)	33.92 (3.32)
CP Loco (± SEM)	0.15 (0.01)	0.15 (0.01)	
PPTg			
Time (proportion; ± SEM)	41.77 (2.48)	29.17 (0.58)	29.06 (2.48)
CP Loco (± SEM)	0.12 (0.05)	0.13 (0.06)	

Non-Deprived

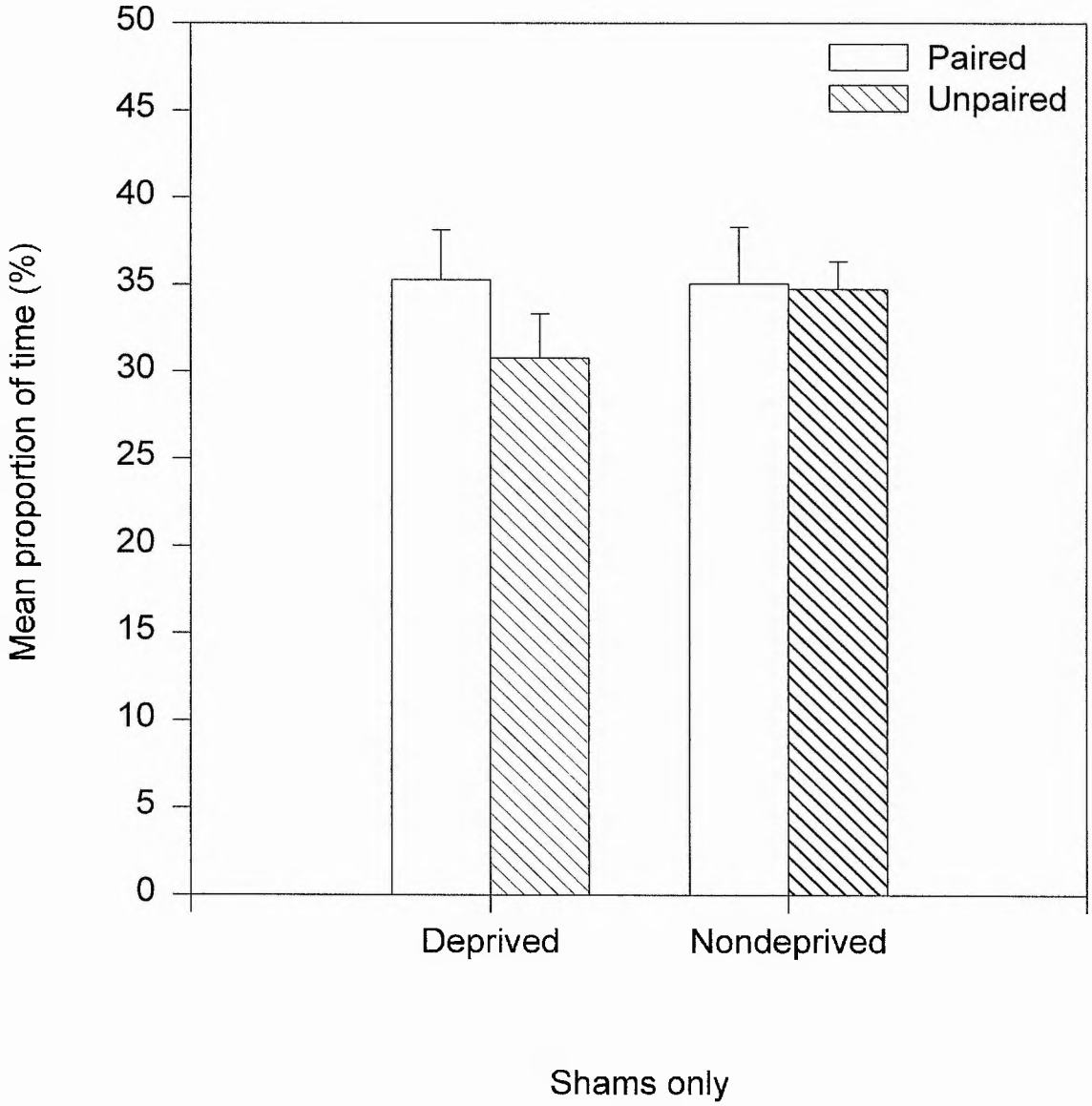
	Paired	Unpaired	Neutral
SHAM			
Time (proportion; ± SEM)	35.07 (3.25)	34.75 (1.59)	30.18 (2.08)
CP Loco (± SEM)	0.11 (0.01)	0.11 (0.01)	
PPTg			
Time (proportion; ± SEM)	35.88 (3.93)	39.25 (5.46)	24.87 (1.57)
CP Loco (± SEM)	0.13 (0.03)	0.12 (0.04)	

significant two-way interaction of group x side ($F_{2,24}$ = 0.27), group x state ($F_{1,12}$ = 1.00) or state x side ($F_{2,24}$ = 0.69) and the three way interaction of group x side x state ($F_{2,24}$ = 1.43) was not significant. Therefore the data from these two groups were combined for further analysis. The main analysis of variance revealed no main effect of group ($F_{1,19}$ < 0.001), or state ($F_{1,19}$ < 0.001), but there was a significant main effect of side ($F_{2,38}$ =3.34, p = 0.046). Post-hoc analysis revealed that the time spent in the paired environment is significantly different than that spent in the neutral environment, but not the unpaired environment and the time spent in the unpaired environment is not statistically different than either the time spent in the paired or neutral environments (Tukey-HSD, p = 0.05). There was no significant two-way interaction of group x side ($F_{2,38}$ = 1.22), group x state ($F_{1,19}$ < 0.001), or side x state ($F_{2,38}$ = 2.21) and the three way interaction of group x side x state ($F_{2,38}$ = 0.52) was not significant. Considering there was no significant difference found between the time spent in the paired environment and the unpaired environment, for sham or ibotenate lesioned rats, seems to again reveal the failure of the rats to form a significant positive conditioned place preference. The performance of the sham rats in either the food deprived or non-deprived state is represented in Figure 4.2.2. As in experiment 1, this lack of effect is uncertain. Having now determined that the lack of preference formation is not due to the lack of environment cue specificity, the role of the reward stimulus will have to be considered, and is discussed further below.

Measures of generalised activity in the number of crosses the rats made in either the paired or unpaired environment were analysed as well (there was no room for crosses in the neutral environment; measurement of the number of rears, as in

Figure 4.2.2

Mean proportion of time spent in the paired and unpaired boxes of CPP procedure 2 following sham lesion of the PPTg. This graph illustrates data for both food deprived and non-deprived animals. As evident from this figure there is no difference in the amount of time spent on either the paired or unpaired side for these animals. See text for statistical details.


50
45
40
35
30
25
20
15
10
5
0
Mean proportion of time (%)
Paired
Unpaired
Deprived
Nondeprived
Shams only

experiment 1, was not available as only the lower portion of the rat's body was visible in the modified boxes). Crosses were calculated as a proportion in relation to the time spent in each environment. For example, the total number of crosses in the reward-paired environment / time spent in reward environment over 15 minute test period and is referred to as conditional probability of locomotion. Means of these for the 4 groups are outlined in Table 4.2.2. Again, first looking at the two sham groups (operated vs. un-operated) revealed no main effect of group ($F_{1,12} = 0.73$), side ($F_{1,12} = 0.10$), or state ($F_{1,12} = 0.73$). The were no significant two-way interactions (group x side [$F_{1,12} = 4.37$; $p > 0.05$], group x state [$F_{1,12} = 0.79$], or side x state [$F_{1,12} = 0.05$]) or three-way interaction of group x side x state ($F_{1,12} = 2.02$). As a result of these analyses, the sham groups were combined for further analysis. The main analysis of variance for the number of crosses revealed no main effect of group ($F_{1,19} = 0.05$), no main effect of side ($F_{1,19} = 0.03$), and no main effect of state ($F_{1,19} = 0.71$). There were no significant two way interactions (group x side [$F_{1,19} = 0.01$], group x state [$F_{1,19} = 0.79$], or side x state [$F_{1,19} = 0.67$]) or three-way interaction of group x side x state ($F_{1,19} = 0.38$). From these data it is clear that there were no differences between the groups in the level of generalised arousal as measured by locomotion. These data do demonstrate that the rats physically explored the environments, but did not display a stimulus reward association in the form of a positive place preference.

Discussion

Similar to the findings of experiment 1, while PPTg lesioned rats failed to form a positive place preference, control rats failed to establish the same preference. Again this result was unexpected, especially as it was anticipated that the

modification to the experimental apparatus would rectify the previous null results. The only suggested interpretation of the finding is that food is simply not rewarding enough to establish a place preference. While food may be more rewarding in an environment than lack of food, it may not be sufficient to establish the formation of a stimulus-environment association. This is suggested as the food reward does not differ from the food pellets the animal receives in its home cage on a daily basis. The lack of a discriminable reward stimulus may make it difficult to appreciate the significance of the pairing box as an associate with the rewarding stimuli leading to a lack of a stimulus-environment association and display of approach behavior indicative of a positive place preference.

To address this problem the use of a more substantial and discriminable reward stimulus was employed. Using the place preference paradigm of White and Carr (1985), 20% sucrose solution was used as a reward stimulus and the number of association pairs was increased to replicate the methodology outlined by White and Carr (1985). Given their success in establishing a positive place preference with a range of sucrose solutions in unlesioned rats it was hoped that this would prove successful in examining any role of the PPTg in CPP formation.

4.3 Experiment 3: Effects of excitotoxic lesions of the pedunculopontine tegmental nucleus on the formation of a sucrose conditioned place preference in food deprived and non-food deprived animals.

Methods

Animals

31 rats were used (Charles River). Mean weight at time of surgery was 342.20 ± 18.60 (SD).

Surgery

Rats were anaesthetised with sodium pentobarbitone (Sagatal, Rhône Mérieux; 60 mg/ml) before being placed in the stereotaxic frame. 14 rats were given a lesion of the PPTg while 17 were given a phosphate buffer control lesion (see General Methods). Of these rats 7 ibotenate lesioned rats and 9 control lesioned rats were in the food deprived condition, while 7 ibotenate lesioned rats and 8 control lesioned rats were in the non-deprived condition. Rats were given at least 2 weeks postoperative care before behavioural testing began.

Behavioural Testing

Rats were trained under the conditioned place preference Procedure 3 as outlined in the General Methods. In this experiment the amount of food and water intake throughout training was measured as was the amount of sucrose reward consumed in the pairing environment.

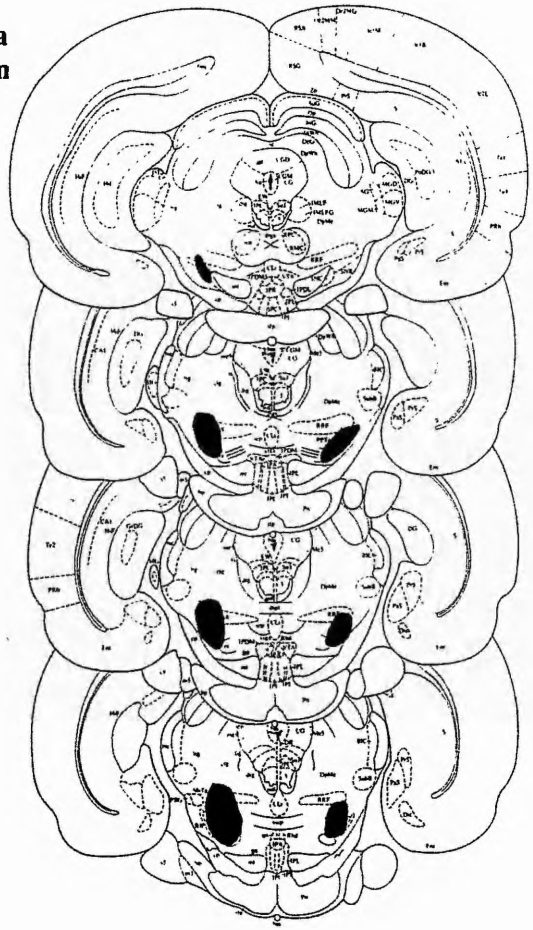
Results

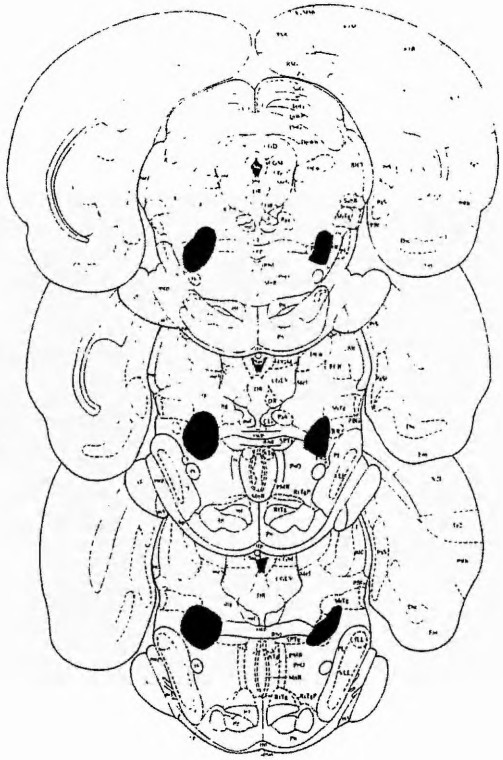
Figure 4.3.1 represents the largest and smallest lesion sizes for the PPTg ibotenate lesioned rats of this experiment. Damage to nearby structures was

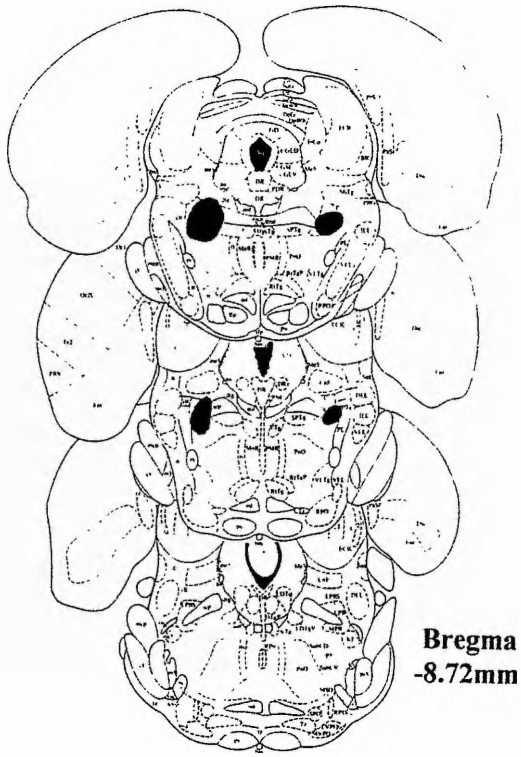
Figure 4.3.1

Representative sections from Bregma -6.30 to -8.72 mm redrawn from the atlas of Paxinos and Watson (1986) which illustrate the largest (left) and smallest (right) lesions for PPTg ibotenic acid lesioned rats in this experiment.

Bregma
-6.30mm







Bregma
-8.72mm

negligible and inconsistent across rats. 3 rats had damage to the deep mesencephalic nucleus, 2 of which were very minimal. 5 rats had damage to the adjoining retrorubral field and 2 rats had damage to the parabrachial nucleus. 1 rat showed very minimal damage to the subpeduncular nucleus. All rats had minimal damage to the superior cerebellar peduncle. The inconsistent damage to these structures did not produce a change in behaviour across the PPTg lesioned rats. This was examined through direct comparison of damage sites and behavioural outcome. No consistent relationship between damage to a structure other than the PPTg and behaviour were found.

Measurement of time spent in the reward paired and unpaired environment were represented as proportion of time. The mean proportion of time spent in all environments for the deprived and non-deprived rats are outlined in Table 4.3.1. Data were analysed using a repeated measures analysis of variance with lesion group (sham vs. ibotenate) and deprivation state (deprived vs. non-deprived) as the between subjects factors and side or compartment (paired, unpaired, and neutral) as the within subjects factor. As mentioned above, this type of repeated measures analysis is commonly used in the conditioned place preference literature and was the analysis used by White and Carr (1985). As this experiment was an almost exact replication of the methodology of White and Carr (1985), this analysis was used in this experiment. Analysis of variance revealed no main effect of group ($F_{1,27} = 0.30$), and no main effect of state ($F_{1,27} = 0.30$), but a significant effect of side ($F_{2,54} = 9.59$, $p < 0.001$). Post-hoc analysis revealed that the time spent in the paired environment was greater than time spent in either the unpaired or neutral environments while these

Table 4.3.1

Conditioned Place Preference: Procedure 3: The mean proportion of time spent in the paired, unpaired and neutral environments for deprived and non-deprived animals as well a measure of locomotion in the paired and unpaired environments.

Deprived

	Paired	Unpaired	Neutral
SHAM			
Time (proportion; ± SEM)	43.32 (2.83)	24.30 (2.39)	32.38 (2.64)
CP Loco (± SEM)	0.20 (0.03)	0.23 (0.02)	
PPTg			
Time (proportion; ± SEM)	40.96 (2.76)	29.83 (2.87)	29.21 (1.99)
CP Loco (± SEM)	0.16 (0.02)	0.19 (0.03)	

Non-Deprived

	Paired	Unpaired	Neutral
SHAM			
Time (proportion; ± SEM)	40.03 (4.68)	29.68 (5.36)	30.29 (2.40)
CP Loco (± SEM)	0.13 (0.01)	0.16 (0.01)	
PPTg			
Time (proportion; ± SEM)	36.58 (3.87)	27.39 (4.16)	36.03 (1.25)
CP Loco (± SEM)	0.14 (0.02)	0.17 (0.03)	

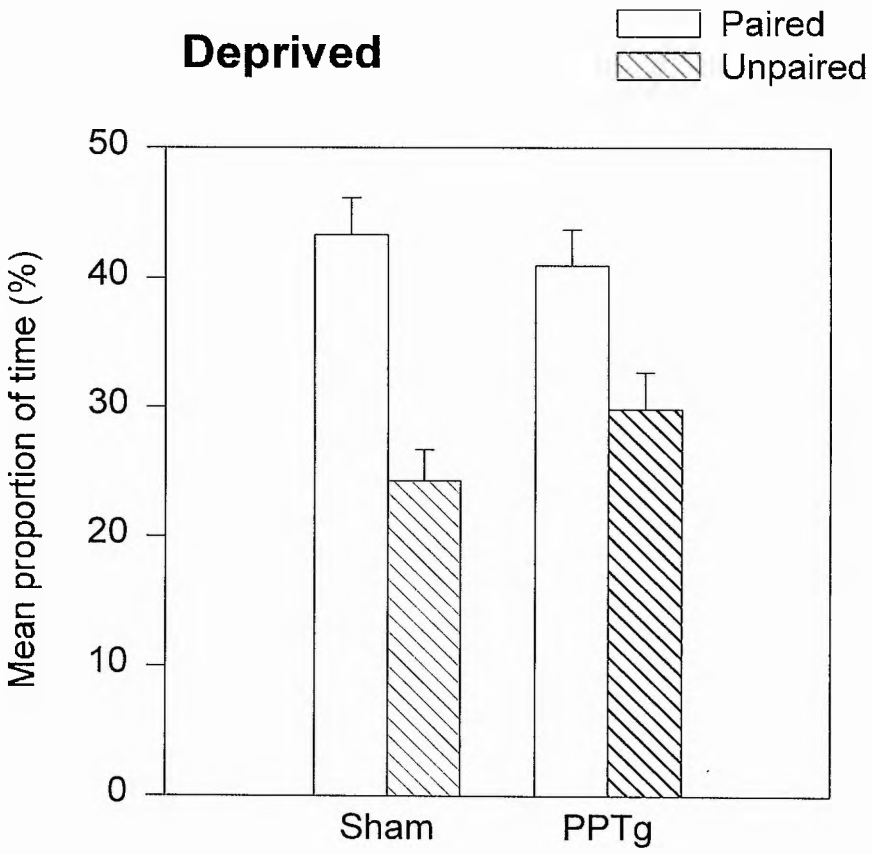
latter times were not statistically different from each other (Tukey-HSD, $p = 0.05$). There were no significant two-way interaction of group x side ($F_{2,54} = 0.38$), group x state ($F_{1,27} = 0.43$), or side x state ($F_{2,54} = 0.68$), or a three way interaction of group x side x state ($F_{2,54} = 1.06$). These results suggest that the rats had formed a place preference and, as demonstrated in Figure 4.3.2, this preference was to the reward paired side. This formation of a place preference was seen in both sham and PPTg lesioned rats, independent of deprivation state. Lesions of the PPTg did not interfere with the rats' ability to form a positive place preference using a 20% sucrose solution reward.

Further analysis of the formation of the place preference over the 15 min test session was examined in dividing the session into separate 5 min time periods. While all groups were able to form a place preference, this analysis was conducted to determine if there was any difference in the pattern of this preference formation over the test session. Data from each time period was analysed using a repeated measures analysis of variance with lesion group and deprivation state as the between subjects factors and side or compartment as the within subjects factor. These data are shown in Table 4.3.2.

Analysis of the 0-5 min period revealed no main effect of group ($F_{1,27} < 0.001$), and no main effect of state ($F_{1,27} < 0.001$), but a main effect of side ($F_{2,54} = 3.91$, $p = 0.026$). Post-hoc analysis revealed that the time spent in the neutral environment was greater than that of the unpaired environment, but was not different than the time spent in the paired environment (Tukey-HSD, $p = 0.05$). This result is

Figure 4.3.2

Mean proportion of time spent in the paired and unpaired boxes of CPP procedure 3 following sham lesion of the PPTg. This graph illustrates data for both food deprived and non-deprived animals. As evident from this figure there is a difference in the amount of time spent on the paired side versus the unpaired side for these animals. See text for statistical details.


Deprived
Paired
Unpaired
Mean proportion of time (%)
50
40
30
20
10
0
Sham
PPTg

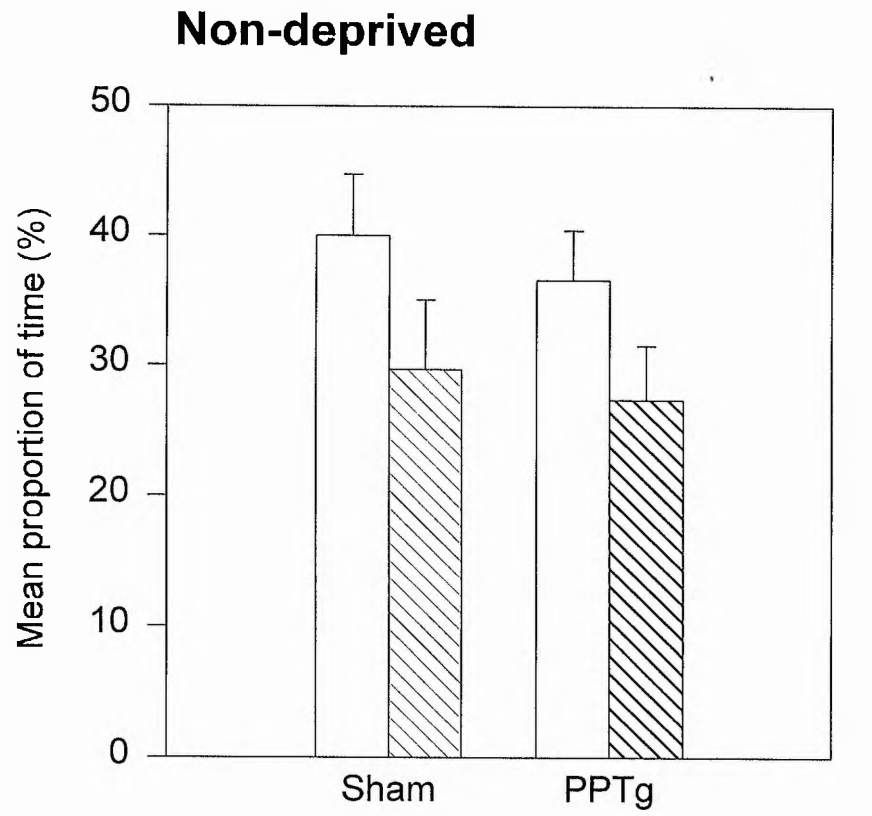

Non-deprived
Mean proportion of time (%)
50
40
30
20
10
0
Sham
PPTg

Table 4.3.2

Conditioned Place Preference: Procedure 3: The mean (± SEM) proportion of time spent in the paired, unpaired and neutral environments for deprived and non-deprived animals during the test session. Session was divided into 3 equal time periods for more discrete analysis. See text for details.

Group	CPP Test Session Divided Into Equal Time Periods								
	0-5 min			5-10 min			0-15 min		
	Paired	Unpaired	Neutral	Paired	Unpaired	Neutral	Paired	Unpaired	Neutral
Sham Deprived	37.61 ± 5.99	28.58 ± 7.01	33.81 ± 3.20	48.64 ± 3.92	20.53 ± 2.25	30.83 ± 4.75	44.22 ± 5.63	23.76 ± 3.20	32.02 ± 4.75
PPTg Deprived	34.71 ± 1.86	25.72 ± 3.24	39.57 ± 2.47	45.17 ± 4.69	31.94 ± 3.62	22.89 ± 1.82	44.01 ± 5.43	32.91 ± 4.12	23.08 ± 2.74
Sham Non-Deprived	31.65 ± 4.51	28.78 ± 4.57	39.57 ± 4.43	40.97 ± 6.40	29.95 ± 6.43	29.08 ± 2.93	47.45 ± 8.50	30.32 ± 7.24	22.23 ± 3.23
PPTg Non-Deprived	34.94 ± 3.84	26.1 ± 3.86	38.96 ± 2.78	37.46 ± 5.85	24.82 ± 5.13	37.72 ± 1.62	37.93 ± 6.33	30.67 ± 6.81	31.4 ± 1.97

not surprising as the animal was coming back in contact with the neutral environment that it had not been exposed to for the past two weeks when the rats were restricted to either their paired or unpaired environments. The novelty this creates results in an equivalent amount of time spent in this environment compared to the environment in which they previously received sucrose reward. There was no significant two-way interaction of group x side ($F_{2,54} = 0.23$), group x state ($F_{1,27} < 0.001$), or side x state ($F_{2,54} = 0.24$), or a three-way interaction of group x side x state ($F_{2,54} = 0.32$).

Analysis of the 5-10 min period revealed no main effect of group ($F_{1,27} < 0.001$), and no main effect of state ($F_{1,27} < 0.001$), but a main effect of side ($F_{2,54} = 9.80$, $p < 0.001$). Post-hoc analysis revealed that the time spent in the paired environment was greater than that spent in either the unpaired or neutral environments, while the time spent in these latter environments were not different than each other. The initial novelty of the neutral environment was now abated and the animal spent more time in the environment in which it previously received sucrose reward, revealing its place preference. Further analysis revealed no significant two-way interaction of group x side ($F_{2,54} = 0.37$), group x state ($F_{1,27} < 0.001$), or side x state ($F_{2,54} = 1.72$), or a three-way interaction of group x side x state ($F_{2,54} = 2.28$).

Finally, analysis of the 10-15 min period revealed no main effect of group ($F_{1,27} < 0.001$), and no main effect of state ($F_{1,27} < 0.001$), but a main effect of side ($F_{2,54} = 6.99$, $p = 0.002$). This result is similar to the 5-10 min period in that post-hoc

analysis revealed that the amount of time spent in the paired environment was greater than that spent in either the unpaired or neutral environments, while the time spent in these latter environments were not different from each other. Simply put, the rats were displaying their place preference in spending more time in the environment in which they previously received sucrose reward. This pattern is not different across sham or PPTg lesioned rats, deprived or non-deprived. Further analysis revealed no significant two-way interaction of group x side ($F_{2,54} = 0.52$), group x state ($F_{1,27} < 0.001$), or side x state ($F_{2,54} = 0.08$), or a three-way interaction of group x side x state ($F_{2,54} = 1.39$).

In summary then, the results of the time period analysis revealed that there was no difference among the groups in the pattern of place preference formation. In the first 5 min period the rats spend an equal proportion of time in the paired and neutral environments, while in the second and third 5 min periods, the rats spend a greater proportion of time in the paired environment over the unpaired and neutral environments. The first period pattern of behaviour reflects the influence of the novelty of the neutral environment as the animal had not had exposure to this environment during the previous 14 days when the rats was either exposed to its paired or unpaired environments. The remainder of the test session the rats' spend a greater proportion of time in the paired environment reflecting their place preference for the environment in which they previously received sucrose reward. These data illustrate that not only were the PPTg rats, deprived or non-deprived, no different than sham rats in their ability to form a place preference, they were no different in the pattern with which this preference is demonstrated.

Repeated measures analysis of locomotion, as measured by number of crosses in both paired and unpaired environments, revealed no main effect of group ($F_{1,27} = 0.74$), no main effect of side ($F_{1,27} = 3.77$, $p > 0.05$), but a significant main effect of state ($F_{1,27} = 5.76$, $p = 0.024$). Overall, rats in the food deprived condition exhibited more crosses than rats in the non-deprived group (Tukey-HSD, $p = 0.05$), but it did not seem to affect the rats' ability to form a positive place preference. There were no significant two-way interaction of group x side ($F_{1,27} = 0.01$), group x state ($F_{1,27} = 1.68$), side x state ($F_{1,27} < 0.001$), or three-way interaction of group x side x state ($F_{1,27} < 0.001$). With no main effect of group or interactions, this analysis basically revealed that there was no difference in any of the rats' ability to explore the environment.

Amount of sucrose consumed over the 7 pairing days was analysed using a mixed between-within subjects repeated measures analysis of variance, with days as the within subjects factor and lesion group (sham vs. ibotenate) and deprivation state (deprived vs. non-deprived) as the between subjects factors. The mean amount of sucrose consumed across groups is demonstrated in Figure 4.3.3. The between subjects analysis reveals a significant main effect of group ($F_{1,27} = 29.68$, $p < 0.001$), a significant main effect of state ($F_{1,27} = 8.89$, $p = 0.006$), and a significant two-way interaction of group x state ($F_{1,27} = 8.27$, $p = 0.008$). The within subjects analysis revealed no main effect of reward pairing (1-7), ($F_{6,162} = 2.03$), and no two-way interactions of group x reward pairing ($F_{6,162} = 0.44$), state x reward pairing ($F_{6,162} = 1.44$), or a three way within subjects interaction of group x state x reward pairings ($F_{6,162} = 0.65$). This analysis demonstrates first that overall PPTg lesioned rats

consumed more of the 20% sucrose solution presented in the pairing environment, and specifically demonstrates that PPTg lesioned rats, in the food deprived condition, consume significantly more sucrose than rats in any of the other groups (Tukey-HSD, $p = 0.05$). This effect is demonstrated graphically in Figure 4.3.3. This effect is very robust and will be explored further in the discussion.

Data from food intake over the training period was analysed using repeated measures analysis of variance with days as the within subjects factor and group and deprivation state as the between subjects factors. This data is demonstrated in Figure 4.3.4. Between subjects analysis revealed a significant main effect of group ($F_{1,27} = 11.26$, $p=0.002$), a significant main effect of state ($F_{1,27} = 438.58$, $p<0.0001$), and a significant two-way interaction of group x state ($F_{1,27} = 8.75$, $p = 0.006$). Within subjects analysis revealed a significant main effect of days ($F_{13,351} = 3.85$, $p < 0.001$), a non-significant two-way interaction of group x days ($F_{13,351} = 0.28$), but a significant two way interaction of state x days ($F_{13,351} = 5.86$, $p < 0.001$), and a significant three way interaction of group x state x days ($F_{13,351} = 1.88$, $p = 0.032$). Essentially, non-deprived rats were consuming more food than deprived rats (which is obvious); sham rats are consuming slightly more than PPTg rats overall; and sham non-deprived rats are consuming most of all (Tukey-HSD, $p = 0.05$). This pattern fluctuates over days (as evident from the pattern of the graphs in Figure 4.3.4) but overall does not seem to influence the rats' formation of a positive place preference. These results may partially reflect the fact that on half the days analysed for food intake rats have another energy source in the form of sucrose in the paired place preference environment. As outlined above, deprived rats consume more sucrose

than non-deprived rats and PPTg rats consume more than sham rats and as such would probably need less energy intake overall.

Data from water intake over the training period was analysed using a repeated measures analysis of variance, with days as the within subjects factor and group and deprivation state as the between subjects factors. These data are demonstrated in Figure 4.3.5. Data from one animal was removed due to missing data points resulting from water bottle spillage. Between subjects analysis reveals a significant main effect of group ($F_{1,26} = 4.91$, $p = 0.036$), a significant main effect of state ($F_{1,26} = 23.80$, $p < 0.001$), but no significant two-way interaction of group x state ($F_{1,26} = 1.79$). The within subjects analysis reveals a significant main effect of days ($F_{13,338} = 4.91$, $p < 0.001$), a significant two-way interactions of group x days ($F_{13,338} = 1.76$, $p = 0.048$) and state x days ($F_{13,338} = 7.53$, $p < 0.001$), but no significant three-way interaction of group x state x days ($F_{13,338} = 1.27$). The pattern is quite similar to food intake in that non-deprived rats are drinking more than deprived rats, sham rats are drinking slightly more than PPTg rats overall, and sham non-deprived rats are drinking most of all (Tukey-HSD, $p = 0.05$). This pattern fluctuated over days (as evident from the pattern of the graphs in Figure 4.3.5) which is undoubtedly in relation to the fact that on half the days the rats have another temporary fluid source in the form of sucrose in their paired place preference environment.

Discussion

As is evident from Figure 4.3.2 and Table 4.3.1, sham rats were able to form a positive place preference when 20% sucrose was used as a reward stimulus. Given

Figure 4.3.3

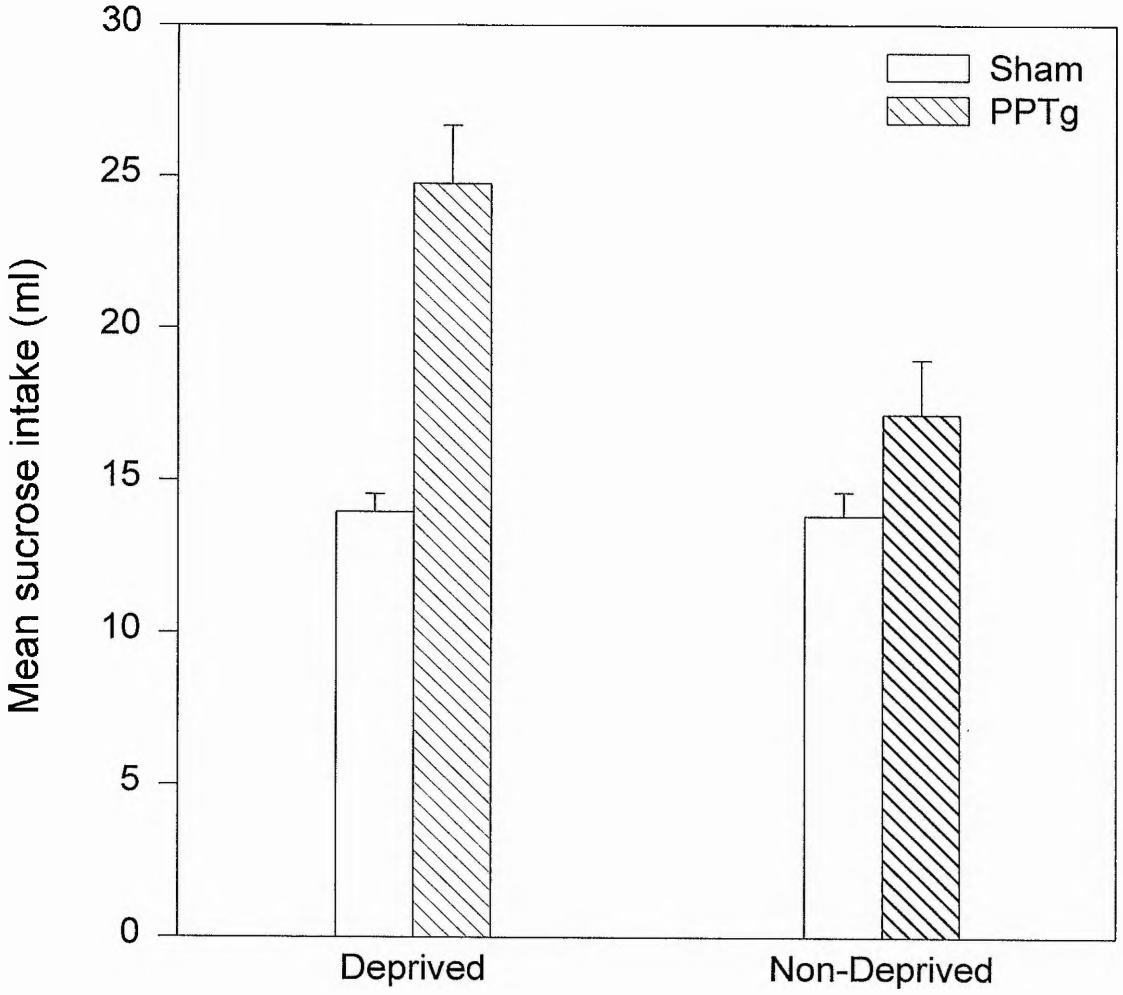
Mean amount of sucrose consumed during each CPP reward pairing session following sham or ibotenic acid PPTg lesions. This graph represents data for both food deprived and non-deprived animals. See text for statistical details.

Figure 4.3.4

Mean amount of home cage food consumed across the reward pairing and non-reward pairing days of CPP training for both food deprived and non-deprived sham and PPTg lesioned animals. See text for statistical details.

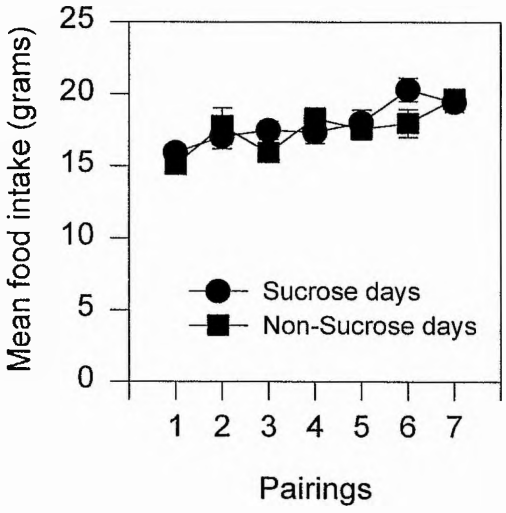
Figure 4.3.5

Mean home cage water intake across the reward pairing and non-reward pairing days of CPP training for both food deprived and non-deprived sham and PPTg lesioned animals. See text for statistical details.

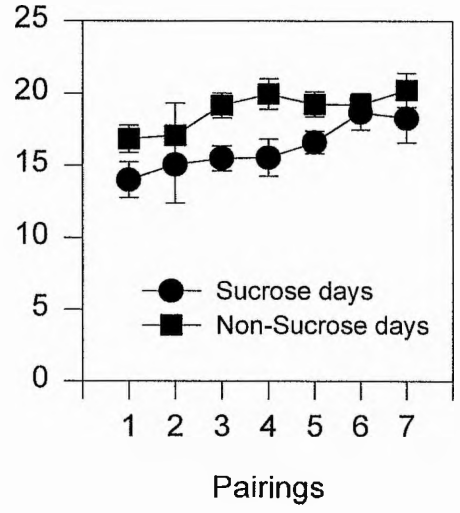

30
25
20
15
10
5
0
Mean sucrose intake (ml)
Sham
PPTg
Deprived
Non-Deprived

Deprived

Sham

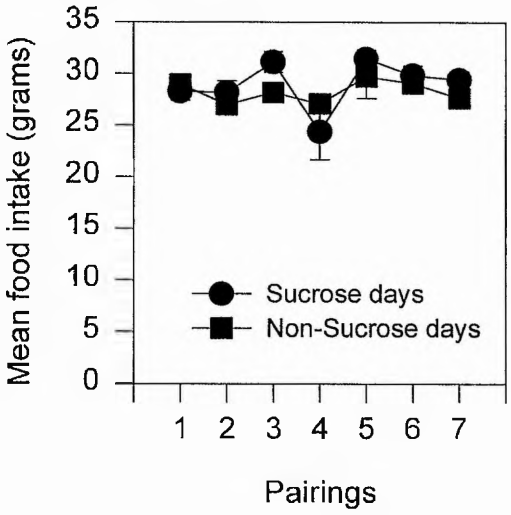


PPTg

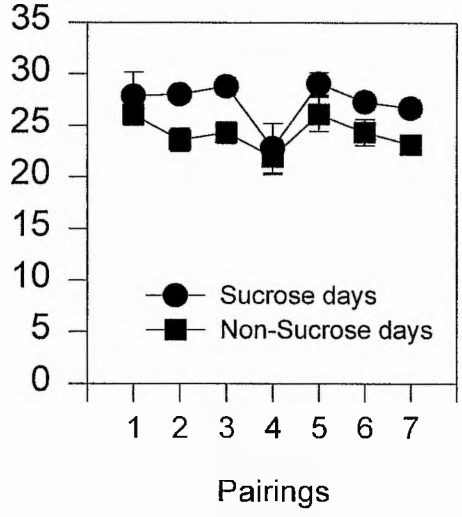


Non-Deprived

Sham

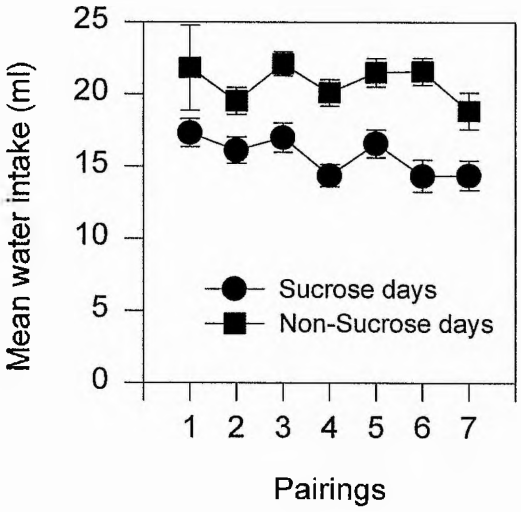


PPTg

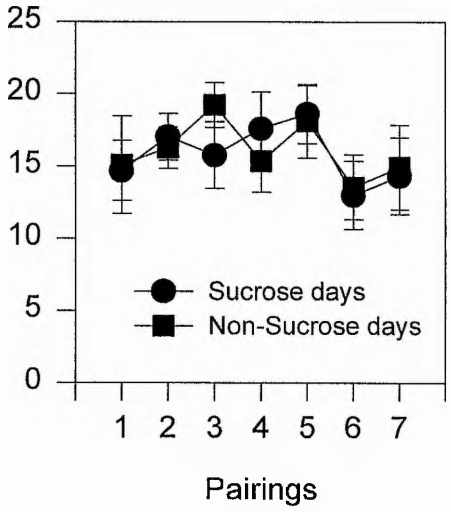


Deprived

Sham

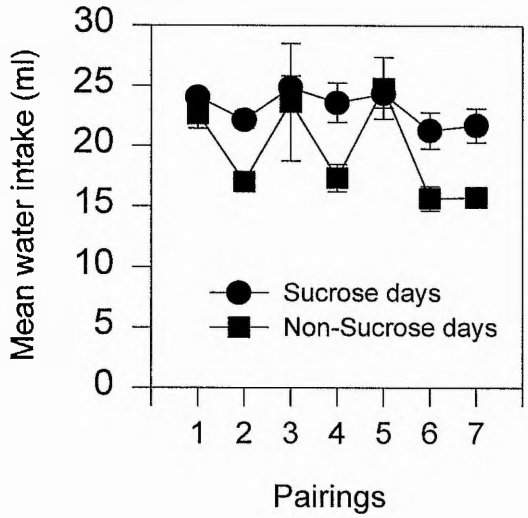


PPTg

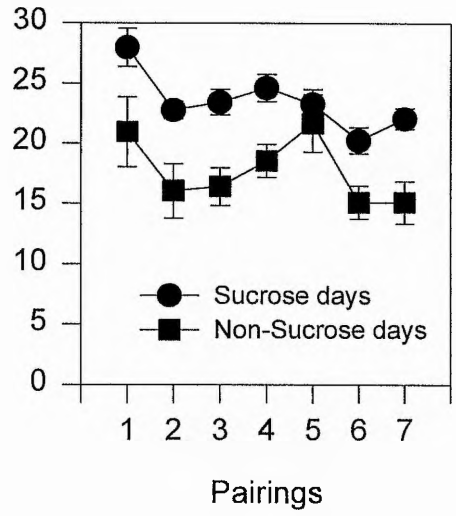


Non-Deprived

Sham



PPTg



the establishment of a control place preference, the effect of excitotoxic lesions of the PPTg on such formation can be examined. From Figure 4.3.2 it can be seen that there was no effect of lesion of the PPTg on the formation of a sucrose place preference in either food deprived or non-deprived rats. Analysis of the time data from this experiment also revealed that there was no difference across groups in the pattern of displaying their place preference across the 15 min test session (as seen in Table 4.3.2 and above). It is suggested that mediation of this reward process must be carried out elsewhere- an intact PPTg is not required. This data would seem contrary to the findings of van der Kooy and his colleagues and methodological differences may be the only explanation for this discrepancy. The effective lesion placements outlined in the work of van der Kooy and his colleagues were more strictly in the ventromedial portion of the PPTg (Bechara and van der Kooy, 1989; 1992b) compared to the lesions in the present experiments which strictly followed the atlas definition of the PPTg as outlined in Paxinos and Watson (1986). In addition, the concentration of ibotenate used by this group was sufficiently high as to warrant the use of tartaric acid as a vehicle for solubility (the vehicle used in the present work was sterile phosphate buffer). The nature of damage this acid vehicle alone would produce on the PPTg and surrounding tissue was not presented, nor was sufficient histology of the nature of toxin spread to surrounding structures from such a concentrated single dose addressed. With such anatomical variation, comparison of data becomes difficult. In this experiment, lesioning of just the PPTg does not affect the formation of a sucrose conditioned place preference. Surrounding structures damaged in the lesion methodology employed by van der Kooy's group may be the

source of mediation of this reward process and would have to be systematically explored for further elucidation.

The other main finding of this experiment was the pattern of sucrose consumption over the pairing days. Intake was found to be greater for PPTg rats and particularly more so for the PPTg deprived rats. Figure 4.3.3 demonstrates the pattern of consumption which reveals the extent to which PPTg deprived rats over-consume compared to rats in the other groups. This finding is curious in that it may seem to say something about the role of response disinhibition and the interplay of this with internal state. Food deprived rats consumed more sucrose, undoubtedly to increase energy intake they are not getting by being food deprived and in this experiment, rats with lesions of the PPTg do this to an even greater extent than control rats. The role of the PPTg in response disinhibition has been addressed to some extent in recent work of Keating and colleagues (1997) in which PPTg lesioned rats disinhibited responding for intravenous self-administration of *d*-amphetamine. What has not been addressed is the role of state or drive in influencing this disinhibition but is explored in Chapter 5 of this thesis.

Summary

The findings of this chapter first revealed that the formation of a positive CPP to a food stimulus was not a straightforward matter and was not replicated in this laboratory. Previous work has outlined the role of the PPTg in formation of a food reward CPP (Bechara and van der Kooy, 1992b), but these results were not replicable in this laboratory. In fact, formation of a food motivated place preference in control rats was not replicated and remains unexplained. A similar methodology to that previously reported (Bechara and van der Kooy, 1992b) was used and so uncertainty exists as to the null results reported in experiment 2 of this chapter.

Secondly, this chapter revealed that formation of a place preference using a sucrose reward was not state dependant, and was not mediated by the PPTg. Previous reports have outlined a state dependant role of the PPTg in mediation of CPP formation using various reward stimuli (Bechara and van der Kooy, 1989; Bechara et al., 1992 and Bechara and van der Kooy, 1992b) and this was not found when using sucrose as a reward stimulus. Both deprived and non-deprived PPTg lesioned rats were able to form a positive place preference using 20% sucrose as the reward stimulus.

Finally, this chapter also revealed that food deprived PPTg lesioned rats disinhibit their responding and over-consume a 20% sucrose solution compared to any of the other rats in this experiment. The role of internal drive in combination with lesions of the PPTg may interplay in degree of motivated responding and was explored further in Chapter 5 of this thesis.

Chapter 5: The role of the PPTg in control of reward-related responding.

5.0 Introduction

Processing of certain reward related responses is attributed to the ventral striatum whose output is in part at least mediated by the PPTg (Inglis and Winn, 1995). The role of the PPTg in appropriate responding to reward stimuli and inhibition of action has been examined indirectly and can be discussed in review of various experimental designs examining the function of the PPTg. Experiments by Dellu and colleagues (1991), looking at water maze performance in rats following PPTg lesions, found that lesioned rats took longer to find the escape platform than controls. Part of the reason the rats were impaired in this task was that they tended to pursue one pathway and did not disengage from it. This may not necessarily represent inappropriate responding but certainly an inability to inhibit a current response. Work by Inglis and colleagues (1994b) demonstrated that PPTg lesioned rats responding in a conditioned reinforcement task were able to respond on the reinforced lever, but responded equally on an unconditioned, non-reinforced lever. In this case, the rats did not seem to learn the value of the separate levers and responded, inappropriately, on both of them equally. The inability of the rats to inhibit responding on the non-reinforced lever was not seen in the sham lesioned rats, lending this behaviour to a feature of the PPTg lesion. More recent work by Keating and colleagues (1997) examined the role of the PPTg in responding for intravenous self-administration of *d*-amphetamine. Rats were initially trained to respond on a fixed ratio task, FR-2, in which rats had to press the lever twice to receive a 0.1 mg/kg intravenous administration of *d*-amphetamine. This responding

could repeat for a maximum of 20 infusions of *d*-amphetamine over a 3hr testing time. In this task PPTg lesioned rats responded quicker than sham rats, receiving most of their amphetamine in the first hour, and received more infusions of *d*-amphetamine as a result of their disinhibited responding. The rats were then trained on a progressive ratio schedule of reinforcement for receipt of *d*-amphetamine in which the rats had to respond an increasing number of times to receive each 0.1 mg/kg infusion. The level of responding for this reward is measured by how far along the progressive schedule the rats are willing to go in order to receive the next infusion of the *d*-amphetamine. The point at which the animal stops responding (lever pressing) for the reward is referred to as the breaking point. In this task the experimenters found that PPTg lesioned rats had a significantly higher breaking point than the sham rats, reaching a breaking point almost three times higher than control rats and thus taking in more infusions than the control rats. In this experiment PPTg lesioned rats were disinhibited in their responding for the *d*-amphetamine reward. This progressive ratio responding for *d*-amphetamine following PPTg lesions, is in contrast to earlier work by Robertson and colleagues (1994) who looked at progressive ratio responding following PPTg lesions but with food as the reward to be worked for. In this study the experimenters found that PPTg lesioned rats did not over-respond on the reinforced lever, but increased responding on the non-reinforced lever and had significantly lower breaking points than control rats. The difference in the responding patterns between both experiments would seem to be related to the reward stimuli used: *d*-amphetamine vs. food. It may be the case that there is a reward stimulus gradient which elicits variable responding in these PPTg lesioned rats.

Given the earlier finding of enhanced consumption and responding to 20% sucrose solution in the conditioned place preference experiment, the role of the PPTg in responding to stimuli along a reward gradient was examined in the following experiments. In this case the reward used was sucrose, but it was examined at varying concentrations to elucidate the nature of PPTg responding to one reward across a gradient. The element of drive related consumptive responding was also examined in including both food deprived and non-deprived rats in the first two experiments.

5.1 Experiment 1: Examination of the consumption response to varying concentrations of a sucrose solution following excitotoxic lesions of the PPTg: 1, 2, 4, 12 and 20% sucrose.

Methods

Animals

31 rats were used (Charles River). Mean weight at time of surgery was 342.20 ± 18.60 (SD). The rats used in this experiment were the same rats used in experiment 3 of the previous chapter (Chapter 4). After CPP experimentation was completed, the rats were randomly re-assigned into food deprived or non-food deprived groups. There was a gap of 1 week after the completion of the CPP testing before this experiment began and the rats were given access to their first concentration of sucrose.

Surgery

Surgery for these rats was performed before the rats began CPP experimentation and is outlined in experiment 3 of the previous chapter. Briefly, rats were anaesthetised with sodium pentobarbitone (Sagatal, Rhône Mérieux; 60 mg/kg) before being placed in the stereotaxic frame. 14 rats were given a lesion of the PPTg while 17 were given a phosphate buffer control lesion (see General Methods). Of these rats 7 ibotenate lesioned rats and 8 control lesioned rats were in the food deprived condition, while 7 ibotenate lesioned rats and 9 control lesioned rats were in the non-deprived condition. Rats were given at least two weeks postoperative care before behavioural testing began.

Sucrose drinking

Rats were given 24 hr home cage access to each of 1, 2, 4, 12, and 20% sucrose solutions. The order of presentation of sucrose was randomised and the rats were given access to one sucrose concentration every other day. Each concentration of sucrose was available only once. Water was available at all times and food was available at all times for the rats in the non-deprived group, while the rats in the food deprived group had access to food for 2 hr/day, delivered at approximately the same time as when the sucrose bottle was either given or taken away.

Results

Figure 5.1.1 represents the largest and smallest lesion sizes for the PPTg ibotenate lesioned rats of this experiment and as the rats of this experiment were the same used in experiment 3 of Chapter 4, this figure is the same as Figure 4.3.1 of the previous chapter. Damage to nearby structures was negligible and inconsistent across rats. 3 rats had damage to the deep mesencephalic nucleus, 2 of which were very minimal. 5 rats had damage to the adjoining retrorubral field and 2 rats had damage to the parabrachial nucleus. 1 rat showed very minimal damage to the subpeduncular nucleus. All rats had minimal damage to the superior cerebellar peduncle. The inconsistent damage to these structures did not produce a change in behaviour across the PPTg lesioned rats. This was examined through direct comparison of damage sites and behavioural outcome. No consistent relationship between damage to a structure other than the PPTg and behaviour were found.

Sucrose intake over the five concentrations was analysed using a repeated measures ANOVA, with group and state as the between subjects factor and

concentrations as the within subjects factor. The results are represented in Figure 5.1.2. Between subjects analysis revealed a significant main effect of group ($F_{1,26}$ = 4.90, p = 0.036), a significant main effect of state ($F_{1,26}$ = 41.94, p < 0.001), but no significant effect of the two-way interaction of group x state ($F_{1,26}$ = 3.53). The within subjects analysis revealed a significant main effect of concentrations ($F_{4,104}$ = 292.76, p < 0.0001), a significant effect of the two-way interactions of group x concentrations ($F_{4,104}$ = 6.02, p < 0.001) and state x concentrations ($F_{4,104}$ = 13.01, p < 0.001), but no significant effect of the three way interaction of group x state x concentrations ($F_{4,104}$ = 1.44). These data demonstrate that PPTg lesioned rats consumed more sucrose than sham rats and deprived rats consumed more than non-deprived rats. Both of these effects became most evident at higher sucrose concentrations, specifically at 12 and 20% (Tukey-HSD post-hoc test, p < 0.05). PPTg deprived rats in particular over-consumed sucrose only at higher concentrations. The increased responding to, in the rats perception, a more 'rewarding' stimulus may be seen as a disinhibited response to motivationally exciting stimuli and will be discussed in more detail below.

Discussion

As evident from Figure 5.1.2, PPTg lesioned rats, in the food-deprived condition, over-consumed sucrose but only when in receipt of higher concentrations. This suggests selective responding to a reward stimulus dependent on its position in a gradient. From Figure 5.1.2 it is clear that increased responding, in the form of consumption, occurred in non-deprived rats at higher concentrations of sucrose, but the true disinhibition of this response only occurred in PPTg lesioned rats that were

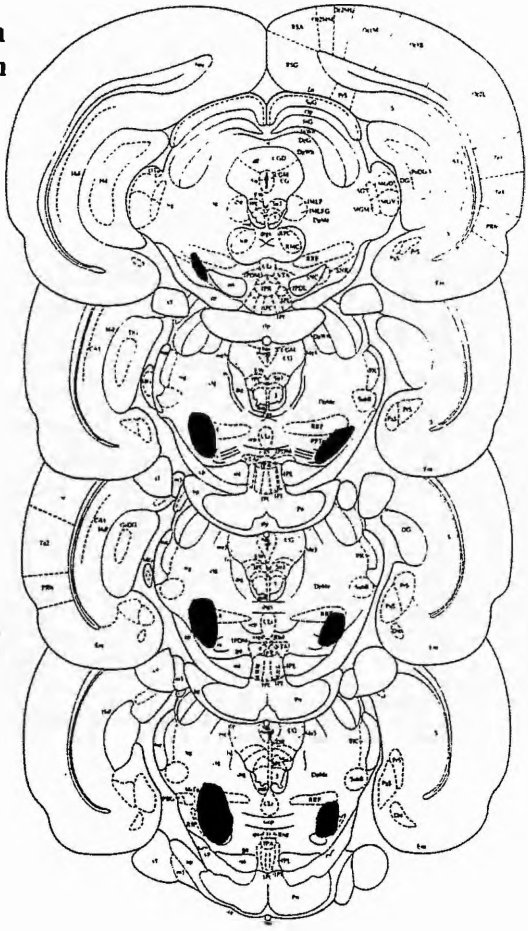
Figure 5.1.1

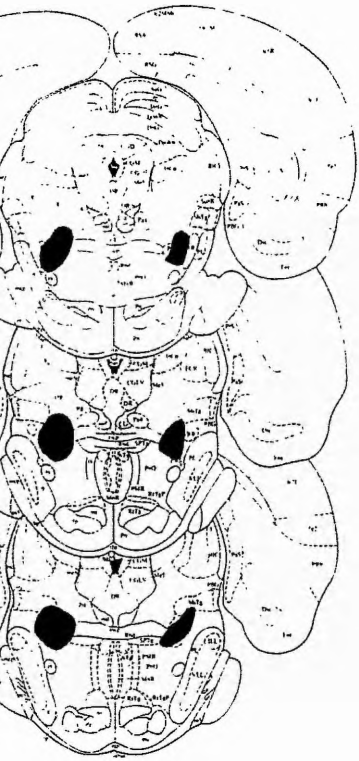
Representative sections from Bregma -6.30 to -8.72 mm redrawn from the atlas of Paxinos and Watson (1986) which illustrate the largest (left) and smallest (right) lesions for PPTg ibotenic acid lesioned rats in this experiment.

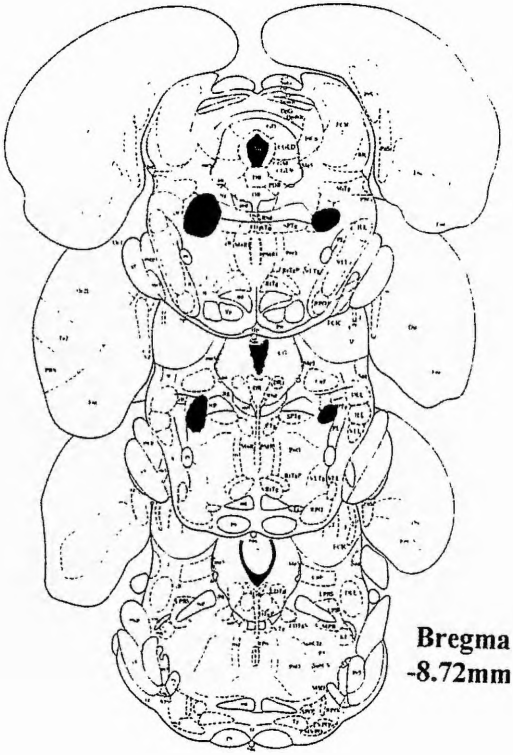
Figure 5.1.2

Mean sucrose intake (grams) for food deprived and non-deprived animals following sham or excitotoxic PPTg lesions.

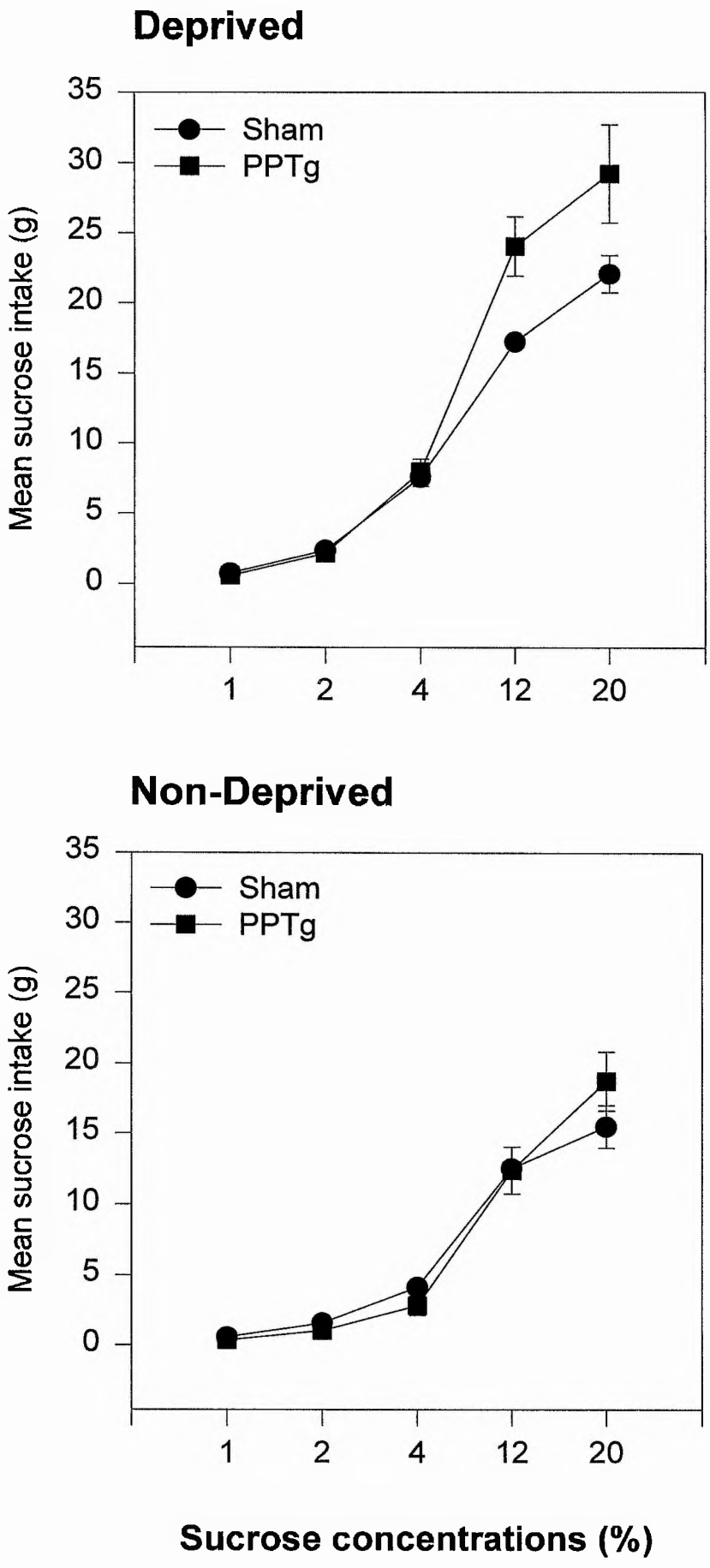
Bregma
-6.30mm







Bregma
-8.72mm


Deprived
Sham
PPTg
Mean sucrose intake (g)
35
30
25
20
15
10
5
0
1
2
4
12
20
Non-Deprived
Sham
PPTg
Mean sucrose intake (g)
35
30
25
20
15
10
5
0
1
2
4
12
20
Sucrose concentrations (%)

food deprived. This type of responding would imply, to some degree, a relationship between the PPTg, incentive stimulus levels and drive state. While both deprived and non-deprived states are sufficient to increase general activity (in the form of drinking), when the stimulus levels are high the resulting activity is increased, perhaps to a degree that is more related to being goal directed than induced by lower stimulus levels (Bindra, 1968). As the stimulus levels used in this experiment were of a limited range in regards to the rats potential responding, further speculation would be meaningless. To further elucidate the value of the stimulus level responding following PPTg lesions a sucrose gradient extending beyond that used in experiment 1 was employed in experiment 2.

5.2 Experiment 2: Further examination of the consumption response to varying concentrations of a sucrose solution following excitotoxic lesions of the PPTg: 4, 12, 24, 40, 60% sucrose.

Methods

Animals

45 rats were used (Charles River). Mean weight at time of surgery was 336.42 ± 51.12 (SD).

Surgery

Rats were anaesthetised with sodium pentobarbitone (Sagatal, Rhône Mérieux; 60 mg/kg) before being placed in the stereotaxic frame. 20 rats were given a lesion of the PPTg while 25 were given a phosphate buffer control lesion (see General Methods). Of these rats 7 ibotenate lesioned rats and 7 control lesioned rats were in the food deprived condition, while 13 ibotenate lesioned rats and 18 control lesioned rats were in the non-deprived condition. Rats were given at least two weeks postoperative care before behavioural testing began.

Sucrose drinking

Rats were given 24 hr home cage access to each of 4, 12, 24, 40, and 60% sucrose solutions. The order of presentation of sucrose was randomised and the rats were given access to the sucrose every other day. Water was available at all times and food was available for the rats in the non-deprived group *ad libitum* while the food deprived rats were maintained at 85% of their free feeding weight (approximately 17.5-20 grams food/day). Food and water intake was recorded along with the amount of sucrose solution consumed.

Results

Figure 5.2.1 represents the largest and smallest lesion sizes for the PPTg ibotenate lesioned rats of this experiment. Damage to nearby structures was negligible and inconsistent across rats. 3 rats had damage to the deep mesencephalic nucleus, 6 rats had damage to the adjoining retrorubral field and 3 rats had damage to the parabrachial nucleus. 8 rats had minimal damage to the superior cerebellar peduncle. The inconsistent damage to these structures did not produce a change in behaviour across the PPTg lesioned rats. This was examined through direct comparison of damage sites and behavioural outcome. No consistent relationship between damage to a structure other than the PPTg and behaviour were found.

Sucrose intake over the five concentrations was analysed using a repeated measures ANOVA, with group and state as the between subjects factor and concentrations as the within subjects factor. The mean sucrose intake is represented graphically in Figure 5.2.2. Between subjects analysis revealed a significant main effect of group ($F_{1,41} = 20.48$, $p < 0.0001$), a significant main effect of state ($F_{1,41} = 24.23$, $p < 0.001$), but no significant effect of the two-way interaction of group x state ($F_{1,41} = 0.82$). The within subjects analysis revealed a significant main effect of concentrations ($F_{4,164} = 64.90$, $p < 0.0001$), a significant effect of the two-way interaction of group x concentrations ($F_{4,164} = 2.85$, $p = 0.026$) but no significant effect of the two-way interaction of state x concentrations ($F_{4,164} = 1.01$), or the three way interaction of group x state x concentrations ($F_{4,164} = 1.45$). From Figure 5.2.2 it is clear that PPTg lesioned rats consumed more sucrose than control rats. It is also clear that food deprived rats consumed more than non-deprived rats and this effect

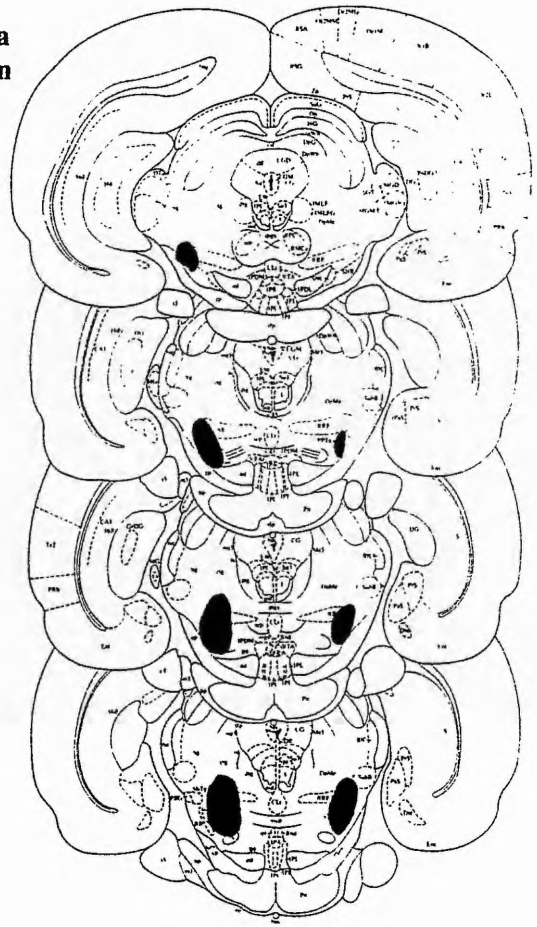
Figure 5.2.1

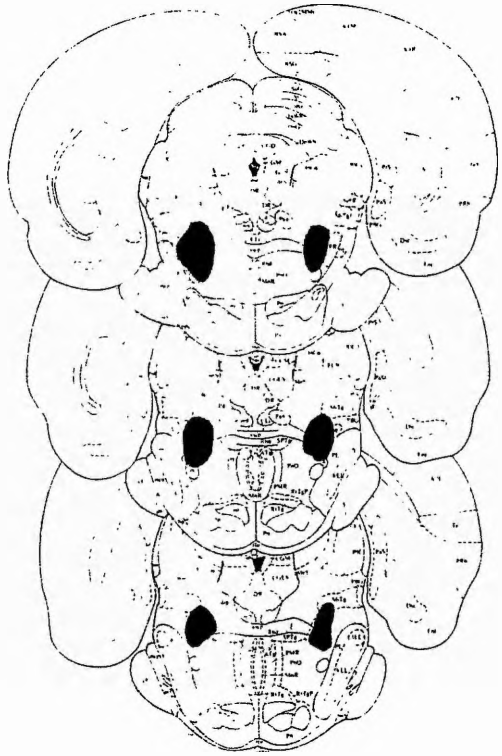
Representative sections from Bregma -6.30 to -8.72 mm redrawn from the atlas of Paxinos and Watson (1986) which illustrate the largest (left) and smallest (right) lesions for PPTg ibotenic acid lesioned rats in this experiment.

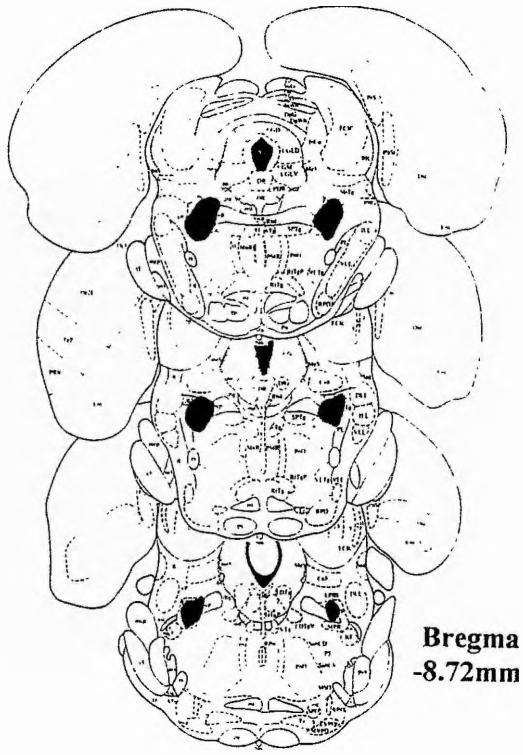
Figure 5.2.2

Mean sucrose intake (grams) for food deprived and non-deprived animals following sham or excitotoxic PPTg lesions.

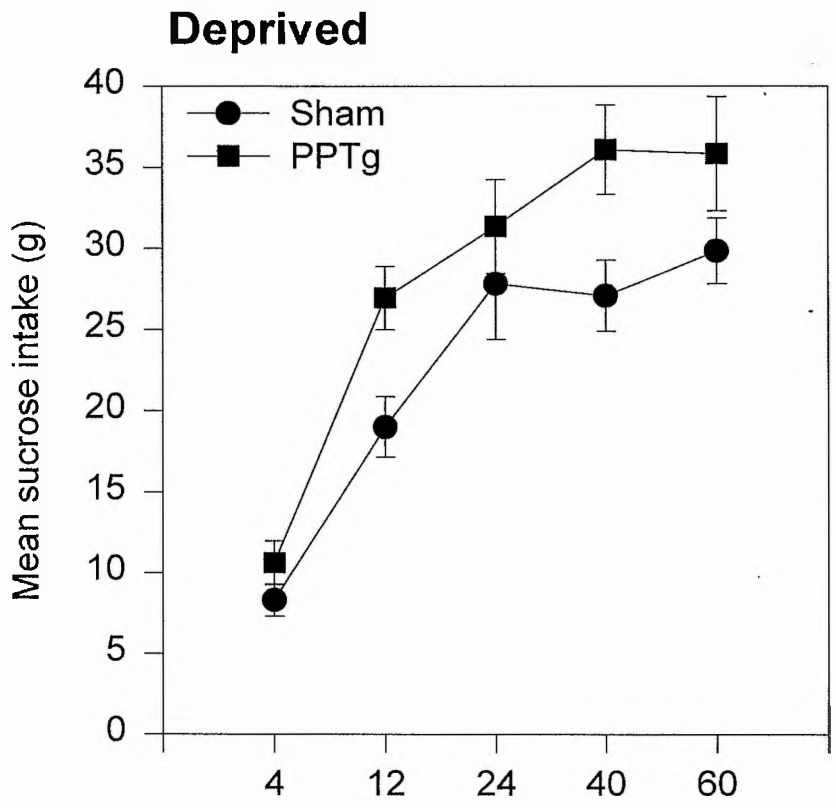
Bregma
-6.30mm

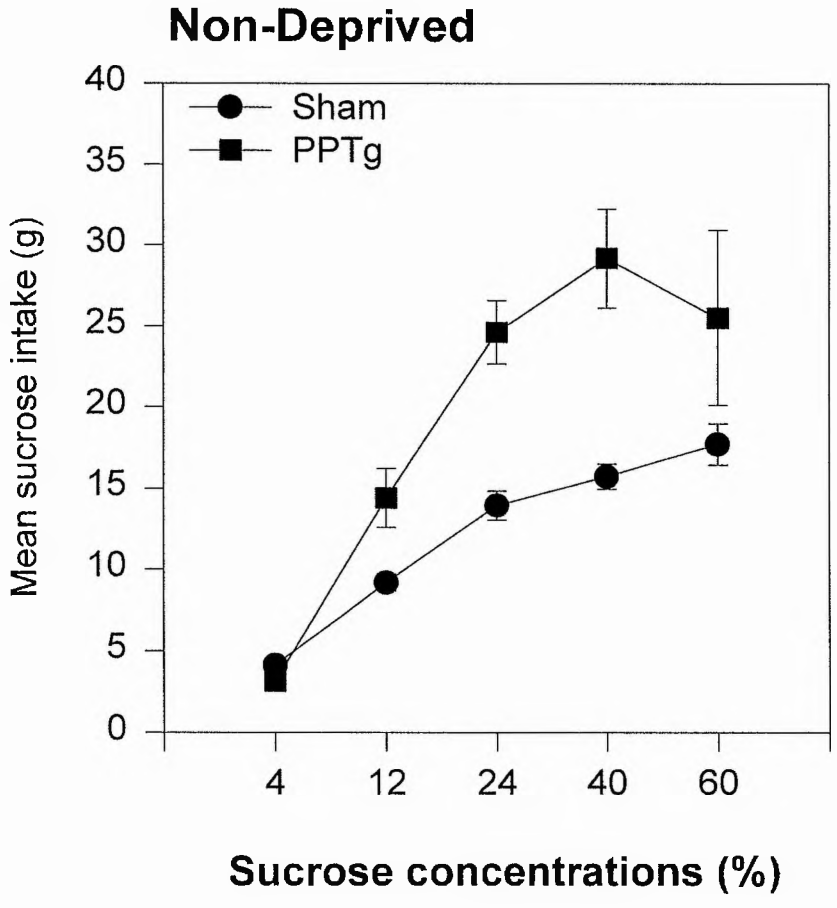






Bregma
-8.72mm


Deprived
Sham
PPTg
Mean sucrose intake (g)
0
5
10
15
20
25
30
35
40
4
12
24
40
60


Non-Deprived
Sham
PPTg
Mean sucrose intake (g)
0
5
10
15
20
25
30
35
40
4
12
24
40
60
Sucrose concentrations (%)

became most evident at higher concentrations of sucrose. While both deprived and non-deprived rats increased consumption at higher concentrations, the data demonstrate that it is PPTg lesioned rats who consume most of all, disinhibiting their responding at higher concentrations of sucrose, specifically at 24, 40 and 60% over 4 and 12% (Tukey-HSD post-hoc test, $p < 0.05$). The nature of this responding will be discussed further below.

Food intake over the five sucrose concentrations was analysed using a repeated measures ANOVA with group and state as the between subjects factor and concentrations as the within subjects factor. These data are represented graphically in Figure 5.2.3. Between subjects analysis revealed a significant main effect of group ($F_{1,41} = 11.59$, $p = 0.001$), a significant main effect of state ($F_{1,41} = 19.49$, $p < 0.001$), but no significant effect of the two-way interaction of group x state ($F_{1,41} = 2.30$). The within subjects analysis revealed a significant main effect of concentrations ($F_{4,164} = 21.55$, $p < 0.001$), a significant effect of the two-way interaction of group x concentrations ($F_{4,164} = 4.69$, $p < 0.001$) and a significant effect of the two-way interaction of state x concentrations ($F_{4,164} = 10.40$, $p < 0.001$), but no significant effect of the three way interaction of group x state x concentrations ($F_{4,164} = 0.72$). The pattern of food intake reflects the nature of what is happening with the sucrose intake. In this case the sham rats are consuming more food than PPTg lesioned rats and, of course, non-deprived rats are consuming more than food deprived rats. As the PPTg lesioned rats had another source of energy intake, in the form of sucrose consumption, their food requirements decreased. This effect was seen across concentrations as PPTg lesioned rats consumed more sucrose at higher

concentrations and as such required even less food intake in relation to the control rats.

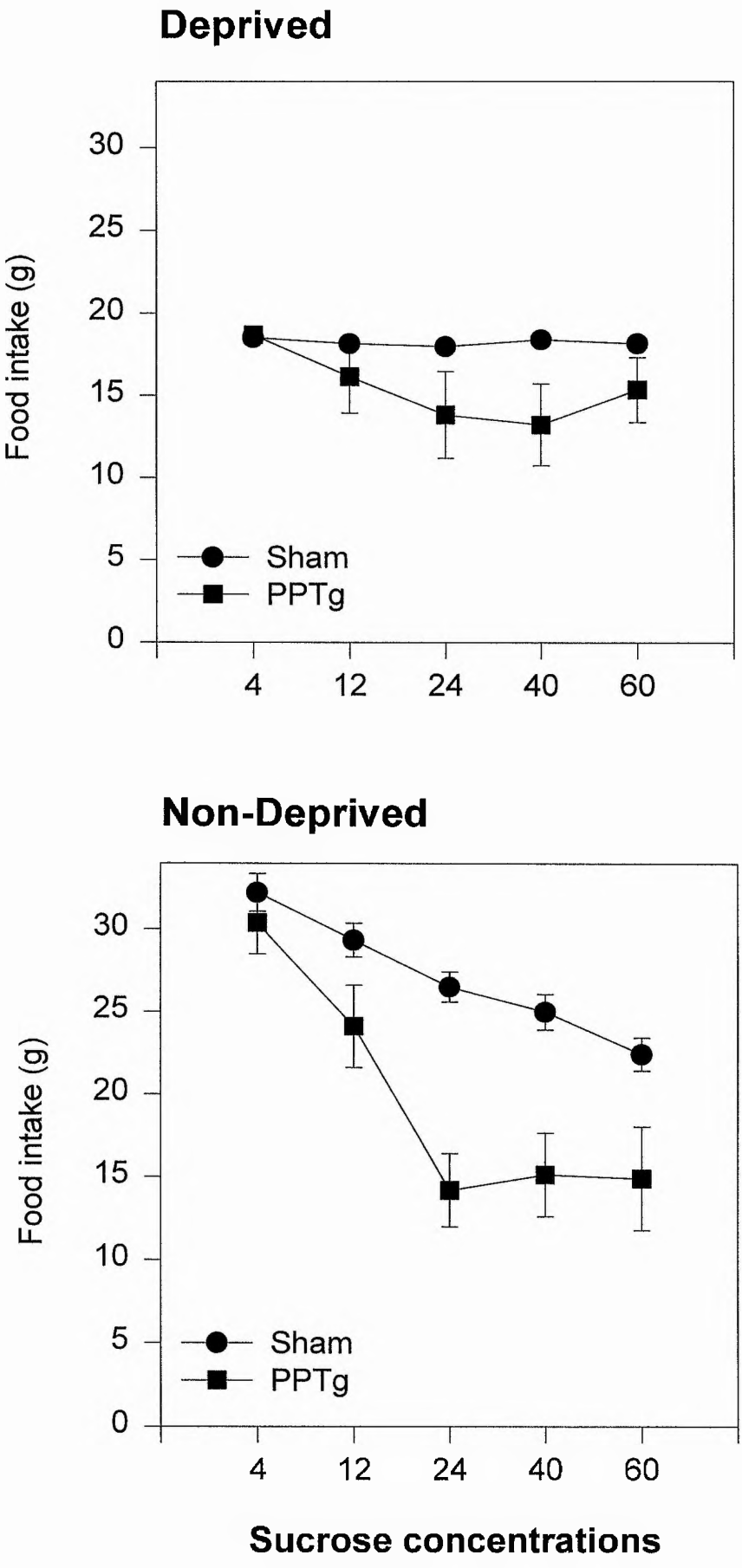
Water intake over the five sucrose concentrations was analysed using a repeated measures ANOVA with group and state as the between subjects factor and concentrations as the within subjects factor. This data is represented graphically in Figure 5.2.4. Between subjects analysis revealed a significant main effect of group ($F_{1,41} = 15.31$, $p < 0.001$), but no significant main effect of state ($F_{1,41} = 2.19$) or of the two-way interaction of group x state ($F_{1,41} = 0.11$). The within subjects analysis revealed a significant main effect of concentrations ($F_{4,164} = 28.69$, $p < 0.001$), a significant effect of the two-way interaction of group x concentrations ($F_{4,164} = 4.77$, $p < 0.001$) but no significant effect of the two-way interaction of state x concentrations ($F_{4,164} = 2.25$) or of the three way interaction of group x state x concentrations ($F_{4,164} = 1.25$). Again this pattern of water intake coincides with the pattern of sucrose intake across the PPTg lesioned and control rats. Water intake is greater across PPTg rats and at the times of higher concentrations of sucrose that reflects the fact that PPTg lesioned rats consume more of the sucrose than control rats, particularly at higher concentrations. A high increase in sugar intake results in the body needing a re-hydrating balance and so the PPTg lesioned rats drank more water than was needed at lower concentrations of sucrose. This pattern holds true for both deprived and non-deprived rats.

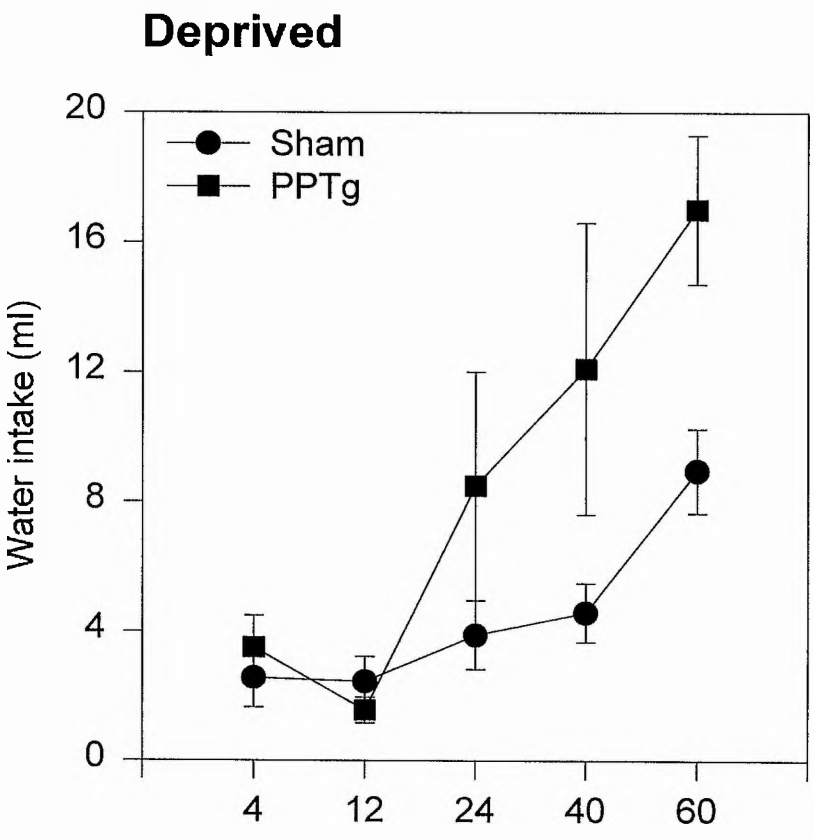
Figure 5.2.3

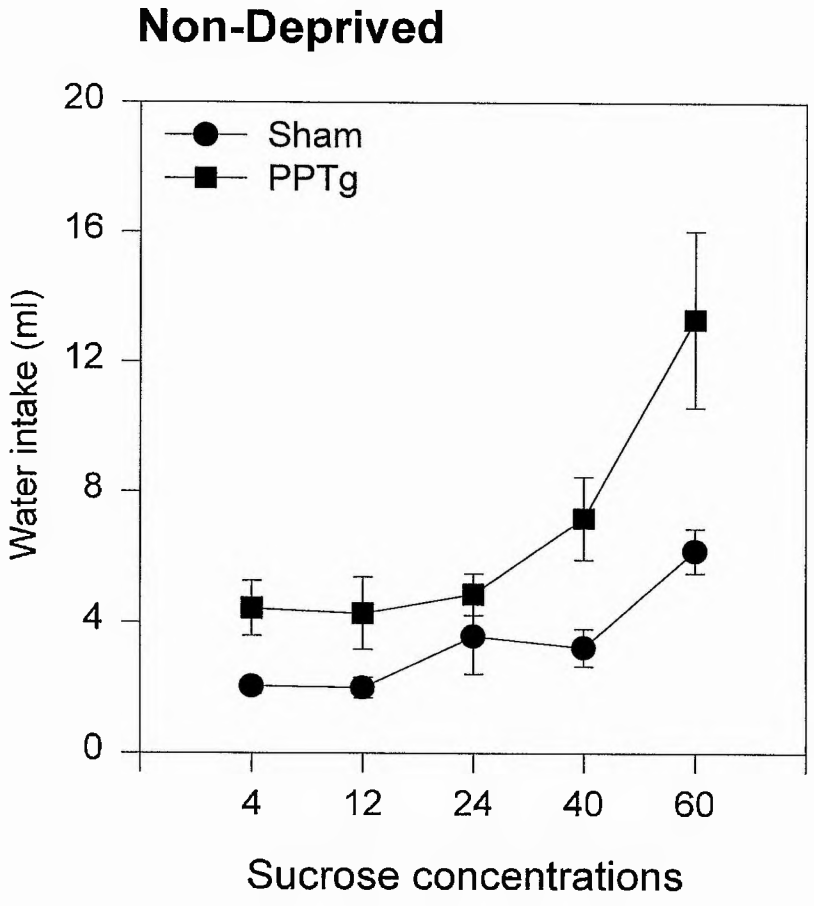
Mean food intake (grams) for food deprived and non-deprived animals on sucrose presentation days following sham or excitotoxic PPTg lesions.

Figure 5.2.4

Mean water intake (ml) for food deprived and non-deprived animals on sucrose presentation days following sham or excitotoxic PPTg lesions.


Deprived
Food intake (g)
30
25
20
15
10
5
0
Sham
PPTg
4
12
24
40
60
Non-Deprived
Food intake (g)
30
25
20
15
10
5
0
Sham
PPTg
4
12
24
40
60
Sucrose concentrations


Deprived
Sham
PPTg
Water intake (ml)
20
16
12
8
4
0
4
12
24
40
60


Non-Deprived
Sham
PPTg
Water intake (ml)
20
16
12
8
4
0
4
12
24
40
60
Sucrose concentrations

Discussion

As evident from Figure 5.2.2 and the above results, the pattern of sucrose intake is increased in PPTg lesioned over control rats, deprived rats over non-deprived rats, and is particularly increased at higher concentrations of sucrose regardless of deprivation state. What this revealed is that PPTg lesioned rats, both in the food deprived and non-deprived condition, over-consume their sucrose intake at particularly high concentrations of sucrose. In some respect these results are similar to the results of experiment 1, but in this case at even higher concentrations of sucrose than used in experiment 1. What has been novel in this experiment is the nature of increased responding, or disinhibition, by both food deprived and non-deprived PPTg lesioned rats at high concentrations of sucrose. This result was not seen in experiment 1 and may be related to fact that this experiment used higher concentrations of sucrose solutions than experiment 1. In this experiment the highest sucrose concentration is three fold higher than used in experiment 1 and it may be that only when the sucrose concentration gets suitably high enough do PPTg lesioned, non-deprived rats over-consume sucrose in the same manner as the deprived rats.

What these data may suggest then, is a reward stimuli gradient over which responding changes in PPTg lesioned rats and a gradient that is slightly different depending on the internal state of the animal. Non-deprived rats may simply need a slightly higher gradient for which disinhibition becomes evident as they do not have the same internal drive to rid of hunger as the food deprived rats do. The physiological drive is not the same and as such requires, what the rats would

consider, even more rewarding stimuli that they will over-respond to. These results again seem to indicate that PPTg lesioned rats are disinhibiting their response to the higher concentrations of sucrose and over-consuming these rewards. The response seems very clear to be along a gradient of reward strength, with increased rewards eliciting more responding, more consumption. This may suggest that signals of internal and external state, deprivation and incentive, respectively, are represented in the PPTg and when this structure is lesioned normal response patterns to such states are disinhibited.

What is not entirely clear from these data is if there are any changes in motivation across these groups and if it is this effect which is affecting sucrose intake. Are PPTg lesioned rats more motivated to consume when presented with a higher concentration of sucrose than with a lower one? Judging by the conditioned place preference data it would be suggested that PPTg lesioned rats do not find sucrose more rewarding than control rats when provided with a high concentration of sucrose. This statement is borne out of the fact that PPTg lesioned rats do not differ in their ability to form a conditioned place preference in comparison to control rats when the reward environment is paired with a 20% sucrose solution. Examining motivation through approach behaviour versus consumptive behaviour responding across a reward stimulus gradient following PPTg lesions is the focus of the next experiment.

5.3 Experiment 3: The role of motivation versus stimuli responding following excitotoxic lesions of the PPTg: Alleyway run speed and sucrose drinking.

Introduction

The early work of Tolman (Tolman and Honzik, 1930a; Tolman and Honzik, 1930b; Tolman, Honzik and Robinson, 1930) revealed that rats' motivation for a reward can be measured by their willingness to complete alley and maze trials and the speed with which this is accomplished can be an indicator of degree of motivation, for example faster completion times equals increased motivation. The role of reward motivation and the formation of place conditioning through a complex pattern of associations has been examined, but a simpler means of inferring the motivation of an animal is to run them in a simple alley/maze paradigm similar to Tolman's. Tolman attempted to assess the role of drive and its influence on subsequent task completion times. In one specific experiment (Tolman and Honzik, 1930a), the experimenters ran four groups of rats, altered in regards to drive and possibility of its satisfaction (hungry-reward; hungry-non-reward; less hungry-reward and less-hungry-non-reward), and examined the time it took these rats to complete maze trials. They discovered that hungry-reward rats were the fastest while less hungry-non-reward were the slowest. Taking away the drive and the possibility of reward dramatically increased the amount of time it took rats to complete the maze trials (mean 24.58 sec vs. 61.9 sec). Using the principles established by Tolman, this experiment attempted to examine the role of incentive stimulus strength on rats' speed to complete a runway task. Here the level of drive has been kept the

same in that all rats were run food deprived, but the value of the reward stimulus was altered. It was also the intention to examine specifically the role of the PPTg in approach and consumption response to incentive stimuli alteration. Recent work by Ikemoto and Panksepp (1996) demonstrated that pharmacological inactivation of the cholinergic neurons of the PPTg with a high dose of atropine resulted in reduced appetitive approach responses and reduced consumption of a sucrose reward in a simple runway task. The previous experimental findings of this chapter would seem to contradict these findings as consumption of sucrose was enhanced following PPTg lesion, certainly not decreased as found by Ikemoto and Panksepp (1996). Clarification of this result was sought through using a simple runway task, similar to that used by Ikeomoto and Panksepp (1996).

In this experiment a simple alleyway was constructed and rats were trained to run the length of the alleyway to receive a sucrose reward. The previous experiments have shown that PPTg lesioned rats consume more sucrose than control rats in the deprived condition, particularly at high sucrose concentrations, but is their motivation to retrieve these rewards any different at low versus high levels of reward? Disinhibited consumption may occur at higher concentrations of sucrose, but is the degree of motivation, judged here by time to run the alleyway to receive the reward, expressed differentially along the same gradient?

Methods

Animals

18 adult male rats (Charles River) were used. Mean weight at surgery time was 381.57 ± 20.92 (SD).

Surgery

Rats were anaesthetised with sodium pentobarbitone (Sagatal, Rhône Mérieux; 60 mg/kg) before being placed in the stereotaxic frame. 9 rats were given a lesion of the PPTg while 9 were given a phosphate buffer control lesion of the PPTg (see General Methods). Of these rats 5 ibotenate lesioned rats and 5 control lesioned had access to 20% sucrose, while 4 ibotenate lesioned rats and 4 control lesioned had access to 4% sucrose; all rats were food deprived. Rats were given at least two weeks post-operative recovery before food deprivation began. Rats were deprived to 85% of their free-feeding weight, receiving access to approximately 17.5-20 grams of food pellets/day.

Behavioural Testing

Rats were trained to receive sucrose reward at the end of the alleyway apparatus as outlined in the General Methods. Rats were randomly assigned to receive access to either 4% or 20% sucrose at the end of the alleyway. Time to run the alleyway and initiate drinking was measured with a stopwatch and the amount of sucrose consumed in the 30 min access period was also recorded.

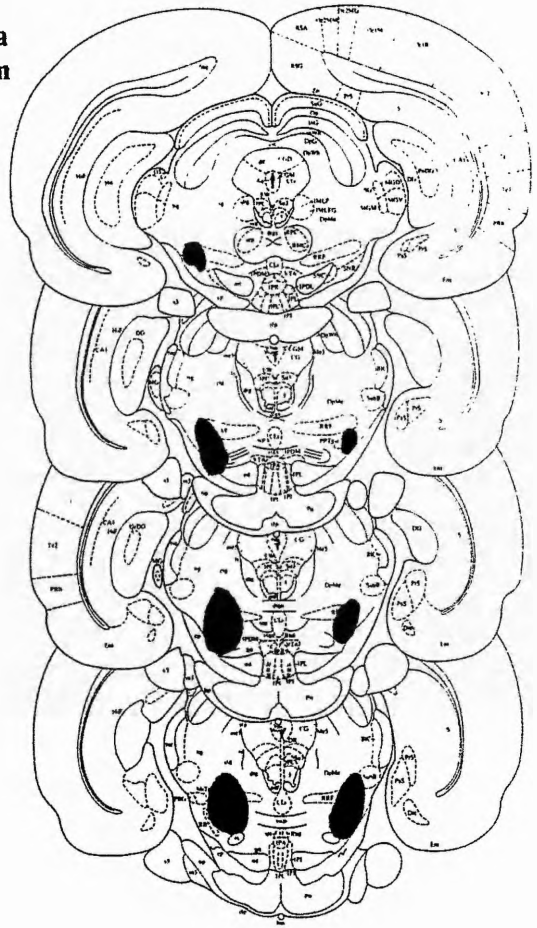
Results

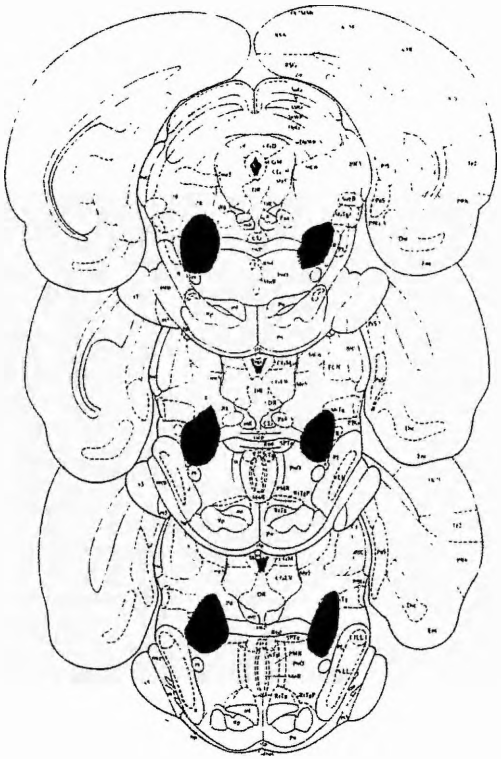
Figure 5.3.1 represents the largest and smallest lesion sizes for the PPTg ibotenate lesioned rats of this experiment. Damage to nearby structures was negligible and inconsistent across rats. 6 rats had damage to the adjoining

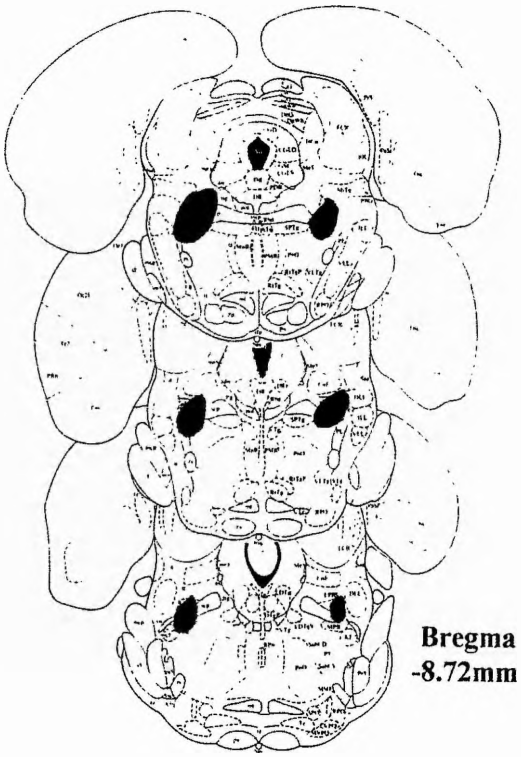
Figure 5.3.1

Representative sections from Bregma -6.30 to -8.72 mm redrawn from the atlas of Paxinos and Watson (1986) which illustrate the largest (left) and smallest (right) lesions for PPTg ibotenic acid lesioned rats in this experiment.

Bregma
-6.30mm







Bregma
-8.72mm

retrorubral field and 3 rats had damage to the deep mesencephalic nucleus. All rats had minimal damage to the superior cerebellar peduncle. The inconsistent damage to these structures did not produce a change in behaviour across the PPTg lesioned rats. This was examined through direct comparison of damage sites and behavioural outcome. No consistent relationship between damage to a structure other than the PPTg and behaviour were found.

Analysis of time to run the complete alleyway and initiate drinking was analysed using a repeated measures analysis of variance with trials (1-7) as the within subjects factor and group (sham vs. PPTg) and concentration of sucrose access (4% vs. 20%) as the between subjects factors. The time data was log transformed to reduce the variance seen in the data. The time to complete trials is displayed graphically in Figure 5.3.2. Between subjects analysis revealed no main effect of group ($F_{1,14} = 1.38$), no main effect of sucrose concentration access ($F_{1,14} = 0.10$), and no significant two-way interaction of group x concentration ($F_{1,14} < 0.001$). Analysis of within subjects data revealed a significant main effect of trials ($F_{6,84} = 2.36$, $p = 0.037$), but no significant two-way interactions of group x trials ($F_{6,84} = 0.11$), concentration x trials ($F_{6,84} = 0.24$), and no significant effect of the three way interaction of group x concentration x trials ($F_{6,84} = 1.49$). From Figure 5.3.2 and these results it is clear that the degree of motivation, as judged by alleyway run speed, is not different between control and PPTg lesioned rats and is not different along the reward gradient as seen by the similar completion times at 4 and 20%. Both at a low (4%) and a high (20%) presentation of sucrose, PPTg lesioned rats did not differ from controls in this measure of motivation. The significant within

subjects effect of trials is reflected in the graph and revealed that the rats, from all groups, simply perform slightly quicker over trials (Tukey-HSD post-hoc test, $p < 0.05$).

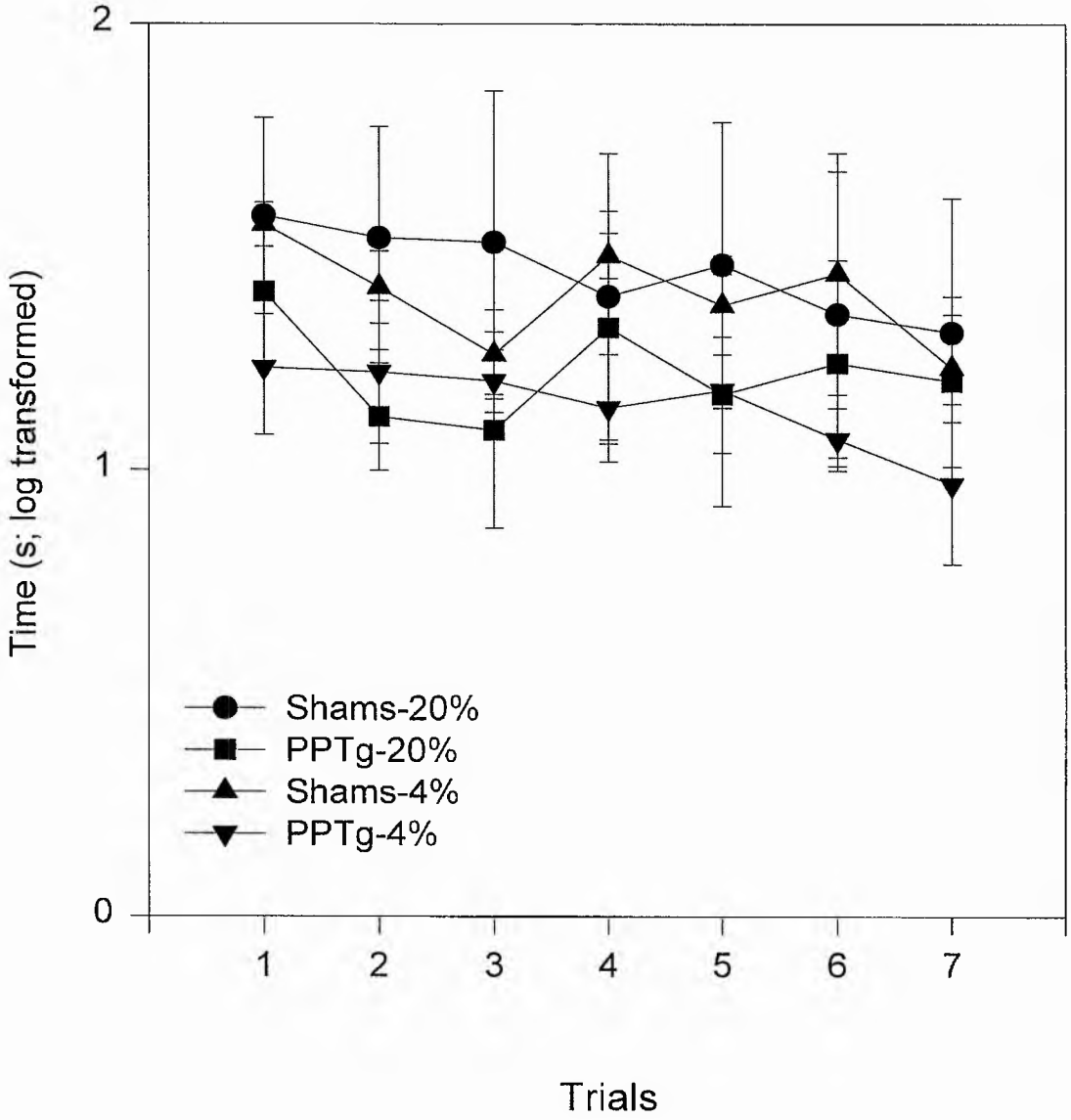
Analysis of amount of sucrose consumed was analysed using a repeated measures analysis of variance with trials (1-7) as the within subjects factor and group (sham vs. PPTg) and concentration of sucrose access (4% vs. 20%) as the between subjects factors. Amount of sucrose consumed by sham and PPTg lesioned rats at 4 and 20% can be seen in Figure 5.3.3. Between subjects analysis revealed no main effect of group ($F_{1,14} = 4.04$; $p = 0.064$), but a significant main effect of sucrose concentration ($F_{1,14} = 77.70$, $p < 0.001$), and a significant two-way interaction of group x concentration ($F_{1,14} = 4.99$, $p = 0.042$). Analysis of within subjects data revealed no significant main effect of trials ($F_{6,84} = 1.03$), no significant effect of the two-way interactions of group x trials ($F_{6,84} = 0.95$), concentration x trials ($F_{6,84} = 0.75$), or the three way interaction of group x concentration x trials ($F_{6,84} = 0.45$). The analysis revealed that while both groups have a similar pattern of sucrose intake (less at 4%, quite a bit more at 20%), PPTg lesioned rats at 20% sucrose drink the most of all, and over-consume in relation to the sham rats at 20%, and sham and PPTg lesioned rats at 4%. Again it seems clear that while motivation itself, as measured by speed of runway completion, is not different across any of the rats, consumption itself is affected with PPTg lesioned rats consuming most of all at 20%. Why this effect is seen only at higher concentrations of sucrose would again seem to be related to a disinhibitory action of PPTg lesioned rats to stimuli of higher reward value.

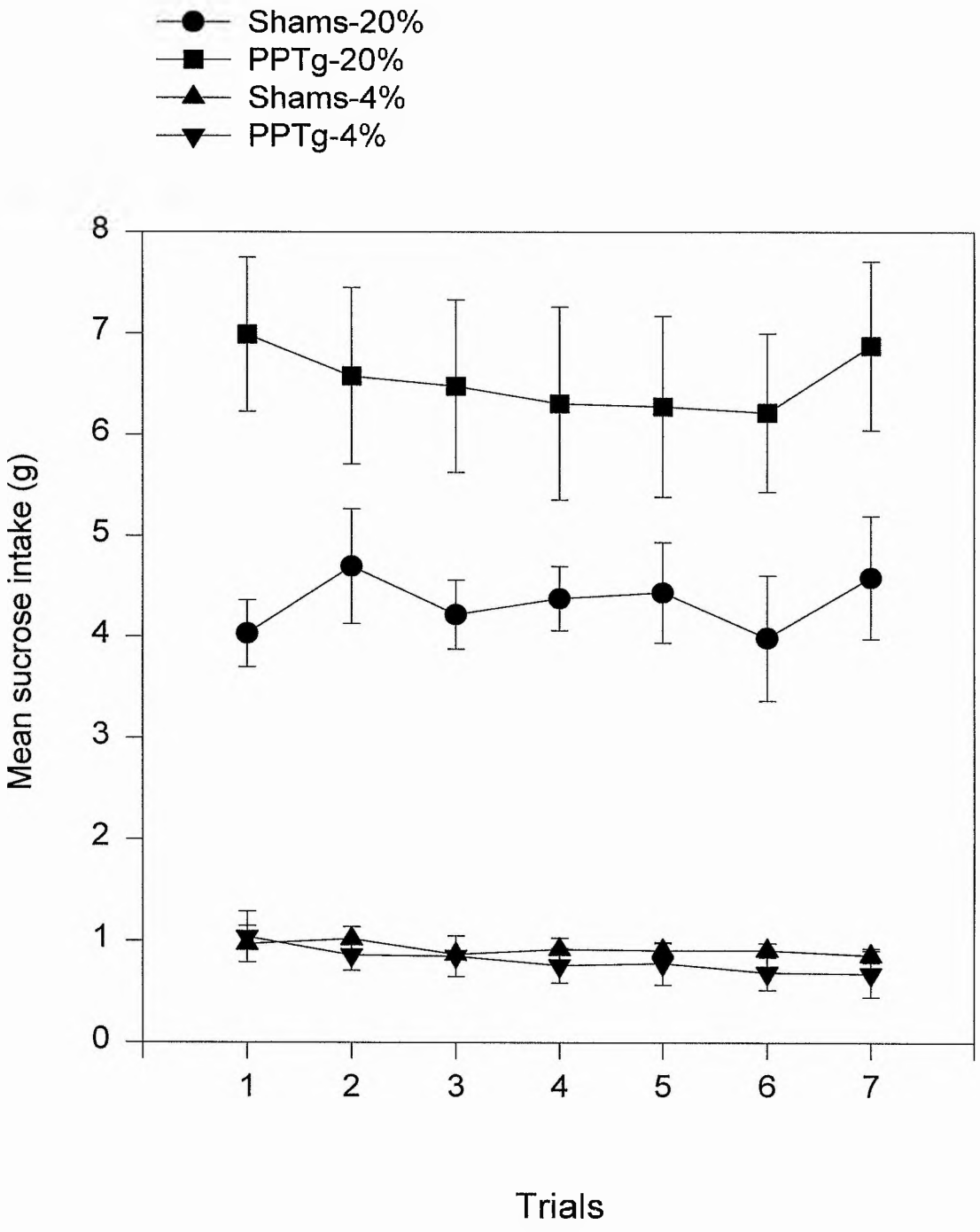
Figure 5.3.2

Mean time to complete runway trials for food deprived animals receiving either 4 or 20% sucrose reward following sham or excitotoxic PPTg lesions. These data were log transformed.

Figure 5.3.3

Mean sucrose intake (grams) for animals receiving either 4 or 20% sucrose reward (30 min access) after completing runway trials following sham or excitotoxic PPTg lesions.


2
1
0
Time (s; log transformed)
1
2
3
4
5
6
7
Trials
Shams-20%
PPTg-20%
Shams-4%
PPTg-4%


Shams-20%
PPTg-20%
Shams-4%
PPTg-4%
Mean sucrose intake (g)
0
1
2
3
4
5
6
7
8
1
2
3
4
5
6
7
Trials

Discussion

The results of this experiment, as outlined above and represented graphically in Figures 5.3.2 and 5.3.3, reveal that the level of motivation in PPTg lesioned rats is not changed in relation to that of control rats, at lower or higher levels of reward. Both control and PPTg lesioned rats are similar in their speed to gain access to the reward, both at low and high concentrations of sucrose. The consumption of the reward itself, however, is variable along this gradient. When presented with higher levels of the reward, PPTg lesioned rats disinhibit their drinking response and over-consume the 20% sucrose reward. The results of sucrose consumption presented here are similar to those reported in the previous experiments. This disassociation between motivation and consumption is intriguing and in these experiments is unique to the PPTg lesioned rats.

These results make no suggestion of the role of reward stimulus levels on performance as the rats performed equally at 4 and 20% sucrose access. The degree of drive, however, was similar for all rats in that they were all food-deprived and thus the incentive levels in all rats was sufficient enough as to not alter reward approach behaviours, regardless of the specific reward stimuli. A future experimental suggestion would be to run this same experiment in both food deprived and non-food deprived rats to vary both drive levels and incentive stimulus levels to examine the role of the PPTg in this responding. This should begin to tease out the inter-play of drive and reward levels. Will a non-deprived animal receiving 20% sucrose respond in a similar fashion as a deprived animal receiving 4% sucrose? Run speed for deprived rats at 4 and 20% are the same but consumption is higher for

the 20% sucrose. Consumption at 20% for a non-deprived animal is higher than 4% (as seen above in experiment 1), but will the drive of the deprived animal, though only receiving 4%, be higher than the non-deprived animal receiving 20%?

Finally, the results of this experiment differ from those of Ikemoto and Panksepp (1996). Their PPTg cholinergic inactivation through the use of a high dose of atropine reduced both the approach response behaviour and sucrose consumption behaviour of their rats. Similarly they found that when a high dose of GABA was injected into the PPTg the rats' run speed was affected, though in this case, not the sucrose reward consumption. These findings differ from the results observed here in that PPTg lesioned rats displayed a similar approach response pattern as control rats and a similar, and in fact enhanced at 20%, consumptive response to the sucrose reward compared to control rats. These differences are undoubtedly a product of distinct methodology in that the work of Ikemoto and Panksepp (1996) used selective pharmacological manipulations through separate and distinct injection of atropine and GABA, while this current experiment removed both cholinergic and non-cholinergic neurons of the PPTg through excitotoxic lesion. The results of Ikemoto and Panksepp (1996), while interesting, do not provide a complete picture of PPTg inactivation and presents a restricted window into their proposed theory of selective neuronal PPTg functioning in approach versus consumptive responding.

Summary

The results of this chapter revealed that the PPTg plays a role in control of responding that may be influenced by internal and external signals. When internal drive and external incentive are modified, the PPTg allows appropriate responding: when this structure is lesioned, responses are disinhibited. This disinhibition of responding makes itself evident along a gradient of stimulus responding such that PPTg lesioned rats that are food deprived disinhibit their responding to sucrose of much lower concentrations than PPTg lesioned rats that are non-food deprived. These non-deprived rats do not begin to exhibit disinhibition of responding until the reward stimulus is particularly motivating. It may be suggested then, that responding in relation to both internal and external state is represented in the PPTg and lesion of this structure results in a disinhibited outcome of such responding. This finding is at variance with those of van der Kooy and colleagues (Chapter 4; Bechara and van der Kooy, 1989;1992b) who have suggested the PPTg to be involved in motivation state dependently.

The other finding of this chapter was that lesions of the PPTg do not alter the rats' approach response behaviours to rewarding stimuli, similar to the findings of the previous chapter when PPTg lesioned rats' ability to exhibit an approach behaviour (i.e. positive place preference) was not interrupted. In this chapter the PPTg lesioned rats were no different in their time to approach reward stimuli compared to control rats, and this did not vary in the rats runway completion times to either 4 or 20% sucrose. Consumption of either the 4 or 20% sucrose was affected

however, as PPTg lesioned rats exhibited disinhibited responding to the 20% sucrose reward. This again confirms a role for the PPTg in control of incentive responding.

Chapter 6: The role of the PPTg in acquisition and retention of a spatial foraging task.

6.0 Introduction

The ability of an animal to forage efficiently and retrieve food successfully is necessary for survival. To avoid both starvation and unnecessary foraging time an animal must learn when and where in space food can be obtained. The radial arm maze is a behavioural tool used for the elucidation of spatial memory abilities of rats. Developed by Olton and Samuelson (1976) it has been used extensively over the last 20 years in cognitive functioning experiments. The standard design of 8 arms radiating out from a central platform forces the animal to make complex series of response choices as it has to return to the centre and is simultaneously faced with all alternative choices in its selection of the next arm. This extends beyond the earlier spatial studies using sequential mazes in that in those tasks each choice - left vs. right - is independent and less taxing. Spatial cues then, while used in both tasks, are critical for correct performance in the radial arm maze such that arm repetition is avoided. Early work of Olton and Samuelson (1976) showed that it was not intra-maze cues that the rats were using successfully to complete the trials, but external spatial maze cues to produce a cognitive map to reference in making subsequent and correct choices. This result was further confirmed in the work of Brown and colleagues (1993) who found that when the radial arm maze was surrounded by plain black curtains, blocking any external spatial cues, rats were unable successfully to perform the task and made significantly more errors in their attempts to find the food pellets. Various studies have examined the behaviour of rats working on the maze.

Arm length preference (Brown and Huggins, 1993) and the pattern of arm selection (Lanke et al., 1993) have been examined, but the critical point about radial arm maze work is that it has been shown reliably to be a useful tool for examining the role of spatial memory foraging. In fact, the reason rats perform so accurately on the radial arm maze is that it mimics natural foraging situations. If an animal is to forage efficiently and avoid predators it must remember food locations already visited and not return to those emptied sites (Yoerg and Kamil, 1982). As observed by Dudai (1989) " *usually animals excel in tasks that have ecological significance and are related to innate response programmes and tendencies* " (p. 33).

The neural substrates underlying spatially mediated foraging behaviour have been extensively examined using radial arm maze procedures. Early work by Olton and Papas (1979) and recently by Jarrard (1993) and McDonald and White (1993) have focused on the role of the hippocampus and found that damage to the hippocampal system consistently produces deficits in task performance. Networks associated with hippocampal function have been explored and a role for cholinergic systems in spatially guided behaviour has been proposed. Unfortunately, a consensus on cholinergic function has not been reached. In a review by Levin (1988), the author outlined that pharmacological blockade of either cholinergic muscarinic or nicotinic receptors, through the use of scopolamine and mecamylamine respectively, impair radial arm maze performance. They suggest that both these cholinergic receptors may act together to produce an overall influence on cholinergic involvement in choice accuracy as when both scopolamine and mecamylamine are co-applied, a greater impairment of choice accuracy was found

than with either of these drugs alone. Basal forebrain-cortical fiber mechanical deafferentation and ibotenic lesions of the nucleus basalis magnocellularis (NBM) have been shown to produce a learning impairment in a spatial radial arm maze task, while quinolinic acid lesions of the NBM on this same task did not result in an impairment (Ammassarie-Teule et al., 1993). IgG-saporin[5] lesions of the medial septum or NBM have been found to not interfere with spatial water maze performance but did produce impairments on the radial arm maze task, but not to the same degree as previous studies using less selective lesions (Dornan et al., 1996). A similar result of IgG-saporin lesions of the medial septum interfering with radial arm maze performance have been reported (Shen et al., 1996), but in this study the authors reported that the lesions interfered with acquisition of a spatial working memory radial arm maze task but not with its retention. Conflicting reports on spatial cognition from non-selective vs. selective cholinergic lesion studies led to the conclusion of Everitt and Robbins (1997) that non-specific basal forebrain lesions are related to disruption of cortico-striato-pallidal pathways.

Understanding the role of striatal pathways, specifically the role of the nucleus accumbens, in spatially mediated behaviour has been examined in studies conducted by Lavoie and Mizumori (1994), Floresco and his colleagues (1996), and Floresco and his colleagues (1997). Experiments in which neuronal activity is recorded while rats perform nondelayed radial arm maze tasks have revealed neurons of the nucleus accumbens that exhibit place, reward and movement specific firing

[5] IgG-saporin, or 192 IgG saporin, is a cytotoxic antibody used for selective lesion of basal forebrain cholinergic neurons. The cytotoxin saporin is coupled to a monoclonal antibody (192 IgG) raised against the low affinity p75 nerve growth factor receptor that is almost uniquely expressed in the basal forebrain cholinergic neurons, thus allowing them to be targeted (Dornan et al., 1996).

(Lavoie and Mizumori, 1994). Pharmacological inactivation of the nucleus accumbens through injection of the dopamine receptor antagonist haloperidol results in an increased number of errors made in a random foraging radial arm maze task, which the authors report increased dose dependently (Floresco et al., 1996). Finally, disconnection lesions of the nucleus accumbens in one hemisphere, and the ventral subiculum portion of the hippocampus in the other hemisphere, was found to result in disruption of foraging in a nondelayed random foraging task (Floresco et al., 1997). In these experiments the rats have no previous information about the location of the food which forces them to forage retrospectively[6] (Cook et al., 1985) to remember where they have been to avoid returning there. Disruption of hippocampal-nucleus accumbens transfer of information results in errors in completing this task which outlines a role for these structures in performance accuracy of ongoing navigational behaviour.

While the above, and work by Kelley and Stinus (1985), may outline a role of the nucleus accumbens in goal directed behaviour, the role of ventral striatal outflow on such behaviours needs to be explored. Ventral striatal outflow to the PPTg in performance of a radial arm maze task has been briefly explored in earlier work by

[6] Retrospective foraging or coding, along with the term(s) prospective foraging or coding, are terms which have been coined by Cook et al., 1985. Retrospective foraging (or to forage retrospectively) refers to an animals' use of information about previously visited arms and choices already made to remember not to return there - to plan with retrospect. This type of foraging is addressed in this chapter and will be referred to in the general discussion. Prospective foraging, which will be addressed in the following chapter and referred to in the general discussion, refers to an animals' advanced planning of anticipated choices. Prospective foraging often comes into use in tasks where a delay element is used. In such a task an animal, for example, will learn over time and trials that some arms are blocked and not accessible before a delay but become open and are the ones that are baited subsequent to the delay. During the delay period an animal can use the gained information to plan the upcoming choices it must make to forage efficiently - to prospectively code anticipated choices.

Dellu and colleagues (1991). They found that, on acquisition of a foraging type task, PPTg lesioned rats were impaired and made more errors in performance than control rats. This might suggest that the PPTg plays a role in rats ability to use current information to retrospectively forage. This study (Dellu et al., 1991), however, provided minimal details of the performance of the PPTg lesioned rats and only examined the role of acquisition of this task following PPTg lesions. The first experiment set out to examine further the performance of PPTg lesioned rats on acquisition of a random foraging radial arm maze task. Following this, the role of the PPTg in retention performance on the same random foraging task was separately examined. In this retention experiment rats first acquired the task and then half of them received bilateral lesions of the PPTg and performance subsequent to the lesion was examined. Details as to the types of errors made, as well as information regarding time to make selection choices and complete trials was fully analysed and explored.

6.1 Experiment 1. Examination of acquisition of a random foraging radial arm maze task following excitotoxic lesions of the pedunculopontine tegmental nucleus.

Introduction

As mentioned above, the work of Dellu and colleagues (1991) briefly examined the role of the PPTg in the acquisition of a random foraging type radial arm maze task. In this experiment the authors baited all 8 arms of the maze and examined the number of errors the rats made in their first 8 choices in attempting to retrieve the pellets, and the number of errors the rats made in retrieving all 8 pellets. They found that PPTg lesioned rats made more errors in their first 8 choices than controls (1.62 vs. 0.95) and more errors in retrieving all 8 pellets than controls (5.36 vs. 2.21). While the authors attribute the impairment as a result of reduced arousal or attention (focusing on the role of the input connections from the PPTg to the thalamus) the results can also be explained as an impairment in response selection and guiding retrospectively planned behaviour into action as lesions of the PPTg interrupt striatal outflow. Considering PPTg lesions remove about 80-90% of the cholinergic neurons of the PPTg and do not interfere with the cholinergic neurons of the LDTg, there is sufficient cholinergic input to thalamus remaining for such a performance deficit to be not likely to be a result of a lack of arousal.

Finally, as the authors ran the task with all the arms baited they were not able to examine the performance of PPTg lesioned rats faced with rewarded vs. un-rewarded arm choices to examine if this might influence the pattern of performance

in completing this task. The aim of the current experiment then was to evaluate the role of the PPTg in performance on a random foraging radial arm maze task in which 4 arms were baited and 4 were left unbaited. The number and types of errors made (re-visits to baited and unbaited arms) in completing the task were scored as well as measures of locomotor and motivational responses.

Methods

Animals

13 rats were used (Charles River). Mean weight at time of surgery was 354.27 ± 21.20 (SD).

Surgery

Rats were anaesthetised with sodium pentobarbitone before being placed in the stereotaxic frame. 6 rats were given a lesion of the PPTg while 7 were given a phosphate buffer control lesion (see General Methods). Rats were given at least two weeks postoperative care before behavioural testing began. In this experiment, rats received bilateral PPTg lesions prior to radial arm maze exposure and training.

Random Foraging

This task is described in General Methods. Following postoperative care rats were food deprived to 85% of their free-feeding weight before habituation trials began. Rats were given 15-20 grams food pellets/day and when reached 85% were maintained on 17.5-20 grams food/day. Water was available *ad libitum* throughout the entire experiment. Once trials began rats were given 1 session/day on the maze

until the sham rats as a group reached a criterion of 1 error or less per day for 3 consecutive days.[7]

Results

Figure 6.1.1 represents the largest and smallest lesion sizes for the PPTg ibotenate lesioned rats of this experiment. Damage to nearby structures was negligible and inconsistent across rats. 1 rat had damage to the deep mesencephalic nucleus, 4 rats had damage to the adjoining retrorubral field and 2 rats had damage to the parabrachial nucleus. 5 rats had minimal damage to the superior cerebellar peduncle. The inconsistent damage to these structures did not produce a change in behaviour across the PPTg lesioned rats. This was examined through direct comparison of damage sites and behavioural outcome. No consistent relationship between damage to a structure other than the PPTg and behaviour were found.

All data were analysed using a repeated measures analysis of variance with group (sham vs. PPTg) as the between subjects variable and days as the within subjects variable. Analysis of the number of errors made had an extra within subjects variable of error type (baited versus unbaited arm re-entries).

The mean number of errors made in task completion across criterion days is shown in Figure 6.1.2. Analysis of errors made revealed a significant main effect of the between subjects factor of group ($F_{1,11} = 10.82$, $p = 0.007$). The within subjects analysis revealed a significant main effect of the factor of days ($F_{8,88} = 2.38$, $p = 0.022$), but no significant main effect of the factor of error type ($F_{1,11} = 0.06$). There

[7] This criterion was established following consultation with Stan Floresco and is based on their random foraging criterion selection (Floresco et al., 1996, 1997).

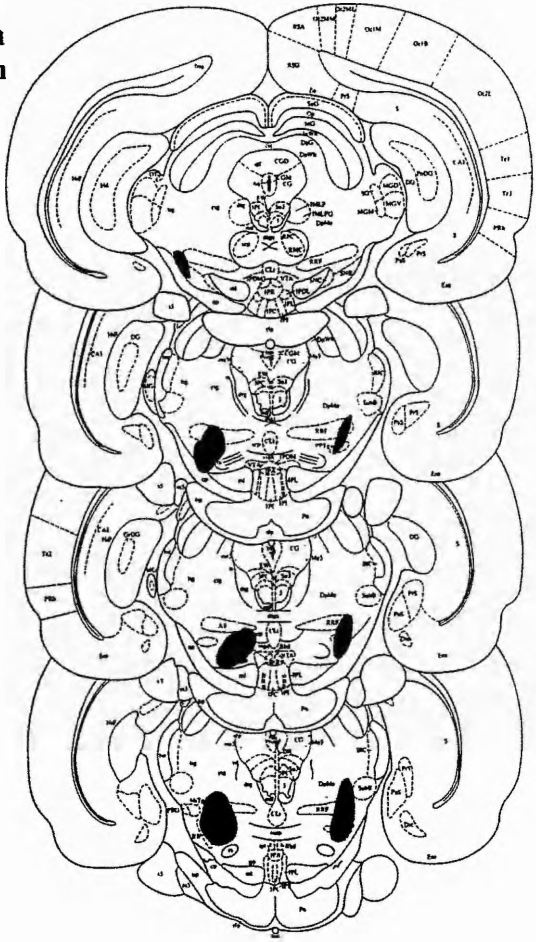
Figure 6.1.1

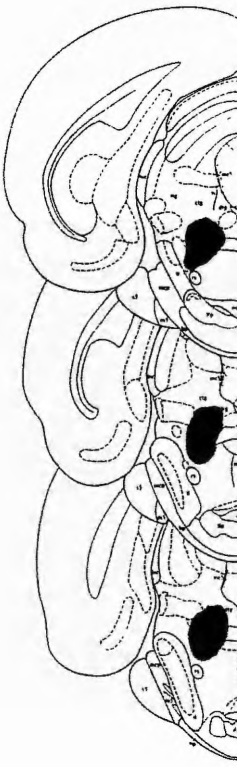
Representative sections from Bregma -6.30 to -8.72 mm redrawn from the atlas of Paxinos and Watson (1986) which illustrate the largest (left) and smallest (right) lesions for PPTg ibotenic acid lesioned rats in this experiment.

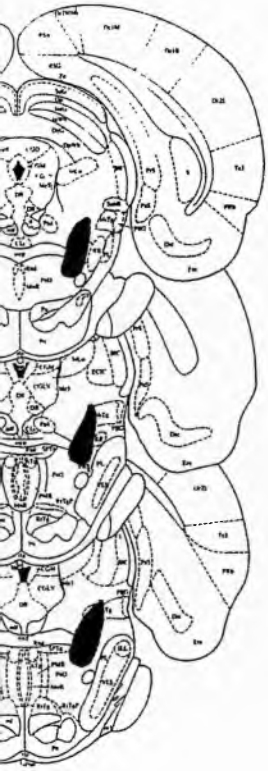
Figure 6.1.2

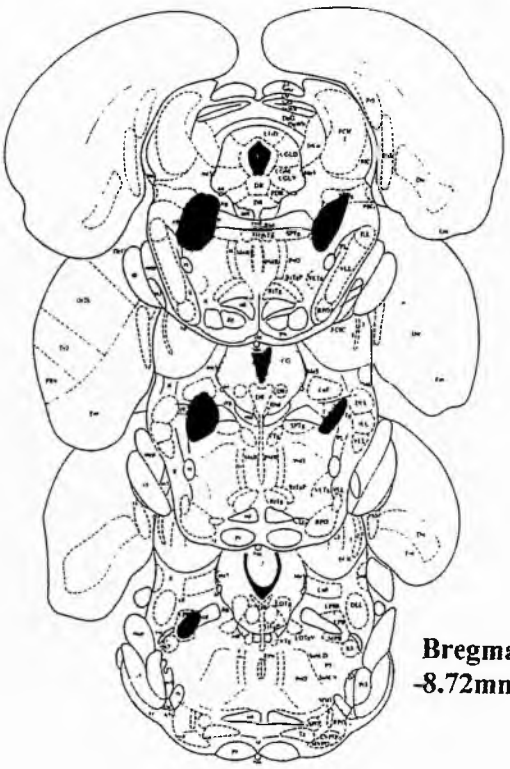
Mean number of errors across the three criterion days for sham and PPTg ibotenic lesioned animals. Inset figure represents the mean errors of the PPTg ibotenic lesioned animals, separated into error type: re-entry to baited (B) and un-baited (U) arms.

Bregma
-6.30mm

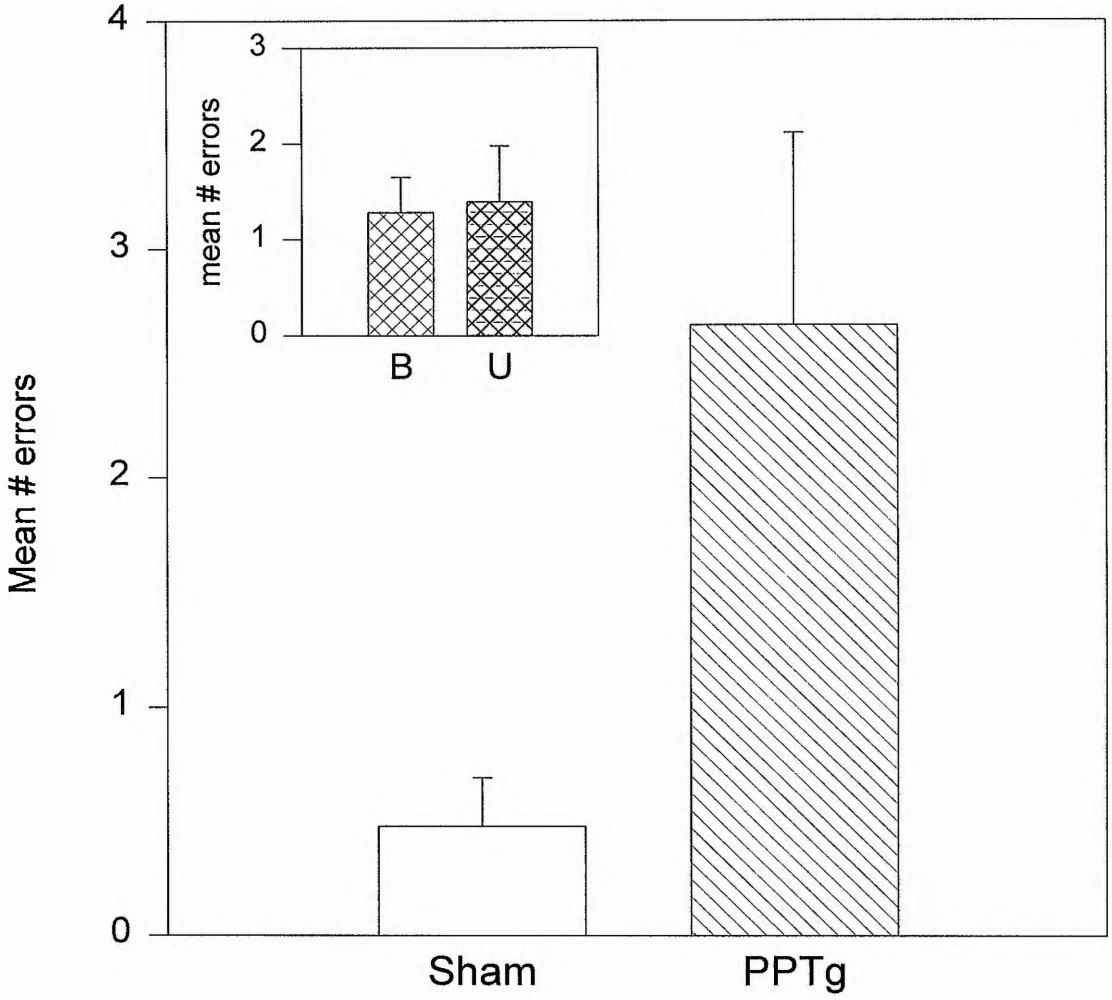








Bregma
-8.72mm

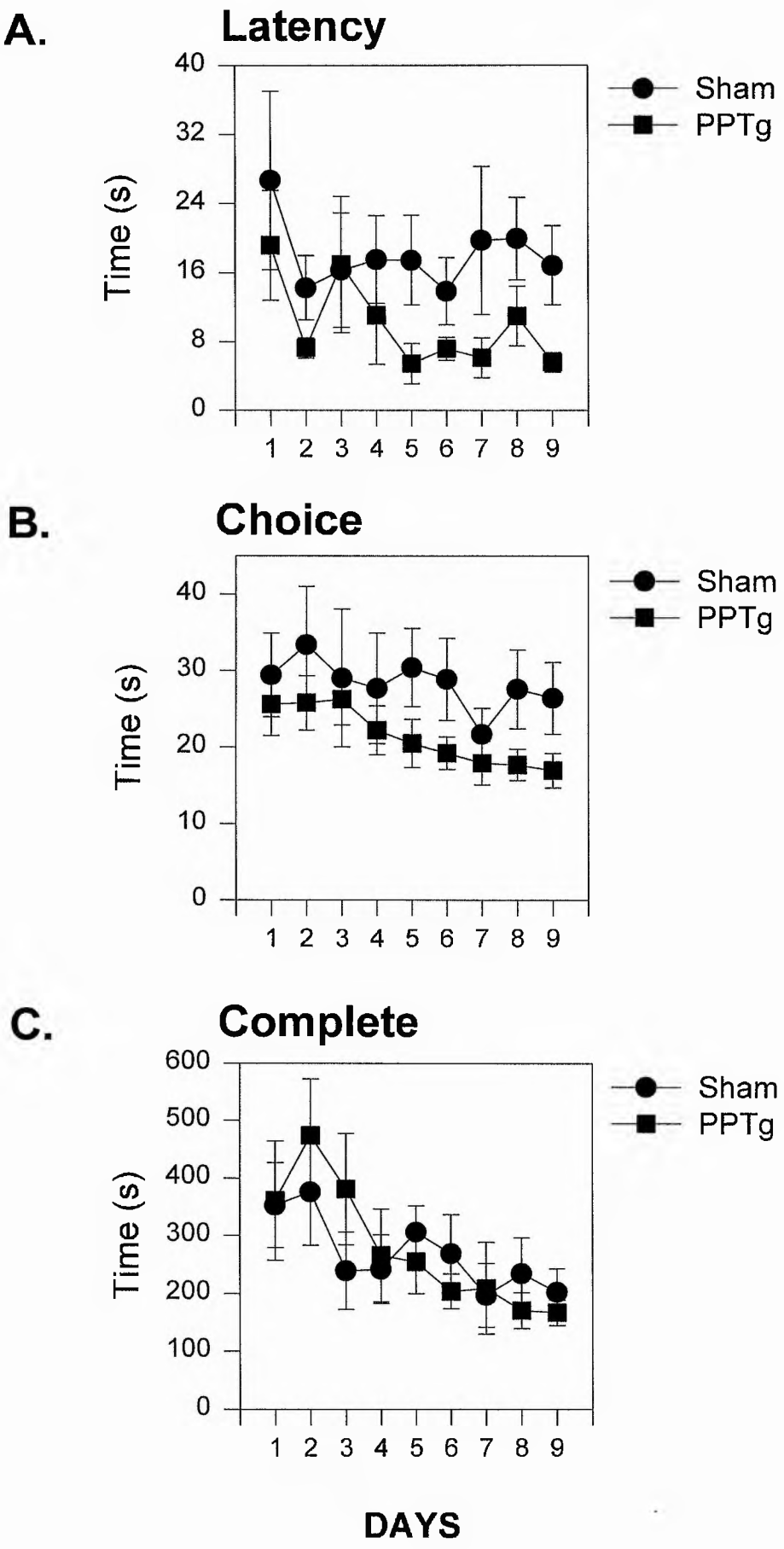

Mean # errors
4
3
2
1
0
Sham
PPTg
mean # errors
3
2
1
0
B
U

was a significant two-way interaction of days x error types ($F_{8,88} = 2.11$, $p = 0.043$), but no significant two-way interaction of group x days ($F_{8,88} = 0.57$), or group x error type ($F_{1,11} = 1.42$), or a three-way interaction of group x days x error type ($F_{8,88} = 1.92$). This analysis revealed that PPTg lesioned rats made significantly more errors than controls in their performance on this task. The analysis of error type revealed that the PPTg lesioned rats made an equal number of both baited and unbaited arm re-entries, but made more of both than control rats. For both groups the number of errors made was more pronounced in the first days of performance on the task, but reduces over days as the rats become more familiar with the task. The types of errors made by both groups over days was variable in that some days more baited re-entries are made than unbaited arm re-entries and vice-versa for other days but overall, there was no main effect of error type or interaction of group and error type.

The time to make the first arm choice across days is shown in Figure 6.1.3 (A). Analysis of these data revealed no significant main effect of the between subjects factor of group ($F_{1,11} = 2.15$). There was also no significant main effect of the within subjects factor of days ($F_{8,88} = 1.84$), or the two-way interaction of group x days ($F_{8,88} = 0.50$). Both groups were similar in their time to initiate the trial and to make their first arm choice. The PPTg lesioned rats were no different to control in mobility or motivation compared to controls. This would seem to indicate that there was no deficit or impairment in arousal in the PPTg lesioned rats compared to controls.

Figure 6.1.3

Measures of the animals physical ability and motivation to perform the task. Each graph represents the trials across days. A. Mean time to initiate trial and make the first arm choice; B. Mean time to make subsequent arm choices; C. Time to complete individual trials.


A.
Latency
Time (s)
40
32
24
16
8
0
1 2 3 4 5 6 7 8 9
Sham
PPTg
B.
Choice
Time (s)
40
30
20
10
0
1 2 3 4 5 6 7 8 9
Sham
PPTg
C.
Complete
Time (s)
600
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9
Sham
PPTg
DAYS

The time to make subsequent arm choices across days is shown in Figure 6.1.3 (B). Analysis of the time to make subsequent choices after trial initiation revealed no significant main effect of the between subjects factor of group ($F_{1,11}$ = 1.39). There was also no significant main effect of the within subjects factor of days ($F_{8,88}$ = 2.01), or the two-way interaction of group x days ($F_{8,88}$ = 0.45). Again, it is shown that the PPTg lesioned rats were no different than controls in their physical ability to perform this task or motivation to continue making arm choices in an attempt to complete the task.

Finally, the time to complete the trials across days is shown in Figure 6.1.3 (C). Analysis of these data revealed no significant effect of the between subjects factor of group ($F_{1,11}$ = 0.01), but a significant main effect of the within subjects factor of days ($F_{8,88}$ = 4.20, $p < 0.001$). This simply reflects the variability in task completion as the rats became more familiar with the task and were able to complete it faster with time (Tukey-HSD, $p = 0.05$). Two-way interaction analysis of group x days was not significant ($F_{8,88}$ = 0.45). These data revealed no difference between the groups in their time to complete trials, again demonstrating the similarity between the groups in all aspects of task performance other than error free foraging performance.

Overall these three measures of task performance: time to initiate session, time to make subsequent choices and time to complete trial revealed that PPTg lesioned rats were no different than controls in their ability to physically perform this task and that they were no different in degree of motivation in that they were as eager

as controls to choose arms and eat all pellets. Results from these three measures are represented in Figure 6.1.3. The difference in these groups was that PPTg lesioned rats were unable to perform the task accurately and thus made more errors than control rats in their completion of the task.

Discussion

The results of this experiment revealed that PPTg lesioned rats were impaired in their acquisition ability to perform a spatially guided random foraging radial arm maze task. Here the rats must forage 'on-line' and retrospectively remember where they've been to know where not to return to. Control rats were successful in this foraging ability while PPTg lesioned rats were quite impaired as seen from the number of errors they made in this task (demonstrated in Figure 6.1.2). The pattern of errors the rats made reveals that they were equally impaired in re-visiting both previously baited as unbaited arms, which may suggest a global disruption in foraging strategy. Here the receipt of reward from baited arms did not influence the type of errors the rats made. This performance impairment could be viewed as mnemonic in their inability to remember the arms visited, but it may also be a problem of response selection and guiding this planned action into behaviour. Work by Floresco and his colleagues (1997) found that lidocaine disconnection lesions of the subiculum in one hemisphere and the nucleus accumbens in the opposite hemisphere resulted in a similar pattern of impaired performance on the random foraging task as that demonstrated here following PPTg lesions. The role of the nucleus accumbens is seen to be important for successful performance in this task and when lesioned, results in an impairment. In this experiment, while the spatially

relevant information can be processed effectively through an intact hippocampal-nucleus accumbens circuitry, the selection of the correct response and the guiding of this planned behaviour into action is halted through arrival of nucleus accumbens outflow to an inactive and lesioned PPTg. With no means to place the retrospectively planned behaviour into an appropriate response choice then the rats make more errors, or inappropriate choices, in their completion of the task. Making inappropriate response choices following PPTg lesions has been seen before in a conditioned reinforcement task (Inglis et al., 1994b). In this task the rats learn that of two levers presented, one provides them with food reinforcement while the other is a non-reinforced lever. Following PPTg lesions, rats respond correctly on the reinforced lever but respond equally, and inappropriately, on the non-reinforced lever. Here, the association of the lever reward value has not been appropriately distinguished producing equal responding to both choices.

Finally, data from this experiment revealed the similarity between the groups in the latency to make their first arm choice, the time to make subsequent arm choices and in the time to complete the trials. This similarity revealed that there was no difference in the rats' ability to physically perform the task, or in their motivation to forage and retrieve pellets. This finding is important for negation of any suggestion that lesions of the PPTg result in locomotor impairments which would hamper with their physical ability to perform the task. The impairment in the rats performance on the task was related to appropriateness of responding and is more likely to be a result of disrupted ventral striatal outflow and its consequent impairment of guiding spatially relevant planned behaviours into appropriate action.

6.2 Experiment 2. Examination of retention of a random foraging radial arm maze task following excitotoxic lesions of the pedunculopontine tegmental nucleus.

Introduction

This experiment examined the role of the PPTg in an rats ability to retain performance on a random foraging radial arm maze task. Experiment 1 outlined that control rats were able to acquire this random foraging task successfully, while rats with lesions of the PPTg were quite impaired in acquisition performance. The following experiment sought to extend this finding by examining the effect of the PPTg lesions on rats' performance once they had successfully acquired the ability to perform the task. This experiment gets at the root of reference memory over time for performance of this task. The rats have acquired the information on successful performance of this task and could use this subsequent to surgery to re-perform the task effectively. The role of the PPTg in this process has not been previously examined.

Methods

Animals

18 rats were used (Charles River). Mean weight at time of surgery was 287.83 ± 21.13 (SD).

Surgery

Rats were anaesthetised with sodium pentobarbitone before being placed in the stereotaxic frame. 8 rats were given a lesion of the PPTg while 10 were given a

phosphate buffer control lesion (see General Methods). Rats were given at least two weeks postoperative care before behavioural testing began.

Random Foraging

This task is described in General Methods. In this experiment all rats were trained on the task before and after surgery. In the before surgery phase, rats were deprived to 85% of their free feeding weight before trials began. Rats were maintained on 17.5-20 grams of food pellets/day. Rats were trained until all rats as a group reached criterion of 1 error or less over 3 consecutive trials. Rats then underwent surgery as described above. Following postoperative care rats were food deprived to 85% of their free-feeding weight before habituation trials began. Rats were maintained on 17.5-20 grams food/day. Water was available *ad libitum* throughout the entire experiment. Once post-surgery trials began rats were given 1 session/day on the maze until the sham rats, as a group, reached a criterion of 1 error or less per day for 3 consecutive days.[8]

Results

All data were analysed using a repeated measures analysis of variance with group (sham vs. PPTg) as the between subjects variable and days as the within subjects variable. Analysis of the number of errors made had an extra within subjects variable of error type (baited versus un-baited arm re-entries).

Pre Surgery

The number of errors made in task completion collapsed across criterion days is shown in Figure 6.2.1. Analysis of errors made revealed no significant main effect

[8] As in experiment 1, this criterion was established following consultation with Stan Floresco and is based on their random foraging criterion selection (Floresco et al., 1996, 1997).

of the between subjects factor of group ($F_{1,16} = 0.20$). The within subjects analysis revealed a significant main effect of the factor of days ($F_{18,288} = 1.83$, $p = 0.021$). This result simply reflects the variability in daily performance as the rats learned the task demands. There was no significant main effect of the factor of error type ($F_{1,16} = 0.39$), or the two-way interaction of group x days ($F_{18,288} = 0.42$), or group x error type ($F_{1,16} = 0.19$), but a significant two-way interaction of days x error type ($F_{18,288} = 2.45$, $p = 0.001$). The three-way interaction of group x days x error type ($F_{18,288} = 0.73$) was not significant. These results revealed that before lesions of the PPTg were induced all rats were equal in their performance on this random foraging task. In addition, there were no overall differences in the types of errors made by any of the rats, just variability in daily performance as the rats learned the task over time.

The time to make the first arm choice across days is shown in Figure 6.2.2 (A). Analysis of the latency to make the first choice revealed no significant main effect of the between subjects factor of group ($F_{1,16} = 2.96$), but a significant main effect of the within subjects factor of days ($F_{18,288} = 8.24$, $p< 0.001$), and no significant two-way interaction of group x days ($F_{18,288} = 0.92$).

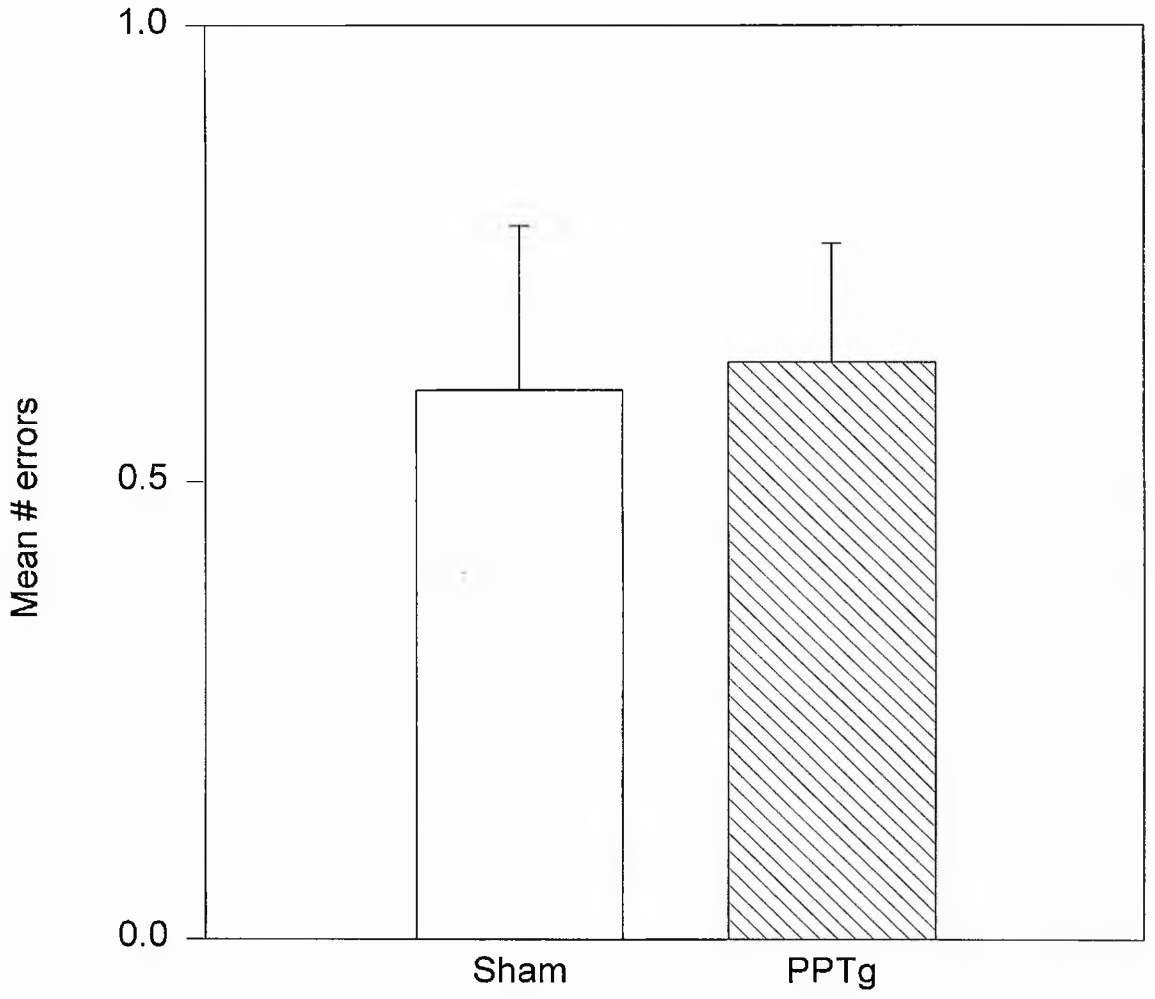
The time to make subsequent arm choices across days is shown in Figure 6.2.2 (B). Analysis of the time to make subsequent choices after trial initiation revealed no significant main effect of the between subjects factor of group ($F_{1,16} = 4.22$), but a significant main effect of the within subjects factor of days ($F_{18,288} =11.61$, $p< 0.001$), and no significant two-way interaction of group x days ($F_{18,288} = 1.15$).

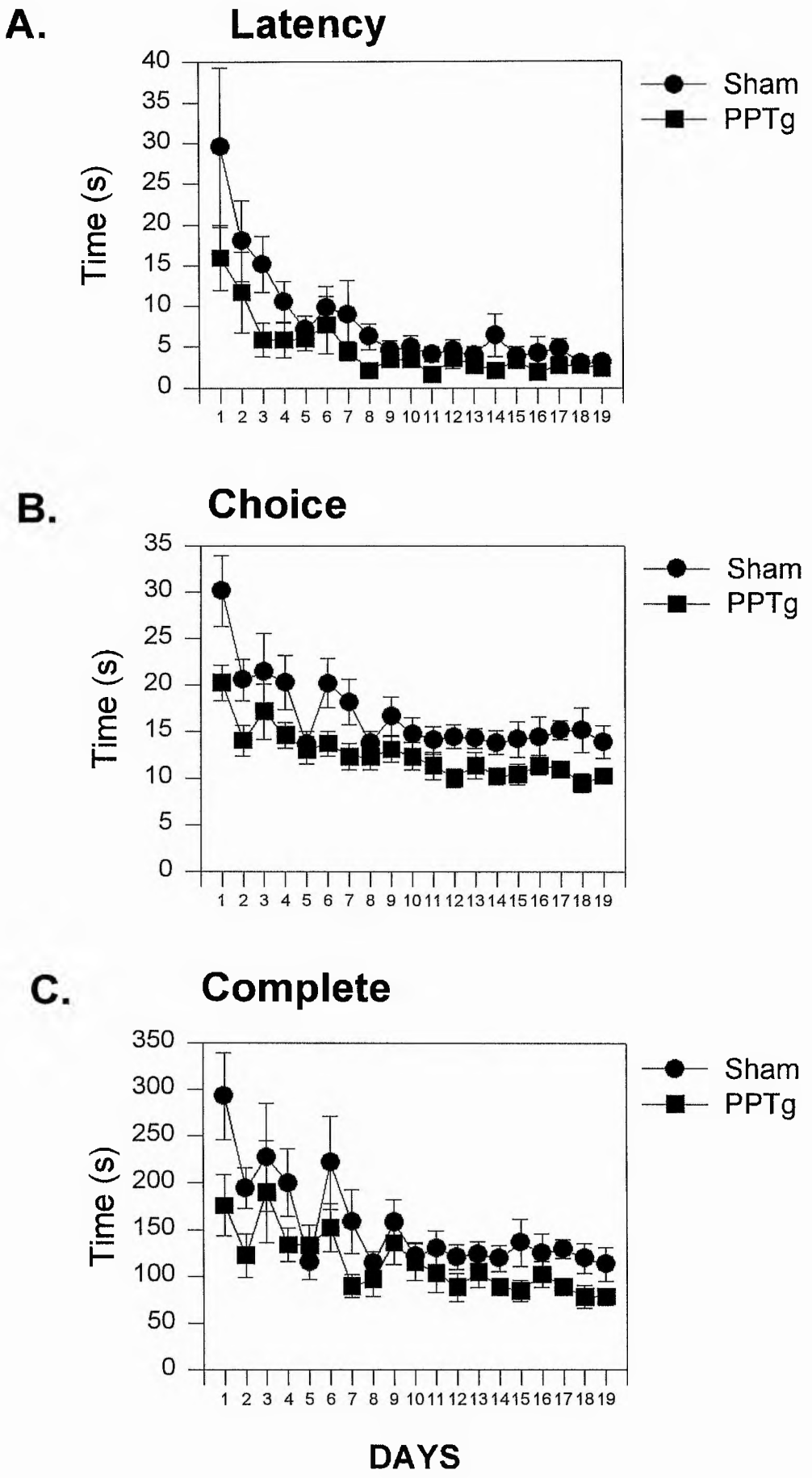
Figure 6.2.1

Pre-surgery data: Mean number of errors across the three criterion days for pre-sham and pre-PPTg ibotenic lesioned animals.

Figure 6.2.2

Pre-surgery data: Measures of the animals physical ability and motivation to perform the task. Each graph represents the trials across days. A. Mean time to initiate trial and make the first arm choice; B. Mean time to make subsequent arm choices; C. Time to complete individual trials.


1.0
0.5
0.0
Mean # errors
Sham
PPTg


A.
Latency
Time (s)
40
35
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sham
PPTg
B.
Choice
Time (s)
35
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sham
PPTg
C.
Complete
Time (s)
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sham
PPTg
DAYS

Finally, the time to complete the session across days is shown in Figure 6.2.2 (C). Analysis of these data revealed no significant main effect of the between subjects factor of group ($F_{1,16}$ = 3.34), but a significant main effect of the within subjects factor of days ($F_{18,288}$ = 6.71, $p < 0.001$), and no significant two-way interaction of group x days ($F_{18,288}$ = 0.94).

The bulk of these analyses revealed that there were no differences amongst any of the rats in their pre-surgery ability to acquire this task. This comparison, and negative outcome, is essential for post PPTg surgery comparisons.

Post Surgery

Figure 6.2.3 represents the largest and smallest lesion sizes for the PPTg ibotenate lesioned rats of this experiment. Damage to nearby structures was negligible and inconsistent across rats. 3 rats had damage to the adjoining retrorubral field and 2 rats had damage to the deep mesencephalic nucleus. 5 rats had minimal damage to the superior cerebellar peduncle. The inconsistent damage to these structures did not produce a change in behaviour across the PPTg lesioned rats. This was examined through direct comparison of damage sites and behavioural outcome. No consistent relationship between damage to a structure other than the PPTg and behaviour were found.

The number of errors made in task completion collapsed across criterion days is shown in Figure 6.2.4. Analysis of errors made revealed a significant main effect of the between subjects factor of group ($F_{1,16}$ = 12.99, p = 0.002). The within subjects analysis revealed no significant main effect of the factor of days ($F_{7,112}$ = 1.61) and no

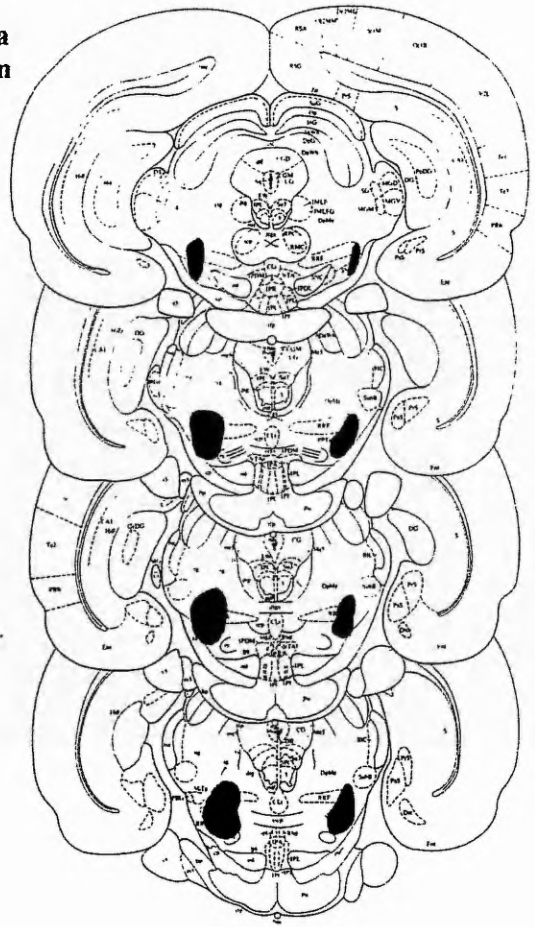
Figure 6.2.3

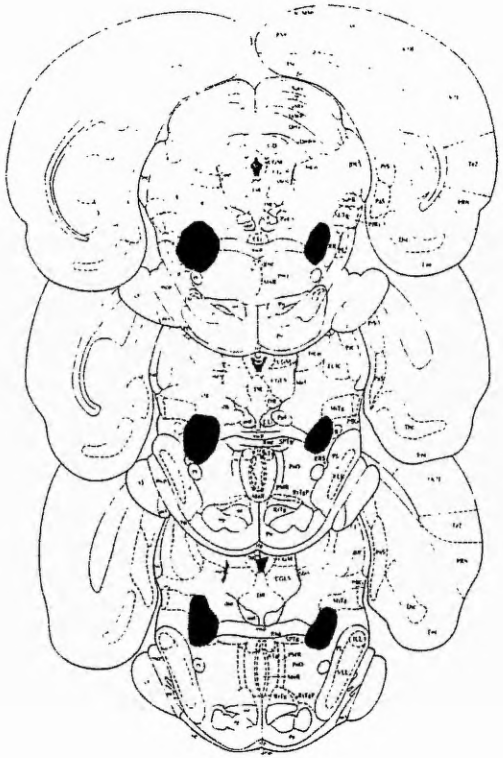
Representative sections from Bregma -6.30 to -8.72 mm redrawn from the atlas of Paxinos and Watson (1986) which illustrate the largest (left) and smallest (right) lesions for PPTg ibotenic acid lesioned rats in this experiment.

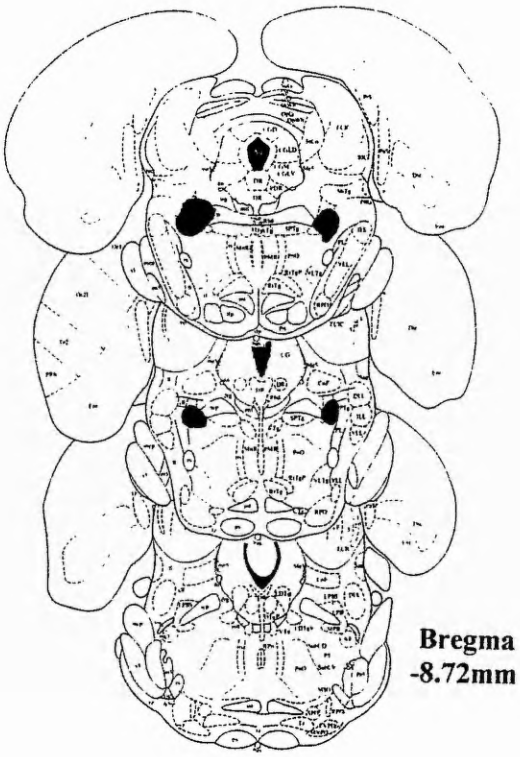
Figure 6.2.4

Mean number of errors across the three criterion days for sham and PPTg ibotenic lesioned animals. Inset figure represents the mean errors of the PPTg ibotenic lesioned animals, separated into error type: re-entry to baited (B) and un-baited (U) arms.

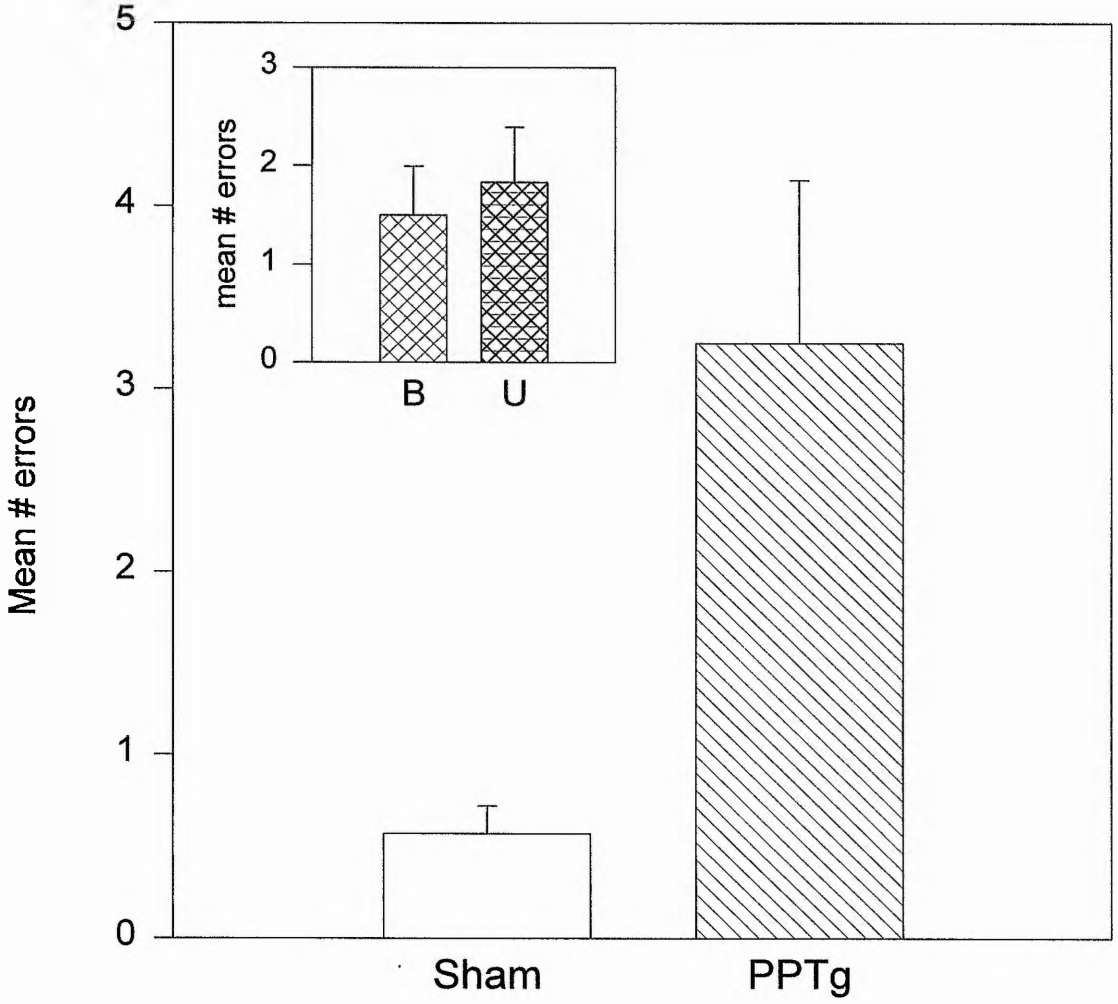
Bregma
-6.30mm







Bregma
-8.72mm


Mean # errors
5
4
3
2
1
0
Sham
PPTg
mean # errors
3
2
1
0
B
U

significant main effect of the factor of error type ($F_{1,16}$ = 0.93). There was no significant two-way interaction of group x days ($F_{7,112}$ = 1.21), group x error type ($F_{1,16}$ = 0.62), or days x error type ($F_{7,112}$ = 1.83), or a three-way interaction of group x days x error type ($F_{7,112}$ = 1.83). This analysis revealed that PPTg lesioned rats made significantly more errors than controls in their performance on this retention task. The analysis of error type revealed that the PPTg lesioned rats made an equal number of both baited and un-baited arm re-entries, just more of them than control rats. This result is also demonstrated in Figure 6.2.4.

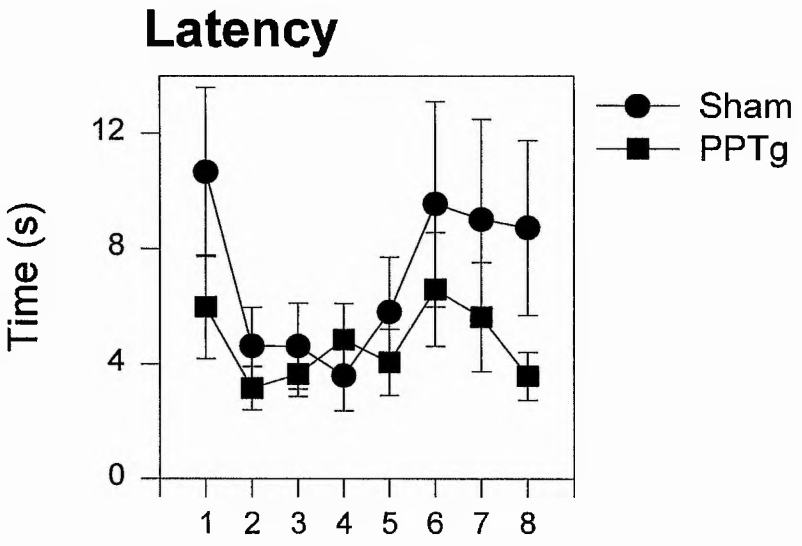
The time to make the first arm choice across days is shown in Figure 6.2.5 (A). Analysis of these latency data revealed no significant main effect of the between subjects factor of group ($F_{1,16}$ = 0.93), but a significant main effect of the within subjects factor of days ($F_{7,112}$ = 3.26, p = 0.003). Finally, there was no significant two-way interaction of group x days ($F_{7,112}$ = 1.06). These data show that both groups were similar in their time to initiate the trial, and make their first arm choice, and thus revealed that the PPTg lesioned rats were no different in mobility or motivation compared to controls.

The time to make subsequent arm choices across days is shown in Figure 6.2.5 (B). Analysis of the time to make subsequent choices after trial initiation revealed no significant main effect of the between subjects factor of group ($F_{1,16}$ = 3.45). There was no significant main effect of the within subjects factor of days ($F_{7,112}$ = 2.08), but a significant two-way interaction of group x days ($F_{7,112}$ = 2.47, p = 0.021). Post-hoc analysis revealed that sham performance on day 6 is slower than sham performance on days 1, 2 and 3; sham performance on day 4 is slower than

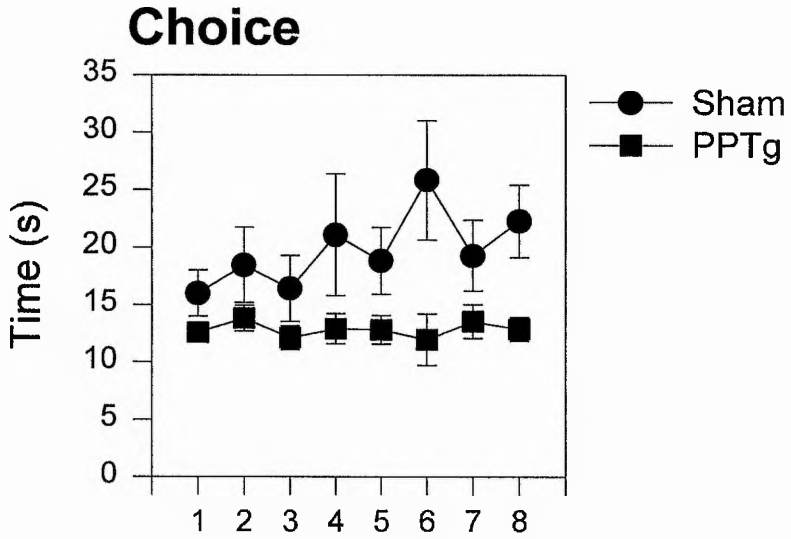
Figure 6.2.5

Measures of the animals physical ability and motivation to perform the task. Each graph represents the trials across days. A. Mean time to initiate trial and make the first arm choice; B. Mean time to make subsequent arm choices; C. Time to complete individual trials.

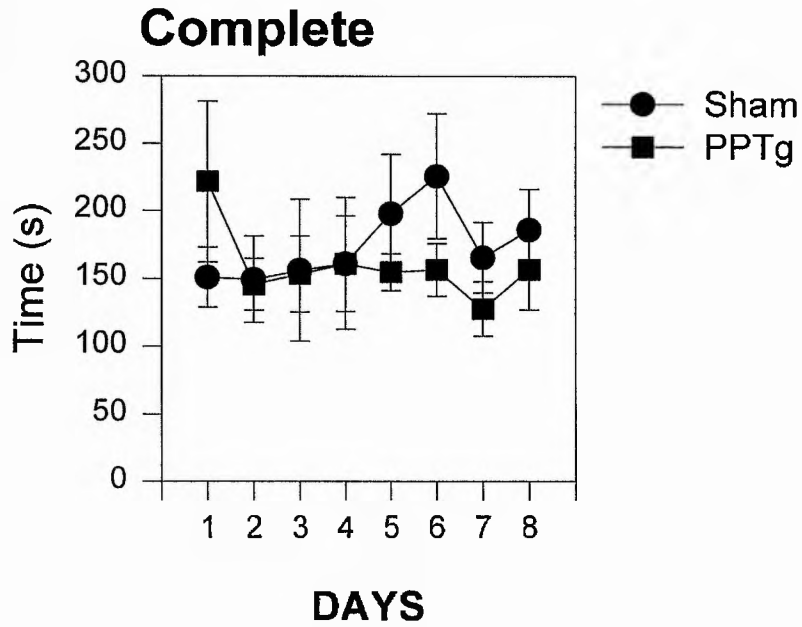
A.


Latency
Time (s)
0
4
8
12
1 2 3 4 5 6 7 8
Sham
PPTg

B.


Choice
Time (s)
0
5
10
15
20
25
30
35
1 2 3 4 5 6 7 8
Sham
PPTg

C.


Complete
Time (s)
0
50
100
150
200
250
300
1 2 3 4 5 6 7 8
DAYS
Sham
PPTg

PPTg performance on days 1, 3, 4, 5, 6 and 8; and sham performance on days 6 and 8 are slower than PPTg performance on days 1-8 (Tukey-HSD post-hoc test, $p < 0.05$).

Finally, time to complete the trials across days is shown in Figure 6.2.5 (C). Analysis of these data revealed no significant effect of the between subjects factor of group ($F_{1,16} = 0.13$). There was no significant main effect of the within subjects factor of days ($F_{7,112} = 0.77$) or of the two-way interaction of group x days ($F_{7,112} = 1.16$). Once again it is demonstrated that the PPTg lesioned rats did not differ from control in their ability to complete the task.

Overall these three measures of task performance- time to initiate session, time to make subsequent choices and time to complete trial- revealed that PPTg lesioned rats were no different than controls in their ability physically to perform this task and that they were no different in degree of motivation in that they were as eager as controls to choose arms and ate all the pellets. The difference between these groups was that PPTg lesioned rats were unable to perform the task accurately and thus made more errors than control rats in their completion of the task.

Discussion

The results of this experiment revealed, for the first time, that PPTg lesioned rats were impaired in their retention of a random foraging radial arm maze task. While being able to acquire this task before their lesion, the rats failed to use this information to aid performance post-surgery. Again it is seen that lesions of the

PPTg interfere with accurate performance of ongoing spatially navigated behaviour. This impairment may be a disruption of reference memory, but it may be more likely to be related to impaired response related processing and a subsequent inability to guide goal related response selections into action. The role of goal directed and planned response behaviour has been proposed to be a function of the nucleus accumbens (Kelley and Stinus, 1985; Mogenson et al., 1980; Mogenson, 1987) and performance on random foraging tasks are impaired when the nucleus accumbens is made inactive (Floresco et al., 1997). As a structure in direct receipt of striatal outflow, the PPTg is undoubtedly influential in goal directed response processing and guiding these responses into action. Once lesioned, then the ability to select and execute appropriate choices is hampered and results in the animal making more errors in foraging performance. These errors are not influenced by reward receipt as the rats made an equal number of re-visits to both baited and un-baited arms, again suggesting a global disruption in foraging strategy.

Finally, the lack of differences between control and PPTg lesioned rats in time to make an initial arm choice, time to make subsequent arm choices and in time to complete trials, reveals that there is no change to locomotion or motivational processes following PPTg lesions. This null result is again relevant to refute any claims that PPTg lesions influence motor capabilities that would hamper the rats ability to perform the task.

Summary

These experiments reveal that excitotoxic lesions of the PPTg impair performance on both acquisition and retention of a random foraging radial arm maze task. These lesions impair rats' ability to efficiently retrospectively forage and result in the rats making significantly more errors relative to control rats. These errors do not seem to be related to the receipt of reward in that the re-visiting errors were made to both baited and un-baited arms. The fact that the rats ate all the pellets and completed the trials revealed that the lesions of the PPTg did not influence their motivation to perform the task. Finally, both experiments revealed no differences between control and PPTg lesioned rats in their time to initiate trials, time to make subsequent arm choices or in time to complete trials, revealing that the lesion did not impair the rats' locomotor ability to physically perform the task. The results of these experiments would seem to suggest a role for the PPTg in appropriate response selection and the execution of appropriately planned behaviours.

Chapter 7. The role of the PPTg in acquisition and retention of a spatial working memory task.

7.0 Introduction

The role of cholinergic systems in memory and cognitive function has been outlined in the previous chapter with a look at the role of hippocampal-nucleus accumbens outflow on performance accuracy during ongoing navigational behaviour in a non-delayed radial arm maze foraging task. While lesions of the PPTg were found to disrupt performance on both acquisition and retention of this task, further exploration of its role in receipt of cortico-hippocampal-striatal outflow and this relationship with performance on memory related tasks needs to be conducted. Previous work has examined the role of the nucleus accumbens in performance on a spatial working memory task in which the animal uses prospective coding for effective foraging strategy and has found different responding than that outlined during performance of on-line foraging tasks where the animal is forced to use retrospective foraging (Cook et al., 1985). In a study by Floresco and his colleagues (1996), the effect of pharmacological inactivation of the nucleus accumbens on a delayed spatial working memory radial arm maze task was examined. In this task the rats had access to 4 baited arms while the other 4 were blocked. Once these pellets were retrieved the animal was taken off the maze for a delay period. Subsequent to the delay the animal was returned to the maze where now all arms were accessible, but only those that were blocked prior to the delay now contained food pellets. The rats were able then to use information acquired before the delay to prospectively guide performance to make choices subsequent to the delay.

Increasing doses of the dopamine receptor antagonist haloperidol were used and the authors found that performance on this delayed task was not affected by any dose. This result was in contrast to the disruption found in performance of a non-delayed task in which the rats must forage retrospectively to complete the task. From this result the authors suggest that the nucleus accumbens is crucial when there is ambiguity about the position of food in space, but not when an animal has previous information about the location of food stimuli. This result was extended when the authors examined the pattern of responding on both a delayed and non-delayed radial arm maze task following disconnection lesions of the nucleus accumbens in one hemisphere and the ventral subiculum portion of the hippocampus in the other hemisphere (Floresco et al., 1997). Again the authors found performance disrupted when the rats were completing a non-delayed spatial task versus when the rats were completing a delayed spatial task where the rats acquired information as to the location of food prior to delay, held it in short-term working memory over the delay and were then able to use to subsequent to the delay to prospectively plan response choices.

These results further inform the findings of examination of the role of the prefrontal cortex-hippocampal pathway in performance of spatial radial arm maze tasks (Floresco et al., 1997). Using lidocaine to induce temporary lesions of the prelimbic portion of the prefrontal cortex in one hemisphere and the ventral subiculum portion of the hippocampus in the other hemisphere, resulted in disrupted performance in a spatial delayed working memory task (as described above) but not disruption in the non-delayed spatial task. In a follow up study to this work

(Seamans et al., 1998), the authors found that the role of the prelimbic portion of the prefrontal cortex in disrupted performance in this delayed spatial working memory task is modulated by D_1 receptors. The authors injected the D_1 receptor antagonist SCH-23390 or the D_2 receptor antagonist sulpiride into the prelimbic region and found that only the D_1 receptor antagonist disrupted performance on the delayed task. These studies reveal a picture of separate, yet complementary activity of the cortico-hippocampal-striatal circuitry in modulating spatially relevant behaviour. The prelimbic-hippocampus portion of the circuitry is important for performance when the animal has previous information as to the location of the food and can prospectively plan response choices whereas the hippocampus-nucleus accumbens circuitry is important for performance when the animal has no previous information to guide choices and is forced to forage retrospectively.

The next step is determining the role of outflow from this circuitry in performance on these delayed and non-delayed spatial tasks. The role of the PPTg in performance on a non-delayed spatial task was examined in the previous chapter. Lesions of the PPTg disrupted performance on both acquisition and retention of a random foraging, ongoing navigational task without affecting any other measures of locomotion or motivation. From this it can be concluded that the ability accurately to guide retrospective response choices is disrupted following lesions of the PPTg. In this chapter, the role of the PPTg in performance on a delayed spatial working memory task will be assessed. Previous work by Dellu and colleagues (1991) examined the role of lesions of the PPTg on performance on a water maze spatial working memory task and found performance was disrupted in lesioned rats.

Lesioned rats took significantly longer (31 s vs. 10 s for controls) to escape to the hidden platform. While this may suggest a role of the PPTg in modulation of a working memory task, use of the water maze to assess performance is considerably different than that of the radial arm maze. The typical water maze task assesses the rat's working memory performance on avoidance behaviour as the rats are trying to escape the water, seeking the safety of the hidden platform. The radial arm maze working memory task, however, examines natural foraging behaviour in a task where the animal acquires information on the location of food in space and must later use this information successfully to plan response choices to acquire food. Here the nature of reward retrieval rather than avoidance behaviour is assessed. The first experiment of this chapter, then, will examine the role of the PPTg on acquisition performance on a delayed spatial working memory radial arm maze task. Both the number and types of errors will be carefully examined as well as measures of locomotion and motivation responding.

7.1 Experiment 1. Examination of acquisition of a delayed spatial win shift radial arm maze task following excitotoxic lesions of the pedunculopontine tegmental nucleus.

Methods

Animals

14 rats were used (Charles River). Mean weight at time of surgery was 314.07 ± 16.84 (SD).

Surgery

Rats were anaesthetised with sodium pentobarbitone (Sagatal, Rhône Mérieux; 60 mg/kg) before being placed in the stereotaxic frame. 7 rats were given a lesion of the PPTg while 7 were given a phosphate buffer control lesion (see General Methods). Rats were given at least two weeks postoperative care before behavioural testing began.

Delayed Spatial Win Shift Task

This task is described in General Methods. Following postoperative care rats were food deprived to 85% of their free-feeding weight before habituation trials began. The rats were given 15-20 grams food pellets/day and when reached 85% were maintained on 17.5-20 grams food/day. Water was available *ad libitum* throughout the entire experiment. Once trials began the rats were given 1 session/day on the maze until the sham rats as a group reached a criterion of 1 error or less per day for 2 consecutive days.[9]

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[9] This criterion was adopted in following the methodological procedures of Floresco et al., 1996; 1997.

Results

Figure 7.1.1 represents the largest and smallest lesion sizes for the PPTg ibotenate lesioned rats of this experiment. Damage to nearby structures was negligible and inconsistent across rats. 2 rats had damage to the cuneiform nucleus. 4 rats had damage to the adjoining retrorubral field and 2 rats had damage to the deep mesencephalic nucleus. All rats had minimal damage to the superior cerebellar peduncle. The inconsistent damage to these structures did not produce a change in behaviour across the PPTg lesioned rats. This was examined through direct comparison of damage sites and behavioural outcome. No consistent relationship between damage to a structure other than the PPTg and behaviour were found.

All data were analysed using a repeated measures analysis of variance with group (sham vs. PPTg) as the between subjects variable and days as the within subjects variable. Analysis of the number of errors made in the test phase had an extra within subjects variable of error type (across phase vs. within phase).

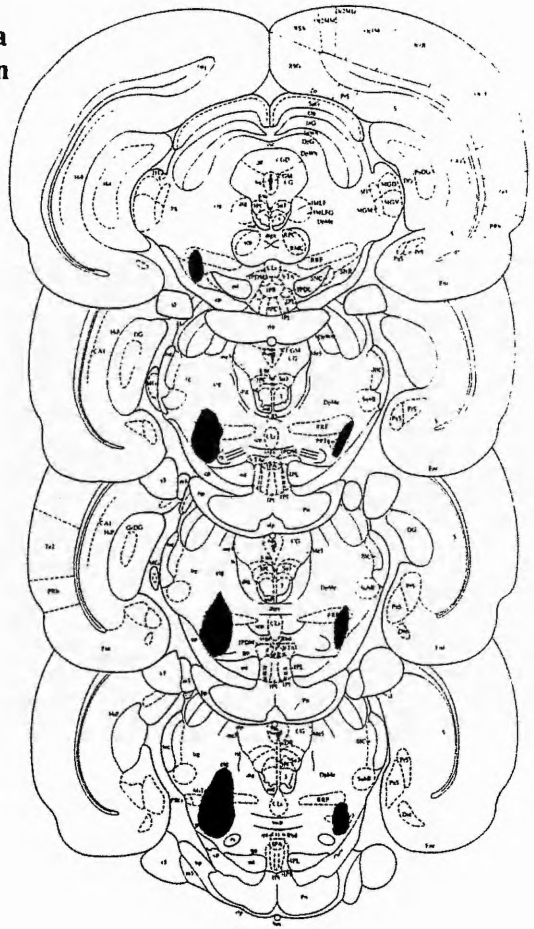
Training phase analysis

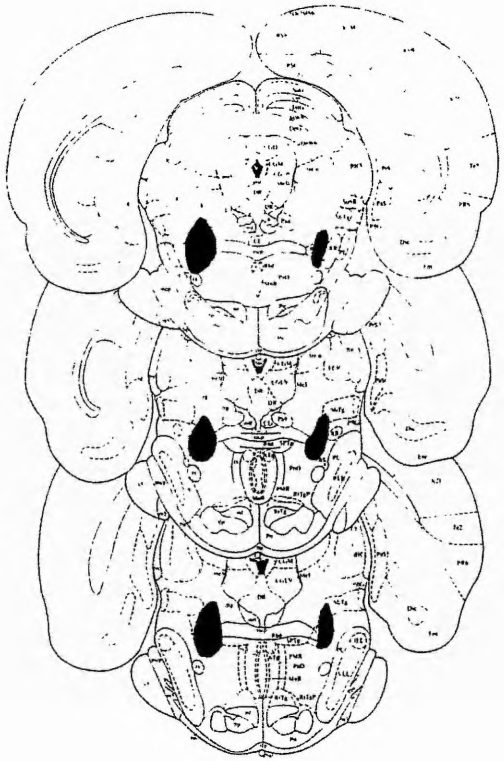
In this portion of the task the rats had access to 4 arms baited with food pellets while the other 4 arms were blocked by the guillotine doors. The mean number of training phase errors across trials is shown in Figure 7.1.2. Analysis of errors made in the training phase revealed no significant between subjects effect of group ($F_{1,12} = 1.54$) and no significant effect of the within subjects factor of days ($F_{9,108} = 1.43$) or the two-way interaction of group x days ($F_{9,108} = 1.83$). This similarity between groups in the training phase portion of the task demonstrated that

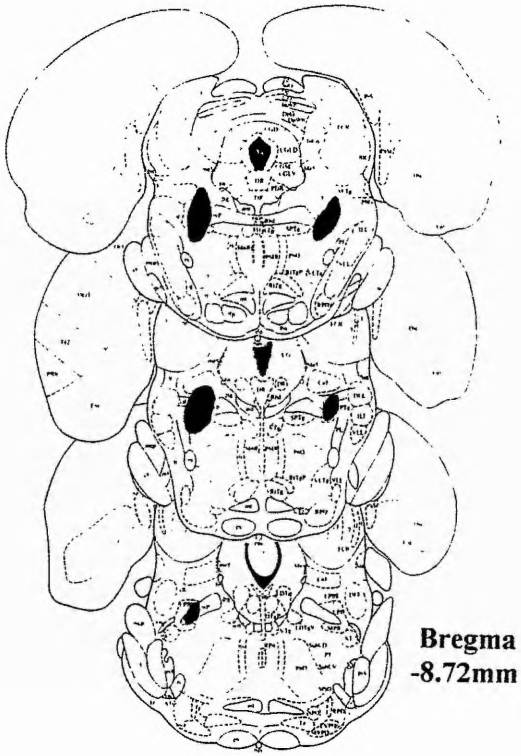
Figure 7.1.1

Representative sections from Bregma -6.30 to -8.72 mm redrawn from the atlas of Paxinos and Watson (1986) which illustrate the largest (left) and smallest (right) lesions for PPTg ibotenic acid lesioned rats in this experiment.

Bregma
-6.30mm







Bregma
-8.72mm

PPTg lesioned rats are able to do simple forms of foraging, in this case retrieve four pellets when only 4 choices are available.

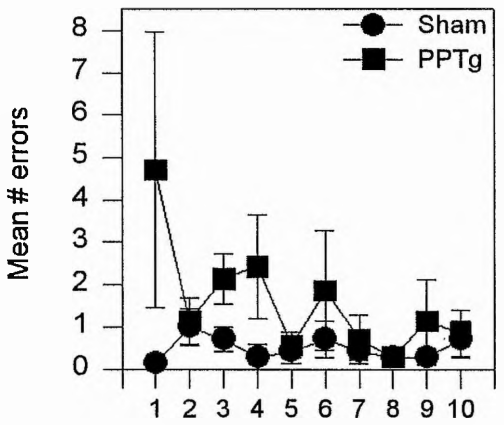
The mean latency time to make the first arm choice is shown in Figure 7.1.2. Analysis of the latency to make the first choice revealed a significant between subjects factor of group ($F_{1,12} = 6.43$, $p = 0.026$) but no significant main effect of the within subjects factor of days ($F_{9,108} = 1.38$), or the two-way interaction of group x days ($F_{9,108} = 1.05$). Here it was found that the PPTg lesioned rats were significantly quicker in initiating their trials and making the first arm choice.

The mean time to make subsequent arm choices is shown in Figure 7.1.2. Analysis of the time to make subsequent choices after trial initiation revealed a significant main effect of the between subjects factor of group ($F_{1,12} = 9.52$, $p = 0.009$), and a significant main effect of the within subjects factor of days ($F_{9,108} = 3.19$, $p = 0.002$), and a significant two-way interaction of group x days ($F_{9,108} = 2.37$, $p = 0.017$). This analysis revealed that the PPTg lesioned rats were quicker to make their subsequent arm choices than the control rats. Post-hoc analysis of the group x days interaction revealed that sham rats on day 2 are slower than PPTg lesioned rats on days 9 and 10; sham rats on day 6 are slower than sham rats on day 10 and slower than PPTg lesioned rats on days 1 and 4-10; sham rats on day 8 are slower than sham rats on days 4, 5, 7, and 10 and PPTg lesioned rats on days 1-10; and sham rats on day 9 are slower than PPTg lesioned rats on day 10 (Tukey-HSD post-hoc test, all $p < 0.05$).

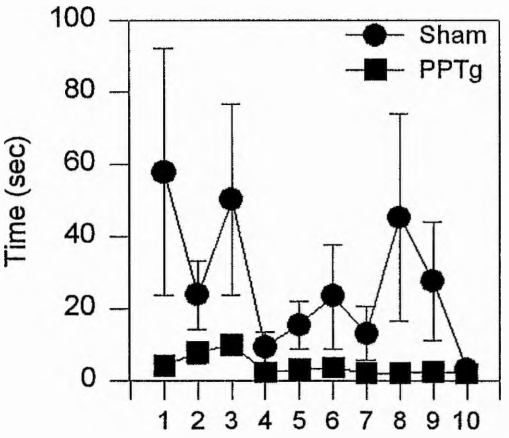
Figure 7.1.2

Training phase data: **Errors**: Mean number of errors across trials for sham and PPTg ibotenic lesioned animals; **Latency**: Mean latency time to make the first choice across trials for sham and PPTg ibotenic lesioned animals; **Subsequent choices**: Mean time to make subsequent arm choices across trials for sham and PPTg ibotenic lesioned animals; **Time to complete**: Mean time to complete the session across trials for sham and PPTg ibotenic lesioned animals.

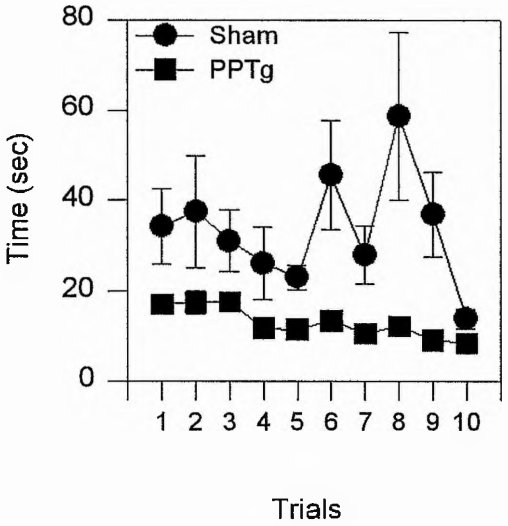
Errors



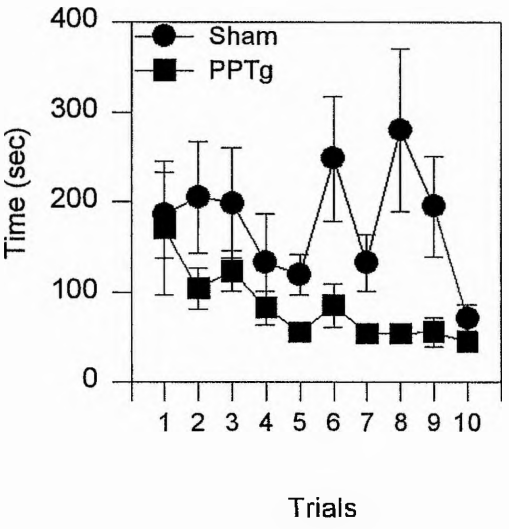
Latency



Subsequent Choices



Time to Complete



Finally, the mean time to complete trials is shown in Figure 7.1.2. Analysis of the time to complete the trial revealed a significant effect of the between subjects factor of group ($F_{1,12} = 5.21$, $p = 0.041$), and a significant main effect of the within subjects factor of days ($F_{9,108} = 2.89$, $p = 0.004$), but no significant two-way interaction of group x days ($F_{9,108} = 1.79$). Again here it was seen that the PPTg lesioned rats were significantly faster than controls to complete the trial and collect all 4 pellets. All rats became quicker in completing the trial over days as the rats became more familiar with the task.

Test phase analysis

The mean number of tests phase errors across the two criterion days are shown in Figure 7.1.3. In this portion of the task, the rats now had access to all 8 arms of the maze but now the arms that were blocked in the training phase were baited while those arms that were baited in the training phase were now blocked. The animal had to shift its response selections to forage correctly and retrieve the food reward. Analysis of errors made in the test phase revealed a significant main effect of the between subjects factor of group ($F_{1,12} = 5.30$, $p = 0.040$). The within subjects analysis revealed a significant main effect of the factor of days ($F_{9,108} = 3.15$, $p = 0.002$), a significant main effect of the factor of error type ($F_{1,12} = 179.98$, $p < 0.0001$), but no significant two-way interactions of group x days ($F_{9,108} = 0.98$), group x error type ($F_{1,12} = 2.49$), or days x error type ($F_{9,108} = 1.95$), or the three-way interaction of group x days x error type ($F_{9,108} = 1.14$). This analysis revealed that PPTg lesioned rats made significantly more errors than controls in their performance on the test phase of this task. In looking at the types of errors made revealed that

both groups made more across phase errors than within phase errors, meaning that they visited and re-visited arms that had been baited in the training phase of the task, but that were no longer baited in the test phase. This effect was more pronounced in the first 4-5 days of performance on the task, but reduced with time as the rats became more familiar with, and learnt the task.

Mean time to make the first arm choice is shown in Figure 7.1.4 (A). Analysis of the latency to make the first choice revealed a significant main effect of the between subjects factor of group ($F_{1,12} = 5.05$, $p = 0.044$), but no significant main effect of the within subjects factor of days ($F_{9,108} = 0.97$), or the two-way interaction of group x days ($F_{9,108} = 0.71$). Here it was found that the PPTg lesioned rats were significantly quicker in initiating their trials and making the first arm choice.

Mean time to make subsequent choices is shown in Figure 7.1.4 (B). Analysis of the time to make subsequent choices after trial initiation revealed a significant main effect of the between subjects factor of group ($F_{1,12} = 11.18$, $p = 0.006$), but no significant main effect of the within subjects factor of days ($F_{9,108} = 1.90$), or the two-way interaction of group x days ($F_{9,108} = 0.81$). Here it is revealed that the PPTg lesioned rats are quicker to make their subsequent arm choices than the control rats.

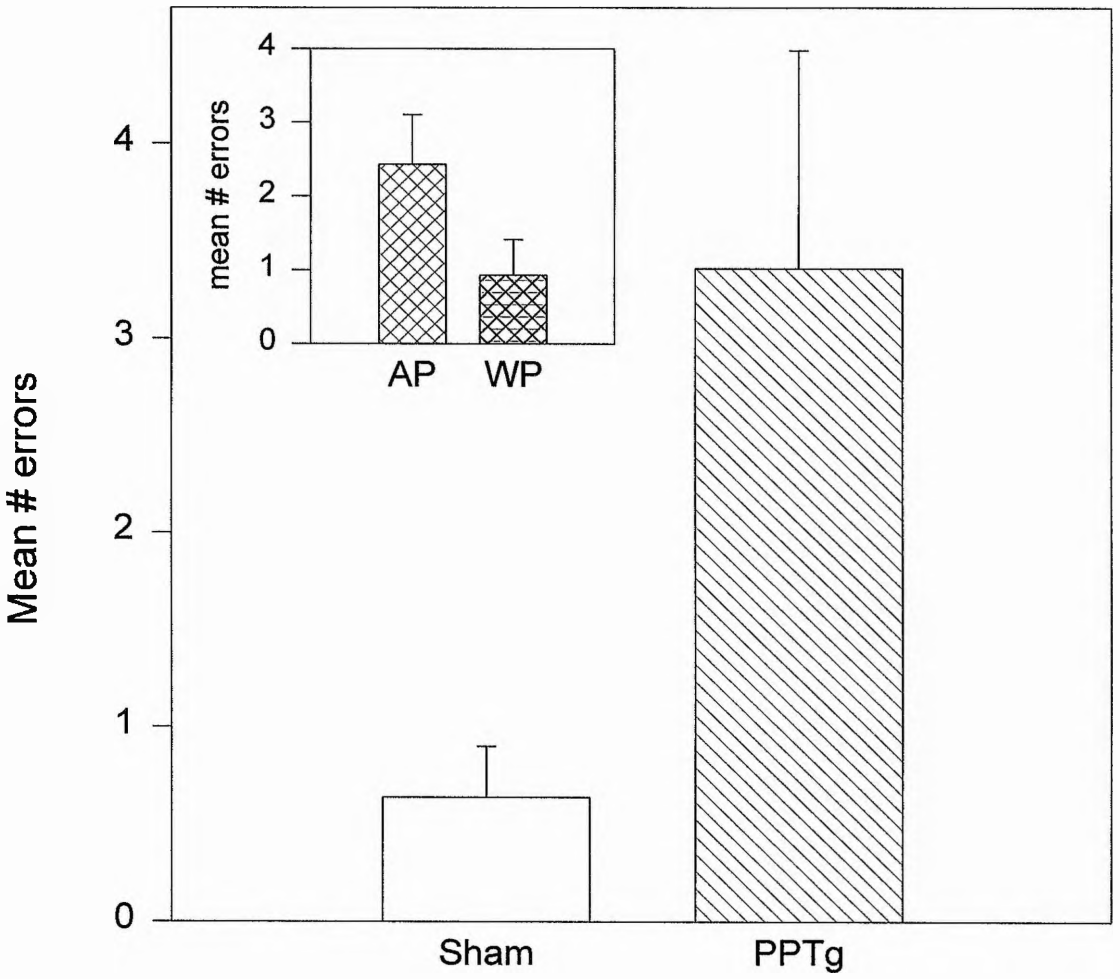
Finally, mean time to complete the trial is shown in Figure 7.1.4 (C). Analysis of the time to complete the trial revealed a significant effect of the between subjects factor of group ($F_{1,12} = 4.83$, $p = 0.048$), and a significant main effect of the

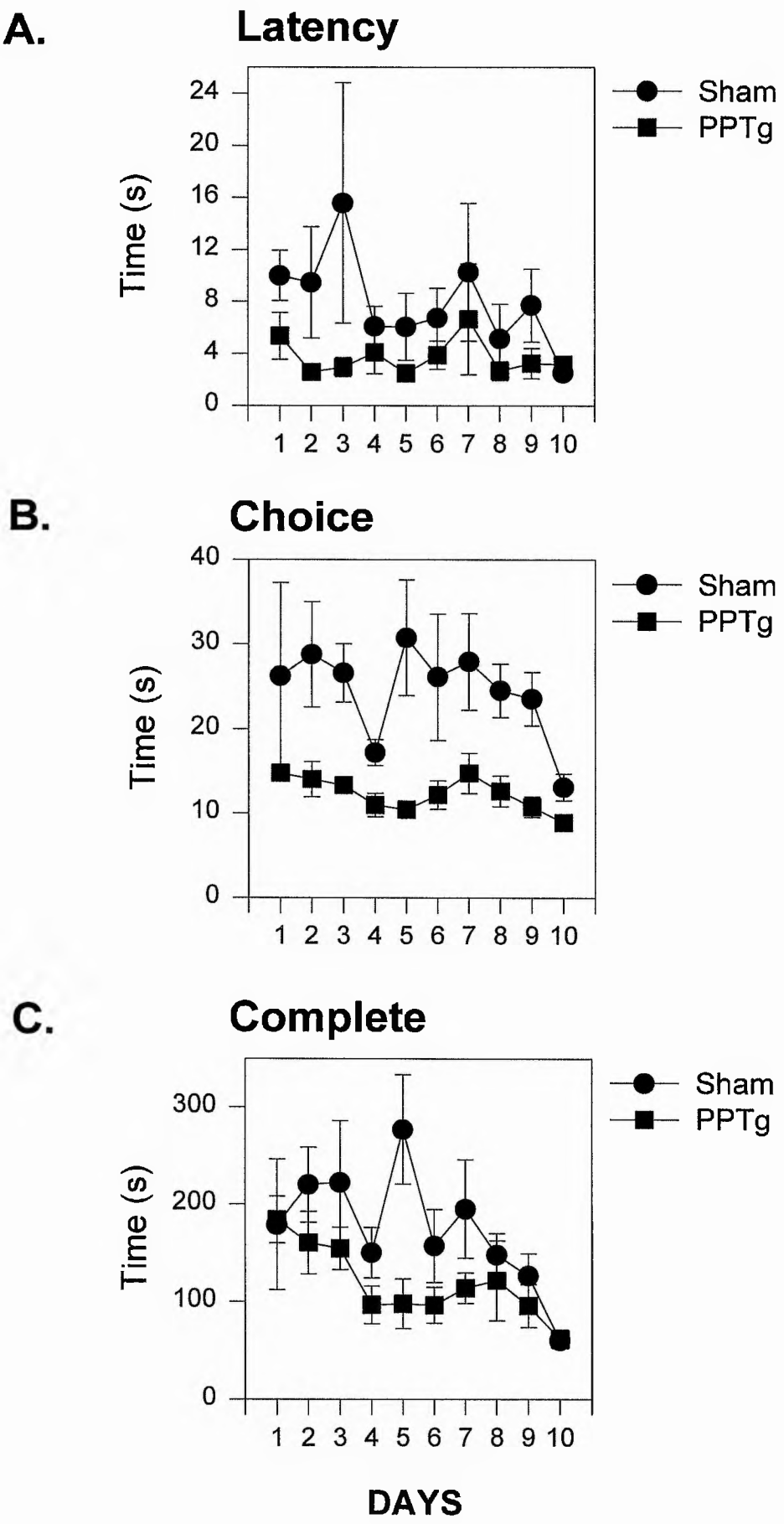
Figure 7.1.3

Mean number of errors for the test phase across the two criterion days for sham and PPTg ibotenic lesioned animals. Inset figure represents the mean errors of the PPTg ibotenic lesioned animals, separated into error type: entry and re-entry to arms baited in the training phase or across phase (AP) errors, and re-entry to test phase baited arms or within phase (WP) errors.

Figure 7.1.4

Measures of the animals physical ability and motivation to perform the task. Each graph represents the trials across days. A. Mean time to initiate trial and make the first arm choice; B. Mean time to make subsequent arm choices; C. Time to complete individual trials.


Mean # errors
0
1
2
3
4
Sham
PPTg
mean # errors
0
1
2
3
4
AP
WP


A.
Latency
Time (s)
24
20
16
12
8
4
0
1 2 3 4 5 6 7 8 9 10
Sham
PPTg
B.
Choice
Time (s)
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10
Sham
PPTg
C.
Complete
Time (s)
300
200
100
0
1 2 3 4 5 6 7 8 9 10
Sham
PPTg
DAYS

within subjects factor of days ($F_{9,108} = 3.51$, $p = 0.001$), but no significant two-way interaction of group x days ($F_{9,108} = 1.32$). Again here it was seen that the PPTg lesioned rats were significantly faster than controls to complete the trial and collect all 4 pellets. All rats became quicker in completing the trial over days as the rats became more familiar with the task.

In both the training phase and test phase of this task PPTg lesioned rats were quicker in the elements of task performance including time to initiate the trial, average time to make subsequent arm choices and time to complete the trial. In the training phase this quicker performance may be related to increased motivational excitement in anticipation of reward. Food deprived PPTg lesioned rats have previously shown enhanced behavioural excitement when presented with reward (as shown in enhanced sucrose drinking compared to controls in Chapters 4 and 5). This may be combined with the fact that in the training phase the rats are faced with access to only 4 arms, each of which is baited with food reward unlike the random foraging task wherein location of reward was uncertain (and PPTg lesioned rats did not show quicker elements of task performance). In the test phase the quicker responding demonstrated by PPTg lesioned rats may be a feature of impulsive anticipation as the rats have been withdrawn from the maze for a delay and are now being re-challenged. This eager responding may make the PPTg lesioned rats vulnerable to unplanned responding which is then reflected in their increased number of errors in completing the task. This is not the entire explanation for increased errors made by the PPTg lesioned rats however, because the pattern of errors made by these rats reflects a more pronounced inability to shift responses to the post delay

appropriate choices and thus results in an increased number of across phase errors compared with within phase errors. A simple impulsivity hypothesis would predict equal distribution of across and within phase errors. This finding will be discussed further below.

Discussion

The results of this experiment show that PPTg lesioned rats were impaired in the acquisition of a spatial working memory radial arm maze task. Control rats were able to hold spatially relevant information in short term memory over a delay and use it to guide prospective behaviour subsequent to the delay in order successfully to complete the task and retrieve food pellets. PPTg lesioned rats were impaired in this ability, viewed both by the number of errors they made in completing the task, and the types of errors made by these rats. Though both groups made more across phase errors than within phase errors, the control rats had reached a performance level such that they were able to complete the task making one error or less (in fact the mean errors across criterion days was 0.64). When they made their one error, it was more likely to be an across phase error than a within phase error. PPTg lesioned rats at this stage however were making a mean average error over 5 times that of control rats (PPTg mean average across the days the control rats reached criterion was 3.35) and of these errors, PPTg lesioned rats made twice as many across phase errors than within phase errors. Thus, while both groups may be demonstrating a degree of response inflexibility in not shifting their arm choices from those that were baited previous to the delay, this effect was more pronounced in PPTg lesioned rats. The impairment may be seen as mnemonic. The PPTg ibotenate lesioned rats may have

been unable to remember which arms had been baited previous to the delay - that is an impairment in their ability to hold this information in short term memory during the delay time. Alternatively it may be considered an impairment in their response flexibility and guidance of planned behaviour into action. The ability to make a prospective plan of appropriate actions may be disrupted and thus response selection is subsequently impaired in rats with lesions of the PPTg. The role of the prefrontal cortex-hippocampus pathway, and subsequent circuitry has been found to be important for spatial delayed radial arm maze tasks (Floresco et al., 1997), and now this experiment also outlined the role of the PPTg in appropriate spatially mediated response selection and execution. The goal directed response choices are inappropriate following lesions of the PPTg and guiding these plans into action are in turn, subsequently disrupted. When the final outflow of spatially mediated processing is sent to choose a response and put this planned behaviour into action, the circuitry finds itself incomplete as the PPTg is lesioned and inactivated. The resulting behaviour then is unguided and inappropriate, resulting in the increased number of errors made by the PPTg lesioned rats in completing this task. Others may suggest a role for lack of attention and/or arousal in performance disruption following lesions of the PPTg as cholinergic input to the thalamus is disrupted (Dellu et al., 1991), but this would seem unlikely. Lesions of the PPTg may diminish cholinergic input to thalamus, but this removal is not total and mesopontine cholinergic input from the adjacent LDTg is not disrupted in this process. What input is lost is made up for in subsequent compensatory mechanisms as seen in the cortical EEG several days post-surgery (Inglis et al., 1995). It would seem unlikely then that the thalamus would be impaired to the degree to affect attentional

performance on this task. As well, arousal does not seem to be affected given that the lesioned rats are willing to carry out the task and consume the food pellets. Impairment in arousal would seem to result in disruption in these processes.

Finally, the other interesting result of this experiment was the finding of quicker latency times to make the first arm choice by PPTg lesioned rats, along with faster subsequent arm choice responses and faster times to complete the trials. This result was seen in the training phase as well and may indicate heightened activity when rats are faced with reward stimuli, or at least the chance to forage for such reward. It does confirm that PPTg lesioned rats are not impaired in either locomotor or motivational responding following their lesions given their ability and willingness to engage in, and complete, the task.

While this experiment examined the role of acquisition of a spatial working memory radial arm maze task, the ability to retain this task also needed to be explored. The following experiment looked at the rats ability to perform a working memory task once they had acquired the task and then a lesion of the PPTg was induced. Further details of this experiment are described below.

7.2 Experiment 2. Examination of retention of a delayed spatial win shift task following excitotoxic lesion of the pedunculopontine tegmental nucleus.

Introduction

The results of experiment 1 revealed that lesions of the PPTg result in impaired performance on acquisition of a spatial working memory radial arm maze task. This adds to the results of the previous chapter which examined the role of the PPTg in accuracy of ongoing navigational behaviour and its retention. Both these experiments found that lesions of the PPTg disrupt appropriate responding and result in the animal making more errors. Together all of these experiments suggest that loss of the PPTg disrupts outflow from the cortico-hippocampal-ventral striatal pathway. Lesions of the PPTg results in impaired response selection and execution of performance on these tasks. While retention of the random foraging task was impaired following lesion of the PPTg, retention performance on the spatial working memory has not been examined. The aim of the following experiment was to examine this: to determine if lesions of the PPTg impair performance once the rats have already acquired the task, and then are given a lesion of the PPTg. This may get at the role of reference memory in performance of this working memory task. Here the rats acquired information on performance of this task and could use it later to aid them in retention performance. This experiment examined what role the PPTg played in the retention performance on a prospectively coded spatial task. Both number and types of errors made were examined for both sham and PPTg ibotenic lesioned rats, as well as measures of locomotion and motivational performance.

Methods

Animals

12 rats were used (Charles River). Mean weight at time of surgery was 344.30 ± 39.63 (SD).

Surgery

The rats were anaesthetised with sodium pentobarbitone (Sagatal, Rhône Mérieux; 60 mg/kg) before being placed in the stereotaxic frame. 5 rats were given a lesion of the PPTg while 7 were given a phosphate buffer control lesion (see General Methods). All rats were given at least two weeks postoperative care before behavioural testing began.

Delayed Spatial Win Shift Task

This task is described in General Methods. In this experiment, as the performance on retention of the task was the focus, all rats were trained on the task before and after surgery. In the before surgery phase, rats were deprived to 85% of their free feeding weight before trials began. The rats were maintained on 17.5-20 grams of food pellets/day. Rats were trained until all rats as a group reached criterion of 1 error or less over 2 consecutive trials. The rats then underwent surgery as described above. Following postoperative care rats were food deprived to 85% of their free-feeding weight before habituation trials began. Rats were given 15-20 grams food pellets/day and when reached 85% were maintained on 17.5-20 grams food/day. Water was available *ad libitum* throughout the entire experiment. Once trials began, the rats

were given 1 session/day on the maze until the sham rats as a group reached a criterion of 1 error or less per day for 2 consecutive days.[10]

Results

All data were analysed using a repeated measures analysis of variance with group (sham vs. PPTg) as the between subjects variable and days as the within subjects variable. Analysis of the number of errors made in the test phase had an extra within subjects variable of error type (across phase vs. within phase).

Pre Surgery

Training phase analysis

The mean number of training phase errors across trials is shown in Figure 7.2.1. Analysis of errors made in the training phase revealed no significant between subjects effect of group ($F_{1,10} = 1.42$) and no significant effect of the within subjects factor of days ($F_{18,180} = 1.62$) or the two-way interaction of group x days ($F_{18,180} = 1.34$).

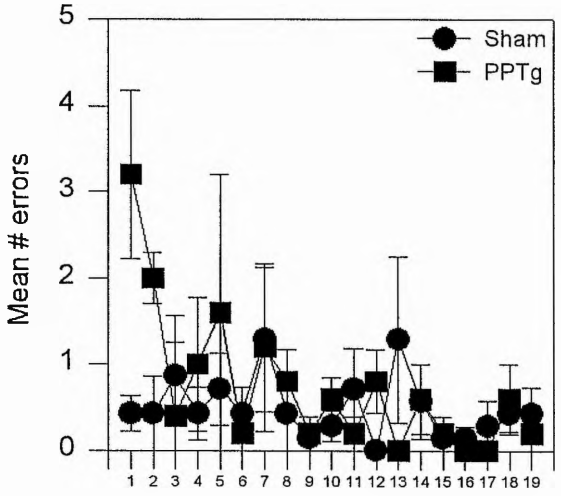
The mean latency time to make the first choice is shown in Figure 7.2.1. Analysis of the latency to make the first choice revealed no significant effect of the between subjects factor of group ($F_{1,10} = 0.02$) and no significant main effect of the within subjects factor of days ($F_{18,180} = 1.24$), or the two-way interaction of group x days ($F_{18,180} = 1.08$).

[10] Again this criterion was adopted in following the methodological procedures of Floresco et al., 1996; 1997.

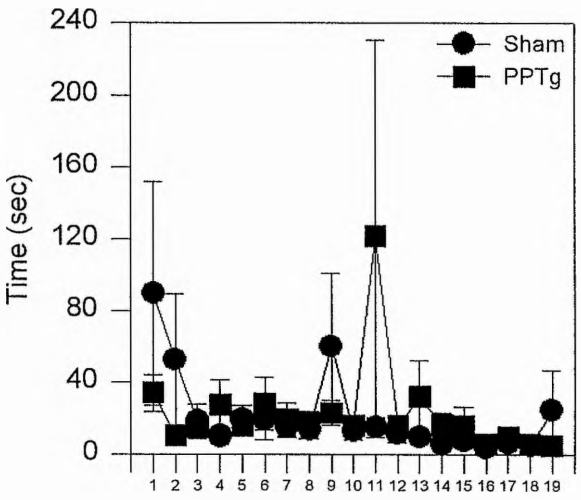
Figure 7.2.1

Pre-surgery training phase data: **Errors**: Mean number of errors across trials for sham and PPTg ibotenic lesioned animals; **Latency**: Mean latency time to make the first choice across trials for sham and PPTg ibotenic lesioned animals; **Subsequent choices**: Mean time to make subsequent arm choices across trials for sham and PPTg ibotenic lesioned animals; **Time to complete**: Mean time to complete the session across trials for sham and PPTg ibotenic lesioned animals.

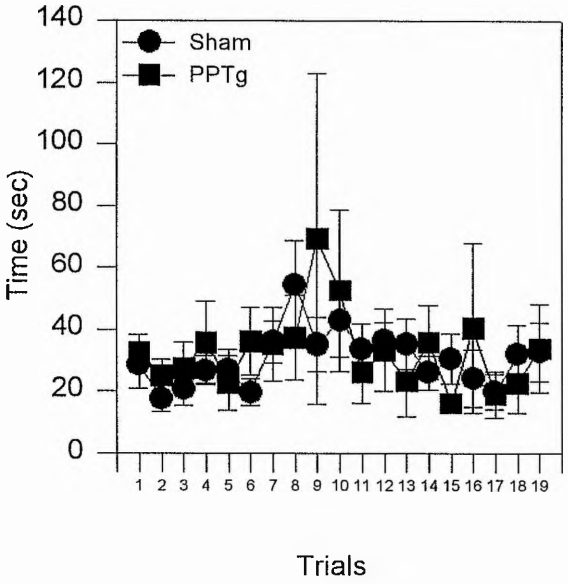
Errors



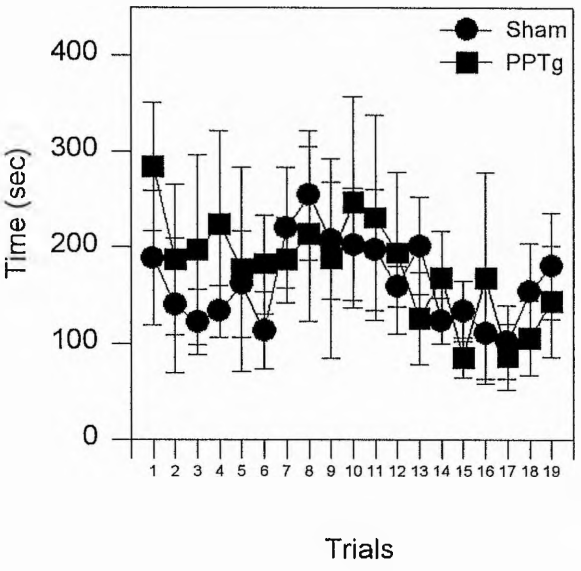
Latency



Subsequent Choices



Time to Complete



The mean time to make subsequent choices is shown in Figure 7.2.1. Analysis of the time to make subsequent choices after trial initiation revealed no significant main effect of the between subjects factor of group ($F_{1,10} = 0.05$), and no significant main effect of the within subjects factor of days ($F_{18,180} = 1.55$), or the two-way interaction of group x days ($F_{18,180} = 0.79$).

Finally, the mean time to complete the training phase is shown in Figure 7.2.1. Analysis of the time to complete the trial revealed no significant effect of the between subjects factor of group ($F_{1,10} = 0.07$), and no significant main effect of the within subjects factor of days ($F_{18,180} = 1.24$) or the two-way interaction of group x days ($F_{18,180} = 0.54$).

Test phase analysis

The number of errors made in task completion collapsed across criterion days is demonstrated in Figure 7.2.2. Analysis of errors made in the test phase revealed no significant main effect of the between subjects factor of group ($F_{1,10} = 2.10$). The within subjects analysis revealed a significant main effect of the factor of days ($F_{18,180} = 10.04$, $p < 0.001$), a significant main effect of the factor of error type ($F_{1,10} = 116.24$, $p < 0.0001$), a significant two-way interaction of group x days ($F_{18,180} = 2.49$, $p = 0.001$), and days x errors ($F_{18,180} = 5.67$, $p < 0.001$), but no significant interaction of group x error type ($F_{1,10} = 0.129$), or the three-way interaction of group x days x error type ($F_{18,180} = 1.57$). The number of errors made during daily trials is presented in Figure 7.2.3. The variability between the groups on performance across days was not systematic and seems to simply reflect random performance fluctuations. It is

evident from this figure, however, that both sham and PPTg pre-surgery groups reach the established performance criterion of making one error or less across two consecutive days before surgery was conducted.

The mean latency time to make the first choice is shown in Figure 7.2.3. Analysis of the latency to make the first choice revealed no significant main effect of the between subjects factor of group ($F_{1,10} = 1.51$) and no significant main effect of the within subjects factor of days ($F_{18,180} = 0.96$), or the two-way interaction of group x days ($F_{18,180} = 1.08$).

Mean time to make subsequent choices is shown in Figure 7.2.3. Analysis of the time to make subsequent choices after trial initiation revealed no significant main effect of the between subjects factor of group ($F_{1,10} = 0.52$), and no significant main effect of the within subjects factor of days ($F_{18,180} = 1.56$), or the two-way interaction of group x days ($F_{18,180} = 1.29$).

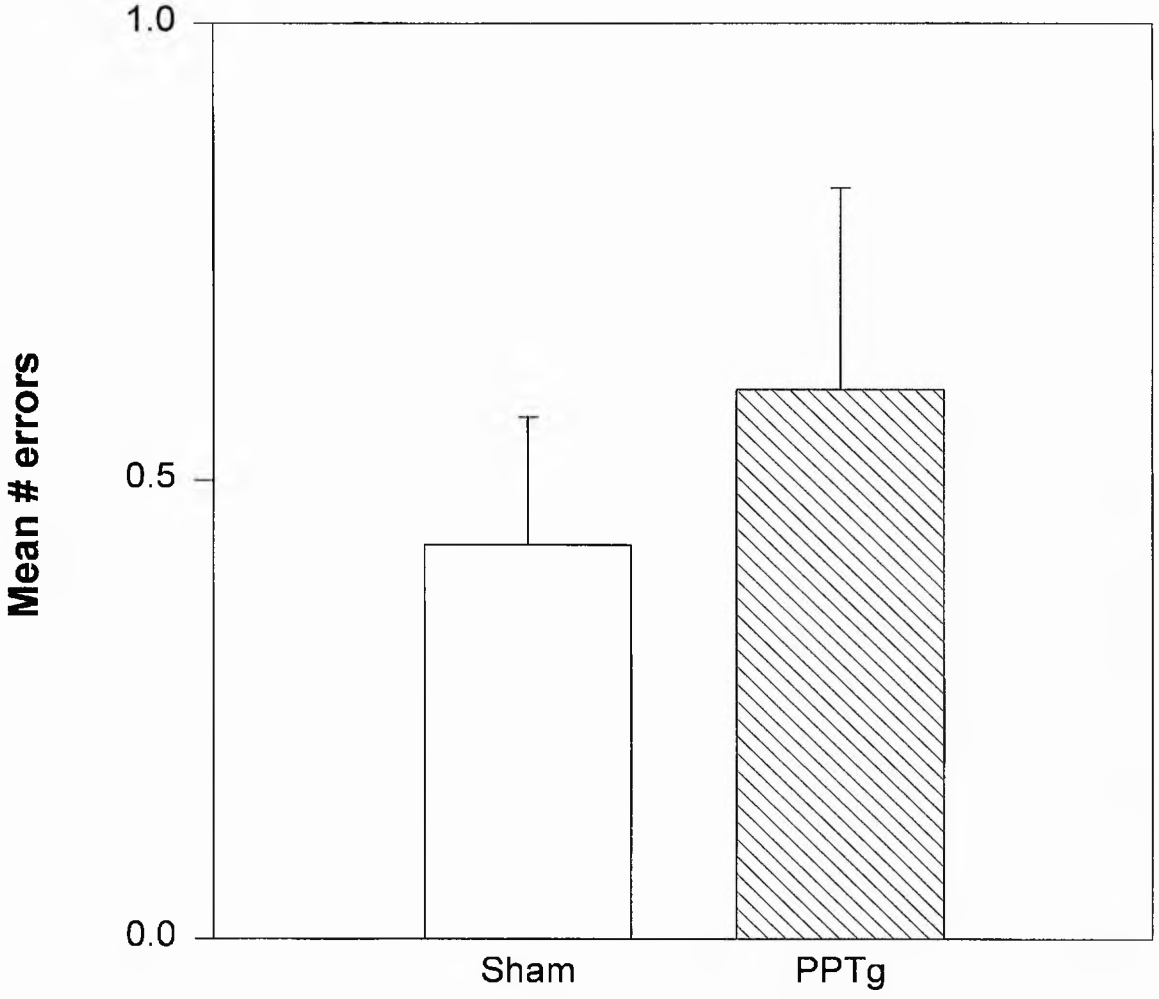
Finally, mean time to complete the test phase trial is shown in Figure 7.2.3. Analysis of the time to complete the trial revealed no significant effect of the between subjects factor of group ($F_{1,10} = 0.90$), but a significant main effect of the within subjects factor of days ($F_{18,180} = 3.06$, $p < 0.001$). This simply reflected the fact that the rats became more familiar with the task over days and subsequently became quicker in their ability to complete the task. There was no significant two-way interaction of group x days ($F_{18,180} = 0.86$).

Figure 7.2.2

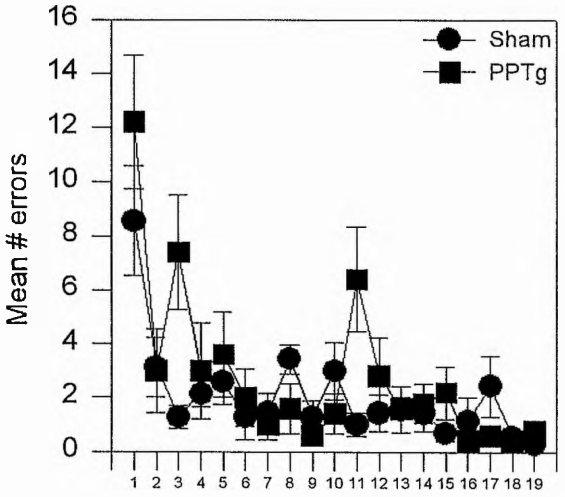
Pre-surgery test phase data: Mean number of errors across the two criterion days for pre-sham and pre-PPTg ibotenic lesioned animals.

Figure 7.2.3

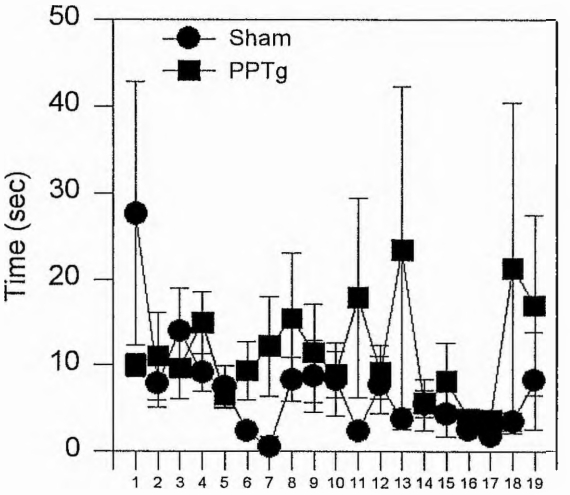
Pre surgery test phase data. Each graph represents the trials across days. **Errors**: Mean number of errors across trials for sham and PPTg ibotenic lesioned animals; **Latency**: Mean latency time to make the first choice across trials for sham and PPTg ibotenic lesioned animals; **Subsequent choices**: Mean time to make subsequent arm choices across trials for sham and PPTg ibotenic lesioned animals; **Time to complete**: Mean time to complete the session across trials for sham and PPTg ibotenic lesioned animals.


1.0
0.5
0.0
Mean # errors
Sham
PPTg

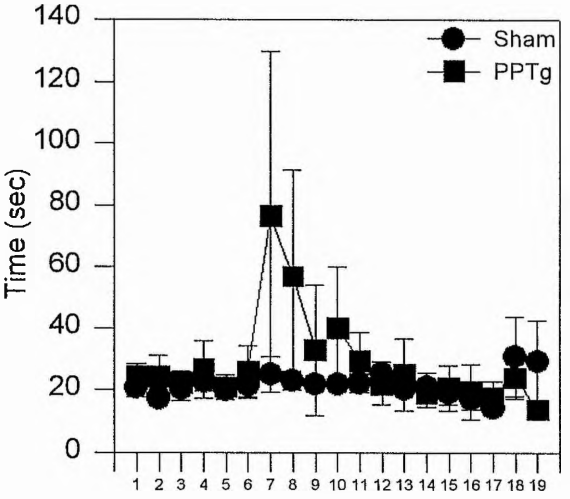
Errors



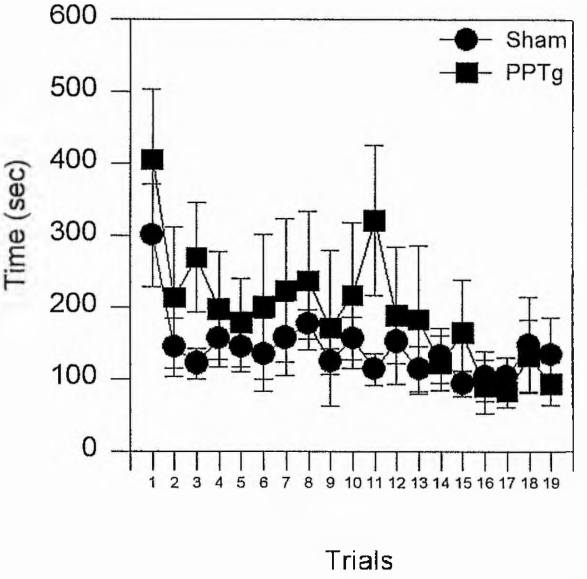
Latency



Subsequent Choices



Time to Complete



These results combined revealed that there was no pre-surgery difference in any of the rats' ability to perform the task. Rats were similar in the number of errors made and times to carry out and complete the trials. This analysis was crucial for post-surgery comparisons.

Post surgery

Figure 7.2.4 represents the largest and smallest lesion sizes for the PPTg ibotenate lesioned rats of this experiment. Damage to nearby structures was negligible and inconsistent across rats. 2 rats had damage to the deep mesencephalic nucleus, with 1 of these being very minimal, and 3 rats had damage to the adjoining retrorubral field. 1 rat showed very minimal damage to the subpeduncular tegmental nucleus. All rats had minimal damage to the superior cerebellar peduncle. The inconsistent damage to these structures did not produce a change in behaviour across the PPTg lesioned rats. This was examined through direct comparison of damage sites and behavioural outcome. No consistent relationship between damage to a structure other than the PPTg and behaviour were found.

Training phase analysis

The mean number of training phase errors across trials are shown in Figure 7.2.5. Analysis of errors made in the training phase revealed no significant between subjects effect of group ($F_{1,10} = 0.45$) but a significant effect of the within subjects factor of days ($F_{4,40} = 13.36$, $p = 0.012$). There was no significant effect of the two-way interaction of group x days ($F_{4,40} = 0.99$). This analysis revealed that the rats make less errors over days as they re-acquire this phase of the task and demonstrated

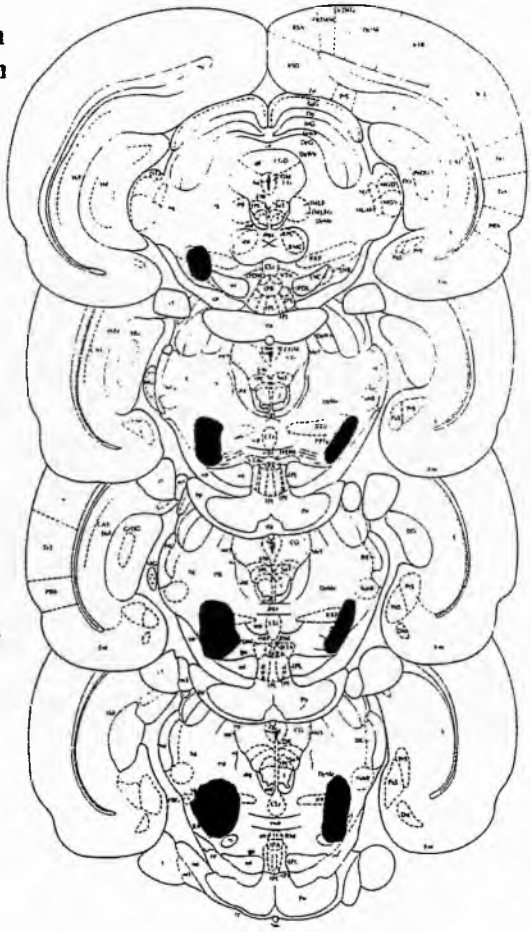
Figure 7.2.4

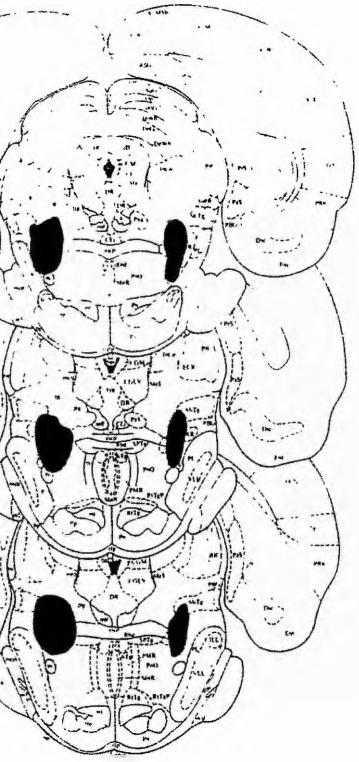
Representative sections from Bregma -6.30 to -8.72 mm redrawn from the atlas of Paxinos and Watson (1986) which illustrate the largest (left) and smallest (right) lesions for PPTg ibotenic acid lesioned rats in this experiment.

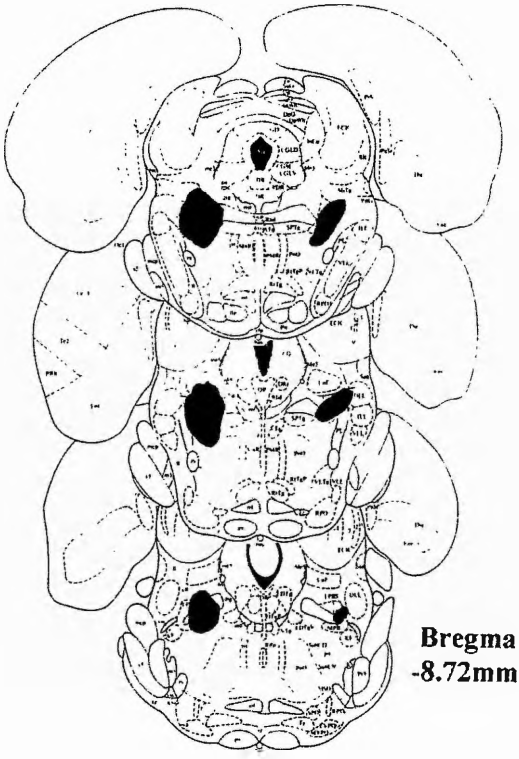
Figure 7.2.5

Post-surgery training phase data: **Errors**: Mean number of errors across trials for sham and PPTg ibotenic lesioned animals; **Latency**: Mean latency time to make the first choice across trials for sham and PPTg ibotenic lesioned animals; **Subsequent choices**: Mean time to make subsequent arm choices across trials for sham and PPTg ibotenic lesioned animals; **Time to complete**: Mean time to complete the session across trials for sham and PPTg ibotenic lesioned animals.

Bregma
-6.30mm







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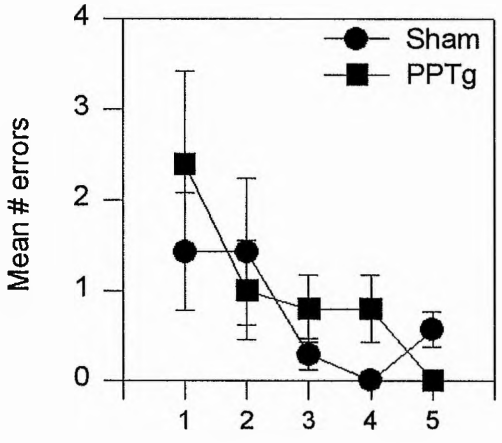
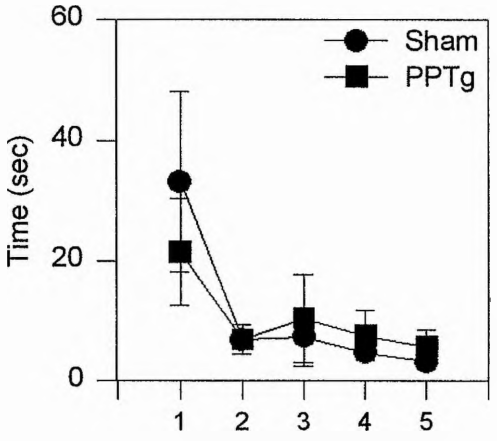
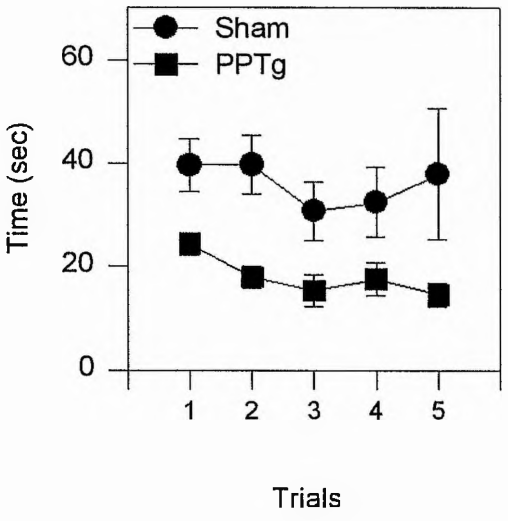
Errors



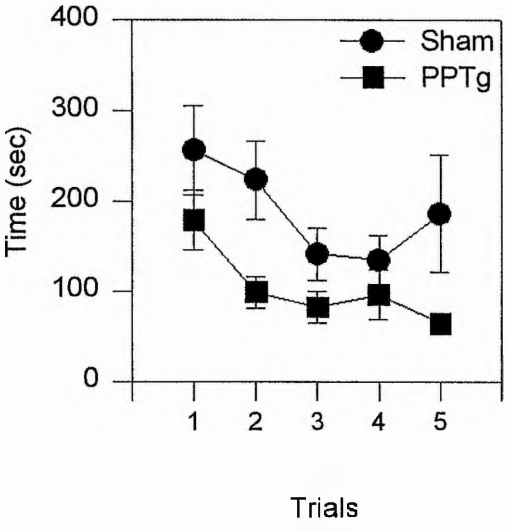
Latency



Subsequent Choices



Time to Complete



that PPTg lesioned rats are able to do simple forms of foraging, in this case retrieve four pellets when only 4 choices are available.

The mean latency time to make the first choice is shown in Figure 7.2.5. Analysis of the latency to make the first choice revealed no significant effect of the between subjects factor of group ($F_{1,10} = 0.01$) but a significant main effect of the within subjects factor of days ($F_{4,40} = 4.55$, $p = 0.004$). There was no significant two-way interaction of group x days ($F_{4,40} = 1.18$).

Mean time to make subsequent choices is shown in Figure 7.2.5. Analysis of the time to make subsequent choices after trial initiation revealed a significant main effect of the between subjects factor of group ($F_{1,10} = 5.75$, $p = 0.037$), but no significant main effect of the within subjects factor of days ($F_{4,40} = 1.18$). There was no significant two-way interaction of group x days ($F_{4,40} = 0.40$).

Finally, the mean time to complete the training phase is shown in Figure 7.2.5. Analysis of the time to complete the trial revealed no significant effect of the between subjects factor of group ($F_{1,10} = 4.74$), but a significant main effect of the within subjects factor of days ($F_{4,40} = 3.49$, $p = 0.015$). There was no significant two-way interaction of group x days ($F_{4,40} = 0.64$).

Test phase analysis

The mean number of test phase errors made are demonstrated in Figure 7.2.6. Analysis of errors made in the test phase revealed no significant main effect of the

between subjects factor of group ($F_{1,10}$ = 4.29, p = 0.065). The within subjects analysis revealed no significant main effect of the factor of days ($F_{4,40}$ = 1.81), but a significant main effect of the factor of error type ($F_{1,10}$ = 46.16, p < 0.001). There was no significant two-way interactions of group x days ($F_{4,40}$ = 1.10), group x error type ($F_{1,10}$ = 2.72), or days x error type ($F_{4,40}$ = 1.77), or the three-way interaction of group x days x error type ($F_{4,40}$ = 1.78). These results revealed that by a small amount, there was no statistical difference between the rats in their retention performance on this delayed spatial task. The graph of these results looks similar to previously presented graphs of errors made in the PPTg rats making more errors than controls, but it is believed that due to high variance and small sample size that the evidence of this result was not significant statistically. This variance is somewhat evident in the inset graph of Figure 7.2.6 which shows the mean errors across all trials for the two groups. These results will be discussed further below.

Mean time to make the first arm choice is demonstrated in Figure 7.2.7 (A). Analysis of the latency to make the first choice revealed no significant main effect of the between subjects factor of group ($F_{1,10}$ = 0.32) and no significant main effect of the within subjects factor of days ($F_{4,40}$ = 1.20), or the two-way interaction of group x days ($F_{4,40}$ = 0.44).

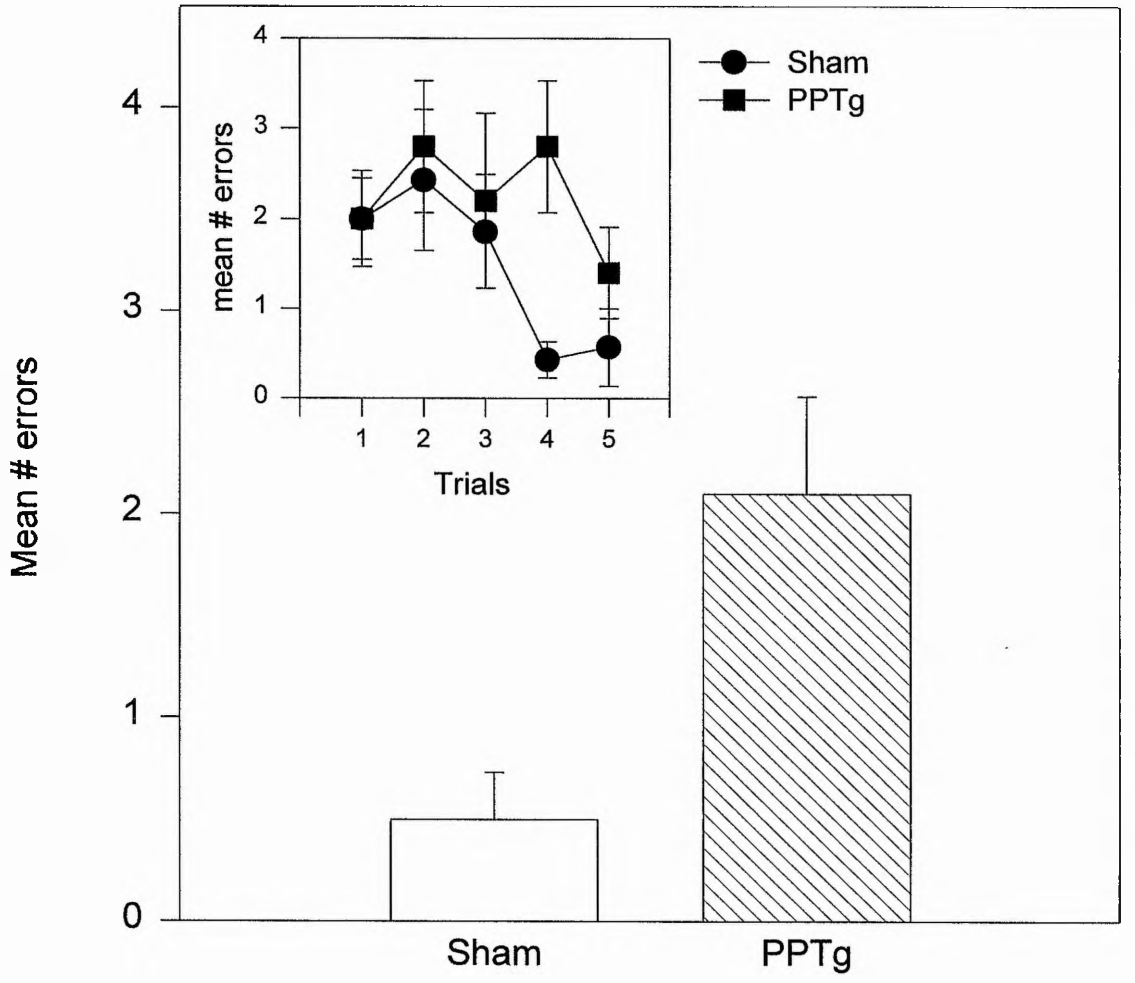
Mean time to make subsequent choices is demonstrated in Figure 7.2.7 (B). Analysis of the time to make subsequent choices after trial initiation revealed no significant main effect of the between subjects factor of group ($F_{1,10}$ = 3.73), and no

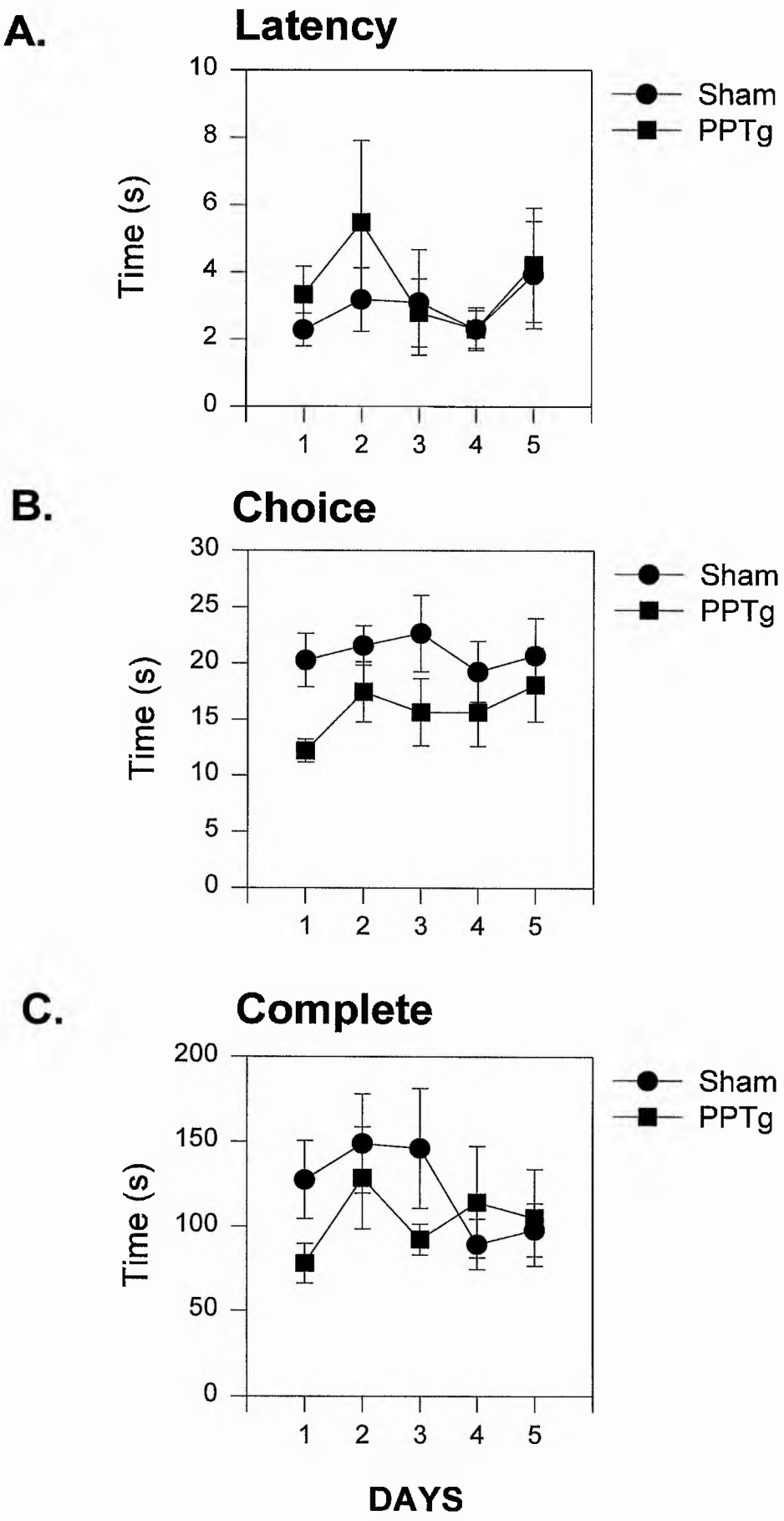
Figure 7.2.6

Mean number of errors across the 2 criterion days for sham and PPTg ibotenic lesioned animals. Inset figure represents the mean errors across all trials and is included to show the variability in responding across the trials.

Figure 7.2.7

Measures of the animals physical ability and motivation to perform the task. Each graph represents the trials across days. A. Mean time to initiate trial and make the first arm choice; B. Mean time to make subsequent arm choices; C. Time to complete individual trials.


Mean # errors
4
3
2
1
0
Sham
PPTg
mean # errors
4
3
2
1
0
1
2
3
4
5
Trials
Sham
PPTg


A.
Latency
Time (s)
10
8
6
4
2
0
1
2
3
4
5
Sham
PPTg
B.
Choice
Time (s)
30
25
20
15
10
5
0
1
2
3
4
5
Sham
PPTg
C.
Complete
Time (s)
200
150
100
50
0
1
2
3
4
5
Sham
PPTg
DAYS

significant main effect of the within subjects factor of days ($F_{4,40} = 0.73$), or the two-way interaction of group x days ($F_{4,40} = 0.48$).

Finally, mean time to complete trials is demonstrated in Figure 7.2.7 (C). Analysis of the time to complete the trial revealed no significant effect of the between subjects factor of group ($F_{1,10} = 0.69$), and no significant main effect of the within subjects factor of days ($F_{4,40} = 1.03$), or of the two-way interaction of group x days ($F_{4,40} = 1.16$).

These three measures revealed that there was no difference in the rats locomotor ability or motivation in completing this task and are represented in Figure 7.2.7.

Discussion

Statistically, these results demonstrated that lesions of the PPTg did not disrupt retention performance on a delayed spatial working memory task. The data did reveal, an only slightly statistically non-significant result ($p = 0.065$ as outlined above) as the power of this experiment was lost in a small sample size and variability in performance of the rats that did survive to perform this task. It is not certain if this is the explanation for this result and so it would be valuable to re-examine this retention performance. Replication of this experiment has begun in this laboratory, but as this procedure is quite lengthy the outcome of such work is not presently available.

It is acknowledged however, that the PPTg may not play a role in the retention of this delayed working memory task. The role of the PPTg in performance on this task may be mediated elsewhere and the PPTg serves to only influence its acquisition, as evident from the first experiment. While lesion of the PPTg disrupts retention of the random foraging task, as reported in the previous chapter, the task requirements for this working memory task are influenced more by the prefrontal cortex-hippocampal pathway and rely on the ability to retain prospective foraging skills which may not be influenced by lesions of the PPTg. Here the influence of reference memory may be differentially mediated depending on the task requirements. When the animal re-performs a non-delayed task in which it must retrospectively code information to forage efficiently, then performance is disrupted if the PPTg is lesioned. However, when the animal re-performs a task in which there is a delay, and the animal has knowledge about the location of the food from before the delay and can now prospectively plan choices to forage efficiently, then performance is not disrupted if the PPTg is lesioned. The PPTg has been found to be important for the acquisition but not retention in other tasks and this may be another case. In acquisition performance on both passive and active avoidance tasks, PPTg lesioned rats are impaired in both tasks (Fujimoto et al., 1992). In the passive avoidance task the lesioned rats do not learn that moving across to a darkened chamber later results in foot shock and after several trials enter this chamber significantly quicker than control rats who have acquired this association. In the active avoidance task, PPTg lesioned rats are impaired in their ability to pair a light-warning tone stimulus pair with upcoming foot shock and fail to jump across to a nearby chamber to avoid the shock. However, when rats have been trained on these

tasks and then receive a lesion of the PPTg, these rats are not impaired in their re-performance on either task and perform as well as unlesioned rats. Here, lesions of the PPTg effect acquisition but not retention and retrieval of these tasks. Similarly, in learning to lever press for administration of brain stimulation of the lateral hypothalamus, NMDA lesions of the PPTg blocked acquisition of this responding (Lepore and Franklin, 1996). When rats were trained to acquire lever pressing and then received a lesion of the PPTg, however, though post-lesion levels of responding were reduced compared to controls and compared to pre-lesion levels, rats still responded for brain stimulation reinforcement (Lepore and Franklin, 1996; Buscher et al., 1989). Again, the rats were impaired in acquisition of this task but demonstrated that PPTg lesions did not disrupt what had already been learned. Finally, a recent study by Olmstead and colleagues (1998) provided another example of impaired acquisition following PPTg lesions, but not task retention. In this study the authors examined the role of the PPTg in acquisition and retention of self-administration responding for intravenous heroin. Here the authors report that on both a fixed ratio schedule (FR-1) of responding and on a progressive ratio schedule of responding some PPTg lesioned rats were impaired in acquisition of this responding and responded at rates significantly lower than control rats. However, when rats were trained to respond for intravenous heroin and were then given a lesion of the PPTg, the rats re-performance of self-administration was not different to controls. Both groups of rats had similar breaking points on the progressive ratio schedule of responding. Basically both groups reached the same point at which they would no longer keep bar pressing to receive intravenous heroin. This experiment demonstrated that lesions of the PPTg effect acquisition but not retention of a

complex responding task. While these tasks are not necessarily similar to that of the delayed spatial working memory task examined here, it does outline that for certain tasks the PPTg may play a role in its acquisition, but not necessarily its retention and this process may be mediated elsewhere.

Summary

The results of this chapter demonstrated that lesions of the PPTg impair acquisition performance on a delayed spatial win shift task. Performance on this task has previously been shown to rely on the integrity of the prelimbic prefrontal cortex-hippocampal pathway (Floresco et al., 1997) and experiment 1 of this chapter revealed that acquisition of this task is impaired following lesions of the PPTg. PPTg lesioned rats make more across phase errors than within phase errors in completing this task indicating that they specifically visit and re-visit arms that were baited before the delay that subsequent to the delay are blocked. This would suggest that PPTg lesioned rats were impaired in their response choice selection and/or execution of goal related responding when choices can be prospectively planned.

The second finding of this chapter was that PPTg lesioned rats were not impaired in retention performance of a delayed spatial win shift task. This result may be an artefact resulting from low animal numbers surviving the post-training surgery and subsequent variable responding of these rats. Further experimentation to re-examine these results would seem warranted, and such re-examination is currently on-going in this laboratory. Alternatively, these results may indeed be genuine and would reflect that retention performance of this task does not require an intact PPTg and is mediated elsewhere.

Prelude to Chapter 8: The effect of basal ganglia outflow modulation on cholinergic neurons of the mesopontine tegmentum, particularly the pedunculopontine tegmental nucleus.

The experimental findings reported up to this point have outlined a role for the PPTg in the control of behavioural processes such as appropriate response selection and execution of goal directed behaviour. These processes have been associated with striatal activity. As the striatum innervates the PPTg through both direct contact and through indirect projections via the globus pallidus and substantia nigra pars reticulata (Jackson and Crossman, 1981a; Rye et al., 1987; Berendse et al., 1992; Zahm and Heimer, 1993; Rye et al., 1996), examination of the effect of this innervation on the anatomical composition of the PPTg seemed appropriate. The effects of striatal outflow on neuronal expression in the mesopontine tegmentum, particularly the PPTg, have not been examined to date and is of importance for further understanding of the role these neurons play in controlling striatal outflow and behavioural responding. Striatal dopamine depletion produces variable effects on dopamine, acetylcholine and neuropeptide expression in the basal ganglia (Schwarting and Huston, 1996), but measures of neurochemical expression in the PPTg, innervated by these areas, has not been examined. Removal of afferentation to other parts of the nervous system has resulted in an increase in NADPH-diaphorase expression (examined in detail below; see Fiallos-Estrada et al., 1993; Yu, 1994; Jia et al., 1994 and Persson et al., 1995) it was hypothesised that changes in the number of neurons expressing NADPH-diaphorase immunoreactivity may be found in the mesopontine tegmentum. Any changes in neurotransmitter expression

in the PPTg would have substantial effects on subsequent behavioural processing and responding and was thus examined.

Chapter 8: The effect of basal ganglia outflow modulation on cholinergic neurons of the mesopontine tegmentum, particularly the pedunculopontine tegmental nucleus.

8.0 Introduction

Recent work by Garcia-Rill and colleagues (1995) has implicated the mesopontine tegmentum, particularly the PPTg, in the clinical disorder of schizophrenia. Schizophrenia is marked by slow wave sleep disturbances with reductions in stage 3 and 4 of slow wave sleep and reduction in REM latency (Tandon and Greden, 1989). As well, hallucinations have been likened to 'bizarre dreaming' episodes (Mamelak and Hobson, 1989) and have been proposed to be due to REM sleep episodes occurring during the waking state in schizophrenic patients (Garcia-Rill, 1991). The PPTg has a substantial role in modulation of sleep-wake cycles and deficits in sleep could seem to implicate the PPTg in the pathogenesis of schizophrenic signs and symptoms. Deficits in sensory gating are another hallmark of schizophrenia (Reese et al., 1995). Habituation tasks using stimulus pairs find normal subjects inhibiting their responding to the second stimulus whereas schizophrenic subjects do not inhibit such responding. For example, looking at the P1 potential, Freedman and colleagues (1983) found that schizophrenic subjects showed decreased habituation to the second click of a two click stimulus pair compared to control subjects. The P1 potential is an auditory middle latency evoked response which shows marked habituation to presentation of stimuli, and is thought to be generated by PPTg neurons projecting to the thalamus (Garcia-Rill et al.,

1995). Such findings again suggest a role of disturbance of the PPTg in schizophrenia. Finally, sensory gating deficits are accompanied by motor and postural anomalies as well as impaired smooth pursuit eye movements (Reese et al., 1995). In the mesopontine tegmentum, both the PPTg and LDTg have substantial connections with motor regions within the medulla and spinal cord (Grofova and Keane, 1991), as well as the superior colliculus (Beninato and Spencer, 1986; Hallanger and Wainer, 1988) and it could be suggested that disturbances of the PPTg and/or LDTg may be involved in marked expressions of schizophrenia.

Garcia-Rill and colleagues (1995) examined the post-mortem brains of 9 schizophrenic patients, 4 psychiatric controls and 5 normal controls and assessed the neuronal content of the mesopontine tegmentum. Their focus was on cell counts of nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase positive neurons of the PPTg, and LDTg and counts of TOH-positive LC neurons that served as a control structure due to its proximity to the PPTg and LDTg in the brainstem. No differences were found between the psychiatric controls and normal controls and thus these were pooled to form one control group. Comparison of cell counts in these areas found no statistical difference in either the LDTg or the LC neuronal number in schizophrenic brains versus controls, but a difference was found between these two groups for cell counts of the PPTg. The mean cell number for the PPTg of schizophrenic subjects was 15, 975 ± 5788, compared to 9781 ± 2961 for the control subjects, representing a 64% increase in PPTg cell number in schizophrenic brains. Lack of change in cell number in the locus coeruleus is suggested to indicate a specific effect on cell number in posterior midbrain cholinergic neurons and not in

noradrenergic neurons. Conclusions drawn from this study include the suggestion that an increase in cholinergic neuronal number in the PPTg could cause a hyperactivity in this area of the system, potentially accounting for some of the signs and symptoms of schizophrenia.

While these data are interesting, there are several elements of concern. The first is the degree of heterogeneity of the cell counts in the schizophrenics' PPTgs. One subject had a count as low as 8494 while another had a count as high as 24, 576, an almost three-fold difference. This degree of difference is reflected in the higher standard deviation of cell counts in the schizophrenic group compared to the control, and is a cause for question. These data are also difficult to reconcile with the report of a marked decrease in ChAT in the mesopontine tegmentum (Karson et al., 1996) combined with the fact that independent confirmation of this increased neuronal number within the PPTg and LDTg using ChAT has not occurred (Rye, 1997). Finally, the effects of a lifetime of antipsychotic drug exposure in these patients serves as a potential confound. The patients used in this study were medicated for an undisclosed amount of time with various antipsychotics, including haloperidol, which are known to act on neurotransmitter systems of the basal ganglia (Ashby and Wang, 1996) which provide input to the mesopontine tegmentum (Jackson and Crossman, 1981a; Rye et al., 1987; Berendse et al., 1992; Zahm and Heimer, 1993). The effects these antipsychotics may produce on these neuronal populations independent of schizophrenia is not known and would need to be clarified before further speculation of these tentative results are brought forth.

The other feature of this work that needs to be explored is whether these data reflect an increase in cell number or a change in neurotransmitter expression, thus leading to the finding of increased number of neurons with neurotransmitter expression. Again the role of the antipsychotic medication in the findings of Garcia-Rill and colleagues (1995) may come into play. These medications provide dopaminergic blockade of the striatum. This will affect striatal output to downstream sites, including the mesopontine tegmentum. Removal of afferent input to other parts of the nervous system has been shown to produce changes on neurotransmitter expression. Persson and colleagues (1995) examined the effects of sciatic nerve transection on expression of cytochemical markers in lumbar dorsal root ganglion cells projecting to the nucleus gracilis. They found nitric oxide synthase-like immunoreactivity in 2% of Flurogold labelled cells in normal rats and in 10% of cells after injury- a 500% increase. Fiallos-Estrada and colleagues (1993) also found increases in neurons expressing NADPH-diaphorase in lumbar 4 and lumbar 5 dorsal root ganglion cells following left sciatic nerve transection in the rat. At normal levels 2.7% of L4/L5 cells were labelled for NADPH, but after 3 days post-injury labelling increased, reaching a maximum of 26.8% of cells at 10 days. This increased expression was found to persist up to 50 days. After 150 days post-injury, 8.7% of L4/L5 neurons were still labelled for NADPH. Carrying out rat pelvic nerve transections, Ando and colleagues (1996) found increases in NADPH diaphorase staining up to 2.2 times that of controls in the lumbrosacral intermediolateral neurons 1 week post injury. These increases were found to return to control levels after 5 weeks post injury and even decreased significantly below controls 10-11 weeks after nerve transection.

In examining NADPH expression changes in cranial visceral components after vagus nerve injury, Jia and colleagues (1994) found NADPH markedly enhanced in the dorsal motor nucleus of the vagus nerve, the ambiguus nucleus, the solitary tract and the nucleus of the tract solitarus 8 days post injury. Yu (1994) found similar increases in NADPH expression following the transection of cranial nerves peripherally. Normally neurons in the hypoglossal nucleus and motor nucleus of the facial nerve are devoid of NADPH-diaphorase positive staining. The same is true in the dorsal motor nucleus of the vagus, except a few in the rostral and caudal poles that stain positively for NADPH. However, 2 weeks after transection of the hypoglossal, vagus or facial nerves about 30-50% of the neuron population on the lesioned side in each of the cranial nuclei studied, were NADPH-diaphorase positive. Upregulation of nitric oxide synthase (NOS), as expressed in increases in the number of NADPH-diaphorase positive staining neurons, seems to be a response to the effects of the removed afferentation. The degree to which this is true to all systems, particularly in regards to afferentation to the mesopontine tegmentum, however, has not been explored.

The object of this first experiment was to examine the role of the antipsychotics haloperidol and clozapine on NADPH-diaphorase neurons of the mesopontine tegmentum. Particular attention was paid to the neurons of the PPTg, as it was here that increases were reported by Garcia-Rill and colleagues (1995). The intake of the antipsychotics was through oral administration to keep in line with the administrative route of these medications in schizophrenic patients. The remaining experiments of this chapter examined the effect of direct surgical removal of

dopaminergic input to the mesopontine tegmentum on NADPH-diaphorase expressing neurons there.

8.1 Experiment 1: Effects of the antipsychotics clozapine and haloperidol on NADPH-diaphorase expression in the mesopontine tegmentum.

Introduction

To examine the role of antipsychotics on NADPH-diaphorase expression in the mesopontine tegmentum, the typical antipsychotic, haloperidol, and the atypical antipsychotic, clozapine, were chosen for administration.

Haloperidol is one of the most commonly used antipsychotics being a strong antagonist at striatal dopamine D_2 and D_4 receptors (Baptista et al., 1993; Deutch et al., 1995). Administration of haloperidol in rats for a 6 week period has been shown to cause an increase in striatal and pallidal D_2-like receptors by 63% and 95%, respectively (Reynolds, 1996). As well, haloperidol has varying effects on cholinergic activity in several brain regions. Short term haloperidol treatment is associated with increases in ChAT and AChE, while chronic haloperidol treatment resulted in significantly decreased ChAT activity in the striatum and hippocampus (Korenovsky et al., 1990). Neuroleptics such as haloperidol influence central cholinergic activity either directly through their anticholinergic action, or indirectly by altering dopaminergic-cholinergic balance. An unfortunate result from haloperidol treatment, however, is that it produces more extrapyramidal side effects than any other neuroleptic, including symptoms such as tremor, peculiar gait and writhing movements (Snyder et al., 1974). It is thought that the incidence of extrapyramidal effects could be the anticholinergic action of certain neuroleptics known as phenothiazines (including haloperidol). These neuroleptics bear

resemblance to drugs such as atropine which block muscarinic cholinergic synapses (Snyder et al., 1974). It seems that the affinity of neuroleptics for muscarinic receptors correlates inversely with their tendency to elicit extrapyramidal side effects (Snyder et al., 1974).

Clozapine, a dibenzodiazepine derivative, is regarded as an atypical antipsychotic because of its chemical effectiveness in the treatment of most negative symptoms of schizophrenia, and through its therapeutic actions in 30-50 % of patients who do not respond to other neuroleptics (Fitton and Heel, 1990; Deutch et al., 1995; Kane, 1996). Clozapine has been found to manifest the lowest incidence of extrapyramidal effects of any antipsychotic drug, while it has the highest relative affinity for muscarinic cholinergic receptors compared with other antischizophrenic drugs (Snyder et al., 1974; Kane, 1996). Fitton and Heel (1990) suggest that clozapine may act preferentially on mesolimbic and amygdaloid pathways rather than neostriatal pathways, and that it is this site specificity that may underlie the dissociation between clozapine's marked antipsychotic activity and its relative absence of extrapyramidal side effects. Potential support for the site specific action of clozapine comes from examination of *c-fos* expression in the forebrain following neuroleptic injections (Robertson and Fibiger, 1992; Kinon and Lieberman, 1996). C-*fos* encodes the protein fos whose immunoreactivity has been suggested to reflect increased activity of a neuron or neuronal population. Following injections of clozapine, *c-fos* expression significantly increased in limbic structures such as the nucleus accumbens and lateral septum (Robertson and Fibiger, 1992), as well as the medial prefrontal cortex and mediodorsal thalamus (Kinon and Lieberman, 1996),

while in the striatum the only finding was a small increase in expression following the highest dose of clozapine (Robertson and Fibiger, 1992). This is in contrast to haloperidol administration after which increases in *c-fos* expression have been seen in both the striatum and nucleus accumbens (Kinon and Lieberman, 1996). It is suggested that the limited elevation of *c-fos* expression in the striatum may provide a neuroanatomical source for clozapine's low incidence of extrapyramidal effects (Robertson and Fibiger, 1992).

Clozapine is also considered atypical because of its pharmacological properties in relation to classic antipsychotics. It provides a more potent blockade of central dopamine D_1 and D_4, muscarinic cholinergic receptors M_1-M_5, serotonergic 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, 5-HT_6, 5-HT_7, histamine H_1 and H_3, and α_1 and α_2- adrenergic receptors (Fitton and Heel, 1990; Baptista et al., 1993; Kane, 1996; Ashby and Wang, 1996). Long term administration of clozapine enhances striatal dopamine D_1 receptor function in rats and results in down regulation of serotonin 5-HT_2 receptors, suggesting that an interaction between these two neurotransmitter systems may be a significant component of the drug's antipsychotic action (Fitton and Heel, 1990). Clozapine provides a very high affinity for the D_4 receptor, 10-15 fold higher than its affinity for D_2. At therapeutic concentrations, clozapine could occupy 75-90% of the D_4 receptors (Ashby and Wang, 1996; Sanyal and van Tol, 1997).

The action of haloperidol and clozapine on the D_1/D_2 and D_4 receptors expressed on the striatal and pallidal pathways, respectively (Rye, 1997) and the potential influence of this blockade on subsequent innervation sites, including the

mesopontine tegmentum (Jackson and Crossman, 1981a; Rye et al., 1987; Berendse et al., 1992; Zahm and Heimer, 1993), was the focus of this experiment. The effect of antipsychotic administration on cholinergic neurons of the mesopontine has not been previously examined. Rats received either haloperidol or clozapine in their drinking supply and subsequent analysis was conducted on the NADPH-diaphorase content of their mesopontine tegmental area.

Methods

Animals

29 rats (Charles River) were used; mean body weight at the beginning of drug administration was 361.07 ± 24.34 (SD). The rats were divided into 3 groups: 10 received clozapine in their drinking water, 10 received haloperidol in their drinking water and 9 controls were maintained on tap water.

Drug Administration

For 39 days, rats were administered either clozapine (18-22 mg/kg/day) or haloperidol (1.5-2.0 mg/kg/day) in their drinking water, which was available continuously. All water bottles were light proof to prevent drug oxidation.

Clozapine (generously donated by Sandoz, Basel, Switzerland) was dissolved in 0.8% acetic acid in distilled water and the pH of the solution was adjusted to 5.5-6.1 with 1 M NaOH (Invernizzi et al., 1995). Haloperidol (Sigma) was dissolved in 0.8% acetic acid in distilled water and the pH of the solution was adjusted to 5.0-5.2 with 1 M NaOH. In both cases fresh solutions were prepared every week.

Histology

Rats were sacrificed 40-41 days after the experiment began; sacrifices from each group were evenly distributed over this period. Tissue sections were stained for NADPH-diaphorase (see General Methods).

Cell Counting

NADPH-diaphorase neurons were viewed under high magnification on a Leitz microscope. The NADPH-diaphorase histochemical procedure yields a dark blue-purple reaction product which helps visualise the cell body of the neuron. Only neurons with NADPH-diaphorase immunoreactive nuclei were included in counts. Individual neurons were counted in both hemispheres in each section. Tissue sections were 50 μm thick, taken 200 μm apart. The mean average diameter of neurons through this area have been found to be approximately 18.9-20.2 μm (caudal-rostral, respectively; Rye et al., 1987) and thus the distance between sections was sufficient as to warrant *not* using a correction factor for cell counts- that is, a correction to guard against double counting of split neurons (Abercrombie correction for instance). A minimum of 9 sections were used from each brain for cell counting. In each case counts were made in accordance with the anatomical guidelines for each structure from Paxinos and Watson (1986).

Results

Figure 8.1.1 is a high magnification, computerised image of NADPH-diaphorase positive neurons of the LDTg, SPTg, and the PPTg. The dark NADPH-diaphorase reaction product which helps visualise individual neurons is evident.

Figure 8.1.2 demonstrates the mean total number of NADPH-diaphorase positive neurons counted in the LDTg, SPTg and the PPTg for control, clozapine and haloperidol treated rats. Cell counts were analysed using a mixed between-within repeated measures ANOVA. The type of treatment (clozapine vs. haloperidol vs. tap water) was the between subjects variable while the location of cell count (LDTg, SPTg and PPTg) and side of cell counting (left vs. right) were the within subjects variables. This analysis of variance revealed no main effect of treatment ($F_{2,26}$ = 2.58), or side of cell counting ($F_{1,26}$ = 0.97), but a main effect of location of cell count ($F_{2,52}$ = 1553.79, $p < 0.0001$). This effect was not surprising given the difference in size of these structures and subsequent neuronal content. Post-hoc analysis confirmed this and shows that counts of the LDTg were larger than the PPTg which was larger than the SPTg (Tukey-HSD post-hoc test, $p = 0.05$). There were no significant two-way interaction of treatment x area ($F_{4,52}$ = 1.04), or location x side ($F_{2,52}$ = 1.26), or the three-way interaction of treatment x location x side ($F_{4,52}$ = 0.41), but there was a significant two-way interaction of treatment x side ($F_{2,26}$ = 9.94, p = 0.001). Post-hoc analysis revealed that this effect was a result of clozapine treated rats having higher cell counts in the left hemisphere than counts from any other rats (counts from clozapine treated rats in the right hemisphere, or haloperidol or control treated rats in the left or right hemisphere; Tukey-HSD post-hoc test, $p = 0.05$). As clozapine was administered via the rats drinking supply and thus the effect not being concentrated to one hemisphere, it is uncertain why this arose and may be considered anomalous.

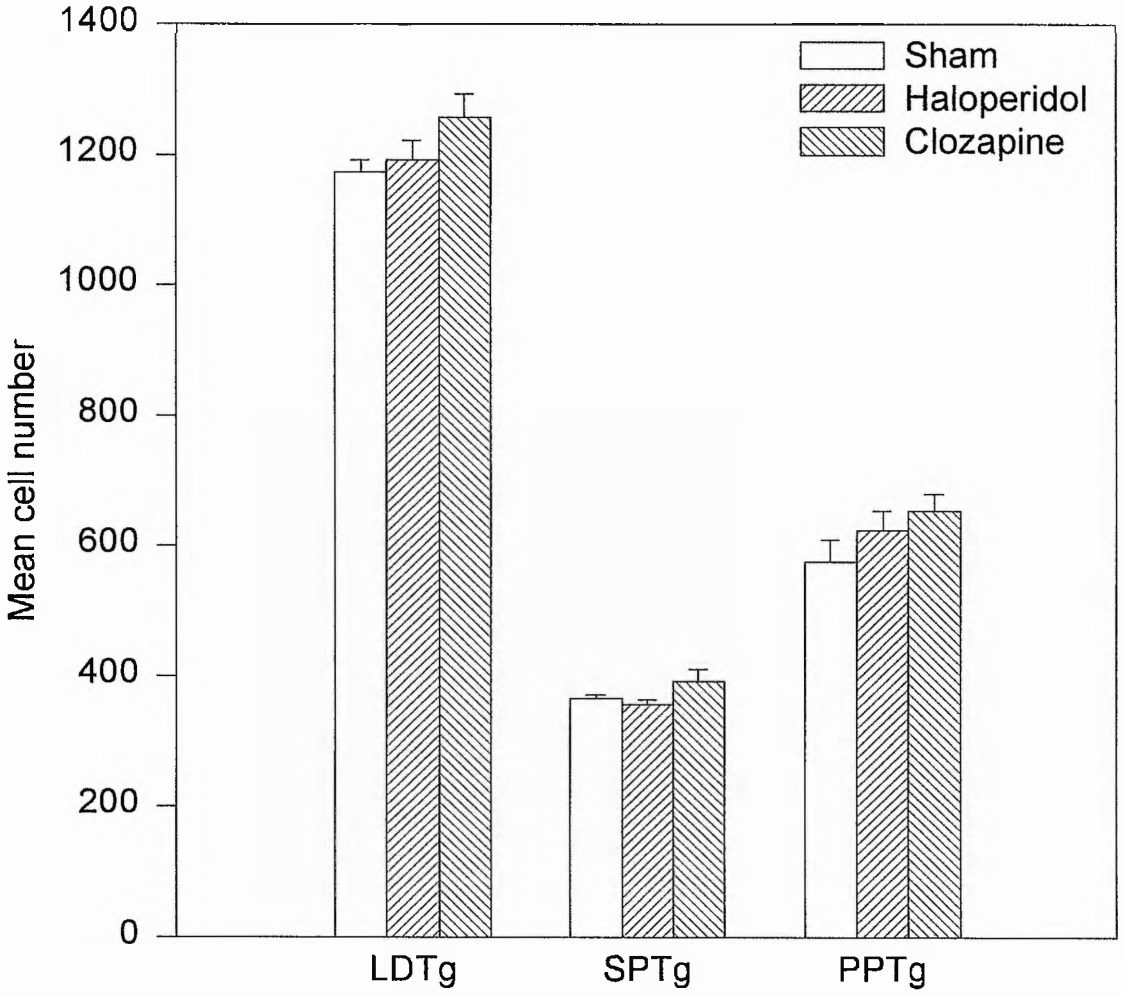
Figure 8.1.1

A magnified computerised image of NADPH-diaphorase positive neurons of the LDTg, SPTg, and the PPTg. The dark NADPH-diaphorase reaction product which helps visualise individual neurons is evident.

Figure 8.1.2

Mean NADPH-diaphorase positive cell counts for the LDTg, SPTg and PPTg in control, clozapine or haloperidol treated animals.


LDTg
SPTg


1400
1200
1000
800
600
400
200
0
Mean cell number
Sham
Haloperidol
Clozapine
LDTg
SPTg
PPTg

Discussion

Though statistical analysis revealed no main effect of haloperidol or clozapine on NADPH-diaphorase expression in the mesopontine tegmentum, individual rats' cell counts demonstrated that 5 of the 10 clozapine maintained rats had cell counts higher than those of control rats (see Table 8.1.1). While this was not statistically significant, the extent of its relevance is not to be discounted. What may be crucial for further experimentation is to examine the effects of these antipsychotics on NADPH-diaphorase expression over longer term administration. The present study kept rats maintained on clozapine (20 mg/kg/day) or haloperidol (1.5-2.0 mg/kg/day) for just under 6 weeks. This time frame was a product of a fixed supply of clozapine which was generously donated from Sandoz, Switzerland and is meagre compared to the 30+ years of probable maintenance in the schizophrenic patients.[11] A longer time frame, equivalent to the human years of medication would be needed to obtain a more realistic effect of these antipsychotics on the mesopontine tegmentum. After only 39 days of administration, the number of NADPH-diaphorase neurons was increased in half of the clozapine treated rats to numbers above the highest cell count obtained in control rats, and so it may only be a matter of longer administration for further increases to become evident.

This study sought to examine the effect of pharmacological blockade of dopamine receptors expressed on the striatal and pallidal output pathways (particularly D_1/D_2 and D_4, respectively) on NADPH-diaphorase expression of a

[11] The duration of illness reported for schizophrenic patients in the Garcia-Rill et al., 1995 study was 33.7 ± 7.2 years of which and undisclosed amount of time was treated with antipsychotic medication.

Table 8.1.1: Mean NADPH-diaphorase positive cell counts for control, clozapine and haloperidol treated animals. Values reflect total cell counts from the LDTg, SPTg and PPTg combined. * indicates cell counts from clozapine treated animals that are higher than those of controls

Control	Haloperidol	Clozapine
1889	1760	1959
1952	2010	2037
1953	2041	2122
2119	2140	2247
2173	2181	2282
2174	2244	2417*
2227	2272	2424*
2256	2279	2498*
2294	2332	2573*
	2462	2555*

downstream innervated structure, namely the PPTg. Its intention was to examine a potential confound of the finding of an increase in NADPH-diaphorase expression in the PPTg of post-mortem schizophrenic brains from patients who had been maintained on antipsychotic medications for an undisclosed number of years. While the results of this experiment are interesting, and provide an avenue for future research, the role of dopamine outflow on NADPH-diaphorase expression in the mesopontine tegmentum requires further experimentation. A more direct approach of afferent input removal may prove useful for such examination. The following experiments employed excitotoxic lesions to examine the effects of the removal of dopamine afferentation on NADPH-diaphorase expression of the mesopontine tegmentum.

8.2 Experiment 2: Effects of 6-OHDA lesions of either the caudate putamen or nucleus accumbens on NADPH-diaphorase expression of the mesopontine tegmentum.

Introduction

The innervation by basal ganglia structures to the mesopontine tegmentum, particularly the PPTg, occurs through either direct contact from the striatum or through indirect projections from the striatum via the globus pallidus and substantia nigra pars reticulata (Jackson and Crossman, 1981a; Rye et al., 1987; Berendse et al., 1992; Zahm and Heimer, 1993; Rye et al., 1996). Dopaminergic denervation of the striatum and related basal ganglia structures results in a series of behavioural and physiological effects (see Dunnett and Robbins, 1992 and Schwarting and Huston,1996, respectively, for reviews). Briefly, behavioural consequences include a decline in spontaneous locomotor activity (Creese and Iversen, 1976), rotation following drug challenge (Dunnett and Robbins, 1992), disruption of escape performance in water maze tasks (Whishaw and Dunnett, 1985) and disruption of random foraging in radial arm maze tasks (Floresco et al., 1997). Unilateral nigrostriatal lesions impair reaching and manipulative tasks using the contralateral hand in marmosets and disrupts task initiation in reaction time tasks in rats (Dunnett and Robbins, 1992), while dopamine depletion in the ventral striatum blocks amphetamine's capacity to act as a self-administered reinforcer (Dunnett and Robbins, 1992) and to act as an unconditioned stimulus in place preference conditioning (Spryaki et al., 1982).

The physiological consequences of dopamine denervation, typically using the catecholaminergic neurotoxin 6-OHDA, are extensive and varied. Extracellular dopamine and dopamine metabolites are drastically reduced or virtually non-detectable following neostriatal denervation, thus reducing dopaminergic stimulation of post-synaptic receptor elements (Schwarting and Huston, 1996). Receptor binding following 6-OHDA lesions of the neostriatum produces variable results, with D_2 receptor binding increased, but D_1 receptor binding producing inconsistent results (Schwarting and Huston,1996). This increase in D_2 receptor binding is usually due to an increase in receptor number, not receptor affinity. Neuropeptides show variable responses to such lesions with reduced immunoreactivity for substance P, dynorphin and cholecystokinin (CCK) but increases for enkephalin, neurotensin and somatostatin (Schwarting and Huston, 1996). Cholinergic activity is also affected following 6-OHDA lesions of the striatum. Nicotinic receptor binding was found to be decreased in the neostriatum, nucleus accumbens and olfactory tubercle following neostriatal lesions, whereas either decreases or no changes in muscarinic receptor binding have been found (Schwarting and Huston, 1996). Extracellular acetylcholine was found to be increased in the denervated neostriatum by 50%, but this was found only when the 6-OHDA lesion was accompanied by administration of desipramine to protect noradrenergic neurons and was not found when 6-OHDA was given alone (Schwarting and Huston, 1996). Dopamine has an inhibitory effect on acetylcholine. Tyrosine hydroxylase and choline acetyltransferase immunoreactive terminals are found in close apposition in the striatum, both found predominantly on dendritic shafts and spines (Stoof et al., 1992). This acetylcholine increase following removal of the dopaminergic inhibition is therefore not surprising. Finally, metabolic activity

as measured by radiolabelled 2-DG uptake has provided inconsistent results for several basal ganglia related areas, including the neostriatum, nucleus accumbens, olfactory tubercle and substantia nigra, but consistent increases in glucose consumption were found following striatal 6-OHDA lesions when the lateral habenula and globus pallidus were analysed (Schwarting and Huston,1996). This increase may reflect altered neuronal activity of striatal efferents that innervate these structures. They, in turn, may then play a role in post 6-OHDA regulation of neuronal activity of sites they innervate, including the PPTg. In fact, the role of basal ganglia dopamine on neuronal activity in related structures was measured in a MPTP induced model of hemi-parkinsonism (Mitchell et al., 1989). Following application of MPTP either through systemic administration in some monkeys or through carotid artery administration in other monkeys, 2-DG uptake was measured and increases were found in all monkeys in several structures including the lateral habenula and globus pallidus, and the single largest increase was seen in the PPTg.

The effect of direct depletion of striatal dopamine on NADPH-diaphorase expression of the mesopontine tegmentum, particularly the PPTg, has not been previously examined and was the focus of study of this chapter. Striatal dopamine depletion produces variable effects on dopamine, acetylcholine and neuropeptide expression in the basal ganglia, but measures of expression in structures innervated by these areas has not been examined. Here the number of cholinergic neurons, as measured by NADPH-diaphorase, were assessed following 6-OHDA lesions of the striatum or medial forebrain bundle. As removal of afferentation to other parts of the nervous system has resulted in an increase in NADPH-diaphorase expression

(outlined above; see Fiallos-Estrada et al., 1993; Yu, 1994; Jia et al., 1994 and Persson et al., 1995) it was hypothesised that changes in the number of neurons expressing NADPH-diaphorase immunoreactivity may be found in the mesopontine tegmentum.

Methods

Animals

21 rats (bred in house) were used. Mean body weight at time of surgery was 351.7 ± 12.33 (SD).

Surgery

Rats were divided into 3 groups: caudate-putamen 6-OHDA (n=8), nucleus accumbens 6-OHDA (n=9) or phosphate buffer control (n=4). 15-30 min prior to surgery rats were pre-treated with 15 mg/kg of the monoamine oxidase (MAO) inhibitor, pargyline hydrochloride (Sigma Chemicals). Rats were anaesthetised with sodium pentobarbitone (Sagatal, Rhône Mérieux; 60 mg/kg) and placed in a stereotaxic frame.

Histology

Rats were sacrificed 23-34 days after surgery; sacrifices from each group were evenly distributed over this period. Tissue sections were stained for NADPH-diaphorase and for TOH (see General Methods).

Cell Counting

NADPH-diaphorase neurons were viewed under high magnification on a Leitz microscope, and counts were taken as outlined above.

Results

Figure 8.2.1 demonstrates the mean total number of NADPH-diaphorase positive neurons for the LDTg, SPTg and the PPTg for sham, caudate-putamen and nucleus accumbens lesioned rats. Cell counts were analysed using a mixed between-within repeated measures analysis of variance. The lesion type (sham vs. caudate-putamen vs. nucleus accumbens) was the between subjects variable and location of cell count (LDTg, SPTg and PPTg) and side of cell counting (ipsilateral to lesion vs. contralateral to lesion) were the within subjects variables. This analysis of variance revealed no main effect of lesion type ($F_{2,18} = 0.55$) or side of counting ($F_{1,18} < 0.001$), but a main effect of location of cell counting ($F_{2,36} = 332.5$, $p < 0.001$). Again this result was not surprising considering the difference in size between these three structures and post-hoc analysis revealed that the number of cells counted in the LDTg was greater than the PPTg which was greater than the SPTg (Tukey-HSD post-hoc test, $p = 0.05$). There was no significant two-way interaction of lesion type x area ($F_{4,36} = 0.42$), lesion x side ($F_{2,18} = 0.20$), or location x side ($F_{2,36} = 0.47$) or the three way interaction of lesion x location x side ($F_{4,36} = 0.25$).

Histological examination of the lesion sites revealed that the 6-OHDA lesions were incomplete as portions of the caudate-putamen and nucleus accumbens were still intact. Figure 8.2.2 is a computerised image of a representative section from an animal that had a left caudate-putamen lesion and Figure 8.2.3 is a computerised image of a representative section from an animal that had a right nucleus accumbens lesion. As can be seen by the brown staining pattern (TOH immunoreactive product) the lesion did not eliminate dopamine completely from these structures.

Figure 8.2.1

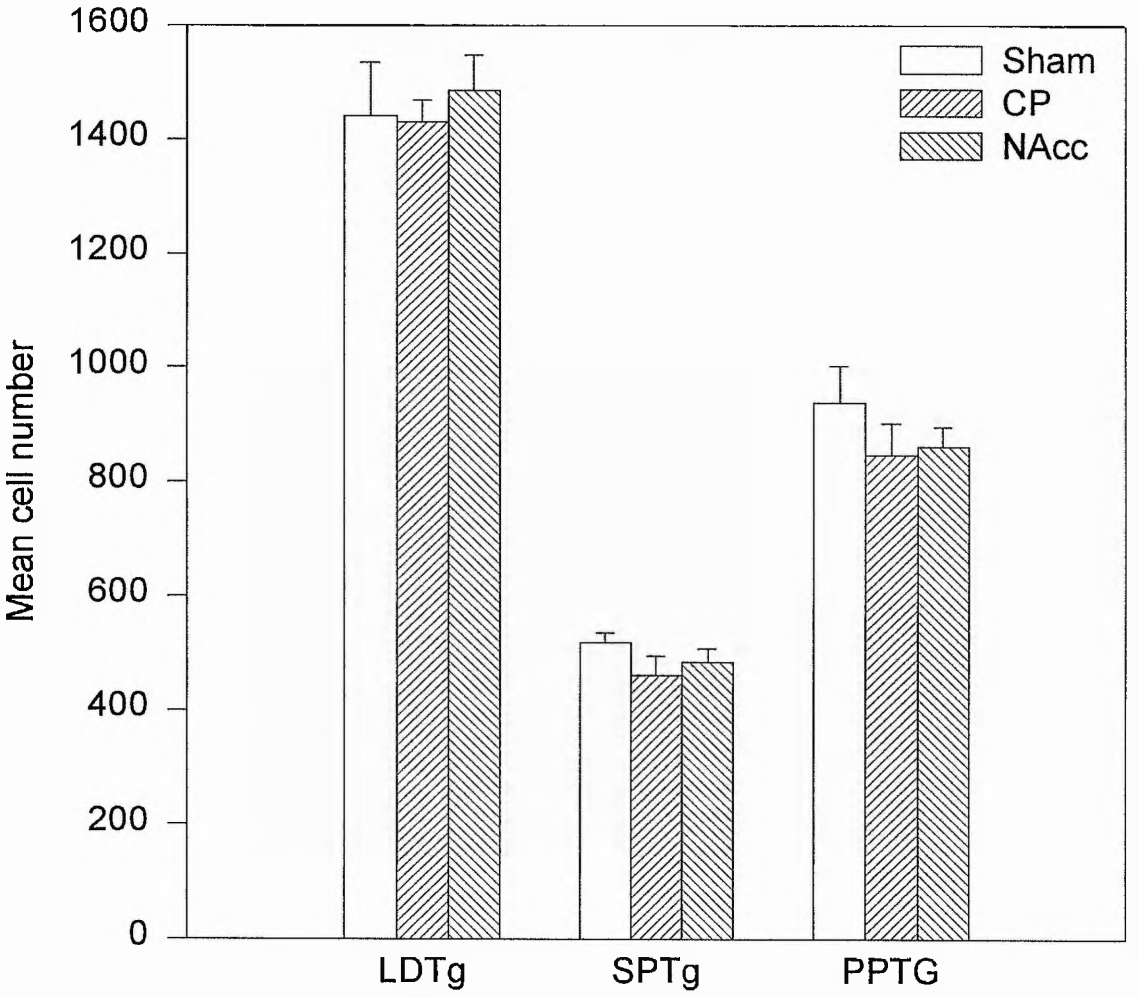
Mean NADPH-diaphorase positive cell counts for the LDTg, SPTg and PPTg following infusion of either phosphate buffer or 6-OHDA into the caudate-putamen or nucleus accumbens in the rat.

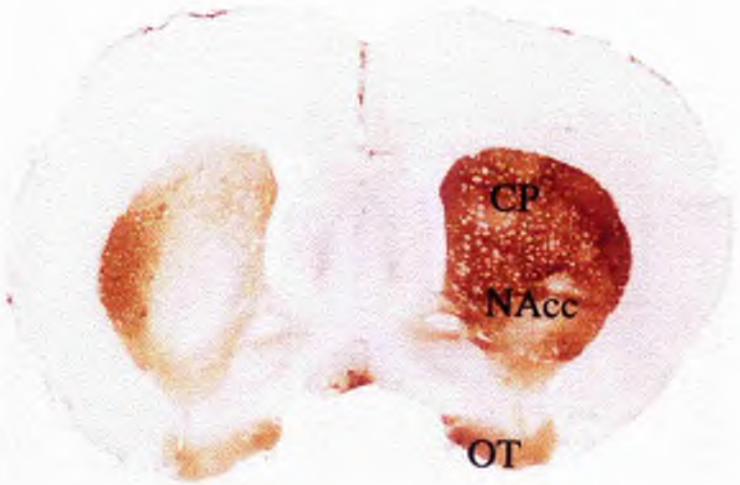
Figure 8.2.2

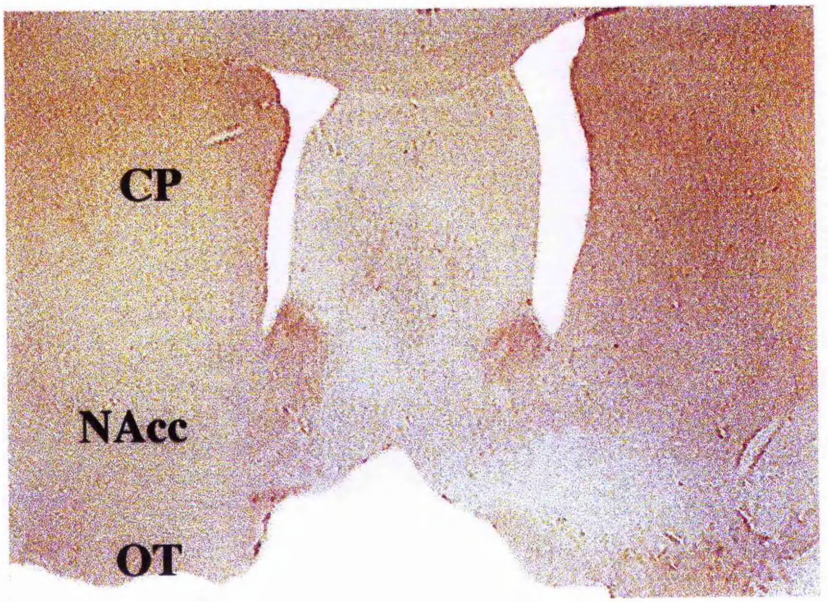
A representative coronal section from a rat following a left 6-OHDA lesion of the caudate-putamen. As can be seen from the remaining brown staining (TOH immunoreactivity product), the caudate-putamen has not been completely eliminated. CP: caudate-putamen; Nacc: nucleus accumbens; OT: olfactory tubercle.

Figure 8.2.3

A representative coronal section from a rat following a right 6-OHDA lesion of the nucleus accumbens. As can be seen form the remaining brown staining (TOH immunoreactivity product), the nucleus accumbens has not been completely eliminayted. CP: caudate-putamen; Nacc: nucleus accumbens; OT: olfactory tubercle. Note: This image was taken at a higher magnification than that in Figure 8.2.2.


1600
1400
1200
1000
800
600
400
200
0
Mean cell number
Sham
CP
NAcc
LDTg
SPTg
PPTG


CP
NAcc
OT


CP
NAcc
OT

Discussion

From Figure 8.2.1 it is evident that lesions of the caudate-putamen or nucleus accumbens did not affect NADPH-diaphorase expression in the mesopontine tegmentum in comparison to controls. Examination of the lesion sites demonstrated that portions of both structures were still intact, particularly in cases of the caudate-putamen lesions. This may be a possible explanation for the negative findings and so it was necessary to examine the effect of more substantial lesions of the dopaminergic innervation to the mesopontine tegmentum. As such, 6-OHDA lesions of the medial forebrain bundle were introduced as the next experiment.

8.3 Experiment 3: Effects of 6-OHDA lesions of the medial forebrain bundle on NADPH-diaphorase expression in the mesopontine tegmentum.

Methods

Animals

13 adult male rats (Charles River) were used. Mean weight at time of surgery was 348.37 ± 14.78 (SD).

Surgery

Rats were randomly divided into 2 groups: 6-OHDA lesion (n=7) or phosphate buffer control (n=6). 15-30 min prior to surgery rats were pre-treated with 15 mg/kg of MAO inhibitor, pargyline hydrochloride (Sigma Chemicals) and 25 mg/kg desmethylimipramine hydrochloride (Sigma Chemicals) to protect noradrenergic neurons. Rats were anaesthetised with sodium pentobarbitone (Sagatal, Rhône Mérieux; 60 mg/kg) and placed in a stereotaxic frame.

Histology

Rats were sacrificed 30-34 days after surgery; sacrifices from each group were evenly distributed over this period. Tissue sections were stained for NADPH-diaphorase and for TOH (see General Methods).

Cell Counting

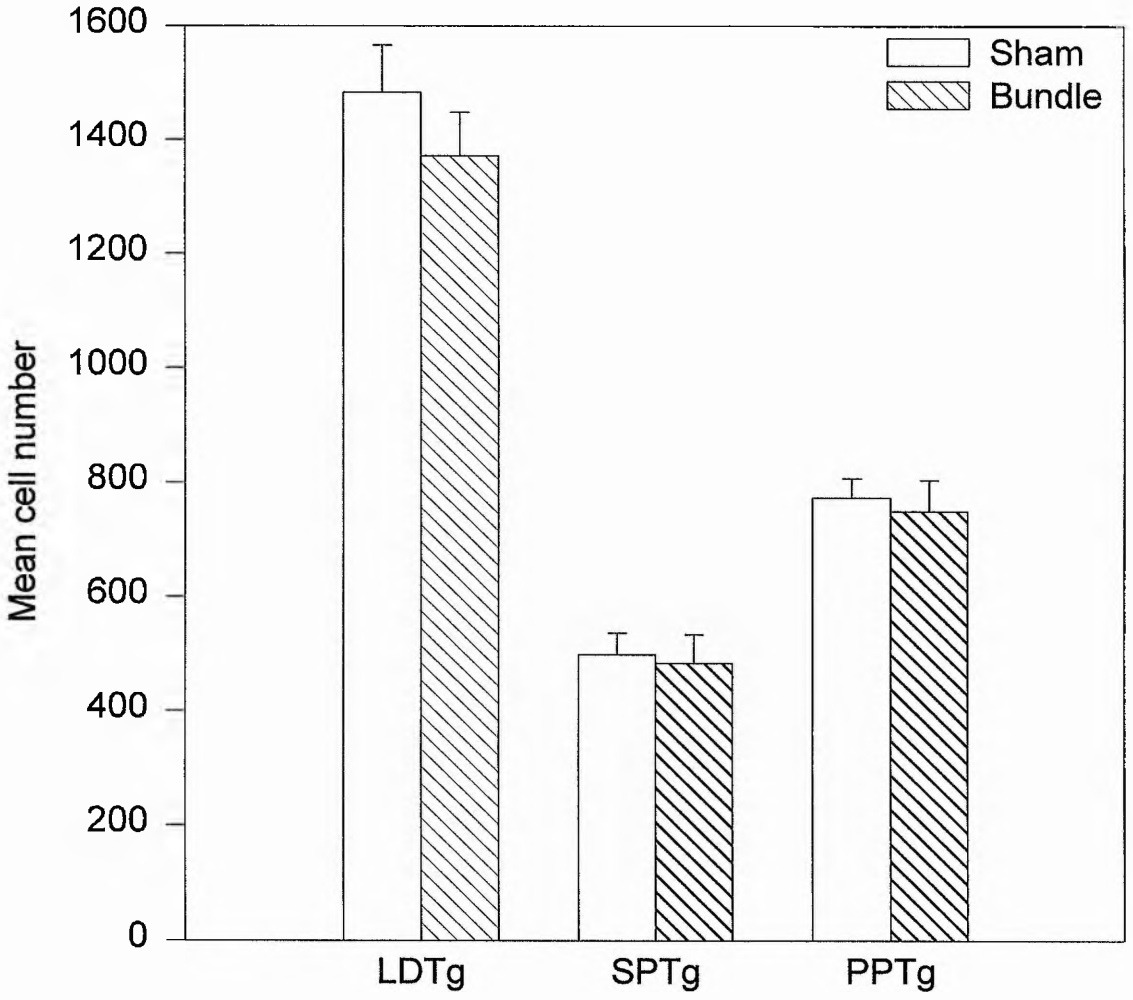
NADPH-diaphorase neurons were viewed under high magnification on a Leitz microscope, and counts were taken as outlined above.

Results

Figure 8.3.1 demonstrates the mean total number of NADPH-diaphorase positive neurons for the LDTg, SPTg and the PPTg for sham and medial forebrain bundle lesioned rats. Cell counts were analysed using a mixed between-within repeated measures analysis of variance. The lesion type (sham vs. medial forebrain bundle) was the between subjects variable and location of cell count (LDTg, SPTg and PPTg) and side of cell counting (ipsilateral to lesion vs. contralateral to lesion) were the within subjects variables. This analysis of variance revealed no main effect of lesion type ($F_{1,11} = 0.40$) or side of counting ($F_{1,11} = 0.39$), but a main effect of location of cell counting ($F_{2,22} = 556.40$, $p < 0.001$). Again this result was not surprising considering the difference in size between these three structures and post-hoc analysis revealed that the number of cells counted in the LDTg was greater than the PPTg which was greater than the SPTg (Tukey-HSD post-hoc test, $p = 0.05$). There was no significant two-way interaction of lesion type x location ($F_{2,22} = 1.69$), lesion x side ($F_{1,11} = 0.45$), or the three way interaction of lesion x location x side ($F_{2,22} = 0.31$), but there was a significant two-way interaction of location x side ($F_{2,22} = 3.83$, $p = 0.037$). Post-hoc analysis revealed that the difference in cell counts was not between sides within a structure, but differences between sides across structures. That is there was no difference in cell counts between the ipsi- and contralateral sides within either the LDTg, SPTg, or PPTg. For this reason, cell counts represented in Figure 8.3.1 are shown combined per structure and not per side per structure. The interaction effect results as there was a statistical difference between the cell counts from the ipsilateral side of the LDTg and the ipsi- and contralateral sides of the SPTg and PPTg and there was a difference between cell counts from the contralateral side

Figure 8.3.1

Mean NADPH-diaphorase positive cell counts for the LDTg, SPTg and PPTg following infusion of either phosphate buffer or 6-OHDA into the medial forebrain bundle in the rat.


1600
1400
1200
1000
800
600
400
200
0
Mean cell number
Sham
Bundle
LDTg
SPTg
PPTg

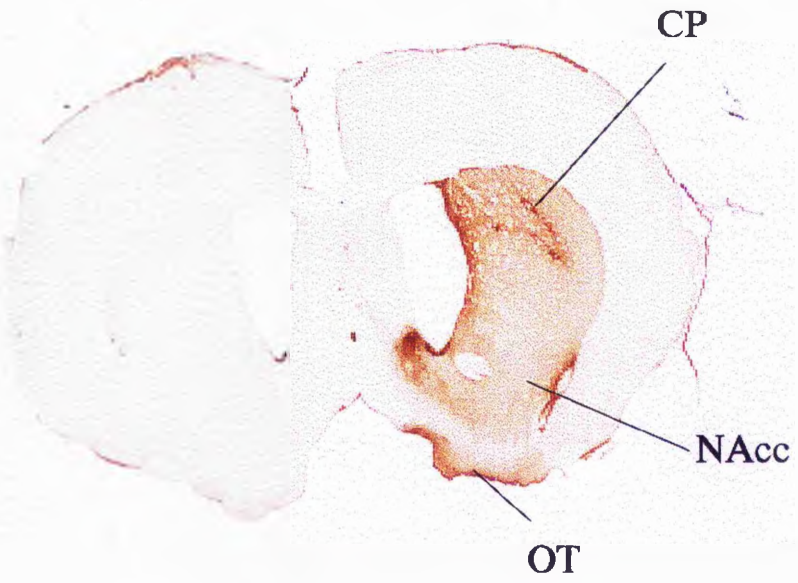
of the LDTg and the ipsi- and contralateral sides of the SPTg and PPTg. Finally there were differences between cell counts from the ipsilateral side of the SPTg and the ipsi- and contralateral sides of the PPTg, and differences between cell counts from the contralateral side of the SPTg and the ipsi- and contralateral sides of the PPTg (all of these results, Tukey-HSD post-hoc tests, $p = 0.05$). This interaction does not reveal a differential effect for one structure independent of the others and thus does not appear to provide specifically relevant information to the study design. The fact that there is no main effect of the lesion variable or its interactions demonstrates that the results of this experiment are null. Histological examination of the lesion sites revealed that the 6-OHDA lesions were complete. Figure 8.3.2 is a representative section from an animal that has had a left medial forebrain bundle lesion. Comparing the staining pattern with the control side revealed that the entirety of the caudate-putamen, nucleus accumbens and olfactory tubercle has been destroyed by the lesion.

Discussion

As is evident from Figure 8.3.1, 6-OHDA lesions of the medial forebrain bundle did not produce an effect on NADPH-diaphorase expression in the mesopontine tegmentum. Examining tissue sections processed for TOH reactivity revealed that the entirety of the caudate-putamen and nucleus accumbens had been removed (see Figure 8.3.2), unlike that of the first experiment. However, as a negative result was again revealed further investigation seems warranted. It has been noted that the areas so far examined provide only indirect or weak direct projections to the mesopontine tegmentum (Rye et al., 1987; Hallanger and Wainer, 1988) and

Figure 8.3.2

A representative coronal section from a rat following a 6-OHDA lesion of the medial forebrain bundle on the left side. As can be seen by the absence of TOH reaction product, the entirety of the caudate-putamen, nucleus accumbens and the olfactory tubercle have been removed. CP: caudate-putamen; Nacc: nucleus accumbens; OT: olfactory tubercle.


CP
NAcc
OT

so it may be necessary to examine the effects on NADPH-diaphorase expression following removal of a more direct innervation to this area. Direct pallidal projections to the mesopontine tegmentum (specifically the PPTg) have been described in the rat (Jackson and Crossman, 1981a) and more recently in the rhesus monkey and in human (Rye et al., 1996). A future experiment then, would be to induce pallidal lesions in the rat and to examine any subsequent changes in NADPH-diaphorase expression in the mesopontine tegmentum.

Summary

The effect of dopamine mediated striatal outflow on NADPH-diaphorase expression in the PPTg had not been previously explored and was examined in this chapter. A recent study reported an increase in NADPH-diaphorase expression in the PPTg in post-mortem brains of schizophrenic patients (Garcia-Rill et al., 1995). As these patients were under long term medication with antipsychotics which provide blockade of striatal dopaminergic output, examination of the effect of blockade of striatal mediated output independent of the disease state was warranted. In addition, removal of afferent input to other parts of the nervous system has been shown to result in changes in neurotransmitter expression and thus it was deemed useful to examine the effect on neurotransmitter expression in the mesopontine tegmentum following removal of dopaminergic afferentation to this area. 1) Dopaminergic blockade of D_1/D_2 and D_4 preferentially expressed on the striatal and pallidal efferent pathways, respectively (Rye, 1997) through application of the antipsychotics haloperidol and clozapine, did not statistically effect NADPH-diaphorase expression in the LDTg, SPTg, or PPTg. Clozapine administration, however, did increase neuronal expression in half of the rats treated to a number higher than the highest expression in control rats. This result needs further examination as the administration of clozapine was limited and a longer time period is needed for more realistic comparisons with human medicated administration periods. 2) Direct dopaminergic denervation of the striatum, specifically the caudate-putamen and nucleus accumbens, was subsequently attempted and no effect on NADPH-diaphorase expression was found. In this experiment, however, it was found that lesions of these sites were incomplete and thus commentary on dopaminergic

denervation is not viable. 3) Finally, more severe dopaminergic denervation through lesion of the medial forebrain bundle also did not effect NADPH-diaphorase expression of the LDTg, SPTg, or PPTg, though removal of the dopamine content of the caudate-putamen, nucleus accumbens and olfactory tubercle were complete. It may be that a more direct innervation site of the mesopontine tegmentum would have greater influence on NADPH-diaphorase expression in the PPTg and pallidal lesions may be a source of further experimentation.

The experiments of this chapter have been limited to a neuroanatomical investigation of the influence of dopamine mediated striatal outflow on mesopontine NADPH-diaphorase expression. Examination of neurochemical changes, as measured by *in vivo* microdialysis or voltammetry, remain to be explored. The influence this output may have on mesopontine NADPH-diaphorase expression in medicated schizophrenic patients remains to be clarified. In addition, the role of this striatal outflow on PPTg function in appropriate responding and goal directed behaviours has been previously outlined (Inglis et al., 1994b; Allen and Winn, 1995; Keating et al., 1996; Keating et al., 1997) and is suggested to play a role in the findings of the previous chapters of this thesis. Elucidating the potential role this outflow may have on the neurochemical, neuroanatomical or neurophysiological expression of the PPTg is necessary if further understanding of this structure is to come forth.

.Chapter 9. General Discussion

9.0 Introduction

The aim of this thesis has been to examine the PPTg as a structure in receipt of basal ganglia, and particularly striatal, outflow. Situated deep within the brainstem this structure has only recently been explored to determine its role in complex behavioural selection and responding processes. This thesis sought to provide further evaluation of these processes to extend existing understanding of the role of the PPTg and striatal outflow processing, and to combine this information with existing findings. This thesis by no means sought to provide the definite answer on the function of the PPTg but hoped to add to the existing collective knowledge and to point to future ways forward.

9.1 Summary of findings and conclusions

Response selection and goal directed behaviours are processes that have been both attributed to ventral striatal control and processing. Work by for example, Mogenson and colleagues (1980), Kelley and Stinus (1985), Mogenson (1987), and more recently Floresco and colleagues (1997), has identified the ventral striatum as a structure influential in making appropriate response choices. Recent work has expanded the work of these authors to outline a role for the PPTg in similar processing. The PPTg is a structure the anatomical connectivity of which allows it to receive ventral striatal and other basal ganglia outflow and then, in turn, to re-

influence these and other forebrain structures, as well as sending output to downstream brainstem and spinal cord structures in direct control of processes such as locomotion. This places the PPTg at a point of potential significant influence for a range of activities, not the least of which is response selection related to motivated behaviours.

Conditioned place preference

Several findings of this thesis extend and clarify existing literature, while other findings are novel. The proposed role of the PPTg in associative learning and subsequent approach behaviour in the conditioned place preference task has been described in relation to deprivation state and the type of reward stimuli used. Derek van der Kooy and his colleagues have carried out most of the research examining the PPTg in relation to CPP performance. Their theory, that the PPTg mediates CPP dependent on deprivation state is not consistent with other studies of PPTg functioning. They maintain that the PPTg mediates CPP responding only when a rat is non-deprived, response performance for rats that are in a deprived state being mediated elsewhere (Bechara and van der Kooy, 1989; 1992b; Sterfurak and van der Kooy, 1994; Parker and van der Kooy, 1995). The results of these place preference experiments do not fit with impaired response performance in other tasks following PPTg lesions in which the rats were kept in a food deprived state (Inglis et al., 1994b; Fujimoto et al., 1992; Olmstead et al., 1998), which thus suggests that the role of PPTg in response performance is not necessarily state dependent as van der Kooy and colleagues outline. Data in this thesis sought to clarify further the role of the PPTg in CPP performance and found that rats with lesions of the PPTg, either

food deprived or non-food deprived, were not impaired in their ability to form a positive place preference when 20% sucrose was used as the reward stimulus. Here, the ability of PPTg lesioned rats to form a place preference was clearly not dependent on state. These rats were able to form appropriate associations regardless of deprivation state when the task demands were kept simple. Work by Fujimoto and colleagues (1989; 1992) found an impairment in associative learning in an active avoidance task following lesions of the PPTg, but in this case the associative learning and appropriate response pattern to be made was more complex. In this task the rat had to associate a light-tone stimulus pair with upcoming negative foot shock and learn to jump across to a nearby safety chamber to avoid the upcoming shock. This task related more to preparing a response and avoidance of negative outcome, and encompassed more complex patterns of associations and appropriate responding than learning that one box had reward and another did not. The novel data from the present CPP experiments came from the finding of increased sucrose reward intake in PPTg lesioned rats that were food deprived compared to both non-deprived lesioned rats and control rats that were either deprived or non-deprived. This finding revealed disinhibited consumption by PPTg lesioned rats in a state of food deprivation without the perception of reward or approach behaviour to said reward (as measured by the CPP) being affected.

Reward related responding

Further experimentation revealed that the disinhibition of consummatory responding in PPTg lesioned rats did not occur at low concentrations of sucrose, but did at higher concentrations. Control rats increased their intake at these higher

concentrations as well, but did not disinhibit their responding in the same way as PPTg lesioned rats did. Initially this increased intake was only found from food deprived PPTg lesioned rats, but when the concentrations of sucrose were raised then non-food deprived PPTg lesioned rats increased their consumption intake as well. These data address the nature of the influence of internal and external motivations and resulting intake responding. When internal drive (in the form of deprivation) was present, disinhibited responding was seen in PPTg lesioned rats as the reward value increased. As this disinhibited responding was not seen at all concentrations of sucrose, the value of external reward must reach a certain level, or threshold of motivating value, before increased or disinhibited responding becomes evident. As this same degree of increased responding was not seen in control rats it suggests a role for the PPTg in this disinhibited response activity. The role of different external rewards, and resulting differential responding from PPTg lesioned rats, has been examined. Work by Robertson and colleagues (1994) examined the nature of responding on a progressive ratio task for food reward and found that PPTg lesioned rats had significantly lower breaking points than controls. These rats stopped working for food reward at a point lower than control rats, somewhat indicative perhaps that the reward they were working for was not motivating enough to maintain lever pressing. This result is in direct contrast to data demonstrating that PPTg lesioned rats worked more quickly, had significantly higher breaking points and thus received more reward when *d*-amphetamine was used as the external reward or motivator (Keating et al., 1997). In this experiment the rats began bar pressing quite quickly and eagerly and thus received most of their infusions of the *d*-amphetamine reward within the first hour of the three hour testing session. These

studies seem to indicate that different external rewards influence responding patterns in rats that have lesions of the PPTg. What is not entirely clear is whether the previous differential responding reflects a response to the *nature* of the reward, or to the *degree* of the reward. Differences between natural rewards (food) versus artificial rewards (drugs) and the degree of these rewards needs to be directly compared. Higher palatable food may produce higher breaking points and persisted lever pressing during progressive-ratio responding, while lower doses of *d*-amphetamine may result in decreased lever pressing and reduced breaking points. These studies would have to be investigated before further comment can be made. Disinhibited intake responding to reward stimuli (increasing concentrations of sucrose) in this thesis did not however, interfere or influence the approach response behaviour to the reward. Control and PPTg lesioned rats faced with either 4 or 20% sucrose at the end of an alleyway did not differ from each other in their approach response time and initiation of sucrose drinking, though the degree of intake responding to the 20% sucrose was significantly increased in PPTg lesioned rats compared to controls and compared to both control and lesioned rats receiving 4% sucrose. Here the dissociation between approach and consumption was clear and indicative that while all rats were as eager to receive the sucrose reward, when the sucrose stimuli was increased then PPTg lesioned rats disinhibited their consumption.

Spatial random foraging

The results of this thesis demonstrate that lesions of the PPTg affect rats' ability to plan and execute appropriate responses in a random foraging radial arm maze task.

Lesions of the PPTg interrupt rats' ability to retrospectively plan choices and execute appropriate response selection in this task. This resulted in lesioned rats making significantly more errors in the task compared with control rats. This finding holds true in both acquisition and retention of a spatial random foraging task. In both cases the rats' motivation to perform the task was not impaired as judged by the lack of difference compared to control rats in their time to initiate their first response choice, their time to make subsequent response choices, and their time to complete the session. The lesioned rats consumed the food pellets available once they reached them, further indicating that they were motivated to complete the task. In these experiments the deficits seen in PPTg lesioned rats lie in their inability to plan and execute appropriate response choices or selections. The impairment in acquisition of this foraging task found in this thesis are comparable to the finding of Dellu and colleagues (1991). In a similar random foraging task this group found that PPTg lesioned rats made an increasing number of errors in their first 8 choices to find food pellets and in the total number of choices required to retrieve all the food pellets compared to control rats. Again these results demonstrate that lesions of the PPTg result in an impaired ability to make accurate and appropriate response choice selections. While the impairment in planning and execution of this random foraging task is novel, the impairment in appropriate responding following lesions of the PPTg are comparable to findings of Inglis and her colleagues (1994b). This study found inappropriate responding in a conditioned reinforcement task in which the rats had to distinguish between two levers, one that provided food reward and the other that was not reinforced. In this task PPTg lesioned rats did not make this distinction and made as many presses on the non-reinforced lever as the reinforced one. Rats

displayed an impairment in their ability to execute appropriate response choice selections and were not able to suppress inappropriate lever responding. The inability of PPTg lesioned rats to adequately retain performance of this random foraging task also demonstrated a role for this structure in reference memory. PPTg lesioned rats were clearly unable to use their previously acquired ability to perform the task at the appropriate point post-surgery.

Spatial working memory task: delayed spatial win-shift

Further examination of the role of the PPTg in spatially mediated appropriate response selection found more impairments. When the rat is faced with a more complex task, one that relies on the rats' ability to hold information in memory over time and to then prospectively plan appropriate responses, PPTg lesioned rats are impaired compared to control rats. Work by Seamans and his colleagues (1996) and Floresco and his colleagues (1997) found that effective foraging and appropriate responding on a working memory delayed spatial win-shift task was dependent on the integrity of the prelimbic prefrontal cortex-hippocampal pathway. The work of this thesis demonstrated that effective foraging and appropriate responding on the acquisition of this working memory task was also dependent on the integrity of the PPTg. Lesions here resulted in rats making significantly more errors than controls in their attempt to complete the task and find all the food pellets. The fact that the lesioned rats were no different than control rats on measures of time to make their first response choices, their time to make subsequent arm choices and their time to complete the session, and in fact to consume the food pellets when found, is indicative that their impaired performance was not related to a lack of motivation or

attention to the task, but to an inability to plan and execute accurate and appropriate response choice selections. Here PPTg lesioned rats displayed a degree of inflexible memory in their inability to shift their responses to make new arm choices after the delay rather than continuing to choose arms that were previously baited. This inflexibility in performance led to the PPTg lesioned rats with an inability to adequately perform this working memory task. Impairments in acquisition of this task and not retention has two possible explanations. It may be the case that retention of this type of prospective foraging working memory task is not dependent on the integrity of the PPTg, and accurate performance may be mediated elsewhere. Impairments in acquisition performance that are not seen in retention performance involving the PPTg have been previously found. Work by Fujimoto and colleagues (1992) found that PPTg lesioned rats were impaired in their acquisition of passive and active avoidance task related to receipt of foot shock, but when rats were trained on these tasks and then received a lesion of the PPTg, the rats' performance was not impaired. This type of impaired acquisition / unimpaired retention was also found in a study in which rats that had to learn to bar press to administer brain stimulation of the lateral hypothalamus (Lepore and Franklin, 1996). PPTg lesioned rats were impaired in acquisition of this task but were able to re-perform this task post-lesion once it had been acquired preoperatively. Finally, recent work by Olmstead and colleagues (1998) outlined an impairment in acquisition performance from PPTg lesioned rats on fixed and progressive ratio bar pressing tasks for intravenous heroin administration. This performance was not impaired when the rats re-performed the task, as lesioned rats showed the same rate of responding, and showed similar breaking points at which they would no longer respond, as control rats. While these

tasks may not be the same as the delayed spatial win-shift task used in this thesis, it does demonstrate that performance on complex tasks can reveal impairments from PPTg lesioned rats on their acquisition but not necessarily on their retention. The second potential explanation for the finding of unimpaired performance from PPTg lesioned rats on retention of this delayed spatial win-shift task is that the result is anomalous resulting from a low number of rats surviving the post-training lesion producing low power in the statistical analysis. Replication of this experiment is currently ongoing in this laboratory and future results may provide clarification of this result.

Striatal outflow and NADPH-diaphorase expression of the mesopontine tegmentum

Finally, examination of the role of dopamine-mediated striatal outflow on neurotransmitter expression in the PPTg revealed null results that require further exploration. Work outlining an increase in NADPH-diaphorase expression in the PPTg in post-mortem brains of schizophrenic patients called into question the potential role of the PPTg in the modified behavioural responses that are common features of schizophrenia. However, examination of the potential role of dopamine mediated striatal outflow on neurotransmitter expression in the PPTg found that neither pharmacological blockade of this input through administration of the D_1/D_2 receptor antagonist haloperidol or the D_4 receptor antagonist clozapine, nor removal of this dopamine afferentation through lesion of the striatum (locally or by lesion of the medial forebrain bundle) produced a change on NADPH-diaphorase expression in the PPTg. It is worth noting that clozapine administration did produce an increase in NADPH-diaphorase expression in 5 of the 10 treated rats above the level of

control rats and so experiments with longer term administration of clozapine are warranted. In addition, it may be useful to remove innervation from structures closer to the PPTg than the striatum to establish neurotransmitter expression changes. More direct removal of sites such as the globus pallidus may produce the changes in NADPH-diaphorase expression that have been shown from removal of direct afferentation on sites in other parts of the nervous system (Fiallos-Estrada et al., 1993; Yu, 1994; Jia et al., 1994 and Persson et al., 1995).

Together then, the findings of this thesis outline a role for the PPTg in goal directed behavioural responding and appropriate response choice planning and execution. These data fall in line with current theory regarding goal directed behaviour. The work of Balleine and Dickinson (1998) outlines the hypothesis that goal directed behaviour is mediated by the processes of contingency learning and incentive learning and that the prelimbic area of the prefrontal cortex and the insular cortex, respectively, control these processes. The data in this thesis, and existing data regarding the role of the PPTg, now extend this theory to report that the PPTg is also involved in these processes. In regards to contingency learning the work of Inglis and her colleagues (1994) and Fujimoto and his colleagues (1989, 1992) demonstrated that lesion of the PPTg interrupts the ability of a rat to associate stimuli with an appropriate response. Inglis and colleagues (1994) showed that rats with PPTg lesions were unable to discriminate between reinforced and non-reinforced levers. In the work of Fujimoto and colleagues (1989, 1992), PPTg lesioned rats were unable to pair a light-tone warning stimulus with upcoming footshock and thus did not move across to a nearby escape box to avoid the shock. In regards to

incentive learning, and incentive value of rewards, lesions of the PPTg play a role in this processing in their consummatory responding to the reward rather than performance for the reward per se. For example, food deprived PPTg lesioned rats did not display differences in approach behaviour to 4% or 20% sucrose reward compared to control rats. Lesioned rats with access to 20% sucrose, however, displayed disinhibited intake responding to this incentive reward compared to lesioned rats with 4% or control rats with either 4 or 20% sucrose. This increased consummatory responding to highly incentive sucrose rewards was also displayed when lesioned rats had access to a gradient of sucrose concentrations. Lesioned rats increased their sucrose intake when presented with concentrations of 24, 40 and 60% sucrose compared to control rats. These results outline a role for the PPTg in appropriate responding to incentive stimuli.

In addition, the degree of influence of the PPTg in behavioural processing is related to task complexity (Inglis and Winn, 1995) and degree of information integration. In approach response tasks related to associative learning, when the task demands are quite simple, lesions of the PPTg do not result in impaired performance. This is seen in simple runway tasks (Chapter 5) and in conditioned place preference tasks (Chapter 4). The PPTg does play a role when the degree of associative learning is more complex as in the active avoidance associative learning paradigm in which the rat must pair light/tone stimuli with an upcoming event (footshock). Appropriate integration of this information to affect pre-emptive and preparatory responding is attributed to the PPTg as lesions here result in impaired and inappropriate responding (in the lack of avoidance responding; Fujimoto et al., 1992.

Finally, the results of this thesis outline a role of the PPTg in behavioural processing that has previously been only attributed to sites higher on the neuraxis. The ability to either prospectively or retrospectively plan and execute appropriate response choices has been found to be dependent on either the prelimbic-hippocampal pathway or the hippocampal-ventral striatal pathway, respectively (Seamans et al, 1996; Floresco et al., 1997). The work of Chapters 7 and 8 of this thesis now outline a role for the PPTg in these behaviours. Removal of the PPTg results in the type of inaccurate and inappropriate response choices in these tasks as have been previously demonstrated following disconnection lesions of either the prelimbic-hippocampal or hippocampal-ventral striatal pathways. These findings suggest mediation of behavioural processing at a point lower in the neuraxis than has been previously accepted and suggests that this structure is more dynamic and complex than previously imagined.

Work on this structure and further elucidation of its function, however is far from complete. While the work of this thesis had provided support and addition to the existing literature, it has also set forth the way for future experimentation.

9.2 Future experiments

The work of White and Carr (1985) has identified an increase in the expression of associative learning with increasing concentrations of sucrose reward. That is, with increasing concentrations of sucrose rats spend more time in the reward associated environment during the test session in the absence of the reward compared to the non-rewarded environment. The data presented in Chapter 4 of this thesis

outlined that lesions of the PPTg do not impair rats' ability to form a positive conditioned place preference compared to controls but demonstrated that there was a disinhibition of responding in regards to consumption of present sucrose reward. It would be of interest to evaluate the effect of varying concentrations of sucrose on the display of a conditioned place preference from PPTg lesioned rats. As data in Chapter 5 outlined that PPTg lesioned rats did not disinhibit their consumption of sucrose reward at low concentrations of sucrose, only at higher concentrations that are presumed to have increased salience and motivational value, would this response affect the performance of PPTg lesioned rats in their ability to form a positive place preference to the environments paired with low concentrations of sucrose reward?

The second set of experiments that would be interesting to pursue relates to the findings of experiment 3 of Chapter 5 in evaluating the disassociation between approach behaviour and consummatory response. All of these rats were food deprived and though displayed no difference in their motivation as revealed by their time to complete the runway and approach the sucrose reward available, did demonstrate a different consumption response to low (4%) versus a high (20%) concentration of sucrose reward with PPTg lesioned rats showing a disinhibition of responding to only the high concentration. As the results of experiments 1 and 2 of that same chapter revealed that internal drive and external stimuli interplay in the resulting intake response it would be interesting to evaluate the pattern of responding in this approach task when there is a difference in internal drive (non-deprived versus deprived rats). This response would be evaluated both in regard to approach response time and consumption of available reward. There was no difference

between PPTg lesioned rats in terms of runway completion times to either 4 or 20% sucrose when the rat was food deprived, but would a dissociation become apparent if the lesioned rat was non-food deprived?

Finally, evaluation of the role of neurochemical changes during behavioural processing is an element of literature that has not been explored in relation to the PPTg. It would be of empirical interest to examine the changes in acetylcholine and dopamine levels in substantia nigra, striatum and thalamus during performance of any of the tasks and related behavioural processing evaluated in this thesis. Microdialysis technology for such neurochemical evaluation in rats performing behavioural tasks is available, and becoming more advanced, and thus only the time and the interest remains to be found for such exploration.

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Appendix 1

References for Table 1.2.1: Connectivity of the PPTg: Again the majority of studies used rodents; * indicates those that used primates and † indicates those that used cats.

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