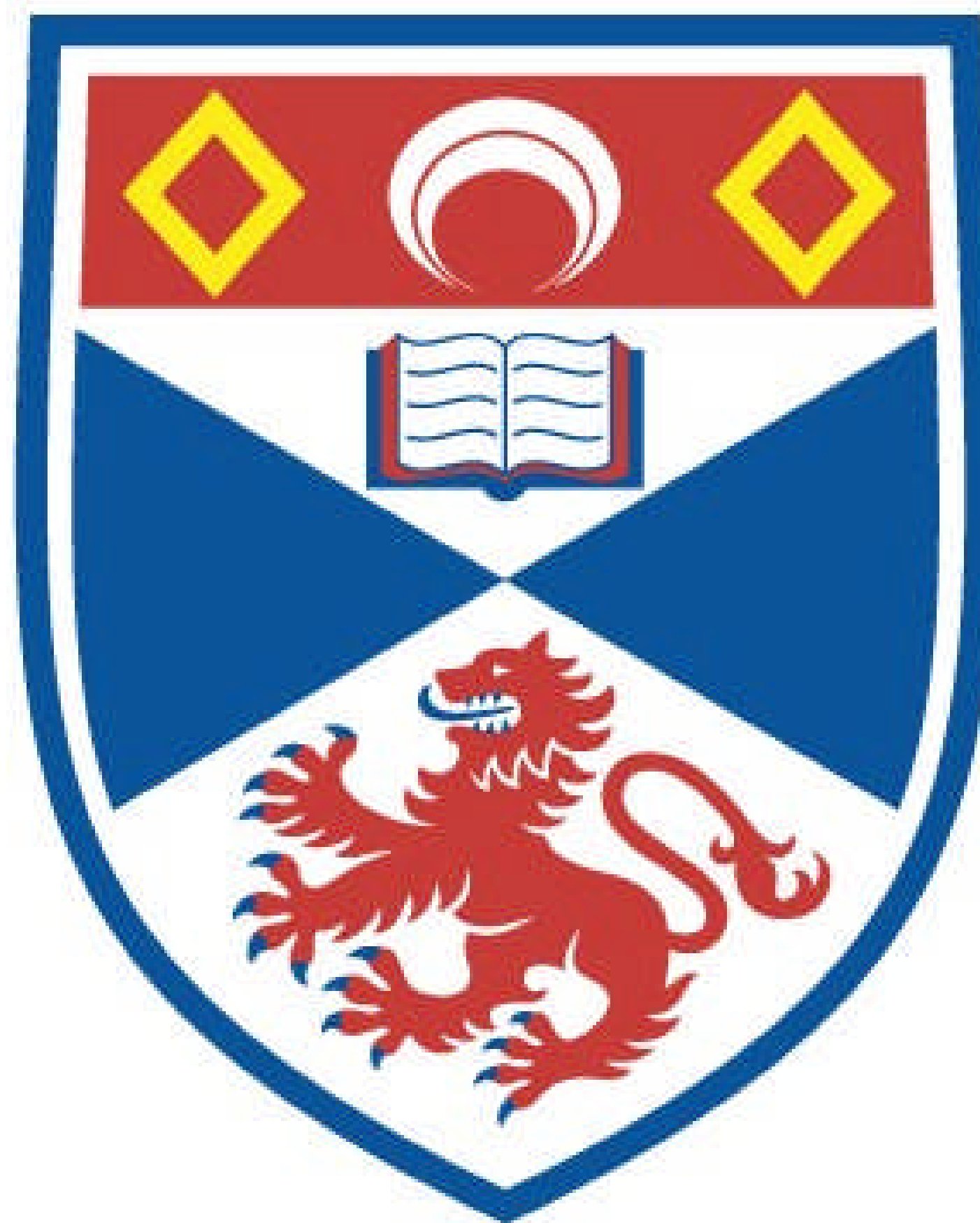


**NEURAL MECHANISMS OF EXECUTIVE FUNCTION : THE
ROLE OF THE PEDUNCULOPONTINE TEGMENTAL
NUCLEUS AND MEDIAL PREFRONTAL CORTEX IN
DELAYED SPATIAL WIN-SHIFT BEHAVIOUR IN THE RAT**

Claire L. Taylor

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



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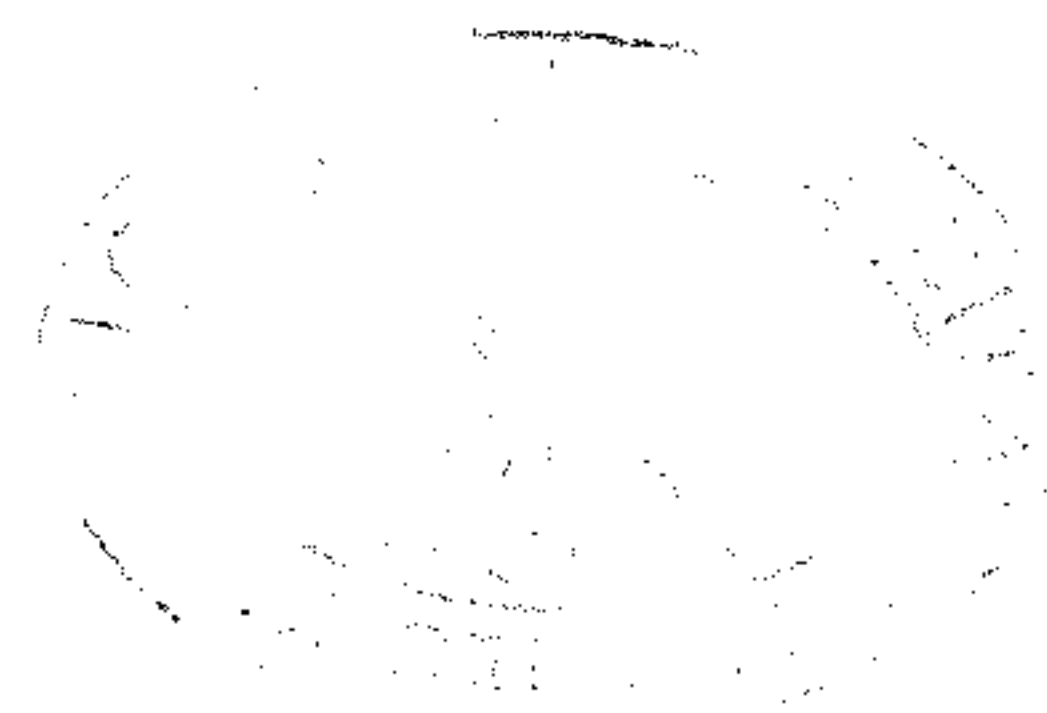
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RAT.**

CLAIRE L. TAYLOR

Submitted for the degree of PhD, December 2001



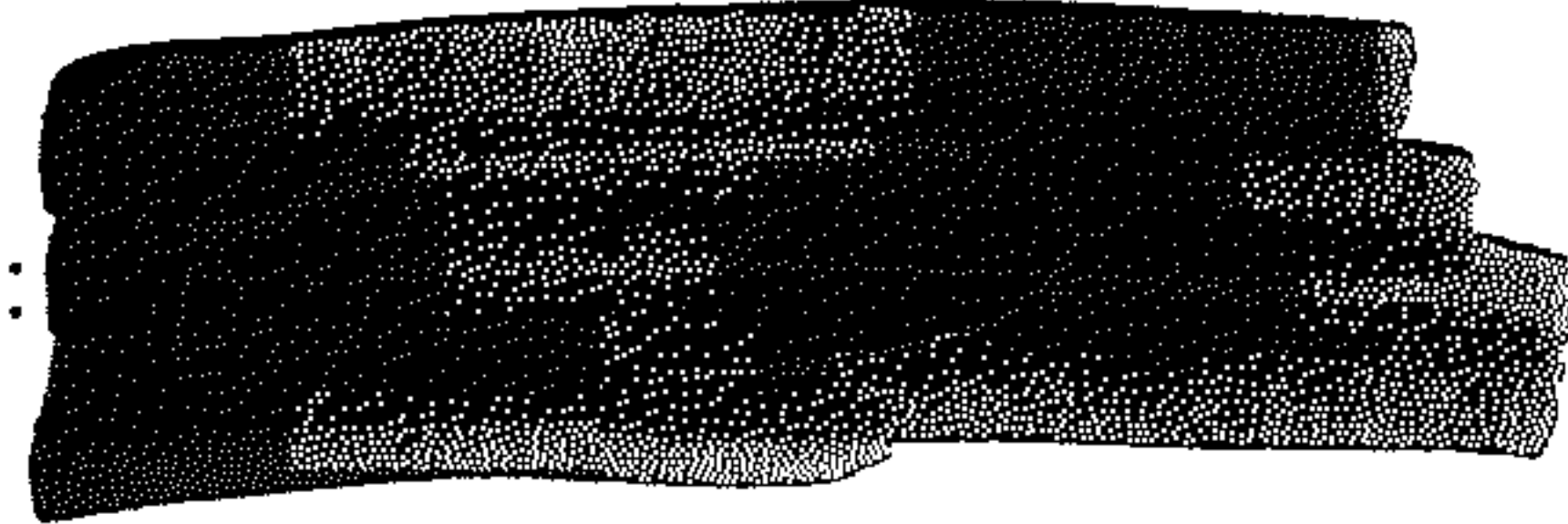
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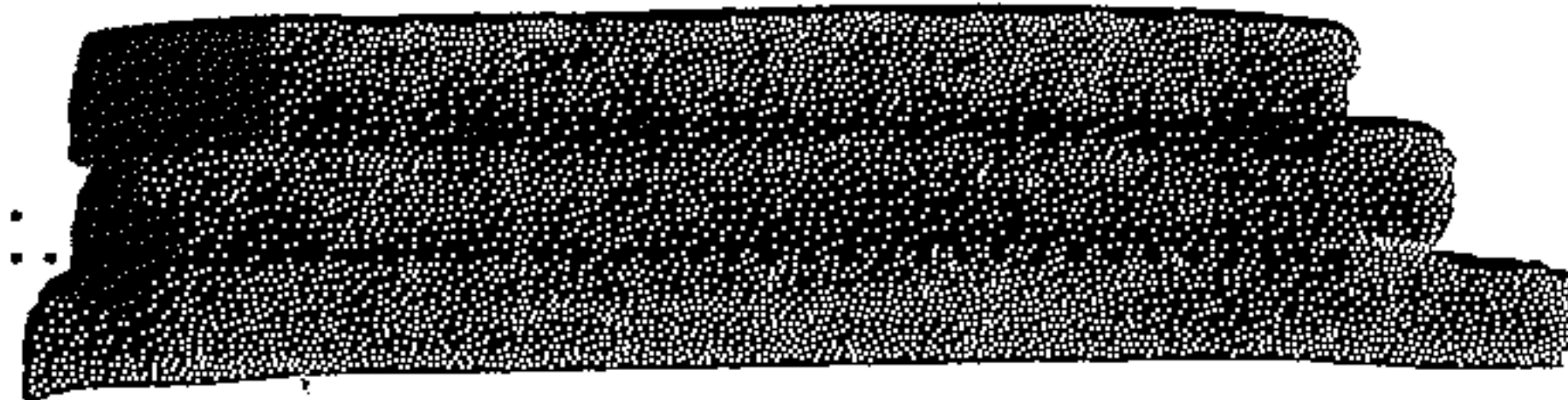
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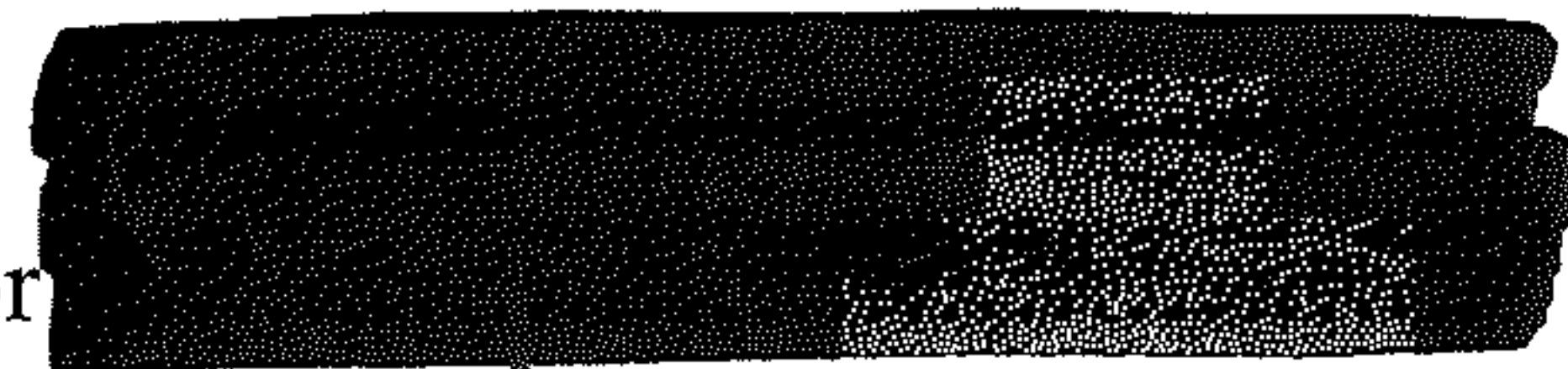
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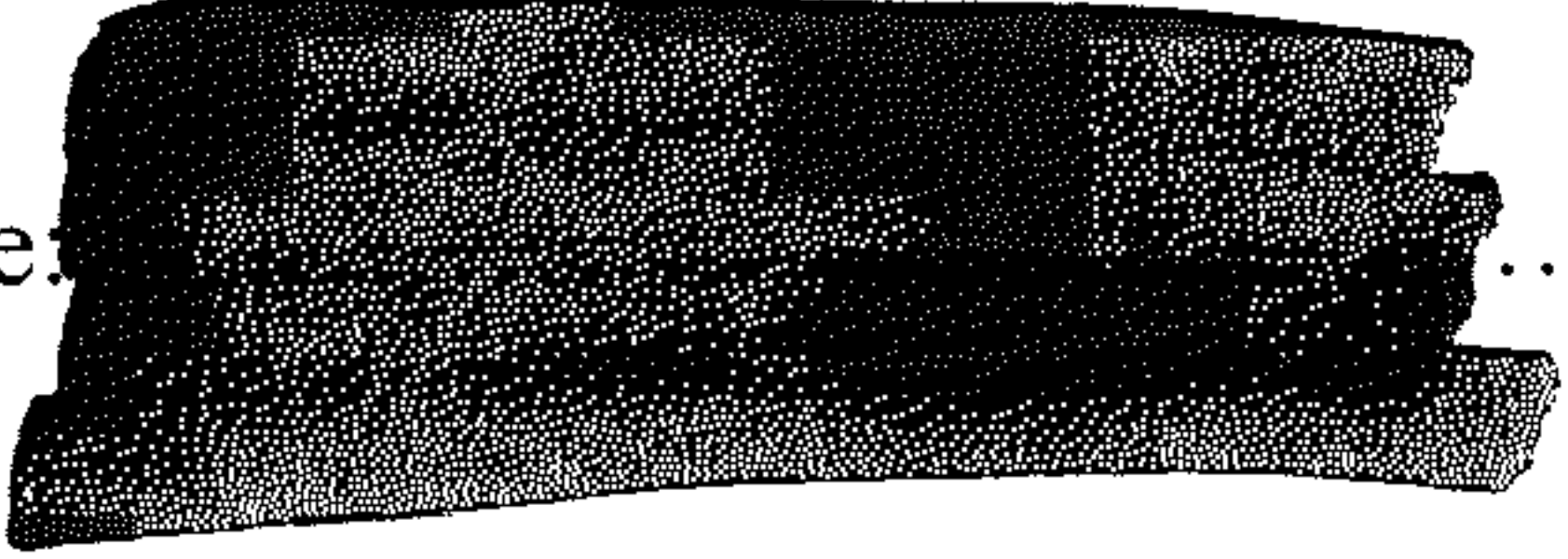


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ACKNOWLEDGEMENTS

Firstly I wish to thank Phil Winn for his patient supervision and sound advice. You've been a great supervisor and I really appreciate being given the opportunity to try out my own ideas and express my occasionally wacky theories! Thanks must go also to Verity Brown, my second supervisor, who provided some much needed advice on the findings of the prefrontal cortex chapter. I'm also indebted to her for the support she gave me following the development of my allergy and for giving me the opportunity to try out some human testing.

Next I have to thank all of the members of the Winn lab and Mary Latimer who provided me with invaluable assistance and support during the final experiment contained in this thesis. Without them it's likely that the experiment would not have been completed. Thank you all. Special mention must also be made for Rouba Kozak who undertook practical supervision of this process and Claire Birch whose ability to listen to all my little problems throughout the duration of my PhD has been very much appreciated!

Last, but by no means least, I wish to thank my family, my friends, and my boyfriend Paul. Their support for me has been tremendous and thoroughly appreciated. Especially the assistance I've been given to ensure that my computer wouldn't keel over and die every time I did any work on my thesis! Thanks guys.....

ABBREVIATIONS IN MAIN BODY OF TEXT

- ABC** Avidin biotin complex
- AC** Anterior cingulate region of the prefrontal cortex
- ACh** Acetylcholine (or cholinergic)
- ADS** Antibody diluting solution
- ANOVA** Analysis of variance
- AP** Across phase test errors
- (aq)** Aqueous solution
- ARAS** Ascending reticular activating system
- CA1** Field 1 of the hippocampus
- ChAT** Choline acetyl transferase
- Ch5** Type 5 cholinergic neurons
- Ch6** Type 6 cholinergic neurons
- CP** Caudate putamen
- CR** Conditioned response
- CS** Conditioned stimulus
- CT** Claire Taylor
- DA** Dopamine (or dopaminergic)
- DAB** Diaminobenzidine
- dAC** Dorsal anterior cingulate region of the prefrontal cortex
- dH₂O** Distilled water
- dIPFC** Dorsolateral prefrontal cortex
- DPX** Distrene plasticiser for xylene
- DSWS** Delayed spatial win-shift

EEG Electroencephalogram

FEF Frontal eye field

Fr2 Premotor region of the prefrontal cortex

GABA Gamma-amino-butyric acid

GP Globus pallidus

GPe External segment of the globus pallidus

GPI Internal segment of the globus pallidus

5HT Serotonin (or serotonergic)

ID Intradimensional

IgG Immunoglobulin G

IL Infralimbic region of the prefrontal cortex

ip. Intraperitoneal

IQ Intelligence quotient

LDTg Laterodorsal tegmental nucleus

LH Lateral hypothalamus

MD Mediodorsal nucleus of thalamus

MLR Mesencephalic locomotor region

mPFC Medial prefrontal cortex

NA Noradrenalin (or noradrenergic)

NADPH Nicotinamide adenine dinucleotide phosphate

NBT Nitro blue tetrazoleum

NeuN Mouse anti-neuronal nuclei

NGF Nerve growth factor

No. Number

Non-ACh Non-cholinergic

6-OHDA 6-hydroxydopamine

omPFC Orbitomedial prefrontal cortex

PAP Peroxidase antiperoxidase

PB Phosphate buffer

PBS Phosphate buffered saline

PC Personal computer

PFC Prefrontal cortex

PL Prelimbic region of the prefrontal cortex

PL/IL Prelimbic-Infralimbic region of the prefrontal cortex

PL/MO Prelimbic-medial orbital region of the prefrontal cortex

PPTg Pedunculo pontine tegmental nucleus

RF Random foraging

RK Rouba Kozak

SGE Scientific Glass Engineering

SI Substantia inominata

SN Substantia nigra

SNc Substantia nigra pars compacta

SNr Substantia nigra pars reticulata

STN Subthalamic nucleus

TRN Thalamic reticular nucleus

US Unconditioned stimulus

VA Ventroanterior thalamic nucleus

vAC Ventral anterior cingulate region of the prefrontal cortex

VA/VL Ventroanterior/ventro-lateral thalamic nuclei

VL Ventrolateral thalamic nucleus

VLCP Ventrolateral caudate putamen

VM Ventromedial thalamic nucleus

VP Ventral pallidum

vsub Ventral subiculum of the hippocampus

VTA Ventral tegmental area

WCST Wisconsin card sorting task

WP Within phase errors

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ABSTRACT

The pedunculo-pontine tegmental nucleus (PPTg) has been argued to be involved in mediating neural processing relating to executive functions (Winn, 1998). Since it shares connections with fronto-striatal circuitry this proposal is not surprising. However, research examining the functions of structures within this system has frequently ignored the contribution of PPTg. The delayed spatial win-shift (DSWS) task is a task that measures spatial working memory and, as such, has been used by Phillips and colleagues to reveal the involvement of ventral striatal structures in this form of “executive” behaviour. This suggests that structures interconnected with PFC share in some way frontal functions. As might be expected from its connections, the involvement of PPTg in the same task has also been demonstrated (Keating & Winn, 2001). The current research was designed to assess further the involvement of PPTg in the DSWS task, and to compare this directly to the involvement of prefrontal cortex (PFC). In order to achieve this, rats with excitotoxic lesions of PPTg and medial PFC were assessed on the DSWS task, in addition to rats with crossed unilateral disconnection of medial PFC/PPTg. Statistically, results demonstrated both PPTg and medial PFC lesions produced similar impairments in the test phase of DSWS, characterised by increased errors, earlier error occurrence, and slower latencies. In contrast, disconnection lesions of medial PFC/PPTg produced an impairment that was fundamentally different. It was concluded that while this supports the importance of PPTg functioning within fronto-striatal systems, the pattern of impairment shown by disconnected rats suggested that this function may not be *executive* per se but might be *necessary* for executive functions to influence behaviour.

1. INTRODUCTION

1.1 What is Executive Functioning?

The term “executive function” is a notoriously difficult one to define. It is often closely linked with the functioning of the frontal cortex and hence disorders of neural processing in this region are synonymously referred to as frontal syndrome or dysexecutive syndrome. However, it is important to note how misleading this concept is since dysexecutive syndrome can be observed in patients without direct damage to PFC. Thus, although the role of the PFC is in executive functioning, executive functions may not be solely restricted to the PFC. Robbins (2000) defines executive functioning as:

“that set of cognitive control processes that serve to optimise performance in complex tasks engaging the dedicated processing modules”¹.

The benefit of this definition is that it can be used to describe executive functioning comparatively. In other words, while there is as yet no clear-cut, precise definition of what an executive function is, there is a better understanding of those functions that do *not* constitute an executive function. By this approach, executive functioning is not just the use of memory or attention (for which there are dedicated processing modules in the brain that can be assessed in simple, yet specifically designed, tasks). Instead it is the *control* of these processes in a situation that requires both complex and flexible responding. The crucial aspect of such a situation is the need for continued monitoring

¹ Robbins, T.W (2000) Chemical Neuromodulation of Frontal-Executive Functions in Humans and Other Animals. *Exp. Brain Res.*, 133, 130-138, p.130.

and updating of information in the functioning of the dedicated processing modules. A more detailed examination of the theoretical issues relating to executive functioning and the PFC in the rat and primate brain can be found in Chapter 3.

1.2 Considering the PPTg in Terms of Executive Functioning

In this thesis it is argued that the PPTg, a term taken to refer to both Ch5 neurons and the adjacent non-cholinergic neurons, is extensively interconnected with basal ganglia structures. It is also suggested that the PPTg has been shown functionally to be in receipt of neural information from both dorsal and ventral striatum. Precise details of these arguments are presented in Chapter 4 in support of the theory that the function of the PPTg should be considered in relation to the functioning of the prefrontal cortex. In particular, striatal structures, in addition to the rest of the basal ganglia and their output structures, have been suggested to enjoy close functional relationships with the frontal cortex (Rolls, 1999). In fact, projections arising from the frontal cortex are shown in Chapter 2 to progress in a series of looped circuits down the neuraxis, to the output structures of the basal ganglia, and back to the cortex via the thalamus. The main argument of this thesis is that the executive functions of the prefrontal cortex may be distributed to other structures involved in the basal ganglia looped circuitry, including the PPTg.

Behavioural evidence for this comes from the fact that the deficits associated with damage to the frontal lobe can be classified as disinhibited, inappropriate and perseverative responding, and that these descriptions can be applied with relevance to the behavioural deficits observed following PPTg lesions (see Winn 1998 and Chapter

4). In light of this idea this thesis extends previous work showing that the random foraging (RF) and delayed spatial win-shift (DSWS) radial maze tasks used by Phillips and co-workers to examine fronto-striatal system involvement in executive cognitive processing have also been used to reveal deficits of a marked nature in PPTg lesioned animals (Keating & Winn 2001). On these tasks, rats with PPTg lesions exhibited what could be described as perseverative responding. That is, they continued to revisit locations positively associated with reward to a far greater extent than sham operated rats, even when they had been pre-surgically trained to an optimal criterion performance, and regardless of whether these locations had to be remembered over a delay period (DSWS) or not (RF).

In terms of executive functioning the eight arm radial maze DSWS task is a valid assessment since it requires memory for the spatial location of food reward in a situation that demands the constant monitoring and updating of progress. Rats must remember in which arms they found food rewards prior to a delay and win-shift from those locations to find food rewards only in the arms that were not previously baited. This process involves the development and online maintenance of a planned foraging strategy in the post-delay phase that is subject to change upon both a successful and an unsuccessful visit to an arm. Therefore it would seem that rats with PPTg lesions experience problems with these aspects of executive function. To date, however, the involvement of the PFC in the DSWS radial maze task appears to have been studied only under a temporary lidocaine procedure (discussed in Chapter 3) making the true extent of this deficit difficult to compare with that found in PPTg lesioned rats by Keating & Winn (2001). The aim of the work presented in this thesis was to understand the involvement of both the rat PPTg and PFC in the DSWS task in more detail, and to

demonstrate using crossed unilateral disconnection lesions of the PPTg and PFC, that the PPTg is in receipt of neural information originating from the PFC.

2. THE BASAL GANGLIA

The basal ganglia are a collection of extensively connected brain nuclei that lie ventral to the cerebral cortex. These nuclei include the globus pallidus, and the caudate nucleus and putamen (more often referred to as a single brain structure by the terms “caudate putamen” or “neo-striatum”) in addition to the nucleus accumbens and the ventral pallidum. Interest in these structures has come about largely as a result of clinical studies indicating that, in humans, lesions have led to various disorders ranging from hypo-kinetic to hyper-kinetic functions. These are apparent in diseases such as Parkinson’s disease and Huntington’s chorea. Despite the obvious motor impairments persisting in such disorders, however, the issue of whether the basal ganglia can be considered to be involved only in motor function has been a subject of much debate (see discussions by Cools, Van den Bercken, Horstink, van Spaendonck, & Berger, 1984, Marsden, 1982, & Oberg & Divac, 1979). This is particularly relevant given that the basal ganglia have been shown to participate in the processing of cortical information of an associational nature, in addition to information arising from cortical motor areas. Given their location, and the fact that they receive such extensive cortical information, it seems reasonable to suggest that the basal ganglia serve to complement this range of cortical functions (Parent & Hazrati, 1995). Precisely how they do this, however, depends on the manner in which neural processing is organised both within the basal ganglia and their input and output structures.

Research into the anatomical organisation of circuitry involving the basal ganglia has produced several influential concepts that have generated many approaches to the consideration of basal ganglia function. These range from the notion of “funnelling”

discussed by Kemp & Powell (1971) to the more widely accepted idea of parallel “loops” pioneered by Alexander, DeLong & Strick (1986). Differences between the approaches discussed below occur largely in the issue of extent of integration of information within identified circuits, in addition to the mechanisms proposed to achieve this integration. On the whole, however, it is generally accepted that some integration of cortical information is necessary in order for coherent behaviour to be produced. Current knowledge regarding basal ganglia circuitry owes much to developments in tracing studies that have dramatically increased the extent to which brain anatomy can be visualised. These developments range from the early Nauta silver stains revealing lesion induced axonal degeneration, to single axon/single cell labelling. With these improvements in methodology, however, the basal ganglia have appeared vastly more complex, consisting of an intricate mosaic of neurotransmitters and interconnections difficult to comprehend. Recent research, for example, has argued that theories of basal ganglia function must take account of the axonal collateralisation existing in projections from output structures of the basal ganglia (Parent *et al.* 2000), this collateralisation being something that was not apparent in the early axonal degeneration tracing studies. Despite this unarguable complexity, however, generalised patterns of anatomy are still accepted as recognisable within the basal ganglia, enabling some, albeit abstract, conclusions regarding basal ganglia function.

2.1 The neo-striatum as a “funnel”

Early attempts to delineate the function and connections of the basal ganglia were concerned with examining the organisation of efferent and afferent projections of the caudate nucleus and putamen, focusing particularly on cortico-striatal connections

(Kemp & Powell, 1970). This study, in the monkey, identified degenerating axons following cortical lesions and confirmed previous work showing that the caudate nucleus and putamen received extensive projections from the entire cortex, which end predominately on dendritic spines (Kemp, 1968). These projections were shown to be ipsilateral (Kemp & Powell 1970, Carman, Cowan, & Powell 1963, & Webster, 1965, 1961) except for a small bilateral projection from the sensori-motor cortex (Kemp & Powell, 1970, & Carman, Cowan, Powell, & Webster 1965). Furthermore, since all areas of the cortex were found to project to both the caudate nucleus and the putamen in this study, it was suggested that these be considered as one undifferentiated structure (something that had remained questionable until this time).

Significantly, Kemp & Powell (1970) demonstrated that the cortico-striatal projections identified in their study were organised in a topographical manner such that the frontal lobe projects largely to the anterior part of the head of the caudate nucleus and the visual cortex at the occipital lobe projects to the posterior part of the striatum. This arrangement exists with a degree of overlap in the striatum of areas of projection from different regions of the cortex, leading Kemp & Powell to conclude that:

"It is unlikely... that any part of the caudate nucleus or the putamen is under the sole influence of one structural or functional area of the cortex" ¹.

Based on these findings, and to explain the diminishing number of neurons occurring from cortex to caudate nucleus and putamen, Kemp & Powell (1971) proposed that the striatum was involved in integrating a diverse range of cortical information and then

¹ Kemp, J. M. & Powell, T.P.S (1970) The cortico-striate projection in the monkey. *Brain*, 93, 525-546, p.539.

funnelling this input, via output structures, to the ventrolateral thalamus and the motor cortex. That is, the function that they proposed for the striatum was the narrowing of the diversity of cortical information rather than simply relaying it on to basal ganglia output structures. In particular, they argued that this provided a route whereby information from cortical association areas could influence motor cortex thereby participating in the initiation and control of movement.

In addition, evidence from studies preceding those of Kemp & Powell, and indeed discussed by them (1970), had shown that the caudate nucleus and the putamen project upon the globus pallidus also in a well-ordered and topographical manner. The lateral sections of the striatum, for example, project to the external segment and the medial sections project to the medial globus pallidus or both (Cowan & Powell 1966, Nauta & Mehler 1966, Voneida, 1960, & Szabo 1969, 1968, 1962). In turn, the projections of the two segments of the globus pallidus also differ since the external segment projects to the subthalamic nucleus, and the internal segment projects to the ventrolateral and centromedian thalamic nuclei, as well as the midbrain tegmentum (Nauta & Mehler, 1966). This emphasis on the *divergence* of projections arising to, and from, the globus pallidus suggests that, although funnelling (and thus integration) occurred at the striatal level, it did not appear to do so at the pallidal level. Indeed, this concept of divergence was later expanded to include segregation at the level of all structures involved in fronto-striatal processing (Alexander *et al.*, 1986).

2.2 The basal ganglia in a three -tier system.

Nauta, (1979) proposed a theory of basal ganglia organisation that was based on a reassignment of the priorities of the criteria used to group and delineate structures. He argued that structures should be classified according to common internal histology, input-output patterns, and neurotransmitters, rather than in relation to obvious landmarks such as the internal capsule. This was an idea that he related specifically to the caudate nucleus and the putamen being considered as a single entity. Based on this system of classification, Nauta's model arranged the cortex-striatum-basal ganglia output structures into 3 tiers, forming the first half of the looped circuitry later identified by Alexander *et al.* (1986), and Joel & Weiner (1994). In particular, Nauta was concerned with identifying structural evidence for the integration of information within his system, which he found in 2/3 of his levels. Tier I consisted of the cortex, at which there is an extensive system of cortico-cortical associations, allowing for some mixing of information within this level. Tier II consisted of the striatum (significantly Nauta also included the nucleus accumbens along with the caudate putamen) with some degree of intercommunication arising from local interneuron connections (although this allowed very short range communication). Finally, Tier III comprised the substantia inominata, globus pallidus external segment, globus pallidus internal segment, and the substantia nigra pars reticulata (without any level of intercommunication he argued).

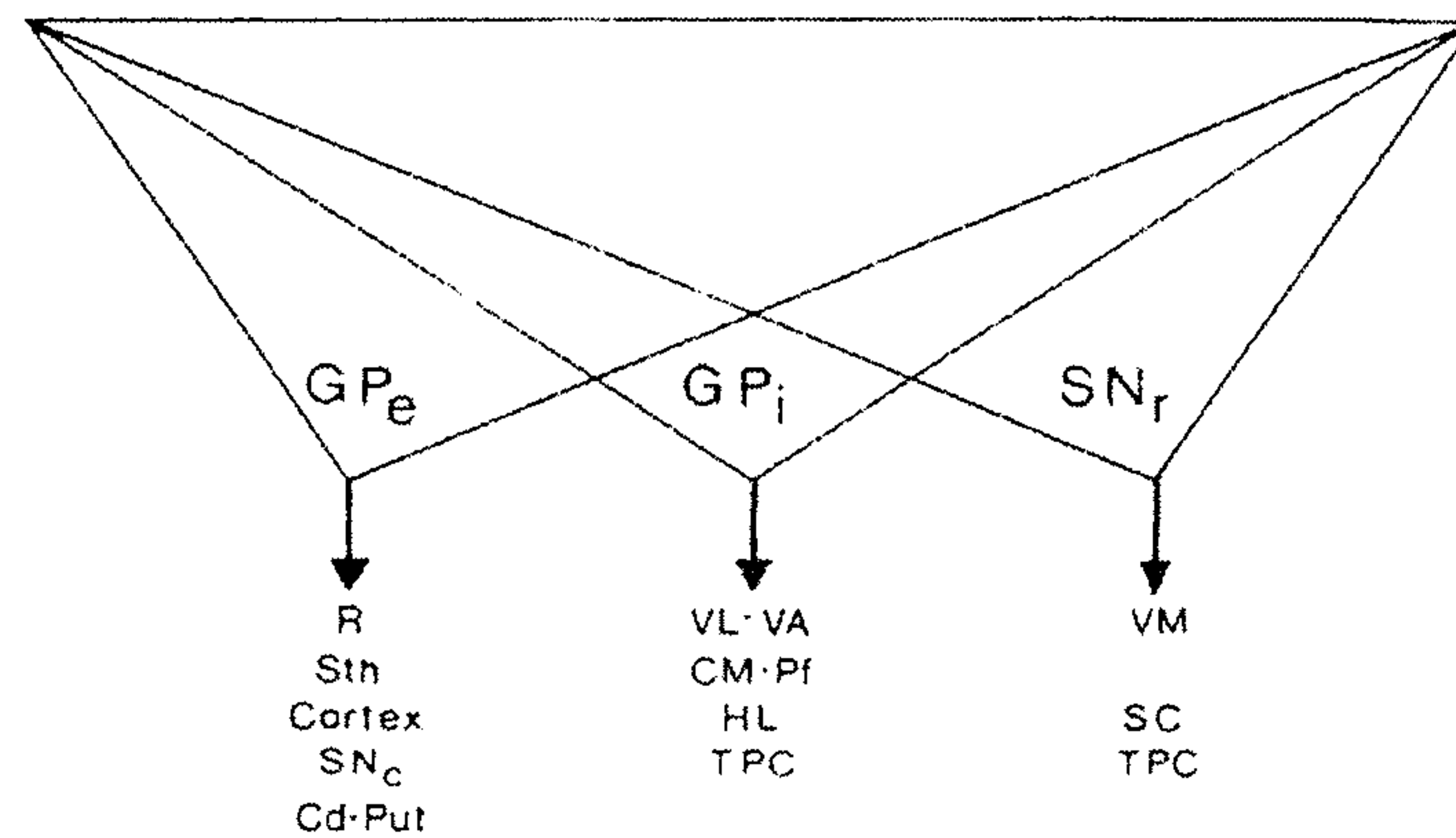
The issue of the extent of integration of information within the circuitry of the basal ganglia is one that has been extensively debated, even recently following the identification of looped circuitry beyond the 3 tiers proposed by Nauta (Joel & Weiner, 1994, & Alexander *et al.*, 1986)). Kemp & Powell's model allowed for integration at

the level of the caudate putamen but did not overtly recognise the divergence occurring beyond this point. The crucial aspect of Nauta's view of the basal ganglia that departs from the concept of funnelling devised by Kemp & Powell is that, although the Tier III structures have common inputs allowing for integration at the level of cortex and striatum, their outputs are distinctive. This led Nauta to propose the existence of three overlapping funnels, and the following metaphor:

"Thus, if the corpus striatum can be compared to a funnel, then one must view it as one with a sprinkler at its spout"²

Interestingly, the model proposed by Nauta (1979) – see Fig. 2.i – includes the pedunculo-pontine tegmental nucleus as a site receiving information processed in 2 of the main proposed overlapping funnels (involving the globus pallidus internal segment and the substantia nigra pars reticulata, but not involving the globus pallidus external segment). This recognition of the PPTg as having an important role in basal ganglia neural information processing is discussed further in Chapter 4, and represents a particular benefit of Nauta's work. This is because an extension of the investigation of the basal ganglia beyond the striatum and its main output structures is something that is likely to be a critical component in the understanding of basal ganglia functioning. Furthermore, the model proposed by Nauta had the additional benefit of extending the number of structures considered part of basal ganglia processing by incorporating the SNr as one of the related structures on Tier III and the nucleus accumbens as related to the caudate putamen in Tier II. The inclusion of these structures in basal ganglia processing is something upheld by most of the models that followed Nauta's.

² Nauta, H. J. W (1979) A proposed conceptual reorganisation of the basal ganglia and telencephalon. *Neuroscience*, 4, 1875-1881, p. 1877.

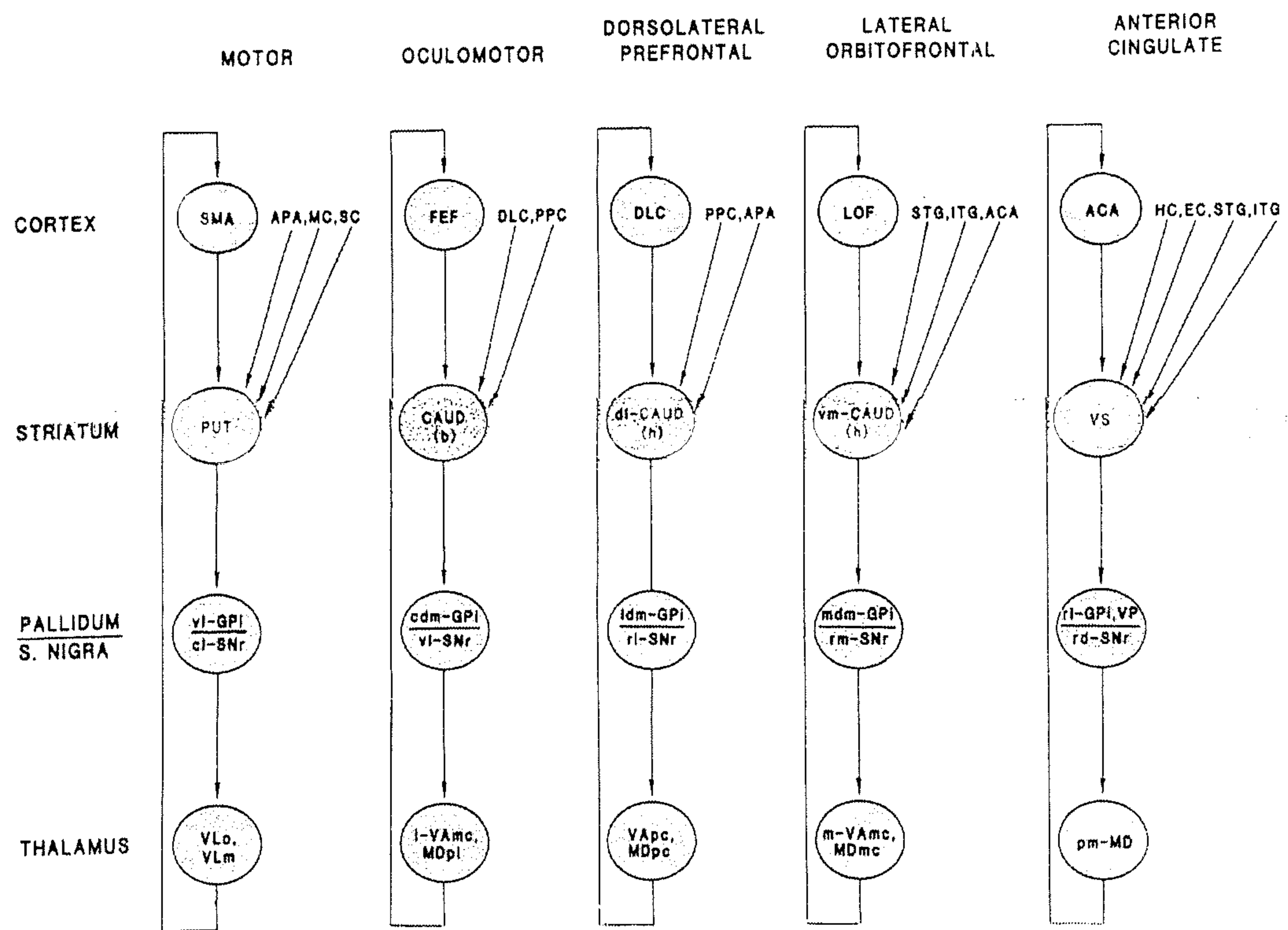
FIGURE 2.i Nauta's Model – Overlapping Outputs of Tier III (1979)

GPe – Globus pallidus external segment, GPi – Globus pallidus internal segment, SNr – Substantia nigra pars reticulata, R – Thalamic reticular nucleus, Sth – Subthalamic nucleus, SNc – Substantia nigra pars compacta, Cd-Put – Caudate putamen, VL-VA – ventrolateral/ventroanterior complex of thalamus, HL – Lateral habenula, TPC – Pedunculopontine tegmental nucleus, VM – ventromedial nucleus of thalamus.

2.3 The parallel organisation of functionally segregated circuits.

Anatomical and physiological findings subsequent to the concepts proposed by Kemp & Powell (1971) & Nauta (1979) argued instead for an apparent maintained segregation of influences, specifically from the sensori-motor cortex and association cortex to the striatum and the rest of the basal-ganglia thalamo-cortical pathway (DeLong & Georgopoulos, 1981). The data presented provided evidence to suggest the existence of at least 2 looped circuits. These comprised a motor loop passing through the putamen

on to premotor cortical areas, and an association loop passing through the caudate nucleus to the prefrontal cortex (DeLong, Georgopoulos & Crutcher, 1983, & DeLong & Georgopoulos, 1981). However, the ignorance of the ventral striatum prevented the inclusion of a third loop in these models. Later work was to extend the number to at least 5 parallel looped circuits, described as the “motor loop”, the “oculomotor loop”, the “dorsolateral prefrontal loop”, the “lateral orbitofrontal loop”, and the “anterior cingulate loop” (Alexander *et al.*, 1986). All of the loops identified were therefore centred on respective parts of the frontal lobe, moving away from the consideration of the striatum as a site integrating input from the entire cortex (as in Kemp & Powell, 1970), since only the frontal lobe receives information in return from the basal ganglia (Groenewegen, Wright & Uylings, 1997). None of the loops, however, appeared to pass through the globus pallidus external segment, an area included in Tier III in Nauta’s model, and only one of them (the anterior cingulate circuit) was identified as passing through the ventral striatum – see Figure 2.ii.

FIGURE 2.ii The 5 basal ganglia loops (Alexander *et al.*, 1986)

ACA – Anterior cingulate area, APA – Arcuate premotor area, CAUD – Caudate, (b) body, (h) head, DLC – Dorsolateral prefrontal cortex, EC – Entorhinal cortex, FEF – Frontal eye field, GPI – Globus pallidus internal segment, HC – Hippocampus, ITG – Inferior temporal gyrus, LOF – Lateral orbitofrontal cortex, MC – Motor cortex, MDpl – Paralamellar segment of mediodorsal thalamic nucleus, MDmc – Magnocellular segment of mediodorsal thalamic nucleus, MDpc – Parvocellular segment of mediodorsal thalamic nucleus, PPC – Posterior parietal cortex, PUT – Putamen, SC – Somatosensory cortex, SMA – Supplementary motor area, SNr – Substantia nigra pars reticulata, STG – Superior temporal gyrus, VAmc – Magnocellular division of ventral anterior thalamic nucleus, VApc – Parvocellular division of ventral anterior thalamic nucleus, VLo – Oral ventrolateral thalamic nucleus, VLm – Medial division of ventrolateral thalamic nucleus, VLo – Oral ventrolateral thalamic nucleus, VP – Ventral pallidum, VS – Ventral striatum, cl – caudolateral, cdm – caudomedial, dl – dorsolateral, l – lateral, ldm – lateral dorsomedial, m – medial, mdm – medial dorsomedial, pm – posteromedial, rd – rostradorsal, rl – rostromedial, rm – rostromedial, vm – ventromedial, vl – ventrolateral.

In their model Alexander *et al.* (1986) accept that each circuit receives partially overlapping corticostriatal inputs that are progressively integrated as they pass through each element of the loop. However, they argue that these areas of overlap are from cortical sites that are *related* in nature, and that each circuit retains a partially closed

component by projecting back to one of these sites. This idea retains the concept of funnelling that was prominent in the work of Kemp & Powell (1971), and Nauta (1979), but adds the rider that integration occurs only *within* the segregated parallel functional pathways. Critically, then, Alexander *et al.* (1986) place their emphasis on the extent of parallel segregation within the loops arguing that:

“The elements of each circuit include discrete, essentially non-overlapping parts of the striatum, globus pallidus, substantia nigra, thalamus and cortex”³.

However, the argument about segregation of functionally distinct information is based on a topographical analysis of projections in the basal ganglia system and has been challenged by analysis at the synaptic level (Percheron, Yelnik, & Francois, 1984). Specifically, neurons in the globus pallidus has large disk shaped dendritic arborisations oriented in an orthogonal plane to incoming striatal fibers and these may serve to integrate the output of putamen neurons. Alexander *et al.* (1986) discuss this aspect of the segregation vs. integration debate by accepting this, but they argue that integration is only of putamen outputs that are related in nature. This argument is based on a cited analysis of the motor loop that suggests that integration is carried out along the lines of individual body parts (DeLong, Crutcher, & Georgopoulos, 1985). Consequently Alexander *et al.* (1986) also propose that the motor circuit may be organised as 3 broad sub-circuits dedicated to the processing of information pertaining to the somatotopic organisation of body parts i.e. the arm, the leg, and the face. This multiple channel organisation of the motor circuit has the benefit of helping to clarify how involuntary movements of a single body part, or impairments of movement in these body parts

³ Alexander, G. E., DeLong, M. R., & Strick, P. L. (1986) Parallel organisation of functionally segregated circuits linking basal ganglia and cortex. *Ann. Rev. Neuroscience*, 9, 357-381. p. 359.

(focal dystonias, or focal dyskinesias) may result from restricted damage to specific areas of the basal ganglia.

A related aspect of the model proposed by Alexander *et al.* (1986) is that, given the parallel nature of the 5 circuits and the corresponding synaptic organisation at each level of these circuits, similar neuronal operations should be performed at comparable stages. This could be argued to conform loosely to Nauta's model (with the addition of further tiers in the current model to form the looped sections of the circuits) since he argued that the structures grouped in each tier have comparable anatomy and inputs (as did DeLong & Georgopoulos, 1981). This means that the processing occurring within the pallidum for the motor circuit could be comparable to, for example, that occurring in the pallidum for the dorsolateral prefrontal circuit. However, since Alexander *et al.* present no specific evidence for this, it is a contention that remains debatable.

A particular benefit of the model of Alexander *et al.*, compared to those of Nauta and Kemp & Powell, may be the consideration that is given to what Alexander *et al.* term "subsidiary circuits". These subsidiary circuits are smaller looped circuits consisting of one or two structures that are connected with the 5 proposed, mainly closed, loop circuits. Alexander *et al.* argue that these subsidiary circuits serve to modify transmission in the main circuits with one of these subsidiary circuits suggested to occur around the PPTg (Parent *et al.* 1983). Again this provides evidence for the recognition of the role of the PPTg in terms of basal ganglia functioning. Unlike Nauta's model (which includes the PPTg as a main outflow site), however, the PPTg is proposed to play a role supplementary to that occurring in the main loops. That the PPTg receives connections that would enable it to process basal ganglia information is clear, although

it would appear from an analysis of its efferent and afferent connections, in addition to the findings of lesion studies, that its primary role is more important than its existence in a purely subsidiary circuit would suggest (see Chapter 4). Furthermore, despite the extension in the Alexander *et al.* model of the concepts proposed by both Kemp & Powell and Nauta, the usefulness of a model that excludes the possible function of the external segment of the globus pallidus remains questionable. More recent work has also cast doubt over the extent to which the looped circuits can be characterised as both “segregated” and “closed”.

2.4 Open and interconnected loops.

2.4.1. Integration: is it an essential component of basal ganglia circuitry?

Given that most of the preceding works on the anatomy of the fronto-striatal loops (including those discussed above) are limited to the primate brain, Joel & Weiner’s model (1994) has the particular benefit of discussing both rat and primate basal ganglia anatomy. Because of this, their model is presented here in greater depth than those discussed previously. In particular Joel & Weiner credit the work of Groenewegen and colleagues who described several parallel circuits involving the ventral striatum in the rat (Groenewegen, Berendse, & Haber, 1993, Groenewegen *et al.*, 1991, & Groenewegen, Berendse, Wolters & Lohman, 1990). In their proposal of the existence of at least 3 “split” circuit loops, Joel & Weiner lend support to Groenewegen’s contention that integration of information arising from different basal ganglia circuits is essential in order to produce coherent behaviour. Working with this idea, and the idea that investigation of basal ganglia function must consequently concern itself with *how*

the basal ganglia achieve this integration of information, Joel & Weiner then diverge from Groenewegen's proposed modes of interaction (cortico-cortical connections, dopaminergic innervation of the striatum, and striatal interneurons) by suggesting that interaction is a *central* feature inherent in the design of the loops. They argue:

"...the central characteristic...is an asymmetry in the frontal cortex - basal ganglia relationships, so that while each frontocortical sub field innervates one striatal region, each striatal region influences the basal ganglia output to two frontocortical sub fields"⁴.

2.4.2 Characterising the striatum

In characterising the striatum as the main input structure of the basal ganglia Joel & Weiner also include the ventral striatum, noting the further division of the nucleus accumbens into core (which more closely resembles the caudate putamen) and shell, a level of detail not achieved in previous studies. Furthermore they also describe, in depth, the classification of the dorsal striatum into patch and matrix compartments that can be distinguished due to immunohistochemical distribution of several markers (enkephalin, substance P, dopamine, opiate receptors and calcium binding protein). This distinction between compartments of the dorsal striatum is important because, although both patch and matrix regions receive inputs from all fronto-cortical areas (albeit from different layers - Parent, 1990 & Gerfen, 1989, 1992), the outputs contribute to two different systems. That is, the patch neurons innervate the DA neurons of the SNc (participating in local basal ganglia circuitry – Gerfen, 1992), and the matrix neurons

⁴ Joel, D. & Weiner, I. (1994) The organisation of the basal-ganglia – thalamocortical circuits: Open interconnected rather than closed segregated. *Neuroscience*, 63 (2), 363-379. p. 364.

participate in the main basal ganglia circuitry by innervating the GABA neurons of the SNr and GP. Similarly the diverging outputs of the nucleus accumbens can also be distinguished as local or main loop circuitry, although the organisation of this is more complex (Meredith, Pennartz, & Groenewegen, 1993, Zahm & Brog, 1992, & Voorn, Gerfen, & Groenewegen, 1989).

2.4.3 Fronto-striatal projections

The delineation of rat and primate frontocortical connections in Joel & Weiner (1994) allows for the tentative classification of topography in the striatum into 3 compartments – motor, association, and limbic. They argue that these regions comprise longitudinal strips situated on a rostro-caudal axis. However, they refrain from discussing whether or not these strips consist of areas of overlap.

In the primate the motor striatum is the region innervated by the primary motor cortex and premotor areas, which are the dorsolateral and lateral regions of the caudate putamen (Alexander, Crutcher, & DeLong, 1990, Parent, 1990, Alexander *et al.*, 1986, & Selemon, & Goldman-Rakic, 1985). In the rat the lateral and medial agranular cortices have been regarded as similar to primate primary motor cortex, with the medial agranular rat cortex suggested to have areas analogous to primate supplementary motor and premotor areas (Fabri, & Burton, 1991). These regions of the rat frontal cortex also innervate the lateral caudate putamen, thereby paralleling the existence of motor striatum in primates (Berendse, Galis-de Graaf, & Groenewegen, 1992, & McGeorge & Faull, 1989).

In contrast the central region of the striatum in both rat and primate forms the association compartment, receiving projections from areas of the prefrontal cortex (Walker's 8, 9, 10 & 46) in the primate, and the anterior cingulate cortex in the rat (Parent, 1990, & Uylings & van Eden, 1990). This is in direct contrast to the idea of Alexander *et al.* (1986) that the anterior cingulate cortex participates in a loop passing through the ventral striatum. In the Joel and Weiner (1994) thesis it is also argued that the ventral striatum, or limbic compartment, receives projections from limbic structures such as the hippocampus and amygdala as well as prefrontal cortical areas such as the orbitofrontal cortex, in both primate and rat, and also the agranular insular areas in the rat (Berendse *et al.* 1992, Deniau, & Chevalier, 1992, Groenewegen, Berendse, Wolters, & Lohman, 1990, & Cicirata, Angaut, Cioni, Serapide, & Papale, 1986).

2.4.4 Striatonigral & Striatopallidal projections

Hedreen & DeLong (1991) demonstrated that, in primate, each striatal area projects to both the GPi and the SNr but that these projections do not arise from the same neurons, suggesting segregation of influence. Furthermore, the projections to the GPi from the motor and association areas do not overlap, although they may do so in the SNr (however, overlap does not demonstrate convergence). In the rat there is evidence that both GPi and SNr also both receive from each striatal area (Gerfen, 1985) with the ventral striatum projecting in addition to the ventral pallidum.

2.4.5 Nigrothalamic & Pallidothalamic projections

In primate the GPi innervates the ventral anterior nucleus of the thalamus (VA) while the SNr primarily innervates the mediodorsal nucleus (MD), with some projections to the magnocellular division of the VA (Illinsky, & Kultas-Illinsky, 1987, Illinsky, Jouandet, & Goldman-Rakic, 1985, & Goldman-Rakic & Porrino, 1985). The ventral pallidum innervates the magnocellular division of MD (Haber, Lynd-Balta, & Mitchell, 1993, & Hreib, Rosene, & Moss, 1988) although this is argued not to be as prominent as the VP innervation of the MD thalamic nucleus in the rat (Haber *et al.*, 1993).

Similarly, in the rat, GPi (entopeduncular nucleus) innervates the VA/VL complex and the lateral subdivision of the ventromedial nucleus. The ventral pallidum innervates the MD nucleus (central and medial segments), and the SNr innervates the lateral and medial parts of MD and the ventromedial nucleus (Groenewegen, *et al.*, 1990, & Groenewegen, 1988).

2.4.6 Thalamocortical projections

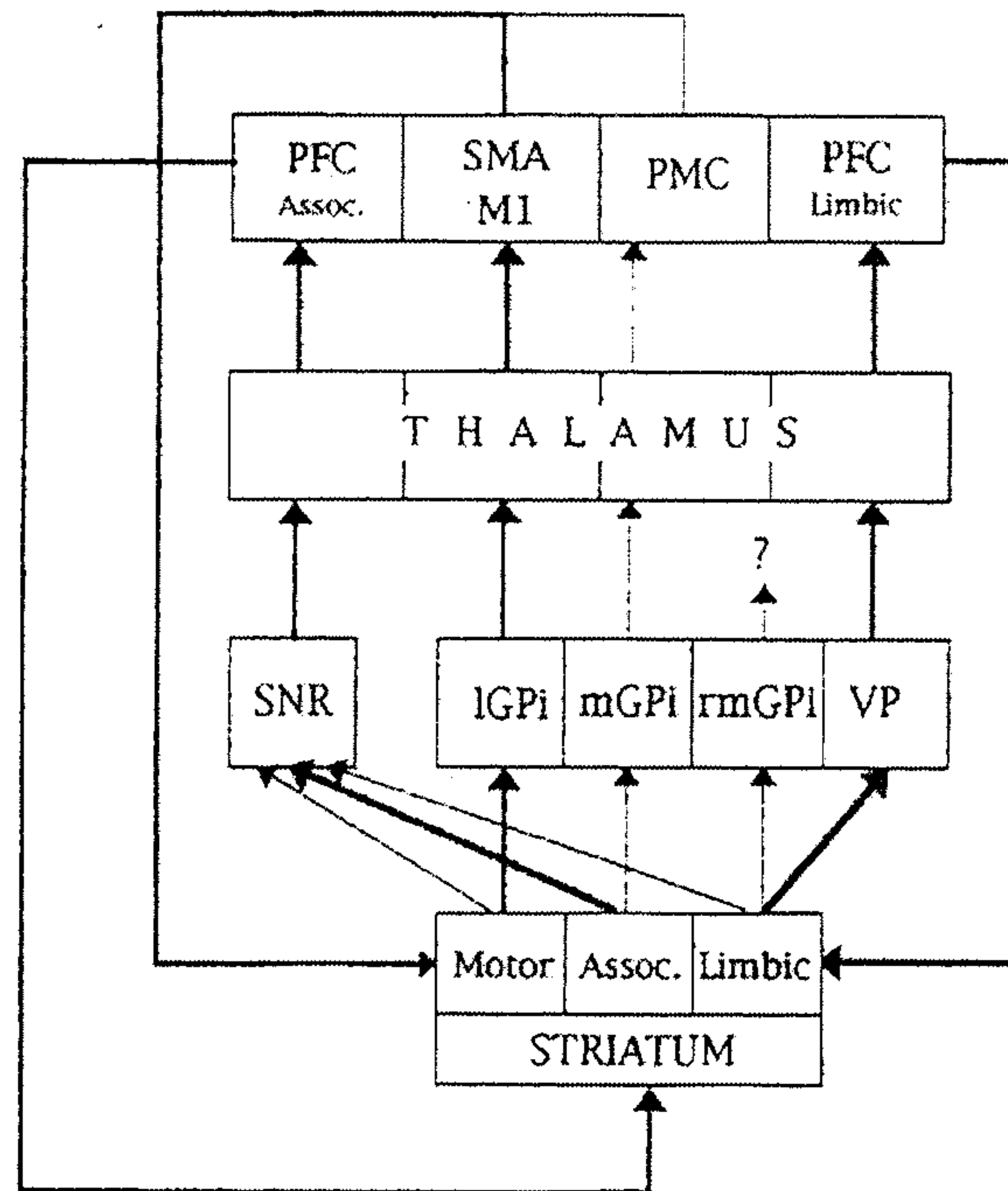
In primate both MD and VA project to partially overlapping regions of the prefrontal cortex (Giguere, & Goldman-Rakic, 1988, & Goldman-Rakic, & Porrino, 1985), although those of MD to the PFC are denser - suggested to comprise 50-80% of thalamic innervation of the PFC (Barbas, Haswell Henion, & Dermon, 1991). In particular the area of VA projecting to the PFC appears to be the magnocellular division, which is innervated, along with the MD, by the SNr. Therefore it would

appear from these connections that the GPi does not innervate the primate PFC via the thalamus (Joel & Weiner, 1994).

In the rat it appears that the entopeduncular nucleus (the primate GPi homologue) influences the motor and premotor areas of the cortex (medial and lateral agranular) through VA/VL and VM (Cicirata, Angaut, Cioni, Serapide, & Papale, 1986, Donoghue, & Parham, 1983, & Jones, & Leavitt, 1974), while the SNr and VP can influence the associative and limbic areas of the PFC respectively through sub-regions of MD (Groenewegen *et al.*, 1990, & Groenewegen, 1988).

2.4.7 Summary of open interconnected circuits

In summary, each striatal region (motor, association or limbic) receives input from a distinct frontocortical sub field and sends output to both the GPi and SNr, with the addition of the VP in the case of the ventral (limbic) striatum. These structures then pass information onto differing thalamic regions that innervate, in turn, differing cortical regions. In addition to this, though, Joel & Weiner note that each striatal region has connections with subcortical areas via the output of the GPi, SNr and VP to structures such as the PPTg, recognising (as did Nauta and Alexander *et al.*) that the PPTg participates in basal ganglia processing.

FIGURE 2.iii Summary of 3 “Split” Loops (Joel *et al.*, 1994)

PFC – Prefrontal cortex, SMA – Supplementary motor area, M1 – Primary motor cortex, PMC – Premotor cortex, SNR – Substantia nigra pars reticulata, IGpi – Lateral globus pallidus internal segment, mGpi – Medial globus pallidus internal segment, rmGpi – Rostromedial globus pallidus internal segment, VP – Ventral pallidum.

Through this divergence of information between the striatum and its output structures each loop originating from one frontocortical region gains access to another cortical region. For example, for the dorsal striatum these include a motor or premotor area (through the GPi), and an associative area (through the SNr). For the ventral striatum these include a limbic area (through the VP), and an associative area (through the SNr), with the possibility of an additional motor area via the GPi. This means that the looped circuitry can therefore be described as cortico-striato-(pallido-thalamo-cortical) + (nigro-thalamo-cortical), as shown in Figure 2.iii.

Thus, interaction comprises the major feature of this model in direct contrast to the emphasis on segregation in Alexander *et al.* (1986) and is argued to occur in the circuits in one of two ways. The open association pathway achieves influence over a motor sub field simply by carrying information up to that area in the open pathway. The motor and limbic open pathways achieve influence over an association area by converging on the SNr (fig. 2.iii). This allows for interaction of information at the level of the cortex and the level of the SNr, rather than at the level of the striatum as in previous models (Kemp & Powell, 1971, & Nauta, 1979).

2.4.8 Functional implications of the open circuit model

Joel & Weiner (1994) argue that their open associative pathway is likely to serve as a feed-forward projection that enables activation of the closed motor circuit by the associative PFC. In contrast, the projections from the SNr of the motor striatum are likely to be feed-backward such that the associative PFC can be updated with the processing occurring in the motor circuit. Such functions could provide the PFC with access to motor systems as well as information concerning ongoing movements. This is something that could be an essential component of the executive functions of the PFC, extending the functional concept of the basal ganglia from one regarding the associational influence on motor function (Kemp & Powell, 1971) to include one of motor influence on association functions.

2.5 Direct & Indirect fronto-striatal pathways

The disappearance of the GPe from among the structures considered responsible for the main looped information flow in the basal ganglia is a result of the recognition of an indirect striatal output pathway – see Figure 2.iv. Following the development of the loops concept the GPe was relegated to performing a subsidiary function, involving the STN, modulating information processing in all loops comprising the main circuitry (Parent, & Hazrati, 1995). However, the role of the GPe may be of greater importance than this simple subsidiary function had originally suggested. The GPe receives massive projections from the GPi & SNr, as well as the thalamic reticular nucleus, reciprocating these projections via the STN. Parent & Hazrati (1995) have argued that this indicates that the GPe may serve as a control structure for the main outputs of the basal ganglia, and this is supported particularly by Parent *et al.* (2000) who have argued that the outputs of the GPe extend far beyond the STN and virtually all striatal-fugal projections go to the GPe. Accordingly, then, Parent *et al.* (2000), propose that the GPe is a....

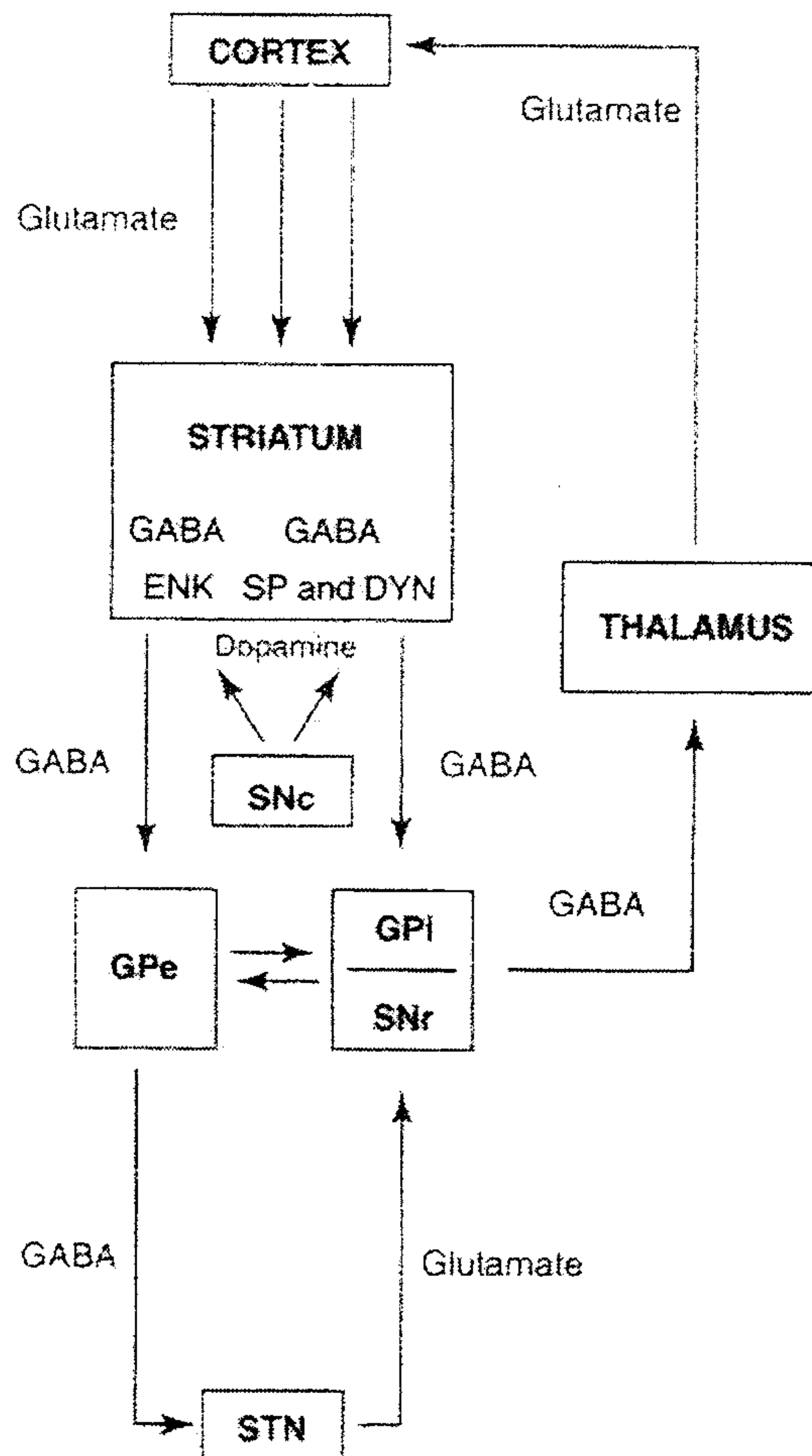
“major integrative nucleus that can affect virtually all components of the basal ganglia”⁵.

Since GPe neurons are GABAergic, the GPe is in a position to inhibit action in both the GPi and SNr, as well as in the thalamic reticular nucleus (TRN). In turn the TRN is known to exert a powerful GABA mediated inhibitory influence over other thalamic

⁵ Parent, A., Sato, F., Wu, Y., Gauthier, J., Levesque, M., & Parent, M. (2000) Organisation of the basal ganglia: the importance of axonal collateralisation. *Trends in Neurosci.* 23(supp) S20-27, S23.

neurons placing the GPe in such a position as to disinhibit these other thalamic neurons, including those receiving output from the GPi and the SNr.

FIGURE 2.iv Direct & Indirect Basal Ganglia Circuits (Parent *et al.*, 2000)



(a) Abbreviations: SNc – Substantia nigra pars compacta, GPe – Globus pallidus external segment, GPi – Globus pallidus internal segment, STN – Subthalamic reticular nucleus, GABA – Gamma-amino-butyric acid, ENK – Enkephalin, DYN – Dynorphin, SP – Substance P.

The functional implications of such an inhibitory action are extremely important since disinhibition of thalamo-cortical VA/VL cells that are kept under tonic inhibition by GPi and SNr in the resting state is essential for the initiation of movement (Chevalier &

Deniau, 1990). This process of disinhibition is ultimately likely to increase the excitability of the frontal cortex, something that is central to the physiology of the basal ganglia. By its excitatory influence on the SNr then, the indirect pathway could participate in the spatio-temporal shaping of this disinhibitory process, contributing to the scaling of movements and the inhibition of competing motor programmes (Mink & Thach, 1993).

Similarly this function could apply in other loops such as the limbic loop. In particular the organisation and function of the ventral striatal loop in the rat, involving the prelimbic (PL) and medial orbital (MO) areas of the prefrontal cortex, has been extensively studied (Montaron, Deniau, Menetrey, Glowinski, & Thierry, 1996, & Deniau, Menetrey, & Thierry, 1994, & Maurice, Deniau, Menetrey, Glowinski, & Thierry, 1997, 1998). Areas PL/MO send an excitatory glutamatergic projection to the core of the nucleus accumbens, which projects, via an indirect and a direct pathway, to the dorsomedial part of the SNr. The indirect pathway in this circuit involves the lateral ventral pallidum and the medial part of the STN. As in motor circuits, the PL/MO direct and indirect pathways could also participate in the shaping of the discharge of SNr neurons, an imbalance in which could be responsible for disturbed prefrontal functions such as perseveration and alterations in attentional and emotional processes (Maurice, Deniau, Glowinski, & Thierry, 1999).

2.6 Basal ganglia function: Integration of motor, associational & limbic information?

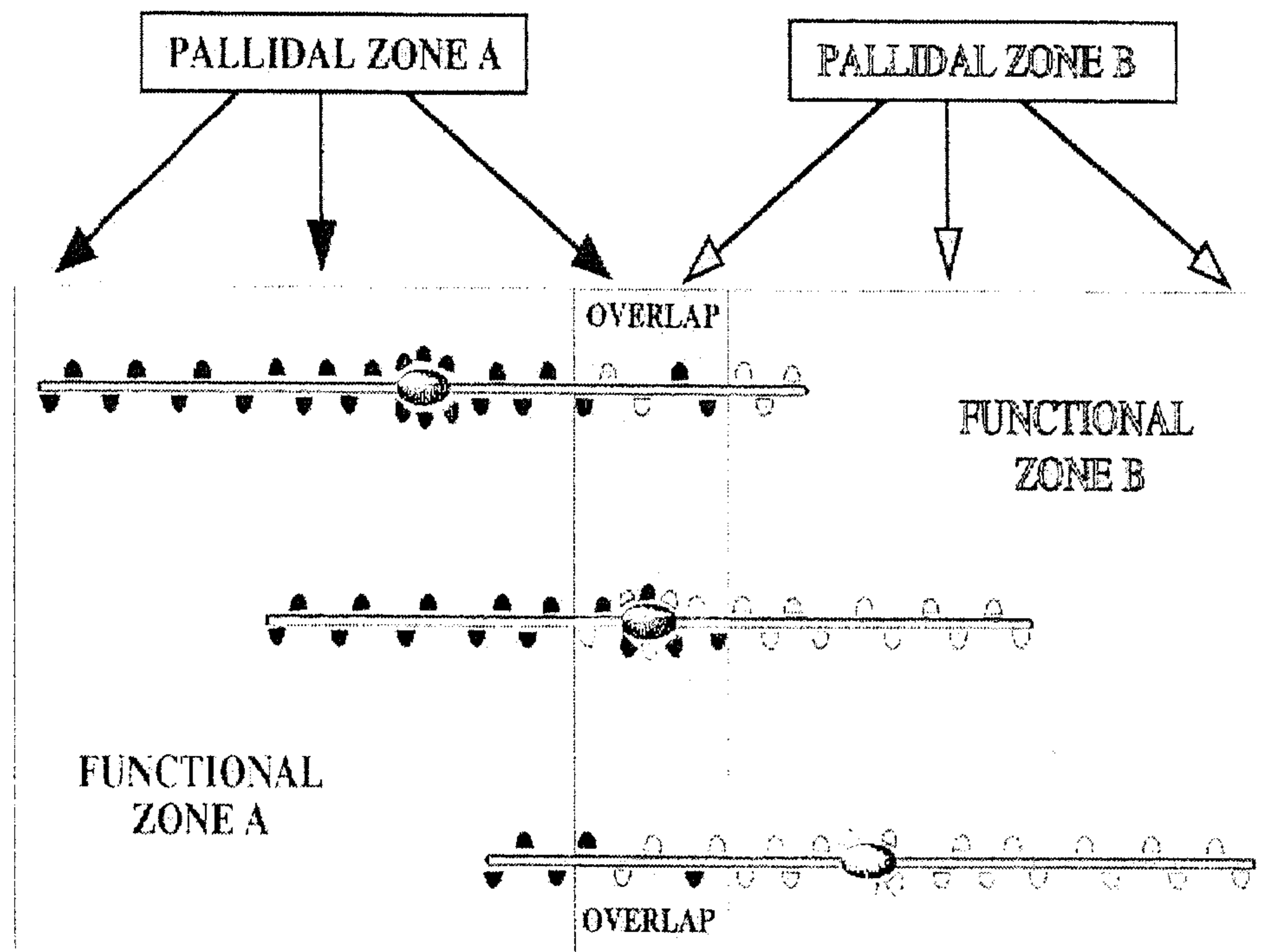
The basal ganglia were initially considered to be the centre for motor activity following the demonstration by Carville & Duret (1875) that a bilateral section through the basal ganglia and internal capsule made an animal both powerless and prostrate. In addition Ferrier's (1876) observations in the rabbit of a striking lack of spontaneous activity on removal of the basal ganglia, led him to conclude that the basal ganglia were the centres of organisation of habitual and automatic movements (both Carville *et al.* and Ferrier cited in Chaudhuri & Behan, 2000). However, extensive research into the anatomical organisation of the basal ganglia has generated much debate over extending their function beyond motor ability. Rolls (1994) noted from his review of neurophysiological investigations into the functions of the striatum that there are differences in neuronal activity that parallel the proposed regions of segregation of function. Furthermore, the signals that activate the striatal neurons in these regions are derived functionally from the cortical region that projects to it (Rolls & Johnstone, 1992). These findings support Parent & Hazrati's view that basal ganglia function complements that of the cortex (1995) such that it can be concluded that the functions of the basal ganglia include functions relating to the processing of limbic and associational information. However, as a result of this diversity of information, Rolls (1994) has concluded that integration must occur in order to produce a coherent stream of behaviour.

The concept of parallel fronto-striatal loops is now widely accepted in literature investigating basal ganglia function, as is the suggestion that these loops have an open

element allowing for the necessary integration of information. However, the apparent complexity of the basal ganglia has meant that a detailed understanding of the functional implication of this integration remains to be achieved. Currently theories relating to the function of the basal ganglia are limited to an abstract level. Nauta (1986), for example, has suggested that the basal ganglia function to link spontaneous motor activity to the executive pathways such that they provide a neurological substrate whereby interoceptive or motivational influences could be channelled to the motor system. This functional suggestion expands on those proposed by Alexander *et al.* (1986) & Joel & Weiner (1994) by emphasising reciprocal associative/motor circuit influences with the addition of motivational information arising from the limbic circuit.

Recently, though, studies employing double labelling techniques have provided evidence to support integration occurring at the level of individual neurons of the basal ganglia, suggesting that there are alternative mechanisms of integration of information beyond that supplied by the split circuitry (Bevan, Clarke, & Bolam, 1997, & Bevan, Smith, & Bolam, 1996). These elegant studies have shown that neurons of functionally diverse regions of the pallidal complex have convergent synaptic contacts in SNr, GPi, STN, and DA neurons of the SNc. Two such mechanisms have been demonstrated including integration at the cell body and proximal dendrites (where information related in nature can be integrated), in addition to convergence at distal dendrites (where more diverse information could be integrated - see Fig 2.v).

FIGURE 2.v Schematic Diagram of Integration of Functionally Distinct Pallidal Information – Bolam *et al.* (1997)



“Schematic summary of the somatic and dendritic modes of synaptic integration of descending, functionally distinct pallidal projections in the subthalamic nucleus revealed by double anterograde labelling and electron microscopy. The pallidal complex provides projections to the subthalamic nucleus that largely maintain the functional topography. Adjacent populations of neurons, illustrated by PALLIDAL ZONE A and PALLIDAL ZONE B and giving rise to black and white boutons, respectively, although mainly innervating separate but adjacent regions of the subthalamic nucleus, also give rise to a region of overlap....” Bolam *et al* (1997) p.322.

However, this convergence of output arising from the pallidum occurs in the *indirect* striatal circuit that is involved (as mentioned previously) in the resting inhibitory output of the basal ganglia. Such findings do not refute the necessity of the split circuit model of Joel & Weiner (1994), therefore, but instead indicate that mechanisms of integration

are also required within control circuits that modulate the activity in the main loops. Despite the importance of this finding though, Bevan *et al.* (1997) do not discuss any functional implications in depth, preferring to argue that:

“the parallel but distributed and partially convergent network of the descending projections of the pallidal complex provides an anatomical basis for the association of functionally diverse information that may be of importance in the production of integrated behaviour and learning”⁶.

In sum, then, it seems that despite developments in both methodology of investigation and resultant knowledge, the complexity of the basal ganglia makes precise conclusions relating to function rather difficult. Rolls (1994) has concluded from his analysis of the functions of the striatum that this structure is involved in switching or altering behaviour as appropriate depending on information received from differing cortical areas. Given the evidence described above however, it would seem that this is a function that could be attributed to the whole of the basal ganglia, including the influence of indirect circuits. This suggests that the basal ganglia are responsible for structuring behaviour, both learned and habitual, such that they provide a mechanism of action generation that, when disrupted, leads to an inability to respond appropriately to external cues on the basis of internally generated coordinated sequences of behaviour. Structures that communicate with the basal ganglia therefore (including the PPTg) are likely to play a significant role in the production of contextually appropriate behaviour. Attempting to understand the details of this involvement would seem to be a useful step in the further delineation of basal ganglia function.

⁶ Bevan, M., D., Clark, N., P., & Bolam, J., P. (1997) Synaptic integration of functionally diverse pallidal information in the entopeduncular nucleus and subthalamic nucleus in the rat. *J. Neurosci.* 17(1): 308-324. p. 322.

3. THE PREFRONTAL CORTEX: ANATOMY & FUNCTION

3.1 Executive functions & issues of debate

In humans, the area of the frontal lobe that can be defined as the prefrontal cortex has reached its most advanced state of evolution, comprising approximately 30% of the total volume of cortex (Uylings, & van Eden, 1990). This fact led early theorists to suggest that the PFC was the seat of human intellect and abstract reasoning processes. However, the involvement of the PFC in such functions was soon doubted since lesions to this area did not seem to produce deficits in IQ. In all species there is a degree of morphological and functional differentiation in the PFC, with this differentiation also reaching its highest level in the human brain. As a result of this differentiation, there still exists a general difficulty in surmising a function for this region of the cortex, especially since it is not directly connected to any primary channels of sensory information, nor to motor neurons (Groenewegen, & Uylings, 2000).

The existing pattern of anatomical connectivity of the PFC (summarised below from Groenewegen & Uylings 2000, & Uylings & van Eden, 1990) has led to many theories regarding PFC function. One of these is that the prefrontal cortex is homogenous in function. For example, the PFC has been proposed to exert an executive influence in the brain such that its primary role is in controlling and directing processing functions that are associated with other brain regions. However, not all homogeneity theories agree with the notion of a “central executive”. For example Fuster (1997) has suggested that the many functions that have been proposed for the PFC can be categorised under a

general function relating to the temporal organisation of goal directed behaviours and this remains a highly influential theory.

The alternative view in this debate is that the PFC is heterogeneous rather than homogenous in function (Roberts, Robbins, & Weiskrantz, 1998). However, this notion further complicates the issue of PFC function by introducing a variety of cognitive components such as working memory, attention, response selection, flexibility, and strategic planning that are extremely difficult to dissociate from one another in an experimental paradigm. Likewise, there are a multitude of theories covering how these processes are organised in the PFC, including the extent to which the PFC is regionally specialised across species, and whether these proposed multiple functions are arranged hierarchically or in parallel.

Interpreting the experimental findings regarding the issue of PFC function is therefore highly problematic. Individual studies usually opt for one or more of the heterogeneous functions listed above although whether these can ultimately all be grouped under a single overriding function remains questionable. In order to begin to review these theories of PFC function, however, it is necessary first to draw comparisons between the definition and connectivity of the PFC in both primates and rat, since these are the species most commonly used in the experimental studies. Furthermore, it is important to demonstrate for the purposes of the current research that the executive functions proposed to exist in the primate brain are to some extent comparable to those of the rat brain. To achieve this aim, what follows is a review of the structural homology/functional analogy debate regarding the link between rat and primate PFC.

3.2 Anatomical delineation of the prefrontal cortex

The prefrontal cortex (PFC) constitutes the cerebral cortical area in the frontal lobe, rostral to the motor and premotor cortices (Groenenwegen & Uylings, 2000, Uylings & van Eden, 1990). Although in most mammalian species a prefrontal cortex can be identified, a single morphological or functional criterion for delineating the prefrontal cortex in all species has not been found. Rose & Woolsey (1948), based on early knowledge of PFC thalamic connectivity, proposed that the PFC could be defined as the area of the cortex that receives connections from the mediodorsal thalamic nucleus (MD), since MD was believed to be the only thalamic nucleus that was connected to PFC. However, following improvements in staining methodology it became clear that other thalamic nuclei, such as the midline and intralaminar nuclei, projected to PFC also. In addition Giguere *et al.* (1988) showed that some MD projections reach areas of the cortex other than the PFC. Given that this is the case, Uylings & van Eden (1990) have suggested that the delineation of PFC based on reciprocal connections with MD might best be categorised as the area of the cortex that receives the *most dense* innervation by MD, and this is the hodological definition adopted in the present research.

According to this delineation, the PFC in the primate may be grossly subdivided into 2 main areas. These divisions include the dorsolateral PFC and the orbitomedial PFC (Passingham, 1993, & Roberts *et al.* 1998), a model that concurs with a general functional distinction of cognitive vs. socio-emotional processing respectively (Rolls, 1999). However, within this general division, sub-divisions are identifiable (Petrides & Pandya, 1994) a factor which becomes apparent in the later discussion of the afferent

and efferent connections and functions of the PFC in the primate. Thus, it should be noted that the division of primate PFC into dlPFC and omPFC suggested here is one example only.

In the rat the PFC can be divided into a medial, a lateral, and a ventral/orbital region. However, as in primates, even smaller subdivisions are recognisable (Groenewegen, 1988, & Krettek & Price, 1997a, 1997b). For example, the medial section contains the anterior cingulate, prelimbic and infralimbic cortices. The premotor region (Fr2) is also sometimes included in this division although it has mixed premotor and prefrontal characteristics. In contrast the lateral section contains the dorsal and ventral agranular insular areas.

3.3 Afferent & efferent connections of the PFC in rat & primate

3.3.1 Cortico-cortical connections

In primates the delineation of the PFC into a dorsolateral and an orbitomedial section can be reflected by differences in the patterns of cortico-cortical connections. In the dorsolateral PFC these have been discussed by Pandya, & Yeterian (1998), and in the orbitomedial PFC they have been investigated by Carmichael & Price (1995a),(1995b), & 1996. The following discussion provides only a brief review of these papers and the reader is referred to the original works for more details.

In the dorsolateral PFC (dlPFC) inputs range from frontal premotor & orbitomedial areas, to parietal, temporal & cingulate cortices. The parietal and temporal areas that

project to the dlPFC themselves receive information from visual areas in the occipital lobe, as well as somatosensory information from the parietal lobe and auditory information from the temporal lobe. Projections from the anterior cingulate cortex and the orbitomedial areas of the cortex may also allow the dlPFC access to somatosensory information at a higher level of integration than that coming from the parietal lobe. Thus the dlPFC receives information from most sensory modalities.

In addition the dlPFC has been suggested to be in receipt of two differing streams of information regarding the senses. These are a dorsal stream and a ventral stream, apparent mostly in the integration of visual and somatosensory information, such that the dlPFC dorsal to the principal sulcus receives information pertaining to “where” (spatial orientation) while the dlPFC ventral to this sulcus receives information relating to “what” (object recognition) – Courtney, Ungerleider, Keil, & Haxby (1996), & Wilson, O’Scalaidhe, & Goldman-Rakic (1993). Pandya and Yeterian (1998) have also shown that these two main areas of the dlPFC have profuse interconnections such that the integration of spatial and non-spatial aspects relating to the perception of the external environment might also occur in dlPFC. This may be particularly important if the PFC is involved in the generation of appropriate behaviour.

Likewise the orbitomedial PFC (omPFC) in primates has been shown to consist also of sub regions, generally divisible on a rostro-caudal axis. Carmichael & Price (1995a) have argued that the distinct patterns of cortico-cortical connections allow the caudal orbital PFC to be classified as involved in visceral and olfactory related processing, while the rostrolateral PFC can be classified as involved in processing of sensory and motor related information. As a whole, the omPFC allows for a high degree of

integration of limbic and sensory information that is supported by a vast connective network of sub-regions with highly specific patterns of overlapping inputs.

In rats the PFC is also extensively interconnected with premotor, somatosensory, auditory, visual, olfactory, gustatory, and limbic cortical areas. However, the patterns of connectivity are less well differentiated than in primate. In the medial PFC a particular subdivision is important. The frontal area Fr2 (Uylings & van Eden, 1990) and the dorsal anterior cingulate area (dAC) can be distinguished from the ventral anterior cingulate area (vAC) and the prelimbic (PL) and infralimbic (IL) areas. As mentioned previously, area Fr2 has mixed premotor and prefrontal characteristics and, along with the dAC, is in receipt of mostly somatosensory and motor information. In contrast the vAC/PL/IL areas receive information from the perirhinal and entorhinal cortices, and the dorsal and ventral agranular insular areas. For example gustatory and olfactory information is projected to the ventral portion of the mPFC via selected agranular insular areas. In addition, in the rat, the orbital PFC can be further subdivided into four main parts with specific patterns of connections with AC, Fr2, parietal, occipital, and granular and agranular insular areas (Reep, Corwin, & King, 1996).

3.3.2 *Cortico-thalamic connections*

The main inputs to the PFC from the thalamus are from the mediodorsal nucleus (MD). However, other thalamic inputs are also observable in both rats and primates. For example PFC areas that receive MD innervation are also projected to by the rostral parts of the ventral complex and the pulvinar (Uylings & van Eden, 1990, & Illinsky, *et al.* 1985)), as well as by the midline and intralaminar nuclei (Berendse, & Groenewegen,

1991, Uylings & van Eden, 1990). Furthermore MD also projects to the premotor and primary motor areas of the cortex (Illinsky *et al.*, 1985, & Goldman-Rakic & Porrino, 1985).

The fact that the PFC also receives projections from other thalamic nuclei is particularly important with regard to function. This is because these thalamic nuclei form relay stations in the basal ganglia thalamo-cortical loops (see Chapter 2). For example the midline and intralaminar nuclei in both rats and primates have very extensive topographically organised projections to the striatum in addition to their cortical projections (Groenewegen & Uylings, 2000). This suggests a comparable level of anatomical organisation between primate and rat at least in the important aspect of PFC functioning, in relation to basal ganglia circuitry.

In primates the MD can be divided into three main segments. These are a medial magnocellular segment that is reciprocally connected to the orbitomedial PFC, a lateral parvicellular and multiform segment that is connected to the dorsolateral PFC, and a paralamellar or densocellular segment that is connected to the peri-arcuate region of the PFC (frontal eye field - FEF). The medial MD segment receives information from olfactory cortex in addition to the basal forebrain, basal amygdaloid nuclei, the medial SNr, and several brainstem nuclei (Illinsky *et al.* 1985, & Gower, 1989). In contrast the lateral MD receives information from the anterolateral SNr, the superior colliculus, the midbrain reticular formation, the medial vestibular nuclei, and the spinal cord (Illinsky *et al.*, 1985, & Harting, Huerta, & Frankfurter, 1980). Finally, the paralamellar MD segment receives input from the deep cerebellar nuclei, the caudal SNr and the spinal cord (Illinsky *et al.*, 1985)

In rats the medial segment of the MD projects primarily to the PL/IL and medial orbital areas of the medial PFC in addition to the agranular insular area in the lateral PFC. It receives information from the basal forebrain, lateral hypothalamus, peribrachial nucleus and nucleus of the solitary tract, the amygdala, entorhinal cortex, subiculum and endopiriform nucleus, allowing it to pass on information of both a limbic and visceral nature to receiving areas of the PFC. A central MD segment is also distinguishable in the rat and this forms a relay for olfactory inputs (via olfactory tubercle and piriform cortex) to the ventral agranular insular area (Groenewegen & Uylings, 2000). Finally the lateral and paralamellar segments primarily relay brainstem inputs (e.g. from superior colliculus, midbrain reticular formation) to the anterior cingulate and Fr2 regions of the PFC.

3.3.3 *Connections with limbic structures*

In primates the PFC is directly and reciprocally connected with both the amygdala and the hippocampus. Hippocampal fibers in primates originate from the rostral hippocampus, terminating in the medial and caudal orbital areas of the PFC. However it is not yet known whether these fibers originate from area CA1, the subiculum, or both (Groenewegen & Uylings, 2000). Projections are also present from the dlPFC to the hippocampus, although these are not strong. The most influential route for the PFC to access the hippocampus is via the parahippocampal cortices (Goldman-Rakic, Selemon, & Schwartz, 1984). In addition the amygdala most strongly projects to the medial and orbital regions of the PFC but there is a lighter innervation occurring to the lateral PFC (Amaral, & Price, 1984). The projections between the PFC and the amygdala are

suggested to be topographically organised and mostly reciprocal (Carmichael & Price, 1995a).

In rats both the CA1 and subiculum regions of the hippocampus project to the medial PFC (PL/IL, medial orbital, and lateral areas) with the strongest innervation occurring from the ventral hippocampus (Groenewegen & Uylings, 2000). The medial and lateral areas of the PFC in the rat also receive projections from the amygdala (stemming primarily from the basal amygdaloid complex – Wright, & Groenewegen, 1995).

3.3.4 *Hypothalamic connections*

Through its direct reciprocal connections with the hypothalamus the PFC is able to exert control over autonomic and endocrine functions. This may be particularly important with regards to its hypothesised involvement in complex behaviour given that these reciprocal connections would provide PFC with interoceptive information in addition to the exteroceptive sensory information received from the cortico-cortical connections. In primates the areas of the PFC that project to the hypothalamus have been shown to be Walker's areas 25 and 32 on the medial wall (which project to the anterior area and ventromedial nucleus), in addition to the caudal part of the orbital PFC (which projects to the posterior hypothalamus). In rats the areas that project to the hypothalamus are PL and IL regions. These provide the densest innervation of the hypothalamus from regions of the PFC and occur to the lateral and medial areas respectively.

The hypothalamus plays a role in various goal directed behaviours that include foraging, eating, drinking, sexual behaviour and stress behaviour. The PFC may therefore

function to supervise hypothalamic involvement in these behaviours, although hypothalamic/cortical connections with areas of the brainstem may also be important (see Groenewegen & Uylings 2000 for a more comprehensive discussion of this).

3.3.5 *Relationships with cholinergic and monoaminergic systems*

The functioning of the PFC can be modulated by inputs from cholinergic cells in the basal forebrain, and from monoaminergic cells in the brainstem (including serotonergic input from the raphe nuclei, noradrenergic input from the locus coeruleus, and dopaminergic inputs from the ventral tegmental area and substantia nigra pars compacta. In return the PFC also projects to these systems.

The dopaminergic innervation of the PFC (from SNc to dorsal PFC and from VTA to ventral PFC) has been particularly important with respect to the consideration of the executive functions of the PFC. However, it should be noted that there is a substantial level of dopamine in other areas of the cortex such as the premotor and primary motor cortices (Gaspar, Stepniewska, & Kaas, 1992, & Berger, Trottier, Verney, Gaspar, & Alvarez, 1988). Indeed, the level of comparable innervation of these areas and the PFC differs greatly across species. In the primate the DA innervation of the premotor and primary motor cortex is denser than that of some areas of the PFC, while in the rat the DA innervation of the PFC is denser (Berger, Gaspar, & Verney, 1991). Significantly, the majority of the DA connections with the PFC have also been shown to be reciprocal such that the PFC is able not only to influence other structures, but to engage in self-regulation.

In both rats and primates the cholinergic innervation of the PFC from the basal forebrain is strongest to the medial PFC, which reciprocates these projections, sending an important source of excitatory information to the basal forebrain (Zaborsky, Gaykema, Swanson, & Cullinan, 1997, Sesack, Deutch, Roth, & Bunney, 1989, Lemann, & Saper, 1985, & Mesulam & Mufson, 1984). In primates, other inputs to the ACh neurons of the basal forebrain have been identified including some from the temporal cortex and the amygdala (Aggleton, Friedman, & Mishkin, 1987, & Mesulam & Mufson, 1984). Furthermore, in rats, there is another important source of ACh innervation to the medial PFC arising from the laterodorsal tegmental nucleus (Semba & Fibiger, 1992, & Satoh & Fibiger, 1986).

In both primates and rats the ventromedial PFC is the area believed to selectively contribute to the innervation of the 5HT neurons of the raphe nuclei, and the medial PFC to the NA neurons of the locus coeruleus.

As a result of their diffuse ascending inputs, the projections PFC shares with all these brain systems enables it to exert a profound control over global influences such as mood, stress, reinforcing feedback, arousal, and on processing within all the main telencephalic structures such as the limbic system, thalamus and striatum as well as the cortex. The nature and extent of this controlling influence therefore makes the PFC a unique brain structure.

3.3.6 *Summary of PFC connections with respect to comparable primate/rat anatomy*

In sum, both primate and rat PFC can be divided into several subdivisions. In primate, on a gross scale, these are the dorsolateral and the medial orbital regions. In the rat medial wall (the area of focus of the rat PFC in the current research) a clear division can be made between the dorsal (Fr2 & dAC) and ventral (PL/IL and MO) regions and these can be related to the primate subdivisions. In terms of connections, the areas of the rat PFC that more closely resemble the omPFC in the primate have extensive reciprocal connections with olfactory, gustatory, and multimodal sensory association cortices, as well as with limbic structures such as the hippocampus and the amygdala. In addition, these are the cortical areas that are involved in the limbic basal ganglia thalamo-cortical circuits – areas PL/IL and MO. In contrast the areas of the rat PFC that more closely resemble the dlPFC have reciprocal connections with neocortical sensory association areas and motor cortex. These are the areas that are involved in the basal ganglia thalamo-cortical circuits using the caudate putamen, termed the motor and association loops. These are areas Fr2 and dAC.

This characterisation of the PFC has direct implications for the widely suggested homology of dlPFC with rat prelimbic area. This is because true homology of brain structures refers to a common evolutionary and embryological developmental origin with comparative cytoarchitectural evidence in support of this. The above review of the anatomical connections gleaned from Groenewegen and Uylings (2000) and Uylings and van Eden (1990) does *not* convincingly support true structural homology. For example, in primate the dlPFC receives most of its cortico-cortical connections from

somatosensory and motor areas. In contrast, area PL in the rat receives its connections from limbic, visceral and autonomic cortical areas. With regards thalamo-cortical connections homology is also not supported. Primate dlPFC receives its MD connections from the lateral parvicellular division while rat PL receives its connections from MD from the medial division. Furthermore, area PL has strong reciprocal connections with limbic structures, and the hypothalamus, while area dlPFC in the primate receives only mild innervation from these limbic structures, and no innervation from the hypothalamus.

3.4 Functions of the rat and primate PFC

3.4.1 Analogy rather than homology

Despite the evidence against a specific anatomical homology of rat area PL and primate dlPFC, general anatomical principles do suggest at least the presence of a region of cerebral cortex in the rat comparable to the PFC in primates. This is in terms of the anatomical connections reviewed in the previous sections. Therefore, generalised principles of function, such as higher order cognitive capacities, should be as observable in the rat as in they are in the primate, supporting this general anatomical parallel. The following section presents evidence for this case and the argument that the rat is therefore a useful model for studying executive functions. Throughout this review evidence of the functional roles suggested for areas PL and dlPFC are also specifically noted (when clearly stated in the studies) to assess the presence of functional analogy rather than the anatomical homology between these areas. It should be noted that this theory of functional analogy is not a new one and has been proposed and supported

previously (Birrell & Brown, 2000), at least with regards attentional functions of the mPFC.

3.4.2 Working Memory Functions

In the rat the area of the PFC most extensively studied for function is the medial wall (areas AC/PL/IL). More often than not, lesions to this area include the prelimbic region precisely because it has been proposed as a homologue for primate dlPFC (Granon *et al.* 2000). Studies examining the functional consequences of damage to the mPFC usually limit their conclusions to one or two of the commonly cited executive functions such as working memory and attention rather than suggesting a single overriding function. As regards the proposed homology of area PL with dlPFC in particular, this arose from indications that the PL region was involved in working memory since lesions to this area produced deficits in a delayed response task (Larsen & Divac, 1978). Later evidence has confirmed the role of PL in these types of task (Brito & Brito, 1990, Bubser & Schmidt, 1990, & Dunnett, 1990) with deficits occurring in the same types of tasks in humans (Owen, Downes, Sahakian, Polkey, & Robbins, 1990, Spinnler, Della Sala, Bandera, & Baddeley, 1988, & Milner, 1964) and in non-human primates (Goldman-Rakic, 1990, Passingham, 1975, & Pribram & Tubbs, 1967). This idea of the involvement of the PFC in working memory is represented by the *domain specificity model* of primate PFC function (Goldman-Rakic, 1995, 1996). It proposes a functional dissociation between the lateral PFC dorsal to the principal sulcus (spatial memory) and that ventral to the principal sulcus (object memory), and this fits well with the dissociation of incoming visual information proposed to exist in these areas (see section 3.3.1). On the basis of this model, dlPFC and area PL analogy would be present if area

PL could be shown to have a role in spatial working memory. However, although evidence for the involvement of area PL of the rat in spatial working memory has accumulated to some extent, it is still surprisingly lacking. Furthermore, when this evidence is present the involvement of this area in working memory does not seem to be easily classified within a neat “spatial” framework.

For example, Ragozzino, Adams, & Kesner, 1998 investigated the role of area PL in a 12-arm radial maze random foraging task. They concluded that area PL was involved in the representation of *allocentric* spatial information in working memory, which at first glance would support a spatial working memory hypothesis. However, area PL was found not to be involved in the processing of *egocentric* spatial information. In contrast, area AC was not necessary for performance on this task. This immediately points to an important functional dissociation between the dorsal and ventral mPFC of the rat that had been suggested previously but in terms of anatomical connectivity. The functions of the rat area AC are therefore discussed later. As regards area PL, further research has shown that the role of this area would not seem to be in the encoding or storage of spatial information but in retrieval of this information (Granon & Poucet, 1995, Seamans, Floresco, & Phillips, 1995, de Bruin, Sanchez-Santed, Heinsbroek, Donker, & Postmes, 1994, & Kesner & Holbrook, 1987). The last of these studies (Seamans *et al.* 1995) is particularly notable as it has been useful for further delineating rat mPFC working memory functions. Because of this it has also formed the basis for the current research.

In an elegantly designed series of studies Anthony Phillips and colleagues (Floresco, Braaksma, & Phillips, 1999, Seamans & Phillips, 1994, & Floresco *et al.* (1995, 1996,

1997) have systematically investigated the involvement of various fronto-striatal loop structures in the solution of the delayed spatial win-shift and random foraging 8-arm radial maze tasks. Specifically these studies have focused on areas AC and PL of the rat mPFC, the involvement of the ventral subiculum of the hippocampus (vsub), PL/vsub & nucleus accumbens/vsub disconnections, and the role of the ventral pallidum and nucleus accumbens using temporary electrolytic lidocaine lesions. Within the present context their 1995 study is important because they used lidocaine infusions of area PL at various stages of the DSWS task to illustrate a specific impairment at the retrieval stage of memory for spatial locations. However, their explanation regarding the role of area PL in working memory was *not* limited to working memory alone. Instead other functions such as response flexibility were also introduced suggesting that the rat mPFC, and indeed area PL, has functions that are not adequately accounted for by the primate domain specificity model.

3.4.3 Response flexibility in strategic responding: updating working memory?

The delayed spatial win-shift radial maze task consists of 2 phases separated by a delay. In the first phase (training phase) the rat is required to forage for 4 food pellets, each placed at the end of one of 4 open arms, randomly selected. In the second phase (test phase) the rat is presented with all 8 arms open, but will only now find a food reward in each of the 4 arms that were previously closed off. The rat must use a win-shift strategy. Seamans *et al.*(1995) specifically showed that rats with PL inactivation prior to the training phase were not impaired in either that phase or the test phase, hence the conclusion that area PL is not essential during encoding. However, infusions of lidocaine made prior to the test phase did produce a deficit in that phase. Furthermore,

the classification of test phase error type into across phase (number of training phase arms entered) and within phase (number of training phase and test phase arms *re-entered*) showed that PL rats made more of both types of these errors, suggesting random responding as a result of an inability to correctly recall previously visited spatial locations. However, rats with PL temporary lesions also made more errors when switched from a win-shift paradigm to a random foraging paradigm, even though they were not impaired at random foraging if they had been exclusively trained on that task.

The above finding led Seamans *et al.* (1995) to propose that area PL may have additional functions beyond spatial working memory including switching to a different strategy when a previously correct strategy is no longer important. This notion of the rat mPFC in flexibility of strategic responding represents the most commonly cited function for this region of the rat PFC (Birrell & Brown, 2000, Ragozzino, Wilcox, Raso, & Kesner, 1999, Li & Shao, 1998, Fritts, Asbury, Horton, & Isaac, 1998, Joel, Weiner, & Feldon, 1997, Porter & Mair, 1997, Delatour & Gisquet-Verrier, 1996, Granon, Vidal, Thinus-Blanc, Changeaux, & Poucet 1994, Neave, Lloyd, Sahgal, & Aggleton, 1994, Poucet, 1990, and Winocur & Moscovitch, 1990), although it should be noted that whether this flexibility is in relation to working memory or attentional functions is not agreed across these studies.

In terms of a working memory hypothesis the above indications that the role of the rat mPFC may be more than this function, fits well with the suggestion that, rather than simply holding information “on-line”, the PFC may also be involved in the *manipulation* (in this case updating) of mnemonic information for the selection of responding. In fact, manipulation of working memory information is an important

aspect of executive functioning (Shimamura, 2000) and indications that the rat PFC can be involved in this bodes well for illustrating comparable functions and the eligibility of the rat as a model of dysexecutive syndrome. For example studies on human and non-human primates with mid-dorsal frontal lobe damage have demonstrated the involvement of this area in manipulation of working memory (Petrides & Milner, 1982). In addition, the manipulation hypothesis has been specifically attached to the dlPFC in the *levels of processing* model of primate PFC function developed by Petrides (1994, 1995) and supported by Owen, Lee, & Williams (2000).

However, a role in response flexibility is not strictly limited to area PL of the rat mPFC. Indeed it is a function more often proposed with reference to area AC, and this can also be supported from Seamans *et al.* (1995). Specifically, they demonstrated that rats with AC lidocaine inactivation were impaired at the DSWS task both following pre-training phase injections, and pre-test phase injections – with a specific increase in across phase errors. In addition, AC rats were also impaired at random foraging on the 8 arm radial maze, producing more errors of entry to baited arms. This suggests the presence of a tendency to perseverate with visits to arms previously associated with reward in rats with AC lesions. Significantly, area AC has also been proposed by some to be a homologue of dlPFC (Kesner, 2000) and this would fit the anatomical conclusions of the preceding section. However, since it is clear in the rat literature that there is functional dissociation between areas AC and PL and a homology argument would necessarily preclude one or the other of these regions this highlights a significant problem with addressing comparable regional specialisation across primate and rat.

3.4.4 A role for area AC in the application of rules: learning, memory & shifting

Winocur & Moscovitch (1990) trained rats to complete a specific Hebb-Williams maze in order to assess the contribution of dorsal mPFC to different memory systems. These Hebb Williams mazes are square closed field tests (76 x 76 cm) with a start box and a goal box located at opposite diagonal corners. Each of these squares is marked into 36 smaller squares that can contain barriers or represent an error zone. Rats must navigate from the start box to the goal box along the path mapped out by the barriers without straying into the error zones. Following surgery the rats were then tested on the same maze and a different maze to differentiate between memory for specific maze information and the learning and transfer of a general maze running skill. On both mazes the rats were tested in comparison to other rats that had received no previous training. Their hypothesis was that trained dorsal mPFC lesioned rats would not differ from untrained dorsal mPFC lesioned rats on performance of the second maze but would show “savings” on the first maze (if they could remember maze specific but not general maze solving information). This hypothesis was supported by a significant maze x training interaction within the dorsal mPFC groups such that the trained dorsal mPFC lesioned rats performed significantly better than their untrained counterparts on the first maze but not the second. However, they were impaired on both mazes relative to controls.

In their paper Winocur & Moscovitch (1990) suggest that their findings can convincingly account for why mPFC lesioned rats are significantly able to improve their performance in some testing situations. This represents a significant benefit over other accounts of mPFC function in the rat that tend to simply relegate a deficit to one of the

favoured executive functions per se, none of which, on their own, can account for this factor (observed for example in Granon *et al.* 1994, & Poucet, 1990). They propose that a significant part of maze learning is to learn not to re-enter previously visited incorrect alleys. Rats with dorsal mPFC lesions in their study, however, showed an exaggerated tendency to perseverate with this type of behaviour, which supports the findings of the Seamans *et al.* (1995) study with AC lesioned rats. Crucially though each alley in the Winocur *et al.* (1990) experiment was not immediately paired with reward suggesting that the presence of reward is not a necessary precursor for perseveration in dorsal mPFC lesioned rats. Based on this Winocur & Moscovitch (1990) proposed that performance in a particular maze is immediately impaired because of the loss of the rule to avoid incorrect alleys. However, performance improves as this perseverative behaviour drops out with practice (which increases the influence of maze specific information), leaving behind behaviour that is guided solely by intact memory for maze-specific information.

This could be taken to suggest that the dorsal mPFC of the rat might be involved in what has been termed procedural memory, in contrast to the hippocampus that appears to mediate episodic memory. On this note, however, it is important to recognise that the involvement of dorsal medial wall areas in rule *learning* has also been documented (Gisquet-Verrier, Winocur, & Delatour, 2000, DeCoteau, Kesner, & Williams, 1997, & Bussey, Muir, Everitt, & Robbins, 1996). However a clear distinction between habit learning (the formation of basic S-R associations) and the learning of complex (more abstract) rules should be made here since the former is usually attributed to the caudate putamen with only the latter attributed to the cingulate areas of the PFC in the rat (Bussey *et al.* 1996). Recent support for the involvement of dorsal mPFC in procedural

memory (memory for *how* to do a task) comes from an electrophysiological recording study (Jung, Qin, Lee, & Mook-Jung, 2000). In this study PFC neuronal activity recorded on an 8 arm radial maze during random foraging showed activity that was selective to various stages of the task. This is contrasted to evidence that the hippocampus has been shown to exhibit place specific firing, paralleling the procedural/episodic memory structural distinction.

However, it should be noted that other work has also shown that hippocampal neurons fire not only to place but also to other task specific components (Eichenbaum, Dudchenko, Wood, Shapiro, & Tanila, 1999, Wiebe & Staubli, 1999, & Wood, Dudchenko, & Eichenbaum, 1999, 2001). Furthermore, the notion that the mPFC is not involved in episodic memory per se does not fit with the highly influential theory of PFC function that the PFC is involved in the *temporal organisation of behaviour* (Fuster, 1989). While this notion of the “temporal organisation of behaviour” could still theoretically apply to the sequencing of new behaviour and not memory for old, there is evidence that lesions to the AC area of the rat mPFC do produce profound deficits in memory for temporal order on a radial maze (Kesner & Holbrook, 1987) and on a Y maze (Johnston, Hart, & Howell, 1974). Unfortunately this precludes a precise conclusion that dorsal mPFC is specifically *not* involved in episodic memory (memory for *events*) since a large part of this function requires memory for “when” events occurred in addition to “what” events occurred. However, what these findings do demonstrate is that the temporal sequencing of events remains an important aspect of PFC functioning in both the primate and the rat.

A later study examining variable delayed alternation and conditional discrimination learning in an operant chamber (Winocur, 1991) confirmed the previous finding that the dorsal mPFC is *not* involved in specific item memory, since rats with these lesions showed learning deficits that were not affected by manipulations of memory load (varied inter-trial intervals - ITIs). Likewise these manipulations show that temporal relationships were not a contributory factor in the observed deficit since the increase in ITIs had no effect. However, although Winocur (1991) claims this is evidence against the theory of Fuster (1989) this is not the case. There is a very important distinction between measuring a deficit that is temporally dependent (or independent) and measuring a deficit that can be shown to occur primarily *in* temporal processing. Indeed the time dependence or independence of mPFC deficits has been much abused in relation to specific theories of mPFC functioning and is a particular problem for interpreting function. For example it has generally been assumed that time dependence of a deficit indicates a memory disturbance while time independence indicates a deficit in mechanisms relating to attention or response selection (see for example Joel, Tarrasch, Feldon, & Weiner, 1997). However, regarding attentional function in particular, time dependence has also been used to suggest problems with sustained attention, since this is arguably a function that also degrades with time (Broersen & Uylings, 1999) in addition to memory.

Regardless of these problems, the involvement of the rat dorsal mPFC in rule-based behaviour may not be limited to the learning or memory of complex rules, especially since a pure procedural memory hypothesis does not account for all findings. A factor that is more frequently cited in relation to this type of responding is the importance of context and the transfer of contextual information from one context to another. This

could equally apply to the results of Winocur and Moscovitch (1990) such that behaviour observed can be more precisely described as a deficit in rule *shifting*. This is a behaviour that fits well with research on human subjects – although this is a function ascribed to the human anterior cingulate cortex rather than to one of the PFC divisions noted in the anatomical section of this chapter. For example Posner (1993) studied changes in metabolic activity in the AC cortex of humans who were over-trained on a task, and who were subsequently asked to perform the same task but with a new set of stimuli. During over-training, Posner (1993) observed a decrease in AC activity but increased activity was again observed once the new set of stimuli was introduced. This has obvious similarities with the updating of working memory theory and indicates that dissociating between the different functions proposed for the PFC on the basis of experimental findings is extremely difficult. It is possible that this difficulty may result from the loss of the same sub-functions that underlie deficits produced in apparently different settings (such as a general mechanism of cognitive flexibility). Evidence that this may be the case comes from the fact that rule shifting is also examined in terms of attentional functions, as set shifting.

3.4.5 Attentional functions

On the whole there are relatively few studies examining the role of the rat mPFC in attentional functions. Most likely this is a result of the difficulties involved in validly assessing specific attentional mechanisms such as sustained, selective, and divided attention, as well as vigilance and set shifting. Given the reciprocal connections the rat mPFC has with the basal forebrain a role in attentional functions would seem rather obvious. However there have been mixed findings in relation to this.

Olton, Wenk, Church, & Meck (1988) specifically demonstrated that rats with mPFC lesions did not have a deficit in their ability to successfully time a stimulus in an operant chamber, apparently refuting the notion that PFC is necessary for temporally related behaviour. However, when these rats were presented with the option of timing 2 stimuli in conjunction they were unable to do this. That is, they successfully timed the second stimulus, presented partway during presentation of the first, but timed the first as if it had been absent during the presentation of the second even though it had not been. This seems like good evidence that the mPFC of the rat is necessary for the ability to focus attention on more than one event – known as divided attention. However, it does not refute Fuster's hypothesis that the PFC is involved in the temporal sequencing of events (i.e. more than one event).

In this study, however, it is also possible that the failure of the mPFC rats was not in allocating attentional resources to the 2 stimuli simultaneously (since both were perceived), but in performing the complexity of the operations needed to time both at the same time – in scheduling multiple operations (which would arguably place heavy demands on working memory in remembering simultaneously how long each stimulus has been presented for). Indeed, the involvement of the rat mPFC in “effortful processing” is well established (Granon *et al.* 1994) and since this argument can not be dissociated from the divided attention conclusions of Olton *et al.* (1988), a firm conclusion that the mPFC is required for divided attention on the basis of these results would seem premature.

Muir, Everitt, & Robbins (1996) conducted perhaps the most commonly cited examination of the mPFC and its role in attention. In this study, rats with mPFC lesions centred on area PL were impaired in a choice reaction time task in response to presentation of a stimulus when there were 5 possible spatial locations in which the stimulus (presentation of a light) could occur. This also seems like good evidence that rats with mPFC lesions are impaired at dividing their attention effectively between several stimulus locations, and could support the conclusions of Olton *et al.* (1988). However, a role for rat mPFC in divided attention is not consistent with reports from human subjects linking the PFC with sustained and selective attentional functions rather than divided attentional functions (Chao & Knight, 1996, & Perret, 1974). Furthermore, the findings of Chudasama & Muir (1997) that intact rats use mediating strategies to perform a 2 choice lever task, while mPFC rats do not, could suggest that the five choice serial reaction time task deficit noted above may be the result of a failure in mPFC rats to organise these mediating strategies and not in dividing attention.

Miner, Ostrander, & Sarter (1997) have investigated the involvement of the whole of the mPFC in what they classified as sustained attention/vigilance. However, this can be immediately criticised on the grounds that the terms “sustained attention” and “vigilance” are not interchangeable. Sustained attention can be classified as a state of alert readiness maintained during a task that is outside of automatic processing. In contrast vigilance refers to the preparedness to focus attention and detect events that are unpredictable. In their experiment rats with complete mPFC lesions were impaired in retention of a lever-pressing task that required the opposite lever press on presentation of the stimulus to that required following a trial in which the stimulus was absent. In contrast to a go/no-go task therefore, mPFC rats were still required to respond when the

stimulus was absent. However the pattern of results obtained was argued not to be consistent with an attentional impairment. Specifically, lesioned rats responded in a random, robust manner (time independent) and were not affected by the presentation of distracters (distracters are often used to test sustained attention). Instead the results were proposed to be more consistent with the disruption of processing of prepositional rules at the point of response selection, which ties in quite neatly with the research discussed above for area AC and its involvement in complex rule behaviour.

Granon, Hardouin, Courtier, & Poucet (1998) also investigated mPFC involvement in sustained attention. In their experiment mPFC lesioned rats required to press a lever in response to brightness discrimination displayed significantly higher omissions than controls, suggesting that they missed the signal. Furthermore, in a related experiment in which a warning tone was given preparing the rats for the signal, mPFC lesioned rats were not impaired. Granon *et al.* (1998) concluded from this that the mPFC was involved in sustained attention. However, since it is likely that the presentation of a warning tone would *not* serve to aid sustained attention (i.e. would not help to maintain attention once focused) but would aid mechanisms of selective/focused attention, it remains questionable as to the precise aspect of attention that was affected by these lesions.

Finally, recent work has demonstrated a very specific impairment in rats with mPFC lesions centred on area PL in the shifting of attention set. This experiment is notable because it used a task that was specifically designed to parallel the Wisconsin Card Sort Test used in clinical settings with human subjects. It is a task known to be sensitive to frontal lobe dysfunction, although explanations for the deficit produced following

frontal lobe damage can be difficult to dissociate (Robbins, 1998). In non-human primates however, it has been shown that the lateral prefrontal cortex mediates the extra dimensional shifting of attention. That is from a discrimination based on one stimulus dimension to another discrimination based on an entirely new dimension – for example from colour to shape (Dias, Robbins, & Roberts, 1996a, 1996b, 1997). In their study Birrell and Brown (2000) used food bowl textures and digging medium textures and odours as stimulus dimensions and clearly indicated that the rat area PL was necessary for extra dimensional shifting but not intradimensional shifting, reversal of responding or the ability to make simple or compound discriminations. Based on these findings it was argued that the impairment that occurred following PL centred lesions in this task could not be attributed to an inability to hold the currently valid dimension “on-line” or in an inability to withhold responding to the previously correct dimension since reversal or ID shifting was not impaired. As suggested previously this work fits well with ideas concerning flexibility of responding and the mPFC, and supports other studies that specifically examined flexible responding in the rat (Joel, Weiner & Feldon, 1997, & Ragozzino, Detrick & Kesner, 1999), although out-with attention functions.

3.5 Conclusions: Does the rat possess executive functions?

The preceding discussion has highlighted some of the many problems associated with addressing the precise cognitive functions of the PFC in any species, in addition to drawing across species comparisons. The review of primate and rat anatomy has shown that there does exist a comparable area of cortex in the rat brain to that of the PFC in the primate. This was a necessary demonstration for the present research to justify the use of a rat model of executive function for assessing the involvement of the PPTg in higher

order capacities. This comparable region of cortex in the rat is distinguishable on the basis of density of projections from the MD nucleus of the thalamus, in addition to paralleled levels of cortico-cortical interconnectivity, and shared projections with basal ganglia circuitry, limbic structures, and major cholinergic and monoaminergic brain systems. At the general level, these comparisons would therefore suggest similar functions for these regions of the cortex in the primate and the rat and this has been supported throughout this chapter. For example the cognitive functions proposed for the PFC in the human and non-human primate range from working memory and attention to response flexibility and these have also been proposed through various experimental paradigms to exist in the rat brain. In addition, although not discussed in the preceding review, there is also evidence to suggest that the functions of the orbitomedial division of the PFC in primates in emotional processing are also present in the rat (Quirk, Russo, Barron, & Lebron, 2000, Sullivan & Gratton, 1999, Jinks & McGregor, 1997, & Sarter & Bruno, 1994).

However, the problem arises when strict homological comparisons are attempted between areas of the PFC in the rat and the primate. The dlPFC and area PL homology was proposed to exist functionally following the discovery that damage to each area produced deficits in delayed responding tasks. This was suggested to indicate a role for these regions in the maintenance of information in working memory during a delay. However, this theory has fallen out of favour and has been extended to include the manipulation of information within this memory system. This model has the benefit of accounting for the issue of flexibility of responding, at least within a working memory hypothesis. To a certain extent, then, the reviewed functional evidence suggests that there may be functional analogy between dlPFC and PL, in updating working memory

and/or as far as attention set shifting is concerned. However, whether these are dissociable functions or tax the same underlying mechanism of cognitive flexibility is another issue. Certainly more work is needed to facilitate comparisons across species in the same tasks since there are relatively few studies that are able to claim direct methodological replication of a primate task for the rat. This will help to answer this open issue of analogy/homology of PFC sub-regions. It is unlikely that an informed conclusion regarding parallels of primate PFC sub regions in the rat can be reached, however, without a clearer understanding of the nature of the processing of neural information occurring in these regions. For example there is still no generally accepted consensus regarding executive functions of the PFC and how these are organised despite the extensive amounts of research to address this. An examination of comparable involvement of the PFC and the PPTg in executive functions therefore requires a clear delineation of PFC functioning in the selected task before such comparisons can proceed.

4. THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS: STRUCTURE & FUNCTION

4.1 Why study the pedunculopontine tegmental nucleus?

The anatomical relationship of frontal systems to the striatum is now widely accepted as being organised into topographical loops – see Chapter 2. These loops allow distinct areas of the cortex to project to specific regions of the dorsal and ventral striatum, which in turn project to specific areas of the pallidum and substantia nigra pars reticulata. These structures then pass neural information back to the cortex via the thalamus. Current thinking regarding these looped circuits is that they are open and interdigitated rather than closed and segregated (Joel and Weiner, 1994). That is, although information that originates from a specific cortical region returns to that same region via the looped circuit, the same information is also passed onto another discrete cortical region. It is also apparent that there is some overlapping of neuronal populations associated with the separate loops within both striatum and the basal ganglia (Joel and Weiner, 1994).

The roles played by such looped circuits in the initiation and maintenance of behavioural output is extremely complex and difficult to understand, and is especially complicated by the high degree of intricacy in the interactions of the structures involved in fronto-striatal processing. This is a problem that is likely to be a direct result of the manner in which parts of the fronto-striatal brain system have evolved. For example, Marin, Smeets, & Gonzalez (1998) have presented evidence to argue that the basal ganglia in tetra pods have evolved as a complete system, with many anatomical and

morphological similarities in the organisation of this system across both amniotic and anamniotic vertebrates. Attempts to delineate the functions of a particular structure within the context of fronto-striatal processing must therefore take account of this organisational evolution, since the manner in which the basal ganglia come to exist in the brain strongly suggests that it is the degree of interaction between structures that is the key to understanding the neural processing carried out there. This is becoming increasingly clear from lesion studies also, as research involving disconnection lesioning procedures has suggested multiple functions for a single structure depending on the communication route that is being examined.

Anthony Phillips and colleagues (1994, 1995, & 1997) have investigated the involvement of fronto-striatal structures in the selection of action on the radial maze. Recently in one study in which they employed a temporary disconnection lesion technique, they have highlighted that the function of hippocampus – nucleus accumbens connections is fundamentally different to the function of hippocampus – prefrontal cortex connections (Floresco *et al.* 1997). This experiment showed that lidocaine disconnection of the prelimbic (PL) region of the PFC and the ventral subiculum (Vsub) of the hippocampus resulted in a selective impairment on the delayed spatial win-shift (DSWS) task where rats had to forage for 4 food pellets on the basis of information about the location of 4 pellets in contrasting locations received prior to a delay. However they also demonstrated that rats with the same disconnection lesions did not have a deficit on the non-delayed random foraging (RF) task in which they had to forage for 4 pellets in one session without prior information on the probable location of the food. In contrast to this pattern of findings for PL/Vsub lesions, lidocaine disconnection of the nucleus accumbens and the ventral subiculum resulted in a

selective impairment on the RF task but not the DSWS task. Floresco *et al.* (1997) argued that their results demonstrated that the connections between the hippocampus and the PL are involved in organising and executing a *prospective* foraging strategy where rats have to use previous information to predict the location of reward. However, the function that is subserved by the connections hippocampus has with the nucleus accumbens appears to be in organising and executing a *retrospective* foraging strategy. That is, in the initiation and guidance of exploratory locomotion when there is no pre-existing information about the location of reward. This dissociation of function between the different connections made by one fronto-striatal structure is something that would not have been apparent from a simple hippocampus lesioning procedure, and has indicated that a disconnection lesion technique may be a particularly useful tool for further delineating fronto-striatal processes.

It is important to note, however, that subsidiary circuits have been described within the fronto-striatal system (Alexander *et al.* 1986) and these have received scant attention in comparison to the main looped cortical re-entrant circuits, even within the work of Phillips and colleagues. For example Alexander *et al.* (1986) have argued that the basal ganglia can participate in several subsidiary circuits, serving to modify transmission through the main basal ganglia – thalamocortical pathways. Each of these circuits also contains the same kind of topographical features that are characteristic of the main looped circuits and have been shown to occur around the main “nodal points” of the subthalamic nucleus (Nauta & Cole, 1978), the dopaminergic nuclei of the mesencephalic tegmentum (Parent, Mackey, & DeBellefeuille, 1983, & Carpenter & Peter, 1972), the intralaminar nuclei of the thalamus (Parent *et al.*, 1983, & Kalil, 1978), and finally, the pedunculopontine tegmental nucleus (Parent *et al.*, 1983). Furthermore,

output from the cortex has been shown to go directly to the pallidum (Naito & Kita, 1994). Since output from the basal ganglia can be processed in structures lower in the neuraxis before returning to the cortex via thalamus, or more notably, before being passed onto motor sites in the spinal cord without reference back to the cortex, a comprehensive understanding of fronto-striatal functioning would be impossible without an understanding of the neural processing that occurs within these subsidiary circuits.

In particular, the pedunculo-pontine tegmental nucleus (PPTg) is a pontine structure that both receives and makes extensive connections with fronto-striatal systems. Indeed it is the lowest site in the neuraxis to receive extensive direct outflow from all the components of the basal ganglia (Groenewegen *et al.*, 1993, Berendse *et al.*, 1992, Semba *et al.*, 1992, Steininger, Rye, & Wainer, 1992, Spann & Grofova, 1991, & Heimer, Zahm, Churchill, Kalivas, & Wohltman, 1991) making it part of an important basal ganglia feedback system. Critically however, it is also a site that provides fronto-striatal processed information with access to lower motor structures, outwit the looped information flow back to the cortex and the importance of these connections has so far been understated. Such connections indicate that the PPTg must have a significant role in relation to striatal processing, yet the necessity of considering the PPTg in light of its close relationship with the striatum has only recently been recognised (Winn, Brown, & Inglis, 1997).

4.2 What, and where, is the Pedunclopontine Tegmental Nucleus?

4.2.1 Anatomical & Morphological Characteristics

The precise anatomy of the PPTg is an unresolved issue. One aspect of its structure that is widely accepted is that it contains a group of large cholinergic (ACh) Ch5 neurons situated in the mesopontine tegmentum (see Figs 4.i & 4.ii). In the rodent brain there are approximately 1600 of these large Ch5 neurons, which have a distinctive columnar organisation within each hemisphere. These neurons extend rostro-caudally from the substantia nigra to the lateral parabrachial nucleus and are bordered dorsally by the deep mesencephalic and cuneiform nuclei and ventrally by the pontine reticular nucleus. The organisation of the Ch5 neurons within the PPTg contributes to the distinct regions of the nucleus observed as pars compacta and the more anteriorly located pars dissipata. However, interdigitated and medial to these Ch5 neurons there are also non-cholinergic, principally GABA containing neurons (Ford, Holmes, Mainville, & Jones, 1995). In the past some authors have used the term “PPTg” to refer only to the Ch5 neurons, preferring to include the non-cholinergic neurons with the adjacent midbrain extrapyramidal area (Rye, Saper, Lee, & Wainer, 1987). However, previous work from this laboratory has argued that the PPTg be considered similar in organisation to the substantia nigra (Inglis, Dunbar, & Winn, 1994, Inglis, Allen, Whitelaw, Latimer, Brace, & Winn, 1994 & Winn, Brown, & Inglis, 1997). This is because the substantia nigra, like the PPTg, is comprised of two neuronal populations that are neurochemically, electrophysiologically, and anatomically different. However no one would suggest that the substantia nigra be considered as two distinct structures. Evidence that the PPTg should be considered in an analogous structural manner to the

Figure 4.i: NADPH diaphorase staining of Ch6 and caudal Ch5 neurons (PPTg is situated on the right, LDTg is situated on the left).

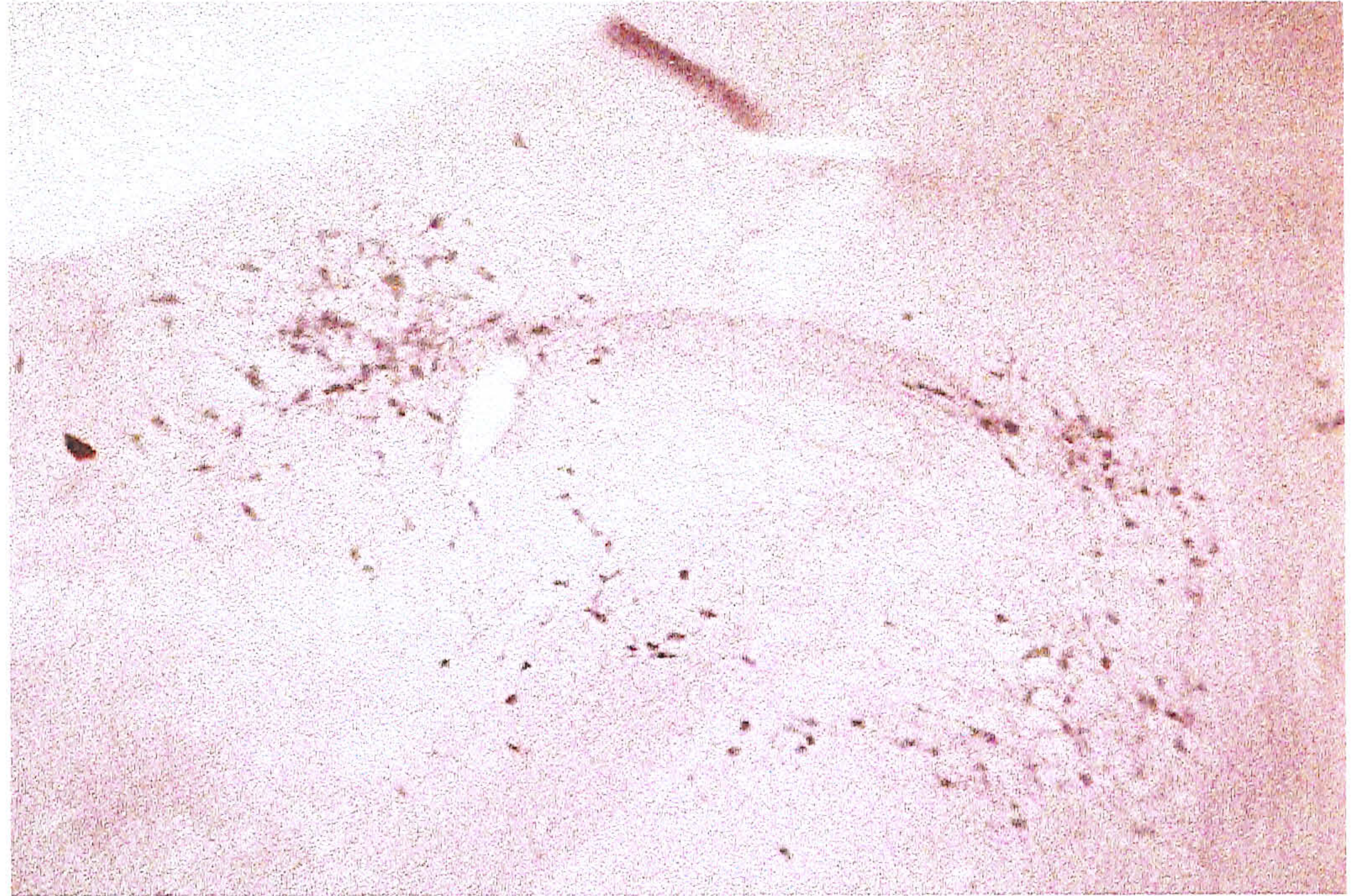
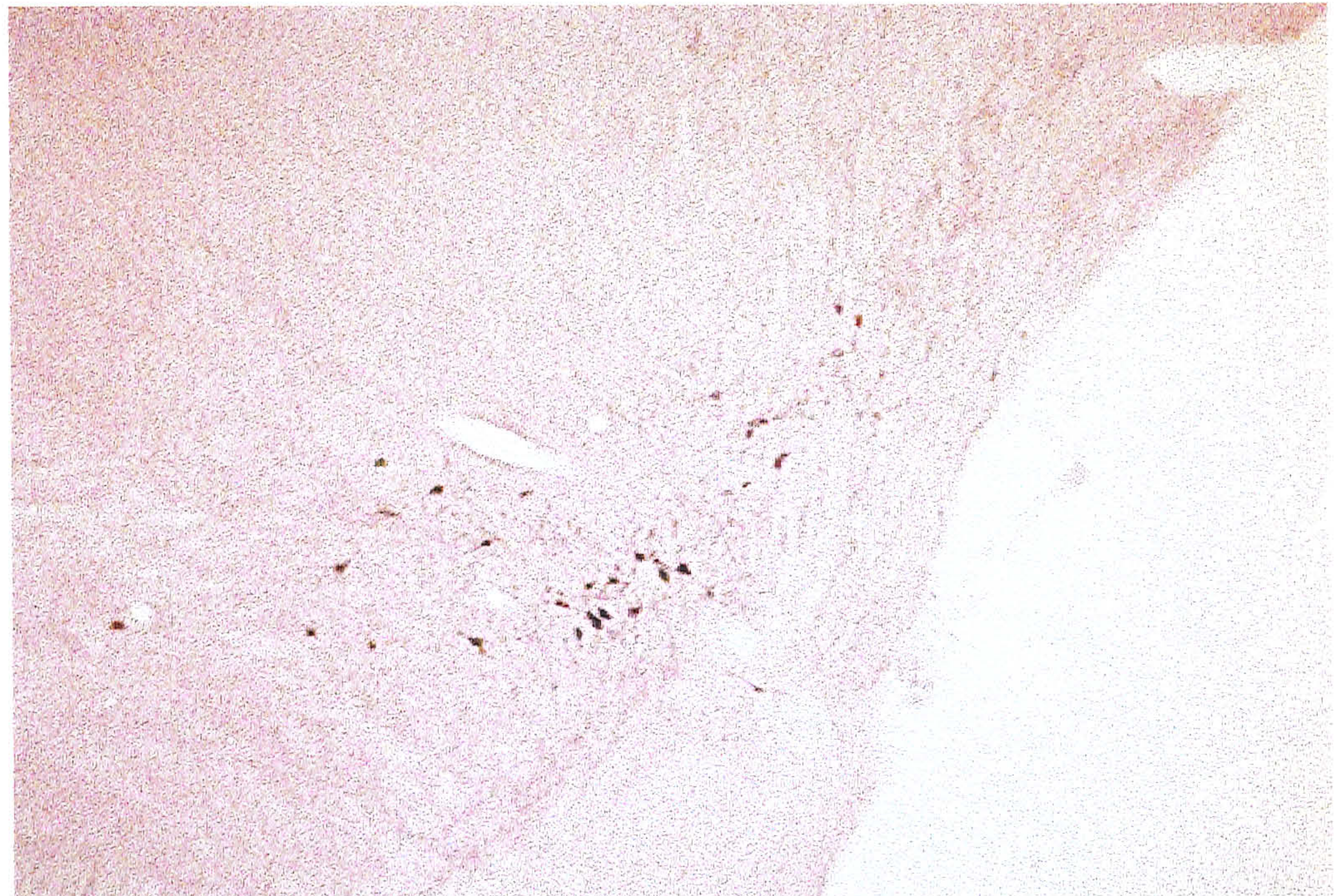


Figure 4.ii: NADPH diaphorase staining of rostral Ch5 neurons of PPTg.



substantia nigra comes from the close relationship between the SN and PPTg in terms of location and connections, and in the brains of chicks (*Gallus domesticus*), the PPTg and the SN are so closely related that they are regarded as one undifferentiated structure (Kuenzel & Masson, 1988).

Such a characterisation of the PPTg does, of course, depend on the existence of functional communication between the distinct neuronal sub-populations and this still remains to be directly demonstrated. However, the length of ACh neuronal dendrites (300µm – Jones, 1990) could provide a route for such communication. Furthermore, (Honda & Semba, 1995) have shown that the ChAT positive neuron somas are often located in direct apposition to both ChAT positive and ChAT negative dendrites, and ChAT positive axons have also often been observed contacting unlabelled somata and dendrites, thereby forming many synaptic specialisations with them. Finally, and perhaps most importantly, Inglis & Winn (1995) have argued that considering the non-ACh neurons as separate from the ACh neurons obscures the likely *functional* importance arising from the interdigitation of these neurons.

4.2.2 Electrophysiological Characteristics

Electrophysiological studies have demonstrated that there are 3 different kinds of neuron in the PPTg and the neighbouring LDTg (Kamondi, Williams, Hutcheon, & Reiner, 1992, and Leonard & Llinas, 1990). However, the precise classification of the 3 neuronal types as cholinergic or non-cholinergic remains debatable. Type III neurons that show the transient outward potassium current (tonic firing) associated with Type II neurons, as well as the low-threshold, rapidly inactivating inward calcium current (burst

firing) associated with the Type I neurons, are generally agreed to be cholinergic. However, it is the proportion of the two remaining neuronal types that are cholinergic or non-cholinergic that remains the contentious issue. This current gap in our knowledge regarding the anatomy of the PPTg illustrates that there is still much to be done in order to increase our understanding of this nucleus. However, it is important to note that this lack of knowledge of PPTg structure has not prevented the investigation of PPTg function to date. Admittedly, however, an adequate delineation of PPTg function does depend on a sound understanding of its structure, and it is highly likely that improvements in our knowledge of PPTg anatomy will be matched by new functional considerations.

4.3 Connections of the PPTg

4.3.1 Cholinergic or non-cholinergic?

In describing the connections of the PPTg, thus defined, some effort has been made to distinguish between the connections of the cholinergic and non-cholinergic neurons. However, it is becoming increasingly clear that such a distinction may be oversimplified as some neurotransmitters can co-exist within single PPTg neurons (Lavoie & Parent, 1994, & Vincent *et al.* 1986). What's more, describing the connections of the PPTg is not a straightforward task since its borders are not clearly defined across studies. Some tracing experiments, for example, have identified connections with neurons known to be in the region of the PPTg but whether these neurons can actually be classified as PPTg neurons is not clear. The issue is further confused both by our lack of knowledge of local circuit connections within PPTg and the incomplete

understanding of the extent of collateralisation of PPTg axons. Any discussion of PPTg connections, therefore, can only be as comprehensive as it is currently possible to make it (Winn *et al.* 1997).

Previous work has suggested that PPTg neurons have quite different patterns of afferent and efferent innervations depending on whether they are cholinergic or not. For example, the cholinergic neurons are usually considered to be part of the ascending reticular activating system (ARAS) and the non-cholinergic neurons, those argued by Rye *et al.* (1987) to be part of the midbrain extrapyramidal area, are generally involved in processing basal ganglia outflow. However, while the distinction between cholinergic and non-cholinergic neurons is preserved to some extent in the discussion below, it is noted that future work may reveal a more complex patterning of sub-regions within the PPTg, perhaps with separate functions based on a more precise understanding of their patterns of afferent and efferent innervations.

4.3.2 Efferent Connections

Cholinergic neurons of the PPTg innervate all of the major thalamic nuclei, making this the largest output of the PPTg. Indeed, Sofroniew & Priestley (1985) estimated that 60% of PPTg neurons were retrogradely labelled following a single large horseradish peroxidase injection into the thalamus. However, studies using such large injections of tracer could not distinguish between the projections of the PPTg to each thalamic nucleus. Later work has rectified this with smaller discrete injections and has revealed particularly strong innervations of the sensory and motor nuclei (Hallanger & Wainer, 1988, & Hallanger, Levey, Lee, Rye, & Wainer, 1987). This pattern of innervation is

different than that of the Ch6 neuronal group of the neighbouring latero-dorsal tegmental nucleus (LDTg) that shows a preference for limbic thalamic nuclei such as the mediodorsal nucleus.

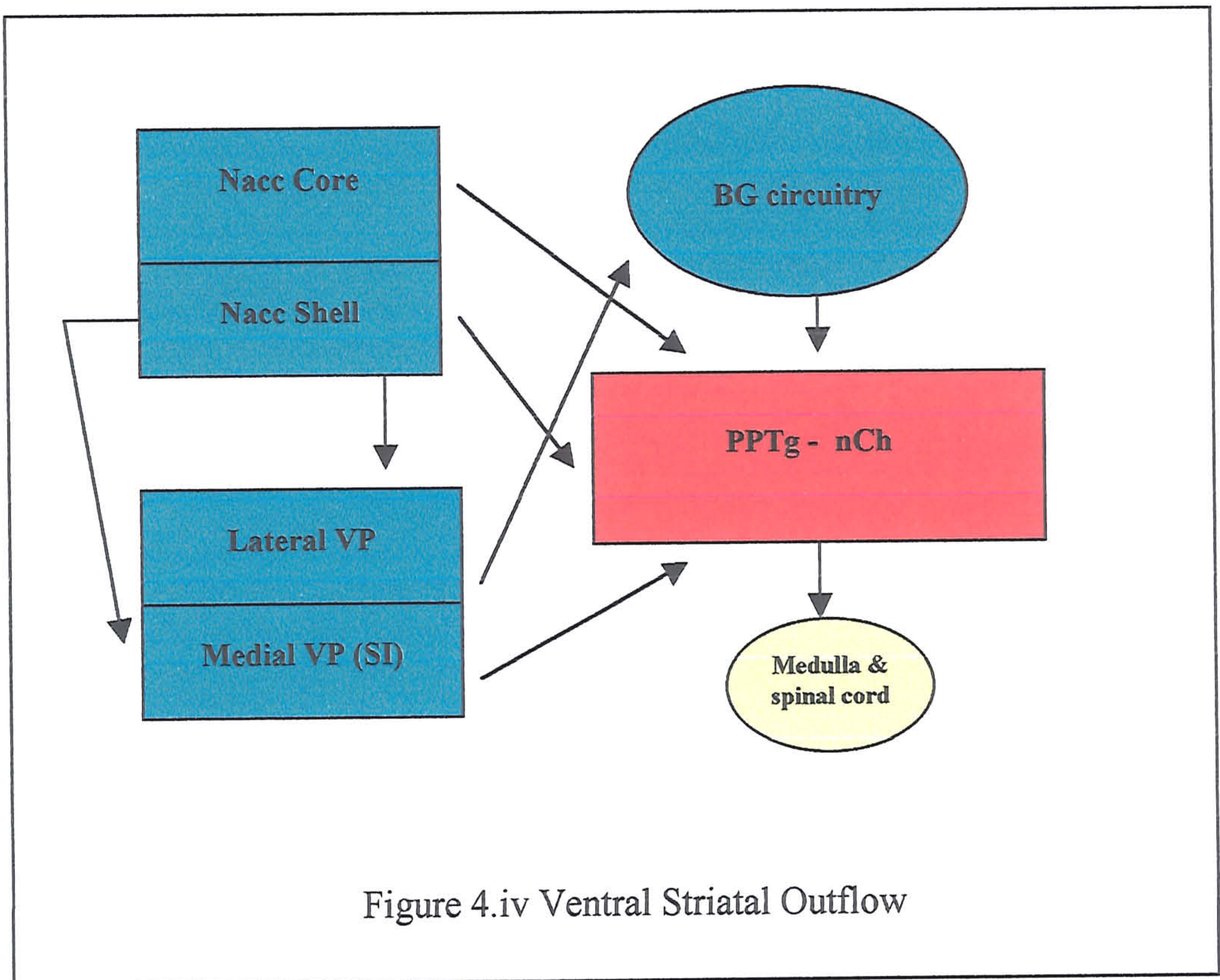
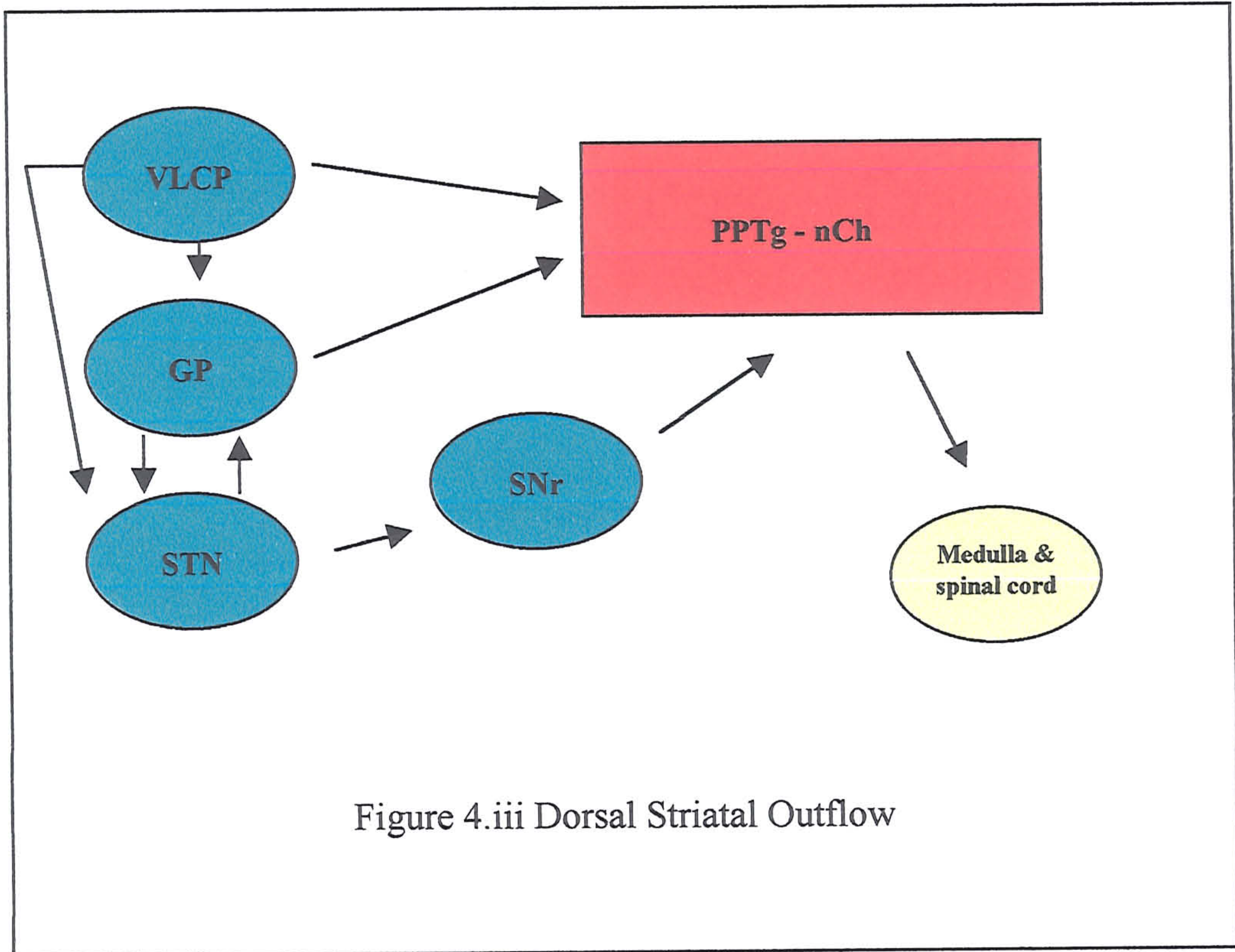
The cholinergic neurons of the PPTg also project to the basal ganglia including excitatory projections to the dopamine neurons of the substantia nigra pars compacta (SNc) (Blaha & Winn, 1993, Bolam, Francis, & Henderson, 1991, & Beninato & Spencer, 1997, 1998). Rye *et al.* (1987), however, have argued that this connection is from non-ACh neurons despite the wealth of evidence to the contrary. There is also a lesser innervation, and from more caudally located PPTg ACh neurons, to the ventral tegmental area (VTA) (Jackson & Crossman, 1983, & Phillipson, 1978). In contrast to this pattern of innervation of mid-brain dopamine neurons shown by the PPTg Ch5 neurons, the Ch6 neurons of the LDTg exhibit more excitatory connections to the VTA (Oakman, Faris, Kerr, Cozzari & Hartman, 1995).

Connections are also present between the PPTg and other sites associated with striatal input/output. For example, possibly both ACh and non-ACh neurons project to the internal segment of the globus pallidus (entopeduncular nucleus in the rat - GPi) (Hallanger & Wainer, 1988, Rye *et al.* 1987, Woolf & Butcher, 1986, & Saper & Loewy, 1982), and there is a non-ACh connection to the subthalamic nucleus (STN) (Bevan & Bolam, 1995, & Lavoie & Parent, 1994) which electrophysiological studies have suggested arises from the collaterals of PPTg efferent connections to the pallidal complex. Finally there is also some evidence of a PPTg ACh connection to the caudate putamen (Hallanger & Wainer, 1988, Woolf & Butcher, 1986, & Saper & Loewy, 1982), as well as to the superior colliculus (Beninato & Spencer, 1986).

Cholinergic connections to other sites than the basal ganglia are to structures that send extensive non-specific input to the cortex. These include the basal forebrain (Hallanger & Wainer, 1988, & Woolf & Butcher, 1986), and the lateral septum, lateral hypothalamus, and zona incerta (Ford *et al.* 1995, & Hallanger & Wainer, 1988). These connections allow (mostly ACh) neurons of the PPTg extensive direct and indirect influence over both cortical and striatal activity. In contrast, the descending connections of the PPTg arise from both the cholinergic and non-cholinergic neurons and include a variety of sites involved in the control of motor and autonomic processes - in the pontine and medullary reticular formation (Rye, Lee, Saper, & Wainer, 1988), and the spinal cord (Skinner, Kinjo, Henderson, & Garcia-Rill, 1990). In fact, Lee Rye, Hallanger, Levey, & Wainer (1988) have shown that the innervation of the spinal cord is most substantial from non-ACh neurons.

4.3.3 Afferent Connections:

Sites that connect to the PPTg may include prelimbic cortex in addition to motor cortex (Zahm – personal communication). Structures associated with dorsal striatal outflow also project there (see Figure 4.iii), including the substantia nigra pars reticulata (the most dense innervation of non-ACh neurons – Spann & Grofova, 1991), globus pallidus, ventrolateral caudate putamen, and STN (Joel & Weiner, 1994, Groenewegen, Berendse, & Hauber, 1993, Berendse, Groenewegen & Lohman, 1992, Steininger, Rye & Wainer, 1992, & Spann, & Grofova, 1991). Although, the STN innervation of PPTg non-ACh neurons is small however (about 1% - Jackson et al, 1983) the STN can also



communicate with the non-ACh neurons of the PPTg via connections with the globus pallidus and SNr (van der Kooy & Hattori, 1980).

In addition the PPTg receives connections from sites associated with ventral striatal outflow (see Figure 4.iv), such as the nucleus accumbens, substantia inominata (part of the ventral pallidum), and again, the substantia nigra pars reticulata (SNr) (Groenewegen *et al.* 1993, Semba & Fibiger, 1992, & Heimer, Zahm, Churchill, Kalivas & Wohltmann, 1991). The afferents arising from nucleus accumbens are particularly notable as they are directly from the rostral core and ventromedial part of the caudal shell. These are areas that are thought to carry information in the parallel looped circuits described previously and they receive prefrontal cortex innervation from the prelimbic and infralimbic cortices (Groenewegen *et al.* 1993). Furthermore, Heimer *et al.* (1991) have argued that the core of the nucleus accumbens sends indirect information to the PPTg via the basal ganglia, making the PPTg in receipt of both core and shell information.

Other limbic structures like the central nucleus of the amygdala, lateral hypothalamus, zona incerta, and the bed nucleus of the stria terminalis also project to the PPTg (Steininger, Rye & Wainer, 1992, & Semba & Fibiger, 1992). Finally, the PPTg receives connections from several elements of the ascending reticular activating system (ARAS). These include serotonergic input from the raphe nuclei, noradrenergic input from the locus coeruleus, and ACh input from the neighbouring LDTg, all of which connect with the Ch5 neurons (Honda, & Semba, 1994, Semba & Fibiger, 1992, Greene & McCarley, 1990, & Jones, 1990).

4.3.4 Integration of Afferent Information:

Given the extent of striatal information that the PPTg receives, it is of interest to consider whether *single* neurons in the PPTg are in receipt of *both* dorsal and ventral striatal information (Winn *et al.* 1997). This is a particularly important point relating to the functions of PPTg connections, as it may provide insight into whether the PPTg performs a role in integrating limbic information from the ventral striatum with motor information from the dorsal striatum. Some research in primates has indicated that there is overlap between the regions of PPTg that receive functionally diverse streams of pallidal outflow (Smith, 1995, as cited in Winn, 1998). However it remains to be demonstrated directly whether single neurons of the PPTg do integrate both limbic and motor information. Whether such neurons are likely to be ACh or non-ACh is also unknown at present. Although the likelihood is that both receive this type of information, the bulk of striatal information appears to be received by those non-ACh neurons argued by Rye and Wainer to be part of the mid-brain extrapyramidal area.

4.4 Behavioural Disturbances following Bilateral PPTg Lesions.

4.4.1 Autonomic Functions:

The functions of the PPTg may be as diverse as the structures with which it communicates. This is because, as was the case for the hippocampus (Floresco *et al.*, 1997), each of the connections of the PPTg may sub-serve quite different functions within what could appear to be a single (subsidiary) brain system. For example, one function commonly associated with the PPTg in terms of its connections with the

thalamus is a role in the induction and/or maintenance of REM sleep (Semba, 1993, Semba, Reiner, & Fibiger, 1990, & Steriade & McCarley, 1990). Cholinergic activity from the PPTg is important in suppressing spindles and delta waves in the thalamus that are characteristic of slow-wave sleep. Indeed, ACh activity is involved in maintaining the thalamus in single spike mode associated with cortical activation. However since the PPTg provides only one half of the ACh modulation of the thalamus (the remainder coming mostly from LDTg, and some from the basal forebrain) it is not surprising that bilateral ibotenic acid lesions of the PPTg appear to produce no lasting sleep disturbances. Since ibotenic acid lesions of the PPTg tend to remove around 70-80% of the PPTg ACh neurons though, it is plausible that there are some significant changes occurring within thalamus as a result of this lesioning procedure. For example, there is some evidence for a temporary fragmentation of the sleep-wake pattern and changes in the component frequencies of cortical EEG as a result of the same PPTg lesions that produce behavioural deficits (Inglis, Thakkar, Rainnie, Greene, McCarley, & Semba, 1995). However, the precise make-up of thalamic changes occurring as a result of PPTg ibotenate lesions is something that remains under investigation at this laboratory.

Aside from the involvement of PPTg neurons in sleep states, the activity displayed by PPTg neurons during the waking state is unclear. It is likely that PPTg firing patterns are determined by a balance of its inputs: monoamine from ARAS connections, glutamate from the STN, and GABA from striatal connections. However, since most significant inputs to the PPTg are inhibitory (aside from the possible glutamatergic input from the contralateral PPTg, ipsilateral LDTg and STN) one plausible hypothesis is that disinhibition is a key characteristic of PPTg neuronal function.

Another possible function of the PPTg ACh neurons stems from their extensive projections to areas involved in autonomic control like the rostral ventrolateral reticular nucleus. This nucleus is believed to be important in driving the respiratory cycle, and in the integration of cardiovascular and respiratory reflexes. Such connections of PPTg with the thalamus and the rostral ventrolateral reticular nucleus could help to explain some of the more immediate consequences of ibotenate PPTg lesions observed at this laboratory. These include the extensive lengthening of unconsciousness resulting from anaesthesia (from approx 3hrs to 9hrs), problems with waking sleeping animals in the first few days following surgery, and problems with the maintenance of respiratory functions (despite strong heartbeat) immediately following infusion of ibotenic acid into the PPTg. These acute effects of ibotenate infusion into the PPTg are short lived, however, and it should be noted that PPTg ibotenate lesioned animals appear essentially normal with respect to control of these functions within approximately 4 hours of surgery for respiratory functions, and 4-7 days of surgery for sleep disturbances. Any behavioural effects observed after this time, therefore (discussed below), are unlikely to be a secondary result of distress caused by, for example, difficulty in inducing or maintaining REM sleep.

4.4.2 Functions related to the processing of dorsal striatal outflow:

Rats with bilateral ibotenic acid lesions of the PPTg display disruption of motor behaviours that are also disrupted following lesions to the dorsal striatum, the ventrolateral caudate putamen (VLCP) in the rat (Inglis & Winn, 1995). This is a fact that is not surprising given that, in the rat, the PPTg is in direct receipt of dorsal striatal outflow from VLCP. For example, injections of fluorogold in and around the PPTg

have been shown to selectively stain the VLCP leaving the rest of the CP unstained (Zahm – personal communication).

6-hydroxydopamine lesions of the VLCP in the rat have caused deficits in tasks that require reaching and grasping with the forepaws (Pisa, 1988), and similar deficits in forepaw reaching (though not grasping) have been observed in a staircase test in PPTg lesioned rats (Dunbar *et al.* 1992). Furthermore, Pisa (1988) and Jicha & Salamone (1991) have shown deficits in oro-motor behaviours, again following 6-OHDA lesions of the VLCP, and some oro-motor behaviour is also disrupted in PPTg lesioned rats (although not feeding which has also been linked to the VLCP – Jicha & Salamone, 1991). For example, Allen & Winn (1995) have shown that bilateral ibotenic acid lesions of the PPTg lead to extreme, enhanced orofacial stereotypies following infusion of d-amphetamine directly into the VLCP. In this experiment the intensification of the licking and biting normally attributed to VLCP d-amphetamine stimulation was so severe that animals had to be removed from the observation cages and in some cases had to be distracted from biting their forepaws by presentation of a pen barrel.

4.4.3 Functions relating to the processing of ventral striatal outflow:

Given the extensive projection of motor cortex and nucleus accumbens to PPTg, as well as PPTg descending projections to the spinal cord, it has been argued that the PPTg has a role in mediating either general locomotor ability, or locomotion stimulated via nucleus accumbens. Regarding the first hypothesis some have argued that the PPTg is a component of the functionally defined mesencephalic locomotor region - MLR (Mogenson, Wu, & Tsai, 1989, Mogenson & Wu, 1988, & Shik, Severin, & Orlovski,

1966), a region thought to be necessary for the production of coordinated stepping movements. Direct electrical stimulation of the PPTg in the rat has been shown to elicit locomotion (Garcia-Rill *et al.* 1990). However, evidence against the MLR hypothesis comes from the finding that bilateral excitotoxic lesions of the PPTg do not produce changes in locomotion measured either acutely over a few hours, or over a more extensive period of 24 hours (see Winn, 1998). In contrast to this, studies that have examined *conditioned* locomotion as a measure of drug reinforcement do show significant effects of bilateral PPTg lesions (Bechara & van der Kooy, 1992a). This pattern of findings might therefore suggest that the PPTg is not involved in the mediation of locomotion per se but that it may be necessary for the expression of learned behaviour. Further examination of the functional role of the PPTg with regards its connections with nucleus accumbens supports this contention.

The earliest examination of the functions of the PPTg in relation to striatal processing, by Mogenson and colleagues, had suggested that the PPTg does mediate locomotor activity stimulated from the nucleus accumbens (Mogenson *et al.* 1989, Mogenson and Wu, 1988, Brudzynski & Mogenson, 1985). However, Mogenson's study had used a procaine anaesthetic procedure for the PPTg (procaine being a non-selective toxin that affects local fiber systems) calling into question whether the effects he obtained could actually be attributed to PPTg damage. In a direct comparison of locomotor ability stimulated by intra-accumbens infusion of d-amphetamine with acquisition of responding for conditioned reinforcement (also stimulated by d-amphetamine) Inglis *et al.* (1994) convincingly demonstrated that the PPTg is involved only in mediating learned incentive behaviour through nucleus accumbens, and not basic locomotor ability stimulated in the same structure. In this study PPTg lesioned rats experienced enhanced

responding attributed to d-amphetamine stimulation in accumbens, as was the case in control rats. However, in PPTg lesioned rats this responding was misdirected. That is, PPTg lesioned animals expressed an increase in responding on both the reinforced and the non-reinforced lever in comparison to control rats that experienced enhanced responding only on the reinforced lever.

The acquisition of responding for conditioned reinforcement has been used to investigate how fronto-striatal systems control the processes by which environmental stimuli shape behaviour (Inglis & Winn, 1995). In this procedure rats are trained to form a first order association between a conditioned stimulus (CS e.g. light/click) and an unconditioned stimulus (US – e.g. food) and are then tested for acquisition of a second order association between pressing of a specific lever and presentation of the CS. Injections of d-amphetamine into the nucleus accumbens reliably produce an increase in responding on the conditioned response (CR) lever in intact rats without any change in responding on the non-reinforced lever (Taylor & Robbins, 1984). PPTg lesioned rats in the Inglis *et al.* (1994) study therefore could not perform the conditioned discriminated responding in response to d-amphetamine stimulation. While it could be argued that a breakdown in second order conditioning could be the result of the loss of incentive value of the compound conditioned stimulus, the results of the Inglis *et al.* (1994) study suggest that this was not the case. Specifically PPTg lesioned rats still acquired the lever press response, and still responded on the CR lever 80% of the time, even though responding on the non-conditioned lever was increased. Thus their behaviour was not entirely random. Taken together these results suggest the existence of a discriminatory incentive learning impairment following bilateral PPTg lesions and this is supported by the finding that bilateral PPTg lesions lead to a loss of acquisition

of conditioned place preferences to morphine and d-amphetamine (Bechara & van der Kooy, 1989). Since conditioned place preferences test the ability to attribute motivational significance to a test environment an intact PPTg would appear to be a necessary prerequisite for the production of appropriate positively conditioned behaviour, a contention that is in support of the position held by other research at this laboratory that the PPTg....

"...has a role very much more complex than the induction of stepping movements" ⁷.

4.4.4 Is the PPTg involved in incentive motivation?

The above findings regarding the role of the PPTg in positively conditioned behaviour are particularly important, not just because they show that the PPTg does mediate ventral striatal behaviour, but also because they challenge the conventional view of incentive motivation as being mediated via the connections nucleus accumbens has with other limbic structures such as the amygdala (Robbins, Cador, Taylor, & Everitt, 1989). Incentive motivation is a construct that is frequently described in terms of its observable effects on behaviour because such an approach avoids the introduction of concepts such as drive (which would complicate matters by requiring some description of the construct of emotion - Salamone, 1992). As a result of this approach, incentive motivation is defined as encouraging or inciting action, and is therefore thought of as the mechanism that underlies both conditioned and unconditioned behaviour. Given that the PPTg has been shown to disrupt mechanisms of conditioned behaviour it is therefore likely that it does have a role in mediating incentive motivation. However,

⁷ Winn, P, Brown, V, J, & Inglis, W, L (1997) On the relationship between the striatum and the pedunculopontine tegmental nucleus *Critical Reviews in Neurobiology*, 11 (4): 241-261 p.242.

rather than being responsible for “producing” incentive motivation levels per se the PPTg would appear to have a role in translating incentive motivation into appropriate action. This is particularly the case given that PPTg deficits in reward related responding have been shown to be *dependent* on the level of incentive motivation induced by the presence or absence of deprivation or manipulation of reward value.

A further study by Derek van der Kooy and colleagues (1992b) has supported the notion that PPTg lesions disrupt conditioned place preference (this time for food and morphine), but in this study disruption occurred only in PPTg lesioned rats experiencing non-deprived levels of incentive motivation. This confirms that the PPTg is a mediating structure crucial for the expression of positively conditioned approach behaviour but adds the rider that this may only be the case under levels of incentive motivation that are not enhanced by food deprivation or morphine withdrawal. Bechara & van der Kooy (1992b) explained their findings as suggesting that separate neural mechanisms subserve deprivation and non-deprivation induced motivation and that the PPTg is a structure crucial for the expression of non-deprived motivation only. In particular they argue that conditioned place preferences in sated rats....

“...reflect the activation of a motivational system underlying exploration and the approach of organisms to resources that have survival value (i.e. food) especially in the absence of an immediate need to alleviate a deprivation condition such as hunger¹”.

¹ Bechara, A, & van der Kooy, D, (1992) A single brainstem substrate mediates the motivational effects of both opiates and food in nondeprived rats but not deprived rats Behavioural Neuroscience, 106 (2): 351-363, p.360.

However, Inglis & Winn (1995) have noted that there are alternative explanations for why a disruption might not occur under deprivation/withdrawal, even though it does occur under non-deprivation/drug naïve conditions. That is, naïve and non-deprived rats will form a conditioned place preference because of an association between a particular environment and positive reinforcement based on reward. In contrast, deprived rats, and rats suffering from drug withdrawal, may form an association between the same environment and positive reinforcement as the alleviation of a negative state (hunger or drug withdrawal). However, one problem with this alternative explanation is that it would seem to suggest that the PPTg is not involved in the expression of the avoidance of negative stimuli, and this is in direct contrast to findings showing PPTg deficits in pre-pulse inhibition and active avoidance (see section 4.4.5 below).

Other work showing differing effects of PPTg lesions depending on the level of incentive motivation come from research conducted at this laboratory. Ainge, Jenkins, Latimer and Winn (1999) & Keating & Winn (1996) have demonstrated that ibotenate bi-laterally lesioned PPTg rats over consume sucrose (under conditions of deprivation) only when the concentration of the sucrose solution is high. While these findings confirm that the PPTg behavioural deficit would appear to be dependent on the level of incentive motivation, the PPTg impairment in this case is worse when the level of incentive motivation is *higher*. Admittedly this finding could indicate that PPTg lesioned rats simply found the higher percentage sucrose to be more rewarding than the control rats – i.e. they had an altered hedonic appreciation of reward. However, when sucrose was used to generate a conditioned place preference, PPTg lesioned rats did not spend any more time in comparison to controls on the side paired with the sucrose

(Keating and Winn, 1996) a fact that may refute a purely hedonic explanation. Furthermore, in Ainge *et al.* (1999) the PPTg rats who over consumed the 20% sucrose did not show a concomitant increase in approach behaviour (a decrease in latency to reach the sucrose-drinking spout from a start position on the runway might be expected if the PPTg rats did find the sucrose more rewarding). This suggests, therefore, that PPTg rats do not have an altered hedonic appreciation of reward, but strongly indicates that they are unable to control their behaviour under conditions of reinforcement. However, the precise nature of this deficit may dissociate between approach and consummatory responding.

Effects of varying incentive motivation level (produced by the presence or absence of food deprivation) on nucleus accumbens DA have also been reported for both consummatory and approach behaviours – although more so for consummatory behaviours. In their study showing these effects (Wilson, Nomikos, Collu, & Fibiger, 1995) concluded that:

“motivational state can influence the magnitude of neurochemical events that are associated with goal directed behaviours²”.

This suggests that mechanisms do exist in the brain to support the idea that motivational state can affect goal directed behaviour, as well as indicating that the effects of motivational state on PPTg deficits may also be mediated by PPTg connections with nucleus accumbens.

² Wilson, C, Nomikos, G, G, Collu, M, & Fibiger, H, C, (1995) Dopaminergic correlates of motivated behaviour: Importance of drive The Journal of Neuroscience 15(7): 5169-5178 p.5169.

4.4.5 The PPTg as a limbic-motor interface

Salamone (1992) argues that the concept of incentive motivation was one that originated from work on the functions of the nucleus accumbens and the notion that this structure was involved in the hedonic appreciation of reward (which was a role that was in direct contrast to the argument that the nucleus accumbens mediated motor ability). It is now recognised, however, that the nucleus accumbens has a function far more complex than either the simple perception of reward or the mediation of locomotor ability since lesions or pharmacological manipulations of the nucleus accumbens do not remove all aspects of motor or reward behaviour (Salamone, 1992). In fact this is precisely why the concept of a limbic-motor interface has been applied to describe its functions (Groenewegen, Wright, & Beijer, 1996 & Mogenson, Jones, & Yin, 1980).

Similarly to the nucleus accumbens, the PPTg has been implicated in both motor and reward related processes, as the preceding discussion has shown. This is likely due to the fact that the PPTg is in receipt of limbic information from the ventral striatum and its associated output structures in addition to motor information from the dorsal striatum and its output structures. Thus the concept of a limbic-motor interface could be applied with equal relevance to the PPTg (see Inglis *et al.*, 1997, & Inglis & Winn, 1995). Such an argument fits well with the proposed involvement of the PPTg in directing incentive related behaviour since this function would require the integration of both limbic and motor information. However the concept of the PPTg as a limbic-motor interface raises important questions as to whether both structures can sub-serve this function as a unit, and if they do, how the role is split between them.

4.4.6 The role of the PPTg in negative reinforcement

Koch, Kungel, & Herbert (1993) have examined the ability of PPTg lesioned rats to inhibit a freezing response based on pre-pulse inhibition. Their findings have demonstrated that the apparent inability of PPTg rats to exert control over behaviour in reinforced conditions also extends to conditions of negative reinforcement (something that has also been recently argued for the involvement of nucleus accumbens in reinforced behaviours – Ikemoto & Panksepp, 1999). In this task the appropriate behaviour involved prediction of a negative event (the presentation of a loud noise) following a preceding warning stimulus of lesser intensity. The results of Koch *et al.* (1993) showed that PPTg lesioned rats exhibited longer freezing times in comparison to control rats that were demonstrably able to use the warning stimulus to reduce their freezing responses. Disruption of pre-pulse inhibition has also been argued to occur when dopamine in the nucleus accumbens is overactive (Swerdlow, Caine, Braff, & Geyer, 1992). What's more the involvement of the PPTg in active avoidance (which also relies on the formation of an association between a warning stimulus and a negative event) also supports the idea that PPTg rats have a deficit that becomes apparent in the expression of conditioned behaviour (Fujimoto, Ikeguchi, & Yoshida, 1992 & Fujimoto, Yoshida, Ikeguchi, & Nijima, 1989).

4.4.7 Is the PPTg involved in attentional processing?

Given the wealth of experimental evidence described above it seems quite reasonable to conclude that the PPTg is responsible, in some part, for the successful initiation and maintenance of voluntary, goal directed behaviour. In particular this has been discussed

in relation to incentive motivation. However, other studies have shown that, following bilateral PPTg lesions, the extent of the disruption that occurs in this type of behaviour may be dependent on the difficulty of the task demands. This has led to the suggestion that deficits in PPTg lesioned animals could result from the disruption of attentional processing (Dellu, Mayo, Cherkaoui, LeMaol, & Simon, 1991).

In their study Dellu *et al.* (1991) examined the effects of PPTg lesions on three different tasks that they argued differed in the degree to which the rats had to structure together continuous choices, thus placing increasing demands on attentional resources. These tasks included reference memory on a plus maze (on which the PPTg lesioned animals were not significantly impaired), an 8-arm baited radial maze foraging task (on which PPTg animals were significantly impaired), and finally a water maze avoidance task (in which the PPTg rats were severely and permanently impaired). Specifically Dellu *et al.* proposed that the PPTg lesioned rats in their study were experiencing a disruption in sustained attention, the ability to direct and maintain neural processing towards particular stimuli.

Also of interest is that Dellu *et al.* (1991) found their lesions of the PPTg resulted in a decrease of ChAT activity (29%) in the thalamus (ventroposteromedial, ventrolateral, ventroposterolateral nuclei), something that they argued could conceivably have produced the reduced attention displayed by PPTg lesioned rats in this experiment. This point is particularly salient given that the functions of the PPTg have been discussed so far largely in relation to basal ganglia processing, ignoring the functional relevance of ascending ACh PPTg connections. However, on their own, the results of Dellu *et al.* should be treated with caution since the PPTg lesions in this experiment

removed only the Ch5 neurons of the compact portion of the PPTg, leaving the posterior dissipata intact. Kessler, Markowitsch, & Sigg (1986) have found similar effects of posterior PPTg lesions on delayed place learning tasks, however, which would suggest that at least some of Deltu *et al.*'s findings can be extended to include the posterior PPTg.

In addition to the findings of Deltu *et al.* (1991), further results of interest from the Inglis *et al.* (1994) study showed that, during the postoperative training phase of the task (first order conditioning), PPTg lesioned animals omitted trials, failing to press a food hopper to collect food pellets when these became available on presentation of a compound light/click stimulus. Indeed, this deficit was so marked, and was without improvement, that PPTg rats had to be transferred to the lever pressing test phase of the experiment after only 8 days of postoperative training. In order to appreciate the salience of this deficit it should be noted that control rats rarely, if ever, omitted a trial in the postoperative training phase in this study (mean omissions of PPTg rats = 13.5 +/- 5.23 & mean omissions of control rats = 0.16 +/- 0.13). Given that performance of this postoperative training phase relied on an intact stimulus – reward association, this might appear at first glance to have deteriorated following PPTg lesions and thus represent a failure of conditioned behaviour. However, evidence against this comes from previous studies that have shown that PPTg lesioned rats can retain incentive associations if they are pre-trained prior to surgery (Bechara and van der Kooy, 1989, and Fujimoto, Ikeguchi & Yoshida, 1992). An alternative explanation for these findings could therefore be that PPTg lesioned rats were experiencing a deficit in sustained attention mechanisms such that they often failed to notice the presentation of the compound stimulus (Inglis *et al.*, 1994).

One problem to note at this point, though, is that of accepting an attentional argument on the basis of results from tasks that were not strictly designed to test for this. This is because a great deal of data could be explained in this way (Inglis & Winn, 1995) making this aspect of PPTg functioning something worthy of further investigation. However, encouraging preliminary findings from this laboratory investigating the role of the PPTg in a vigilance task do support the involvement of the PPTg in some aspects of attentional function (Kozak & Winn – unpublished).

4.5 Summary of PPTg functions

Although the preceding discussion has treated the involvement of the PPTg in the behavioural expression of incentive motivation and attention as if they were separable components of PPTg function, this is not necessarily the case. For example the aspect of the PPTg that is most likely to provide a clue to its involvement in behaviour is the interdigitation of both ACh and non-ACh neurons (Honda & Semba, 1995, & Grofova & Zhou, 1993, as cited in Winn, 1988). Because of this organisation therefore, it is possible that descending information from the striatum (to non-ACh PPTg neurons), possibly regarding learned associations and sequences of actions, interact with ascending connections to forebrain systems (from ACh PPTg neurons) mediating processes of arousal and attention. Such a contention is purely hypothetical at this stage, however, since it remains unclear as to the precise contributions of PPTg ACh and non-ACh neurons to the processes discussed. Selective lesioning techniques for PPTg ACh neurons would be a highly beneficial procedural development for resolving this issue, as would techniques that would enable the consideration of whether ACh and

non-ACh neurons communicate directly with each other. Until such time as these techniques are available, the issue of defining a precise function for the PPTg remains open. However attempts to resolve it in the meantime will be necessarily shaped by the functions of the systems with which the PPTg is connected.

5. STATEMENT OF THE AIMS OF THE THESIS

The purpose of the current research was to demonstrate that the PPTg is involved in executive functioning. Thus one aim was to extend the findings of Phillips and colleagues, by addressing PFC involvement in DSWS behaviour following permanent excitotoxic lesions. In addition, given the startling extent of the DSWS deficit in PPTg lesioned rats, and the fact that this task has been so successfully used to examine the contributions made by other fronto-striatal structures to performance of DSWS behaviour, we aimed to investigate more precisely the nature of the PPTg involvement in the DSWS task. This was especially true given the evidence to suggest that PPTg lesion deficits could be modulated by variations in reward value. We hoped to demonstrate, through a comparative study of PPTg DSWS and PFC DSWS lesion deficits, that the functions of the PPTg are closely related to the executive functions more commonly attributed to the PFC, in this case the medial PFC of the rat. Finally, since the disconnection lesion technique has proved so insightful for Phillips *et al.* with regards functional structural communication routes, it was hoped that the same technique could be used to demonstrate *directly* the involvement of the PPTg in the processing of neural information originating from the cortex.

In line with these aims, the following hypotheses were advanced for the experiments contained in the relevant chapters:

Chapter 8: Rats with permanent excitotoxic *bilateral* lesions of the medial wall of the prefrontal cortex would show significantly *more* test phase errors in *retention* of the DSWS task in comparison to sham lesioned rats. This was hypothesised based on the

findings of Seamans *et al.* (1995) since rats with lesions of area AC and area PL made separately had been shown to exhibit test phase error impairment in DSWS retention in this study.

Chapters 9 & 10: Rats with permanent excitotoxic *bilateral* lesions of the PPTg would show significantly *more* test phase errors in *retention* of the DSWS task in comparison to sham lesioned rats. This was hypothesised based on the findings of Keating & Winn (2001). In addition, rats with *bilateral* PPTg lesions would *not* show a further increase in test phase errors following an increase in the incentive value of the reward available in the DSWS task. This was hypothesised based on the assumption that the DSWS impairment in PPTg lesioned rats reflects a disruption of executive functions rather than a disruption of the processing of purely motivational information.

Chapter 11: Rats with permanent excitotoxic *unilateral* lesions of the PPTg and mPFC would *not* show significantly more test phase errors in *acquisition* of the DSWS task in comparison to sham lesioned rats. This was hypothesised based on the assumption that damage to these structures in only one hemisphere would not be sufficient to produce loss of function, and relates directly to our aim to use crossed unilateral disconnection lesions to show PPTg involvement in the processing of neural information originating from the cortex. In addition, rats with *bilateral* excitotoxic mPFC lesions would show a significant *increase* in test phase errors in *acquisition* of the DSWS task. This hypothesis was based on the assumption that a deficit in executive functioning following bilateral mPFC lesions, if demonstrated during DSWS retention, should also be apparent in an acquisition paradigm. This would especially be the case if the mPFC deficit was one of working memory or response inhibition.

Chapter 12: Rats with permanent excitotoxic *unilateral* lesions of the PPTg and mPFC would *not* show significantly increased test phase errors in *retention* of the DSWS task in comparison to sham lesioned rats. The rationale for this hypothesis is as for the first hypothesis of Chapter 11. In addition, rats with crossed unilateral excitotoxic *disconnection* lesions of the PPTg and mPFC would show significantly *increased* test phase errors in *retention* of the DSWS task in comparison to sham lesioned rats. This was hypothesised based on the finding that bilateral damage sustained to each structure produced increased test phase errors in this task (Keating & Winn, 2001, and Seamans *et al.*, 1995), and that lidocaine disconnection lesions of regions of mPFC with other structures of the ventral striatal system also produce increases in test phase errors in the DSWS task (Floresco *et al.*, 1997).

Chapter 13: Rats with unilateral lesions of the PPTg and mPFC made in the *same hemisphere* would *not* show significantly increased test phase errors in *retention* of the DSWS task in comparison to sham lesioned rats. This was hypothesised based on the assumption that *disconnection* of two structures must occur following unilateral damage to these structures in order for function to be impaired.

6. GENERAL METHODS

6.1 Subjects

Adult male Lister Hooded rats (Charles River, Margate, Kent, UK) were used weighing approximately 300 – 400 g at the time of surgery. Approximate weights for each experiment are given in the relevant chapters. All rats were initially housed in pairs but were separated immediately prior to imposition of food restriction. Once this was imposed, rats were fed 12g of lab chow daily in order to restrict body weight to 85% of free feeding weight (defined as weight on the day prior to food restriction). On reaching this target, behavioural training began and rats were given 18-20g lab chow daily in order to stabilise and maintain weight; feeding occurred once all rats had completed DSWS trials for the day. Rats were housed in 25 x 45 x 15 cm plastic cages in temperature and humidity controlled rooms with a 12:12 hr light/dark cycle. The light period coincided with the times of behavioural testing. All guidelines and requirements set out in the *Principles of Laboratory Animal Care* (National Institutes of Health, Publication No. 86-23, revised 1985) and the UK Animals (Scientific Procedures) Act 1986 were followed.

6.2 Apparatus

All behavioural training and testing was carried out on an elevated 8 arm, wooden radial maze. The central platform and arms were grey in colour with each arm containing a white plastic food cup 3 cm in diameter and 1.5 cm deep. The food cups were glued

onto the surface of the arms approximately 1 cm from the end of each arm. The entrances to the arms could be blocked by white, plastic, guillotine doors. The central platform measured 45 cm in diameter, and the arms measured 68 cm in length and 10 cm in width. Once the doors were raised, the entryways to the arms were 10 cm in height and 9 cm separated each one. The maze was elevated 50 cm from floor level and was situated in a well-lit “L” shaped room. The maze apparatus was located in one arm of the “L” and the observation area in the other. Various extra-maze cues surrounded the apparatus including furniture and 4 posters. The experimenter observed and recorded the sessions from a PC using the Observer 2.0 package (Noldus Information Technology). The PC was located approximately 2 m from the maze.

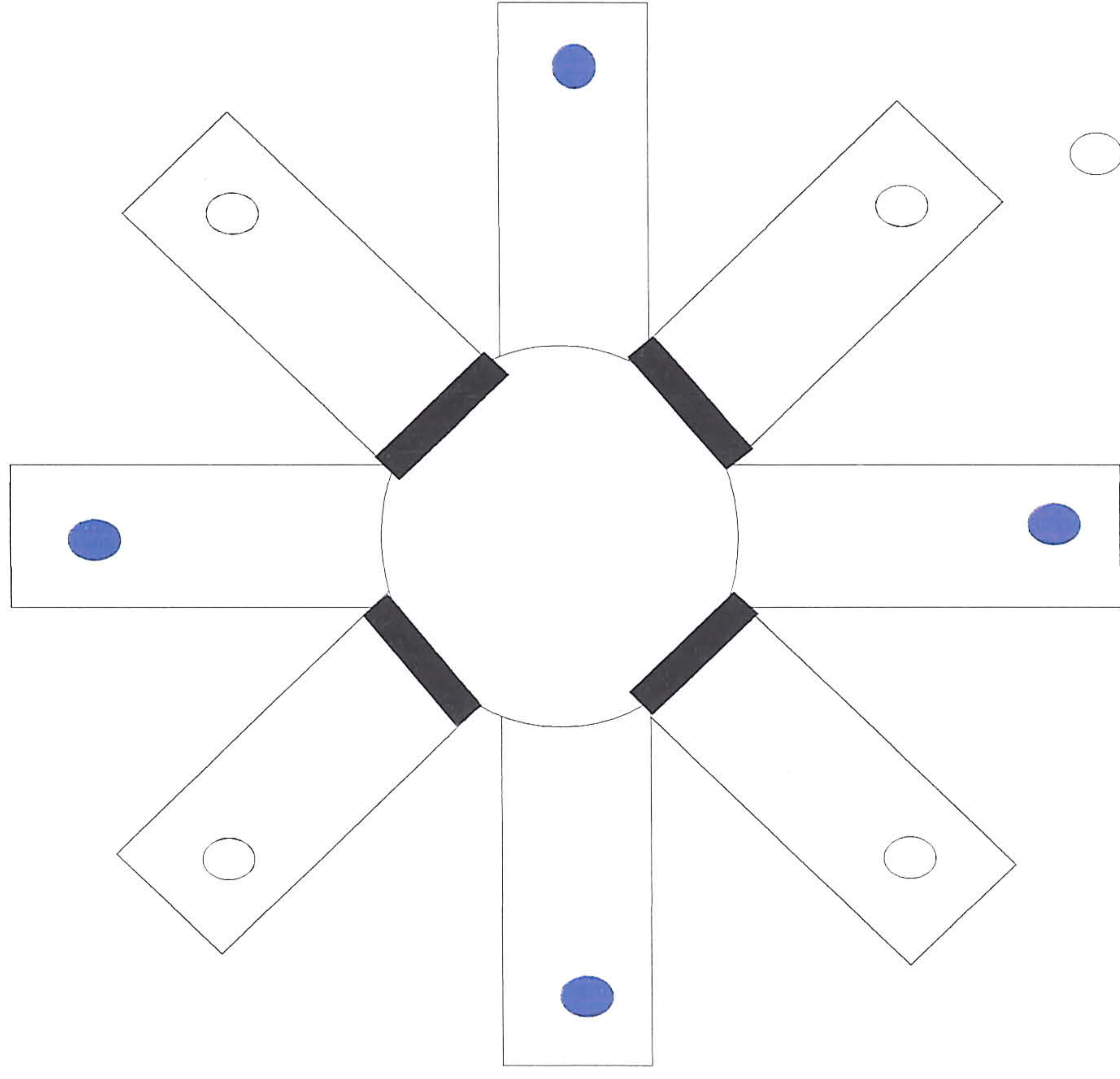
6.3 Behavioural Training and Testing

The DSWS task was adapted from Seamans, Floresco and Phillips (1995). It was developed to test the ability to select correct responses whilst inhibiting incorrect responses based on spatial information from extra-maze cues (Olton & Samuelson, 1976). All animals were given 2 habituation sessions to the maze, each consisting of 10 mins free access to all arms, where no food reward was available. The sessions occurred over 2 consecutive days immediately prior to beginning the DSWS procedure.

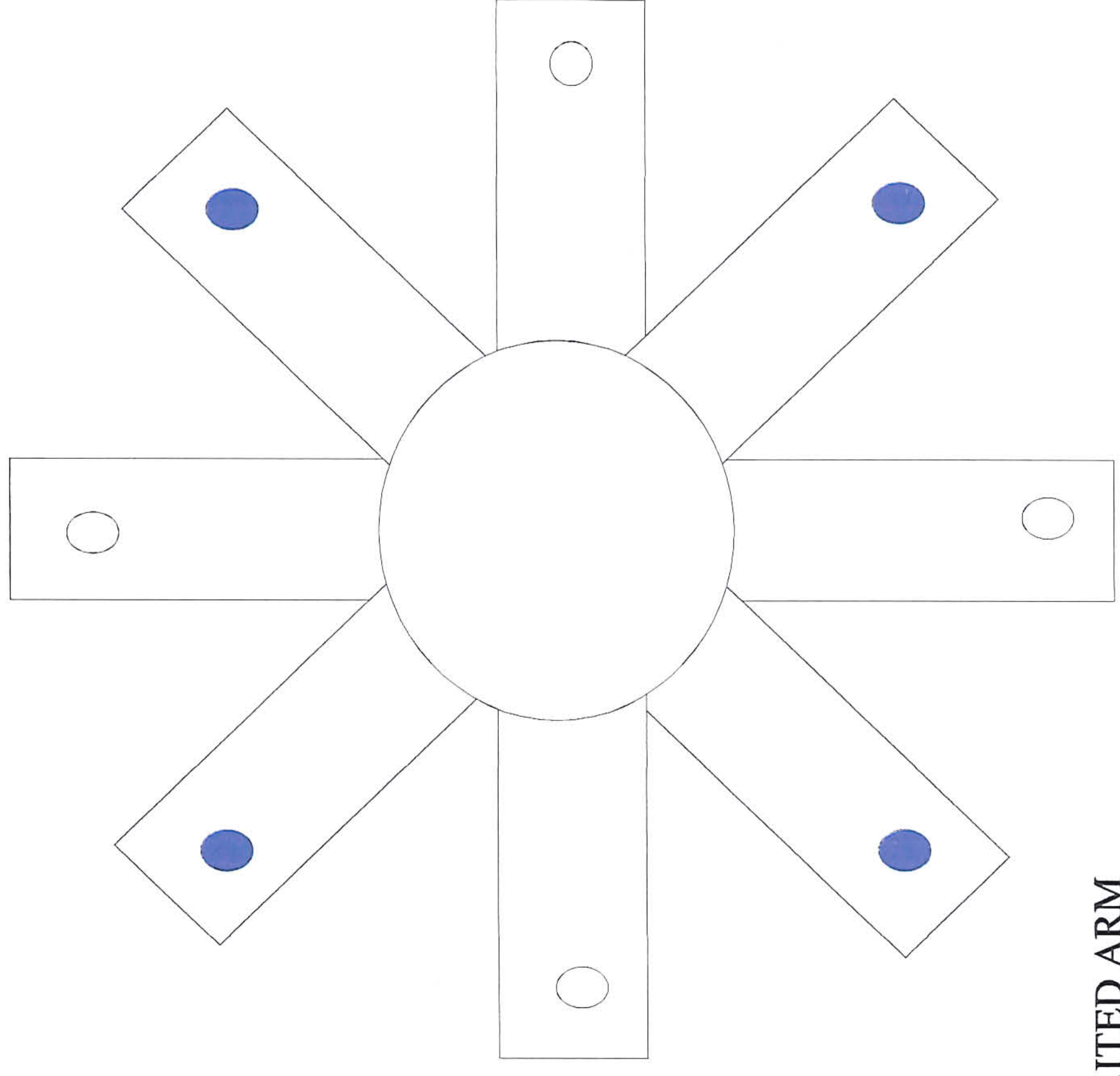
A representation of the DSWS task can be seen in Figure 6.i. Rats were given one trial of the DSWS task per day. Each trial consisted of 2 phases separated by a delay of 5 mins, referred to as the training phase and the test phase respectively. At the start of each phase the rats were placed on the central platform, facing away from the experimenter. Each phase provided the opportunity to forage for 4 x 45 mg food pellets

FIGURE 6.i THE DSWS TASK

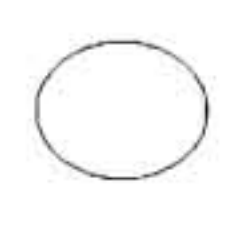
TRAINING PHASE



TEST PHASE



UNBAITED ARM



BAITED ARM



BLOCKED ARM



with 1 pellet per food cup. Timing for each phase began once the rat had been placed in the centre of the maze and ended once all 4 pellets were collected, or when 10 mins had expired (whichever was sooner). At the end of each phase the rat was removed from the maze and returned to the home cage.

The training phase of the task allowed the rat to choose 4/4 available arms with a new arm combination selected each day. Arm combinations were prohibited from including more than 2 consecutive arms such that arms 1,2,4,7 would be a valid combination while arms 1,2,3,6 would not be. The test phase required the rat to choose 4/8 available arms such that it would find a food reward in each of the 4 arms that had *not* been previously visited in the training phase. For example, if arms 1,2,4,7 had been baited in the training phase then food rewards would be found in arms 3,5,6,8 in the test phase. This constituted the win-shift component of the task.

In experiments where rats were pre-trained this training occurred 7 days a week until acquisition of the task. Acquisition criterion was defined as a mean error score of 1.0 or less across all subjects over 2 consecutive days, in each phase. This criterion was identical to that of Seamans *et al.* (1995). Post-surgical experimental testing in the same experiments occurred until the sham groups regained the criterion score.

An arm entry was recorded once the animal had reached the food cup at the end of the arm and an arm exit was recorded once the animal had all 4 paws on the central platform. There were 4 measures used in statistical analysis that were common to both phases. These were the number of errors made, the number of correct choices made before the first error, latency to reach the first arm food cup from the central platform,

and mean arm choice time (defined as: time to complete the phase/number of choices made). In the test phase the error score was further divided into “across phase” and “within phase” errors. Across phase errors were defined as the number of training phase arms returned to in the test phase. This measure therefore had a ceiling score of 4. Within phase errors were defined as re-entry to any arm previously visited in the test phase.

Given that there are a large number of dependent variables for the DSWS task it should be noted that performance on some of these is not likely to be entirely independent of performance on the others. Within both the training and test phases it is probable that both the latency and the mean arm choice time measures covary to some extent since both assess mechanisms of run speed responding. However, it should be noted that the mean arm choice time measure includes time to consume the food reward while latency does not. Furthermore, since the AP and WP error scores are derived from the total test phase error score it can be inferred that performance on each error type covaries with performance on the total score. Finally, and perhaps most importantly, it is unlikely that the accuracy measures covary *across* the training and test phases given that pre-surgical training to criterion takes much longer on the test phase in comparison to the training phase (approximately 3 times as long). Furthermore, there is evidence in support of this assumption since deficits on the DSWS task can occur in the test phase without impairment in the training phase (Keating & Winn, 2001). Thus it would seem that there are cognitive mechanisms accessed during that test phase that are not required during the training phase for accurate responding.

6.4 Data analysis

All data analyses were conducted using SPSS version 10. For all pre-surgery data, two-way mixed ANOVAs (within subjects on day and between subjects on group) were conducted on each of the measures in the training and test phase of the task to test for pre-existing between group differences. When necessary all analyses were subjected to the Huyn Feldt correction following violation of the sphericity assumption. None of these ANOVAs revealed significant differences between the surgical groups prior to surgery.

All postsurgical data was initially analysed using the criterion days method used by Keating & Winn (2001). This involved calculating a mean value on each measure for each rat across the 2 days at which sham rats regained criterion. These values were then analysed using one-way between subjects ANOVAs. Analysing the obtained data by this method addresses the very specific question of whether lesioned rats are impaired *by the time sham rats regain asymptotic performance*. Additional two-way mixed ANOVAs were conducted on the data from all postsurgical trials only where observation of data from earlier trials indicated that significant effects might be present. Where these analyses were conducted they were also corrected using the Huyn Feldt correction following observation of heterogeneity of variance. The Huyn Feldt correction was adopted rather than the Greenhouse-Geisser correction because the Greenhouse-Geisser correction can be overly conservative, especially with small group sizes. In cases where the Huyn Feldt correction has been used, corrected numerator and denominator degrees of freedom are reported.

For all experiments the data for the measures of latency to the first arm and mean arm choice time were log transformed. This transformation was performed since it is an effective method for normalising distributions that have positive skew, something that occurs frequently in time scale dependent variables (Winer, 1971). Thus the transformation was limited only to the time scale variables, and the ANOVAs were performed on the log-transformed data.

In the graphical presentation of data error bars reflect standard error and not standard deviation for the means. In experiments where there were significant group effects for the test phase error score the mean percentage of the total test phase errors that were AP and WP were calculated and presented graphically. This was done to parallel the approach of Phillips and colleagues whereby the error patterning of significant lesion effects are described to maximise understanding of the lesioned group's deficit (Seamans *et al.*, 1995). As such therefore, this descriptive approach was conducted only in experiments where significant test phase error effects were found. For analytical purposes however, the question of whether groups made significantly more across phase than within phase errors was addressed using obtained error score values rather than percentage scores since some rats were excluded from the percentage analysis because they did not make any errors.

6.5 Surgery

All surgical procedures were carried out with the authority of the appropriate UK Home Office project and personal licences. Furthermore the guidelines set out in the European Communities Council Directive of 24 November 1986 (86/609/EEC) were followed.

Rats were assigned to surgical groups with matching mean bodyweights. They were anaesthetised with ip sodium pentobarbital (Sagatal -1 ml/kg, 50:50 sterile water), with the depth of anaesthesia monitored by the paw withdrawal reflex and the eye blink reflex. No surgical procedures were conducted until both reflexes were absent. Once anaesthesia was at a suitable level the rats had their heads shaved and were placed in a Kopf stereotaxic frame. A midline incision was made and the scalp retracted to expose the skull.

Surgery was conducted using SGE syringes mounted in a manual microdrive on the stereotaxic frame. Lesions were made using ibotenic acid (Tocris Cookson) or quinolinic acid (Sigma) with sham operated controls receiving infusions of sterile phosphate buffer. To allow for diffusion the needle was left in situ for the same amount of time as that required to infuse the substances. When bevelled needles were used care was taken to ensure that the bevelled face was always pointing forwards. Following infusions the incisions were closed with surgical clips and recovery was monitored to check for problems with breathing or regulation of body temperature. Rats were allowed 7 days recovery from the date of the last surgery with ad libitum access to food before re-imposition of food restriction.

6.6 Histology

6.6.1 Tissue Preparation

Following completion of behavioural testing rats were given a lethal i.p. injection (150 mg) of sodium pentobarbital (Euthatal – 200mg/ml). They were perfused transcardially

with 0.9% phosphate buffered saline followed by 4% paraformaldehyde in 0.1M phosphate buffer. mPFC lesioned brains were removed and placed in 4% paraformaldehyde overnight to ensure adequate level of fixing. Following this, mPFC brains were stored in 20% sucrose in 0.1M phosphate buffer (PB) until further processing. PPTg lesioned brains were immediately placed in 20% sucrose in 0.1M PB following removal.

Brains with mPFC lesions were placed in egg yolk prior to cutting to prevent the cortex becoming separated from the rest of the brain. First, the front third of each brain was separated and washed in dH₂O to remove excess sucrose. These were then carefully dried with absorbent tissue. Small plastic trays with wells approximately 1.5cm in diameter and 1.5cm deep were lightly greased with WD40. The front portions of the brains were then placed in the wells such that the cut edge rested on the bottom of the well. Using a Pasteur pipette, egg yolk was piped around these until they were completely covered. Care was taken to prevent the formation of bubbles. Following this the trays were placed in a basin containing 20mls 40% paraformaldehyde, with a large plastic bag sealed around the basin to prevent fumes escaping. The basin was then left for 24h until the egg yolk had set. Once removed from the trays, the brains were encased in a rubbery egg layer and were stored in 20% sucrose solution until cutting. On cutting with the freezing microtome care had to be taken to ensure that the freezing occurred quickly otherwise ice crystals formed in the tissue.

All sections were cut at 50 μ m on the freezing microtome in the coronal plane. 1 in 4 sections were retained for each stain (NADPH diaphorase, cresyl violet and NeuN) for histological verification of lesion location.

6.6.2 Cresyl Violet Staining

The cresyl violet staining solution was prepared prior to the staining procedure. 0.5g cresyl fast violet acetate (aq) was dissolved in 475ml water and 25ml glacial acetic acid using an ultrasonic bath. This took approximately 60min. Following this the pH was adjusted to 3.5 using sodium acetate solution. Sections were mounted onto slides using water and were left to dry for 12h at room temperature. The slides were then placed in a formalin gas bath for a minimum of 30 min to fix the sections in place.

Slides were then placed in xylene (2 min) to defat them and rehydrated using graded alcohols (100% then 50%) and then tap water. They were then placed in the cresyl fast violet solution for approximately 2 min or until the staining was judged to be at an appropriate level. Following this the slides were rinsed in running tap water for 5 mins and then differentiated in graded alcohols (50% then 100%). Finally they were cleared in xylene and cover-slipped using DPX. Cresyl violet stains nissl substance a purple/blue colour and nuclei and some cytoplasmic processes of neurons a pale blue colour.

6.6.3 Nicotinamide Adenine Dinucleotide Phosphate (NADPH diaphorase) Staining

For this procedure sections were cut into 0.1M PB and then sorted into net baskets with 20% sucrose in 0.1M PB. A 50ml solution of 10% Triton X in distilled water was prepared prior to staining. 1.5ml of this solution was made into 0.3% Triton X by adding 48.5ml 0.1M PB. 1ml of this 0.3% Triton X in 0.1M PB was then added to a

10mg vial of Nitro Blue Tetrazolium (NBT) concentrate, stored in the fridge. 10mg of b-NADPH (tetrasodium salt type 1) was then weighed into a disposable vial. 9.9ml of the 0.3% Triton X in 0.1M PB and 0.1ml NBT concentrate was then added to the vial. Once this solution was made it was put into tissue culture plates with wells 2cm in diameter and 2 cm deep. With 0.4ml per well this amount of solution enabled staining of 6 brains, each brain occupying a row of 4 wells, 4-5 sections per well. The sections were carefully removed from the net basket and were transferred to the wells. The tissue culture plates were then kept at 37° centigrade in a thermostatically controlled water bath for 20min, or until staining was judged to be at an adequate level. The sections were then removed from the wells and stored in net baskets in 0.1M PB until they could be mounted. Sections were mounted onto gelatin-coated slides from water and left to dry overnight at room temperature. They were then cover-slipped using glycerol gelatin. NADPH diaphorase is a stain specific for NOS positive neurons, and stains approximately 90% PPTg ACh cell bodies a deep purple colour (Inglis et al 1994).

6.6.4 NeuN Standard Procedure

As for NADPH diaphorase staining, sections for this stain were sorted into a net basket in 20% sucrose in 0.1M PB following cutting. They were then washed in 0.1M phosphate buffered saline (PBS) 4x5min on a shaker. Following this they were placed in 1% sodium borohydride in distilled water at room temperature for 15 mins, a procedure that removes un-reacted formaldehyde from the tissue. They were then washed at 5 mins intervals in 0.1M PBS on a shaker until there were no bubbles being formed. The sections were then transferred to NeuN primary antibody made up for use

(1:1000 in Antibody diluting solution - ADS) 0.4ml per well in a 24 well tissue culture plate (4-5 sections per well). They were then left on a shaker in the fridge (4°C) overnight. The next day the sections were removed from the tissue culture plate, transferred to a net basket, and washed in 0.1M PBS 4x5 min on a shaker. Following this they were placed in the first, secondary antibody (anti-mouse IgG 1:200 in ADS), 0.4ml per well in a tissue culture plate, and incubated at room temperature on a shaker for 60min. Again they were washed after this in 0.1M PBS 4x5min, and then transferred to the second, secondary antibody (mouse PAP 1:200 in ADS) 0.4ml per well. They were incubated at room temperature for 60min on a shaker and washed 4x5min with 0.1M PBS following this. During the last wash cycle 2 x diaminobenzidine (DAB) tablets were removed from the fridge and added to 30ml of distilled water (15ml per tablet). Sections were then incubated at room temperature in the DAB solution on a shaker until depth of staining was judged acceptable. Finally they were transferred to a net basket and washed at room temperature 3x4min in 0.1M PBS, mounted onto gelatin coated slides and left to dry overnight at room temperature. The slides were then cover-slipped from xylene using DPX. NeuN is a vertebrate neuron-specific nuclear protein (Chemicon International Inc. CAT No. MAB377) that reacts with most neuronal cell types throughout the nervous system. It primarily stains the nucleus of neurons with lighter staining in the cytoplasm. With DAB tablets the stain can be visualised as a mid-brown colour.

6.6.5 Microscopy and lesion mapping

During lesion examination all lesions were defined according to areas of cell loss and reactive gliosis and were assessed using light microscopy (Leitz "Diaplan" microscope

fitted with a Sony DXC-3000P video camera). Each lesion was mapped individually from the combination of neuronal stains visualised on a high-resolution monitor. Damage to surrounding non-focal structures was also tabulated according to percentage loss. Lesion diagrams were then collated and the smallest and largest lesions were selected and mapped using Corel Draw (version 3.0). Representative lesion diagrams are therefore included for each group in each chapter showing the smallest and largest lesions within these groups. The smallest mapped lesion therefore represents the complete histology of a single animal, as does the largest. The lesions included in the analyses for each group therefore vary between the two extremes of damage mapped in the diagrams. Photographic representations of brain sections were captured with a Pixera camera (PVC 100C) connected to a Power Macintosh 7300/200 using the Pixera VCS 1.2 program.

7. LESIONING THE mPFC: DEVELOPMENT OF THE EXCITOTOXIC LESIONING PROCEDURE

7.1 Introduction

The aim of the current research was to assess the involvement of the PPTg in executive functions originating from the mPFC. To achieve this aim it was necessary to develop an excitotoxic lesioning technique for the mPFC that would enable a detailed study of the effects of permanent mPFC lesions in DSWS behaviour. This would facilitate comparisons with the effects of PPTg lesions in this task and would also enable an investigation of DSWS performance following disconnection lesions of the PPTg and mPFC.

A review of literature employing mPFC lesioning techniques showed a preference for electrolytic lesions (produced via various methods) that inactivate fibers of passage (Broersen *et al.*, 1999, Fritts *et al.*, 1998, Mair *et al.*, 1998, Joel *et al.*, 1997, & Seamans *et al.* 1995). However, average coordinates were borrowed from these studies, in addition to being based on an assessment of location and size of areas PL and AC in the stereotaxic atlas of Paxinos & Watson (1997).

7.2 Method

6 adult male Lister Hooded rats were used weighing approximately 350g at the time of surgery. Rats were housed according to the conditions set out under “General Methods”, and were subjected to food restriction (12g daily) 48h prior to surgery. All rats received bilateral infusions of ibotenate made using a manually driven microdrive. Infusions were made at the following coordinates: 2.6mm anterior to bregma, 0.7mm laterally to the midline sinus, and 3.8mm ventral to the surface of the skull.

3 rats received lesions using a 5 μ l syringe mounted on the stereotaxic frame. Of these, 2 animals were infused with 1.0 μ l 0.12M ibotenate and 1 with 1.0 μ l 0.06M ibotenate. The other 3 animals received lesions using a 1.0 μ l syringe mounted on the stereotaxic frame. 1 was infused with 0.5 μ l 0.12M ibotenate, 1 with 0.75 μ l 0.06M ibotenate, and 1 with 0.5 μ l 0.06M ibotenate. Care was taken to ensure that the bevel of the needle was always pointing forward. Infusions took approximately 6 min with the needle left in situ for a further 6 min to allow for diffusion of the toxin. The rats were sacrificed within 24h according to the procedure laid out under “General Methods” and histology was conducted using cresyl violet stain for nissl substance.

7.3 Results

Figure 7.i shows a comparison of all of the lesions at +2.70mm from bregma (the approximate centre of the lesions). Lesion size was defined using areas of cell loss and reactive gliosis. All lesions showed good anterior/posterior spread stretching between +4.70mm from bregma and +1.70mm from bregma. The largest lesion, 1.0 μ l of 0.12M ibotenate, produced considerable tissue collapse. However this was reduced in line with the nmol amount of toxin used. For the smallest two lesions, 0.75 μ l and 0.5 μ l 0.06M ibotenate, there was no tissue collapse evident. Behaviourally, all subjects appeared to exhibit enhanced responding to overhead threat (informally observed during recovery from surgery) that lessened between time of surgery and time of sacrifice. It was expected that this symptom would therefore extinguish with the extensive handling of subjects required during the DSWS procedure.

7.4 Discussion

Based on the results shown in Figure 7.i it was concluded that the lesion made using 0.5 μ l 0.06M ibotenate was the most acceptable, invading less of the pre-motor areas of the mPFC. However, since this procedure was made using only one animal it was decided that a slightly larger infusion of toxin would be more likely to ensure this level of damage in larger subject numbers. Since the 0.75 μ l 0.06M lesion appeared to be too large, however, the procedure selected for the DSWS mPFC lesions was 0.6 μ l 0.06M ibotenate (an approximate average of the two tested techniques).

8. DSWS RETENTION FOLLOWING EXCITOTOXIC LESIONS OF THE MEDIAL PREFRONTAL CORTEX IN THE RAT

8.1 Introduction

The involvement of prefrontal cortex (PFC) in the processing of neural information has been extensively studied (see Chapter 3). The picture that has emerged is of a brain region involved in a variety of higher order cognitive processes necessary for the selection of behaviour. The term executive function is generally used to refer to these processes, although its application is not strictly limited to PFC since dysexecutive syndrome has been observed in humans without direct damage to PFC. However, clarifying the precise nature of processes that sub serve PFC executive function has proved problematic, most likely a result of the complex organisational nature of this region of the brain. Issues still under debate include whether PFC can be considered to be unitary or heterogenous in function, as well as the related issue of appropriate compartmentalisation of structure and function, which is further complicated by between-species differences. Despite these problems however, some processes are regularly associated with PFC across species, including working memory, attention and response inhibition.

In humans clinical studies have revealed overwhelming evidence to support a role for PFC in some form of mnemonic processing. For example, patients with lesions or disorders of the frontal lobes exhibit impairments in tests of memory examining working memory (Owen *et al.* 1990, Spinnler *et al.* 1988, & Milner 1964), and temporal organisation of memory (Fuster, 1989). These memory functions have generally been

attributed to the dorsolateral region of PFC (dlPFC) with similar findings occurring in dlPFC lesioned non-human primates in delayed response tasks (Goldman-Rakic, 1990, Passingham, 1975, & Pribram & Tubbs, 1967). More recent electrophysiological evidence also suggests that primate dlPFC exhibits delay activity in response to spatial cues (Wilson *et al.*, 1993) adding further credence to a mnemonic function role.

As well as the suggested involvement of PFC in mnemonic functions, emphasis has also been placed on the use of attention mechanisms to guide complex and flexible behavioural strategies. For example, the Wisconsin Card Sorting Test (WCST) in humans revealed deficits in frontal patients in switching to a new card sort strategy (Milner, 1963). This is an impairment characterised by a lack of inhibition of responding to the old, but irrelevant, card sort strategy. A WCST deficit has been argued to occur as a result of an inability to shift attention set by some authors (the shifting of the allocation of attention from a particular feature or class of features to a new set of features) whilst others have argued that it is a result of an inability to hold the currently valid card sorting principle in working memory (Robbins, 1998). Dissociating between the executive functions of working memory, attention and response inhibition can prove difficult. However, since a frontal lesion deficit on the WCST task is specific to the extra-dimensional shift stage, with subjects able to perform intra-dimensional shifts and reversals of card sorting principles, an argument for the PFC WCST deficit in terms of working memory seems less likely (Robbins, 1998). Such an argument might also apply to the consideration of response inhibition impairment.

Experiments conducted on rodents have the benefit of being able to make controlled lesions in large subject groups. However this can be offset by the problem of the

homology of related PFC structures. Despite the absence of a granular layer IV casting doubt on the existence of PFC in the rat, prelimbic region (PL) of rat PFC has often been proposed as a homologue of primate dlPFC by virtue of extensive reciprocal connections with mediodorsal nucleus (MD) of thalamus (Kolb, 1990, & Groenewegen, 1988 - see Chapter 3 for further discussion of the homology debate). Area PL in the rat extends from the frontal pole through the genu of the corpus callosum just ventral to the anterior cingulate cortex. As well as connections with MD, area PL shares direct connections with hippocampus (Jay, Glowinski, & Thierry, 1989) such that hippocampal activity is capable of eliciting long-term potentiation in area PL (Mulder, Arts, & Lopes da Silva, 1997). Consistent with these connections, and the above findings for human and non-human primate dlPFC, several lesion studies on rat PFC, with damage extending to area PL, support its role in response selection based on spatial and/or temporal working memory (Ragozzino *et al.*, 1998, Granon *et al.*, 1994, & Kesner & Holbrook, 1987). This could suggest at the very least functional analogy, if not anatomical homology, between rat area PL and primate dlPFC.

Analyses of working memory and response inhibition deficits in the rat are commonly achieved through 2 choice lever or maze tasks (Neave *et al.*, 1994, Poucet & Herrman, 1990, Eichenbaum, Clegg, & Feeley, 1983, & Johnston *et al.*, 1974). However, the delayed spatial win-shift (DSWS) radial arm maze task may be more relevant for assessing these PFC deficits as it is a more complex task requiring strategic responding between 8, as opposed to 2, choices. This makes increased demands on the processing capacity of higher functions, a factor that may feature strongly in the detection of mPFC deficits in the rat (Porter & Mair, 1997, & Granon *et al.*, 1994).

Floresco *et al.* (1996, 1997), & Seamans *et al.*, (1994, 1995) used the DSWS task to analyse the involvement of structures associated with ventral striatal processing in the organisation of behaviour. They have revealed deficits in the use of memory for previously encountered spatial locations to guide foraging following lidocaine inactivation of the anterior cingulate region (AC), and area PL of the rat mPFC (Seamans *et al.*, 1995). Specifically, the design of this study was such that the role of area PL in spatial memory could be linked to retrieval processes rather than encoding processes. However, despite this elegant design, there are several important caveats that apply to the use of a temporary lesion methodology, analysing behavioural deficits produced in a single trial. For example, functional spread of lidocaine is difficult to calculate and cannot be confirmed in the same way that permanent tissue damage can be. Also, lidocaine is non-selective and the possibility exists that the effects observed were due to inactivation of fibre systems, as is the case with electrolytic lesions. Finally, a lidocaine study can only provide a “snapshot” of any observable deficit, a point that is particularly problematic considering that some research has suggested that PFC deficits in rats, including those observed in the radial maze, may only be transient in nature (Porter *et al.*, 1997, & Granon *et al.*, 1994). This is a factor which should be taken into account when inferring the functions of the PFC. The current study was therefore conducted to examine the effects of permanent selective damage to cell bodies in the medial wall of the PFC using a similar version of the DSWS task employed in the (Seamans *et al.*, 1994, 1995, & Floresco *et al.*, 1996, 1997) lidocaine studies. It was hoped that extended analysis across trials of the pattern of responding produced by rats with mPFC damage would help to extend understanding of mPFC function beyond that gained from previous research, including the lidocaine study of Floresco and colleagues (1995).

8.2 Method

19 naïve male Lister Hooded rats (Charles River, Margate, Kent, UK) were pre-trained for 18 days on the DSWS task according to the procedure discussed in “General Methods”. The rats weighed approximately 330g at time of surgery and were housed and fed in line with the protocol laid out in “General Methods”. mPFC lesions were made using the surgical procedure developed in the preceding chapter. In all cases the midline sinus was exposed to ensure accurate midline measurement. Rats were given 7 days recovery from the date of the last surgery with ad libitum access to food and water. Following this all rats were returned to food restriction conditions with the 85% target weight calculated with weight on day 7 as 100% weight. Postsurgical training occurred for a total of 10 days within which time the sham lesioned rats regained criterion. Perfusion and histology was as discussed in “General Methods”. Lesions were assessed using cresyl violet staining for nissl substance

Postsurgical data was analysed using two-way mixed ANOVAs (between subjects on surgical group and within subjects on day). This was done specifically because it allowed analysis of performance over all trials in order to extend the single DSWS trial findings of Seamans *et al.* (1995). Where necessary these ANOVAs were corrected using the Huyn Feldt correction for heterogeneity of variance.

8.3 Results

8.3.1 Histology

Following analysis of the cresyl violet staining, 3 rats in the lesion group were excluded, two of which each had a unilateral lesion, and one because the bilateral lesion was too small. This meant that, in total, there were 7 mPFC lesioned rats and 9 sham-operated controls retained for the data analysis. Fig 8.i shows the extent of the largest and smallest lesions included in the analysis. The lesion area was defined as an area of neuron loss and reactive gliosis. Cresyl violet staining revealed extensive loss of neurons within the lesion boundaries although some tissue collapse was evident in a small proportion of subjects. All lesions included in the analysis had damage to the prelimbic (PL) and anterior cingulate (AC) regions of the medial wall. Larger lesions also extended into the pre-motor area Fr2 (according to nomenclature listed by Paxinos and Watson, 1997). One of the lesions also extended ventrally to include most of the infralimbic area (IL). However, across the group as a whole, the damage to the PL and AC areas of the medial wall was bilaterally symmetrical, with no consistent damage to other areas. The lesions extended rostrally to include most of area PL (as it first appears in Paxinos and Watson, 1997 at AP + 4.70mm from Bregma), and caudally to include areas ACd and ACv at their most rostral aspect (again as in Paxinos and Watson, 1997 at AP + 1.70mm from Bregma). There was no invasion of the corpus callosum.

8.3.2 The DSWS task

Figure 8.ii shows the mean and standard error for the number of errors made in the training phase for all days. The two-way mixed ANOVA showed that there was no significant main effect of group $F(1,14) = 2.36$ or of day $F(7.85,109.84) = 1.40$. The group x day interaction was also not significant $F(7.85,109.84) = 0.74$.

FIGURE 8.ii TRAINING ERRORS

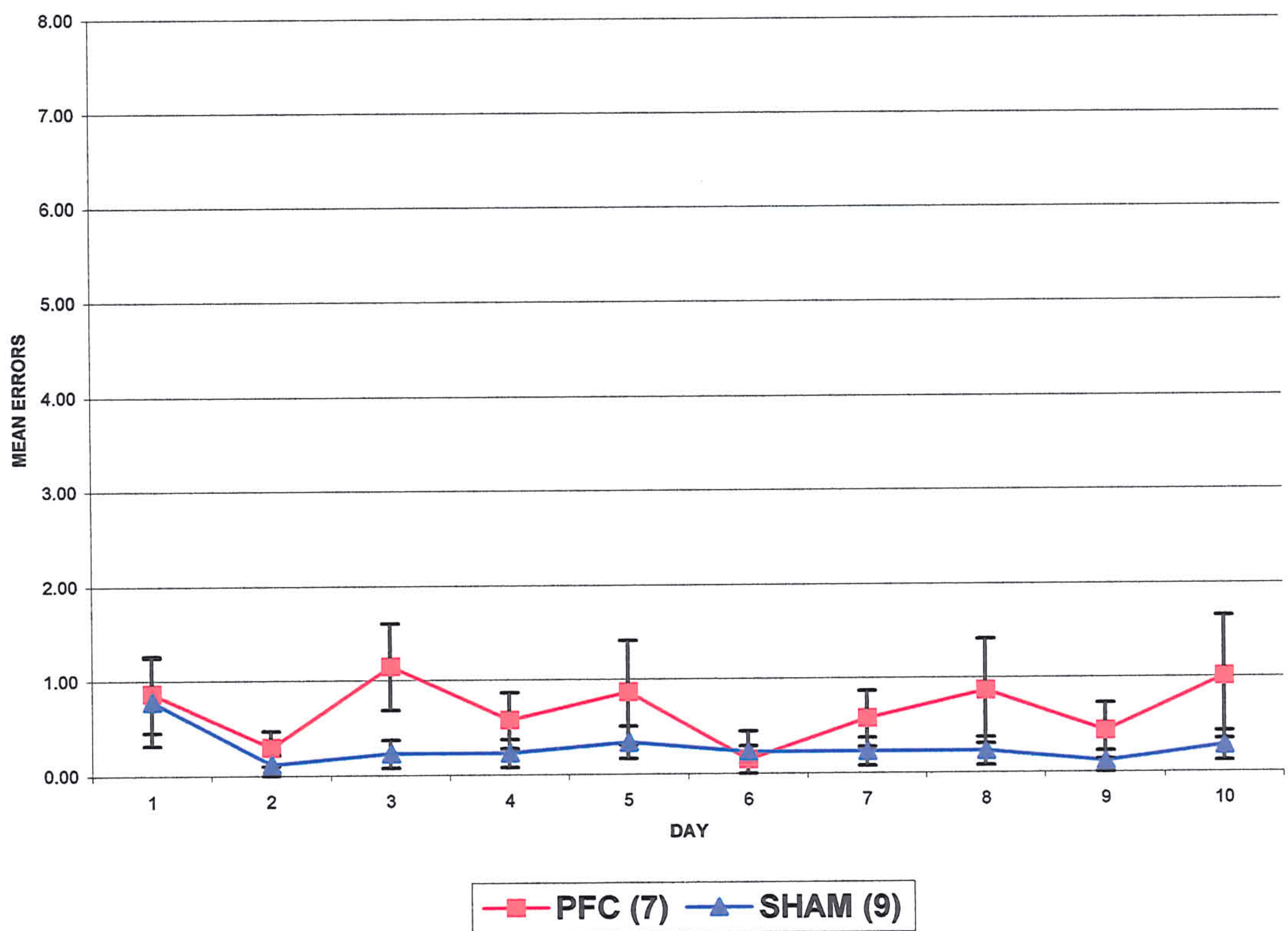
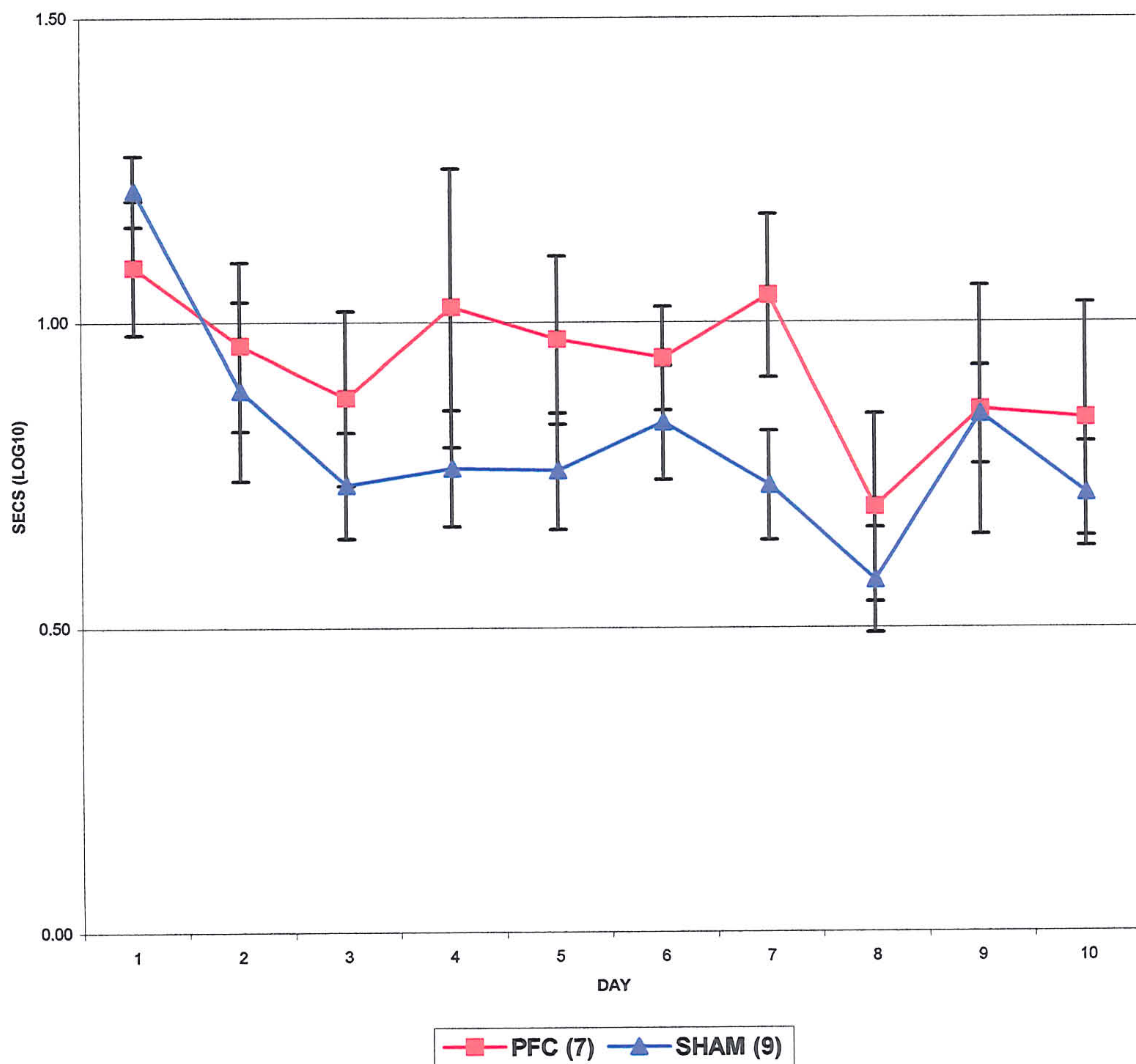


Figure 8.iii shows the mean and standard error for the number of correct responses made before the first error in the training phase for all days. The ANOVA confirmed that there was no significant main effect of group $F(1,14) = 2.45$, or of day $F(9,126) = 1.21$. The interaction was also not significant $F(9,126) = 0.42$.



The mean and standard error for the latencies to the first arm for each group in the training phase on all days can be seen in Figure 8.iv. The ANOVA showed that there was no significant main effect of group $F(1,14) = 1.10$. The main effect of day was significant $F(8.65,121.13) = 3.92$ $p < 0.001$ reflecting the fact that both groups got faster with experience. There was not a significant group x day interaction $F(8.65,121.13) = 0.93$.

FIGURE 8.iv TRAINING LATENCY



The mean and standard error for the arm choice times in the training phase on all days are shown in Figure 8.v. The two-way mixed ANOVA conducted on this data showed that there was a significant main effect of day $F(9,126) = 1.95$ $p=0.05$. The main effect of group was not significant $F(1,14) = 2.37$, and neither was the interaction $F(9,126) = 0.65$.

FIGURE 8.v TRAINING CHOICE TIMES

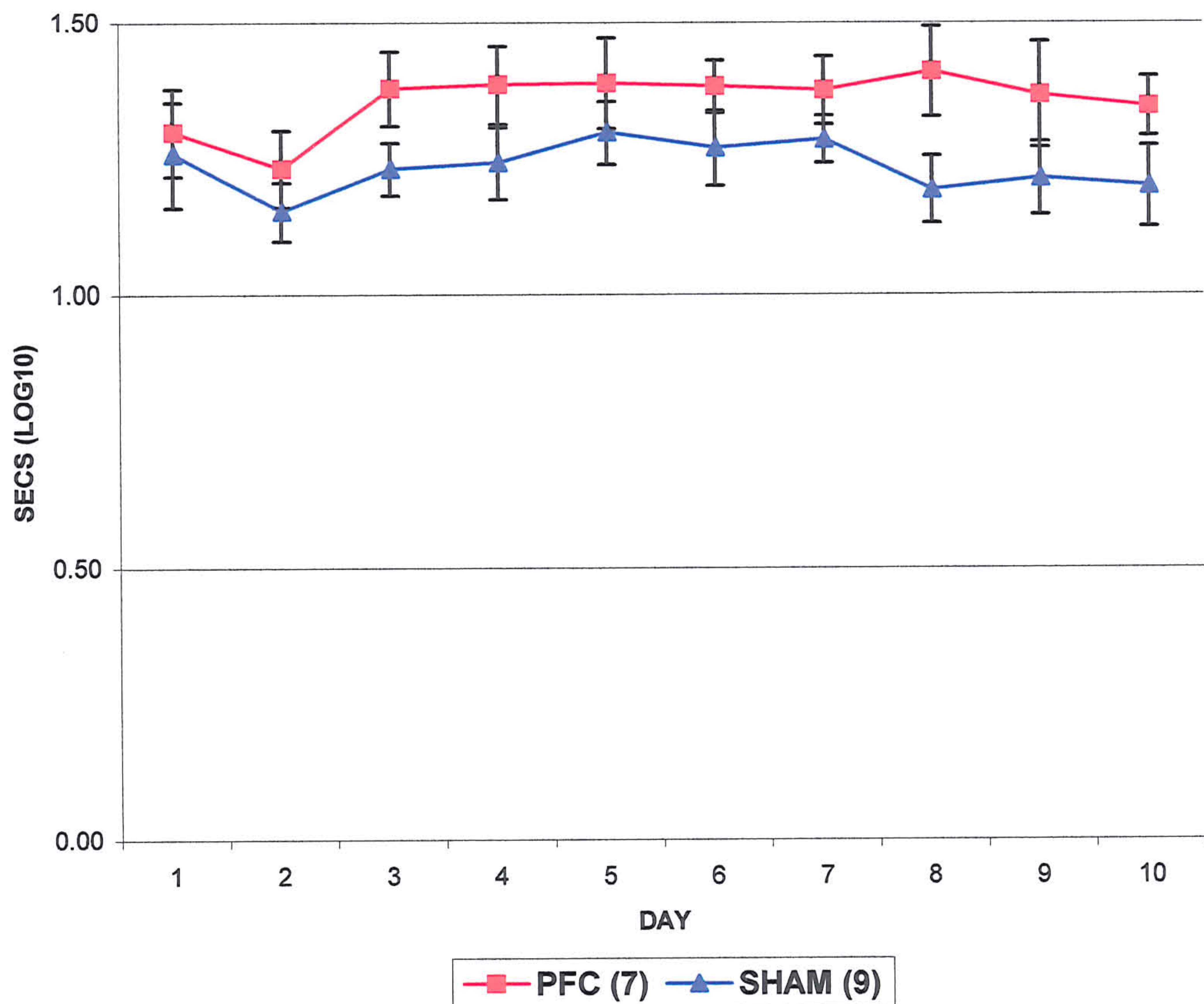
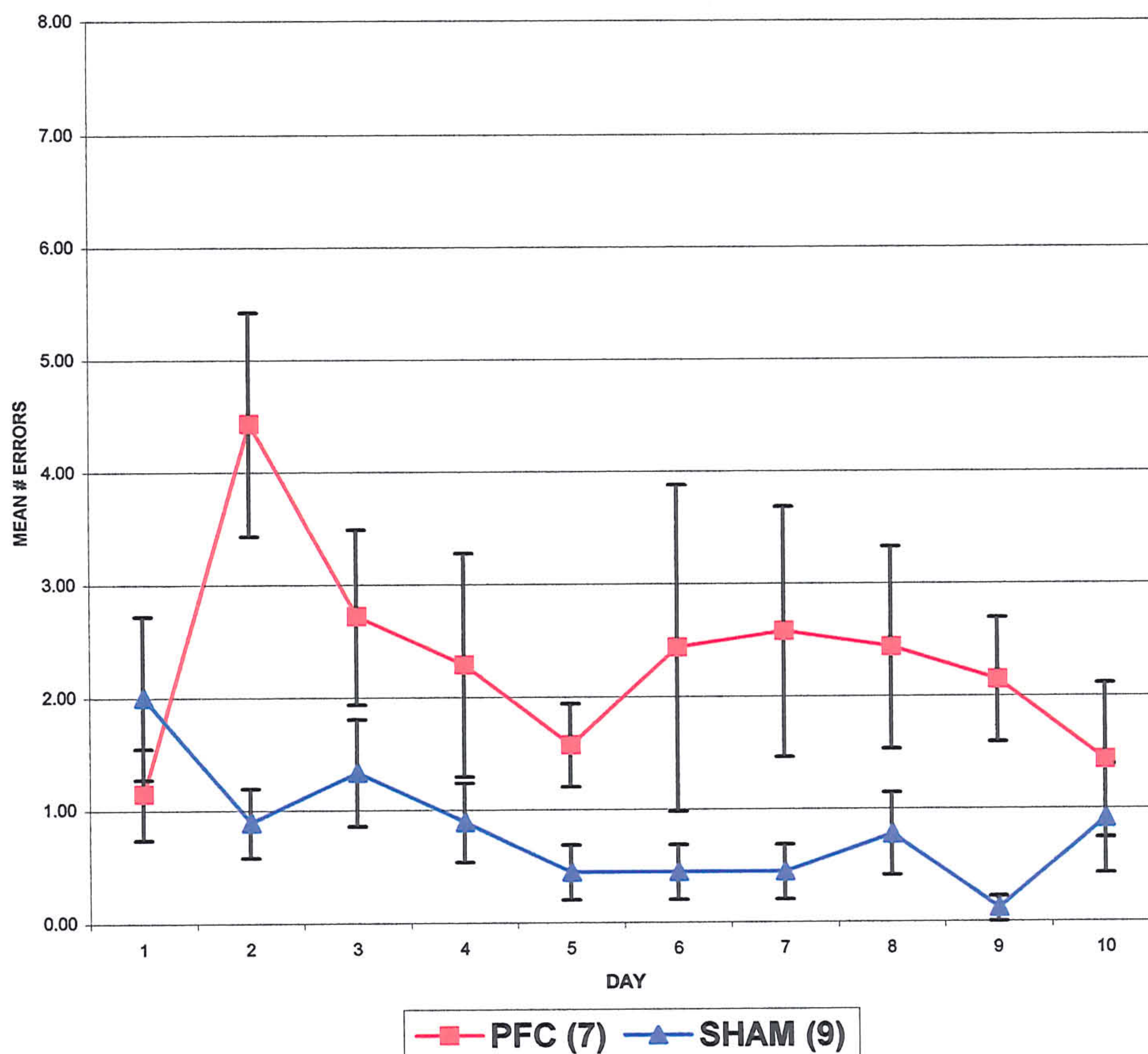


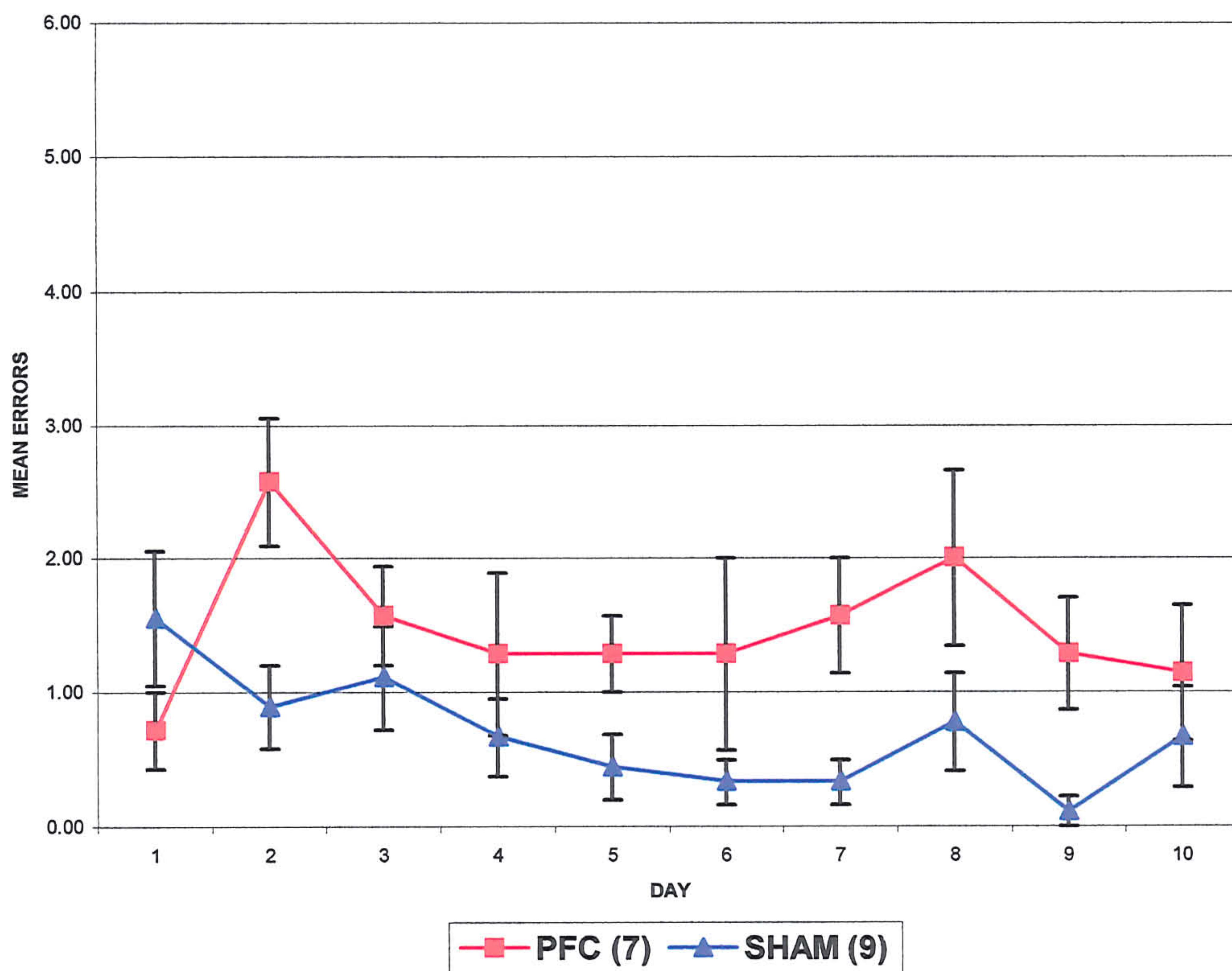
Figure 8.vi shows the mean and standard error for the number of errors made in the test phase on all days. The two-way mixed ANOVA confirmed that there was a significant main effect of group $F(1,14) = 13.01$ $p < 0.01$ such that mPFC lesioned rats made significantly more errors on this phase than sham lesioned rats. Partial eta squared for this effect was 0.48 with an observed power of 0.92. There was not a significant main effect of day $F(6.97,97.60) = 1.39$ or a significant group x day interaction $F(6.97,97.60) = 1.87$ (although the interaction narrowly missed the significance level of $p < 0.05$ at $p = 0.08$ with an observed power of 0.72).

FIGURE 8.vi TEST PHASE ERRORS



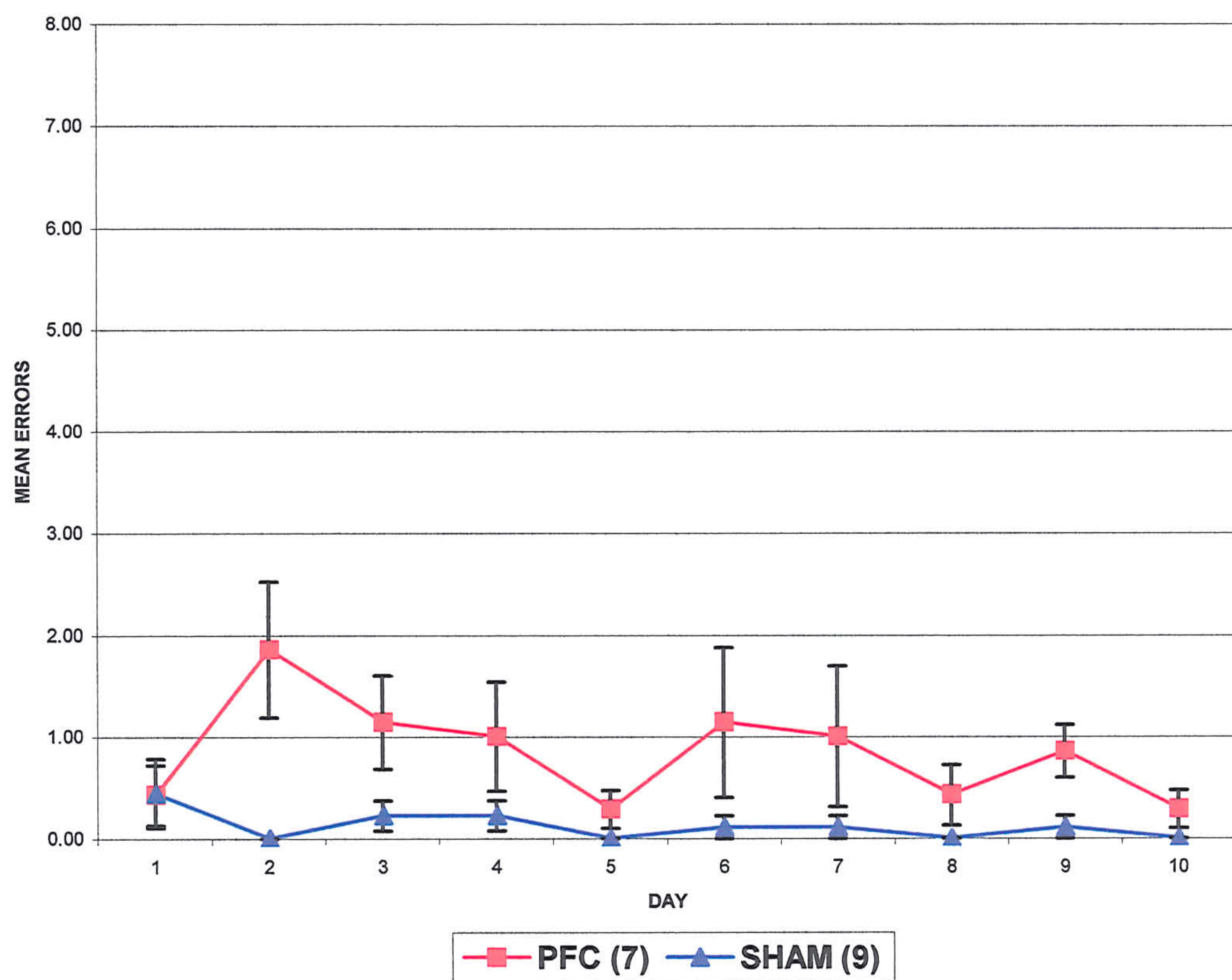
The mean and standard error for the number of across phase errors made by both groups over all days can be seen in Figure 8.vii. The ANOVA showed that there was a significant main effect of group on this measure $F(1,14) = 13.82$ $p < 0.01$. Partial eta squared for this effect was 0.50 with an observed power of 0.93. There was not a significant main effect of day $F(8.78,122.91) = 1.35$, or a significant group x day interaction $F(8.78,122.91) = 1.57$.

FIGURE 8.vii ACROSS PHASE ERRORS



The mean and standard error for the number of within phase errors made by both mPFC and sham lesioned rats across all days can be seen in Figure 8.viii. The two-way mixed ANOVA confirmed that there was a significant main effect of group $F(1,14) = 8.91$ $p=0.01$. Partial eta squared for this effect was 0.39 with observed power of 0.79. The main effect of day was not significant $F(5.51,77.07) = 1.67$ and neither was the interaction $F(5.51,77.07) = 1.75$.

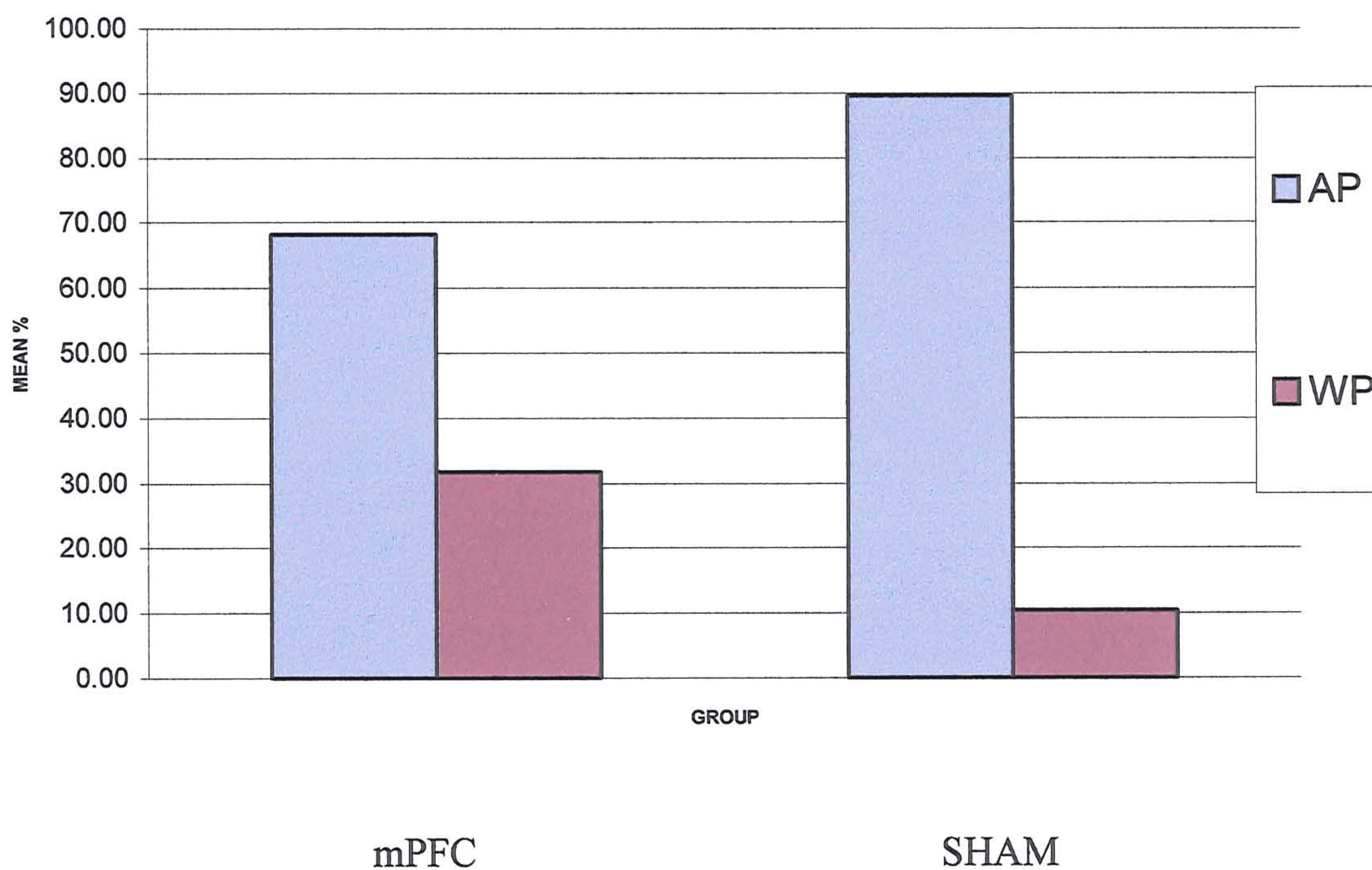
FIGURE 8.viii WITHIN PHASE ERRORS



In order to understand the impaired test phase DSWS responding of mPFC lesioned rats in more detail, further two-way within-subjects ANOVAs were conducted for each group to compare patterning of errors. A two-way within-subjects ANOVA conducted on sham lesioned rats' test phase error rates across all days revealed that they made significantly more across phase than within phase errors, $F(1,8) = 96.33$ $p < 0.001$. However, there was not a significant day effect, $F(9,72) = 1.93$, and the error x day interaction was also not significant $F(9,72) = 1.30$. This indicates that sham lesioned rats did not significantly improve on their rates of both error types throughout the duration of the testing period in this analysis. The same two-way within-subjects ANOVA conducted for mPFC lesioned rats test error performance across all days also showed a significant main effect of error type $F(1,6) = 11.11$ $p < 0.05$. There was not a significant main effect of day $F(9,54) = 1.29$ and the error x day interaction was also not significant $F(9,54) = 1.30$. This suggests that, while mPFC lesioned rats made more errors they made these errors in a pattern that was similar to the way in which sham lesioned rats made their errors.

The above proposed similarity in responding between sham and mPFC lesioned rats can be seen in Figure 8.ix where the mean number of AP and WP errors made over the entire test period are expressed as a percentage of the total test phase errors. However, this figure shows that while mPFC lesioned rats made significantly more AP than WP errors, the difference in the percentages of their errors types is reduced in comparison to sham lesioned rats. In addition to this, the two way within-subjects ANOVA also shows that mPFC lesioned rats were unable to overcome their deficit in test phase accuracy of responding, despite repeated exposure to the DSWS task, since the ANOVA conducted on their error rates did not reveal a significant day effect or a significant group x day interaction.

FIGURE 8.ix MEAN % OF TOTAL TEST ERRORS THAT WERE AP AND WP



The mean and standard error for the number of correct responses made before error in the test phase over all days can be seen in Figure 8.x. There was a significant main effect of group on this measure $F(1,14) = 12.49$ $p < 0.01$ showing that mPFC lesioned

rats made their test phase errors significantly earlier in their choice sequence compared to shams. Partial eta squared for this effect was 0.47 with power observed at 0.91. The main effect of day was also significant $F(9,126) = 2.08$ $p < 0.05$ however the magnitude of this effect was smaller with partial eta squared at 0.19. There was not a significant group x day interaction $F(9,126) = 1.46$. Examination of the data contained in Figure 8.ix indicates that, in sham lesioned rats, a significant day effect occurred due to improvement across trials. For mPFC rats, however, the graph suggests that significant day effects occur between days 1 and 2, and over days 5,6, and 7. Despite the pattern of findings shown by the graph, however, the lack of a significant group x day interaction on this measure suggests that the day effects are not significantly different in each group. Thus the mPFC day effect is also likely to be the result of across trial improvement.

FIGURE 8.x TEST PHASE # CORRECT BEFORE ERROR

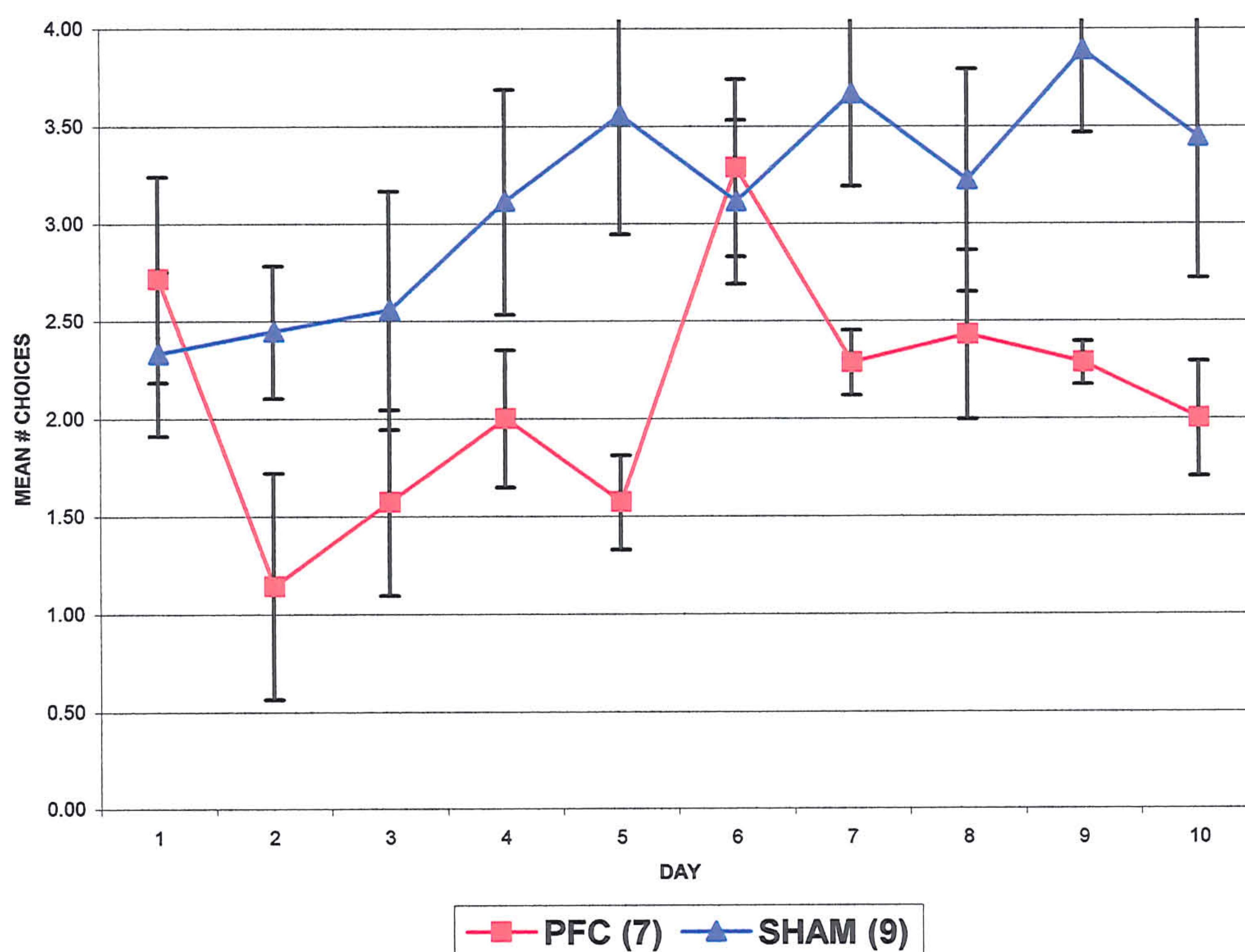
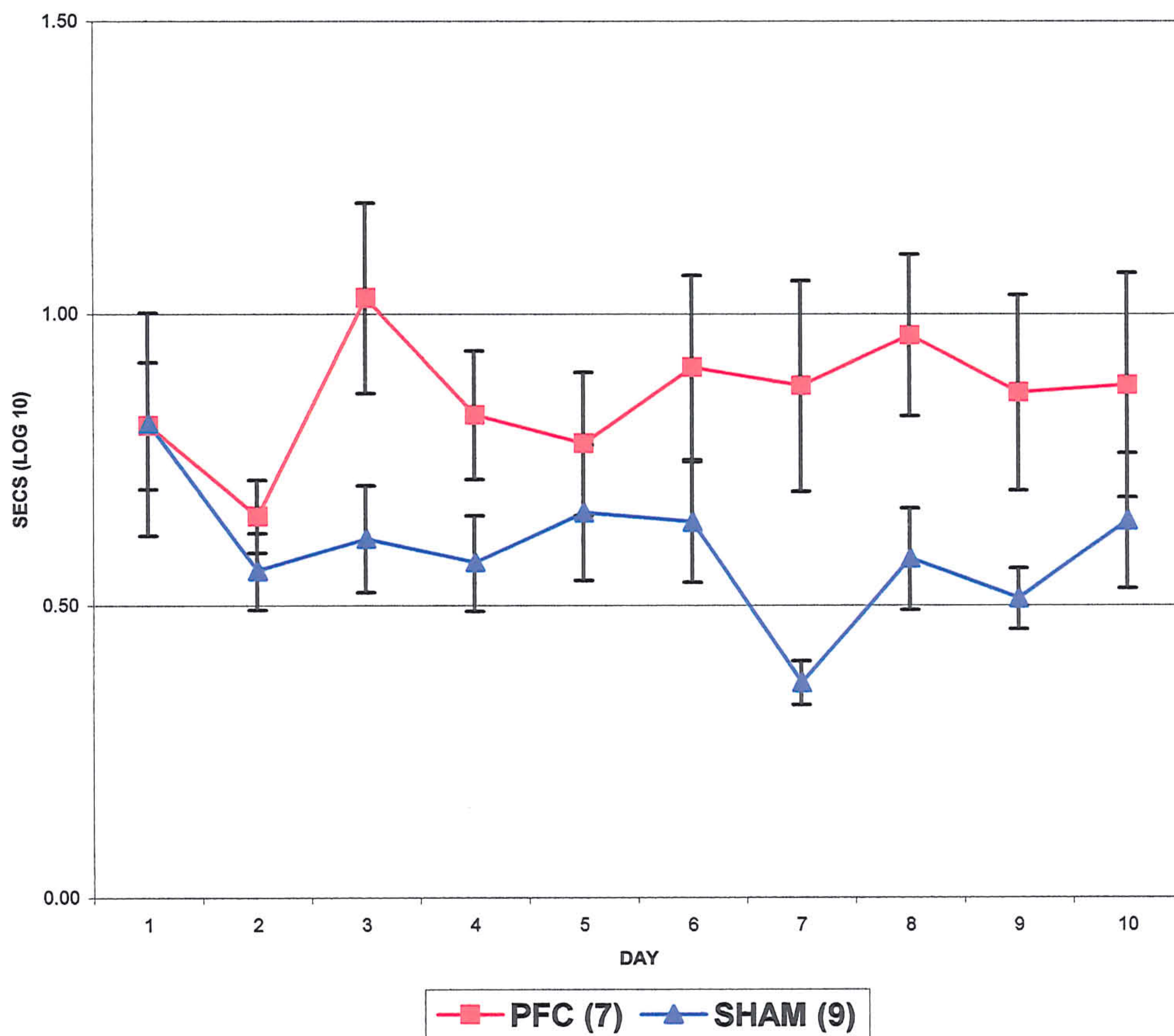
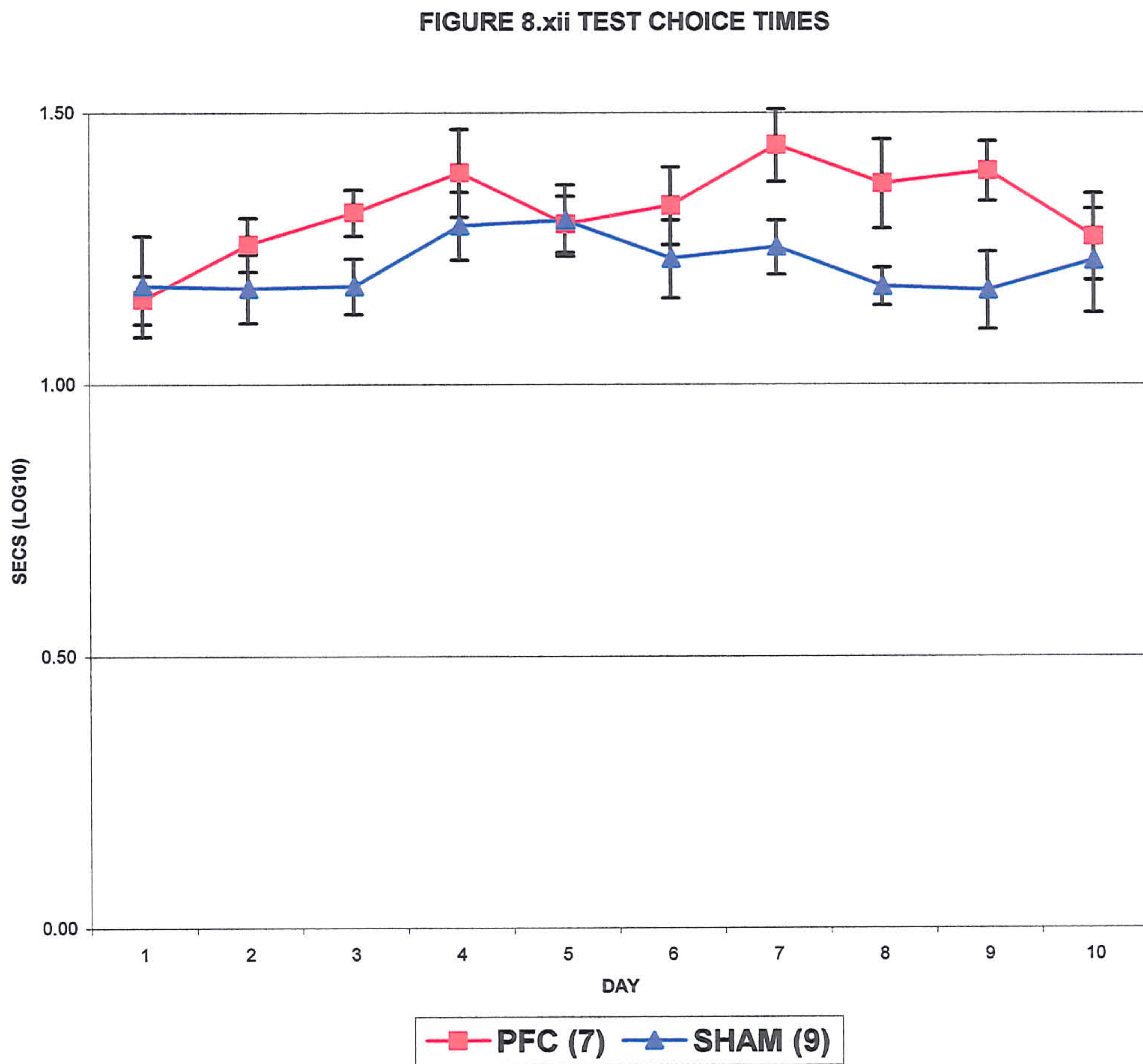


Figure 8.xi shows the mean and standard error for the latencies to the first arm in the test phase over all days. The ANOVA conducted on these results showed that there was a main effect of group on this measure $F(1,14) = 5.90$ $p < 0.05$. Partial eta squared for this effect was 0.30 with power observed at 0.62. The main effect of day was not significant $F(7.65,107.15) = 1.70$, and neither was the group x day interaction $F(7.65,107.15) = 1.23$.

FIGURE 8.xi TEST LATENCY



Finally Figure 8.xii shows the mean and standard error for the arm choice times in the test phase over all days. There was not a main effect of group on this measure $F(1,14) = 1.75$, despite the trend shown by the graph. The main effect of day was significant $F(9,126) = 3.22$ $p < 0.01$ but the interaction narrowly missed significance at $F(9,126) = 1.92$ $p = 0.055$.



In summary Table 8a below presents a review of the findings of this chapter for ease of comparison with the other experimental findings relating to lesion effects contained in this thesis.

Table 8a. Summary of Results

	Lesion	Day	Lesion x Day
Training Errors	ns	ns	ns
Training Correct	ns	ns	ns
Training Latency	ns	p<0.001 Both groups faster	ns
Training Choice Times	ns	p=0.05 Both groups slower	ns
Test Errors	p<0.01 PFC > Sham	ns	ns
AP	p<0.01 PFC > Sham	ns	ns
WP	p=0.01 PFC > Sham	ns	ns
AP > WP?	Yes for both	-	-
Test Correct	p<0.01 PFC < Sham	p<0.05 Both groups increase	ns
Test Latency	p<0.05 PFC slower	ns	ns
Test Choice Times	ns	p<0.01 Both slower	ns

8.4 Discussion

Rats with mPFC lesions (including damage to areas PL and AC) were not significantly impaired relative to shams on the training phase of the DSWS task. However, they were clearly and significantly impaired on the test phase. This impairment was characterised by an increase in total test phase errors, along with an increase in AP and WP errors when these were assessed separately. The lack of any significant main effects of day, or group x day interactions, indicates that this part of the mPFC impairment was stable and did not improve with experience. Furthermore, and perhaps most interestingly, mPFC lesioned rats displayed a pattern of error that was the same as that shown by intact rats. That is, the number of across phase errors made by mPFC lesioned rats was significantly different to the number of within phase errors for mPFC lesioned rats, as was the case for the numbers of these errors for the sham group. This finding parallels the data of Seamans *et al.* (1995) for AC lesioned rats but not that for PL lesioned rats. This suggests that damage to AC regions of the PFC in this experiment was more significant in producing the deficit observed, and this is supported by that fact that rats with smaller mPFC lesions did show more extensive damage to AC in comparison to PL.

Rats with lesions of the mPFC in this experiment also made their test phase errors significantly earlier in their choice sequence (something not assessed by Seamans *et al.*, 1995), and displayed significantly slower latencies to the first arm in the test phase. The mean number of responses correct before error in the test phase also showed a significant main effect of day, indicating that rats with mPFC lesions could improve on

their performance on this measure. Taken together these results suggest that, while mPFC rats could delay the intrusion of errors into their choice sequence, they could not ultimately prevent them from happening. It is possible therefore that the mechanisms responsible for structuring correct choices in the DSWS task, may be dissociable from those responsible for preventing error.

One interesting point to note from the current results is that there was a trend of a change in performance between days 1 and 2 on all the other measures on which mPFC lesioned rats were impaired (although it should be noted that this trend was not significant in these measures). However given the multivariate nature of the trend it does suggest the intriguing possibility of a lack of lesion effects on the first day of postsurgical testing and perhaps future experiments with larger group numbers will prove this to be the case (this may be particularly true for test phase errors for example since the group x day interaction only narrowly missed the significance margin). One hypothesis that could account for such a pattern of responding would be interference effects across trials. Such a hypothesis would suggest that mPFC rats are able to win-shift, as would appear to be the case from their performance on day 1. However, when day 2 requires updating and reorganising the pattern of arms in relation to training and test phases, this is where the mPFC lesion takes effect. The existence of such an effect would find support within PFC literature (Delatour *et al.*, 1996, Granon *et al.*, 1994, & Poucet, 1990) and fits well with the notion of PFC involvement in flexibility of responding. Furthermore it is interesting to note that such a lack of lesion effects (if they exist) would not have been apparent in the work of Seamans and colleagues (1995) due to the manner in which that study was designed. Specifically, the Seamans *et al.* (1995) study included post-surgical re-training to criterion prior to the lidocaine

infusion trials such that there was always a preceding trial to interfere with the lidocaine trials.

This trend aside, the lack of any significant effects of permanent damage to the medial wall of the rat PFC on any of the measures in the training phase supports the findings of Seamans *et al.* (1995) obtained using temporary inactivation of areas PL and AC separately. In that study a within subjects comparison of training phase data revealed no significant increases in latency, choice times, or errors on lidocaine infusion trials when compared to saline infusion, or no infusion trials. It has been suggested that tasks that fall under the influence of executive function are those that are non-routine or novel (Rabbitt, 1997, Knight, 1984, & Shallice, 1982). Therefore, over-training might conceivably result in a lack of executive processing involvement in performance. Analysis of pre-surgical data from this experiment could support a hypothesised lack of involvement of executive function in the training phase: subjects reached criterion performance within 3 trials on the training phase, yet took 15 more trials to reach criterion in the test phase.

As regards the pattern of results obtained for mPFC lesioned rats in the test phase, results are also consistent with executive dysfunction. Increased errors (both AP and WP) coupled with earlier error occurrence in the choice sequence, is indicative of a failure to control the selection of appropriate behaviour. In line with this conclusion mPFC rats showed some evidence of slowness of information processing (measured, combined with run speed, by latency to the first arm) which is also a characteristic of executive dysfunction (Rabbitt, 1997). It is possible that the significant increase in latency to the first arm in the test phase could be the result of a motor deficit following

extension of lesions into the pre-motor area Fr2. However, such a deficit would be expected to be apparent in all measures dependent on motor ability. Thus the absence of significant increases in run speed measures in the training phase refutes a conclusion in favour of motor impairment. It should be borne in mind, though, that the slowness observed on the test phase latency measure may also be the result of lower levels of motivation in mPFC lesioned rats following their poor performance in this phase. If this were the case it follows that the latency effect would not be apparent either on the first day of postsurgical testing, or crucially, if mPFC lesions do not take effect until day 2, the second day of postsurgical testing. Examination of Figure 8.x does indicate that the slower latency deficit of mPFC rats in the test phase does not occur until day 3. However, since this effect was also not significant the hypothetical lack of lesion effects on days 1 and 2 remains speculative.

In the Seamans *et al.* (1995) study the temporary lesion methodology had the benefit of enabling observation of deficits at different stages of the DSWS task, something that could not be achieved with the permanent lesion methodology employed here. However, despite this benefit, it is difficult to dissociate executive function components in the deficits observed in that study. Involvement of area PL in the retrieval of trial unique information was explained as problems with the use of spatial memory to prospectively plan a sequence of responses. In contrast, involvement of area AC at both the acquisition and retrieval stage, producing a bias in responses towards across phase errors, was suggested to indicate a role for this brain region both in memory encoding and in response flexibility. In the current study it is impossible to separate the functions of the PL and AC PFC sub-regions since both area PL and area AC were damaged in the same animals. This makes comparisons between the Seamans *et al.* (1995) study

and the current study problematic. However the results of the current study, with lesions centred on area PL but extending into AC, do at least offer support regarding a proposed role of the mPFC of the rat in mnemonic function.

9. EFFECTS OF VARYING REWARD VALUE ON THE PPTg DSWS TASK DEFICIT

9.1 Introduction

Bilateral ibotenate lesions of the PPTg made using the standard excitotoxic PPTg lesioning technique developed at this laboratory (Rugg, Dunbar, Latimer, & Winn, 1992) have been shown to produce lasting deficits in the DSWS task. This impairment occurs whether PPTg lesioned rats have undergone pre-surgical training to criterion or not (Keating & Winn, 2001). The impairments in a retention paradigm have been characterised by increased total test phase errors but with the pattern of more across phase than within phase errors shown by sham rats also observed in PPTg lesioned rats. Furthermore, average arm choice speed in PPTg lesioned rats is faster than in shams, although PPTg lesioned rats are slower in the test phase in comparison to the training phase while sham operated controls show the opposite pattern of responding. Table 9a shows precise values for the deficit obtained by Keating and Winn (2001).

Table 9a. Data from Keating and Winn (2001) Means and Standard Error

	PPTg	Sham	Significant?
Training Errors	0.93 (+/- 0.44)	0.15 (+/- 0.11)	No
Training Latency	4.65 (+/- 1.34)	15.85 (+/- 5.73)	No
Training Choice Times	4.61 (+/- 1.01)	27.94 (+/- 14.83)	Yes
Test Errors	3.57 (+/- 1.12)	0.60 (+/- 0.27)	Yes
AP	2.86 (+/- 0.96)	0.71 (+/- 0.29)	Yes
WP	0.45 (+/- 0.19)	0.15 (+/- 0.11)	Yes
AP > WP?	Yes	Yes	-
Test Latency	1.70 (+/- 0.35)	1.51 (+/- 0.32)	No
Test Choice Times	14.43 (+/- 0.93)	15.00 (+/- 0.80)	Yes

The findings regarding test phase error patterning parallel data obtained in a similar task using lidocaine lesions of the ventral pallidum (Floresco *et al.*, 1999), which projects monosynaptically to the PPTg (Semba & Fibiger, 1992). The ventral pallidum represents one stage in the ventral striatal loop system (also comprising area PL of the mPFC, the hippocampus, the nucleus accumbens, and the medial dorsal nucleus of the thalamus). As such it is likely to be a disruption of this stream of information processing that produces the DSWS deficit following VP and PPTg inactivation (Keating & Winn, 2001, & Floresco *et al.* 1999,). However, as yet the deficit following PPTg lesions cannot be reliably located to either the ACh or non-ACh neurons (or both) since selective lesioning techniques for the PPTg are not possible. For example, 192

IgG saporin is not effective since the PPTg does not have NGF receptors (Knusel & Hefti, 1988). Despite this limitation to the development of knowledge in terms of the precise neural locus of the PPTg DSWS deficit, there remains scope for expanding the understanding of the nature of the PPTg involvement in the neural processing required by the DSWS task.

In particular there have been several other studies implicating the PPTg in reward related processes (see chapter 4) along with evidence that suggests that the extent of PPTg deficits in reward related responding may be variable depending on:

- i) Level of deprivation (Bechara & van der Kooy, 1992)
- ii) Reward value (Ainge *et al.*, & Keating *et al.*, 1996).

This suggests an impairment that is linked to motivational state.

The study of Ainge *et al.* (1999) is particularly notable regarding the PPTg deficit and responding to variations in reward value. In this task PPTg and sham lesioned rats under food deprivation were placed on a 3m runway with a sucrose solution contained in a drinking spout/burette delivery system. Simple approach and consummatory responses were assessed for 7 days, by placing the rats at the very end of the runway and recording how long it took them to reach the spout and how much sucrose they consumed in a 30 mins period once there. For these 7 days half of the lesioned and sham rats received 4% sucrose solution and half received 20 %. These percentage solutions were then reversed within the groups for a further 7 days.

This experiment showed that PPTg lesioned rats significantly over-consumed the sucrose solution in comparison to shams only when the percentage of sucrose was high (20%). Furthermore this pattern occurred even when the rats had previous experience of the other solution. Analysis of the run speed patterns of responding (approach behaviour) also suggested a PPTg deficit that could be specific to high levels of reward. Sham rats displayed the expected shift in run speed predicted on the basis of reward value when reward was increased: they ran faster for the higher percentage sucrose than for the lower percentage sucrose. However, when sham rats were reversed from 20% to 4%, their run speed did not alter. Arguably this suggests that sham rats were running anticipating 20 % sucrose at the end of the runway instead of 4%. If this was the case, it would imply a level of responding for the 4% sucrose in the reward decrease condition that was contingent on the initial presentation of the 20% sucrose. This is particularly important for the interpretation of the PPTg deficit in the approach phase of this task.

When comparing PPTg and sham rats reversed from 4% to 20% the data showed that PPTg lesioned rats were not impaired in approach behaviour if the reward was low (4%) but their behaviour was not appropriate at the higher level of reward (20%) since they did not increase their run speed. In addition a comparison of the PPTg and sham rats reversed from 20% to 4% showed that PPTg rats were always slower than the shams. This pattern of results could be the product of an impaired initial response in PPTg lesioned rats to the 20% sucrose that carries over to the 4% sucrose. In fact this is supported by the fact that PPTg lesioned rats were not impaired in approach behaviour for the 4% sucrose when it was presented first. Thus it would seem that PPTg rats are impaired, at least in a runway task, when the reward level is high

In addition, in Ainge *et al.*, PPTg lesioned rats did not simply respond variably on the basis of reward value alone. Rather, the direction of impairment in PPTg lesioned rats in response to increased reward showed *enhanced* unconditioned behaviour but *reduced* conditioned behaviour. This led Ainge *et al.* (1999) to conclude that responding for reward value in PPTg lesioned rats might dissociate depending on the *type* of behaviour being assessed (approach/conditioned vs. consummatory/unconditioned behaviour – see chapter 4).

The DSWS task is a more complex task than the runway task of Ainge *et al.* (1999) and it requires executive functioning. However it is also a task of conditioned behaviour. Given that Keating & Winn (2001) have revealed a DSWS deficit in PPTg lesioned rats when a standard food reward is used (Noyes precision food pellets) the current study was conducted to address whether this impairment would be adversely affected by the presentation of a more positive reward (as could be expected based on Ainge *et al.*). Furthermore it also aimed to replicate the PPTg DSWS findings of Keating & Winn (2001) with food reward such that comparisons between mPFC and PPTg responding could be made within the current work. Based on these aims the experiment was designed so that pre-surgical training paralleled that of Keating & Winn (Noyes food pellet reward only) with magnitude of reward value manipulated post-surgery as in Ainge *et al.* (1999), in this case using Noyes food pellets vs. “Supercook” chocolate drops.

9.2 Methods

40 Adult male Lister Hooded rats weighing approximately 360g at the time of surgery were used for pre-surgical DSWS training. They were housed and fed in line with the methodology set out under “General Methods”. Training was divided between two experimenters (CT and RK) such that one trained 20 rats in the morning and one trained 20 rats in the afternoon. This meant that the groups of rats were trained at the same time each day by a particular experimenter. Inter rater reliability was assessed prior to beginning training by testing 4 rats (not used in this experiment) on the training phase of the DSWS task. Each experimenter observed and recorded behaviours simultaneously. The records of performance of the rats on the three measures were then correlated using a Spearman rank order correlation for matched data. Mean inter rater reliability was then calculated from these values.

The use of “Supercook” chocolate drops as the preferred reward type was determined prior to beginning the pre-surgery testing by examining consummatory responding in the home cage to different reward examples. This testing was conducted on 8 rats not used in this experiment. Reward types assessed included Mini Smarties, Mini M&Ms, and Cadbury’s Chocolate Buttons, in addition to the “Supercook” chocolate drops. Both Mini Smarties and Mini M&Ms were rejected since the sugar coating meant that the reward required greater than 30 seconds to consume on all occasions of presentation. Given that the Noyes food pellets can be eaten within 1-2 seconds on the radial maze task the lengthy eating time resulting from the sugar coating was not desirable. In addition the chocolate buttons were too large to be consumed in the required time period, and attempts to cut them into halves or quarter sections produced inconsistent

sizes of reward. The “Supercook” chocolate drops were chosen because they overcame the limitations of the other reward types examined. Preference for these chocolate drops in relation to the Noyes food pellets was confirmed through examining which of the reward types was consumed first during home cage presentation.

All training and testing for this experiment was conducted on the elevated 8 arm wooden radial maze described in “General Methods”. Rats required 15 days to reach criterion prior to surgery using the standard Noyes precision food pellets (45mg) as reward.

PPTg lesions were made over 2 weeks such that the bilateral lesion was made 7 days after the first hemisphere was lesioned. This is standard procedure since rats receiving bilateral PPTg lesions made in the same surgical session do not survive. Infusions were made at the following coordinates:

- i) 0.8mm anterior to the interaural line, 1.6mm lateral to the midline, and 7.0mm ventral to skull surface.
- ii) 1.5mm anterior to the interaural line, 1.7mm lateral to the midline, and 7.8mm ventral to the surface of the skull.

In the first week PPTg lesions were made using 1 x 0.2 μ l 0.12M ibotenate at each set of coordinates with sham operated rats receiving infusions of 0.2 μ l sterile PB. In the second week the lesions were made using infusions of 1 x 0.165 μ l 0.12M ibotenate at each set of coordinates with sham rats receiving equal infusions of PB. This procedure

was used since histology from other experiments at this laboratory had shown that lesions made in the second week were always slightly larger than those made in the first week. The adapted procedure aimed to reduce this inconsistency.

Infusions took approximately 5 mins using the manual microdrive with the needle left in situ for a further 5 mins. All rats were monitored closely up to 8 h following surgery for changes in body temperature and for breathing problems. In particular PPTg rats were wrapped in bubble wrap and placed under 60W incandescent lamps to keep them warm as rapid decline in body temperature often occurs and can be fatal.

A total of 10 rats died as a result of surgery over the two weeks. The rest were closely monitored for feeding and drinking problems and loss of bodyweight for 7 days after the second surgery. If PPTg rats were found not to be eating (usually after the second surgery) they were first offered “wet mash” – standard lab chow mixed with water. Those losing more than 8g bodyweight in a 24 hr period were given “strawberry dream” baby food (Boots plc) as a last resort in an attempt to encourage them to eat. Presentation of these alternative foodstuffs usually had to be made on a spoon directly to the rat such that it was aware that these were available. Otherwise they tended to ignore the presence of the food bowl in their home cages.

Food restriction was imposed following 7 days recovery when all rats were eating normally. The remaining 30 rats were then tested 7 days a week post-surgery on the DSWS task once they had regained their target weight. Since all rats were fed at the end of a test day and were maintained at 85% free feeding bodyweight for the duration of the testing period, it can be assumed that rats were hungry while they underwent all

DSWS postsurgical trials. Testing was divided between the experimenters such that each tested the surviving rats from their original group of 20.

Analysis of results was conducted as in Keating and Winn (2001) using a mean score of performance over the two criterion days for each rat on each measure. Since sham operated rats are also initially impaired through normal mechanisms of forgetting in a retention paradigm this analysis enables a comparison of sham and lesioned rats performance at the point in time by which sham rats have returned to asymptote performance level. These criterion scores were then subjected to univariate two way between subjects ANOVAs with lesion and reward as the between groups independent factors.

9.3 Results

9.3.1 Histology

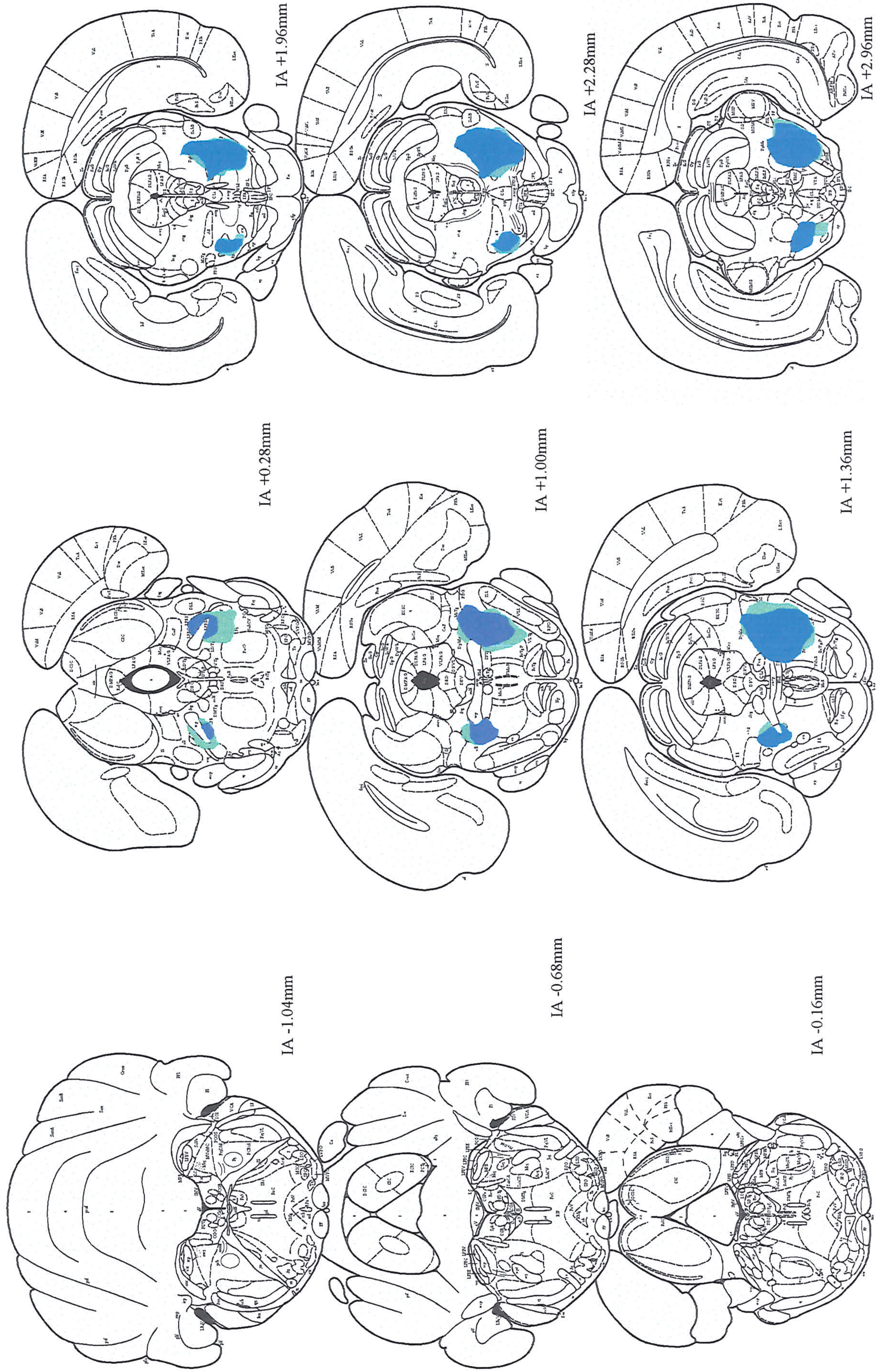
Analysis of NADPH diaphorase and cresyl violet stained sections indicated that most PPTg lesions were bilaterally symmetrical and centred over the PPTg. There was considerable variation in size between the smallest and largest lesions, although most lesions tended to be very large. These larger lesions extended over an area much wider than the PPTg such that damage was frequently observed in additional structures such as the retrorubal field, the LDTg, and the substantia nigra. A total of 4 rats were excluded from the lesioned groups; 1 because the extent of rostral survival was greater than 40% and 3 because the lesions appeared to be unilateral. The remaining lesions included in the analysis had greater than 90% loss of the PPTg including total destruction of ACh neurons. Given the extensive size of all these lesions this was the

only criterion required for inclusion. Of the 12 remaining lesioned animals for example, 7 had bilateral invasion of the SN, and 4 had unilateral invasion of this structure with only 1 PPTg lesioned rat sustaining no damage to the SN in either hemisphere. A representation of the smallest and largest lesions for each PPTg group can be seen in Figure 9.i. Smallest and largest lesions were determined by assessing the size of each hemispheric lesion individually across all rats in a group. It should be noted that there was little difference in size of lesion or extent of invasion of other structures between the PPTg groups. In total there remained 7 rats in the PPTg chocolate group and 5 rats in the PPTg food group. For the shams there were 7 rats in the chocolate group and 7 rats in the food group.

9.3.2 Inter rater reliability

Mean inter rater reliability was calculated at $r_s = +0.98$.

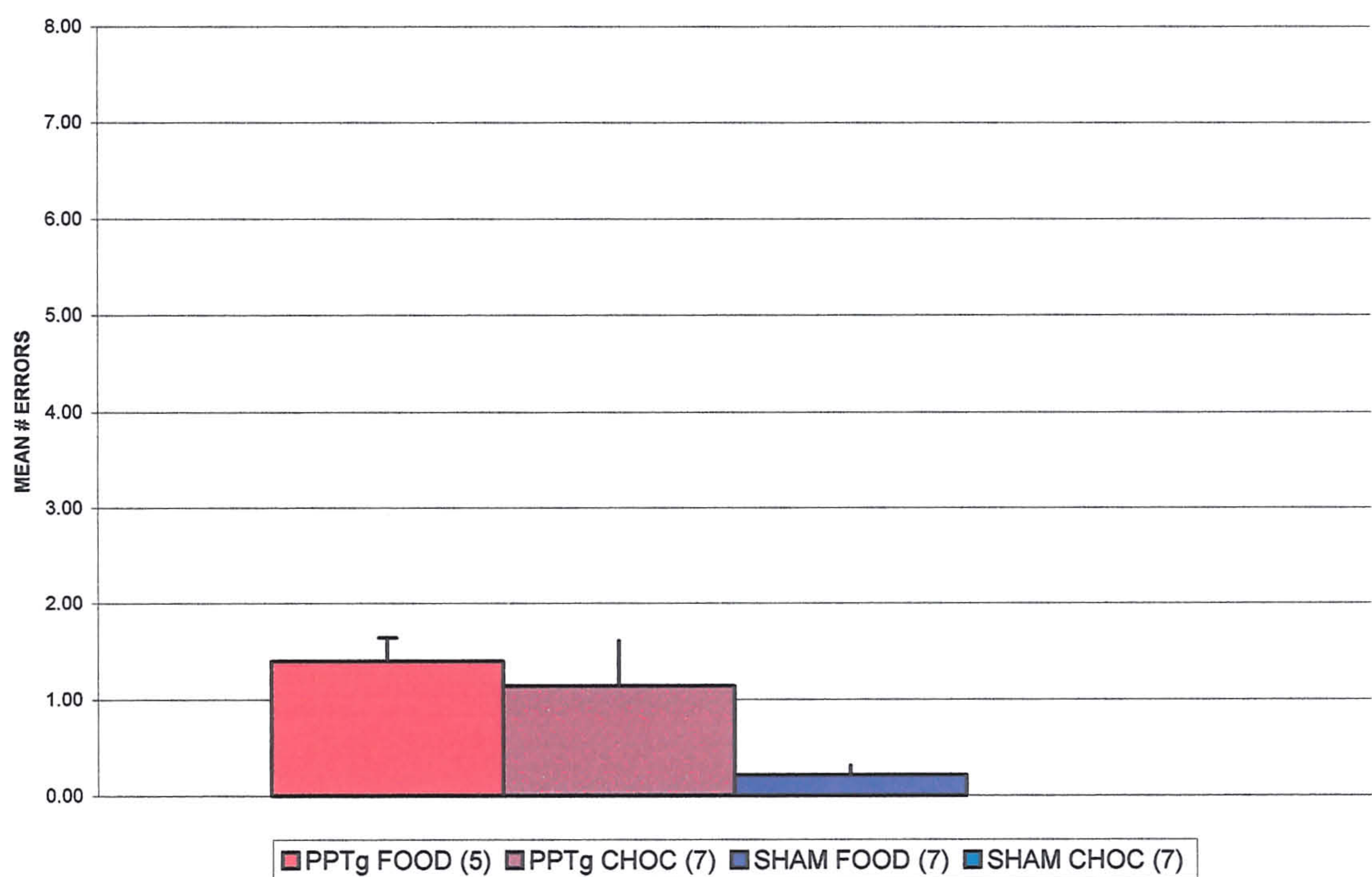
Figure 9.i Lesions of the PPTg - smallest lesion is depicted on the left and largest on the right. Blue represents PPTg-Food group while green represents PPTg-Chocolate group



9.3.3 The DSWS Task

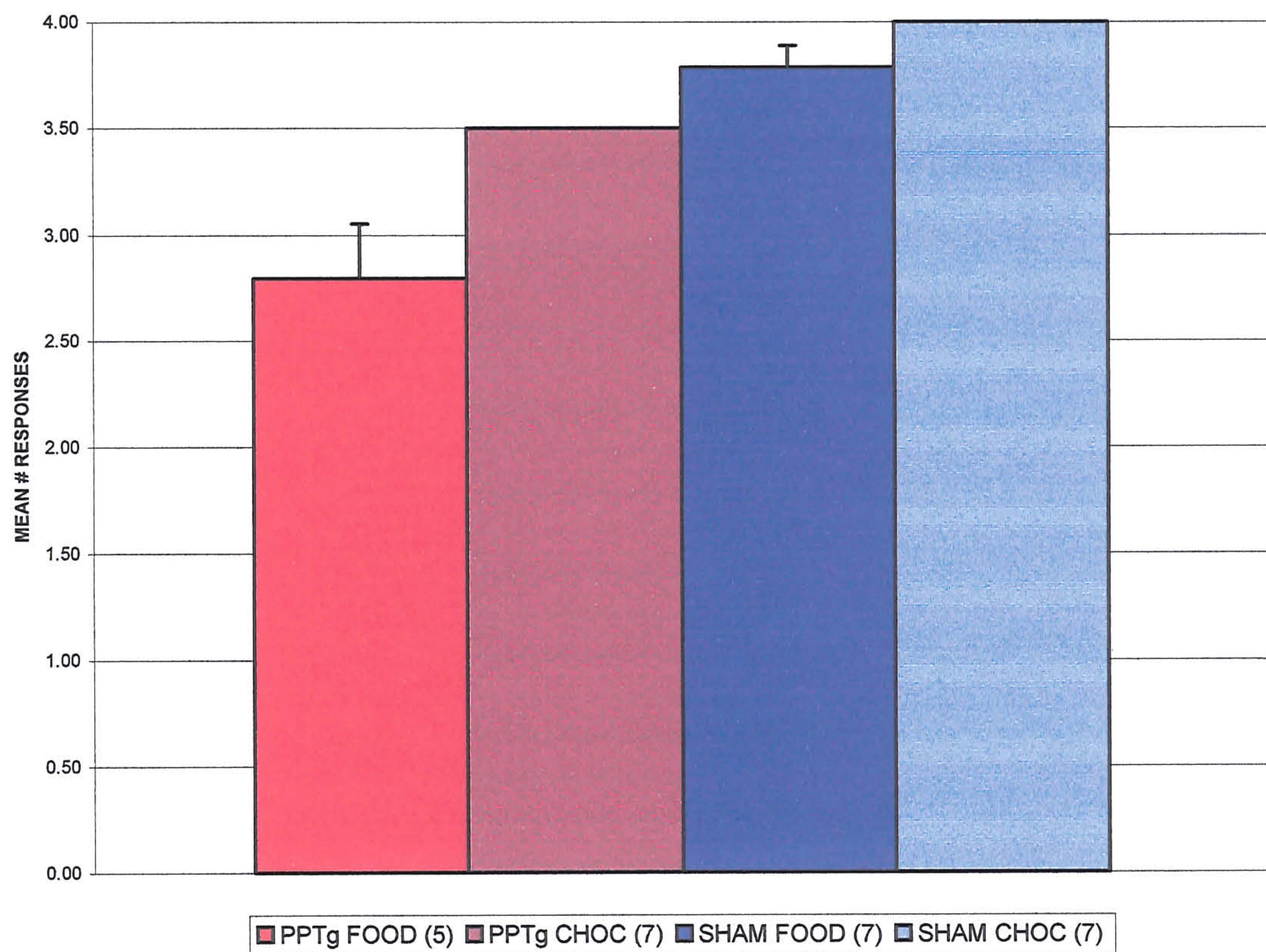
Figure 9.ii shows the mean and standard error for the number of errors made in the training phase over the criterion days (6+7). The univariate 2 way ANOVA conducted on these scores confirms that there was a main effect of lesion $F(1,22) = 17.28$ $p < 0.01$ but no main effect of reward $F(1,22) = 0.71$ or reward x lesion interaction $F(1,22) = 0.006$. Partial eta squared for the main effect of lesion was 0.44 with power observed at 0.98. These results indicate that rats with bilateral PPTg lesions in this experiment made significantly more errors in this phase.

FIGURE 9.ii TRAINING ERRORS



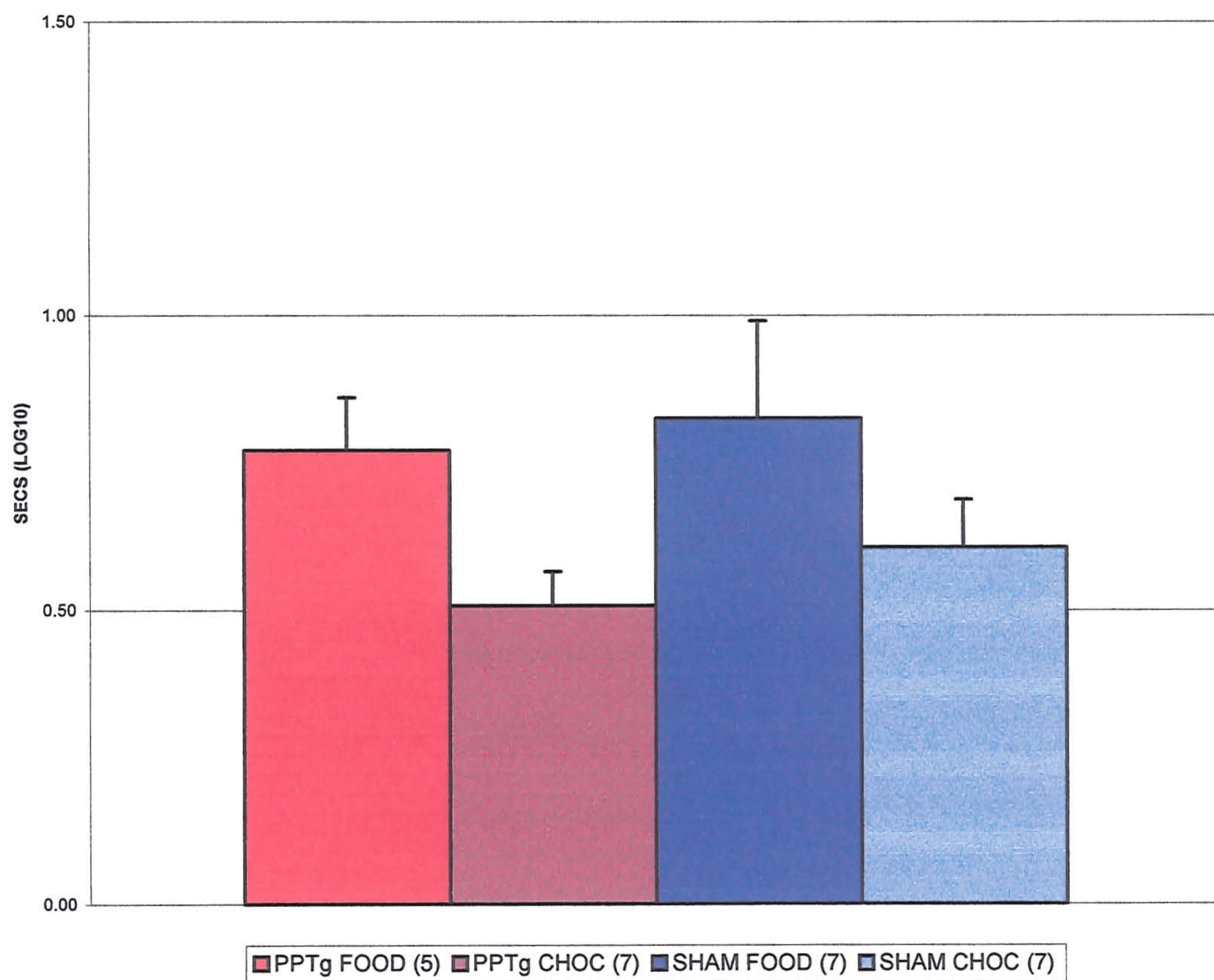
As for the means and standard error for the number of correct responses made before error in this phase, these results can be seen in Figure 9.iii. The ANOVA indicates that there were significant main effects of lesion $F(1,22) = 23.93$ $p < 0.01$, and of reward $F(1,22) = 9.06$ $p < 0.01$, but no significant lesion x reward interaction $F(1,22) = 2.56$. Partial eta squared for the main effect of reward was 0.29 with power observed at 1.0, while for the main effect of lesion it was 0.52 with experimental power observed at 0.82. This shows that rats with PPTg lesions made their errors significantly earlier than rats with sham lesions, but also that rats receiving chocolate made errors significantly later than those receiving food.

FIGURE 9.iii TRAINING # CORRECT RESPONSES BEFORE ERROR



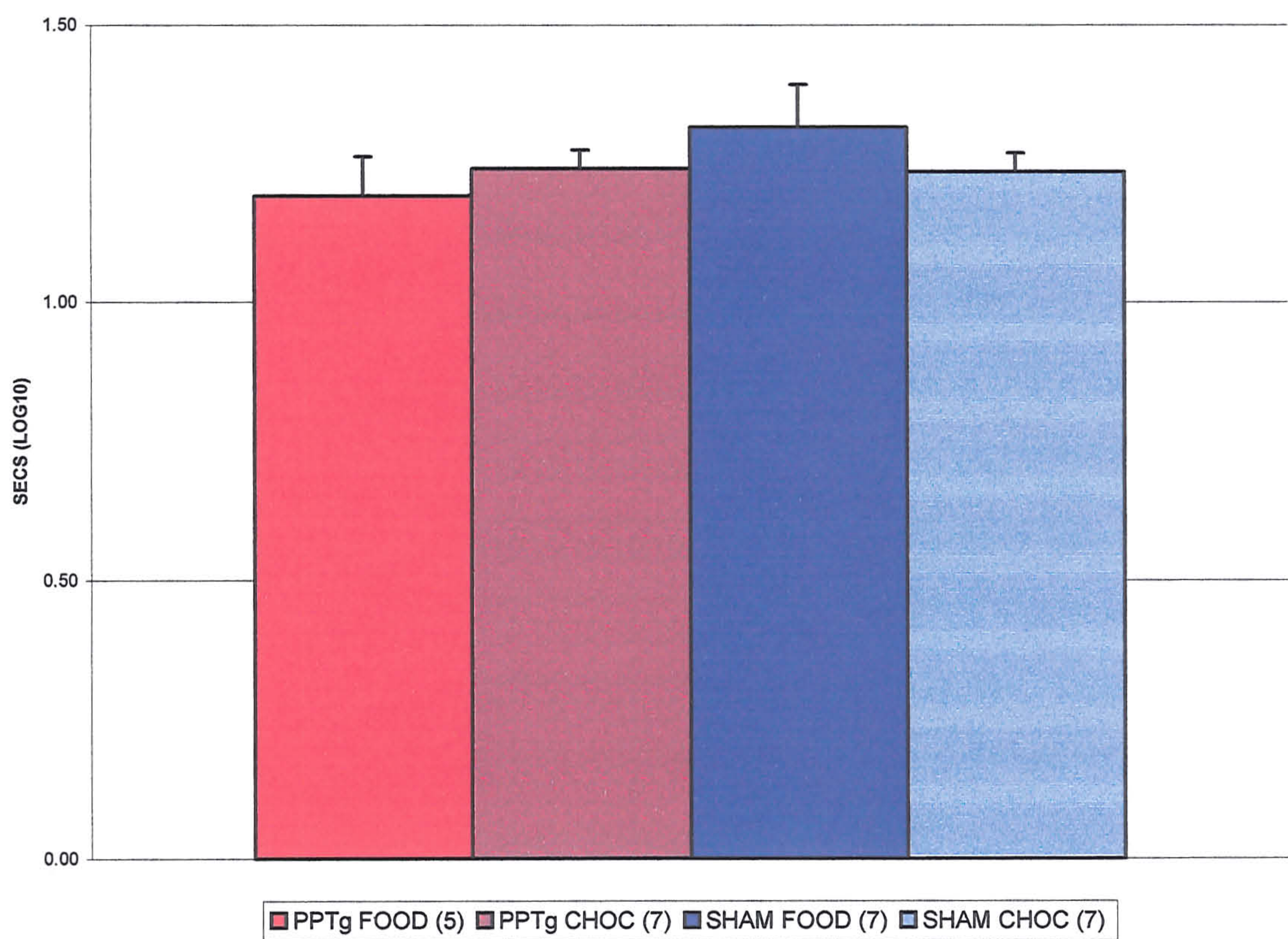
The means and standard error for the training phase latencies for each group over the criterion days can be seen in Figure 9.iv. A univariate 2 way ANOVA conducted on these results showed a main effect of reward $F(1,22) = 4.74$ $p < 0.05$, but no main effect of lesion $F(1,22) = 0.50$, or lesion x reward interaction $F(1,22) = 0.04$. This indicates that both PPTg lesioned rats and sham rats increased their speed of responding if chocolate was available.

FIGURE 9.iv TRAINING LATENCY (LOG10)



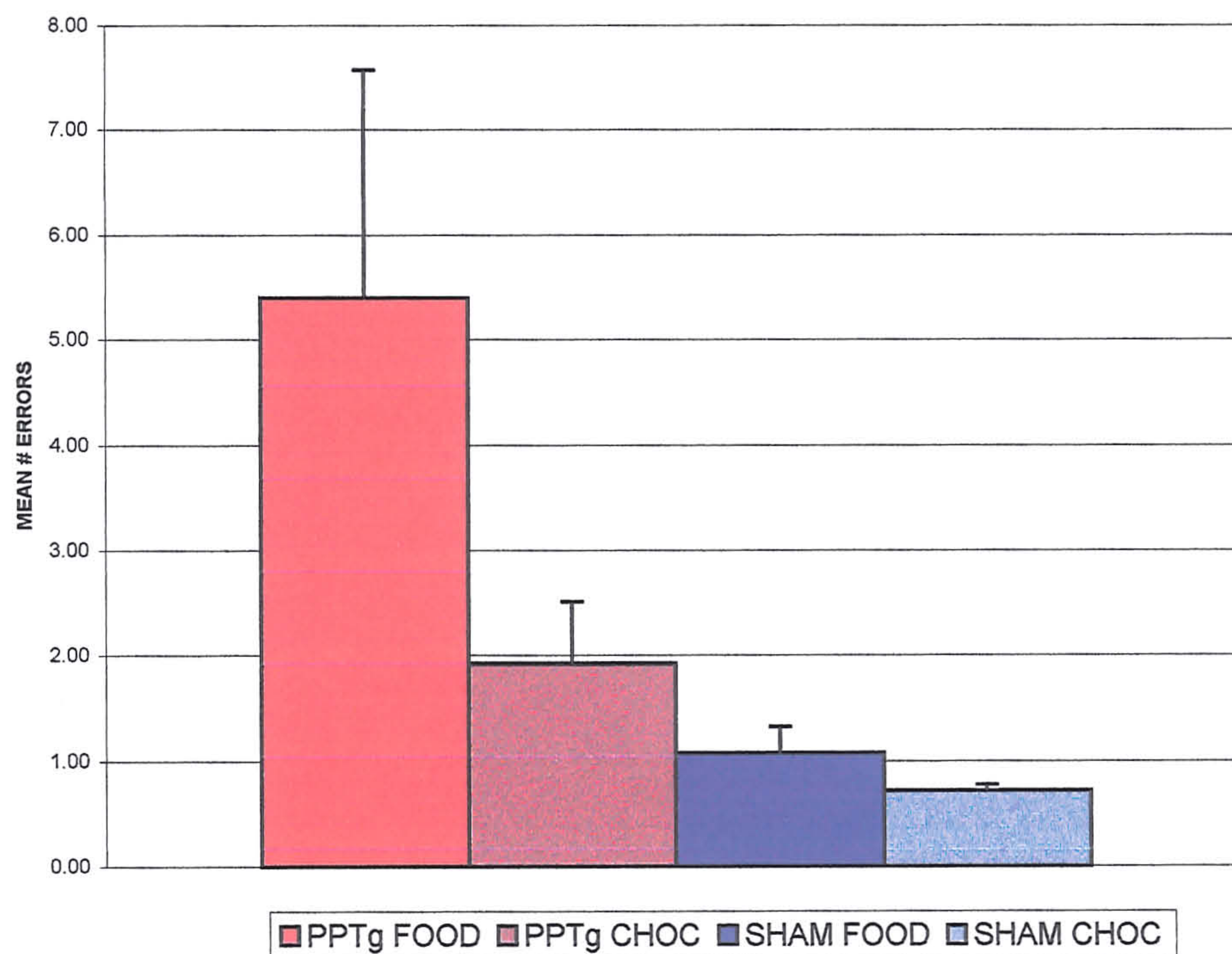
The means and standard error for the training phase choice times can be seen in Figure 9.v. The ANOVA conducted on these results revealed no significant main effects or interaction. Main effect of lesion $F(1,22) = 1.06$, main effect of reward $F(1,22) = 0.07$, and lesion x reward interaction $F(1,22) = 1.33$.

FIGURE 9.v TRAINING CHOICE TIMES (LOG10)



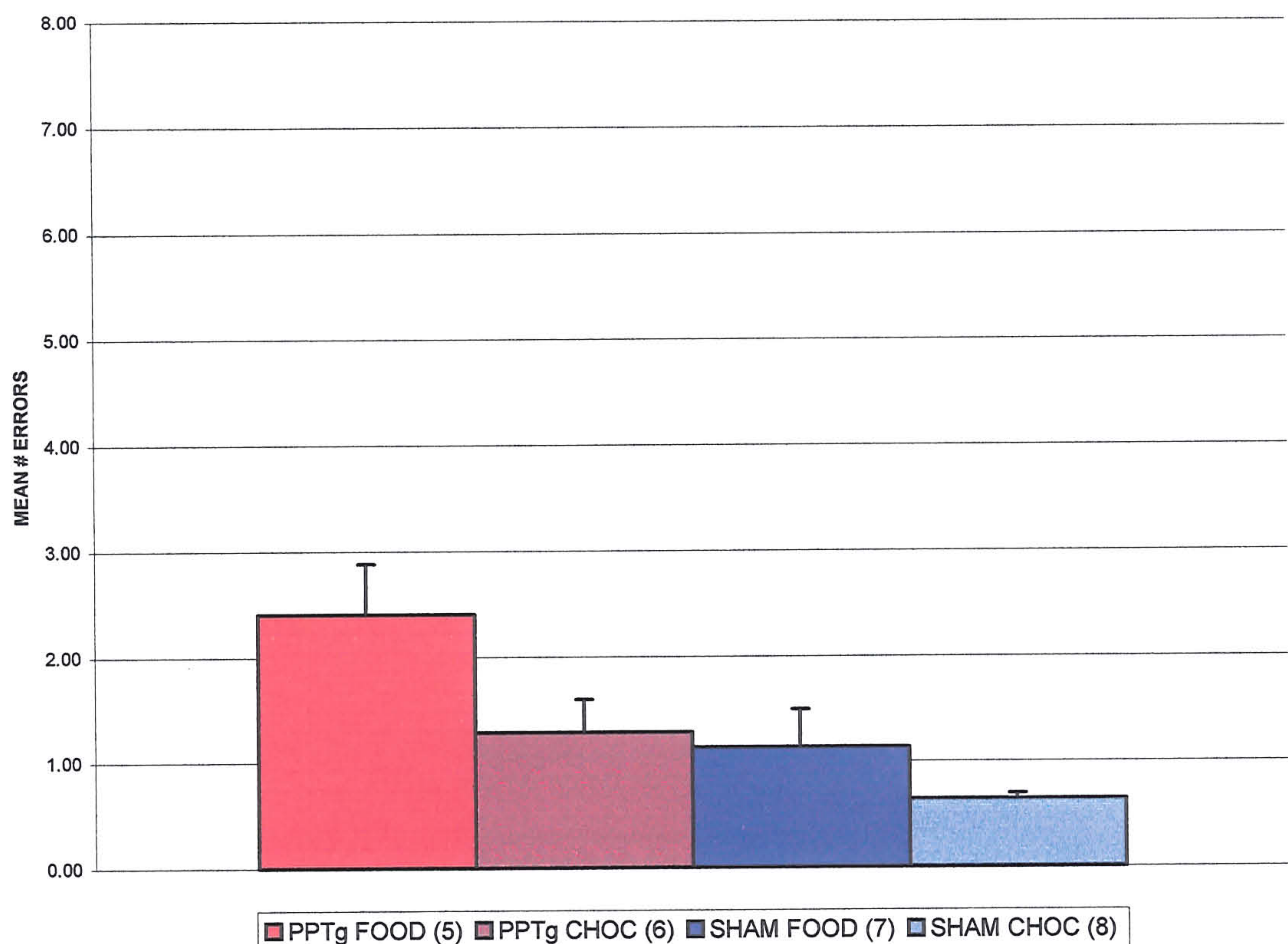
The mean and standard error for the number of errors made over the criterion days can be seen in Figure 9.vi. A 2 way univariate ANOVA calculated on these results indicated that there were significant main effects of lesion $F(1,22) = 6.59$ $p < 0.05$, and reward $F(1,22) = 5.17$ $p < 0.05$. Partial eta squared for the main effect of lesion was 0.23 with observed power at 0.69. For the main effect of reward partial eta squared was 0.19 with power observed at 0.58. The interaction was not significant $F(1,22) = 1.73$, although power was much lower for the interaction at 0.24. This shows that PPTg lesioned rats made significantly more errors than rats with sham lesions, and that the presence of a chocolate reward improved performance in both of these groups.

FIGURE 9.vi TEST ERRORS



The error score in the test phase was further divided into across phase and within phase errors. The mean and standard error for the number of across phase errors made over the criterion days can be seen in Figure 9.vii. The ANOVA conducted on these results showed similar findings as for overall number of errors in the test phase. There was a main effect of lesion $F(1,22) = 8.24$ $p < 0.01$, and of reward $F(1,22) = 5.95$ $p < 0.05$. Partial eta squared for the main effect of lesion was 0.27 with power observed at 0.78. For the main effect of reward partial eta squared was 0.21 with power at 0.65. The interaction of lesion x reward was not significant $F(1,22) = 0.86$, although observed power was very low at 0.14. These results show that PPTg lesioned rats make significantly more across phase errors than sham, and that having a chocolate reward can significantly decrease the number of these errors in both groups.

FIGURE 9.vii ACROSS PHASE ERRORS



The means and standard error for the within phase errors made over the criterion days can be seen in Figure 9.viii. A 2 way univariate ANOVA conducted on these results indicated that there was a significant main effect of lesion $F(1,22) = 4.37$ $p < 0.05$, but not a significant main effect of reward $F(1,22) = 3.59$, or a significant lesion x reward interaction $F(1,22) = 1.72$. Observed power for the lesion effect was quite low at 0.52 with partial eta squared at 0.17. Unlike across phase errors therefore, the presence of a chocolate reward did not improve the number of these errors being made in either group. However, as for AP errors, PPTg lesioned rats made significantly more WP errors than shams.

FIGURE 9.viii WITHIN PHASE ERRORS

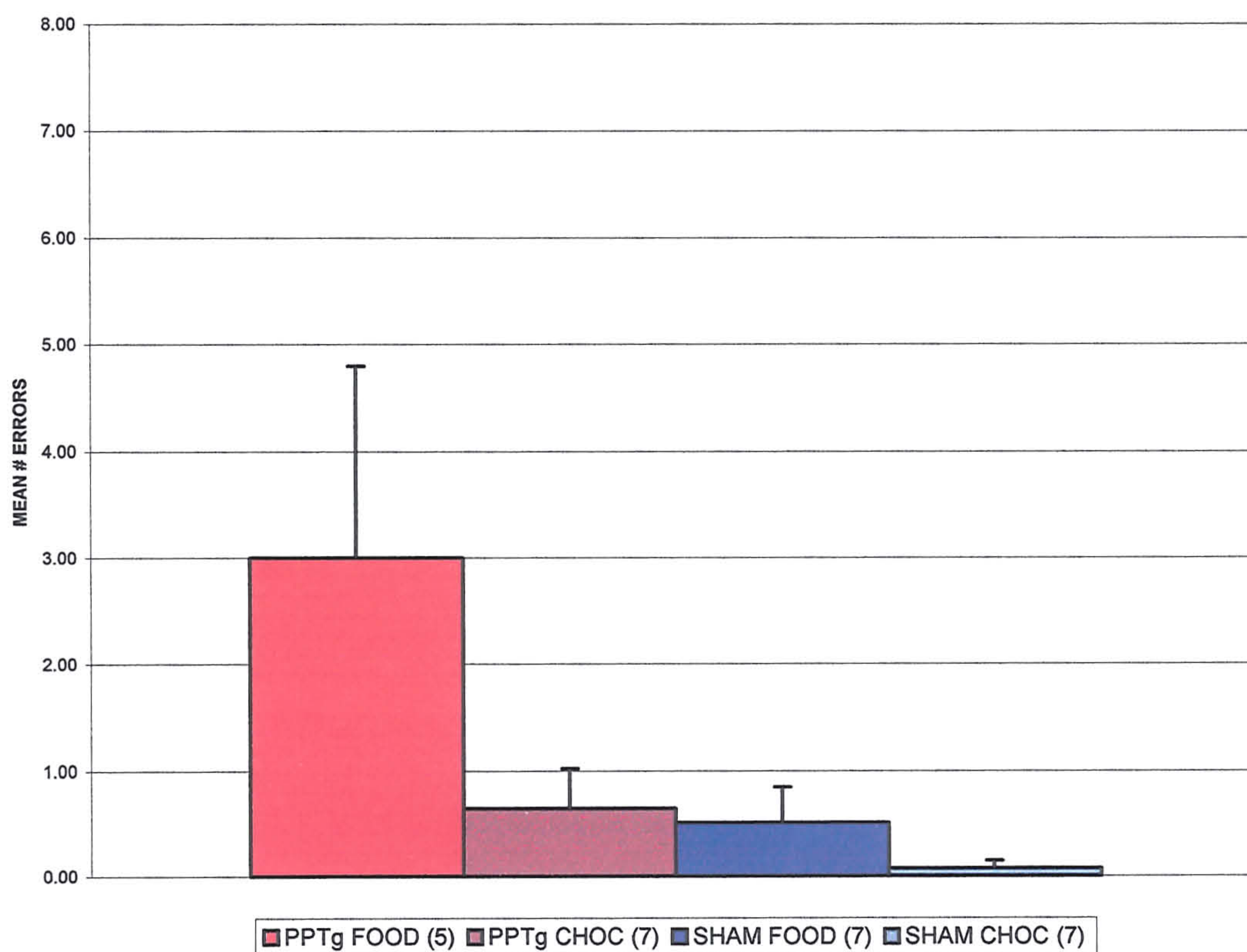


FIGURE 9.ix. MEAN % TOTAL TEST PHASE ERRORS THAT WERE AP AND WP

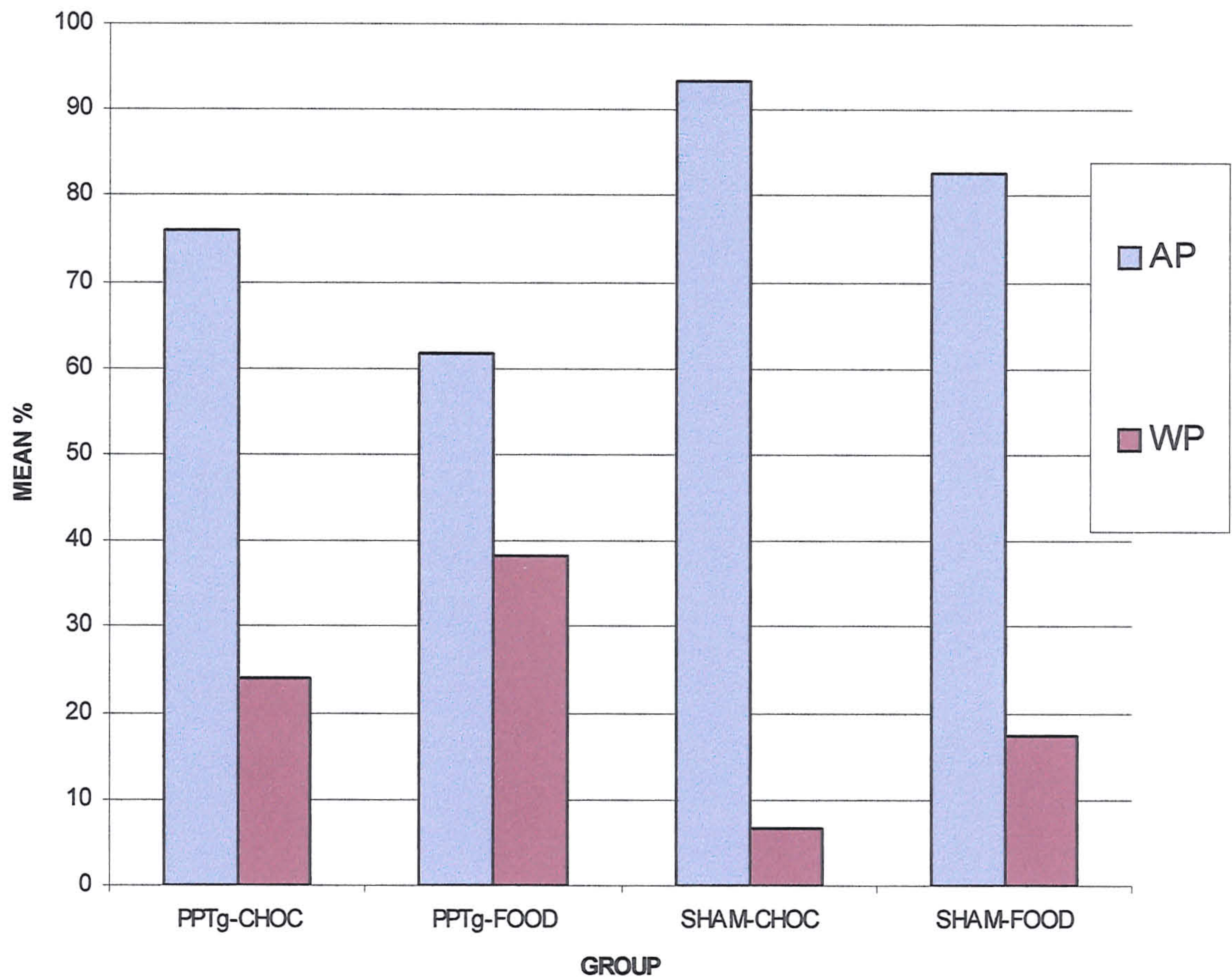


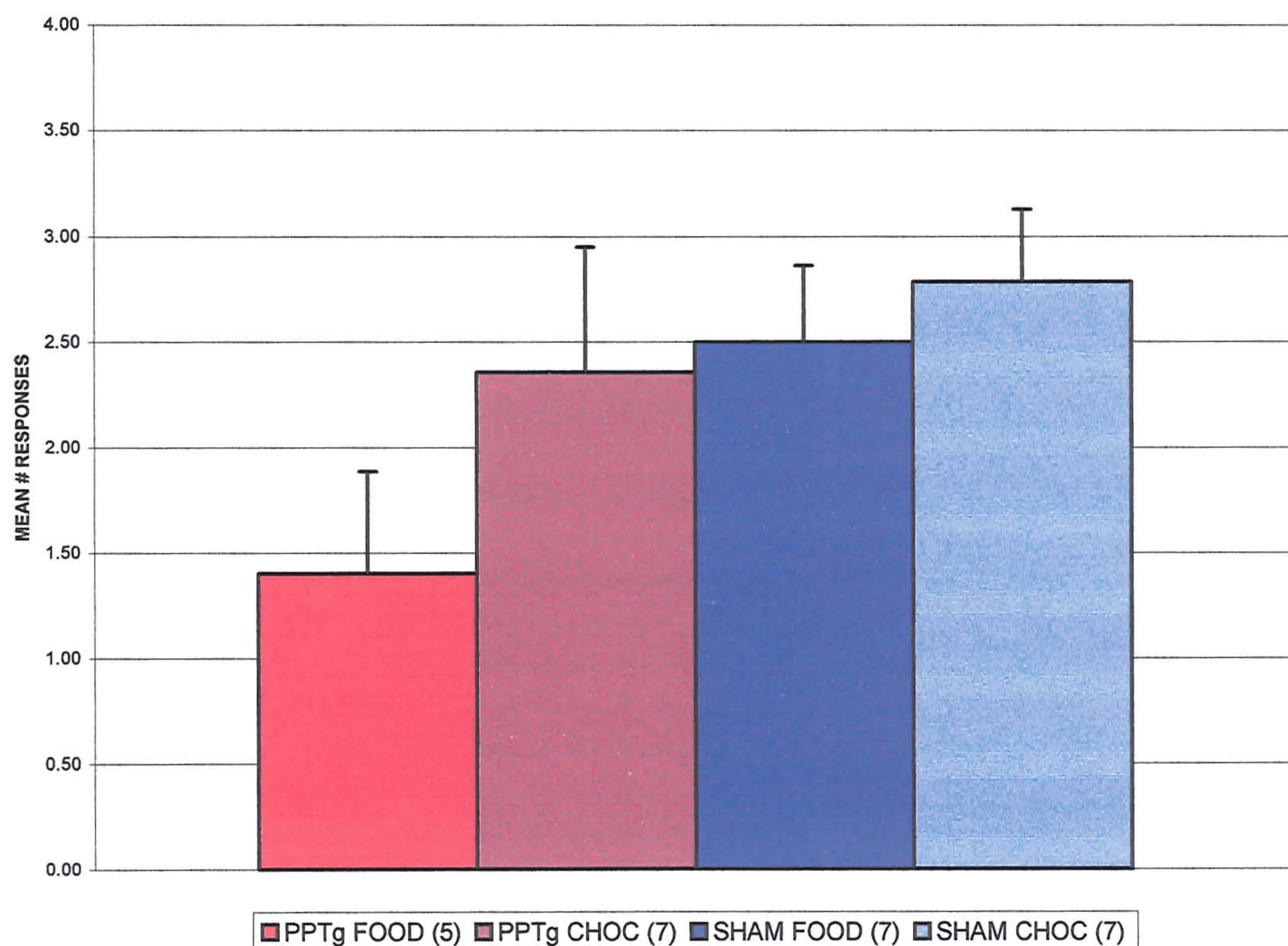
Figure 9.ix shows the mean percentage of total test phase errors over the criterion days that were AP and WP. This figure is included for comparison with the data from previous chapter. However, it should be noted that data from the current experiment represents criterion days' performance and, as such, limits the validity of the comparison. This is because the percentage scores are calculated for each rat. In this experiment, however, there were a large proportion of rats that did not make any errors over the criterion period. Since percentage scores cannot be calculated when the total

error score is zero, the above graph represents only the data from each group for rats that did make errors. The number of rats from each group that were included were; for the PPTg-food group 5/5 rats, for the PPTg-choc group 6/7 rats, for the sham-food group 6/7 rats, and for the sham-choc group 5/7 rats. This data suggests that only the PPTg-Food group display the reduced disparity between percentages of AP vs. WP errors for those rats that made any errors.

In order to compare statistically whether each group made significantly more AP than WP errors further within-subjects t-tests were conducted on the number of within and across phase errors made on the criterion days for each group. For the PPTg-Choc group $t(6) = -2.75$ $p < 0.05$, two-tailed. For the PPTg-Food group $t(4) = 1.77$ ns, two-tailed. For the Sham-Choc group $t(6) = -3.57$ $p < 0.05$, two-tailed. For the Sham-Food group $t(6) = -4.26$ $p < 0.01$, two-tailed. These results confirm the findings suggested by Figure 9.ix since only the PPTg-Food group made approximately equal numbers of across phase and within phase errors.

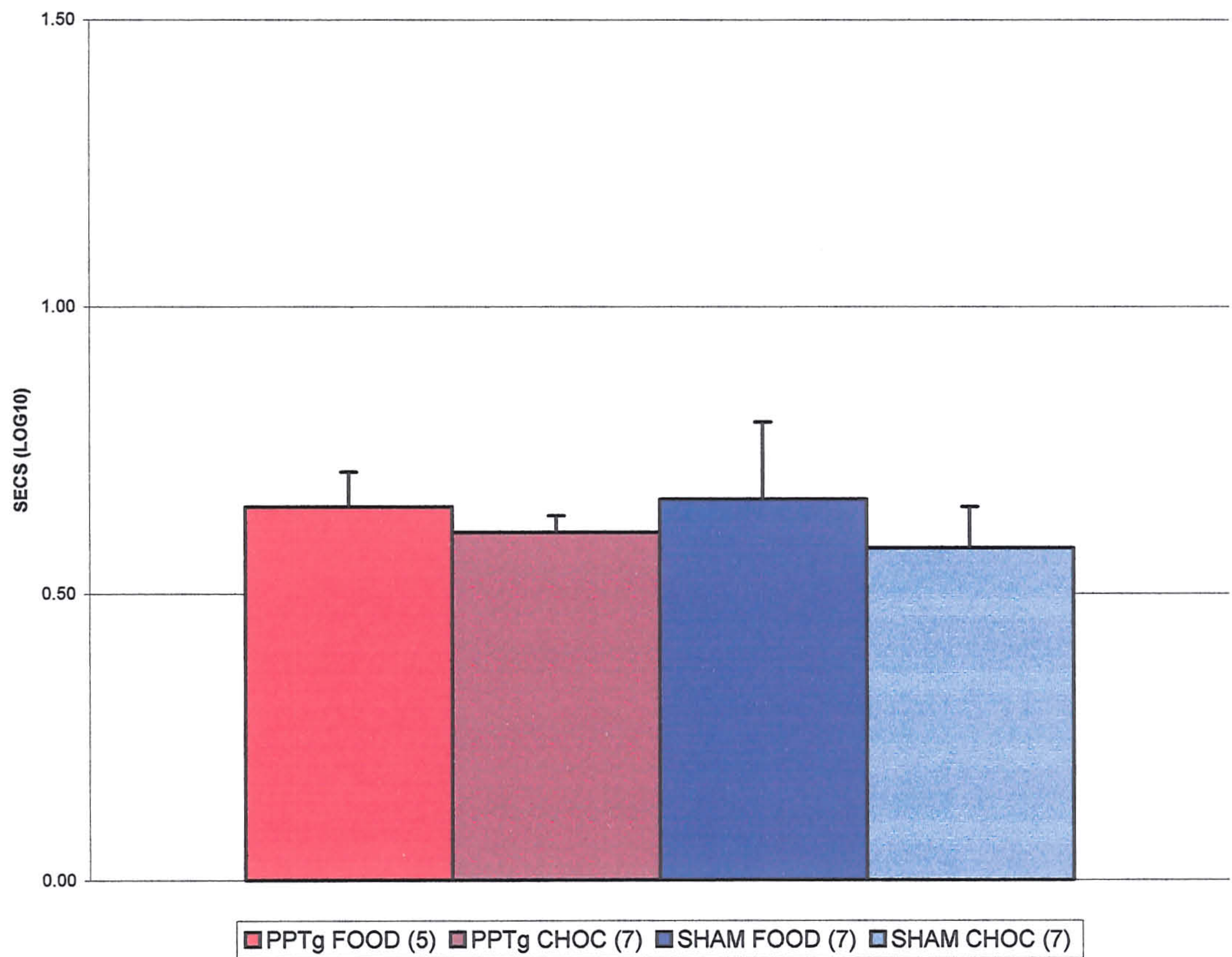
The mean and standard error for the number of correct responses made before error over the criterion days can be seen in Figure 9.x. The ANOVA conducted on these results showed that there were no significant main effects. Main effect of lesion $F(1,22) = 2.73$, and main effect of reward $F(1,22) = 1.80$. The interaction of lesion x reward was also non-significant $F(1,22) = 0.53$. This illustrates that while PPTg lesioned rats were impaired in the test phase through making more errors, they did not make these errors earlier than the shams. This suggests that PPTg rats tended to make two correct responses before beginning to make their errors. However, once they had made an error, they had a significantly greater tendency to make more errors after this in comparison to shams.

FIGURE 9.x TEST # CORRECT RESPONSES BEFORE ERROR



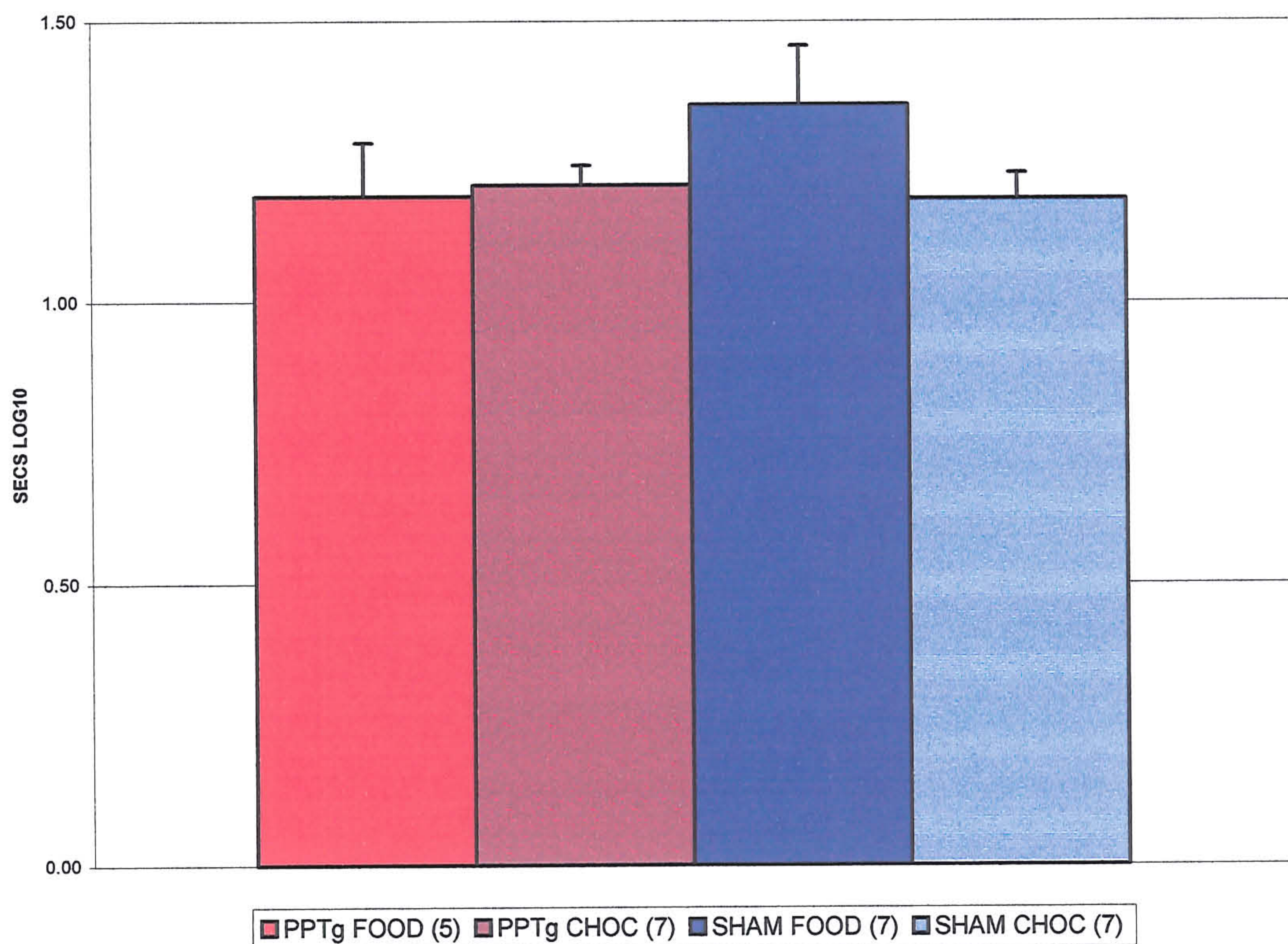
The mean and standard error for the latency to the first arm over the criterion period can be seen in Figure 9.xi. Unlike for the training phase latencies however there was no main effect of reward $F(1,22) = 0.42$. The main effect of lesion and the lesion x reward interaction were also non-significant $F(1,22) = 0.007$, and $F(1,22) = 0.04$ respectively.

FIGURE 9.xi TEST LATENCY (LOG10)



Finally the mean and standard error for the test phase choice times can be seen in Figure 9.xii. As for test phase latency there were no significant effects revealed by the ANOVA that was conducted on these results. Main effect of lesion $F(1,22) = 0.80$, main effect of reward $F(1,22) = 0.97$, and lesion x reward interaction $F(1,22) = 1.58$.

FIGURE 9.xii TEST CHOICE TIMES (LOG10)



In summary the table presented below (Table 9b) shows a review of the findings of the current experiment.

Table 9b. Summary of Results

	Lesion	Reward	Lesion x Reward
Training Errors	p<0.01 PPTg > Sham	ns	ns
Training Correct	p<0.01 PPTg < Sham	p<0.01 Food < Choc	ns
Training Latency	ns	p<0.05 Choc Faster	ns
Training Choice Times	ns	ns	ns
Test Errors	p<0.05 PPTg > Sham	p<0.05 Food > Choc	ns
AP	p<0.01 PPTg > Sham	p<0.05 Food > Choc	ns
WP	p<0.05 PPTg > Sham	ns	ns
AP > WP?	Yes for PPTg – Choc And both sham groups	-	-
Test Correct	ns	ns	ns
Test Latency	ns	ns	ns
Test Choice Times	ns	ns	ns

9.4 Discussion

9.4.1 *Where were the lesion effects?*

Significant lesion effects occurred in most of the accuracy measures but none of the speed measures. Rats with lesions centred on the PPTg were significantly impaired in both the training and test phases of the DSWS task as they made a greater number of errors than shams. However, these errors occurred significantly earlier in the choice sequences in the training phase but not in the test phase. Furthermore rats with lesions centred on the PPTg made significantly more of both types of error in the test phase. However the disparity between the percentages of the types of error was not the same for both PPTg groups. These findings did not directly replicate those of Keating and Winn (2001) since rats with PPTg lesions in that experiment made significantly more errors in the test phase only, with the pattern of AP/WP errors showing more AP than WP. One immediate problem with this comparison between the results of Keating & Winn (2001) and the present experiment is that it may be confounded by the chocolate condition in the current experiment. However, since the omnibus ANOVAs showed no significant interaction between the lesion effects and the effect of reward in the accuracy measures (indeed in any of the measures) this would not seem likely. Specifically, the lack of interaction indicates that the lesion effect shown by the omnibus ANOVAs did not change between the two reward conditions. That is, the PPTg/sham comparisons were not significantly differentially affected by the presence of the chocolate condition refuting a possible confound. However, given that rats with damage extending into the SN were excluded from the analyses of Keating *et al.* the two sets of results are not directly comparable on the basis of lesion extent.

9.4.2 Where were the reward effects?

Significant reward effects occurred in the early stages of the training phase (training latency to first arm and number of arms correct before error), and in the total number of test errors and number of across phase errors. Given that the decrease in test phase errors was the result of a decrease in across phase errors only it is likely that the effects of reward seen in the training phase were directly linked to these effects in the test phase. Specifically, if chocolate enhanced responding, perhaps by increasing attention or motivation throughout the early stages of the training phase, it would be more likely that the training phase arms would be recalled successfully in the test phase. In addition it should be noted that, since only the PPTg-Food group displayed a pattern of error that was significantly different to the shams, the chocolate reward might have served to significantly change the PPTg lesion deficit. There were also further suggestions in the test phase accuracy graphs that only the PPTg-Food group displayed a deficit in responding, although none of the interactions were significant for these measures. However one final point to note on this issue is that observed power for the interactions was typically low and it could be that increased group numbers for future experiments would significantly alter the findings reported here.

9.4.3 Did manipulations in reward value affect PPTg and sham groups differentially?

Given that there were no significant interactions between lesion and reward in any of the measures, manipulations of reward value in this experiment did not have any different effects on rats with lesions centred on the PPTg in comparison to the effects

shown in shams. In particular the results for the training phase latency measure, a measure most comparable to the run speed measure of Ainge *et al.* (1999), showed that rats with PPTg centred lesions *did* respond appropriately to an increase in reward. While this appeared to be in contrast to the findings of the runway experiment of Ainge *et al.* (1999) there are several salient differences between that experiment and the current one that impede a direct comparison. The first is obviously the size and location of the lesions. In the present experiment structures other than the PPTg were damaged such that the effects observed could not be reliably linked to PPTg neuronal loss.

The second difference is that run speed measured in the runway task is a simple run speed measure of approach behaviour. DSWS performance on the latency measure is more complex than this due to several factors. Specifically, rats run the required distance on the runway purely to get reward. This is the only aim of the runway rat and the only outcome to his running behaviour. In contrast, rats running the first arm of the DSWS task are immediately presented with a choice of arms. In addition they have to be aware of where they are running to when making their arm choice by encoding the motor response within working memory. The outcome of this response relates to the next arm choice. It is therefore likely that the neural mechanisms accessed by the rat to run the DSWS task are not directly comparable to those accessed during the runway task. A dissociation of the PPTg impairment in conditioned reward related responding between simple and complex (executive) tasks would be extremely intriguing especially in relation to PPTg involvement in executive function. However, again a conclusion in this regard would only be possible if the current results could be directly linked to PPTg damage.

Alternatively, one further locus of the differing results of this experiment and Ainge *et al.* (1999) was that the DSWS rats did not have access to as much reward as they required at the end of their running response. That is, the reward is limited to a finite amount in the DSWS task. Based on this it may be that if PPTg lesioned rats obtain the same amounts of a more positive reward as shams their subsequent approach behaviour is not affected. Put simply, the fact that PPTg rats drink significantly more of the 20% sucrose may be what causes their change in approach behaviour. However, why their approach behaviour should be negatively affected by greater consumption of reward is puzzling. One possibility could be malaise such that they do not run as fast as shams for the 20% sucrose because it has negatively reinforcing properties in addition to its positively reinforcing properties associated with alleviation of food deprivation. Given the extent of the lesions in the current experiment however, this remains speculative but is something that could be investigated further. One way would be to repeat the runway experiment but with control over the reward intake of shams and PPTg rats.

9.4.4 Conclusions

This experiment aimed to investigate the nature of the PPTg deficit in an executive task and the possible variability of this depending on reward value given the recent findings of Ainge *et al.* (1999) in a simple runway task. However, as a result of the extent of damage to structures other than the PPTg in the lesioned groups conclusions relating to the PPTg specifically could not be made. Comparisons between the findings of Keating & Winn (2001) & Ainge *et al.* (1999) and the current experiment suggest that the invasion of other structures may have contributed something more to the DSWS task deficit than would perhaps have been apparent following lesions restricted to the PPTg.

However the true extent of this confounding effect remains questionable until the experiment can be repeated with more restricted lesions.

Despite this problem the current experiment has indicated either that the PPTg decline in conditioned behaviour following reward value increase in a simple task is not paralleled in a more complex task, or that there may be an alternative explanation for the results of Ainge *et al.* (1999). Both of these have important implications for an interpretation of the role of the PPTg in reward related responding and should therefore be investigated further.

However, for a repeated DSWS experiment to parallel the simple runway behaviour measured in Ainge *et al.* (1999) as closely as possible there is a required modification to its design. Ainge *et al.* (1999) conducted their experiment to test whether the PPTg lesion effects based on reward value would also occur if the rats had prior experience of the other reward value (i.e. the effects of increasing and decreasing reward were investigated). In the current experiment there was only an increase in reward value since rats were pre-trained with food and the food/choc manipulation was introduced post-surgery. A suggested design modification for the next experiment was therefore that the food/choc conditions be reversed so that the effects of reward decrease on DSWS performance could also be studied.

10. EFFECTS OF VARYING REWARD VALUE ON THE PPTg DSWS TASK DEFICIT (2)

10.1 Introduction

Several studies have implicated the PPTg in responding for reward (Keating & Winn, 2001, Ainge *et al.*, 1999, Keating *et al.*, 1996, Inglis *et al.*, 1994, Bechara *et al.*, 1992, & Bechara *et al.*, 1989). However, the precise involvement of the PPTg in such behaviour remains unresolved because of the complexity of the deficits observed following PPTg inactivation. For example the runway study of Ainge *et al.* (1999) has indicated that impairment in responding for reward in PPTg lesioned rats is variable depending on the level of reward offered and the type of behaviour being observed. That is, PPTg lesioned rats' behaviour was enhanced for consummatory responding but decreased for approach responding, in comparison to shams, following the presentation of a more positive reward (20% sucrose as opposed to 4%). However, the responding required in this runway task is of a simple nature in comparison to the executive behaviour required for successful solution of the DSWS task.

PPTg lesioned rats are known to be impaired in the DSWS task (Keating & Winn 2001) but whether the decline in conditioned behaviour observed following an increase of reward level in the runway task would also be observed in a complex task in which PPTg lesioned rats were already impaired (with a standard food reward) was unknown. The experiment discussed in Chapter 9 was conducted to investigate this possibility. However, since the results did not appear to support those of Ainge *et al.* (1999) they suggested either dissociation between simple and complex conditioned behaviour PPTg

deficits or an alternative explanation for the findings of Ainge *et al.* (1999) – see Chapter 9. Problems with the extension of the lesions produced in that experiment meant, however, that neither of these possibilities could be validly proposed on the basis of those results.

The current experiment aimed to overcome these problems in order to confirm or refute the apparent disparity between the runway and DSWS PPTg deficit in conditioned responding for reward. Furthermore, the design of the present experiment was such that it more closely paralleled that of Ainge *et al.* (1999) with both a reward increase and a reward decrease condition. However, observations from the preceding DSWS experiments had shown that day-to-day performance on the DSWS task (perhaps because of its complexity) is highly variable both within individuals and within groups, and changes due to re-learning of the task when assessed in a retention paradigm. As a result of this a full or restricted groups analysis over all days would not allow a reliable conclusion that a day effect was the result of reward reversal (as is conducted in the runway task of Ainge *et al.*, 1999). The data from the present experiment was therefore analysed in three separate stages with the aim of answering the following specific questions:

- 1) Does the PPTg DSWS deficit vary depending on level of reward?
- 2) What are the immediate effects of reversing (increasing or decreasing) this reward on PPTg lesioned animals?
- 3) What are the long-term effects of reversing (increasing or decreasing) this reward on PPTg lesioned animals? In other words, are the immediate effects

present in the long term and are there long-term effects that are not present immediately?

10.2 Method

40 adult male Lister Hooded rats weighing approximately 330g at the time of surgery were pre-trained to criterion on the DSWS radial maze task. They were housed and fed as discussed previously (see General Methods). As with the prior experiment training was conducted (CT & RK) so that each group of 20 rats was trained by one of the experimenters at a particular time of the day. Rats took 10 days to reach criterion using Noyes food pellets (45mg) as reward, following which they were assigned to surgical groups with matching mean bodyweights.

The surgical procedure was as described in Chapter 9 with three exceptions. The first was that the infusions were made using a step-down procedure (0.01 μ l / 10 secs), a procedure shown to produce more controlled, restricted lesions of the PPTg in other work at this laboratory. The needle was left in situ for a further 5 minutes. The second was that the second hemisphere infusions were of equal volume to the first hemisphere infusions (0.02 μ l), again in line with the procedures used in other work from this lab. In addition however, experimenter error meant that 3 animals were infused in the second week with 0.12M quinolinate instead of 0.12M ibotenate. See results section for analysis of impact of this error on quality of lesions produced. Following surgery the post-surgical care was as described previously (Chapter 9). A total of 18 PPTg lesioned and 15 sham lesioned rats survived surgery.

Postsurgical testing occurred for 9 days (to parallel length of time to criterion in the previous experiment) before reversal of the food reward. Again each experimenter (CT & RK) tested the surviving rats from their original group of 20. Rats were then tested for a further 9 days with rewards reversed. The effect of varied reward level on the PPTg DSWS deficit was analysed as in the previous experiment to facilitate comparison between the two. Data for the criterion days (7+8) was used to calculate a mean score of performance for each rat on each measure over the two days. These were then subjected to two-way univariate ANOVAs. The immediate effects of reward reversal were assessed by comparing mean performance on the last three days of testing prior to reversal (including the criterion days 7+8), defined as the “pre-reversal period”, with that on the first three days following reversal, defined as the “post-reversal period”. In this case lesion and reward factors were collapsed into a single between subjects factor of “group”, with “reversal” acting as a within subjects factor. This data was analysed using two-way mixed ANOVAs. The long-term effects of reward reversal were analysed by comparing performance on the last day of the pre-reversal period with that on the last day of the post-reversal period. Again the factors of lesion and reward were collapsed into a single between subjects factor of “group” with “reversal” acting as a within subjects factor. This data was also analysed using two-way mixed ANOVAs.

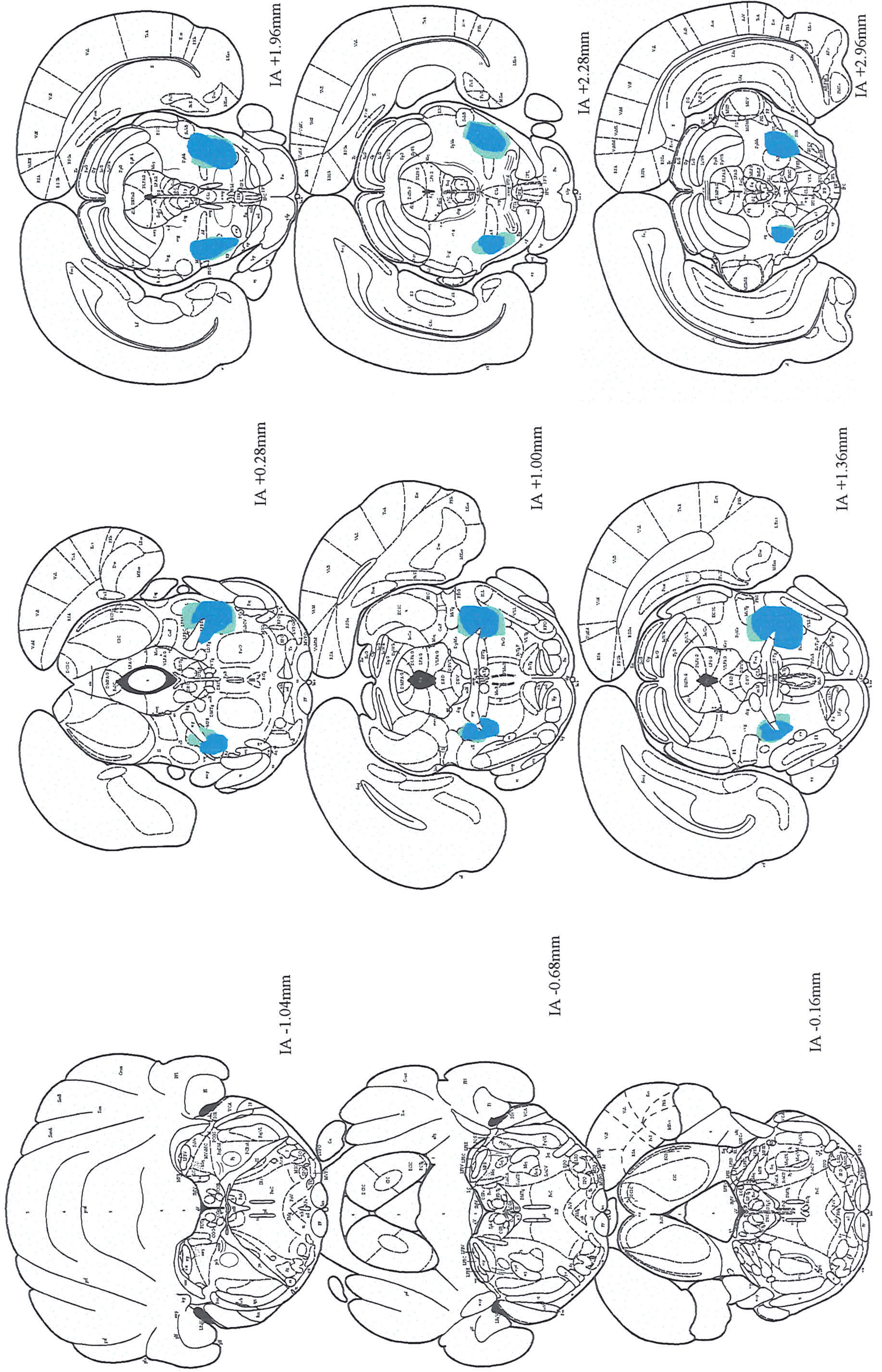
10.3 Results

10.3.1 Histology

Analysis of NADPH diaphorase and cresyl violet stained sections revealed that, in contrast to the lesions produced in the preceding experiment, PPTg lesions in this experiment were largely confined to the PPTg. These lesions were not as varied in size as those produced using the manual microdrive infusion technique and there was only very minor invasion of the substantia nigra in the largest lesions of each group. Figure 10.i illustrates the extent of the lesions included in the analyses for both the chocolate and the food groups (defined according to first postsurgical reward received). Damage to the PPTg was extensive both rostrally and caudally with more than 80% neuronal loss occurring in this structure in the animals included in the analyses. 4 animals were excluded because they had received only unilateral damage and a further 3 animals were excluded on the basis of incomplete PPTg loss (either rostral or caudal survival) in one of the lesioned hemispheres. In total there were 6 rats remaining in the PPTg choc/food group, 5 rats in the PPTg food/choc group, 8 rats in the sham choc/food group, and 7 rats in the sham food/choc group.

When all lesions were rated blindly, and independently, by both experimenters (CT & RK) the quinolinate lesions were not identifiable as distinct from the ibotenate lesions. This finding is supported by the work of Rugg *et al.* (1992) who used comparable toxin volume (1x 0.5 μ l 0.12M) infusions of quinolinate and ibotenate in the PPTg in a step-down procedure. Thus the lesions made with quinolinate were not separated from the ibotenate lesions in the DSWS analyses.

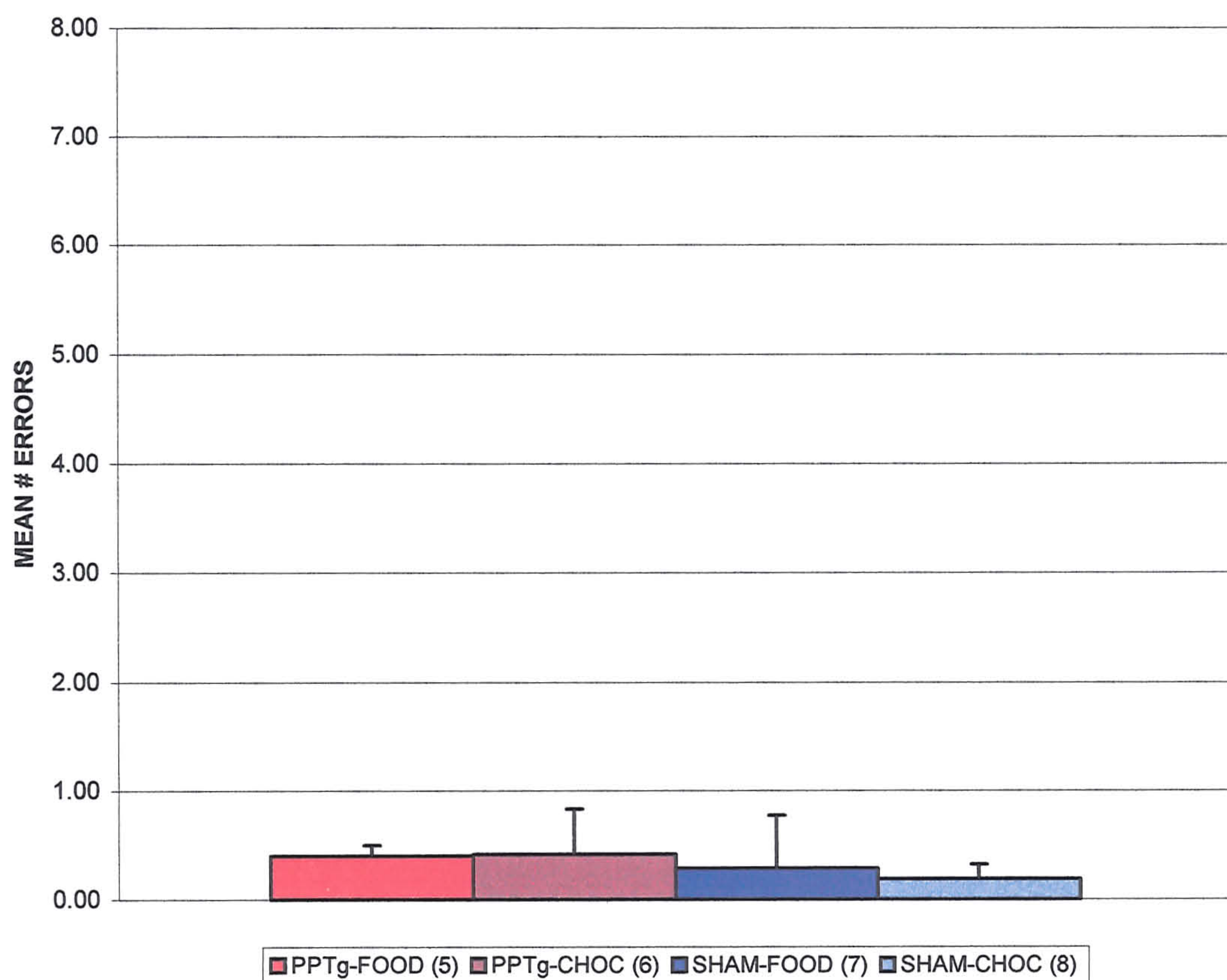
Figure 10.i Lesions of the PPTg - smallest is depicted on the left and largest on the right. Blue represents the PPTg-Food group and green represents the PPTg-Chocolate group



10.3.2 *The DSWS Task (1): Effects of varied reward level on the PPTg deficit*

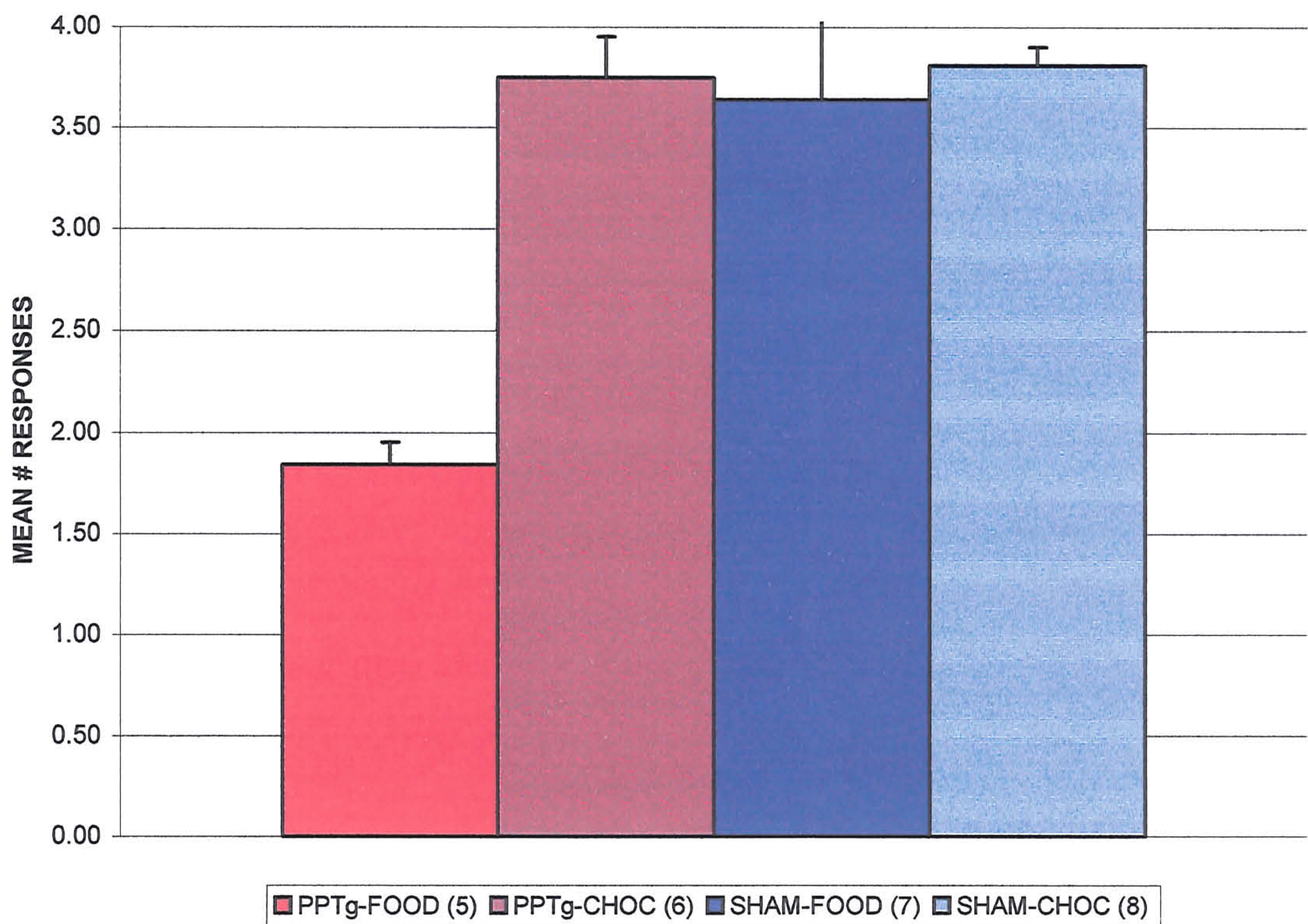
The mean and standard error for the number of errors made in the training phase over the 2 criterion days can be seen in Figure 10.ii. The univariate 2 way ANOVA conducted on the results showed that there were no significant main effects of lesion: $F(1,22) = 0.52$, or reward $F(1,22) = 0.03$. The interaction was also non-significant $F(1,22) = 0.06$.

FIGURE 10.ii TRAINING ERRORS



The mean and standard error for the number of correct responses made before error in the training phase over the criterion days can be seen in Figure 10.iii. As with number of errors, there were no significant effects on this measure. Main effect of lesion $F(1,22) = 0.001$, main effect of reward $F(1,22) = 0.48$, and lesion x reward interaction $F(1,22) = 0.14$. Although the graph suggests the presence of an interaction, observed power for this was very low at 0.07.

FIGURE 10.iii TRAINING # CORRECT RESPONSES BEFORE ERROR



The mean and standard error for the latencies in the training phase over the 2 criterion days can be seen in Figure 10.iv. The 2 way univariate ANOVA showed a main effect of reward $F(1,22) = 8.74$ $p < 0.01$ but no main effect of lesion $F(1,22) = 2.09$. The lesion x reward interaction was also non-significant $F(1,22) = 0.87$. These results confirm that all rats receiving chocolate ran significantly faster in the training phase to the first arm when compared to those receiving the standard food reward.

FIGURE 10.iv TRAINING LATENCY (LOG10)

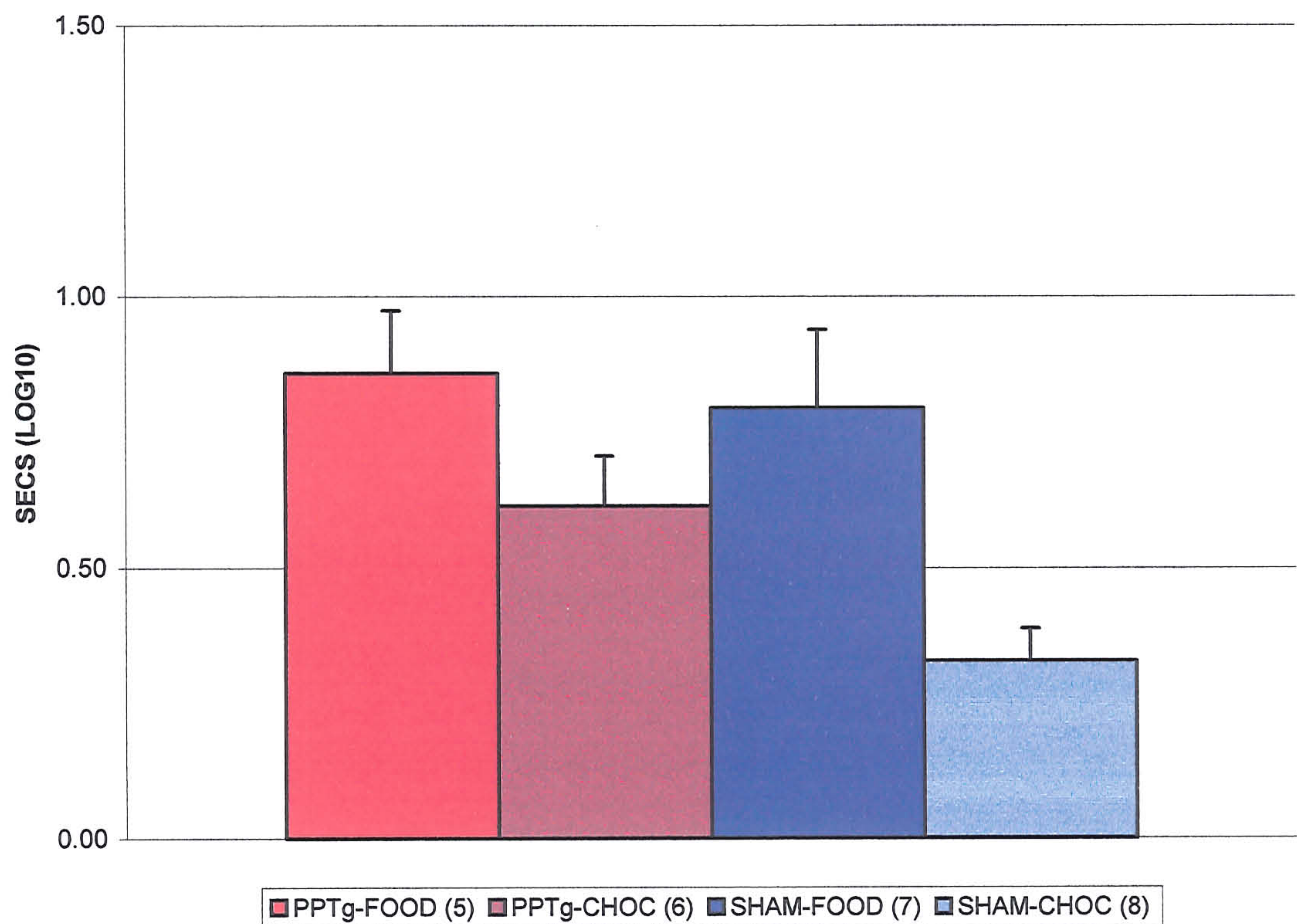
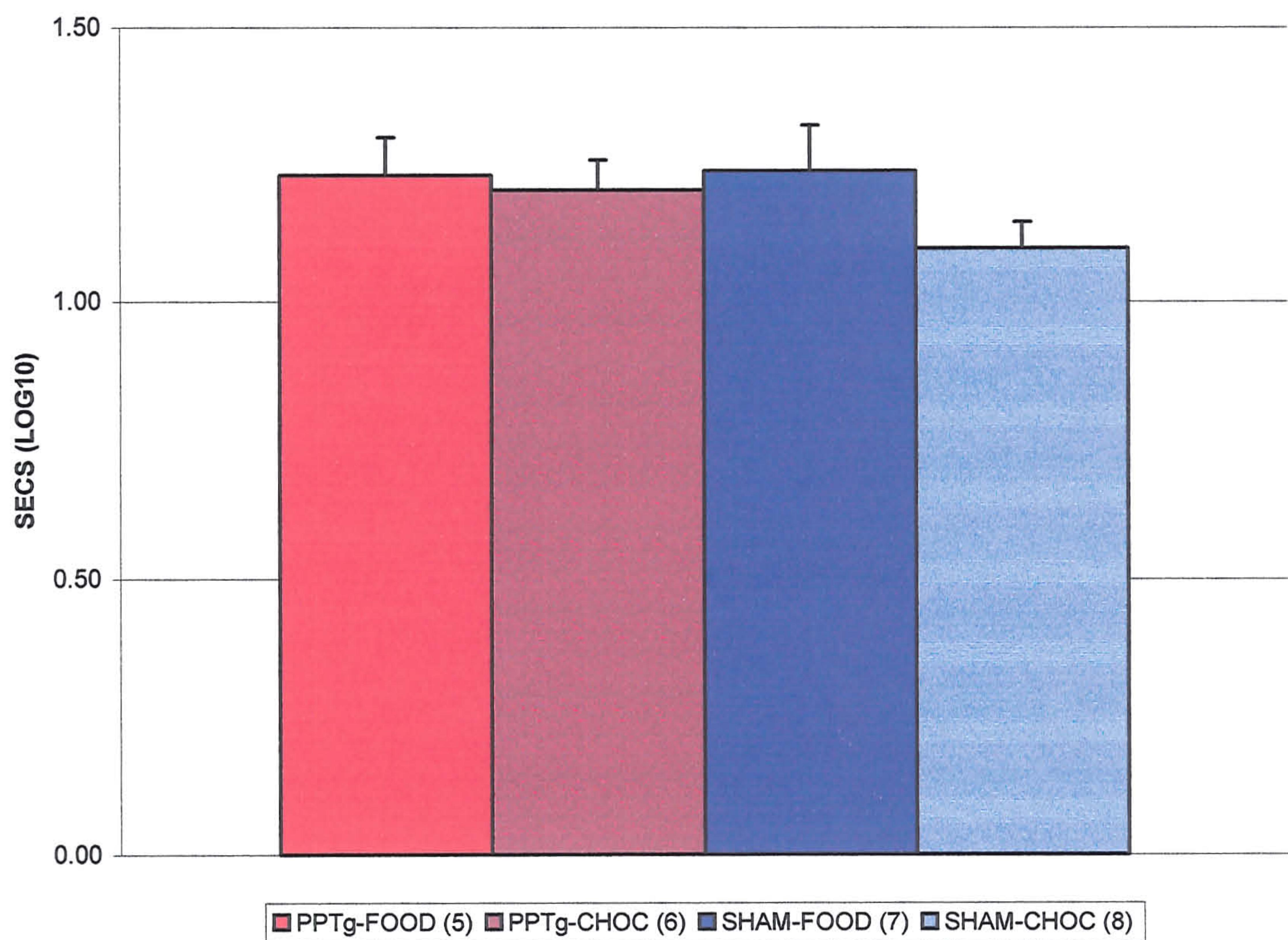


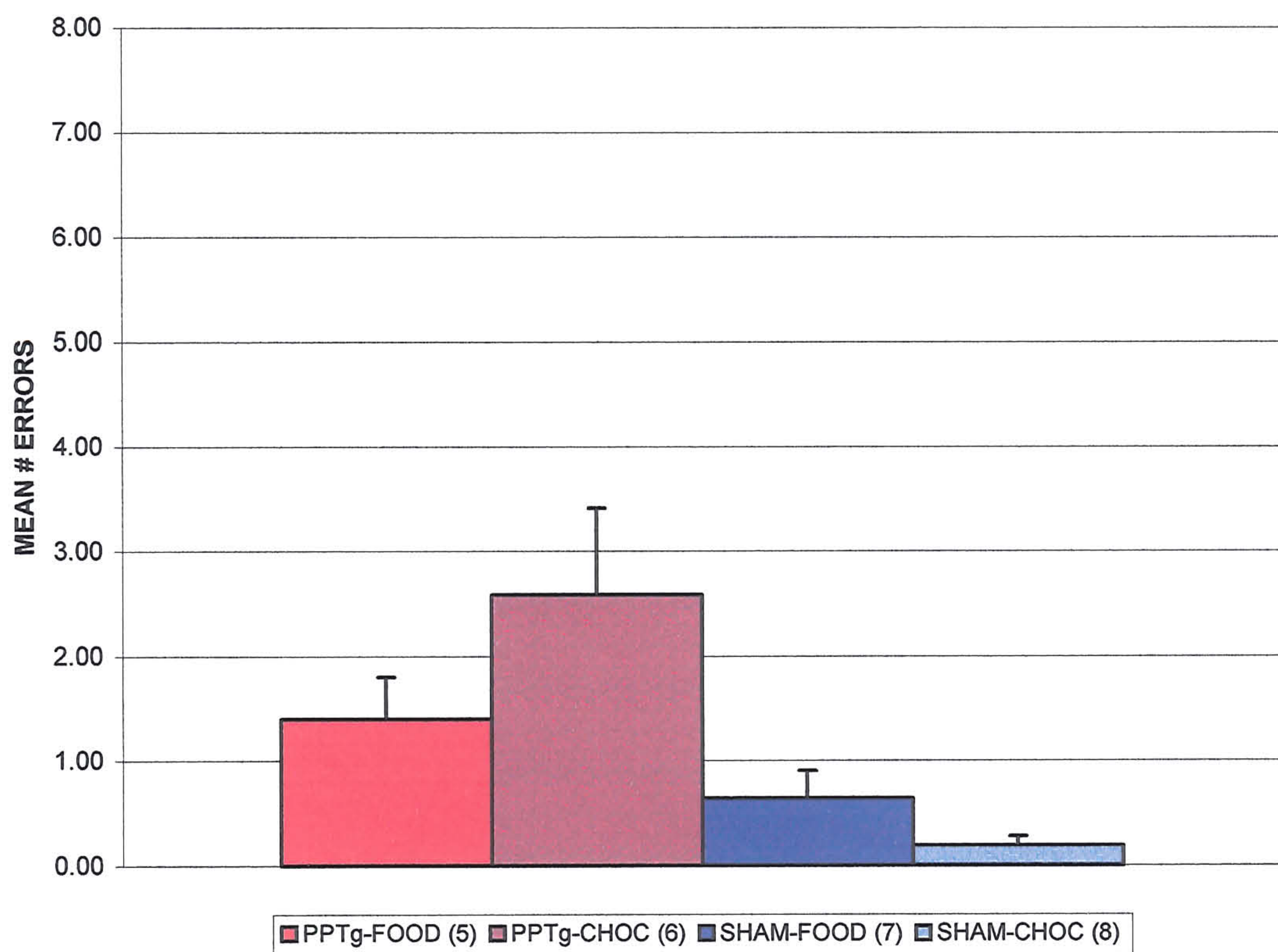
Figure 10.v shows the mean and standard error for the time to make arm choices in the training phase for each group over the 2 criterion days. The ANOVA revealed no significant main effects or interaction. Main effect of lesion $F(1,22) = 0.44$, main effect of reward $F(1,22) = 1.26$, and lesion x reward interaction = 0.58.

FIGURE 10.v TRAINING CHOICE TIMES (LOG10)



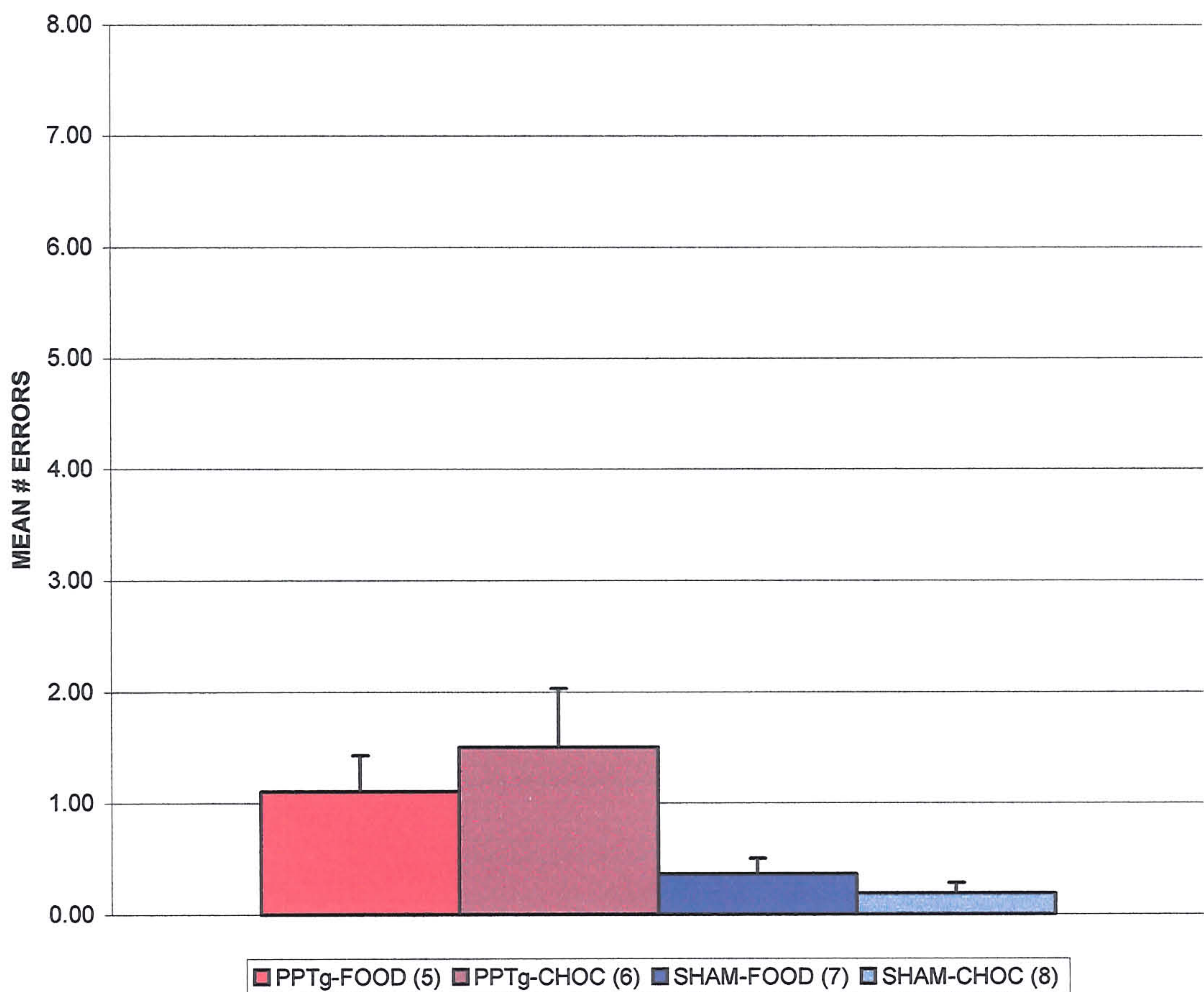
The mean and standard error for the number of errors made in the test phase over the 2 criterion days can be seen in Figure 10.vi. A 2 way univariate ANOVA conducted on these results showed a main effect of lesion $F(1,22) = 12.66$ $p < 0.01$, but no main effect of reward $F(1,22) = 0.68$, or lesion x reward interaction $F(1,22) = 3.42$. Partial eta squared for the main effect of lesion was 0.37 with power observed at 0.93. These results show that bilateral PPTg lesions in this experiment led to significantly increased numbers of errors in the test phase of the DSWS task.

FIGURE 10.vi TEST ERRORS



The means and standard error for the across phase errors over the criterion days can be seen in Figure 10.vii. The ANOVA conducted on these results showed that there was a main effect of lesion $F(1,22) = 12.18$ $p < 0.01$ but no main effect of reward $F(1,22) = 0.15$. The reward \times lesion interaction was also non-significant $F(1,22) = 0.94$. Partial eta squared for the main effect of lesion was 0.36 with power observed at 0.92. These results confirm that rats with PPTg lesions make significantly more across phase errors than shams.

FIGURE 10.vii ACROSS PHASE ERRORS



The means and standard error for the within phase errors over the criterion days can be seen in Figure 10.viii. The 2 way univariate ANOVA conducted on these results revealed a main effect of lesion $F(1,22) = 4.67$ $p < 0.05$ with a partial eta squared of 0.17 and observed power at 0.52. There was also a significant lesion x reward interaction $F(1,22) = 4.43$ $p < 0.05$. The main effect of reward was not significant $F(1,22) = 0.96$. Post hoc Tukey tests for each group revealed a significant effect of reward in the sham but not the PPTg lesioned animals. These results show that presentation of a preferred reward can improve WP error performance in the sham rats but not the PPTg rats, who are significantly impaired relative to the shams. Further analysis (related t-tests) addressing whether PPTg lesioned rats made more AP than WP errors in their pattern of responding did not reveal this to be the case. When the PPTg groups were combined; $t(10) = 1.66$ ns two-tailed. When assessed separately PPTg-choc; $t(5) = 0.69$ ns two-tailed and PPTg-food; $t(4) = 2.14$ ns two-tailed.

FIGURE 10.viii WITHIN PHASE ERRORS

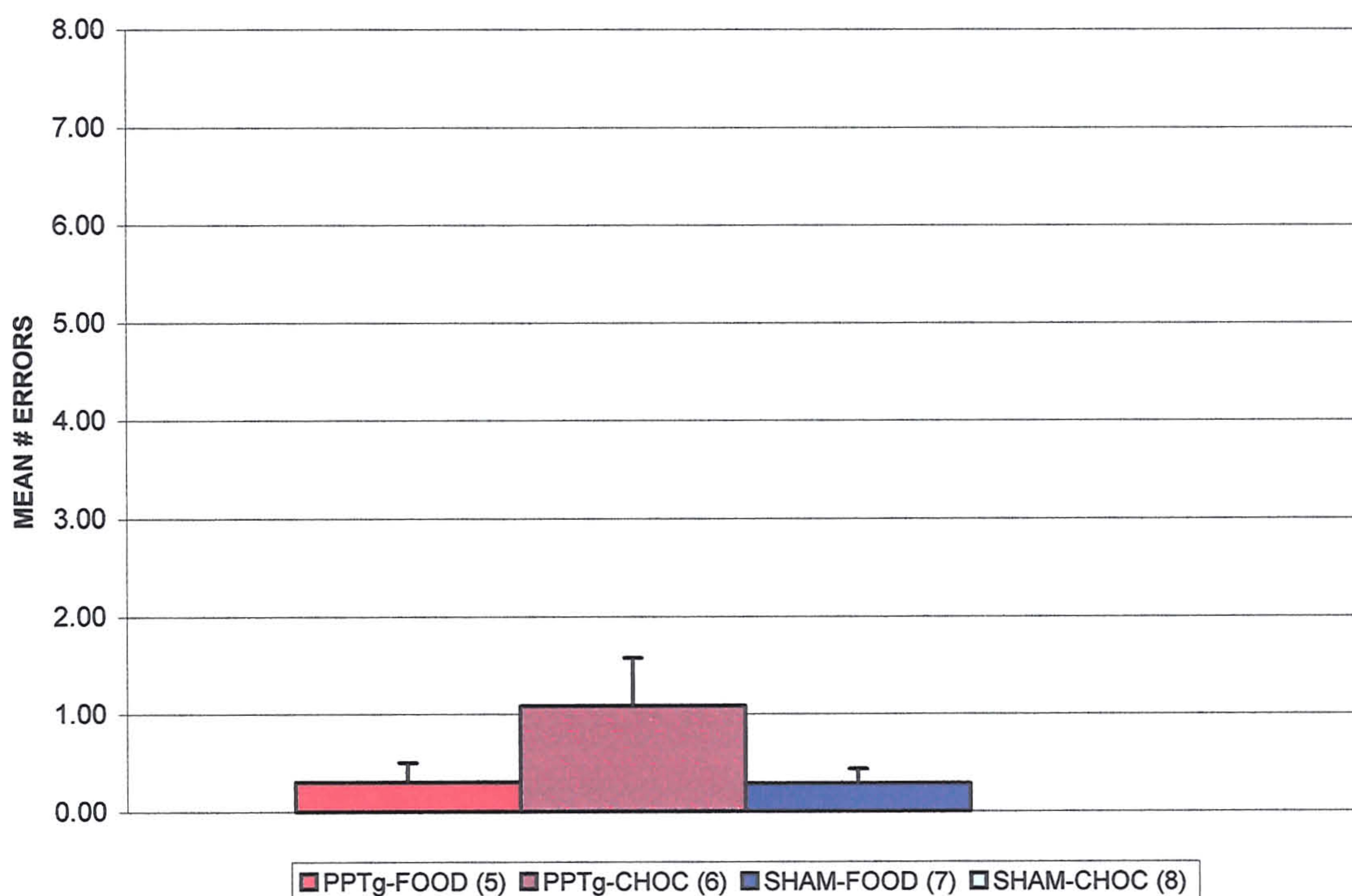
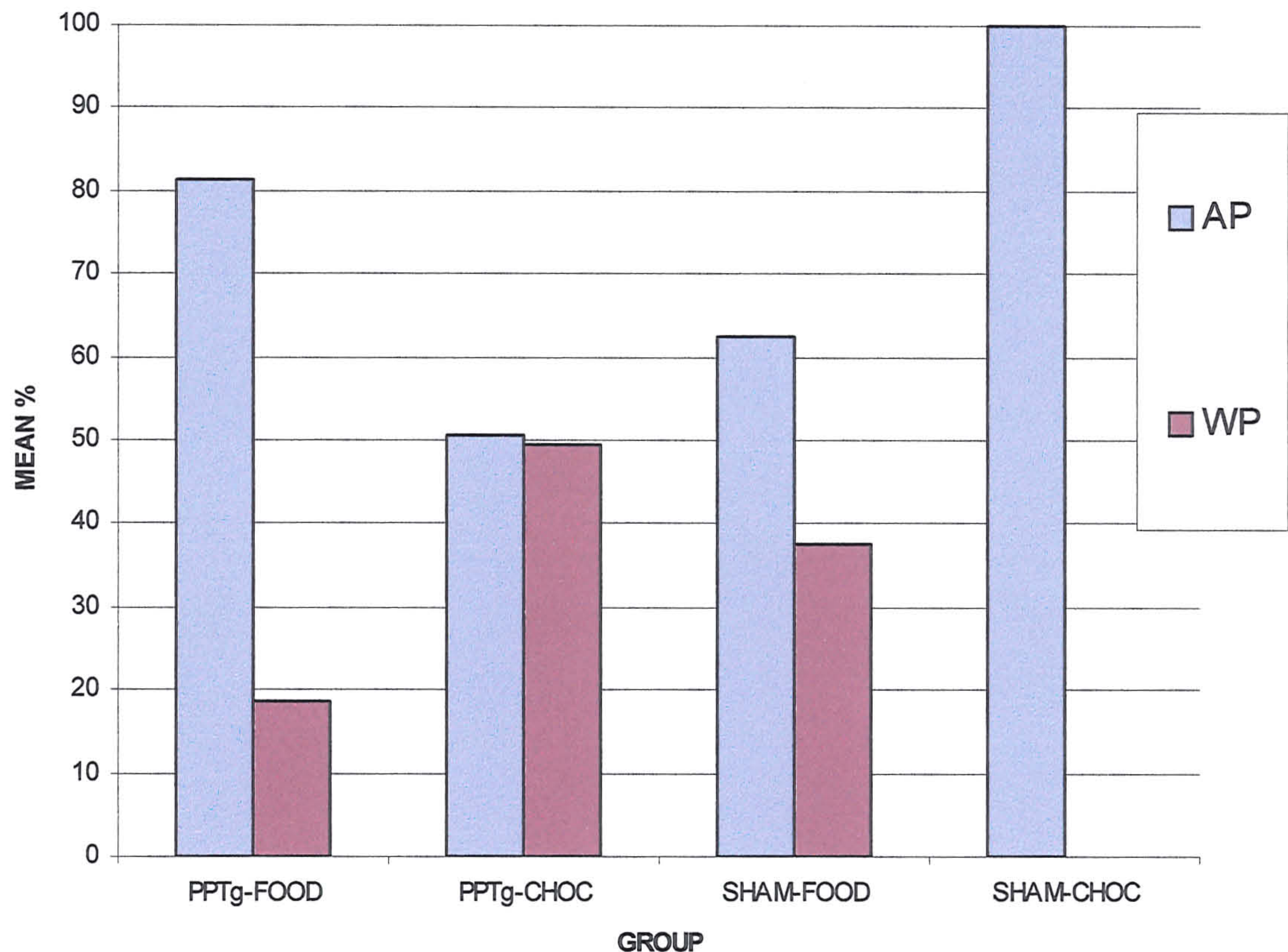


FIGURE 10.ix. MEAN % TEST PHASE ERRORS THAT WERE AP AND WP



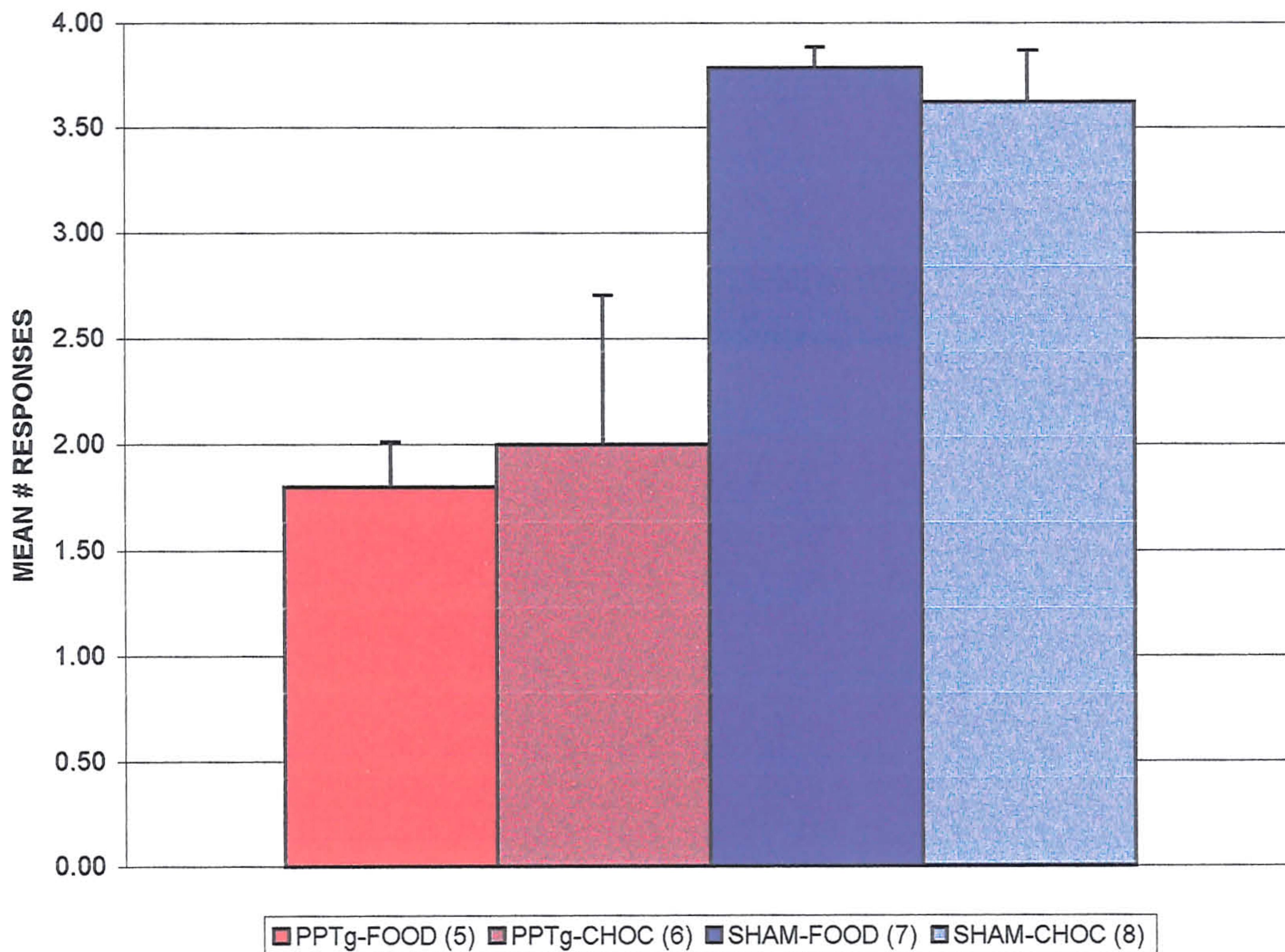
However, as for the preceding experiment the AP and WP error rates are expressed as a percentage of the total test phase errors in the graph above (Figure 10.ix). Again, just as in the preceding experiment, this data was calculated from the criterion days only, excluding those rats that did not make any test phase errors. The number of rats included in each group were; for the PPTg-Food group 4/5, for the PPTg-Choc group 6/6, for the Sham-Food group 4/7, and for the Sham-Choc group 3/8. Figure 10.ix shows that, while PPTg-Choc rats did display a similar error patterning to the PPTg-Food group in the previous experiment, the PPTg-food rats did not. Infact, their percentages of AP and WP errors more closely matched those of the sham groups from

this and the previous experiment (although the sham-food group also showed reduced AP/WP percentage disparity in this experiment).

In order to analyse the relationship of AP and WP errors further for each group related t-tests were conducted on the number of within and across phase errors made over the criterion days. For the PPTg-Food group $t(4) = -2.14$ ns, two-tailed. For the PPTg-Choc group $t(5) = -1.03$ ns, two-tailed. For the Sham-Food group $t(6) = 0.58$ - ns, two-tailed. For the Sham-Choc group $t(7) = -0.58$ ns, two-tailed. Thus, none of the groups in this experiment made significantly more AP than WP errors. However, one possible reason for this finding, particularly in the sham groups, is that actual numbers of errors during the criterion period in this experiment were extremely low. For this reason the percentage graph is misleading because it excludes those rats that made no errors and, through its representation of the data as percentage values, suggests that the Sham-Choc group in particular displayed a huge disparity between AP and WP errors. In reality this group only made a total of 1.5 AP errors between them.

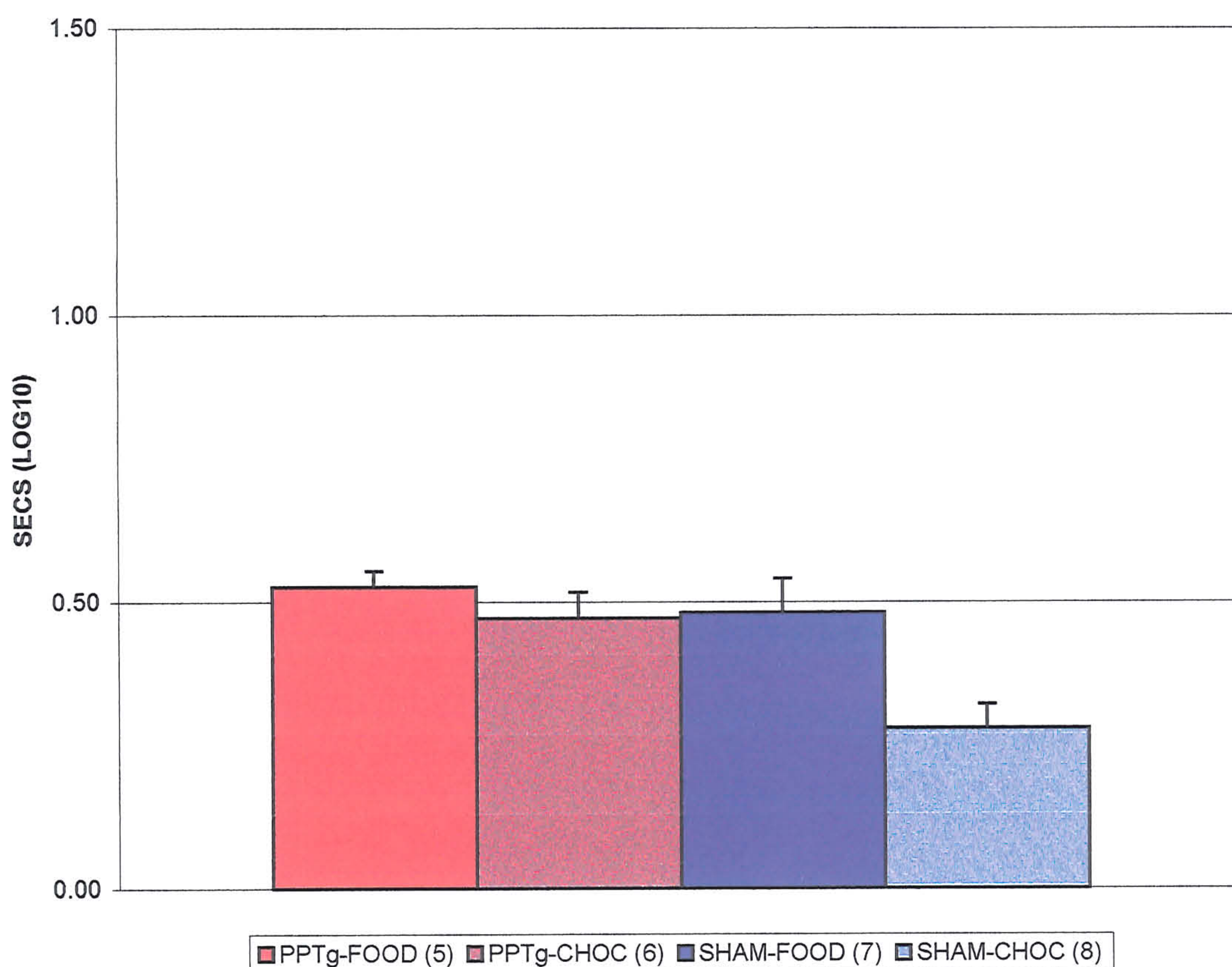
The mean and standard error for the criterion days' data for the measure of number of correct responses before error in the test phase can be seen in Figure 10.x. The ANOVA conducted on these results revealed a significant main effect of lesion $F(1,22) = 10.06$ $p < 0.01$, but not a significant main effect of reward $F(1,22) = 2.62$, or a significant reward x lesion interaction $F(1,22) = 1.31$. Partial eta squared for the main effect of lesion was 0.31 with power observed at 0.86. These results show that rats with bilateral PPTg lesions in this experiment were impaired in the test phase not only by making more errors, but also by making their errors significantly earlier in their choice sequence than shams.

FIGURE 10.x TEST PHASE # CORRECT RESPONSES BEFORE ERROR



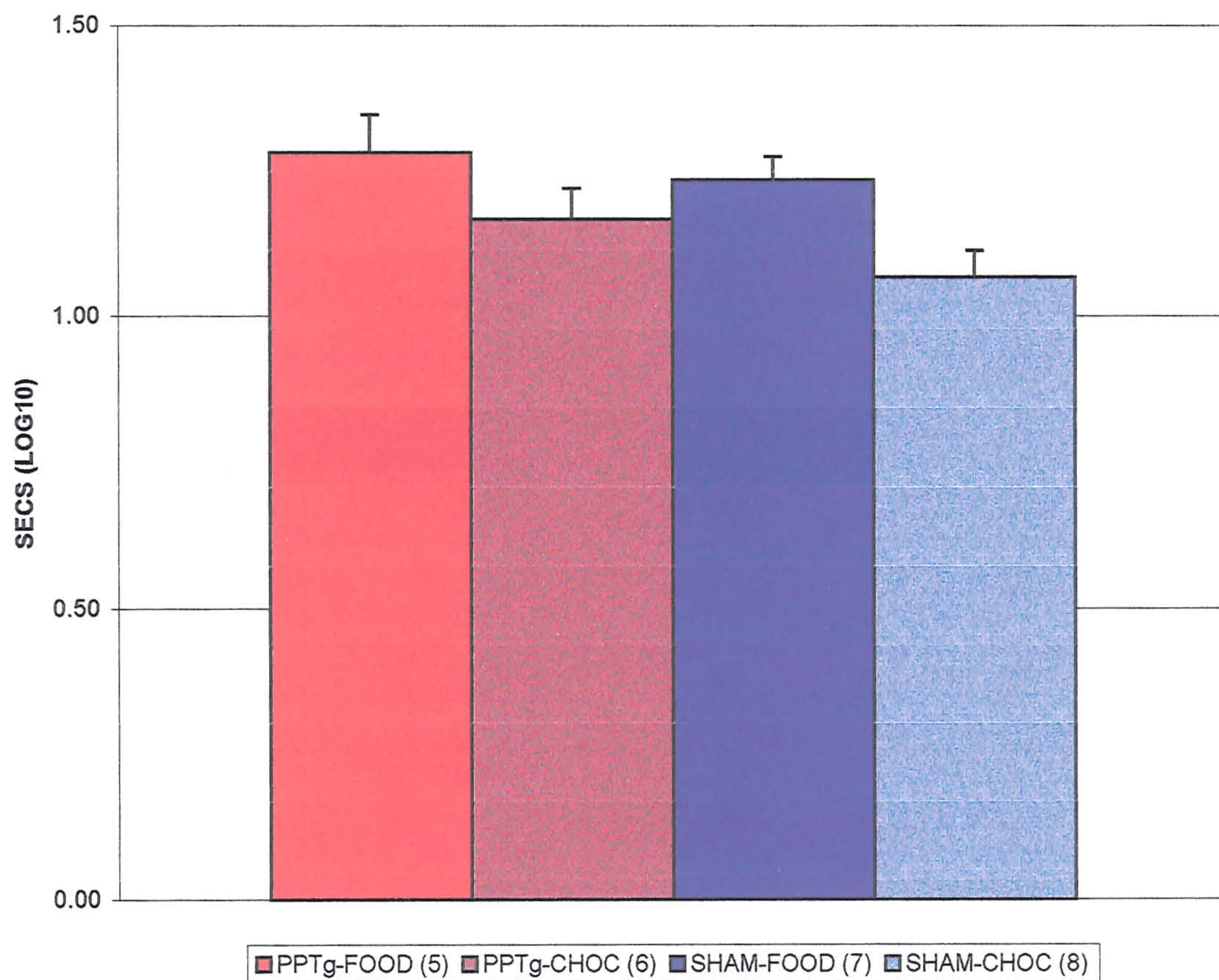
The mean and standard error for the test phase latencies over the criterion days can be seen in Figure 10.xi. The ANOVA for these results showed a significant main effect of lesion $F(1,22) = 5.40$ $p < 0.05$, and of reward $F(1,22) = 6.15$ $p < 0.05$. The lesion \times reward interaction was not significant $F(1,22) = 1.94$. Partial eta squared for the main effect of lesion was 0.20 with an observed power of 0.60. For the main effect of reward partial eta squared was 0.22 with observed power at 0.66. These results show that PPTg lesioned rats were significantly slower than sham lesioned rats to initiate the test phase of the DSWS task, but that chocolate could improve the speed of both groups of animals.

FIGURE 10.xi TEST LATENCY (LOG10)



The mean and standard error for the choice times for the criterion days can be seen in Figure 10.xii. A 2 way univariate ANOVA conducted on these results revealed that there was a significant effect of reward $F(1,22) = 5.59$ $p < 0.05$ but not of lesion $F(1,22) = 1.42$. The reward x lesion interaction was also non-significant $F(1,22) = 0.18$. These results show that both PPTg lesioned and sham lesioned animals can improve their choice times if chocolate is the reward available on the maze in the DSWS task.

FIGURE 10.xii TEST CHOICE TIMES (LOG10)



10.3.3 Summary (1):

A summary of the results of the criterion analyses is presented below in Table 10a.

Table 10a. Summary of Criterion Analyses

	Lesion	Reward	Lesion x Reward
Training Errors	ns	ns	ns
Training Correct	ns	ns	ns
Training Latency	ns	p<0.01 Choc Faster	ns
Training Choice Times	ns	ns	ns
Test Errors	p<0.01 PPTg > Sham	ns	ns
AP	p<0.01 PPTg > Sham	ns	ns
WP	p<0.05 PPTg > Sham	ns	p<0.05 Reward only in Shams
AP > WP	No	-	-
Test Correct	p<0.01 PPTg < Shams	ns	ns
Test Latency	p<0.05 PPTg Slower	p<0.05 Choc Faster	ns
Test Choice Times	ns	p<0.05 Choc Faster	ns

PPTg lesioned rats in this experiment were not impaired on any of the accuracy measures in the training phase part of the DSWS task by the time that sham rats had reached asymptote performance in the test phase. Neither were there any effects of varying reward value on these measures. Likewise, PPTg lesions had no effects on the latency or choice time measures in the training phase on the sham criterion days. The only effect of varying reward value on the training phase was to increase the speed at which both PPTg lesioned and sham lesioned rats initiated the task (made their first arm response). Thus in this phase there was no evidence that the chocolate reward significantly worsened the performance of PPTg lesioned rats. Furthermore PPTg lesioned rats responded in the same manner as shams in the training phase to an increase in reward value.

In the test phase PPTg lesioned rats were impaired on the accuracy of performance of the DSWS task by the time sham rats had reached criterion. They made significantly more errors (both across phase and within phase) and made their errors significantly earlier in their choice sequences when compared to shams. Presentation of a preferred reward significantly decreased the number of within phase errors made by sham rats on the criterion days but had no effect on PPTg lesioned rats on these days. Although both PPTg lesioned groups in this experiment did not make significantly more AP than WP errors, neither did the sham groups. While in the sham groups this is likely to be a direct result of the very low numbers of errors made by these rats this finding unfortunately precludes any conclusions regarding PPTg deficits in error patterning in this experiment.

PPTg lesioned rats were impaired relative to shams on the test phase latency to first arm since they were significantly slower on the criterion days. However, as in shams, presentation of a preferred reward significantly improved this slowness in speed. PPTg lesioned rats were not significantly slower overall at making their test phase arm choices, though, despite being slower to make the first arm choice. Furthermore chocolate significantly increased the speed at which test phase arm choices were made in both groups.

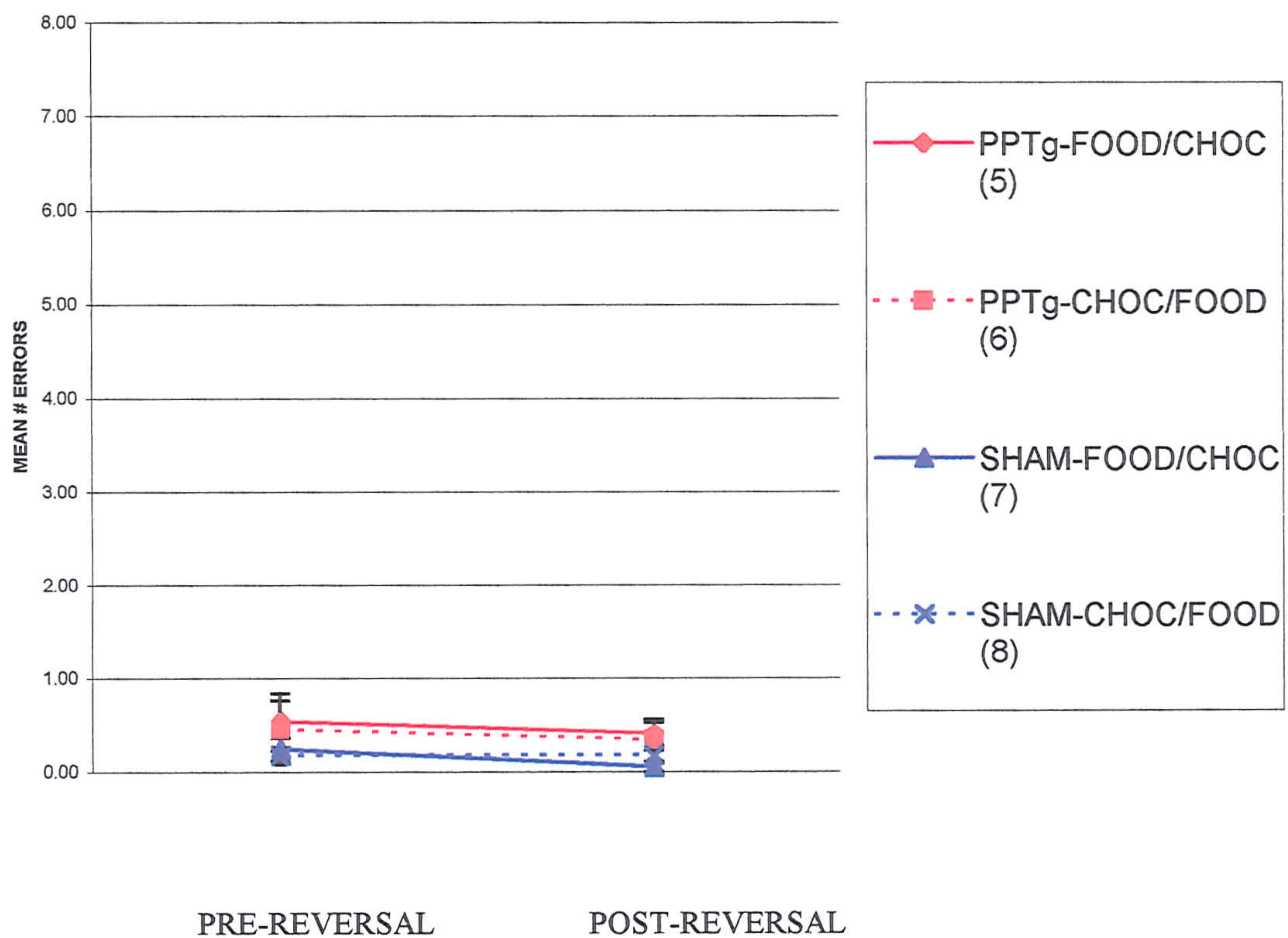
Although there was a trend in the test phase errors score indicating that chocolate had increased the number of errors made in PPTg lesioned rats in comparison to PPTg rats receiving a food reward this was not significant. Also, as in the training phase, the PPTg rats responded in the same manner as shams to increased reward value on run speed measures. The only measure on which PPTg lesioned rats did not respond similarly to shams to an increase in reward was on the WP error score. On this score the sham/PPTg comparison in the chocolate groups reveals a larger deficit than the same comparison in the food groups. However, since this is the result of a sham-choc improvement rather than a PPTg-choc decline in performance this is not evidence to support the hypothesised deterioration in PPTg level of responding in response to a preferred reward.

10.3.4 *The DSWS Task (2): Immediate Effects of Reversal of food reward*

In all graphs in this section the label “pre-reversal” refers to the mean for the last 3 days of testing prior to reversal, while the label “post-reversal” refers to the mean for the first 3 days of testing following reversal.

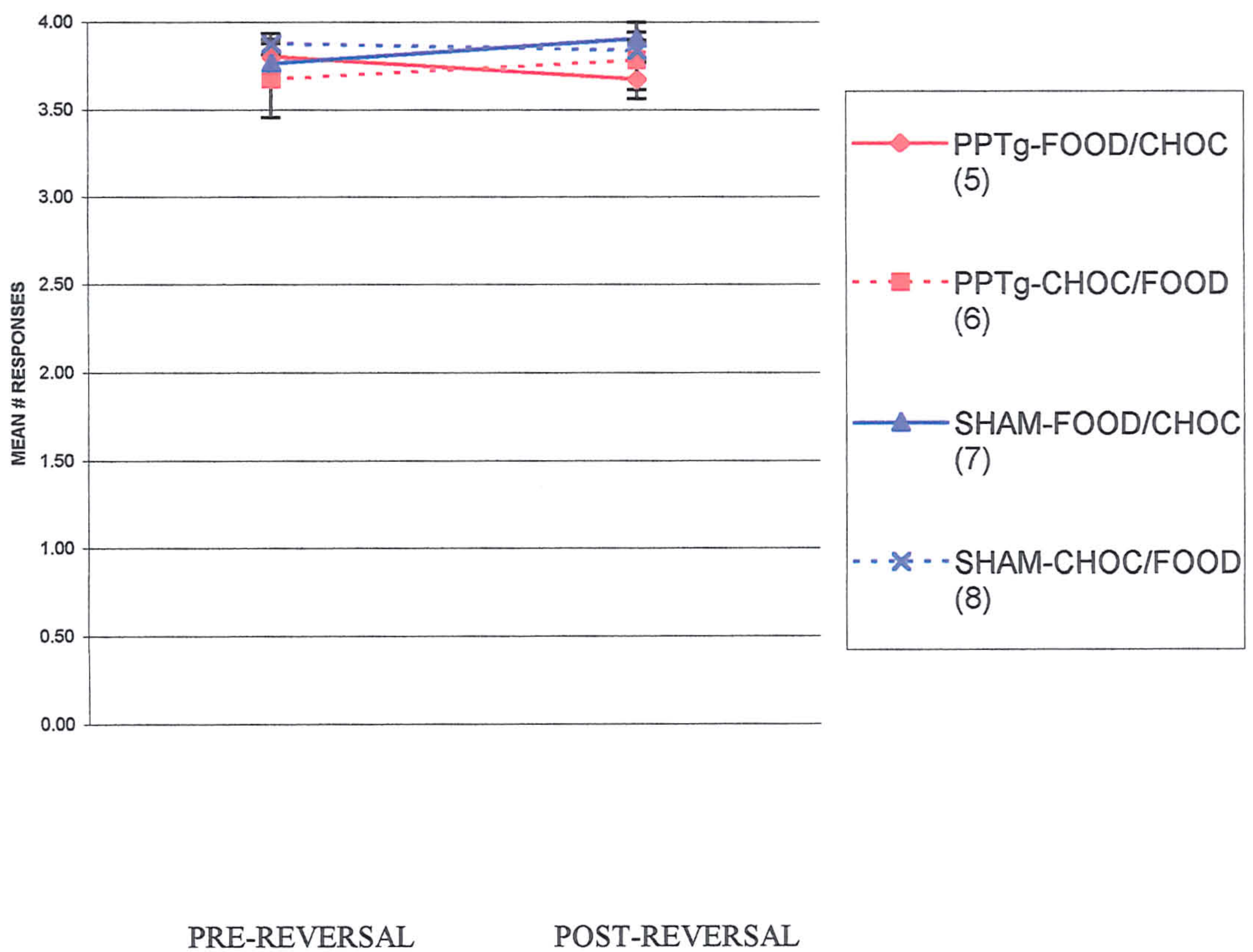
The mean and standard error for the number of errors made in the training phase for the selected pre and post reversal periods can be seen for each group in Figure 10.xiii. A 2 way mixed ANOVA conducted on these results showed no main effects of group $F(3,22) = 1.38$, or of reversal $F(1,22) = 1.06$. The group x reversal interaction was also non-significant $F(3,22) = 0.17$. There were therefore no significant immediate effects of reversing the food reward on this measure.

FIGURE 10.xiii TRAINING ERRORS



The mean and standard error for the number of correct responses made before error in this phase for the pre and post reversal periods can be seen in Figure 10.xiv. The 2 way ANOVA revealed that there were no significant effects. Group $F(3,22) = 0.37$, reversal $F(1,22) = 0.17$, and group x reversal $F(3,22) = 1.81$.

FIGURE 10.xiv TRAINING # CORRECT RESPONSES BEFORE ERROR



The mean and standard error for the training phase latencies for pre and post reversal can be seen in Figure 10.xv. The 2 way mixed ANOVA conducted on these results showed that there was a main effect of group $F(3,22) = 3.57$ $p < 0.05$ and of reversal $F(1,22) = 11.53$ $p < 0.01$. However the interaction was non-significant $F(3,22) = 1.50$ although observed power was quite low at 0.30. Bonferroni pairwise comparisons conducted on the significant main effect of group showed that this was because the PPTg-Food group differed significantly from the Sham-Choc group at $p < 0.05$. For interpreting the effects of reversal these results show that all rats improved their run speeds when the reward was reversed, independent of having a PPTg lesion, or whether they were switched to a chocolate or food reward.

FIGURE 10.xv TRAINING LATENCY (log 10)

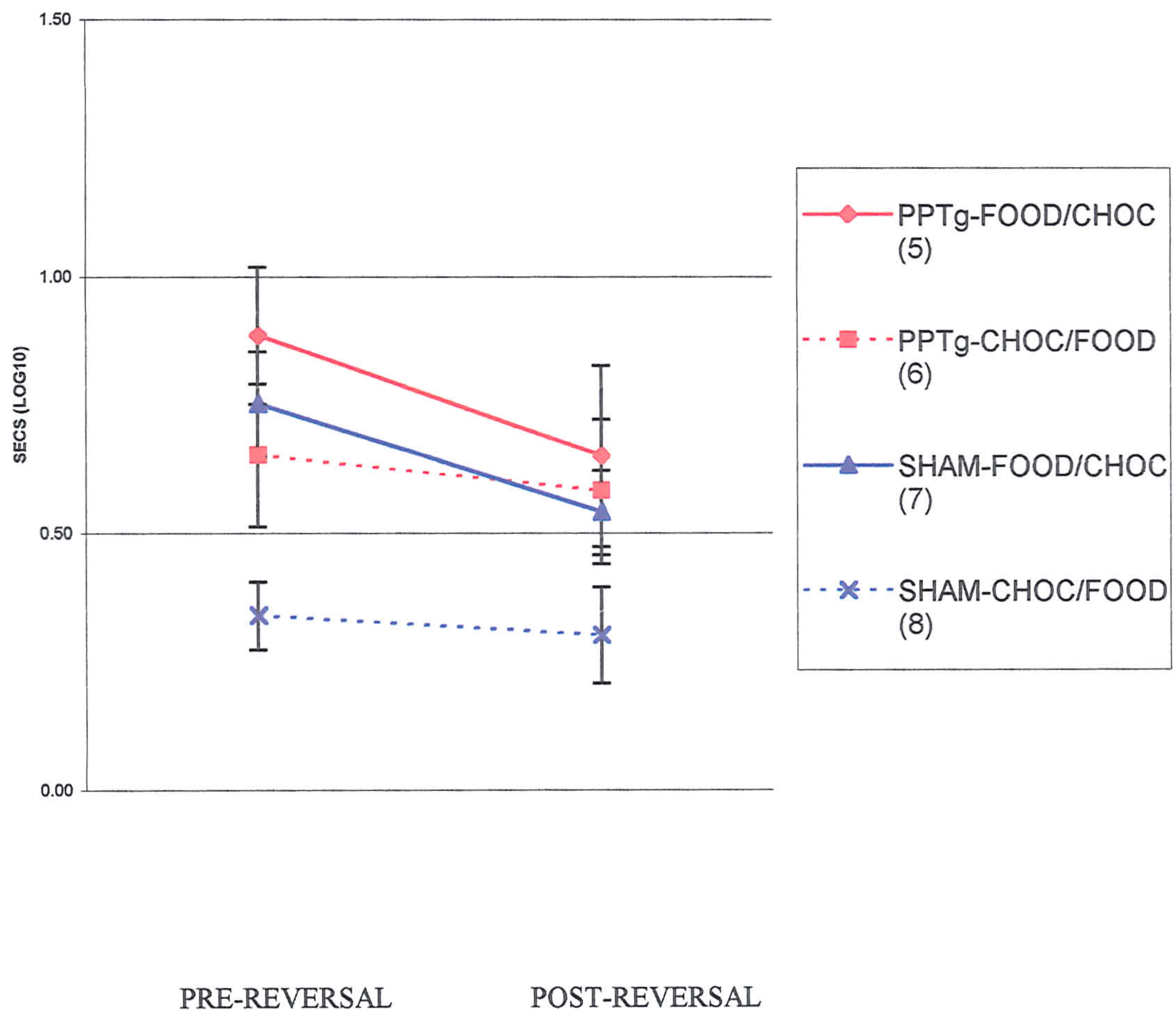
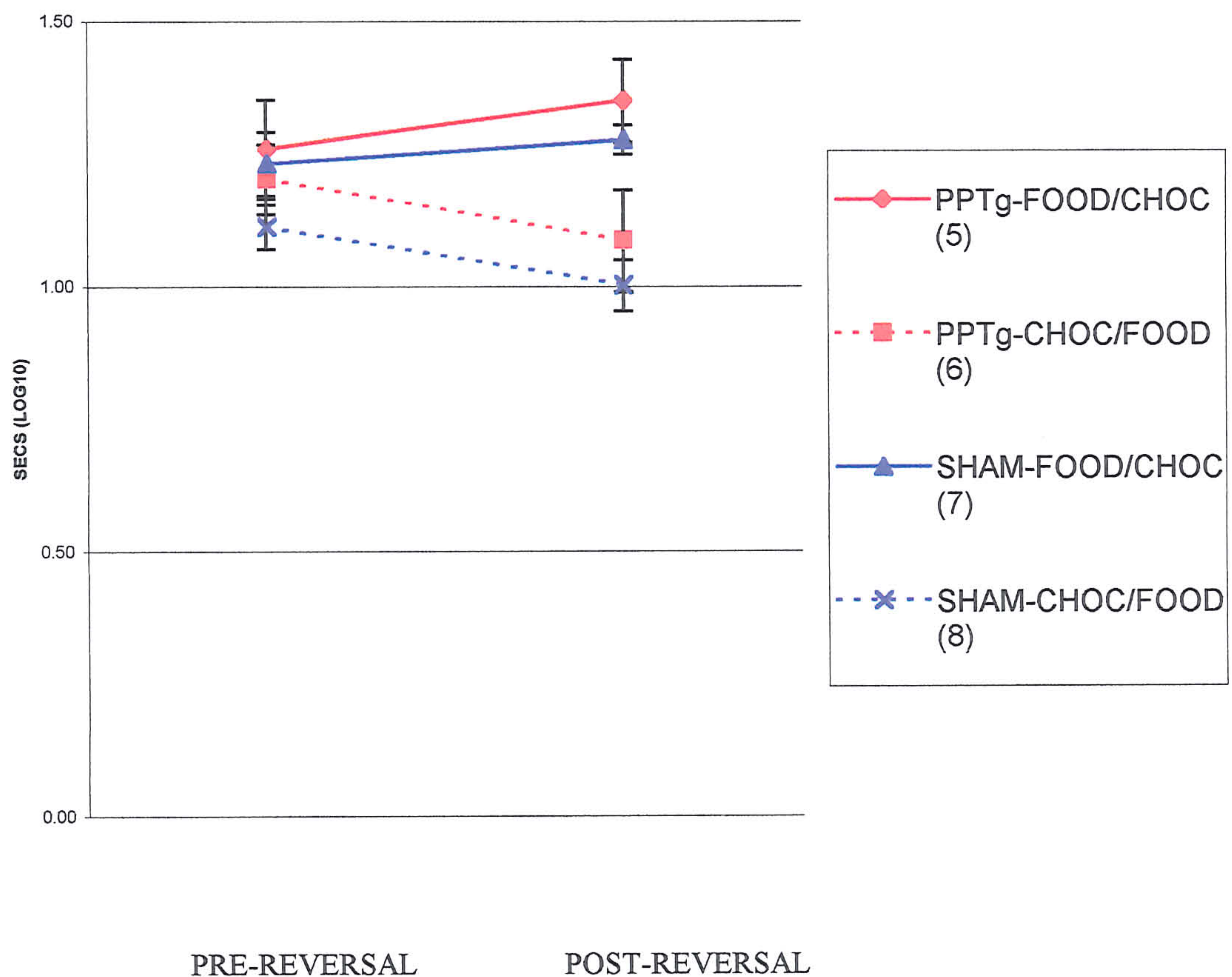


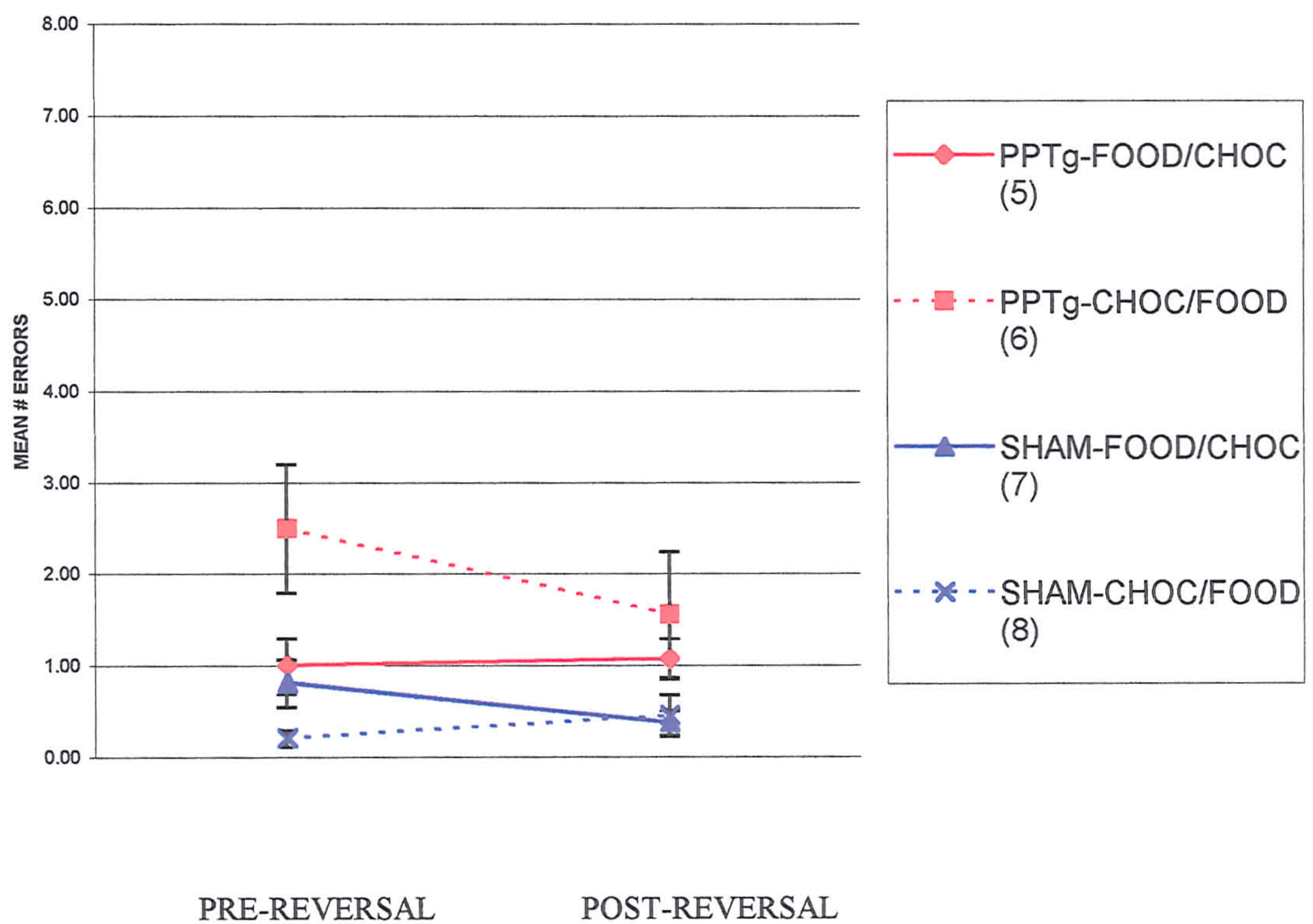
Figure 10.xvi shows the mean and standard error for the training phase choice times for pre and post reversal. A 2 way mixed ANOVA revealed a significant reversal x group interaction $F(3,22) = 5.65$ $p < 0.01$ and a significant main effect of group $F(3,22) = 3.64$ $p < 0.05$. There was not a significant main effect of reversal $F(1,22) = 1.19$. Analysis of the interaction addressing the simple main effect of reversal in each group showed that the sham choc/food group were significantly faster at making their arm choices post reversal in comparison to their pre-reversal arm choice time $t(7) = 4.04$ $p < 0.01$ two-tailed. None of the other groups showed a significant simple main effect of reversal: PPTg food/choc $t(4) = -2.51$ ns two-tailed, PPTg choc/food $t(5) = 2.10$ ns two-tailed, sham food/choc $t(6) = -0.88$ ns two-tailed.

FIGURE 10.xvi TRAINING CHOICE TIMES (log 10)



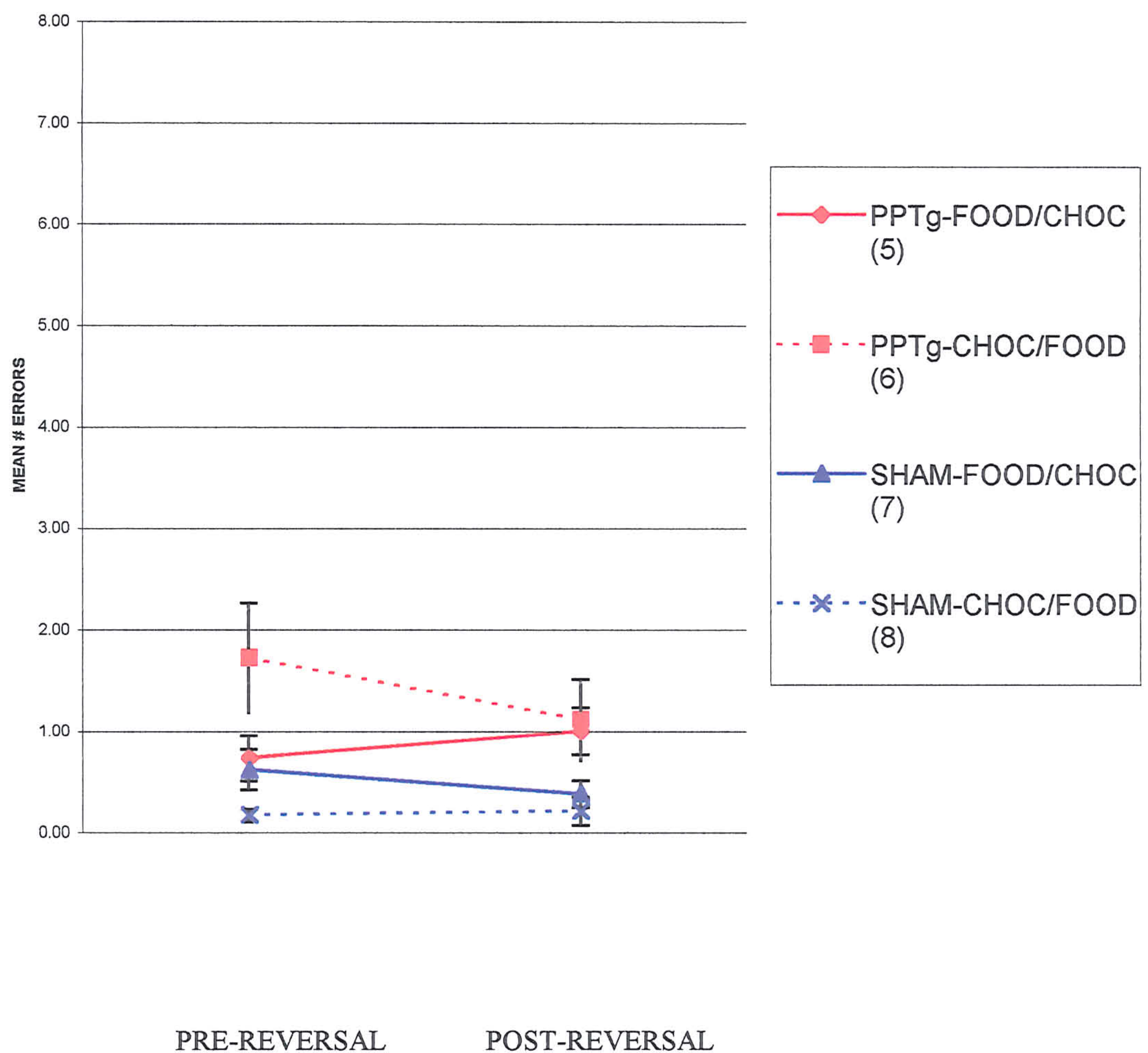
The mean and standard error for the number of test errors made over the pre and post reversal sessions for each group can be seen in Figure 10.xvii. A 2 way mixed ANOVA revealed that there was a significant interaction $F(3,22) = 4.26$ $p < 0.05$ and a main effect of group $F(3,22) = 4.72$ $p < 0.05$. The main effect of reversal was non-significant $F(1,22) = 4.02$. A further analysis conducted on the interaction addressing the simple main effect of reversal in each group showed that this occurred as a result of the PPTg-choc/food group making significantly less errors post reversal in comparison to their pre-reversal performance $t(5) = 3.40$ $p < 0.05$ two-tailed. None of the other groups displayed a simple main effect of reversal: PPTg food/choc $t(4) = -0.23$ ns two-tailed, sham food/choc $t(6) = 1.53$ ns two-tailed, and sham choc/food $t(7) = -1.22$ ns two-tailed.

FIGURE 10.xvii TEST ERRORS



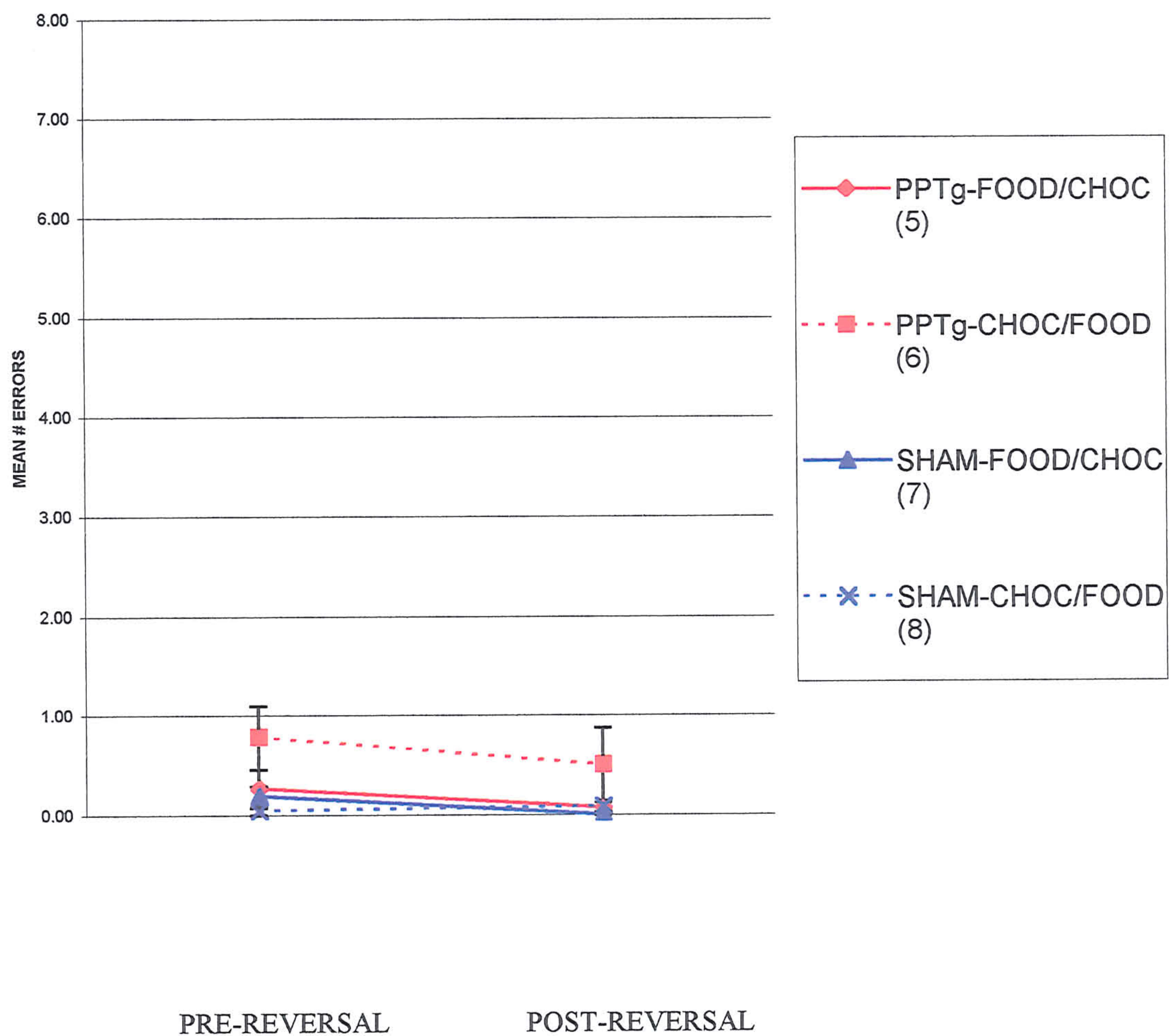
Pre and post reversal means and standard error for across phase test errors can be seen in Figure 10.xviii. The 2 way mixed ANOVA showed that there was a main effect of group $F(3,22) = 5.05$ $p < 0.01$ but not a main effect of reversal $F(1,22) = 1.65$. The reversal x group interaction was also non-significant $F(3,22) = 2.97$. Bonferroni pairwise comparisons conducted on the main effect of group revealed that only the PPTg-Choc group differed significantly from the Sham-Choc group at $p < 0.01$. These results indicate that there were no significant effects of reversing the food reward on this measure.

FIGURE 10.xviii ACROSS PHASE ERRORS



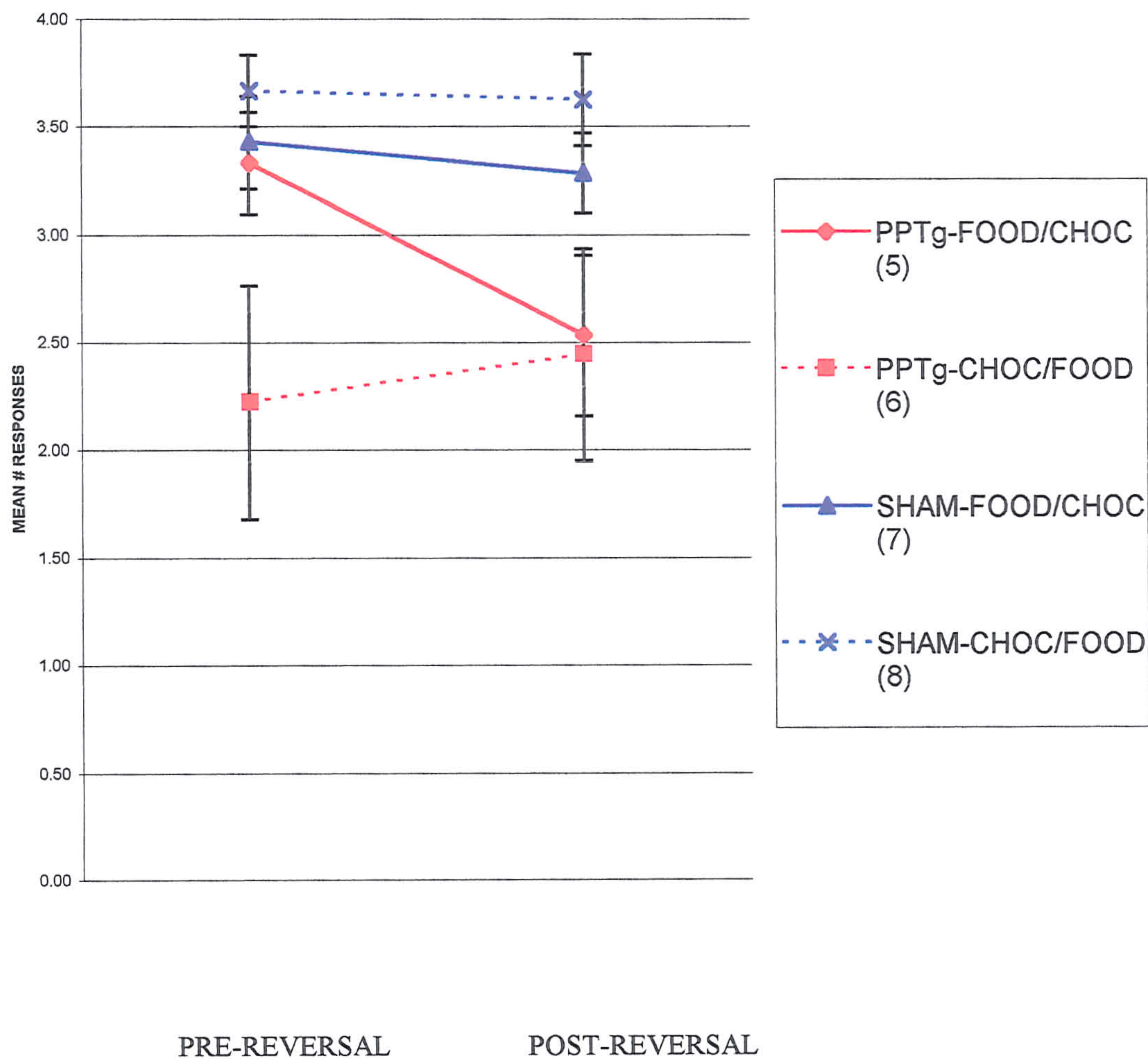
Pre and post reversal means and standard error for the within phase errors can be seen in Figure 10.xix. The ANOVA conducted on these results revealed a main effect of reversal $F(1,22) = 7.42$ $p < 0.05$ but not a main effect of group $F(3,22) = 2.58$, or a group x reversal interaction $F(3,22) = 1.65$. These results show that all groups improved their within phase error score following reversal of the reward regardless of whether they were PPTg lesioned or shams, and whether they were switched to a more or less positive reward.

FIGURE 10.xix WITHIN PHASE ERRORS



The mean and standard error for the number of correct responses prior to error occurrence in the test phase for the defined pre and post reversal sessions can be seen in Figure 10.xx. A 2 way mixed ANOVA showed a main effect of group $F(3,22) = 4.30$ $p < 0.05$ but no main effect of reversal $F(1,22) = 1.93$, or significant reversal x group interaction $F(3,22) = 2.10$. Although the graph suggests a possible significant interaction for this measure the observed power was low at 0.50. Bonferroni pairwise comparisons conducted on the main effect of group showed that only the PPTg-Choc group differed from the Sham-Choc group at $p < 0.05$. Overall these results show that reversing the food reward had no immediate effects on this measure.

FIGURE 10.xx TEST # CORRECT RESPONSES BEFORE ERROR



The mean and standard error for the test phase latencies are shown in Figure 10.xxi for the pre and post reversal periods. The ANOVA conducted on these results showed that there were no main effects of group $F(3,22) = 2.09$, or reversal $F(1,22) = 0.07$, and that the group x reward interaction was not significant $F(3,22) = 0.19$. Reversing the food reward had no immediate effects on this measure.

FIGURE 10.xx TEST LATENCY (log 10)

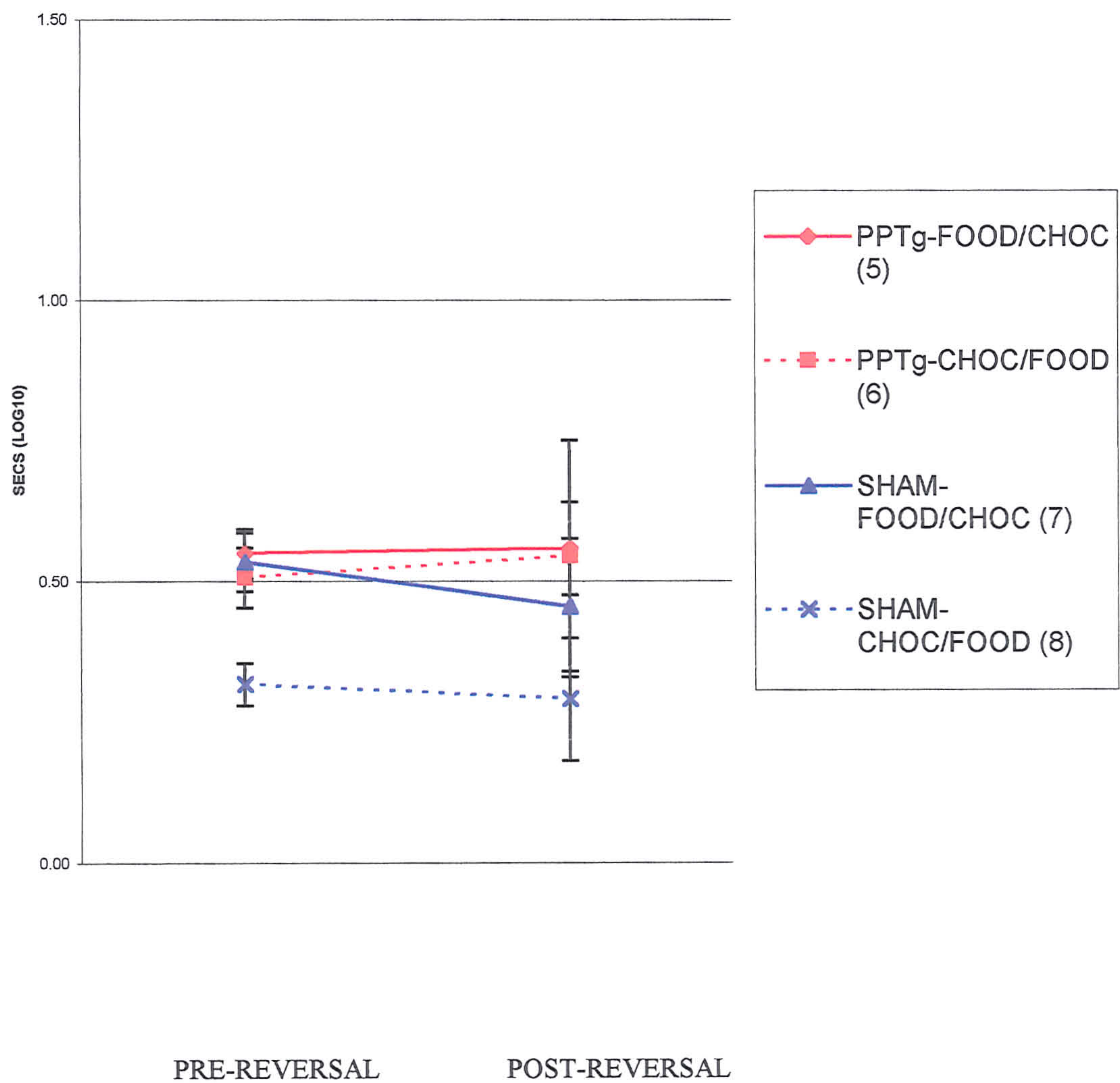
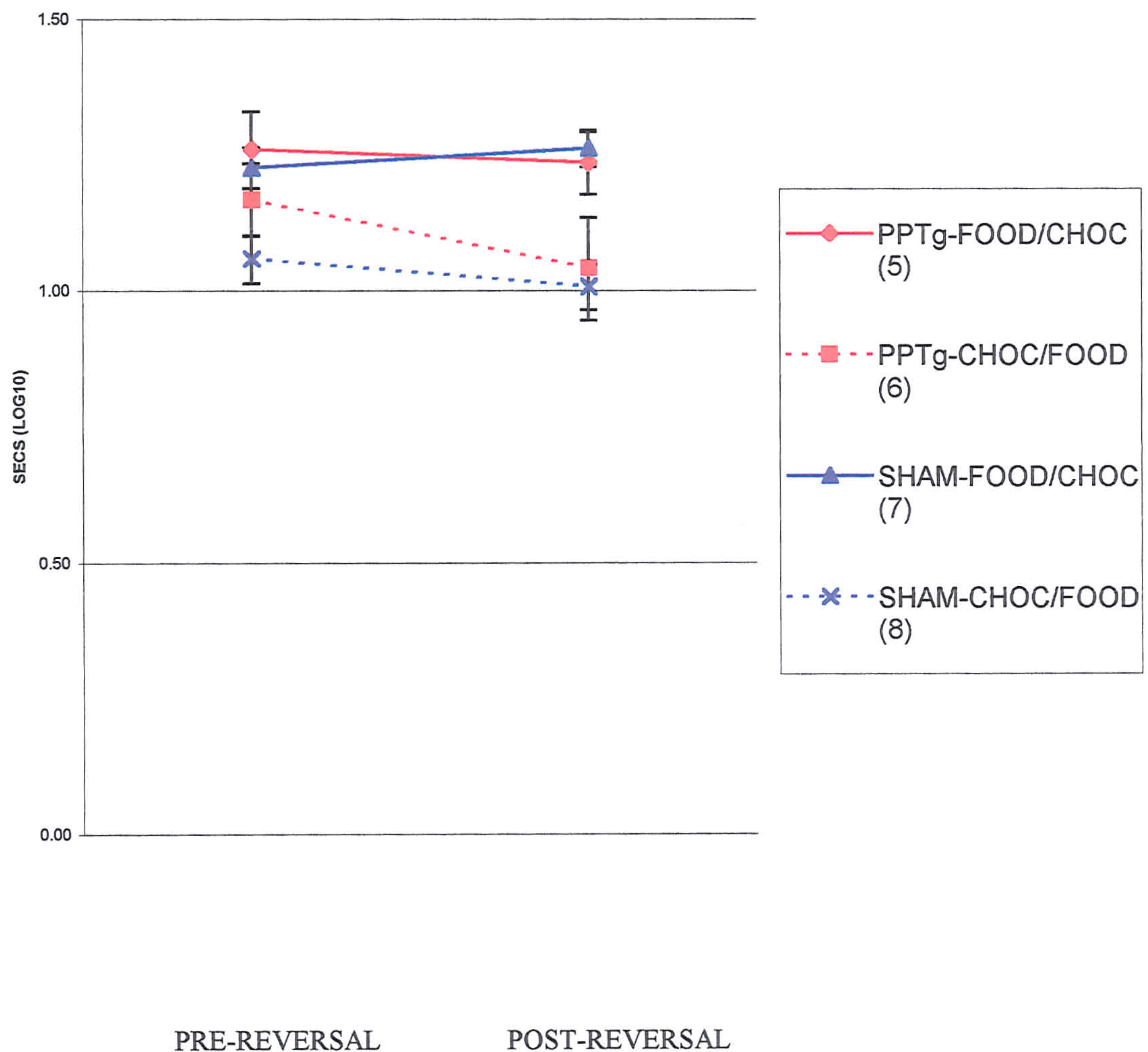


Figure 10.xxii shows the mean and standard error for the test phase choice times prior to and post reward reversal. A 2 way mixed ANOVA conducted on the results showed that there was a main effect of group $F(3,22) = 4.51$ $p < 0.05$, but not a main effect of reversal $F(1,22) = 3.19$. The reversal x group interaction was also non significant $F(3,22) = 2.05$. Bonferroni pairwise comparisons conducted on the significant main effect of group showed that the Sham-Choc group differed significantly from the Sham-Food group at $p < 0.05$. None of the other groups differed from each other. As for latency in this phase, reversing the food reward had no immediate effects on this measure.

FIGURE 10.xxii TEST CHOICE TIMES (log 10)



10.3.5 Summary (2):

The results for the analyses addressing the immediate effects of reversing the food reward are summarised in Table 10b.

Since this section was concerned with analysing the immediate effects of reversing the food reward the focus was restricted either to main effects of reversal or reversal x group interactions. These findings are summarised below.

The immediate effects of reversing the food reward occurred for all groups in the training phase for the latency measure. That is, changing the reward available on the radial maze (whether to a more or less preferred reward) had the effect of increasing the speed at which all rats began the task by making their first arm choice. However, when all of the arm choice times are taken into account in the training phase the effect of reversing the food reward occurred only in the sham rats who went from a more preferred to a less preferred reward (they slowed down significantly). Thus PPTg lesioned rats did not respond in the same way as shams to a decrease in reward value on this measure.

The immediate effect of reversing the food reward in the test phase occurred for all groups on the number of within phase errors, significantly reducing the number of these being made whether the reversal occurred to a more or less positive reward. For overall test phase errors switching to a food reward from a chocolate reward significantly improved the performance of the PPTg lesioned rats. This is indirect evidence that the presence of a preferred reward has a detrimental effect on PPTg lesioned animals since

they significantly immediately improved their performance on this measure when the reward value was decreased.

Table 10b. Summary of Immediate Effects of Reversal Analyses

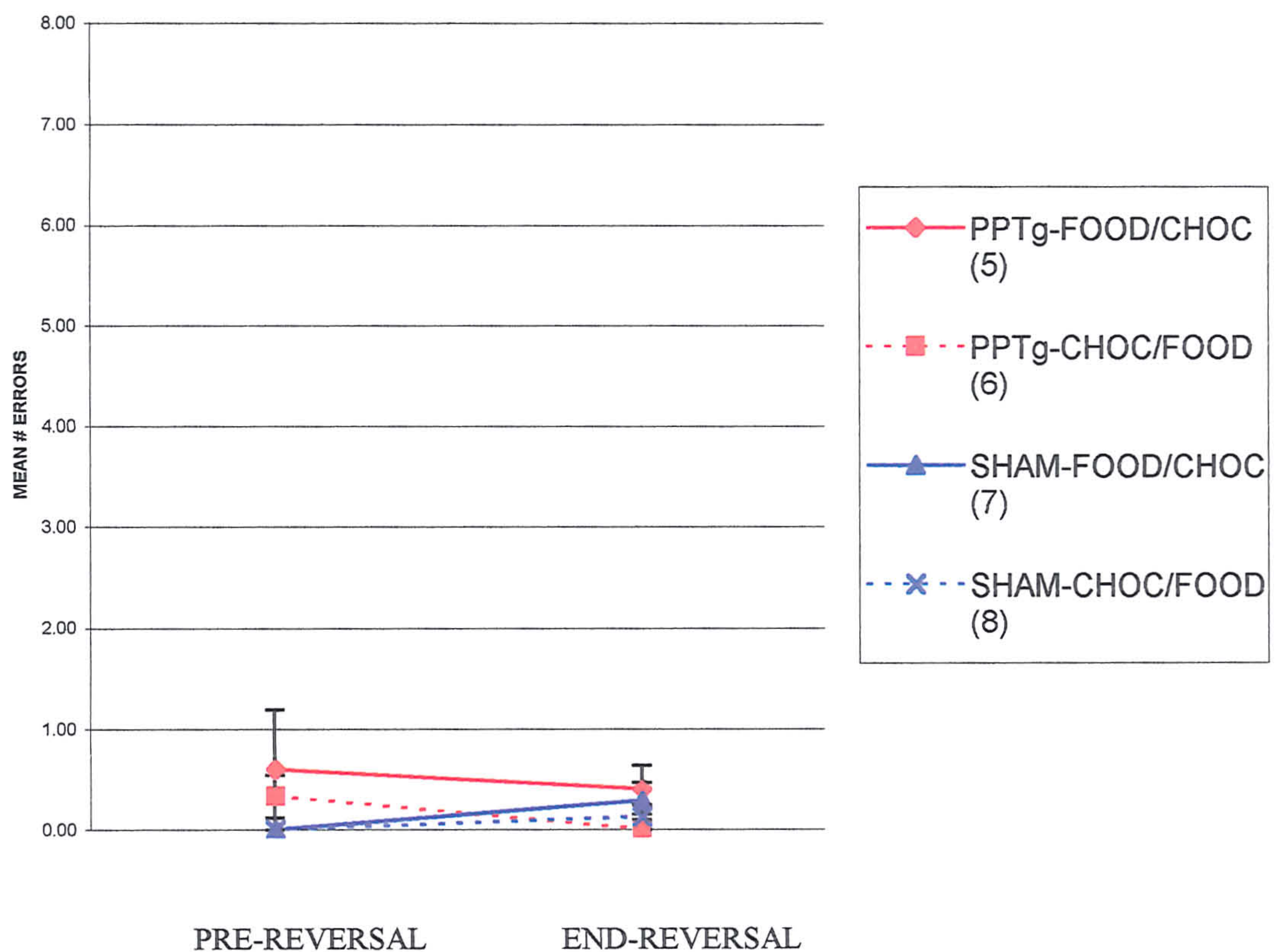
	Group	Reversal	Group x Reversal
Training Errors	ns	ns	ns
Training Correct	ns	ns	ns
Training Latency	p<0.05 PPTg-F/C slower than Sham-C/F	p<0.01 Post rev all faster	ns
Training Choice Times	p<0.05 PPTg slower	ns	p<0.01 Sham-C/F faster post rev
Test Errors	p<0.05 PPTg > Sham	ns	p<0.05 PPTg-C/F less post rev
AP	p<0.01 PPTg-C/F > Sham-C/F	ns	ns
WP	Ns	p<0.05 All less post	ns
Test Correct	p<0.05 PPTg-C/F < Sham-C/F	ns	ns
Test Latency	Ns	ns	ns
Test Choice Times	p<0.05 Sham-C/F faster than Sham-F/C	ns	ns

10.3.6 *The DSWS Task (3): Long Term Effects of Reversing the Food Reward*

In all graphs in this section the label “pre-reversal” refers to performance on the last day of testing prior to reversal. The label “end-reversal” refers to the score of performance on the last day of the post reversal session.

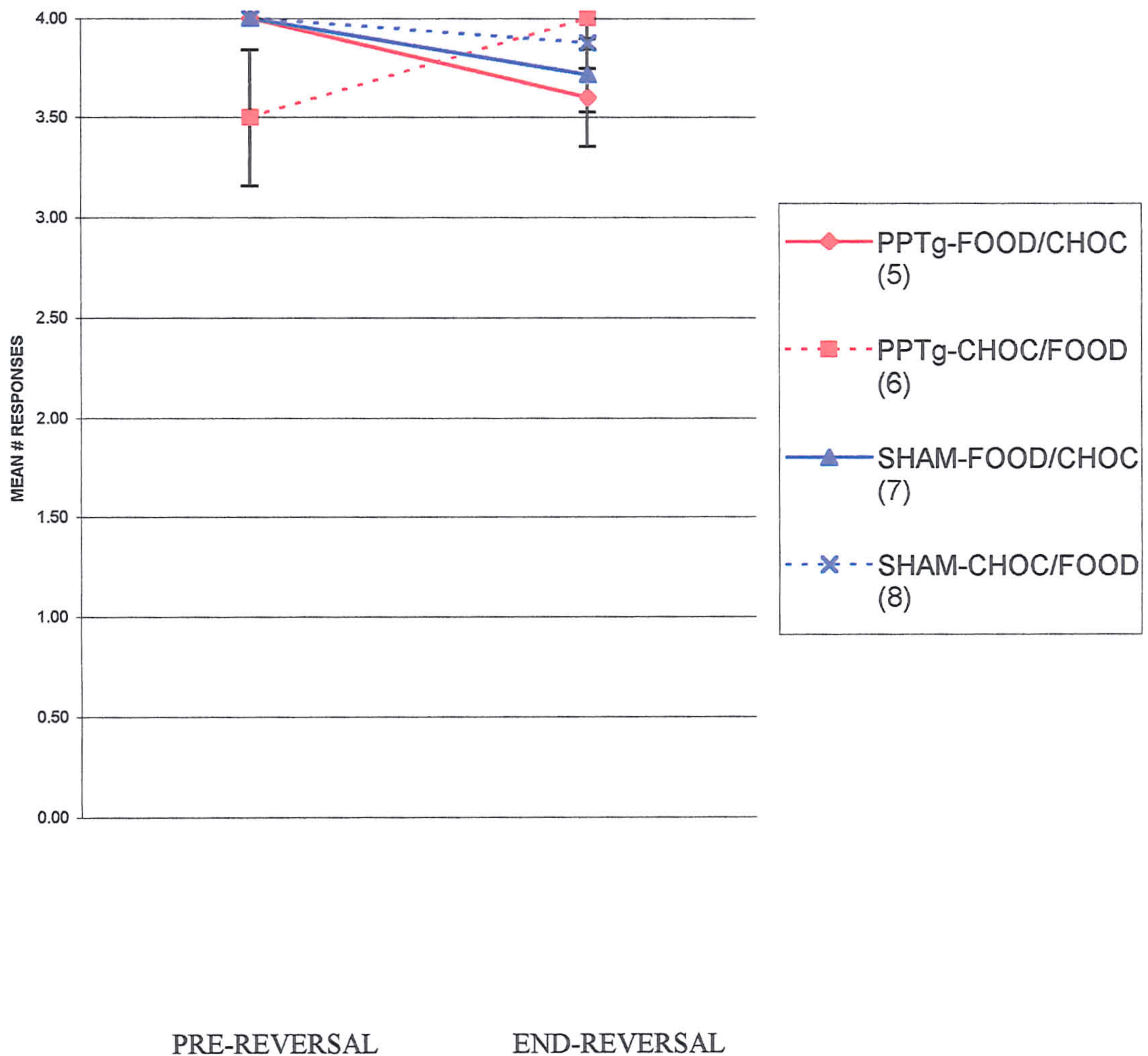
The mean and standard error for the number of errors made in the training phase on the last day of the pre-reversal session and the last day of the post-reversal session can be seen in Figure 10.xxiii. A 2 way mixed ANOVA conducted on these results revealed no main effects of reversal $F(1,22) = 0.06$, or of group $F(3,22) = 1.18$. The reversal x group interaction was also non-significant $F(3,22) = 1.36$. These results show that there were no long-term effects of reversing the food reward on this measure.

FIGURE 10.xxiii TRAINING ERRORS



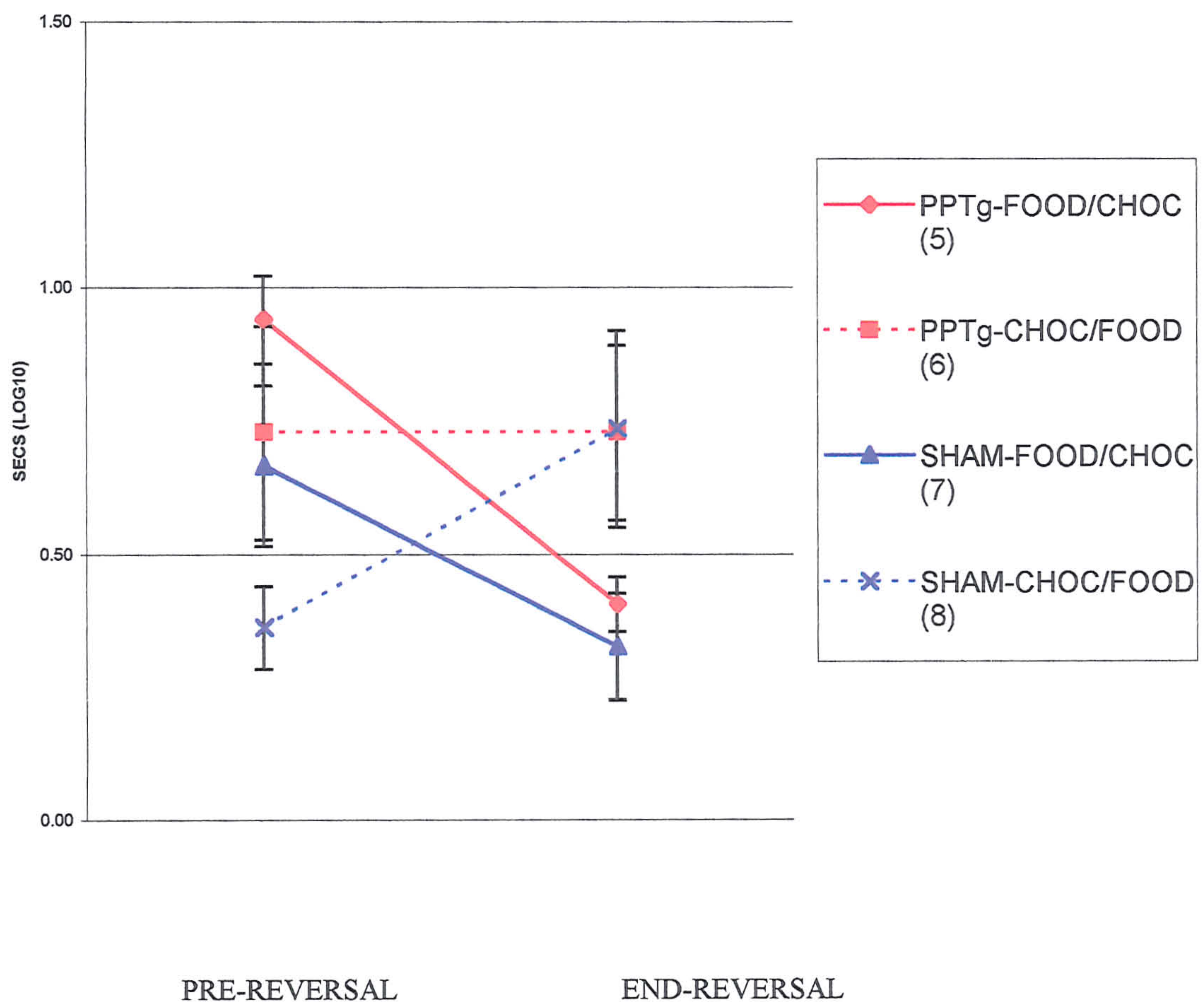
The mean and standard error for the number of correct responses before error in the training phase on the last day of the pre-reversal session and the last day of the post reversal session can be seen in Figure 10.xxiv. A 2 way ANOVA revealed that there were no significant main effects: reversal $F(1,22) = 0.48$, and group $F(3,22) = 0.56$. The interaction of group x reversal was also non significant $F(3,22) = 2.98$. Again there were no long-term effects of reversing the food reward on this measure.

FIGURE 10.xxiv TRAINING # CORRECT RESPONSES BEFORE ERROR



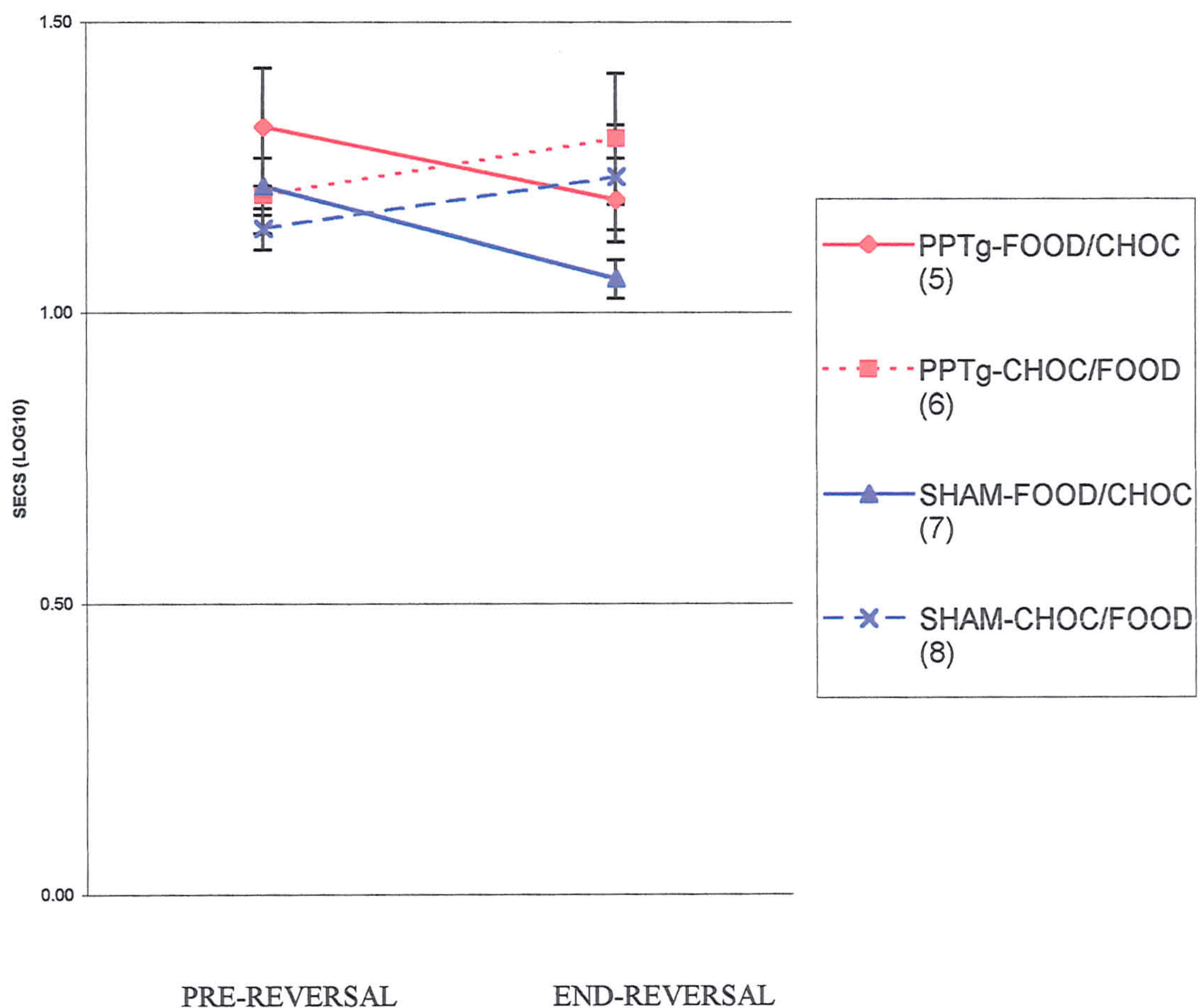
The mean and standard error for the training phase latency to the first arm on the last day of the pre-reversal session and the last day of the post-reversal session can be seen in Figure 10.xxv. A 2 way mixed ANOVA conducted on these results showed a significant reversal x group interaction $F(3,22) = 5.98$ $p < 0.01$. There were no significant main effects: reversal $F(1,22) = 2.20$ and group $F(3,22) = 0.82$. Analysis on the interaction addressing the simple main effect of reversal at each level of group using paired t-tests revealed that PPTg-food/choc group had a significantly faster latency post reversal in comparison to their pre-reversal speed $t(4) = 4.01$ $p < 0.05$ two-tailed. None of the other groups showed a significant main effect of reversal: PPTg choc/food $t(5) = 0.00$ two-tailed, sham food/choc $t(6) = 1.95$ two-tailed, and sham choc/food $t(7) = -2.12$ two-tailed.

FIGURE 10.xxv TRAINING LATENCY (log 10)



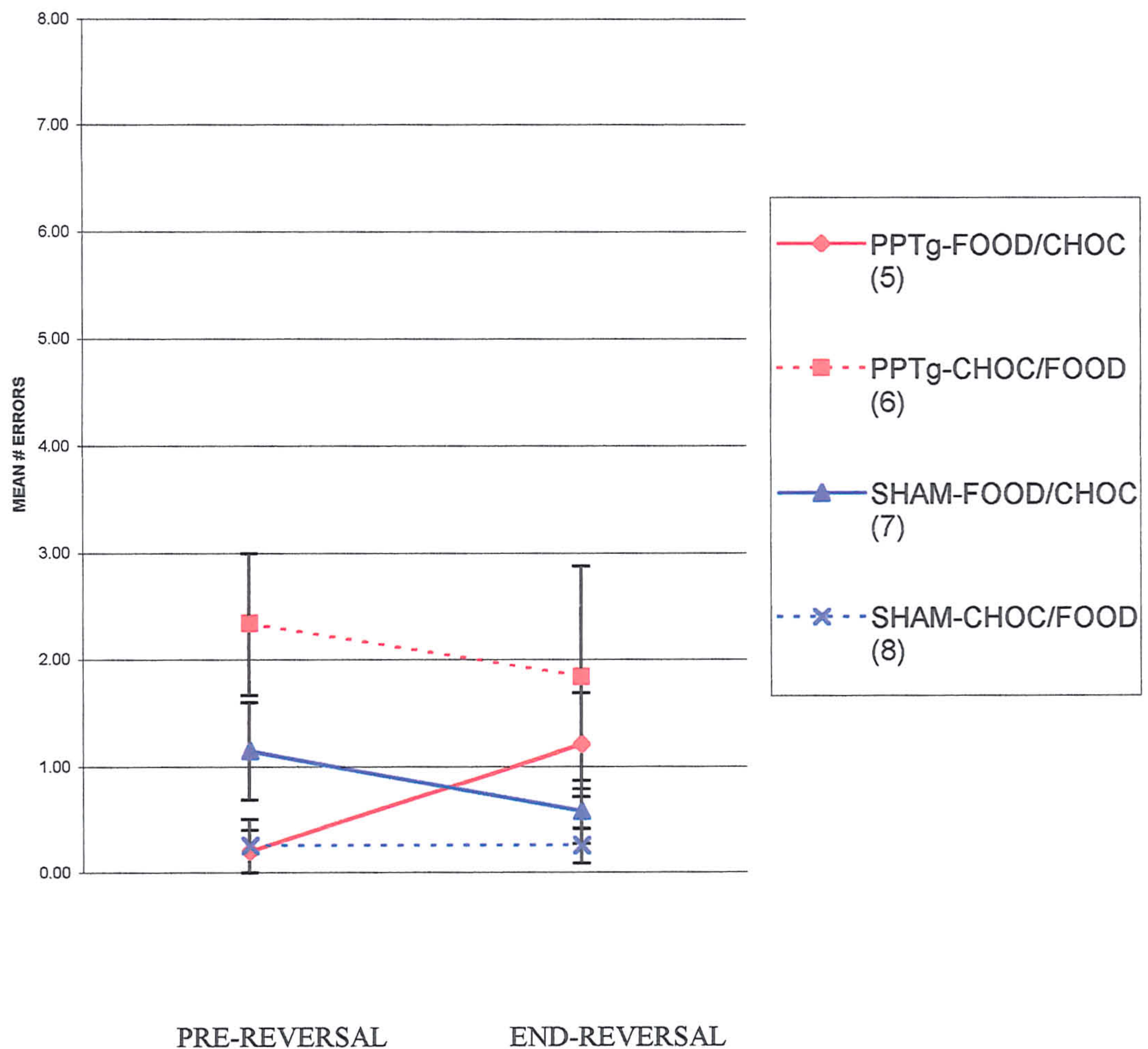
The mean and standard error for the training phase arm choice times on the last day of the pre-reversal session and the last day of the post-reversal session can be seen in Figure 10.xxvi. A 2 way ANOVA revealed no significant main effects: group $F(3,22) = 0.85$, and reversal $F(1,22) = 0.36$. However the reversal x group interaction was significant $F(3,22) = 4.19$ $p < 0.05$. Analysis of the interaction using paired t-tests to address the simple main effect of reversal for each group revealed that both the sham and the PPTg groups reversing from food to chocolate were significantly faster at making their arm choice times in the post reversal session compared to the pre-reversal session. For PPTg food/choc $t(4) = 3.11$ $p < 0.05$ two-tailed, for PPTg choc/food $t(5) = -1.53$ ns two-tailed, for sham food/choc $t(6) = 3.89$ $p < 0.01$, and for sham choc/food $t(7) = -1.08$ ns.

FIGURE 10.xxvi TRAINING CHOICE TIMES (log 10)



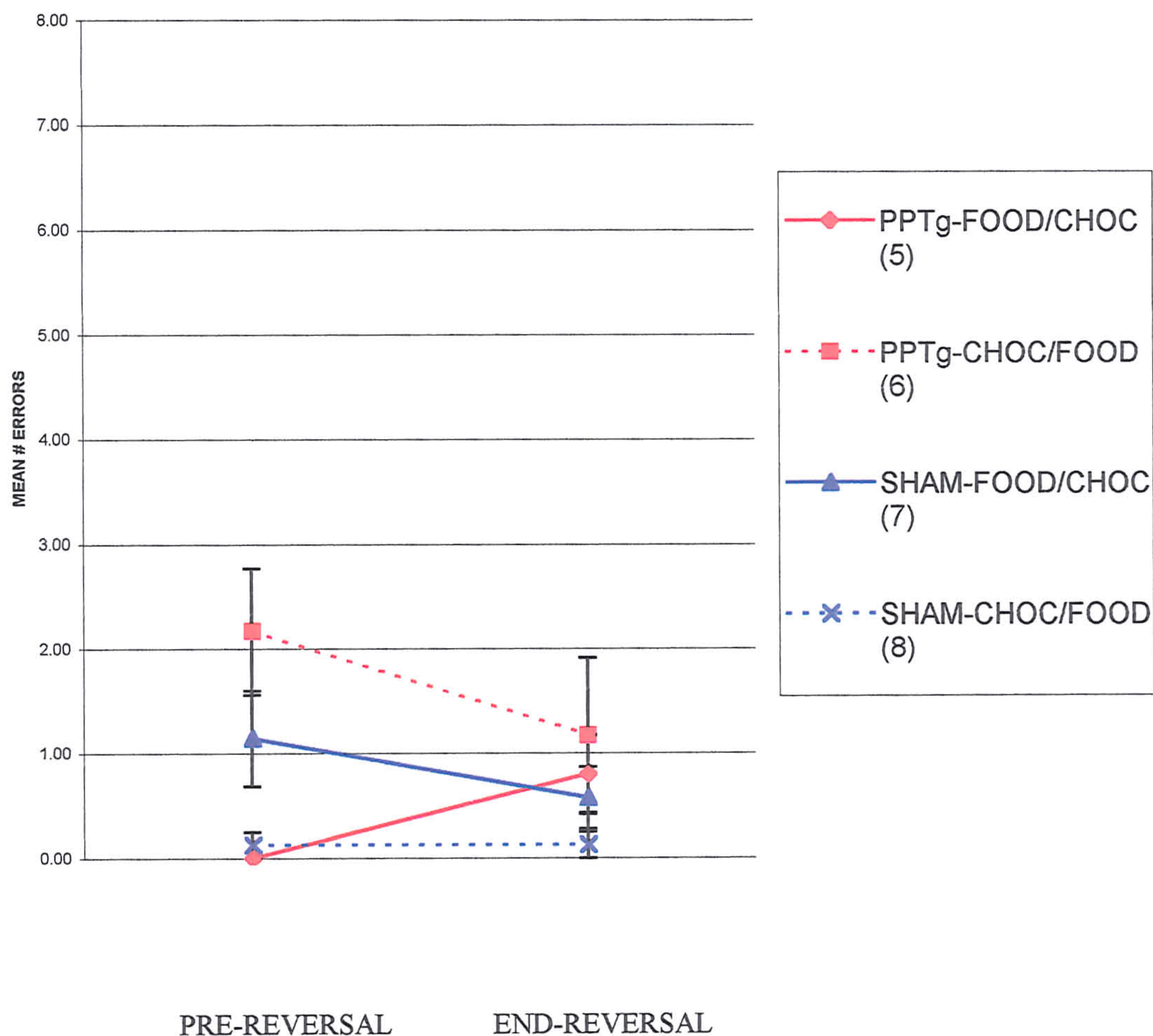
The mean and standard error for the number of test errors made on the last day of the pre-reversal session and the last day of the post-reversal session can be seen in Figure 10.xxvii. A 2 way mixed ANOVA revealed a significant main effect of group $F(3,22) = 5.06$ $p < 0.01$. The main effect of reversal was not significant $F(1,22) = 0.003$, and neither was the reversal x group interaction $F(3,22) = 0.92$. Bonferroni pairwise comparisons conducted on the main effect of group showed that the PPTg-Choc group were significantly different from the Sham-Choc group at $p < 0.01$. None of the other groups differed significantly from each other. These results show that there were no significant long-term effects of reversing the reward on this measure.

FIGURE 10.xxvii TEST ERRORS



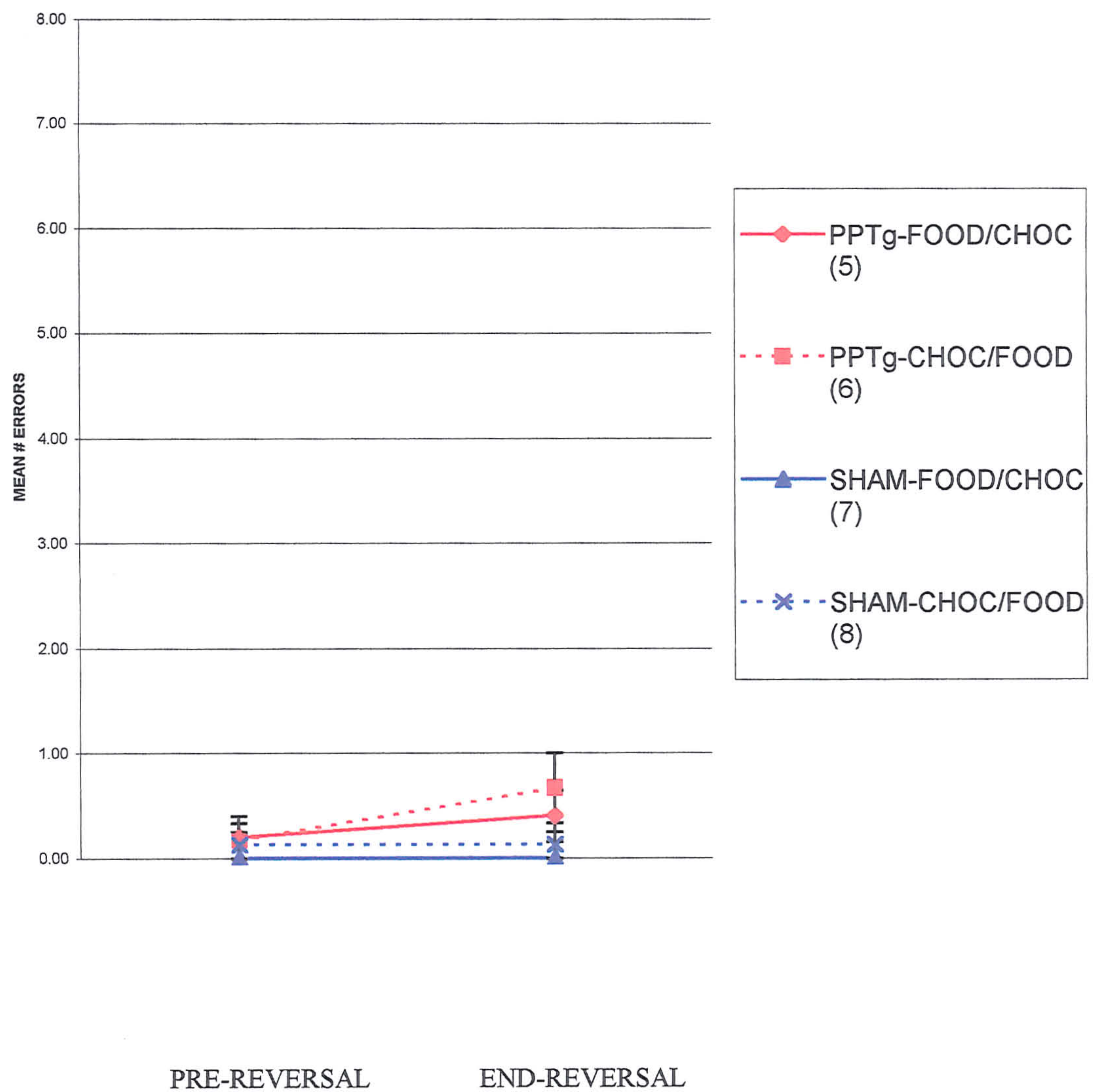
The mean and standard error for the across phase error data for the last day of the pre-reversal session and the last day of the post-reversal session can be seen in Figure 10.xxviii. The 2 way ANOVA conducted on these results showed a significant main effect of group $F(3,22) = 6.32$ $p < 0.01$ but not a significant main effect of reversal $F(1,22) = 1.53$. The reversal x group interaction was also non significant $F(3,22) = 1.53$. Bonferroni pairwise comparisons conducted on the main effect of group showed that the PPTg-Choc group were significantly different from the PPTg-Food group at $p < 0.05$ and from the Sham-Choc group at $p < 0.01$. None of the other groups differed significantly from each other. These results indicate that there were no long-term effects of reversing the food reward on this measure.

FIGURE 10.xxviii ACROSS PHASE ERRORS



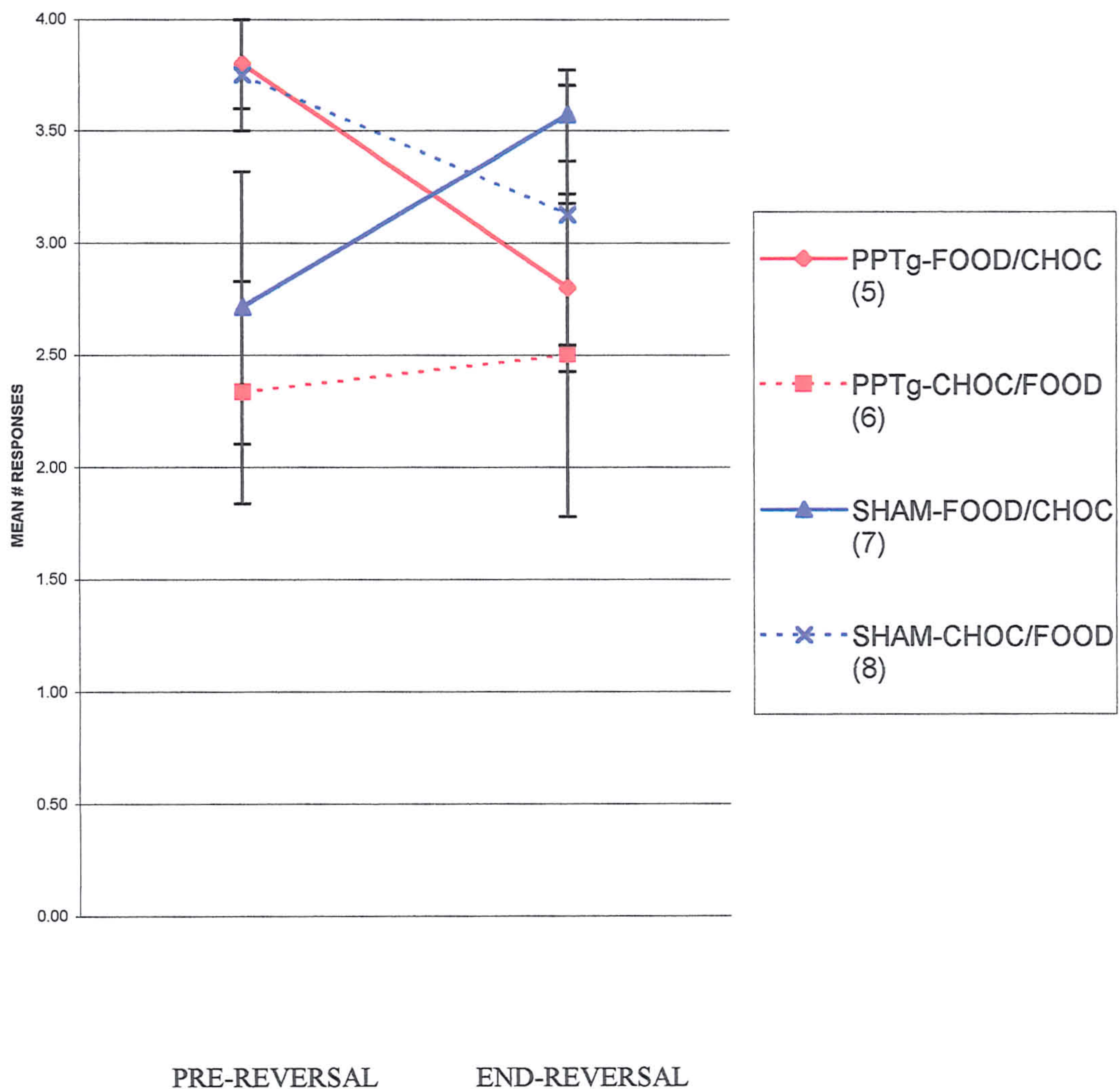
The mean and standard error for the data for within phase errors can be seen in Figure 10.xxix. A 2 way mixed ANOVA revealed that there were no significant main effects of group $F(3,22) = 2.55$, or reversal $F(1,22) = 2.05$. The reversal x group interaction was also non significant $F(3,22) = 0.96$. Again these results show that there were no long-term effects of reversing the food reward on this measure.

FIGURE 10.xxix WITHIN PHASE ERRORS



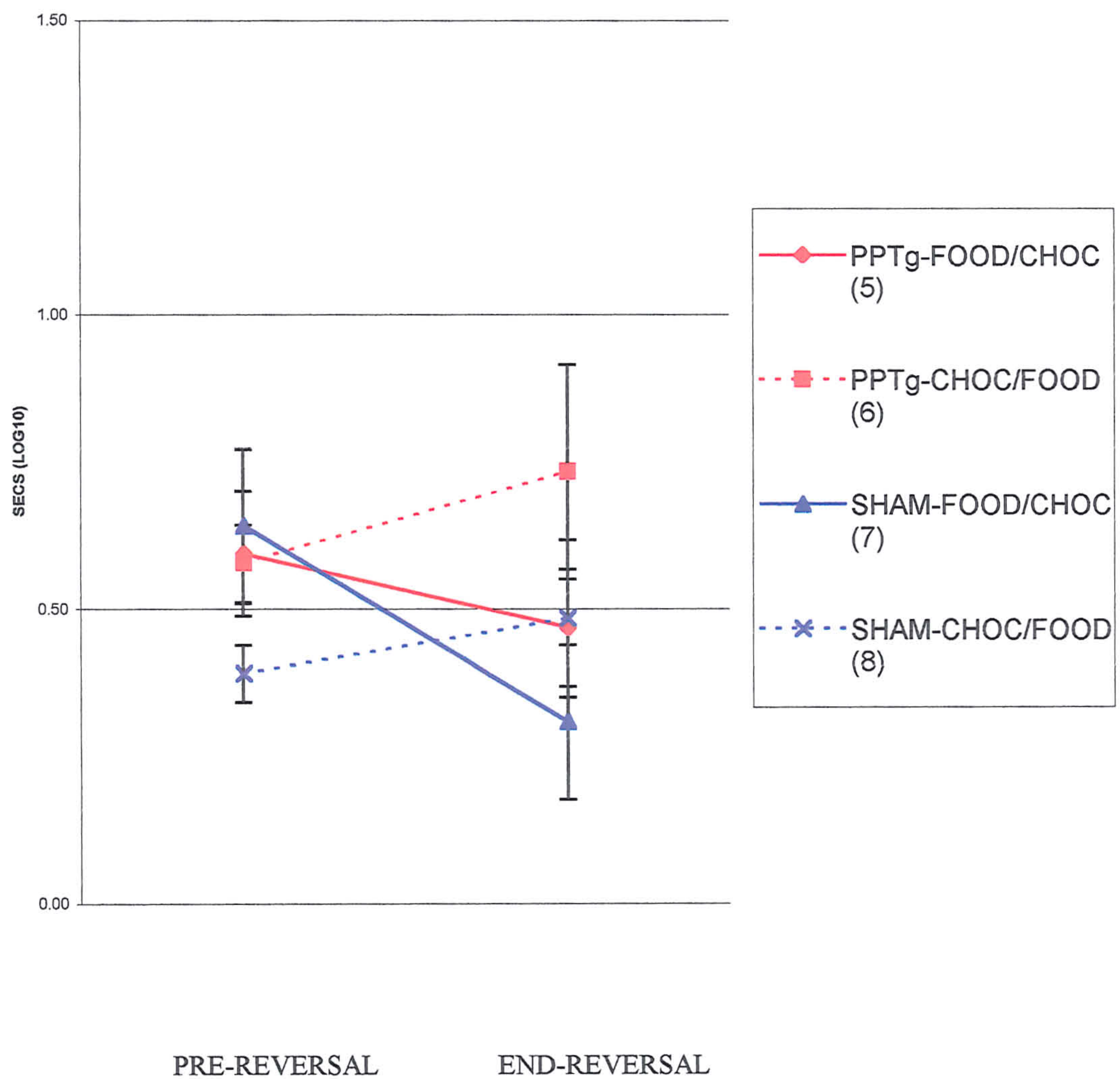
The mean and standard error for the number of correct responses before error in the test phase on the last day of the pre-reversal session and the last day of the post-reversal session can be seen in Figure 10.xxx. A 2 way mixed ANOVA showed that there were no significant main effects of group $F(3,22) = 1.28$, or of reversal $F(1,22) = 0.30$. The reversal x group interaction was also non significant $F(3,22) = 2.31$. There were no long-term effects of reversing the food reward on this measure.

FIGURE 10.xxx TEST # CORRECT RESPONSES BEFORE ERROR



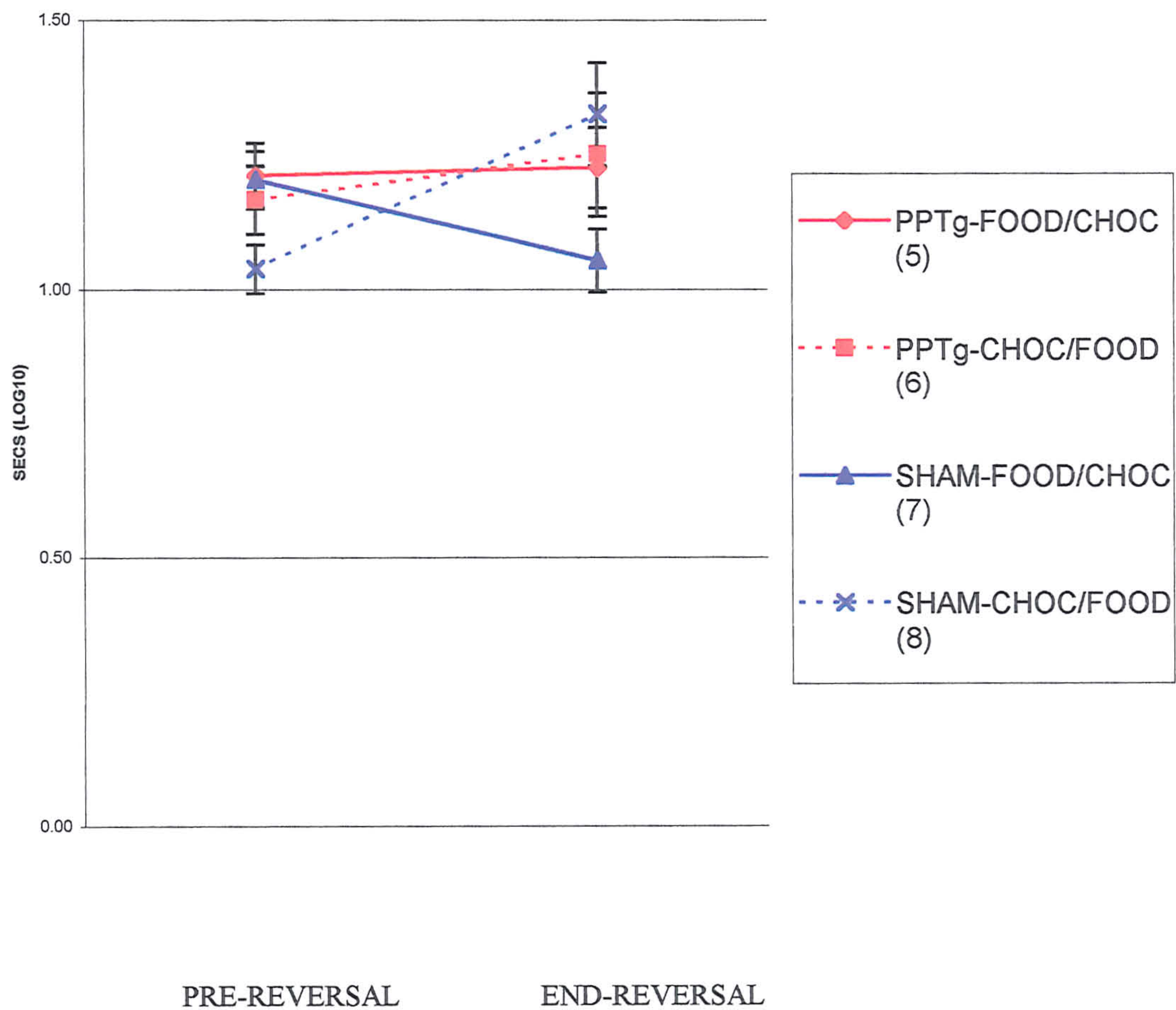
The mean and standard error for the test phase latencies for the last day of the pre-reversal session and the last day of the post-reversal session can be compared in Figure 10.xxxi. The ANOVA conducted on these results showed that there were no significant main effects: group $F(3,22) = 0.97$ and reversal $F(1,22) = 0.51$. The interaction between reversal and group was also non significant $F(3,22) = 2.63$. There were no long-term effects of reversing the food reward on this measure either.

FIGURE 10.xxxi TEST LATENCY (log 10)



The means and standard error for the test phase choice times data can be compared in Figure 10.xxxii for the last day of the pre-reversal session and the last day of the post-reversal session. The 2 way mixed ANOVA conducted on these results showed a significant group x reversal interaction $F(3,22) = 7.50$ $p < 0.01$. There was not a main effect of group $F(3,22) = 0.37$, or of reversal $F(1,22) = 2.58$. Analysis of the interaction addressing the simple main effect of reversal in each group revealed that the sham choc/food group were significantly slower at making their arm choices post reversal in comparison to pre-reversal $t(7) = -4.32$ $p < 0.01$ two-tailed. None of the other groups showed a simple main effect of reversal: PPTg food/choc $t(4) = -0.17$ two-tailed, PPTg choc/food $t(5) = -1.30$ two-tailed, and sham food/choc $t(6) = 1.99$ two-tailed.

FIGURE 10.xxxii TEST CHOICE TIMES (log 10)



10.3.7 Summary (3):

Table 10c presents the results for this section in summary form

Table 10c. Summary of Findings for Long Term Reversal Analyses

	Group	Reversal	Group x Reversal
Training Errors	ns	ns	ns
Training Correct	ns	ns	ns
Training Latency	ns	ns	p<0.01 PPTg-F/C faster post
Training Choice Times	ns	ns	p<0.05 Both F/C groups Faster post
Test Errors	p<0.01 PPTg-C/F > Sham-C/F	ns	ns
AP	p<0.01 PPTg-C/F > F/C & Sham-C/F	ns	ns
WP	ns	ns	ns
Test Correct	ns	ns	ns
Test Latency	ns	ns	ns
Test Choice Times	ns	ns	p<0.01 Sham – C/F slower post

Again as this section was concerned only with the long-term effects of reversal of food reward this summary relates specifically to main effects of reversal or reversal x group interactions.

The long-term effects of reversing the food reward occurred in the training phase only on the speed measures for the PPTg lesioned rats switching from a food reward to a chocolate reward. These rats significantly improved both the time taken to make their first arm choice and the mean time taken to make all their arm choices after the reversal.

The long-term effects of reversing the reward in the test phase occurred only for sham rats on the mean arm choice time measure if they were reversed from a more preferred to a less preferred reward – they significantly slowed down their choice times post reversal.

These results show that only the PPTg-food/choc group upheld the increase in run speed to the first arm shown by all rats immediately after reversal. Interestingly, sham rats reversed from food to chocolate did not show this pattern in the long term. As expected sham rats decreased their mean arm choice time when reversed from a more preferred to a less preferred reward. PPTg lesioned rats did not parallel this change.

10.4 Discussion

10.4.1 Where did PPTg lesion impairments occur?

Lasting PPTg lesion impairments occurred in this experiment only in the test phase of the DSWS task. This finding is more in line with the results of Keating & Winn (2001) in contrast to those of the preceding experiment. This suggests that the analyses of the previous experiment examining the PPTg DSWS deficit may not have been entirely supportive of Keating & Winn (2001) due to the extensive invasion of the lesion beyond the borders of the PPTg. In the current experiment the PPTg lesion impairment was shown in all test phase accuracy measures including the number of correct responses before error. This finding therefore extends the work of Keating & Winn (2001) by illustrating that, not only do PPTg lesioned rats make more errors in the test phase, but also they make these errors much earlier in their choice sequence. Furthermore PPTg lesioned rats were also impaired in the time taken to make the first arm choice in the test phase (to initialise this part of the task). Their slowness to engage in the test phase could be a direct result of their poor performance on this phase. Specifically PPTg lesioned rats experience a significantly greater number of arms not paired with reward in comparison to shams in the test phase so it is perhaps not surprising that they seem less willing to begin this phase. This is supported by the fact that they are not significantly slower on the training phase latency measure, refuting an explanation relating to a motor impairment for the test phase latency measure finding.

10.4.2 Where did the initial chocolate effects occur?

Chocolate effects occurred in both sham and PPTg lesioned animals on most speed measures (training phase latency, test phase latency and test phase choice times). The only measure on which the chocolate effect diverged across the lesion factor was on within phase errors: presentation of chocolate was able to significantly reduce the number of these errors being made in sham rats but not in PPTg lesioned rats. The existing PPTg lesion impairments in accuracy of test phase performance were not therefore significantly worsened by the initial presentation of a more positive reward. This refutes the hypothesis generated from the results of Ainge *et al.* (1999) that the PPTg DSWS performance would deteriorate if a more positive reward were used in this task.

10.4.3 What were the immediate effects of reversing the reward?

A simple change in the reward available, regardless of the direction of change (more or less positive), was observed for both sham and PPTg lesioned rats in the training latency measure and the number of within phase errors in the test phase. Given that the improvements made in performance on these measures occurred regardless of the direction of change in reward these findings are likely to be the result of the “surprise” element of reward change. Thus PPTg lesioned rats responded normally to this unexpected environmental change.

A particular point of interest regarding the training latency measure is that the sham-choc/food group appeared to be responding faster than all of the other groups. While

this corresponds to the significant group effect observed on this measure in the second series of analyses the same effect did not occur in the criterion day analysis of this measure (it would have been apparent as a significant lesion x reward interaction). However, this might be because the extra days' data included in the immediate effects of reversal analysis increase the power for revealing a significant effect. This finding does therefore offer some support for the results of Ainge *et al.* since the PPTg rats reversed from 20% to 4% in the runway task continued to respond at a slower speed than shams under the same conditions.

The divergent effects of reversal of reward across the lesion factor in this experiment (and varying according to direction of change) occurred in the training phase choice times and the test phase errors. On the training phase choice times (a measure on which PPTg rats were not impaired and initial level of reward had no effect) changing the reward to a less positive one immediately decreased the speed of responding of shams only. Thus, at least in this measure, PPTg lesioned rats did not respond appropriately to a decrease in reward. On the test phase errors (a measure on which PPTg lesioned animals were impaired but level of reward had no effect) changing the reward to a less positive reward immediately improved the performance of PPTg lesioned rats. However, as argued previously this does not constitute indirect evidence to support the hypothesis that chocolate worsens PPTg lesioned rats performance in this task.

10.4.4 What were the long-term effects of reversing the reward?

None of the measures on which there were long-term effects of reversing the food reward were those on which PPTg lesioned rats were impaired. Therefore the immediate effect of reversing to a less positive reward (see above) on test error scores of PPTg rats was only transient. Indeed, long-term effects of reward reversal occurred only on speed measures.

For training latency the initial effect of chocolate (analysed by a criterion days ANOVA) had been to improve the run speed of both sham and PPTg lesioned rats. However the long-term effects of *reversing* to a chocolate reward from a food reward improved the run speed only of PPTg lesioned rats. This is interesting because all rats initially speeded up their training latency time simply in response to reversal in general (regardless of direction) but it seems that only the PPTg food/choc group maintained this speed change long-term. However, while not significant in the criterion ANOVA, the graph (10.xxiv) showing the long-term effects of reversal on this measure suggests that this disparity between PPTg and sham lesioned rats reversing from food to chocolate could be the result of the PPTg rats running at a slightly slower speed initially for the food reward. Thus the magnitude of run speed change in response to chocolate is significantly greater than for shams.

For training choice times there had been no initial effect of presenting chocolate compared to the effect of the standard food reward. There was also no immediate effect of reversal to chocolate but a long-term effect in both PPTg and shams such that both these groups improved their speed. In this case, therefore, PPTg rats did not respond

differently to the sham pattern of responding following an increase in level of reward available in the DSWS task.

For test choice times the initial presentation of chocolate increased the speed of responding in both PPTg and sham lesioned animals. In addition, while there was no immediate effect of reversal to a food reward on either of these groups, the long-term effects showed that only sham rats significantly slowed down their test phase mean arm choice time. Thus PPTg lesioned rats did not show the same pattern of slowing of responding in response to a reward decrease. Again however, the graph (10.xxxi) indicates that this is due to an impaired initial response to chocolate such that the magnitude of change in the sham rats was significantly greater than in PPTg rats. However this impaired initial response was not confirmed in the criterion days ANOVA on this measure.

10.4.5 Conclusions

The only firm conclusion that can be drawn from this experiment is that PPTg lesioned rats are permanently impaired in their performance of the DSWS task when trained to asymptote performance prior to surgery. The pattern of deficit produced in this experiment was similar to that produced in Keating & Winn (2001) suggesting that the PPTg impairment in the DSWS task arises due to disruption of the flow of information processing within the ventral striatal system. This is behavioural evidence that confirms PPTg involvement in the processing of information believed to be reserved for the basal ganglia loops. Furthermore it suggests that the PPTg contributes in some way to executive functioning originating from the frontal cortex.

There was no firm, direct evidence that offering a preferred reward in this task would further deteriorate the level of performance of the already impaired PPTg rats. For example the significant improvement in the number of test phase errors observed in immediate response to switching to a food reward from a chocolate reward was not upheld in the long term ANOVA. Furthermore there were no other effects of presenting chocolate on the performance of PPTg lesioned rats on the measures on which they were impaired. Thus, the impairment shown by PPTg lesioned rats appears to be largely separate from possible problems adapting their behaviour to change in reward.

Evidence supporting this impairment in PPTg lesioned rats in adaption of conditioned responding to a change in reward level was limited. There were frequent trends in the “long-term effects of reward reversal” graphs indicating this was the case. For example in the training latency, test latency and test choice times graphs sham rats clearly display the expected crossover in responding following reward reversal. In contrast PPTg rats did not mirror this pattern. However, these trends for impairments were not upheld by the ANOVAs that were conducted on these measures. Given the complex nature of the DSWS task, along with the very specific design of the current experiment, it is likely that it was not ideal for revealing these deficits should they occur. Indeed the simple runway task of Ainge *et al.* (1999) is best designed to achieve this and indeed did confirm a deficit in PPTg rats of this nature.

11. ACQUISITION OF THE DSWS TASK

11.1 Introduction

Bilateral ibotenate lesions of the mPFC have been shown to produce impairment in retention of the DSWS task (see Chapter 8). The deficit of mPFC lesioned rats is characterised by increased total test phase errors (with more across phase than within phase errors but a significant increase in both), earlier test phase error occurrence, and increased test phase latency. This deficit is similar in nature to that produced following bilateral ibotenate lesions of the PPTg (Keating & Winn 2001 and Chapter 10), and lidocaine inactivation of the VP (Floresco, *et al.*, 1999), suggesting that the DSWS deficit results from general disruption to the ventral striatal stream of information processing. This indicates that the PPTg is intimately involved in the processing of information within this circuit that originated from the mPFC, in addition to the structures usually included within this basal ganglia loop.

Bilateral ibotenate lesions of the PPTg have also been shown to produce similar impairment in an acquisition paradigm to that which occurs in a retention paradigm (Keating & Winn 2001). However, the effect of bilateral mPFC lesions on acquisition of the DSWS has not been investigated. In addition, whether the same impairments produced by bilateral lesions of the PPTg and mPFC can be produced using unilateral lesions of the same structures is not known. Given that the aim of this work was to undertake disconnection lesions of the mPFC and the PPTg it was important to investigate this possibility. The current study was therefore undertaken to examine the effects of bilateral and unilateral mPFC lesions, along with unilateral PPTg lesions, on

acquisition of the DSWS task. The effects of unilateral lesions to both these structures in a retention paradigm can be seen in Chapter 12.

11.2 Method

All procedures for housing and care of the 28 rats used as subjects in this experiment were as discussed under “General Methods”. In this experiment rats were handled daily before surgery to familiarise them to contact with the experimenter. This aimed to parallel the amount of contact rats in a retention paradigm receive in their pre-surgical training sessions. All rats were separated with one rat per home cage 2 days prior to the start of surgery. They weighed approximately 400g at this time. mPFC surgery was conducted as stated in Chapter 8 (manually infused using the microdrive) and PPTg surgery was as stated in Chapter 10 (step-down procedure). Rats were placed on food restriction following a 7-day recovery period from surgery. On reaching their 85% target weight (assessed by using body weight on day 7 as 100% weight) rats were tested for impairment in acquisition of the DSWS task under the DSWS procedure laid out in “General Methods”. In this experiment testing on the DSWS task continued until sham rats reached criterion (a total of 8 days). Histology was conducted as described previously (General Methods). Data was converted as in the experiments presented in Chapters 9 and 10 by calculating mean scores for each group on each measure over the two criterion days. These scores were then analysed using one-way between subjects ANOVAs (SPSS version 10). This was done because the experiment specifically assessed task acquisition and the criterion days’ analysis is more suited to reveal impairment in task acquisition given that sham rats also perform poorly during initial stages of task learning.

11.3 Results

11.3.1 *Histology*

Unilateral and bilateral lesions of the mPFC assessed using cresyl violet staining revealed variable damage to regions AC and PL of the mPFC. In all cases accepted for statistical analysis, damage to area AC was extensive (approximately 80%) but damage to area PL was more than 50% complete in only 4/6 bilateral PFC rats and in 4/5 unilateral PFC rats. However, in all cases accepted, the level of damage observed in AC/PL was consistent in the anterior posterior plane and was centred along the medial wall. 2 rats with mPFC lesions were excluded due to restricted anterior-posterior spread and 3 were excluded due to misplacement – they were situated too laterally, in the motor area M1 (as defined by Paxinos and Watson, 1997). A representation of the lesions included in the analysis can be seen in Figure 11.i. There were a total of 5 unilateral mPFC lesioned rats and 6 bilateral mPFC lesioned rats included in the analyses.

PPTg lesions assessed using both cresyl violet and NADPH staining revealed large lesions extending beyond the boundaries of the PPTg. However, within the group as whole there was no consistent, complete neuronal loss to surrounding structures (such as the cuneiform nucleus, retrorubal field or the pontine reticular nucleus). Frequent damage to the LDTg was observed within the group. Given the extent of this damage a lesion was accepted for analysis if it included more than 70% loss of the PPTg (including the ACh neurons) but not more than 50% LDTg ACh loss. In no cases did damage extend inside the borders of the substantia nigra. Representative PPTg lesions

FIGURE 11.i Bilateral and Unilateral lesions of the mPFC. Bilateral lesions are shown on the left where red shows the largest lesion and blue shows the smallest lesion. Unilateral lesions are shown on the right where the largest lesion is represented on the right hemisphere and the smallest on the left hemisphere.

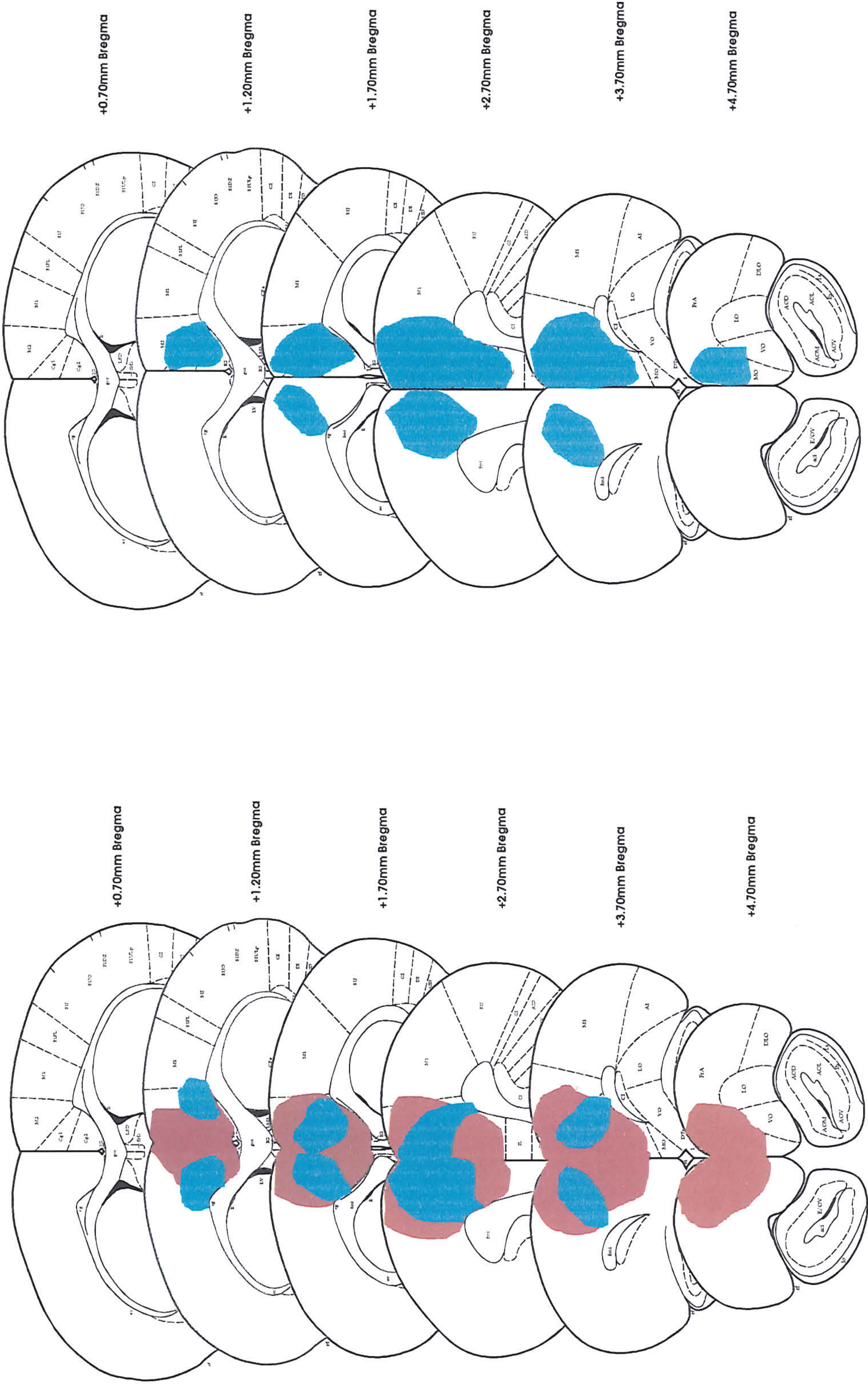
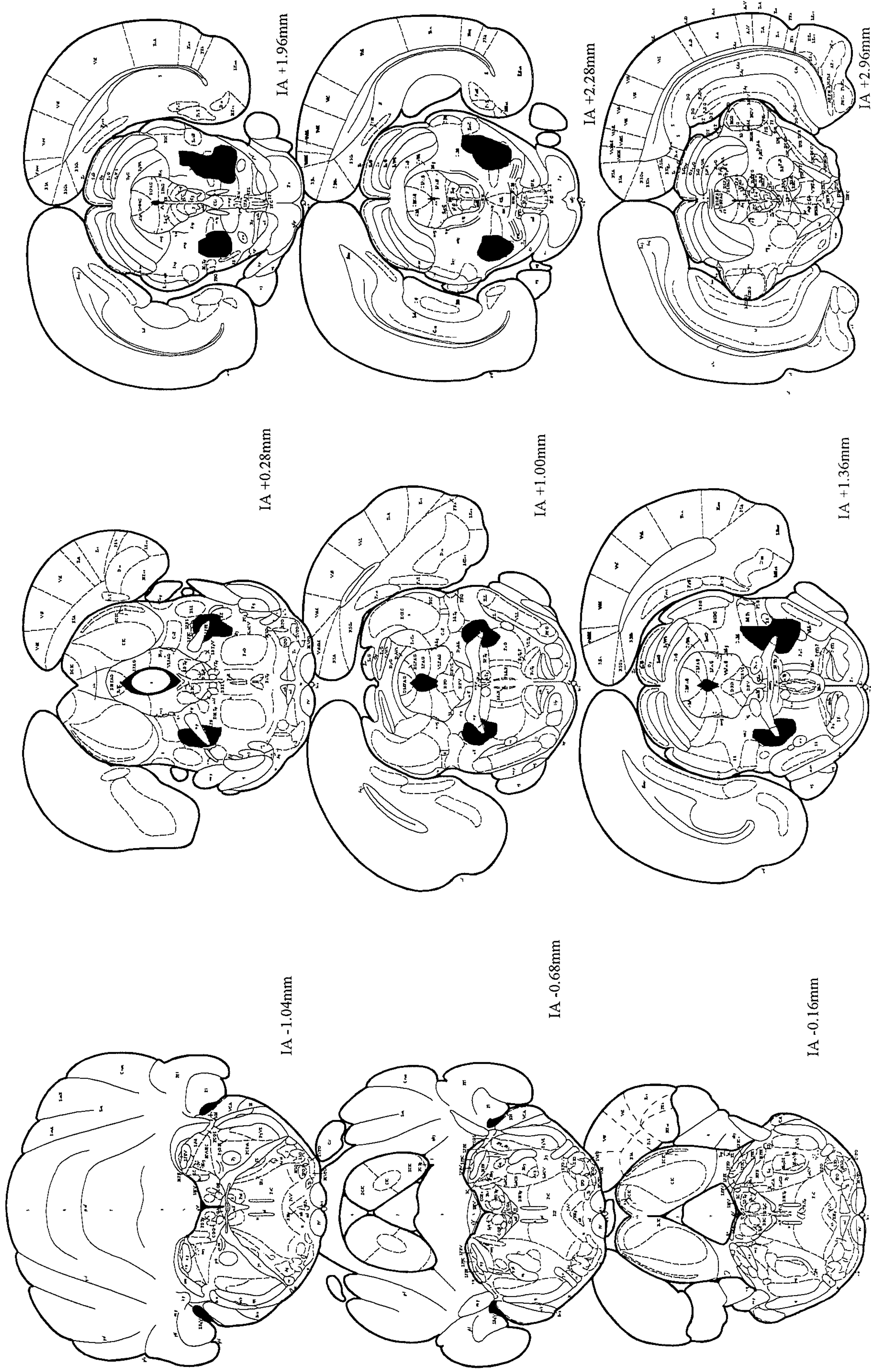


FIGURE 11.ii Unilateral lesions of the PPTg - smallest is shown on the left and largest on the right.



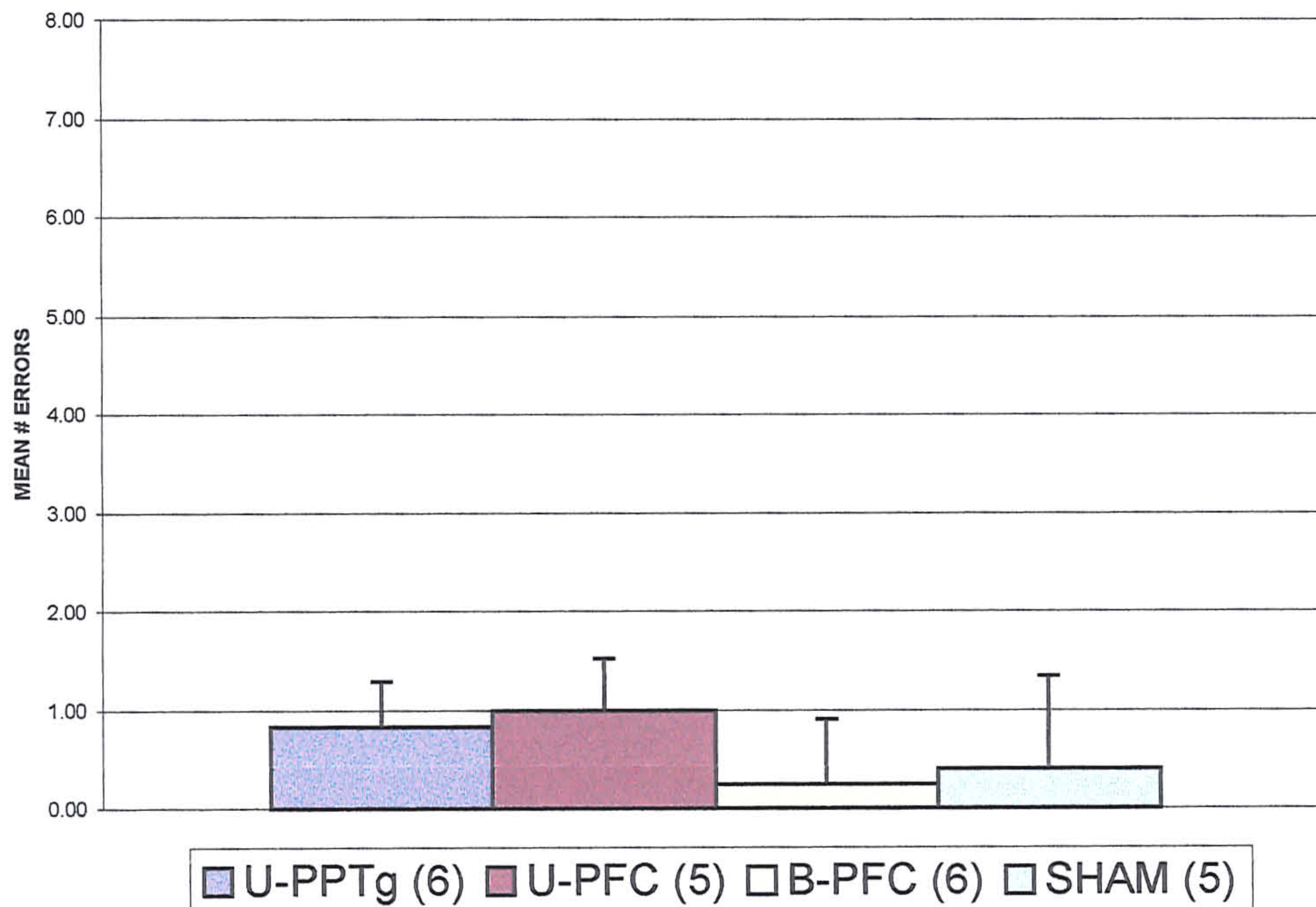
can be seen in Figure 11.ii. There were a total of 6 unilateral lesioned rats included in the analyses along with 5 sham-operated controls.

11.3.2 *The DSWS Task*

In the graphs that follow the label “U-PPTg” refers to the unilateral PPTg lesioned group, the label “U-PFC” refers to the unilateral mPFC lesioned group, the label “B-PFC” refers to the bilateral mPFC lesioned group, and finally, the label “SHAM” refers to the sham operated controls.

The mean and standard error for the number of training phase errors made by each group over the criterion days can be seen in Figure 11.iii. The ANOVA indicated that there were no significant effects of any of the lesions on this measure $F(3,21) = 0.96$.

FIGURE 11.iii TRAINING PHASE ERRORS



The mean and standard error for the number of correct responses before error in the training phase over the criterion days can be seen in Figure 11.iv. There were no significant effects of any of the lesions on this measure $F(3,21) = 1.28$.

FIGURE 11.iv TRAINING PHASE # CORRECT BEFORE ERROR

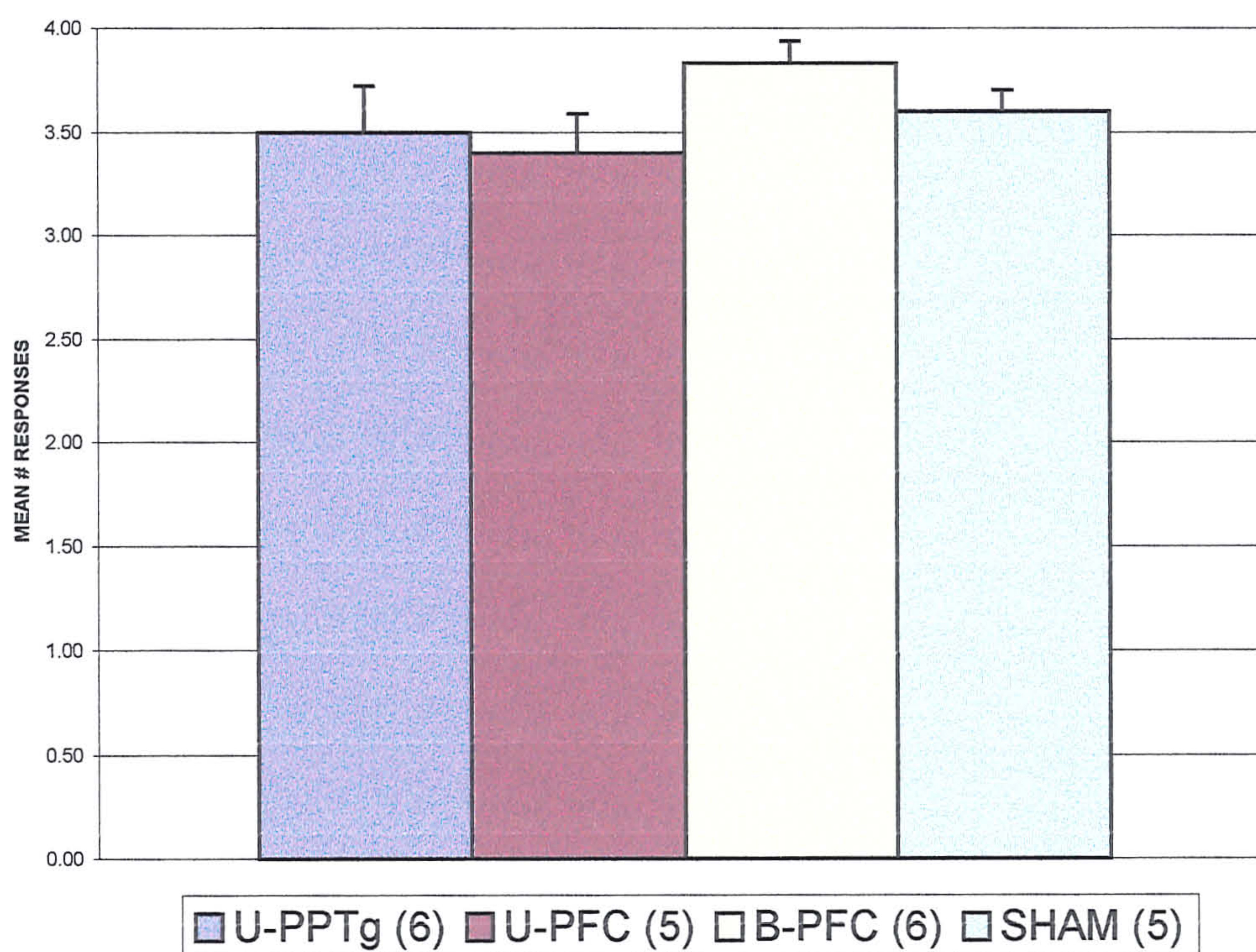


Figure 11.v shows the mean and standard error for the latency for each group over the criterion days and Figure 11.vi shows the mean arm choice times. There were no significant effects of the lesions on either of these measures in the training phase $F(3,21) = 0.29$ and $F(3,21) = 0.78$ respectively.

FIGURE 11.v TRAINING LATENCY (LOG10)

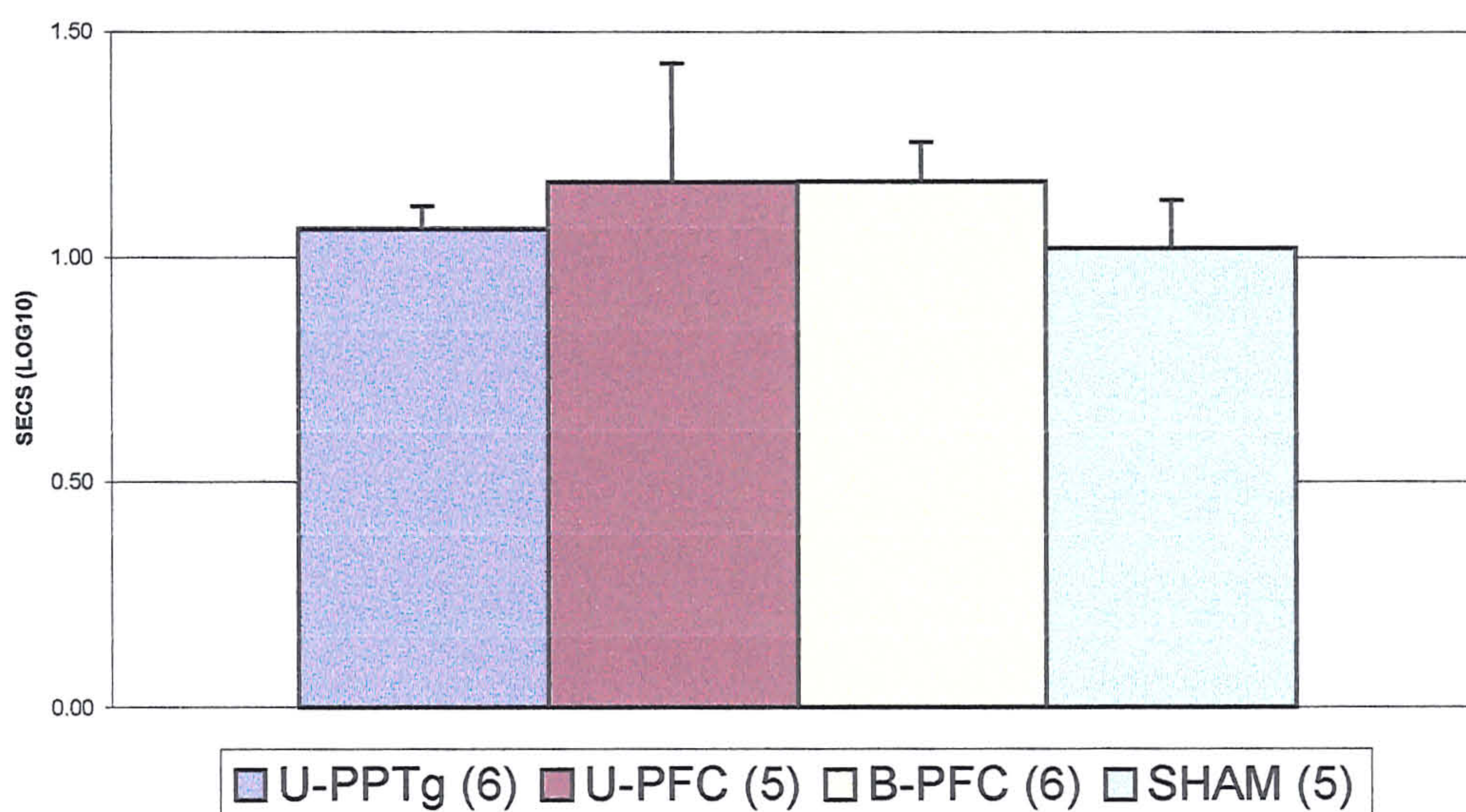
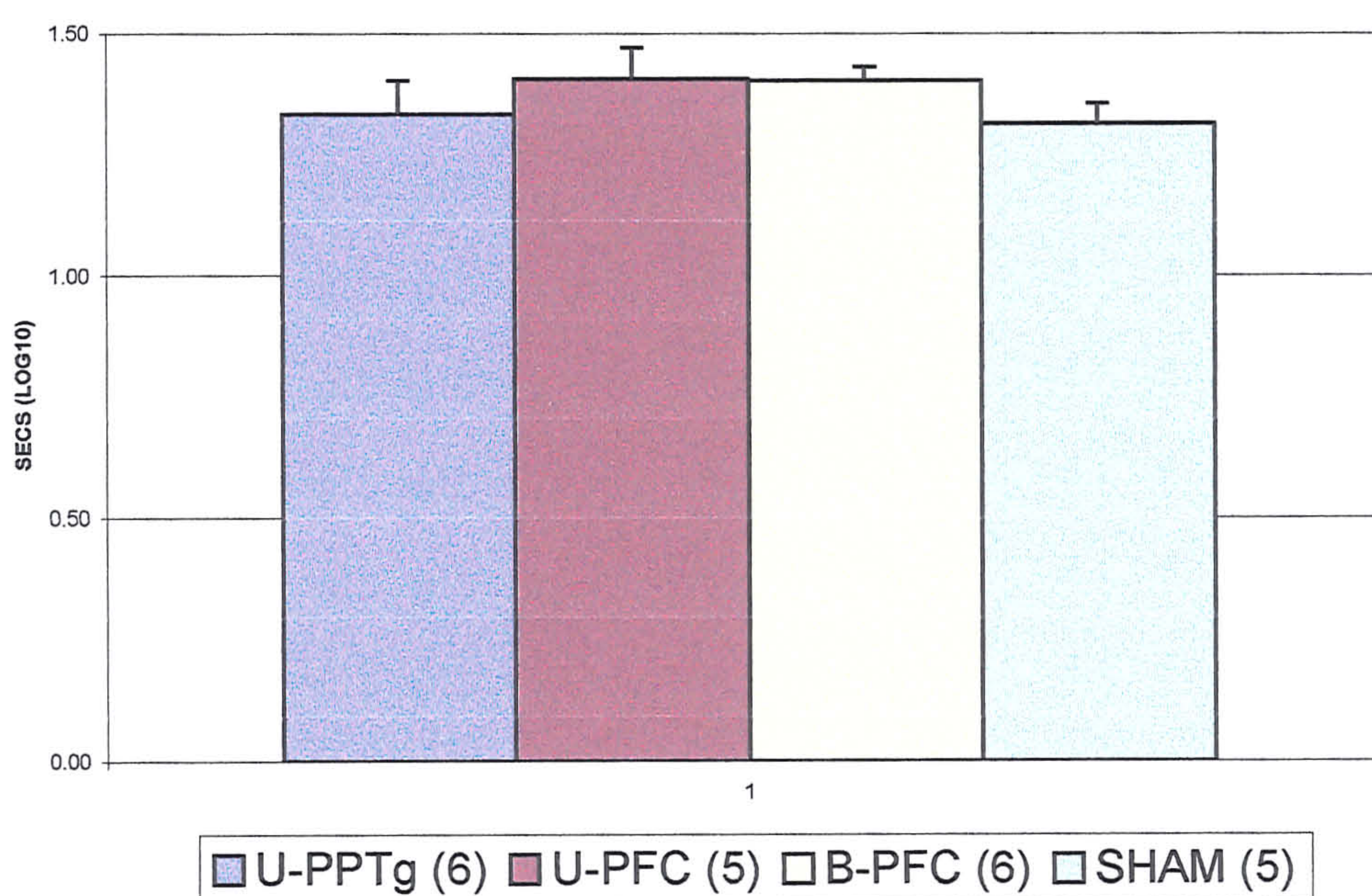


FIGURE 11.vi TRAINING CHOICE TIMES (LOG10)



The mean and standard error for the number of errors made by each of the groups in the test phase over the criterion days can be seen in Figure 11.vii. As with the number of training phase errors there was not a significant effect of any of the lesions on this measure $F(3,21) = 1.01$.

FIGURE 11.vii TEST PHASE ERRORS

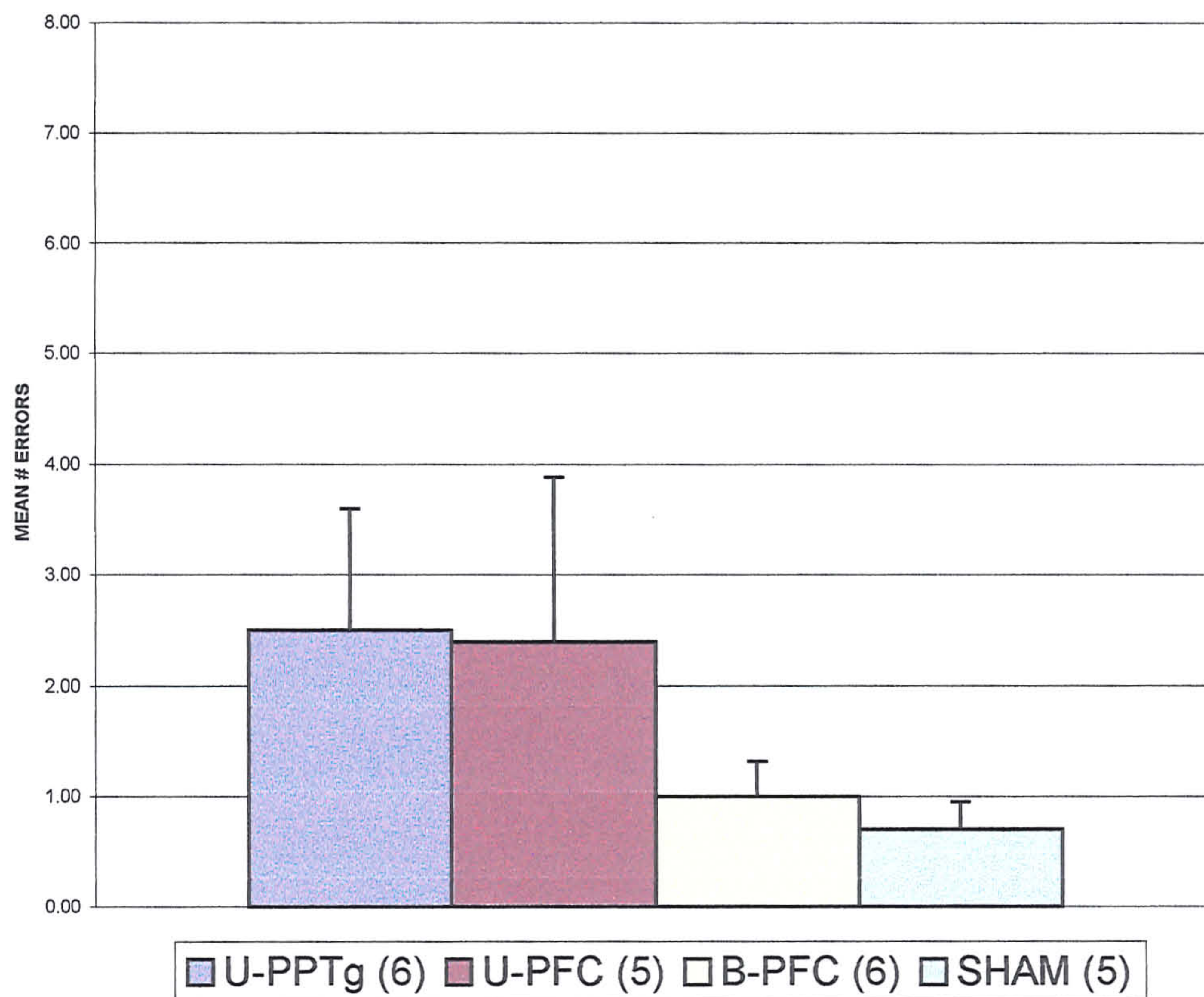


Figure 11.viii shows the mean and standard error for the number of across phase errors and Figure 11.ix shows the mean number of within phase errors made over the criterion days. There were no significant effects of the lesions on these measures $F(3,21) = 0.69$, and $F(3,21) = 1.02$ respectively.

FIGURE 11.viii ACROSS PHASE ERRORS

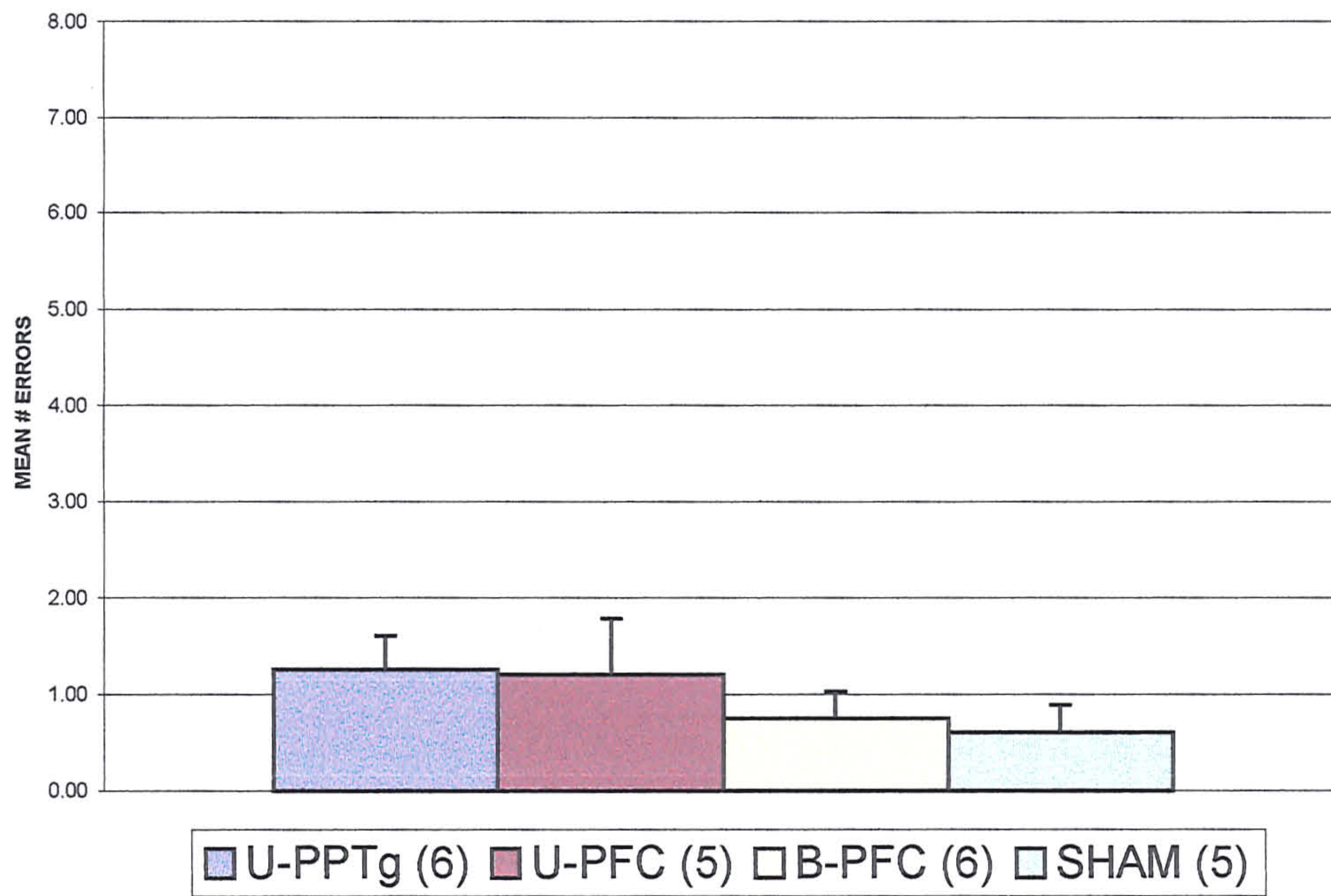


FIGURE 11.ix WITHIN PHASE ERRORS

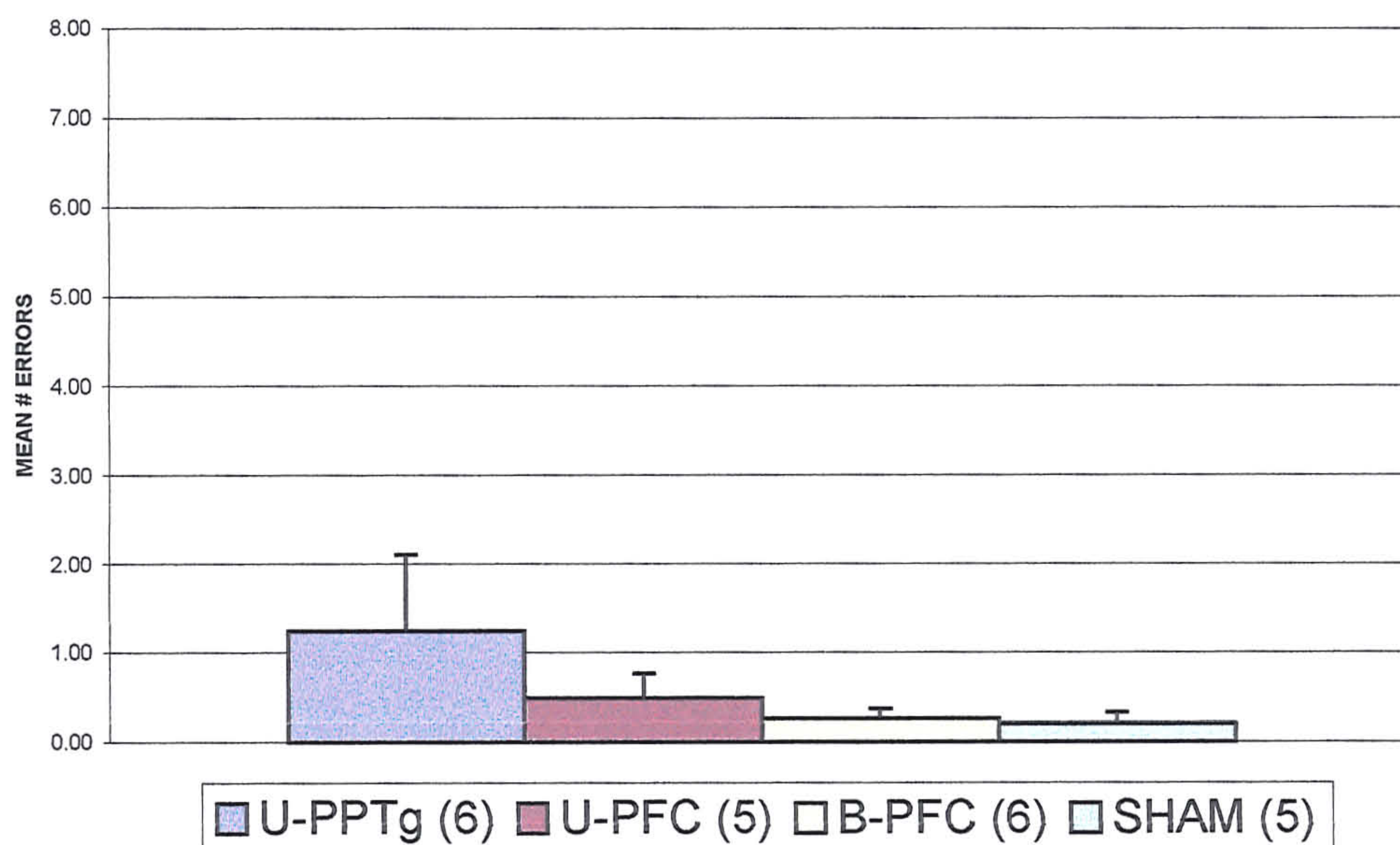


Figure 11.x shows the mean and standard error for the number of correct responses before error for the test phase over the criterion days. The ANOVA showed that there were no significant effects of any of the lesions $F(3,21) = 0.74$.

FIGURE 11.x TEST PHASE # CORRECT BEFORE ERROR

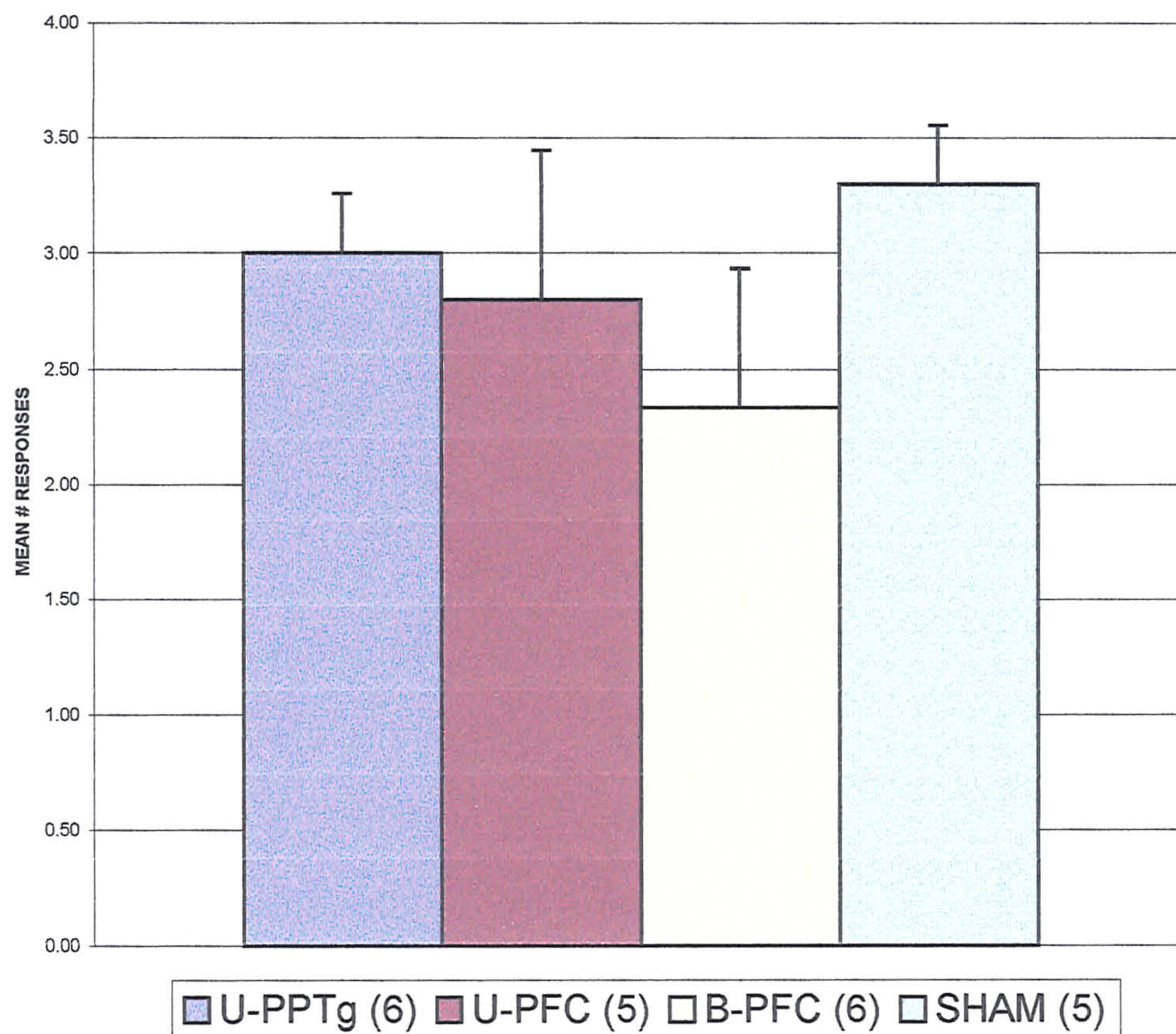
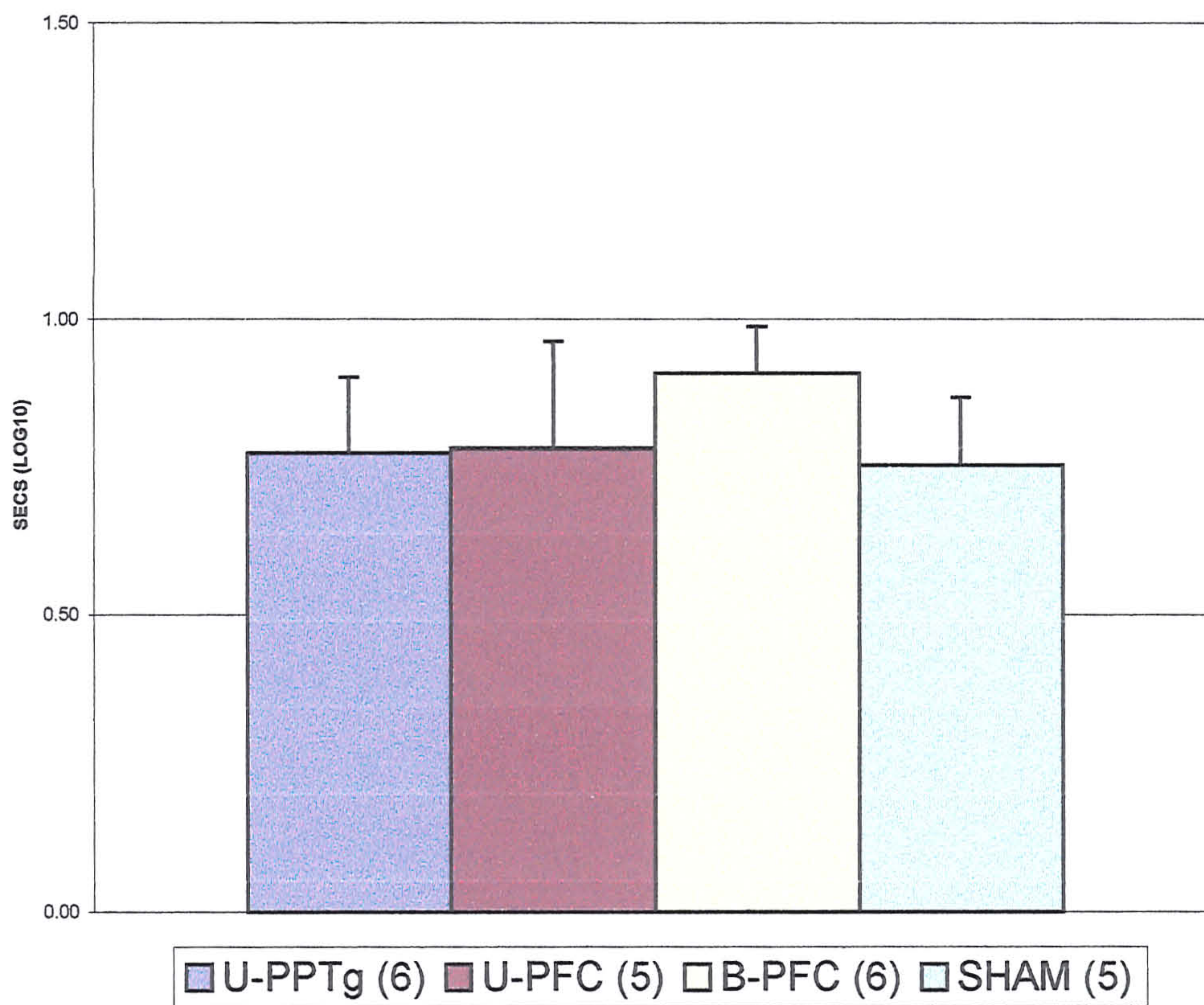
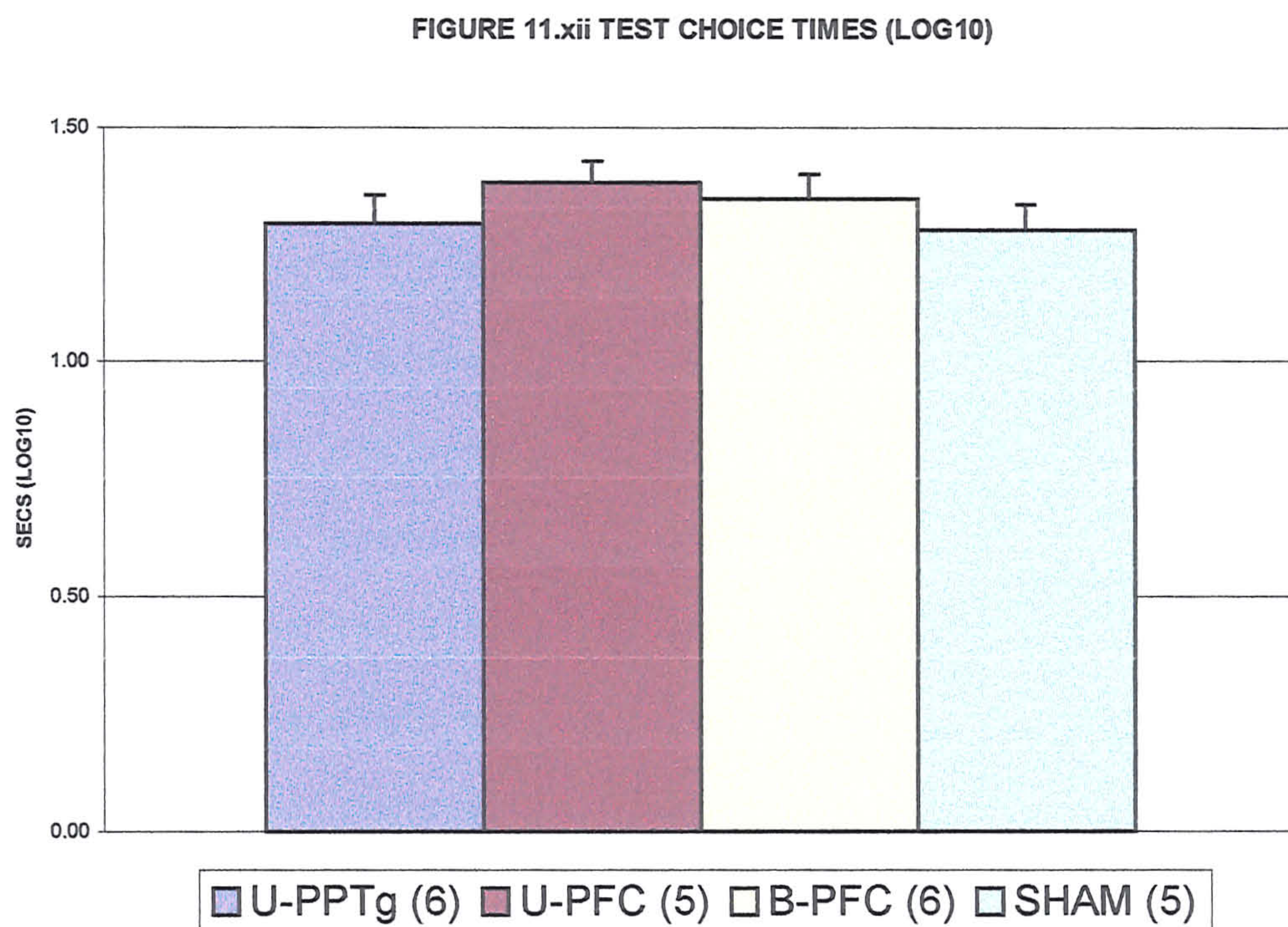


Figure 11.xi shows the mean and standard error for the latency to the first arm for each group over the criterion days. There was not a significant effect on this measure for any of the lesions $F(3,21) = 0.34$.

FIGURE 11.xi TEST LATENCY (LOG 10)



Finally Figure 11.xii shows the mean and standard error for the arm choice times over the criterion days for each group. As can be seen in the graph there was not a significant effect of the lesions $F(3,21) = 0.74$.



Given that there were no significant effects in the criterion analyses conducted for this experiment, the data has not been presented in summary format.

11.4 Discussion

There were no significant effects of any of the lesions on acquisition performance of this task. While none of the lesion groups had reached criterion by the same time that shams had reached criterion (days 7+8) their performance was nonetheless not significantly different to the performance of the sham rats on these days.

The mPFC lesions produced in this experiment varied in the amount of damage sustained to area PL, a region of the rat mPFC that is often found to produce the same effects as larger mPFC lesions (Brito & Brito, 1990). In the experiment presented in Chapter 8, where bilateral ibotenate mPFC lesions produced a DSWS retention deficit, damage was sustained to both areas AC and PL. Thus it is possible that if bilateral damage to the PL region of the medial wall of the PFC is not sufficient then DSWS effects are not observed. Given the small group sizes in the current experiment, however, a contrast between performance of mPFC lesioned rats with and without PL damage was not possible in order to determine whether this might be the case. Seamans *et al.* (1995) have specifically investigated retention of the DSWS task in rats with temporary bilateral AC inactivation using infusions of lidocaine. Their results showed a significant impairment in this task indicating that AC damage may be sufficient to produce a DSWS deficit. However there are problems with a lidocaine methodology since functional spread can only be estimated rather than confirmed. Furthermore some of the placements indicated in the Seamans *et al.* (1995) paper suggest inactivation of area Fr2 in addition to AC. Evidence from other research is mixed regarding impairments in working memory tasks following AC lesions with or without limited PL

damage (see for example Ragozzino *et al.*, 1998, Neave *et al.*, 1994, Poucet, 1990, & Kesner, Farnsworth, & DiMattia, 1989). Therefore it remains speculative whether the rat mPFC is necessary for acquisition of the DSWS task, either intact bilaterally or unilaterally.

Unilateral lesions of the PPTg were, however, effective in producing extensive damage to this structure. Using the same procedure, bilateral PPTg lesions have been shown in the context of the current research to produce a DSWS retention deficit (Chapter 10). Furthermore lesions made using the same volume of toxin infused at the same coordinates have also been shown to produce a deficit in acquisition of the DSWS task (Keating & Winn, 2001). Given this supporting evidence it seems reasonable to conclude that unilateral PPTg lesions are not sufficient to produce the effects seen in acquisition of this task with bilateral PPTg damage.

12. DSWS RETENTION FOLLOWING CROSSED UNILATERAL DISCONNECTION LESIONS OF THE PPTg AND mPFC (1)

12.1 Introduction

Traditionally behavioural neuroscience has applied lesioning techniques to a structure and identified impaired cognitive components from an observable behavioural deficit. This technique has proved very valuable and has revealed much about the functions of particular brain structures. However, it is a technique that relies on a very direct and simple assumption that a brain structure can be tagged with a single function. Given the extensively interconnected nature of brain structures and systems it is quite likely that this approach “misses the point” in assessing how the brain operates. That is, a structure may have more than one function based on which of its many connections are activated at a particular time.

The PPTg is argued in this thesis to be an essential component in the ventral striatal system. The research discussed in Chapters 2,3 & 4 suggests that this is a very complex brain system and that the extent of the interconnectivity of the system components may reflect this. Thus it is not surprising that the ventral striatal system is a brain system hypothesised to play a role in the generation of behaviour based on higher order, or executive, functions. Indeed, the very nature of this system, and the fact that it is involved in such complex behaviour, would suggest that alternative methods of study are necessary to move our level of understanding beyond that gained by the more traditional methodologies.

Disconnection lesions are used to identify the components of a neural circuit that are vital for a specific behaviour to occur. The crossed unilateral lesions disrupt the serial transmission of information bilaterally by blocking the origin of neural activity in one hemisphere and disrupting the receipt of this information in the other. Thus a disconnection lesion prevents the serial transmission of information from one structure to another, in parallel (see Figure 12.i) and therefore enables the study of the function of *connections* rather than of structures. Significantly, this technique has shown that the function of a structure in the ventral striatal system (the hippocampus) can vary depending on which connecting structure it is “disconnected” from (Floresco *et al.*, 1997). Therefore, there is evidence to suggest that the disconnection technique is a technique particularly applicable to the study of this system.

A direct connection between the medial wall of the rat PFC and the region of the PPTg has been demonstrated previously (Zahm personal communication). Furthermore, the PPTg is known to be in receipt of limbic ventral striatal information originating from the PL region of the mPFC. Thus the PPTg is well located for processing complex neural information associated with the PFC and its executive functions. This proposal is controversial because it challenges the traditional view that these higher cognitive processes are “situated” in the frontal cortex. If the PPTg could be directly demonstrated to process information arising from a specific frontal cortical region known to be involved in executive functioning, this would support the view that such functions are widely distributed throughout the fronto-striatal system and that the PPTg is very much involved.

In addition the experiment discussed here also provided the opportunity to assess, as a means of control, whether unilateral lesions of either the mPFC or PPTg would produce the DSWS retention deficit seen following bilateral lesions of either structure.

12.2 Methods

A total of 30 rats began the DSWS training prior to surgery (approximate weight at time of surgery was 320g). All rats were housed and fed as stated in “General Methods”. However 3 were excluded from the group within the first week of pre-surgical training: 1 who would not eat any of the food rewards available on the maze, despite eating the same pellets when given in the home cage, and 2 who stopped running the task (they remained sitting in the centre portion throughout the 10 minute duration periods). The remaining 27 rats required 15 days training to reach acquisition criterion.

Surgery was conducted using the coordinates and procedures stated previously (General Methods and Chapters 8 and 10). The disconnection lesioned group rats received crossed unilateral lesions of the mPFC and PPTg with contralateral infusions of sterile PB in each structure. The unilateral lesioned groups each received unilateral lesions of the corresponding structure with contralateral infusions of sterile PB in the same structure and bilateral infusions of PB in the other structure. Thus for example a unilateral mPFC lesioned rat would receive a right ibotenate infusion in the mPFC, a left PB infusion of the mPFC, and bilateral infusions of PB in the PPTg. All mPFC lesions were made in the first week of surgery and PPTg lesions were made in the second week of surgery. This order was chosen since PPTg surgery is more severe and survival might have been reduced if PPTg lesions were incurred first and the lesioned

rats had to undergo another surgery for mPFC lesions in the second week. In total all rats received 3 infusions per hemisphere (2 for the PPTg and 1 for the mPFC). One rat died during surgery following anaesthesia.

The 26 surviving rats received 7 days recovery from the date of the last surgery before food restriction was re-imposed. 85% free feeding weight was calculated using the weight on day 7 as the 100% weight. Once rats had reached this target DSWS retention testing began and was conducted as discussed in “General Methods”. Testing continued until sham lesioned rats reached criterion. Perfusion and histology was as stated previously (General Methods). PPTg lesions were assessed using NADPH diaphorase, cresyl violet staining and NeuN immunohistochemistry, mPFC lesions by cresyl violet and NeuN staining. NeuN immunohistochemistry was conducted using several procedures:

- Standard primary/secondary antibody procedure with DAB stain, discussed under “General Methods”.
- ABC procedure with DAB stain. This was conducted in the same manner as above but with a biotinylated secondary antibody (100 μ l:10ml ADS) and a preformed avidin and biotinylated horseradish peroxidase macromolecular complex (10ml PB to 100 μ l solution A and 100 μ l solution B). The biotinylated secondary antibody and the A and B solutions were obtained from a VECTASTAIN elite ABC kit.

These multiple NeuN procedures were conducted in order to assess the best protocol for visualising mPFC and PPTg lesions using NeuN immunohistochemistry.

Data for this experiment was first analysed according to the method described for the previous experiment (Chapter 11) in keeping with the preceding work and Keating et al (2001). In cases where non-significant lesion effects were shown on the criterion days but the graphs of data for all trials indicated that this might not be the case during the earlier test trials, omnibus ANOVAs were also calculated to reveal whether significant group effects were present at the start of the postsurgical period that were not present by the point in time that sham lesioned rats had reached criterion. These ANOVAs were two-way mixed ANOVAs; within subjects on the factor of Day and between subjects on the factor of Group. All ANOVAs were corrected when necessary using the Huynh Feldt correction.

12.3 Results

12.3.1 *Histology*

Both sets of lesions (PPTg and mPFC) produced in this experiment varied in size. For the PPTg, damage was usually extensive, invading a large proportion of surrounding structures including the substantia nigra in some cases. Examples of extent of substantia nigra damage can be seen in Figures 12a and 12b. One rat in the unilateral PPTg group was excluded due to poor rostral damage, and 2 rats in the disconnection group were excluded due to more than 40% PPTg cholinergic neuron survival. In total there were 5 unilateral PPTg lesions included in the analyses and 5 disconnection lesions. 4/5 of these disconnection lesions also sustained invasion of the SN along with 3/5 unilateral PPTg lesions. In addition 2/5 disconnection lesions and 2/5 unilateral PPTg lesions sustained greater than 50% loss of LDTg ACh neurons. Low group numbers, however, prevented the exclusion of these animals. In all cases included in the analyses PPTg damage was greater than 70%. A representation of the unilateral PPTg lesions can be seen in Figure 12.ii. Furthermore the unilateral PPTg lesions of the disconnection group can be seen in Figure 12.iii.

mPFC lesions varied considerably in the extent of damage sustained to areas AC and PL. Two rats were excluded from the unilateral mPFC group due to very poor damage to both AC and PL – one lesion was centred too far laterally and in both animals damage was not sustained in area PL or to the caudal mPFC to include good anterior posterior spread of AC loss. Again small group numbers meant that inclusion of lesions

Figure 12a: NeuN stained section to illustrate nigral damage resulting from PPTg lesioning procedure. Intact hemisphere is on left side (+2.20mm from Interaural line).

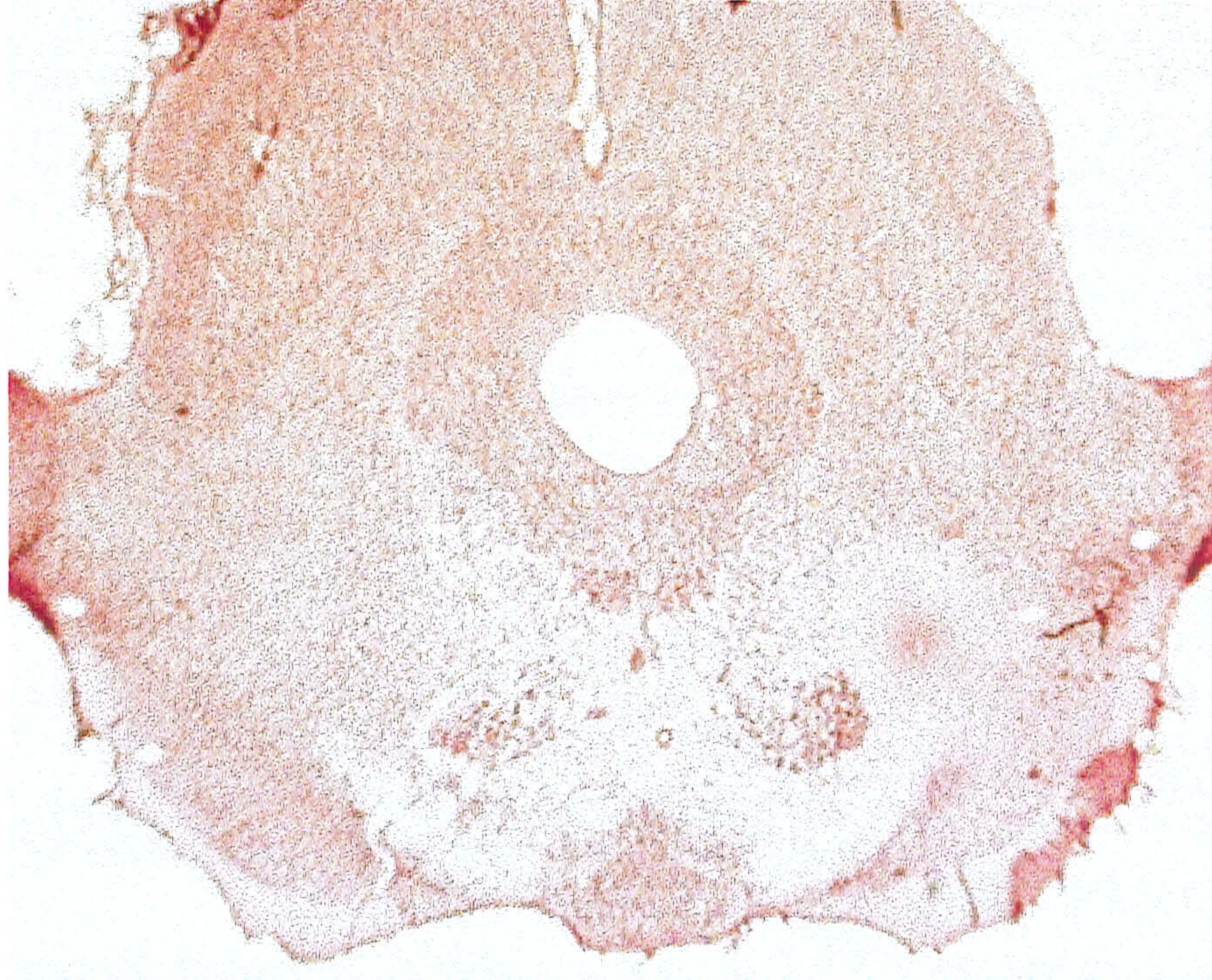


Figure 12b: NeuN stained section to illustrate nigral damage resulting from PPTg lesioning procedure. Intact hemisphere is on right side (+2.20mm from Interaural line).

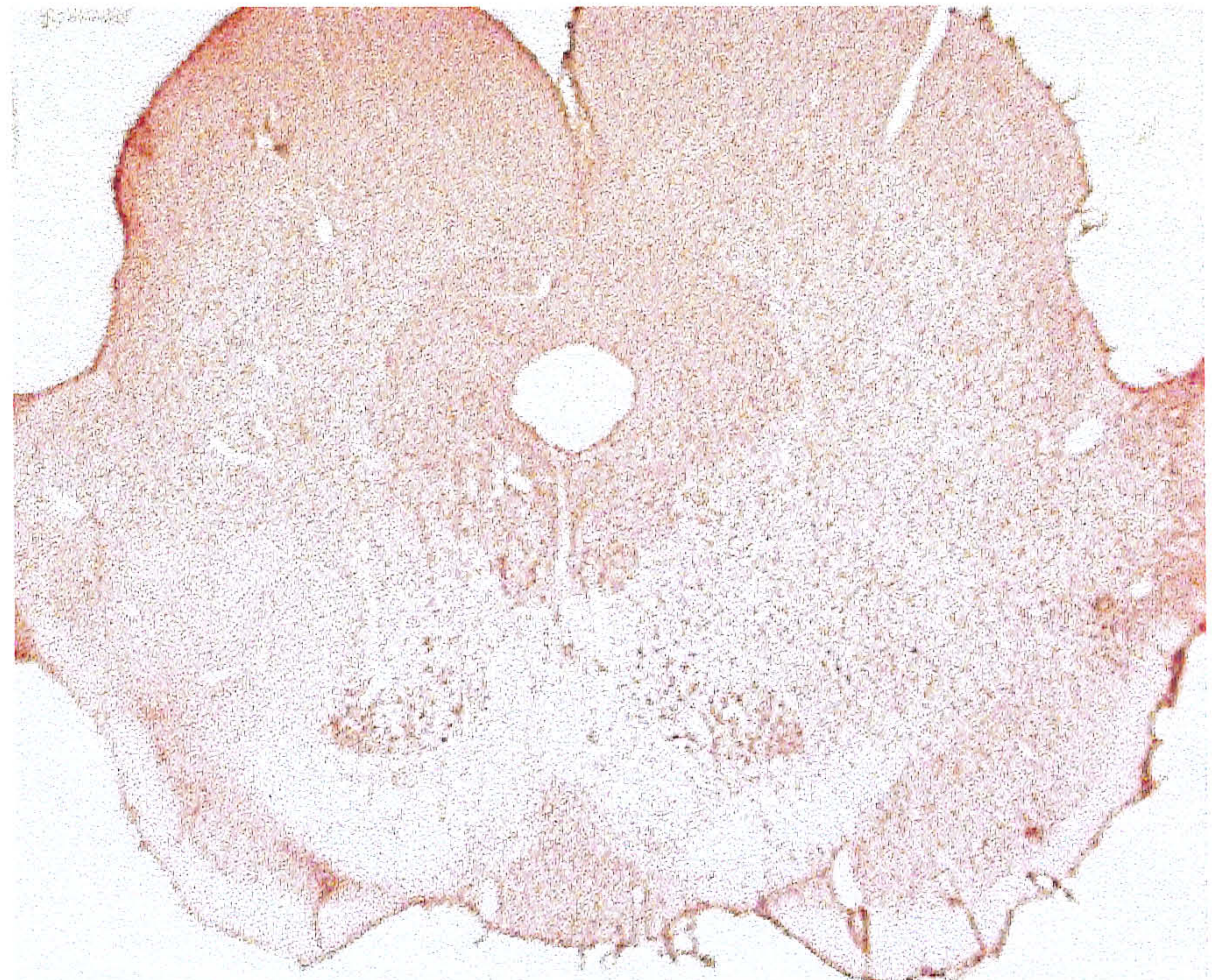


FIGURE 12.ii UNILATERAL LESIONS OF THE PPTg - SMALLEST IS REPRESENTED ON THE LEFT AND LARGEST ON THE RIGHT.

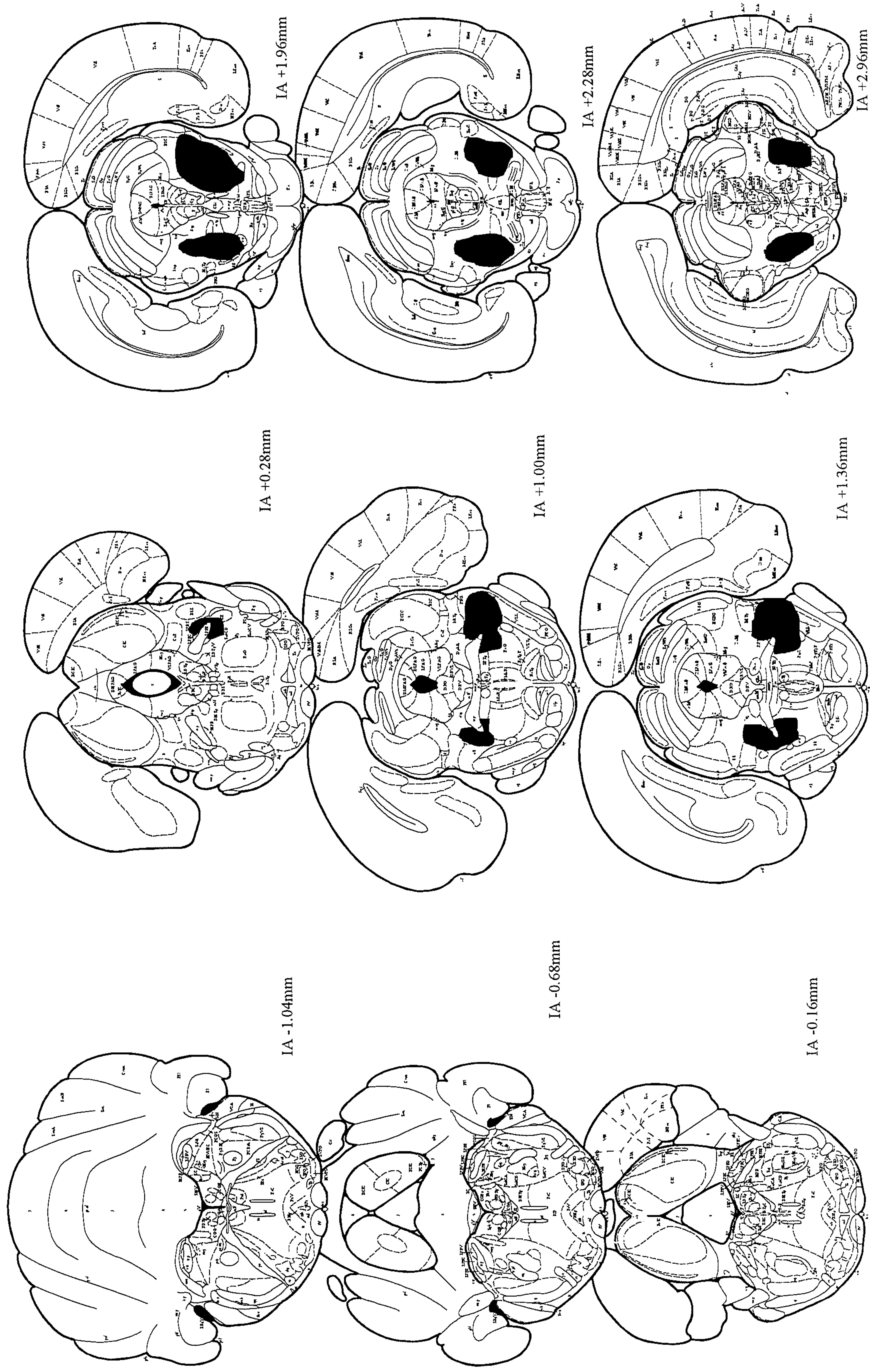
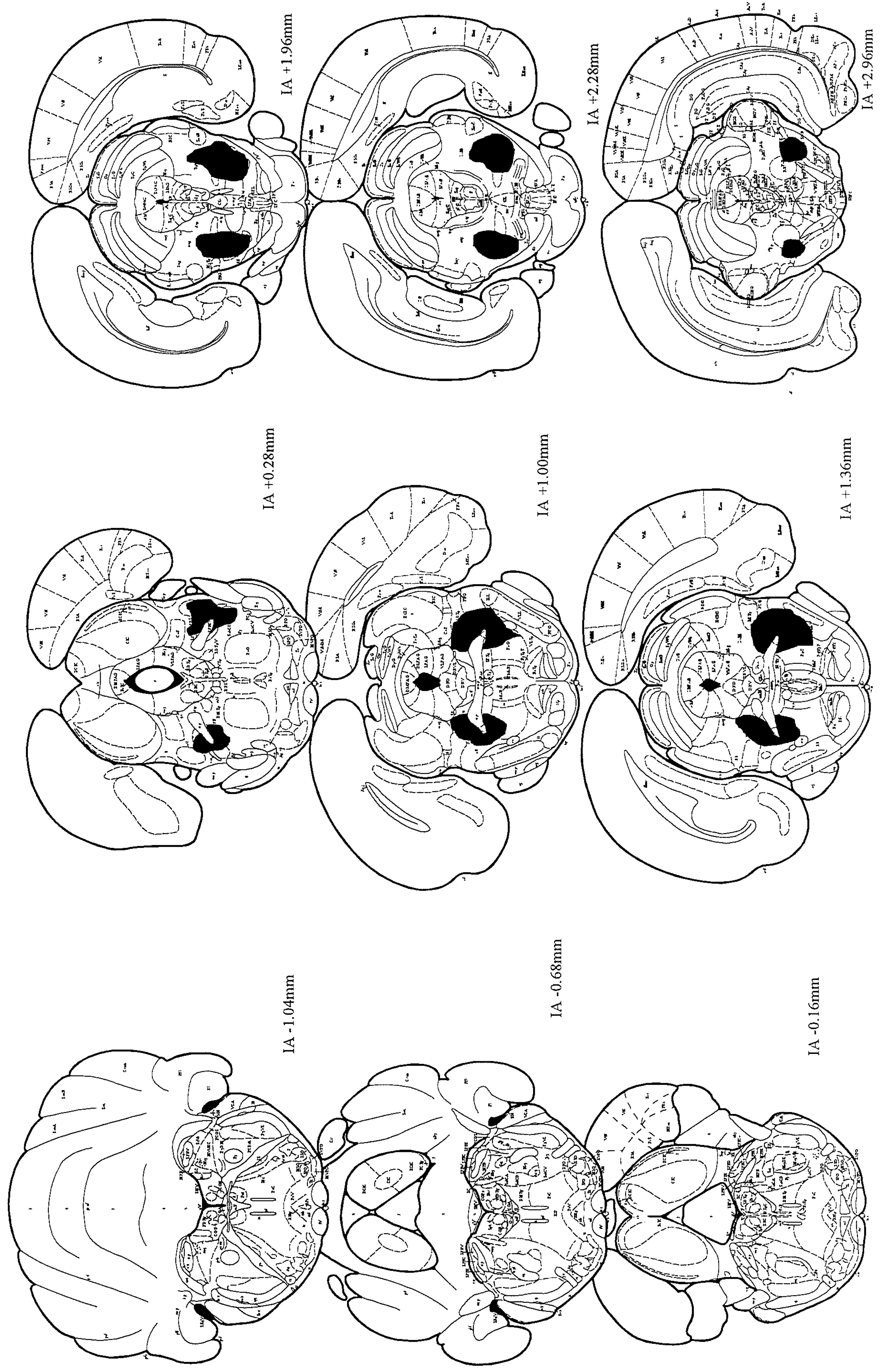


FIGURE 12.iii UNILATERAL LESIONS OF THE PPTg FOR THE DISCONNECTION GROUP - SMALLEST IS SHOWN ON THE LEFT AND LARGEST ON THE RIGHT.



with only good (more than 70%) of both AC and PL damage was not possible. Therefore all mPFC lesions included in the analysis had sustained greater than 70% loss to either area AC or PL or both. Thus there were a total of 5 rats included in the unilateral mPFC group. The 5 rats included in the disconnection group on the basis of PPTg damage were also acceptable according to the criteria for mPFC damage. These unilateral mPFC lesions of the disconnection group are shown in Figure 12.iv. The unilateral lesions in the U-PFC group can be seen in Figure 12.v. In total there were 5 disconnection lesioned rats, 5 unilateral PPTg lesioned rats, 5 unilateral mPFC lesioned rats, and 6 sham operated control rats included in the analyses.

A comparison of NeuN staining techniques revealed that, while the standard procedure with DAB staining was sufficient to visualise the PPTg lesions (see Figure 12.c), the level of staining produced in the mPFC using this technique was very light (Figure 12.d) and virtually impossible to see. However, the ABC method, which produces a greater number of binding sites for the DAB stain, was suitable for assessing the extent of mPFC lesions (see Figure 12.e).

Figure 12c: NeuN standard procedure with DAB stain for PPTg lesions (+1.70mm from Interaural line).

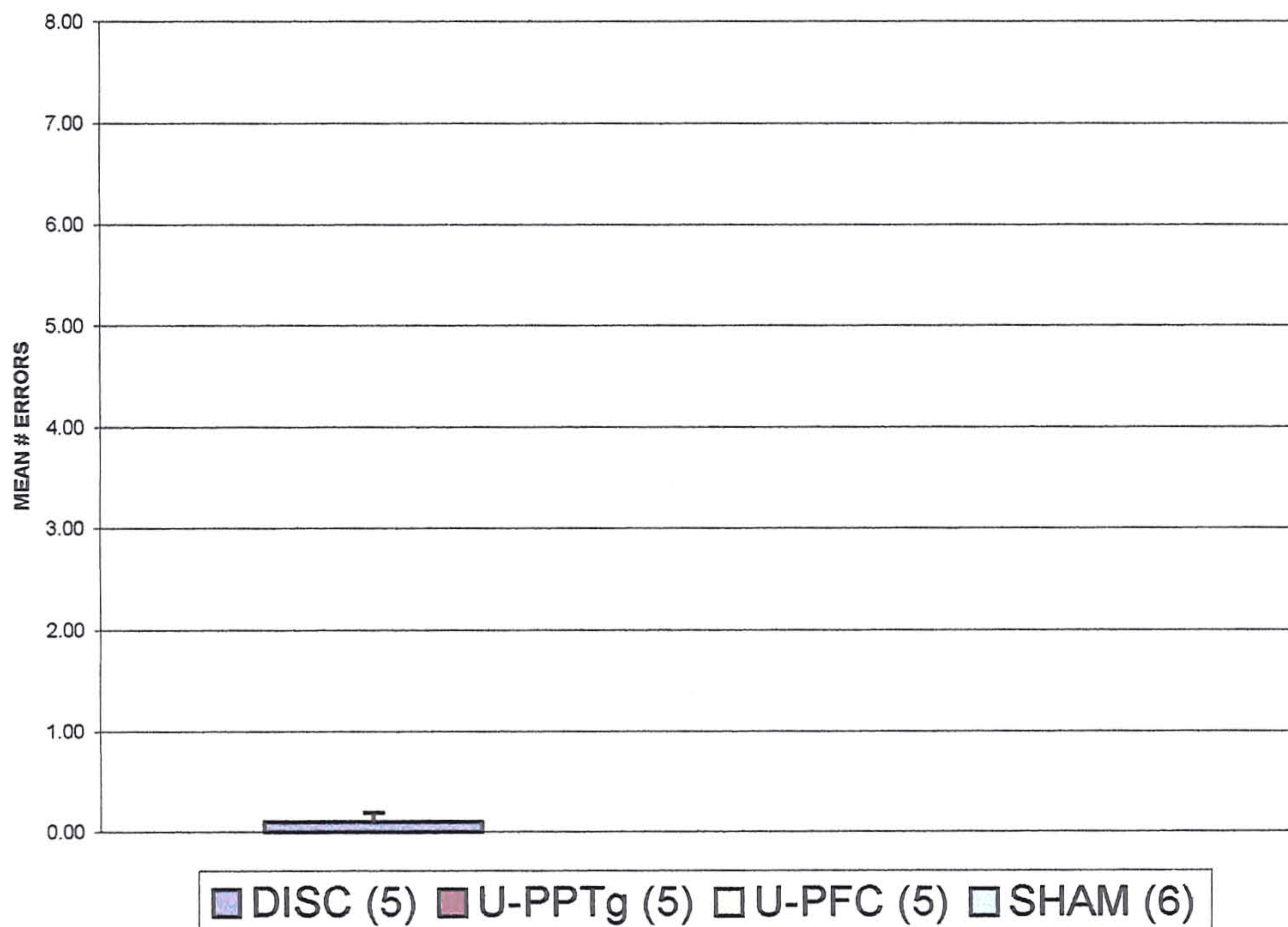


12.3.2 *The DSWS Task: Criterion Days Analysis*

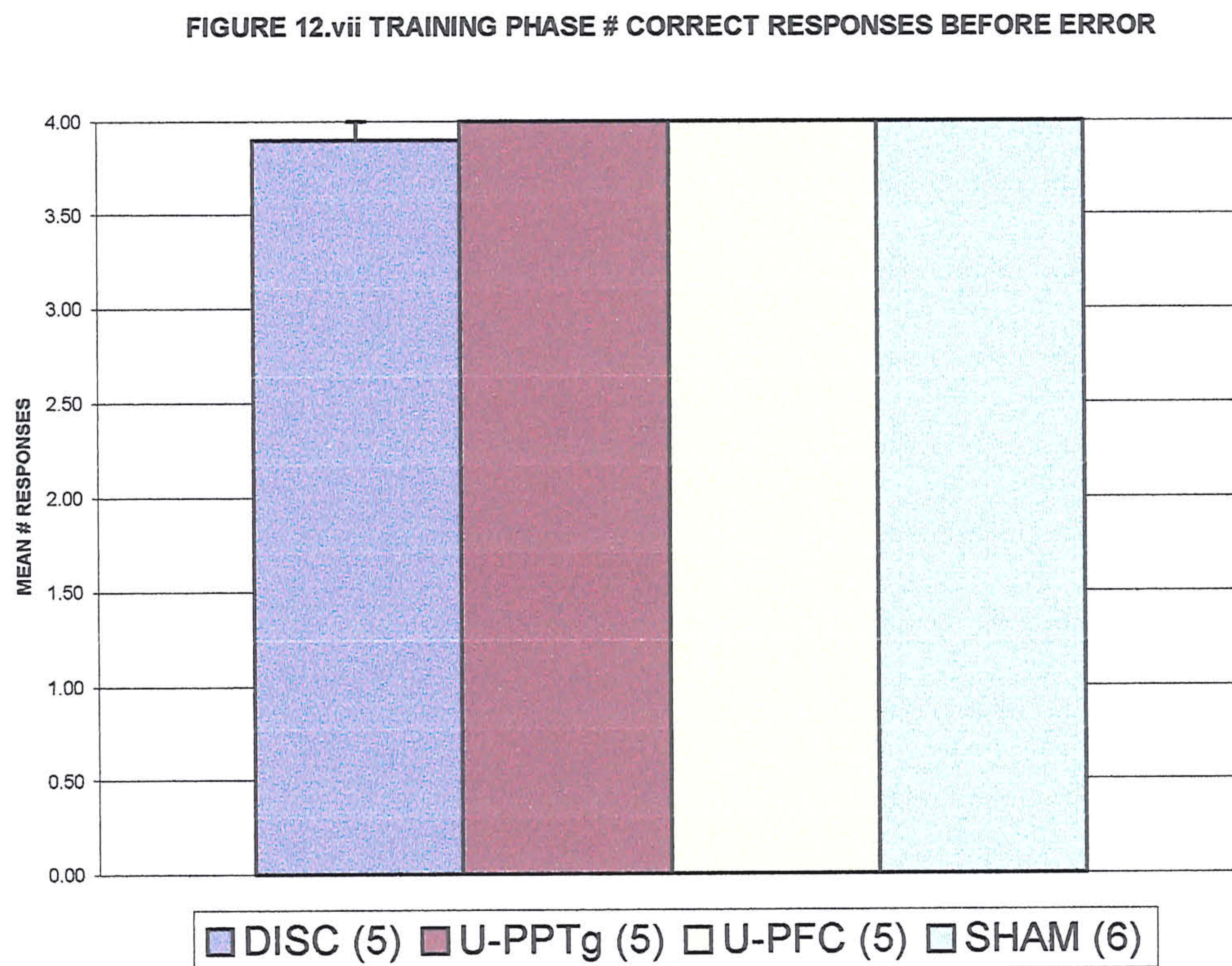
In the graphs that follow the label “DISC” refers to the disconnection lesioned group, the label “U-PPTg” to the unilateral PPTg lesioned group, the label “U-PFC” to the unilateral mPFC lesioned group, and finally, the label “SHAM” represents the sham operated control group.

The data for the mean and standard error for the number of errors made over the criterion days in the training phase are shown in Figure 12.vi. The one-way between subjects ANOVA showed that there were no significant effects of any of the lesions $F(3,17) = 1.08$.

FIGURE 12.vi TRAINING PHASE ERRORS

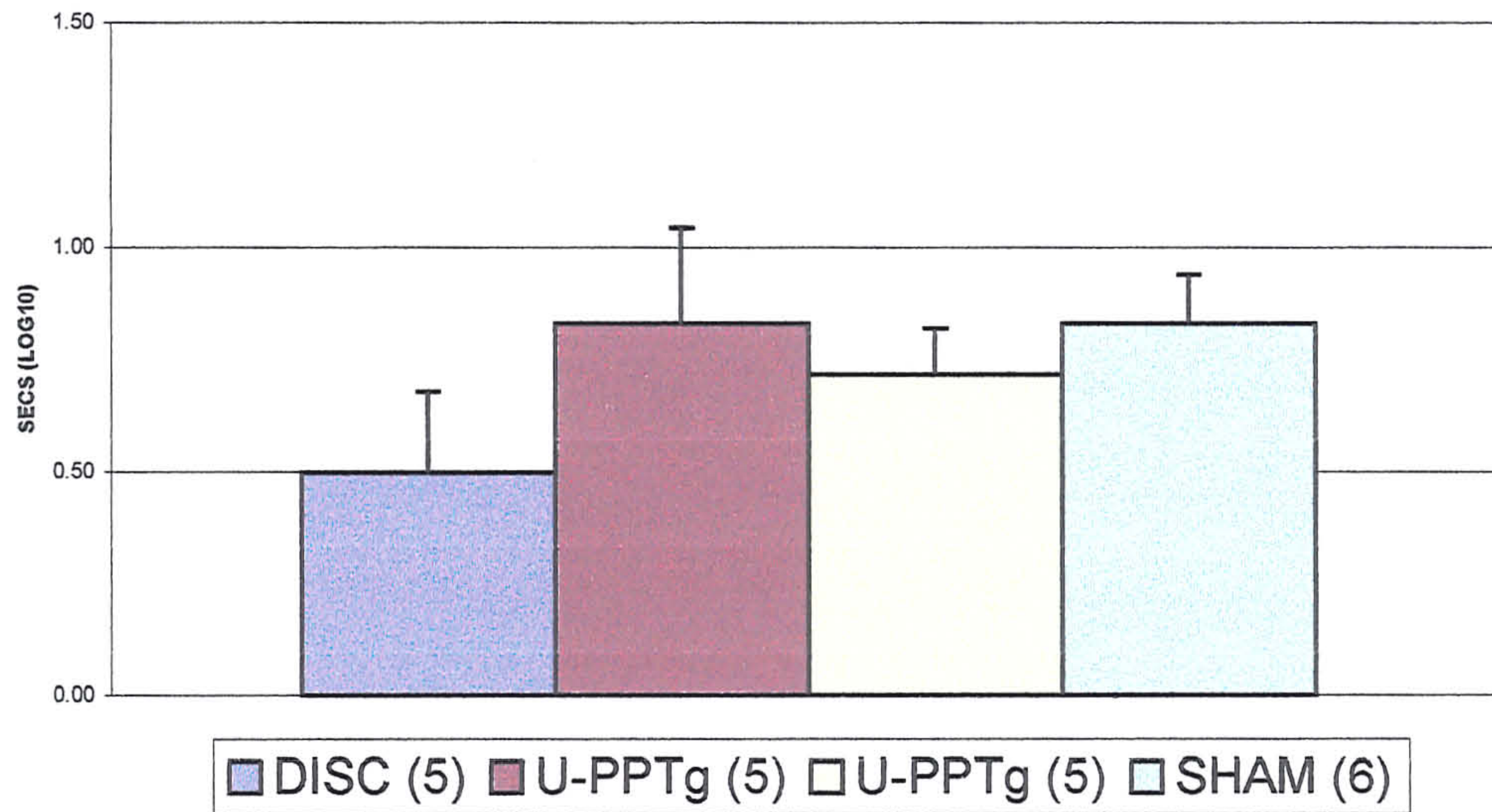


The mean and standard error for the number of correct responses made before error in the training phase over the criterion days can be seen in Figure 12.vii. The ANOVA showed that there were no significant effects of any of the lesions on this measure $F(3,17) = 1.54$.



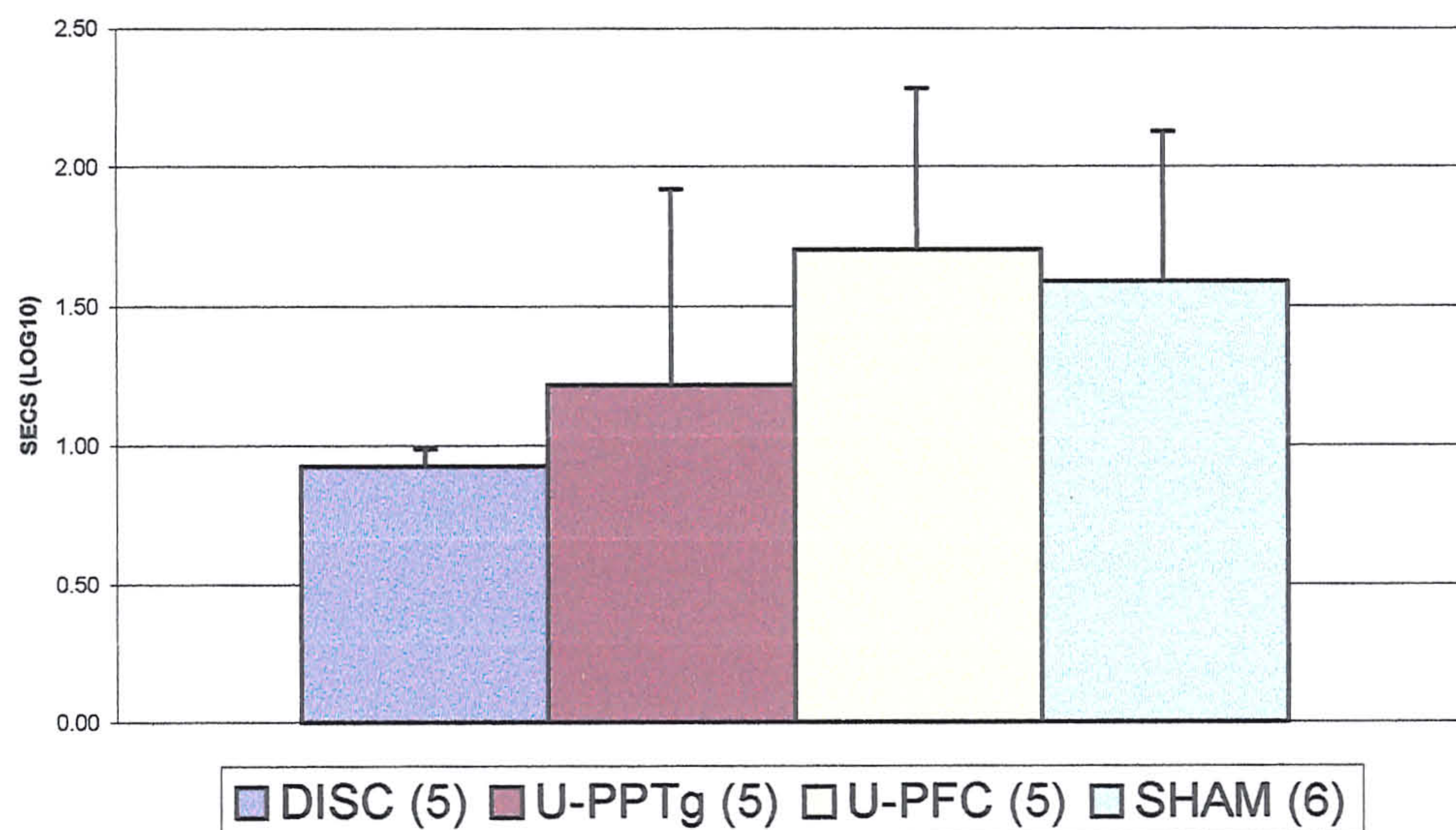
The mean and standard error for the training phase latency to first arm over the criterion days can be seen in Figure 12.viii. There were no significant lesion effects on this measure $F(3,17) = 1.37$.

FIGURE 12.viii TRAINING LATENCY (LOG10)



The mean and standard error for the arm choice time in the training phase over the criterion days can be seen in Figure 12.vix. None of lesions had any significant effects on this measure $F(3,17) = 2.43$.

FIGURE 12.ix TRAINING CHOICE TIMES (log 10)



The mean and standard error for the number of errors made in the test phase over the criterion days can be seen in Figure 12.x. The one-way between subjects ANOVA conducted on this measure revealed that there were no significant lesion effects on this measure $F(3,17) = 1.57$.

FIGURE 12.x TEST PHASE ERRORS

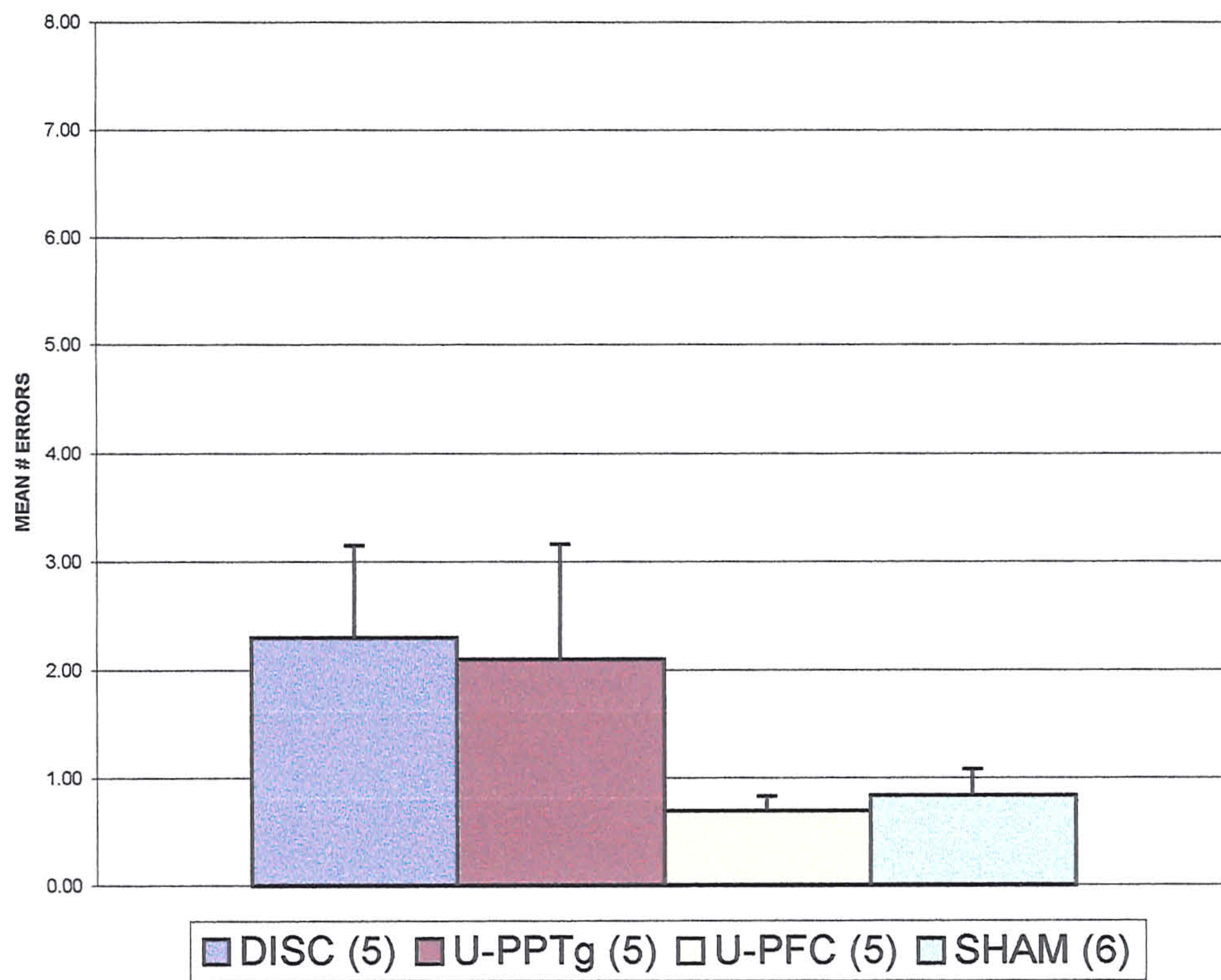
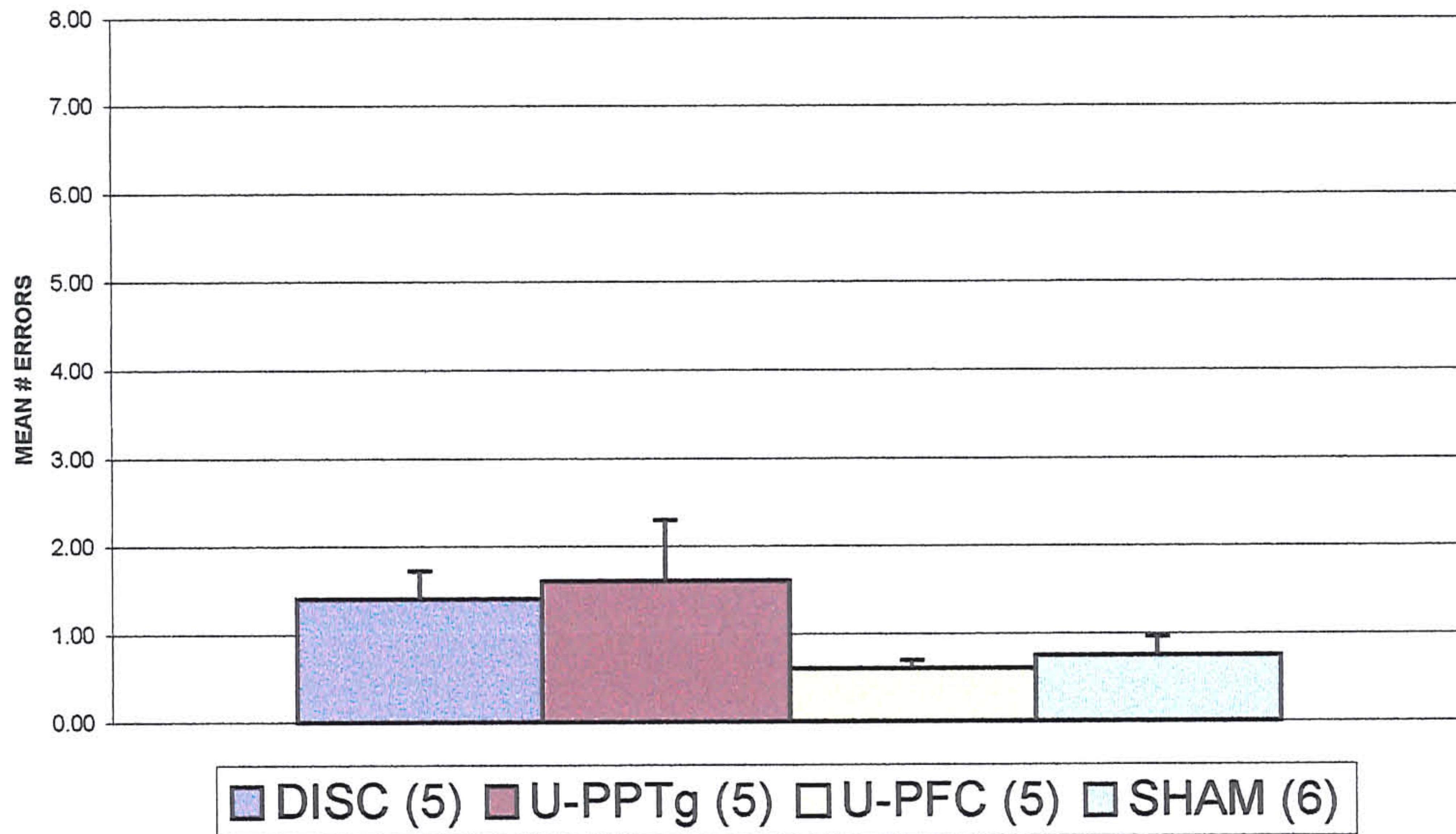


Figure 12.xi shows the mean and standard error for the number of across phase errors made in the test phase over the criterion days. The ANOVA showed that there were no significant effects of any of the lesions $F(3,17) = 1.08$.

FIGURE 12.xi ACROSS PHASE ERRORS



The mean and standard error for the number of within phase errors over the criterion days can be seen in Figure 12.xii. There were no significant lesion effects on this measure $F(3,17) = 1.59$.

FIGURE 12.xii WITHIN PHASE ERRORS

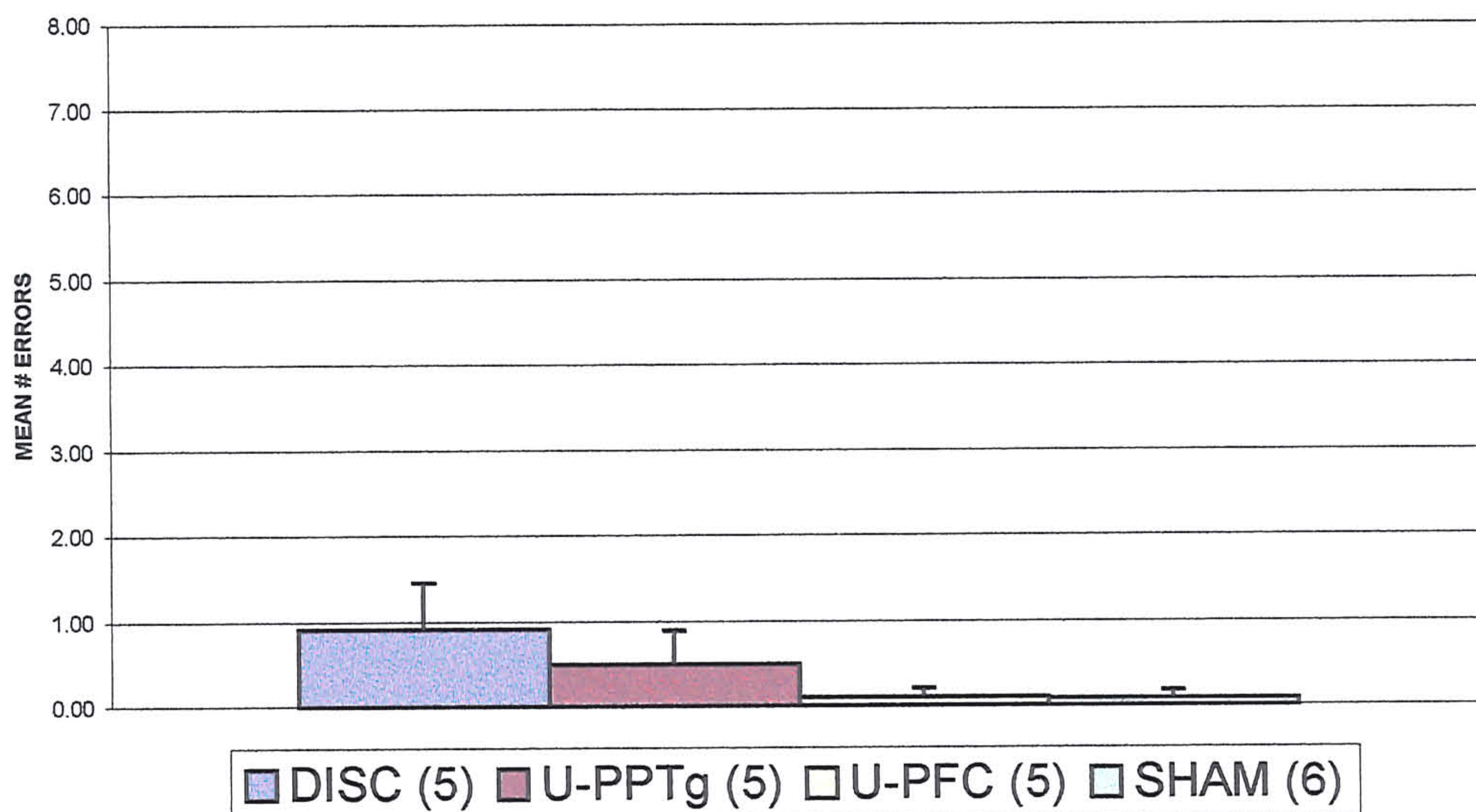


Figure 12.xiii shows the mean and standard error for the number of correct responses before the first error in the test phase over the criterion days. There were no significant lesion effects on this measure either $F(3,17) = 1.38$.

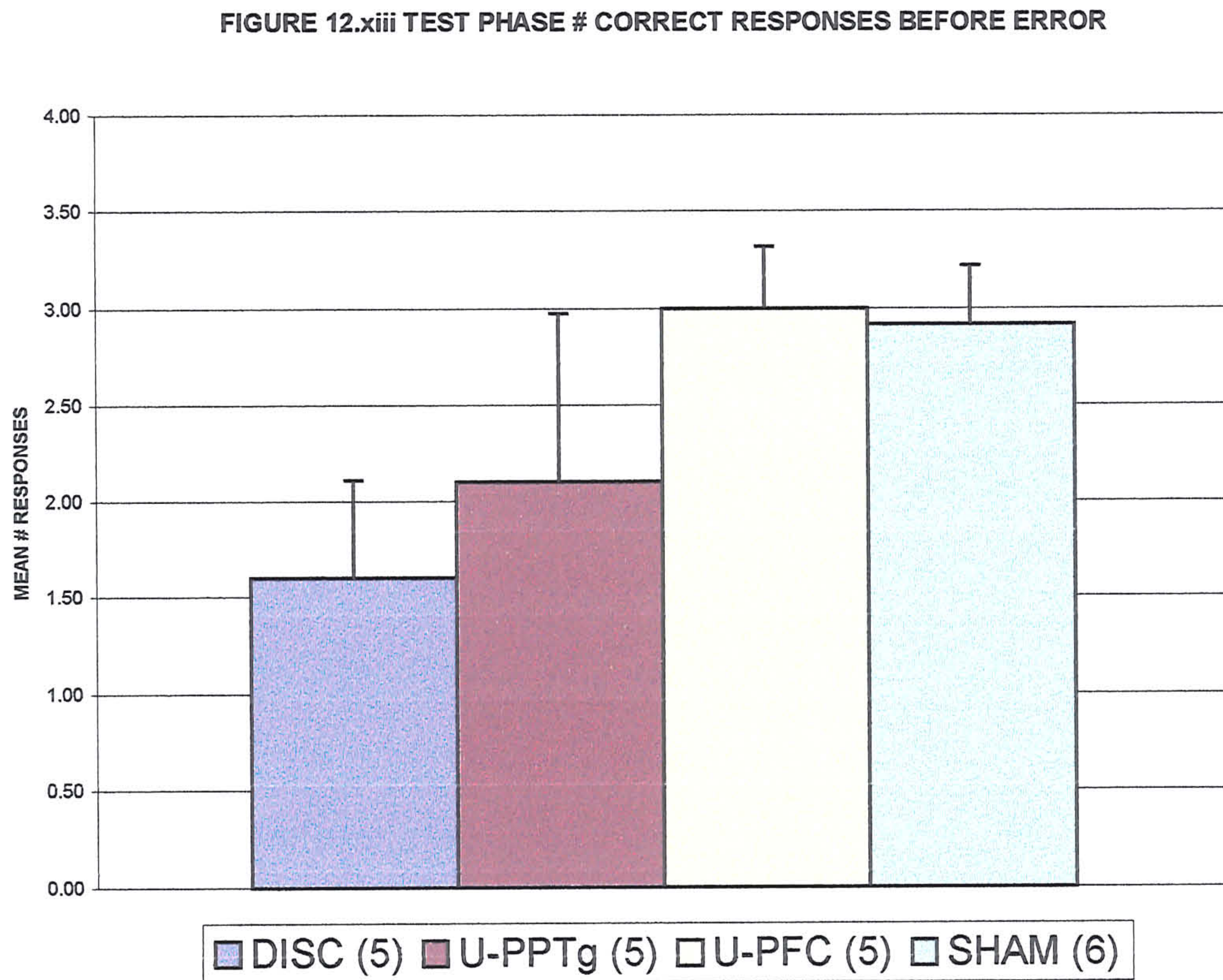
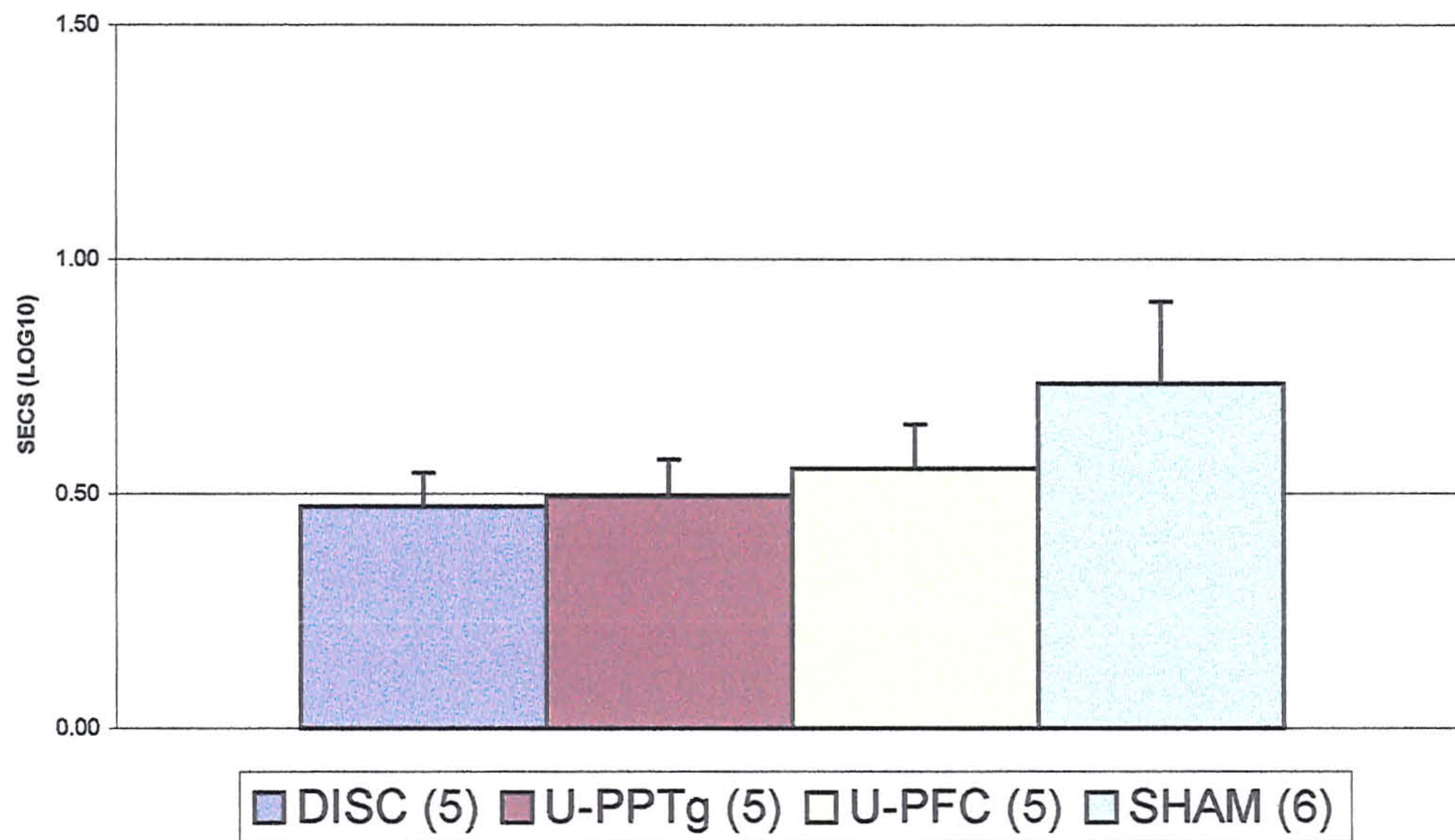


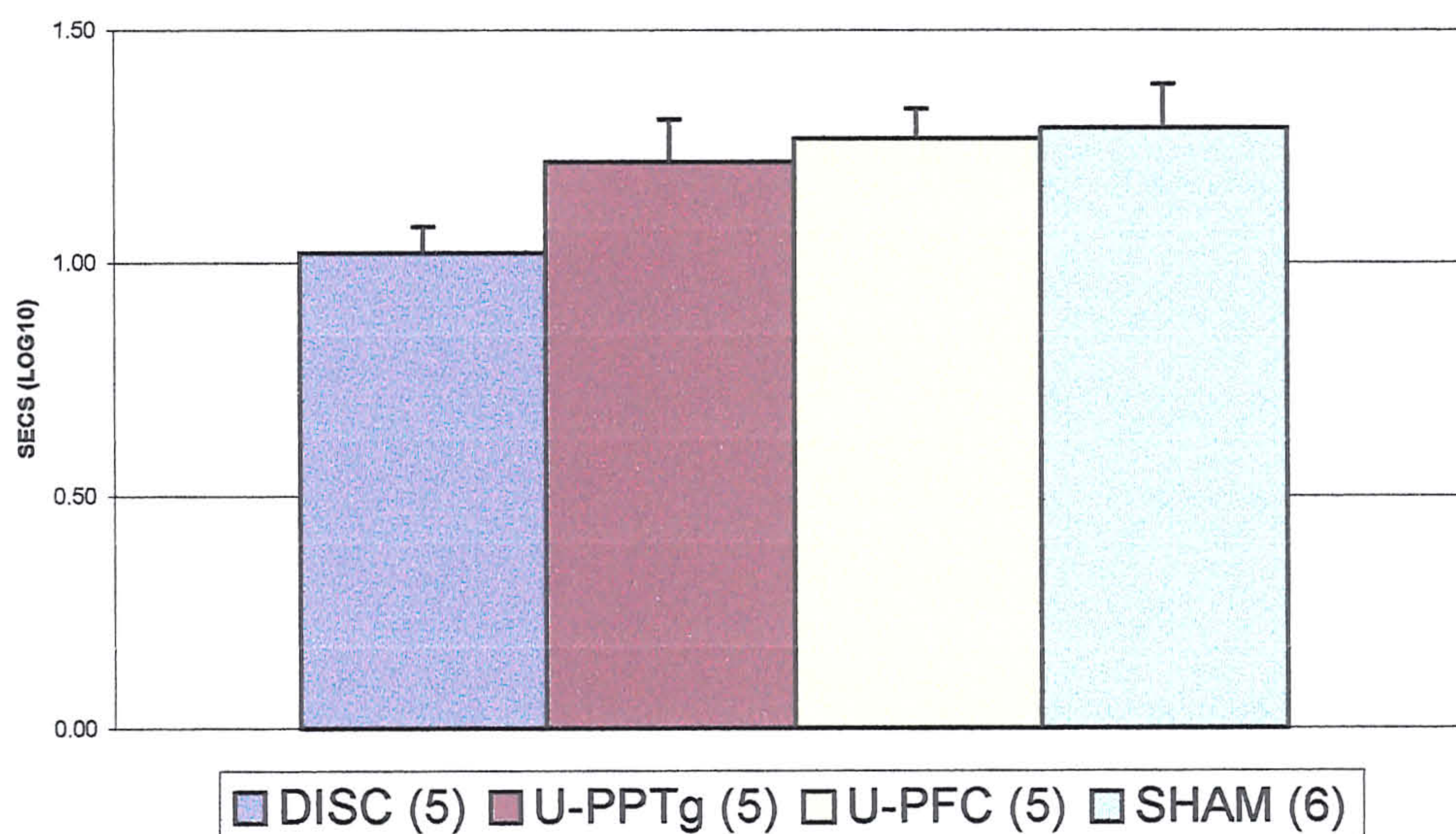
Figure 12.xiv shows the mean and standard error for the latency to the first arm in the test phase over the criterion days. The ANOVA confirmed that there were no significant lesion effects on this measure $F(3,17) = 1.02$.

FIGURE 12.xiv TEST LATENCY (LOG10)



Finally the mean and standard error for the arm choice time over the criterion days in the test phase can be seen in Figure 12.xv. There were no significant lesion effects on this measure either $F(3,17) = 2.28$.

FIGURE 12.xv TEST CHOICE TIMES (log10)



12.3.3 *Summary of Criterion Analysis*

This analysis performed on the criterion days' data indicates that, despite the trends to the contrary in some measures, there were no significant effects of mPFC/PPTg disconnection, or of unilateral PPTg or mPFC lesions on this task. As a result of this the findings of these analyses have not been presented in a summary table. In this experiment, therefore, by the time sham rats had regained asymptote performance none of the lesion groups were significantly impaired. However, this analysis is a very specific analysis and does not address whether lesioned rats were impaired earlier in the post-surgical testing period. To see whether there were impairments limited to the earlier test trials, omnibus ANOVAs were also conducted on the data for all trials.

12.3.4 *The DSWS Task: Omnibus ANOVAs for all trials*

In the graphs that follow the label "DISC" refers to the disconnection lesion group, the label "U-PPTg" to the unilateral PPTg lesion group, the label "U-PFC" to the unilateral mPFC lesion group, and the label "SHAM" to the sham operated controls.

Figure 12.xvi shows the means and standard error obtained for the training phase error measure across all trials. The two-way mixed ANOVA conducted on this data revealed a significant main effect of day $F(5.62,95.49) = 2.25 p < 0.05$. The main effect of group was not significant $F(3,17) = 2.90$ and neither was the interaction $F(16.85, 95.49) = 0.91$.

FIGURE 12.xvi TRAINING PHASE ERRORS

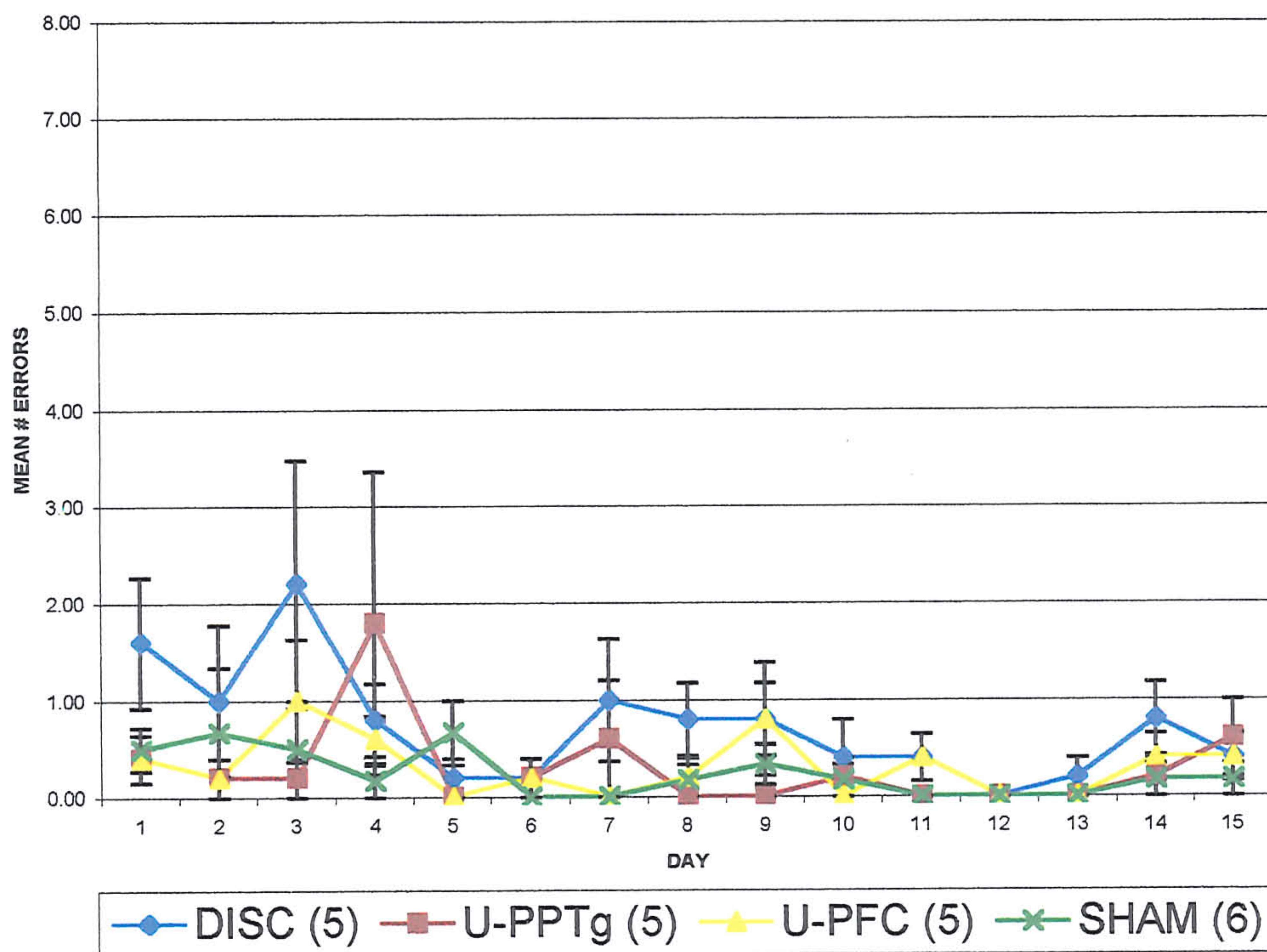
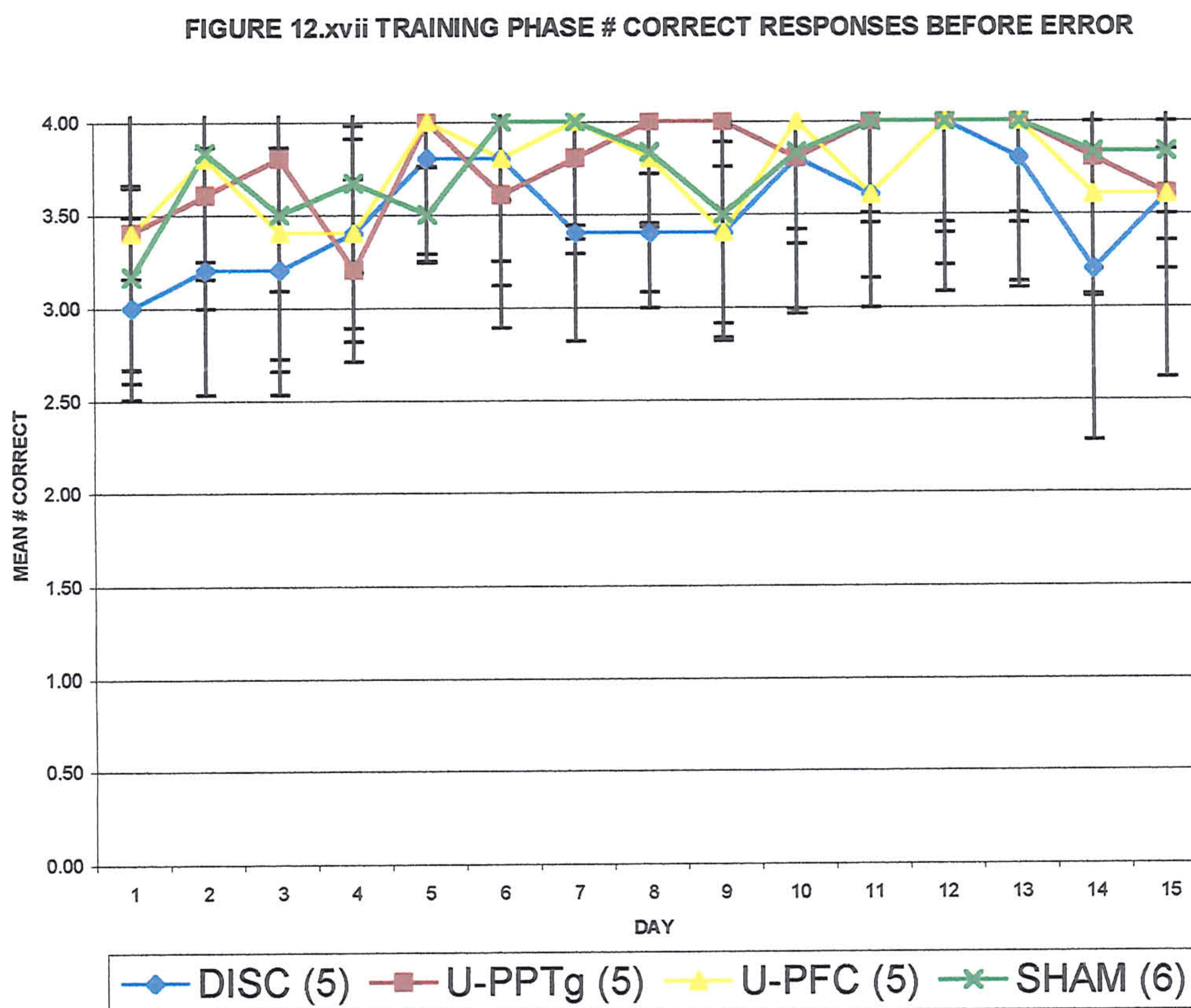


Figure 12.xvii shows the mean and standard error for the number of correct choices before error in the training phase for all trials. The ANOVA conducted on this data showed that there was a main effect of day $F(8.38, 142.40) = 2.63$ $p < 0.01$. The main effect of group was not significant $F(3,17) = 1.67$, and neither was the group x day interaction $F(25.13, 142.40) = 0.51$.



The mean and standard error for the training phase latencies across all trials can be seen in Figure 12.xviii. The two-way mixed ANOVA showed that there was a main effect of day $F(14,238) = 6.32$ $p < 0.01$ but not a main effect of group $F(3,17) = 0.90$. The group \times day interaction was also not significant $F(42,238) = 0.55$.

FIGURE 12.xviii TRAINING PHASE LATENCIES (LOG10)

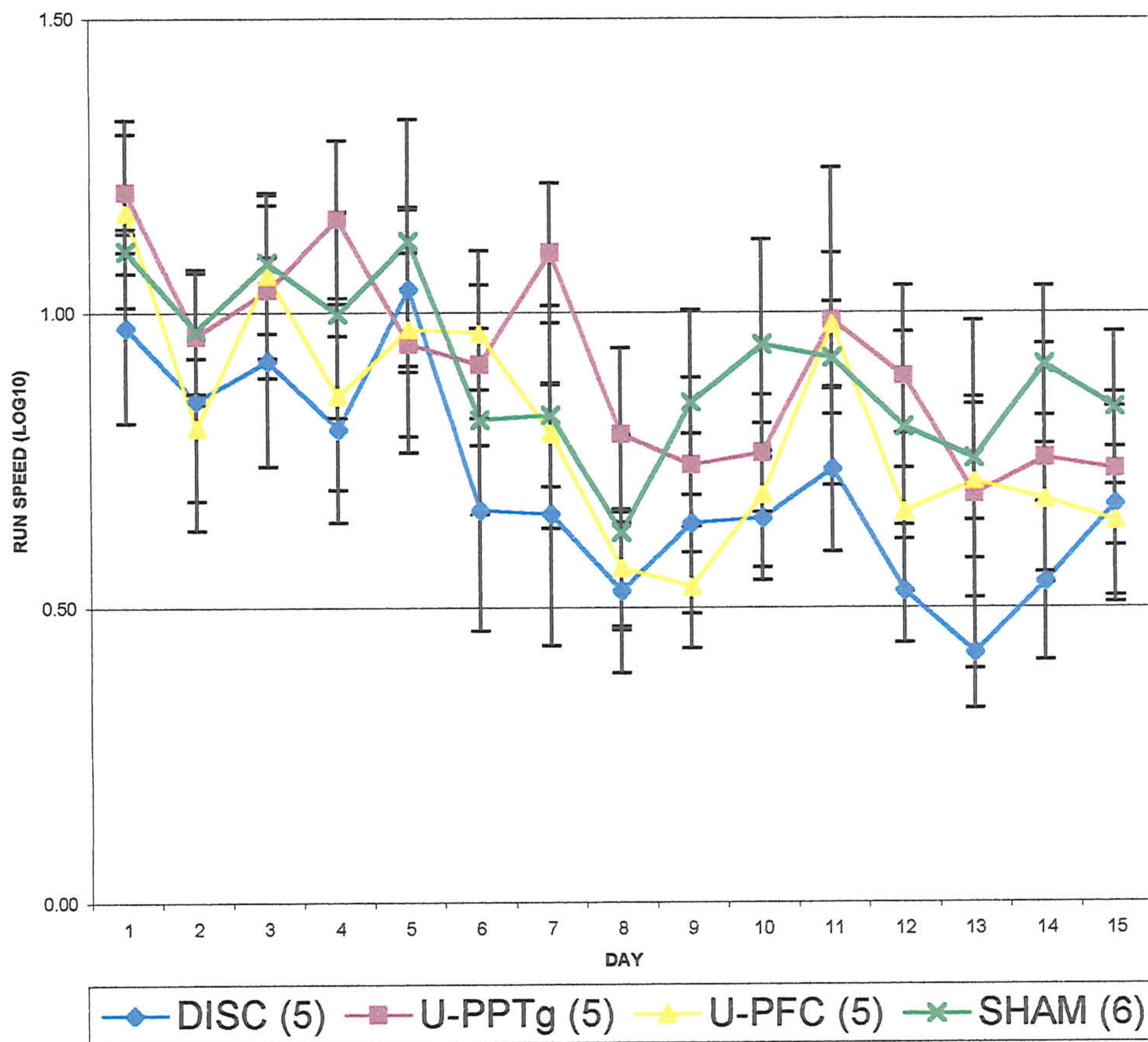
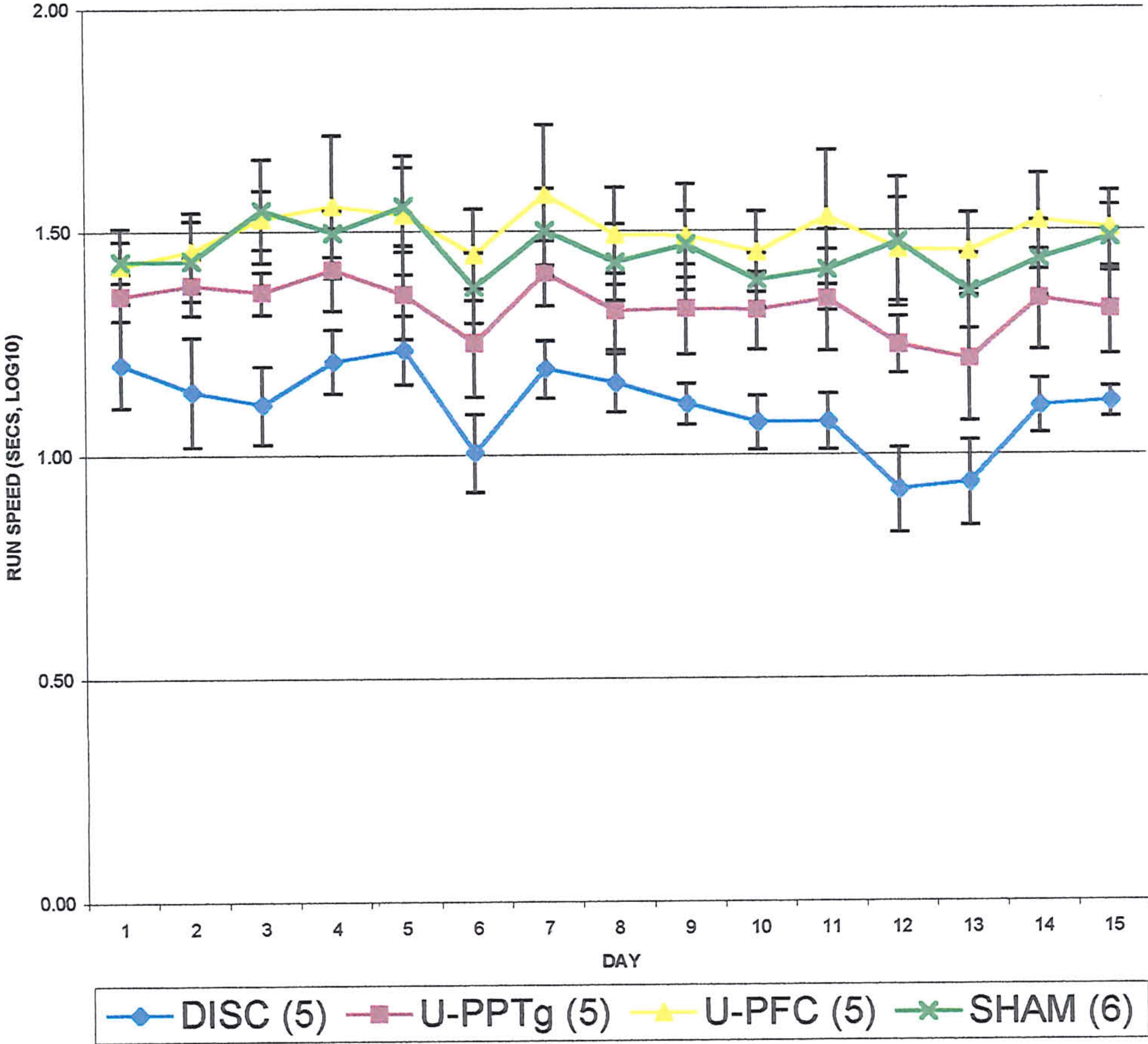


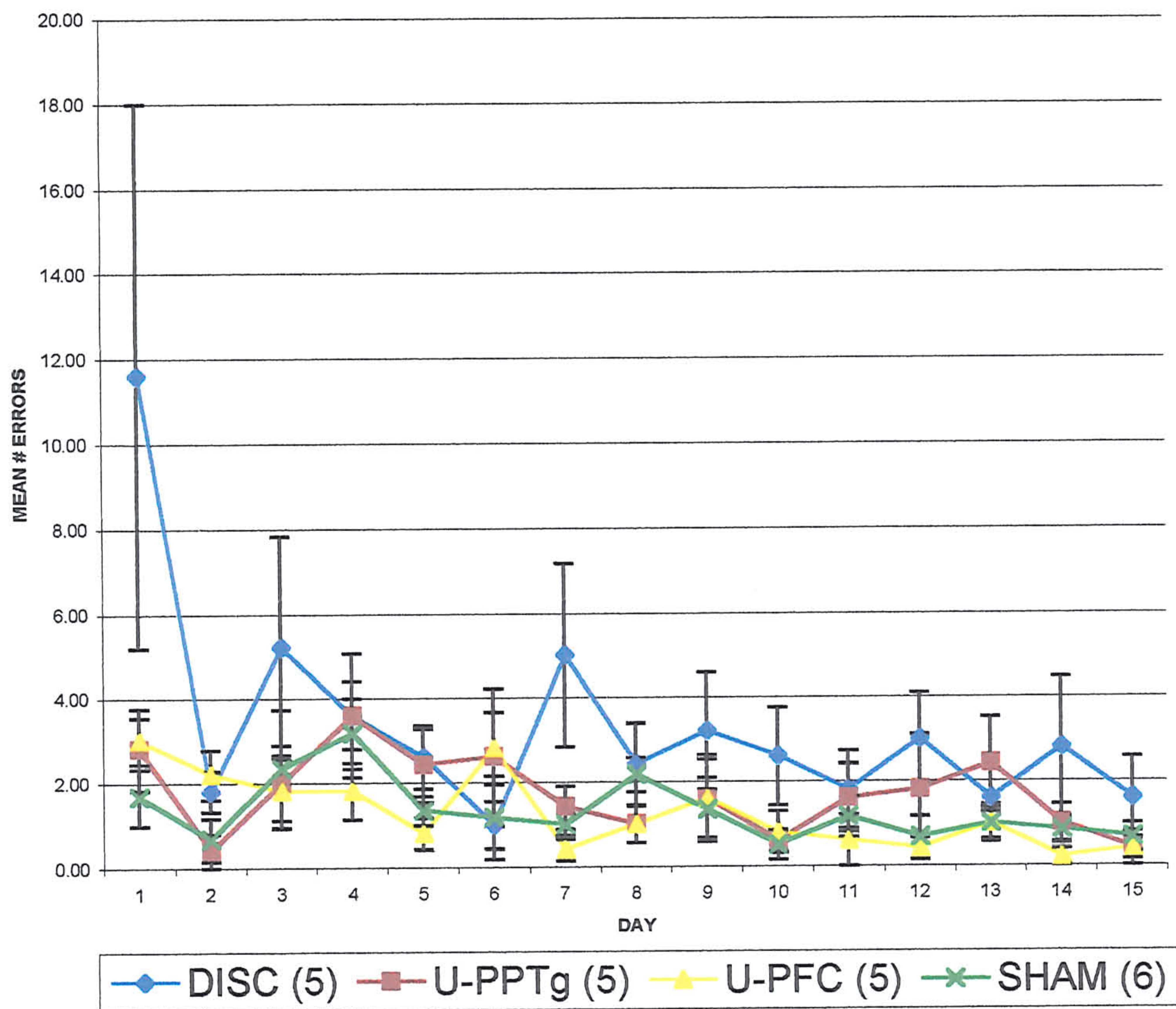
Figure 12.xix shows the means and standard error for the training phase choice times for all trials. The ANOVA showed that there was a main effect of day $F(14,238) = 3.92$ $p < 0.01$ but not a main effect of group $F(3,17) = 1.70$, despite the graph suggesting that the disconnection lesion group are significantly faster than the other groups. However observed power was quite low for the effect of group at 0.37. The group x day interaction was also not significant $F(42,238) = 0.72$.

FIGURE 12.xix TRAINING PHASE CHOICE TIMES (LOG10)



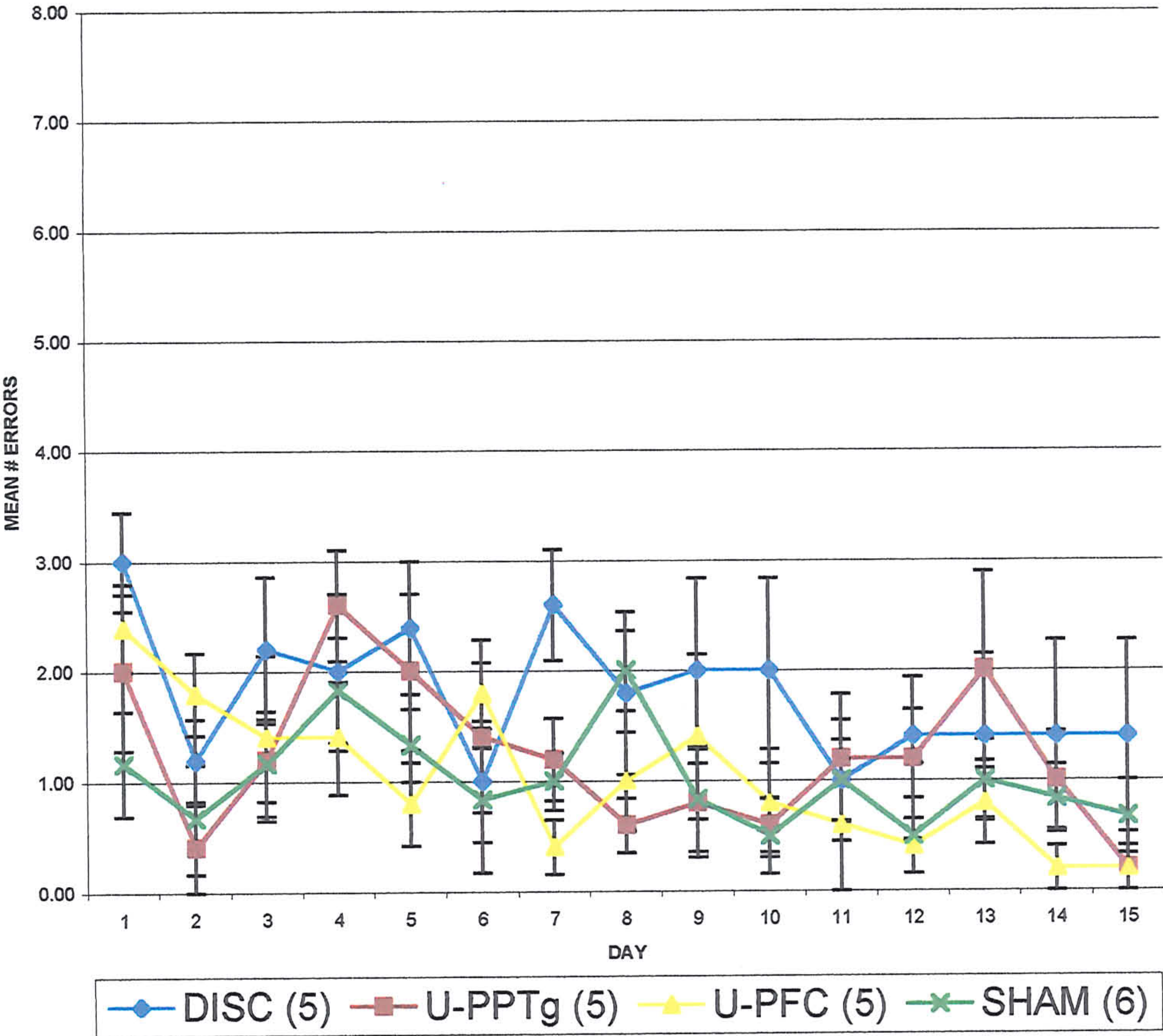
The mean and standard error for the number of test phase errors made for all trials can be seen in Figure 12.xx. The ANOVA showed that there was a main effect of group $F(3,17) = 3.38$ $p < 0.05$, and a main effect of day $F(4.23,71.94) = 3.35$ $p < 0.01$. The group x day interaction was not significant $F(12.70, 71.94) = 1.35$. Bonferroni pairwise comparisons conducted on the main effect of group revealed no significant differences, although the Disc/Sham and Disc/U-PFC comparisons had a significance level of $p = 0.08$. This finding could be due to the low observed power for the group effect at 0.66.

FIGURE 12.xx TEST PHASE ERRORS



The mean and standard error for the number of across phase errors for all trials can be seen in Figure 12.xxi. The two-way mixed ANOVA showed that there was a main effect of day for this measure $F(14,238) = 2.54$ $p < 0.01$, but not a main effect of group $F(3,17) = 2.45$. The group x day interaction was also not significant $F(42,238) = 1.00$.

FIGURE 12.xxi ACROSS PHASE ERRORS



The mean and standard error for the number of within phase errors for all trials can be seen in Figure 12.xxii. The two-way mixed ANOVA conducted on this measure showed, in contrast to the number of across phase errors, that there was a main effect of group $F(3,17) = 3.32$ $p < 0.05$. There was not a main effect of day $F(2.05,34.89) = 2.19$, or a significant interaction $F(6.16, 34.89) = 1.36$. Bonferroni pairwise comparisons showed no significant differences, however the Disc/Sham and Disc/U-PFC comparisons had a significance level of $p = 0.09$. Again observed power was quite low in this experiment since, for the group effect, power was at 0.65.

FIGURE 12.xxii WITHIN PHASE ERRORS

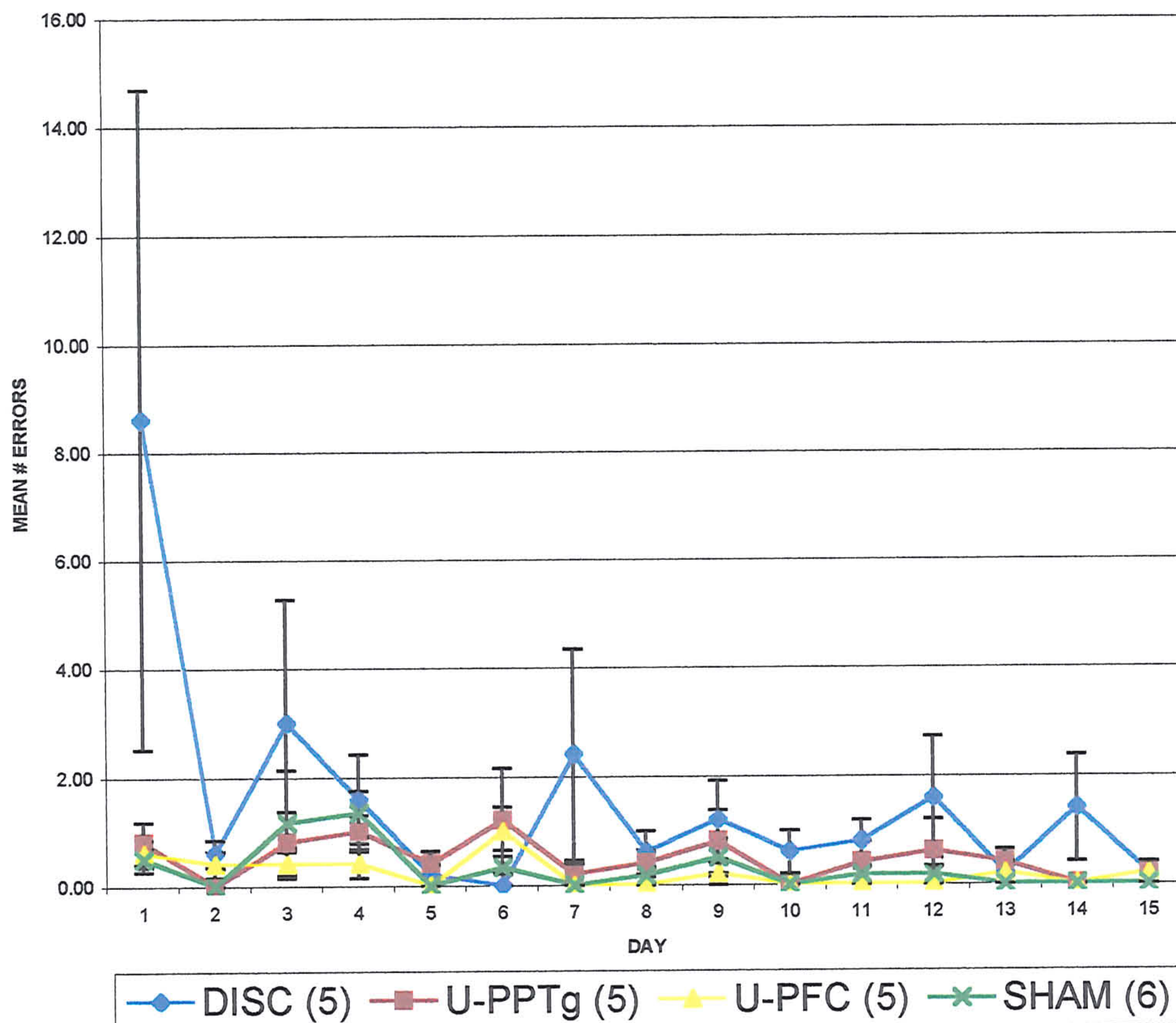


FIGURE 12.xxiii MEAN % OF TOTAL TEST ERRORS THAT WERE AP AND WP

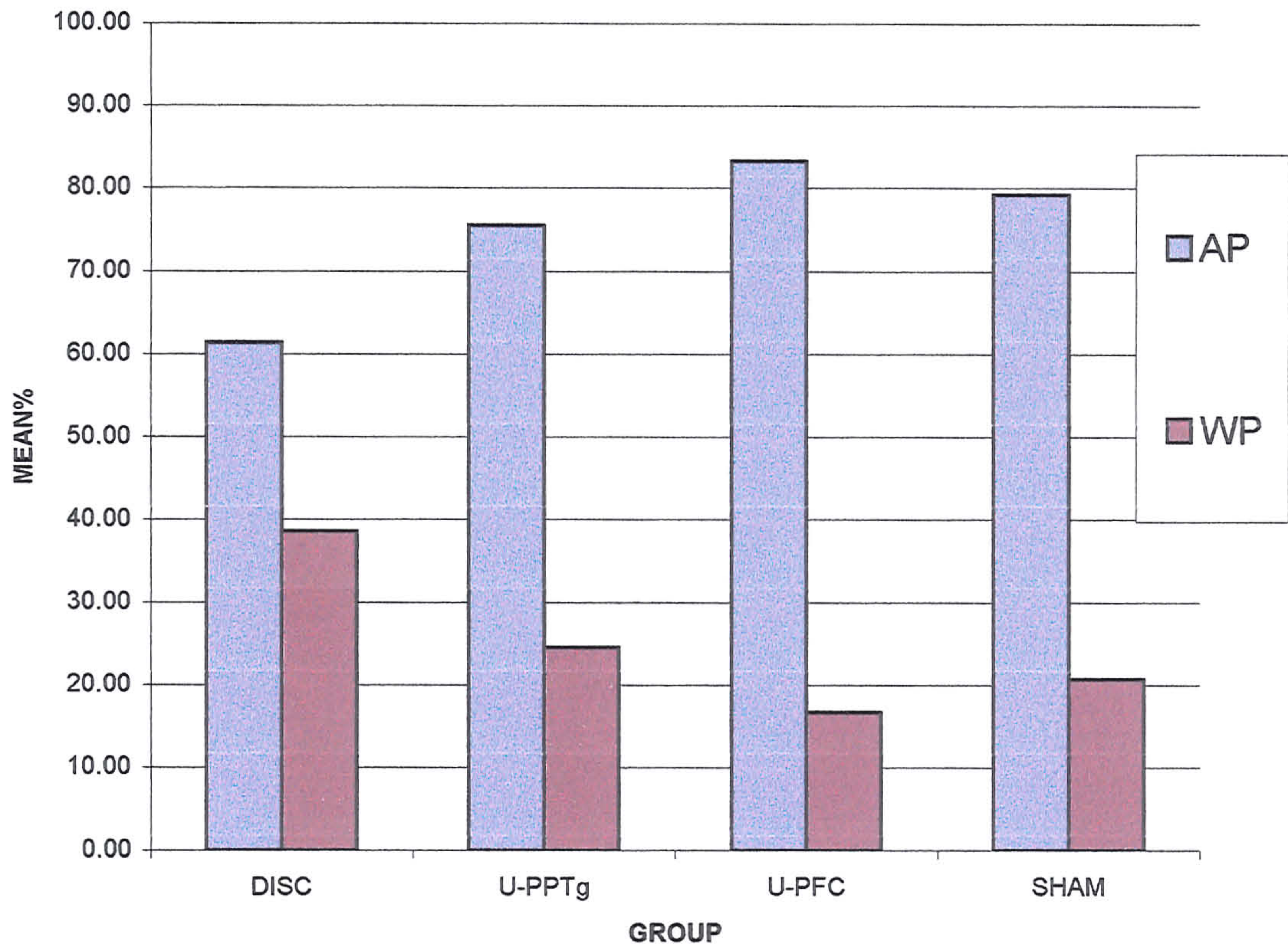


Figure 12.xxiii shows the mean percentage of total test phase errors that were AP and WP for each group, for comparison with the experiments contained in Chapters 8, 9, & 10 where there were also significant lesion effects. This data was calculated across all test days so there were no rats excluded. The data indicates that only the disconnection lesioned group showed a reduced percentage AP/WP disparity in comparison to the sham group. Two-way within subjects ANOVAs conducted for each group on the number of across and within phase errors over all days revealed that there was not a significant effect of error in the Disc group $F(1,4) = 0.43$. In contrast, for the Sham group there was a significant effect of error $F(1,5) = 34.90$ $p < 0.01$, and there was also a significant effect of error in the U-PPTg group $F(1,4) = 56.00$ $p < 0.01$, and in the U-PFC

group $F(1,4) = 205.71$ $p < 0.0001$. Thus the two-way within subjects ANOVAs confirmed the data shown in the percentage graph.

The mean and standard error for the number of correct responses before error for the test phase, across all trials, can be seen in Figure 12.xxiv. The two-way mixed ANOVA showed that there was not a significant main effect of group $F(3,17) = 1.25$, or a significant main effect of day $F(14,238) = 1.68$. The group x day interaction was also not significant $F(42,238) = 1.06$.

FIGURE 12.xxiv TEST PHASE # CORRECT RESPONSES BEFORE ERROR

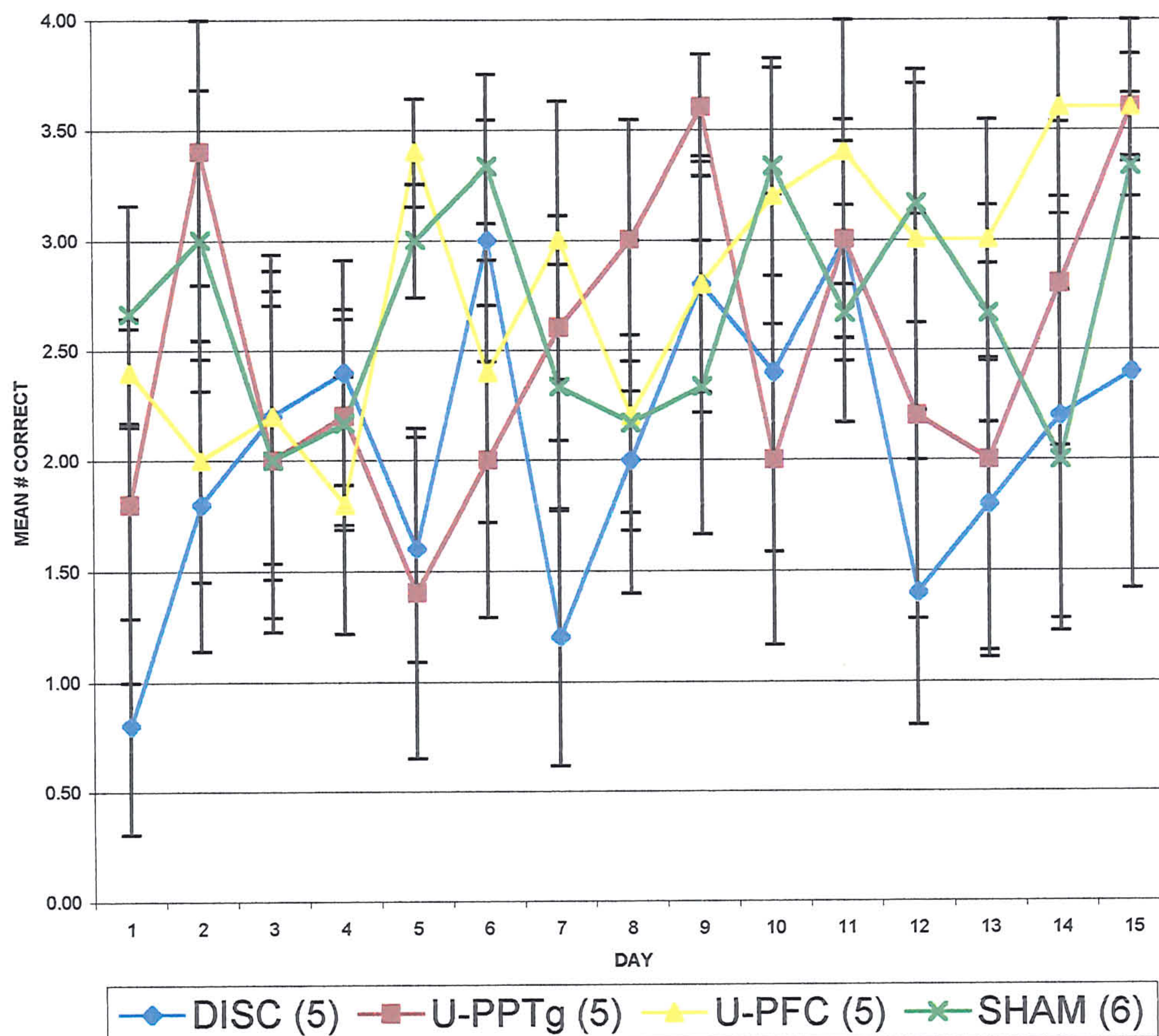
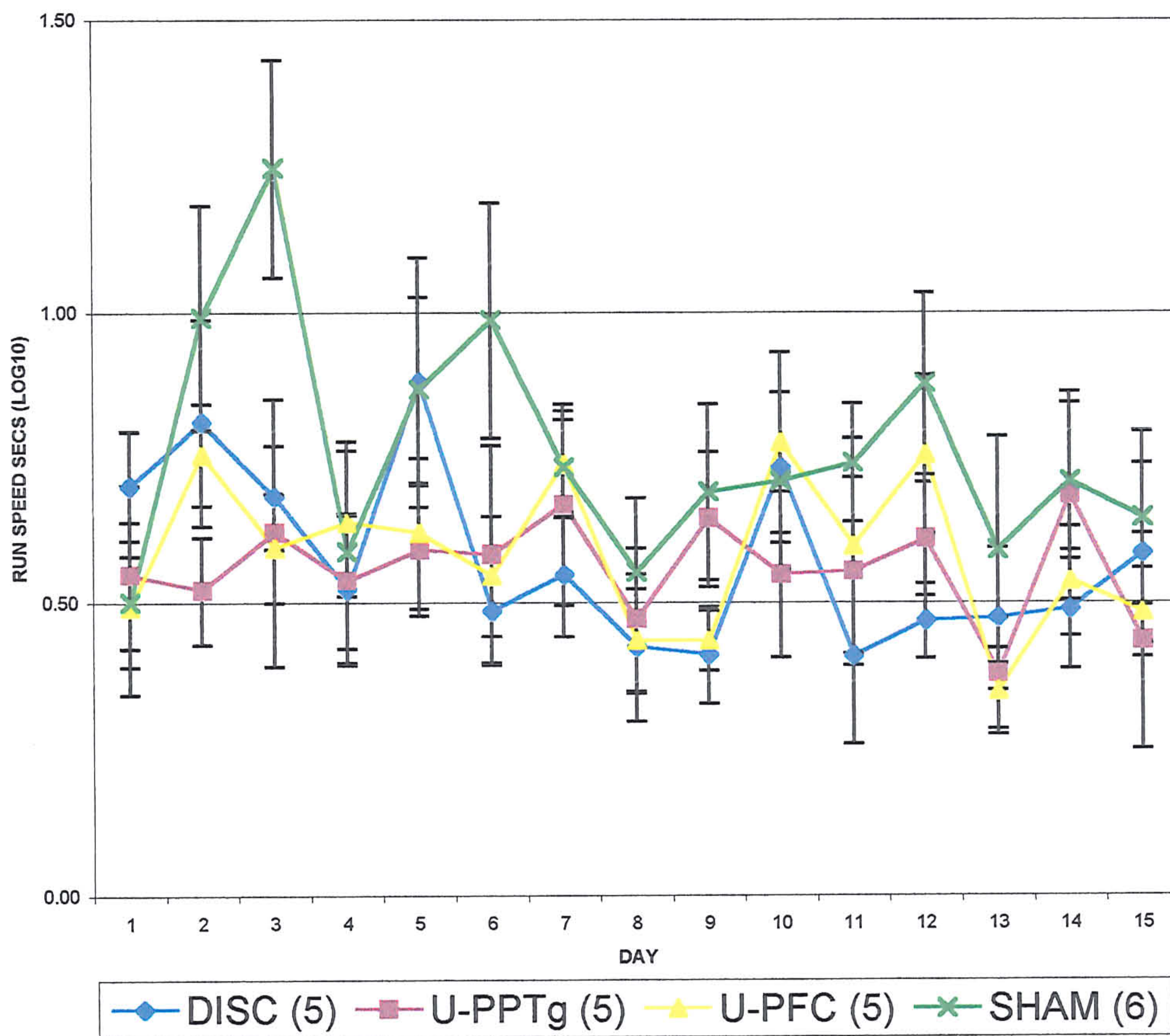


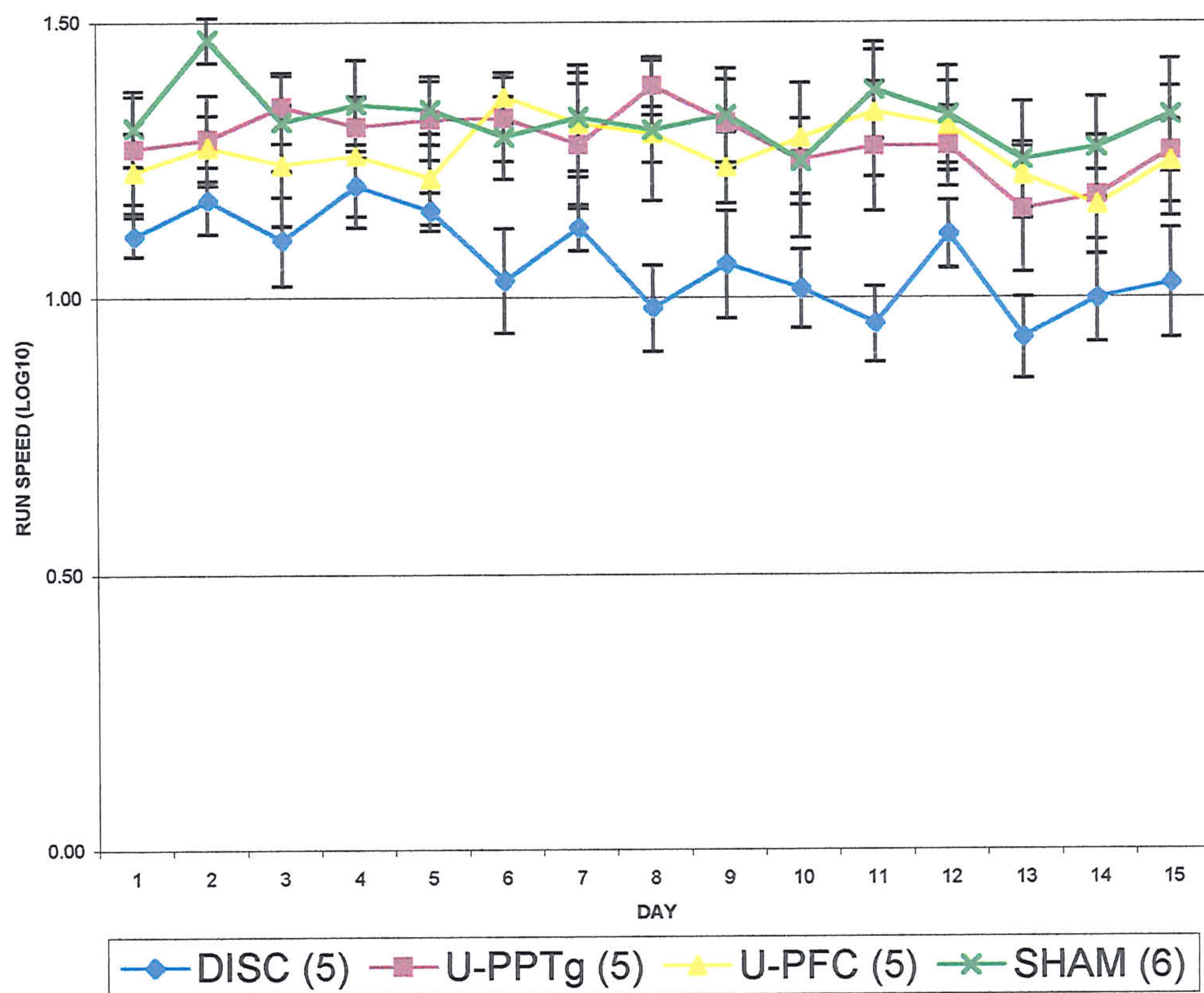
Figure 12.xxv shows the means and standard error for the latencies to the first arm in the test phase across all trials. The ANOVA showed that there was not a main effect of group $F(3,17) = 1.31$. However, there was a main effect of day $F(14,238) = 3.21$ $p < 0.01$. The day x group interaction was not significant $F(42,238) = 1.17$.

FIGURE 12.xxv TEST PHASE LATENCIES (LOG10)



The mean and standard error for the arm choice times for the test phase across all trials can be seen in Figure 12.xxvi. The two-way mixed ANOVA confirmed that there was a main effect of group $F(3,17) = 3.37 p < 0.05$. There was also a main effect of day $F(14,238) = 2.00 p < 0.05$. The group x day interaction was not significant $F(42,238) = 0.78$. Bonferroni pairwise comparisons confirmed that the Disc group were significantly different from the Sham group at $p = 0.05$. Again power was quite low for this group effect at 0.66.

FIGURE 12.xxvi TEST PHASE CHOICE TIMES (LOG 10)



These findings are presented below in a summary table, Table 12a.

Table 12a. Summary of Omnibus ANOVAs

	Lesion	Day	Lesion x Day
Training Errors	ns	p<0.05 All <	ns
Training Correct	ns	p<0.01 All >	ns
Training Latency	ns	p<0.01 All faster	ns
Training Choice Times	ns	P<0.01	ns
Test Errors	p<0.05	p<0.01 All <	ns
AP	ns	p<0.01 All <	ns
WP	p<0.05	ns	ns
AP > WP?	Yes, all but Disc	-	-
Test Correct	ns	ns	ns
Test Latency	ns	p<0.01 All faster	ns
Test Choice Times	p<0.05 Disc faster than Shams	p<0.05 All faster	ns

12.4 Discussion

Although there were no significant impairments in this experiment for any of the lesion groups (disconnection of PPTg/mPFC, unilateral PPTg, or unilateral mPFC) in retention of the DSWS task when assessed via a criterion days' analysis, the omnibus ANOVAs conducted for all trials did find evidence of a significant disconnection lesion deficit. This was characterised by increased test phase errors, a result of increased WP errors only, and faster mean arm choice times in the test phase. Furthermore, the disconnection lesioned group additionally showed a pattern of error that was different to that shown by the other unilaterally lesioned groups and the shams. In contrast the disconnection lesion group did not show any significant evidence of impairment in the training phase of the DSWS task. This pattern of results can be compared with the DSWS retention deficits obtained in bilaterally lesioned PPTg and mPFC rats where there was a significant increase in both AP and WP errors, in addition to the earlier occurrence of errors in the choice sequence, and slower test phase latency to the first arm (Chapters 8 and 10). Thus the disconnection impairment seen in the current experiment does not parallel the impairments produced by bilateral lesions of either structure. This is likely to be the result of the fact that disconnection lesions very specifically investigate the function of one connection of each structure, and each structure has varied connections, and possibly varied functions, depending on the connections activated at a particular time (this may be particularly true for the PPTg - see Chapter 4).

The findings of the current experiment indicate that the disconnection lesion deficit, such as it is, occurs due to a lack of executive control over behaviour, since it appears

only in the more complex test phase where responding at a basic level is not sufficient to solve the task correctly. For example mPFC lesion deficits have often proved sensitive to the number of possible response choices in a task (Porter *et al.*, 1997, & Granon *et al.*, 1994) and it could be the contrast between 4 choices vs. 8 choices that produces the selective test phase impairment (and selective executive function involvement). Alternatively, as suggested for the bilateral mPFC lesion deficit in this task (discussed in Chapter 8), it could be pre-surgical over-training on the training phase due to a disparity in criterion acquisition times that executive involvement is not required in the training phase.

At the point in time where executive functions are needed (the test phase) it is important to note that PPTg/mPFC disconnection lesioned rats do not make significantly more across phase errors, and do not make their errors any earlier. This is vital for the interpretation of the current experimental findings because it could suggest that the working memory component of the task (the win-shift stage) is intact in these rats. However, the very specific increase in WP errors, bringing these into line with AP error amounts, suggests a problem with response inhibition to *recently* baited arms within the test phase. Thus it seems that the performance of PPTg/mPFC disconnection lesioned rats is characterised by a selective response pattern that could indicate an inability to prevent responding based at a simple pre-potent stimulus-reward level at a time when executive control over behaviour should be activated. The fact that they do not make a significantly greater number of across phase errors suggests that the executive working memory function is operational in these animals, and indeed does function during the initial stages of the test phase. However, once reward has been received and the animal is faced with the next complex arm choice the pre-potent response tendency to repeat a

response recently associated with reward frequently overrides the learned win-shift rule. Contrary to the original hypothesis therefore, the current findings indicate that the PPTg may not function *per se* in executive functions, but that it is *necessary* for executive functions to occur. Its role might be best characterised then, at least in this task, as the withholding of simple response tendencies such that executive control over behaviour can take place. This explanation fits well with the finding that the disconnection lesion group also have significantly faster choice times, a factor that cannot be explained by heightened motivation since the latency to the first arm in either phase was not affected by the disconnection lesions.

Given the importance of the current experimental findings, it is also reassuring to note that unilateral lesions of either the PPTg or the mPFC were not sufficient to produce the disconnection lesion effect in retention of the DSWS seen in the current experiment, or the bilateral lesion effects seen in Keating & Winn (2001) or Chapters 8 and 10. This combines well with the findings of the previous experiment (Chapter 11) showing that unilateral lesions of the mPFC or PPTg are also not sufficient to produce and impairment in DSWS task acquisition.

13. DSWS RETENTION FOLLOWING CROSSED UNILATERAL DISCONNECTION LESIONS OF THE PPTg AND mPFC (2).

13.1 Introduction

The findings of the preceding chapter provided some evidence that the PPTg is involved in mediating executive behaviours previously associated with the mPFC. In that experiment, rats with crossed unilateral disconnection lesions of the PPTg and mPFC were shown to be impaired in retention of the DSWS task, a validated task of working memory for spatial location (Olton and Samuelson, 1974). This impairment was characterised differently to the impairments produced following bilateral lesions of either the mPFC or the PPTg, similar in nature to each other. In the previous experiment PPTg and mPFC disconnected rats showed elevated WP error scores but not elevated AP error scores, accompanied with faster arm choice times in the test phase. Furthermore, in that experiment unilateral lesions to either structure individually were not found to be sufficient to produce either the effects seen in retention of the DSWS task following bilateral lesions, or disconnection lesions. However, group numbers in the preceding experiment were low and thus did not permit the optimal exclusion of animals with damage extending to structures surrounding the PPTg and the mPFC. In addition, while unilateral controls were used to assess the effects of placing lesions of the PPTg or the mPFC in one hemisphere, the effects of making unilateral lesions in both structures *in the same hemisphere* were not investigated. The current experiment was therefore conducted with two aims. The first was to increase the existing disconnection lesion subject sample from the preceding experiment. In addition to carrying out the standard analyses between the current experimental groups it was

hoped that this experiment would enable a combined analysis across both experimental disconnection lesion groups such that only those subjects with damage restricted to the PPTg and mPFC could be included. The second aim was to address whether the disconnection effects shown in the preceding chapter would also be seen following lesions of similar magnitude and location but that did not specifically “disconnect” the PPTg and mPFC.

13.2 Methods

A total of 30 rats began DSWS pre-surgical training to criterion. However, 2 were excluded within the first 7 days of training because they ceased to make arm responses. Although both rats had begun the pre-surgical training performing similarly to the other rats, and thus were not obviously distinguishable from them, they were excluded from the experiment when each had spent 2 consecutive complete trials (a total of 4 placements in the maze) sitting in the central platform without attempt to investigate the arms. The retained group of 28 rats took a total of 17 days to reach acquisition criterion (defined in “General Methods”). The remaining rats weighed approximately 320g at the time of surgery.

Surgery was conducted using the coordinates and procedures stated previously (General Methods and Chapters 8 and 10). Rats were allocated into 3 groups with matching mean body weights. Those in the disconnection lesion and sham lesion groups received infusions as discussed in the preceding chapter (Chapter 12). The “lesion control” group received a similar pattern of infusions as the disconnection group except that the unilateral lesions of the PPTg and mPFC were in the same, rather than the opposing,

hemisphere. As before, mPFC surgery was conducted in the first week and PPTg surgery in the following week. A total of 27 rats survived surgery. All rats were allowed 7 days recovery from the date of the final surgery before re-imposition of food restriction (100% weight was calculated as weight on day 7). Body weight and food and water intake were assessed closely during this time to monitor progress of recovery. Once all rats reached their 85% target weight postsurgical testing began and was conducted as discussed under “General Methods”. Testing continued until sham lesioned rats regained criterion performance.

Perfusion and histology was as stated previously (General Methods). PPTg lesions were assessed using NADPH diaphorase and NeuN immunohistochemistry with a cresyl counter stain. mPFC lesions were assessed using the NeuN/cresyl counter stain. The NeuN/cresyl counter stain technique had been developed at this laboratory by M.P. Latimer immediately prior to this experiment and had been shown to be an excellent method for visualising lesions of the central and basolateral nucleus of the amygdala. For this technique, all sections were processed first for NeuN immunohistochemistry using the optimal procedures for PPTg and mPFC selected on the basis of the results of Chapter 12. Once mounted onto slides these NeuN stained sections were then processed for cresyl violet staining using the cresyl method discussed in “General Methods”.

In line with the previous chapters, data for this experiment was first analysed using one way between subjects ANOVAs conducted on the criterion days mean values. Given the restrictions that accompany this method of analysis (see Chapter 12) the data was also then analysed using omnibus two-way mixed ANOVAs on the data for all trials.

This was done to facilitate comparisons with the data from the preceding chapter. Where necessary the ANOVAs were corrected using the Huynh Feldt correction.

13.3 Results

13.3.1 *Histology*

Both mPFC and PPTg lesions were clearly visible under the NeuN/cresyl counter stain technique. Furthermore, the boundaries of the damage could be more easily distinguished than when using either stain on its own (as in the previous experiment). Figure 13a shows an example of a PPTg lesion revealed by the NeuN/cresyl counter stain and Figure 13b shows an example of a mPFC lesion. Comparison with Figures 13c and 13d that show cresyl violet stained lesions of the PPTg and mPFC respectively, along with Figures 12a and 12b from the preceding chapter, illustrates the relative benefit of combining both stains.

Both sets of lesions in this experiment varied considerably in the amount of damage that occurred to surrounding structures. PPTg lesions were extremely large and damage was frequently observed in the substantia nigra, LDTg, cuneiform nucleus and retro-rubal field. In contrast mPFC lesions were usually too small, or were not ideally situated to include acceptable loss of AC, PL or both. Given these problems, but in conjunction with the requirement to create reasonable group sizes, animals were excluded on the basis of mPFC lesions only. This was because, despite the amount of damage sustained to structures neighbouring the PPTg, loss of the PPTg was frequently complete while mPFC damage was not. Lesions were therefore included in the analyses if there had

Figure 13a: Neun ABC with DAB stain plus cresyl violet counterstaining for PPTg lesions (+1.70mm from Interaural line).



Figure 13b: Neun ABC with DAB stain plus cresyl violet counterstaining for mPFC lesions (+2.70mm from Bregma).

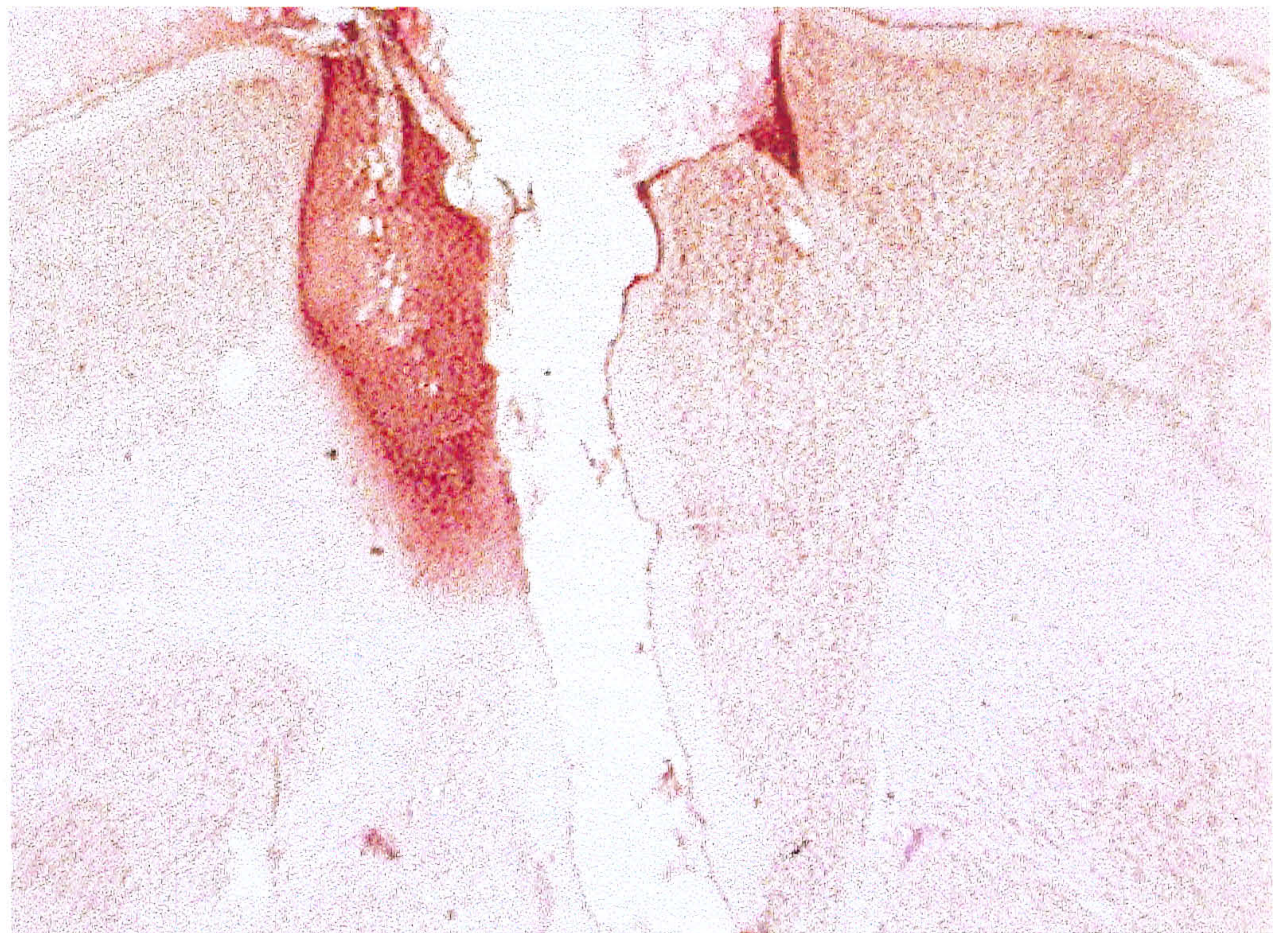


Figure 13c: Example of cresyl violet staining for PPTg lesions (+0.28mm from Interaural line).

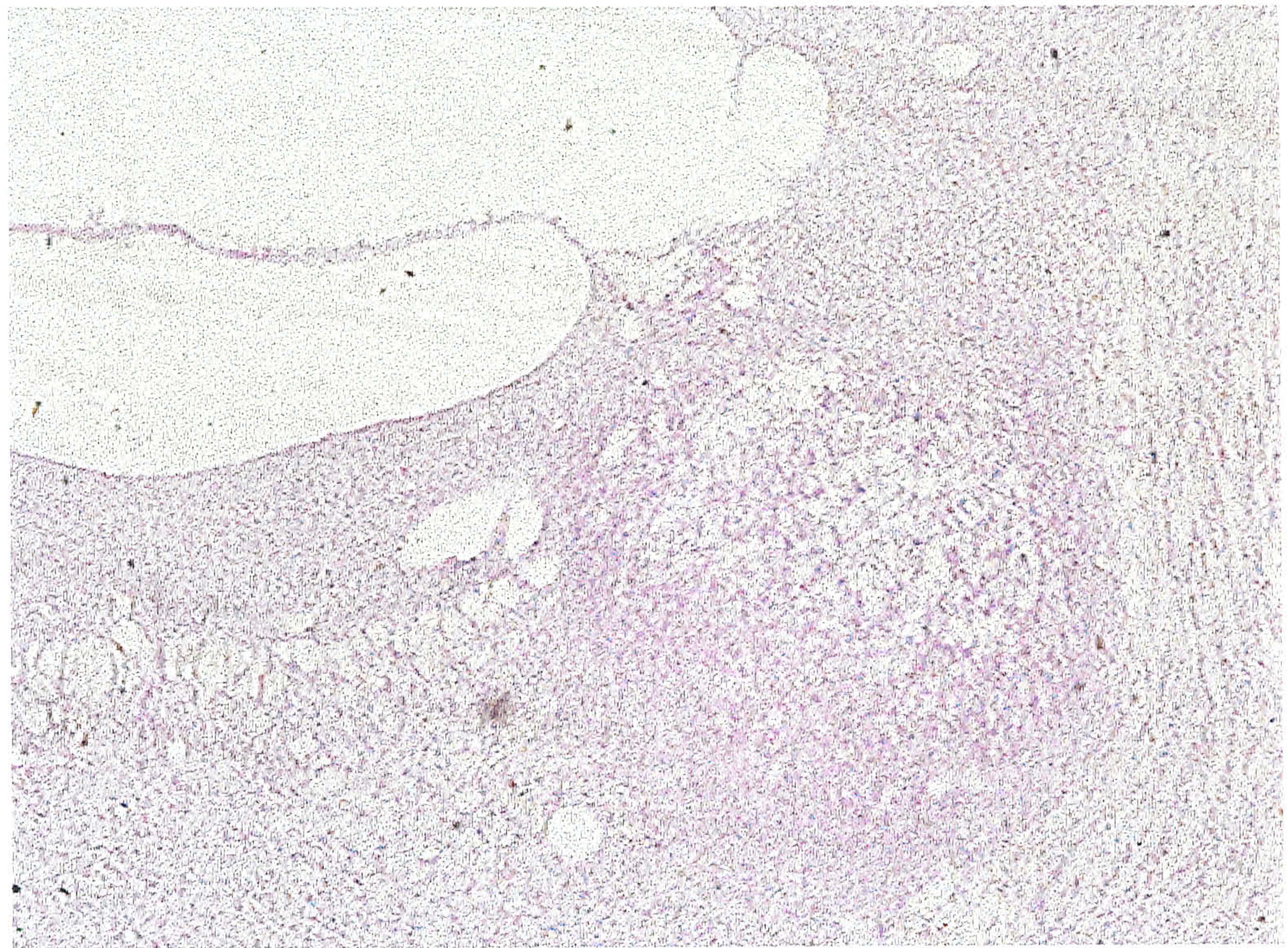
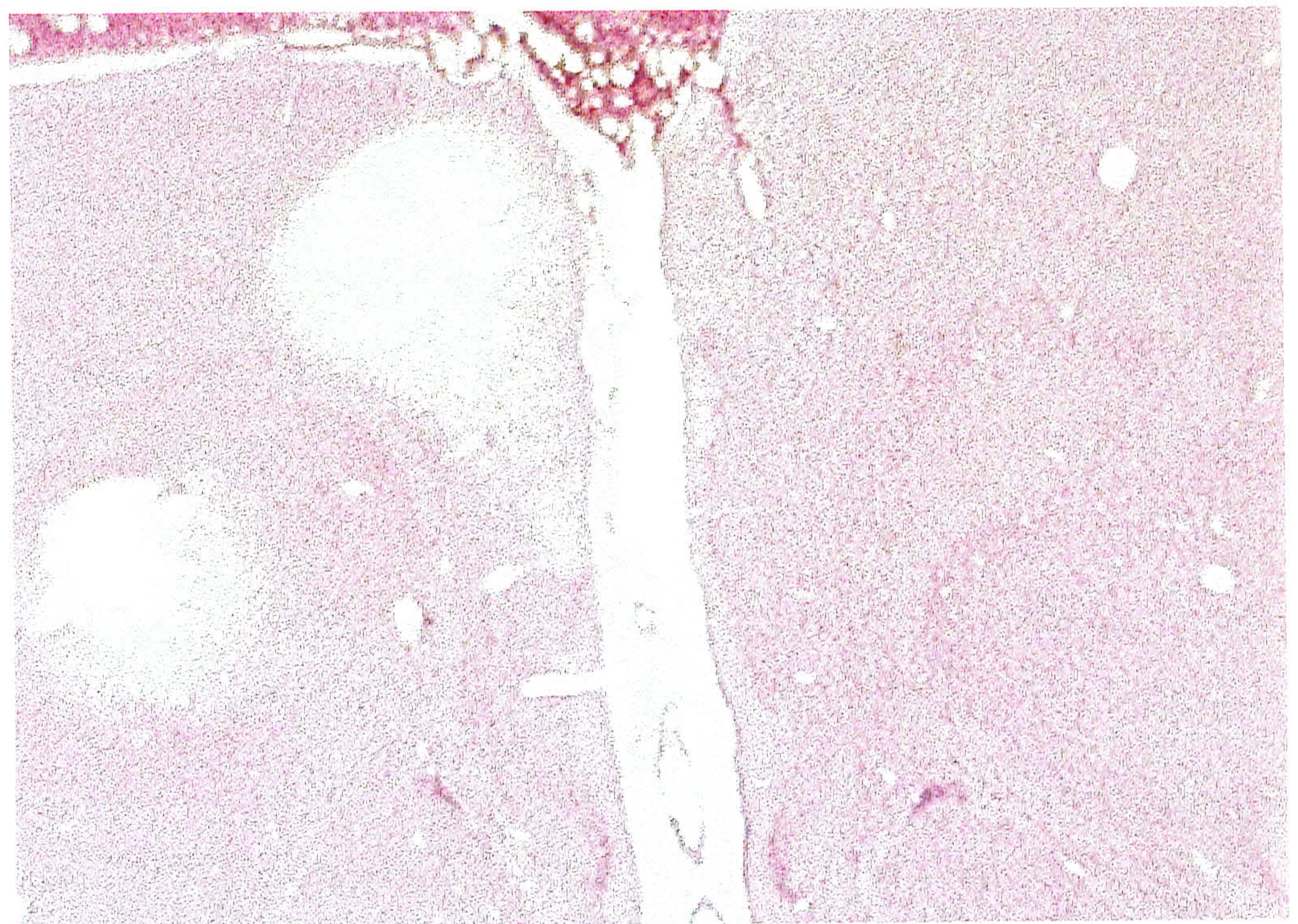


Figure 13d: Example of cresyl violet staining for mPFC lesions (+2.70mm from Bregma).



been more than 80% loss of PPTg neurons both caudally and rostrally, and there had been greater than 70% loss of either AC or PL (or both), either in the caudal or the rostral portion. This criterion was established on the basis that anterior posterior spread of ibotenate in the mPFC in this experiment was often not complete. A total of 2 animals were excluded from the disconnection group, 1 because the mPFC infusion had appeared to have occurred down the mid-line and had resulted in bilateral loss of medial wall neurons, and 1 because the mPFC lesion was situated too laterally and too caudally. A total of 6 animals were excluded from the lesion control group. 2 of these rats appeared to have no visible mPFC damage despite sustaining acceptable PPTg loss and 4 rats had damage of the PFC that was either too lateral, or too small.

A representation of the PPTg lesions included in the disconnection group can be seen in Figure 13.i. Likewise the PPTg lesions included in the lesion control group can be seen in Figure 13.ii. Figure 13.iii shows the mPFC lesions included in the disconnection group and Figure 13.iv shows the lesions of the mPFC included in the lesion control group. Of the included lesions, 4/5 disconnection lesions had extensive damage to the substantia nigra at its caudal most aspect, along with 4/6 lesion controls. In comparison only 1/5 disconnection lesions and 2/6 lesion controls sustained damage to the LDTg. Given the extent of lesion problems in this experiment, therefore, a combined analysis of disconnection groups from this and the preceding experiment was not conducted. In sum there were 5 disconnection lesioned rats, 6 lesion control rats, and 7 sham-operated controls included in the analyses.

**FIGURE 13.i UNILATERAL LESIONS OF THE PPTg FOR DISCONNECTION GROUP -
SMALLEST IS SHOWN ON THE RIGHT AND LARGEST IS SHOWN ON THE LEFT.**

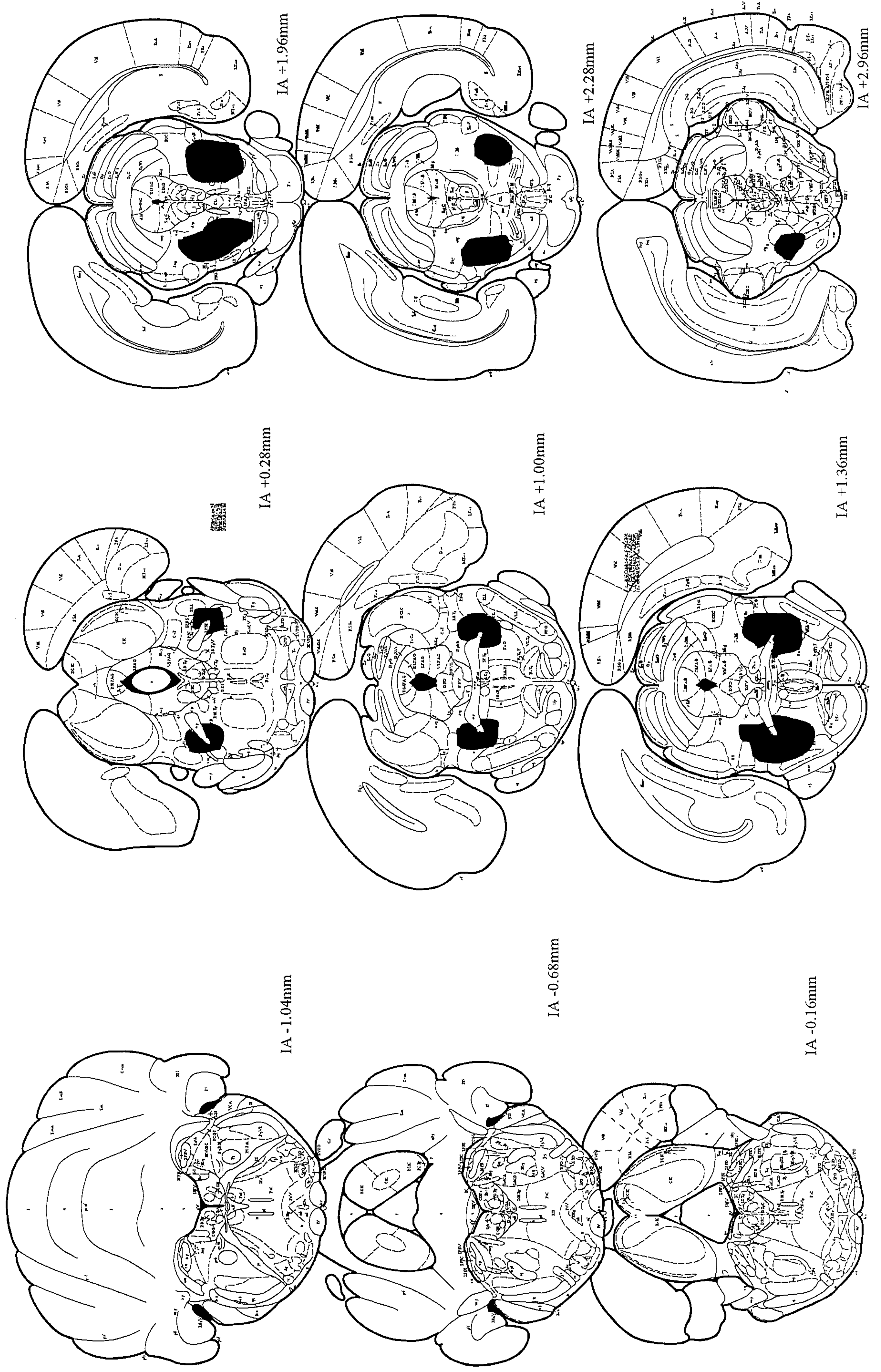


FIGURE 13.ii UNILATERAL LESIONS OF THE PPTg FOR LESION CONTROL GROUP - SMALLEST IS SHOWN ON THE RIGHT AND LARGEST IS SHOWN ON THE LEFT.

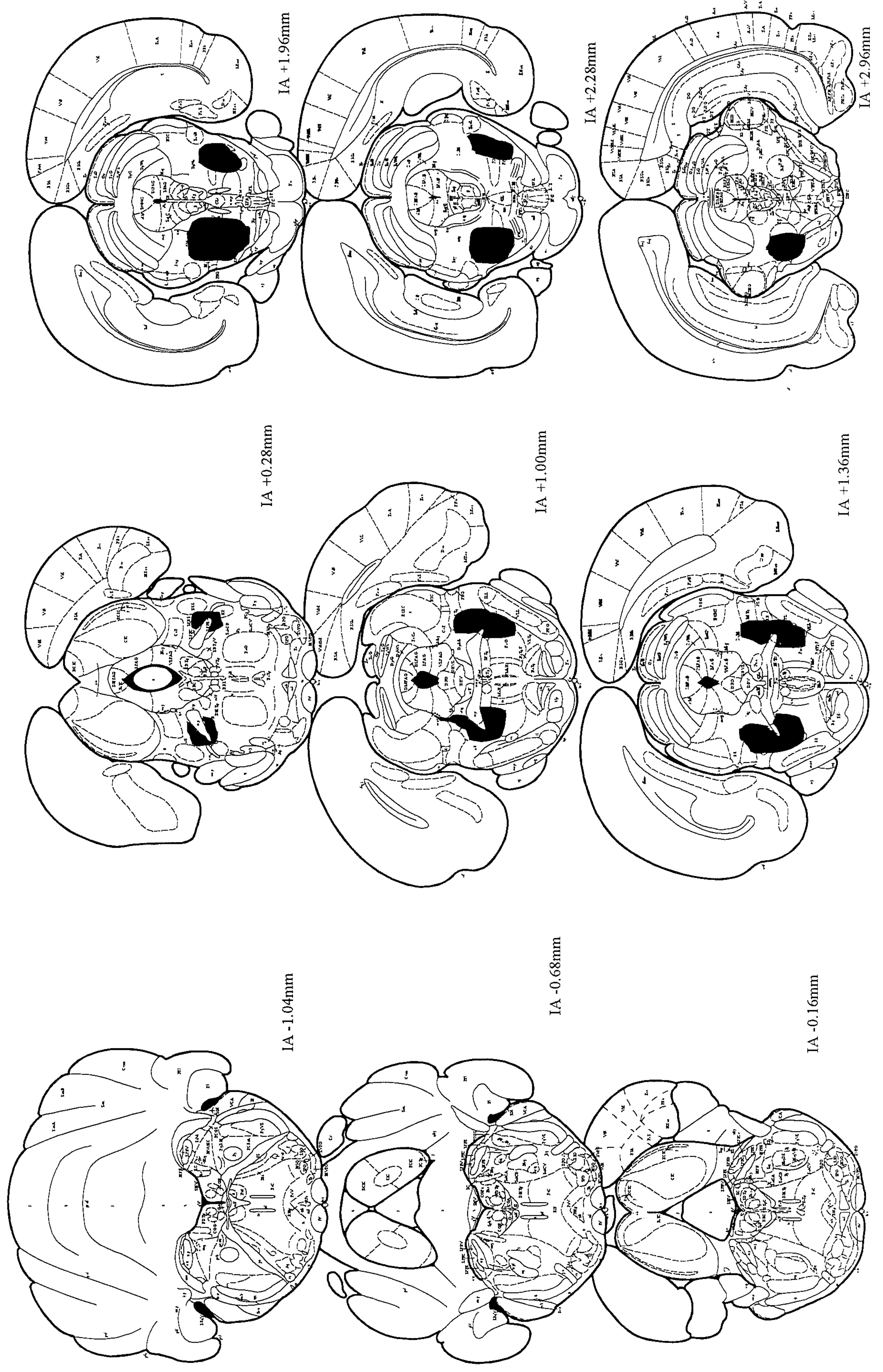


FIGURE 13.iii UNILATERAL LESIONS OF THE mPFC FOR DISCONNECTION GROUP - SMALLEST IS SHOWN ON THE RIGHT AND LARGEST ON THE LEFT.

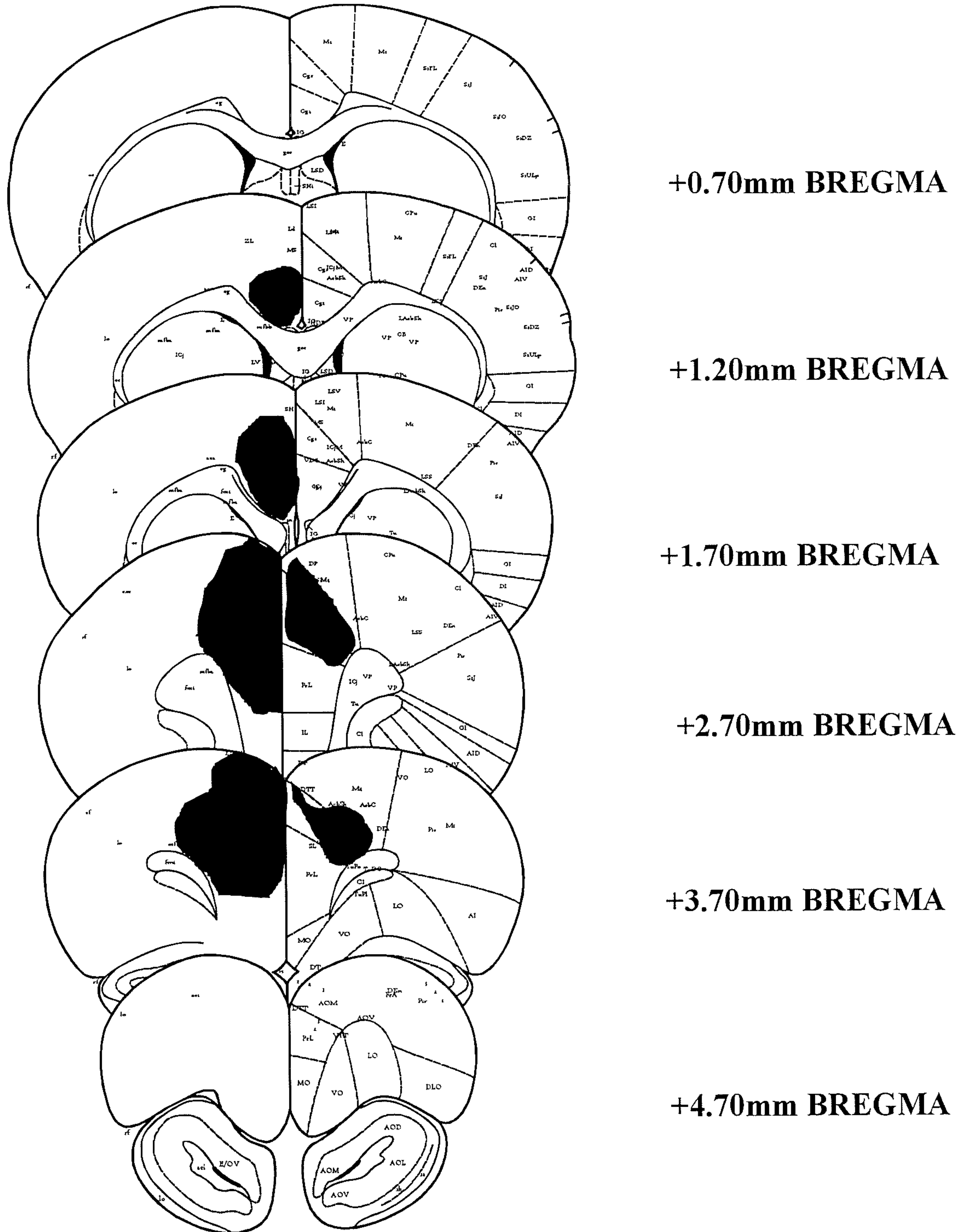
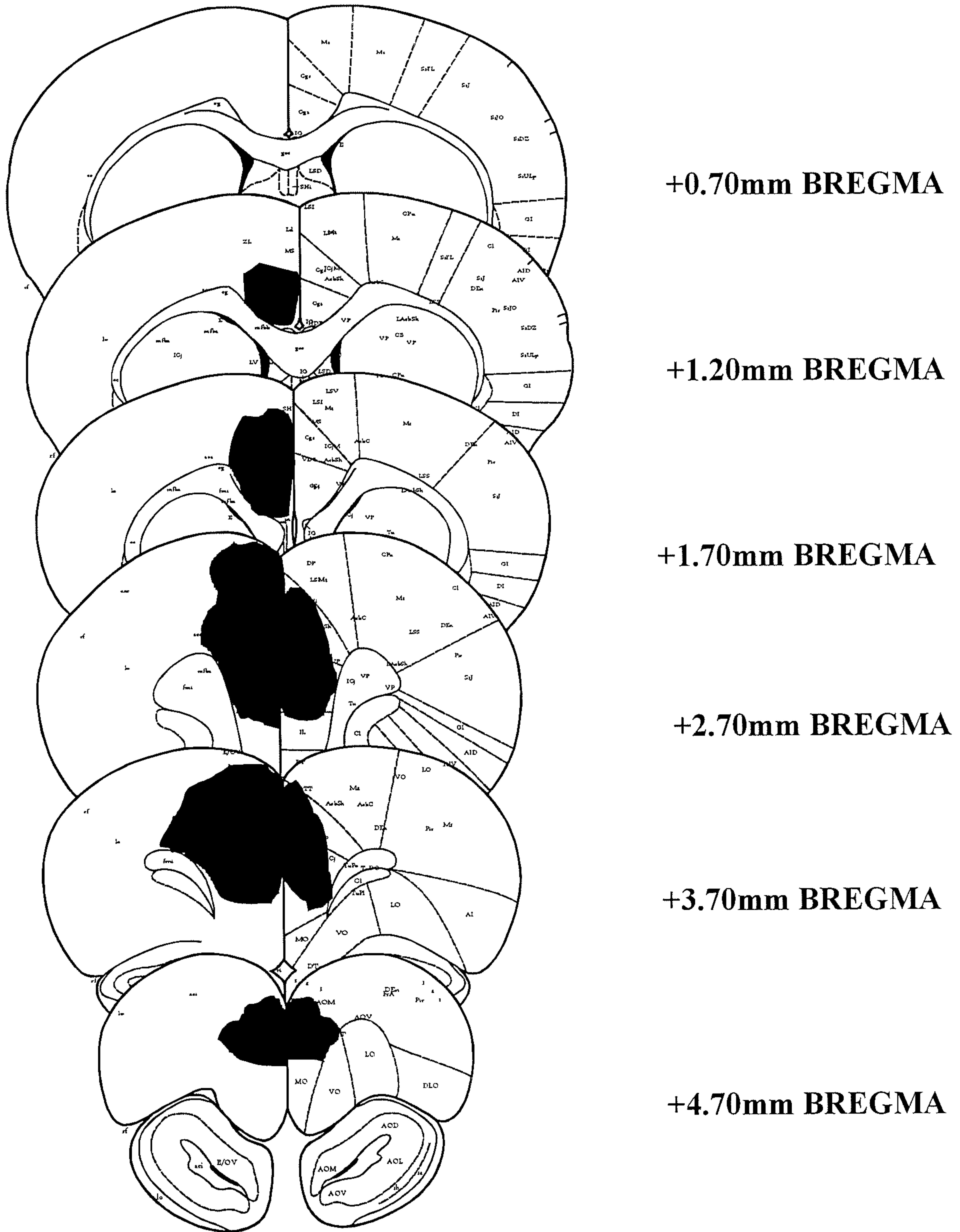


FIGURE 13.iv UNILATERAL LESIONS OF THE mPFC FOR LESION CONTROL GROUP - SMALLEST IS SHOWN ON THE RIGHT AND LARGEST ON THE LEFT.

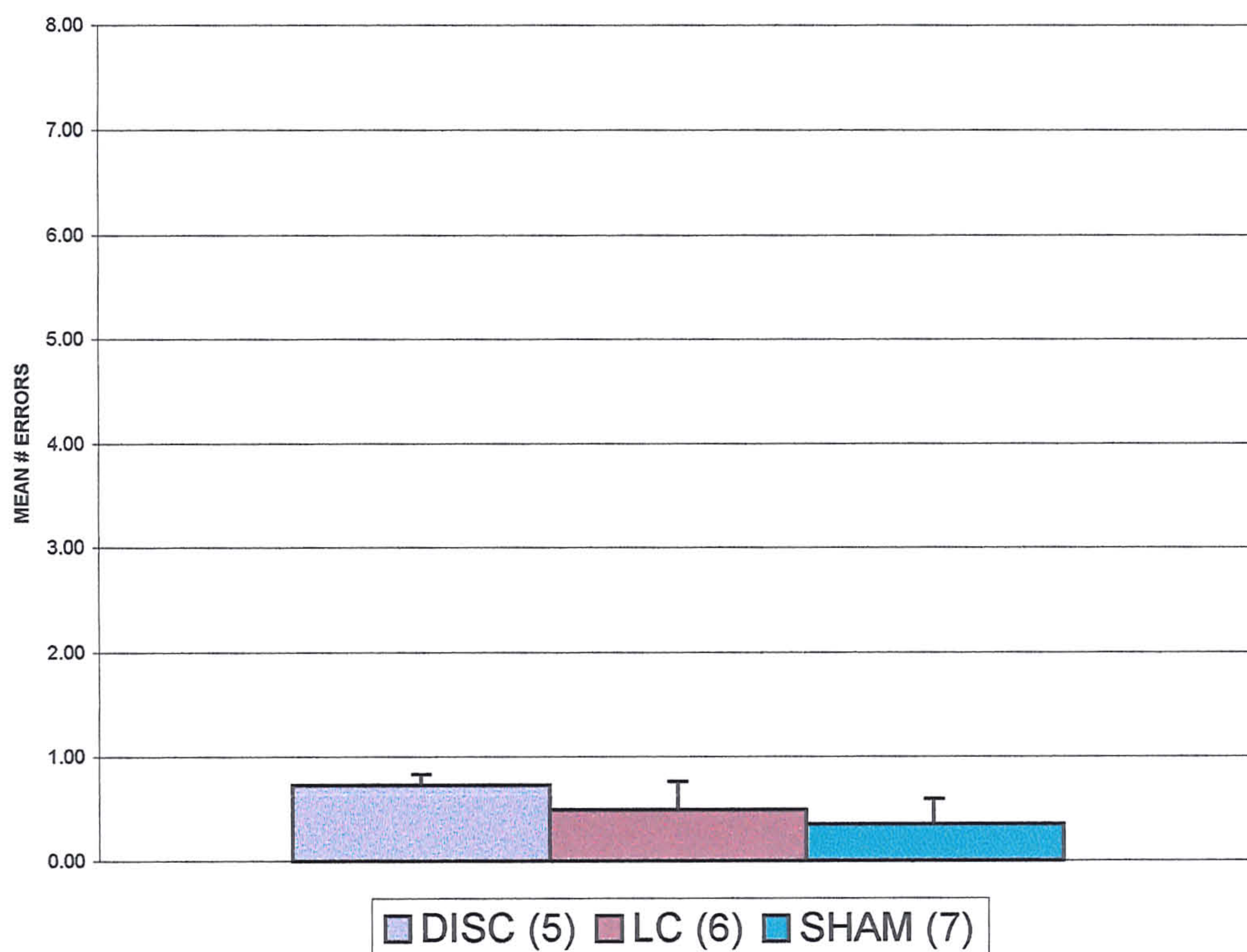


13.3.2 The DSWS Task: Criterion Days Analysis

In the figures that follow the label “DISC” refers to the disconnection lesion group, the label “LC” refers to the lesion control group, and the label “SHAM” refers to rats receiving sham lesions.

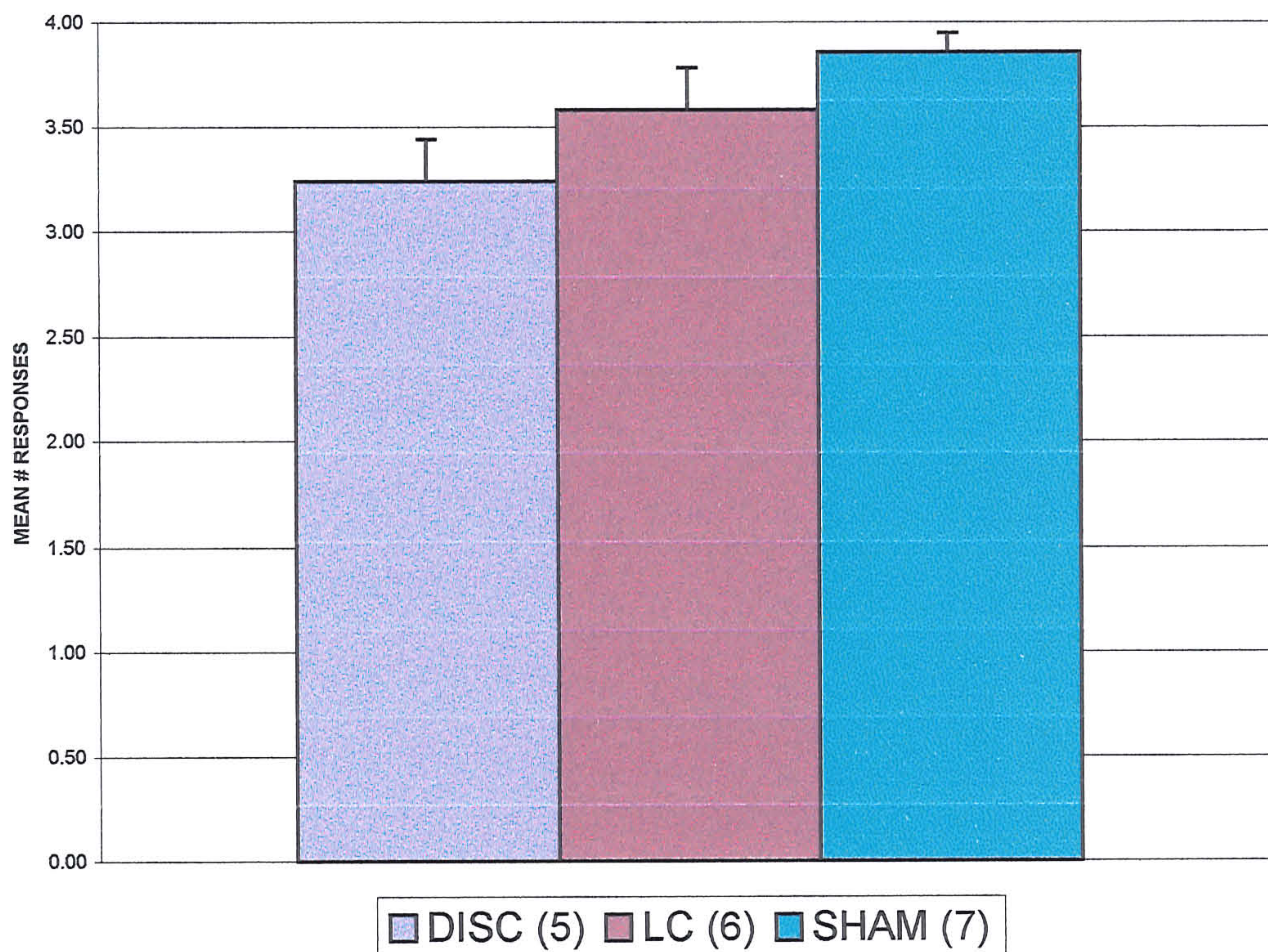
The mean and standard error for the number of errors made in the training phase over the criterion days can be seen in Figure 13.v. The one-way between subjects ANOVA showed that there was not a significant effect of any of the lesions on this measure $F(2,15) = 0.73$.

FIGURE 13.v TRAINING PHASE ERRORS



The mean and standard error for the number of correct responses made before the first error for the training phase over the criterion days can be seen in Figure 13.vi. Despite the trend indicating that the disconnection group were worse than the shams there was not a significant effect of group on this measure $F(2,15) = 0.82$.

FIGURE 13. vi TRAINING # CORRECT RESPONSES BEFORE ERROR



The mean and standard error for the training phase latency to the first arm over the criterion days can be seen in Figure 13.vii. The ANOVA showed that there was not a significant effect of the lesions on this measure $F(2,15) = 0.03$.

FIGURE 13.vii TRAINING LATENCY

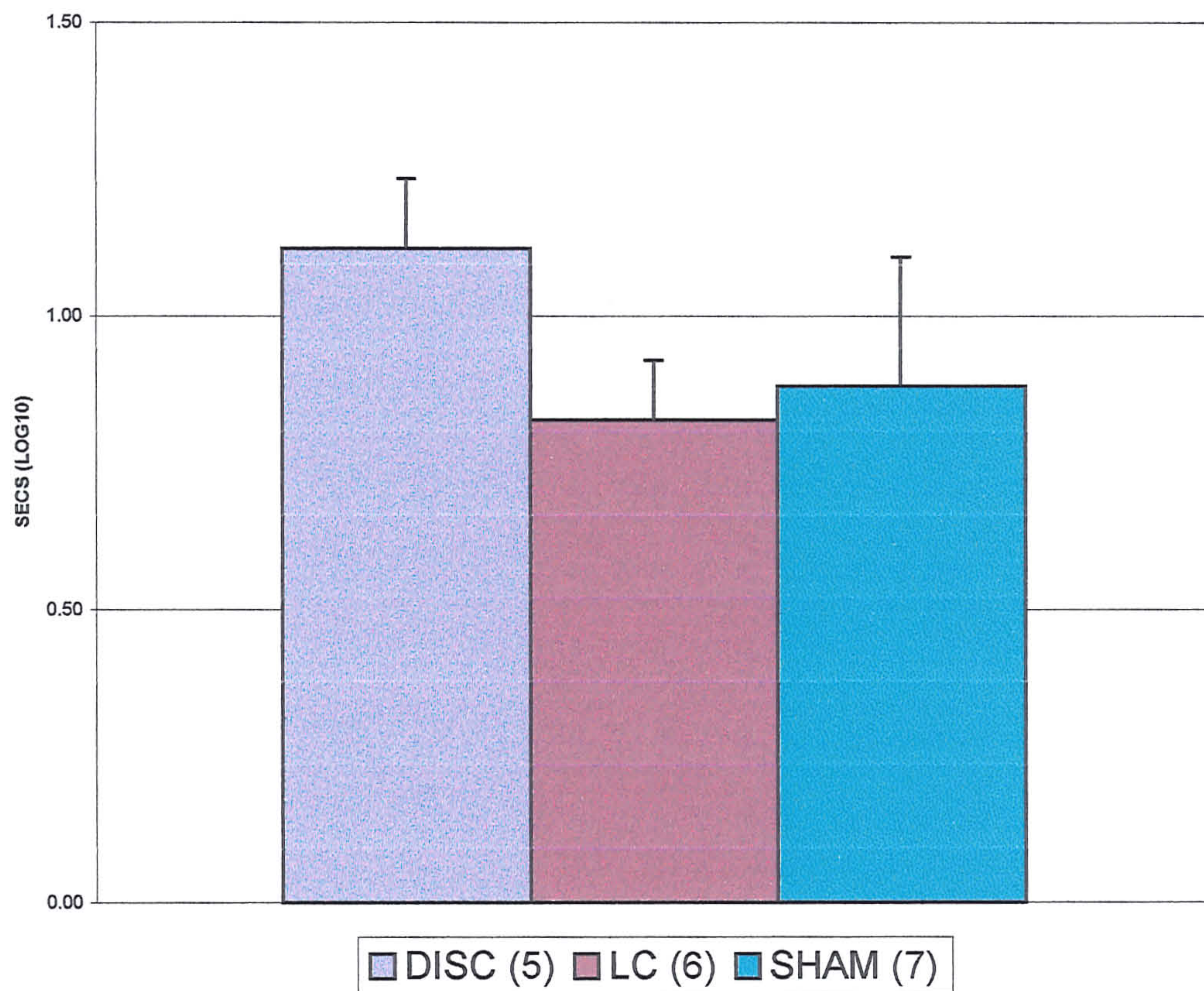
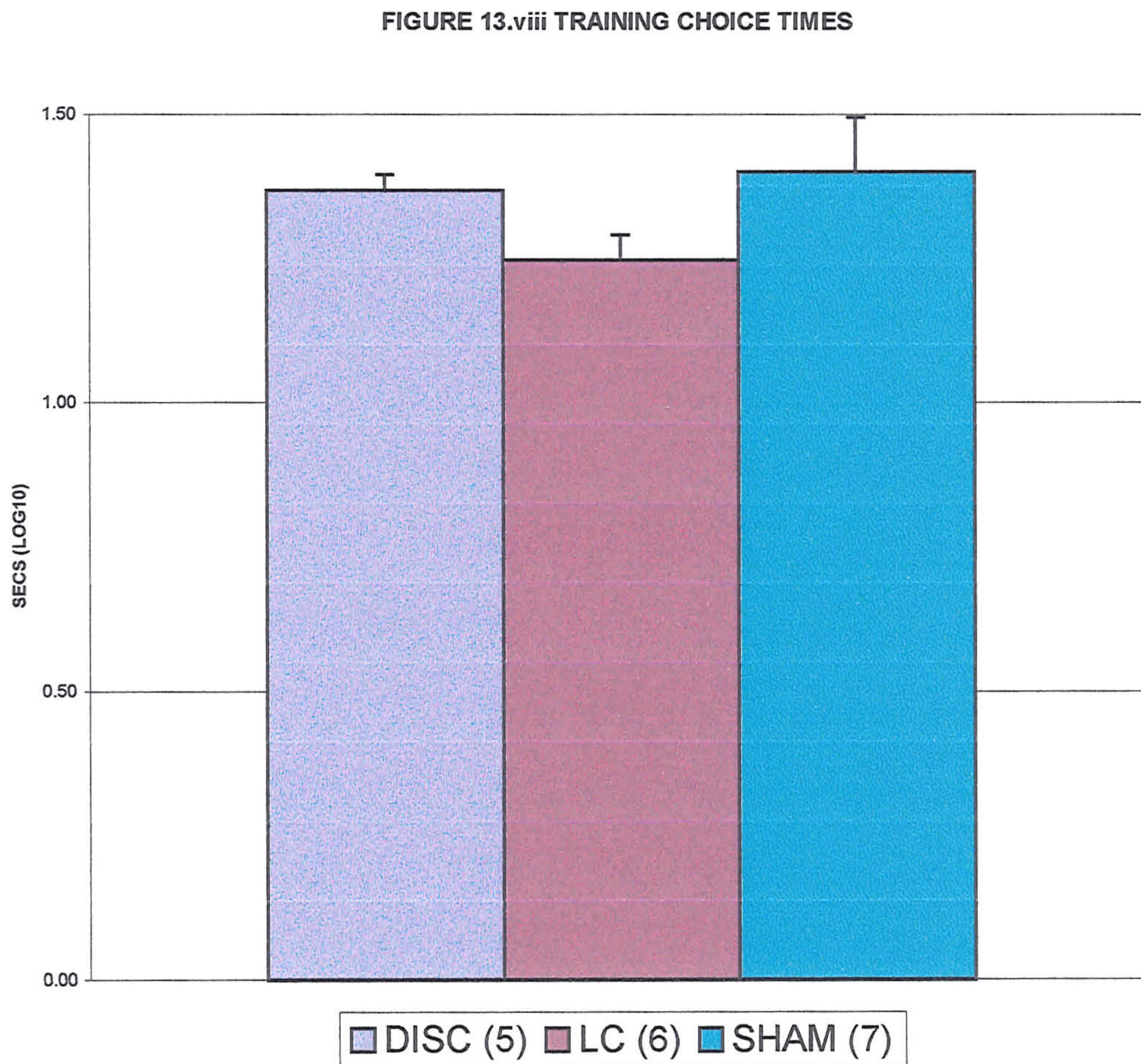


Figure 13.viii shows the mean and standard error for the arm choice times over the criterion days for the training phase. There was not a significant effect of any of the lesions on this measure $F(2,15) = 1.82$.



The mean and standard error for the number of errors made in the test phase over the criterion days can be seen in Figure 13.ix. Despite the trend indicating otherwise the ANOVA conducted on these results showed that there was not a significant effect of the lesions on this measure $F(2,15) = 0.08$.

FIGURE 13.ix TEST PHASE ERRORS

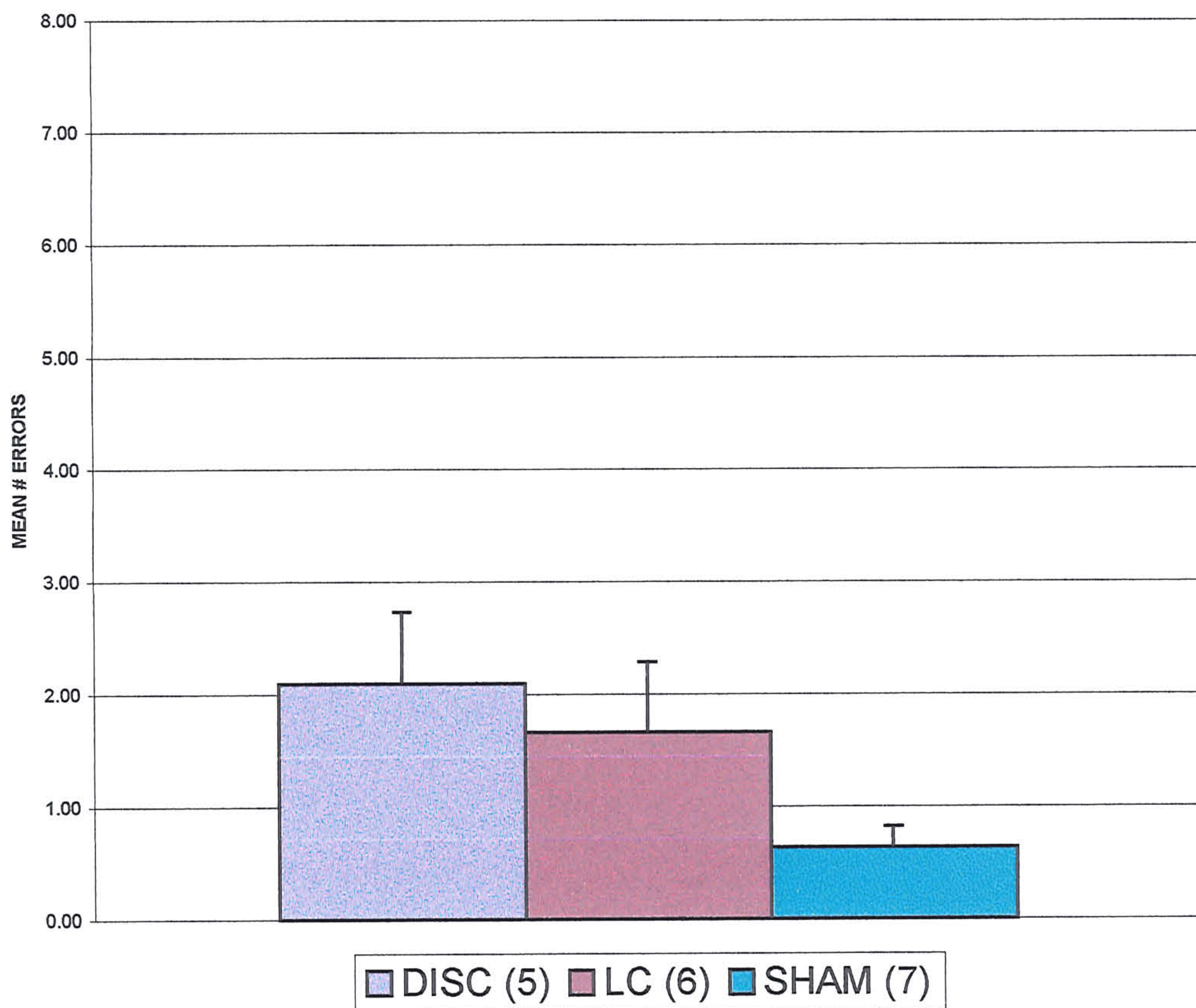
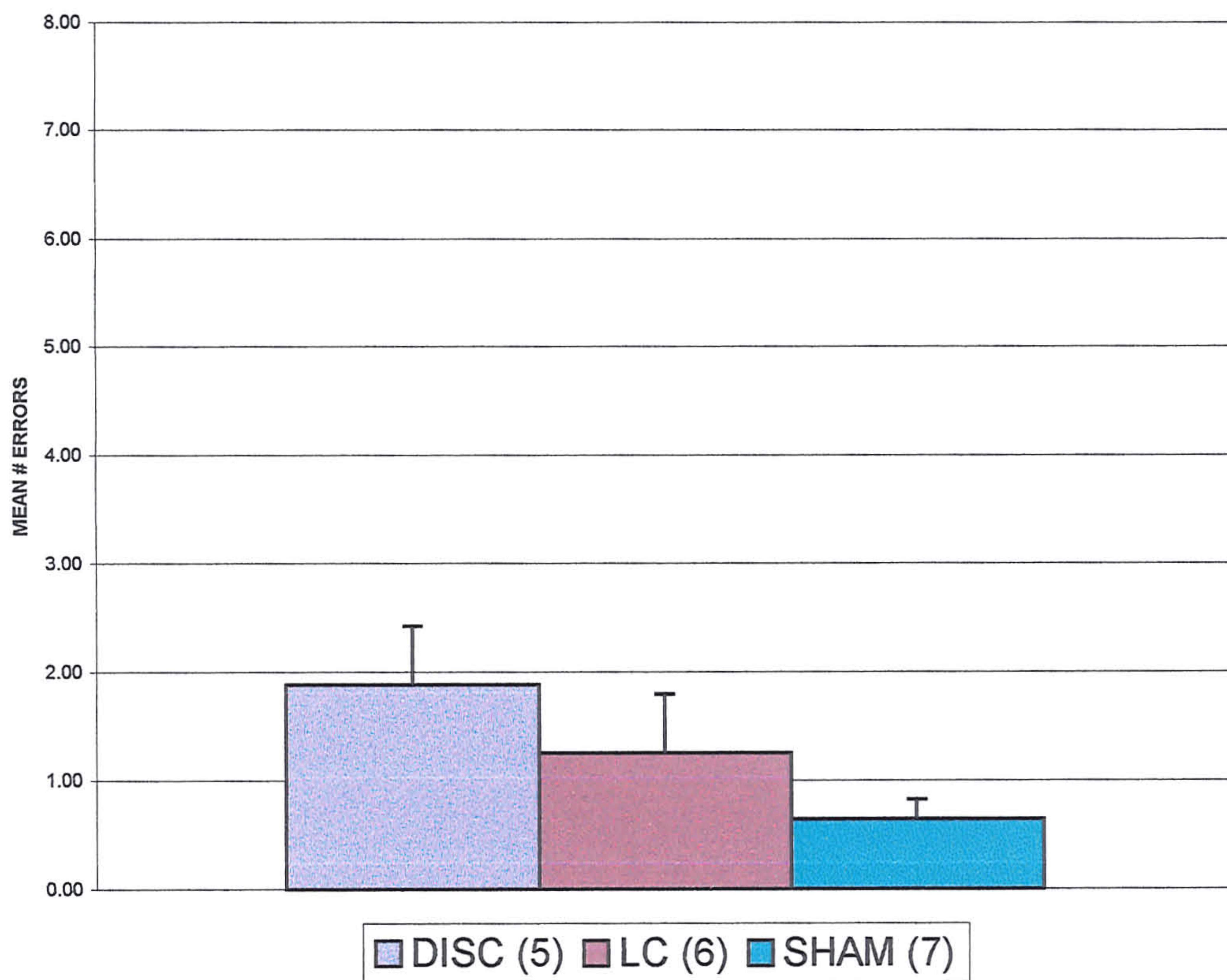


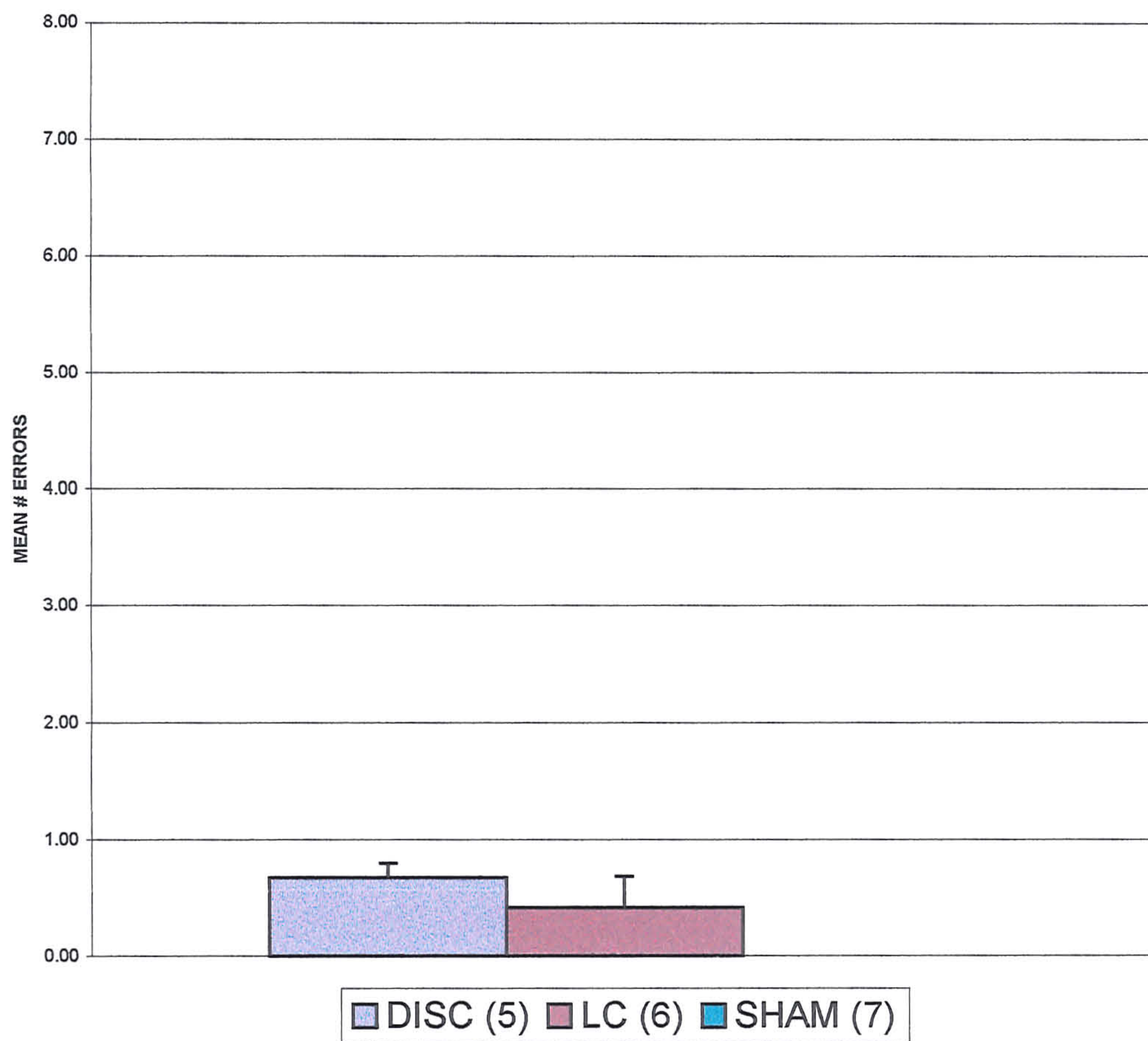
Figure 13.x shows the mean and standard error for the number of across phase errors made in the test phase over the criterion days. Again there was not a significant effect of group on this measure $F(2,15) = 1.58$.

FIGURE 13.x ACROSS PHASE ERRORS



The mean and standard error for the number of within phase errors made over the criterion days can be seen in Figure 13.xi. As for the number of within phase errors there was not a significant effect of group on this measure $F(2,15) = 1.68$.

FIGURE 13.xi WITHIN PHASE ERRORS



The mean and standard error for the number of correct responses made before the first error in the test phase over the criterion period can be seen in Figure 13.xii. There were no significant effects of the lesions on this measure $F(2,15) = 0.08$.

FIGURE 13.xii TEST # CORRECT RESPONSES BEFORE ERROR

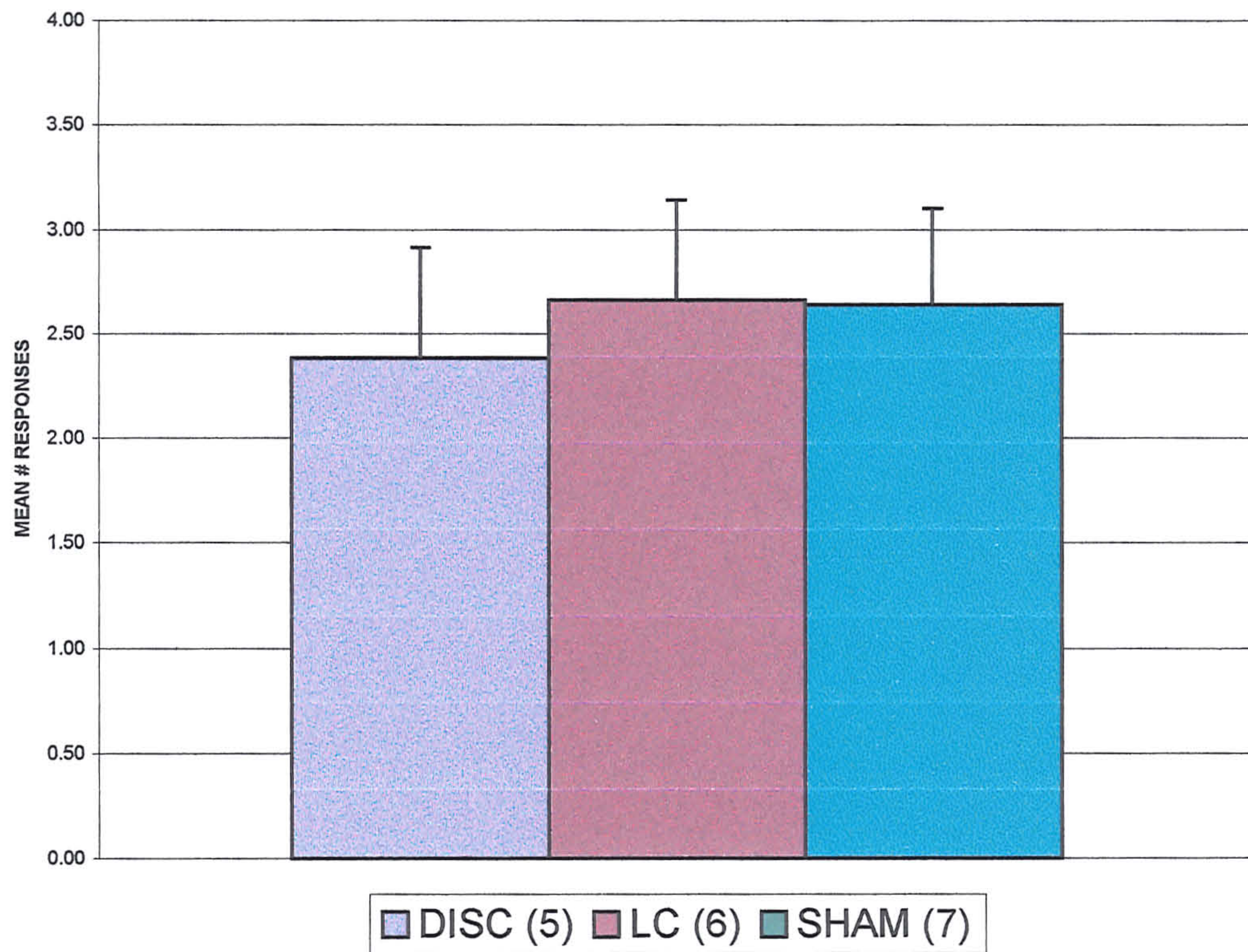
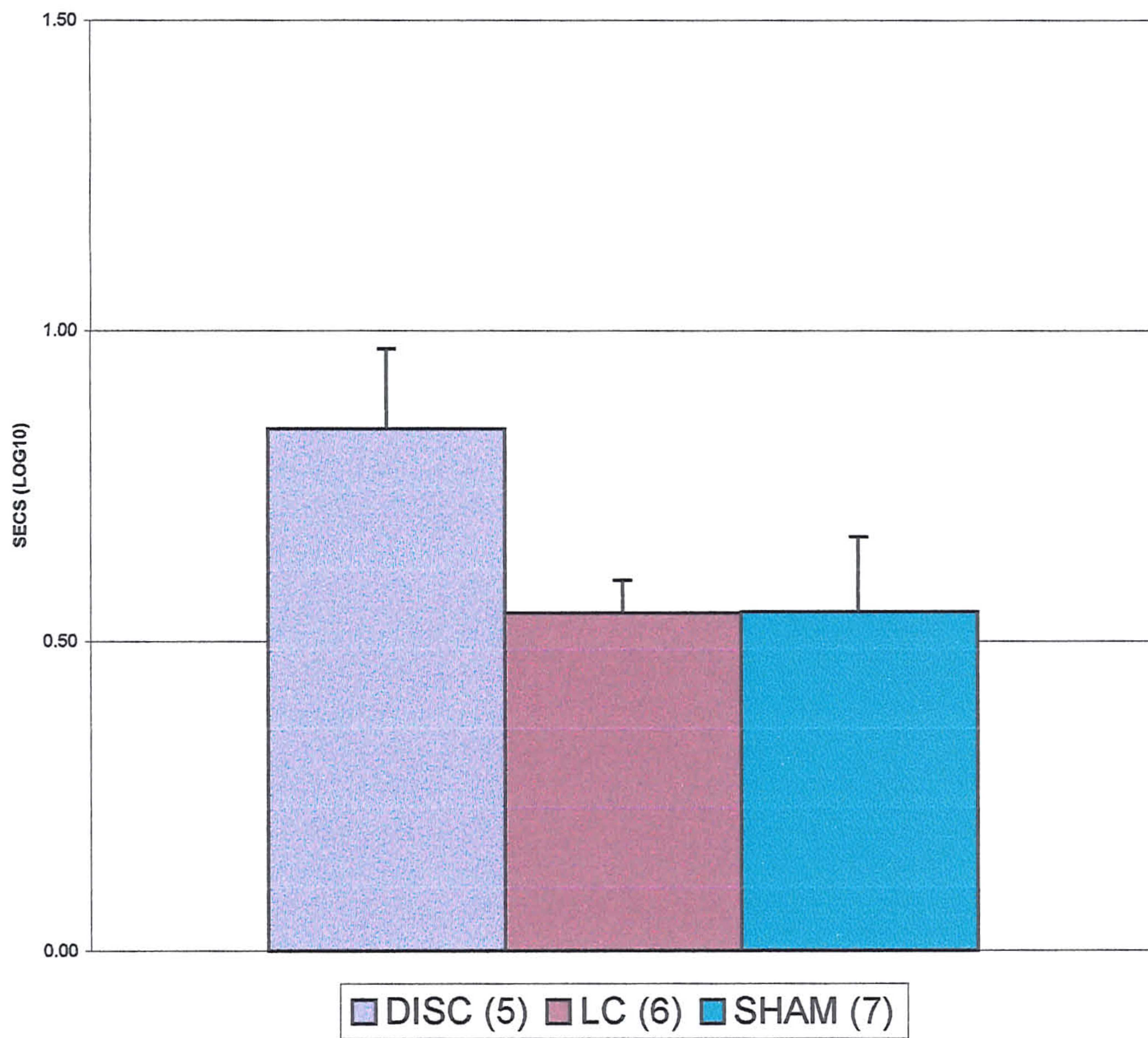


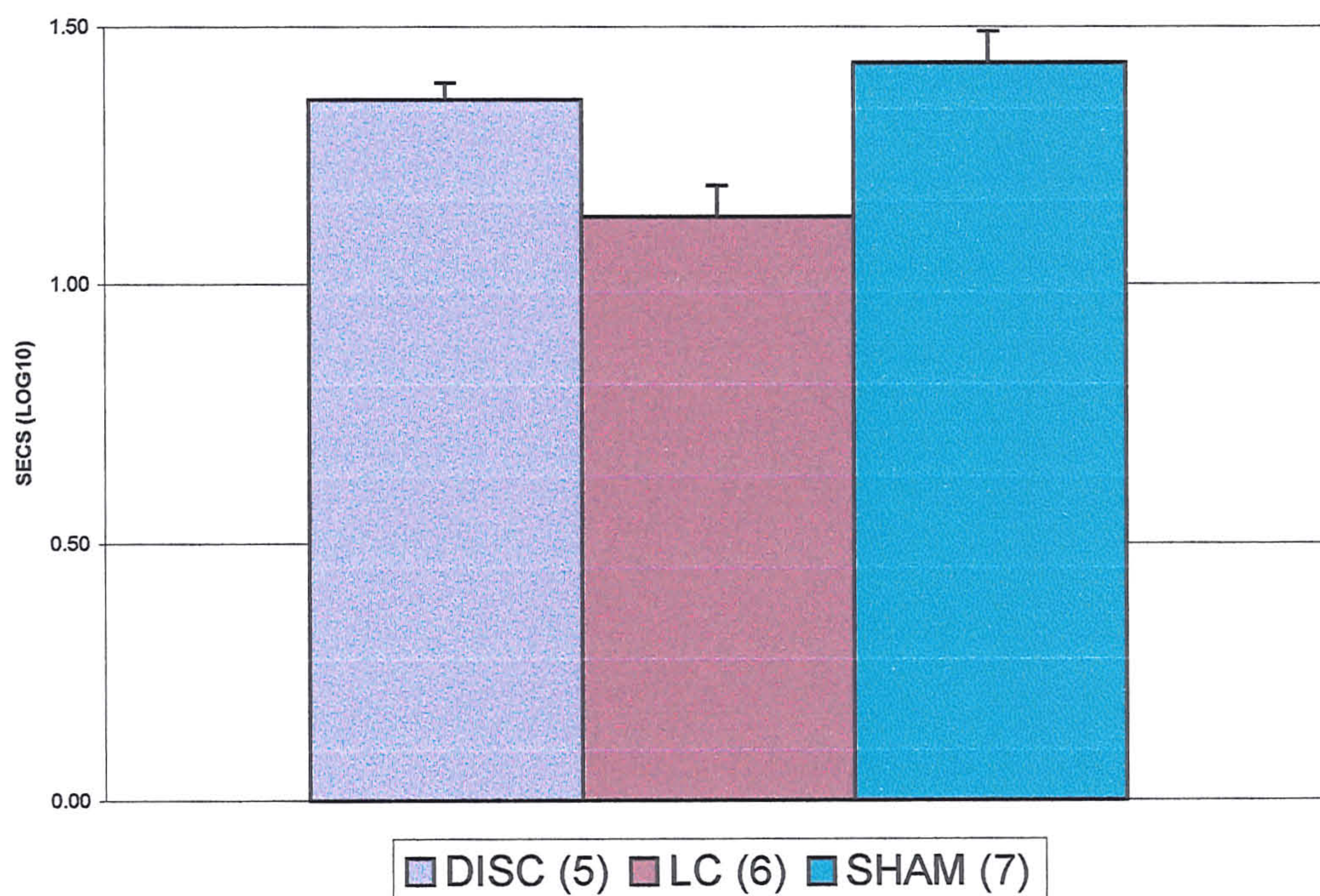
Figure 13.xiii shows the mean and standard error for the latency to the first arm in the test phase over the criterion period. Despite the trend suggesting that the disconnection group were significantly slower in comparison to the other groups there was not a significant effect of any of the lesions on this measure $F(2,15) = 0.40$.

FIGURE 13.xiii TEST LATENCY



Finally, Figure 13.xiv shows the mean and standard error for the arm choice times over the criterion period in the test phase. The one-way between subjects ANOVA conducted on this measure showed that there was a significant effect of group $F(2,15) = 7.18$ $p < 0.05$. Eta squared for this effect was high at 0.49. A post hoc Tukey test confirmed that the significant difference was between the lesion controls and the shams. None of the other comparisons were significant.

FIGURE 13.xiv TEST CHOICE TIMES



13.3.3 *Summary of Criterion Analysis*

This analysis, performed on the data obtained for the criterion days only, showed that there were no significant effects of disconnection lesions of the mPFC and PPTg by the point in time that sham rats had regained asymptote performance. This finding is in agreement with the results of the criterion analysis in the previous experiment. However, lesion controls in the present experiment displayed significantly faster arm choice times in the test phase in comparison to sham-operated controls, despite not making more errors in that phase. As a result of the limited significant findings of these criterion analyses this data has not been presented in summarised form. In the preceding experiment, despite a trend that disconnection lesion rats were making their test choice times faster, this did not reach significance in the criterion analysis. However the omnibus ANOVAs showed that the effect of faster arm choice times was significant in some of the earlier trials. Given that the current finding suggested that the effect may be paralleled with lesions of the PPTg and mPFC in the same hemisphere, it was important to assess whether the same speed effect was apparent in the earlier experimental trials in the lesion control group. Furthermore, as there was evidence that the speed effect can be reproduced following lesions of the mPFC and PPTg in the same hemisphere it was vital to assess whether the effect on total test phase errors and within phase errors seen in disconnection lesioned rats in Chapter 12 could also be reproduced in the lesion controls in the omnibus ANOVAs.

13.3.4 The DSWS Task: Omnibus ANOVAs for all trials

Again in the figures that follow the label “DISC” refers to the disconnection lesioned group, the label “LC” to the lesion control group, and the label “SHAM” to the sham operated controls.

Figure 13.xv shows the mean and standard error for the number of training phase errors made across all trials in the post-surgical testing period. The omnibus two-way mixed ANOVA conducted on this data showed that there was not a main effect of group $F(2,15) = 2.63$ or of day $F(8,120) = 0.71$. The day x group interaction was also not significant $F(16,120) = 0.82$.

FIGURE 13.xv TRAINING PHASE ERRORS

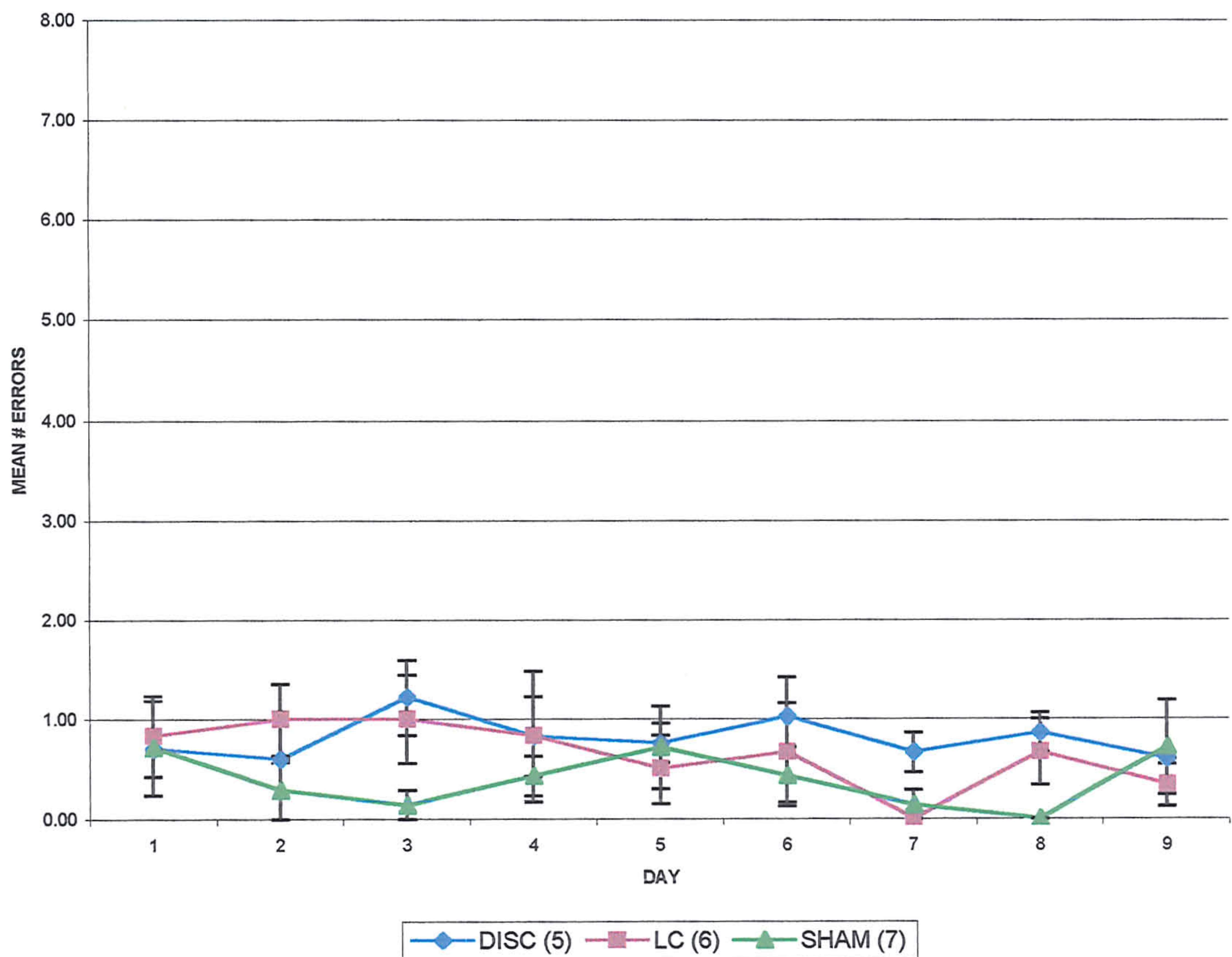
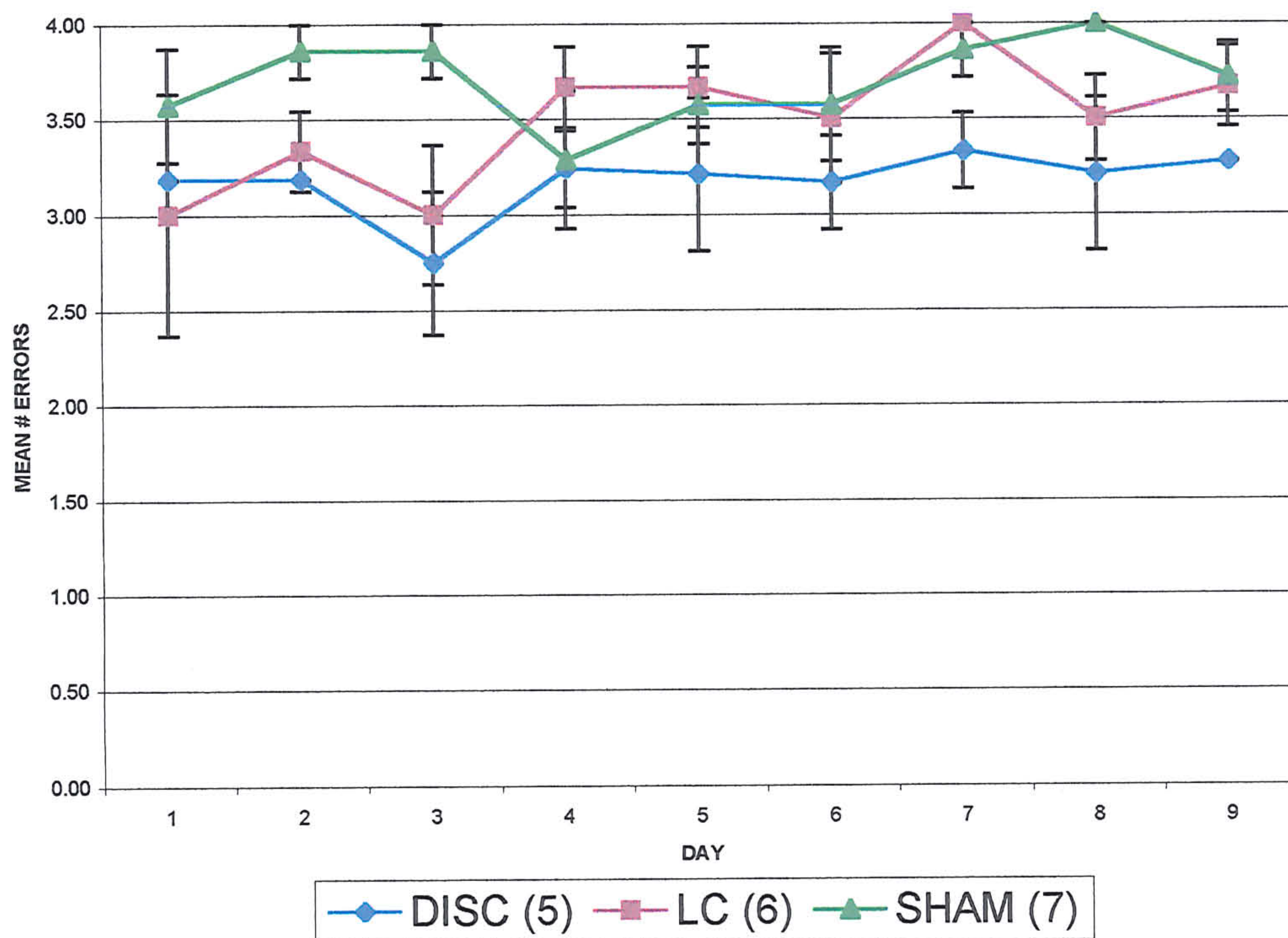


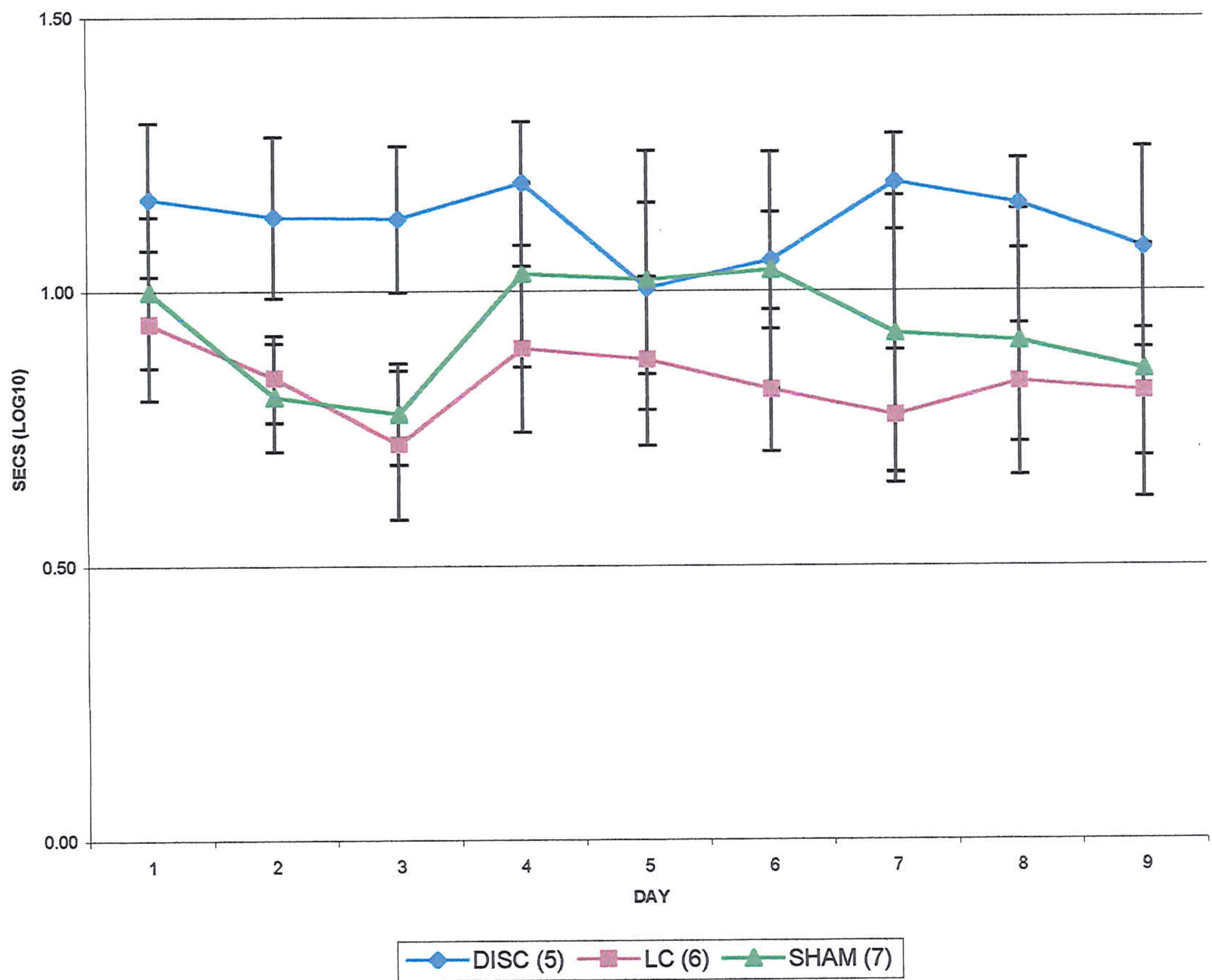
Figure 13.xvi shows the mean and standard error for the number of correct responses before error in the training phase for all trials. The ANOVA showed that there was not a main effect of group $F(2,15) = 1.47$ or of day $F(7.34,110.16) = 1.14$. The interaction between group and day was also not significant $F(14.69,110.16) = 1.15$.

FIGURE 13.xvi TRAINING PHASE # CORRECT RESPONSES BEFORE ERROR



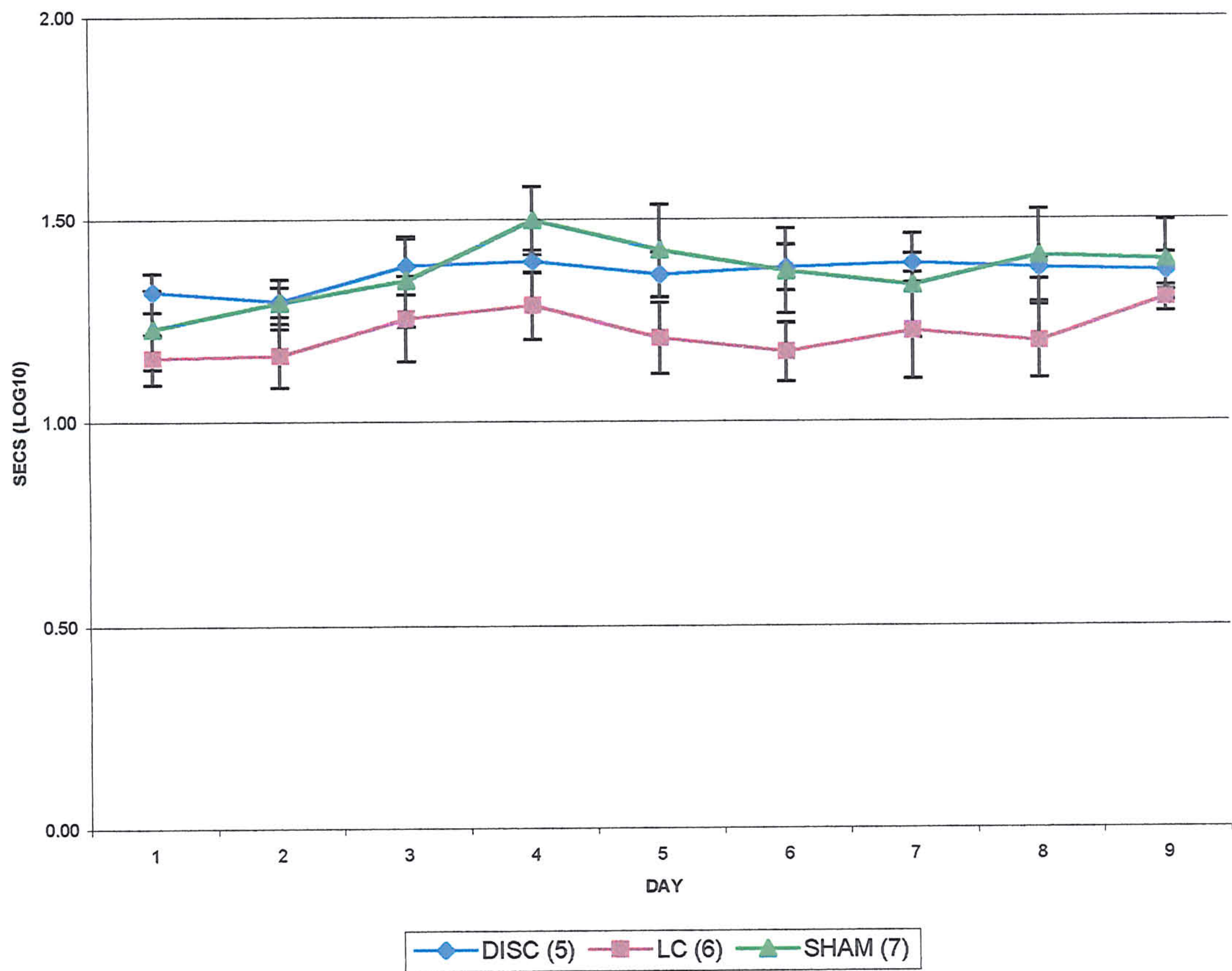
The mean and standard error for the latency to the first arm in the training phase over all trials can be seen in Figure 13.xvii. The two-way mixed ANOVA showed that there was not a main effect of group $F(2,15) = 0.19$, or day $F(8,120) = 0.57$. The group x day interaction was also not significant $F(16,120) = 0.83$.

FIGURE 13.xvii TRAINING LATENCY (LOG10)



The mean and standard error for the arm choice times in the training phase for all trials can be seen in Figure 13.xviii. The ANOVA conducted on this data showed that there was not a main effect of group $F(2,15) = 1.64$ or of day $F(7.22,108.31) = 2.81$. The group x day interaction was also not significant $F(14.44,108.31) = 0.54$.

FIGURE 13.xviii TRAINING CHOICE TIMES (LOG10)



The mean and standard error for the number of errors made in the test phase across all trials can be seen in Figure 13.xix. The two-way mixed ANOVA showed that there was not a main effect of group $F(2,15) = 0.08$, or of day $F(8,120) = 2.01$. The interaction was also not significant $F(16,120) = 0.67$.

FIGURE 13.xix TEST ERRORS

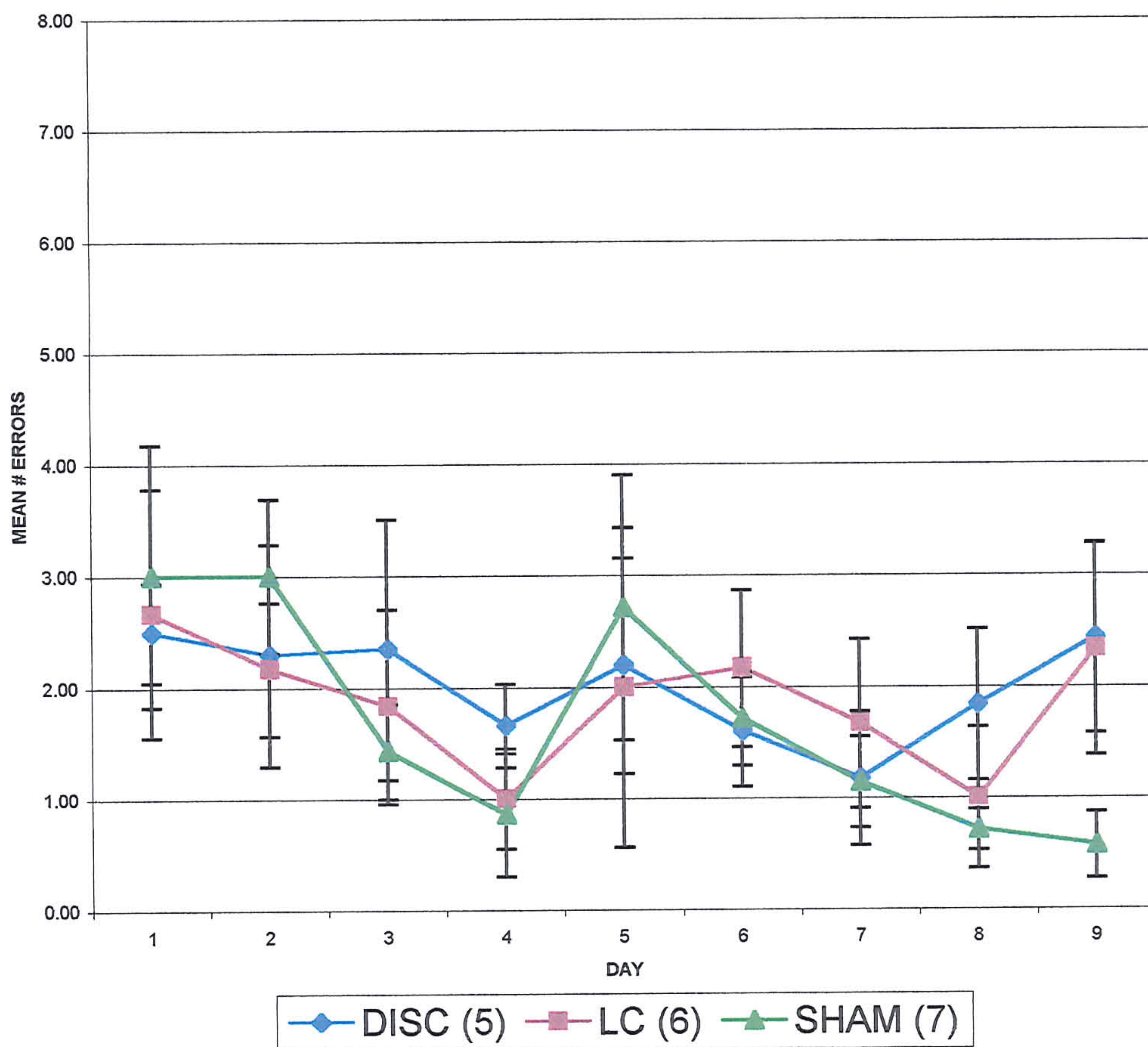


Figure 13.xx shows the mean and standard error for the number of across phase errors made in the test phase for all trials. The ANOVA showed that there were no significant main effects or interaction. Main effect of group $F(2,15) = 0.12$, main effect of day $F(8,120) = 1.93$, and group x day $F(16,120) = 1.01$.

FIGURE 13.xx ACROSS PHASE ERRORS

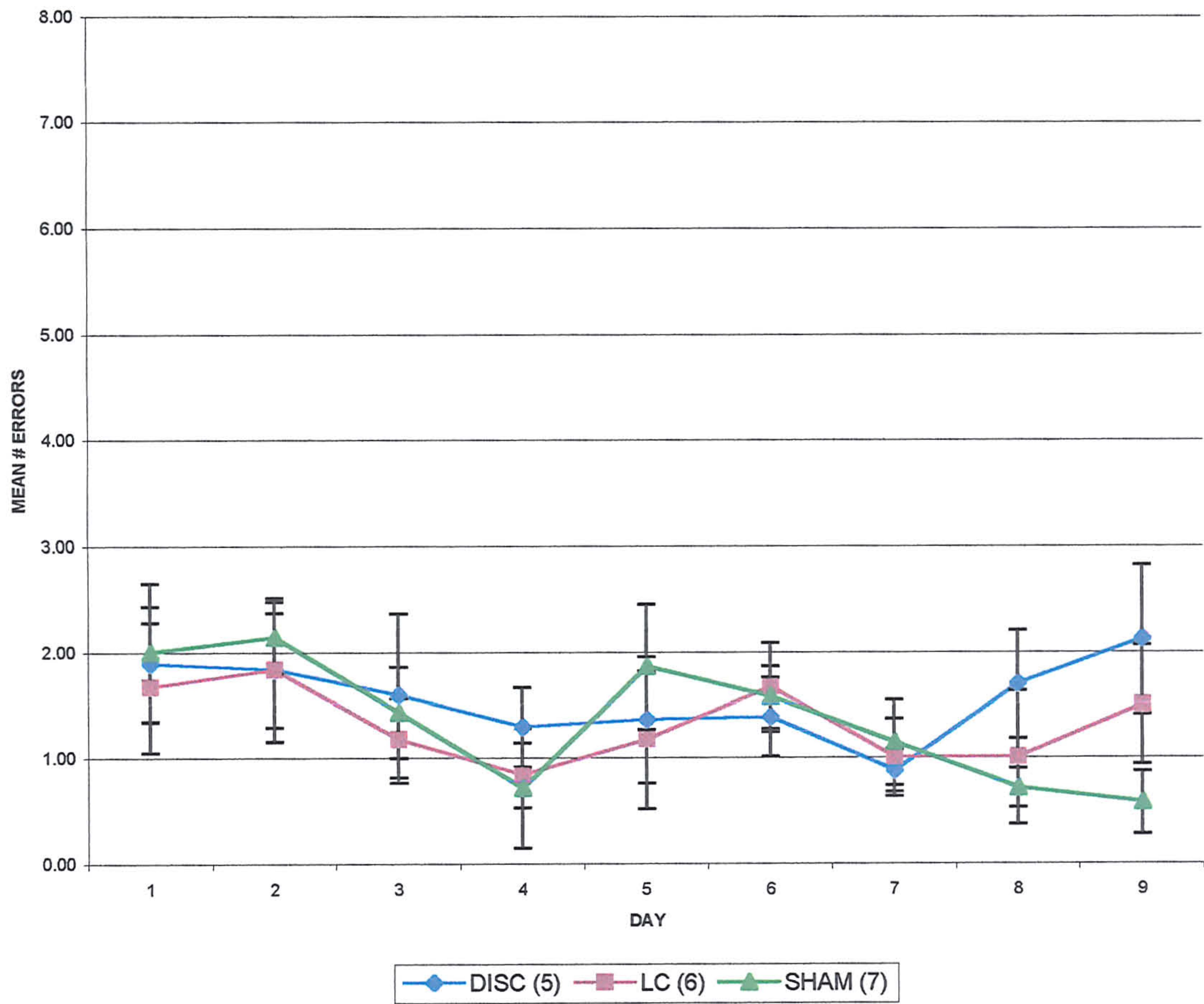
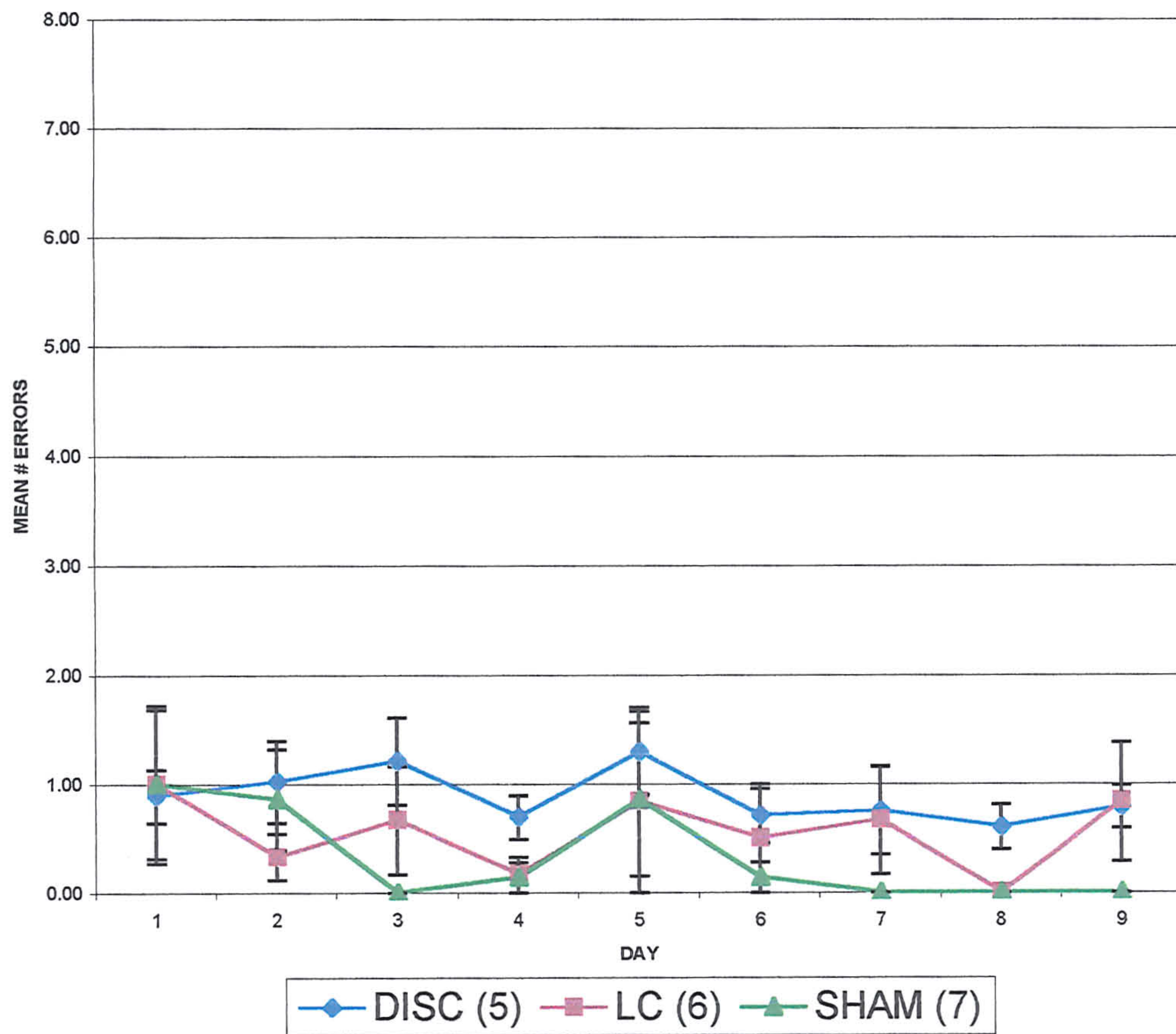


Figure 13.xxi shows the mean and standard error for the number of within phase errors made by each group in the test phase over all postsurgical trials. There was not a main effect of group on this measure $F(2,15) = 0.45$. The main effect of day was also non-significant $F(5,74.98) = 1.21$, as was the group x day interaction $F(10,74.98) = 0.48$.

FIGURE 13.xxi WITHIN PHASE ERRORS



The mean and standard error for the number of correct responses before error in the test phase, for all trials, can be seen in Figure 13.xxii. The two-way mixed ANOVA conducted on this data showed that there was not a main effect of group $F(2,15) = 0.61$. The main effect of day was also not significant $F(8,120) = 0.87$, and neither was the group x day interaction $F(16,120) = 1.48$.

FIGURE 13.xxii TEST PHASE # CORRECT RESPONSES BEFORE ERROR

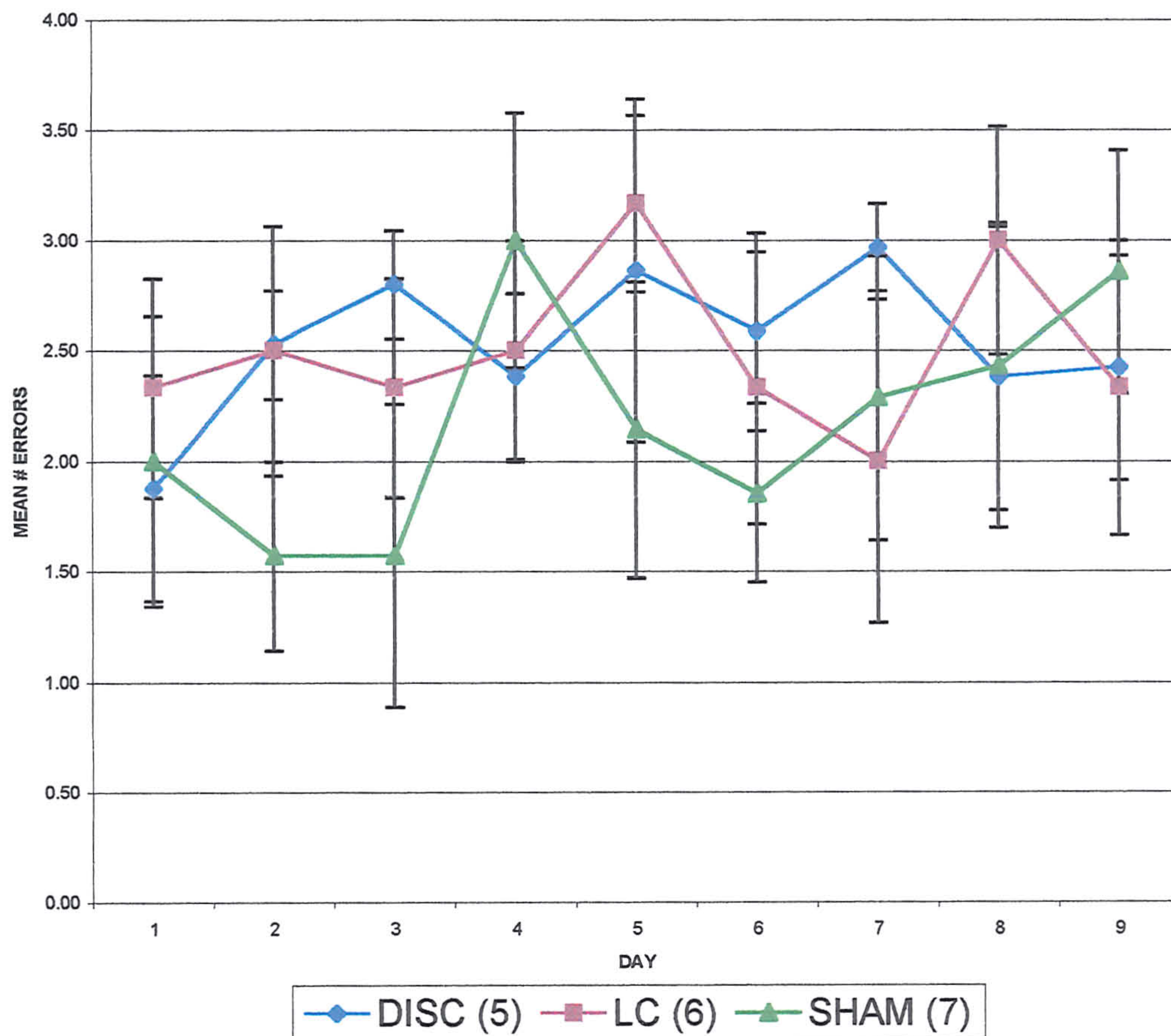


Figure 13.xxiii shows the mean and standard error for the latency to the first arm in the test phase across all trials. The ANOVA conducted on this data showed that there was not a main effect of group on this measure $F(2,15) = 0.85$ or a main effect of day $F(8,120) = 1.57$. The group x day interaction was also not significant $F(16,120) = 0.75$.

FIGURE 13.xxiii TEST LATENCY (LOG10)

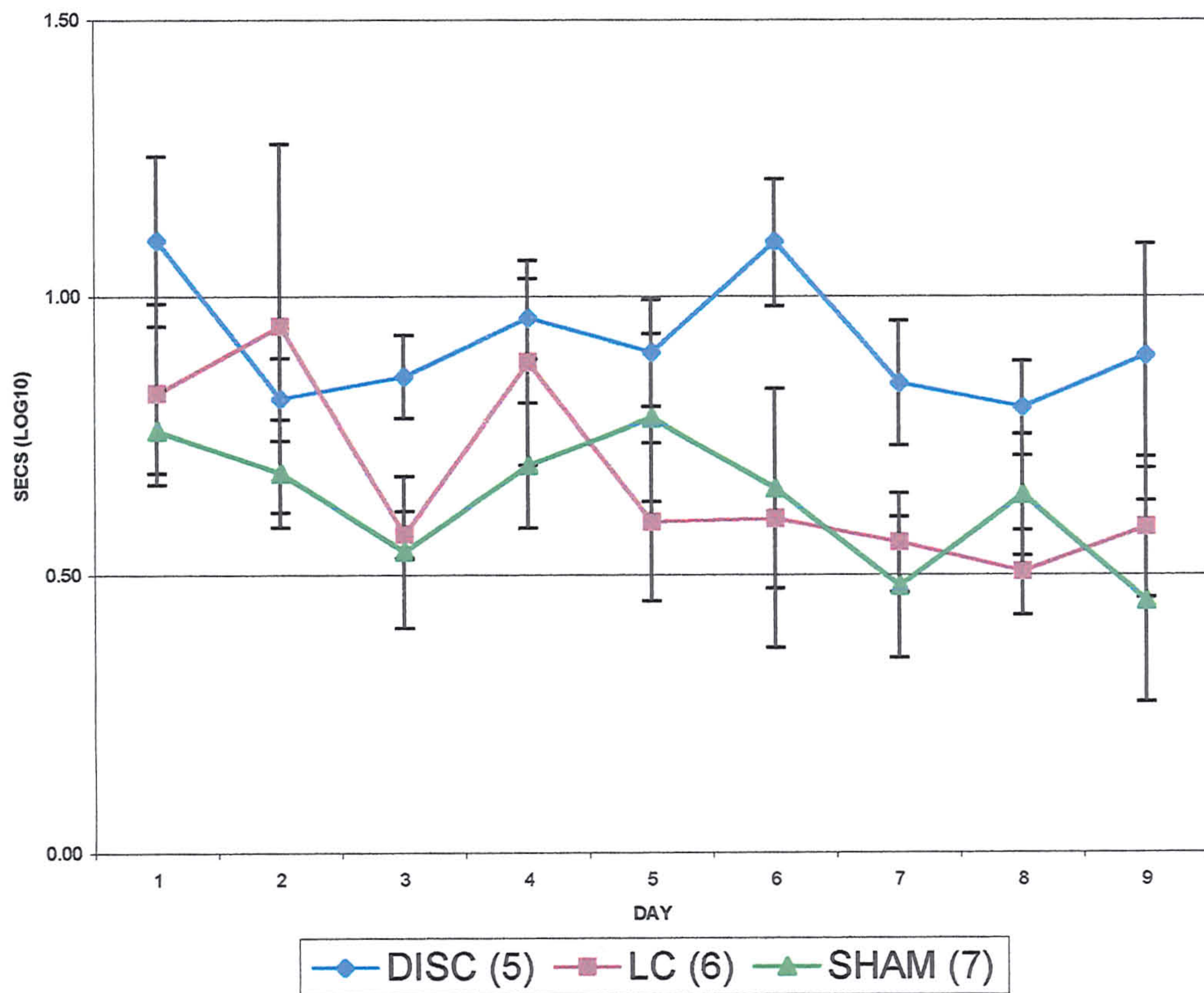
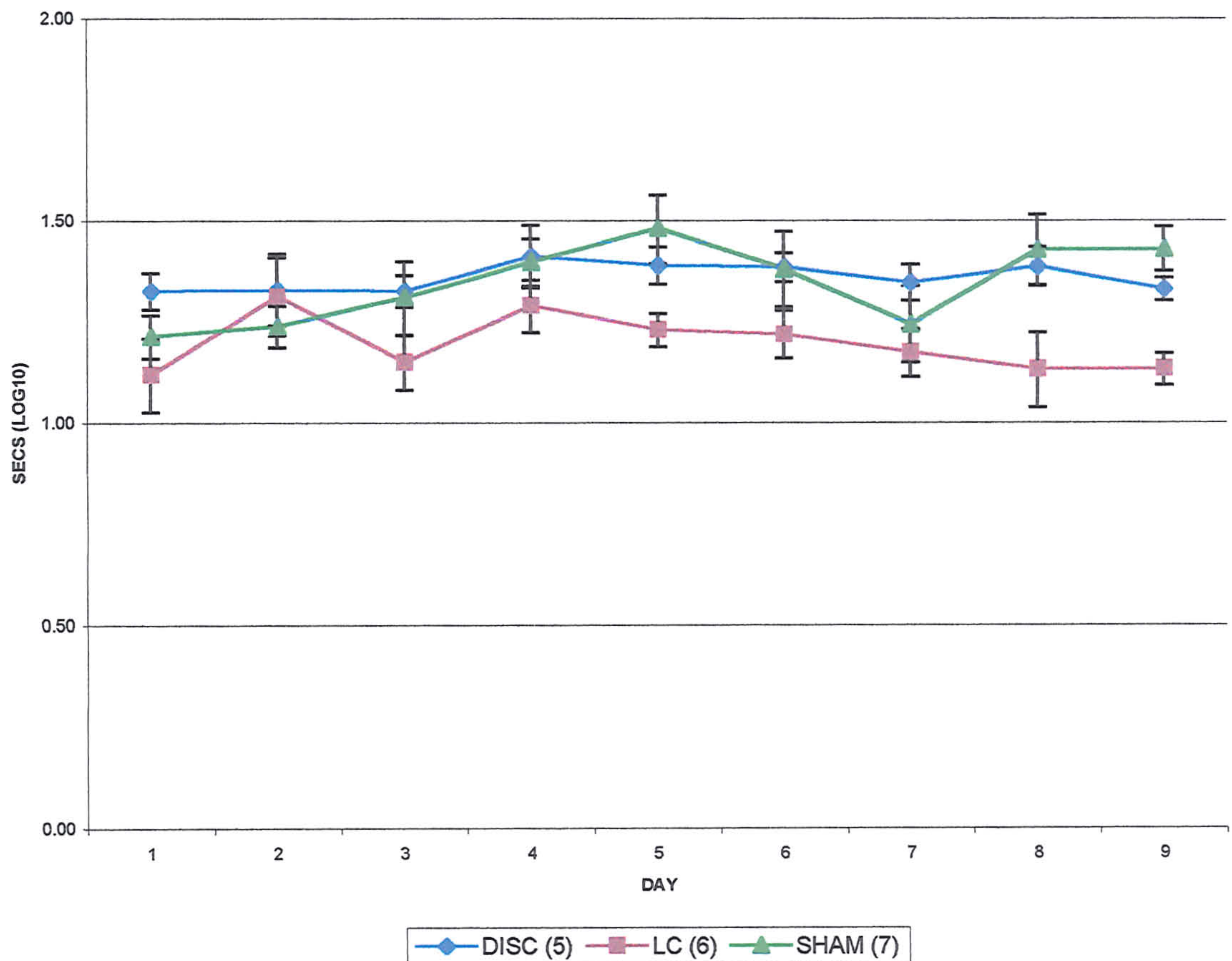


Figure 13.xxiv shows the mean and standard error for the arm choice times for the test phase across all postsurgical trials. The two-way mixed ANOVA showed that there was not a main effect of group $F(2,15) = 3.57$ but there was a main effect of day $F(7.05,105.75) = 2.55$ $p < 0.05$. The group x day interaction was not significant $F(14.10,105.75) = 1.36$. Thus, although the lesion control group had been shown to make their choice times significantly faster when the analysis was restricted to the criterion days, the data for all trials did not reveal a significant effect of this nature across all days (critical F for group at $p < 0.05 = 3.68$).

FIGURE 13.xxiv TEST CHOICE TIMES



13.4 Discussion

The current experiment was conducted with two main aims. The first was to assess, again, the effects of disconnection of the PPTg and mPFC. This was designed to add to the findings of the experiment presented in Chapter 12 such that a combined disconnection lesion effect could be obtained with lesions restricted to the PPTg and mPFC. However, given the extensive problems with the lesions produced in this experiment it was not possible to achieve this aim, and indeed the combined analysis has not been conducted.

The second aim was to investigate the effects of damage to the PPTg and mPFC in the same hemisphere with the hope of illustrating that the disconnection lesion effects seen in the preceding chapter were the result of PPTg/mPFC *disconnection*, rather than simply the result of having sustained a certain amount of neuronal loss in both of these structures in combination. It is difficult to draw precise conclusions with regard to this issue since the lesions produced in the current experiment in the lesion control group did not parallel the lesions produced in the disconnection group in the last one. Specifically, mPFC loss in the present experiment was poor and very often did not include damage to area PL. Furthermore, damage sustained to area AC was not sufficient to include this region throughout the entire anterior posterior axis. As a result of these problems, comparisons with the findings of Chapter 12 are necessarily limited.

There were significant effects in the criterion analysis in the current experiment for the lesion control group. Perhaps worryingly, this effect occurred in one of the measures on which there was a significant disconnection lesion effect in Chapter 12: test choice

times. However, the implication of this result for the interpretation of the PPTg/mPFC disconnection effect remains questionable. For example, the extent of damage seen in both the PPTg and the mPFC in the lesion control and disconnection groups of the current experiment was similar. Therefore, if the lesion control effect does mirror the disconnection lesion effect of the preceding experiment the same effect would also be expected to be present in the disconnection group of the current experiment. Certainly the lesion control finding is enough to warrant further investigation of the issue, although the fact that it was not significantly apparent throughout the earlier test trials, while the disconnection lesion effect of Chapter 12 was, might suggest that the lesion control effect on test choice times in this experiment is a spurious result. The alternative explanation could be, though, that high variability in the mPFC lesion quality masked the effect in the omnibus ANOVA.

Precisely why the mPFC lesions in this experiment were poor is unclear since the developed technique (Chapter 5) did not prove to be a problem in the previous experiments. Re-current use and damage to syringes is one possibility given that a substantial number of mPFC lesions were either not present or too small. The large nature of the PPTg lesions and the number of cases of invasion of the substantia nigra has, however, been an undesirable outcome occurring with particular frequency. The specific pattern of damage external to PPTg boundaries would suggest that it is the rostral infusion that creates most problems (given the high vs. low invasion of SN and LDTg respectively). As the PPTg does change in both shape and size along the rostro-caudal axis, with its rostral portion being smaller than its caudal portion, perhaps less nigral damage would be observed with a technique that reduces the volume/concentration of toxin infused at the two sites, in line with this change. Indeed,

if further investigation of the PPTg/mPFC disconnection effect was to occur, reducing the amount of variability of the lesion areas at both sites would seem to be a necessary prerequisite for ensuring larger group sizes and greater confidence in the disconnection lesion effects produced.

14. GENERAL DISCUSSION

14.1 Considering the functions of the PPTg in relation to fronto-striatal processing

The PPTg is a collection of cholinergic and non-cholinergic neurons situated in the mesopontine tegmentum. It is a structure that is bordered dorsally by the cuneiform and deep mesencephalic nuclei, laterally by the lemniscal fibres, caudally by the parabrachial nucleus, and rostrally by the substantia nigra. Defined in this way, the PPTg has an extensive array of connections that include a close relationship with the basal ganglia and thus fronto-striatal systems. These connections include the receipt of both dorsal and ventral striatal outflow, in addition to reciprocal connections with basal ganglia output structures such as the globus pallidus, ventral pallidum, and substantia nigra. Furthermore the PPTg is thought to be in direct receipt of both motor (Hartmann-von Monakow, Akert, & Kunzle, 1979) and limbic (Zahm, personal communication) cortical information. As a result of the abundance of PPTg connections with fronto-striatal circuitry it is likely that the behavioural output of this system depends, in some way, on the neural processing occurring in PPTg. This is especially the case given that PPTg provides fronto-striatal processed information with access to mechanisms of motor control in the medulla and spinal cord out with the “looped” circuitry returning to cortex via thalamus.

Early studies examining the functions of the PPTg had suggested that it was involved in some way in the production of motor behaviour, for example coordinated stepping movements (Mogenson *et al.* 1989, & Mogenson & Wu, 1988). While direct electrical

stimulation of the PPTg in the rat has been shown to elicit locomotion (Garcia-Rill *et al.*, 1990), lesions of the PPTg, also in the rat, have not been shown to produce changes in locomotion *per se* (see Winn 1998). Rather, the changes in locomotor behaviour that have been observed occur in conditioned locomotion when it is used as a measure of drug reinforcement (Bechara *et al.*, 1992a). Further examination of the effects of PPTg lesions (see Chapter 4) have suggested that the PPTg is involved in the production of behaviours rather more complex than locomotion itself. Infact, lesions of the PPTg have been suggested to produce behavioural disorders that might best be characterised as disinhibited, perseverative and inappropriate responding (Winn, 1998 and Chapter 5). As these are phrases more commonly employed to describe the behavioural consequences of damage to the frontal lobe (although they do not describe this syndrome in its entirety) it was of interest to investigate the involvement of PPTg in fronto-striatal processing further.

14.2 Basal ganglia evolution and disconnection lesions

Marin *et al.* (1998) have presented evidence to show that the basal ganglia evolve in amniotic and anamniotic vertebrates with many anatomical and morphological similarities. This suggests that the basal ganglia have developed within the brain as a whole, rather than as a collection of individual structures with unique functions that can exist incompletely. Thus the high level of interaction occurring both within the basal ganglia and the structures with which they are connected may be the key to understanding the function of this complex brain system. As a direct result of this, a methodology that enables the function of *connections* to be understood rather than the function of *structures* would be particularly appropriate.

Evidence in support of this position comes from the work of Anthony Phillips and co-workers who have used a lidocaine disconnection lesion technique to study the involvement of various structures in the ventral fronto-striatal system in the performance of the same radial maze tasks. One of these tasks is the DSWS task. Significantly they have shown that the connection of hippocampus with nucleus accumbens performs a function different to that of hippocampus connections with the prefrontal cortex (see section 4.1). Crucially, this is not something that would have been apparent from an examination of the behavioural consequences of hippocampal lesions alone, supporting the contention that a disconnection lesion can be a particularly useful tool when applied to assess the functions of the fronto-striatal system. In light of this, the aim of the research discussed here was to apply this procedure to study the functional role of the PPTg in relation to its extensive connections with fronto-striatal structures.

14.3 Executive functions and the role of the mPFC in the DSWS task

Attempting to analyse the behavioural consequences of PPTg lesions in the rat in terms of executive functions is highly problematic not least because no one can say with surety precisely what “executive functions” are. They have been characterised for the present research as higher cognitive functions requiring some form of control over processes normally carried out by specialised areas of the brain connected with PFC. These higher cognitive functions can include working memory, attention, planning and response inhibition components but this is by no means a definitive list. There are many other proposals regarding the nature of executive functions and these have been

discussed previously (see Chapters 3 & 8). Furthermore, the existence of these higher cognitive functions and related deficits have been demonstrated in the rat (Chapter 3), making this an appropriate model for studying these functions despite the fact that there remains a question over homology of primate and rodent regions of PFC.

The involvement of the rat mPFC in the DSWS task had been demonstrated prior to the current research by the work of Seamans *et al.* (1995). This cleverly designed study revealed that areas PL and AC may have separate roles in DSWS performance, with area PL suggested to be necessary for the retrieval of trial unique information acquired prior to a delay. This supports the contention that rat area PL may be functionally analogous to primate dlPFC because of its involvement in working memory. However, there were important limitations to the Seamans *et al.* (1995) lidocaine study that were required to be resolved prior to the application of a permanent excitotoxic disconnection lesion technique for mPFC and PPTg. The experiment presented in Chapter 8 aimed to extend the findings of Seamans *et al.* (1995) by enabling an analysis of DSWS behaviour across more than a single trial (with permanent lesions) to assess whether significant improvement on the DSWS task would occur with practice in rats with bilateral mPFC lesions centred on area PL but with damage extending to area AC. The findings of that experiment did support the work of Seamans *et al.* (1995) since rats with ibotenate mPFC lesions were significantly impaired on the DSWS task. They also displayed a pattern of responding that resembled the AP/WP error comparisons presented in Seamans *et al.* (1995) for rats with AC but not PL lesions. Furthermore, mPFC lesioned rats did not significantly improve their performance in terms of the number of errors made in the test phase, although they were able to significantly delay the intrusion of errors into their choice sequence.

Of particular interest in this experiment, however, was the intriguing trend of a “lack” of impairment in mPFC lesioned rats on the first day of DSWS post-surgical testing. This trend occurred on all measures on which rats with mPFC lesions were found to be impaired. Thus although the trend did not reach significance, its robust nature could indicate that rats with mPFC lesions can perform the DSWS task in a single trial. However their impairment becomes apparent when the next day’s trial requires a reorganisation of the arm pairings with reward. Such an effect would be consistent with theories of PFC function centred on the notion of flexibility of responding and would also be in line with recent findings suggesting a role for rat mPFC in attention set shifting (Birrel & Brown 2000), since aspects of the reorganisation of arm pairing may parallel a set shift. Given that the Seamans *et al.* (1995) study was designed in such a way that this “lack of effect” would not have been apparent, it is something that is worthy of future study. It is also something that, until it is clarified, prevents a precise conclusion regarding the psychological function of the mPFC in the DSWS task.

Given the suggested involvement of rat mPFC in memory for trial unique information in the DSWS task, whether this is, or is not limited to the second trial onwards, it should be the case that impairment is also observed in DSWS acquisition. However this did not occur following bilateral mPFC lesions in the experiment presented in Chapter 11. The low group numbers in that experiment, and the fact that the smaller lesions included in the analysis sustained limited damage to area PL may explain this finding. However, it would be expected that bilateral lesions of area AC alone would also produce a significant deficit on acquisition of the DSWS task given the findings of Seamans *et al.* (1995). More specifically, area AC in the Seamans *et al.* (1995) study was proposed to play a role in response flexibility since rats with AC lesions were impaired in both the

DSWS task and the random foraging task. The AC impairment in both tasks was characterised by the persistent revisiting of arms previously baited with reward, something that should also occur during DSWS task acquisition. A future assessment of whether or not rats with restricted AC lesions in a retention paradigm could be shown to improve significantly over the testing period may be the next logical step in determining AC involvement in DSWS performance. This is because an impairment that is apparent only in the first few days of testing during acquisition could be masked by the equally poor performance of sham operated rats.

14.4 The functions of the PPTg

The involvement of the PPTg in DSWS behaviour had also been demonstrated prior to the research presented here. Keating & Winn (2001) selected the DSWS task for an assessment of PPTg functions precisely because of the findings of Seamans *et al.* (1995). It was their aim to demonstrate that rats with bilateral PPTg lesions would exhibit impairments in the same tasks in which rats with mPFC lesions exhibit impairments, thus extending to PPTg the argument that structures connected with the frontal cortex share in some way frontal functions. Their study showed that rats with bilateral PPTg lesions were impaired in retention and acquisition of the DSWS task with the nature of the impairment in PPTg lesioned rats being similar to that described in the current research for mPFC lesioned rats. That is, rats with mPFC lesions made significantly more AP errors than WP errors in the current research, and rats with PPTg lesions in Keating and Winn (2001) also made this pattern of more across phase than within phase errors (a pattern of responding that is similar to the responding of sham operated controls).

Further experiments (see Chapters 4 and 9) have shown that the PPTg deficit in tasks that require responding for reward may be variable depending on the strength of the reward that is used. That is, under conditions of increasing motivational excitement PPTg lesioned rats seem to suffer a higher level of impairment. However, this deficit had been studied in simple consummatory and approach behaviours only (see for example Ainge *et al.*, 1999). Given that PPTg lesioned rats had already been shown to be impaired in the executive DSWS task the question arose as to whether this impairment would be worsened following the introduction of a “nicer” reward and thus help to establish the psychological involvement of PPTg in this task in relation to the proposals made for mPFC.

The experiments presented in Chapters 9 & 10 examined this hypothesis in detail but did not find conclusive evidence to suggest that PPTg DSWS impairments could be worsened if a more positive reward was used. Thus the modulation of level of motivational excitement in PPTg lesioned rats did not have significant effects on their DSWS performance. In addition to this there was no significant evidence to suggest that PPTg lesioned rats suffered impairment in speed of responding to a change in reward, which did appear to be the case from the runway results of Ainge *et al.* (1999). This suggests that the problems experienced by PPTg lesioned rats in the DSWS task may be separate to those experienced on the runway task of Ainge *et al.* (1999). Given the complex nature of the DSWS task it is likely that the findings of Ainge *et al.* best represent PPTg deficits in simple reward related responding. However it is still possible that effects on approach behaviour in the runway task differ from those in the DSWS task because finite amounts of reward are available on the DSWS task (see section 9.4.3) and this requires further examination also.

Taken together the results of the experiments presented in Chapters 9 & 10 provided mixed support for the work of Keating & Winn (2001). Rats with PPTg lesions in the first experiment (Chapter 9) experienced problems in the training phase of the DSWS task in addition to the test phase. Extension of lesions beyond the boundaries of the PPTg in that experiment could conceivably have contributed to these additional effects. This is especially the case given that lesions invaded parts of the SNc. Dopaminergic neurons of the SNc have been argued to be involved in the prediction of reward (Brown, Bullock, & Grossberg, 1999) making damage to this region cause for concern in an experiment that manipulates reward level unexpectedly. However, the fact that rats with PPTg lesions in this experiment experienced no akinesia suggests that SN damage may not have been significant.

The second experiment (Chapter 10) with more controlled damage to PPTg did show that PPTg lesioned rats were impaired only in the test phase, and this was on all measures on which mPFC lesioned rats were impaired. However, rats with lesions restricted to PPTg in Chapter 10 did not make significantly more AP than WP errors, producing a pattern of responding on the DSWS measures that differed from that found in mPFC lesioned rats in Chapter 8. Although this does not directly support the pattern of responding in PPTg lesioned rats found in Keating & Winn (2001), the data in Chapter 10 did show a trend in support of their findings. Repeated experimentation will help to clarify more precisely which pattern of responding PPTg lesioned rats exhibit. However, with regards the aim of the current research it is interesting to note that PPTg rats may exhibit a DSWS deficit that mirrors the impairments shown by mPFC lesioned rats on this task.

14.5 Disconnection of PPTg and mPFC

The experiment presented in Chapter 11 provided evidence to suggest that unilateral lesions of either mPFC or PPTg were not sufficient to produce impairment in acquisition of the DSWS task. Furthermore, Chapter 12 additionally demonstrated that unilateral lesions of either structure did not lead to deficits in DSWS retention. Thus it can be concluded that impairments shown by rats with crossed unilateral disconnection lesions of the mPFC and PPTg were not a result of either PPTg or mPFC unilateral lesions in isolation.

Chapter 12 presented some evidence to support the contention that the transfer of neural information between PPTg and mPFC is important for DSWS retention during the early stages of postsurgical testing. In this experiment rats with crossed unilateral disconnection lesions did not show any impairment when analysis was restricted to the sham criterion days. However, additional analyses across all trials did show that disconnection lesioned rats performed significantly differently to shams on three of the test phase measures. These were the number of total test phase errors, the number of within phase errors, and the mean arm choice time. Furthermore, rats with disconnection lesions in this experiment were the only group not to make significantly more AP than WP errors, a finding that is likely to be a result of their restricted increase in WP errors only. These results are represented in Table 14.a for ease of comparison with findings for PPTg (Chapter 10) and mPFC (Chapter 8).

Table 14.a A comparison of DSWS deficits for PPTg, mPFC and Disconnection lesioned rats.

	PPTg	MPFC	DISC
<i>Training Errors</i>	NONE	NONE	NONE
<i>Training No. Correct</i>	NONE	NONE	NONE
<i>Training Latency</i>	NONE	NONE	NONE
<i>Training Choice Times</i>	NONE	NONE	NONE
<i>Test Errors</i>	>	>	>
<i>AP</i>	>	>	NONE
<i>WP</i>	>	>	>
<i>Test No. Correct</i>	<	<	NONE
<i>Test Latency</i>	>	>	NONE
<i>Test Choice Times</i>	NONE	NONE	<

NB. The deficits shown in the table for PPTg rats were calculated from the criterion days' analyses while the deficits shown for mPFC and DISC rats were calculated from extended analyses across trials.

Given that the overall aim of this research was to employ the disconnection lesion technique to demonstrate directly PPTg involvement in the processing of fronto-striatal information, the results of the experiments presented in Chapters 12 & 13 were disappointing. Problems with the lesion techniques in Chapter 13 made these results particularly problematic to interpret. However, the results of Chapter 12 do suggest that the application of disconnection lesions between PPTg and mPFC, and indeed perhaps other sites in the ventral striatal system, is worthy of future study. One point of interest may be to study the effects of disconnection of structures lower in the neuraxis than mPFC, such as VP and PPTg, since VP has also been shown to be involved in DSWS behaviour. However, if future disconnection lesions were attempted between mPFC and PPTg the focus must undoubtedly be on the perfection of reliable lesion techniques for mPFC and PPTg. The possibility of a large degree of variation in the extension of each unilateral lesion beyond the borders of the target structures makes finding pure cases of disconnection extremely difficult and this is a particular problem for any study attempting disconnection lesions. However, the very complex and interactive nature of the fronto-striatal system does make it an invaluable tool for neuroscience research.

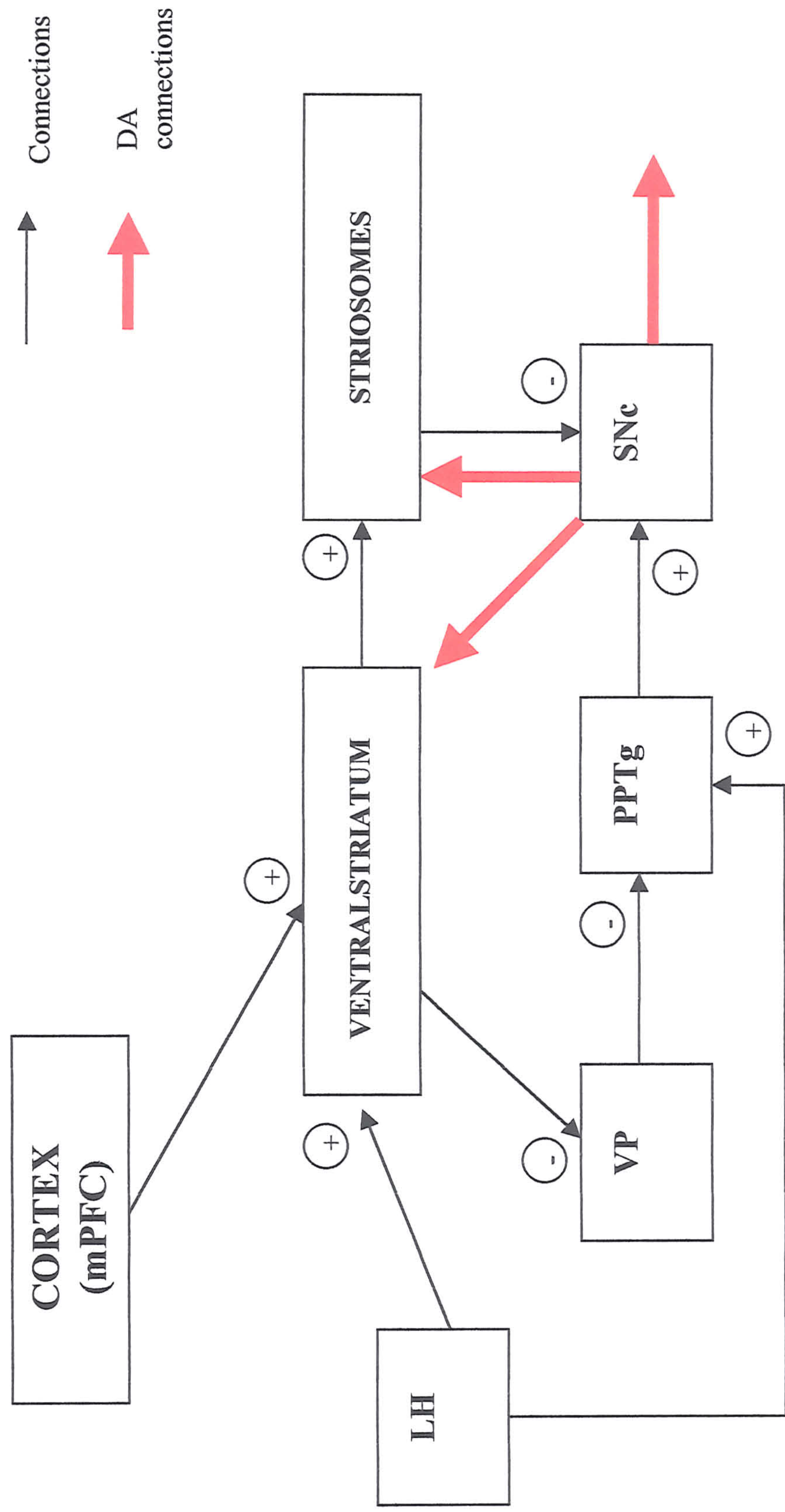
14.6 A model for the involvement of PPTg and mPFC in responding for reward?

Brown *et al.* (1999) have presented a model of ventral striatal involvement in responding for conditioned reinforcement that includes a very precise role for PPTg and can be related to the findings of the current research. In this model PPTg seems to mediate information about a conditioned stimulus originating from cortex and passing to ventral striatum, and its output structure the VP, in relation to a primary reward signal

that PPTg receives from its connections with lateral hypothalamus. The connections of this system can be seen in Figure 14.i. The role of PPTg in this system is related to inducing phasic bursts of activity in SNc in response to the receipt of a primary reward or reward predicting conditioned stimuli. This is important because Berridge & Robinson (1998) have argued that increases in DA phasic activity endow immediate salience to biologically relevant stimuli prompting the selection of appropriate patterns of behaviour. In this model a CS is assumed to activate a sustained working memory input from limbic cortex to the ventral striatum. A subsequent primary reward signal triggers a dopamine burst that augments the weights between the working memory site and the ventral striatum. Thus future presentations of the CS elicit an immediate excitatory prediction of reward. In addition the CS also activates a population of lagged inhibitory signals from striosomes to the SNc such that when a DA burst occurs at a sufficient time lag after the CS, it strengthens the subset of lagged inhibitory signals that are active at that time. Thus parallel types of learning in this system enable a CS to generate an immediate reward-predicting signal but also to cancel subsequent SNc excitation that would otherwise be caused by the predicted reward related signals. Lesions of the PPTg could conceivably, therefore, be expected to prevent the generation of the reward-predicting signal and impair reward related learning.

Given the suggestions made in this model concerning the roles of the mPFC and the PPTg in reward related responding, and thus DSWS behaviour, the two structures would appear to function in a complimentary fashion. The provision of working memory for the CS is the role proposed for the mPFC while the mediation of this information with that received from the lateral hypothalamus regarding the US would mean that PPTg is involved in learning to successfully predict the occurrence of reward.

FIGURE 14.i Model for PPTg and mPFC involvement in responding for reward



These ideas fit very well with current knowledge of both mPFC involvement in working memory and the findings that PPTg lesioned rats do not respond in the same manner to sham rats in instrumental tasks. However, it should be borne in mind that this model is necessarily restricted in terms of an assessment of PPTg function since it includes only connections PPTg shares with VP, LH and SNc. The direction of future research must therefore be in ascertaining a function for PPTg that accounts for all of its many and varied connections.

14.7 Some conclusions

Given the problems associated with defining executive functions in the rat perhaps the best way to assess these would be in a task that has been derived from one frequently used to reveal frontal dysfunction in humans. In this way more obvious parallels could be drawn across species. The attention set shifting task for rats developed by Birrell and Brown (2000) may be one that is ideally placed to do this, and would usefully extend the research presented here. This is especially the case given the interesting trend displayed by mPFC lesioned rats in Chapter 8 since attention set shifting directly measures mechanisms of cognitive flexibility. Thus, although the findings of the DSWS task statistically show similar impairments for PPTg and mPFC lesioned rats, the trend shown by mPFC lesioned rats in Chapter 8 suggests that there may be more to their deficit than is revealed by the ANOVAs. An assessment of PPTg and mPFC involvement in this task may therefore prove particularly insightful. One further piece of evidence to support the proposed use of the attention set shifting task is the nature of the DSWS task and related problems with its use with permanent lesions. Specifically within subject variability in performance on the DSWS task can be high and this is not

desirable because it has the potential to mask lesion effects. Thus while the DSWS task may be acceptable for the single trial lidocaine studies of Phillips and colleagues it is not ideally suited to research that examines performance across a number of trials.

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