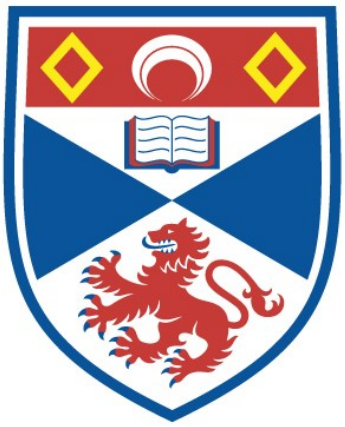
THE ASSESSMENT OF BEHAVIOURAL DEFICITS FOLLOWING FOCAL CEREBRAL ISCHEMIA

Nicholas M. Ward

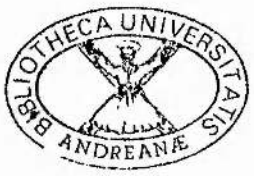
A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1997

The Assessment of Behavioural Deficits Following Focal Cerebral Ischemia

Nicholas M. Ward



Submitted to the University of St. Andrews for the Degree of Ph.D.

August 1997

Declaration

i) I, Nicholas Matthew Ward, hereby certify that this thesis, which is approximately 37,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

Date: 1/8/97 Signature of candidate:

ii) I was admitted as a research student in October 1994 and as a candidate for the degree of Ph.D. in October 1994; the higher study for which this is a record was carried out in the University of St Andrews between 1994 and 1997.

Date: 1/8/97 Signature of candidate:

iii) I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Ph.D. in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

Date: 1/8/97 Signature of supervisor:

Copyright

Unrestricted

In submitting this thesis to the University of St Andrews I understand that I am giving permission for it to be made available for use in accordance with the regulations of the University Library for the time being in force, subject to any copyright vested in the work not being affected thereby. I also understand that the title and abstract will be published, and that a copy of the work may be made and supplied to any bona fide library or research worker.

Date: 1/8/97 Signature of Candidate:

Acknowledgements

This research was made possible by a Medical Research Council (UK) Collaborative Studentship (G78/4236) in association with the Fujisawa Institute of Neuroscience (FINE), Department of Pharmacology, University of Edinburgh.

Expert advice has been gratefully received from Dr. John Sharkey (FINE), Dr. Eric Bowman and Dr. Hugh Marston (FINE) who have helped to keep me on the narrow path towards a Ph.D. I would also like to thank Dr. Janice Phillips and Dr. Colin Davidson for assistance with the behavioural testing and the technicians of the Animal House and Workshop of the School of Psychology for their expertise at all stages of this work. In particular a special mention should go to Mary Latimer for her expert tuition and valuable suggestions in the craft of histology. Beyond the advice and support for the work conducted in this thesis, a very special thanks should go for everyone's personal encouragement, which has been so important during the last 3 years.

Finally my supervisor Dr. Verity Brown deserves a special commendation for her exceptional work guiding me through the research maze towards a Ph.D.

This thesis is dedicated to my family: Dad, Mum, Simon, Richard, Phillip and my fiancee Kathryn

List of Contents

Papers and Abstracts (Derived from research conducted during the Ph.D.)

Papers

- Ward, N.M., & Brown, V.J. (1996). Covert orienting of attention in the rat and the role of striatal dopamine. **Journal of Neuroscience**, 16, 3082-3088.

- Ward, N.M., Sharkey, J., & Brown, V.J. (1997). Assessment of sensorimotor neglect following occlusion of the middle cerebral artery in the rat. **Behavioral Neuroscience**, 111, 1133-1145.

- Ward, N.M., & Brown, V.J. **(in press)**. Deficits in response initiation, but not attention, following excitotoxic lesions of posterior parietal cortex in the rat. **Brain Research**.

- Ward, N.M., Sharkey, J., & Brown, V.J. **(submitted)**. Simple and choice reaction time performance after occlusion of the anterior cerebral arteries in the rat. **Experimental Brain Research**.

Abstracts

- Marston, H.M., Sharkey, J., Ward, N.M., & Good, M.A. (1995). Comparison of excitotoxic hippocampal lesions and mid-line ischaemic damage on delayed non-matching to place in the rat. **European Journal of Neuroscience**, Supplement 8, 136.

- Ward, N.M., Sharkey, J., Latimer, M., & Brown, V.J. (1996). Assessment of the volume of ischaemic damage occurring after occlusion of the middle cerebral artery in the rat: a comparison of cresyl violet and glial fibrillary acidic protein (GFAP) staining. **Brain Research Association Abstracts**, 13, 64.

- Ward, N.M., Sharkey, J., & Brown, V.J. (1996). Reaction time performance in the rat after occlusion of the anterior cerebral arteries. **Society for Neuroscience Abstracts**, 22, part 1, 446.

Abstract

Evaluating the efficacy of neuroprotective drugs in rat models of focal cerebral ischemia has involved histological and behavioural batteries to examine pathology and sensorimotor function. However, the behavioural tests used provide little insight into the nature of the neurological impairments. In an effort to gain further insight into the behavioural impairment following ischemic lesions, a battery of tasks were used. The tasks included tests of sensorimotor, motor (paw use), motivation, sensory and attentional function.

The use of the potent vasoconstrictor endothelin-1 has allowed cerebral arteries to be occluded. This can be used to occlude the MCA (which is a common target of ischemia research), as well as other arteries, such as the ACA. Typically quantitative volumetric analysis has used nissl stains to assess lesion extent. However, alternative markers of tissue dysfunction are available including GFAP to assess the astroglial response to ischemia. Consequently cresyl violet and GFAP were compared along with different methods for calculating lesion volume. The boundaries of the lesion identified using the two stains corresponded closely providing care was taken when calculating lesion volume to avoid distortion from histological procedures and edema.

Following MCA occlusion the rats displayed unilateral somatosensory and motor deficits, however there was no evidence of attentional dysfunction. Performance in the covert orienting task was compared with striatal dopamine depletion and with a posterior parietal cortical lesion. Neither of these manipulations resulted in deficits of covert orienting. Furthermore, the behavioural consequences of ACA occlusion were studied in two experiments using reaction time tasks designed to dissociate response impairments from dysfunction of motivation and attention. The ACAo ischemic damage did not disrupt motivation or attention, however, the results were consistent with an impairment in selecting and initiating responses.

Chapter 1:

General Introduction

1.1. Stroke

The brain is highly sensitive to it's metabolic environment: it is not only dependent on the continual supply of glucose, oxygen and other nutrients through the vascular system to maintain stability, but is also vulnerable to the build-up of toxic metabolic by-products. If blood supply is reduced, within minutes a cascade of events may begin, with cell death if supply is not restored. Such an interruption of blood supply to the brain has commonly been referred to as a 'stroke' and may arise due to a variety of causes which may co-occur, including cardiac arrest, embolisms, thrombus and haemorrhage. Stroke is a major neurological disease in the United Kingdom, with a first stroke striking 180/100,000 of the population each year. Of those who survive a first stroke, a further 25 % will have another stroke and this culminates in approximately 1 % of the United Kingdom population suffering from neurological impairment caused by a stroke (Marsden & Fowler, 1989). Unfortunately stroke remains amongst the most prominent causes of death and disability in the Western world (Heros, 1994; Millikan et al., 1987a). Of the mechanisms causing disturbances in blood supply to the brain occlusion of a cerebral artery is the most common form accounting for 70 % of strokes (Millikan et al., 1987). Occlusive stroke in the experimental research literature has been referred to as focal cerebral ischemia, and this will be the focus of the research reported in this thesis.

1.2. Focal Cerebral Ischemia and the Human Arterial Cerebrovasculature

The onset of focal cerebral ischemia is typically preceded by a narrowing of the vasculature due to atherosclerosis and thrombus, which may combine with the formation and detachment of embolic material to cause arterial occlusion (Millikan et al., 1987d). In humans approximately 70 % of infarcts occur in the territory of the carotid artery. Of these 96 % involve the vascular territory of the MCA and 3 % occur in the territory of the ACA (Bogousslavsky et al., 1988). Consequently as the MCA is the most common location of infarction it provides a focus of interest for those investigating focal cerebral ischemia. The middle cerebral artery (MCA) arises from the internal carotid artery after it has penetrated the dura and formed the anterior input to the Circle of Willis (Figure 1.1). The course of the MCA takes it out from the Circle of Willis and up, passing between the inferior and medial/superior temporal cortex along the lateral fissure. The internal carotid arteries, once in the skull, divide to form the anterior portion of the Circle of Willis

giving off the anterior cerebral arteries (ACA) which are joined by the anterior communicating artery. The posterior cerebral arteries (PCA) along with the posterior communicating arteries provide the posterior portion of the Circle of Willis (Bannister, 1979; Brust, 1991). Occlusion of the MCA or the ACA (beyond the anterior communicating artery) cannot be compensated by redirection of supply through the Circle of Willis. However, the arterial system is able to provide some compensation if blood supply from the vertebral or internal carotid arteries is interrupted with redirected flow through the Circle of Willis. Indeed occlusion of the Internal Carotid Artery may not result in an infarction if collateral blood supply is normal (Millikan et al., 1987c).

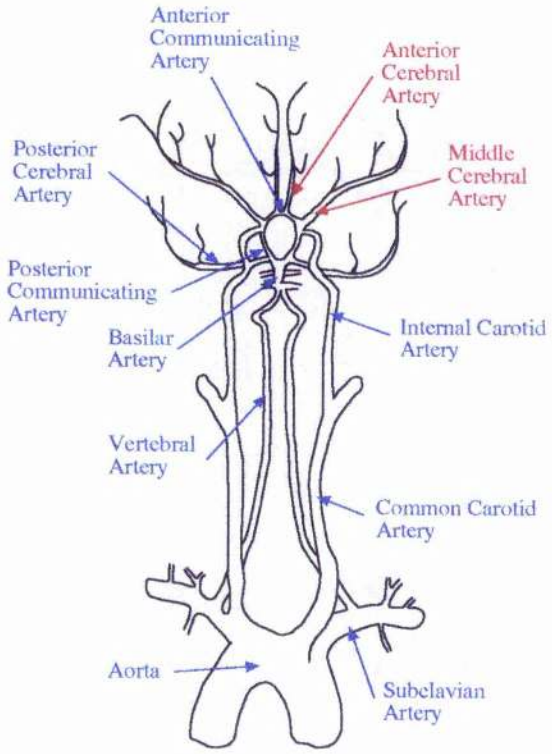


Figure 1.1. *An illustration of the principal arteries arising from the aorta that supply the cerebral arteries. The major cerebral arteries are interlinked to form the Circle of Willis, which may compensate for an interruption of blood supply following occlusion of an artery (adapted from Brust, 1991). The experiments reported in this thesis involve occlusion of the middle and anterior cerebral arteries in the rat. The structure of cerebrovasculature is similar in the rat, although there is some variation, with the anterior communicating artery absent in many rats (Scremin, 1995).*

The MCA covers the lateral surface of the cortex supplying blood to lateral frontal, insula, lateral parietal, superior temporal and posterior association cortex (Figure 1.2). The penetrating branches of the MCA supply blood to subcortical structures including the body of the caudate, putamen, external globus pallidus and posterior fibres of the internal capsule (Brust, 1991; Van der Zwan et al., 1992).

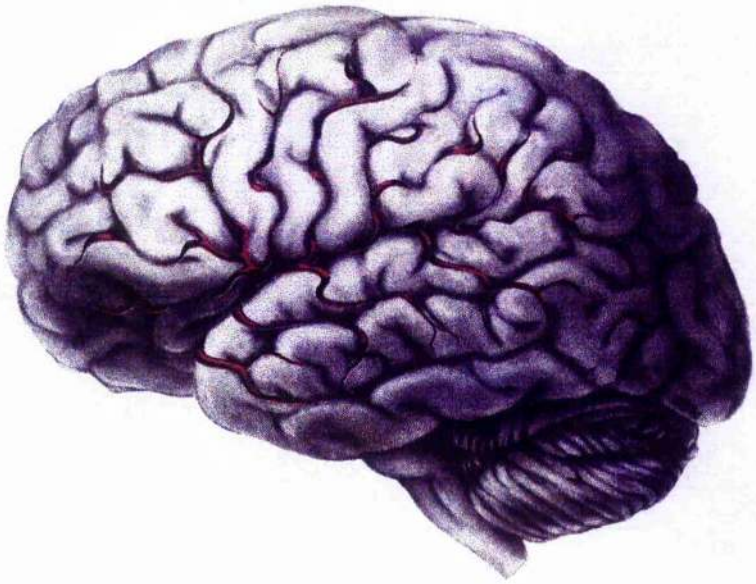


Figure 1.2. *The middle cerebral artery (visible in red) arises along the lateral fissure and supplies the lateral cortex (parietal, temporal, lateral frontal and association cortex) and portions of the basal ganglia (from Heimer, 1983).*

There are two other major arteries which branch off from the Circle of Willis on each side; the anterior cerebral and posterior cerebral arteries. The inferior frontal lobe and medial surface of the cortex extending back to the occipital lobe, underlying white matter and anterior corpus callosum receive their blood supply from the ACA. Penetrating branches of the ACA supply blood to cingulate cortex, head of the caudate and anterior portion of the fibres of the internal capsule (Brust, 1991; Van der Zwan et al., 1992). The posterior cerebral artery supplies blood to the inferior temporal, medial occipital cortex and posterior corpus callosum. Branches of the PCA penetrate down into subcortical tissue to supply blood to the thalamus and subthalamic nuclei (Brust, 1991; Van der Zwan et al., 1992). The major cerebral arteries (MCA, ACA and PCA) are connected with each other at the surface of the brain via anastomoses. These connections

at arterial border zones provide an alternative supply source if cerebral blood flow fails in an adjacent artery. The penetrating branches of the major cerebral arteries supplying subcortical tissue do not have interconnections with adjacent arteries and so are regarded as end arterial zones without alternative blood supply sources (Bannister, 1979).

The sizes of territory supplied by the major cerebral arteries are variable, any of the major cerebral arteries may supply larger or smaller territories than the pattern described above. However, the differences in vascular territories do not reflect fundamental changes in distribution, but rather variation in the supply patterns at border zones. For example the MCA artery at the lateral surface supplies a core area radiating out from the lateral fissure in all brains studied by Van der Zwan et al. (1992). The variation exists with the extent of the territory supplied outside the cortex surrounding the lateral fissure. However, the most common arterial distribution pattern for the MCA corresponds to the distribution described previously.

1.3. Impairment Following Focal Cerebral Ischemia in Humans

The disturbance of neuronal function due to focal cerebral ischemia is also associated with neurological symptoms (Brust, 1991). Dependent on the severity of the ischemia, location and extent of neural tissue affected the consequence can be diverse ranging from death to mild neurological impairment with full recovery. Neurological patterns of impairment following occlusion of different arteries are distinguishable to the extent that function can be localised within the brain.

The territories of the MCA and ACA include such a large proportion of cortical and subcortical areas that to detail the full range of deficits in humans would describe nearly the entire field of neuropsychology. The extent of the brain involved is evident when the different areas of cortex and subcortex supplied are considered. The regions of brain supplied by the middle cerebral artery include the cortical areas: dorsolateral, orbital, premotor, frontal eye fields, insula, Broca's area, motor strip for the face and mouth, precentral gyrus, postcentral gyrus, posterior parietal, supramarginal angular gyrus, parietal occipital and posterior temporal junctions and include the subcortical areas: striatum, internal capsule and external globus pallidus. The anterior cerebral artery supplies medial cortex including: cingulate gyrus, supplementary motor area, medial motor strip and extends subcortically to supply the anterior corpus callosum and internal

capsule. The anterior communicating artery supplies subcortical structures including the septum, nucleus accumbens and diagonal band of Broca. The range of impairments which may follow occlusion of the middle and anterior cerebral artery in humans include disruption of language, memory, reasoning, personality, movement, co-ordination and perception (see Tables 1.1 and 1.2). Given that the behavioural consequences can be so devastating, the amelioration of these neurological deficits remains the primary objective in developing treatment for focal cerebral ischemia. In order to develop an informative model of focal cerebral ischemia in the rat the focus is directed towards functions which can be assessed in both humans and rats. Areas of impairment particularly relevant are motor and sensory functions, and capacities including attention, executive control, spatial processing, learning and memory.

Brain Regions Supplied by the MCA Branch	Branches of the MCA	Clinical Signs
Dorsolateral, orbital, premotor and frontal eye fields	Orbitofrontal artery	Abnormal social/sexual behaviour [1] Risk taking [1] Impaired strategy formation/divergent thought [1,2] Impaired cognitive flexibility [2] Impaired temporal memory [3,4] Oral apraxia (LH) [5] Apraxia (LH) [6] Impaired contralateral conjugate gaze [7,8] Impaired eye movements [26] Forced grasping [9] Hemineglect, motor (RH) [9,10,11,25] Anosognosia (RH) [9]
Insula cortex, Broca's area and motor strip for face	Operculofrontal artery	Broca's type aphasia/agraphia (LH) [7,8,12] Facial/tongue weakness [1]
Precentral and postcentral gyrus	Central sulcus artery	Hemiplegia/hemiparesis contralateral hand, arm, face, hip and partial leg [7,8,13] Somatosensory tactile, temperature and pain loss contralateral hand, arm, face, hip and partial leg [7,14,15]
Posterior parietal, supramarginal, angular gyrus, parietal-occipital and posterior-temporal border zones	Posterior parietal, angular and posterior temporal arteries	Wernicke's type aphasia (LH) [7,12] Acalculia, alexia, agraphia, finger agnosia, left-right confusion (LH) [8,13,16] Impaired memory for language (LH) [16] Hemineglect/hemi-inattention (RH) [11,17,18,25] Anosognosia (RH) [7,19] Topograhagnosia (RH) [7] Apraxia (LH) [6,20,21] Dressing apraxia (RH) [7] Constructional apraxia (RH) [7,8,13] Homonymous hemianopsia [7,11] Cross modal deficits e.g. astereognosia [14] Impaired proprioception [7]
Striatum, internal capsule and external globus pallidus	Lenticulostriate arteries	Broca's type aphasia (anterior LH) [22,23] Wernicke's type aphasia (posterior LH) [24] Impaired cognitive flexibility [2] Contralateral hemiparesis [23] Hemineglect (RH) [23] Apraxia (LH) [23,24]

Table 1.1. *Neurological deficits arising from an infarct in the territory of the middle cerebral artery. Some deficits are associated with lesions in a particular hemisphere, although this association is not always true. The usual hemisphere involved is indicated by LH-left hemisphere or RH-right hemisphere.*

[1]Waddington & Ring, 1968; [2]Eslinger & Gratton, 1993; [3]Goldman-Rakic, 1987; [4]Stuss & Benson, 1986; [5]Mazzoni et al., 1992; [6]Alexander et al., 1992; [7]Brust, 1991; [8]Millikan et al., 1987b;

[9]Heilman & Valenstein, 1972; [10]Kinsella et al., 1993; [11]Mattingley et al., 1994; [12]Geschwind, 1974; [13]Caplan & Stein, 1986; [14]Bassetti et al., 1993; [15]Derouesne et al., 1984; [16]Takayama et al., 1994; [17]Halligan & Marshall, 1993; [18]Bisiach et al., 1981; [19]Starkstein et al., 1992; [20]Harrington & Haaland, 1992; [21]De Renzi et al., 1983; [22]Alexander et al, 1987; [23]Donnan et al., 1991; [24]Naeser et al., 1982; [25]Daffner et al., 1990; Rivaud et al., 1994.

Brain Regions Supplied by the ACA	Branches of the ACA	Clinical Signs
Medial cortex from the frontal pole to medial parietal cortex includes cingulate gyrus, supplementary motor area, medial motor strip, anterior corpus callosum and internal capsule	Main ACA artery	Mental confusion [1,4] Apathy [4,16] Transcortical motor aphasia (LH) [1,4,5,6,7,8,11,12,16,17] Hemiparesis (lower extremities) [1,4,5,6,9,10,11,12,13,14,16,17] Hemisensory loss (lower extremities) [1,4,5,16] Alien hand [2,14] Forced grasping [1,2,3,4,10,12,14] Impaired response preparation and inhibition [2] Ideomotor apraxia [1,2,5,10,14] Incontinence [1,2,4,5,6,14,16] Hemineglect [2,4,11] Failure to maintain attention [2] Akinetic mutism [2,4,14,15]
Septum, nucleus accumbens, diagonal band of Broca	Anterior communicating artery	Retrograde and anterograde amnesia [2] Fluent but incoherent confabulation [2] Apathy [2,18]

Table 1.2. *Neurological deficits arising from an infarct in the territory of the anterior cerebral artery.*

[1]Critchely, 1930; [2]Bogousslavsky, 1994; [3]De Renzi & Barbieri, 1992; [4]Bogousslavsky & Regli, 1990; [5]Caplan & Stein, 1986; [6]Rubens, 1975; [7]Alexander & Schmitt, 1980; [8]Racy et al., 1979; [9]Yamadori et al., 1980; [10]Watson et al., 1986, [11]Brust et al., 1982; [12]Freedman et al., 1984; [13]Bogousslavsky et al., 1992; [14]Chan & Ross, 1988; [15]Devinsky et al., 1995; [16]Brust, 1991; [17]Ross, 1980; [17]Marsden & Fowler, 1989.

Focal cerebral ischemia is a dynamic process in which a diverse range of factors can affect the outcome of an interruption of blood supply. Ischemia may be aggravated or ameliorated in humans by a variety of factors including the pattern of arterial supply, prevailing physiological conditions, cause and location of occlusion. The extent of behavioural recovery from focal cerebral ischemia is also affected by many factors including age, intelligence, sex, diaschisis, personality and handedness (Kolb & Whishaw, 1990). As a consequence of the variability in the clinical condition in humans, systematic study is difficult. Consequently animal models of focal cerebral ischemia have been developed to provide the opportunity to conduct studies utilising a variety of

behavioural, biochemical and histological techniques for investigating cerebral ischemia under controlled conditions. In terms of experimental control in focal cerebral ischemia the location (Markgraf et al., 1992; 1994; Marston et al., 1995b), duration (Bartus et al., 1994; Hong et al., 1994; Longa et al., 1989), prevailing physiological conditions (Duverger & MacKenzie 1988; Folbergrova et al., 1992; Memezawa et al., 1992), temperature (Chen et al., 1991; Bartus et al., 1994; Morikawa et al., 1992), time elapsed after occlusion and presence of other diseases can be controlled, and, depending on the suitability of the model, manipulated. In terms of behavioural consequences, age, sex, recovery environment and time can also be manipulated (Grabowski et al., 1995). Finally a variety of treatment regimes maybe employed and evaluated under controlled conditions and with the appropriate experimental techniques the neuroprotective mechanisms investigated (Bartus et al., 1994; Dirnagl et al., 1990; Hong et al., 1994; Nagafuji et al., 1992; Sharkey & Butcher, 1994). The ability to conduct studies with such experimental control has undoubtedly advanced our understanding of the pathophysiology of focal cerebral ischemia immensely.

1.4. The Suitability of the Rat for Studying Focal Cerebral Ischemia

The rat has proved to be particularly suited to elucidating the processes operating in focal cerebral ischemia. The main reasons for examining MCA occlusion in the rat are listed below.

1. The MCA is a common location of occlusion in humans (Hunter et al., 1995).

2. The rat possesses a greater similarity to the human cerebrovasculature compared to gerbils, cats or dogs (Macrae, 1992, Scremin, 1995). The pattern of arterial supply to the brain is similar to that shown in Figure 1.1. For example the middle cerebral artery in the rat arises from the Circle of Willis and follows a course over the surface of the allocortex and then lateral cortex (see Figure 1.3) beneath the temporal bone (Scremin, 1995). Penetrating arteries (e.g., lenticulostriate arteries) branch off from the MCA to supply the striatum. Consequently MCA occlusion in the rat results in an infarct involving lateral cortex and lateral striatum. The anatomical pathology thus bears some resemblance to that observed in humans after MCA occlusion.

3. The relative accessibility of the MCA for occlusive surgery.

4. The lissencephalic cortex of the rat simplifies evaluation of the histopathological outcome of MCA occlusion.

5. An extensive range of tests have been developed to evaluate behavioural function in the rat.

6. The financial and ethical constraints are comparatively lower compared to alternative species such as nonhuman primates. This widens the opportunity for researchers to conduct, refine and replicate experiments.

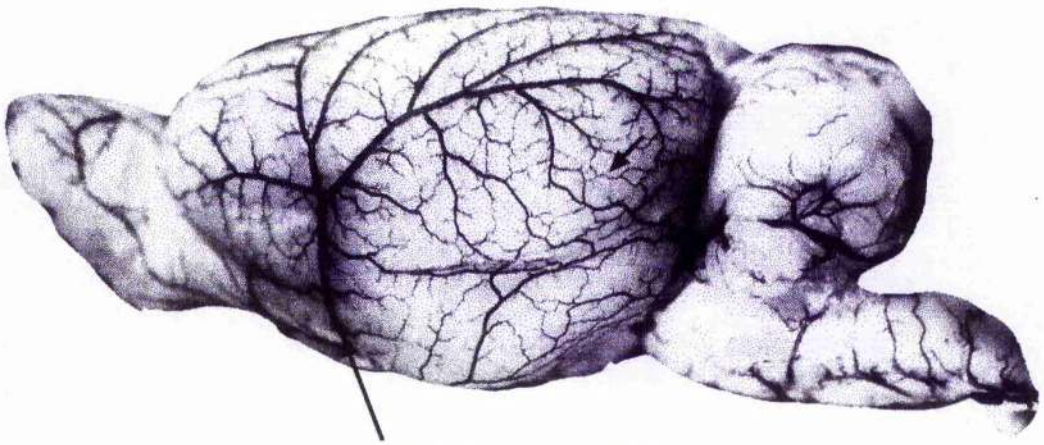


Figure 1.3. *A photograph of a rat brain which has been perfused with indian ink and latex. The course of the middle cerebral artery over the surface of the rat neocortex and allocortex is clearly visible, along with the anastomoses (e.g., indicated by the arrow) interconnecting the posterior and middle cerebral arteries (adapted from Scremin, 1995).*

There are a variety of techniques for inducing focal cerebral ischemia, with each offering a series of benefits and costs (see Table 1.3 and Macrae, 1992). However, recently a model of MCA occlusion has been developed which involves the stereotaxic application of the potent vasoconstrictor endothelin-1 in close proximity to the artery to be occluded (Sharkey et al., 1993). Endothelin-1 induces vasoconstriction by initiating vascular smooth muscle contraction (Rubanyi & Polokoff, 1994) in the location of intracerebral injection (Fuxe et al., 1992). This technique has allowed arterial occlusion by less invasive and more flexible techniques, and is the technique which will be used for inducing focal cerebral ischemia in the experiments which follow. The primary benefits include

1. The ability to avoid a subtemporal approach and the inevitable damage to the temporalis muscle, with the consequential disturbance of feeding.

2. The capacity to occlude any arteries for which reliable stereotaxic co-ordinates can be established.

Method of Occlusion	Benefits	Disadvantages
Ligation/clipping/ electrocoagulation of the MCA after subtemporal craniotomy [1]	Artery can be reversibly occluded (except electrocoagulation)	Artery may be mechanically damaged Subtemporal craniotomy is technically difficult Postsurgical feeding problems
Endothelin-1 applied to the exposed MCA after subtemporal craniotomy [2]	Avoids mechanical damage to vessel	Subtemporal craniotomy technically difficult Postsurgical feeding problems
Stereotaxic injection of endothelin-1 close to the MCA [3]	Avoids mechanical damage to vessel No postsurgical feeding problems Utilises standard stereotaxic techniques Any stereotaxically accessible cerebral artery can be occluded Ischemia can be induced in a conscious animal avoiding the use of anaesthetics	Craniotomy required Duration of occlusion cannot currently be experimentally manipulated The injection needle causes cortical damage
Photochemically induced thrombosis of the MCA [4]	No postsurgical feeding problems Selective parts of the cortex can be irradiated to induce ischemia	Irradiation can cause thermal damage to vessel and surrounding tissue initiating extraischemic pathology
Introduction of clotted blood/microspheres to the cerebral vasculature through the CCA [5,6]	The occlusion is reversible with the use of anticoagulants Craniotomy unnecessary No postsurgical feeding problems Clinically more realistic as occlusion in humans often due to cardiac emboli	Infarction volume and location highly variable
Advancement of a filament through the CCA and ICA up to the origin of the MCA [7]	The occlusion is reversible with removal of the filament Craniotomy unnecessary No postsurgical feeding problems Ischemia can be induced in a conscious animal avoiding the use of anaesthetics	Mechanical damage to the lumen of the CCA, ICA and the Circle of Willis The filament can puncture arteries resulting in haemorrhage

Table 1.3. *The benefits and disadvantages of various techniques which have been developed to occlude the MCA in the rat. (ICA-internal carotid artery, CCA-common carotid artery).*

[1]Tamura et al., 1981; [2]Robinson et al., 1990; [3]Sharkey et al., 1993; [4]Watson et al., 1985; [5]Papadopoulos et al., 1987; [6]Zivin et al., 1987; [7]Longa et al., 1989.

The rat has proved to be a highly popular choice of species in which to conduct research examining the consequences of focal cerebral ischemia. However, the histopathological consequences of MCA occlusion in the rat varies, important factors that have been identified include the method of occlusion (Macrae, 1992), physiological parameters, source of rats (Oliff et al., 1995a; 1995b) and strain of rat (Duverger & MacKenzie, 1988; Van der Staay et al., 1996a). The volume of ischemic damage has been found to be dependent on the strain of rat employed, in particular hypertensive strains (Stroke-Prone Spontaneously Hypertensive and Spontaneously Hypertensive Rats) sustain larger lesions following MCA occlusion than normotensive strains (Fischer 344, Sprague Dawley and Wistar/Kyoto). However, in normotensive strains there is also some variation, the infarct volume of Wistar Kyoto rats significantly differs from Fischer 344 and Sprague Dawley strains, although the latter two do not differ significantly from one another. Thus, between strains (e.g., normotensive and hypertensive) the volume of infarct can vary. Selection of a particular strain of rat has depended on the question posed by the experimenter, thus a rat may be selected to reflect pre-existing risk factors such as hypertension (Duverger & MacKenzie, 1988; Grabowski et al., 1991; 1993) or to maximise the incidence of uniform infarcts after MCA occlusion in examining treatment efficacy. However, little consideration has been given to the choice of rat strain when undertaking behavioural assessment following MCA occlusion.

The most popular strain of normotensive rat has been the Sprague Dawley, which has proved to be highly suited to focal cerebral ischemia research as a strain in which infarcts are consistent in extent and pattern (Duverger & MacKenzie, 1988; Osborne et al., 1987; Sharkey et al., 1993). The Sprague Dawley has also been a prominent choice in studies examining the behavioural consequences of MCA occlusion (Bederson et al., 1986; Hirakawa et al., 1994; Markgraf et al., 1994; 1992; Seren et al., 1994; Tominaga & Ohnishi, 1989; Wahl et al., 1992). The behavioural testing so far has been limited to testing sensorimotor function, spatial learning in maze tasks (Markgraf et al., 1994; 1992), paw reaching (Grabowski et al., 1993) and active/passive avoidance learning (Hirakawa et al., 1994; Seren et al., 1994). Moreover, the suitability of strains for behavioural testing varies (Van der Staay et al., 1996a), notably the Wistar Kyoto strain has proved difficult to test in sensorimotor tasks due to a general inactivity after MCA occlusion (Van der Staay et al., 1996b).

Furthermore, in validating the endothelin-1 model for MCA occlusion, demonstrating comparability between normotensive strains is desirable. For these reasons an early requirement in examining the behavioural consequences of MCA occlusion will be to characterise the neuropathological outcome in the Lister hooded strain. The Lister hooded rat has been successfully employed in behavioural tasks similar to those used for the current research (Brown et al., 1996; Brown & Robbins, 1989a; 1989b), but has rarely been employed in models of MCA occlusion.

1.5. The Pathophysiology of Focal Cerebral Ischemia

The development of an in vivo model of focal cerebral ischemia in the rat has contributed immensely to current understanding of the pathophysiological mechanisms involved in ischemia. These advances have permitted the development of strategies for therapeutic intervention aimed at arresting cell death in the acute stages of ischemia and subsequently these treatments have been evaluated in rat models of focal cerebral ischemia. The deprivation of blood supply to neurons and glia after occlusion of a cerebral artery results in the loss of critical supplies of glucose, oxygen, nutrients and the build up of toxic metabolic by-products both intracellular and extracellular. It is primarily a havoc wreaked by the loss of the ability of cells to regulate their internal and external environment. The depth of understanding of the pathophysiology of focal cerebral ischemia has made tremendous progress in recent years with a multitude of mechanisms identified which contribute to cell death. The range of dysfunction and responses in the cell's internal and external environment to ischemia are extensive. These include acidosis from anaerobic glycolysis (Obrenovitch et al., 1988), loss of ion homeostasis, active transport failure (Yang et al., 1992), uncontrolled extracellular accumulation of glutamate and consequently glutamate receptor activation (Hillered et al., 1989), deleterious protein activation (Wang & Yuen, 1994), the production of free radicals, damage to DNA, breakdown of membrane integrity and an immune system response which is augmented by an influx of leukocytes following disruption of the blood brain barrier. The events leading to cell death are summarised in Figure 1.4 many of these interact with one another to further aggravate the events leading to cell death.

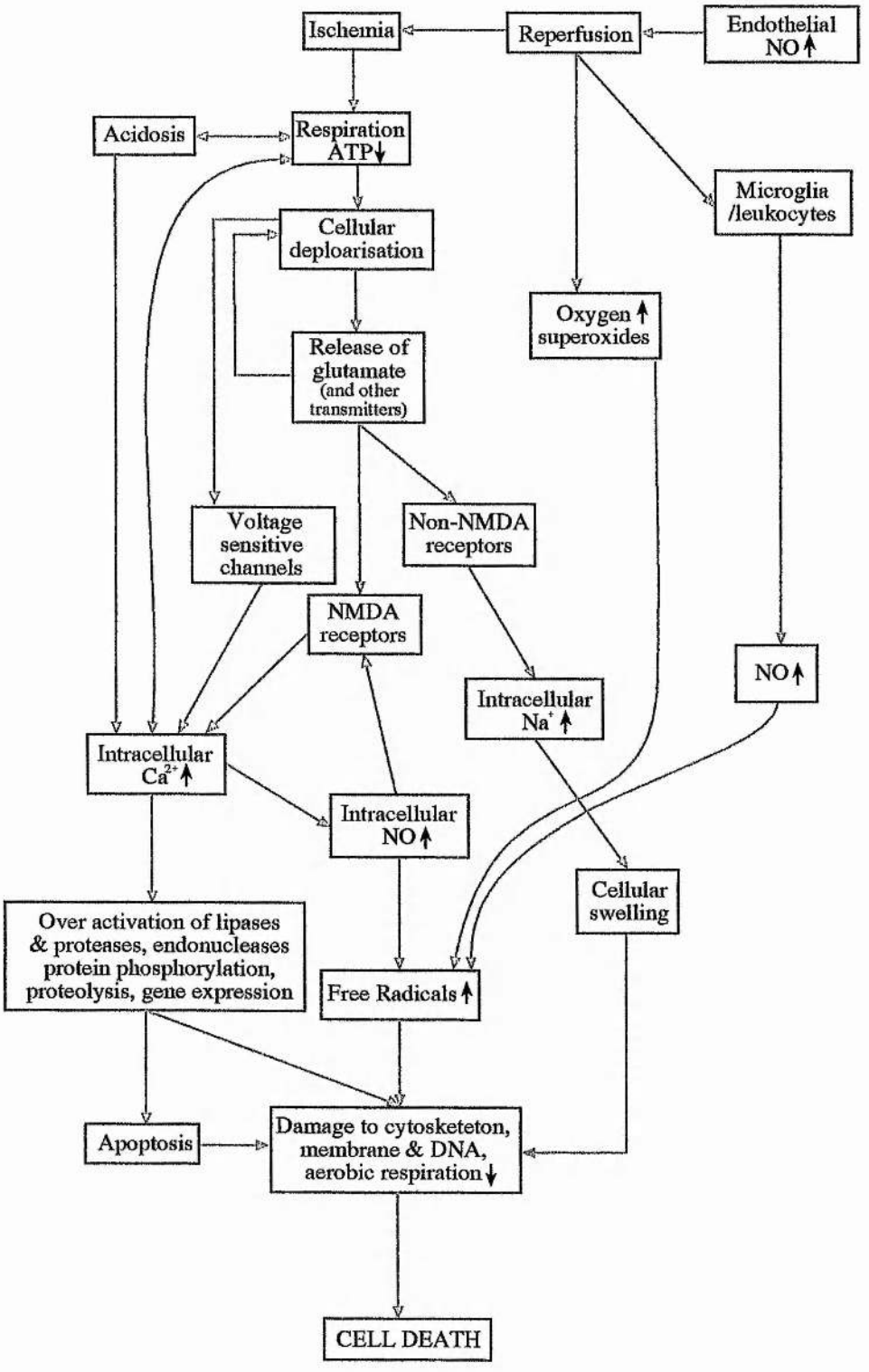


Figure 1.4. *A summary of the key intra and extracellular events following focal cerebral ischemia that contribute to cell death (NO-nitric oxide). Some factors may inhibit as well as advance the evolution of the infarct (indicated by red arrows).*

A key consequence of a loss of blood supply is the depletion of ATP (adenosine triphosphate) which occurs in the cell (Folbergrova et al., 1992; Obrenovitch et al., 1988). There are two important repercussions for energy production during focal cerebral ischemia. First, the neurons are deprived of their source of glucose for energy metabolism. The effects are particularly acute in neurons which unlike many other cells do not possess any glucose reserves. For a short period the cell is able to synthesise ATP from ADP (adenosine diphosphate) using creatine phosphate catalysed by creatine kinase (Obrenovitch et al., 1988). However, this route for ATP production is short lived as the reaction soon exhausts available supplies of creatine phosphate. Many important cellular processes require energy to sustain function and thus the cell starts to deteriorate. Amongst the cellular processes dependent on energy from ATP are active transport at the membrane which maintains ion homeostasis, protein synthesis of microfilaments and microtubuli required to maintain internal cell structure and lipids critical to membrane integrity. The second consequence concerns the deterioration of ion homeostasis, which is critical to the normal function of neurons. Uncontrolled depolarisation of the cell triggers the unregulated activation or interference of cellular processes dependent on the intracellular presence of calcium. The release of glutamate activates both NMDA and Kainate/AMPA ionotropic receptors resulting in an intrusion of Na^+ (sodium) and extrusion of K^+ (potassium). Initially a rise in extracellular K^+ occurs gradually before ATP levels have fallen below 50 %. Following the relatively small change in extracellular K^+ follows a rapid exchange of ions as the membrane depolarises and becomes permeable to K^+, Ca^{2+} (calcium), Na^+ and Cl^- (chloride). Accompanying the rapid ion movement is a transient rise in extracellular pH which Siesjo (1992) suggested may be due to a brief temporary cellular influx of H^+ or reverse flow of HCO^{3-}. The increase in membrane permeability aggravates the active transport failure by exhausting vital remaining ATP supplies in an ill-fated attempt to restore the electrochemical distribution across the membrane.

In addition to partial responsibility for an intracellular rise in calcium the inability to control electrochemical ion fluxes is also the primary factor in the evolution of edema with the osmotic induced accumulation of intracellular water. The failure of the Na^+/K^+ pump arises directly from the depletion of ATP which fuels the energy dependent enzyme Na^+, K^+-ATPase. The consequence for the cells is an influx of Na^+ and outflow of K^+. The increase in Na^+:K^+ ratio has been observed to coincide with a rise in water

content in the lesion area starting 1 hour after occlusion and a fall in Na^+, K^+-ATPase activity beginning 30 minutes after permanent occlusion of the MCA (Yang et al., 1992).

Following the loss of oxygen and exhaustion of creatine phosphate reserves, anaerobic glycolysis in the cytosol is the sole origin for ATP production from the available glucose. This method of energy production is highly inefficient yielding only two ATP molecules as compared with 36 ATP molecules per glucose molecule at the end of the oxidative phosphorylation process (Sherwood, 1993). Despite such inefficiency glycolysis can out produce the full oxidative phosphorylation cycle due to the speed at which ATP can be produced, but only if large supplies of glucose are available. However, the reliance on anaerobic glycolysis brings about an accumulation of pyruvic acid which under aerobic conditions would proceed to the citric-acid cycle but in the absence of oxygen is converted to lactic acid by pyruvate reductase. Without a functioning circulation the lactic acid builds up, thereby lowering pH. Consequently although hyperglycaemia may allow continued production of ATP it is at the cost of further aggravating the lowering of tissue pH by providing additional glucose for anaerobic glycolysis (Folbergrova et al., 1992). The lowering of pH has a deleterious effect on mitochondrial function and interferes with the sequestration of Ca^{2+}. Furthermore, the efficiency of oxidative phosphorylation in mitochondria is effected by the cytosolic concentration of Ca^{2+}. Excess intracellular calcium is taken up by mitochondria reducing the ability to produce ATP while oxygen is still available increasing acidosis, however, when oxygen is depleted the mitochondria can no longer absorb excess Ca^{2+} which is then dislocated by H^+ to return to the cytosol. Thus an early result of ischemia is a depletion of ATP with cellular demands outstripping supply and the build up of harmful metabolic by-products from the only available energy production process.

The intracellular rise of calcium could be regarded as the single most important event during ischemia. That is not to dismiss other factors which contribute immensely to the developing infarct, however, intracellular calcium plays a key role in the regulation of cellular function under normal conditions.

A cytosolic increase in calcium can originate from two sources. First, the extracellular fluid contains Ca^{2+} which may enter the cell via a variety of receptor operated (NMDA receptors) and voltage sensitive channels. Second there are

intracellular stores of Ca^{2+} in the sarcoplasmic reticulum (Godfraind & Govoni, 1995). Activation of the metabotropic glutamate receptor triggers the release of intracellular calcium via the activation of phospholipase C (Umemura et al., 1992). The sequence of events are believed to precede with the depolarisation of the presynaptic cell membrane with Na^{+}, this progresses to the presynaptic boutton activating voltage sensitive calcium channels, allowing Ca^{2+} to enter the boutton and activate the release of excitatory amino acids such as glutamate (Siesjo & Bengtsson, 1989). Due to energy depletion the rise in intracellular calcium cannot be readjusted by the energy dependent Na^{+}-Ca^{2+} exchanger. This loss of calcium homeostasis is then believed to account for the rise of calcium which can continue in the absence of the original agonist. Experiments conducted in vitro that block the entry of extracellular Ca^{2+} have demonstrated only partial protection from excitotoxic cell death (Dubinsky, 1993) this in part may reflect the importance of intracellular stores of calcium.

The rise in intracellular calcium has a variety of consequences for the cell, which include lipolysis resulting in increased free fatty acids and lysophospholipid induced membrane damage, protein phosphorylation, proteolysis resulting in enzyme conversion and damage to the cytoskeleton (Siesjo, 1992b). Membrane channel functions can also be altered by the activation of protein kinase C. While the induction of phospholipases launch a cascade of events resulting in disruption of the blood brain barrier and the attraction of leukocytes. The abnormal activation of intracellular calcium pathways may also induce apoptosis.

Furthermore, calcium increases the production of nitric oxide through the activation of calmodulin-dependent nitric oxide synthase in neurons and the endothelium. This initial rise in nitric oxide (NO) production is limited as important substrates for the production process are limited in ischemic conditions. Neuronal NO contributes to the regulation of the glutamate receptor via inhibitory feedback reducing the influx of calcium and consequently the production of NO. However, NO may also increase glutamate release and inhibit glutamate re-uptake. A further and more prominent source of NO is provided by the activation of microglia and leukocytes which synthesise NO by inducible NO synthase. Production of NO from glia can be initiated by cytokines and NO production maybe sustained over days and once initiated is independent of intracellular calcium levels. NO is known to mediate the destruction of neurons and glia by microglia and thus the latter source of NO contributes to cell death.

Alterations in NO levels appear to be capable of both aggravating and ameliorating ischemic injury (Dawson, 1994). However, it has become apparent that the impact of NO in the evolution of the infarct changes over time (Dawson, 1994). The deleterious action of NO is mediated by the free radical form of NO which in combination with superoxide forms peroxynitrite and hydroxyl radicals (Chan, 1994) which are believed to be the primary cause of cellular damage. The Cell membrane and DNA within the nucleus can both be subjected to peroxidative damage by free radicals (Chan, 1994), while DNA synthesis, glycolysis and oxidative phosphorylation are also disrupted by NO (Dawson, 1994). Nitric oxide may, however, also ameliorate the consequences of ischemia through vasodilation improving perfusion and via modulation of the glutamate receptors in the early stages of focal cerebral ischemia. However, the return of blood flow to a region in which energy depletion and cellular dysfunction is prevalent contributes to what is known as the reperfusion injury. Mitochondria constantly produce superoxide as a metabolic by-product which is normally scavenged by superoxide dismutases and other antioxidants. The ischemic tissue is unable to maintain control over the production of superoxide due to the inactivation of superoxide dismutase and depletion without re-supply of antioxidants. Consequently the return of oxygen can fuel the production of superoxide if the tissue is sufficiently compromised and ultimately increase free radical induced injury.

The spread of ischemic damage to adjacent tissue is an issue of considerable contention as efforts proceed to identify the mechanisms involved. The core region of ischemic cell death is surrounded by tissue which is experiencing conditions of reduced perfusion and consequently cells are metabolically abnormal, however, the cells are sufficiently intact to potentially survive. This region surrounding the ischemic core has been referred to as the penumbra. Acute pharmacological intervention has been successful in salvaging tissue in the penumbra, and consequently interrupting processes which recruit tissue into the infarct remains a high priority (Koroshetz & Moskowitz, 1996). Repeated depolarisation induced by ischemic conditions in the core may mediate the recruitment of cells into the infarct zone (Hossman, 1994). Cells surrounding the core of the lesion have to endure depolarisation, which demands increased metabolism in cells with an inadequate oxygen supply to cope. Consequently there is a gradual and cumulative deterioration of cellular function leading to cell death.

The diverse cascade of events which focal cerebral ischemia triggers offers a diverse range of opportunities for acute pharmacological intervention. Antagonists have been employed to counter the extracellular rise in glutamate and block voltage sensitive channels for calcium and sodium (Koroshetz & Moskowitz, 1996). Efforts have also preceded with both NO agonists and antagonists to improve perfusion and counter the action of free radicals (Dawson, 1994).

1.6. The Assessment of Focal Cerebral Ischemia in the Rat

Considerable insight into the pathophysiology of focal cerebral ischemia has been achieved using rodent models, which have also assisted the development of strategies for pharmacological interventions (Buisson et al., 1993; Dalkara et al., 1994; Dawson, 1994; Gerlach et al., 1995; Graham et al., 1993; Hossmann, 1994; Morley et al., 1994; Siesjo, 1992a; 1992b). It is probably the case that neuroprotective drugs act by preventing the growth of the lesion. Consequently drug intervention within the first few hours of the onset of focal cerebral ischemia is capable of saving cells and reducing lesion size (Gerlach et al., 1995; McCulloch et al., 1992; Sharkey & Butcher, 1994). In the evaluation of drug treatment success, quantitative histological measures of the infarct volume following treatment have been used as an analysis endpoint (Chen et al., 1993; Osborne et al., 1987; Sharkey & Butcher, 1994; Sharkey et al., 1994). Beyond a reduction in lesion volume, however, the ability of drug treatment to resolve behavioural deficits which follow focal cerebral ischemia has received rather limited attention.

The range of options for ameliorating the consequences of focal cerebral ischemia are growing rapidly. Acute intervention aimed at reducing the infarct volume has been a prominent approach. However, this solution is limited by the necessity to intervene rapidly prior to cell death. Furthermore, acute intervention at present saves tissue in the penumbra of an ischemic insult and does not prevent cell death in the core. There are, however, alternative therapeutic strategies that can be used to improve outcome beyond the point for halting cell death. The options at present include neural transplants (Grabowski et al., 1995), the use of growth factors promoting neural sprouting (Kawamata et al., 1996) and physiotherapy as an integral part of promoting recovery and neural reorganisation. These treatments may also augment acute intervention as well as treat those arriving too late to benefit from treatments aimed at arresting acute cell death. These treatment strategies are capable of reducing behavioural

impairment without reducing infarct volume (Grabowski et al., 1995; Kawamata et al., 1996).

Therefore, the assessment of treatment efficacy using behavioural criterion will increase the ability to detect beneficial outcome for treatments which improve neurological function without altering infarct size. The opportunity to combine treatment strategies also raises another prominent reason for using behavioural outcome as a measure of treatment success. The use of behavioural tests will permit direct comparison between acute intervention and late intervention by the same criterion. The comparison will, by necessity be restricted to comparing treatments at late stage, to allow treatments using grafts and growth factors time to become functionally integrated. This, however, would provide a view of the relative success of late intervention and it's value in augmenting acute intervention, which would be difficult to achieve using standard volumetric analysis. To achieve this objective requires an extensive understanding of the behavioural consequences of focal cerebral ischemia across a variety of tests which reflect the range of impairment which may follow ischemia.

1.7. The Functions of the Rat Brain Supplied by the Middle Cerebral Artery

The ischemic lesion following MCA occlusion encompasses a significant proportion of the rat lateral cortex including Par1, Par2, FL, HL, Fr1, Fr3, Oc2L, allocortex and subcortically the lateral striatum. The core of the lesion is somatosensory cortex (Par1, Par2, FL) and striatum, while the extent of involvement of motor (Fr1 and Fr3) and association cortex (Oc2L) is more variable. A first step in understanding the behavioural deficits which might follow MCA occlusion in the rat can be aided by considering the behavioural consequences of selective lesions (non-ischemic) of cortex and striatum supplied by the MCA, which are listed in Table 1.4.

Brain Area Zilles's cortical areas	Test
Fr1, Fr3 (motor)	Postural reflex [1] Paw reaching [12,13] Reaching wet mash with tongue [1] Latch box task [1]
Par1, Par2 (somatosensory)	Tactile stimulation [2] Orienting to vibrissal stimulation [27] Gap-crossing task [15] Cheese board task [3] Maier 3-Table task [22] 8 Arm radial maze (win-stay and win-shift) [24] 8 Arm radial maze (two choice spatial discrimination) [25] 8 Arm radial maze (item memory) [26]
FL	Paw reaching [12,13] Somatosensory asymmetry test [4,8] Tactile discrimination [17] Tactile stimulation [2] Limb placement [4,8,17] Beam walking [5]
HL	Beam walking [1,4] Tactile stimulation [2] Limb placement [17]
Oc2L (lateral posterior parietal cortex)	Morris water maze [18,19,21,23,28] Landmark test [18,28] Multimodality orienting [9,10] Cheese board task [20] Maier 3-Table task [22]
Striatum	Choice reaction time task [11] Morris water maze [16,29,32] Paw reaching [13,14,30,31] Reaching wet mash with tongue [30] Tactile stimulation [6] Rotation [7,31]

Table 1.4. *The behavioural consequences of selective lesions (the lesion techniques include aspiration, electrolytic and excitotoxins) involving brain areas supplied by the MCA in the rat.*

[1]Kolb & Whishaw, 1983; [2]Glassman, 1994; [3]Kesner et al., 1989; [4]Kozlowski et al., 1996; [5]Soblosky et al., 1996; [6]Dunnett & Iversen, 1982; [7]Glick & Cox, 1978; [8]Barth et al., 1990; [9]Corwin et al., 1996; [10]King & Corwin, 1993; [11]Brown & Robbins, 1989b; [12]Castro, 1972; [13]Whishaw et al., 1986; [14]Marston et al., 1995a; [15]Hutson & Masterson, 1968; [16]Block et al., 1993; [17]Finger &

Frommer, 1968; [18]Kolb & Walkey, 1987; [19]Kolb et al., 1994; [20]King & Corwin, 1992; [21]Save & Moghaddam, 1996; [22]Thinus-Blanc et al., 1996; [23]DiMattia & Kesner, 1988a; [24]DiMattia & Kesner, 1988b; [25]Cho & Kesner, 1996; [26]Kesner & Gray, 1989; [27]Crowne et al., 1986; [28]Crowne et al., 1992; [29]Whishaw et al., 1987; [30]Pisa, 1988; [31]Dunnett et al., 1988; [32]Selden et al., 1990.

The fundamental pattern of impairment following injury to somatosensory cortex (Par1, Par2, FL and HL) includes a reduction in orienting to contralateral tactile stimulation to the body, vibrissa and limbs (Glassman, 1994). Performance in maze tasks is also disrupted by parietal lesions. The capacity to solve many maze tasks can in fact be mediated by a number of different strategies (Kesner et al., 1989) including information learned from locomotion around the maze. Thus the impairment following a lesion to somatosensory cortex could reflect the importance of kinesthetic cues in spatial learning (Save & Moghaddam, 1996).

A lesion of Zilles's (1990) area FL also results in a reduction in successful paw reaching. Paw reaching represents a skilled learned sensorimotor task in which the rat must extend the forepaw towards the pellet and grasp the pellet to retract it to the mouth. The success of the action depends on the generation of an accurate reach and sensory feedback to confirm that the pellet has been grasped before withdrawing the forepaw. Careful video analysis and force platforms have also indicated that posture is also important in assisting reaching (Miklyaeva et al., 1996; Whishaw & Pellis, 1990). The consequence of lesions may thus be to disturb any aspect of the reach, grasp or position to reduce the efficiency of paw reaching. Castro (1972) has reported that large bilateral motor cortex lesions result in a reduction in reaching with a fall in the number of attempted reaches resulting in contact with a pellet. Following the recovery of reaching attempts, tactile deficits became apparent as successful grasps were aborted and unsuccessful grasps continued. The occurrence of motor and somatosensory impairment reflects the extent of the lesion which involved both motor cortex and somatosensory FL. Furthermore, where the size of a unilateral lesion has been varied to include FL and also adjacent sensorimotor cortex the magnitude of the ipsilateral paw preference increases and accuracy with the contralateral forepaw decreases (Whishaw et al., 1986). The increasing effect of damage extending outside of FL may reflect the importance of sensorimotor cortex in controlling posture to permit effective reaching.

A consistent feature of posterior parietal lesions in the rat has been the impairment of maze navigation (Crowne et al., 1992; King & Corwin, 1993; Kolb &

Walkey, 1987; Save & Moghaddam, 1996; Spangler et al., 1994), which has been attributed to a difficulty in selecting an initial trajectory (Foreman et al., 1992; Kolb & Walkey, 1987; Save & Moghaddam, 1996). These results have been interpreted as a failure to use extrapersonal visuospatial cues to orient the head and body before the execution of the motor plan to reach the goal (Kolb, 1990b). Multimodal 'neglect' has also been reported in the rat after posterior parietal lesions (Corwin et al., 1996; King & Corwin, 1993), using tasks which assess the ability to orient to lateralised stimuli in different modalities. A putative circuit for directing spatial attention has been identified (King & Corwin, 1993) which is thought to be equivalent to the distributed cortical attentional system proposed in primates (Mesulam, 1981). The circuit in the rat is thought to involve medial agranular (AGm) (King & Corwin, 1993), ventrolateral orbital (Corwin et al., 1994; King et al., 1989) and posterior parietal cortex (Corwin et al., 1996; King & Corwin, 1993). To draw further comparisons between rodent and primate attentional systems, it would be useful to specify in greater detail the behavioural characteristics of posterior parietal lesions, using tests of sensorimotor and attentional function.

Excitotoxic injury to the striatum also causes a variety of deficits including a bias to respond to ipsilateral tactile stimulation (Dunnett & Iversen, 1982) and drug induced ipsilateral rotation (Dunnett et al., 1988). Paw reaching is also disturbed with a reduction in the number of pellets recovered with the contralateral forepaw in the staircase reaching task (Marston et al., 1995a) and a reduction in paw reaching success in a free reaching task with the contralateral forepaw (Dunnett et al., 1988). Maze navigation also appears to be impaired with an increase in the time taken to find the platform in the Morris water maze task due to both an increase in path length and decrease in swimming speed (Block et al., 1993; Selden et al., 1990; Whishaw et al., 1987). It has also been demonstrated that subregions of the striatum make distinct contributions to the initiation of action (Brown & Robbins, 1989b). Lesions of the striatum result in response related impairments, however, the medial and lateral striatum can be dissociated in a choice reaction time task (Brown & Robbins, 1989b). A lesion of the lateral striatum results in an ipsilateral response bias while medial striatal lesions increase contralateral reaction time and cause a relatively smaller ipsilateral response bias.

1.8. Impairment Following Focal Cerebral Ischemia in the Rat

Following occlusion of the MCA in the rat a variety of behavioural tests have been employed to demonstrate neurological impairment. The deficits which have been identified have primarily been sensorimotor and impaired memory function (see Table 1.5).

	Test	Time course of recovery where assessed (days)
Tests of sensorimotor function	Somatosensory asymmetry test [1,2,3] (after Schallert et al., 1982)	Partial recovery (30) [2] No recovery (30) [3]
	Orienting to tactile stimulus [4,5,6,7, 8]	Partial recovery (90) [6] Partial recovery (61) [8]
	Gap-crossing task [8]	Full recovery (46) [8]
	Limb placement [2,3,7,9,10,11,12,13,36, 45]	Full recovery (30) [2] Partial recovery (30) [3] No recovery (21) [10,11]
	Spontaneous limb use [3]	Full recovery (25) [3]
	Beam walking (foot faults) [2]	Partial recovery (30) [2]
	Beam balance/rotarod [4,7,12,42,44]	
	Paw reaching [6,7,14,15] (after Montoya et al., 1991)	Partial recovery (60-90) [6]
	Postural reflex [2,4,9,12,13,16,17,18,19, 20,21,22,23,33,34,35,36,37,38,39,40,41,42,43,44, 45,46,47,48,49,50,51	Full recovery (23) [2] Full recovery (19) [3] Partial recovery (28) [21,22]
	Amphetamine induced rotation [5,6]	
	Vertical screen/prehensile strength test [26,40,41,42,	
Tests of cognitive function	Active avoidance [24] (retention)	
	Passive avoidance [4,21,22,23,24,25, 26,27,28,44] (retention and acquisition)	
	Morris Water maze [2,28,29,30,44,45] (Spatial memory)	
	8-arm radial maze [31,32] (Spatial memory)	Partial recovery (90) [31,32]

Table 1.5. *The range of behavioural impairments demonstrated following MCA occlusion in the rat.*

[1]Andersen et al., 1991; [2]Markgraf et al., 1992; [3]Markgraf et al., 1994; [4]Borlongan et al., 1995; [5]Grabowski et al., 1991; [6]Grabowski et al., 1993; [7]Grabowski et al., 1995; [8]Hurwitz et al., 1990; [9]Alexis et al., 1996; [10]De Ryck et al., 1989; [11]De Ryck et al., 1992; [12]Kawamata et al., 1996; [13]Van der Staay et al., 1996b; [14]Marston et al., 1995a; [15]Sharkey et al., 1996; [16]Bederson et al., 1986; [17]Garcia et al., 1995; [18]Kawai et al., 1996; [19]Persson et al., 1989; [20]Van der Staay et al., 1996a; [21]Yamamoto et al., 1988; [22]Yamamoto et al., 1989; [23]Yamaguchi et al., 1995; [24]Hirakawa et al., 1994; [25]Seren et al., 1994; [26]Tominaga & Ohnishi, 1989; [27]Wahl et al., 1992; [28]Yonemori et al., 1996; [29]Lyden & Hedges, 1992; [30]Lyden & Lonzo, 1994; [31]Okada et al., 1995a; [32]Okada et al., 1995b; [33]Katsuta et al., 1996; [34]Park & Hall, 1994; [35]Smith & Meldrum, 1995; [36]Barone et al., 1993; [37]Hara et al., 1997; [38]Jiang et al., 1996; [39]Kawai et al., 1997; [40]Lin & Phillis, 1992; [41]Lin & Phillis, 1991; [42]Ridenour et al., 1991; [43]Roussel et al., 1991; [44]Smith et al., 1997; [45]Markgraf et al., 1997; [46]Kawazura et al., 1997; [47]Yanaka et al., 1997; [48]Yanaka et al., 1996a; [49]Yanaka et al., 1996b; [50]Yanaka et al., 1996c; [51]Yanaka et al., 1996d.

Occlusion of the MCA results in a lesion encompassing primary sensorimotor cortex and consequently hemiplegia has frequently been observed. The main manifestation of hemiplegia has been the observation that the contralateral limbs are adducted when the rat is suspended 1 m above a surface and that the capacity to resist a lateral push across a flat surface with the contralateral limbs is reduced. Infarcts involving sensorimotor cortex also result in an impairment of tactile and proprioceptive limb placement (Alexis et al., 1996; Barone et al., 1993; De Ryck et al., 1989; 1992; Grabowski et al., 1995; Kawamata et al., 1996; Markgraf et al., 1992; 1994; Van der Staay et al., 1996b). Deficits in limb placement appear to be most pronounced in the absence of information from the vibrissa or the visual system (De Ryck et al., 1989). Limb use deficiencies appeared to be due to impaired somatosensory function, rather than a disruption of integrated behaviour. Tests examining motor strength have also identified impaired performance immediately (within 4 days) following MCA occlusion using an inclined plane and prehensile tests (Lin & Phillis, 1992; Lin & Phillis, 1991; Ridenour et al., 1991; Tominaga & Ohnishi, 1989). However, an impairment of motor strength appears to be transient, as reports of testing which started one week or more after surgery have not found an impairment (Andersen et al., 1991; Markgraf et al., 1992; 1994; Smith et al., 1997; Van der Staay et al., 1996b). The transient impairment may be attributable to the effects of edema as Tominaga & Ohnishi (1989) observed that a composite motor score correlated with brain edema at its peak 72 hours following surgery. Similarly tests of locomotor function have not identified an impairment (Grabowski et al., 1991; Yamaguchi et al., 1995; Yamamoto et al., 1988), although there have also been claims that right but not left MCA occlusion results in hyperactivity (Robinson, 1979; Robinson & Coyle, 1980).

The sensorimotor tests which have been used by researchers examining the consequences of MCA occlusion have had the advantage of requiring only the simplest

testing apparatus and an observer to score performance. The tests have also been quick to administer and often do not require extensive training. The simplicity of the tests, however, are also their limitation. The resolution of the tests in demonstrating dysfunction are limited by the keen eye of the observer (video analysis being rarely employed). Furthermore, it is recognised that one of the problems in making neurological evaluation is the stability of the behavioural deficit. It is apparent that motor functions return first (e.g., motor strength, postural reflex, spontaneous limb use) and these are the primary tests employed to evaluate the consequence of MCA occlusion. It has been suggested that tests of complex cognitive or skilled motor function may be more stable and informative (see Marston et al., 1995a; Sharkey et al., 1996), and, with recent advances in the development of less invasive techniques for arterial occlusion (Sharkey et al., 1994; 1993) operant behavioural tests have become more feasible.

The investigation of cognitive function following MCA occlusion has focused on memory. Following MCA occlusion, rats are impaired in both acquisition and retention of passive avoidance (Hirakawa et al., 1994; Seren et al., 1994; Smith et al., 1997; Tominaga & Ohnishi, 1989; Wahl et al., 1992; Yamaguchi et al., 1995; Yamamoto et al., 1988). The Morris Water Maze has also been used to demonstrate an impairment in spatial learning following MCA occlusion in rats (Lyden & Hedges, 1992; Markgraf et al., 1992; Smith et al., 1997). The lesioned rats are able to swim as fast as controls and do learn, albeit more slowly than controls during the task. Furthermore, the routes undertaken are less efficient than those used by controls. However, as yet the characteristics of the cognitive deficit in the Morris water maze are unknown, although, the deficit is known to persist for at least eight weeks postsurgery (Markgraf et al., 1992; Yonemori et al., 1996). Distal occlusion of the MCA which leaves the striatum intact does not disturb performance in the Morris water maze (Markgraf et al., 1994), which provides some evidence that the deficit may reflect the involvement of the striatum. However, the effects of differences in the extent of cortical damage and occlusion technique have not been discounted. Detailed analysis of the performance to identify specifically why the routes followed appear to be inefficient has not been reported. A variety of errors could account for the poor performance including perseveration or failure to orient correctly to the extramaze cues. There is some tentative support for the latter from Smith et al. (1997) who found that rats following MCA occlusion are slower to improve their initial heading angle in the maze. The infarct following occlusion of the MCA does not, however, disturb working memory as tested by delayed nonmatching-to-

place in a Y-maze (Wahl et al., 1992) or delayed nonmatching-to-position in a Skinner box (Hugh Marston, personal communication, July 1997).

Although efforts have been made to characterise the cognitive deficits following MCA occlusion, the sensorimotor deficits remain to be investigated and the extent to which they represent dysfunction in motor response, sensory perception or integrative function are yet to be established. The use of more sensitive tests may provide a detailed understanding of the consequences of MCA occlusion and could increase the capacity to detect more subtle deficits. Animal models of neurodegenerative diseases (i.e., Parkinson's and Alzheimer's disease) offers a parallel field in which the tests have become progressively more sophisticated. The investigation of behavioural dysfunction following various lesions has benefited from the use of carefully devised tests and multiple performance measures to dissect behavioural dysfunction and specify it's characteristics (Muir, 1996; Robbins & Brown, 1990).

Amongst the sensorimotor deficits following occlusion of the middle cerebral artery in the rat are some deficits which have been referred to as 'hemineglect' (Andersen et al., 1991; Markgraf et al., 1994; 1992). These deficits, however, could arise from one or more functional deficits. In particular, it is necessary to exclude primary sensory or motor impairments. Any attentional deficit could arise from an integrative sensorimotor dysfunction, and the challenge is to distinguish among these primary and/or attentional deficits. The typical lesion observed following MCA occlusion in the rat includes primary sensory (Par1/FL) and, to a lesser extent primary motor (posterior Fr1/3) areas. The periphery of the lesion includes lateral Oc2M/2L, an area which is thought to be homologous to primate posterior parietal cortex (Chandler et al., 1992; Kolb, 1990b; Kolb & Walkey, 1987; Kreig, 1946; Reep et al., 1994). Aspirative lesions of area Oc2M/2L disrupt spatial performance, impairing initial orientation in mazes (King & Corwin, 1992; Kolb & Walkey, 1987; Save & Moghaddam, 1996) and result in multimodality 'hemineglect' (King & Corwin, 1993). This raises the possibility that the lesion following MCA occlusion may result in disruption of attention in addition to other primary sensory and motor deficits.

Behavioural tests have focused on the assessment of sensorimotor function and have not attempted to qualify the characteristics of the resulting impairment. Consequently an objective for the current research has been to apply tests capable of dissociating sensorimotor function. Carli et al. (1989; 1985) demonstrated that it is possible to dissociate sensory and motor aspects of so called sensorimotor 'neglect', and Brown et al. (1991) extended the same rationale to characterise the 'neglect' following unilateral lesions of medial agranular frontal cortex as being response-related deficits. Brown et al. (1991) used a selection of tests which have in common that they are sensitive to sensorimotor asymmetry following unilateral lesions, but which also enable the separation and dissociation of primary sensory, primary motor, or integrative sensorimotor or attentional deficits. As might be relevant, for example, for investigating premotor theories of attention which propose a common neural linkage between motor preparatory processing and attention (Rizzolatti et al., 1987; see also Tipper et al., 1992). A similar approach can be adopted to examine the consequences of MCA occlusion. The intention for the current research has been to adapt one of these tests to examine the covert orienting of attention.

1.9. Statement of Aims

Chapter 2 (General Methods) details the methods employed to complete the experiments detailed in Chapters 3, 4, 5, 6 & 7. The methods include a description of the behavioural testing apparatus used and an account of the behavioural tests administered. The various performance measures collected are also specified along with the statistical tests employed to analyse them. Finally, a description of the surgical and histological procedures used for inducing and assessing the ischemic and neurotoxic lesions are reported.

In vivo models of focal cerebral ischemia should be robust if they are to be reliable for detecting clinically important neuroprotective compounds. Therefore, the model should be reproducible in different laboratories and it would be desirable to establish if the model can be used with different strains of rat. The stereotaxic application of endothelin-1 for occluding the MCA has been developed using the Sprague Dawley strain, but has not been employed with other strains. The task in Experiment 1 *(Middle cerebral artery occlusion in the Lister hooded and Sprague Dawley rat strains: Chapter 3)*, therefore, was to establish that the model of MCA occlusion could be successfully

used with a different strain of normotensive rat (Lister hooded). Furthermore, different methods of quantitative volumetric analysis were compared using cresyl violet and immunohistochemistry for glial fibrillary acidic protein (GFAP) as markers of ischemic damage.

Experiment 2 *(Covert orienting in the rat: 1) Demonstration of the phenomenon, 2) The effects of striatal dopamine depletion: Chapter 4)* has two objectives; 1) to establish that the rat is capable of undertaking visual covert orienting and 2) to investigate the contribution of striatal dopamine to task performance. Previous research examining the contribution of striatal dopamine in the rat on a similar task has identified a response impairment (Brown & Robbins, 1989a; Carli et al., 1989). Similarly the early stages of Parkinson's Disease, which involves depletion of striatal dopamine, has also identified a response impairment on the covert orienting task (Rafal et al., 1984). However, more controversially there has also been reports that the capacity to maintain attention in an endogenous covert orienting task is reduced in some Parkinson's patients (Bradshaw et al., 1993; Wright et al., 1990; Yamada et al., 1990) and following a systemically administered dopamine antagonist in an endogenous (Clark et al., 1989) and peripheral (Witte et al., 1992) covert orienting task. Consequently the lesion will be used to further qualify the contribution of dopamine in covert orienting. Furthermore, the ischemic lesion following MCA occlusion involves the striatum. Thus investigating striatal involvement in the covert orienting task may contribute to understanding any deficits which may follow MCA occlusion.

The unilateral excitotoxic lesion of posterior parietal in Experiment 3 *(Covert orienting following excitotoxic lesions of posterior parietal cortex: Chapter 5)* was undertaken to investigate the degree of functional similarity between rodent and primate posterior parietal cortex. A lesion of posterior parietal cortex in the rat permitted the profile of impairment in the covert orienting task to be compared with the known outcome in humans. Damage to posterior parietal cortex in humans results in an increase in the validity effect, which is thought to be due to a difficulty disengaging attention from an invalid cue (so increasing the costs), with no change in the benefit of a valid cue (Petersen et al., 1989; Posner et al., 1984). The adaptation of this test for use with the rat is an opportunity to begin to identify a posterior attentional system in rodents which may be similar to the primate system involving posterior parietal, superior colliculus and the lateral pulvinar (for review see Posner & Petersen, 1990). The circuit in the rat may

include posterior parietal cortex, the superior colliculus and the lateral posterior nucleus of the thalamus, which might be homologous to primate pulvinar (Takahashi, 1985). Further tests of somatosensory asymmetry and paw reaching were also used to establish if the 'neglect' reported following a rodent posterior parietal lesion was indeed multimodal (King & Corwin, 1993) and whether reaching would be disrupted by the lesion.

The ischemic lesion following MCA occlusion in Experiment 4 *(Assessment of sensorimotor neglect following occlusion of the middle cerebral artery: Chapter 6)* results in damage to lateral cortex (including parietal) and striatum. The consequence of parietal injury in humans has been characterised as a difficulty in disengaging attention and consequently provides the predicted outcome for parietal injury in the rat. The behavioural consequences of unilateral MCA occlusion were investigated using the covert orienting task to assess sensorimotor and attentional function. Further tests were also employed to investigate orienting to somatosensory stimulation and fine motor control (somatosensory asymmetry test and paw reaching respectively) which have previously been used to investigate the outcome of MCA occlusion.

Experiment 5 *(Simple and choice reaction time performance following occlusion of the anterior cerebral arteries: Chapter 7)* was undertaken to exploit the versatility of the stereotaxic technique for inducing focal cerebral ischemia by occluding the anterior cerebral arteries. The ischemic lesion which follows bilateral ACA occlusion includes mediofrontal cortex and midline anterior basal forebrain. A simple reaction time task was used to investigate motivation, memory and learning and a choice reaction time task was used to assess the covert orienting of attention.

The general discussion considers the empirical work in the context of the use of the rat as a model of injury following focal cerebral ischemia. Performance in the behavioural tasks following the various lesions employed in the thesis will be reviewed to determine what has been established by their use. Finally in undertaking a review of the results attained in this thesis consideration will also be given to possible future research which may both extend and verify the findings.

Chapter 2:

General Methods

2.1. Animals

Lister hooded rats (supplied by in-house breeding program, School of Psychology, University of St. Andrews or from Charles River UK, Ltd, Margate, UK) were used during each study with the exception of one experiment which also used the Sprague Dawley strain. The rats were maintained on a 12 hour light/dark cycle (lights on 0800) with free access to water and a restricted diet of 15-20 g of sucrose pellets and standard laboratory chow per day.

Behavioural testing was conducted during the light phase of the cycle and did not begin within 1 hour of the lights coming on. The extensive training undertaken for operant procedures allowed all animals to be habituated to handling for at least one month before the collection of any presurgical behavioural data.

The requirements of the UK Animals (Scientific Procedures) Act, 1986, were adhered to throughout each study.

2.2. Behavioural Tests

2.2.1. Nine-Hole Box Apparatus

Four 'Nine-hole boxes' controlled by a 'Spider' operating system (Paul Fray Ltd, Cambridge, UK), were used for operant testing. Figure 2.1 illustrates the basic design of each aluminium test chamber.

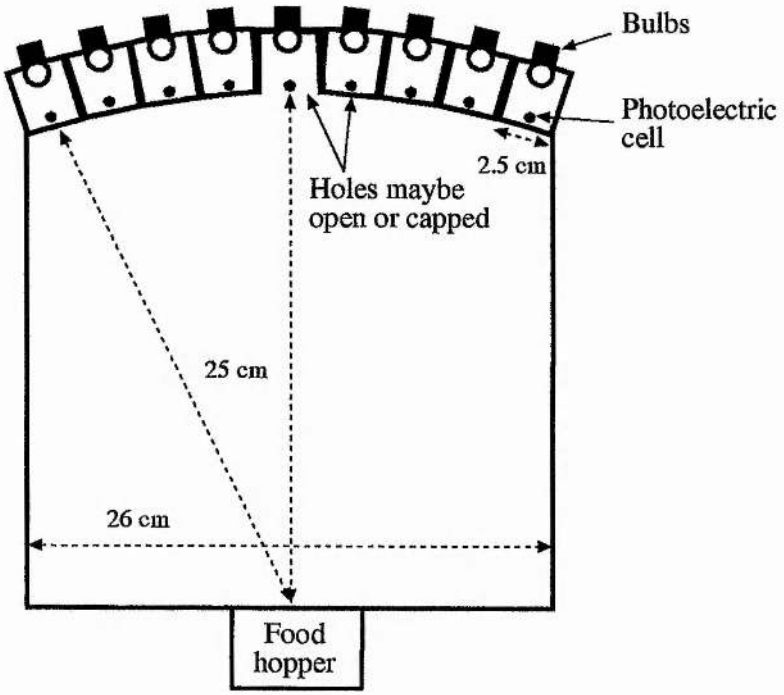


Figure 2.1. *Schematic representation of the 'Nine-hole box' apparatus. In the rear wall 1.5 cm above the wire grid floor are nine square holes (2.5 cm^2 opening and 4 cm deep) each containing a 3 (W) bulb at the rear and photoelectric cell at the entrance to the hole. The entrance to each hole could be closed off with a transparent cover. The bulbs at the rear of each hole could be switched on and off at different levels of illumination (3 W or 1.5 W) under computer control to generate localised visual stimuli. The photoelectric cells were also monitored by a computer and could effectively be used during trials to maintain a rat in a specific location by requiring a sustained nose-poke into a hole. The photoelectric cells allowed reaction times to be calculated (accuracy of 10 ms) for withdrawal from a hole after imperative signals and record subsequent actions in response holes included in a task. In the centre of the front wall was the food hopper into which 45 mg pellets (45 mg sucrose, BioServ Inc., Frenchtown, NJ) were delivered by a silent operation automatic pellet dispenser. The entrance to the food hopper was covered by a hinged Perspex door which, when opened, triggered a microswitch. The food hopper could be illuminated by a 3 W bulb. Above the food hopper was a panel door through which the rat was introduced in to the chamber. In the centre of the ceiling was a houselight (3 W) and beside that a loud speaker, which could be used to generate white noise or imperative signals. The entire chamber was encased in a sound attenuating box with a fan which generated low-level background noise and circulated air through the box.*

2.2.2. Covert Orienting of Attention

2.2.2.i. Training Regime

The rats were placed on a restricted diet 24 hours before the commencement of training. Testing was conducted in the 'Nine-hole box' apparatus which has previously been employed in similar visual reaction time tasks (Brown & Robbins, 1989a; Carli et al., 1989). Training began with habituation to the test apparatus for 1 hour, with standard laboratory food pellets placed in the hopper.

In the first training program, the rat pushed the panel door open to receive a food pellet. During this training, a light in the food hopper was activated with each panel press. Once a rat was able to gain 100 pellets in 15 minutes (typically, within two 30 minute training sessions) they progressed to the next stage of training.

In the next stage of training, the central hole was uncapped. To receive a pellet, the rat now had to place its nose in the central hole in response to the hole light coming on, and maintain it there for a brief delay, after which the hopper light came on and a food pellet reward was delivered into the food hopper. Premature withdrawal from the central hole resulted in the house light in the chamber switching off for a 'time-out' punishment and no food reward: after 1 sec the house light and the light in the food hopper were activated. To initiate a new trial the rat pushed open the panel door of the food hopper. After 5 days of this training, the rats were able to wait for foreperiods of up to 400 ms, at which point they progressed to the testing paradigm.

2.2.2.ii. Test Description

Figure 2.2 illustrates the order of trial events. The variable foreperiods preceding the target light were gradually increased until they were 200, 400, 600 and 800 ms. Each testing session lasted until completion of 120 correct trials or for 30 minutes.

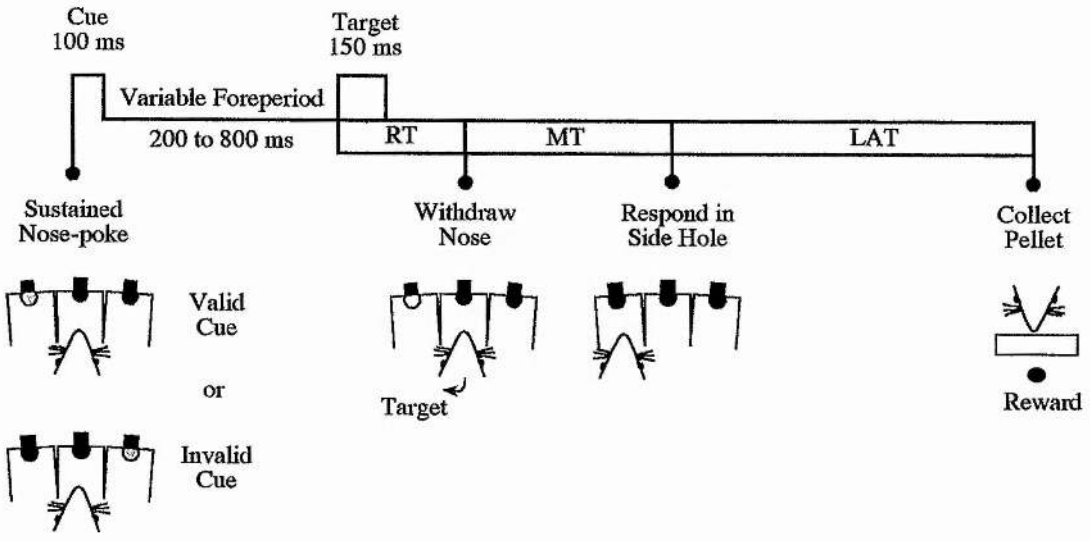


Figure 2.2. *A schematic representation of the covert orienting test in the 'Nine-hole box'. The central three holes in the chamber were uncapped, and the rat was required to sustain a nose poke in the central hole, initiating the onset of a peripheral cue. The cue was the brief (100 ms) dim illumination of the bulb in the hole to the left or right of the central hole. The nose poke also initiated one of four discrete foreperiods (200-800 ms in 200 ms increments) at the end of which the target (the bright 150 ms illumination, of the bulb in one of the 2 peripheral holes) was presented. The rat had to withdraw from the central hole and respond in the target hole. Successful completion of a trial was rewarded with a 45 mg sucrose pellet (BioServ, Frenchtown, NJ). The cue correctly indicated the side of the target on 80 % of the trials (valid cue). Invalid cues, in which the cue light appeared on the opposite side to the target, comprised the remaining 20 % of the trials. The order of valid and invalid trials and the variable foreperiods between cue and target lights were randomised. The time to complete various components of the task are defined in the figure; reaction time (RT) was the time from onset of the target until withdrawal of the nose from the central hole; movement time (MT) included the time from withdrawal of the nose from the central hole until responding in the target hole; and latency to collect reward (LAT) was the time elapsed between withdrawal of the nose from the target hole until response at the food hopper panel.*

Eye movements are commonly monitored in studies using human subjects, to ensure that the task measures the covert rather than overt orienting of attention. Similar precautions in the rat are not necessary due to the configuration of the rat's eye. The eyes of a rat are lateral in the head, such that when the head is still and central, the eyes point to the lateral compartments. As such, it is impossible for the eyes to be directed to only one side. Furthermore, the distribution of ganglion cells across the retina is relatively even (the difference between ganglion cell density in the highest and lowest density areas is only 5:1) thus, exploratory fixation (i.e., eye movements bringing the image of an object of interest to a region of high resolution) is thought to be unlikely to occur (Sefton & Dreher, 1995). For confirmation of covert orienting of attention it is therefore most important that the head of the rat is maintained centrally and still, which is controlled in this task.

2.2.2.iii. Definition of Measures

Various chronometric and accuracy measures were recorded to assess performance in the task (chronometric measures are defined in Figure 2.2). Reaction time was the time from target stimulus onset to the complete withdrawal of the rat's head from the central hole, measured with an accuracy of 10 ms. In adopting this measure, it was accepted that there was a movement time component included in the measure (see Carli et al. 1989), with the reaction times extended by the time taken from the start of head withdrawal to the point at which the photoelectric cell beam was no longer interrupted by the head, but this was minimised by the use of non-mechanical switches. The time taken to reach the target hole after withdrawal from the central hole was recorded as Movement Time. Latency to collect reward was the time from withdrawal of the head from the target hole until the food hopper panel was pushed open.

Three types of errors could occur in the test. Anticipatory errors involved withdrawal from the central hole before or within 100 ms of the target light onset. Responding in the opposite hole to the target light was recorded as an incorrect error. A movement time greater than 2000 ms was recorded as a late error. Trials with reaction times greater than 1500 ms (but which were otherwise completed correctly) were also classified as late errors for subsequent analysis, but were rewarded and advanced during testing. The occurrence of errors during testing resulted in 1500 ms of darkness in the

chamber ('time-out'), no food reward and repetition of the trial until successfully completed.

2.2.2.iv. Simultaneous Bilateral Cues

In two experiments following the collection of the postsurgical data for the covert orienting task, a block of five sessions (a total of 600 trials per rat) were run in which the dim cues were presented bilaterally. Other than the addition of simultaneous bilateral cues the program remained unchanged and the description provided for the visual covert orienting task remains applicable. The modified task was intended to test for the presence of hemineglect arising from 'extinction' of the contralateral cue when presented simultaneously with the ipsilateral cue.

2.2.3. Simple Reaction Time Task Performance, Under a Visually Cued Multiple-Ratio Schedule of Reinforcement

2.2.3.i. Training Regime

The program of training used for the covert orienting test described previously was adhered to with the following modifications. During training to sustain a nose poke, the foreperiods before the occurrence of an imperative signal (tone) were increased up to 500 ms before progressing to the next stage of the training. The food pellet was not delivered into the hopper until the panel press. In addition the rats were introduced to the visually cued multiple ratio schedule of reward. Thus during these trials the four holes either side of the central hole were either switched on (3 W) or off during each trial to indicate one trial before reward. Once a rat could complete a 100 trials in 30 minutes, the maximum number of trials to complete before reward was increased. At first to two (indicated by the dim illumination of the cue holes 1.5 W) and then finally three trials before reward (indicated by either the bright illumination (3 W) or no lights in the cue holes, corresponding to the initial cue order introduced earlier in the training).

2.2.3.ii. Test Description

In the 'Nine-hole box', only the central hole was uncapped, which contained a light at the rear and a photocell beam at the front of the hole. The simple reaction time task included a multiple-ratio schedule of reward and has been previously employed to investigate motivational processes in rats (Brown et al., 1996; Bowman & Brown, 1996) and monkeys (Bowman et al., 1996). Visual cues were used to indicate the position in the schedule of work. The four holes to either side of the central hole were capped with transparent covers allowing the cue lights in the rear of each hole to be seen when activated. Rats initiated a trial by pressing the panel door of the food hopper, which activated the light in the central hole and a cue which was dependent on the position in the current schedule. Figure 2.3 summarises the order of trial events.

2.2.3.iii. Definition of Measures

Performance was assessed using chronometric measures which are defined in Figure 2.3 and performance accuracy. Reaction time included the period of time elapsing between the imperative signal and withdrawal of the nose from the central hole. Movement time was the time between withdrawal from the central hole until pressing open the food hopper panel. Two types of mutually exclusive errors could occur; anticipatory and late errors. Errors resulted in 1500 ms of darkness in the chamber ("time-out") and the trial was repeated. Anticipatory errors were counted if the rat withdrew its nose from the central location before, or within 100 ms of, the onset of the tone. Late errors were trials on which the movement time to the food hopper exceeded 1500 ms.

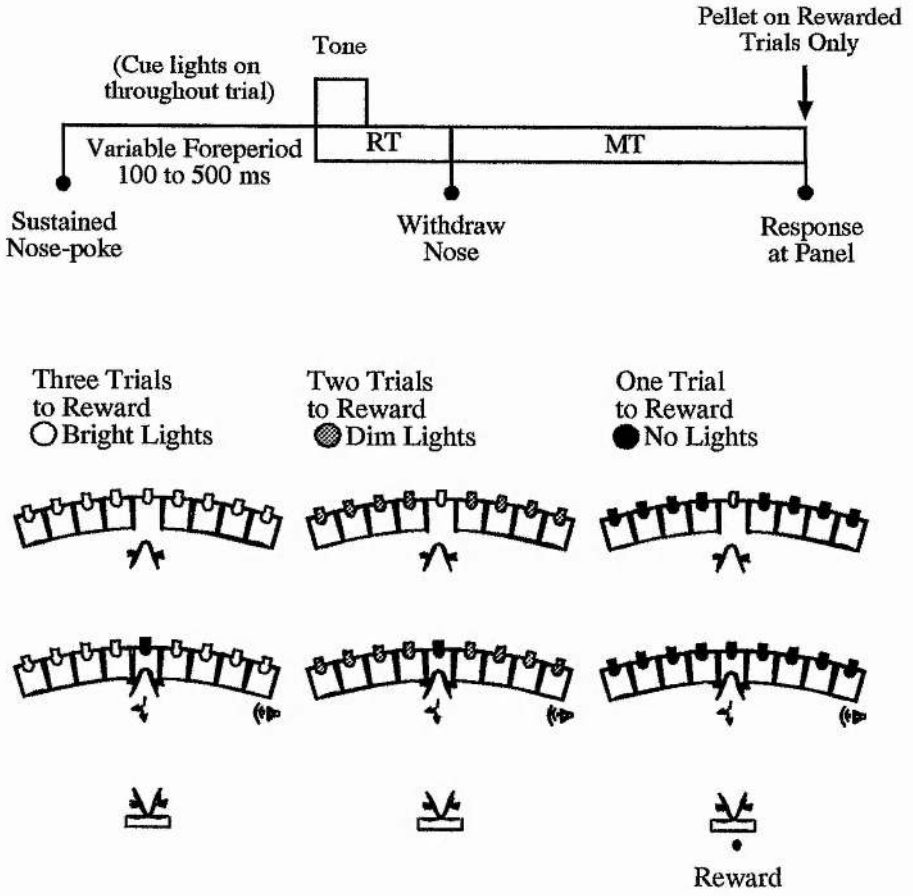


Figure 2.3. *The simple reaction time task required the rat to maintain a sustained nose-poke in the central hole for a variable foreperiod (100 to 500 ms, 5 foreperiods in 100 ms increments) before an auditory tone. The trial was completed by withdrawing the nose from the central hole and pushing open the food hopper panel at the front of the box. The multiple-ratio schedule of reward required the completion of either 3, 2 or 1 correct trials before the reward of a pellet (45 mg sucrose; BioServ, Frenchtown, NJ), which was delivered into the food hopper on the opening of the hopper panel. The position in the multiple-ratio reward schedule was indicated continuously throughout the trial by cue lights in the holes to the left and right of the central hole. There were three lighting conditions in the side holes; bright cue, dim cue and lights off. The cues signalled 3 trials, 2 trials and 1 trial to reward, respectively. The cue order was reversed for half of the rats to counterbalance for the possible differential salience of the brightness of the cues. The starting position in the three schedules was varied by the controlling computer in a pseudorandom order.*

2.2.3.iv. Removal and Reversal of Visual Cues

In addition to examining the postsurgical performance on a visually cued multiple-ratio reward schedule, two further behavioural manipulations were undertaken. 1) The visual cues were absent (all cue lights switched off), but otherwise the task remained the same (~600 trials per rat, starting 3 weeks postsurgery). 2) The cue lights were reinstated, but the meaning of the cue lights were reversed (six blocks (480 trials per block), data was collected starting 3½ weeks postsurgery for two weeks).

2.2.4. Somatosensory Asymmetry Test

A test of somatosensory 'neglect' (Schallert et al., 1982) was used to assess the capacity to detect and overtly respond to a tactile stimulus. Testing took place on an observation pedestal (platform 29 cm in diameter) providing a clear view of the rat during testing. Before any testing began the rats were habituated to the pedestal. On each forelimb a white self-adhesive paper patch (circular, 13 mm in diameter) was attached, with the order of attachment reversed for each consecutive trial. Once the patches had been attached both forelimbs were pressed simultaneously. The rat was returned to the observation pedestal and an observer positioned in front of the pedestal recorded the time to contact the patches on each paw. A trial lasted for two minutes or until both patches had been contacted. If one or both patches fell off prior to any contact the trial was disregarded and repeated. Each rat was tested for ten trials per day. 'Neglect' would be expected to take the form of an asymmetry in the order in which the patches are contacted and/or a deficit in the latency to contact the contralateral patch.

2.2.5. Paw Reaching

A paw reaching task was used to examine forepaw motor competence. Rats were placed in a cage (25 cm by 38 cm) with a tray attached to the front, initial training included overnight sessions before progressing to 15 minute sessions. The tray contained twenty food pellets (45 mg sucrose pellets) in shallow wells, evenly spaced along the cage front (MCA occlusion experiment). In the experiment following the excitotoxic posterior parietal lesion unlimited food pellets were available during training and testing. The food pellets could be obtained with the forepaws, but could not be reached with the

tongue. The rats were tested during 15 minute sessions. Testing was conducted in red light, with the observer (blind to surgical outcome) seated in front of the cage, at a distance of one metre. The number of successful and unsuccessful reaches with each paw were recorded during each testing session. Extension of the paw through the cage bars past the wrist was regarded as a reach. Successful consumption of the grasped food pellet was recorded as a successful reach. Reaches that ended with the withdrawal of the paw without a food pellet or dropping of the food pellet were noted as a failed reach. In addition the tray containing the pellets was divided into four sections which were used to score the position of the rat in the cage every minute during the testing sessions. Of interest was spontaneous paw use as well as reaching accuracy with the contralateral paw.

2.3. Statistical Analysis

2.3.1. Reaction Time Data

Raw data were processed to extract mean reaction times, movement time, latency to collect reward, and error type frequency for each rat. Accuracy of performance was assessed as the proportion of correct responses as a function of all trials (correct and all error responses). Each type of error was also examined separately as a percentage of all trials. The percentage of contralateral responses were also calculated, as a measure of response asymmetry. Mean reaction time, movement time, latency to collect reward, percentage correct and percentage of errors by type were analysed by repeated-measures Analysis of Variance.

The assumption of homogeneity of covariance required by repeated-measures ANOVA was tested using Mauchly's test of sphericity. If this test indicated that the assumption had been violated, then the F ratio was evaluated with more conservative degrees of freedom adjusted by the Huynh-Feldt correction factor (Howell, 1987). When appropriate, further investigation of significant interactions ($p < 0.05$) were conducted using *post hoc* Newman-Keuls comparisons.

Probability density distributions (Silverman, 1986; see Bowman et al., 1993) of reaction times were produced to gain insight into the nature of the significant changes in the mean reaction times as indicated by the ANOVA. The distributions were computed

by replacing each reaction time with a Gaussian kernel ($\sigma = 40$ ms) centred on the reaction time. The kernels were then summed across trials and the probability of a response per millisecond was plotted against reaction time bins of 10 ms.

2.3.2. Asymmetry Test

Paw reaching (numbers of reaches) and the somatosensory asymmetry test (latency to contact) were also analysed using repeated-measures ANOVA. In addition, an asymmetry score was calculated as the proportion of responses to the contralateral side as a function of total responses, expressed as a percentage where 50 % is no asymmetry, and 100 % is an exclusive contralateral asymmetry.

2.3.3. Controls

Following unilateral 6-OHDA striatal dopamine depletion, unilateral MCA occlusion and excitotoxic unilateral posterior parietal lesion the intact contralateral hemisphere served as a control. Ischemic lesions (MCA and ACA occlusion) also included an operated control group in the analysis.

2.4. Surgery

2.4.1. Anaesthesia

Initial anaesthesia was induced in an anaesthetic chamber using halothane at 4-5 %, delivered in a nitrous oxide/oxygen (3:1) mix. When the pedal reflex could no longer be elicited the rat was placed in a stereotaxic frame with 'atraumatic' ear bars (Kopf, Tujunga, CA) and the nose bar set appropriately for the surgery undertaken. Halothane was then reduced to 1.5-2 % for the duration of the surgical procedure. Normal body temperature (37 ± 1 °C) was maintained during surgery using a thermostatically regulated heating blanket (Harvard Apparatus Ltd, Edenbridge, Kent, UK).

2.4.2. Dopamine Depleting Lesions (6-hydroxydopamine) of the Striatum

The rats were pre-treated with an intraperitoneal injection of the monoamine oxidase inhibitor, pargyline (50 mg/kg in warm sterile 0.9 % saline, Sigma Chemical Co.,

Poole, UK) before surgery to enhance the efficacy of 6-OHDA (Breese & Traylor, 1971). Twenty minutes after the injection of pargyline, anaesthesia was induced. The rats were then placed into a stereotaxic frame with the nose bar set at + 5 mm. A midline incision was made along the scalp and the skin and fascia were retracted to reveal the skull. A hole was then drilled in the skull at the co-ordinates AP + 2.0 mm and L ± 3.0 mm to bregma (lesions were unilateral). A 30 gauge injection cannula containing 8 μg of 6-OHDA base in 2 μl of ascorbate acid saline was then lowered to 6.5 mm below skull, and the 6-OHDA was manually infused at a rate of 0.1 μl every 10 seconds. The cannula was left in place for 3 minutes, before being slowly withdrawn. The incision in the scalp was then closed using sterilised metal clips. Finally the animal was placed in a warm cage to recover before transfer back to a home cage.

2.4.3. Excitotoxic Lesions of Posterior Parietal Cortex

The anaesthetised rat was placed into a stereotaxic frame with the nose plate set at - 3.3 mm. A midline incision was made to permit retraction of the scalp and fascia to expose the skull. A craniotomy was performed on one side of the skull (lesions were unilateral) which exposed the brain between L ± 1.5, AP - 3.8 to - 5.8 mm and at L ± 6.0 mm, AP - 4.3 to - 5.8 mm relative to bregma. Quinolinic acid (Sigma Chemical Co., Poole, UK) was sprinkled directly on to the exposed cortex using a fine brush. The scalp incision was closed and the rat was placed into a warm environment to recover.

2.4.4. Occlusion of the Middle Cerebral Artery - MCAo

The nose plate was set to - 3.7 mm for the procedure. A dorsal craniotomy was performed to allow an injection cannula to be lowered to AP + 0.9 mm, 5.2 mm lateral and - 8.8 mm (- 8.7 mm, comparison of rat strain) at skull below bregma. Injection was accomplished using a 5 μl syringe filled with sterile water and attached to a 31 gauge injection cannula via a polythene tube. A 0.2 μl air bubble was drawn into the tube before the potent vasoconstrictor endothelin-1 was taken up. The position of the air bubble was marked and the movement of the bubble monitored to confirm successful injection. Occlusion of the right MCA was then achieved by manual intracerebral injection of the endothelin-1 (150 pmol in 3 μl 0.9 % sterile saline, Novabiochem, Nottingham, UK) adjacent to the MCA (Sharkey et al. 1993). The endothelin-1 was administered as three injections of 1 μl, each separated by a 90 second delay. In the experiment examining rat

strain endothelin-1 was administered as two injections of 1 µl separated by a 90 second delay (100 pmol in 2 µl 0.9 % sterile saline). The cannula was then left in situ for 5 minutes before being slowly withdrawn. After completely withdrawing the injection cannula the patency of the line was verified. Finally the burr hole in the skull was filled with bone wax and the incision in the scalp closed using sterilised metal clips (suture in experiment examining rat strain). During recovery the rat was placed in a warm environment to maintain normothermia throughout the recovery period.

All the rats in the study received an intracerebral injection of endothelin-1. Operated controls arise from the surgery as those rats with only needle tract damage and only a small lesion at the site of injection. Any animal that displayed difficulty in feeding or gross abnormality was humanely killed. The importance of high blood glucose levels in aggravating the evolution of an ischemic lesion has been well established in the literature (Duverger & MacKenzie, 1988 & Slivka, 1991) thus rats were always maintained on a standard laboratory diet for at least 48 hours after surgery.

The use of a craniotomy has been criticised for exposing the brain tissue, thereby potentially allowing a fall in brain temperature. A fall as small as 2^{oC} in brain temperature has been demonstrated to reduce the severity of ischemic injury (Ginsberg et al., 1992). However, Henshall et al. (1995) has recently demonstrated that intracerebral temperature does not fall after the dorsolateral craniotomy performed for stereotaxic administration of endothelin-1.

2.4.5. Occlusion of the Anterior Cerebral Arteries - ACAo

The procedure for occlusion of the middle cerebral artery applicable to chapter 6 was adhered to with the following modifications. The nose plate was set at - 3.3 mm and the injection cannula was positioned according to the co-ordinates AP + 3.0 mm, L 0.0 mm and V - 6.5 mm at skull below bregma. The burr hole was covered with sterile gelfoam rather than bone wax.

2.5. Histology

2.5.1. Perfusion

The rats were deeply anaesthetised by intraperitoneal injection of pentobarbitone (0.7 ml at 200 mg/ml). The rats were then intracardially perfused with phosphate buffer for two minutes followed by 4 % paraformaldehyde in phosphate buffer for 10 minutes at 10 ml/min. The brains were then carefully removed and placed into a 20 % sucrose/4 % paraformaldehyde phosphate buffer solution for storage. Coronal sections 50 μm thick, were cut using a freezing microtome, and a section every 200 μm was taken for staining with cresyl violet. Additional adjacent sections (50 μm) were taken in some experiments for immunohistochemistry (Glial Fibrillary Acidic Protein (GFAP); tyrosine hydroxylase). The brains were also photographed prior to being cut for staining in some experiments.

2.5.2. Immunohistochemistry

Immunohistochemistry was performed according to the peroxidase-antiperoxidase (PAP) protocol of Côté et al. (1993). The antibodies used in Côté et al.'s (1993) protocol included a primary antibody either Monoclonal anti-GFAP (Affiniti Research, Exeter, UK) or anti-tyrosine hydroxylase (Sigma Chemical Co., Poole, UK) as appropriate to the immunohistochemistry undertaken. The two secondary antibodies used in the protocol were Mouse clonoPAP® and Goat antimouse IgG (antibodies were supplied by Affiniti Research, Exeter, UK).

2.5.3. Quantification of Lesions

The sections were examined using a light microscope (Leitz Diaplan microscope), and the damage was annotated onto paper depicting the actual sections obtained with an image scanner. To establish the area of lesion cresyl violet sections were examined for the absence of neurons, the presence of microglia and abnormal cell morphology. The extent of the infarct assessed by GFAP was determined by the presence of GFAP positive astroglia, evident under the light microscope. Sections stained for tyrosine hydroxylase were examined for the absence of positive staining in the striatum.

Once the boundaries to the lesion were established, the area of lesion, intact contralateral and intact ipsilateral tissue were then quantitatively measured using *NIH Image Analysis* (NIMH, Bethesda, MD, USA; version 1.57), which was calibrated for measuring distances in both planes for the 2-dimensional sections. Following unilateral MCA occlusion and posterior parietal lesions the area of intact ipsilateral and contralateral tissue was measured to permit the calculation of the area of lesion, while avoiding the measurement of the actual damaged tissue, which maybe distorted by edema and handling for histology (Lin et al., 1993). For comparative purposes the actual area of lesion was used to calculate MCA lesion volume in the experiment examining the effect of rat strain. Following ACA occlusion the bilateral lesion did not leave an intact hemisphere to measure, consequently the damage was annotated onto standardised sections taken from Paxinos & Watson (1986). The area of dopamine depletion and the ipsilateral striatum were measured to allow a percentage of dopamine depletion in the striatum to be calculated. One section every millimetre (or every 400 μm CPu 6-OHDA lesion) was selected for measurement from the appropriate region for each lesion (ACA and MCA occlusion from bregma + 5.0 to - 7.0 mm and CPu 6-OHDA from bregma + 1.4 to - 2.4 mm). The volume was then calculated by integrating the cross-sectional areas with the distances separating them.

Chapter 3:

Middle cerebral artery occlusion in the Lister hooded and Sprague Dawley rat strains

3.1. Introduction

The Sprague Dawley strain of rat has been used to develop a model of MCA occlusion in which the vasoconstrictor endothelin-1 has been used to induce ischemia (Sharkey et al., 1993). The model of focal cerebral ischemia has subsequently been used to evaluate neuroprotective agents in the Sprague Dawley rat according to histopathological criteria (Sharkey & Butcher, 1994; Sharkey et al., 1994). However, it remains desirable to establish how robust the model is. It is important to ensure that unforeseen anomalies in a rat strain do not give rise to misleading results. One approach to this problem includes using a different strain of rat. This experiment will therefore compare two strains of rat (Sprauge Dawley and Lister hooded) and verify that the endothelin-1 model of MCA occlusion can be used with the Lister hooded strain.

In addition to comparing rat strain, histological techniques for evaluating the extent of ischemic damage were examined using two different stains and by comparing different methods for calculating the volume of damage. A common technique for evaluating the histological outcome of focal cerebral ischemia has involved the use of nissl stains, such as cresyl violet (Borlongan et al., 1995; Katsuta et al., 1995; Sharkey & Butcher, 1994) and eosin (Garcia et al., 1995; Katsuta et al., 1995; Sharkey et al., 1993). These stains have relied on gross morphological changes in the neurons and neuronal loss as indicators of the extent of the ischemic lesion.

However, focal cerebral ischemia sets off a cascade of changes in the normal brain which are indicative of metabolic stress and neuronal cell death, including astrocytosis. It is possible to visualise the astrocyte response by immunohistochemistry for glial fibrillary acidic protein (GFAP). An increase in GFAP immunoreactivity and induction of mRNA for GFAP after focal cerebral ischemia has been reported previously (Chiamulera et al., 1993; Fuxe et al., 1992; Garcia et al., 1993; Post et al., 1996; Schroeter et al., 1995; Yamashita et al., 1996). Schroeter et al. (1995) reported a distinct astroglial response to focal cerebral ischemia after photochemically induced thrombosis, in which the border zone of the infarct expresses GFAP. They suggest that it is around the infarct that proliferation occurs in response to invading macrophages that release interleukin-1, a factor known to activate astrocytosis (Giulian et al., 1988). Furthermore, ischemia is known to induce molecules associated with reactive astrocytes (Eddleston & Mucke, 1993).

Thus the second objective of the experiment was to compare the extent of ischemic damage identifiable after MCA occlusion with the potent vasoconstrictor endothelin-1 (Sharkey et al., 1993), using a nissl stain (cresyl violet) and immunoreactivity for GFAP as an indicator of the short term astrocyte response to ischemia. It was hoped that undertaking this comparison would clarify the suitability of GFAP for use in assessing the consequence of MCA occlusion by perivascular administration of endothelin-1.

3.1.1. Hypotheses

Previous research has found that although some strains of normotensive rat do not differ in terms of the infarct volumes resulting from MCA occlusion this does not hold for all normotensive rat strains (Duverger & MacKenzie, 1988; Van der Staay et al., 1996a). However, the lesion resulting from MCA occlusion in the Lister hooded rat should not differ from the Sprague Dawley strain if vascular and physiological parameters are comparable. Furthermore, increased GFAP immunoreactivity at the boundary of an ischemic infarct has been reported (Schroeter et al., 1995), and thus assessment of lesion extent for GFAP and cresyl violet should correspond.

3.2. Materials and Methods

Twelve Lister hooded (274-312 g) and nine Sprague Dawley rats (282-305 g) were used in the study. The rats underwent unilateral middle cerebral artery occlusion according to the protocol detailed in section 2.4.4.

3.2.1. Collection of Data

The rats were allowed to survive for three days before perfusion, during which time they had free access to standard laboratory chow and water. Serial coronal sections 50 μm thick were cut using a freezing microtome and two adjacent sections every 200 μm were subsequently taken for staining with cresyl violet and immunohistochemistry for GFAP. Volumetric analysis was conducted according to the protocol detailed in section 2.5.3. Two measures were used to quantitatively assess the extent of ischemic damage. Absolute area of tissue damage was calculated according to area of actual

tissue damage and by a second method assessing tissue damage by subtracting the area of intact ipsilateral tissue from the area of contralateral tissue. One section every millimetre was selected for measurement from bregma + 5 to bregma - 7 mm.

3.3. Results

3.3.1. Description of the Extent of Lesion

The extent of the ischemic damage following MCA occlusion is presented in Table 3.1 and was heterogeneous in both strains. The following description of the lesion location is based on Paxinos & Watson (1986) and the cortical structural divisions identified by Zilles (1990) and refers to both strains. Cortical lesions above 170 mm^3 (Sprague Dawley, n = 3) included near complete destruction of the cortex anterior to bregma + 5 mm and an infarct involving as much as 76 % of neocortex posterior to bregma - 7 mm. In addition there was damage throughout the lateral olfactory tract/anterior olfactory nucleus and neuronal loss and microglia evident in Cg 1-3 contralateral to the side of lesion. The bilateral damage in one animal also extended into the septum. Outside of the frontal cortex there was severe neuronal loss throughout Par1, Par2, FL, HL and allocortex. Posterior to bregma - 4 mm, extensive damage could be identified in Te1/3 and Oc2M/L to bregma -7 mm. Striatal damage in these animals was complete, with severe neuronal loss and infiltration of microglia throughout the striatum. Further subcortical damage was apparent in some animals with increased GFAP immunoreactivity in the ipsilateral globus pallidus.

The cortical lesions smaller than 55 mm^3 (Sprague Dawley, n = 2 and Lister hooded, n = 3) were confined to lateral cortex and did not extend into Cg 1-3 or contralateral cortex. The core region of the infarct was between bregma + 1 mm and bregma - 1 mm, including PAR 1, 2, FL and allocortex. The infarct beyond these levels gradually reduced occupying a progressively more dorsolateral location in the cortex at coronal levels beyond bregma + 3 mm and up to bregma - 6 mm. Subcortical damage was mainly located in the dorsolateral striatum, although the striatum was intact in one Lister hooded rat.

Rat	Fr1	Fr2	Fr3	Par 1/2	FL	HL	Oc 2M	Oc 2L	Te1	Te2	Te3	Allo-cortex	CPu	Other	Volume Cortex/CPu (mm^3)
94601	√	√	√	√	√	√	√	√	√	√	√	√	√	Cg1/2(ic), Oc1B/M	217/33
94602	√	√	√	√	√	√		√	√	√	√	√	√	Oc1B/M	136/28
94604	√	√	√	√	√	√	√	√	√	√	√	√	√	LS/MS, Cg1/2/3(ic), Oc1B/M, GP (GFAP)	195/28
94605				√	√			√	√		√	√	√		40/9
94606				√	√							√	√		38/18
95601	√	√	√	√	√	√	√	√	√	√	√	√	√	Cg1/2/3(ic), Oc1B/M GP (GFAP)	185/36
95602	√		√	√	√	√		√	√		√	√	√		83/33
95604	√	√	√	√	√			√				√	√		96/26
95605	√		√	√	√			√				√			31/0
95610	√	√	√	√	√	√	√	√	√	√	√	√	√	Cg 1/2 (i), Oc1B/M	137/25
95613	√	√		√								√	√		27/21
95614	√	√	√	√	√			√	√		√	√	√	GP (GFAP), Acbc/AcbSh	138/30
95616	√		√	√					√		√	√	√		53/5

Table 3.1. *The extent of the ischemic lesion following MCA occlusion assessed by cresyl violet and GFAP (the nomenclature and areas correspond to Paxinos & Watson (1986)). The volume of lesion in the cortex and striatum was calculated using the intact contralateral and ipsilateral tissue to calculate the lesion area from cresyl violet stained sections. 94601-95602 Sprague Dawley, 95604-95616 Lister hooded, i - ipsilateral, c - contralateral.*

3.3.2. Comparison of Ischemic Lesion after MCA Occlusion in the Lister Hooded and Sprague Dawley Rat Strains

The success rate in occluding the MCA in the Lister hooded (n = 6) and Sprague Dawley (n = 7) rat strains did not differ significantly with the sample sizes used in the study (n = 12 and n = 9 respectively) as determined by Fisher's Exact Test (one-tail $p < 0.2$). Operated controls had less than 6 mm^3 of cortical damage associated with the cannula tract around bregma 0.

The volume of ischemic damage (calculated using intact tissue) in the two strains of rat did not differ significantly from one another in either the cortex (Strain, $F(1,11) = 2.11$, ns) or striatum (Strain, $F(1,11) = 1.8$, ns). Similarly more detailed comparison of the area of ischemic damage at coronal levels did not identify any significant difference between the two strains in the extent of cortical damage (Cortex, Strain by Coronal $F(5,59) = 1.08$, ns). The area of damage in the striatum did reveal a nearly significant difference between the two strains, with smaller infarct areas in the main body of the striatum (bregma + 1.0, 0.0 and - 1.0) in Lister hooded compared with the Sprague Dawley strain (Striatum, Strain by Coronal $F(2,22) = 2.97$, $p < 0.07$). The core of the lesion was evident around bregma 0 and gradually decreased as it progressed from the core, which was reflected by a significant main effect for coronal level for both cortex (Coronal $F(5,59) = 23.61$, $p < 0.001$) and striatum (Coronal $F(2,22) = 50.18$, $p < 0.001$).

3.3.3. Comparison of Infarct Assessed by Cresyl Violet and GFAP

The average area of ischemic damage identified in the cortex and striatum using GFAP and cresyl violet at fixed coronal levels revealed a close correspondence between the two stains around the periphery of the lesion illustrated in Figure 3.1.

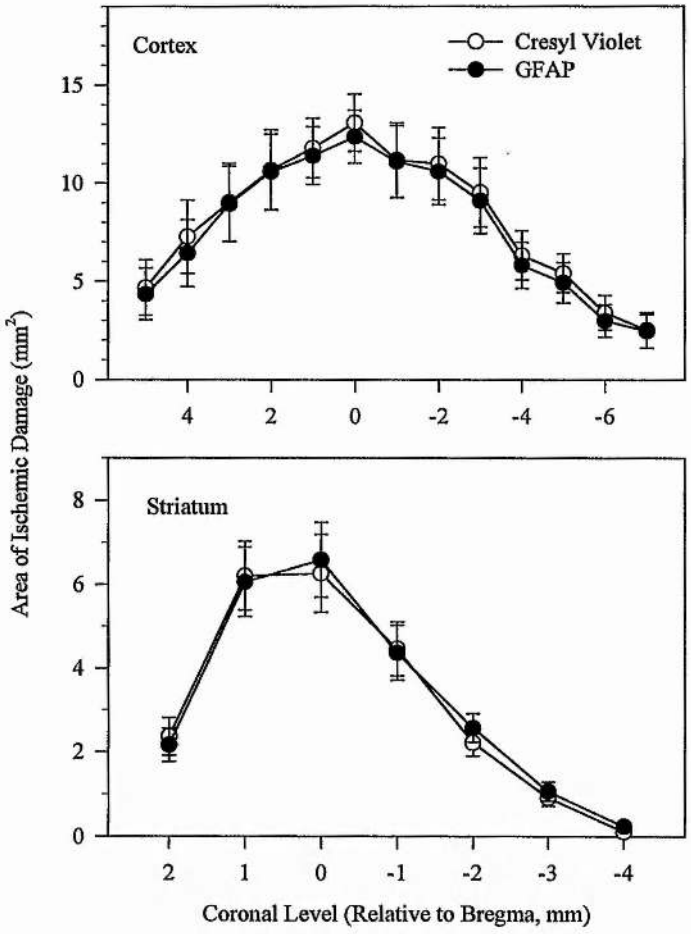


Figure 3.1. *The extent of damage (mean ± sem, n = 13) assessed by cresyl violet and GFAP revealed a close correspondence at adjacent coronal levels in both the cortex and striatum. The area of intact contralateral and ipsilateral tissue was used to calculate the lesion area.*

This finding applied for both the cortex and striatum, if the area of damage was calculated by using the intact contralateral and ipsilateral tissue (Cortex, Stain by Coronal, $F(9,101) = 1.09$, ns; Striatum, Stain by Coronal, $F(3,30) = 1.53$, ns). A comparison of the volumes of damage calculated from cresyl violet and GFAP sections illustrates close equality between each estimate (Figure 3.2).

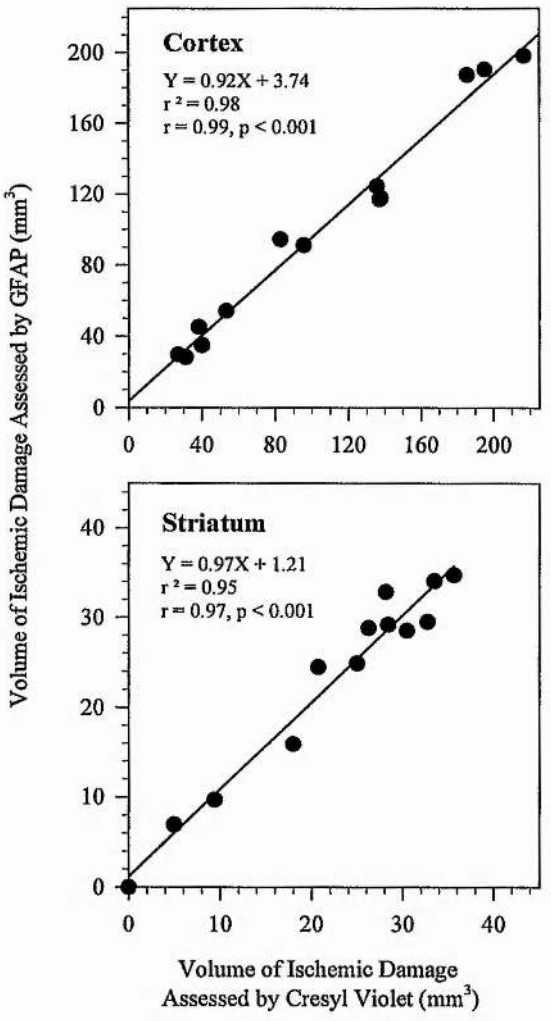


Figure 3.2. *The volume of ischemic damage assessed by cresyl violet and GFAP concur for both the cortex and striatum (n = 13). The volume was derived from the integrated area of tissue damage at each coronal level assessed by measuring the intact contralateral and ipsilateral tissue to calculate the lesion area.*

However, it is evident that for the cortex in Figure 3.3 measuring the actual area of ischemic tissue produced a significantly smaller area for GFAP than was observed with cresyl violet in the core (bregma + 2.0, + 1.0 and 0) of the lesion (Stain by Coronal, $F(7,74) = 2.33$, $p < 0.035$).

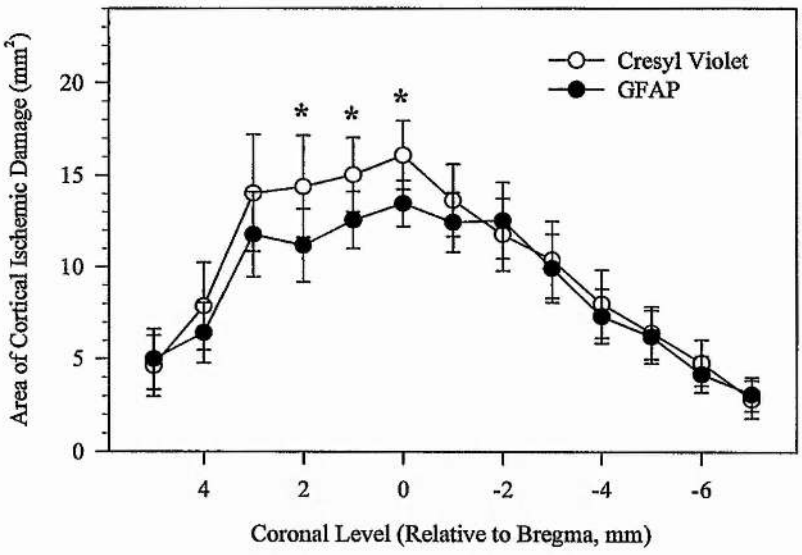


Figure 3.3. *The absolute area (mean ± sem, n = 13) of cortical ischemic damage (actual damaged tissue measured) in the core of the lesion was underestimated by GFAP when compared with cresyl violet (the asterisks indicate a significant difference between cresyl violet and GFAP, $p < 0.05$).*

Figure 3.4 illustrates a strong correlation with the underestimation of cortical ($r = 0.9$, $p < 0.001$) volume by GFAP as the lesion volume increases, when compared with measurements taken on cresyl violet sections.

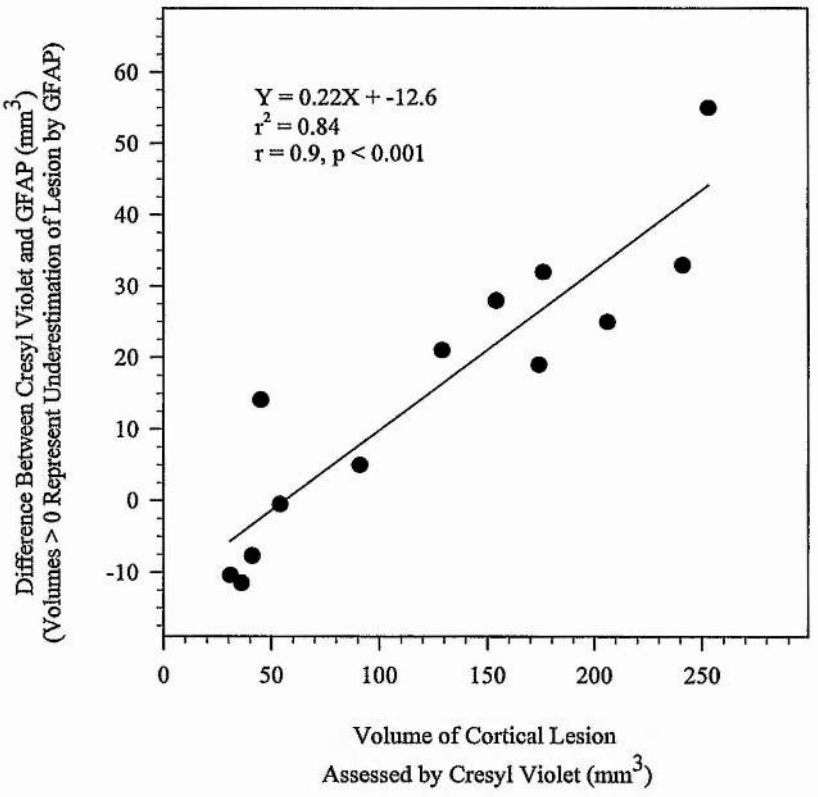


Figure 3.4. *As the absolute volume of ischemic tissue damage in the cortex increased (assessed by cresyl violet) the extent of underestimation of cortical lesion volume by GFAP also increased (n = 13). These figures arise from measuring the actual area damaged rather than intact tissue.*

The actual lesion boundaries illustrated by cresyl violet and GFAP correspond closely with each other (Figure 3.5). GFAP positive astroglia were evident in the globus pallidus in some animals (Figure 3.5c), indicating an area experiencing metabolic stress. However, this supplementary information could not be discerned examining the globus pallidus in adjacent coronal sections stained with cresyl violet, which appeared to be normal (Figure 3.5d).

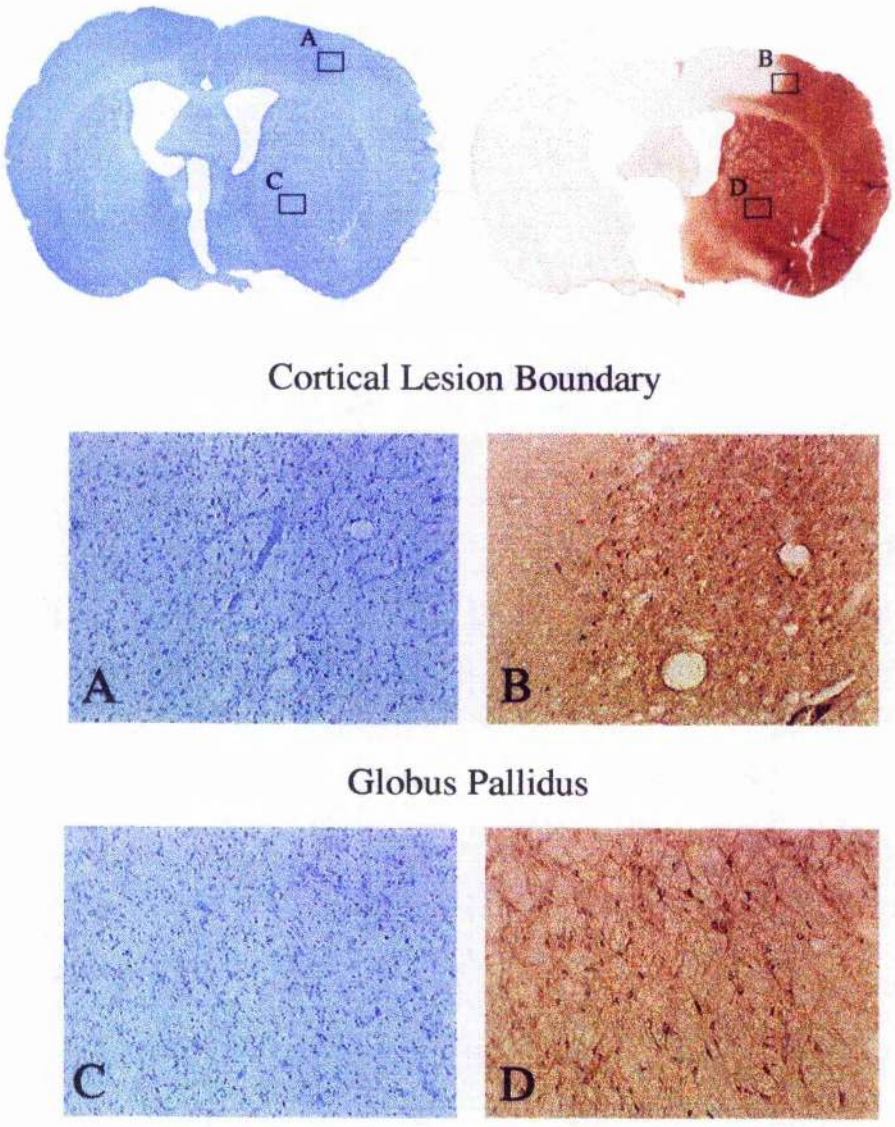


Figure 3.5. *The figure illustrates two adjacent coronal sections (bregma - 0.8 mm) stained for cresyl violet and GFAP 72 hours following MCA occlusion (rat #95614). The cresyl violet section is larger than the GFAP section, which underwent immunohistochemistry as a free floating section, possibly causing some distortion. Subsequent enlargements are taken from the cortical lesion boundary (A and B) and the globus pallidus (C and D). The lesion boundaries correspond for cresyl violet and GFAP. Astroglia are evident in the globus pallidus (GFAP D), which may indicate an area experiencing metabolic stress. The corresponding section stained with cresyl violet illustrates the typically chaotic appearance of the globus pallidus following nissl stains and does not differ in appearance from controls.*

3.4. Discussion

The neuropathological consequences of MCA occlusion did not significantly differ between Lister hooded and Sprague Dawley strains. Examination of coronal sections revealed that 72 hours after ischemic injury, GFAP immunoreactivity corresponded to the same boundaries identified using cresyl violet sections. Cresyl violet provided an easy method for identifying gross change after ischemia in the cortex and striatum. GFAP immunohistochemistry, however, suggested more subtle secondary changes in some animals with damage through the entire extent of the striatum, where there was also an indication that cells were experiencing metabolic stress in the globus pallidus. The globus pallidus in adjacent sections stained with cresyl violet appeared normal.

While GFAP did underestimate the size of the lesion when compared with cresyl violet, this only arose when the absolute area of tissue damage was measured. The underestimation was probably attributable to tissue loss and collapse when free floating sections were processed for GFAP and was most pronounced amongst the largest lesions, which would also be associated with the most delicate tissue. Thus caution must be exercised if absolute volume is used to assess the extent of ischemic damage. The use of actual area of tissue damage to assess lesion extent is also prone to overestimation in the early stages of the evolution of an infarct due to the inclusion of tissue swollen by edema (Lin et al., 1993). In addition, following longer survival times (+ 1 month) atrophy may further lead to an underestimation if actual area of visibly damaged tissue is used to quantitatively assess damage extent (Hara et al., 1993).

The current study did not identify a significant difference between Sprague Dawley (n=7) and Lister hooded (n=6) rats, although the lesions were heterogeneous and the sample sizes were small. However, since this experiment, others have repeated MCA occlusion in Lister hooded rats with consistent results (Marston et al., 1995a; Sharkey et al., 1996). There was one anomaly indicating (close to significance $p < 0.07$) that the area of striatal lesion was smaller in the main body of the striatum in Lister hooded when compared to the Sprague Dawley strain. Sharkey et al. (1996) whilst examining the efficacy of FK506 has reported significant neuroprotection in the striatum in the Lister hooded strain, which is rarely protected by acute intervention after MCA occlusion in other strains. An explanation for these apparent differences in the effects of MCA

occlusion on the striatum in Lister hooded rats maybe due to a difference in cerebrovasculature architecture, with the presence of subcortical interconnections between the ACA and MCA (Sharkey et al., 1996). The importance of these anastomoses may become more prominent during neuroprotection.

Astroglia are believed to be important in a variety of roles by providing support for neuronal activity and interacting with the Blood Brain Barrier. Specifically, astrocytes are believed to be important in regulating the local extracellular environment (Bignami, 1991; Walz, 1989). A prominent early consequence of ischemia is a rise in extracellular potassium as the membrane depolarises, preceding the catastrophic failure of ATP dependent ion pumps and efflux of excitotoxic levels of glutamate (Siesjo, 1992a). Possibly consistent with this proposed homeostatic role, astrocytes during ischemia appear to proliferate and sequestrate glutamate (Swanson et al., 1994; Torp et al., 1991). Despite the sequestration of glutamate by astroglia, it is unlikely to be beneficial in the ischemic brain. Apart from the likelihood that the homeostatic function of astroglia is overwhelmed in the ischemic brain (Barbour et al., 1988; Torp et al., 1991) astroglia are also immunocompetent cells (Piani et al., 1994; Wekerle, 1995) and as such contribute to the immune response involving leukocytes. Furthermore, astroglia are capable of producing nitric oxide (Galea et al., 1992) through inducible Nitric Oxide Synthase (iNOS). Nitric oxide maybe beneficial as a vasodilator in the initial stages of ischemia. However, the production of nitric oxide has also been implicated in the production of free radicals which cause oxidative damage to cellular membranes and the nucleus, and nitric oxide can also increase glutamate release (Dawson, 1994). These reactions are deleterious to neuronal survival in ischemia.

GFAP positive astroglia were found in the globus pallidus of some animals without any discernible indication of abnormality on corresponding cresyl violet stained sections. Without any verification it was not possible to conclude that the globus pallidus was irreversibly damaged at 72 hours after MCA occlusion. However, the increase in GFAP reactivity would suggest a region under metabolic stress. Damage in the substantia nigra has previously been reported (Hara et al., 1993; Tamura et al., 1990) and it was suggested that excitotoxicity arising from the loss of inhibitory GABAergic input from the striatum accounted for the mechanism of damage. Similarly the globus pallidus receives GABAergic inhibitory input from the striatum, and thus the astrocytosis in the globus pallidus could have arisen due to the stress of excess excitatory activation and not

directly due to ischemia. Although GFAP immunoreactivity was not evident in the substantia nigra of the rats that displayed increased GFAP in the globus pallidus, the time course for increased GFAP immunoreactivity could vary.

Chapter 4:

Covert orienting in the rat: 1) Demonstration of the phenomenon, 2) The effects of striatal dopamine depletion

4.1. Introduction

The appearance of a visual target is often associated with the movement of the head and eyes toward it's location; however, shifts of attention can occur in the absence of such overt orienting (Ericksen & Hoffman, 1972; Jonides, 1981). Posner (1980) devised a task to define operationally, in the laboratory, covert orienting of attention: with a subject fixating centrally, a peripheral cue was presented followed by a target light. For 80 % of the trials the cue corresponded (valid cue) to the side of the subsequent target. The remaining 20 % of trials, the cue was presented on the opposite side (invalid cue). Reaction times for trials with a valid cue were faster than for trials with invalid cues. The difference in reaction time between validly cued and invalidly cued trials is referred to as the 'validity effect'. This task can be used to quantify attentional function, in terms of the costs and benefits of peripheral visual cues, which direct or misdirect attention toward the location of subsequent peripheral visual targets. The covert orienting task has been extensively employed to examine reaction time performance after a variety of brain injuries in humans. As a consequence, the task has successfully identified the organisation of a distributed attentional network in the human brain (Table 4.1).

Posner & Petersen (1990) have proposed that there are three fundamental components to covert orienting and these have been associated with different brain regions; disengagement of attention (parietal cortex: Petersen et al., 1989; Posner et al., 1984), shifting of attention (superior colliculus: Posner et al., 1982; Rafal et al., 1988; Robinson & Kertzman, 1995) and engagement of attention at the new location (lateral pulvinar of the thalamus: Petersen et al., 1987; Rafal & Posner, 1987). Additional experimental and clinical work has also suggested the involvement of anterior cingulate, lateral frontal cortex and the basal ganglia in performance of the task, with these systems thought to contribute variously to target detection and response preparation (Posner & Petersen, 1990; Posner & Driver, 1992). The design of the covert orienting task is particularly effective for investigating the consequence of unilateral lesions due to the various combinations of side to which the cue, target and response may occur and so can be used to define the cause of a variety of deficits.

Lesion/Disease/ Drug	Deficit	Contralateral Valid	Contralateral Invalid	Ipsilateral Valid	Ipsilateral Invalid
Unilateral parietal cortex	Disengagement of attention [1,2,3*,4*]	-	↑	-	-
Unilateral thalamic haemorrhage	Engagement of attention [5]	↑	↑	-	↓
Unilateral lateral pulvinar	Engagement of attention [6]	↑ (muscimol) ↓ (bicuculline)	↑ (muscimol) ↓ (bicuculline)	-	↓ (muscimol) ↑ (bicuculline)
Progressive Supranuclear Palsy/unilateral superior colliculus	Movement of attention [7,8,9] (vertical orientation)	↑	↓	↑	↓
Unilateral excitotoxic lesion-lateral CPu / 6-OHDA lesion CPu	Response initiation [10,11,12]	↑?	↑?	-	-

↑ Increase in reaction time; ↓ Decrease in reaction time; - No change in reaction time

Table 4.1. *Reaction time performance on the covert orienting task following a variety of pathologies and pharmacological manipulation in humans and nonhuman primates. The first four rows have provided evidence for a distributed neural system for controlling the covert deployment of attention (Posner & Petersen, 1990). The final row illustrates a response initiation impairment, which has previously been observed in rats in a choice reaction time task similar to the covert orienting task used in this thesis. * Alzheimer's disease has also been observed to result in a difficulty disengaging attention bilaterally.*

[1]Petersen et al., 1989; [2]Posner et al., 1984; [3]Rafal & Posner, 1987; [4]Maruff & Currie, 1995; [5]Parasuraman et al., 1992; [6]Petersen et al., 1987; [7]Posner et al., 1982; [8]Rafal et al., 1988; [9]Robinson & Kertzman, 1995 [10]Brown & Robbins, 1989b; [11]Carli et al, 1989; [12]Carli et al, 1985

Measuring covert shifts in attention, in the absence of overt orienting, has been possible in primates (Bowman et al., 1993; Petersen et al., 1987; Witte et al., 1997), but to date has only very recently (and, indeed, only during the course of this work) been reported in the rat (Rosner & Mittleman, 1996). Previous research in pigeons (Shimp & Friedrich, 1993) and the rat (Bushnell, 1995; Bushnell & Oshiro, 1994) did not control movement during the presentation of cues in an orienting task and as a result were only successful in demonstrating *overt* orienting. Thus, the first experimental aim is to provide a description of a paradigm which allows the measurement of covert orienting to peripheral cues in the rat.

The covert orienting task developed for the rat has been devised from one of a pair of tasks originally employed to distinguish between impairment of sensory and motor functions in the rat following striatal lesions. The tests achieved this by varying the requirement to respond toward or away from an imperative visual signal (Brown & Robbins, 1989b; Carli et al., 1985; 1989). In the rat unilateral striatal dopamine depletion slows contralateral response initiation but leaves the execution of a response intact and results in a bias to respond ipsilateral to the side of lesion. These impairments occur regardless of the side of presentation of the imperative signal and thus demonstrate that the impairment is a response rather than sensory deficit (Carli et al., 1985; 1989). Furthermore, excitotoxic lesions of the striatum have identified distinct response impairments corresponding to the location of the striatal lesion (Brown & Robbins, 1989b). Lesions of lateral striatum result in an ipsilateral response bias but leave the initiation of contralateral responses intact. In contrast lesions of medial striatum slow the initiation of contralateral responses and induce a comparatively smaller ipsilateral response bias. These results provided additional support to the conclusion that the 'neglect' following striatal lesions reflects an impairment in response initiation, rather than sensory processes. The covert orienting task differs from that used by Brown & Robbins (1989b) by the addition of visual cues prior to the imperative signal.

Recent research conducted with Parkinson's patients (Wright et al., 1990) and with a systemically administered dopamine antagonist (Clark et al., 1989) has suggested

that there may be an impairment in maintaining endogenous attention in covert orienting tasks. Parkinson's patients do not appear to incur the cost associated with the invalid cue when compared to a neutral cue condition (Clark et al., 1989; Wright et al., 1990). However, the overall validity effect (without comparison with a neutral cue) does not appear different between patients and controls (Wright et al., 1990). Furthermore, it is not clear why under conditions of abnormal dopamine function there should only be a benefit and no cost associated with a cue. The period during which maintenance of attention to the cue occurs, precedes the onset of the target, it is only with the appearance of the target that the cue proves either to have misdirected or correctly directed attention. In conclusion a limited capacity to attend to the cue should apply equally to valid and invalid trials. A similar failure to maintain attention has been reported in Rhesus monkeys following pharmacologically induced catecholamine depletion in a covert orienting task using peripheral cues (Witte et al., 1992). However, in contrast, Bennett et al. (1995) and Rafal et al. (1984) found a global increase in reaction time of both valid and invalid trials for Parkinsonian patients, but with no change in the magnitude of the validity effect. Table 4.2 summarises the impairments which have been observed following Parkinson's disease and systemic dopamine antagonists. The apparently inconsistent results might be accounted for by heterogeneity of the patient groups, for example, the presence of extra-striate pathology (Agid et al., 1990; Fahn, 1986; Javoy-Agid et al., 1981). The development of a rat model of covert orienting will consequently provide an opportunity to conduct a systematic investigation of the role of dopamine in covert orienting.

The ischemic lesion following MCA occlusion includes cortex and striatum. Therefore, before examining the consequences of an ischemic lesion it was desirable to examine covert orienting in the rat after lesions which have been investigated in humans using similar tests. This will facilitate distinguishing between behavioural impairments attributable to functionally distinct areas within the territory of the MCA. The consequences of Parkinson's disease and systemic droperidol has implicated the dopaminergic system in covert orienting. A separate dopamine depleting lesion of the striatum will thus provide the opportunity to rule out striatal involvement in covert orienting and allow any change following MCA occlusion to be attributed to cortical damage.

Disease/Drug	Deficit	Valid cue	Invalid cue
Parkinson's Disease (early stage)	Response initiation [1,2*,3*]	↑	↑
Parkinson's Disease (late stage)	Failure to maintain attention [3*,4*]	-	↓
Systemic Droperidol /Clonidine	Failure to maintain attention [5,6*]	-	↓

↑ Increase in reaction time; ↓ Decrease in reaction time; - No change in reaction time

Table 4.2. *Reaction time performance on the covert orienting task following Parkinson's/Alzheimer's disease and systemic drug administration. * Central cues precede target (not peripheral cues).*

[1]Rafal et al., 1984; [2]Bennett et al., 1995; [3]Yamada et al., 1990; [4]Wright et al., 1990; [5]Witte et al., 1992; [6]Clark et al., 1989

4.1.1. Hypotheses

By using a unilateral model each rat served as its own control: reaction time for contralateral responses were compared with ipsilateral responses. It is possible to make predictions about the pattern of reaction times expected with different deficits and, on the basis of previous neuropsychological studies, many of these are already associated with particular anatomical regions (see Table 4.1). Two hypothesise are of primary interest in the current experiment (see Table 4.2). 1) Striatal dopamine may play a role in the maintenance of attention. 2) Depletion of striatal dopamine might result in only a response related deficit.

4.2. Materials and Methods

Twenty, pair-housed rats were used during the study (weight range, 215-280 g at the start and 373-432 g at completion of the study). The rats were tested pre and postsurgery on the covert orienting task (see section 2.2.2). All rats received dopamine depleting lesions of the dorsal striatum (see section 2.4.2).

4.2.1. Collection of Data

Once all the rats had reached the performance criterion of 120 correct trials within 30 minutes, presurgical data were collected over 5 days for 10 test sessions (approximately 1200 trials). On completion of the collection of presurgical data, the rats were assigned to receive a unilateral intrastriatal infusion of the neurotoxin 6-hydroxydopamine (6-OHDA, Sigma Chemical Co., Poole, UK). The side lesioned was determined by presurgical task performance. If there was an asymmetry in performance the side contralateral to the strongest validity effect was lesioned ($n = 10$). Where there was no asymmetry, the side of lesion was assigned randomly. After two weeks recovery from surgery, the rats were tested for three weeks. A total of approximately 3900 correct trials were collected for each rat over this period. Seven weeks following surgery the rats were killed humanely and perfused.

4.3. Results

4.3.1. Histological Results

Tyrosine hydroxylase depletion was not evident in the striatum of one rat and therefore this rat was excluded from subsequent analysis. In the remaining rats (n = 19), the percentage area of tyrosine hydroxylase depletion in the striatum ranged from 19 % to 91 % (mean 54 % ± 5.4 sem). Figure 4.1 shows tissue sections stained for tyrosine hydroxylase from the cases with the largest and smallest lesions. The depletion was evident in the body of the striatum between bregma + 1.4 and - 0.6, and, in the larger lesions, extended into the ventral striatum. The smallest lesion was located centrally in the striatum. Inspection of the cresyl violet sections did not reveal any evidence of damage outside of the striatum except for some limited cortical damage attributable to the cannula tract.

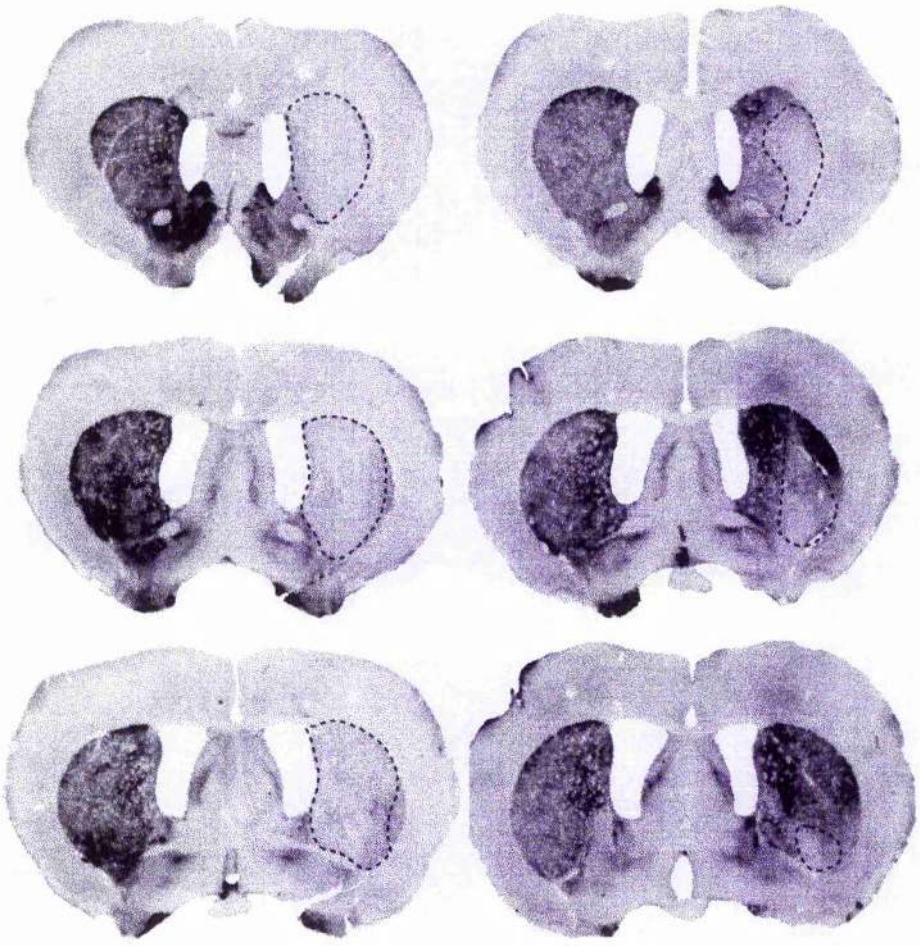


***Figure 4.1**. Digitised coronal sections illustrating the extent of tyrosine hydroxylase depletion in the striatum following an intrastriatal injection of the neurotoxin 6-hydroxydopamine. The largest lesion is depicted in the left panel (Rat #289; dopamine depletion in the striatum included 91 % of striatal volume) and the smallest lesion in the right panel (Rat #291; dopamine depletion in the striatum included 19 % of striatal volume).*

4.3.2. Covert Orienting of Attention

4.3.2.i. Reaction Time, Movement Time and Latency to Collect Reward

There was a significant validity effect at 200 (61 ms ± 17.7, 95 % confidence interval) and 400 ms (38 ms ± 15.7, 95 % confidence interval) foreperiods, which was irrespective of side of response (Validity by Foreperiod: $F(3,54) = 10.63$, $p < 0.0001$). The validity effect was not significant at the longer foreperiods of 600 and 800 ms.

Figure 4.2 illustrates the effects of the unilateral striatal dopamine depletion on reaction time. There was no change in the magnitude of the validity effect after surgery (Surgery by Side by Validity: $F(1,18) = 0.64$, ns; Surgery by Validity: $F(1,18) = 1.6$, ns). However, the mean reaction time increased by an average of 73 ms (± 29.4 sem) for all responses initiated contralateral to the side of lesion. Ipsilateral reaction times, by contrast, were faster postoperatively, with a decrease in the mean of 54 ms (± 23.7 sem) (Surgery by Side, $F(1,18) = 12.45$, $p < 0.01$).

Data analysis was undertaken to establish the relationship between lesion size and reaction time deficit. The postoperative change in reaction time was calculated using both change in contralateral and ipsilateral performance following surgery. The calculation assessed a reaction time deficit as a slowing of contralateral responses postsurgery relative to presurgical performance and improved ipsilateral responses postsurgery. The data was unsuitable for analysing using a Pearson correlation due to heterogeneous variance of reaction time data for lesions exceeding 50 %. However, analysis using Spearman's Rank correlation did reveal that increasing lesion size was associated with an increasing reaction time deficit (Spearman correlation coefficient = 0.48, $p < 0.04$, $n = 19$).

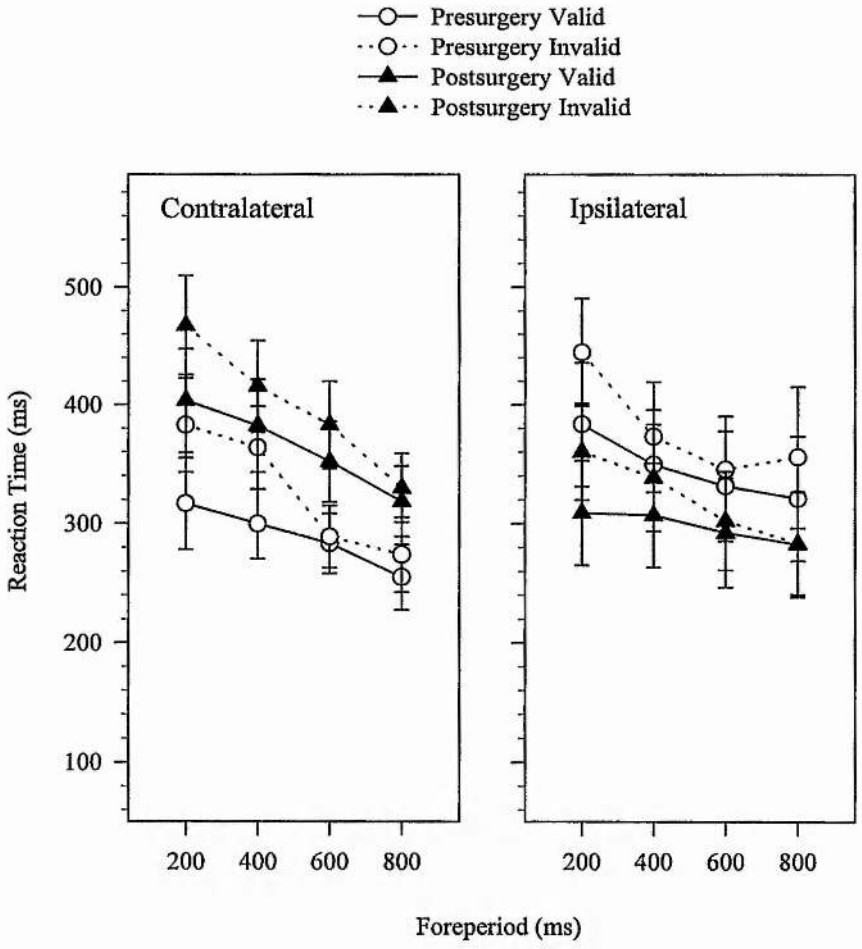


Figure 4.2. *Mean (± sem, n = 19) of reaction times, before and after surgery for validly and invalidly cued trials to each side. Presurgical reaction times are plotted with respect to the side of the subsequent lesion. Mean reaction time was lengthened for contralateral responses postsurgery. Reaction times were longer following invalid as compared with valid cues and this pattern did not change following unilateral dopamine depletion in the striatum.*

Figure 4.3 shows the probability of a response as a function of reaction time. Although there was an increase in mean contralateral reaction time, the average modal reaction time did not change after surgery, remaining at 175 ms. However, the reaction time distribution for contralateral responses postsurgery displays a downward shift in the probability of a response at the mode. It is apparent that the reason for the significant postsurgery increase in mean reaction time is that the relative frequency of reaction times around the mode has decreased, resulting in the slower reaction times in the tail of

distribution increasing the mean. The reaction time distributions for responses to the ipsilateral side display smaller changes, with a decrease in both the mode (- 10 ms) and an increase in the probability of a modal response.

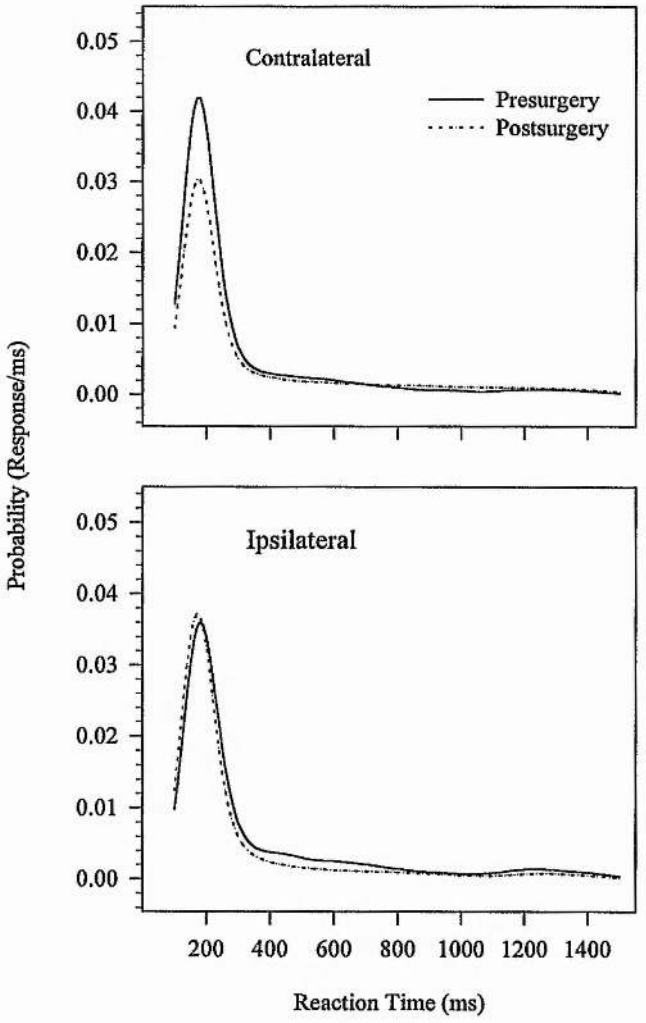


Figure 4.3. *The mean (n=19) probability density distributions, pre- and postsurgery are plotted as a function of response side and include both validity conditions and all foreperiods. The increase in mean reaction time for responses made contralateral to the side of lesion was attributable to a fall in the probability of responses occurring at the mode rather than a lateral shift in the distribution.*

The effect of cue validity at foreperiods of 200 and 400 ms (illustrated in Figure 4.4) also results in a small downward shift in the probability of a modal reaction time for invalid as compared with validly cued trials. This pattern is true for both sides and also both presurgery and postsurgery. In addition, there is an increase in the mode of the

reaction time distribution as the mode increases from 170 ms for valid trials to 190 ms for the invalid trials. Thus, the significant increase in mean reaction times for invalidly cued trials is due to a decreased probability of occurrence of responses at the mode and a slight lengthening of reaction times globally.

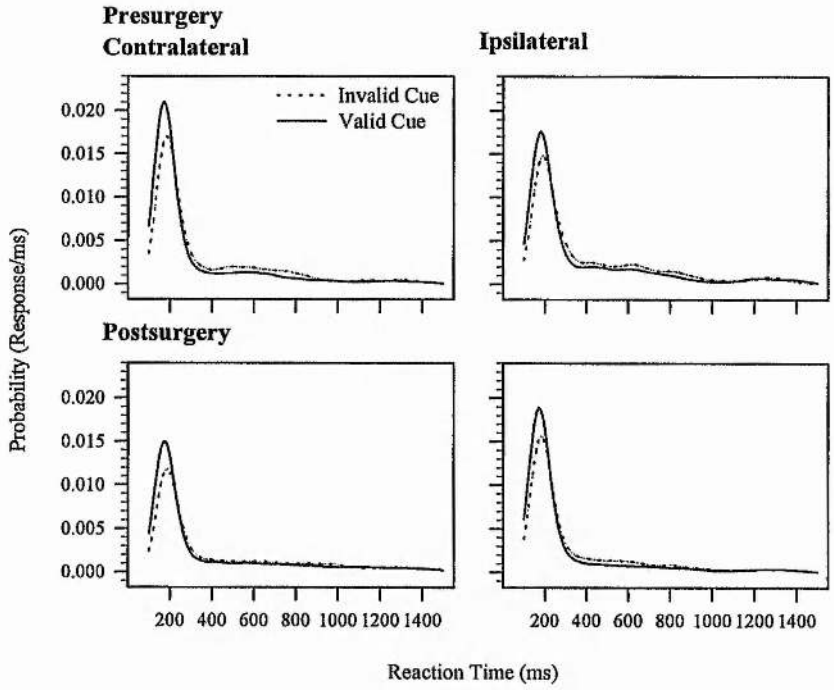


Figure 4.4. *The mean (n=19) probability distributions for the first two foreperiods, plotted as a function of Surgery, Response Side and Validity. The validity effect results in a change in probability of a response at the mode and also a shift in the distribution. This pattern is independent and additive with the effect of Surgery.*

In contrast to the increase in postoperative reaction time for responses to contralateral targets, movement time did not change (Surgery by Side, $F(1,18) = 0.15$, ns). However, postsurgery the latency to collect reward did increase significantly at the two shortest foreperiods (200 and 400 ms) by 275 ms (± 51 sem) and 229 ms (± 55 sem) respectively (Figure 4.5, Surgery by Foreperiod, $F(3,54) = 12.95$, $p < 0.001$).

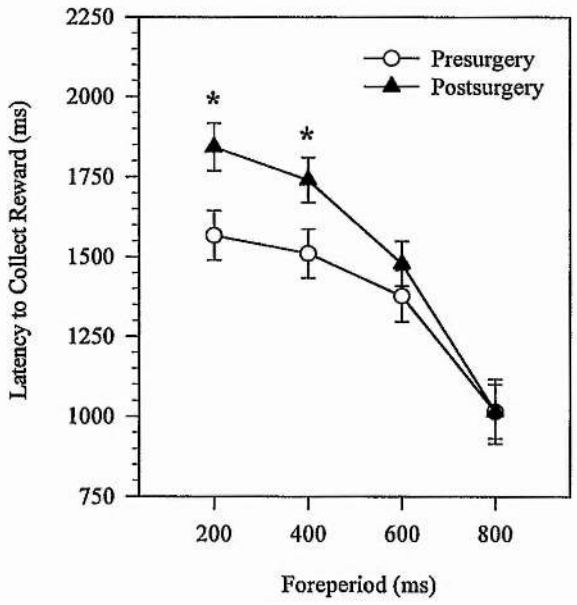


Figure 4.5. *Following surgery the latency to collect reward (± sem, n = 19) increased significantly at the earliest foreperiods (200 and 400 ms, the asterisks indicate significant increases, p < 0.05).*

4.3.2.ii. Accuracy

The percentage correct fell from 72 % (1.2 ± sem) presurgery to 57 % (1.4 ± sem) postsurgery for responses to targets contralateral to the side of lesion. This was greater than the fall in percentage correct postsurgery for ipsilateral responses to the side of lesion (from 69 % (1.4 ± sem) to 62 % (1.3 ± sem); Surgery by Side, $F(1,18) = 6.53$, $p < 0.02$). The fall in percentage correct was independent of the validity of the cue, with no significant interactions of Surgery with Validity (Surgery by Side by Validity: $F(1,18) = 0.66$, ns.; Surgery by Validity: $F(1,18) = 0.34$, ns). The percentage correct also fell as a function of increasing foreperiod ($F(3,54) = 213.96$, $p < 0.0001$), due to an increase in anticipatory errors as a function of foreperiod.

The significant interaction between Surgery and Side for percentage correct was further investigated by examining the percentage of anticipatory, incorrect and late errors. Not surprisingly, there were no interactions of Surgery with Validity and/or Side for anticipatory errors (Surgery by Side by Validity ($F(1,18) = 0.06$, ns; Surgery by Side: $F(1,18) = 0.36$, ns; Surgery by Validity: $F(1,18) = 0.0003$, ns). However, the interaction

of Surgery and Side was also not significant for late (Surgery by Side: $F(1,18) = 1.8$, ns) or incorrect ($F(1,18) = 1.59$, ns) errors. Nevertheless, as is apparent in Figure 4.6, the origin of the significant Surgery by Side interaction for overall percentage correct is a cumulative effect of an increase in the percentage of both late and incorrect errors postsurgery for responses to the side contralateral to the lesion.

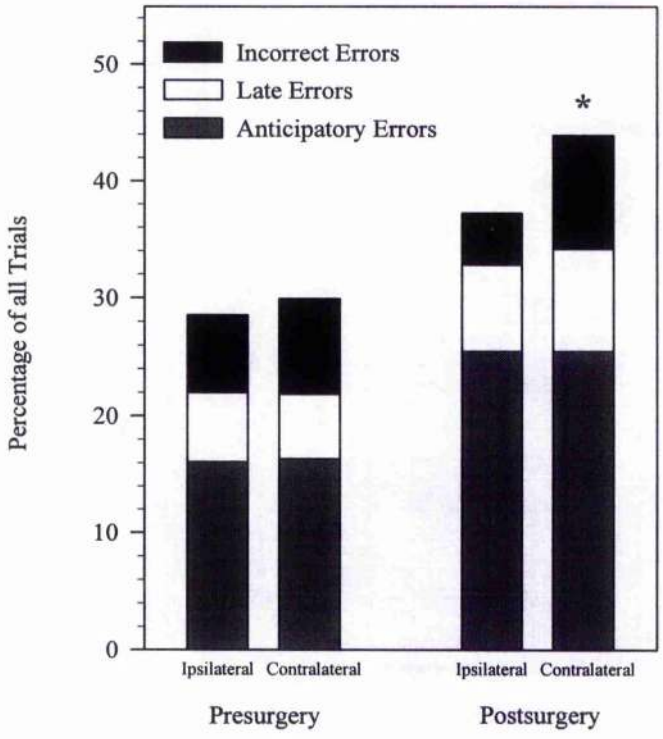


***Figure 4.6**. Bar graph showing error type as a percentage of all trials. The significant interaction for percentage correct between Side and Surgery can be attributed to the cumulative effect of an increase in both late and incorrect errors for responses to the contralateral side (the asterisk indicates a significant increase, $p < 0.05$).*

4.4. Discussion

In this study, it has been possible to measure covert orienting in the rat, so extending previous demonstrations of covert orienting of attention in humans (Posner, 1980; Posner et al., 1984) and in nonhuman primates (Bowman et al., 1993; Witte et al., 1996) to the rat. There was a significant increase in reaction time of responses to targets preceded by an invalid rather than valid cue. The use of this task in the rat provides a

reliable model in which the neural basis of covert orienting can be further investigated. Although caution must be exercised in assuming that the rat and primate use the same behavioural processes and neural systems in performing this task, nevertheless the similarity of their behaviour in this task suggests that this possibility should be explored.

Following unilateral striatal dopamine depletion, there was an increase in mean reaction time of responses made to the side contralateral to the lesion (with a corresponding decrease in ipsilateral reaction time), which was irrespective of the validity of the preceding cue. Furthermore, movement time did not change following surgery, which suggests that the reaction time impairment reflects a disruption of motor initiation and not execution. The magnitude of the validity effect did not change after dopamine depletion, which supports the hypothesis that dopamine in the striatum is important for response processes and does not play a role in mechanisms of directed attention.

These results are consistent with, and extend the findings of, Carli et al. (1989) and Brown & Robbins (1989b). Using similar reaction time paradigms, involving responses either away from or towards lateralised sensory stimuli, they reported the consequences of unilateral dopamine depletion or excitotoxic lesion of the striatum: a bias to respond to the side ipsilateral to the lesion and, following the dopamine depleting lesion, a lengthening of reaction time for responses to the contralateral side. These effects were irrespective of the side of sensory stimulus. Carli et al. (1989) did suggest that there may be attentional changes following the dopamine depleting lesion: after the lesion some rats employed an attentional strategy, attending preferentially to a contralateral stimulus which governed an ipsilateral response. Although this observation could be taken as evidence for the "premotor theory" of attention, with attention deriving from motor preparatory processing (Sheliga et al, 1995), the results from the present study, which specifically manipulated attentional processes, have demonstrated that a dopamine depleting lesion in the striatum has no adverse effect on the covert orienting of attention. Thus, changes in attention following a dopamine depleting lesion are more likely to be secondary behavioural compensation for lesion effects and are not primary deficits. The present results provide further evidence that the deficits of orientation which have been termed 'neglect' (e.g., Marshall & Teitelbaum, 1974) commonly observed after depletion of dopamine in the striatum are attributable to response deficits rather than to disturbances in sensory perception or attentional processes. Furthermore, the current

findings that striatal dopamine depletion does not disrupt disengaging from a visual stimulus extends previous observations. Schallert & Hall (1988) have also reported that there was no difficulty in disengaging attention from ongoing behaviour and orienting toward tactile stimuli following striatal dopamine depletion, although they do report a deficit when the rat was engaged in consummatory behaviour.

The increase in mean reaction time does not cause a shift in the entire reaction time distribution, rather there is a decreased probability of responses at the mode of the distribution. This finding, of a fall in the probability of a response at the mode of the distribution, has previously been reported in Parkinsonian patients tested with and without L-dopa therapy (Brown et al., 1993). The reaction time distributions of controls and patients with Parkinson's disease were distinguished on the basis of an increase in the proportion of responses with longer reaction times and a decrease in the probability of a response with the modal reaction time. This would suggest that dopamine is particularly significant for fast reaction times.

The suggestion that there might be a role for dopamine in covert orienting has support from several sources of evidence examining covert orienting using central and peripheral cues. In normal subjects, Clark et al. (1989) reported that the dopamine antagonist, droperidol, resulted in decreases in the cost of an invalid cue compared with a neutral cue. Similarly, in the Rhesus monkey systemic droperidol results in a reduction in the magnitude of the validity effect in a covert orienting task using peripheral cues (Witte et al., 1992). However, the specificity of these systemic drug effects has not been determined. Furthermore, Clark et al. (1989) and Witte et al. (1992) also demonstrated that blockade of noradrenergic function, using clonidine, resulted in similar effects. In patients with Parkinson's disease, Bradshaw et al. (1993) and Wright et al. (1990; 1993) suggest that there is a difficulty in maintaining attention, which is manifest as a reduction in the lengthening of reaction time by an invalid cue. However, their conclusions were based on a comparison of reaction times in a so-called 'neutral cue' condition with those following an invalid cue. Unfortunately, the use of a neutral cue is problematic (see Jonides & Mack, 1984); although not itself informative about target location, the neutral cue may make additional processing demands and may not elicit neutral behavioural strategies in the subject. It is not clear whether the effects reported in the patients are due to a reduction in reaction time in the invalid condition or to an increase in reaction time in the neutral condition. Notwithstanding these difficulties, the deficits of the patients

may indeed be due to a difficulty in maintaining attention, but one arising from dysfunction outside of the striatum as the disease progresses. Consistent with this suggestion, Yamada et al. (1990) reported attentional deficits in patients with Parkinson's disease, but only in patients in the advanced stages. The present results demonstrate that it is also unlikely that dopamine in the striatum contributes to the covert orienting of attention.

Chapter 5:

Covert orienting following excitotoxic lesions of posterior parietal cortex

5.1. Introduction

In primates, injury to posterior parietal cortex results in disturbances of spatial processing, causing deficits such as 'neglect' (Anderson, 1987; Bisiach et al., 1981; Bisiach & Luzzatti, 1978; Daffner et al., 1990; Petersen et al., 1989; Posner et al., 1984) and disruption of visually guided reaching (Anderson, 1987; Jakobson et al., 1991; Jeannrod et al., 1994; Johnson et al., 1993; Nixon et al., 1992). The area of rat cortex which is homologous to primate posterior parietal cortex has been established using axonal tracers to demonstrate thalamocortical (Chandler et al., 1992; Kolb & Walkey, 1987) and corticocortical (Chandler et al., 1992; Kolb & Walkey, 1987; Reep et al., 1994) connections. By this connectional anatomical criterion, rat posterior parietal cortex is located on the dorsolateral aspect of the cortex between somatosensory and secondary occipital cortex (-3.5 to 5.0 mm AP to bregma and 1.5 to 5.0 mm lateral to bregma (Chandler et al., 1992)).

Rosner & Mittleman (1996) examined the consequences of an aspirative lesion of posterior parietal cortex on a visual reaction time test of covert orienting (Posner, 1980). Although Rosner & Mittleman (1996) found no evidence of attentional impairment following the posterior parietal lesion, the reaction time results must be regarded with some caution. There was no reaction time deficit postoperatively, rather, the reaction time of ipsilateral responses became faster, while those of contralateral responses did not change. This result was interpreted by the authors as arising from a deficit in contralateral responses, which was masked by a continued improvement following surgery as a result of continued training. Therefore, ensuring that asymptotic performance was attained prior to surgery is important in this experiment. The interpretation of Rosner & Mittleman's (1996) study is further confounded by the aspirative lesion used in the experiment. Aspiration has been used frequently in experiments examining posterior parietal function in the rat (Crowne et al., 1992; King & Corwin, 1993, Kolb & Walkey, 1987; Save & Moghaddam, 1996), however, apsiration may also remove fibres passing through posterior parietal. The aspirative lesion may thus have disrupted task performance as a consequence of disconnecting visual from frontal cortex. The current experiment employed the excitotoxin quinolinic acid to destroy neurons in posterior parietal cortex while avoiding nonspecific damage to fibres of passage.

Furthermore, posterior parietal in humans and primates contributes to reaching accuracy and grasping (Jakobson et al., 1991; Jeannrod et al., 1994; Johnson et al., 1993; Nixon et al., 1992), which may also be practically assessed in the rat. Tests of paw reaching have been used to investigate cortical (Brown et al., 1991; Castro, 1972; Whishaw et al., 1986), subcortical (Whishaw et al., 1986) and neurochemical (Miklyaeva et al., 1994; Whishaw et al., 1986) contributions to reaching in the rat. The effects of posterior parietal lesions have not been examined. There are grounds on which to suspect posterior parietal cortex is not important for skilled paw reaching. Goodale & Carey (1990) have argued that because the rat does not possess significant foveal vision, the rat visual system operates to orient but not guide specific spatial interactions with the environment. Furthermore, it has been argued that paw reaching in the rat is mediated by olfactory rather than visual guidance (Whishaw & Tomie, 1989). Nevertheless, the involvement of posterior parietal cortex in the rat in reaching has not been assessed.

The objectives for the current experiment were to test whether damage to the homologue of primate posterior parietal cortex in the rat would disturb similar functions to those disrupted in humans and primates (i.e., covert orienting and reaching) and to extend our understanding of deficits following this lesion, which have been termed 'neglect' (King & Corwin, 1993). Three tests were used. They were selected as they required discrete responses from the rat which could be measured automatically or easily rated by an observer, thus avoiding the need to make subjective judgements. The first test was the visual reaction time test of covert orienting as a replication and extension of Rosner & Mittleman (1996). There was also a test of somatosensory 'neglect', in which bilateral stimuli (self-adhesive paper patches) are applied to the forepaws and the order and latency of contact is noted. The final test, was a test of free paw reaching, in which a tray of food pellets is positioned outside the cage, from which pellets can only be recovered by extending the paw through the bars of the cage (Whishaw et al., 1986). The paw reaching task can be employed to examine fine motor control and can be used as a measure of 'neglect'.

5.1.1. Hypotheses

Table 5.1 illustrates the pattern of reaction time performance which might be expected following the posterior parietal lesion. Based on evidence from humans, an attentional impairment would be expected to take the form of a difficulty disengaging

attention from invalid ipsilateral cues (those preceding contralateral targets) (Petersen et al., 1989; Posner et al., 1984). The task may also be used to distinguish between an attentional impairment, as is seen in humans, and a response deficit or sensory impairment. In patients, there is also a bilateral increase in reaction time when diffuse (i.e., not spatially informative) cues precede the target, which is most pronounced for responding to contralateral targets (Petersen et al., 1989). The diffuse cues are thought to either freeze attention centrally or direct attention to the ipsilateral side which had attained processing dominance following the lesion. Thus on the bilateral simultaneous cue task an increase in reaction time to contralateral targets and an ipsilateral response bias might be expected.

Deficit	Contralateral Valid	Contralateral Invalid	Ipsilateral Valid	Ipsilateral Invalid
Disengagement of Attention	-	↑	-	-
Sensory Deficit/ Engagement of Attention	↑	↑	-	↓
Response Deficit	↑	↑	-	-

↑ Increase in reaction time; ↓ Decrease in reaction time; - No change in reaction time

Table 5.1. *The pattern of reaction time deficits which may follow a unilateral lesion. The profile of reaction time performance changes according to the hypothesised deficit. The first deficit has been associated with unilateral damage of posterior parietal cortex in humans (Petersen et al., 1989; Posner et al., 1984).*

5.2. Materials and Methods

Twelve, pair-housed male rats were used during the study (weight range during study; start 330-410 g; completion 420-480 g). The rats were tested on the covert orienting task, somatosensory asymmetry test and paw reaching (see sections 2.2.2, 2.2.4 and 2.2.5). All the rats received unilateral excitotoxic lesions of posterior parietal cortex (see section 2.4.3).

5.2.1. Collection of Data

Presurgery, 1200 correct trials of the basic covert orienting task were collected for each rat over ten days. One week following surgery, 2400 correct trials were collected per rat over 20 days. Four weeks after surgery the rats were tested in a modified version of the covert orienting test in which the dim cues were presented bilaterally. Data were collected over three days (a total of 600 trials per rat).

Data for the somatosensory asymmetry test were collected for three days presurgery and testing commenced again four days after surgery for a further three days. Testing on the paw reaching task was conducted for three, fifteen minute sessions presurgery and was repeated one week following surgery. Six weeks following surgery the rats were humanely killed and perfused.

5.3. Results

5.3.1. Histological Results

Figure 5.1 illustrates the extent of the largest, smallest and typical lesion with the corresponding coronal section illustrating the extent of the excitotoxic damage to posterior parietal cortex. The cresyl violet stained sections revealed that the unilateral excitotoxic lesions caused destruction of posterior parietal cortex between bregma AP -4 mm to -6 mm and laterally from 1.5 to 5.5 mm (corresponding to Oc2MM, Oc2ML, medial Oc2L (Paxinos & Watson, 1986)). The mean volume of cortical damage resulting from the lesion was 36 mm^3 (± 4.6 sem). The damage in posterior parietal extended ventrally to the underlying white matter. Outside of posterior parietal cortex the extent of additional damage was limited to parts of adjacent cortical areas (HL, dorsal Par1, Oc1B and Oc1M (Paxinos & Watson, 1986)) and included the ipsilateral CA1 hippocampal cells directly beneath the lesion. The smallest lesion also included posterior HL and anterior Oc1M but damage was limited to the superficial layers.

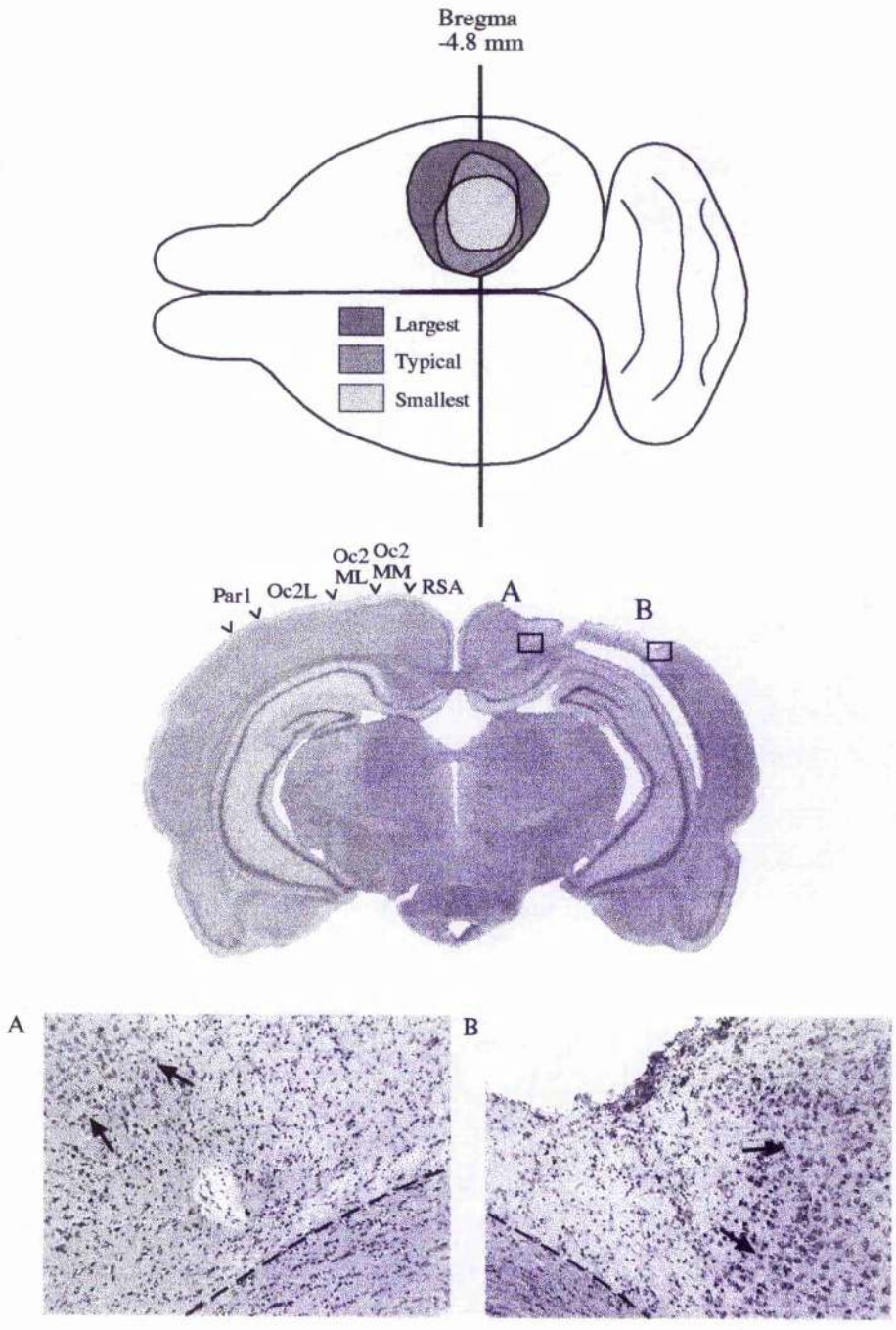


Figure 5.1. *A schematic of the rat brain illustrates the consequence of excitotoxic damage, displaying the largest (Rat #96177), typical (Rat #96192) and smallest (Rat #96184) lesions. A typical cresyl violet stained coronal section and enlargements from the lesion boundaries (indicated by arrows) illustrates the depth of damage, which extended down to the underlying white matter. The lesions include the region defined by the thalamocortical connections as posterior parietal.*

5.3.2. Behavioural Observation

Postsurgery, all but two of the twelve rats displayed an unusual behaviour in their home cages. The bedding in the cages was piled up to the front of the cage and usually predominantly to one side, which varied from day to day within rats, the behaviour had disappeared by the end of the first week. The rats otherwise appeared normal during handling.

5.3.3. Covert Orienting of Attention

5.3.3.i. Reaction Time, Movement Time and Latency to Collect Reward

Faster reaction times were recorded for trials in which the target was preceded by a valid (286 ms ± 17 sem) rather than invalid cue (312 ms ± 19 sem), providing a reliable measure of attentional function (Validity, $F(1,11) = 56.9$, $p < 0.001$). Figure 5.2 illustrates that, there was no deficit in disengaging attention postoperatively, with no alteration in the magnitude of the contralateral validity effect following the posterior parietal lesion (Surgery by Side by Validity, $F(1,11) = 0.82$, ns; Surgery by Side by Validity by Foreperiod, $F(3,33) = 1.2$, ns).

There was an increase in reaction time to contralateral targets (302 ms ± 23 sem presurgery increased to 336 ms ± 23 sem postsurgery) which was irrespective of the validity of the preceding cue (Surgery by Side, $F(1,11) = 5.68$, $p < 0.04$). Reaction time to ipsilateral targets did not change significantly postsurgery.

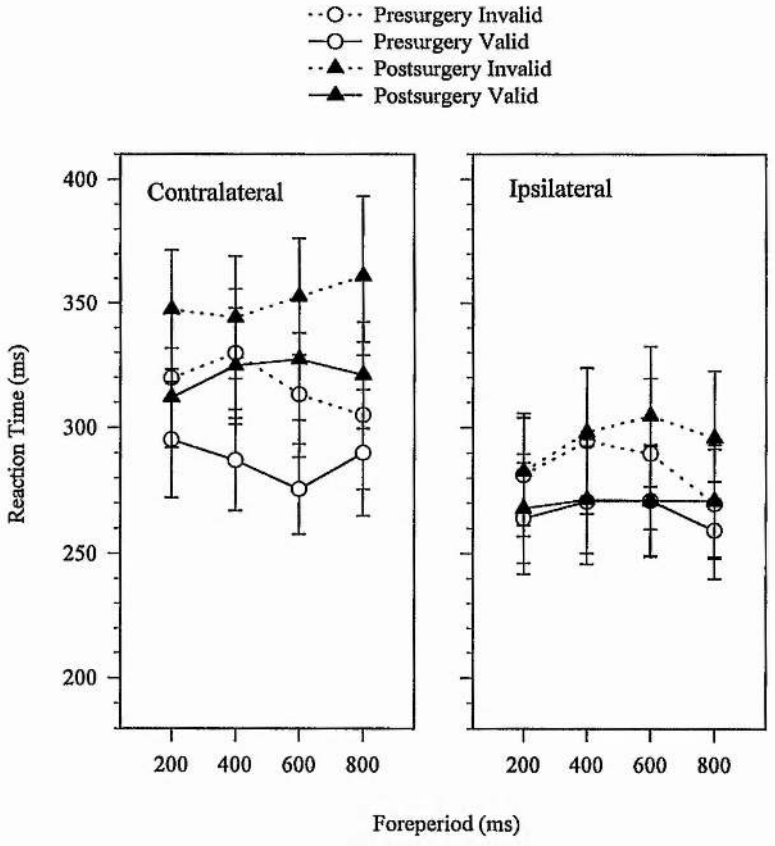


Figure 5.2. *Reaction time performance (mean ± sem, n = 12) illustrating faster detection of validly cued trials compared with invalidly cued trials. Postsurgery the magnitude of the validity effect did not change and the capacity to disengage attention during invalidly cued contralateral trials was unaffected. However, there was a slowing in reaction time to contralateral targets irrespective of validity.*

There have been some reports of hemispheric specialisation in rats (Crowne et al., 1987; King & Corwin, 1992). Consequently, to exclude the possibility of lateralised attentional function, Side of Lesion was also examined as a factor. There were, however, no difference in performance between rats with right or left posterior parietal lesions (Lesion by Surgery by Side by Validity, $F(1,10) = 0.22$, ns; Lesion by Surgery by Side by Validity by Foreperiod, $F(3,33) = 0.62$, ns).

There was an increase for movement time towards contralateral targets (128 ms ± 6 sem increasing to 139 ms ± 12 sem postsurgery) and a decrease in movement time to ipsilateral targets (139 ms ± 18 sem decreasing to 126 ms ± 8 sem postsurgery) which approached significance (Surgery by Side, $F(1,11) = 4.56$, $p < 0.06$). There was also a

tendency for the latency to collect reward to increase postsurgery (1630 ms ± 87 sem increasing to 1718 ms ± 84 sem), but again this only approached significance (Surgery, $F(1,11) = 4.11$, $p < 0.07$).

5.3.3.ii. Accuracy

Postoperatively, there was no evidence of a response asymmetry (Figure 5.3, Surgery, $F(1,11) = 0.00$, ns). The incidence of incorrect errors was higher for invalid compared to valid trials (2.6 % ± 0.9, 95 % confidence interval, for validly cued trials and 4.1 % ± 1.5, 95 % confidence interval, for invalidly cued trials, Validity, $F(1,11) = 23.15$, $p < 0.001$). There was, however, no increase in incorrect errors following surgery either as a function of Side or Validity (Surgery by Side, $F(1,11) = 2.01$, ns; Surgery by Side by Validity, $F(1,11) = 0.8$, ns). Late errors also did not change following surgery (Surgery by Side, $F(1,11) = 0.41$, ns; Surgery by Side by Validity, $F(1,11) = 0.21$, ns) and neither did anticipatory errors (Surgery, $F(1,11) = 2.16$, ns).

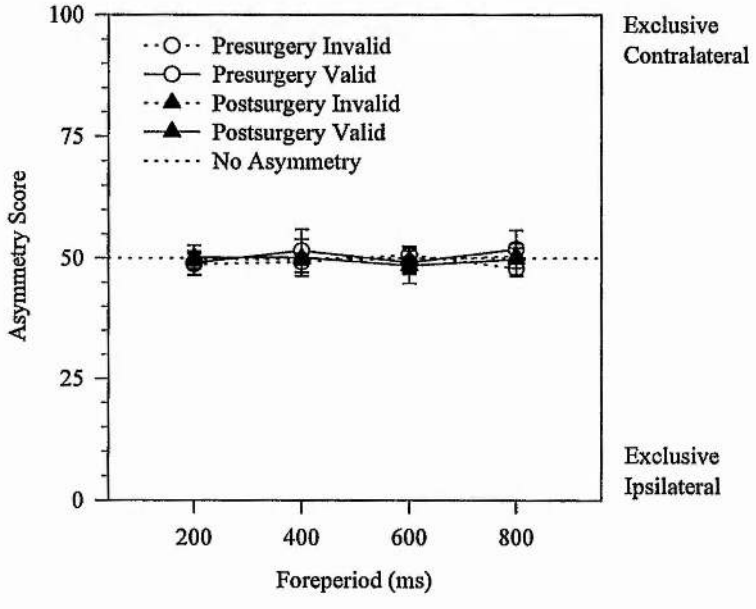


Figure 5.3. *The posterior parietal damage did not induce a response asymmetry (mean ± 95 % confidence interval, n = 12) either to cue validity or the side of target occurrence.*

5.3.3.iii. Simultaneous Bilateral Cues

Four weeks following surgery the rats were tested in the task with bilateral cues. Reaction time was still slower to contralateral (317 ms ± 20 sem) than to ipsilateral (261 ms ± 20 sem) targets (Figure 5.4, Side, $F(1,11) = 4.9$, $p < 0.049$). Compared to pre and postsurgical performance the difference was no greater than the reaction time deficit found postsurgery. The difference between contralateral and ipsilateral reaction time was 53 ms (± 27 sem) postsurgery and 56 ms (± 25 sem) in the simultaneous bilateral cues task compared with only 27 ms (± 28 sem) presurgery.

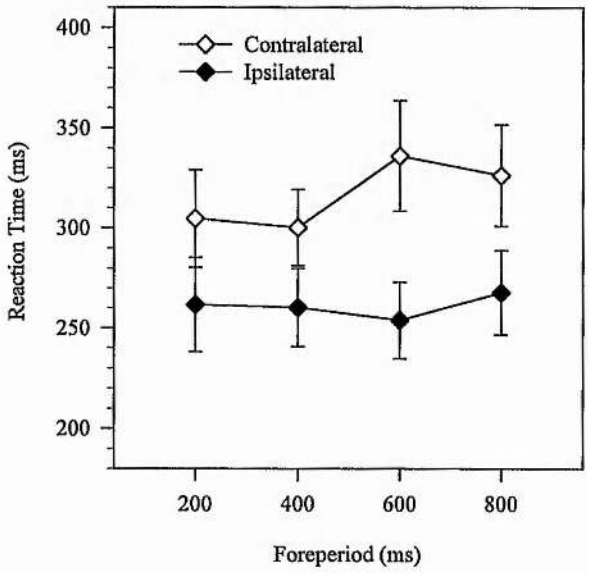


Figure 5.4. *Reaction time (mean ± sem, n = 12) was slower to contralateral than ipsilateral targets in the simultaneous bilateral cue task. However, the inclusion of bilateral cues did not increase the reaction time deficit already evident postsurgery.*

The bilateral cues also did not increase the incidence of incorrect errors (Side, $F(1,11) = 0.62$, ns) or induce an ipsilateral response asymmetry (Figure 5.5).

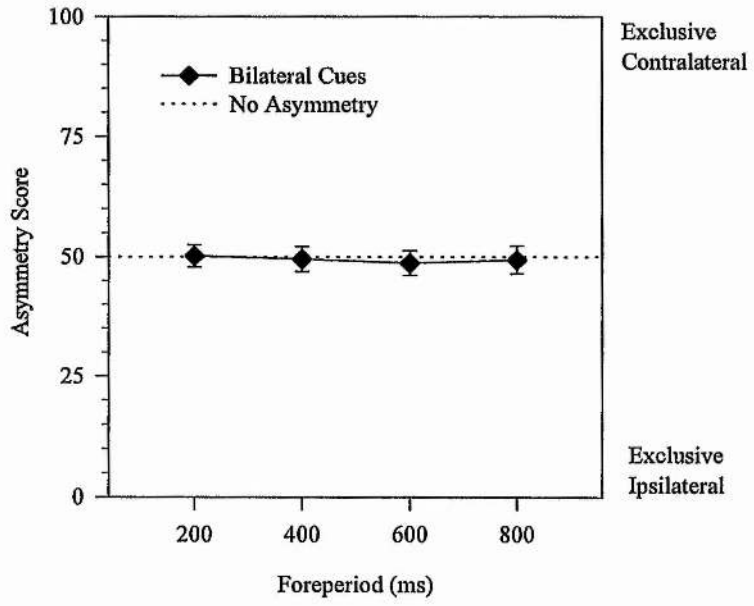


Figure 5.5. *Bilateral cues did not induce a response asymmetry (mean ± 95 % confidence interval, n = 12) in task performance.*

5.3.4. Somatosensory Asymmetry Test

Postsurgery, the latency to contact the patch on the contralateral forepaw did not increase significantly, either when that patch was contacted first (Surgery by Forepaw, $F(1,11) = 2.51$, ns) or second (Surgery by Forepaw, $F(1,11) = 1.85$, ns). Consistent with the absence of a change in latency to contact the contralateral patch, there was also no postsurgical change in the asymmetry score which reflects the order of contact (t-test $(11) = -1.29$, ns).

5.3.5. Paw Reaching

One rat failed to acquire the paw reaching task during training and was subsequently excluded from the data analysis. Figure 5.6 illustrates a fall in the total number of attempted reaches with the contralateral paw postoperatively (Surgery by Paw, $F(1,10) = 5.48$, $p < 0.041$).

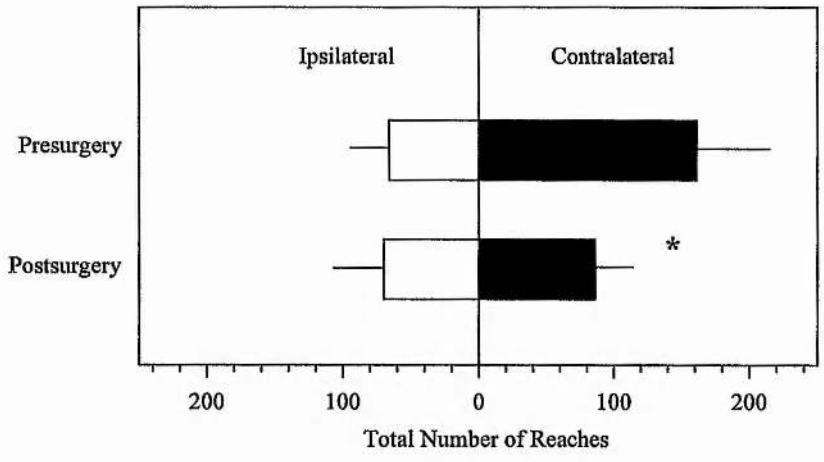


Figure 5.6. *The total number of attempted contralateral reaches (mean ± sem, n = 11) fell postsurgery, while ipsilateral reaches remained unchanged (the asterisk indicates a significant decrease, $p < 0.05$).*

This was accompanied by a postoperative reduction in the time spent in the contralateral half of the cage, which approached significance (Figure 5.7, Surgery by Side, $F(1,10) = 4.5$, $p < 0.06$). However, the accuracy of reaching did not change postsurgery (Surgery by Paw, $F(1,10) = 0.45$, ns).

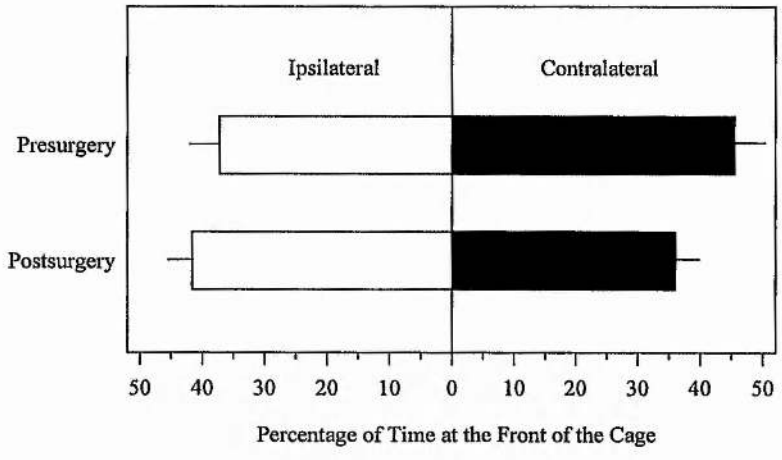


Figure 5.7. *The time spent in each side of the cage was assessed by recording position every minute during testing. The figure illustrates the percentage (mean ± sem, n = 11) of time spent in each half of the cage (ipsilateral + contralateral does not equal 100%, as position in the cage was only included when at the food tray). Postsurgery there was a tendency for the rats to spend less time in the front contralateral half of the cage.*

5.4. Discussion

Quinolinic acid-induced lesions of posterior parietal cortex resulted in a constellation of impairments and intact performance which permits more detailed specification of the characteristics of posterior parietal lesions in the rat. In the reaction time task, the lesion resulted in slower reaction times to contralateral targets irrespective of preceding cue validity. Contrary to the expectation from lesion studies in humans (Petersen et al., 1989; Posner et al., 1984), but consistent with a previous report (Rosner & Mittleman, 1996), the ability to disengage attention remained intact following the lesion. Furthermore, the testing of extinction by bilateral cues did not increase the magnitude of the reaction time deficit found in the covert orienting task, nor did it induce a response asymmetry, indicating the lesion did not result in an ipsilateral processing advantage. Following surgery, there was also a fall in the number of attempted reaches with the contralateral paw and less time was spent in the contralateral half of the paw reaching cage. However, in a test of somatosensory 'neglect' which did not involve an overt head or body orienting response, there was no evidence of asymmetry or latency impairment.

An increase in reaction time to contralateral targets could arise from either a sensory or a response deficit. Although it is possible to dissociate sensory and response-related deficits by varying the response requirements as either toward or away from the side of an imperative signal (Brown & Robbins, 1989b; Carli et al., 1989), in the current task the response was always towards the imperative signal. However, it is still possible in this task to make an assessment of sensory and response factors. Trials in which the ipsilateral target was preceded by an invalid cue required detection of that cue by the damaged contralateral hemisphere. If there were difficulty in visual detection, this would be expected to apply (perhaps, especially so) to the dimmer cues. Such an impairment would therefore have decreased the magnitude of the ipsilateral validity effect (see Table 5.1). Consequently, the results suggest that the postoperative increase in reaction time was due to a response impairment rather than difficulty in perceiving the visual targets. This conclusion is further supported by the absence of an increase in the magnitude of the reaction time deficit in the simultaneous bilateral cue task, which imposes a greater demand on sensory processing but leaves response requirements unchanged.

In primates, posterior parietal cortex forms part of a distributed neural network subserving visuospatial attention (Mesulam, 1981; Posner & Petersen, 1990). King & Corwin (1993) have proposed a similar attentional network in the rat based on a multimodal 'neglect' following cortical damage including posterior parietal. However, in light of the reaction time deficit reported here and by Rosner & Mittleman (1996), it is likely that previous impairments in orienting tasks which have been considered evidence of 'neglect' following posterior parietal lesions are not attentional in origin. Although, it should be conceded that the current task assesses only the visual modality and did not vary the stimulus distance, which could prove important if posterior parietal cortex were involved in extrapersonal, but not peripersonal, visuospatial processing.

The consequences of posterior parietal lesions are distinct from an ischemic lesion of more anterolateral cortex corresponding to Zilles's (1990) Par1/2 and FL, which does not impair response initiation but does result in a transient impairment in the execution of lateralised movement (Ward et al., 1997, Experiment 4, *Assessment of sensorimotor neglect following occlusion of the middle cerebral artery: Chapter 6*). This Par1/2 lesion does not result in an impairment in covert orienting (Ward et al., 1997, Experiment 4, *Assessment of sensorimotor neglect following occlusion of the middle cerebral artery: Chapter 6*) or signal detection or vigilance (Muir et al., 1996b).

There was a reduction in the use of the contralateral paw to reach for food pellets. However, there was no change in the percentage of successful contralateral reaches, indicating no gross deficit in reaching accuracy and grasping (although it should be born in mind that the rats were reaching into a tray full of pellets and therefore the task did not require any degree of targeted reaching). One possible explanation of the paw reaching deficit could be the inclusion of HL in the lesion. Video analysis of paw reaching has emphasised the importance of posture in reaching (Miklyaeva et al., 1994). It is therefore possible, that damage to HL might disrupt reaching, although the expectation would be of a general decline in reaching ability rather than a fall in contralateral paw use. Furthermore, damage to FL results in reductions in the success of contralateral reaches rather than just a decline in contralateral paw use (Ward et al., 1997, Experiment 4, *Assessment of sensorimotor neglect following occlusion of the middle cerebral artery: Chapter 6*; Whishaw et al., 1986).

The fall in the use of the contralateral paw for reaching was also accompanied by a reduction in the time spent in the contralateral half of the cage, suggesting an alteration in spatial behaviour. The unusual home-cage behaviour, in which bedding was piled in one place, may indicate a spatial disturbance, although there was no systematic side bias for this behaviour. The variation on which side the rats piled bedding could be attributable to the orientation of the rat which, obviously, was unconstrained. Systematic observation of the rats in the home-cage was not undertaken.

Brown et al. (1991) reported intact accuracy on a paw reaching task with a fall in contralateral reaches following an apirative lesion of AGm, which they concluded indicated that sensory processes remained intact, since successful manipulation of food pellets would be expected to be dependent on intact sensory feedback (Sabol et al., 1985). In primates following posterior parietal damage reaching deficits are commonly manifested as poor accuracy and grasping, however, there has been a report of a decrease in attempted reaches with the contralateral limb (Deuel et al., 1993) which our results extend to the rat. Thus there is some support for the contention that posterior parietal cortex in the rat is involved in the initiation of reaching. This task may not, however, be sufficiently sensitive to assess guidance or grasping accuracy. Although there was no inversion of paw preference following surgery (ipsilateral reaches remain unchanged), there was a reduction in time spent in the contralateral half of the cage, which was also reported following AGm lesions (Brown et al., 1991). The implication of this impairment might be spatial 'neglect', but the underlying cause remains unclear and it might be secondary to paw use.

The somatosensory asymmetry test was used as a test of sensorimotor 'neglect' which does not involve a lateralised head or body orienting response (Schallert et al., 1982). This is in contrast to the other tasks used in this experiment and also the tests of 'neglect' used in previous studies of posterior parietal cortex (Corwin et al., 1996; King & Corwin, 1993). The absence of an impairment in the somatosensory asymmetry test following the posterior parietal cortex lesion distinguishes between this posterior parietal lesion and an aspirative lesion of AGm (Brown et al., 1991) or an ischemic lesion of Par1/2/FL (Ward et al., 1997, Experiment 4, *Assessment of sensorimotor neglect following occlusion of the middle cerebral artery: Chapter 6*) both of which result in an asymmetry in this task. Thus, the 'neglect' reported here appears to be predominantly confined to the initiation of contralateral responses. The magnitude of the reaction time

impairment is smaller (34 ms compared with 80 ms) than unilateral AGm lesions and the impairment is also limited to contralateral responses, which contrasts with the bilateral impairment observed following unilateral AGm lesions. The paw reaching deficit and tendency to be in the ipsilateral half of the cage is also less pronounced. Therefore it is possible that the 'neglect' is more subtle than that following AGm lesion and so not detected in the somatosensory test. Alternatively, the difference in the magnitude of the impairment following the two lesions could be attributable to the more extensive damage to fibres of passage following aspiration compared with an excitotoxic lesion.

Goodale & Carey (1990) have argued that orienting in the rat does not serve the function of foveation of an object for identification (inferred from retinal characteristics (Sefton & Dreher, 1995)). Instead orienting under visual guidance positions the rat toward the target for tactile and olfactory exploration of it. The deficits reported here, do not resemble 'neglect' as seen following lesions of primate posterior parietal cortex and might be regarded as 'attentional' only in so far as they impair overt orienting.

Chapter 6:

Assessment of sensorimotor neglect following occlusion of the middle cerebral artery

6.1. Introduction

To investigate the characteristics of a possible 'neglect' syndrome following middle cerebral artery occlusion a selection of tests were used to investigate sensorimotor and attentional function. The tests used were as follows:

1) A test of reaching to assess paw use, after Whishaw et al. (1986). Grabowski et al. (1993) and Marston et al. (1995a) have reported that occlusion of the middle cerebral artery in the rat results in impairments in skilled paw use, specifically in the ability to recover small food pellets which are located with increasing distance at either side of the rat. These impairments of skilled paw use were shown to be bilateral. The test used here is a mainly a test of paw preference and use (with the rat able to reach the food pellets with either paw), rather than an assessment of preserved skill, as when each paw must be used in order to reach all pellets.

2) A somatosensory test for 'hemineglect', after Schallert et al. (1982). The task has previously been used to demonstrate what is described as a sensorimotor impairment after middle cerebral artery occlusion in the rat (Andersen et al., 1991; Markgraf et al., 1992; 1994) and was included here for comparison with other tests and to replicate these previous results.

3) A visual reaction time task, after Carli et al. (1985; 1989). In the current study, the basic task (which involved orienting towards a lateralised visual stimulus) was modified to be a test of covert orienting of attention (after Posner, 1980; see Ward & Brown, 1996). Visual cues preceded the targets, either correctly (valid cue) or incorrectly (invalid cue) predicting the side of the subsequent target.

6.1.1. Hypotheses

The damage arising from MCA occlusion in the rat includes extensive lateral neocortex and lateral striatum. The failure to respond to contralateral stimulation has been referred to as 'neglect' and could be attributable to a variety of causes including impaired sensory, motor or attentional processing. The covert orienting task and

sensorimotor tests used in this experiment can be used to distinguish between these various impairments (see Table 5.1 & Table 6.1). Attentional dysfunction in the task could arise as a difficulty in disengaging attention from an invalid cue (or the ipsilateral cue in the simultaneous bilateral cues task) processed by the intact hemisphere (similar to the deficit observed in humans with parietal damage (Petersen et al., 1989; Posner et al., 1984)). Furthermore, a response impairment may also result in a bias to respond to ipsilateral targets, this has previously been observed following excitotoxic lesions of the lateral striatum (Brown et al., 1989b). The somatosensory asymmetry test has previously identified a bias to respond to ipsilateral stimulation and an increase in time taken to contact the contralateral stimulus following MCA occlusion (Andersen et al., 1991; Markgraf et al., 1992; 1994). Finally, the paw reaching task employed in this experiment allows freedom to use either forepaw for reaching. Unilateral lesions of FL have been demonstrated to reduce successful reaches with the contralateral forepaw and result in an increase in the use of the ipsilateral forepaw in this task (Whishaw et al., 1986).

	Covert Orienting of Attention		Simultaneous Bilateral Cues		Somatosensory Asymmetry Test		Paw Reaching	
Deficits	Contra-lateral RT/MT	Ipsilateral bias	Contra-lateral RT/MT	Ipsilateral bias	Contra-lateral contact time	Ipsilateral bias	Successful reaches contra-lateral	ipsi-lateral
Attentional	RT↑ invalid cue	-	RT↑	↑	-	-	-	-
Response Initiation	RT↑ all cues	↑	RT↑	↑	-	-	-	-
Motor	MT↑	-	MT↑	-	-	-	↓	↑
Somatosensory	-	-	-	-	↑	↑	↓	↑

Table 6.1. *The table illustrates hypothesised changes in performance which would indicate impaired attention, response initiation, motor and somatosensory function (RT-reaction time, MT-movement time).*

6.2. Materials and Methods

Twenty four, pair-housed male rats were used during the study (weight range during the study; start 255-335 g; completion 350-435 g). The rats were tested on the covert orienting task, somatosensory asymmetry test and paw reaching (see sections 2.2.2, 2.2.4 and 2.2.5). The rats underwent surgery to unilaterally occlude the right middle cerebral artery (see section 2.4.4).

6.2.1. Collection of Data

Presurgical data were collected over a period of ten days with one session run per day (~ 1200 trials per rat). After one week of recovery, postsurgical data were collected for 20 days (~ 2400 trials per rat). After the collection of the postsurgical data for the visual reaction time task (four weeks after surgery), five sessions (600 trials per rat) were run in which the cues were presented bilaterally on every trial.

At the conclusion of each day's visual reaction time testing the rats were tested using the somatosensory asymmetry test. Data were collected for five days presurgery and for five days starting one week after surgery. In the paw reaching task testing was conducted over five, 15 minute sessions presurgery and was repeated starting two weeks following surgery. Six weeks following surgery the rats were deeply anaesthetised and perfused.

6.3. Results

6.3.1. Histological Results

Three rats were dropped from the study due to postsurgical complications. An additional rat, with an ischemic infarct which was as large as the largest lesion (see Figure 6.1), was also excluded from all data analysis as this rat failed to generate data in the reaction time and paw reaching tasks postoperatively. The performance of this rat is

detailed in Table 6.2: for the test of somatosensory 'neglect', the rat had a large ipsilateral asymmetry, which was consistent with the performance of the remaining rats.

Measure	Presurgery (mean ± sem)	Postsurgery (mean ± sem)
Asymmetry	50 % ± 3	3 % ± 1
Latency to contact: ipsilateral	28.6 ± 3.4	12.6 ± 2.2 (↓)
Latency to contact: contralateral	24.7 ± 4.3	37.2 ± 3.9 (↑)

Table 6.2. *This rat (#95065) did not generate any data after surgery in the reaction time and paw reaching tasks and therefore all data from this rat were excluded from analyses. The rats performance on the somatosensory asymmetry test is summarised above and illustrates a change in performance consistent with the main lesion group. The extent of the lesion in this rat was similar to rat 95077, illustrated in Figure 6.1.*

Examination of the cresyl violet sections of the remaining rats revealed a heterogeneous lesion group consisting of seven rats with a mean cortical lesion volume of 55.3 mm^3 (± 5.7 sem). The extent of the largest and smallest lesions is illustrated in Figure 6.1. Using the nomenclature of Zilles (Paxinos & Watson, 1986), the area of damage common to all 7 rats included Par 1, anterior Par 2, FL and allocortex. The lesion did not extend into Oc2M/dorsal Oc2L (The area which Kolb & Walkey (1987) referred to as being analogous to primate posterior parietal cortex). The presence of damage in the lateral neostriatum was variable, but six of the seven rats presented with a pattern of infarct which was typical of this lesion (Sharkey et al., 1994). The injection site was located at the core of the ischemic infarct in the lesioned animals, and thus it was not possible to distinguish between injection site damage and the ischemic damage six weeks postsurgery. Not all injections result in arterial occlusion, and it is these which serve as operated control subjects. Thirteen rats served as operated controls, as they did not sustain occlusion of the middle cerebral artery. The damage in this group was restricted to the needle tract and minor damage at the site of injection in piriform cortex.

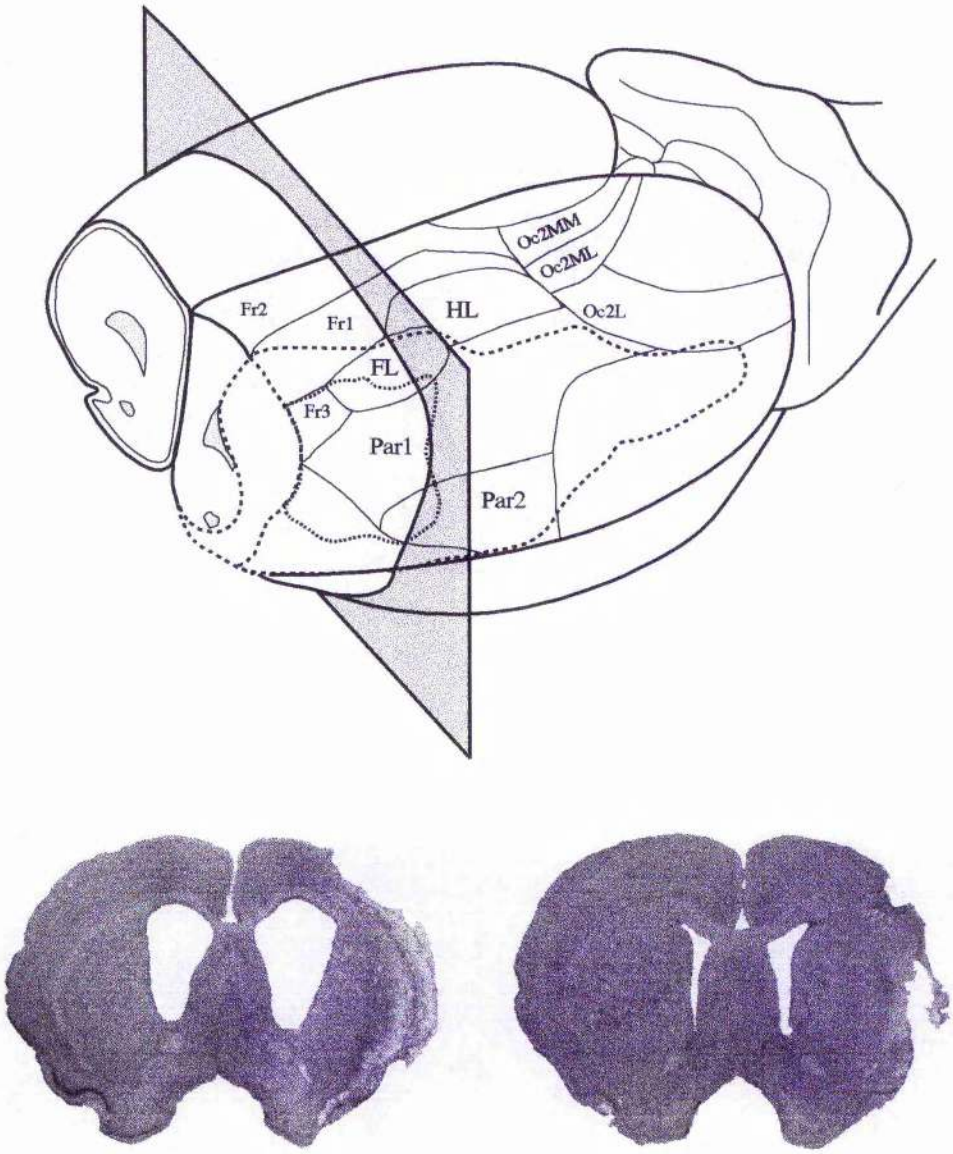


Figure 6.1. *A 3-dimensional projection of the extent of the largest (rat #95077) and smallest (rat #95062) lesions following focal cerebral ischemia induced by occlusion of the middle cerebral artery by injection of the vasoconstrictor endothelin-1. The core region of damage includes area FL, Par1 and anterior Par2. The lower portion of the figure shows corresponding coronal sections (from bregma 0) stained for cresyl violet.*

6.3.2. Covert Orienting of Attention

6.3.2.i. Reaction Time, Movement Time and Latency to Collect Reward

The mean reaction time to targets preceded by an invalid cue (mean of 311 ms ± 10.1 sem) was significantly slower than to targets preceded by a valid cue (mean of 277 ms ± 8.7 sem) giving rise to a significant validity effect irrespective of lesion group, side of target, or foreperiod (Validity, $F(1,18) = 132$, $p < 0.001$). Following surgery, the validity effect did not change for either lesion group, or side of target, as shown in Figure 6.2 (Group by Surgery By Side by Validity, $F(1,18) = 1.82$, ns). Furthermore, following surgery there was no overall increase in reaction time for contralateral targets, irrespective of validity (Group by Surgery by Side, $F(1,18) = 2.01$, ns).

In contrast to reaction time, the ischemic lesion resulted in an increase in movement time to the contralateral side (Group by Surgery by Side, $F(1,18) = 7.3$, $p < 0.01$). This movement time impairment was restricted to the lateralised movement of the response: the latency to collect reward, a measure of locomotion without a lateralised component, remained unchanged postsurgery for both groups (Group by Surgery, $F(1,18) = 1.05$, ns).

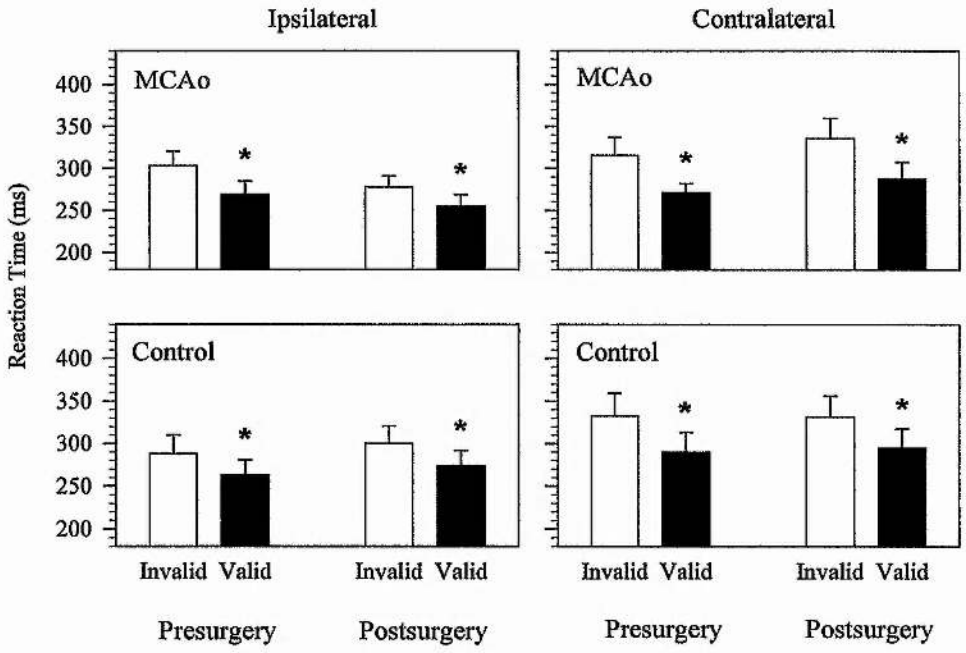


Figure 6.2. *Following surgery, reaction time performance on the covert orienting task did not change; there was neither an increase in reaction time to contralateral targets, nor was there a change in the magnitude of the validity effect. The reaction time to targets preceded by invalid cues was slower than to targets preceded by valid cues. This 'validity effect' reflects both the costs and benefits of covert orienting of attention. The graphs show the mean (± sem, MCAo = 7, controls = 13) reaction time to validly and invalidly cued targets presented to the ipsilateral and contralateral sides (the asterisks indicate a significant difference between valid and invalid trials, $p < 0.05$).*

6.3.2.ii. Accuracy

The percentage correct fell from 77 % (± 1.9 sem) to 71 % (± 2.6 sem) for the lesion group after surgery (Group by Surgery, $F(1,18) = 9.55$, $p < 0.01$). As percentage correct was a composite score of anticipatory, incorrect and late errors, it might have reflected a change in any one or all types of errors. Therefore errors were analysed by type. Figure 6.3 shows errors by type for each group, before and after surgery.

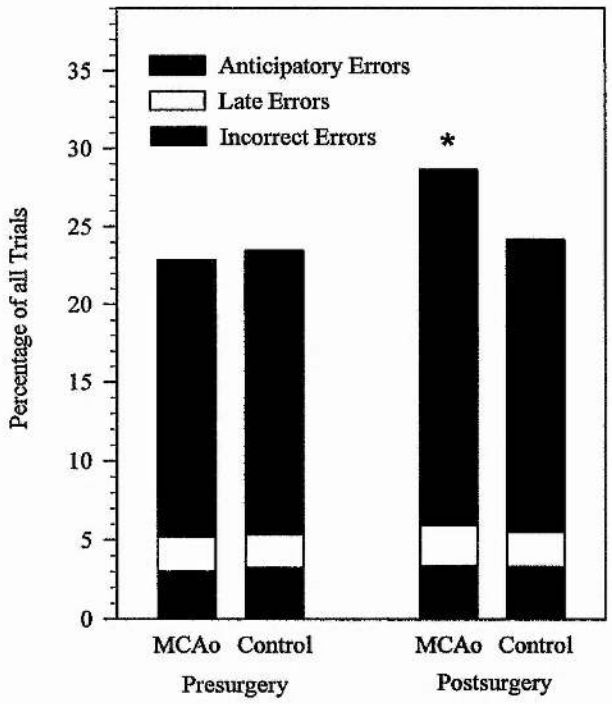


Figure 6.3. *Postsurgery, the number of late errors and incorrect errors did not increase. There was, however, an increase in the number of anticipatory errors in the MCA occlusion group (the asterisk indicates a significant increase, $p < 0.05$). The stacked bar graph shows the mean number of errors (MCAo = 7, controls = 13).*

The percentage of incorrect errors was higher for the invalidly cued as compared with the validly cued trials (Validity, $F(1,18) = 23.4$, $p < 0.001$). However, the lesion did not result in any changes in the percentage of incorrect errors as a function of group, surgery, side or validity (Group by Surgery by Side by Validity, $F(1,18) = 0.58$, ns; Group by Surgery by Side, $F(1,18) = 0.51$, ns). This was also reflected in the asymmetry score which remained unchanged (Group by Surgery by Validity, $F(1,18) = 0.14$, ns; Group by Surgery, $F(1,18) = 0.06$, ns).

The percentage of late errors also did not increase significantly as a function of surgery for either group, side or validity (Group by Surgery by Side by Validity, $F(1,18) = 0.42$, ns; Group by Surgery by Side, $F(1,18) = 0.45$, ns).

Anticipatory errors are responses which are made before the rat can evaluate whether the cue correctly predicts the subsequent target, and thus this measure does not

reflect validity or side. However, these errors may indicate a reduced capacity to sustain attention to the task. Anticipatory errors for the lesion group increased as a percentage of all trials from 18 % (± 1.8 sem) to 23 % (± 1.9 sem) following surgery, whereas the control group remained unchanged (anticipatory errors, Group by Surgery, $F(1,18) = 13.8$, $p < 0.01$). It is apparent from Figure 6.3 that the overall fall in percentage correct originates from the increase in anticipatory errors in the lesion group postsurgery.

6.3.2.iii. Simultaneous Bilateral Cues

The bilateral cues did not reveal an impairment in the performance of the lesion group compared with the control group. In particular, there was no evidence to suggest that the lesion group had a subtle deficit revealed as extinction: Figure 6.4 illustrates that reaction time was not longer to contralateral targets in the lesion group (Group by Side, $F(1,18) = 0.00$, ns).

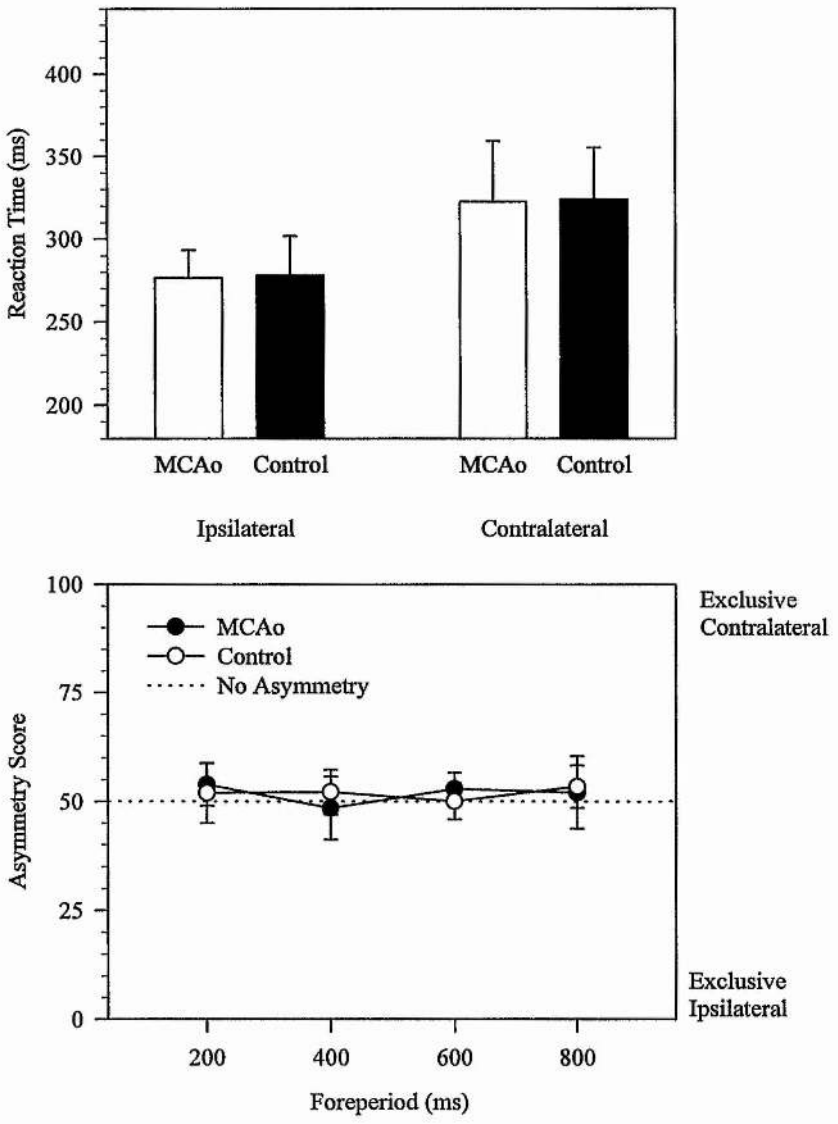


Figure 6.4. *The inclusion of bilateral cues preceding the target did not detrimentally affect the performance of the MCA occlusion group relative to the control group. The figure shows the means (± 95 % confidence interval, MCAo = 7, controls = 13) for reaction time and asymmetry score; there were no significant differences.*

Similarly, there was no asymmetry to respond preferentially to ipsilateral targets (Group, $F(1,18) = 0.00$, ns) or an increase in the incidence of other errors (late errors, Group by Side, $F(1,18) = 0.11$, ns; incorrect errors, Group by Side, $F(1,18) = 2.31$, ns). However, the movement time impairment which was observed postoperatively was not observed during this testing (Group by Side, $F(1,18) = 0.92$, ns).

6.3.4. Somatosensory Asymmetry Test

Postsurgery, the lesion group showed an increase in asymmetry for the order of patch contact. The mean asymmetry score changed from 58 % (± 15.7, 95 % confidence interval) presurgery to 33 % (± 10.8, 95 % confidence interval) postsurgery, reflecting an increased preference to contact the ipsilateral patch first (Figure 6.5; Group by Surgery interaction, $F(1,18) = 7.28$, $p < 0.01$).

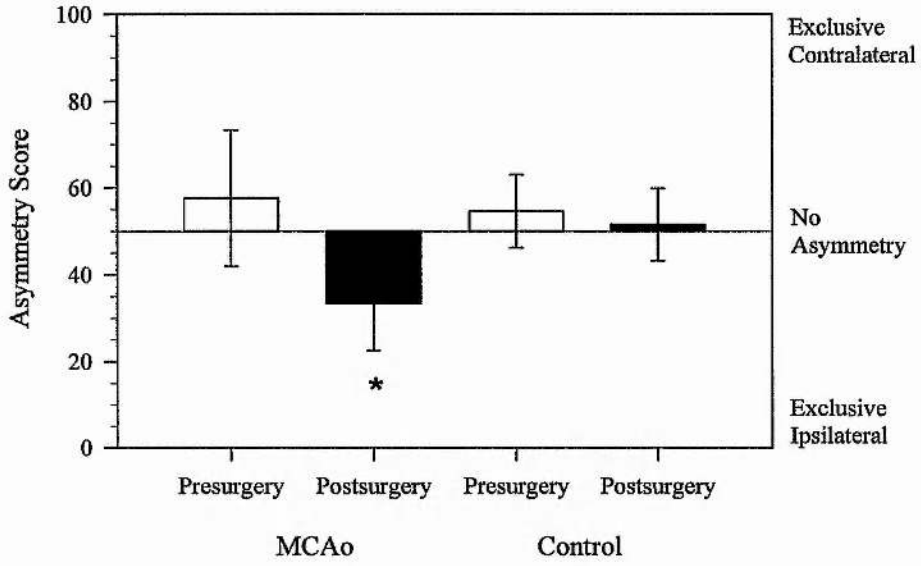


Figure 6.5. *The unilateral lesion resulted in a postsurgical preference to contact first the ipsilateral forepaw, which reversed the chance presurgical preference for contacting the contralateral forepaw. The figure shows the mean (± 95 % confidence interval, MCAo = 7, controls = 13) asymmetry score (the asterisk indicates the significant increase in the asymmetry score, $p < 0.05$).*

The change in preference for the order of contact was also accompanied by an increase in the latency to contact the contralateral patch in the lesion group. Although this increase in latency to contact the contralateral patch might be expected given the increase in asymmetry (the second patch to be contacted will necessarily have a greater contact latency), the increased latency was observed even when the time to contact the

ipsilateral patch was subtracted (Figure 6.6; Group by Surgery by Forelimb, $F(1,18) = 21.5$, $p < 0.001$).

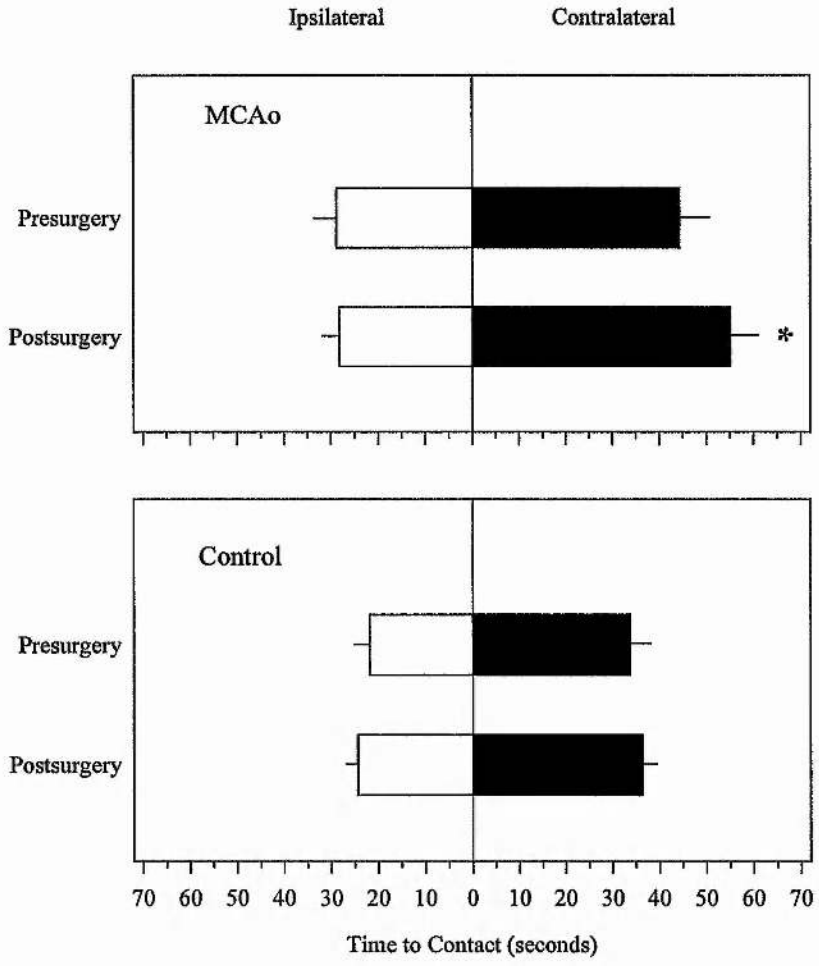


Figure 6.6. *The latency to contact the contralateral patch increased following surgery for the MCA occlusion group (the asterisk indicates a significant increase, $p < 0.05$). The figure shows the mean (± sem, MCAo = 7, controls = 13) of the latency to contact the contralateral patch following contact of the ipsilateral patch, in those trials in which the ipsilateral patch was contacted first.*

6.3.5. Paw Reaching

Figure 6.7 illustrates a decrease in the number of successful paw reaches with the contralateral forepaw (down from a mean of 31.7 ± 7.1 sem presurgery to 18.4 ± 7 sem postsurgery) and an increase in the number of successful paw reaches with the ipsilateral

forepaw (a mean of 11.3 ± 5.2 sem presurgery rising to 17.4 ± 7.6 sem postsurgery) for the lesion group. In contrast, the forepaw use of the control group remained unchanged, giving rise to a significant Group by Surgery by Forelimb interaction ($F(1,18) = 6.82$, $p < 0.05$).

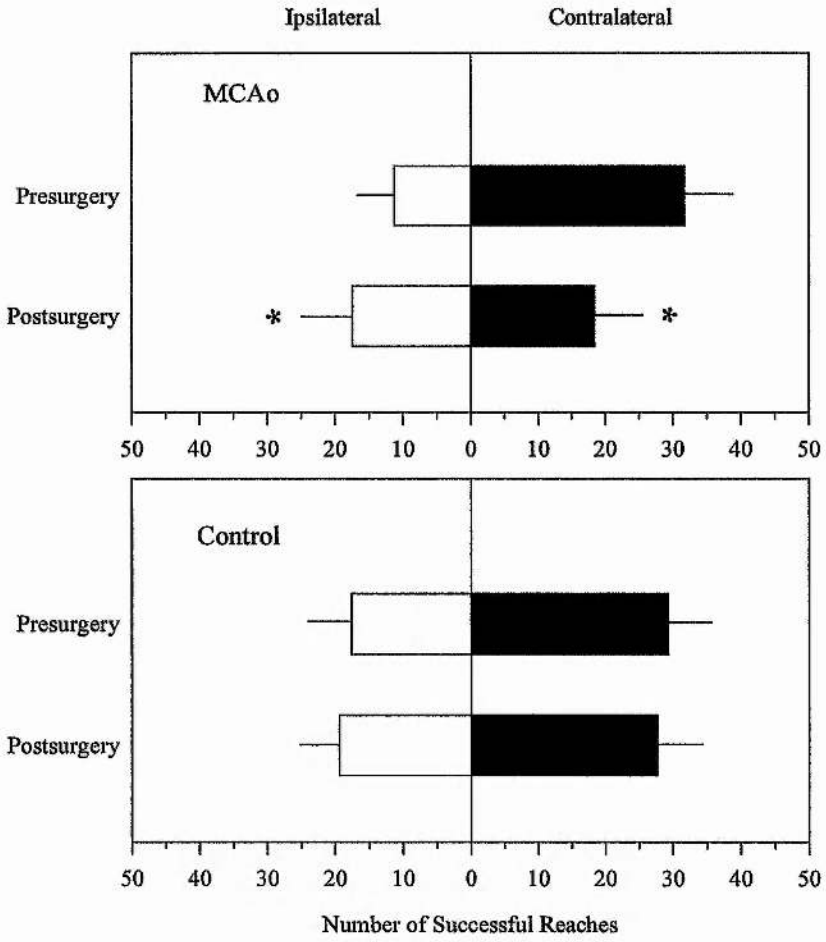


Figure 6.7. *The mean (±sem, MCAo = 7, controls = 13) number of successful paw reaches with each limb is shown for each group. Postsurgery for the MCA occlusion group the number of successful reaches with the contralateral paw decreased, and the number of successful reaches with the ipsilateral paw increased (the asterisks indicate the significant postoperative changes, $p < 0.05$).*

The motivation to attempt reaches remained intact postsurgery in the lesion group; indeed, there was an overall increase in the total number of reaches (from 177 ± 8.6 sem to 201 ± 13.5 sem after surgery) which was irrespective of side (Group by Surgery, $(F(1,18) = 4.61, p < 0.05)$).

6.4. Discussion

The infarct arising from the endothelin-1 induced occlusion of the middle cerebral artery was heterogeneous in size, however, there was an extensive area of damage common to all rats which included the parietal cortex (Par1 and anterior Par2) of Zilles and the forelimb somatosensory area. Consequently, it is perhaps not surprising that there was evidence of a contralateral deficit in the somatosensory test, in which stimuli were applied to the forelimbs and the time taken to contact the stimuli was recorded. There was also an asymmetry of paw skill, with a decrease in successful reaches with the contralesional paw and an increase in successful reaches with the ipsilateral paw. Previous researchers have used such evidence of asymmetry to infer an attentional deficit such as 'neglect'. However, it is not necessary to invoke 'neglect' to account for this deficit.

Indeed, in the visual reaction time task, the rats showed normal reaction times to lateralised stimuli and, furthermore, showed no evidence of an impairment in covert orienting of attention, with a normal magnitude of 'validity effect' for the lateralised cues which preceded the targets. Using a different kind of reaction time task, which might be characterised as a test of vigilance, Muir et al. (1996b) have reported that lesions of Par1 and Par2 spare attentional function. From the data, performance in the covert orienting of attention task appears to be intact following a lesion of Par1 and Par2. There was an increase in anticipatory responding which could be viewed as abortive errors attributable to a difficulty in sustaining attention to the task. In the Muir et al. (1996b) study, there was no increase in anticipatory responding (nose pokes before any stimulus). However, their vigilance task had different demands and a deficit in sustaining attention to their task was more likely to be reflected in an increase in errors of omission (failure to respond to the target). However, the salience of the target in their task, would elicit an orienting response, and this is a function which appears to be intact in the current study.

Testing for extinction (i.e., when stimuli are presented simultaneously to both sides, on the assumption that the stimulus processed by the intact hemisphere will gain a processing advantage) is a technique used in the neurological clinic to reveal subtle

deficits of hemi-inattention (Petersen et al., 1989). However, when the visual cues in the reaction time task were presented simultaneously, the lesion group did not exhibit an asymmetry to respond preferentially to the side ipsilateral to the lesion, nor did they display an increase in reaction time and/or late errors to contralateral target lights. Therefore, it is possible to conclude, that even when challenged in this way, there is no evidence of 'neglect' following this lesion.

This result might be considered somewhat surprising in view of the evidence that links the parietal cortex to attentional functions. However, Par1 and Par2 contain a topographical somatosensory representation of the body, head and whiskers (Chapin & Lin, 1990; Hall & Lindholm, 1974) which is a characteristic of the primary somatosensory cortex rather than the association cortex. Although there have been reports that damage in Par1 and Par2 disrupts performance in some spatial tasks, notably navigation through mazes, this navigational deficit may reflect the importance of somatosensory cues derived from locomotion for learning routes through mazes (Save & Moghaddam, 1996) rather than a deficit in visuospatial processing or a spatial 'neglect'. The area which is homologous to the posterior parietal cortex in primates is more likely to be lateral Oc2M/2L (Chandler et al., 1992; Kolb, 1990b; Kolb & Walkey, 1987; Kreig, 1946; Reep et al., 1994). Nevertheless, recent reports (Rosner & Mittleman, 1996) and Experiment 3 reported in this thesis *(Covert orienting following excitotoxic lesions of posterior parietal cortex: Chapter 5)* suggest that covert orienting was intact even following an aspirative lesion of lateral Oc2M/2L (Rosner & Mittleman, 1996).

Although attentional processes remained intact following the ischemic lesion, movement time was lengthened for responses to the contralateral side. The latency to collect reward did not increase, excluding the possibility of a generalised motor impairment, which is consistent with research indicating intact locomotion following MCA occlusion (Grabowski et al., 1991; Yamaguchi et al., 1995; Yamamoto et al., 1988). It is possible that this movement time impairment is another indication of the problem with contralateral paw use, which might result in impaired turning ability. Nevertheless, this deficit was assumed to have recovered by four weeks post surgery, as it was not found during the testing for extinction, in which the response requirements of the covert orienting task remained the same and only the cues changed.

In the somatosensory test, the change in likelihood of contacting the ipsilateral stimulus postsurgery in the lesion group is indicative of an underlying sensory deficit due to damage extending into the forelimb region (FL). The finding is consistent with Barth et al. (1990) and Markgraf et al. (1992; 1994) who reported an ipsilateral preference asymmetry after damage to area FL. The change in the order of contact (with an increase in the likelihood that the ipsilateral forepaw is contacted first) would support the conclusion that the deficit is a consequence of a sensory impairment: a motor impairment would not be expected to result in a change in the order of contact (Schallert et al., 1982). As such, this deficit probably reflects somatosensory impairment and does not reflect hemi-inattention.

Similarly, the fall in the mean number of successful reaches with the contralateral forepaw is consistent with other reports (Whishaw & Coles, 1996) after lesions to Zilles's region FL. Although there is an asymmetry in the change in paw skill, the results do not conflict with previous reports of bilateral deficit in skilled reaching after middle cerebral artery occlusion (Grabowski et al., 1993; Marston et al., 1995a; Sharkey et al., 1996). Whishaw and colleagues have emphasised the importance of the non-reaching forelimb in aiding posture and manipulation of the food pellet during reaching (Milkyaeva et al., 1994; Whishaw & Coles, 1996; Whishaw & Pellis, 1990), thus, a bilateral deficit might follow a unilateral lesion. Grabowski et al. (1993) and Marston et al. (1995) used the 'staircase' reaching task, the performance of which might be particularly dependant on the use of the non-reaching limb for balance. Although ostensibly paw reaching is a test of motor skill, previous authors have noted that tests of paw preference and paw skill are dependant on intact somatosensory function: impaired proprioceptive feedback is likely to result in the appearance of motor incompetence (Sabol et al., 1985). Therefore, it is not necessary to invoke an additional deficit, over and above a somatosensory impairment, to account for this reaching deficit.

Excitotoxic lesions of the lateral striatum result in an asymmetry towards the side of the lesion (Brown & Robbins, 1989b). Although middle cerebral artery occlusion did not result in a response asymmetry in the covert orienting task, the lesions of the striatum following MCA occlusion were small and incomplete in comparison to the excitotoxic lesions obtained by Brown & Robbins (1989b). Since there was no response asymmetry in the operant tasks, this provides further evidence that the deficit in the somatosensory asymmetry test was more likely the result of a deficit in somatosensory

processing rather than due to a response asymmetry. Reaction time deficits in this and similar tasks are associated with lesions of the medial striatum (Brown & Robbins, 1989b) or depletion of striatal dopamine (Ward & Brown, 1996) and not lateral striatal lesions.

The experiment did not specifically examine the time course of recovery following MCA occlusion. The somatosensory asymmetry and paw reaching deficit were present at two and three weeks postoperatively, respectively (see Figure 6.8). Markgraf et al. (1992; 1994) reported recovery of asymmetry in some animals by four weeks. The movement time deficit recovered by four weeks postsurgery, which is consistent with reports of an improvement in beam walking over 30 days (Markgraf et al., 1992). The duration of the somatosensory deficits reported in the current experiment remains to be established, but the deficits would be suitable for testing the efficacy of acute intervention with neuroprotective agents, a task which is already underway (Sharkey et al., 1996).

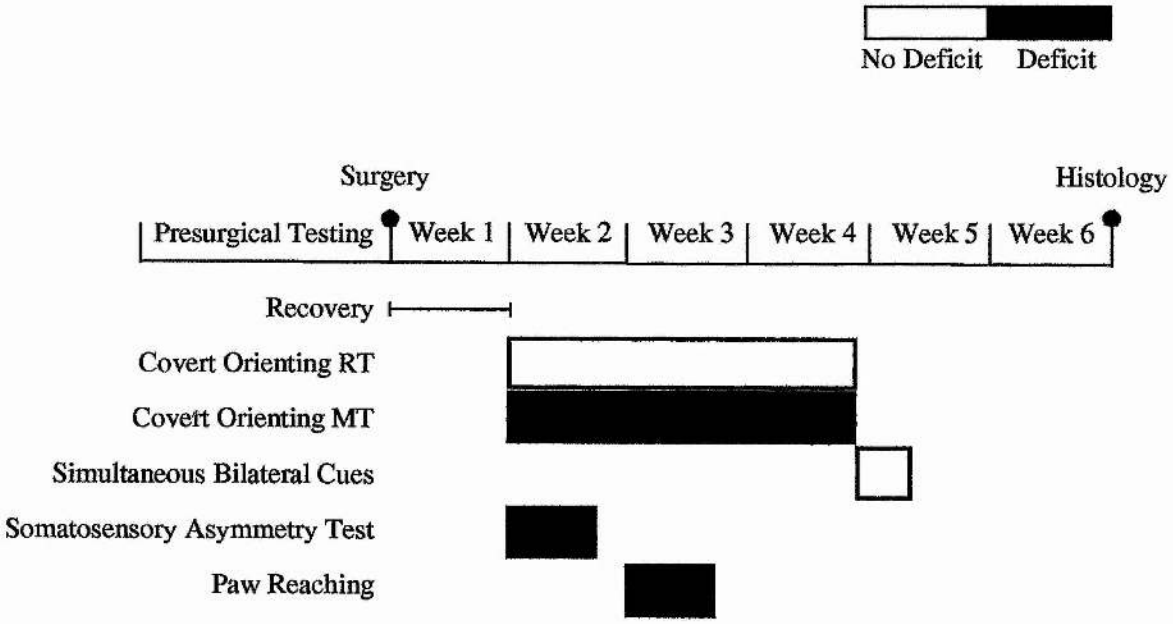


Figure 6.8. *Details of the time course of postsurgical testing and the known duration of the presence or absence of an impairment in test performance.*

The covert orienting task, somatosensory asymmetry test and paw reaching tasks were successfully employed to investigate the behavioural consequences of MCA occlusion. The results indicated there was a unilateral sensorimotor impairment but that attentional processes remained intact. The unilateral deficit was indicated by difficulty executing contralateral movements, a bias to respond to ipsilateral tactile stimulation and a change in successful paw reaching. The results demonstrate that 'neglect' following MCA occlusion is not attributable to dysfunctional attention or response initiation but instead suggests a somatosensory impairment.

The opportunity to evaluate putative neuroprotective agents in rat models of MCA occlusion has provided a valuable advance for preclinical research. Employing behavioural assessment in addition to quantitative volumetric analysis has demonstrated that reductions in lesion volume following pharmacological treatment are accompanied by a reduction in behavioural impairment. However, the reliability of neuroprotective agents could be evaluated further by developing a model of focal cerebral ischemia in a vascular territory outside of the MCA. Diversifying the range of models used to evaluate pharmacological treatments may help to verify that the mechanism of action of a neuroprotective agent is not unique to the prevailing physiological conditions in the territory of the MCA.

Chapter 7:

Simple and choice reaction time performance following occlusion of the anterior cerebral arteries

7.1. Introduction

Models of focal cerebral ischemia in the rat have primarily focused on the consequences of MCA occlusion. However, the stereotaxic injection of the potent vasoconstrictor endothelin-1 in close proximity to the artery (Sharkey et al., 1993) provides a flexible method for inducing arterial occlusion. The procedure affords the opportunity to occlude any artery for which reliable stereotaxic co-ordinates can be determined. To exploit this versatility, a model of anterior cerebral artery occlusion has been developed, using stereotaxic injection of endothelin-1 to bilaterally occlude the ACA (Marston et al., 1995b), which results in an 80 % reduction of blood flow in the territory of the ACA (Sharkey et al., 1997). The development of ACA occlusion will permit the opportunity to verify the efficacy of various treatments in arterial territories other than the MCA.

Investigation of the behavioural consequences of middle cerebral arterial occlusion have provided a criterion in addition to quantitative histopathological assessment, by which to evaluate neuroprotective efficacy. Treatments such as neural transplants, enriched laboratory housing environments and growth factors have been demonstrated to facilitate behavioural recovery after the acute stage of ischemic cell death in the absence of a decrease in the extent of the infarct (Grabowski et al., 1995; Kawamata et al., 1996). Therefore, understanding of the behavioural consequences of MCA occlusion has an important role in establishing the characteristics of the model and response to a variety of treatment strategies. Similarly, in characterising the ACA occlusion model, an important objective is to establish the behavioural impairments following ACA occlusion.

The vascular territory of the ACA encompasses Zilles's areas Cg1, Cg2, Cg3, IL, MO, VO, anteromedial Fr2 (Zilles, 1990) and extends subcortically to include septum, medial striatum and vertical diagonal band of Broca (Scremin, 1995). Occlusion of the ACA does not typically result in damage to primary motor (Fr1 and Fr3) or lateral/posterior Fr2. The ischemic lesion following bilateral ACA occlusion involves medial prefrontal cortex in the rat, cortex which receives input from the mediodorsal thalamus (Groenewegen, 1988; Krettek & Price, 1977; Leonard, 1969), which has been used to define the rat homologue of primate prefrontal cortex (Kolb, 1990a; Van Eden et al., 1992).

Selective lesions of rat mediofrontal cortex have identified a range of deficits in spatial and delayed stimulus-response tasks (De Bruin et al., 1994; Granon & Poucet, 1995; Granon et al., 1994; Kolb, 1990a; Kolb et al., 1994; Kolb & Cioe, 1996; Mogensen & Holm, 1994) which are consistent with mediofrontal involvement in response inhibition (Sokolowski & Salamone, 1994), selection (Granon et al., 1996; Sarter et al., 1996) and response flexibility (Kolb et al., 1994). Selective excitotoxic lesions of anteromedial prefrontal cortex also result in response perseveration (Kolb et al., 1994; 1974; Muir et al., 1996b; Sanchez-Santed et al., 1997), while a more posterior prefrontal lesion has been reported to cause an increase in anticipatory responses, which is perhaps indicative of a failure to inhibit responses (Muir et al., 1996b). Response initiation (but not execution) also appears to be impaired in reaction time tasks, following prefrontal dopamine depletion (Hauber et al., 1994) and prefrontal excitotoxic lesions (Muir et al., 1996b). These results in rodents are consistent with the observed consequences of prefrontal lesions in man, which include impulsive behaviour, slowed response initiation, increased distractibility and a tendency to perseverate (Alivisatos & Milner, 1989; Bogousslavsky, 1994; Degos et al., 1993; Rueckert & Grafman, 1996; Viallet et al., 1995). The behavioural consequences of ACA occlusion will thus, be investigated using simple and choice reaction tasks which assess a variety of functions including response selection, motivation, memory, learning and attention. The use of reaction time to measure performance provides a particularly sensitive measure of brain function (Godefroy et al., 1994) in addition to accuracy of performance measures.

The first experiment assesses the capacity to interpret motivationally salient cues, using a simple reaction time task (see Brown et al. 1996). The simple reaction time task used in the current experiment includes cues indicating proximity of reward. The task design maintains the same response requirements for every trial while varying the cues to indicate proximity of reward. Difficulty in initiating or executing movement can be distinguished from a deficit specific to the disruption of response vigour (Brown & Bowman, 1995) influenced by the visual cues. The test will permit an investigation of the involvement of mediofrontal cortex (which includes anterior cingulate) in motivational processes. Devinsky et al. (1995) in a recent review of the functions of primate anterior cingulate concludes "Overall, anterior cingulate appears to play a crucial role in initiation, motivation and goal directed behaviours." In the rat cells in anterior cingulate appear to be active in anticipation of reward (Takenouchi et al., 1996) and may

participate in a limbic-motor circuit underlying motivation to action. Following postoperative testing, performance was also examined in the same multiple ratio schedule without the cues, to confirm that the cues continued to influence performance in the task. Finally the ability to learn the meaning of cues during reversal was also assessed.

The second experiment assesses attentional function using a test of covert orienting (analogous to the test for humans developed by Posner (1980)). The covert orienting task is capable of detecting a variety of attentional impairments including difficulty in the covert engagement, maintenance, disengagement and shifting of attention (Posner & Petersen, 1990). The task used a choice reaction time test, with peripheral visual cues preceding peripheral visual targets to which the rat was required to make a lateralised response.

Damage to mediofrontal cortex in the rat has impaired performance in a task demanding sustained attention (Muir et al., 1996). Furthermore, Parkinson's disease and droperidol (a dopamine antagonist) have been demonstrated to reduce the effect of invalid cues in humans (Clark et al., 1989; Wright et al., 1990; Yamada et al., 1990) and monkeys (Witte et al., 1992). Wright et al. (1990) and Yamada et al. (1990) have speculated that a failure to maintain attention in the covert orienting task is due to prefrontal dopamine depletion.

7.2. Experiment 1: Effects of ACA occlusion on simple reaction time performance, in which visual cues indicate the availability of reward

7.2.1. Hypotheses

The motivational task is capable of distinguishing between a motivational and response deficit. The hypothesised outcomes can be specified as 1) a failure to respond to the motivational information provided by the visual cues will result in a differential change in response vigour or 2) a response initiation deficit will appear as a global rise in reaction time irrespective of the proximity of reward (see Figure 7.1). Removal of the visual cues will provide an indication of their impact on response vigour and accuracy.

Finally, the rats were also tested to assess their capacity to adapt to a reversal of cue meanings. Difficulty in adapting to a new stimulus-reward association should become evident as a failure to alter response vigour and accuracy to reflect the new meaning of the cues or a slower rate of adaptation in comparison with the control group.

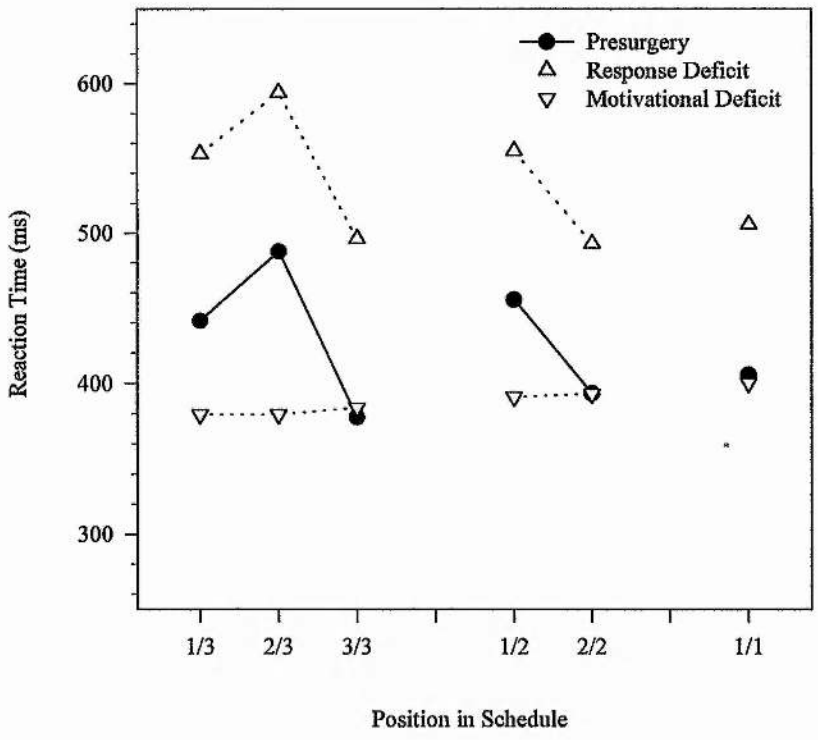


Figure 7.1. *The graph illustrates faster responses to rewarded over nonrewarded trials as a measure of motivation. A failure to respond or perceive the motivational salience of the visual cues is also illustrated along with an impairment in response initiation.*

7.2.2. Materials and Methods

Eighteen male rats were used during the study (weight range during study; start 290-355 g; completion 355-410 g). The rats were tested on a series of simple reaction time tasks (see section 2.2.3). The anterior cerebral arteries were bilaterally occluded according to the protocol detailed in section 2.4.5.

7.2.2.i. Collection of Data

Presurgical data were collected over a period of eight days (~1400 correct trials per rat). Two weeks after surgery the postsurgical data were collected over a further eight days. At conclusion of this testing, two further behavioural manipulations were undertaken. 1) The visual cues were removed, but otherwise the task remained the same (~600 trials per rat, starting 3 weeks postsurgery). 2) The cue lights were reinstated, but the meaning of the cue lights was reversed and the data collected over two weeks starting 3½ weeks postsurgery. Six weeks following surgery the rats were perfused.

7.2.3. Results

7.2.3.i. Histological Results

Four rats failed to recover from surgery. A further two rats did not sustain bilateral ischemic damage to medial prefrontal cortex and were removed from all analysis. Examination of the remaining twelve rats identified seven rats with bilateral ischemic damage and five controls who had undergone an identical surgical procedure but who sustained no damage. The extent of the largest and smallest lesions are illustrated in Figure 7.2a. The following description of the lesion location uses the nomenclature of Zilles (1990) and Paxinos & Watson (1986). The core of the ischemic lesion extended through medial prefrontal cortex involving cortical regions Cg1, Cg2, Cg3, IL and anteromedial Fr2. Five of the seven rats sustained damage to the corpus callosum underlying the ischemic damage to medial cortex between bregma + 1.6 and 0. Subcortical damage included the septum (LSV, LSD, SHi, MS, LS) and the vertical diagonal band of Broca (VDB). The two most extensive lesions extended ventrally to include orbital cortex (MO/VO) and caudally to include retrosplenial cortex (RSG/RSA). The mean volume of ischemic damage in the lesioned rats was 66.7 mm^3 (± 10.8 sem). In the rats who sustained no damage the only detectable damage was small, resulting from the needle tract. These rats formed the control group.

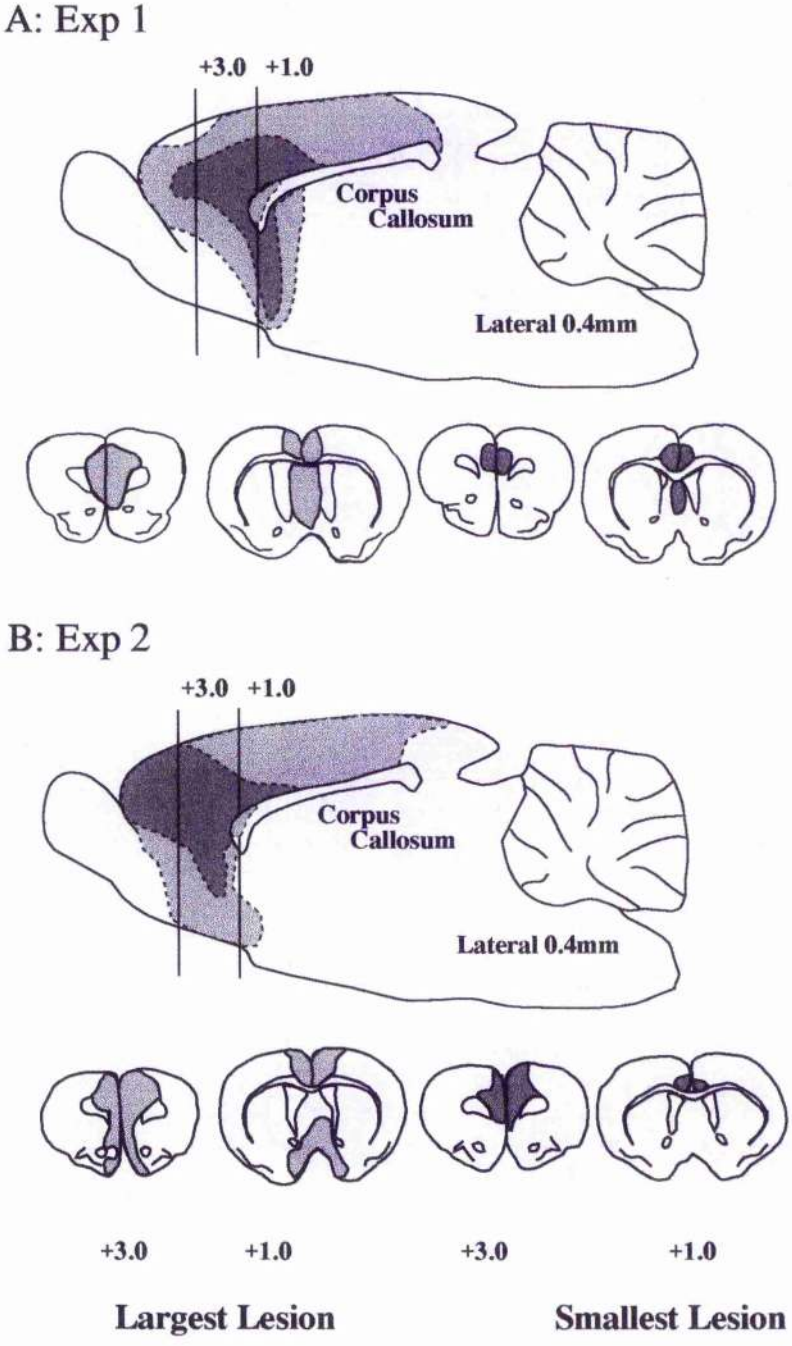


Figure 7.2. *A: The largest and smallest lesions illustrate the extent of ischemic damage following ACA occlusion in Experiment 1. Schematic sagittal and coronal sections illustrate the extent of ischemic damage in medial prefrontal cortex, which included Cg1, Cg2, Cg3, IL, anteromedial Fr2, MO/VO, septum and vertical diagonal band of Broca. B: ACA occlusion in Experiment 2 also resulted in extensive tissue loss in medial prefrontal cortex and anteromedial basal forebrain.*

7.2.3.ii. Behavioural Observations

Four of the rats with ACA occlusion displayed hyper-reactivity to auditory, visual and tactile stimuli, making the rats difficult to handle, this resolved within two weeks of surgery. These observations are consistent with previous reports of a syndrome referred to as 'septal rage', which includes hyper-emotionality, increased aggression and hyper-reactivity to stimulation, including handling (Brady & Nauta, 1953; Goodlett et al., 1982). However, despite the presence of septal damage in all of the rats, 'septal rage' was only observed in four rats.

7.2.3.iii. Visually Cued Multiple-Ratio Schedule

7.2.3.iii.i. Reaction Time and Movement Time

The reaction time was significantly faster on rewarded trials compared with when reward was two, or three, trials away (Position in Schedule $F(4,36) = 23.16$, $p < 0.0001$). As the foreperiod before the tone lengthened, reaction time significantly decreased (Foreperiod $F(1,13) = 162.23$, $p < 0.0001$). There was no effect of the lesion on reaction time performance (Group by Surgery $F(1,10) = 0.38$, ns). In particular, the ischemic lesion did not alter performance on the task as a function of position in the schedule, which is illustrated in Figure 7.3 (Group by Surgery by Position in Schedule by Foreperiod $F(15,147) = 1.35$, ns; Group by Surgery by Position in Schedule $F(4,39) = 0.96$, ns).

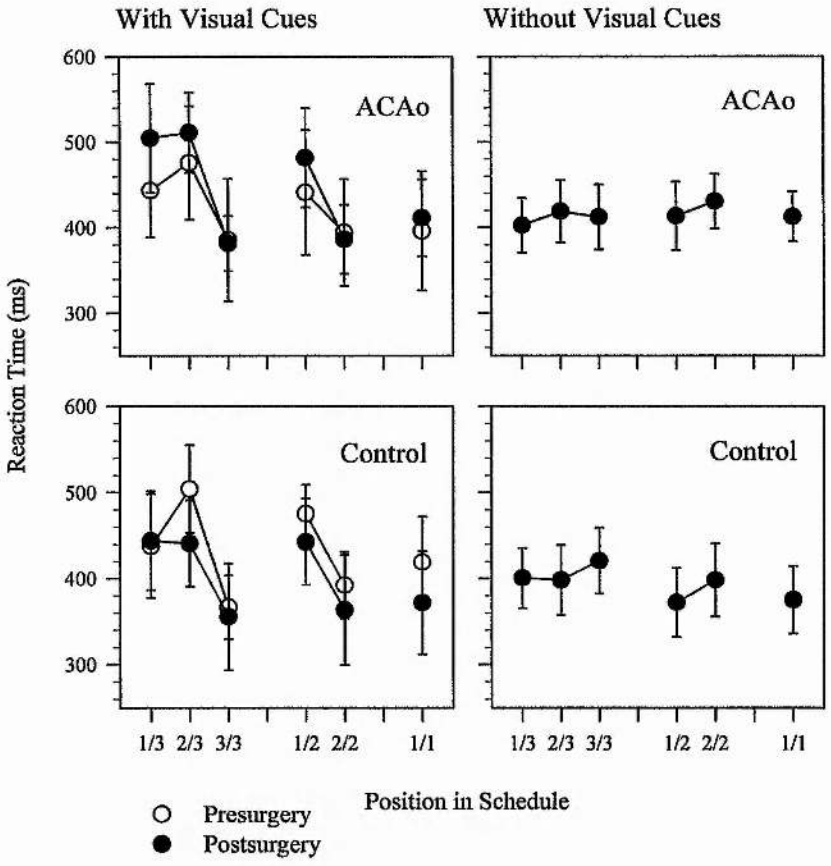


Figure 7.3. *Mean reaction times (± sem, ACAo = 7, controls = 5) when the cue indicated one trial to reward were faster than when the cues indicated two or three trials before reward. Ischemic damage to medial prefrontal cortex following ACA occlusion did not alter response to the cues or globally slow the motor response. Reaction time (mean ± sem, ACAo = 7, controls = 5) no longer reflected proximity to reward following removal of the visual cues.*

Movement to the food hopper was quicker for rewarded as compared with non rewarded trials (Position in Schedule $F(2,17) = 19.04$, $p < 0.0001$) . As the pellet was not delivered into the hopper until the opening of the hopper door, the faster reward collection time was governed solely by expectation of reward and is thus an instrumental rather than consummatory response. Following the ischemic lesion, the mean movement time continued to reflect position in the schedule for both groups (Group by Surgery by Position in Schedule by Foreperiod $F(14,145) = 0.86$, ns; Group by Surgery by Position in Schedule $F(3,29) = 1.1$, ns). Although movement time increased postsurgery from 846

ms (36 ± sem) to 1247 ms (81 ± sem) (Surgery $F(1,10) = 31.65$, $p < 0.0001$) this change was irrespective of group (Group by Surgery $F(1,10) = 1.21$, ns).

7.2.3.iii.ii. Accuracy

The two types of errors, either premature withdrawal from the central hole (anticipatory error) or failure to move quickly to the food hopper panel (late error) were analysed separately.

The number of anticipatory errors increased as a function of foreperiod (Foreperiod $F(1,12) = 50.26$, $p < 0.0001$). There were very few anticipatory errors when one or two trials from reward and anticipatory errors occurred with greatest frequency on trials three responses from reward (illustrated in Figure 7.4). Following surgery, the number of anticipatory errors at the two longest foreperiods increased for the controls, but decreased for the ACA occlusion group (Group by Surgery by Foreperiod $F(2,17) = 4.56$, $p < 0.03$).

The number of late errors increased as a function of the number of trials to reward. Late errors did not increase following the ischemic damage (Figure 7.4, Group by Surgery by Position in Schedule by Foreperiod $F(13,130) = 1.31$, ns; Group by Surgery by Position in Schedule $F(2,24) = 0.88$, ns).

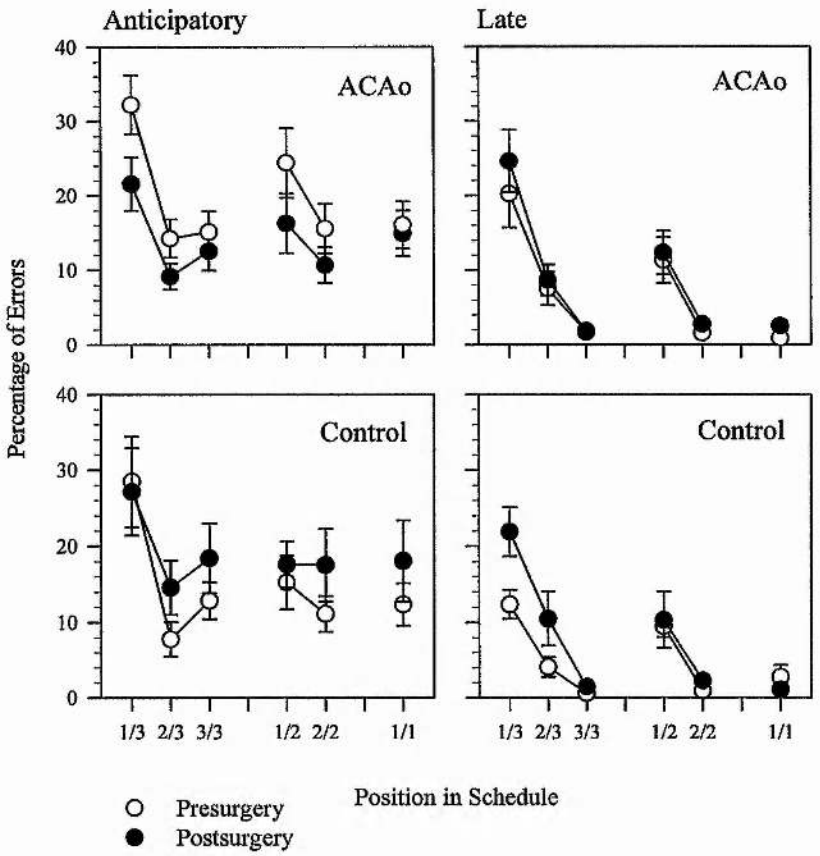


Figure 7.4. *The occurrence of anticipatory errors was higher for trials cued as three trials from reward than those cued as one or two trials from reward (mean ± sem, ACAo = 7, controls = 5). The incidence of late errors (mean ± sem, ACAo = 7, controls = 5) reflected the proximity of reward indicated by the cues, as the number of trials before reward increased the occurrence of late errors also increased. Ischemic damage following ACA occlusion did not alter the accuracy of response to the cues.*

7.2.3.iv. Multiple-Ratio Schedule without Visual Cues

7.2.3.iv.i. Reaction Time and Movement Time

The cues which indicated the position in the schedule were removed for one block of testing. When the rats were tested without visual cues, reaction time continued to reflect foreperiod as expected (Foreperiod, $F(3,28) = 91.97$, $p < 0.0001$) but it no longer reflected position in the schedule (Figure 7.3, Position in Schedule $F(3,33) = 0.65$, ns). This effect was observed in both groups (Group by Position in Schedule by Foreperiod $F(19,192) = 0.59$, ns; Group by Position in Schedule $F(3,33) = 0.27$, ns).

Although reaction time no longer reflected position in schedule, movement time was significantly faster for later trials compared with the first trials in each schedule (Position in Schedule $F(5,50) = 4.32$, $p < 0.002$), an effect which was seen in both groups (Group by Position in Schedule by Foreperiod $F(9,85) = 1.63$, ns; Group by Position in Schedule $F(5,50) = 0.5$, ns).

7.2.3.iv.ii. Accuracy

Despite the removal of the visual cues, anticipatory errors still indicated position in schedule at the later foreperiods (300 - 500 ms). The number of anticipatory errors was greater for the initial trials (i.e., trials following rewarded trials) in each schedule as compared to the later trials in each schedule (Position in Schedule by Foreperiod $F(20,200) = 3.35$, $p < 0.0001$). Figure 7.5 illustrates that the ischemic lesion and control groups did not differ with respect to their progression through the schedule (Group by Position in Schedule by Foreperiod $F(20,200) = 1.14$, ns; Group by Position in Schedule $F(5,50) = 0.42$, ns).

Without visual cues, late errors no longer reflected position in schedule (Position in Schedule $F(5,50) = 1.19$, ns) for either group (Group by Position in Schedule by Foreperiod $F(17,166) = 1.17$, ns; Group by Position in Schedule $F(5,50) = 1.24$, ns).

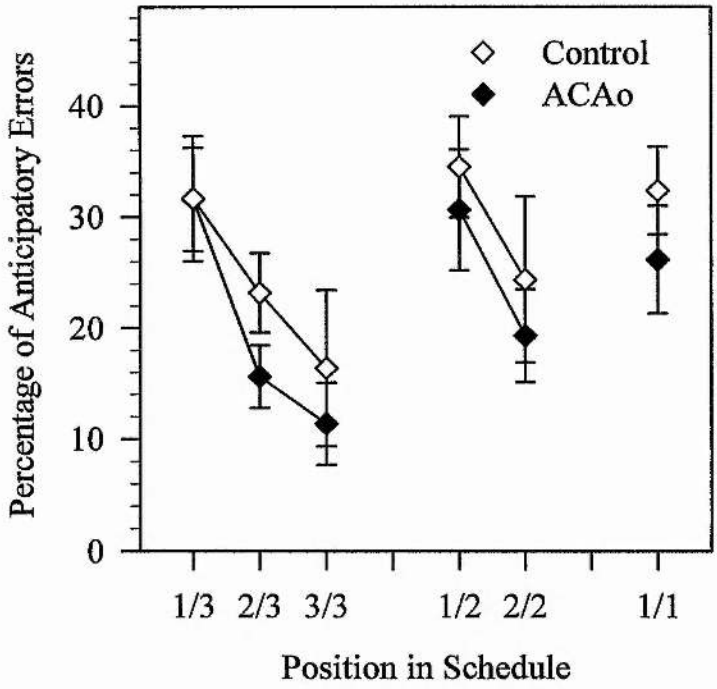


Figure 7.5. *Without visual cues memory for the proximity of the last reward became important. The propensity to abort trials early (mean ± sem, 300 to 500 ms only, ACAo = 7, controls = 5) was higher for trials immediately following reward than trials occurring later in each schedule. Both the ACA occlusion and control groups displayed this pattern. This pattern probably arises due to the probability of reward increasing with progression through each schedule.*

7.2.3.v. Multiple-Ratio Schedule after Reversal of Visual Cues

The first session following reversal was characterised by a vigour of responding (Reaction Time) to the cues according to their earlier association (Figure 7.6, Reversal by Position in Schedule $F(3,35) = 13.95$, $p < 0.001$). By the sixth session responses reflected the new meaning of the cues and there was no difference between the ACA occlusion and control groups (Group by Reversal by Position in Schedule $F(5,50) = 1.31$, ns). The initial response to the reversal observed for reaction time was also seen for the movement time (Reversal by Position in Schedule $F(2,18) = 14.52$, $p < 0.001$) and by the sixth session again reflected the new relationship (Group by Reversal by Position in Schedule $F(2,20) = 0.79$, ns). The pattern of late errors also demonstrated the same effect of reversal (Reversal by Position in Schedule $F(4,36) = 19.3$, $p < 0.001$) and the rate of

adaptation was the same for both groups (sixth session following reversal, Group by Reversal by Position in Schedule $F(2,19) = 0.34$, ns).

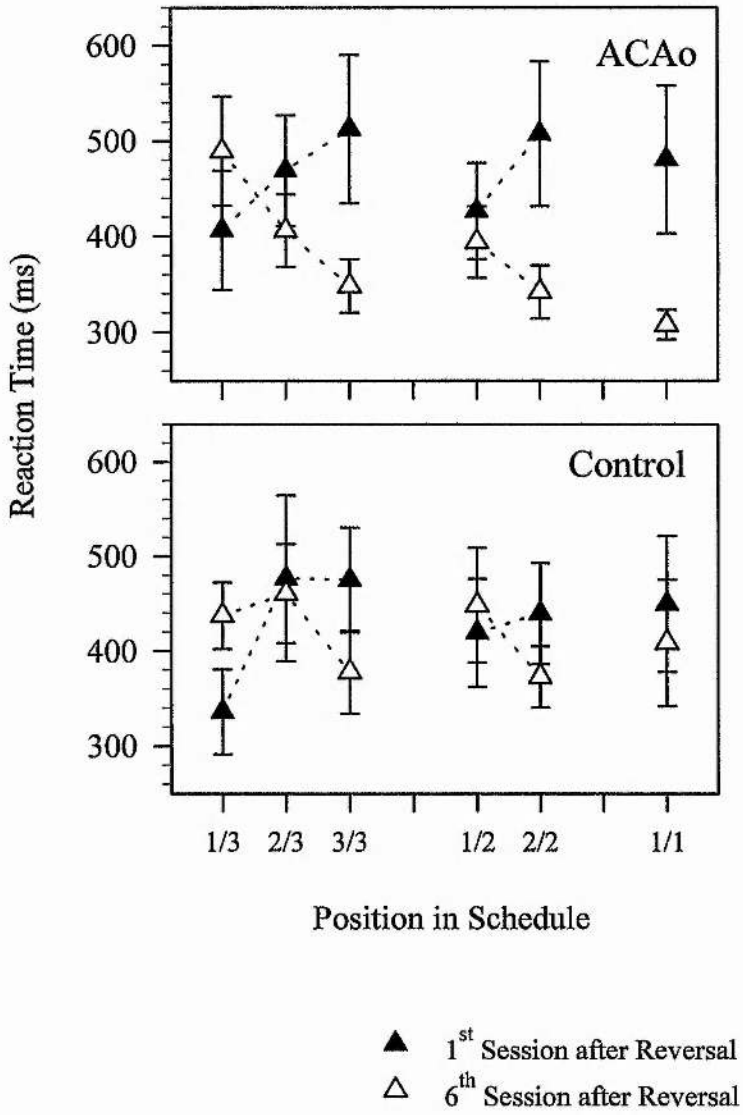


Figure 7.6. *Initially following reversal of the cue meanings, the ACA occlusion and control groups responded to the cues with a vigour consistent with their old associations. Within six sessions following reversal the ACA occlusion and control groups had adapted to the new relationship between the cues and proximity of reward (mean ± sem, ACAo = 7, controls = 5).*

7.2.4. Discussion

The vigour and accuracy of responses in the simple reaction time task illustrated that the visual cues indicating proximity of reward were used to govern responses on rewarded and nonrewarded trials. The ischemic lesion did not prevent the use of visual cues to determine vigour and accuracy of response, according to the proximity of reward. For comparison the reaction time performance following removal of the visual cues indicated the pattern of response vigour, if there had been a failure to interpret or respond to the cues. Thus medial prefrontal cortex, which was damaged by the ischemic lesion, is neither crucial to the initiation of movement weighted by the motivational salience of a signal or is it required for interpretation of the valence of that sensory signal. The ischemic lesion also did not result in an increase in reaction time and therefore it is possible to conclude that response initiation in the simple reaction time task remained intact.

Following removal of the visual cues response vigour no longer indicated position in schedule in either group. However, position in the schedule still had an effect on other performance measures, which reflected the expectation of an increasing probability of reward at each trial as the rat progressed through the schedule. Consequently, for position in the schedule to have an effect demands intact working memory for proximity of the last rewarded trial. There was no difference in performance between the ACA occlusion and control groups: both groups displayed fewer anticipatory errors and faster movement times as they progressed through later trials in each schedule. Response adaptability was also unaffected by ACA occlusion, as the lesioned rats adapted to the new relationship between stimulus and reward when the meaning of the visual cues was reversed as rapidly as controls.

7.3. Experiment 2: Effects of ACA occlusion on choice reaction time performance in a test of covert orienting

7.3.1. Hypotheses

Tables 4.1 and 4.2 outline the variety of response and attentional impairments which may follow lesions, disease and drug administration in the covert orienting task. Alterations in postsurgical performance indicating 1) a failure to maintain attention (suggested by changes in the magnitude of the validity effect) or 2) the time taken to initiate a response were the hypothesised outcome of ACA occlusion.

7.3.2. Materials and Methods

Eighteen, pair-housed male rats were used in the study (weight range during study; start 305-435 g; completion 405-490 g). The rats were tested on the covert orienting test (see section 2.2.2). The anterior cerebral arteries were bilaterally occluded according to the protocol detailed in section 2.4.5.

7.3.2.i. Collection of Data

Presurgical data were collected over a period of ten days with one session run per day (~ 1200 trials per rat). Three weeks following surgery data were collected for fourteen days (up to 1680 trials per rat). Following completion of postsurgical testing (six weeks postsurgery) the rats were perfused.

7.3.3. Results

7.3.3.i. Histological Results

Two rats were dropped from the study due to postsurgical complications and a further two lesioned rats were inactive during behavioural testing postsurgery. As these four rats failed to generate any postoperative data they were excluded from the data analysis. The size and location of lesion in these rats was comparable to that of the largest lesion illustrated in Figure 7.2b. Examination of the remaining fourteen rats identified eight with bilateral ischemic damage. Figure 7.2b shows the extent of the largest and smallest lesions resulting from the ischemic insult following bilateral occlusion of the ACA. The ischemic lesion included anterior cingulate (Cg1, Cg2 and Cg3), anteromedial Fr2 (anterolateral and posterior Fr2 was spared in all but the largest lesion), infralimbic (IL), and orbital cortex (MO/VO). The largest lesions extended caudally to include retrosplenial cortex (RSG/RSA). Six of the rats also sustained damage to the corpus callosum beneath medial cortex between bregma + 1.6 and 0. Subcortically the lesion extended to include the anterior septum (LSV, LSD, SHi, LS, MS) and vertical diagonal band of Broca. The mean volume of ischemic damage following ACA occlusion in Experiment 2 was 68.6 mm^3 (± 10.1 sem). Figure 7.7 illustrates there was no significant difference between the area of ischemic damage arising from ACA occlusion in Experiment 1 and 2 either overall (Experiment, $F(1,13) = 0.02$, ns) or at each coronal level (Experiment by Coronal Level, $F(5,64) = 1.41$, ns). The remaining six rats did not sustain any ischemic damage and were used as operated controls.

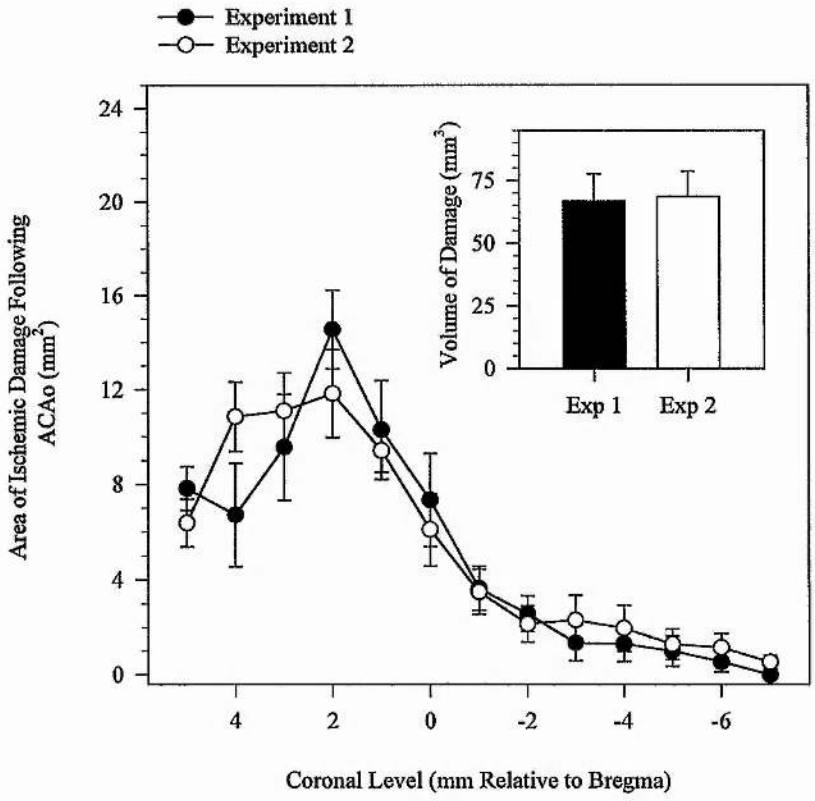


Figure 7.7. *The graph illustrates the area of ischemic (mean ± sem, ACAo = 8, controls = 6) damage over 13 coronal sections between + 5 and - 7 mm relative to bregma and the volume of ischemic damage in Experiments 1 and 2. The extent of the infarct following ACA occlusion did not differ between Experiments 1 and 2.*

7.3.3.ii. Covert Orienting of Attention

7.3.3.ii.i. Reaction Time, Movement Time and Latency to Collect Reward

The reaction times to targets following valid cues were on average 24 ms (± 2.7 sem) faster than trials following invalid cues (Validity, $F(1,12) = 72.96$, $p < 0.0001$). There was an interaction between Validity and Foreperiod, with a significant validity effect at 200, 600 and 800 ms (Validity by Foreperiod, $F(3,36) = 4.09$, $p < 0.013$), but reaction time did not decrease with increasing foreperiod (Foreperiod, $F(3,36) = 1.4$, ns). Following surgery, the ischemic lesion group still responded to the targets preceded by valid cues significantly faster than targets preceded by invalid cues, and there was no

decrease or increase in the magnitude of the validity effect (Group by Surgery by Validity, $F(1,12) = 0.05$, ns; Group by Surgery by Validity by Foreperiod, $F(3,36) = 1.04$, ns). The ischemic lesion did, however, result in a global increase in reaction time (Figure 7.8), rising from 263 (± 12 sem) to 361 ms (± 29 sem) postsurgery, in contrast the reaction time of the control group did not change significantly (Group by Surgery, $F(1,12) = 6.44$, $p < 0.026$).

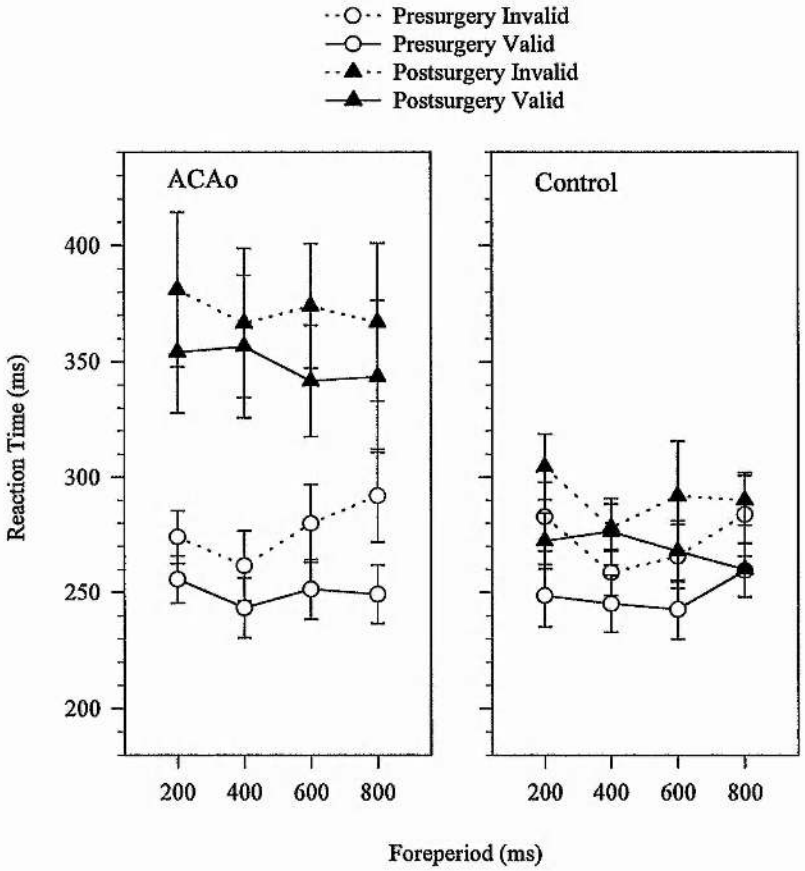


Figure 7.8. *Reaction time was significantly faster following a valid cue compared to an invalid cue. Postsurgery the magnitude of the validity effect did not change for either group. There was, however, a global increase in reaction time to initiate a response to the target by the ACA occlusion group postsurgery, this was irrespective of cue validity. The controls did not change significantly. The graph illustrates reaction time (mean ± sem, ACAo = 8, controls = 6) performance for each group.*

The increase in mean reaction time postsurgery was further analysed by examining the reaction time distribution illustrated in Figure 7.9. Mean reaction time

increased postsurgery for the ACA occlusion group due to a fall in the probability of a response at the modal reaction time and a 10 ms increase in the modal reaction time.

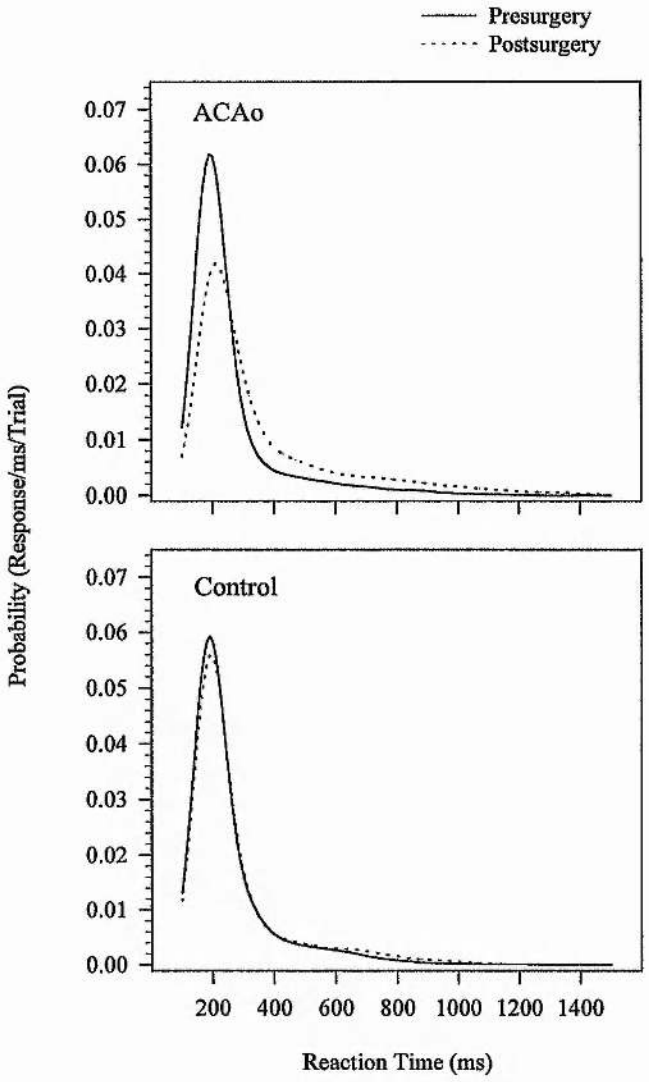


Figure 7.9. *The graph illustrates the reaction time distribution for the ACA occlusion and control groups pre- and postsurgery. It is evident that the fall in mean reaction time for the ACA occlusion group postsurgery was due to a fall in the probability of the fastest reaction times occurring.*

Movement time did not change following surgery (Group by Surgery, $F(1,12) = 1.54$, ns). But, there was an increase in the latency to move from the target hole to the food hopper postsurgery for the ACA occlusion group (Figure 7.10, Group by Surgery, $F(1,12) = 8.0$, $p < 0.015$).

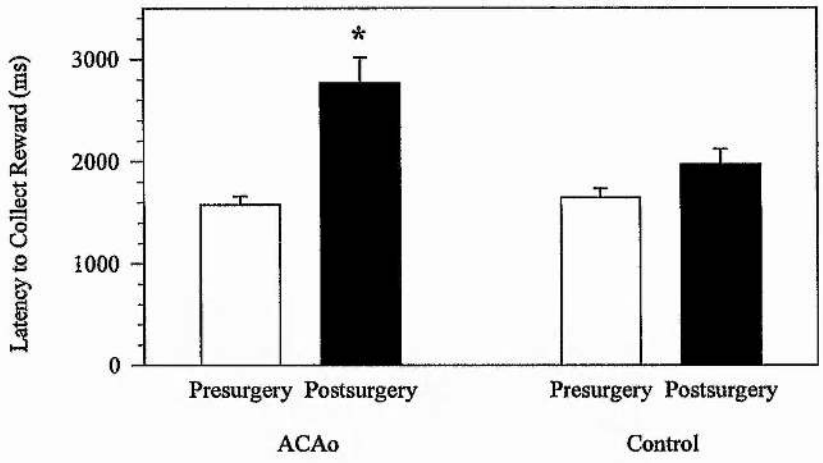


Figure 7.10. *Postsurgery the latency to move to the food hopper and collect the pellet reward increased significantly for the ACA occlusion group (the asterisk indicates the significant increase, $p < 0.05$). The figure shows the latency to collect a pellet (mean ± sem, ACAo = 8, controls = 6).*

To prevent the selective aborting of trials, trials in which an error occurred were not advanced until successfully completed. However, this raises the possibility that performance in a trial following an error could differ from trials correct on the first attempt. There was, however, no significant difference in the pattern of reaction time on trials following an error compared to trials correct on the first attempt (Trial Type), and furthermore the results did not interact with validity (Trial Type by Validity, $F(2,24) = 0.01$, ns) nor did they change as a result of surgery (Group by Surgery by Trial Type by Validity, $F(2,24) = 0.31$, ns; Group by Surgery by Trial Type, $F(2,24) = 2.3$, ns).

7.3.3.ii.ii. Accuracy

Postsurgery there was no increase in anticipatory errors (Group by Surgery, $F(1,12) = 1.05$, ns) or late errors (Group by Surgery, $F(1,12) = 2.71$, ns).

Trials in which the target light was preceded by an invalid cue, were more often incorrect than validly cued trials (Validity, $F(1,12) = 25.02$, $p < 0.0001$). Following surgery, the percentage of incorrect errors increased for the ACA occlusion group (Group

by Surgery, $F(1,12) = 4.99$, $p < 0.045$), but Figure 7.11 illustrates that this increase did not interact with validity (Group by Surgery by Validity, $F(1,12) = 1.65$, ns).

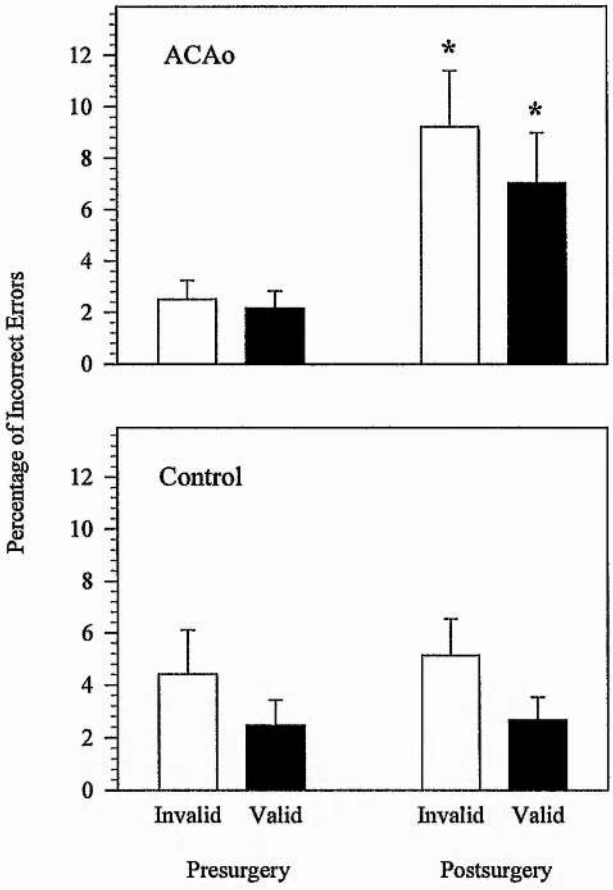


Figure 7.11. *The percentage of incorrect errors increased postsurgery for the ACA occlusion group, but remained unchanged for the control group (the asterisks indicate the significant increases, $p < 0.05$). The graph displays the percentage of incorrect errors (mean ± sem, ACAo = 8, controls = 6) for each group pre and postsurgery for Validity.*

7.3.4. Discussion

Attentional function was assessed in a choice reaction time task following ACA occlusion. Reaction time was faster when responding to the targets cued by valid rather than invalid cues, providing a reliable measure of attentional function. Occlusion of the ACA resulted in extensive damage throughout medial prefrontal cortex and caused a global increase in reaction time to targets irrespective of the validity of the preceding cue.

Movement time did not change significantly postoperatively, which taken together with the reaction time impairment suggests an impairment in response initiation, but intact motor execution. The postsurgery increase in mean reaction time was attributable to a decrease in the probability of the fastest reaction times. However, the ischemic lesion did not alter the magnitude of the validity effect, which indicated that the rats were still maintaining attention to the valid and invalid cues. In addition, the incidence of incorrect errors increased for the ischemic lesion group following surgery, suggesting difficulty in response selection.

7.4. General Discussion

In both experiments, occlusion of the rat ACA resulted in an infarct, which was confined to the vascular territory of the vessel. The resulting ischemic lesion extended through medial prefrontal cortex to include ventral (IL, Cg1 and Cg3) and dorsal anterior cingulate (Cg1 and Cg2), anteromedial Fr2 and orbitofrontal cortex (MO and VO), but did not include posterior lateral Fr2. Furthermore, the lesion extended subcortically to include the septum and vertical diagonal band of Broca which is also the origin of cholinergic neurons projecting to medial prefrontal cortex including anterior cingulate (Marston et al., 1994; Muir et al., 1996a). The two behavioural tasks employed revealed a pattern of intact and impaired performance which can be used to delineate the characteristics of a response impairment following ACA occlusion. Although the behavioural consequences of ACA occlusion have not been comprehensively investigated, the results could be compared with selective lesions of the cortical midline. Nevertheless, caution must be exercised, in consideration of the extent of the ACA lesion.

There was an increase in choice reaction time (covert orienting task) following ACA occlusion but not in simple reaction time (motivational task). However, the two tasks differed not only in being simple vs. choice reaction time but also in the modality of the imperative signal (auditory vs. visual respectively). Thus, it is possible that a sensory impairment in detecting the visual stimulus could account for the slowing in reaction time in the choice reaction time task. Nevertheless, the absence of any change in response vigour to the visual cues in the motivational task or in the covert orienting effect suggests that there was no difficulty discriminating visual cues per se. Furthermore, in the covert orienting task the peripheral attentional cues were dimmer than the imperative signal but

were still effective in directing attention following the lesion. Therefore it is unlikely that a visual deficit can account for the reaction time impairment. One further difference was the nature of the cues in the two tasks. In the choice reaction time task, the cues served as a 'ready' signal prior to the target, whereas in the simple reaction time task the cues were always present. Alivisatos & Milner (1989) have reported frontal patients were impaired in the use of a 'ready' signal. This is consistent with the current results.

The increase in reaction time in the choice reaction time task occurred without any increase in movement time. There was also an increased incidence of incorrect errors postoperatively. The results are consistent with the disruption of the central programming of movement, rather than a deficit in the execution of movement. However, contrary to this conclusion there was also an increase in the latency to collect reward in the covert orienting task. The increase in latency to collect reward could reflect impaired motor execution or even motivational deficits. The absence of any alteration in movement time following ACA occlusion would suggest that motor execution was intact. Furthermore, the results from the simple reaction time task (designed to test for deficits in motivation) has indicated that motivation remained intact. However, collecting reward in the latter task could be considered to be instrumental behaviour, as reward only became available once the food hopper was opened. In contrast, in the covert orienting task, reward was delivered into the food hopper when there was a correct response to a target, thus the response in the food hopper could be regarded as consummatory behaviour. Consequently the increase in the latency to collect reward in the covert orienting task may indicate an impairment in motivation under conditions of reward availability.

The results of occlusion of the ACA differ from those due to ischemic damage in the territory of the MCA which has also been investigated using the covert orienting task. While bilateral ACA occlusion has identified a response impairment, unilateral MCA occlusion caused only transient disturbance of contralateral motor execution, leaving response initiation intact (Ward et al., 1997, Experiment 4, *Assessment of sensorimotor neglect following occlusion of the middle cerebral artery: Chapter 6*). The results also differ from a dopamine depleting lesion of prefrontal cortex which impairs response initiation in a simple reaction time task (Hauber et al., 1994). However, the ischemic mediofrontal lesion left simple reaction time intact and impaired choice reaction time response. The results suggest that ACA occlusion disturbs the initiation of a response only when the task requires the selection of a response.

The ischemic damage includes dorsomedial prefrontal cortex which projects bilaterally to the dorsomedial striatum overlapping with the projection from visual cortex (McGeorge & Faull, 1989). There is no suggestion that this may indicate a modality specific disruption in the simple and choice reaction time tasks since the ischemic lesion also includes ventromedial prefrontal cortex which projects to ventromedial striatum overlapping with the projection from auditory cortex (McGeorge & Faull, 1989). Previous unilateral excitotoxic lesions of the dorsomedial striatum have resulted in response deficits including an increase in reaction time and a response bias (Brown & Robbins, 1989b). Thus the increase in reaction time and incorrect errors following ACA occlusion appears similar to the consequences of a lesion of dorsomedial striatum (Brown & Robbins, 1989b) to which dorsomedial prefrontal cortex projects.

There was no increase in the incidence of anticipatory responses following the ischemic lesion in either the simple or choice reaction time tasks. Previously, lesions of anterior cingulate have been reported to cause an increase in anticipatory responses in a vigilance, (signal detection) task (Muir et al., 1996b), which would support a role for anterior cingulate influencing response selection through response inhibition. Despite the extensive lesion to anterior cingulate in the current experiment the anticipatory error rate did not increase. However, the nature of so-called 'anticipatory' responding is quite different in the two tasks. The test of covert orienting was self-paced in terms of trial initiation, unlike the vigilance task of Muir et al. (1994) when the wait period for a target was much longer. Furthermore, in the choice reaction time task the occurrence and location of a response following anticipatory withdrawal from the central hole was not recorded, and thus any influence the cue may have had on 'time-out' responding was not recorded. Although the incidence of incorrect errors increased significantly, this was not selective for invalidly cued trials, which would have been expected if there were difficulty inhibiting a response to the cue.

Current proposals concerning the neural basis of motivational processes indicate the involvement of the limbic-basal ganglia circuitry in determining a motor response to stimulus-reward relationships (Robbins & Everitt, 1996). Although there was no evidence of motivational impairment, the results do not exclude the possibility that medial prefrontal cortex maybe involved when differential responses must be selected. With visual cues indicating reward removed performance reflected working memory for

the proximity of the last reward. However, the ACA occlusion group did not differ from controls in their response to progression through the schedule and so working memory appeared to be intact. The lack of a deficit may, reflect an insufficient temporal gap (as such being not sufficiently challenging to reveal a working memory deficit) between rewarded reference trials and further progression through the schedule, which was not under experimental control.

Recently Bussey et al. (1996) have demonstrated the involvement of anterior cingulate in the early stage of acquiring a conditional visual discrimination. That study also noted that following a lesion of anterior cingulate, responding continued under extinction conditions for a longer period when compared with controls or a more posterior lesion. Although the task differed in a number of respects to the current task, arguably the most important difference was the requirement to select a response according to the visual stimuli presented. Their task therefore required the acquisition of a new stimulus-response solution. In the current reversal task, however, it was a new stimulus-reward association which had to be learned. The cue reversal may thus have been not sufficiently challenging, given that it did not require any novel response selection or adaptation to novel stimuli. However, Dias et al. (1996) has demonstrated that lesions of orbitofrontal cortex in marmosets slows adaptation to a reversal in stimuli-reinforcement associations in an attentional set shifting procedure. In contrast the capacity to form a new stimulus-reward association was not impaired by the orbitofrontal lesion. The intact performance in the simple reaction time reversal reported here may have reflected the limited ischemic damage to the rat homologue of primate orbitofrontal cortex.

Granon et al. (1994) has reported a variety of impairments in spatial working memory tasks (matching-and nonmatching-to-sample in a T-maze) following medial prefrontal lesions. The impairments identified differed between the matching and nonmatching-to-sample tasks, performance accuracy in the matching-to-sample was impaired, while accuracy was intact in the nonmatching-to-sample task. It was proposed that the natural tendency for the rat to engage in a win-shift strategy makes the matching-to-sample task the more difficult of the two. Therefore the consequence of the prefrontal lesion was to disrupt response selection when greater effort is required to initiate a response. Bilateral occlusion of the ACA does, however, disrupt acquisition (Marston et al., in press) and trained performance (Marston et al., 1995b) in a delayed nonmatching-

to-position task in a Skinner box. Performance is poor across all delays but the impairment can be partially ameliorated at the earliest delays by acute drug treatment (Marston et al., in press). However, in a task examining spontaneous spatial exploration but not requiring explicit response selection a medial prefrontal lesion had no effect (Granon et al., 1996). Recently, questions have been raised about the validity of the nonmatching-to-position task (Chudasama & Muir, in press) making these results in need of verification.

In summary the behavioural consequences of ACA occlusion were assessed using a series of reaction time tasks to specify a profile of impaired and intact function. The ischemic lesion included medial prefrontal cortex and anterior basal forebrain. The extensive lesion did not disturb motivational and attentional processes, which were assessed in a cued multiple ratio schedule task and a test of covert orienting. However, reaction time and the incidence of incorrect responses increased in the choice but not simple reaction time tasks. The results appear similar to the consequences of medial prefrontal lesions which disrupt selection and initiation of responses.

Chapter 8:

General Discussion

8.1. Summary of Results

Occlusion of the MCA *(Experiment 4, Assessment of sensorimotor neglect following occlusion of the middle cerebral artery: Chapter 6)* and ACA *(Experiment 5, Simple and choice reaction time performance following occlusion of the anterior cerebral arteries: Chapter 7)* has been made possible using the potent vasoconstrictor endothelin-1, providing a flexible model with which to study the behavioural sequelae following focal cerebral ischemia. The model of MCA occlusion was validated in the Lister hooded rat, which although commonly employed in behavioural studies had not been used in studies of MCA occlusion *(Experiment 1, Middle cerebral artery occlusion in the Lister hooded and Sprague Dawley rat strains: Chapter 3).*

The behavioural consequences of ACA and MCA occlusion were explored using a variety of tasks including a test of somatosensory asymmetry (MCA), paw reaching (MCA), test of motivational response (ACA) and a test of covert orienting (MCA and ACA) adapted for the rat. The test of covert orienting involved peripheral visual valid and invalid cues, which directed and misdirected attention respectively to subsequent visual targets. Reaction time performance in the covert orienting test confirmed that targets preceded by valid cues were detected earlier than those preceded by invalid cues, resulting in a validity effect and measure of covert attentional function.

The primary consequence of MCA occlusion was a profile of impairment consistent with somatosensory dysfunction, the deficit observed was compatible with the involvement of Par1/2/FL in the lesion. The ischemic lesion following ACA occlusion involved medial prefrontal cortex and anteromedial basal forebrain and caused an impairment in response initiation and selection in the choice (covert orienting) but not simple (motivational) reaction time tasks. However, neither MCA nor ACA occlusion disturbed covert attention and the capacity to respond to motivationally salient cues remained intact following ACA occlusion. The neural basis to the covert orienting test was investigated further using an excitotoxic lesion of posterior parietal and a dopamine depleting lesion of the striatum. Both lesions resulted in deficits consistent with impaired response initiation but left covert attention intact. The effects of the lesions on reaction time performance in the covert orienting task are summarised in Table 8.1.

Chapter	Lesion	Deficit	Contralateral Valid	Contralateral Invalid	Ipsilateral Valid	Ipsilateral Invalid
4	Unilateral 6-OHDA CPu	Response initiation	↑	↑	-	-
5	Unilateral excitotoxin PPC	Response initiation	↑	↑	-	-
6	Unilateral MCAo Par1/2/FL		-	-	-	-
7	Bilateral ACAo mPFC	Response initiation	↑	↑	↑	↑

↑ Increase in reaction time; ↓ Decrease in reaction time; - No change in reaction time

Table 8.1. *A summary of changes in reaction time performance in the covert orienting task following different lesions employed in this thesis. The results are consistent with impairment of response initiation but intact covert attention (except following MCA occlusion, which transiently increased the execution of contralateral responses).*

The operant tasks which have been used during the course of testing have included a variety of chronometric and accuracy measures which permit specification of the characteristics of the impairments identified following each lesion. Previous behavioural testing in models of MCA occlusion have not used such a diverse range of indicators to assess task performance, relying instead on composite gross neurological scores indicating an unspecified impairment (Alexis et al., 1996; Bederson et al., 1986; Garcia et al., 1995; Persson et al., 1989). It has been possible using the 'Nine-hole box' apparatus to distinguish between impaired initiation or execution of a response. Furthermore, a breakdown of the different types of errors (incorrect, anticipatory and late) have permitted response selection (incorrect), inhibition (anticipatory) and stimulus detection (late) to be assessed. Further detail could also be obtained by extending the behavioural responses that are recorded to include actions outside of initial responses, such as 'time-out' responses, which can be used to highlight perseverative responding in the choice reaction time task.

The performance in the paw reaching and somatosensory asymmetry tests have augmented the results obtained in the covert orienting task permitting a profile of impairment to be identified following MCA occlusion and a lesion of posterior parietal. The paw reaching task identified distinct outcomes following each lesion. The ischemic injury to parietal cortex (Par1/2/FL) resulted in a reduction in successful contralateral reaches consistent with an impairment of somatosensory function. In contrast, a posterior parietal lesion caused a reduction of contralateral reaches but did not effect accuracy. The effect of a posterior parietal lesion was distinct from the consequences in primates which often include deficiencies in reaching accuracy and grasping. However, the results do suggest that posterior parietal contributes to the initiation of reaching. The results from the posterior parietal lesion in particular are consistent with an emphasis on action oriented systems, in which cortical organisation integrates sensory processes with action (Tipper et al., 1992).

8.2. In Vivo Focal Cerebral Ischemia: Diversifying Assessment

The objective in conducting the current research was to diversify the methods for evaluating neuroprotective agents by examining the consequences of focal cerebral ischemia with novel histopathological and behavioural approaches. This was achieved by using a novel strain of rat, immunohistochemistry, operant procedures and a newly developed model of focal cerebral ischemia.

The endothelin-1 model of MCA occlusion proved to be amenable to use with a new strain of rat (Lister hooded) in which the pattern of infarct involving lateral cortex and striatum was concordant with previous research in other strains. However, although lesion volume was not significantly different between the two strains, there was a nearly significant effect of strain for the striatal lesion. Furthermore, the extent of lesion could be assessed successfully by both cresyl violet and GFAP, if care was taken in calculating lesion volume. In particular GFAP suggested the presence of metabolic stress in the globus pallidus of rats with large striatal lesions, information which was not discernible from cresyl violet stained sections. A program of research establishing the comprehensive impact of a neuroprotective agent could benefit from such additional measures of tissue dysfunction. These findings were in concordance with previous research which has also emphasised the importance of factors such as strain, histology,

atrophy and edema when undertaking volumetric analysis. An increase in the diversity of histological and biochemical markers may also provide an opportunity to identify predictors of functional recovery.

The degree of similarity between impairments apparent following focal cerebral ischemia in humans and rats is important for demonstrating clearly the potential of treatments screened in the rat for therapeutic success in humans. In the series of experiments conducted for this thesis it was possible to demonstrate somatosensory (MCA occlusion) and response (ACA occlusion) impairments in the rat not unlike that previously seen in humans (see Tables 1.1 and 1.2). However, covert attention remained intact with no alteration in the magnitude of the validity effect *(Experiment 4, Assessment of sensorimotor neglect following occlusion of the middle cerebral artery: Chapter 6; Experiment 5, Simple and choice reaction time performance following occlusion of the anterior cerebral arteries: Chapter 7)*. Investigating covert attention and motivation in focal cerebral ischemia provided detailed testing of psychological processes not previously attempted. The results are important as they rule out impairments of attention and motivation following MCA and ACA occlusion in the rat. Furthermore, they might have more wide applicability for interpreting other deficits. For example, intact covert orienting following MCA occlusion suggests that impaired learning in the Morris Water Maze is not attributable to a failure to orient to maze cues.

Bilateral occlusion of the ACA has provided a new model of focal cerebral ischemia. An alternative in vivo model to MCA occlusion is a significant advance as it offers the opportunity to establish how reliably a treatment will provide neuroprotection. The simple reaction task used to examine the consequences of ACA occlusion illustrated intact performance concerning response initiation and motivational processes, despite extensive damage to medial cortex. Nonetheless, the results are informative when compared to the reaction time impairment found in the choice reaction time task (covert orienting). Additional testing which could be undertaken includes using the 5 choice serial reaction time task (analogous to the continuous performance test for humans) to assess sustained and divided attention. Performance in this test is already known to be disrupted by lesions of medial cortex (Muir et al., 1996).

The principle of systematic and thorough investigation of deficits could be extended to the somatosensory asymmetry test and paw reaching task, which identified

unilateral impairment following MCA occlusion. The use of video, with the ability to conduct kinematic analysis (Miklyaeva et al., 1994; Whishaw & Coles, 1996; Whishaw & Pellis, 1990; Whishaw et al., 1991) could prove useful for further investigation of the characteristics of both impairments. The data collection for the paw reaching task could be improved by discriminating between and recording different types of failed reaches. Thus, reaches which fail to make contact with a pellet may reflect a failure in the execution of a reach. in contrast reaches in which the forepaw is retracted to the mouth empty having contacted but never grasped a pellet or where pellets are grasped but then dropped may indicate a somatosensory failure. The use of force platforms may also provide an insight into changes in posture which may follow MCA occlusion.

Establishing a comprehensive understanding of the behavioural impairments which may follow focal cerebral ischemia in the rat remains an important objective if the optimal power for detecting neuroprotective agents is to be achieved. The principle that a reduction in infarct volume translates into a decline in the extent of neurological impairment is an important indicator that the arrest of neuropathological processes can provide functional benefit. Furthermore, the long term profile of recovery remains to be established in the tests currently used. There was only a transient impairment of movement execution in the MCA occlusion experiment, however, recovery of somatosensory function in paw reaching and the somatosensory asymmetry test remains unspecified beyond one month. In addition, following ACA occlusion the extent and rate of recovery for the choice reaction time impairment also remains to be investigated. The stability of impairment will be specifically important for assessing the non-acute intervention strategies such as nerve growth factors and grafts.

8.3. The Covert Orienting of Attention in the Rat

An initial attempt to study covert orienting in the rat was confounded by a failure to maintain the rat's head in a central location relative to the stimuli (Bushnell, 1995; Bushnell & Oshiro, 1994). Consequently, the test could only be used to demonstrate overt orienting. Fortunately, it has been possible to overcome this problem by using the 'Nine-hole box' apparatus, which consists of nose-poke holes that can be used to maintain the rat in a central location.

A test of covert orienting has been devised in parallel with the test reported here by Rosner & Mittleman (1996). Their task included a greater range of foreperiods (50 to 1500 ms), and also established a validity effect (~85-125 ms) which decreased with increasing foreperiod. The design is essentially similar to the test reported here using a response choice procedure. Although in the experiments conducted for this thesis the validity effect is smaller (24-61 ms) this is within the range reported in monkeys and humans (Petersen et al., 1987; Petersen et al., 1989; Posner et al., 1982; Rafal et al., 1988; Rafal et al., 1984). The difference in the magnitude of the validity effect reported by Rosner & Mittleman (1996) and the results reported here may be attributable to the overlap between the cue and target, which is present in Rosner & Mittleman's (1996) task but not the task reported here. Furthermore, the consequences of a posterior parietal lesion have been examined using both covert orienting tests with essentially similar results (see Rosner & Mittleman, 1996 *and Experiment 3, Covert orienting following excitotoxic lesions of posterior parietal cortex: Chapter 5*), suggesting that each test despite some design differences is assessing equivalent processes.

The covert orienting task designed for use with the rat in the series of experiments reported here shares a great deal of similarity with that employed in humans. The rat is able to undertake a test in which peripheral visuospatial cues predict the location of a subsequent target for 80 % of trials, a design which is analogous to that used with humans (Posner, 1980). However, there are also some differences, the covert orienting task used in this thesis involves a choice rather than a simple reaction time design. The choice reaction time task permits a directional response, allowing a response bias to be detected, but using this design also raises the possibility that response preparation could account for the validity effect. Evidence counter to this argument is provided in Experiment 2 *(Covert orienting in the rat: 1) Demonstration of the phenomenon, 2) The effects of striatal dopamine depletion: Chapter 4)*, which found a decrease in the validity effect with increasing foreperiod. Increasing foreperiod should permit better response preparation not a deterioration (Rosner & Mittleman, 1996). Moreover, the unilateral lesions *(Experiment 2, Covert orienting in the rat: 1) Demonstration of the phenomenon, 2) The effects of striatal dopamine depletion: Chapter 4; Experiment 3, Covert orienting following excitotoxic lesions of posterior parietal cortex: Chapter 5)* resulted in an impairment of response initiation without disrupting the ipsilateral validity effect, which is in part due to a contralateral invalid cue processed by the lesioned hemisphere. If motor preparation accounted for the validity

effect, then a failure to prepare a response should have been apparent from the decreased magnitude of the ipsilateral validity effect.

The validity effect with peripheral cues has been observed to decrease as foreperiod increases, which is believed to reflect the initial automatic grasping of attention by the cue, with the effect fading over time as attention moves back toward the centre. This effect was observed in Experiment 2 *(Covert orienting in the rat: 1) Demonstration of the phenomenon, 2) The effects of striatal dopamine depletion: Chapter 4)* and by Rosner & Mittleman (1996). However, where there is high expectation of the predictive value of a cue it may also be expected that a slower engagement of directed attention under endogenous control may occur at the later delays (Muller & Rabbitt, 1989). This may account for the validity effect at longer delays in subsequent experiments (*Experiment 3, Covert orienting following excitotoxic lesions of posterior parietal cortex: Chapter 5; Experiment 4, Assessment of sensorimotor neglect following occlusion of the middle cerebral artery: Chapter 6; Experiment 5, Simple and choice reaction time performance following occlusion of the anterior cerebral arteries: Chapter 7)*. The task employed in human studies has also illustrated some variability in the occurrence of a decreasing validity effect with increasing foreperiod (Petersen et al., 1989; 1987; Posner et al., 1984; Rafal et al., 1988; Robinson & Kertzman, 1995). The dynamics of this effect could be investigated by varying the probability of valid cues and examining performance over longer foreperiods than currently used. Further research could also be undertaken to investigate how attention moves across space by utilising all nine holes in the test apparatus to examine the effects of cues placed beyond and before the subsequent target in each hemispace over different foreperiods.

Lesions of rat posterior parietal and subsequently parietal did not result in any difficulty in disengaging attention. These results are contrary to those found in primates and humans in which the neural loci involved in attentional operations undertaken during covert orienting have been established *(see General Introduction: Chapter 1)*. The rat and primate visual systems do differ significantly. In primates receptor density from the centre to the periphery of the retina decreases by the order of 300:1. Consequently the primate visual system performs two operations through detection of targets in peripheral vision and orienting to permit identification using foveal vision. This contrasts with the retina of the rat over which the receptor density varies by only 5:1. It is questionable that

the visual system would be organised with the same objectives as in the primate. Species specific behaviour would also suggest a different organisation would be appropriate for the rat. The rat is dependent on olfactory (Whishaw & Tomie, 1989) and tactile senses and has relatively poor vision. However, orienting behaviour in the rat would still be important to facilitate movement toward visual stimuli to investigate through olfactory and tactile modalities (Goodale & Carey, 1990) and so does not negate the role of visual orienting.

8.4. Future Research Using the Covert Orienting Task for the Rat

The response requirements of the task could be altered. The task could be simplified, with only withdrawal of the head from the central hole to report detection of the target. Conversion of the covert orienting test to a simple reaction time task would provide further evidence that the validity effect does not reflect movement preparation in response to the cue, since response would not differ according to the cue in the simple reaction time version of the task. This manipulation could also prove important for clarifying the consequences of ACA occlusion which resulted in a response impairment in a choice reaction time (covert orienting task) but not a simple reaction time (motivational) task. The task designs were unfortunately not directly comparable. The simple reaction time design would also allow systemic drug administration as a method of evaluating the involvement of selective neurotransmitters.

The lesions used in this thesis have primarily involved excitotoxic mechanisms of cell death (*Experiment 3, Covert orienting following excitotoxic lesions of posterior parietal cortex: Chapter 5; Experiment 4, Assessment of sensorimotor neglect following occlusion of the middle cerebral artery: Chapter 6; Experiment 5, Simple and choice reaction time performance following occlusion of the anterior cerebral arteries: Chapter 7)*, however, the manipulation of neurochemical systems initially using systemically administered agonists and antagonists may direct future research into the contribution of dopaminergic, noreadrenergic, serotonergic and cholinergic systems. This approach has made considerable progress in specifying the contribution of each system in vigilance tasks (Brockel & Fowler, 1995; Carli et al., 1983; Cole & Robbins et al., 1989; Holley et al., 1995; Jones & Higgins, 1995; McGaughty & Sarter, 1995; Muir et al., 1994; Muir et

al., 1995; Steckler & Sahgal et al., 1995; Turchi et al., 1995) and has subsequently been followed by restricted cortical lesions selected according to the neurochemical innervation of the cortex (Muir et al., 1996; Miner et al., 1997; Sarter et al., 1996).

Previous research examining the contribution of the cholinergic system has found that nicotine, a cholinergic agonist decreases the magnitude of the validity effect (Marrocco & Witte, 1993; Witte & Marrocco, 1993), which is the opposite effect of Alzheimer's disease on covert orienting (Parasuraman et al., 1992). While noreadrenaline contributes to arousal and an alerting response, and might be expected to be involved in modulating the orienting response to cues, which may be distinct from its role in sustained attention (Marrocco et al., 1994). Manipulating arousal could be undertaken to challenge the rats further by varying the duration and/or brightness of the cue or target, which will increase the task difficulty and assess the capability of the cue to grasp attention. This strategy has been successfully employed by Carli et al. (1983) to validate the 5-choice serial reaction time task in rats and has revealed impairments which initially were not apparent in a treatment control group during testing to assess the success of cholinergic grafts (Muir et al., 1992).

Covert attention was examined following striatal dopamine depletion, the primary consequence was slowed response initiation but intact covert attention and was in agreement with previous experiments examining vigilance following manipulation of the dopaminergic system, which have also indicated a role mediating motor output but not attentional processes (McGaughty & Sarter, 1995). Haloperidol has been found to increase reaction time and errors of omission in a sustained attention task, which was reversed by amphetamine, this impairment was interpreted as evidence for both motor and attentional impairment (Brockel & Fowler, 1995). Amphetamine administered alone has also been found to increase errors of omission and reduce reaction time (Steckler & Sahgal, 1995). There is evidence suggesting that dopamine and noreadrenaline may be important for maintaining attention during covert orienting to central (Clark et al., 1989) and peripheral cues (Witte et al., 1992). It is clear that striatal dopamine depleting lesions in the rat do not appear to alter covert attention. Moreover, although the neural site of action of dopamine is not yet established it has been suggested that the dopaminergic prefrontal innervation could be important (Wright et al., 1990; Yamada et al., 1990). Counter to this suggestion, Experiment 5 *(Simple and choice reaction time performance*

following occlusion of the anterior cerebral arteries: Chapter 7) did not alter attentional function while causing extensive damage to prefrontal cortex in the rat that receives a dopaminergic input (Berger et al., 1976; Lindvall et al., 1974).

The development of the touch sensitive screen for rodent testing (Bussey et al., 1996; Steckler & Sahgal, 1995) will provide a particularly flexible apparatus with which to further investigate the covert orienting task, permitting symbolic cues (central cues) and variations in the vertical and horizontal planes of stimuli presentation. The ability to vary the plane of stimulus presentation will prove particularly important for studying the effects of a lesion of the superior colliculus in which the greatest deficit has been observed for the vertical orientation (Posner et al., 1982; Rafal et al., 1988). Although the ideal method for maintaining the rats head in a central and perpendicular orientation relative to the screen will require investigation and may require the addition of a nose poke hole similar to those used in the 'Nine-hole box'. The extent of similarity between the test designed for the rat and the equivalent in use with humans permits ease of comparison between species, however, Steckler & Muir (1996) have emphasised that within this concept the task design should take account of morphological differences between species to allow optimal performance (D'Mello & Steckler, 1996). Therefore, careful consideration will also have to be given to the position and type of central cues which are used, which must take into account the visual field of the rat.

The development of an endogenously controlled version of the task will raise important issues in attempting comparative studies between rats and humans. The extent of practice remains an important factor as humans typically undertake 200+ trials (Petersen et al., 1989; Posner et al., 1984), while the rat undertakes staged training for operant tasks before completing hundreds of practice trials before testing. This raises the possibility that the rat may conduct the task with a different degree of cognitive control (Steckler & Muir 1996). The importance of this difference in covert orienting is already suggested by comparative studies between humans and monkeys (Bowman et al., 1993; Witte et al., 1997). There are differences between humans and monkeys on the endogenous covert orienting test, emphasising that the degree of cognitive control in covert orienting varies across species (Bowman et al., 1993; Witte et al., 1996). Although monkeys are able to use symbolic cues to direct attention, unlike humans they appear insensitive to changes in the probability of valid peripheral cues (Bowman et al., 1993).

The ability of rats to direct attention under endogenous control remains to be established. One prominent issue in investigating the degree of cognitive control is likely to be training history and the extent to which responses have become automatic as a result of training (Bowman et al., 1993) which may confound demonstrating cognitive control in the task.

8.5. Simple Reaction Time Performance, Under a Visually Cued Multiple-Ratio Schedule of Reinforcement

The simple reaction time task employed in Experiment 5 *(Simple and choice reaction time performance following occlusion of the anterior cerebral arteries: Chapter 7)* has been developed recently for use in the monkey (Bowman et al., 1996) and then adapted for the rat (Bowman et al., 1996; Brown et al., 1996). The task was designed to dissociate motivation from motor response. A prominent difficulty in studying processes of appetitive motivation has been task design, which has relied on the testing environment requiring increasing motor output to attain a goal and thereby infer motivation from the trade-off between action and the availability of reward. The simple reaction time task currently used provides the benefit that motor response is held constant during the task, while the visual cues indicate proximity of reward. Preoperative performance illustrated increased vigour and accuracy in response to the cues, which was consistent with previous testing in the monkey (Bowman et al., 1996). Subsequent modification of the task by removing or reversing the meaning of visual cues also successfully illustrated alterations in behavioural performance consistent with self initiated adaptation to the changing motivational information available in the tasks.

The response in the task would be expected to involve a hypothesised limbic-motor interface. However, lesions of the nucleus accumbens (Bowman & Brown, 1996) or medial prefrontal cortex after ACA occlusion *(Experiment 5, Simple and choice reaction time performance following occlusion of the anterior cerebral arteries: Chapter 7)* do not affect motivational performance in the task used. Unfortunately the neural systems which assign motivational valence and determine vigour of response to the visual cues remain unknown. However, the task has previously been used successfully to investigate the effects of systemic amphetamine, which was demonstrated to decrease reaction time in a manner consistent with the increasing motor readiness observed with lengthening foreperiod. Amphetamine did not alter the effect of the cues on motivation,

which displayed a pattern independent of either the effect of amphetamine or foreperiod (Brown et al., 1996). Previous tasks have been unable to clearly make this distinction.

8.6. Conclusion

The thesis has adapted a number of original techniques to explore both the consequences of focal cerebral ischemia and the neural basis to covert attention and motivation in the rat. Endothelin-1 was used to occlude the middle cerebral artery, proving to be an adaptable technique across different strains of rat and permitting occlusion of the ACA. Previous experiments examining the consequences of focal cerebral ischemia have focused on sensorimotor or memory performance employing maze tasks. The results of the current series of experiments has extended the range of tests used to examine the consequences of focal cerebral ischemia to include tests of reaction time with a test of covert orienting which is comparable with that employed in humans and a recently devised test of motivational function. Although there was no disruption to covert attention or motivation following focal cerebral ischemia the operant tasks were able in conjunction with the other tests employed to specify a profile of impaired and intact function. However, the sensorimotor tasks which have identified unilateral impairment could be investigated further to distinguish more clearly somatosensory and motor dysfunction. The potential for adapting the covert orienting test to both validate and extend understanding of covert attention in the rat remains high, and should provide a useful model with which to investigate covert attention, which until recently has been a research exercise restricted to primates.

References

Agid. Y., Ruberg, M., Raisman, R., Hirsch, E., & Javoy-Agid, F. (1990). The Biochemistry of Parkinson's Disease. In **Parkinson's Disease,** Stern, G.M. (Ed), London, Chapman and Hall Medical, pp 99-125.

Alexander, M.P., Baker, E., Naeser, M.A., Kaplan, E., & Palumbo, C. (1992). Neuropsychological and neuroanatomical dimensions of ideomotor apraxia. **Brain**, 115, 87-107.

Alexander, M.P., Naeser, M.A., & Palumbo, C. (1987). Correlations of subcortical CT lesion sites and aphasia profiles. **Brain**, 110, 961-991.

Alexander, M.P., & Schmitt, M.A. (1980). The aphasia syndrome of stroke in the left anterior cerebral artery territory. **Archives of Neurology**, 37, 97-100.

Alexis, N.E., Back, T., Zhao, W., Dietrich, W.D., Watson, B.D., & Ginsberg, M.D. (1996). Neurobehavioral consequences of induced spreading depression following photothrombotic middle cerebral artery occlusion. **Brain Research**, 706, 273-282.

Alivisatos, B., & Milner, B. (1989). Effects of frontal or temporal lobectomy on the use of advance information in a choice reaction time task. **Neuropsychologia**, 27, 495-503.

Andersen, C.S., Andersen, A.B., & Finger, S. (1991). Neurological correlates of unilateral and bilateral "strokes" of the middle cerebral artery in the rat. **Physiology and Behavior**, 50, 263-269.

Anderson, R.A. (1987). Inferior parietal lobule function in spatial perception and visuomotor integration. In Mountcastle, V.B., Plum F., & Geiger, S.R. (Eds), **Handbook of Physiology, Section I, The Nervous System**, Bethesda MD, American Physiological Society, pp 483-518.

Bannister, R. (1969). Disorders of the Cerebral Circulation. **Brain's Clinical Neurology** (3rd ed), London, Oxford Medical Publications, pp 213-239.

Barbour, B., Brew, H., & Attwell, D. (1988). Electrogenic glutamate uptake in glial cells is activated by intracellular potassium. **Nature**, 335, 433-435.

Barone, F.C., Clark, R.K., Price, W.J., White, R.F., Feuerstein, G.Z., Storer, B.L., & Ohlstein, E.H. (1993). Neuron-specific enolase increases in cerebral and systemic circulation following focal ischemia. **Brain Research**, 623, 77-82.

Barth, T.M., Jones, T.A., & Schallert, T. (1990). Functional subdivisions of the rat somatic sensorimotor cortex. **Behavioural Brain Research**, 39, 73-95.

Bartus, R.T., Baker, K.L., Heiser, A.D., Sawyer, S.D., Dean, R.L., Elliott, P.J., & Straub, J.A. (1994). Postischemic administration of AK275, a calpain inhibitor, provides substantial protection against focal ischemic brain damage. **Journal of Cerebral Blood Flow and Metabolism**, 14, 537-544.

Bassetti, C., Bogousslavsky, J., & Regli, F. (1993). Sensory syndromes in parietal stroke. **Neurology**, 43, 1942-1949.

Bederson, J.B., Pitts, L.H., Tsuji, M., Nishimura, M.C., Davis, R.L., & Bartkowski, H. (1986). Rat middle cerebral artery occlusion: Evaluation of the model and development of a neurologic examination. **Stroke**, 17, 472-476.

Bennett, K.M.B., Waterman, C., Scarpa, M., & Castiello, U. (1995). Covert visuospatial attentional mechanisms in Parkinson's disease. **Brain**, 118, 152-166.

Berger, B., Thierry, A.M., Tassin, J.P., & Moyne, M.A. (1976). Dopaminergic innervation of the rat prefrontal cortex: Fluoresence histochemical study. **Brain Research**, 106, 133-145.

Bignami, A. (1991). Glial cells in the central nervous system. **Discussions in Neuroscience**, 8, 11-45.

Bisiach, E., Capitani, E., Luzzatti, C., & Perani, D. (1981). Brain and conscious representation of outside reality. **Neuropsychologia**, 19, 543-551.

Bisiach, E., & Luzzatti, C. (1978).Unilateral neglect of representational space. **Cortex**, 14, 129-133.

Block, F., & Kunkel, M., & Scharwz, M. (1993). Quinolinic acid lesion of the striatum induces impairment in spatial learning and motor performance in rats. **Neuroscience Letters**, 149, 126-128.

Bogousslavsky, J. (1994). Frontal Stroke Syndromes. **European Neurology**, 34, 306-315.

Bogousslavasky, J., Martin, R., & Moulin, T. (1992). Homolateral ataxia and crural paresis: A syndrome of anterior cerebral artery territory infarction. **Journal of Neurology, Neurosurgery and Psychiatry**, 55, 1146-1149.

Bogousslavasky, J., & Regli, F. (1990). Anterior cerebral artery territory infarction in the Lausanne Stroke Registry: Clinical and eitological patterns. **Archives of Neurology**, 47, 144-150.

Bogousslavasky, J., Van Melle, G., & Regli, F. (1988). The Lausanne Stroke Registry: Analysis of 1,000 consecutive patients with first stroke. **Stroke**, 19, 1083-1092.

Borlongan, C.V., Martinez, R., Shytle, R.D., Freeman, T.B., Cahill, D.W., & Sanberg, P.R. (1995). Striatal Dopamine-Mediated Behavior is altered following occlusion of the middle cerebral artery. **Pharmacology Biochemistry and Behavior**, 52, 225-229.

Bowman, E.M., Aigner, T.G., & Richmond, B.J. (1996). Neural signals in the monkey ventral striatum related to motivation for juice and cocaine rewards. **Journal of Neurophysiology**, 75, 1061-1073.

Bowman, E.M., & Brown, V.J. (1996). Lesions of the rat ventral striatum change performance in a progressive fixed-ratio schedule of reinforcement without affecting reaction times when visual cues indicate reward cost. **Society for Neuroscience Abstracts**, 22, part 1, 446.

Bowman, E.M., Brown, V.J., Kertzman, C., Schwarz, U., & Robinson, D.L. (1993). Covert orienting of attention in macaques I. Effects of behavioral context. **Journal of Neurophysiology**, 70, 431-443.

Bradshaw, J.L., Waterfall, M.L., Phillips, J.G., Iansek, R., Mattingley, J.B., & Bradshaw, J.A. (1993). Re-orientation of attention in Parkinson's disease: An extension to vibrotactile modality. **Neuropsychologia**, 31, 51-66.

Brady, J.V., & Nauta, W.J.H. (1953). Subcortical mechanisms in emotional behavior: Affective changes following septal forebrain lesions in the albino rat. **Journal of Comparative Physiological Psychology**, 46, 339-346.

Breese, G.R., & Traylor, T.D. (1971). Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. **British Journal of Pharmacology**, 42, 88-99.

Brockel, B.J., & Fowler, S.C. (1995). Effects of chronic haloperidol on reaction time and errors in a sustained attention task: Partial reversal by anticholinergics and by amphetamine. **Journal of Pharmacology and Experimental Therapeutics**, 275, 1090-1098.

Brown, V.J., Bowman, E.M., & Robbins, T.W. (1991). Response-related deficits following unilateral lesions of the medial agranular cortex of the rat. **Behavioral Neuroscience**, 105, 567-578.

Brown, V.J., Brasted, P.J., & Bowman, E.M. (1996). The effects of systemic d-amphetamine on motor versus motivational processes in the rat. **Psychopharmacology**, 128, 171-180.

Brown, V.J., & Robbins, T.W. (1989a). Deficits in response space following unilateral striatal dopamine depletion in the rat. **Journal of Neuroscience**, 9, 983-989.

Brown, V.J., & Robbins, T.W. (1989b). Elementary processes of response selection mediated by distinct regions of the striatum. **Journal of Neuroscience**, 9, 3760-3765.

Brown, V.J., Schwarz, U., Bowman, E.M., Fuhr, P., Robinson, D.L., & Hallett, M. (1993). Dopamine dependent reaction time deficits in patients with Parkinson's disease are task specific. **Neuropsychologia**, 31, 459-469.

Brust, J.C.M. (1991). Cerebral Circulation: Stroke, In E.R. Kandel, J.H. Schwartz, & T.M. Jessell, (Eds), **Principles of Neural Science** (3rd ed), CT, Appleton and Lange, pp 1041-1049.

Brust, J.C.M., Plank, C., Burke, A., Guobadia, M.M.I., & Healton, E.B. (1982). Language disorder in a right-hander after occlusion of the right anterior cerebral artery. **Neurology**, 32, 492-497.

Buisson, A., Margaill, I., Callebert, J., Plotkine, M., & Boulu, R.G. (1993). Mechanisms involved in the neuroprotective activity of a nitric oxide synthase inhibitor during focal cerebral ischemia. **Journal of Neurochemistry**, 61, 690-696.

Bushnell, P.J. (1995). Overt orienting in the rat: Parametric studies of cued detection of visual targets. **Behavioral Neuroscience**, 109, 1095-1105.

Bushnell, P.J., & Oshiro, W.M. (1994). Overt orienting in the rat: Validation of methods and the effects of cholinergic drugs on selective attention. **Society for Neuroscience Abstracts**, 20, 577.

Bussey, T.J., Muir, J.L., Everitt, B.J., & Robbins, T.W. (1996). Dissociable effects of anterior and posterior cingulate cortex lesions on the acquisition of a conditional visual discrimination: Facilitation of early learning vs. impairment of late learning. **Behavioural Brain Research**, 82, 45-56.

Caplan, L.R., & Stein, R.W. (1986). Large-Vessel Occlusive Disease of the Anterior Circulation. In Caplan, L.R., & Stein, R.W. (Eds). **Stroke A Clinical Approach**, Boston MA, Buttersworth, pp 123-138.

Carli, M., Evenden, J. L., & Robbins, T. W. (1985). Depletion of unilateral striatal dopamine impairs initiation of contralateral actions and not sensory attention. **Nature**, 313, 679-682.

Carli, M., Jones, G.H., & Robbins, T.W. (1989). Effects of unilateral dorsal and ventral striatal dopamine depletion on visual "neglect" in the rat: A neural and behavioural analysis. **Neuroscience**, 29, 309-327.

Carli, M., Robbins, T.W., Evenden, J.L., & Everitt, B.J. (1983). Effects of lesions to ascending noradrenergic neurones on performance of a 5-choice serial reaction time task in rats: Implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. **Behavioural Brain Research**, 9, 361-380.

Castro, A.J. (1972). The effects of cortical ablations on digital usage in the rat. **Brain Research**, 37, 173-185.

Chan, P.H. (1994). Oxygen radicals in focal cerebral ischemia. **Brain Pathology**, 4, 59-65.

Chan, J.L., & Ross, E.D. (1988). Left-handed mirror writing following right anterior cerebral artery infarction: Evidence for nonmirror transformation of motor programs by right supplementary motor area. **Neurology**, 38, 59-63.

Chandler, H.C., King, V., Corwin, J.V., & Reep, R.L. (1992). Thalamocortical connections of rat posterior parietal cortex. **Neuroscience Letters**, 143, 237-242.

Chapin, J.K., & Lin, C.S. (1990). The somatic sensory cortex of the rat. In Kolb, B. & Tees, R.C. (Eds), **The Rat Cerebral Cortex**, Cambridge MA, MIT Press, pp 341-380.

Chen, H., Chopp, M., & Welch, K.M.A. (1991). Effect of mild hyperthermia on the ischemic infarct volume after middle cerebral artery occlusion in the rat. **Neurology**, 41, 1133-1135.

Chen, J., Graham, S., Moroni, F., & Simon, R. (1993). A study of the dose dependency of a glycine receptor antagonist in focal ischemia. **Journal of Pharmacology and Experimental Therapeutics**, 267, 937-941.

Chiamulera, C., Terron, A., Reggiani, A., & Cristofori, P. (1993). Qualitative and quantitative analysis of the progressive cerebral damage after middle cerebral artery occlusion in mice. **Brain Research**, 606, 251-258.

Cho, Y.H., & Kesner, R.P. (1996). Involvement of entorhinal cortex or parietal cortex in long-term spatial discrimination memory in rats: Retrograde amnesia. **Behavioral Neuroscience**, 110, 436-442.

Chudasama, Y., & Muir, J.L. (in press). A behavioural analysis of the delayed nonmatching-to-position task: The effects of scopolamine, lesions of the fornix and of the prelimbic region on mediating behaviours by rats. **Psychopharmacology**.

Clark, C.R., Geffen, G.M., & Geffen, L.B. (1989). Catecholamines and the covert orienting of attention in humans. **Neuropsychologia**, 27,131-139.

Cole, B.W., & Robbins, T.W. (1989). Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi on performance of a 5 choice serial reaction time task in rats: Implications for theories of selective attention and arousal. **Behavioural Brain Research**, 33, 165-179.

Corwin, J.V., Burcham, K.J., & Hix, G.I. (1996). Apomorphine produces an acute dose-dependent therapeutic effect on neglect produced by unilateral destruction of the posterior parietal cortex in rats. **Behavioural Brain Research**, 79, 41-49.

Corwin, J.V., Fussinger, M., Meyer, R.C., King, V.R., & Reep, R.L. (1994). Bilateral destruction of the ventrolateral orbital cortex produces allocentric but not egocentric spatial deficits in rats. **Behavioural Brain Research**, 61, 79-86.

Côté, S.L., Ribeiro-da-Silva, A., & Cuello, A.C. (1993). Current Protocols for Light Microscopy Immunocytochemistry. In Cuello, A.C. (Ed). **Immunohistochemistry II. IBRO Handbook Series: Methods in the Neurosciences**, Vol 14, Chichester, John Wiley and Sons Ltd, pp 147-168.

Critchley, M. (1930). The anterior cerebral artery and its syndromes. **Brain**, 53, 120-165.

Crowne, D.P., Novotny, M.F., Maier, S.E., & Vitols, R. (1992). Effects of unilateral parietal lesions on spatial localization in the rat. **Behavioral Neuroscience**, 106, 808-819.

Crowne, D.P., Richardson, C.M., & Dawson, K.A. (1987). Lateralization of emotionality in right parietal cortex of the rat. **Behavioral Neuroscience**, 101, 134-138.

Crowne, D.P., Richardson, C.M., & Dawson, K.A. (1986). Parietal and frontal eye field neglect in the rat. **Behavioural Brain Research**, 22, 227-231.

Daffner, K.R., Ahern, G.L., Weintraub, S., & Mesulam, M.M. (1990). Dissociated neglect following sequential strokes in the right hemisphere. **Annals of Neurology**, 28, 97-101.

Dalkara, T., Yoshida, T., Irikura, K., & Moskowitz, M.A. (1994). Dual role of nitric oxide in focal cerebral ischemia. **Neuropharmacology**, 33, 1447-1452.

Dawson, D.A. (1994). Nitric oxide and focal cerebral ischemia: Multiplicity of actions and diverse outcome. **Cerebrovasculature and Brain Metabolism Reviews**, 6, 299-324.

De Bruin, J.P.C., Sanchez-Santed, F., Heinsbroek, R.P.W., Donker, A., & Postmes, P. (1994). A behavioral analysis of rats with damage to the medial prefrontal cortex using the morris water maze: Evidence for behavioral flexibility, but not for impaired spatial navigation. **Brain Research**, 652, 323-333.

de Courtney-Myers, G.M., Kleinholz, M., Wagner, K.R., & Myers, R.E. (1994). Normoglycemia (not hypoglycemia) optimizes outcome from middle cerebral artery occlusion. **Journal of Cerebral Blood Flow and Metabolism**, 14, 227-236.

Degos, J.D., Dafonseca, N., Gray, F., & Cesaro, P. (1993). Severe frontal syndrome associated with infarcts of the left anterior cingulate gyrus and the head of the right caudate nucleus - a clinicopathological case. **Brain**, 116, 1541-1548.

Dias, R., Robbins, T.W., & Roberts, A.C. (1996). Dissociation of prefrontal cortex of affective and attentional shifts. **Nature**, 380, 69-72.

D'Mello, G.D., & Steckler, T. (1996). Animal models in cognitive behavioural pharmacology: An overview. **Cognitive Brain Research**, 3, 345-352.

Derouesne, C., Mas, J.L., Bolgert, F., & Castaigne, P. (1984). Pure sensory stroke caused by a small cortical infarct in the middle cerebral artery territory. **Stroke**, 15, 660-662.

De Renzi, E., & Barbieri, C. (1992). The incidence of the grasp reflex following hemispheric lesion and its relation to frontal damage. **Brain**, 115, 293-313.

De Renzi, E., Faglioni, P., Lodesani, M., & Vecchi, A. (1983). Performance of left brain-damaged patients on imitation of single movements and motor sequences. Frontal and parietal-injured patients compared. **Cortex**, 19, 333-343

De Ryck, M., Van Reempts, J., Borgers, M., Wauquier, A., & Janssen, P.A.J. (1989). Photochemical stroke model: Flunarizine prevents sensorimotor deficits after neocortical infarcts in rats. **Stroke**, 20, 1383-1390.

De Ryck, M., Van Reempts, J., Duytschaever, H., Van Deuren, B., & Clincke, G. (1992). Neocortical localization of tactile/proprioceptive limb placing reactions in the rat. **Brain Research**, 573, 44-60.

Deuel, R.K., & Farrar, C.A. (1993). Stimulus cancellation by macaques with unilateral frontal or parietal lesions. **Neuropsychologia**, 31, 29-38.

Devinsky, O., Morrell, M.J., & Vogt, B.A. (1995). Contributions of anterior cingulate cortex to behaviour. **Brain**, 118, 279-306.

DiMattia, B.D., & Kesner, R.P. (1988a). Spatial cognitive maps: Differential role of parietal cortex and hippocampal formation. **Behavioral Neuroscience**, 102, 471-480.

DiMattia, B.D., & Kesner, R.P. (1988b). Role of the posterior parietal association cortex in the processing of spatial event information. **Behavioral Neuroscience**, 102, 397-403.

Dirnagl, U., Tanabe, J., & Pulsinelli, W. (1990). Pre- and post-treatment with MK-801 but not pretreatment alone reduces neocortical damage after focal cerebral ischemia in the rat. **Brain Research**, 527, 62-68.

Donnan, G.A., Bladin, P.F., Berkovic, S.F., Longley, W.A., & Saling, M.M. (1991). The stroke syndrome of stiatocapsular infarction. **Brain**, 114, 51-70.

Dubinsky, J.M. (1993). Examination of the role of calcium in neuronal death. **Annals of the New York Academy of Sciences**, 679, 34-42.

Dunnett, S.B., & Iversen, S.D. (1982). Sensorimotor impairments following localized kainic acid and 6-hyrdroxydopamine lesions of the neostriatum. **Brain Research**, 248, 121-127.

Dunnett, S.B., Isacson, O., Sirinathsinghji, D.J.S., Clarke, D.J., & Bjorklund, A. (1988). Striatal grafts in rats with unilateral neostriatal lesions. III. Recovery from dopamine-dependent motor asymmetry and deficits in skilled paw reaching. **Neuroscience**, 24, 813-820.

Duverger, D., & MacKenzie E.T. (1988). The quantification of cerebral infarction following focal ischemia in the rat: Influence of strain, arterial pressure, blood glucose concentration, and age. **Journal of Cerebral Blood Flow and Metabolism**, 8, 449-461.

Eddleston, M. & Mucke, L. (1993). Molecular profile of reactive astrocytes-implications for their role in neurologic disease. **Neuroscience**, 54, 15-36.

Eriksen, C.W., & Hoffman, J.E. (1972). Temporal and spatial characteristics of selective encoding from visual displays. **Perceptual Psychophysics**, 12, 201-204.

Eslinger, P.J., & Grattan, L.M. (1993). Frontal lobe and frontal striatal substrates for different forms of human cognitive flexibility. **Neuropsychologia**, 31, 17-28.

Fahn, S. (1986). Parkinson's Disease and Other Basal Ganglion Disorders. In **Diseases of the Nervous System: Clinical Neurobiology**, Volume II, Asbury, A.K., McKhann, G.M., & McDonald, W.I. (Eds), London, William Heinemann Medical Books, pp 1217-1228

Finger, S., & Frommer, G.P. (1967). Effects of somatosensory thalamic and cortical lesion on roughness discrimination in the albino rat. **Physiology and Behavior**, 3, 83-89

Folbergrova, J., Memezawa, H., Smith, M., & Siesjo, B.K. (1992). Focal and perifocal changes in tissue energy state during middle cerebral artery occlusion in normo- and hyperglycemic rats. **Journal of Cerebral Blood Flow and Metabolism**, 12, 25-33.

Foreman, N., Save, E., Thinus-Blanc, C., & Buhot, M.C. (1992). Visually guided locomotion, distractibility, and the missing-stimulus effect in hooded rats with unilateral or bilateral lesions of parietal cortex. **Behavioral Neuroscience**, 106, 529-538.

Freedman, M., Alexander, M.P., & Naeser, M.A. (1984). Anatomic basis of transcortical motor aphasia. **Neurology**, 34, 409-417.

Fuxe, K., Kurosawa, N., Cintra, A., Hallstrom, A., Goiny, M., Rosen, L., Agnati, L.F., & Ungerstedt, U. (1992). Involvement of local ischemia in endothelin-1 induced lesions of the neostriatum of the anaesthetised rat. **Experimental Brain Research**, 88, 131-139.

Galea, E., Feinstein, D.L., & Reis, D.J. (1992). Induction of calcium-independent nitric oxide synthase activity in primary rat glial cultures. **Proceedings of the National Academy of Sciences USA**, 89, 10945-10949.

Garcia J.H., Yoshida, Y. Chen, H., Zheng, Y.L., Zhang, G., Lian, J. Chen, S., & Chopp, M. (1993). Progression from ischemic-injury to infarct following middle cerebral artery occlusion in the rat. **American Journal of Pathology**, 142, 623-635.

Garcia, J.H., Wagner, S., Liu, K.F., & Hu, X.J. (1995). Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats. **Stroke**, 26, 627-635.

Gerlach, M., Riederer, P., Youdim, M.B.H. (1995). Neuroprotective therapeutic strategies - comparison of experimental and clinical results. **Biochemistry and Pharmacology**, 50, 1-16.

Geschwind, N. (1974). The Organization of Language and the Brain. In Cohen, R.S., & Wartolsky, M.W. (Eds), Boston Studies in the Philosophy of Science Vol 16, **N Geschwind Selected Papers on Language and the Brain,** Dorderecht Holland, D Reidel Publishing Company, pp 452-466.

Ginsberg, M.D., & Busto, R. (1989). Rodent models of cerebral ischemia. **Stroke**, 20, 1627-1642.

Ginsberg, M.D., Sternau, L.L., Globus, M., Dietrich, W.D., & Busto, R. (1992). Therapeutic modulation of brain temperature: Relevance to ischemic brain injury. **Cerebrovasculature and Brain Metabolism Reviews**, 4, 189-225.

Giulian, D., Woodward, J., Young, D., Krebs, J.F., & Lachman, L.B. (1988). Interleukin-1 injected into mammalian brain stimulates astrogliosis and neovascularization. **Journal of Neuroscience**, 8, 2485-2490.

Glassman, R.B. (1994). Behavioral effects of SI versus SII cortex ablations on tactile orientation-localization and postural reflexes of rats. **Experimental Neurology**, 125, 125-133.

Glick, S.D., & Cox, R.D. (1978). Nocturnal rotation in normal rats: Correlation with amphetamine-induced rotation and effects of nigrostriatal lesions. **Brain Research,** 150, 149-161.

Godefroy, O., Lhullier, C., & Rosseaux, M. (1994). Reliability of reaction time measurements in brain-damaged patients. **Journal of Neurological Science**, 126, 168-171.

Godfraind, T., & Govoni, S. (1995). Recent advances in the pharmacology of Ca2+ and K+ channels. **Trends in Pharmacological Science**, 16, 1-4.

Goodale, M.A., & Carey, D.P. (1990). The role of cerebral cortex in visuomotor control. In Kolb, B., & Tees, R.C. (Eds), **The Rat Cerebral Cortex**, Cambridge MA, MIT Press, pp 309-340.

Goodlett, C.R., Engellenner, W.J., Burright, R.G., & Donovick, P.J. (1982). Influences of environmental rearing history and postsurgical environmental change on the septal rage syndrome in mice. **Physiology and Behavior**, 28, 1077-1081.

Goldman-Rakic, P.S. (1987). Circuitry of primate prefrontal cortex and regulation of behavior by representational memory. In Mountcastle, V.B., Plum F., & Geiger, S.R. (Eds), **Handbook of Physiology, Section I, The Nervous System**, Bethesda MD, American Physiological Society, pp 373-417.

Grabowski, M., Brundin, P., & Johansson, B.B. (1993). Paw reaching, sensorimotor, and rotational behavior after brain infarction in rats, **Stroke**, 24, 889-895.

Grabowski, M., Nordborg, C., & Johansson, B.B. (1991). Sensorimotor performance and rotation correlate to lesion size in right but not left hemisphere brain infarcts in the spontaneously hypertensive rat. **Brain Research**, 547, 249-257.

Grabowski, M., Sorensen, J.C., Mattsson, B., Zimmer, J., & Johansson, B.B. (1995). Influence of an enriched environment and cortical grafting on functional outcome in brain infarcts of adult rats. **Experimental Neurology**, 133, 96-102.

Graham ,S.H., Chen, J., Sharp, F.R., & Simon, R.P. (1993). Limiting ischemic injury by inhibition of excitatory amino acid release. **Journal of Cerebral Blood Flow and Metabolism**, 13, 88-97.

Granon, S., & Poucet, B. (1995). Medial prefrontal lesions in the rat and spatial navigation: Evidence for impaired planning. **Behavioral Neuroscience**, 109, 474-484.

Granon, S., Save, E., Buhot, M.C., & Poucet, B. (1996). Effortful information processing in a spontaneous spatial situation by rats with medial prefrontal lesions. **Behavioural Brain Research**, 78, 147-154.

Granon, S., Vidal, C., Thinus-Blanc, C., Changeux, J.P., & Poucet, B. (1994). Working memory, response selection, and effortful processing in rats with medial prefrontal lesions. **Behavioral Neuroscience**, 108, 883-891.

Groenewegen, H.J. (1988). Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to the mediodorsal-prefrontal topography. **Neuroscience**, 24, 379-431.

Gross, C.G., Chorover, S.L., & Cohen, S.M. (1965). Caudate, cortical, hippocampal and dorsal thalamic lesions in rats: Alternation and Hebb-Williams Maze performance. **Neuropsychologia**, 3, 53-68.

Hall, R.D., & Lindholm, E.P. (1974). Organization of motor and somatosensory neocortex in the albino rat. **Brain Research**, 66, 23-38.

Halligan, P.W., & Marshall, J.C. (1991). Left neglect for near but not far space in man. **Nature**, 350, 498-500.

Hara, H., Friedlander, R.M., Gagliardini, V., Ayata, C., Fink, K., Huang, Z., Shimizu-Sasamata, M., Yuan, J., & Moskowitz, M.A. (1997). Inhibition of interleukin 1β converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. **Proceedings of the National Academy of Sciences USA**, 94, 2007-2012.

Hara, H., Harada, K., & Sukamoto, T. (1993). Chronological atrophy after transient middle cerebral artery occlusion in rats. **Brain Research**, 1993, 251-260.

Harrington, D.L., & Haaland, K.Y. (1992). Motor sequencing with left-hemisphere damage- are some cognitive deficits specific to limb apraxia. **Brain**, 115, 857-874.

Hauber, W., Bubser, M., & Schmidt, W.J. (1994). 6-Hydroxydopamine lesion of the rat prefrontal cortex impairs motor initiation but not motor execution. **Experimental Brain Research**, 99, 524-528.

He, Y.Y., Li, H.Y., Zhang, S.X., Dong, G., Shi, R., Wei, L., Fan, G.S., Xu, X.M., & Hsu, C.Y. (1996). Endovascular injury in suture MCAo stroke models. **Society for Neuroscience Abstracts**, 22, part 3, 2145.

Heilman, K.M., & Valenstein, E. (1972). Frontal lobe neglect in man. **Neurology**, 22, 660-664.

Heimer, L. (1983). First dissection, Meninges and subarachnoid cisterns, superficial arteries, vertebral-basilar system, internal carotid system, basal surface and cranial nerves. **The Human Brain and Spinal Cord Functional Neuroanatomy and Dissection Guide**, New York NY, Springer-Verlag, pp 53-64.

Henshall, D.C., Butcher, S.P., & Sharkey, J. (1995). Middle cerebral artery occlusion is not associated with a reduction in brain temperature. **Brain Research Association Abstracts**, 13, 64.

Heros, R.C. (1994). Stroke: Early pathophysiology and treatment. Summary of the Fifth Annual Decade of the Brain Symposium. **Stroke**, 25, 1877-1881.

Hillered, L., Hallstrom, A., Segersvard, S., Persson, L., & Ungerstedt, U. (1989). Dynamics of extracellular metabolites in the striatum after middle cerebral artery occlusion in the rat monitored by intracerebral microdialysis. **Journal of Cerebral Blood Flow and Metabolism**, 9, 607-616.

Hirakawa, M., Tamura, A., Nagashima, H., Nakayama, H., & Sano, K. (1994). Disturbance of retention memory after focal cerebral ischemia in rats. **Stroke**, 25, 2471-2475.

Holley, L.A., Turchi, J., Courtney A., & Sarter, M. (1995). Dissociation between the attentional effects of infusions of a benzodiazepine receptor agonist and an inverse agonist into the basal forebrain. **Psychopharmacology**, 120, 99-108.

Hong, S., Goto, Y., Lanzino, G., Soleau, S., Kassell, N.F., & Lee, K.S. (1994). Neuroprotection with a calpain inhibitor in a model of focal cerebral ischemia. **Stroke**, 25, 663-669.

Hossmann, K.A. (1994). Glutamate-mediated injury in focal cerebral ischemia: The excitotoxin hypothesis revised. **Brain Pathology**, 4, 23-36.

Howell, D.C. (1987). Repeated-Measures Designs. **Statistical Methods for Psychology** (2nd ed), Boston MA, PWS-Kent, pp 413-462.

Hsu, C.Y. (1993). Criteria for valid preclinical trials using animal stroke models. **Stroke**, 24, 633-636.

Hunter, A.J., Green, R., & Cross, A.J. (1995). Animal models of acute ischemic stroke: Can they predict clinically successful neuroprotective drugs? **Trends in Pharmacological Science**, 16, 123-128.

Hurwitz, B.E., Dietrich, W.D., McCabe, P.M., Watson, B.D., Ginsberg, M.D., & Schneiderman, N. (1990). Sensory-motor deficit and recovery from thrombotic infarction of the vibrissal barrel-field cortex. **Brain Research**, 512, 210-220.

Hutson, K.A., & Masterson, R.B. (1986). The sensory contribution of a single vibrissa's cortical barrel. **Journal of Neurophsyiology**, 56, 1196-1223.

Jakobson, L.S., Archibald, Y.M., Carey, D.P., & Goodale, M.A. (1991). A kinematic analysis of reaching and grasping movements in a patient recovering from optic ataxia. **Neuropsychologia**, 29, 803-809.

Javoy-Agid, F., Ploska, A., & Agid, Y. (1981). Microtopography of tyrosine hydroxylase, glutamic acid decarboxylase, and choline acetyltransferase in the substantia nigra and ventral tegmental area of control and Parkinsonian brains. **Journal of Neurochemistry**, 37, 1218-1227.

Jeannrod, M., Decety, J., & Michel, F. (1994). Impairment of grasping movements following a bilateral posterior parietal lesion. **Neuropsychologia**, 32, 369-380.

Jiang, N., Chopp, M., Stein, D., & Feit, H. (1996). Progesterone is neuroprotective after transient middle cerebral artery occlusion in male rats. **Brain Research**, 735, 101-107.

Johnson, P.B., Ferraina, S., & Caminiti, R. (1993). Cortical networks for visual reaching. **Experimental Brain Research**, 97, 361-365.

Jones, D.N.C., & Higgins, G.A. (1995). Effects of scopolamine on visual attention in rats. **Psychopharmacology**, 120, 142-149.

Jonides, J. (1981). Voluntary versus automatic control over the mind's eye's movement. In **Attention and Performance IX**. Long, J., & Baddeley, A. (Eds), Hillsdale, Erlbaum, pp 187-203.

Jonides, J., & Mack, R. (1984). On the cost and benefit of cost and benefit. **Psychological Bulletin**, 96, 29-44.

Katsuta, K., Nakanishi, H., Shirakawa, K., Yoshida, K., Takagi, K., & Tamura, A. (1995). The neuroprotective effects of the novel noncompetitive NMDA antagonist, FR115427 in focal cerebral ischemia in rats. **Journal of Cerebral Blood Flow and Metabolism**, 15, 345-348.

Kawai, H., Nakai, H., Suga, M., Yuki, S., Watanabe, T., & Saito, K.I. (1997). Effects of a novel free radical scavenger, MCI-186, on ischemic brain damage in the rat distal middle cerebral artery occlusion model. **Journal of Pharmacology and Experimental Therapeutics**, 281, 921-927.

Kawai, H., Yuki, S., Sugimoto, J., & Tamao, Y. (1996). Effects of a thrombin inhibitor, argatroban, on ischemic brain damage in the rat distal middle cerebral artery occlusion model. **Journal of Pharmacology and Experimental Therapeutics**, 278, 780-785.

Kawamata, T., Alexis, N.E., Dietrich, W.D., & Finklestein, S.P. (1996). Intracisternal basic fibroblast growth factor (bFGF) enhances behavioral recovery following focal cerebral infarction in the rat. **Journal of Cerebral Blood Flow and Metabolism**, 16, 542-547.

Kawazura, H., Takahashi, Y., Shiga, Y., Shimada, F., Ohto, N., & Tamura, A. (1997). Cerebroprotective effects of a novel pyrazoline derivative, MS-153, on focal ischemia in rats. **Japanese Journal of Pharmacology**, 73, 317-324.

Kesner, R.P., Farnsworth, G., & DiMattia, B.V. (1989). Double dissociation of egocentric and allocentric space following medial prefrontal and parietal cortex lesions in the rat. **Behavioral Neuroscience**, 103, 956-961.

Kesner, R.P., & Gray, M.L. (1989). Dissociation of item and order memory following parietal cortex lesions in the rat. **Behavioral Neuroscience**, 103, 907-910.

King, V.R., & Corwin, J.V. (1993). Comparisons of hemi-inattention produced by unilateral lesions of the posterior parietal cortex or medial agranular prefrontal cortex in rats: Neglect, extinction, and the role of stimulus distance. **Behavioural Brain Research**, 54, 117-131.

King, V.R., & Corwin, J.V. (1992). Spatial deficits and hemispheric asymmetries in the rat following unilateral and bilateral lesions of posterior parietal or medial agranular cortex. **Behavioural Brain Research**, 50, 53-68.

King, V.R., Corwin, J.V., & Reep, R.L. (1989). Production and characterization of neglect in rats with unilateral lesions of ventrolateral orbital cortex. **Experimental Neurology**, 105, 287-299.

Kinsella, G., Oliver, G., Kim, N., Packer, S., & Stark, R. (1993). Analysis of the syndrome of unilateral neglect. **Cortex**, 29, 135-140.

Kolb, B. (1990a). Prefrontal Cortex. In Kolb, B., & Tees, R.C. (Eds), **The Rat Cerebral Cortex**, Cambridge MA, MIT Press, pp 437-458.

Kolb, B. (1990b). Posterior Parietal and Temporal Association Cortex. In Kolb, B. & Tees, R.C. (Eds), **The Rat Cerebral Cortex**, Cambridge MA, MIT Press, pp 459-471.

Kolb, B., Buhrmann, K., McDonald, R., & Sutherland, R.J. (1994). Dissociation of the medial prefrontal, posterior parietal, and posterior temporal cortex for spatial navigation and recognition memory in the rat. **Cerebral Cortex**, 6, 664-680.

Kolb, B., & Cioe, J. (1996). Sex related differences in cortical function after medial frontal lesions in rats. **Behavioral Neuroscience**, 110, 1271-1281.

Kolb, B., Nonneman, A.J., & Singh, R.K. (1974). Double dissociation of spatial impairments and perseveration following selective prefrontal lesions in rats. **Journal of Comparative Physiological Psychology**, 87, 772-780.

Kolb, B., & Walkey, J. (1987). Behavioural and anatomical studies of the posterior parietal cortex in the rat. **Behavioural Brain Research**, 23, 127-145.

Kolb, B., & Whishaw, I.Q. (1990). Recovery of Function. In Kolb, B. & Whishaw I.Q. (Eds) **Fundamentals of Human Neuropsychology** (3rd ed), New York NY, Freeman, pp 712-745.

Kolb, B., & Whishaw, I.Q. (1983). Dissociation of the contributions of the prefrontal, motor, and parietal cortex to the control of movement in the rat: An experimental review. **Canadian Journal of Psychology**, 37, 211-232.

Koroshetz, W.J., & Moskowitz, M.A. (1996). Emerging treatments for stroke in humans. **Trends in Pharmacological Science**, 17, 227-233.

Kozlowski, D.A., James, D.C., & Schallert, T. (1996). Use-dependent exaggeration of neuronal injury after unilateral sensorimotor cortex lesions. **Journal of Neuroscience**, 16, 4776-4786.

Kreig, W.J.S. (1946). Connections of the cerebral cortex. I. The albino rat. B. Structure of the cortical areas. **Journal of Comparative Neurology**, 84, 277-321.

Krettek, J.E., & Price, J.L. (1977). The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. **Journal of Comparative Neurology**, 171, 157-192.

Laing, R.J., Jakubowski, J., & Laing, R.W. (1993). Middle cerebral artery occlusion without craniectomy in rats, which methods works best? **Stroke**, 24, 294-298.

Leonard, C.M. (1969). The prefrontal cortex of the rat. I. Cortical projections of the mediodorsal nucleus. II. Efferent connections. **Brain Research**, 12, 321-343.

Lin, T.N., He, Y.Y., Wu, G., Khan, M., & Hsu, C.Y. (1993). Effect of brain edema on infarct volume in a focal cerebral ischemia model in rats. **Stroke**, 24, 117-121.

Lin, Y., & Phillis, J.W. (1992). Deoxycoformycin and oxypurinol: Protection against focal ischemic brain injury in the rat. **Brain Research**, 571, 272-280.

Lin, Y., & Phillis, J.W. (1991). Oxypurinol reduces ischemic brain injury in the rat. **Neuroscience Letters**, 126, 187-190.

Lindvall, O., Bjorklund, A., Moore, R.Y., & Stenevi, U. (1974). Mesencephalic dopamine neurons projecting to neocortex. **Brain Research**, 81, 325-331.

Longa, E.Z., Weinstein, P.R., Carlson, S., & Cummins, R. (1989). Reversible middle cerebral artery occlusion without craniectomy in rats. **Stroke**, 20, 84-91.

Lyden, P.D., & Hedges, B. (1992). Protective effects of synaptic inhibition during cerebral ischemia in rats and rabbits. **Stroke**, 23, 1463-1470.

Lyden, P.D., & Lonzo, L. (1994). Combination therapy protects ischemic brain in rats, a glutamate antagonist plus a γ-aminobutyric acid agonist. **Stroke**, 25, 189-196.

Macrae, M. (1992). New models of focal cerebral ischemia. **British Journal of Clinical Pharmacology**, 34, 302-308.

Markgraf, C.G., Green, E.J., Hurwitz, B.E., Morikawa, E., Dietrich, W.D., McCabe, P.M., Ginsberg, M.D., & Schneiderman, N. (1992). Sensorimotor and cognitive consequences of middle cerebral artery occlusion in rats. **Brain Research**, 575, 238-245.

Markgraf, C.G., Green, E.J., Watson, B., McCabe, P.M., Schneiderman, N., Dietrich, W.D., & Ginsberg, M.D. (1994). Recovery of sensorimotor function after distal middle cerebral artery photothrombotic occlusion in rats. **Stroke**, 25, 153-159.

Markgraf, C.G., Johnson, M.P., Braun, D.L., & Bickers, M.V. (1997). Behavioral recovery patterns in rats receiving the NMDA receptor antagonist MDL 100,453 immediately post-stroke. **Pharmacology, Biochemistry and Behavior**, 56, 391-397.

Marrocco, R.T., & Witte, E.A. (1993). Systemic nicotine from cigarette smoking modifies covert orienting in human subjects. **Society for Neuroscience Abstracts**, 19, part 1, 561.

Marrocco, R.T., Witte, E.A., & Davidson, M.C. (1994). Arousal systems. **Current Opinion in Neurobiology**, 4, 166-170.

Marsden, C.D., & Fowler, T.J. (1989). Cerebrovascular Disease. In **Clinical Neurology**. London, Edward Arnold, pp 378-398.

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

Marston, H.M., Collins, S.E., Butcher, S.P., & Sharkey, J. (1997). Impaired operant learning following anterior cerebral artery occlusion: Attenuation by MK801. **Brain Research Association Abstracts**, 14, 69.

Marston, H.M., Faber, E.S.L., Crawford, J.H., Butcher, S.P., & Sharkey, J. (1995a). Behavioural assessment of endothelin-1 induced middle cerebral artery occlusion in the rat. **Neuroreport**, 6, 1067-1071.

Marston, H.M., Collins, S.E., Butcher, S.P., Sharkey, J. (In Press). Cognitive deficits associated with anterior cerebral artery occlusion in the rat: Amelioration by dizocilpine. **Journal of Cerebral Blood Flow and Metabolism.**

Marston, H.M., Sharkey, J., Ward, N.M., & Good, M.A. (1995b). Comparison of excitotoxic hippocampal lesions and mid-line ischaemic damage on delayed non-matching performance in the rat. **European Journal of Neuroscience**, Supplement, 8, 136.

Marston, H.M., West, H.L., Wilkinson, L.S., Everitt, B.J., & Robbins, T.W. (1994). Effects of excitotoxic lesions of the septum and vertical limb nucleus of the diagonal band of Broca on conditional visual discrimination: Relationship between performance and choline acetyltransferase activity in cingulate cortex. **Journal of Neuroscience**, 14, 2009-2019.

Maruff, P., & Currie, J. (1995). An attentional grasp reflex in patients Alzheimer's disease. **Neuropsychologia**, 33, 689-701.

Mattingley, J.B., Bradshaw, J.L., Bradshaw, J.A., & Nettleton, N.C. (1994). Recovery from directional hypokinesia and bradykinesia in unilateral neglect. **Journal of Clinical and Experimental Neuropsychology**, 16, 861-876.

Mazzoni, M., Vista, M., Pardossi, L., Avila, L., Bianchi, F., & Moretti, P. (1992). Spontaneous evolution of aphasia after ischemic stroke. **Aphasiology**, 6, 387-396.

McCulloch, J., Bulloch, R., & Teasdale, G.M. (1992). Excitatory amino acids antagonists: Opportunities for the treatment of ischaemic brain damage in man. In Meldrum B.S. (Ed), **Excitatory Amino Acids Antagonists**, Oxford, Blackwell Scientific Publications, pp 287-326

McGaughty, J., & Sarter, M. (1995). Behavioral vigilance in rats: Task validation and effects of age, amphetamine and benzodiazepine receptor ligands. **Psychopharmacology**, 117, 340-357.

McGeorge, A.J., & Faull, R.L.M. (1989). Organization of the projection from the cerebral cortex to the striatum in the rat. **Neuroscience**, 29, 503-537.

Memezawa, H., Smith, M., & Siesjo, B.K. (1992). Penumbral tissues salvaged by reperfusion following middle cerebral artery occlusion in rats. **Stroke**, 23, 552-559.

Mesulam, M.M. (1981). A cortical network for directed attention and unilateral neglect. **Annals of Neurology**, 10, 309-325.

Miklyaeva, E.I., Castaneda, E., & Whishaw, I.Q. (1994). Skilled reaching deficits in unilateral dopamine-depleted rats: Impairments in movement and posture and compensatory adjustments. **Journal of Neuroscience**, 14, 7148-7158.

Miklyaeva, E.I., Nikiforov, E.G., Tompkins, G.J., Ioffe, M.E., Woodward, N.C., & Whishaw, I.Q. (1996). Postural strategies during reaching in unilateral dopamine depleted rats: Use of force platforms for measuring ground reaction forces. **Society for Neuroscience Abstracts**, 22, part 2, 1319.

Millikan, C.H., McDowell, F., & Easton, J.D. (1987a). The Epidemiology of Stroke. In Millikan, C.H., McDowell, F., & Easton, J.D. (Eds). **Stroke**, Philadelphia PA, Lea Febiger, pp 63-70.

Millikan, C.H., McDowell, F., & Easton, J.D. (1987b). Neurovascular Anatomy (Arterial and Venous). In Millikan, C.H., McDowell, F., & Easton, J.D. (Eds). **Stroke**, Philadelphia PA, Lea Febiger.

Millikan, C.H., McDowell, F., & Easton, J.D. (1987c). Completed Stroke. In Millikan, C.H., McDowell, F., & Easton, J.D. (Eds). **Stroke**, Philadelphia PA, Lea Febiger, pp 131-158.

Millikan, C.H., McDowell, F., & Easton, J.D. (1987d). General Pathophysiology and Neuropathology of Stroke. In Millikan, C.H., McDowell, F., & Easton, J.D. (Eds). **Stroke**, Philadelphia PA, Lea Febiger, pp 33-61.

Miner, L.A., Ostrander, M., & Sarter, M. (1997). Effects of ibotenic acid-induced loss of neurons in the medial prefrontal cortex of rats on behavioral vigilance: Evidence for executive dysfunction. **Journal of Psychopharmacology**, 11, 169-178.

Mogenson, J., & Holm, S. (1994). The prefrontal cortex and variants of sequential behaviour: Indications of functional differentiation between subdivisions of the rat's prefrontal cortex. **Behavioural Brain Research**, 63, 89-100.

Montoya, C.P., Campbellhope L.J., Pemberton, K.D., & Dunnett, S.B. (1991). The staircase test a measure of independent forelimb reaching and grasping abilities in rats. **Journal of Neuroscience Methods**, 36, 219-228.

Morikawa, E., Ginsberg, M.D., Dietrich, W.D., Duncan, R.C., Kraydieh, S., Mordecai, Y., Globus, T., & Busto, R. (1992). The significance of brain temperature in focal cerebral ischemia: Histopathological consequences of middle cerebral artery occlusion in the rat. **Journal of Cerebral Blood Flow and Metabolism**, 12, 380-389.

Morley, P., Hogan, M.J., & Hakim, A.M. (1994). Calcium-mediated mechanisms of ischemic injury and protection. **Brain Pathology**, 4, 37-47.

Muir, J.L. (1996). Attention and stimulus processing in the rat. **Cognitive Brain Research**, 3, 215-225.

Muir, J.L., Bussey, T.J., Everitt, B.J., & Robbins, T.W. (1996a). Dissociable effects of AMPA-induced lesions of the vertical limb diagonal band of Broca on performance of the 5-choice serial reaction time task and on the acquisition of a conditional visual discrimination. **Behavioural Brain Research**, 82, 31-44.

Muir, J.L., Dunnett, S.B., Robbins, T.W., & Everitt, B.J. (1992). Attentional functions of the forebrain cholinergic systems: Effects of intraventricular hemicholinium, physostigmine, basal forebrain lesions and intracortical grafts on a multiple-choice serial reaction time task. **Experimental Brain Research**, 89, 611-622.

Muir, J.L., Everitt, B.J., & Robbins, T.W. (1996b). The cerebral cortex of the rat and visual attentional function: Dissociable effects of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions on a five-choice serial reaction time task. **Cerebral Cortex**, 6, 470-481.

Muir, J.L., Everitt, B.J., & Robbins, T.W. (1995). Reversal of visual attentional dysfunction following lesions of the cholinergic basal forebrain by physostigmine and nicotine but not by the 5HT3 receptor agonist, onanestron. **Psychopharmacology**, 118, 82-92.

Muir, J.L., Everitt, B.J., & Robbins, T.W. (1994). AMPA-induced excitotoxic lesions of the basal forebrain: A significant role of the cortical cholinergic system in attentional function. **Journal of Neuroscience**, 14, 2313-2326.

Muller, H.J., & Rabbitt, P.M. (1989). Reflexive and voluntary orienting of visual attention: Time course of activation and resistance to interruption. **Journal of Experimental Psychology Human Perception and Performance**, 15, 315-330.

Nagafuji, T., Matsui, T., Koide, T., & Asano, T. (1992). Blockade of nitric oxide formation by N-nitro-L-arginine mitigates ischemic brain edema and subsequent cerebral infarction in rats. **Neuroscience Letters**, 147, 159-162.

Naeser, M.A., Alexander, M.P., Helm-Estabrooks, N., Levine, H.L., Laughlin, S.A., & Geschwind, N. (1982). Aphasia with predominantly subcortical lesion sites. **Archives of Neurology**, 39, 2-14.

Nixon, P.D., Burbaud, P., & Passingham, R.E. (1992). Control of arm movement after bilateral lesions of area 5 in the monkey. **Experimental Brain Research**, 90, 229-232.

Nowicki, J.P., Assumel-Lurdin, C., Duverger, D., & MacKenzie, E.T. (1988). Temporal evolution of regional energy metabolism following focal cerebral ischemia in the rat. **Journal of Cerebral Blood Flow and Metabolism**, 8, 462-473.

Obrenovitch, T.P., Garofalo, O., Harris, R.J., Bordi, L., Ono, M., Momma, F., Bachelard, H.S., & Symon, L. (1988). Brain tissue concentrations of ATP, phosphocreatine, lactate and tissue pH in relation to reduced cerebral blood flow following experimental acute middle cerebral artery occlusion. **Journal of Cerebral Blood Flow and Metabolism**, 8, 866-874.

Okada, M., Nakanishi, H., Tamura, A., Urae, A., Mine, K., Yamamoto, K., & Fujiwara, M. (1995a). Long-term spatial cognitive impairment after middle cerebral artery occlusion in rats: No involvement of the hippocampus. **Journal of Cerebral Blood Flow and Metabolism**, 15, 1012-1021.

Okada, M., Tamura, A., Urae, A., Nakagomi, T., Kirino, T., Mine, K., & Fujiwara, M. (1995b). Long-term spatial cognitive impairment following middle cerebral artery occlusion in rats. A behavioral study. **Journal of Cerebral Blood Flow and Metabolism**, 15, 505-512.

Oliff, H.S., Weber, E., Eilon, G., & Marek, P. (1995a). The role of strain/vendor differences on the outcome of focal cerebral ischemia induced by intraluminal middle cerebral artery occlusion in the rat. **Brain Research**, 675, 20-26.

Oliff, H.S., Weber, E., Miyakai, B., & Marek, P. (1995b). Infarct volume varies with rat strain and vendor in focal cerebral ischemia induced by transcranial middle cerebral artery occlusion. **Brain Research**, 699, 329-331.

Osborne, K.A., Shigeno, T., Balarsky, A.M., Ford, I., McCulloch, J., Teasdale, G.M., & Graham, D.I. (1987). Quantitative assessment of early brain damage in a rat model of focal cerebral ischaemia. **Journal of Neurology, Neurosurgery and Psychiatry**, 50, 402-410.

Papadopoulos, S.M., Chandler, W.F., Salamat, M.S., Topol, E.J., & Sackellares, J.C. (1987). Recombinant human tissue - type plasminogen activator therapy in acute thromboembolic stroke. **Journal of Neurosurgery**, 67, 394-398.

Parasuraman, R., Greenwood, P.M., Haxby, J.V., & Grady, C.L. (1992). Visuospatial attention in dementia of the Alzheimer type. **Brain**, 115, 711-733.

Park, C.K., & Hall, E.D. (1994). Dose-response analysis of the effect of 21-aminosteroid tirilazad mesylate (U-74006F) upon neurological outcome and ischemic brain damage in permanent focal cerebral ischemia. **Brain Research**, 645, 157-163.

Paxinos, G., & Watson, C. (1986). **The Rat Brain in Stereotaxic Coordinates** (2nd ed), San Diego CA, Academic Press.

Persson, L., Hardemark, H.G., Bolander, H.G., Hillered, L., & Olsson, Y. (1989). Neurologic and neuropathologic outcome after middle cerebral artery occlusion in rats. **Stroke**, 20, 641-645.

Petersen, S.E., Robinson, D,L,, & Currie, J.N. (1989). Influences of lesions of parietal cortex on visual spatial attention in humans. **Experimental Brain Research**, 76, 267-280.

Petersen, S.E., Robinson, D.L., & Morris, J.D. (1987). The contribution of the pulvinar to visual spatial attention. **Neuropsychologia**, 25, 97-105.

Piani, D., Constam, D.B., Frei, K., & Fontana, A. (1994). Macrophages in the brain: Friends or enemies. **News in Physiological Sciences**, 9, 80-84.

Pisa, M. (1988). Motor functions of the striatum in the rat: Critical role of the lateral region in tongue and forelimb reaching. **Neuroscience**, 24, 453-463.

Posner, M.I. (1980). Orienting of attention. **Quarterly Journal of Experimental Psychology**, 32, 3-25.

Posner, M.I., Cohen, Y., & Rafal, R.D. (1982). Neural systems control of spatial orienting. **Philosophical Transactions of the Royal Society of London Biology**, 298, 187-198.

Posner, M.I., & Driver, J. (1992). The neurobiology of selective attention. **Current Opinions in Neurobiology**, 2, 165-169.

Posner, M.I., & Petersen, S.E. (1990). The attention system of the human brain. **Annual Review of Neuroscience**, 13, 25-42.

Posner, M.I., Walker, J.A., Friedrich, F.J., & Rafal, R.D. (1984). Effects of parietal injury on covert orienting of attention. **Journal of Neuroscience**, 4, 1863-1874.

Posner, M.I., Walker, J.A., Friedrich, F.J., & Rafal, R.D. (1987). How do the parietal lobes direct covert attention? **Neuropsychologia**, 25, 135-145.

Post, C., Salvati, P., Schafer, M.K.H., Schaeble, W., Cini, M., Calabresi, M., Vaghi, F., Wong, E.H.F., & Weihe, E. (1996). Early upregulation of complement factors and astrocyte dysfunction in animal models of global ischemia and stroke. **Society for Neuroscience Abstracts**, 22, part 3, 2143.

Racy, A., Jannotta, F.S., & Lehner, L.H. (1979). Aphasia resulting from occlusion of the left anterior cerebral artery: Report of a case with an old infarct in the left rolandic region. **Archives of Neurology**, 36, 221-224.

Rafal, R.D., & Posner, M.I. (1987). Deficits in human visual spatial attention following thalamic lesions. **Proceedings of the National Academy of Sciences USA**, 84, 7349-7353.

Rafal, R.D., Posner, M.I., Friedman, J.H., Inhoff, A.W., & Bernstein, E. (1988). Orienting of visual attention in progressive supranuclear palsy. **Brain**, 111, 267-280.

Rafal, R.D., Posner, M.I., Walker, J.A., & Friedrich, F.J. (1984). Cognition and the basal ganglia. **Brain**, 107, 1083-1094.

Reep, R.L., Chandler, H.C., King, V., & Corwin, J.V. (1994). Rat posterior parietal cortex: Topography of corticocortical and thalamic connections. **Experimental Brain Research**, 100, 67-84.

Ridenour, T.R., Warner, D.S., Todd, M.M., & Baker, M.T. (1991). Effects of ketamine on outcome from temporary middle cererbal artery occlusion in the spontaneously hypertensive rat. **Brain Research**, 565, 116-122.

Rivaud, S., Muri, R.M., Gaymard, B., Vermersch, A.I., & Pierrot-Deseilligny, C. (1994). Eye movement disorders after frontal eye field lesions in humans. **Experimental Brain Research**, 102, 110-120.

Rizzolatti, G., Riggio, L., Dascola, I., & Umilta, C. (1987). Reorienting attention across the horizontal and vertical meridians: Evidence in favor of a premotor theory of attention. **Neuropsychologia**, 25, 31-40.

Robbins, T.W., & Brown, V.J. (1990). The role of the striatum in the mental chronometry of action: A theoretical review. **Reviews in the Neurosciences**, 2, 181-213.

Robbins, T.W., & Everitt, B.J. (1996). Neurobehavioural mechanisms of reward and motivation. **Current Opinions in Neurobiology**, 6, 228-236.

Robinson, R.G. (1979). Differential behavioral and biochemical effects of right and left hemispheric cerebral infarction in the rat. **Science**, 205, 707-710.

Robinson, R.G., & Coyle, (1980). Differential effect of right versus left hemispheric cerebral infarction on catecholamines and behavior in the rat. **Brain Research**, 188, 63-78.

Robinson, D.L., Kertzman, C. (1995). Covert orienting of attention in macaques. III. Contributions of the superior colliculus. **Journal of Neurophysiology**, 74, 713-721.

Robinson, M.J., Macrae, I.M., Todd, M., Reid, J.L., & McCulloch, J. (1990). Reduction in local cerebral blood flow to pathological levels by endothelin-1 applied to the middle cerebral artery in the rat. **Neuroscience Letters**, 118, 269-272.

Rosner A.L., & Mittleman, G. (1996). Visuospatial attention in the rat and posterior parietal cortex lesions. **Behavioural Brain Research**, 79, 69-77.

Ross, E.D. (1980). Left medial parietal lobe and receptive language functions: Mixed transcortical aphasia after left anterior cerebral artery infarction. **Neurology**, 30, 144-151.

Roussel, S., Pinard, E., & Seylaz, J. (1991). Focal cerebral ischemia in chronic hypertension: No protection by (R)-phenylisopropyladenosine. **Brain Research**, 545, 171-174.

Rubanyi, G.M., & Polokoff, M.A. (1994). Endothelins: Molecular biology, biochemistry, pharmacology, physiology and pathophysiology. **Pharmacological Reviews**, 46, 325-415.

Rubens, A.B. (1975). Aphasia with infarction in the territory of the anterior cerebral artery. **Cortex**, 11, 239-250.

Rueckert, L., & Grafman, J. (1996). Sustained attention deficits in patients with right frontal lesions. **Neuropsychologia**, 34, 953-963.

Sabol, K.E., Neill, D.B., Wages, S.A., Church, W.H., & Justice, J.B. (1985). Dopamine depletion in a striatal subregion disrupts performance of a skilled motor task in the rat. **Brain Research**, 335, 33-43.

Sanchez-Santed, F., De Bruin, J.P.C., Heinsbroek, R.P.W., & Verwer, R.W.H. (1997). Spatial delayed alternation of rats in a T-maze: Effects of neurotoxic lesions of the medial prefrontal cortex and of T-maze rotations. **Behavioural Brain Research**, 84, 73-79.

Sarter, M., Holley- Miner, L.A., & Ostrander, M. (1996). Effects of medial prefrontal neuronal loss on sustained attention performance. **Society for Neuroscience Abstracts**, 22, part 3, 1857.

Save, E., & Moghaddam, M. (1996). Effects of lesions of the associative parietal cortex on the acquisition and use of spatial memory in egocentric and allocentric navigation tasks in the rat. **Behavioral Neuroscience**, 110, 74-85.

Schallert, T.J., & Hall, (1988). 'Disengage' sensorimotor deficit following apparent recovery from unilateral dopamine depletion. **Behavioural Brain Research**, 30, 15-24.

Schallert, T., Upchurch, M., Lobaugh, N., Farrar, S.B., Spirduso, W.W., Gilliam, P., Vaughn, D., & Wilcox, R.E. (1982). Tactile extinction: Distinguishing between sensorimotor and motor asymmetries in rats with nigrostriatal damage. **Pharmacology, Biochemistry and Behavior**, 16, 455-462.

Schallert, T., & Whishaw, I.Q. (1984). Bilateral cutaneous stimulation of the somatosensory system in hemidecorticate rats. **Behavioral Neuroscience**, 98, 518-540.

Schroeter, M., Schiene, K., Kraemer, M., Hagamann, G., Weigel, H., Eysel, U.T., Witte, O.W., & Stoll, G. (1995). Astroglial responses in photochemically induced focal ischemia of the rat cortex. **Experimental Brain Research**, 106, 1-6.

Scremin, O.U. (1995). Cerebral Vasculature System. In Paxinos, G., (Ed), **The Rat Central Nervous System** (2nd ed), London, Academic Press, pp 3-39.

Sefton, A.J., & Dreher, B. (1995). Visual System. In Paxinos, G. (Ed), **The Rat Central Nervous System** (2nd ed), New York NY, Academic Press, pp 883-898.

Selden, N.R., Cole, B.J., Everitt, B.J., & Robbins, T.W. (1990). Damage to ceruleo-cortical noradrenergic projections impairs locally cued but enhances spatially cued water maze acquisition. **Behavioural Brain Research**, 39, 29-51.

Seren, M.S., Lazzaro, A., Yang, C.L., Canella, R., Bassan, M., Zanoni, R., & Manev, H. (1994). Orally administered glycolipid derivative LIGA20 reduces infarct volume and behavioural impairment after focal cerebral ischemia. **The Journal of Pharmacology and Experimental Therapeutics**, 268, 460-465.

Shapiro, H.M. (1985). Barbituates in brain ischemia. **British Journal of Anaesthesia**, 57, 82-95.

Sharkey, J., & Butcher, S.P. (1994). Immunophilins mediate the neuroprotective effects of FK506 in focal cerebral ischaemia. **Nature**, 371, 336-339.

Sharkey, J., Butcher, S.P., & Kelly, J.S. (1994). Endothelin-1 induced middle cerebral artery occlusion: Pathological consequences and neuroprotective effects of MK801. **Journal of the Autonomic Nervous System**, 49, S177-S185.

Sharkey, J., Crawford, J.H., Butcher, S.P., & Marston, H.M. (1996). Tacrolimus (FK506) ameliorates skilled motor deficits produced by middle cerebral artery occlusion in rats. **Stroke**, 27, 2282-2286.

Sharkey, J., Marston, H.M., Pollock, J.M., Henshall, D.C., & Butcher, S.P. (1997). Anterior cerebral artery occlusion in the rat: Model development. **Journal of Cerebral Blood Flow and Metabolism**, 17, Supplement 1, S307.

Sharkey, J., Ritchie, I.M., & Kelly, P.A.T. (1993). Perivascular microapplication of endothelin-1: A new model of focal cerebral ischaemia in the rat. **Journal of Cerebral Blood Flow and Metabolism**, 13, 865-871.

Sheliga, B.M., Riggio, L., & Rizzolatti, G. (1995). Spatial attention and eye movements. **Experimental Brain Research**, 105, 261-275.

Sherwood, L. (1993). **Human Physiology from Cells to Systems**. (2nd Ed), New York, West Publishing Company.

Shimp, C.P., & Friedrich, F.J. (1993). Behavioral and computational models of spatial attention. **Journal of Experimental Psychology Animal Behavior**, 19, 26-37.

Siesjö, B.K. (1992a). Pathophysiology and Treatment of Focal Cerebral Ischemia. **Journal of Neurosurgery**, 77, 169-184.

Siesjo, B.K. (1992b). Pathophysiology and treatment of focal cerebral ischemia. Part II: Mechanisms of damage and treatment. **Journal of Neurosurgery**, 77, 337-354.

Siesjo, B.K., & Bengtsson, F. (1989). Calcium fluxes, calcium antagonists and calcium related pathology in brain ischemia, hypoglycemia and spreading depression a unifying hypothesis. **Journal of Cerebral Blood Flow and Metabolism**, 9, 127-140.

Silverman, B.W. (1986). **Density Estimation for Statistics and Data Analysis**, London, Chapman and Hall.

Slivka, A.P. (1991). Hypertension and hyperglycemia in experimental stroke. **Brain Research**, 562, 66-70.

Smith, S.E., Hodges, H., Sowinski, P., Man, C.M., Leach, M.J., Sinden, J.D., Gray, J.A., & Meldrum, B.S. (1997). Long-term beneficial effects of BW619C89 on neurological deficit, cognitive deficit and brain damage after middle cerebral artery occlusion in the rat. **Neuroscience**, 77, 1123-1135.

Smith, S.E., & Meldrum, B.S. (1995). Cerebroprotective effect of lamotrigine after focal ischemia in rats. **Stroke**, 26, 117-122.

Soblosky, J.S., Matthews, M.A., Davidson, J.F., Tabor, S.L., & Carey, M.E. (1996). Traumatic brain injury of the forelimb and hindlimb sensorimotor areas in the rat: Physiological, histological and behavioral correlates. **Behavioural Brain Research**, 79, 79-92.

Sokolowski, J.D., & Salamone, J.D. (1994). Effects of dopamine depletions in the medial prefrontal cortex on DRL performance and motor activity in the rat. **Brain Research**, 642, 20-28.

Spangler, E.L., Heller, B., Hengemihle, J., Muth, N.J., Jones, B.E., Garofalo, P., & Ingram, D.K. (1994). Thrombosis of parietal, but not striate, cortex impairs acquisition of a 14-unit T-maze in the rat. **Physiology and Behavior**, 56, 95-101.

Starkstein, S.E., Fedoroff, J.P., Price, T.R., Leiguardia, R., & Robinson, R.G. (1992). Anosognosia in patients with cerebrovascular lesions- a study of causative factors. **Stroke**, 23, 1446-1453.

Steckler, T., & Muir, J.L. (1996). Measurement of cognitive function: Relating rodent performance with human minds. **Cognitive Brain Research**, 3, 299-308.

Steckler, T., & Sahgal, A. (1995). Psychopharmacological studies in rats responding at touch-sensitive devices. **Psychopharmacology**, 118, 226-229.

Stuss, D.T., & Benson, F. (1986). **The Frontal Lobes**. New York NY, Raven Press.

Swanson, R.A., Chen, J., & Graham, S.H. (1994). Glucose can fuel glutamate uptake in ischemic brain. **Journal of Cerebral Blood Flow and Metabolism**, 14, 1-6.

Takahashi, T. (1985). The organization of the lateral thalamus of the hooded rat. **Journal of Comparative Neurology**, 231, 281-309.

Takayama, Y., Sugishita, M., Akiguchi, I., & Kimura, J. (1994). Isolated Acalculia due to left parietal lesion. **Archives of Neurology**, 51, 286-291.

Takenouchi, K., Nishijo, H., Uwano, T., Takigawa, M., & Ono, T. (1996). Neural representation of reward contingency in the rat anterior cingulate cortex. **Society for Neuroscience Abstracts**, 22, part 2, 1390.

Tamura, A., Graham, D.I., McCulloch, J., & Teasdale, G.M. (1981). Focal cerebral ischaemia in the rat: 1. Description of technique and early neuropathologic consequences following middle cerebral artery occlusion. **Journal of Cerebral Blood Flow and Metabolism**, 1, 53-60.

Tamura, A., Kirino, T., Sano, K., Takagi, K., & Oka, H. (1990). Atrophy of the ipsilateral substantia nigra following middle cerebral artery occlusion in the rat. **Brain Research**, 510, 154-157.

Thinus-Blanc, C., Save, E., Poucet, B., & Foreman, N. (1996). Effects of parietal cortex lesions on spatial problem solving in the rat. **Behavioural Brain Research**, 81, 115-121.

Tipper, S.P., Lortie, C. & Baylis, G.C. (1992). Selective reaching: Evidence for action-centered attention. **Journal of Experimental Psychology: Human Perception and Performance**, 18, 891-905.

Tominaga, T., & Ohnishi, S.T. (1989). Interrelationship of brain edema, motor deficits and memory impairment in rats exposed to focal ischemia. **Stroke**, 20, 513-518.

Torp, R., Andinem, P., Hagberg, H., Karagulle, T., Blackstad, T.W., & Otterson, O.P. (1991). Cellular and subcellular redistribution of glutamate-, glutamine-, and taurine-like immunoreactivities during forebrain ischemia: A semiquantitative electron microscopic study in rat hippocampus. **Neuroscience**, 41, 433-447.

Turchi, J., Holley, L.A., & Sarter, M. (1995). Effects of nicotinic acetylcholine receptor ligands on behavioral vigilance. **Psychopharmacology**, 118, 195-205.

Ueki, A., Rosen, L., Andbjer, B., Anganti, L.F., Hallstrom, A., Goiny, M., Tanganelli, S., Ungerstedt, U., & Fuxe, K. (1993). Evidence for preventive action of the vigilance-promoting drug modafinil against striatal ischemic injury induced by endothelin-1 in the rat. **Experimental Brain Research**, 96, 89-99.

Umemura, A., Mabe, H., & Nagai, H. (1992). A phospholipase C inhibitor ameliorates postischemic neuronal damage in rats. **Stroke**, 23, 1163-1166.

Van der Staay, F.J., Augstein, K.H., & Horvath, E. (1996a). Sensorimotor impairments in rats with cerebral infarction, induced by unilateral occlusion of the left middle cerebral artery: Strain differences and effects of the occlusion site. **Brain Research**, 735, 271-284.

Van der Staay, F.J., Augstein, K.H., & Horvath, E. (1996b). Sensorimotor impairments in Wistar Kyoto rats with cerebral infarction, induced by unilateral occlusion of the middle cerebral artery: Recovery of function. **Brain Research**, 715, 180-188.

Van der Zwan, A., Hillen, B., Tulleken, C.A.F., Dujovny, M., & Dragovic, L. (1992). Variability of the territories of the major cerebral arteries. **Journal of Neurosurgery**, 77, 927-940.

Van Eden, C.G., Lamme, V.A.F., & Uylings, H.B.M. (1992). Heterotopic cortical afferents to the mediofrontal cortex in the rat. A combined retrograde and anterograde tracer study. **European Journal of Neuroscience**, 4, 77-97.

Viallet, F., Vuillon-Cacciuttolo, G., Leglallet, E., Bonnefoi-Kyriacou, B., & Trouche, E. (1995). Bilateral and side-related reaction time impairments in patients with unilateral cerebral lesions of a medial frontal region involving the supplementary motor area. **Neuropsychologia**, 33, 215-223.

Waddington, M.M., & Ring, B.A. (1968). Syndromes of Occlusions of the Middle Cerebral Artery Branches. **Brain**, 91, 685-696.

Wahl, F., Allix, M., Plotkine, M., & Boulu, R.G. (1992). Neurological and behavioural outcome of focal cerebral ischemia in rats. **Stroke**, 23, 267-272.

Walz, W. (1989). The role of glial cells in the regulation of the brain ion microenvironment. **Progress in Neurobiology**, 33, 309-333.

Wang, K.K.W., & Yuen, P.W. (1994). Calpain inhibition: An overview of its therapeutic potential. **Trends in Pharmacological Sciences**, 15, 412-419.

Ward, N.M., & Brown, V.J. (1996). Covert orienting of attention in the rat and the role of striatal dopamine. **Journal of Neuroscience**, 16, 3082-3088.

Ward, N.M., Sharkey, J., & Brown, V.J. (1997). Assessment of sensorimotor neglect following occlusion of the middle cerebral artery in the rat. **Behavioral Neuroscience**, 111, 1133-1145.

Warner, D.S., McFarlane, C., Todd, M.M., Ludwig, P., & McAllister, A.M. (1993). Sevoflurane and halothane reduce focal cerebral ischemic brain damage in the rat. **Anesthesiology**, 79, 985-992.

Watson, B.D., Dietrich, W.D., Busto, R., Wachtelms, M., & Ginsberg, M.D. (1985). Induction of reproducible brain infarction by photochemically initiated thrombosis. **Annals of Neurology**, 17, 497-504.

Watson, R.T., Fleet, W.S., Gonzalez-Rothi, L., & Heilman, K.M. (1986). Apraxia and the supplementary motor area. **Archives of Neurology**, 43, 787-792.

Wekerle, H. (1995). Antigen presentation by central nervous system glia. In Kettenmann, H., Ransom, B.R., (Eds), **Neuroglia**, Oxford University Press, Oxford, pp 685-699.

Whishaw, I.Q., & Coles, B.L.K. (1996). Varieties of paw and digit movement during spontaneous food handling in rats: Postures, bimanual coordination, preferences, and the effect of forelimb cortex lesions. **Behavioural Brain Research**, 77, 135-148.

Whishaw, I.Q., Mittleman, G., Bunch, S.T., & Dunnett, S.B. (1987). Impairments in the acquisition, retention and selection of spatial navigation strategies after medial caudate-putamen lesions in rats. **Behavioural Brain Research**, 24, 125-138.

Whishaw, I.Q., O'Connor, W.T., & Dunnett, S.B. (1986). The contributions of motor cortex, nigrostriatal dopamine and caudate-putamen to skilled forelimb use in the rat. **Brain**, 109, 805-843.

Whishaw, I.Q., & Pellis, S.M. (1990). The structure of skilled forelimb reaching in the rat: A proximally driven movement with a single distal rotatory component. **Behavioural Brain Research**, 41, 49-59.

Whishaw, I. Q., & Pellis, S. M., Gorny, B.P., & Pellis, V.C. (1991). The impairments in reaching and the movements of compensation in rats with motor cortex lesions: An endpoint, videorecording, and movement notation analysis. **Behavioral Brain Research**, 42, 77-91.

Whishaw, I.Q., & Tomie, J.A. (1989). Olfaction directs skilled forelimb reaching in the rat. **Behavioural Brain Research**, 32, 11-21.

Witte, E.A., Lickey, M.E., & Marrocco, R.T. (1992). Pharmacological depletion of catecholamines modifies covert orienting in rheses monkey. **Society for Neuroscience Abstracts**, 18, part 1, 537.

Witte, E.A., & Marrocco, R.T. (1993). Pharmacological manipulation of brain cholinergic activity modifies covert orienting in rhesus monkeys. **Society for Neuroscience Abstracts**, 19, part 1, 562.

Witte, E.A., Villareal, M., & Marrocco, R.T. (1996). Visual orienting and alerting in rhesus monkeys: Comparison with humans. **Behavioural Brain Research**, 82, 103-112.

Wright, M.J., Burns, R.J., Geffen, G.M., & Geffen, L.B. (1990). Covert orientation of visual attention in Parkinson's disease: An impairment in the maintenance of attention. **Neuropsychologia**, 28, 151-159.

Wright, M.J., Geffen, G.M., & Geffen, L.B. (1993). Event-related potentials associated with covert orientation of visual attention in Parkinson's disease. **Neuropsychologia**, 31, 1283-1297.

Yamada, T., Izyuuinn, M., Schulzer, M., & Hirayama, K. (1990). Covert orienting of attention in Parkinson's disease. **Journal of Neurology, Neurosurgery and Psychiatry**, 53, 593-596.

Yamadori, A., Osumi, Y., Ikeda, H., & Kanazawa, Y. (1980). Left unilateral agraphia and tactile anomia. **Archives of Neurology**, 37, 88-91.

Yamaguchi, T., Suzuki, M., & Yamamoto, M. (1995). YM796, a novel muscarinic agonist, improves the impairment of learning behavior in a rat model of chronic focal cerebral ischemia. **Brain Research**, 669, 107-114.

Yamamoto, M., Tamura, A., Kirino, T., Shimizu, M., & Sano, K. (1988). Behavioral changes after focal cerebral ischemia by left middle cerebral artery occlusion in rats. **Brain Research**, 452, 323-328.

Yamamoto, M., Tamura, A., Kirino, T., Shimizu, M., & Sano, K. (1989). Effects of a new thyrotropin-releasing hormone derivative on behavioral changes after focal cerebral ischemia in rats. **Stroke**, 20, 362-366.

Yamashita, K., Vogel, P., Fritze, K., Back, T., Hossmann, K.A., & Wiessner, C. (1996). Monitoring the temporal and spatial activation pattern of astrocytes in focal cerebral ischemia using in situ hybridization to GFAP mRNA: Comparison with sgp-2 and hsp70 mRNA and the effect of glutamate receptor antagonists. **Brain Research**, 735, 285-297.

Yanaka, K., Camarata, P.J., Spellman, S.R., McCarthy, J.B., Furcht, L.T., & Low, W.C. (1997). Antagonism of leukocyte adherence by synthetic fibronectin peptide V in a rat model of transient focal cerebral ischemia. **Neurosurgery**, 40, 557-564.

Yanaka, K., Camarata, P.J., Spellman, S.R., McCarthy, J.B., Furcht, L.T., Low, W.C., & Heros, R.C. (1996a). Neuronal protection from cerebral ischemia by synthetic fibronectin peptides to leukocyte adhesion molecules. **Journal of Cerebral Blood Flow and Metabolism**, 16, 1120-1125.

Yanaka, K., Camarata, P.J., Spellman, S.R., McCarthy, J.B., Furcht, L.T., Low, W.C., & Heros, R.C. (1996b). Synthetic fibronectin peptides and ischemic brain injury after transient middle cerebral artery occlusion in rats. **Journal of Neurosurgery**, 85, 125-130.

Yanaka, K., Spellman, S.R., McCarthy, J.B., Low, W.C., & Camarata, P.J. (1996c). Reduction of brain injury using heparin to inhibit leukocyte accumulation in a rat model of transient focal cerebral ischemia. II. Dose-response effect and the therapeutic window. **Journal of Neurosurgery**, 85, 1108-1112.

Yanaka, K., Spellman, S.R., McCarthy, J.B., Oegema, T.R., Low, W.C., & Camarata, P.J. (1996d). Reduction of brain injury using heparin to inhibit leukocyte accumulation in a rat model of transient focal cerebral ischemia. I. Protective mechanism. **Journal of Neurosurgery**, 85, 1102-1107.

Yang, G.Y., Chen, S.F., Kinouchi, H., Chan, P.H., & Weinstein, P.R. (1992). Edema, cation content, and ATPase activity after middle cerebral artery occlusion in rats. **Stroke**, 23, 1331-1336.

Yonemori, F., Yamada, H., Yamaguchi, T., Uemura, A., & Tamura, A. (1996). Spatial memory disturbance after focal cerebral ischemia in rats. **Journal of Cerebral Blood Flow and Metabolism**, 16, 973-980.

Zhang, Z.G., Zhang, R.L., Chopp, M., Jiang, Q., Raman, S.B.K., & Cantwell, L. (1996). A new rat model of thrombotic focal cerebral ischemia. **Society for Neuroscience Abstracts**, 22, part 3, 2144.

Zilles, K. (1990). Anatomy of the Neocortex: Cytoarchitecture and Myeloarchitecture. In Kolb, B., & Tees, R.C. (Eds), **The Rat Cerebral Cortex**, Cambridge MA, MIT Press, pp 77-112.

Zilles, K., & Wree, A. (1995). Cortex: Areal and Laminar Structure. In Paxinos G. (Ed) **The Rat Central Nervous System** (2nd ed), London, Academic Press, pp 649-685.

Zivin, J.A. (1987). A model for quantitative evaluation of embolic stroke. **Brain Research**, 435, 305-309.

Appendix

(Computer programs for controlling tests conducted in the 'Nine-hole box' apparatus)

Program for Covert Orienting Test (POSNER, choice reaction time task)

```
*SPIDER
AUTO
:
REM 9-HOLE ARRAY TEST BOXES : POSNER TASK by Verity J. Brown: 9/21/93
REM Trial is only advanced if the response is correct on the previous trial
:
*SRDATA 4
*SRDATA 5
*SRDATA 6
*SRDATA 7
ON ERROR PROCdisaster
KILL ALL
FREE SWITCH 0 TO 19
GOVN SWITCH 20 TO 63
:
REM Inputs
DIM rh%(1),wh%(1),ch%(1),panel%(1)
rh%(0)=1 : rh%(1)=11 : wh%(0)=1 : wh%(1)=11
ch%(0)=5 : ch%(1)=15 : panel%(0)=0 : panel%(1)=10
:
REM Outputs
DIM hse%(1),r_lmp%(1),l_lmp%(1),tim%(4),c_lmp%(1),tr_lmp%(1),disp%(1)
hse%(0)=60 : hse%(1)=62 : tim%(0)=1 : tim%(1)=2 : tim%(2)=3 : tim%(3)=4
c_lmp%(0)=45 : c_lmp%(1)=55 : tr_lmp%(0)=20 : tr_lmp%(1)=30
disp%(0)=40 : disp%(1)=50
r_lmp%(0)=26 : l_lmp%(0)=24 : r_lmp%(1)=36 : l_lmp%(1)=34 : cue%=20
:
REM data arrays,counters and pointers
DIM
data%(1,7),lat1%(1),lat2%(1),lat3%(1),lat4%(1),l%(1),val%(1),v%(1),ptr%(1),side%(1),c
ue%(1)
DIM res%(1),D(5),d%(5),T%(5),fail%(1),out1%(7),out2%(7),end%(1)
DIM corr_cnt%(1),earl_cnt%(1),late_cnt%(1),inc_cnt%(1)
fail%(0)=FALSE : fail%(1)=FALSE : end%(0)=FALSE : end%(1)=FALSE
corr_cnt%(0)=-1:corr_cnt%(1)=-1:earl_cnt%(0)=0:earl_cnt%(1)=0
late_cnt%(0)=0:late_cnt%(1)=0:inc_cnt%(0)=0:inc_cnt%(1)=0
ptr%(0)=0 : ptr%(1)=32000
:
REM Data: saved in array data%(B%,x) where B% is the box reference and x is as
follows:
REM   0 - trial count
REM   1 - Side of response (1=right,2=left)
REM   2 - Cue (-1=valid,0=invalid)
REM   3 - Delay (csecs)
REM   4 - Trial type (0=correct, 1=early, 2=late)
REM   5 - Reaction time
REM   6 - Movement time
REM   7 - Latency to initiate nose poke
:
```

```
MODE 3
PROCparameters
T%(0)=T%(1)+T%(2)+T%(3)+T%(4)
DIM delay%(T%(0))
PROCtrialsort
IF box$="B" OR box$="b" THEN PROCrew(1) ELSE PROCrew(0)
IF box$="C" OR box$="c" THEN PROCrew(1)
WAIT
END

DEF PROCparameters
PRINT TAB(0,1) STRING$(23,"*");" 9-HOLE ARRAYS : TASK 1 : 'DELAY'
";STRING$(23,"*")
PRINT TAB(0,3) "Do you wish to use : A-box 1, B-box 2, or C-both boxes ?"
REPEAT
INPUT TAB(0,4) "Please enter A, B or C : "box$
IF box$<>"A" AND box$<>"a" AND box$<>"B" AND box$<>"b" AND box$<>"C"
AND box$<>"c" THEN PROCerror(1)
UNTIL box$="A" OR box$="a" OR box$="B" OR box$="b" OR box$="C" OR
box$="c"
D(1)=0.2 : D(2)=0.4 : D(3)=0.6 : D(4)=0.8
REPEAT
PRINT
TAB(32,7)SPC(4);TAB(32,8)SPC(4);TAB(32,9)SPC(4);TAB(32,10)SPC(4);TAB(32,11)
SPC(4)
PRINT TAB(0,6) "Variable delay before 'RESPOND' signal (Seconds) : "
PRINT TAB(0,7) "Preferred absolute values : A = ";D(1)
PRINT TAB(28,8) "B = ";D(2);TAB(28,9) "C = ";D(3);TAB(28,10) "D = ";D(4)
PRINT TAB(28,11) SPC(10);"Do you wish to change these ?"
REPEAT
INPUT TAB(0,12) "Please enter Y or N : "new$
IF new$<>"Y" AND new$<>"y" AND new$<>"N" AND new$<>"n" THEN
PROCerror(2)
UNTIL new$="Y" OR new$="y" OR new$="N" OR new$="n"
IF new$="Y" OR new$="y" THEN PROCchange
UNTIL new$="N" OR new$="n"
FOR loop%=1 TO 4
d%(loop%)=D(loop%)*100 : REM centiseconds
NEXT loop%
FOR loop%=1 TO 4:T%(loop%)=30: NEXT
REPEAT
INPUT TAB(0,18)"Please enter the percentage of trials which will be INVALIDLY cued:
"ivalid%
IF ivalid% >100 OR ivalid%<0 THEN PROCerror(4)
UNTIL ivalid%<=100 AND ivalid%>=0
CLS
PRINT TAB(0,1) STRING$(23,"*");" 9-HOLE ARRAYS : POSNER TASK
";STRING$(23,"*")
IF box$="A" OR box$="a" OR box$="C" OR box$="c" THEN PROCratone
IF box$="B" OR box$="b" OR box$="C" OR box$="c" THEN PROCrattwo
ENDPROC
```

```
DEF PROCchange
PRINT TAB(0,14) "Please enter new values to a maximum accuracy of two decimal
places :"
tab%=0
FOR loop%=1 TO 4
IF loop%=1 THEN n$="A" ELSE IF loop%=2 THEN n$="B" ELSE IF loop%=3 THEN
n$="C" ELSE IF loop%=4 ELSE n$="D"
REPEAT
PRINT TAB(tab%,15) n$;" = "
INPUT TAB(tab%+4,15)del
IF del<0 OR del>3 THEN PROCerror(3)
UNTIL del>=0 AND del<=3
D(loop%)=del
tab%=tab%+15
NEXT loop%
PRINT TAB(0,16) "These values will be reset for this session only."
PRINT "Press the SPACE BAR to continue."
REPEAT UNTIL GET=32
PRINT TAB(0,14) STRING$(750," ")
PRINT STRING$(160," ")
ENDPROC

DEF PROCtrialsort
CLS
PRINT TAB(0,1) STRING$(23,"*");" 9-HOLE ARRAYS : POSNER TASK ";
STRING$(23,"*")
FOR loop%=1 TO T%(0)
REPEAT
t%=RND(4)
delay%(loop%)=d%(t%)
UNTIL T%(t%)>0
T%(t%)=T%(t%)-1
NEXT loop%
PRINT TAB(0,3) "Place rats in boxes now, then press the SPACE BAR to start the
session."
REPEAT UNTIL GET=32
TIME=0
|(TIMER 7,180000)PROCstop
 PRINT TAB(0,11)"BOX 1: Cnt  Sid  V/I  Del  Resp  RT  MT  Lat"
 PRINT TAB(0,14)"BOX 2: Cnt  Sid  V/I  Del  Resp  RT  MT  Lat"
IF box$="B" OR box$="b" THEN SWITCH ON 62 ELSE SWITCH ON 60
IF box$="C" OR box$="c" THEN SWITCH ON 62
ENDPROC

DEF PROCratone
PRINT TAB(0,3) "Please enter rat No. for box 1 this session : "
REPEAT
INPUT TAB(46,3)rat1%
IF rat1%<1 OR rat1%>999 THEN PROCerror(8)
UNTIL rat1%>=1 AND rat1%<=999
ENDPROC
```

```
 DEF PROCrattwo
  PRINT TAB(0,14) "Please enter rat No. for box 2 this session : "
  REPEAT
  INPUT TAB(46,14)rat2%
  IF rat2%<1 OR rat2%>999 THEN PROCerror(8)
  UNTIL rat2%>=1 AND rat2%<=999
ENDPROC

 DEF PROCrew(B%)
  lat3%(B%)=TIME
  KILL SWITCH rh%(B%):KILL SWITCH wh%(B%)
  |(TIMER tim%(B%),10)PROCrewnxt(B%)
  |(SWITCH panel%(B%),ON)PROCoff(B%)
  fail%(B%)=FALSE
  SWITCH OFF r_lmp%(B%),l_lmp%(B%)
  SWITCH ON disp%(B%),tr_lmp%(B%)
  data%(B%,6)=lat3%(B%)-lat2%(B%):data%(B%,4)=0
  corr_cnt%(B%)=corr_cnt%(B%)+1
 ENDPROC


DEF PROCrewnxt(B%)
  SWITCH OFF disp%(B%)
  l%(B%)=RND(2):val%(B%)=RND(100)
  IF val%(B%)<=ivalid% THEN v%(B%)=FALSE ELSE v%(B%)=TRUE
  IF corr_cnt%(B%)>=T%(0) THEN PROCend(B%)
ENDPROC

 DEF PROCoff(B%)
  IF data%(B%,0)>0 AND B%=0 PROCpr1(B%)
  IF data%(B%,0)>0 AND B%=1 PROCpr2(B%)
   lat4%(B%)=TIME:data%(B%,0)=data%(B%,0)+1
  |(SWITCH ch%(B%),ON)PROCdel(B%)
  SWITCH OFF tr_lmp%(B%):SWITCH ON c_lmp%(B%):KILL SWITCH panel%(B%)
  IF fail%(B%)=FALSE THEN data%(B%,7)=lat4%(B%)-lat3%(B%) ELSE
data%(B%,7)=0
  data%(B%,1)=l%(B%):data%(B%,3)=delay%(corr_cnt%(B%)):data%(B%,2)=v%(B%)
 ENDPROC

DEF PROCpr1(B%)
FOR N%=0 TO 7:
out1%(N%)=data%(B%,N%):P.TAB((N%*5)+7,12);data%(B%,N%);SPC(2):NEXT
P.TAB(35,(B%*3)+13)"Correct:  ";corr_cnt%(B%)
SRWRITE ADDR(out1%(0)), SIZE(out1%()),ptr%(B%)
ptr%(B%)=ptr%(B%)+32
ENDPROC

DEF PROCpr2(B%)
FOR N%=0 TO 7:
out2%(N%)=data%(B%,N%):P.TAB((N%*5)+7,15);data%(B%,N%);SPC(2):NEXT
P.TAB(35,(B%*3)+13)"Correct:  ";corr_cnt%(B%)
SRWRITE ADDR(out2%(0)), SIZE(out2%()),ptr%(B%)
```

```
ptr%(B%)=ptr%(B%)+32
ENDPROC

 DEF PROCdel(B%)
  |(TIMER tim%(B%+2),10)PROCcueoff(B%)
  |(TIMER tim%(B%),delay%(data%(B%,3)))PROClmpon(B%)
  |(SWITCH ch%(B%),OFF)PROCf1(B%)
  SWITCH OFF c_lmp%(B%)
  IF(l%(B%)-v%(B%)=2) THEN SWITCH ON (r_lmp%(B%)+cue%) ELSE SWITCH
ON (l_lmp%(B%)+cue%)
 ENDPROC

 DEF PROCcueoff(B%)
  SWITCH OFF (l_lmp%(B%)+cue%),(r_lmp%(B%)+cue%)
 ENDPROC

 DEF PROCf1(B%)
  KILL SWITCHch%(B%):KILL SWITCH panel%(B%):data%(B%,4)=2
  |(TIMER tim%(B%),200)PROCf1nxt(B%)
  SWITCH OFFhse%(B%),r_lmp%(B%),l_lmp%(B%)
  data%(B%,5)=0:data%(B%,6)=0:earl_cnt%(B%)=earl_cnt%(B%)+1
  P.TAB(0,(B%*3)+13)"Early:  ";earl_cnt%(B%)
 ENDPROC

 DEF PROCf1nxt(B%)
  SWITCH ON hse%(B%),tr_lmp%(B%):fail%(B%)=TRUE
  |(SWITCH panel%(B%),ON)PROCoff(B%)
 ENDPROC

DEF PROClmpon(B%)
  lat1%(B%)=TIME
  |(TIMER tim%(B%+2),15)PROClmpoff(B%)
  |(TIMER tim%(B%),10)PROCresp(B%)
  IF l%(B%)=1 THENrh%(B%)=6+(B%*10):wh%(B%)=4+(B%*10):SWITCH ON
r_lmp%(B%) ELSE rh%(B%)=4+(B%*10):wh%(B%)=6+(B%*10):SWITCH ON
l_lmp%(B%)
 ENDPROC

DEF PROClmpoff(B%)
 SWITCH OFF r_lmp%(B%),l_lmp%(B%),(l_lmp%(B%)+cue%),(r_lmp%(B%)+cue%)
ENDPROC

 DEF PROCresp(B%)
  |(SWITCH ch%(B%),OFF)PROCrt(B%)
  |(TIMER tim%(B%),200)PROCf3(B%)
 ENDPROC

DEF PROCrt(B%):lat2%(B%)=TIME:data%(B%,5)=lat2%(B%)-lat1%(B%)
  KILL SWITCH ch%(B%)
  |(SWITCH rh%(B%),ON)PROCrew(B%)
  |(SWITCH wh%(B%),ON)PROCf2(B%)
ENDPROC
```

```
DEF PROCf2(B%)
 lat3%(B%)=TIME:data%(B%,4)=1
 KILL SWITCH rh%(B%):KILL SWITCH wh%(B%)
 KILL SWITCH ch%(B%):KILL SWITCH panel%(B%)
 |(TIMER tim%(B%),150)PROCf2nxt(B%)
 data%(B%,6)=lat3%(B%)-lat2%(B%):inc_cnt%(B%)=inc_cnt%(B%)+1
 SWITCH OFF hse%(B%),r_lmp%(B%),l_lmp%(B%)
P.TAB(11,(B%*3)+13)"Incorrect:  ";inc_cnt%(B%)
ENDPROC

DEF PROCf2nxt(B%)
 fail%(B%)=TRUE
 SWITCH ON hse%(B%),tr_lmp%(B%)
 |(SWITCH panel%(B%),ON)PROCoff(B%)
ENDPROC

DEF PROCf3(B%)
 data%(B%,6)=0:data%(B%,4)=3:late_cnt%(B%)=late_cnt%(B%)+1
 KILL SWITCH rh%(B%):KILL SWITCH wh%(B%)
 KILL SWITCH ch%(B%):KILL SWITCH panel%(B%)
 |(TIMER tim%(B%),150)PROCf3nxt(B%)
 SWITCH OFF hse%(B%),r_lmp%(B%),l_lmp%(B%)
 P.TAB(25,(B%*3)+13)"Late:  ";late_cnt%(B%)
ENDPROC

DEF PROCf3nxt(B%)
 fail%(B%)=TRUE
 SWITCH ON hse%(B%),tr_lmp%(B%)
 |(SWITCH panel%(B%),ON)PROCoff(B%)
ENDPROC

DEF PROCend(B%)
 KILL SWITCH ch%(B%):KILL SWITCH panel%(B%)
 SWITCH OFF hse%(B%),tr_lmp%(B%),c_lmp%(B%)
 SWITCH OFF r_lmp%(B%),l_lmp%(B%)
 end%(B%)=TRUE
 IF B%=0 THEN b%=1 : box%=1: PROCpr1(B%) ELSE b%=0 : box%=2 :
PROCpr2(B%)
 IF box$<>"C" AND box$<>"c" THEN end%(b%)=TRUE
 PRINT TAB(0,25+box%) "Session completed in box ";box%;" at ";RIGHT$(TIME$,8)
 IF end%(B%)=TRUE AND end%(b%)=TRUE THEN PROCstop
ENDPROC

DEF PROCstop
 PRINT CHR$(7)
 SWITCH OFF 20 TO 62
 KILL ALL
 IF box$="A" OR box$="a" OR box$="C" OR box$="c" PROCbox1
 IF box$="B" OR box$="b" OR box$="C" OR box$="c" PROCbox2
 PROCsumm
 END
```

```
ENDPROC

DEF PROCbox1
in_size1%=ptr%(0)/4
IF in_size1%>7 THEN PROCsave1
ENDPROC

DEF PROCbox2
in_size2%=(ptr%(1)-32000)/4
IF in_size2%>7 THEN PROCsave2
ENDPROC

DEF PROCsave1
CLS
PRINT TAB(0,0) "Please enter a filename for Box 1 data: "
REPEAT
INPUT TAB(0,1) "(Max. 7 characters)    Filename = "name1$
IF LEN(name1$)>7 THEN PROCerror(11)
UNTIL LEN(name1$)<=7
PRINT "Saving data from Box 1 to C:\termem\data\posner\"name1$"".";rat1%
PRINT "Press the SPACE BAR when ready to continue"
REPEAT UNTIL GET=32
CLS
in_size1%=ptr%(0)/4
DIM in1%(in_size1%)
N%=0
SRREAD ADDR(in1%(0)),SIZE(in1%()),0
OSCLI("*SPOOL-FSLINK-
C:\TERMEM\DATA\POSNER\"+name1$+"."+STR$(rat1%))
PRINT rat1%," Percentage of invalid trials: ",ivalid%
REPEAT
PRINT
in1%(N%),in1%(N%+1),in1%(N%+2),in1%(N%+3),in1%(N%+4),in1%(N%+5),in1%(N
%+6),in1%(N%+7)
N%=N%+8
UNTIL N%=in_size1%
*SPOOL
ENDPROC

DEF PROCsave2
CLS
PRINT TAB(0,0) "Please enter a filename for Box 2 data: "
REPEAT
INPUT TAB(0,1) "(Max. 7 characters)    Filename = "name2$
IF LEN(name2$)>7 THEN PROCerror(11)
UNTIL LEN(name2$)<=7
PRINT "Saving data from Box 2 to C:\termem\data\posner\"name2$"".";rat2%
PRINT "Press the SPACE BAR when ready to continue"
REPEAT UNTIL GET=32
CLS
ptr%(1)=ptr%(1)-32000
in_size2%=ptr%(1)/4
```

```
DIM in2%(in_size2%)
N%=0
SRREAD ADDR(in2%(0)),SIZE(in2%()),32000
OSCLI("*SPOOL-FSLINK-
C:\TERMEM\DATA\POSNER\"+name2$+"."+STR$(rat2%))
PRINT rat2%," Percentage of invalid trials: ",ivalid%
REPEAT
PRINT
in2%(N%),in2%(N%+1),in2%(N%+2),in2%(N%+3),in2%(N%+4),in2%(N%+5),in2%(N
%+6),in2%(N%+7)
N%=N%+8
UNTIL N%=in_size2%
*SPOOL
ENDPROC

DEF PROCsumm
CLS
P.TAB(0,1)"SUMMARY OF SESSION IN BOX1"
P.TAB(0,4)"Correct Trials:"corr_cnt%(0)
P.TAB(0,6)"Incorrect:"inc_cnt%(0)
P.TAB(0,8)"Early errors:"earl_cnt%(0)
P.TAB(0,10)"Late errors:"late_cnt%(0)
P.TAB(0,13)"SUMMARY OF SESSION IN BOX2"
P.TAB(0,16)"Correct Trials:"corr_cnt%(1)
P.TAB(0,18)"Incorrect:"inc_cnt%(1)
P.TAB(0,20)"Early errors:"earl_cnt%(1)
P.TAB(0,22)"Late errors:"late_cnt%(1)
ENDPROC

 DEF PROCerror(n%)
  A$="Invalid character. Please enter A, B or C ONLY."
  B$="Invalid character. Please enter Y or N ONLY."
  C$="Number out of range. 0 < delay <= 3."
  D$="Number out of range. 0 <= number <= 100."
  E$="Number out of range. 0 < choice <= 5."
  F$="Number out of range. 1 <= choice <= 4."
  G$="Number out of range. 6 <= choice <=9."
  H$="Number out of range. 0 < rat No. <=1000."
  I$="Session code too long. Max length = 10 characters."
  J$="Invalid character. Please enter 1 or 3 ONLY."
  K$="Filename too large. Max length = 7 characters."
  L$="Filename already exists. Re-enter using new name or drive."
  IFn%=1THENm$=A$ELSE IFn%=2THENm$=B$ELSE IFn%=3THENm$=C$ELSE
IFn%=4THENm$=D$ELSE IFn%=5THENm$=E$ELSE IFn%=6THENm$=F$ELSE
IFn%=7THENm$=G$ELSE IFn%=8THENm$=H$ELSE IFn%=9THENm$=I$ELSE
IFn%=10THENm$=J$ELSE IFn%=11THENm$=K$ELSEm$=L$
  FOR X=1 TO 5
  PRINT TAB(0,23)m$
  FOR Y=1 TO 1000
  NEXT Y
  PRINT TAB(0,23)STRING$(78," ")
  FOR Y=1 TO 200
```

```
 NEXT Y
 NEXT X
ENDPROC

DEF PROCdisaster
 err%=ERR : erl%=ERL
 IF err%=190 THEN message$="Too many files exist at chosen drive. This has caused
program failure." ELSE IF err%=198 THEN message$="Data disc has been completely
filled, causing program failure."
 secondmessage$="Please type KILL ALL (enter) to exit program, then re-RUN using
other side of,  or a new, data disc."
 IF err%=190 OR err%=198 THEN PRINT message$ : PRINT secondmessage$ ELSE
PROCalarm(err%,erl%)
ENDPROC

DEF PROCalarm(err%,erl%)
 PRINT "Error number ";err%;" at line ";erl%
 PRINT "PROGRAM ERROR"
 STOP
ENDPROC
```

Program for Simple Reaction Time Task with a Visually Cued Multiple-Ratio Schedule of Reinforcement (SCHED)

```
*SPIDER
AUTO
REM 9-HOLE ARRAY TEST BOXES : SRT WITH CUED VARIABLE SCHEDULE
:
*SRDATA 4
*SRDATA 5
*SRDATA 6
*SRDATA 7
ON ERROR PROCdisaster
KILL ALL
FREE SWITCH 0 TO 19
GOVN SWITCH 20 TO 63
:
REM Inputs
DIM ch%(1),panel%(1)
ch%(0)=5 : ch%(1)=15 : panel%(0)=0 : panel%(1)=10 :
:
REM Outputs
DIM hse%(1),tone%(1),cue%(1,6),tim%(1),c_lmp%(1),tr_lmp%(1),disp%(1)
hse%(0)=60 : hse%(1)=62 : tone%(0)=61 : tone%(1)=63 : tim%(0)=1 : tim%(1)=2
c_lmp%(0)=25 : c_lmp%(1)=35 : tr_lmp%(0)=20 : tr_lmp%(1)=30
disp%(0)=40 : disp%(1)=50
:
REM data arrays,counters and pointers
DIM schedule%(1),n_sched%(1,180),del%(180),lmps%(1)
DIM data%(1,6),lat1%(1),lat2%(1),lat3%(1),lat4%(1),ptr%(1)
DIM corr_cnt%(1),earl_cnt%(1),late_cnt%(1),I%(1)
DIM res%(1),D(5),d%(5),T%(1,5),fail%(1),out1%(6),out2%(6),end%(1)
ptr%(0)=0 : ptr%(1)=32000 : I%(0)=0 : I%(1)=0
fail%(0)=FALSE : fail%(1)=FALSE : end%(0)=FALSE : end%(1)=FALSE
REM late% = time after which response is considered a late error
late%=1000
corr_cnt%(0)=0:corr_cnt%(1)=0:earl_cnt%(0)=0:earl_cnt%(1)=0:late_cnt%(0)=0:late_cnt
%(1)=0:
REM Data: saved in array data%(B%,x) where B% is the box reference and x is as
follows:
REM   0 - trial count
REM   1 - number in schedule (responses before reward)
REM   2 - Delay (csecs)
REM   3 - Trial type (0=correct, 1=early, 2=late)
REM   4 - Reaction time
REM   5 - Movement time
REM   6 - Latency to initiate nose poke
:
MODE 3
PROCparameters
PROCtrialsort
|(TIMER 7,180000)PROCstop
IF box$="B" OR box$="b" THEN PROCstart(1) ELSE PROCstart(0)
```

```
IF box$="C" OR box$="c" THEN PROCstart(1)
WAIT
END
:
DEF PROCparameters
PRINT TAB(0,1) STRING$(23,"*");" 9-HOLE ARRAYS : TASK 2 : 'SRT'
";STRING$(23,"*")
PRINT TAB(0,3) "Do you wish to use : A-box 1, B-box 2, or C-both boxes ?"
REPEAT
INPUT TAB(0,4) "Please enter A, B or C : "box$
IF box$<>"A" AND box$<>"a" AND box$<>"B" AND box$<>"b" AND box$<>"C"
AND box$<>"c" THEN PROCerror(1)
UNTIL box$="A" OR box$="a" OR box$="B" OR box$="b" OR box$="C" OR
box$="c"
D(1)=0.1 : D(2)=0.2 : D(3)=.3 : D(4)=0.4 : D(5)=0.5
REPEAT
PRINT
TAB(32,7)SPC(4);TAB(32,8)SPC(4);TAB(32,9)SPC(4);TAB(32,10)SPC(4);TAB(32,11)
SPC(4)
PRINT TAB(0,6) "Variable delay before Imperative signal (Seconds) : "
PRINT TAB(0,7) "Preferred absolute values : A = ";D(1)
PRINT TAB(28,8) "B = ";D(2);TAB(28,9) "C = ";D(3);TAB(28,10) "D = ";D(4)
PRINT TAB(28,11) "E = ";D(5);SPC(10);"Do you wish to change these ?"
REPEAT
INPUT TAB(0,12) "Please enter Y or N : "new$
IF new$<>"Y" AND new$<>"y" AND new$<>"N" AND new$<>"n" THEN
PROCerror(7)
UNTIL new$="Y" OR new$="y" OR new$="N" OR new$="n"
IF new$="Y" OR new$="y" THEN PROCchange
UNTIL new$="N" OR new$="n"
FOR loop%=1 TO 5
d%(loop%)=D(loop%)*100 : REM centiseconds
NEXT loop%
PRINT TAB(0,1) STRING$(23,"*");" 9-HOLE ARRAYS : 'SRT' ";STRING$(23,"*")
IF box$="A" OR box$="a" OR box$="C" OR box$="c" THEN PROCratone
IF box$="B" OR box$="b" OR box$="C" OR box$="c" THEN PROCrattwo
CLS
ENDPROC
:
DEF PROCchange
PRINT TAB(0,14) "Please enter new values to a maximum accuracy of two decimal
places :"
tab%=0
FOR loop%=1 TO 5
IF loop%=1 THEN n$="A" ELSE IF loop%=2 THEN n$="B" ELSE IF loop%=3 THEN
n$="C" ELSE IF loop%=4 THEN n$="D" ELSE n$="E"
REPEAT
PRINT TAB(tab%,15) n$;" = "
INPUT TAB(tab%+4,15)del
IF del<0 OR del>3 THEN PROCerror(3)
UNTIL del>=0 AND del<=3
D(loop%)=del
```

```
tab%=tab%+15
NEXT loop%
PRINT TAB(0,16) "These values will be reset for this session only."
PRINT "Press the SPACE BAR to continue."
REPEAT UNTIL GET=32
PRINT TAB(0,14) STRING$(750," ")
PRINT STRING$(160," ")
ENDPROC
:
DEF PROCratone
CLS
PRINT TAB(0,1) STRING$(23,"*");" 9-HOLE ARRAYS : 'SRT' ";STRING$(23,"*")
PRINT TAB(0,3) "                SESSION INFORMATION FOR BOX 1"
PRINT TAB(0,6) "Please enter rat No. for box 1 this session : "
REPEAT
INPUT TAB(46,6)rat1%
IF rat1%<1 OR rat1%>999 THEN PROCerror(4)
UNTIL rat1%>=1 AND rat1%<=999
PRINT TAB(0,9) "Brightness of cue lights for Box 1 :"
REPEAT
INPUT TAB(0,10) "Please enter (B)rightening or (D)imming with reward schedule:
"cue1$
IF cue1$<>"B" AND cue1$<>"b" AND cue1$<>"D" AND cue1$<>"d"  THEN
PROCerror(2)
UNTIL cue1$="B" OR cue1$="b" OR cue1$="D" OR cue1$="d"
IF cue1$="B" OR cue1$="b" THEN lmps%(0)=1 ELSE lmps%(0)=2
cue%(0,1)=21:cue%(0,2)=41:cue%(0,3)=21
PRINT TAB(0,13) "Max. number of responses per reward: 1, 2 or 3 (in random series)?"
REPEAT
INPUT TAB(0,14) "Please enter a number from 1 to 3:  "schedule%(0)
IF schedule%(0)<1 OR schedule%(0)>3 THEN PROCerror(3)
UNTIL schedule%(0)=1 OR schedule%(0)=2 OR schedule%(0)=3
FOR N%=1 TO 5 : T%(0,N%)=24 : NEXT
T%(0,0)=T%(0,1)+T%(0,2)+T%(0,3)+T%(0,4)+T%(0,5)
ENDPROC
:
DEF PROCrattwo
CLS
PRINT TAB(0,1) STRING$(23,"*");" 9-HOLE ARRAYS : 'SRT' ";STRING$(23,"*")
PRINT TAB(0,3) "                SESSION INFORMATION FOR BOX 2"
PRINT TAB(0,6) "Please enter rat No. for box 2 this session : "
REPEAT
INPUT TAB(46,6)rat2%
IF rat2%<1 OR rat2%>999 THEN PROCerror(4)
UNTIL rat2%>=1 AND rat2%<=999
PRINT TAB(0,9) "Cue lights for Box 2:"
REPEAT
INPUT TAB(0,10) "Please enter (B)rightening or (D)imming with reward schedule:
"cue2$
IF cue2$<>"B" AND cue2$<>"b" AND cue2$<>"D" AND cue2$<>"d"  THEN
PROCerror(2)
UNTIL cue2$="B" OR cue2$="b" OR cue2$="D" OR cue2$="d"
```

```
IF cue2$="B" OR cue2$="b" THEN lmps%(1)=1 ELSE lmps%(1)=2
cue%(1,1)=31:cue%(1,2)=51:cue%(1,3)=31
PRINT TAB(0,13) "Max. number of responses per reward: 1, 2 or 3 (in random series)?"
REPEAT
INPUT TAB(0,14) "Please enter a number from 1 to 3:  "schedule%(1)
IF schedule%(1)<1 OR schedule%(1)>3 THEN PROCerror(3)
UNTIL schedule%(1)=1 OR schedule%(1)=2 OR schedule%(1)=3
REM Set numnber of trials here, where total = 5 x T%(1,N%)
FOR N%=1 TO 5 : T%(1,N%)=24 : NEXT
T%(1,0)=T%(1,1)+T%(1,2)+T%(1,3)+T%(1,4)+T%(1,5)
ENDPROC
:
DEF PROCtrialsort
CLS
PRINT TAB(0,1) STRING$(23,"*");" 9-HOLE ARRAYS : TASK 1 : 'SRT' ";
STRING$(23,"*")
p%=0 : q%=0
FOR loop%=0 TO 180
t%=RND(5) : del%(loop%)=d%(t%)
  IF (schedule%(1)>1 AND p%=0) THEN p%=RND(schedule%(1)) ELSE IF
(schedule%(1)=1) THEN p%=1
  IF (schedule%(0)>1 AND q%=0) THEN q%=RND(schedule%(0)) ELSE IF
(schedule%(0)=1) THEN q%=1
n_sched%(1,loop%)=p%:p%=p%-1: n_sched%(0,loop%)=q%:q%=q%-1
NEXT loop%
PRINT TAB(0,6) "The total number of trials in Box 1 for this session will be ";T%(0,0)
PRINT TAB(0,7) "The total number of trials in Box 2 for this session will be ";T%(1,0)
PRINT TAB(0,10) "Place rats in boxes now, then press the SPACE BAR to start the
session."
IF box$="B" OR box$="b" THEN SWITCH ON 62 ELSE SWITCH ON 60
IF box$="C" OR box$="c" THEN SWITCH ON 62
REPEAT UNTIL GET=32
ENDPROC
:
DEF PROCstart(B%)
  CLS
  P.TAB(0,1) STRING$(20,"*");"  SIMPLE R.T. : SESSION RUNNING  ";
STRING$(20,"*")
  PRINT TAB(0,11)"BOX 1: Cnt  Rew  Del  Resp RT   MT   Lat"
  PRINT TAB(0,14)"BOX 2: Cnt  Rew  Del  Resp RT   MT   Lat"
  data%(B%,1)=n_sched%(B%,0)
  PROCrew(B%)
ENDPROC

DEF PROCrew(B%)
  lat3%(B%)=TIME
  |(TIMER tim%(B%),10)PROCrewnext(B%)
  SWITCH OFF cue%(B%,data%(B%,1)) TO cue%(B%,data%(B%,1))+8
  SWITCH ON tr_lmp%(B%)
  IF data%(B%,1)=1 THEN SWITCH ON disp%(B%)
 ENDPROC
```

```
DEF PROCrewnext(B%)
 SWITCH OFF disp%(B%)
 data%(B%,5)=lat3%(B%)-lat2%(B%):data%(B%,3)=0
 fail%(B%)=FALSE
corr_cnt%(B%)=corr_cnt%(B%)+1
PROCoff(B%)
 IF corr_cnt%(B%)=T%(B%,0) THEN PROCend(B%)
ENDPROC

REM : Note - the trial only advances if the response is correct (i.e., neither early nor late).
Otherwise,
REM : an error is recorded and the trial (same delay, same cue) is repeated.

DEF PROCoff(B%)
 IF data%(B%,0)>0 AND B%=0 PROCpr1(B%)
 IF data%(B%,0)>0 AND B%=1 PROCpr2(B%)
 SWITCH ON c_lmp%(B%)
 data%(B%,0)=data%(B%,0)+1
data%(B%,1)=n_sched%(B%,corr_cnt%(B%)):data%(B%,2)=del%(corr_cnt%(B%))
 |(SWITCH ch%(B%),ON)PROCdel(B%)
 IF (lmps%(B%)=1 AND data%(B%,1)>1) OR (lmps%(B%)=2 AND data%(B%,1)<3)
THEN SWITCH ON cue%(B%,data%(B%,1)) TO cue%(B%,data%(B%,1))+8
 KILL SWITCH panel%(B%) : SWITCH OFF tr_lmp%(B%)
ENDPROC

DEF PROCpr1(B%)
FOR N%=0 TO 6:
out1%(N%)=data%(B%,N%):P.TAB((N%*5)+7,12);data%(B%,N%);SPC(2): NEXT
SRWRITE ADDR(out1%(0)), SIZE(out1%()),ptr%(B%)
ptr%(B%)=ptr%(B%)+28
ENDPROC

DEF PROCpr2(B%)
FOR N%=0 TO 6:
out2%(N%)=data%(B%,N%):P.TAB((N%*5)+7,15);data%(B%,N%);SPC(2): NEXT
SRWRITE ADDR(out2%(0)), SIZE(out2%()),ptr%(B%)
ptr%(B%)=ptr%(B%)+28
ENDPROC

DEF PROCdel(B%)
 lat4%(B%)=TIME
 IF fail%(B%)=FALSE THEN data%(B%,6)=lat4%(B%)-lat3%(B%)ELSE
data%(B%,6)=0
 |(TIMER tim%(B%),data%(B%,2))PROCresp(B%)
 |(SWITCH ch%(B%),OFF)PROCf1(B%):SWITCH OFF c_lmp%(B%),
c_lmp%(B%)+20
ENDPROC

DEF PROCf1(B%)
 |(TIMER tim%(B%),150)PROCf1next(B%)
 SWITCH OFF hse%(B%)
 KILL SWITCH ch%(B%)
```

```
 data%(B%,3)=1:data%(B%,4)=0:data%(B%,5)=0:earl_cnt%(B%)=earl_cnt%(B%)+1
 P.TAB(0,(B%*3)+13)"Early:  ";earl_cnt%(B%)
ENDPROC

DEF PROCf1next(B%)
 SWITCH ON hse%(B%),tr_lmp%(B%)
 fail%(B%)=TRUE
 |(SWITCH panel%(B%),ON)PROCoff(B%)
ENDPROC

DEF PROCresp(B%)
 lat1%(B%)=TIME
 |(SWITCH ch%(B%),OFF)PROCrt(B%)
 |(TIMER tim%(B%),10)PROCtone(B%)
 SWITCH ON tone%(B%)
ENDPROC

DEF PROCtone(B%):SWITCH OFF tone%(B%):|(TIMER
tim%(B%),late%)PROCf2(B%):ENDPROC

DEF PROCrt(B%):lat2%(B%)=TIME:data%(B%,4)=lat2%(B%)-lat1%(B%)
 |(SWITCH panel%(B%),ON)PROCrew(B%)
ENDPROC

DEF PROCf2(B%)
 KILL SWITCH panel%(B%)
 data%(B%,3)=2:data%(B%,5)=0:late_cnt%(B%)=late_cnt%(B%)+1
 |(TIMER tim%(B%),150)PROCf2next(B%)
 SWITCH OFF hse%(B%)
 P.TAB(11,(B%*3)+13)"Late:  ";late_cnt%(B%)
ENDPROC

DEF PROCf2next(B%)
 fail%(B%)=TRUE
 SWITCH ON hse%(B%),tr_lmp%(B%)
 |(SWITCH panel%(B%),ON)PROCoff(B%)
ENDPROC

DEF PROCend(B%)
 KILL SWITCH panel%(B%)
 KILL SWITCH ch%(B%)
 KILL TIMER tim%(B%)
 SWITCH OFF hse%(B%),tr_lmp%(B%),c_lmp%(B%),disp%(B%),cue%(B%,1) TO
cue%(B%,1)+8,cue%(B%,2) TO cue%(B%,2)+8
 end%(B%)=TRUE
 IF B%=0 THEN b%=1 : box%=1 : PROCpr1(B%) ELSE b%=0 : box%=2 :
PROCpr2(B%)
 IF box$<>"C" AND box$<>"c" THEN end%(b%)=TRUE
 PRINT TAB(0,13+(box%*3)) "Session completed in box ";box%;" at
";RIGHT$(TIME$,8)
 IF end%(B%)=TRUE AND end%(b%)=TRUE THEN PROCstop
ENDPROC
```

```
DEF PROCstop
PRINT CHR$(7)
SWITCH OFF 20 TO 62
KILL ALL
IF box$="A" OR box$="a" OR box$="C" OR box$="c" PROCbox1
IF box$="B" OR box$="b" OR box$="C" OR box$="c" PROCbox2
PROCsumm
ENDPROC

DEF PROCbox1
in_size1%=ptr%(0)/4
IF in_size1%>6 THEN PROCsave1
ENDPROC

DEF PROCbox2
in_size2%=(ptr%(1)-32000)/4
IF in_size2%>6 THEN PROCsave2
ENDPROC

DEF PROCsave1
CLS
PRINT TAB(0,0) "Please enter a filename for Box 1 data: "
REPEAT
INPUT TAB(0,1) "(Max. 7 characters)   Filename = "name1$
IF LEN(name1$)>7 THEN PROCerror(11)
UNTIL LEN(name1$)<=6
PRINT "Saving data from Box 1 to C:\termem\data\sched\"name1$;cue1$".";rat1%
PRINT "Press the SPACE BAR when ready to continue"
REPEAT UNTIL GET=32
CLS
DIM in1%(in_size1%)
N%=0
SRREAD ADDR(in1%(0)),SIZE(in1%()),0
OSCLI("*SPOOL-FSLINK-
C:\TERMEM\DATA\SCHED\"+name1$+cue1$+"."+STR$(rat1%))
IF lmps%(0)=1 PRINT "Cues brightened" ELSE P."Cues dimmed"
REPEAT
PRINT
in1%(N%),in1%(N%+1),in1%(N%+2),in1%(N%+3),in1%(N%+4),in1%(N%+5),in1%(N
%+6)
N%=N%+7
UNTIL N%=in_size1%
*SPOOL
ENDPROC

DEF PROCsave2
CLS
PRINT TAB(0,0) "Please enter a filename for Box 2 data: "
REPEAT
INPUT TAB(0,1) "(Max. 7 characters)   Filename = "name2$
IF LEN(name2$)>7 THEN PROCerror(11)
```

```
UNTIL LEN(name2$)<=6
PRINT "Saving data from Box 2 to C:\termem\data\sched\"name2$;cue2$".";rat2%
PRINT "Press the SPACE BAR when ready to continue"
REPEAT UNTIL GET=32
CLS
DIM in2%(in_size2%)
N%=0
SRREAD ADDR(in2%(0)),SIZE(in2%()),32000
OSCLI("*SPOOL-FSLINK-
C:\TERMEM\DATA\SCHED\"+name2$+cue2$+"."+STR$(rat2%))
IF lmps%(1)=1 PRINT "Cues brightened" ELSE P."Cues dimmed"
REPEAT
PRINT
in2%(N%),in2%(N%+1),in2%(N%+2),in2%(N%+3),in2%(N%+4),in2%(N%+5),in2%(N
%+6)
N%=N%+7
UNTIL N%=in_size2%
*SPOOL
ENDPROC

DEF PROCsumm
CLS
P.TAB(0,1)"SUMMARY OF SESSION IN BOX1"
P.TAB(0,4)"Correct Trials:"corr_cnt%(0)
P.TAB(0,6)"Early errors:"earl_cnt%(0)
P.TAB(0,8)"Late errors:"late_cnt%(0)
P.TAB(0,13)"SUMMARY OF SESSION IN BOX2"
P.TAB(0,16)"Correct Trials:"corr_cnt%(1)
P.TAB(0,18)"Early errors:"earl_cnt%(1)
P.TAB(0,20)"Late errors:"late_cnt%(1)
ENDPROC

 DEF PROCerror(n%)
  A$="Invalid character. Please enter A, B or C ONLY."
  B$="Invalid character. Please enter B or D ONLY."
  C$="Number out of range. 1 < pellets < 4"
  D$="Number out of range. 0 < rat No. <=999."
  E$="Invalid character. Please enter 1 or 3 ONLY."
  F$="Filename too large. Max length = 7 characters."
  G$="Invalid character. Please enter Y or N ONLY."
  H$="You have made an error"
  IFn%=1THENm$=A$ELSE IFn%=2THENm$=B$ELSE IFn%=3THENm$=C$ELSE
IFn%=4THENm$=D$ELSE IFn%=5THENm$=E$ELSE
IFn%=6THENm$=F$ELSEIFn%=7THENm$=G$ELSE m$=H$
  FOR X=1 TO 3
  PRINT TAB(0,23)m$
  FOR Y=1 TO 100
  NEXT Y
  PRINT TAB(0,23)STRING$(78," ")
  FOR Y=1 TO 100
  NEXT Y
  NEXT X
```

```
ENDPROC

DEF PROCdisaster
 err%=ERR : erl%=ERL
 PRINT "Error number ";err%;" at line ";erl%
 REPORT
 SWITCH OFF 0 TO 63
 KILL ALL
 END
ENDPROC
```