Neuroimaging of function in Alzheimer's disease and other dementias

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To my mother and father

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Statement of authorship

This thesis has been composed by myself and the studies presented are the result of my own independent investigation, apart from the contributions referred to in the acknowledgements. None of the material has been submitted for other degrees or professional qualifications.

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SUMMARY

Interest in Alzheimer's disease (AD) has increased considerably in the last decade. As a result of demographic changes the number of AD sufferers is steadily increasing and AD is recognised as a major public health challenge. This has been paralleled by recent advances in understanding the biology of AD, particularly using neuroimaging and molecular biological methods. This thesis describes an investigation using functional brain imaging techniques of presenile AD patients, and two control groups, Korsakoff's psychosis (KOR) and healthy subjects.

Current understanding of the concept of AD is reviewed, together with the contribution of clinical and neuropathological findings to the establishment of diagnostic criteria. The experimental chapters describe investigations carried out on *presenile* AD patients, therefore the relationship of early onset AD to that presenting at an older age is also discussed.

The rationale of using cerebral blood flow (CBF) as an index of brain metabolism rests on evidence that supports the hypothesis that there is close coupling of CBF and brain metabolism during 'resting' conditions, and possibly also under at least some conditions of brain activation. The development of methods for measuring CBF, from the early estimates of global CBF provided by the Kety-Schmidt technique to the introduction of functional tomographic imaging, such as single

photon emission computed tomography (SPECT) and positron emission tomography (PET), in the last decade is described. The contribution of these techniques to understanding the brain dysfunction of AD is critically reviewed.

A method for measuring the cerebral vascular transit time of pertechnetate (^{99m}Tc), after intravenous bolus injection using a gamma camera is described. Estimates of net mean transit time (net MTT) in a planar non-tomographic vertex projection of brain were determined in 17 patients with presenile AD, 9 abstinent patients with Korsakoff's psychosis (KOR), and 10 healthy matched controls. The CAMDEX was used to assess patients and the CAMCOG to describe cognitive function. In both AD and KOR groups net MTTs were significantly lengthened compared with controls, although without information on volume of distribution it is not possible to relate net MTT results to CBF. There was no significant difference between net MTT results of AD and KOR groups. More importantly, examination of individual net MTT scores in the three groups showed some degree of overlap. Using net MTT data from healthy control subjects, 'normal' cut-off values of net MTT for all regions were established, and positive predictive values calculated. For posterior cortical regions in AD patients, positive predictive values were greater than 70% suggesting that for this region the method may have some degree of clinical utility. The pattern of cognitive function was differentially impaired in AD and KOR groups and there was a tendency for estimates of net MTT to be inversely associated with CAMCOG measures. The prolongation of transit times in cortex of KOR patients suggests cortical involvement in a disorder usually considered primarily subcortical. Positive predictive value of net MTT in posterior regions of interest in AD patients support the case for further investigation of the clinical utility of the method.

The radiotracer ^{99m}Tc-HMPAO was used with SPECT to determine patterns of regional uptake of radiolabel into brain regions of patients with presenile Alzheimer's disease and Korsakoff's psychosis, and age-matched controls. Using occipital cortical uptake as a reference area, the pattern of relative regional cerebral blood flow (rCBF) was determined in other cortical areas and basal ganglia. In Alzheimer's disease reduction in rCBF occurred in posterior temporal and parietal areas. By contrast, in Korsakoff's psychosis posterior temporal rCBF was maintained, although there was a trend to reduced tracer uptake in other cortical areas. There was considerable overlap of individual relative rCBF values in both patient groups and controls. As a result positive predictive values are low and only greater than 50% in posterior temporal regions of AD patients. The degree of overlap of individual test results of different diagnositc groups casts doubt on the diagnostic utility of SPECT imaging using HMPAO. These impairments of flow were correlated with impairments of neuropsychological function. In Alzheimer's disease left posterior temporal and left parietal regions in particular showed rCBF to be correlated with most aspects of cognitive function. In Korsakoff's psychosis, however, impaired flow in frontal regions was correlated with impaired performance on tests of memory and orientation. The findings in Alzheimer's disease show parallels with quantitative studies using PET, and help extend our understanding of the relationship between cognition and regional brain function in dementia. The findings in Korsakoff's psychosis offer evidence linking frontal lobe dysfunction with the cognitive impairment seen clinically in the disorder. The complementary nature of SPECT imaging and structural imaging techniques, such as x-ray CT is illustrated in a case of the rare presenile dementia, Creutzfeldt-Jacob Disease.

^{99m}Tc-HMPAO SPECT was used to study the effects of physostigmine, a drug known to potentiate cholinergic function, on patterns of rCBF in patients with presenile Alzheimer's disease. Relative regional CBF appeared to increase in left cortex relative to right, with the most significant effect detected in left frontal and higher frontal regions. This study shows that measures of regional brain function, such as SPECT, may be a useful complement to psychological test batteries in understanding the effects in brain of putative anti-dementia drugs, such as physostigmine. Using this type of approach, SPECT brain imaging could be used to extend our understanding of the action of other psychotropic drugs in major psychiatric illnesses.

A SPECT technique using a split-dose of the tracer ^{99m}Tc-HMPAO, that permits the effects of physiological, pharmacological or psychological challenge on patterns of rCBF to be imaged is described. In a group of 9 patients with different brain pathologies the test-retest reliability of the method was measured. Precision values varied from +/- 3.6% in superior frontal cortex to 15.9% in basal ganglia. The split-dose paradigm may extend the use of the radiotracer HMPAO from basal SPECT imaging of brain states to investigation of the effects of challenge on patterns of rCBF. Other sources of error in test-retest SPECT paradigms were investigated in a series of experiments to assess: errors in data acquisition and image reconstruction, the effect of head movement during a scan, the effect of re-aligning the patient in the scanner, and serial scanning after one week, as in the physostigmine experiment.

Functional imaging techniques, such as SPECT, have only been available for a relatively short period of time. In neurodegenerative conditions such as AD or KOR, as well as in the

normal brain, they offer new opportunities to study brain metabolism *in vivo*. Initial studies have focussed on basal brain function and its relationship to cognitive impairment, however it is the potential of these techniques to image change in brain function in response to challenge, either pharmacological or neuropsychological, together with receptor imaging studies, that should contribute to future understanding of the biological basis of AD.

CHAPTER 1

THE NATURE OF ALZHEIMER'S DISEASE

1.1 Introduction

Alzheimer's disease (AD) is the commonest dementia, accounting for at least half of all cases of dementia examined at necropsy. It is a primary disorder of nerve cells leading to neuronal cell death and extensive brain atrophy. Although it is over eighty years since Alzheimer presented his case report, the disease that bears his name has only assumed major importance in the last decade.

The prevalence of AD increases with age, affecting at least 5% of those aged over 65 and as many as 25% of those over 80. As the numbers of elderly increase, AD will become a common cause of death, especially in developed countries. In both developed and less developed countries the proportion of elderly in the population is increasing at an unprecedented rate. In 1950 there were 214 million persons aged 60 years and over, by 2025 there will be 1 billion (Henderson, 1986). This will result in a large increase in the number of AD sufferers, many of whom will live longer as a result of improved medical and nursing care. The coming epidemic of AD is therefore a major challenge for public health services in terms of provision of adequate care and the economic resources needed to provide it. In the UK such resources are limited at the present time, and this is despite 80% of sufferers living in the community rather than in nursing homes or hospital. Major economic resources are needed for the care of Alzheimer patients. For example, in the USA it has been estimated that of the 21 billion dollars spent on nursing care in 1982, over 10 billion was spent on care of patients with AD (Terry & Katzman, 1983).

In recent years the application of biochemical and molecular genetic approaches to AD has increased understanding at a cellular level. Along with these advances in basic science, there have also been advances in the clinical understanding of AD. The importance of proper clinical evaluation and investigation of patients presenting with signs of dementia, in order to

establish the likely type of dementing process, is now widely recognised. Definitive diagnosis, however, requires histological examination, which is usually only possible post-mortem as brain biopsy is usually difficult to justify clinically. With more accurate diagnosis, better evaluation of management approaches and service planning is possible, as well as research into pathogenesis.

1.2 Concept of Alzheimer's disease

In November 1906 at a meeting of the South West German Society of Alienists, Alois Alzheimer presented a clinico-neuropathological report of a 51 years old woman whose symptoms began with loss of memory and disorientation. Later she suffered from depression, hallucinations and behavioural disturbance, and in four years profound dementia and death. At autopsy, her brain was found to be atrophied and the cerebral cortex contained miliary lesions ('herdchen'). Alzheimer also noted, using a silver impregnation method, a distortion and clumping of the cortical neurofibrils.

Alzheimer's presentation was published by title only in the *Neurologische Zentralblatt* in 1906 and his case report, 'Uber eine eigenartige Erkankung der Hirndrinde' ('on a peculiar disease of the cerebral cortex') in *Allgemeine Zeitschrift fur Psychiatrie und Psychisch-gerichtliche Medizin* in 1907. In 1910 the Italian, Perusini, described a further four cases of this disorder, which he believed was distinct from senility (Perusini, 1910). The following year Kraepelin proposed that the condition be called Alzheimer's disease, in recognition of Alzheimer's contribution. This tradition continues to be recognised and today the terms primary degenerative dementia of the Alzheimer type (DSM-III-R, 1987) and Alzheimer's disease (AD) are widely used.

However, more than 70 years earlier in England, James Cowles Prichard had described a syndrome that he called 'senile dementia' or 'incoherence', which he said was characterised by 'forgetfulness of recent impressions, while the memory retains a comparatively firm hold of ideas laid up in the recesses from times long past' (Prichard, 1835). As is now recognised, Prichard's senile dementia and that described by Alzheimer in a younger case share a similar neuropathology, and the consensus of opinion at present, is that they represent the same disease process (Roth 1978a).

The neuropathological changes in AD are characteristic: neuronal loss, senile plaques, and neurofibrillary tangles. It has therefore been argued that AD is a single disease entity, with its own causes and natural course. Such a conclusion gained support from the discovery of the cholinergic deficit in AD. As central cholinergic systems had already been implicated in pharmacological models of memory, it seemed plausible that the memory deficits of AD could be caused by selective loss of cholinergic neurones (Coyle et al 1983). However other neurochemical deficits (Hardy et al 1985) also characterise AD, and the disease process is more complex than previously thought . It is not known if the different patterns of neurochemical deficit represent different stages of a single disease process or different processes. The cause of neuronal death in AD is not known, but there does appear to be a consistent pattern of cell death leading to a characteristic clinical syndrome. AD may be the endpoint of many distinct causal pathways, each of which can lead to the pattern of neuronal damage found in AD.

The existence of AD as a distinct disease entity has been questioned, however, and it has been viewed as part of the normal aging process (Brody, 1982). Support for this view comes from studies in which screening tests aimed at detecting individuals at high risk of AD in the community

showed a unimodal rather than bimodal distribution (Katzman, 1988). However this work can be criticised as selecting out only those individuals able to answer the questionnaire, and not the more severely impaired. Perhaps a more interesting question is the way in which aging and AD interact, but this has received relatively little study (Lancet, 1989).

1.3 Clinical features, course and outcome

The onset of AD, although difficult to determine with any precision, is usually after the age of 40 years. Insidious memory impairment, dysphasia, dyspraxia, and visuospatial abnormalities and the progressive development of global dementia are usually recognised as the cardinal features of AD, although it is well recognised that behavioural, affective, psychotic and neurological symptoms may also occur (Blessed, 1980).

Memory difficulties occur as the earliest feature more often in AD than in any other type of presenile dementia (Sim et al 1966). The National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and Alzheimer's Disease and Related Disorders Association (ADRDA) work group on the diagnosis of AD (McKhann et al 1984) have defined dementia as: 'the decline of memory and other cognitive functions in comparison with the patient's previous level of function as determined by a history of decline in performance and by abnormalities noted from clinical examination and neuropsychological tests'. Furthermore, a diagnosis of dementia cannot be made when consciousness is impaired by delirium, drowsiness, stupor, or coma or when other clinical abnormalities prevent adequate evaluation of mental status.

The studies of Sjogren (1950) and Sjogren et al (1952) provide a clear account of the course of AD. Although a degenerative continuum, the progression of AD can be considered as three broad, but merging stages.

In the first stage, considerable social competence is often preserved along with gradual memory failure, muddled mental inefficiency for simple matters and spatial and temporal disorientation. Mood disturbances are well recognised at this time, but psychotic symptoms rarely occur. The change in mood may be manifest as agitation or restless perplexity or alternatively as apathy and lack of interest.

In the next phase memory failure progresses until the patient has little recognition of the major events in his or her life and may even fail to recognise close relatives. Along with intellectual and personality deterioration, focal symptoms appear. Language dysfunction is common with the development of expressive, and later receptive dysphasia. Parietal lobe dysfunction leads to dyspraxia and agnosia, although neuronal loss and tangle formation are often less in parietal regions than in frontal and temporal areas. However parietal association cortex has major connections with superior and inferior temporal cortex and with prefrontal cortex (Goldman-Rakic, 1984) and therefore structural and neurochemical deficits in frontal cortex may contribute to dyspraxia (Roth 1986).

As the patient becomes more demented, insight is lost and the emotions often become shallow and the patient apathetic. Psychotic symptoms may also occur at this stage and in one series, delusions occurred in 30% of AD patients (Cummings et al 1987). Delusions of theft and infidelity are common themes and accusatory beliefs can cause serious difficulties for carers (Rabins et al 1982). Hallucinations are less frequent, occurring in about 25% of subjects (Merriam et al 1988; Burns et al 1990a). Visual hallucinations tend to be more common than auditory hallucinations and may reflect the presence of an intercurrent delirium. Misidentifications also occur, including misidentifications of the subject's own mirror image and of other people. There are however difficulties associated with the elucidation of phenomenology in demented patients and many studies have

relied on reports of symptoms from relatives and caregivers which might ideally have been obtained through a direct interview with the patient (Rabins et al 1982; Merriam et al 1988; Burns et al 1990a). In future, more sensitive instruments might be developed to assess psychotic features in the cognitively impaired.

In the final stage of AD, the patient has severe intellectual impairment, and often finally becomes bedridden and doubly incontinent. Language function declines to a few meaningless utterances and comprehension of speech is lost. Neurological disability develops with increased tone, myoclonus, Parkinsonian features and extensor plantar reflexes. Walking may become increasingly unsteady, until the patient is finally bedridden with severe rigidity, muscle wasting and flexor contractures. Grand mal seizures also occur in the later stages of AD in about 10% of cases. The most frequent final cause of death is registered as bronchopneumonia often in bedridden patients. The final period of the illness is usually one of inexorable decline.

Thus the course of AD is one of progressive decline, with death occurring between two and ten years after onset. Death may occur earlier in presenile cases (Seltzer & Sherwin, 1983). The life expectancy of Alzheimer patients is reduced compared with age-matched controls (Roth 1978a, 1978b). There is some evidence that AD patients may be surviving longer; in 1955, Roth reported a 55% mortality of demented patients within 6 months of hospital admission (Roth, 1955), whereas two recent studies reported a lower mortality at six months: 32% (Blessed & Wilson, 1982) and 25% (Christie, 1982).

1.4 Diagnostic criteria

A variety of criteria have been proposed over the past few years in order to improve the clinical diagnosis of AD. This is important for several reasons; firstly, accurate diagnosis is a prerequisite for efficient management, and although specific treatments for AD are not yet available, there are many aspects of management, such as outcome planning that are improved by knowing which disease process is present; secondly, clinical diagnosis is important for research purposes and in the evaluation of putative new treatments. Essentially all the diagnostic schema for AD select for cases with the typical insidious onset of memory impairment and steady progression of cognitive decline and exclude, through detailed clinical assessment and biochemical, and radiological investigation, other possible causes of dementia (Glen & Christie, 1979; McKhann et al 1984).

The work group on the diagnosis of AD established by NINCDS and ADRDA (see section 1.5) has proposed the following criteria for the clinical diagnosis of *probable AD* (McKhann et al 1984): 1) dementia established by clinical examination and documented by the Mini-Mental State Examination test (Folstein et al 1975), Blessed Dementia Scale (Blessed et al 1968) or similar examination, and confirmed by neuropsychological tests; 2) deficits in two or more areas of cognition; 3) progressive worsening of memory or other cognitive functions; 4) no disturbance of consciousness; 5) onset between the ages of 40 and 90, but more often after the age of 65 years; 6) absence of systemic disorders or other brain diseases that could result in progressive cognitive decline. A diagnosis of probable AD is supported by: progressive deterioration of specific cognitive functions such as language (aphasia), visuospatial motor skills (apraxia), and perception (agnosia); impaired activities of daily living and altered patterns of behaviour; a family history of such disorders, particularly if confirmed neuropathologically; and the following laboratory results: normal

CSF after lumbar puncture, normal pattern or only non-specific changes in the EEG, and evidence of cerebral atrophy on X-ray CT neuroimaging with progression documented by serial observation. Neuropathological confirmation is important for the audit of clinical diagnosis and essential before a diagnosis of *definite AD* can be made. Guidelines for the pathological diagnosis of AD have also been suggested by the Medical Research Council Working Group on Alzheimer's disease, which are consistent with the above NINCDS and ADRDA criteria (Report from the Medical Research Council Alzheimer's disease workshop, 1987).

The variable clinical presentations of AD are taken into account by the NINCDS-ADRDA criteria, which are a useful guide for correctly classifying the probability of making a correct diagnosis of AD. However these guidelines are not a means of staging the disease. For example, even in the very early stages of AD, a diagnosis of *definite* AD could be established if confirmed by brain biopsy. On the other hand, a patient with severe AD may still be classified as *possible* AD if there are other disorders present that might cause the dementia. The variable onset, clinical features, disease progression and pattern of cerebral metabolic disturbance in AD make diagnosis in the early stages difficult. It is not yet clear how best to detect the early signs of AD changes, but most of the available cognitive function screening tests are probably inadequate. As improved treatment becomes more likely, the importance of early and accurate detection of AD will be even more important, therefore more sensitive screening tests are clearly required.

1.5 Prevalence and incidence

The prevalence of AD is not known with any precision. Many surveys of prevalence have not differentiated between types of dementia

and estimates vary considerably ranging from 1.5% to 21.9% (Henderson, 1986). These differences are largely accounted for by differences in the populations studied, differences in diagnostic and assessment criteria for dementia, and the proportion of the very old included. There are no reliable estimates of prevalence of dementia, let alone AD, in the under 65 age group (Mortimer, 1983). Furthermore, most epidemiological studies do not differentiate between AD and multi-infarct dementia (MID). The prevalence of severe dementia in the over 65 population is between 3% and 5%, increasing to greater than 20% in the population aged over 85 years (Kay & Bergmann 1980; Mortimer et al 1981; Gurland & Cross, 1982).

In chronic disease, such as AD, incidence data are preferable to prevalence data which may be confounded by factors associated with differential survival. Incidence data however take long periods to collect, as they call for examination of the study population at one time and re-examination after some interval of time to determine who has developed the condition. There are very few studies of incidence of AD. In the Swedish Lundby Study (Hagnell et al 1983), the incidence of senile dementia, presumably of the Alzheimer type, was 0.7% and 0.5% for men and women respectively, aged 70-79 years, and 1.9% and 2.5% respectively for men and women over 80 years. In the Baltimore Longitudinal Study, the incidence rates were similar (Mortimer et al 1981). In a well defined US community, Schoenberg et al 1987, found that the annual incidence of AD rose with age from 0.004% in the 30-59 age range, to 0.096% in the seventh decade, and 1.43% in the ninth decade. With age adjustments there were no differences between the sexes.

1.6 Neuropathological features

Just as normal anatomy and physiology underpins normal brain function, neuropathological abnormality underlies abnormal brain function and cognitive deficit in AD. Therefore it is essential to review the neuropathological features of AD before studying biological and cognitive dysfunction. Although brains from AD patients often show fronto-temporal atrophy, with enlargement of lateral ventricles (Perry 1984), a pathological diagnosis cannot be made on the macroscopic appearances of the brain and microscopic examination is necessary. The histopathological hallmarks of AD are senile plaques and neurofibrillary tangles (Selkoe, 1986).

Bloq and Marinesco in 1892, first identified plaques in human brain. Plaques are usually associated with AD and the aging human brain, as similar structures only rarely occur in other conditions. Plaques are extracellular, spherical or ovoid structures of about 15-200μm in diameter, composed of abnormal nerve processes (neurites) containing paired helical filaments, glial cells and processes and amyloid fibrils (Tomlinson & Corsellis, 1984). In some plaques, but not all, an amyloid core 5-10μm diameter is present (Wisniewski & Terry, 1973). The amino acid sequence of the protein (A4 or beta protein) that constitutes the amyloid core has recently been elucidated (Masters et al 1985a). Antibodies have been prepared to the A4 protein; such antibodies recognise not only typical, neuritic plaques, but also more diffuse plaque-like structures which are not stained by the histological stains used to visualise plaques. These plaque-like structures are widely distributed throughout the central nervous system and may represent preliminary amyloid deposits which only develop into neuritic plaques in certain areas of the brain, including basal ganglia, cerebellum, periaqueductal and periventricular areas, hippocampus and spinal cord (Ogomori et al 1989; Bugiani et al 1989).

The neocortical tangle was described first by Alzheimer in his description of the disease (Alzheimer, 1907). In contrast to plaque formation, tangles are not found to any significant extent in the neocortex of non-demented brains. Tangles are usually intraneuronal, but can be extracellular. Chemical analysis of tangles is technically very difficult but molecular biological approaches look promising. Recent work suggests that the microtubule-associated protein, tau, is likely to be a significant component of tangles (Wischik et al 1988), and at least part of the tau molecule seems closely bound to paired helical filaments (Kondo et al 1988). The A4 protein also appears to be a component of tangles (Masters et al 1985b; Guiroy et al 1987). Another component of tangles is ubiquitin protein (Perry et al 1987), which appears to have a role in the response of cells to injury.

Plaques and tangles, however, are not confined to AD. In normal aging, plaques are found in the temporal and frontal cortex and hippocampus, whilst tangles are usually restricted to the temporal lobe, in particular the hippocampus . In AD the number of plaques and tangles in these areas is markedly increased compared with controls, and tangles appear in cortical areas and the olfactory system which are rarely affected in normal aging. Blessed et al (1968) showed that the number of cortical plaques in the brains of elderly people correlated with their degree of cognitive impairment. However, although the presence of neocortical tangles was accepted by the MRC Workshop on Alzheimer's disease (1987) as being essential for a diagnosis of AD, recent work suggests that there are elderly cases of AD with large numbers of plaques in temporal and frontal cortex but no tangles in frontal cortex (Terry et al 1987; Katzman et al 1988). There are surprisingly few studies of the relationship of plaque and tangle counts to severity of dementia. Tangle density has been reported to

be directly proportional to degree of dementia (Wilcock et al 1982), but not always (Mann et al 1988a).

It appears that over a small range of plaque and tangle numbers, there is a positive correlation with degree of dementia, but it is likely that a minimum number of plaques and tangles are required before dementia is apparent clinically and above a certain number there is no measurable increase in dementia (Wilcock et al 1982; Blessed et al 1968). Thus plaque and tangle density may not always be useful as a measure of severity of dementia. Plaque and tangle formation are probably separate, though related, degenerative processes which may occur separately in AD.

Tangles are not confined to cerebral cortex and hippocampus, but also occur in subcortical nuclei, such as the basal forebrain nuclei, locus coereleus, raphe nuclei and the tuberomamillary nucleus of hypothalamus (German et al 1987), which project diffusely to cerebral cortex. The association of cortical and subcortical pathology in AD is shown in the observation that neuronal loss in a particular part of the basal forebrain nucleus is related to the number of plaques in the area of cortex targeted by the forebrain nucleus (Arendt et al 1985). The presence of tangles in enterorhinal cortical neurones projecting to the hippocampus, and in neurones from the hippocampus, may isolate hippocampus from the rest of brain and suggests at least one possible mechanism of memory impairment (Hyman et al 1984).

1.7 Relationship of early and late forms of Alzheimer's disease

Historically, dementia occurring after the age of 65 years has been considered separately from that occurring in younger patients. The separate development of clinical services for older patients has reinforced

this practice and the term 'senile dementia' (SD) has tended to acquire a separate nosological status through usage, rather than from weight of scientific evidence. There are two main problems with this term. Firstly, as in younger patients, there are many different types of dementia that can occur in older subjects, apart from AD, and diagnosis can only be determined with certainty at necropsy. Secondly, the use of the term 'SD' has obscured the relationship of the commonest form of senile dementia with the commonest early dementia, viz Alzheimer's disease. At autopsy of elderly patients who have died with a clinical diagnosis of SD, the most frequently detected pathology is very similar to, if not quite identical with, that of Alzheimer's disease in younger patients. As a result, this clinico-pathological condition is now usually referred to as 'senile dementia of the Alzheimer type' (SDAT) or Alzheimer's disease Type 1 (AD-1), early onset AD being referred to as Type 2 (AD-2) (Roth, 1986; Bondareff et al 1987). This reflects the current view that both conditions are likely to be similar manifestations of a single disease process, and, while age of onset may be associated with different clinical and neurochemical characteristics, it is nosologically accurate to consider the two types as variants of *Alzheimer's disease* (Lauter & Meyer, 1968; Terry & Katzman, 1983).

The pathological changes in early onset AD appear to be more severe than in SDAT. Detailed comparisons between the pathology of younger and older patients, show that reduction in brain weight is greater in presenile cases compared with SDAT (Sourander & Sjogren, 1970). In studies of cell loss from nucleus locus coeruleus, the distribution of loss of cells was bimodal (Bondareff et al 1987; Mann et al 1988b) with less cell death in AD-1 (29-30%) compared with AD-2 (70-80%). Further support for the subdivision comes from differences in neocortical noradrenaline concentrations, choline acetyl transferase activity, plaques and tangles.

Similarly, neurotransmitter deficits in post-mortem brain are more pronounced and more widespread in brains from younger patients with an earlier onset who die before the age of 70-80 years, than in brains from older, late onset patients who die after the age of 70-80 years (Yates et al 1983; Rossor et al 1984). Choline acetyl transferase (ChAT) was reduced in temporal and frontal cortex in younger cases, but reduced only in temporal cortex of older cases. Greater reductions in the concentration of noradrenaline (NA), 5-hydroxytryptamine (5-HT), gamma amino butyric acid (GABA), somatostatin, and in the number of 5-HT receptors were observed in post-mortem brain from cases of Alzheimer disease dying at 70-80 years than in older cases dying after 70-80 years (Yates et al 1983; Bowen et al 1983; Rossor et al 1984; Cross et al 1984; Francis et al 1985; Pierotti, 1986). The neurochemical deficits of earlier onset AD tend to be predominantly in fronto-temporal cortex, while in older cases are more confined to temporal cortex (Rossor et al 1984). Neurochemical and histological differences between AD types 1 and 2 have been used to argue for a broad classification of AD into two groups, with the early-onset (AD-2) showing the more severe biochemical change (Bondareff et al 1987). Thus, the neurochemical evidence tends to support the neuropathological evidence that early and late onset cases of AD at least form a continuum. One explanation of the differences between the two groups may be that patients with SDAT (or AD-1) die at an earlier stage in the disease process than those with AD-2. Alternatively it could be argued that although the pathological process may be the same or similar in AD-1 and AD-2, an increased rate of progression in younger patients accounts for the increased severity and more widespread involvement. Why the disease should progress faster in younger patients is not clear, although there are examples of similar phenomena in other disorders. Although there is evidence that the clinical course of AD-1 and AD-2 have particular features,

there has been too little systematic investigation of the progression of different AD types to enable firm conclusions to be drawn. In a comparative study of early- and late-onset cases, Seltzer & Sherwin (1983) found a higher prevalence of speech disorder, gait disturbance, selective dysfunction of left hemisphere and shorter life expectancy in the earlier-onset cases but no differences in dyspraxia in the two groups. Longitudinal studies have also been conducted to identify clinical features that may predict or influence the course of the disease. In one recent study, the rate of progression was found to be greater in patients older than 65 years at onset, compared with those younger than 65 years (Huff et al 1987). However, Heyman et al (1987) found that young age at onset and the presence of aphasia and severity of impairment predicted a rapid course. There is clearly a need for further prospective studies to investigate and compare the clinical, cognitive, neuropathological and neurochemical changes that take place in AD occurring at both younger and older ages.

The experimental studies described in this thesis were carried out on AD-2 early-onset patients. The decision to investigate this group was based on several considerations. Firstly, younger patients with AD are generally in better physical health than the older group, who often have other coexistent illness, which may, or may not be detected, and can therefore introduce additional variance into the measurements. Secondly, age-related complications and pathologies often require medication, with further possible confounding effects on brain function, and such patients are best excluded if possible. The lack of other pathology in combination with detailed supporting history from the patient's spouse, also helps increase diagnostic accuracy. In younger patients, the cognitive and behavioural changes resulting from AD are often recognised as abnormal by the patient or his family at an earlier stage, and, as a result, AD-2 patients often present early and are highly motivated to undergo investigation and co-operate with

research procedures. This may also have implications for studies of therapeutic intervention. There is also a greater likelihood of AD-2 patients living at home with their family, rather than in institutions, and all the AD patients investigated in this study lived at home.

1.8 Aims of the present study

As the above introduction illustrates, knowledge of the biological basis of brain disturbance in Alzheimer's disease, and how this translates into altered mental function, is still at an early stage. Until the development of brain imaging techniques, there were only very limited means of investigating brain structure and function *in vivo*, and for most of the twentieth century psychiatrists retreated from the brain. There are two broad categories of brain imaging: structural techniques and functional techniques. Although structural imaging methods, such as x-ray computed tomography (CT) or magnetic resonance imaging (MRI), are able to visualise in some detail the anatomical structure of brain, they provide little information on brain function. The introduction of techniques that permit brain activity to be measured *in vivo* has facilitated the investigation of metabolic disturbance in neurodegenerative disorders such as AD. The studies discussed in this thesis describe the contribution to understanding AD, and other neurodegenerative disorders, from functional brain imaging of cerebral blood flow (CBF).

The main aims of the work were as follows:

1. To briefly review the concept of AD, its clinical manifestations and underlying neuropathology.

2. To consider the evidence for a relationship between CBF and brain metabolism, and review the utility of CBF as a valid and reliable index of brain metabolism or function.

3. To review the historical development of the main techniques available for measuring CBF in human subjects, and discuss critically their contribution to understanding AD.

4. To measure the vascular transit time of pertechnetate in probable presenile AD and two control groups: patients with Alcoholic Korsakoff Syndrome (KOR), and healthy volunteers. In particular, to assess the utility of the method for investigating cortical function in AD, where the recognised lesions are predominantly cortical, and in KOR, where the major lesions are usually regarded as subcortical. To investigate and describe neuropsychological performance in the two disorders, and its relationship to cortical transit time.

5. To use single photon emission tomography (SPECT) and the radiopharmaceutical, ^{99m}Tc-HMPAO, to investigate the pattern of regional cerebral blood flow (rCBF) in patients with probable presenile AD and KOR and to determine its relation to neuropsychological function in such patients. The central questions were:

(a) What is the pattern of perfusion deficit using SPECT in patients with AD and KOR, and in particular is there evidence in KOR of cortical dysfunction?

(b) Are there relationships between rCBF, a presumptive measure of neuronal function, and neuropsychological performance?

6. To use two-dose SPECT imaging techniques to measure the effects on rCBF of cholinergic potentiation using the anticholinesterase drug physostigmine in presenile AD patients.

7. To develop and describe a method that may allow SPECT imaging to be used to investigate the effects of challenge (eg drug or psychological)

on patterns of rCBF during a single imaging session, using a 'split-dose' of the flow tracer ^{99m}Tc-HMPAO. To assess the reproducibility of ^{99m}Tc-HMPAO uptake measurements using the split-dose paradigm in the absence of challenge.

8. To examine the effect of several different sources of reproducibility error during repeat SPECT scanning, (a) data acquisition and image reconstruction, (b) movement of patient in scanner, (c) patient re-alignment, and (d) serial scanning after one week.

CHAPTER 2

THE MEASUREMENT OF CEREBRAL BLOOD FLOW IN ALZHEIMER'S DISEASE: A LITERATURE REVIEW

2.1 Relationship between CBF and metabolism

In this chapter, the use of cerebral blood flow (CBF) measurements in the study of brain abnormality in AD will be reviewed. Before doing this however, it is necessary to discuss briefly the rationale of using CBF as an index of brain metabolism.

2.1.1 Normal energy sources in brain

In brain, as in any steady-state system, the metabolic rate (ie rate of energy production) is closely correlated with physiological function (ie rate of energy utilization). Since adenosine triphosphate (ATP) is required as the energy source for vital cellular processes, the more functionally active a cell, the more ATP it will require. As glucose is the major source of ATP for the central nervous system (CNS), the functional activities of the various brain regions should be reflected in their rates of regional glucose utilization. Over 95% of normal energy requirements of cerebral tissue are derived from oxidative phosphorylation in mitochondria. Glycolysis in the cytosol generates only a small fraction of the total ATP available compared to that for complete oxidative metabolism of glucose. While glycolysis is much less efficient than oxidative metabolism in terms of ATP yield per molecule of glucose, it has the advantage of rapidly producing ATP in the cytosol, which may be important during intense short-term stimulation. Glycolysis in the presence of adequate oxygen ('aerobic glycolysis') has been demonstrated in stimulated brain structures (Collins et al 1987; Fox et al 1988; Pulsinelli and Kraig 1988; Ueki et al 1988; Blomquist et al 1989), and aerobic glycolysis may be the main supplementary ATP source during stimulated conditions.

2.1.2 Coupling of cerebral blood flow and metabolism

Since the investigations of Roy and Sherrington (1890), it has been generally accepted that an intimate coupling between blood flow and tissue metabolic rate is dynamically maintained in normal brain (eg Siesjo, 1978). Local modulations in neuronal electrical activity are thought to be associated with similar modulations in local metabolism, leading in turn, to alterations in local blood flow. Blood flow and metabolism have therefore been considered to change in unison, with increases in flow reflecting increases in neuronal metabolism, and vice versa, both providing indirect indices of brain work (Roy and Sherrington 1890; Frackowiak et al 1981). The mechanism by which CBF and metabolic rate are coupled remains poorly understood and is outwith the scope of this review.

This close relationship between blood flow and metabolic rate seems to hold in resting brain, and structures having high blood flow (eg grey matter) tend also to have high metabolic rates, while structures having lower blood flow (eg white matter) have lower metabolic rates. Thus there are excellent regional correlations between CBF and regional cerebral metabolic rate of oxygen utilization ($rCMRO_2$), (Raichle et al 1976; Baron et al 1984; Fox and Raichle 1986) and between CBF and regional cerebral metabolic rate of glucose utilization (rCMRglc) (Baron et al 1984; Fox et al 1988). Coupling of CBF and $rCMRO_2$ has also been demonstrated in resting subjects with dementia (Frackowiak et al 1981), but in other pathological states the relationship between CBF and metabolic rate may be more complex.

2.1.3 Changes in energy demands during stimulation

The coupling of CBF and metabolism (CMRglc and $CMRO_2$) which occurs in the 'resting state', is less evident during active brain stimulation.

For example, the clear correlations between CBF and $CMRO_2$ detected using positron emission tomography (PET) in resting healthy human subjects, are not found during somatosensory stimulation (Fox and Raichle 1986). Upon activation, CBF increased by about 30% while the metabolic increase ($CMRO_2$) was only 5%. In a later study by Fox et al (1988) using PET to measure regional cerebral blood flow (rCBF), rCMRglc and $rCMRO_2$ in visual cortex of healthy volunteers during visual stimulation, there was only a small increase in O_2 utilization (5%), but glucose uptake and rCBF both increased proportionately by much more (51% and 50% respectively). For glucose metabolism to rise in excess of oxygen utilization, suggests that glucose is metabolised via glycolysis with production of lactate. This has been confirmed in two independent studies using animals. Using tissue fluoroscopy, Hossman and Linn (1987) noted an increase in tissue lactate in rat somatosensory cortex during forepaw stimulation, while Prichard et al (1987) used magnetic resonance spectroscopy to show that tissue lactate increased in rabbit visual cortex during optic nerve stimulation. In a different study, photic stimulation resulted in increased lactate in the superior colliculus of rats (Pulsinelli and Kraig ,1988). These results suggest that increased transient metabolic needs in the CNS are apparently met with non-oxidative glucose metabolism, namely glycolysis, even in the presence of sufficient oxygen.

Despite the large increase in glucose uptake observed, the actual energy yield in ATP may be much less, even if all the oxygen uptake is consumed by glucose oxidation and the remainder of glucose converted by glycolysis to lactate. In total this would yield only an increase of about 8% in ATP production. However if some of the glucose taken up is converted to glycogen, ATP production would be even less.

After neuronal stimulation there may only be a small increase in energy requirements above base levels because the additional ion fluxes across the

cell membrane probably represent only a small fraction of total cellular activity. It has been estimated that less than 3% of cortical energy consumption would be required for such evoked activity in cortical nerve cells (Creutzfeldt et al 1975). Membrane bound ion pumps and transmitter release systems may depend on ATP generated from aerobic glycolysis. During basal conditions, enzymic oxidative capacity of neurones may be functioning near maximal capacity, and therefore the additional energy demands imposed by stimulation may have to be met by glycolysis (Barrere et al 1989).

2.1.4 Studies using labelled glucose to image stimulation

The deoxyglucose (DG) method of determination of the local cerebral glucose metabolic rate (LCMRglc) developed by Sokoloff (1977) has proven to be a useful and hence widely employed method for evaluation of cerebral function. The use of ^{14}C-DG and ^{18}F-fluorodeoxyglucose (FDG) in autoradiographic studies in animals and ^{18}F-FDG in PET studies in humans (Phelps et al 1982) have been particularly effective in mapping the effects of physiological and pathological stimulation. Pronounced regional increases in DG or FDG-based LCMRglc have been found in seizures, pharmacological interventions, sensory stimulations and motor activity. DG and FDG have become the practical standards of evaluation of cerebral metabolism, despite the development of alternative methods. Quantitative methods for the measurement of local cerebral oxygen metabolism (LCMRO_2) using $^{15}O_2$ and positron emission tomography (PET) scanning have also been developed (Frackowiak et al 1980). However DG and FDG appear to be more suitable than other techniques for imaging increases in brain function associated with stimulation. Using an elegant double tracer technique, Ackermann and Lear (1989) used the radiolabels ^{18}F-FDG and ^{14}C-6-glucose (GLC) in rats to estimate the relative size of contribution to ATP production of the Kreb's Cycle

and glycolytic pathway. The rationale of this approach is that, while FDG is trapped in brain prior to the divergence of oxidative and glycolytic pathways, and thus reflects total (ie, oxidative and glycolytic) glucose metabolism, the label from ^{14}C-GLC is retained only in oxidative metabolic pools and therefore reflects only *oxidative* metabolism. Via glycolysis, the label, ^{14}C, from GLC will proceed to lactate, which will be lost out of the brain. This elegant method was used to study oxidative and glycolytic metabolism during Kainic acid-induced seizures in rats, demonstrating that glycolysis increases markedly in limbic structures during seizures. A similar conclusion can be drawn from single label experiments, and Collins et al (1987) found only a 30% increase of GLC-based LCMRglc compared to a 200% increase of DG-based LCMRglc in superior colliculus after visual stimulation in rats.

2.1.5 CBF and metabolism: conclusions

There is good evidence that under resting conditions CBF and metabolic rate are coupled. However, when stimulated, neurones require additional supplies of ATP, and this is normally met by a substantial increase in glucose utilization but only a small increase in oxidative metabolism. Thus it appears likely that additional ATP requirements are met largely from glycolysis, rather than oxidative intermediary metabolism, which may in the 'resting state' be operating near maximal levels. In this way, the ATP supply may increase during stimulation by about 8%. The work of Fox et al (1988) shows that after photic stimulation, rCBF and rCMRglc increased by 50%, suggesting that in terms of ATP production, they may overestimate energy requirements.

2.2 Methods of measuring CBF and metabolism and their application in AD

2.2.1 Methodological problems

The development of methods for measurement of brain perfusion and metabolism *in vivo* have allowed CBF and metabolism to be studied in Alzheimer's disease (AD). Although many of the principles for CBF and cerebral metabolic rate (CMR) measurement were established some time ago, technical sophistication has improved in the last 40 years, with the introduction of methods which provide regional information and later, tomographic reconstruction. Technical development has been accompanied by improved understanding of the nature and concept of AD, and both ante-mortem clinical, and post-mortem neuropathological, diagnostic criteria have been established (see Chapter 1). Determination of type of dementing illness is now recognised as essential but in earlier imaging studies the need for careful case definition was poorly appreciated or ignored and resulted in findings that are often difficult to evaluate. In many studies, patients were often simply described as having 'dementia', without description of diagnostic criteria and little attempt made to exclude other types of pathological process that could confound results. As described in section 1.4, agreed criteria for clinical diagnosis of probable AD have only been accepted relatively recently (eg McKhann et al 1984; Roth et al 1986; MRC Report from the Alzheimer's Disease Workshop, 1987). While assessment of neuropsychological function has improved with the introduction of a number of valid and reliable instruments for use in AD (eg Blessed et al 1968; Blackburn & Tyrer, 1985; Roth et al 1986), there is still a need to develop more sensitive instruments for assessment of cognitive function in dementia. Although the technical

sophistication of imaging has improved greatly in the last thirty years, this has not been paralleled by similar developments in improved neuropsychological assessment.

Throughout the development of techniques for functional brain imaging, two broad approaches have been taken; firstly, measurement of CBF and/or CMR in demented patients as an index of changed brain function; and secondly, attempts have been made to examine the relationship between changes in CBF/CMR and impaired neuropsychological performance.

2.2.2 The Kety-Schmidt Technique: Inert tracer wash-out

2.2.2.1 Methodology and principles

These early methods are important because they laid the foundations for the development of future methods of measurement of CBF and CMR in clinical and experimental situations. Kety and Schmidt (1945,1948a,1948b) introduced the inert tracer wash-out method using a non-radioactive tracer, nitrous oxide, but subsequent clinical applications used radioactive tracers. The method derives from the Fick Principle that, in a system at equilibrium, and if the tracer is delivered at a measured rate, flow can be calculated from the total quantity of tracer removed from the blood (or 'washed-out') divided by the net mean concentration difference between arterial and venous blood, provided blood flow is constant. In the original Kety-Schmidt method the subject inhaled nitrous oxide and the arterio-venous concentration difference was determined from samples of arterial and jugular venous blood removed over the measurement period.

Using the Fick Principle, perfusion (P) or flow per unit weight of brain is given by:

$$P = Q_t/W \int_0^t (C_a - C_v)\, dt \quad \text{(equation 1)}$$

where Q_t is the quantity of nitrous oxide taken up by the whole brain in time t, measured from the start of inhalation, and C_a and C_v, the arterial and venous concentrations of nitrous oxide respectively, and W the weight of the brain. An important assumption of the method is that the tracer should be freely diffusible in order that its concentration in the venous outflow is in equilibrium with that in the brain. A partition coefficient, λ, expresses the ratio of solubility of tracer in brain to that in blood and can be expressed as,

$$\lambda = (Q_t/W)/C_{v(t)}$$

where Q_t is the quantity of tracer in the brain at time t, W is the brain weight, and $C_{v(t)}$, the venous concentration of tracer at time t. Thus, substituting for Q_t in equation 1 gives,

$$P = C_{v(t)}\lambda \,/ \int_0^t (C_a - C_v)\, dt \quad \text{(equation 2)}$$

Perfusion (P) is thus given by the venous concentration of tracer at time t multiplied by the partition coefficient, to take account of differences in solubility, and divided by the quantity of tracer extracted from the blood during the same time interval.

When a freely diffusible tracer, which does not become bound, is introduced as an instantaneous bolus, equilibrium of tracer concentrations between blood and brain is soon established and the rate at which the tracer

is subsequently washed out (dC_t/d_t) is given by differentiating equation 2 and rearranging,

$$dC_t/dt = k (\lambda C_a - C_v)$$

Furthermore, if it is assumed that after bolus injection the arterial concentration rapidly becomes negligible, then integrating gives,

$$C_t = C_o e^{-kt}$$

and in the case of a constant infusion of tracer where the arterial concentration remains constant,

$$C_t = \lambda C_a (1-e^{-kt})$$

Thus, there are four parameters which can be measured and from which flow can be derived; the rate of wash-in and wash-out of tracer, the total quantity of tracer delivered to the region under study, and the change in the concentration of tracer with time (Zierler 1965). These principles have been described in some detail, because many of the methods for measuring regional cerebral blood flow in both experimental animals and man were developed from them.

2.2.2.2 Global CBF in AD

Using the Kety-Schmidt technique (Kety and Schmidt, 1945,1948a,1948b) described above, measurements of global CBF and $CMRO_2$ were undertaken in patients with dementing illness. Initially, the inhalation of inert nitrous oxide gas, was used, but this was soon replaced by inert radioactive gases in order to take advantage of accurate methods of

scintillation counting. The Kety-Schmidt method gives absolute mean values for whole brain blood flow per unit weight of tissue, conventionally per 100g of tissue. Although non-regional and one-dimensional, it is a valuable reference method against which other CBF methods can be compared.

The earliest observation that CBF and $CMRO_2$ are reduced in senile dementia was that of Freyhan et al in 1951. Studies of demented patients using the same technique have consistently demonstrated similar reductions in CBF and $CMRO_2$ (Lassen et al 1957; Lassen et al 1960). In these early studies it was noted that reductions in global cerebral blood flow tended to be associated with increasing mental deterioration and Lassen et al (1960) reported that this relationship was stronger for CBF in the dominant rather than non-dominant hemisphere. The later development of techniques that allowed regional brain function to be determined confirmed these early findings.

2.2.3 Measurement of regional CBF using xenon

Ideally, tracers used for the measurement of CBF *in vivo* should be metabolically inert and not undergo chemical alteration in the body. Furthermore, in order that equilibrium is rapidly established, the tracer should be lipophilic and therefore pass rapidly from blood into brain. A number of substances meet these criteria reasonably well and have proved useful in clinical practice. Xenon (^{133}Xe) is the isotope most commonly used, but unfortunately has a rather low gamma ray energy of 80keV which results in the count rate being significantly reduced by intervening tissue. This results in measurements that are often biased in reflecting the more superficial parts of the cortex rather than deeper structures.

2.2.3.1 The intra-arterial method

When a bolus of ^{133}Xe dissolved in normal saline is injected into the carotid artery, it is largely delivered during the first pass to the ipsilateral hemisphere. From the rate of wash-out and the partition coefficient, the perfusion of various cortical regions can be determined. The wash-out rate of tracer from brain can be measured using a scintillation detector positioned over the head and, depending on the number of detectors used, hemispheric or regional measures of perfusion can be determined (Lassen & Ingvar 1972).

Regional measurement of CBF using the intra-arterial injection technique was introduced to clinical research in 1961 by Lassen and Ingvar in a method based on the injection of inert radio-active ^{85}Krypton gas. However ^{133}Xe soon became the preferred tracer and after intra-carotid injection, the instantaneous arrival and subsequent clearance of tracer can be measured by multiple external scintillation detectors, each measuring the activity in a different brain region. In one analysis, three different flow compartments can be recognised from which perfusion in grey and white matter and extra-cranial structures can be determined (Obrist et al 1967). In normal subjects the distinction between compartments is usually fairly clear with higher flow in grey matter (the so-called 'fast' component) compared with other compartments, but in pathological states, such as dementia, the distinction can be much more difficult. The rate of disappearance of ^{133}Xe from each region is proportional to the level of perfusion in that region and absolute flow values can be calculated from the exponential clearance curves. Thus grey and white matter blood flow in a particular brain region can be calculated from the fast and slow components of the wash-out curve respectively.

Unfortunately the method has the major drawback of the need for carotid arterial puncture and therefore measurements can only be performed on one occasion, and on one hemisphere.

2.2.3.2 The application of intra-arterial methods in AD

Using the above technique, absolute estimates of regional cortical perfusion have consistently been shown to be reduced in presenile and senile dementia, although type of dementia was often poorly defined, and often presumed to be AD without the use of rigorous operational criteria (Ingvar and Gustafson, 1970; Gustafson and Risberg, 1974; Hagberg and Ingvar, 1976). In presenile dementia patients average flow values were found to be 20-25% lower than in controls. While a fronto-temporal pattern of CBF reduction was reported in patients with 'senile dementia', in presenile patients more widespread flow reductions involving posterior temporal regions were reported (Ingvar & Gustafson, 1970; Obrist et al 1970). Hagberg and Ingvar (1976) found that the pattern of cognitive deficits in each of four groups of increasingly demented patients was associated with reductions in left hemisphere CBF, although no perfusion measurements were made on the right side.

In that study early cases of dementia with only memory impairment had greater flow reductions in anterior temporal regions than elsewhere in left cortex. Severely demented patients had more extensive reductions of rCBF involving other cortical areas, including posterior temporal and parieto-occipital cortex. Although these findings were of some interest, uncritical patient selection and the limited number of regions of interest in only one cortex (left) restricts the conclusions that can be drawn.

A close association between rCBF and cognitive symptoms was found in a large study of fifty presenile patients in whom dementia had presumably started between the ages of 40 and 65 years (Gustafson and Risberg, 1974). In the study, psychiatric symptoms were not rated using standardised or accepted criteria, but on the basis of 'clinical impression' by a trained psychiatrist. Ratings for sixty-seven symptoms were factor analysed and the fourteen resultant factors correlated with rCBF in eight regions of 'dominant'

hemisphere. The results showed an inverse relationship between amnesia, apraxia, agraphia and aphasia, and rCBF in posterior temporal and occipito-parietal regions. This study illustrates several methodological problems that occurred in many early imaging studies that make interpretation of findings difficult. Although the authors excluded, 'dementia secondary to psychosis, apoplexy, and alcohol abuse', inclusion criteria were not described and the sample may well have been quite heterogeneous. More than half the patients were on medication and a few had even received electroconvulsive therapy, yet there is no discussion of how these factors may have affected perfusion. In the study, as in nearly all those using the intra-carotid method, measurements were made only in the 'dominant' hemisphere, yet no information is given on handedness or how the authors determined hemispheric dominance. It is also assumed that pathological changes in 'dementia' are symmetrical, and therefore rCBF measurements need be made only in one hemisphere, without considering the influence of type or stage of illness. As later studies with PET imaging have shown, there is considerable evidence that pathological changes in AD are often asymmetric (see section 2.2.6.3), indicating the importance of studying both hemispheres.

Some of these methodological problems were addressed by Perez et al (1977), who used stricter diagnostic criteria and separated patients into three groups: AD patients, multi-infarct dementia (MID) patients, and a control group with 'various neurological disorders' that were not characterised. Using intra-arterial ^{133}Xe, rCBF was measured in right sided cortical grey and white matter and assessed using a standardized neuropsychological battery, the Wechsler Adult Intelligence Scale (WAIS). Significant reduction in mean rCBF was found for both AD and MID groups, compared with the control group, however no correlation between psychological performance and rCBF was detected. Although the study might have been improved by the inclusion

of a group of healthy control subjects, this was not possible as the intra-arterial technique is invasive and potentially hazardous.

2.2.3.3 Inhalational and intra-venous methods

The invasiveness of the intra-arterial injection technique seriously limited its usefulness for clinical research and led to the development of methods in which ^{133}Xe could be administered intravenously or by inhalation. These were first suggested by Conn in 1955, subsequently developed into a practical method by Mallett and Veall (1965), and the analysis refined by Obrist (Obrist et al 1967,1975).

The technique is carried out using a closed circuit from which the subject breathes oxygen, and to which a small quantity of tracer, usually ^{133}Xe of activity 20MBq/l, is introduced while the subject continues to breathe normally. After rebreathing for a few minutes, equilibrium is established and the concentration of ^{133}Xe in brain is constant. The closed circuit is then opened and the subject breathes room air normally with the exhaled ^{133}Xe collected in a suitable trap. As in the intra-arterial method of measuring rCBF, scintillation detectors over the head record the rate of wash-out of ^{133}Xe from brain tissue. Corrections are made for scattered radiation from the lungs entering the field of the brain detectors, and for recirculation of isotope. The number of detectors positioned over each hemisphere is variable, but commonly 16 detectors are positioned over each side of the head, allowing regional information to be obtained (Obrist et al 1975; Risberg, 1980; Kanno et al 1981). Resolution is limited by the radiation dose given to the patient (which determines count rates at each detector), the amount of scatter (which is relatively high, as pulse height analysers cannot effectively eliminate Compton scatter at this energy), and the time available for imaging which is limited when dynamic (eg wash-out) methods are used. This is a planar or 2-

dimensional technique and therefore cortical perfusion values dominate the information obtained.

In order to overcome the major drawbacks of contamination of clearance curves from scalp and other extracerebral sources, Obrist et al (1967) proposed a three compartment analysis of the wash-out curves. Unfortunately, adequate separation of the the cerebral and extracerebral components required extended periods of recording, which was not ideal for some clinical studies. Later, the same group proposed a two compartment model in which a faster clearing component, presumed to be grey matter, is identified from the slower white matter and extracerebral component (Obrist et al 1975). Although restricted to providing measures of grey matter rCBF, the method has the advantage of requiring much shorter periods of data collection (about 10 minutes) which make it more suitable for psychiatric research. Wyper et al (1975) have also described a 'two-minute slope' method which requires only a brief period for monitoring xenon wash-out and which can be used with either single channel detectors or a gamma camera. The method has many advantages for the study of uncooperative or restless patients and in experiments involving transient physiological stimulation. The intracarotid and inhalational methods have been compared in a study of patients undergoing cerebral angiography, and there was good correspondence between measurements of CBF from both methods (Wyper et al 1975). Reivich et al (1975) compared the 10 minute, two compartment inhalation technique of Obrist et al (1975) with the intra-carotid method and found high correlation for grey matter regions.

2.2.3.4 Inhalational methods in AD

The inhalational method found ready acceptance in clinical research as no carotid arterial puncture was necessary, and quantitative measurements

could be made simultaneously in both hemispheres and repeated later if necessary. Moreover, healthy subjects could now be ethically investigated as a control for patient groups. Despite these advantages however, methodological problems related to extracranial contamination and recirculation of isotope retarded the usefulness of the technique until improved analysis was developed in the mid seventies.

In a study of healthy controls and demented patients, Obrist et al (1975) described a new method of estimating the clearance rate and fractional blood flow using a ^{133}Xe inhalational method. As explained above, a two-compartment model was used, in which a fast (assumed to be grey matter) component, was separated from a slower component, assumed to be white matter and extracerebral tissue. The chief advantage of the method was that only the first 10 minutes of xenon clearance were required for data collection, rather the longer periods needed with three compartment models. Using this approach, rCBF in frontal and parietal regions was measured in young healthy adults and patients with 'organic dementia' (sic), and although no diagnostic criteria are given, 14 of the group of 20 were considered to have 'Alzheimer's disease'. Little or no overlap in measures of rCBF occurred between the two groups, with demented patients having significantly reduced flow.

Several other studies have reported significant reductions in rCBF in demented patients using the xenon inhalation method (Gustafson & Risberg,1979; Risberg, 1980; Zemcov et al 1985). Risberg (1985) used the method to compare patients with different types of dementia, including a group of younger Alzheimer patients, mean age 64 years, and an older Alzheimer group, mean age 76 years, many of whom had a pathologically confirmed diagnosis. The pattern of flow was similar in both AD groups, with apparently 'characteristic' low flow areas in posterior temporal and parietal cortex. In older AD patients the impairment of flow appeared more widespread, but this could reflect increased severity, rather than a different

pathological process. Yamaguchi et al (1980) has emphasised the need for appropriate control groups. Using rigorous diagnostic criteria for probable AD, they found bilateral symmetrical reductions in grey matter CBF that correlated with degree of atrophy measured using x-ray computed tomography. Although handedness was not reported, rCBF in left hemisphere appeared more reduced than in the right side. Unfortunately no regional CBF data is presented in the paper although the use of 16 scintillation detectors per hemisphere would have made this possible. In the putative AD group, mental functioning was assessed in some patients using the WAIS, although this may not be suitable as an instrument for detection of cognitive impairment in dementia as it was designed for use in non-demented patients. In particular, WAIS scores do not necessarily reflect severity of individual impairments in dementia. Unfortunately WAIS scores were not presented in the paper, nor regional CBF-cognitive associations, but the authors commented that, 'severity of dementia' correlated with bilateral CBF reduction. A more appropriate battery of psychological tests was used in conjunction with xenon inhalation in a study of well defined AD and multi-infarct dementia MID patients matched for age, sex, and degree of impairment as judged from neuropsychological performance (Deutsch & Tweedy, 1987). Mean hemispheric CBF was reduced in the AD patient group, aged from 52-81 yrs, compared with MID and healthy subjects. In AD, CBF tended to be reduced bilaterally, in frontal, temporal and parietal areas, with the greatest absolute reductions in parietal regions, especially in the left side. Unfortunately there again was no comment on association of cognitive and CBF measures.

A less commonly used variation of the inhalational technique is to administer the ^{133}Xe tracer by intra-venous injection (Lassen & Ingvar, 1972). This method may be more convenient for patients who find the inhalational technique uncomfortable, or who have limited cooperativity, and it can also be

used repeatedly, even in normal subjects. Like inhalational methods, two-dimensional brain information is obtained that reflects mainly cortical CBF. The method is of limited value in comparing inter-individual differences, as the precise input function of ^{133}Xe to the brain cannot be determined accurately (Lassen, 1987).

2.2.4 First pass methods

A number of methods have been developed for examining the first pass of a bolus of tracer through an organ. The concept of mean transit time (MTT) was proposed by Stewart (1894) who pointed out that the blood flow to any organ could be calculated if the volume of blood within it and the mean transit time of a tracer through it could be measured. Formal proof of this relationship was later provided by Zierler (1962). The vascular mean transit time (MTT) is the volume of blood in the organ under investigation divided by the rate of blood flow through it.

MTT = BLOOD VOLUME / BLOOD FLOW

The reciprocal ratio Blood Flow / Blood Volume (ie 1/MTT) is also referred to as the 'perfusion reserve' (Gibbs et al 1984), and may give some indication of the extent to which cerebral perfusion is being maintained by reflex vasodilatation. The physical meaning of MTT for an intra-vascular tracer such as a red blood cell can be expressed as the mean time for that particle to pass from arterial to venous side of the organ. It has been argued that transit times longer than 10 seconds indicate impending failure of the normal compensatory mechanisms (Powers et al 1984).

The first passage of radioactivity (ie, without recirculation) through a blood vessel can be described by a gamma-variate function of the form:

$$C(t) = k.(t - T)^{a}e^{-(t - T)/b}$$

where C(t) is the tracer concentration (eg in counts per minute) at time t after injection, T is the appearance time (ie the time at which counts are first detected over the region of interest) and k, a and b are constants (Merrick et al 1991). No physiological meaning was ascribed to the parameters a and b, until Davenport (1983) proposed a model in which the circulation was considered as a series of mixing chambers where a + 1 is the number of mixing chambers and b the mean time to empty each chamber. Thus MTT of the radioactivity at any particular detection point can be estimated from,

$$MTT = b\ (a + 1)$$

in order to measure the MTT of an intravenous bolus as it passes through the head and subtract from this the MTT of the bolus entering the head at, for example, the aortic arch. Sodium pertechnetate is a reasonably good tracer for this purpose as it remains intra-vascular. The practical procedures involved in MTT measurement are described in detail in chapter 4 in a study of their application to the investigation of presenile AD and Korsakoff's patients.

First pass methods have a number of disadvantages and limitations. In particular the relationship of MTT to CBF is dependant upon the volume into which the tracer diffuses and this is discussed in chapter 4 in relation to AD and Korsakoff's patients. Also any condition which tends to prolong the central circulation such as a right-to-left or left-to-right shunt, aortic or mitral stenosis or incompetence, or low output failure may prolong the first pass over such a long period that it cannot be distinguished from recirculation. In such

circumstances the method is not applicable although in psychiatric practice these restrictions are likely to apply to only very few patients.

2.2.5 Tomographic imaging

The term tomography refers to scanning techniques that produce images of slices or sections through the body. In tomographic imaging, a series of planar projections are acquired at different points around the patient from which cross-sectional images are computed. Although originally developed using external X-ray sources (transmission computed tomography or CT), the principles have been applied in isotope imaging to enable sectional images to be constructed (emission computed tomography) and also to magnetic resonance signals.

The limitations of the planar scintigraphic methods of imaging described above, such as the xenon inhalational technique, were an important spur for the development of emission tomographic techniques. In emission tomography, cross-sectional images are reconstructed from projections, allowing regional CBF data to be obtained from sectional brain images. Using tomography, better anatomical localisation of cortical regions of interest is possible and, for the first time, sub-cortical structures can also be studied. Two types of emission tomography are currently available, single photon emission tomography (SPECT), using isotopes that emit single photon radiation, and positron emission tomography (PET), using positron emitting isotopes.

2.2.5.1 Single photon emission computed tomography

Single photon emission computed tomography employs gamma emitting radioisotopes such as technetium (^{99m}Tc), iodine (^{123}I), and ^{133}Xe.

The data from the administered isotope can be collected by either a single gamma camera or by systems using more than one detector. The realisation that improvements in resolution necessitate a loss of sensitivity (i.e. a further reduction in the fraction of the emitted gamma rays contributing the image process) has led to the development of SPECT systems with more than one gamma camera or multiple detectors around the patient's head. The increasing tendency towards the introduction of multidetector systems has resulted in improved sensitivity. Several multidetector brain SPECT systems are now available. The experimental studies described in chapters 6 and 7 were undertaken using the Strickman Medical Equipment SME 810, a dedicated brain system that scans one slice at a time. The SME 810 has twelve detectors 30 degrees apart arranged around a circle. Each detector is fitted with a point-focused collimator and during single slice scanning each detector moves in a rectiinear course. The reconstructed spatial resolution is of the order of 8mm. Although associated with high sensitivity a significant fraction of the counts of each measurement originate from slices other that the one being scanned and a reconstruction algorithm is necessary to deal with this problem. Other multi-detector brain SPECT systems include the GE/CGR Neurocam, in which three gamma cameras form a triangular aperture for the patient's head, the Osaka/Hitachi SPECT 2000 utilising four compact gamma cameras rigidly fixed in a square arrangement, the Shimadzu Headtome SET-031 with three ring detectors, and the Picker PRISM and Triomix TRIAD systems each comprising three rectangular detectors arranged as a triangle.

Multidetector SPECT systems offer a substantial increase in sensitivity compared with a single rotating gamma camera. Collimator choice is one of the most important factors determining image quality. While a compromise is necessary between resolution and sensitivity, with multidetector systems, such as the SME 810, improvement in resolution can be achieved with acceptable loss of sensitivity.

A number of SPECT techniques have been proposed which are analogous to methods of measuring rCBF in animals using injection of labelled microspheres. The method employs soluble tracers that can be injected intravenously, have almost 100% extraction during first pass, and remain trapped *in situ* long enough for their distribution to be measured tomographically. The underlying assumption of these methods is that the tracer is retained in the brain in proportion to rCBF. This technique has been applied in SPECT and although a number of compounds have been developed, two in particular have been widely used: ^{123}I-N-isopropyl-amphetamine (IMP) and ^{99m}Tc-d,1 hexamethyl propyleneamine oxime (HMPAO) (Holman et al 1982; Nowotnik et al 1985; Blau 1985; Neirinckx et al 1987). An important assumption of the method is that the tracer is trapped in tissue, but the mechanism by which this occurs is not established for either IMP or HMPAO.

Uptake of IMP is non-saturable and is, therefore, probably not related to specific receptors (Winchell et al 1980). Furthermore the rate of wash-out of IMP varies for different brain regions (Creutzig et al 1986). Although immediately after injection the distribution of IMP is largely a function of blood flow and closely resembles the distribution of perfusion as measured by x-ray xenon enhanced computed tomography (Hellman et al 1986), at later times redistribution of IMP may occur (Royal et al 1985) and the relationship of IMP uptake and blood flow is more variable. Images obtained immediately after injection of IMP and HMPAO are very similar (Leonard et al 1986; Podreka et al 1987). The use of ^{123}I has, however, various drawbacks related to costs, delivery timing and dosimetry. The method is non-quantitative and the behaviour of IMP is not well understood, such that CBF distribution cannot always be imaged reliably (Kuhl et al 1982b; Lassen et al 1983; Creutzig et al 1986).

A ^{99m}Tc-labelled flow tracer, such as HMPAO, has been sought for several years because the radiation characteristics of ^{99m}Tc have many advantages for SPECT imaging. After reconstitution with pertechnetate the resultant ^{99m}Tc-HMPAO complex is relatively unstable *in vitro* and therefore must be administered to the patient within 30 minutes of preparation. The ^{99m}Tc-HMPAO complex is lipophilic and readily crosses the blood brain barrier. The first pass extraction of brain and fatty deposits is close to 100% (Ell et al 1987), but that of other tissues is lower and more variable and as a result there is some degree of recirculation. The cerebral distribution of HMPAO is principally a function of rCBF but other factors such as lipid mass and red cell volume may contribute. However, once the ^{99m}Tc-HMPAO complex has crossed the blood brain barrier, its fate is determined by competition between rapid conversion to a non-diffusible form and washout into the blood. It is thought that after entry to the cell, ^{99m}Tc-HMPAO is rapidly converted to a hydrophilic form, perhaps by reacting with glutathione, and consequently cannot readily diffuse out through the cell membrane (Neirinckx et al 1988). Thus, HMPAO behaves as a chemical microsphere trapped in the brain. However, residue curves obtained after intra-carotid injection studies show that steady-state net extraction of HMPAO is reduced in high flow regions (Lassen et al 1988). Positron emission tomography has also shown that the relationship between HMPAO uptake into brain and rCBF is not always linear (Yonekura et al 1988). Over low and middle range CBF values, there is reasonable linear correspondence between uptake and flow, however, at high flows, HMPAO uptake tends to plateau as flow increases. The main reason may be that as tracer wash-out is flow-dependent, there may be greater wash-out from brain tissue at higher flows. A linearisation algorithm has been suggested by Lassen et al (1988) and has been shown using PET to provide a better linear relationship between HMPAO uptake and rCBF (Inugami et al 1988; Yonekura et al 1988).

Like SPECT using IMP, the method does not provide absolute measures of perfusion as the amount of active ^{99m}Tc-HMPAO available to the brain for extraction and local extraction efficiencies in brain are not known. Consequently only semi-quantitative, relative measures of perfusion are available by using indices such as the ratio of ^{99m}Tc-HMPAO uptake in the region of interest to that in cerebellum or to that in occipital region (Burns et al 1989; Montaldi et al 1990).

2.2.5.2 Application of SPECT in AD

Kuhl first described a method for the analogue back projection technique, leading to the development of emission tomography for brain imaging (Kuhl & Edwards, 1963). The ^{133}Xe inhalation technique was modified by Lassen and colleagues (Stokely et al 1980) to enable single photon emission tomography of CBF to be carried out. However as xenon arrives and washes out in a short time interval, a specialised rapidly rotating detector system with high sensitivity was developed, giving a spatial resolution of about 1.7cm in the same plane as the image and 2.0cm axially across the image. This degree of resolution means that a resolution element is likely to contain a mixture of tissue types. Moreover, Compton scatter from other parts of the brain unavoidably influences the counting rate from a given resolution element . Thus ^{133}Xe, a soft gamma emitter, with radiation of only 81keV is not particularly well suited for SPECT imaging when resolution is important, as in dementia. Furthermore, the need for rapidly rotating detectors, limits the number of centres able to use the method. However, ^{133}Xe inhalation SPECT has proved useful as a quantitative reference method against which other qualitative SPECT methods, such as ^{123}Iodine-isopropyl-iodoamphetamine, can be compared (Lassen et al 1983). Using the xenon SPECT method, nineteen patients with probable AD, diagnosed according to the NINCDS-ADRDA criteria (McKhann et al 1984), were studied by Bonte et

al (1986). Compared with age and sex matched control subjects, AD patients showed reduced rCBF in bilateral temporal regions, and in some this was more marked on the left side. In more severely affected patients, left frontal, and bilateral parietal and occipital reductions in flow were also detected.

In order to overcome the problems discussed above with xenon SPECT, radioligands such as the lipophilic amine, ^{123}Iodine-isopropyl-iodoamphetamine (IMP), were developed. As explained above, IMP behaves as a 'chemical microsphere', with high first pass extraction by brain (about 90%) and slow wash-out, resulting in an initial distribution that correlates well with rCBF distribution (Holman et al 1982). One particular advantage of IMP, compared to the inhalational methods described above, is its ease of administration as a single intravenous injection. The utility of IMP for SPECT in dementia was assessed in a small study of five patients with probable AD (pAD) and diffuse symmetrical reductions in relative ^{123}I-IMP uptake, particularly in parieto-occipital areas, were demonstrated (Cohen et al 1986). In a larger study in Aberdeen (Sharp et al 1986a) that included fourteen senile patients with pAD, SPECT scanning was used to determine relative ^{123}I-IMP uptake in ten symmetrical regions. In all the pAD patients there was bilateral reduction of uptake in temporo-parieto-occipital regions and in many patients posterior frontal flow deficits were also detected. Interestingly, in 64% of the pAD group, magnetic resonance imaging (MRI) was normal in posterior regions, suggesting that SPECT provides different information from MRI.

Using the McKhann et al (1984) standardised diagnostic criteria, fifteen patients with pAD were compared with a group of nine, age matched controls using ^{123}I-IMP and a rotating gamma camera SPECT system (Johnson et al 1987). The patient group consisted of mixed presenile and senile pAD types with onset of illness after 65 years in eight patients, and before 65 years in the remainder. Marked reductions in cortical / cerebellum ratios of tracer uptake occurred in all cortical areas of the AD group compared with controls, but were

most marked (20-30% decrease) in posterior parietal, posterior temporal and frontal areas. Overall, no asymmetries were detected, but marked side to side differences did occur in individual cases. In a different study (Jagust et al 1987), nine patients with pAD were compared with five non-matched control subjects using ^{123}I-IMP SPECT, including neuropsychological testing. In all pAD patients there was marked reduction in relative IMP uptake in bilateral temporo-parietal cortex and this was significantly associated with severity of dementia, as measured by Mini-Mental State Examination score. Rather ambitiously, on the basis of this study, Jagust et al (1987) concluded that the IMP SPECT method was able to distinguish AD from control subjects, even in very early stages of the disease. The authors also emphasised the importance of impaired temporo-parietal rCBF and speculated about its significance in the pathogenesis of AD, and possible utility for staging the progression of the disease.

Experience with ^{123}I-labelled amines, and in particular IMP, showed that microsphere analogue techniques for SPECT imaging of rCBF in humans had many advantages. It was recognised, however, that both ^{123}I and IMP have a number of features that limit their usefulness for SPECT. Iodine-123 is a cyclotron-produced radionuclide, with a short half-life of thirteen hours and as a result, is not readily available, is expensive, and deliveries must be carefully timed to coincide with imaging. As described above in section 2.2.5.1, the behaviour of IMP is poorly understood and is not consistently reliable for imaging CBF distribution. On the other hand, a microsphere analogue flow marker, based on technetium (^{99m}Tc), has several advantages that it make it more suitable for SPECT.

Technetium has better imaging and dosimetry characteristics than ^{123}I, and, as it is routinely used for isotopic imaging, it is widely available at low cost . Therefore the development in 1986 of a hexamethyl derivative of propylene amine oxime (HMPAO), that readily complexed with ^{99m}Tc,

facilitated the use of SPECT for investigation of rCBF distribution (Neirinckx et al 1987). There is high correlation between relative rCBF, measured using ^{99m}Tc-HMPAO SPECT, and corresponding absolute rCBF values, measured with ^{133}Xe inhalation, in both normal subjects and pAD patients (Andersen et al 1988b). Furthermore, in dementia and other neuropsychiatric disorders, ^{99m}Tc-HMPAO SPECT has an advantage in that the tracer is administered intravenously and has a distribution in brain that is relatively stable, thus allowing sufficient time for imaging and patient cooperation.

In a qualitative study using ^{99m}Tc-HMPAO, Neary et al (1987) examined 21 patients with presumed AD and 9 others with 'frontal lobe dementia', which the authors believe may be distinct from AD. The tomograms were evaluated by inspection, and classified according to whether they had anterior or posterior areas of reduced uptake. In the pAD group, 33% had a posterior deficit, a similar percentage a mixed anterior and posterior deficit, and the remainder had either reduced anterior uptake or no detectable abnormality. In two thirds of the pAD patients, visuo-spatial impairment was associated with reduced posterior flow. In patients classified as having 'frontal dementia' on the basis of clinical features, 80% had, not unexpectedly, reduced frontal flow. In a comparable qualitative study of 17 pAD patients, and 10 MID patients, Gemmell et al (1987) used ^{99m}Tc-HMPAO SPECT to investigate whether groups could be distinguished by pattern of uptake. Tomograms were divided into ten regions and their degree of abnormality scored from one to three. All patients also had magnetic resonance imaging, and no focal abnormalities were detected in the pAD patients. Reduced HMPAO uptake was more common in the AD group than the MID group, especially in bilateral temporo-parieto-occipital regions. In both studies it was suggested that inspection of SPECT images may permit better diagnosis of dementia type and that pAD patients tended to have bilateral posterior deficits. Reduced flow in bilateral temporal and posterior

parietal regions was also found in older pAD patients with aphasia and apraxia, and associatlons found between memory loss and temporal lobe activity, and between language and left sided tracer uptake (Burns et al 1989). In another SPECT study of 26 older pAD patients, there was reduced rCBF in cortical regions, particularly in bilateral posterior temporal, but also in left temporal, left parietal and bilateral frontal regions, with relative sparing of occipital regions (Montaldi et al 1990). Numerous associations between cognitive performance and rCBF were found, which may reflect the severity of patient group or the non-specificity of the cognitive battery. The fact that no associations between memory and rCBF were detected is not surprising, in view of the pronounced floor effects that occur in pAD with most simple tests of memory. The correspondence between neuropsychological deficits and SPECT in AD, was investigated by Goldenberg et al (1989), and the results interpreted in terms of two dimensions of organisation, along which rCBF measures and cognitive test scores co-varied in a systematic fashion. The first dimension was said to correspond to the fronto-temporo-parietal axis, with naming, constructional praxis and visual recognition memory associated with parieto-temporal regions, and semantic memory and behaviour more associated with frontal areas. In a second left-right axis of organisation, the ordering of test results was partly consistent with what is known about the lateralization of neuropsychological deficits from studies of localised lesions.

2.2.6 Positron emission tomography

Positron emission tomography is the most sophisticated technique for the study of brain function. Any review of functional brain imaging in dementia would be incomplete without at least some discussion of the principles of PET and the results obtained from its use. In PET, positron emitting isotopes are used to construct 3-dimensional functional brain images. Positrons are β-particles which carry a positive rather than a negative charge,

and which, on collision with an electron, emit a pair of γ-ray photons at approximately 180 degrees (a 'coincidence-pair'), each of 511KeV energy. These photons can be detected by positioning a pair of detectors on either side of the patient and selectively only detecting those γ-rays which are simultaneously counted by both detectors. Such simultaneous detection allows the anatomical origin of the positron to be determined and thus eliminates the need for the lead grid collimators which are required with all SPECT instruments, and which reduce sensitivity by 1-2 orders of magnitude. Unlike the microsphere analogue tracers used in SPECT (eg HMPAO), true quantification is also possible using PET. However, PET cameras have the disadvantage that many accidental coincidences cannot be eliminated, and consequently PET images contain considerable background noise which reduces the accuracy of measurements.

The use of a labelled ligand to measure a physiological process depends on an understanding of the fate of the injected molecule and a description of the whole process in mathematical terms. The versatility of PET is shown by the wide range of variables that have been measured in humans including local tissue perfusion, blood volume, glucose and oxygen consumption, as well as the fractional extraction of various metabolites which have been accurately imaged quantitatively (Phelps et al 1986; Frackowiak 1988). Positron emitting forms of the 'physiological' elements carbon (^{11}C), oxygen (^{15}O), and nitrogen (^{13}N) can be substituted for the same stable elements in molecules to be studied, thus avoiding the conformational alterations of normal metabolites that can result when other isotopes, such as ^{123}I, are used to label molecules. As the brain uses oxygen and glucose almost exclusively as the substrates for metabolic energy, PET measurements of oxygen and glucose consumption accurately reflect neuronal metabolism. Apart from metabolic and transmitter studies, PET can be used to measure

tissue concentrations of any labelled substance or drug and its rate of change with time (Baron et al 1983).

The short half-life of PET radioligands requires considerable on-site technical expertise including a cyclotron for their manufacture. For example, the half-life of ^{15}O is 2.1 minutes and that of ^{11}C, 20 minutes, therefore the newly generated isotopes have to be used quickly. Fluorine-18 (^{18}F) has been used in many PET studies and can be readily incorporated into small molecules (eg as ^{18}F-fluorodeoxyglucose) behaving as a proton and therefore it can be used as a hydrogen substitute. Furthermore, it has a comparatively longer half-life of 110 minutes, making it potentially transportable from site of production to PET facility. After generation in a cyclotron, the radioisotopes are incorporated into the pharmaceutical or ligand and only then is the resulting radio-pharmaceutical administered to the subject. Thus, it is necessary to have a skilled chemistry laboratory on site in order that the short half-life PET isotopes can be quickly incorporated into the ligand of interest.

2.2.6.1 PET measurements of CBF, $CMRO_2$ and CMRglc

There are several ways in which PET has been used to measure cerebral blood flow. The inert gas wash-out method, described earlier, can be used with several positron emitters including ^{77}Kr, ^{11}C, ^{18}F (eg Yamamoto et al 1980). The method was further developed using continuous inhalation by Jones et al (1976), in which the patient inhales air containing carbon dioxide (CO_2) labelled with ^{15}O. In the lungs, $C^{15}O_2$ is rapidly converted by carbonic anhydrase to carbonic acid and $H_2{}^{15}O$. Thus ^{15}O-labelled water, assumed to be freely diffusible between blood and brain, is used as the tracer. After about 7 minutes, (or about three half-lives of ^{15}O), a steady state is established with the rate of arrival of labelled water in brain equal to the rate of loss by wash-out and radioactive decay. Thereafter the concentration and distribution of radioactivity in the brain remains constant for imaging. Thus, if

in the steady-state the arterial (delivery) concentration and tissue concentration can be measured, CBF can be calculated from the following equation (Frackowiak et al 1980), where perfusion (P) is given by,

$$P = \frac{K}{[(H_2O)_a / (H_2O)_b] - 1}$$

and K is the radioactive decay constant for oxygen, $(H_2O)_a$ the arterial concentration of $H_2{}^{15}O$ in an arterial blood sample and $(H_2O)_b$ the concentration of $H_2{}^{15}O$ in brain as measured by PET. There is a reasonably good correlation between CBF measured using this technique and that obtained with intra-arterial microspheres in baboons for perfusion in the range 10-100ml/100mg/min (Steinling et al 1985). The method is more accurate for measuring low or normal flow (eg patients with dementia, Frackowiak et al 1981) but may be misleading in situations where flow is greater than 100ml/100mg/min. However, such situations are quite unusual eg ictal epilepsy or encephalitis and the errors involved may not be crucial. If the subject is made to breath labelled molecular oxygen ($^{15}O_2$), ^{15}O-labelled oxyhaemoglobin is synthesized in lung and, after arterial distribution, ^{15}O is available for tissue oxidative metabolism. This results in the tissue synthesis of $H_2{}^{15}O$, which can be measured, and the oxygen extraction ratio (OER) can be calculated. After corrections are made for blood volume and the recirculating $H_2{}^{15}O$ component, the product of CBF, OER and stable arterial oxygen content is the $CMRO_2$, the rate of oxygen utilization by the brain. Thus, one label (^{15}O), and essentially one tracer ($H_2{}^{15}O$), though generated in two ways, can be used to measure three variables, CBF, OER, and $CMRO_2$.

The advantages of this method are that it is conceptually and mathematical simple and highly reproducible in practice.

A second and entirely different strategy is used to measure cerebral glucose consumption (CMRglc). This is an extension of the techniques originally developed for use with autoradiographic measurements in animals. When glucose is taken up into brain and enters the glycolytic pathway at hexokinase, the vast majority passes into the Kreb's cycle for production of ATP. The product of this pathway is CO_2 which is rapidly cleared from tissue. This explains why ^{11}C-labelled glucose is difficult to use.

An alternative approach is to use an analogue of glucose to study glycolytic flux. Deoxyglucose (DG) can be used as a glucose analogue as it uses the same blood brain barrier uptake system and acts as a substrate for hexokinase. The product of this reaction is deoxyglucose 6-phosphate which cannot be metabolised further and is therefore trapped in the cerebral tissues. Thus measurement of DG consumption can be used as a measure of CMRglc. The relationship between DG handling and glucose metabolism is termed the lumped constant because it incorporates terms relating the different rates of transport and phosphorylation of the analogue and glucose. The technique is practical and straightforward and, because ^{18}F can be used to label DG, it has been widely used.

2.2.6.2 The effect of aging measured by PET

Positron emission tomography (PET), is probably the most elegant technique for imaging *in vivo* brain function. Although several PET studies have confirmed that rCBF, rCMRglc and $rCMRO_2$ are reduced in cortical brain regions in AD compared with age-matched healthy controls, there have been conflicting results regarding the effects of age on normal cerebral metabolism. Kuhl et al (1982a) studied 17 men and 23 women between the ages of 18 and 78 years and found a small but significant decrease in regional glucose

consumption with age. However Duara et al (1984) repeated the study in a more detailed way in 40 healthy men aged from 21 to 83 years, and the mean cerebral metabolic rate did not change significantly with increasing age, although there was a minor trend towards lower values in older men. Several smaller studies also failed to demonstrate significant changes in brain glucose metabolism with advancing age. For example de Leon et al (1983a), used the tracer ^{18}F-2-deoxy-2-fluoro-D-glucose (^{18}F-FDG) with PET to compare glucose metabolism in 15 young and 22 elderly normal subjects. No significant differences in CMRglc were detected between the two groups.

There have been fewer studies of $rCMRO_2$ in humans that addressed this question. Frackowiak et al (1980), using continuous inhalation of ^{15}O-labelled CO_2 and O_2, showed a decrease in rCBF and $rCMRO_2$ with increasing age in grey but not white matter in normal volunteers aged from 26 to 74 years. Lebrun-Grandie et al (1983) confirmed this finding with a similar technique in three regions of cortex in a group of 19 normal subjects. However Frackowiak et al (1983) subsequently reanalysed their PET data and concluded that CBF, but not $CMRO_2$ was age related, due to increases in the oxygen extraction ratio.

It is difficult to reconcile these findings, especially in view of the apparent coupling of CBF, $CMRO_2$ and CMRglc in resting subjects. Some of the observed differences are probably due to variations in methodology eg in Kuhl et al (1982a) subjects were examined with eyes open, while Duara et al (1984) examined subjects with eyes closed and ears plugged in conditions of minimal sensory input. As it is likely that any age-related effect is probably small, the standardization of experimental conditions is essential before studies can be meaningfully compared. Furthermore the putative age-related changes in brain function may only become apparent if subjects are examined under conditions of activation, rather than in the 'resting state'. In reviewing the contribution of PET to the study of the effect of age on normal brain

metabolism, Dastur (1985) concludes that most evidence suggests that CBF and metabolism are not changed, except for a small but significant decrease in glucose utilization.

If age-related changes in CBF and metabolism are important, there is clearly a need for careful age-matching of control subjects and patients.

2.2.6.3 Glucose and oxygen metabolism in AD

Several studies have confirmed that rCMRglc is reduced in cortical areas in senile dementia, compared with age-matched controls. Ferris et al (1980) were the first to report preliminary PET findings in 7 patients with senile AD and 3 normal controls. These authors found significantly reduced rCMRglc in bilateral frontal and temporal cortex and in bilateral caudate and thalamus, but reported the results cautiously because of concerns about the poor resolution of the PET imager. Later, studies with larger series of patients and better instrumentation confirmed this general finding (Farkas et al 1982; Foster et al 1983; Friedland et al 1983; and Cutler et al 1984). Measurements of $rCMRO_2$ and rCBF in dementia were also consistent with these reductions in glucose metabolism (Frackowiak et al 1981).

As the quality of imaging investigation improved, a number of focal patterns of regional metabolism in AD were demonstrated, rather than the generalised reductions noted in earlier studies. Reductions in rCMRglc appeared to be smaller in perirolandic and occipital areas than in association cortices (Benson et al 1983; Haxby et al 1985), while temporo-parietal association areas showed the greatest reductions in mild to moderate AD, with comparatively less reduction in frontal metabolism (Frackowiak et al 1981; Foster et al 1983; Friedland et al 1983). In severe AD however, marked involvement of bilateral frontal cortex was also observed (Frackowiak et al 1981; Benson et al 1983; Duara et al 1986). It is likely that as Alzheimer's disease progresses, other brain regions become involved, and therefore the

pattern of metabolic impairment observed is closely related to the disease stage. As might be expected, patients with mild or early dementia have metabolic rates that overlap with those of controls, but as severity increases there is clear distinction from healthy controls (Duara et al 1986). One common pattern of progression appears to be initial involvement of temporo-parietal regions, with subsequent involvement of frontal regions (Friedland et al 1983; Kuhl et al 1985; Benson, 1988). However, atrophy of frontal and temporal lobes is often a distinctive macroscopic feature of AD pathology (Tomlinson & Corsellis, 1984), and frontal decrements in metabolism and CBF have been found in AD patients of all grades of severity (Duara et al 1986).

Studies of the relation of cerebral metabolic rates to severity of dementia in AD have yielded less consistent results. Earlier studies, found that $rCMRO_2$ was lower in more severely demented patients than in moderately demented patients (Frackowiak et al 1981), and Ferris et al (1983) found significant correlations between rCMRglc and measures of global impairment and memory. On the other hand, in a report by Foster et al (1983) there was no relation between rCMRglc and global severity, but in 13 AD patients there was a postive association with reaction time. In an increased sample, Foster et al (1984) found that rCMRglc values in mild and severe AD were not significantly different from each other. One reason for such inconsistencies may be the large variation in normal values of rCMRglc in healthy adults. Thus, any relation between severity and glucose metabolism may be masked by the variation in premorbid levels of rCMRglc. In order to reduce variance that is not disease related, indices of rCMRglc pattern, such as the ratio of, or difference between, regional values and whole brain rCMRglc, have been used. Only when this analysis was used to compare matched AD and healthy controls, were differences in rCMRglc between groups detected (Haxby et al 1985).

Cerebral atrophy complicates the interpretation of PET (and SPECT) images in two respects. Firstly, the measuring instruments suffer from partial volume effects, that is their spatial resolution is inadequate to resolve cortical structures. Thus measurements from cortical regions will include data from cortex, white matter and cerebrospinal fluid spaces. In addition, interpretational problems also result from the significant cell loss in AD. For example in advanced cases there may as much as 46% neuronal loss in temporal, and 40% loss in frontal cortex (Terry et al 1981) leading to uncertainty about whether estimates of reduced CBF or metabolism reflect neuronal cell death, reduced functional activity of preserved tissue, or, as seems likely, some combination of both. The spatial resolution of PET, typically of the order of 1 cm, may be at the limit of some anatomical examination. Functional imaging of medial temporal structures such as hippocampus that are usually, perhaps always, involved in AD (section 1.6) may potentially help in early AD case detection.

Initial studies of inter-regional metabolic differences suggested that antero-posterior changes were more prominent than left-right hemispheric differences. Friedland et al (1983), for example, reported that frontal to temporo-parietal ratios of CMRglc were greater than right to left ratios in 10 patients with mild to moderate pAD reflecting reduced CMRglc in temporo-parietal regions. However in this study, right to left frontal ratios were strongly correlated with severity of dementia and verbal IQ, and right to left temporo-parietal ratios with performance IQ. Increased right-left asymmetry of rCMRglc has been found in mild to severe AD cases (Foster et al 1983; Friedland et al 1985; Haxby et al 1985; Duara et al 1986), but with no characteristic direction to the asymmetry, ie in some patients there were greater reductions in left rather than right side, but other patients showed the opposite pattern. In mild to moderately severe AD, measurements of rCMRglc in frontal, temporal and parietal association cortices were significantly more laterally asymmetrical

than healthy controls (Haxby et al 1985). Furthermore in this study, lateral asymmetry of rCMRglc in AD correlated with impaired language and visuo-spatial function, in that greater impairment of left sided rCMRglc was associated with relatively greater impairment of language, and right-sided rCMRglc impairments with increased visuo-spatial dysfunction. In a study of 12 AD patients, of whom 4 could be classed as severe, Duara et al (1986) concluded that right to left asymmetry occurs early in the course of AD, and becomes more prominent as the disease progresses. It has also been suggested that metabolic and neuropsychological asymmetries persist at least until the disease becomes very severe (Grady et al 1986).

The nature of the association between mental function and regional metabolism in AD has been addressed in a number of studies. In mild and moderate cases of probable AD, metabolism in specific brain regions has been correlated with some cognitive measures (Foster et al 1983; Haxby et al 1985; Riege et al 1985). Not all studies however used homogeneous patient populations, or adjusted significance levels to take account of multiple correlations. With absolute measures of rCMRglc correlations were not always detected (Cutler et al 1984), and patterns of association have been more often detected between regional CMRglc corrected for whole brain CMRglc or left-right asymmetry, and specific cognitive deficits (Riege et al 1985). Foster et al (1983, 1986) and Chase et al (1987) studied clinically diagnosed pAD patients who were selected as having disproportionate language or visuoconstructive impairments. Those with disproportionate language deficits tended to have relatively lower left hemisphere rCMRglc, whereas patients with predominantly visuospatial deficits had relatively lower right hemisphere metabolism. Furthermore, language performance correlated highly with left perisylvian rCMRglc, and performance with visuospatial consructional tests correlated with posterior parietal rCMRglc. However, as patients were preselected for the presence of particular

neuropsychological deficits, the generality of the conclusions may be uncertain.

All the PET studies reviewed above were carried out under basal or 'resting conditions' and at a different time from assessment of neuropsychological function. The criticism could be made that brain energy demands during 'resting' conditions might not be expected to be closely correlated with mental task performance. It could be argued that more meaningful relationships might exist between task performance and brain metabolism during the task. There are practical difficulties in undertaking such studies but investigation of how brain metabolism changes during psychological activation will be an important direction for this work in future studies.

2.3 Conclusions from literature review on functional imaging in Alzheimer's disease

In summary, the literature reviewed above illustrates the following:

1. A close relationship exists between CBF and metabolism, during both 'basal' and activated conditions of brain function. This is important support for the use of CBF as an index of brain function in the study of AD.

2. Since the introduction of techniques for the measurement of CBF in the 1940s, sophistication has improved from the early estimates of global CBF provided by the Kety-Schmidt method to the tomographic regional values of CBF and metabolism available from PET.

3. Early studies demonstrated reductions in global CBF that were significantly correlated with cognitive impairment. Later, these findings were confirmed by intra-arterial techniques that permitted different cortical regions in one hemisphere to be studied. These studies indicated hypoperfusion of temporal regions in AD, with more widespread cortical perfusion deficits as severity increases.

4. The introduction in the 1960s of the more acceptable inhalational methods of measuring CBF enabled quantitative regional measurements to be made on both hemispheres, and suggested that impaired cortical perfusion in AD is often bilateral.

5. The advantage of tomographic imaging methods, such as SPECT and PET, is that they allow subcortical and cortical regions to be studied and patterns of functional deficit to be identified. Severity of dementia appears to be a major determinant of severity of metabolic impairment and pattern of reduced cerebral function. Several patterns of functional deficit have been recognised using PET and SPECT, suggesting that AD may be more heterogeneous than indicated by the early techniques. Although many, and perhaps most, patients with AD show characteristic metabolic reductions in temporo-parietal regions, other patients show marked frontal deficits, and often not necessarily as a concomitant of severe dementia.

6. As with earlier two-dimensional measurements of CBF, there are often difficulties in the interpretation of SPECT and PET data in patients with significant cortical atrophy. This is mainly the result of partial volume effects due to limited resolution and consequent uncertainty about whether reduced CBF or metabolism results from reduced function or reduced tissue mass.

7. Although PET provides estimates of several metabolic parameters in addition to CBF, there are some advantages associated with SPECT. These include readily available radiopharmaceuticals, low cost isotopes, comparatively lower cost SPECT imagers, and limited technical support required. These factors suggest that SPECT imaging may be performed on large numbers of patients at reasonable cost. Moreover moderate resolution SPECT is potentially available to many researchers and clinicians.

CHAPTER 3

GENERAL METHODS

3.1 Introduction

This chapter describes the main methods used in the experimental sections. Two control groups were studied for comparison with AD patients: matched healthy control subjects and patients with chronic Korsakoff's syndrome. There are several reasons why the study of patients with Korsakoff's syndrome is appropriate and relevant to an investigation of AD. Firstly in Korsakoff's syndrome memory impairment over-shadows other aspects of cognitive failure, in contrast to AD where, although dysmnesia may be an initial feature, other cognitive deficits are usually also prominent. This provides an important opportunity to examine the changes in brain function associated with the two different patterns of cognitive impairment. Secondly, while in AD it is not disputed that cortical pathology makes a significant contribution to the pattern of cognitive deficit, in Korsakoff's syndrome the main lesions have traditionally been considered to be subcortical, and attention at autopsy focussed on basal brain regions. This has continued despite accumulating neuropathological and encephalographic evidence that prolonged alcohol abuse can result in diffuse brain damage (Ron, 1977). Thus the inclusion in the study of a control group with chronic cognitive impairment, possibly attributable to subcortical pathology, was an appealing idea. More important, however, was the opportunity to investigate the functional integrity of cortical regions in Korsakoff patients who had abstained from alcohol for some time in order to assess the contribution of cortical dysfunction to the syndrome. Finally, there is another reason for interest here in Korsakoff syndrome. In a detailed comparison of the memory problems of AD and Korsakoff's syndrome, Kopelman (1985) concluded that they have many deficits in common, with particular difficulty in getting information into long-term storage. There is evidence that damage to a number of neurotransmitter systems, including cholinergic and noradrenergic, are implicated in both disorders (Hardy et al 1985; Kopelman & Corn, 1988;

McEntee et al 1984). In many ways Korsakoff's syndrome may prove to be a useful 'model' system in which to study the way in which neurotransmitter failure produces cognitive failure.

Studies of isotope imaging necessarily involve exposure to ionising radiation. In general, different patient and control groups were recruited for the studies described in chapters 4, 5 and 6, in order to limit the exposure of patients and control subjects to radiation. No control subject was imaged more than once, as it was considered unethical to expose them to this amount of additional radiation. Imaging facilities for measuring MTT were located at the Western General Hospital, Edinburgh, and for SPECT at the Institute of Neurological Sciences, Glasgow. Patients were transported to the two sites using a comfortable car, in the company of the investigator (RH), and often the patient's relative. At both sites patients were given time to relax after the journey, before the experimental session.

3.2 Patient selection

3.2.1 Alzheimer's dementia

Patients with AD were recruited for the studies described in this thesis from clinical referrals to the MRC Brain Metabolism Unit of patients thought to have pre-senile dementia. In the main, referrals were from consultant colleagues in neurology and psychiatry in Lothian, although a few patients were referred to the Unit directly by their general practitioner. From such referrals only those patients meeting recently agreed criteria for probable Alzheimer's disease, as laid down by National Institute of Neurological and Communicative Disorders Stroke (NINCDS) and Alzheimer's Disease and Related Disorders Association (ADRDA) (McKhann et al 1984), were recruited for study. The cardinal features of these criteria, which were satisfied for all AD patients studied, include:

a) onset of symptoms before the age of 65 years of age, with dysmnesia as the initial presenting feature.
b) clinical course characterised by steadily progressing dementia.
c) absence of a history suggestive of an another type of dementia.
d) absence of focal neurological signs.
e) no evidence of hypertension or other cardio-vascular abnormalities and normal electrocardiogram.
f) X-ray computed tomography (CT) scan normal or showing only generalised cortical atrophy and no additional pathology.
g) no focal abnormalities on the electroencephalogram (EEG).
h) all patients had a comprehensive range of blood investigations, including full blood count, erythrocyte sedimentation rate, urea, creatinine and electrolytes, liver function tests, thyroid function tests, serum vitamin B12 and folic acid, and VDRL (Venereal Disease Research Laboratory) and TPA (Treponema Pallidum Agglutination test).
i) all patients had lumbar puncture performed as part of their diagnostic assessment and were not included in the study unless cerebrospinal fluid (CSF) investigations were within normal limits.

These criteria form part of the Cambridge Diagnostic Examination for the Elderly (CAMDEX), developed by Roth et al (1986), which was completed for all patients studied and was used to exclude other psychiatric disorders, such as alcohol abuse or major depressive disorder, or physical illness such as malignancy, diabetes or other major systemic illness, and hence improve the quality of the probable diagnosis of AD.

Figure 3.1 The CAMDEX schedule

Section A Structured psychiatric interview.

Information about present mental and physical state, in particular symptoms suggestive of organic psychoses, depression and functional paranoid psychoses. Past medical and family history.

Section B CAMCOG - the cognitive examination.

Section C Interviewer's observations.

Observations of present mental state, including appearance, behaviour, mood, speech, mental slowing, thought processes and level of consciousness.

Section D Physical examination.

Section E Results of laboratory and radiological investigations.

Section F Current medication.

Section G Additional information.

Section H Structured interview with informant.

Incorporates items on personality change, difficulties in everyday functioning, signs of impaired cognition, evidence of depressive or paranoid phenomenology. Corroboration and elaboration of past medical and family history.

3.2.2 Alcoholic Korsakoff syndrome

Patients with alcoholic Korsakoff Syndrome (KOR) were recruited from the long-stay wards or nursing-supervised hostel accommodation of the Royal Edinburgh Hospital, and in a few cases from other psychiatric hospitals in Lothian. All met DSM-III-R (Diagnostic and Statistical Manual, third edition, revised) criteria for Korsakoff syndrome. The chief criteria used to select Korsakoff patients were as follows:

a) Definite history of prolonged alcohol abuse, usually in excess of 20 years.
b) Severe anterograde memory deficit, out of all proportion to other disturbances of intellect and behaviour.
c) No history of progressive dementia.
d) Definite history of past episodes of either acute confusion or Wernicke's encephalopathy (with or without peripheral neuropathy).
e) No documented history of 'significant' head injury.
f) EEG normal or showing no focal activity.
g) Normal haemotological and biochemical tests as outlined in 3.2.1(h) above.
h) No alcoholic beverage taken for at least 6 months.

Recruitment of KOR patients according to the above criteria presented several difficulties. Firstly, patients with a 'case notes' diagnosis of 'Korsakoff's psychosis, alcoholic' based on the International Classification of Diseases, Ninth Revision (ICD-9, World Health Organisation, 1978), did not always satisfy the above operational criteria, and were therefore not included in the studies. One reason for this is that the diagnostic label 'Korsakoff's psychosis', (ICD-9 category 291.1) has tended in the past to be applied rather widely by some clinicians to incorrectly include any patient with a history of prolonged heavy alcohol intake and signs of dementia. Many patients labelled as 'alcoholic dements' (ICD-9 category 291.2) are in fact suffering from Korsakoff's psychosis, while others may be merely displaying the

profound social and mental disorganisation due to continued heavy drinking, or have coincidental Alzheimer's disease or multi-infarct dementia. However, there is evidence that prolonged ingestion of alcohol can give rise to varying degrees of cognitive impairment and, in some patients, dementia characterised by progressive memory failure and deterioration of personality and intellect (Wilkinson et al 1971). Another difficulty for researchers trying to define a homogeneous group of KOR patients is that many patients who have a long history of alcohol abuse have also sustained head injuries which could also contribute to organic brain damage. Establishing whether or not a patient has such a history is often very difficult but attempts were made to discover a history of head trauma using case notes, informants and by interviewing the patient. No patient with a documented history of significant head injury was included in the KOR group studied.

3.2.3 Healthy control subjects

Healthy control subjects were recruited from local voluntary organisations in Edinburgh including the Hospital League of Friends and the Womens Royal Voluntary Service, and in some cases the patient's spouse also volunteered. All volunteers were carefully interviewed and examined to exclude physical or psychiatric illness. The CAMDEX schedule was also used to detect past or present mental or physical disorder in controls. Although this use of CAMDEX can be questioned, the chief aim was to ensure that significant illness was not present in subjects recruited for the control group. For this limited purpose, CAMDEX formed the basis of a reasonable screening instrument. All subjects had blood taken for full blood count, erythrocyte sedimentation rate, urea, creatinine and electrolytes, liver function tests, thyroid function tests, serum vitamin B12 and folic acid, and VDRL (Venereal Disease Research Laboratory) and TPA (Treponema Pallidum

Agglutination test), and were only included in the study if values were within normal laboratory limits.

3.2.4 Handedness

Handedness may affect the regional localisation of function in brain and hence rCBF. In order to control for the possible confounding effect of handedness, only patients and control subjects who fulfilled criteria for right-handedness were included in the studies. The Edinburgh Inventory for determining handedness was used (Oldfield, 1971).

3.2.5 Age and sex

As discussed in chapter 2 there appears to be a small, but significant reduction in glucose metabolism and probably CBF with aging (eg see Cutler et al 1984). Similarly, it is likely that sex may also affect rCBF. Little is known about the magnitude of such effects but in order to reduce possible confounding age or sex effects, attempts were made to match healthy control subjects for age and sex with AD patients. Efforts to do the same for the Korsakoff group were less successful, mainly because Korsakoff's psychosis is not a common condition, and most sufferers are male with a greater age range than for AD. Recruiting healthy males of late middle age to act as controls proved more difficult than recruiting healthy females. Hence the matching of controls to AD patients was more satisfactory than the matching to KOR patients.

3.2.6 Concomitant medication

None of the patients or control subjects had taken psychotropic medication for at least six months before the study. As all AD patients lived at home under the care of their spouse, and all KOR patients lived in supervised

hospital accomodation, it was possible to be reasonably sure that no unreported medication was taken during the study.

3.3 Consent

Patients and control subjects gave informed consent to take part in the studies and where there was doubt about a patient's understanding of what was entailed, the agreement of a close relative, or independent nurse, who could act as an advocate for the patient, was consulted before the patient was included. The studies were all approved by the Ethics of Medical Research Sub-Committee for Psychiatry and Psychology of the Lothian Health Board and in the case of the SPECT project, consent was also obtained from the Ethics of Medical Research Committee of the Institute of Neurological Sciences, Southern General Hospital, Glasgow. The use of sodium pertechnetate and ^{99m}Tc-hexamethyl propyleneamine oxime (^{99m}Tc-HMPAO) was approved by the Administration of Radioactive Substances Advisory Committee (ARSAC) of the Department of Health.

3.4 Neuropsychological assessment

The CAMDEX was developed for the diagnosis and measurement of dementia (Roth et al 1986) and was completed for all patients in the study. The interval between cognitive assessment and imaging was never more than one week. The CAMDEX provides a useful instrument for assembling information on patients suspected of having a dementing illness and contains the following sections: 1) a structured psychiatric interview with the respondent, incorporating questions regarding the present mental disorder, the past history and family history; 2) the objective evaluation of a broad range of neuropsychological functions; 3) a standardised schedule for recording observations of the present mental state together with appearance and demeanour; 4) physical examination including neurological

examination; 5) a structured interview with a relative or other informant able to provide independent information regarding the respondent's present state, changes in personality and activities of daily living, and to corroborate or complete, the past medical, personal and family history; 6) investigation results and drug history where applicable. The CAMDEX schedule is shown in more detail in Figure 3.1. The CAMDEX section for neuropsychological assessment is the CAMCOG, which has subtests for orientation, comprehension, expression, language, memory (remote, recent, learned and total), attention, praxis, calculation, abstraction and perception. The 19 items which comprise the Mini Mental State Examination (MMSE) of Folstein et al (1975) are incorporated into CAMCOG, allowing MMSE scores to be calculated, however, 43 additional items make the CAMCOG more comprehensive than the MMSE and allow different subtests to be assessed. The total CAMCOG score (maximum 106) and MMSE (maximum score 30) can be used as a measure of overall cognitive function. Administration of CAMCOG takes about 45 minutes, depending on the degree of impairment of the patient.

3.5 Measurement of vascular transit time

The principles of MTT have been summarised in section 2.2.4. Measurements of MTT for cortical blood flow in resting conditions were undertaken in the Department of Nuclear Medicine, Western General Hospital, Edinburgh. The patient was asked to lie supine on a high couch with their head extended over a small field of view (FOV) gamma camera with very high sensitivity collimator. The camera was postioned to exclude the trunk from the FOV and the patient's skull oriented with both orbito-meatal lines parallel to and equidistant from the face of the collimator, which was in contact with the vertex. An 18G Venflow cannula attached to a short length of wide-bore

Figure 3.2

The time-activity curve (TAC) obtained from detector placed over the aortic arch in a normal subject (red line).
The first peak is activity passing through the superior vena cara and the second, through the aortic arch. The third ill-defined peak of the red line is due to recirculation. By fitting a gamma variate to the aortic curve, the MTT from arm to the aortic arch (MTT s-a) can be calculated as described in the text (section 3.5). By subtracting MTTs-a from the MTTs calculated at each pixel of the head measured with a gamma camera (MTT from arm to internal jugular vein) net MTT through the cerebral circulation can be calculated for each pixel.

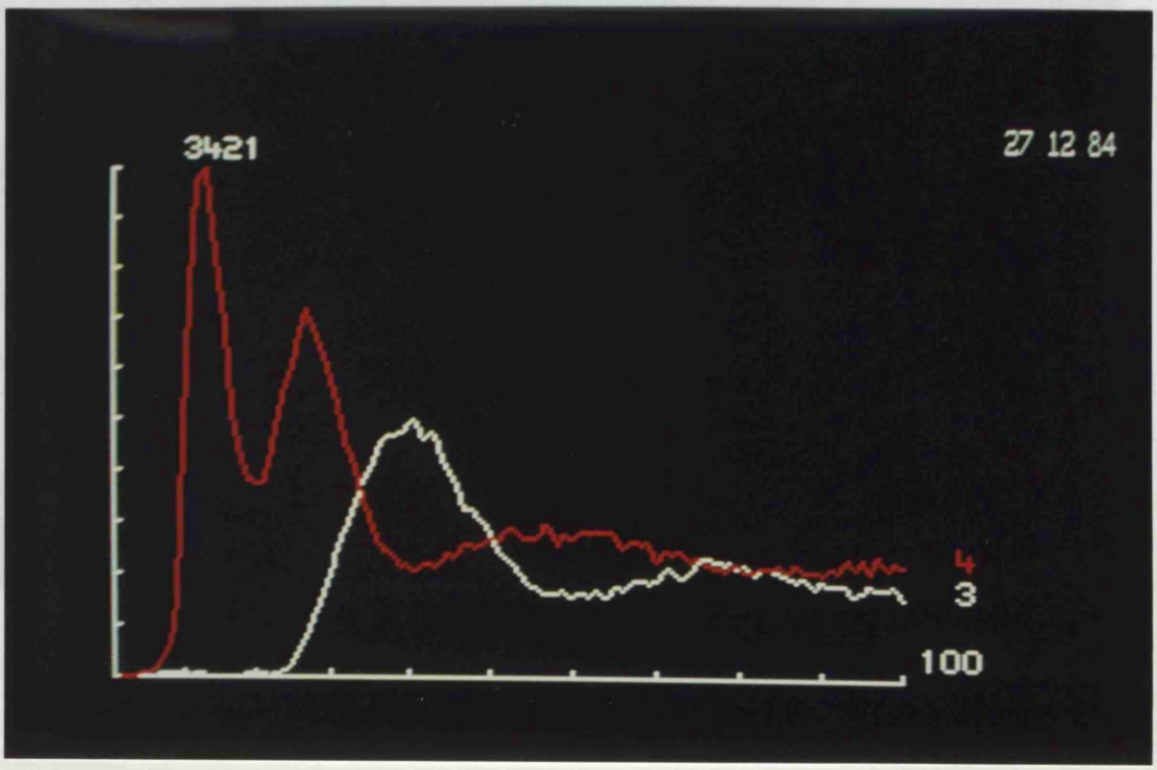
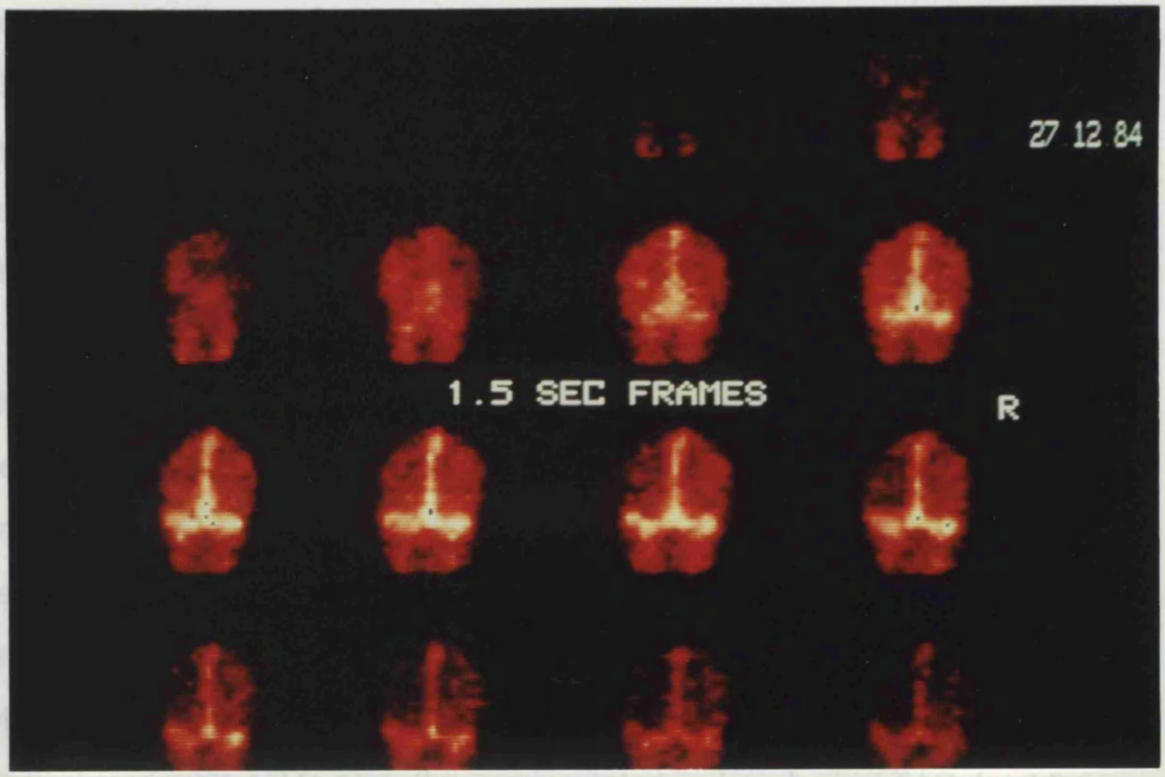


Figure 3.3 The transit of isotope tracer through the brain

A series of vertex images showing (from left to right, and top row down), the passage of pertechnetate (^{99m}Tc) through the brain. Each image has a frame duration of 1.5 seconds, and the activity increases to a maximum (at image number 8, from top left), then decreases as the activity is washed out. For each pixel in the image the MTT from arm to internal jugular vein can be calculated as described in the text.

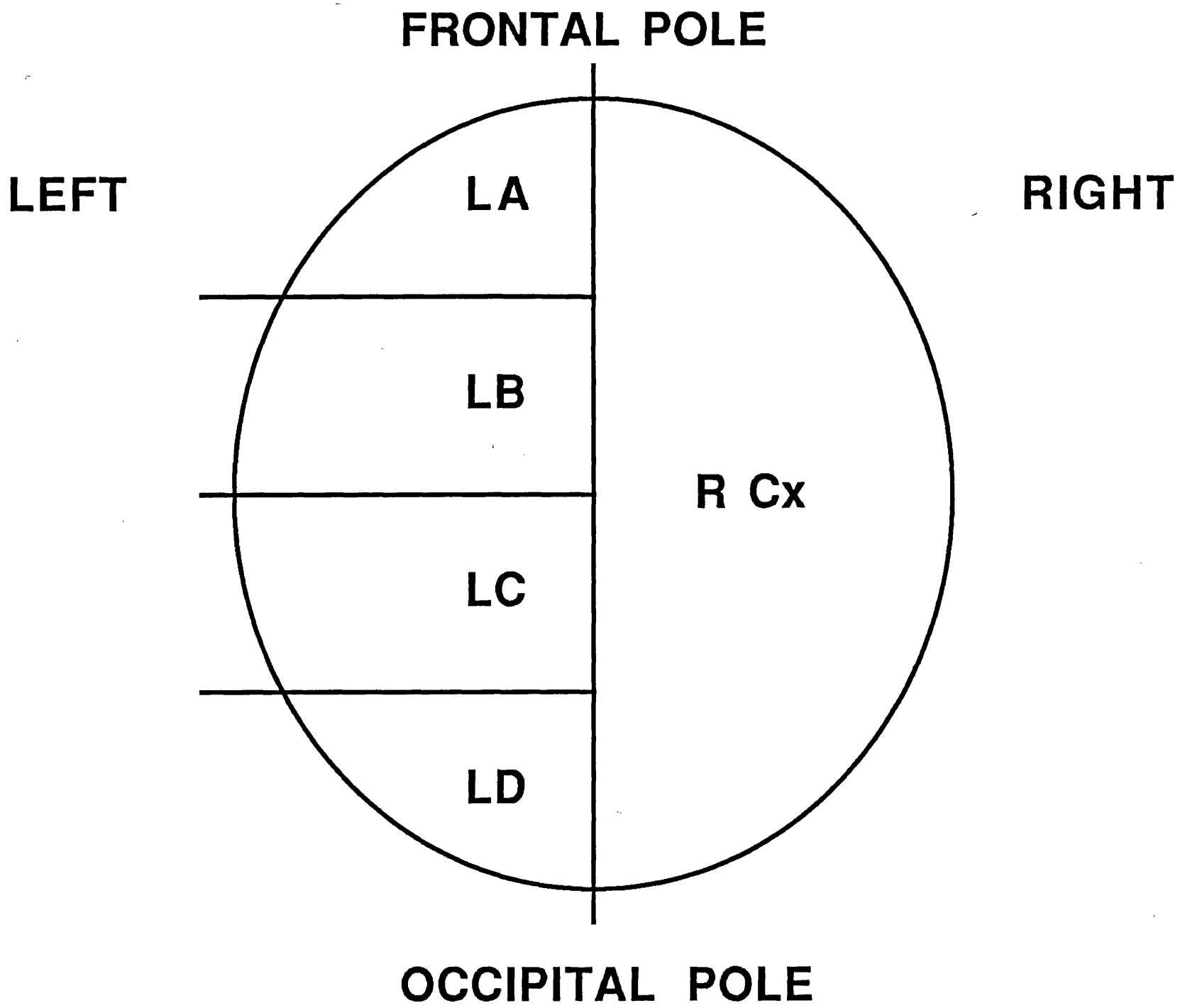


tubing and 3-way tap was inserted into an antecubital vein. The radioactivity (700MBq sodium pertechnetate, ^{99}mTc) in volume of less than 1.5 ml was introduced through the side port of the tap without spilling into the vein. A 1cm diameter scintillation detector fitted with a simple cylindrical collimator was placed 2cm distal to the sterno-manubrial joint, directed dorsally at right angles to the sternum to view the aortic arch. The computer was started and the injection flushed in by the rapid hand injection of 10ml of normal saline. Camera images (eg Figure 3.3) and the probe time-activity curve (TAC; eg see Figure 3.2) were collected simultaneously at a rate of 3 frames per second for 60 seconds, using a 32x32 matrix (pixel area approximately 80mm^2). The first peak of the probe TAC (Figure 3.2), due to activity tranversing the superior vena cava (SVC), was fitted by a gamma variate function (Thomson et al 1964), the start and end points for fitting being set manually on the computer display and the fitted curve subtracted from the raw data to reveal the aortic curve, which was then fitted similarly to exclude recirculation and obtain the MTT from the arm to the aortic arch (MTTs-a).

The frames corresponding to the first pass through the head were identified on a TAC of total count rate. To improve the statistical quality, this part of the study was summed to produce a 20 frame condensed view of the first pass and smoothed. In normal adults the usual frame length was 0.9 seconds, but the optimum probably lies between 0.3 and 2.1 seconds. This produces a 3-dimensional matrix of 32x32 x approximately 20 pixels. Pixels lying outside an elliptical region of interest that defined the outline of the cortical area were set to zero, the matrix rotated and each pixel TAC dead-time corrected. Data points between the first rising and seventieth falling fractile were identified and MTT from the arm to internal jugular vein (MTT s-j) calculated for each pixel by the same method as described above for MTTs-a. The MTT through the cerebral circulation (net MTT) at each pixel can be calculated from the difference between the MTT s-j values and MTT s-a. The

Figure 3.4 Net MTT regions of interest

Diagram showing the position of the regions of interest in relation to the cortical image. On the left side, the regions LA, LB, LC and LD are shown, and on the right side, the hemi-cortical region, R Cx.


FRONTAL POLE
LEFT
RIGHT
LA
LB
R Cx
LC
LD
OCCIPITAL POLE

matrix of net MTTs can be used to construct a cerebral transit time image (eg Figures 4.2, 4.3, 4.4). A second matrix can be produced of the reciprocal of net MTT at each pixel (the cerebral vascular reserve (CVR) or so-called 'fractional flow').

The data from this image can be examined as net MTT for right (RCx) and left (LCx) sides of the midline, or by subdividing each side of the image into four equal regions of interest, A, B, C, and D (see Figure 3.4). It is important to emphasise again however, that a planar imaging system, like the MTT method, does not allow tomographic or anatomical identification of regions. In the study described in Chapter 4, regions of interest A, B, and C are emphasised, as MTT values for posterior region D are likely to be contaminated by neck blood flow. All patients and controls were given a unique code number, and calculation of cerebral net MTT values and regional analysis of the image, was carried out blind to the identity or diagnosis of the subject.

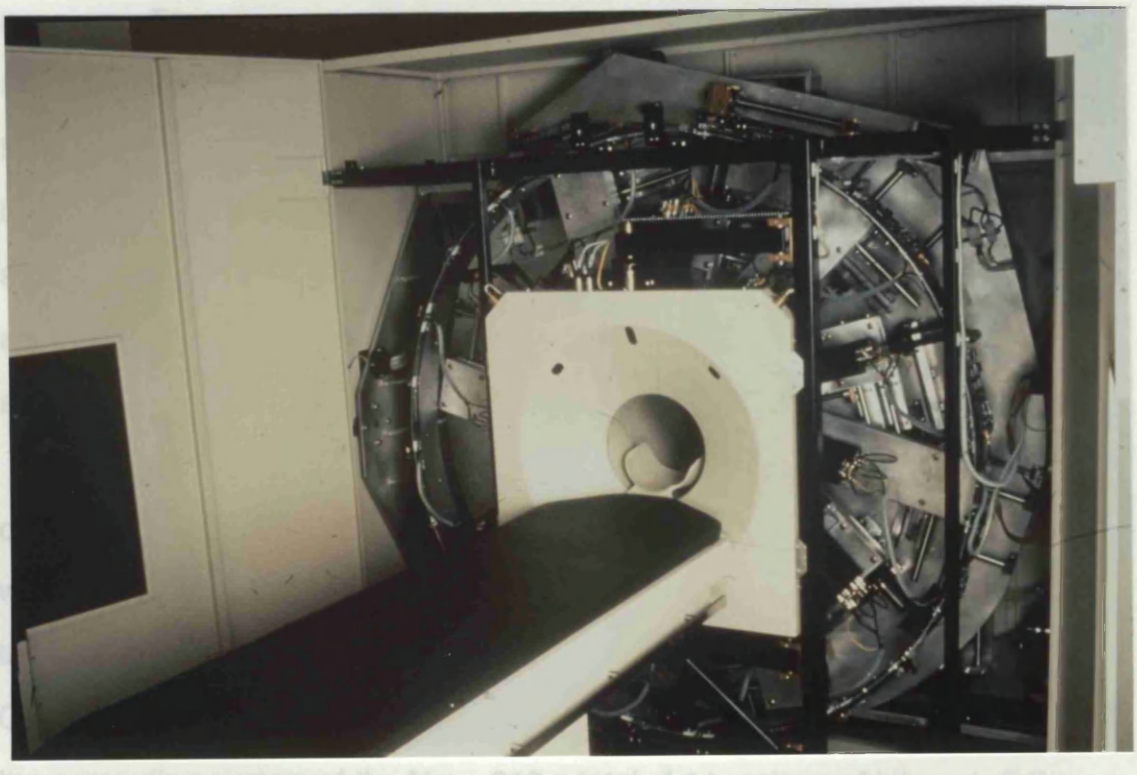
3.6 Single photon emission computed tomography measurements

Single photon emission computed tomography (SPECT) using the flow tracer ^{99m}Tc-hexamethyl propyleneamine oxime (^{99m}Tc-HMPAO) was carried out at the Institute of Neurological Sciences, Southern General Hospital, Glasgow . HMPAO is well suited for SPECT because it shows high fractional uptake into brain with a distribution that reflects regional cerebral blood flow (Neirinckx et al 1987). As it is retained in the brain for many hours without apparent redistribution, there is adequate time for SPECT to be carried out. This has been discussed in section 2.2.5.1 .

HMPAO (Exametazime or 'Ceretec', Amersham International plc, UK Product Licence No. PL0221/0-091) was supplied as a freeze-dried formulation and reconstituted with sodium pertechnetate solution. Within 15

Figure 3.5 The Novo 810 dedicated tomographic brain imager

Figure 3.6 The Novo 810 SPECT imager with covers removed to show the Harvard multi-detector scanning system with 12 sets of scintillation detectors and focussing collimators



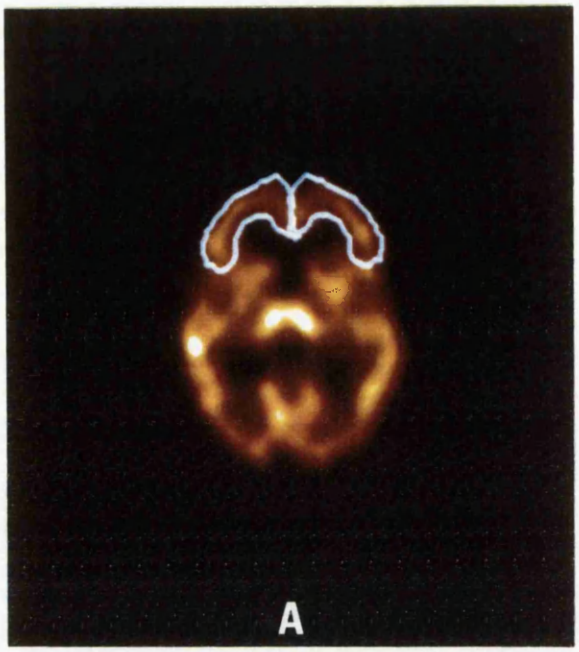
minutes of preparation, subjects were administered 2.5ml (500 MBq) of ^{99m}Tc-HMPAO complex as a single intravenous bolus via a right antecubital vein. The injection was administered in a quiet room with subjects resting, eyes closed and ears unplugged. Imaging was carried out within 30-90 minutes using the Novo 810 (Strichman Medical Equipment Inc.), a dedicated brain SPECT system (Figure 3.5). The Novo 810 imager uses the Harvard multi-detector scanning system with 12 sets of scintillation detectors, each with focussing collimator, arranged at 30 degrees around the field of view (Figure 3.6). Each detector scans both tangentially and radially to the field of view resulting in relatively uniform spatial resolution, sensitivity and slice thickness throughout each slice.

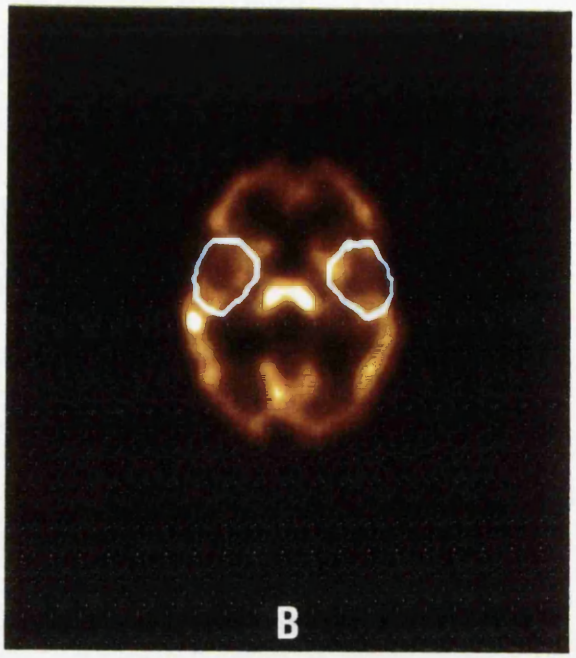
For imaging, subjects were positioned supine on a comfortable tomography table and a light source used to align the scanner parallel to the orbitocanthal meatal (OM). Head support was obtained using polystyrene wedges to ensure that the head remained in the standard configuration during imaging. Transverse sectional images at +30, +40, +50, +60 and +70mm superior to the OM line were constructed. A brain atlas was used to assign the five transverse slice images to particular anatomical levels and to define brain regions. SPECT images of severely demented patients are less anatomically distinct than those of controls, and care must be taken in assigning slice levels and defining anatomical regions. Definition of ROI and/or slice location is a potential error source.

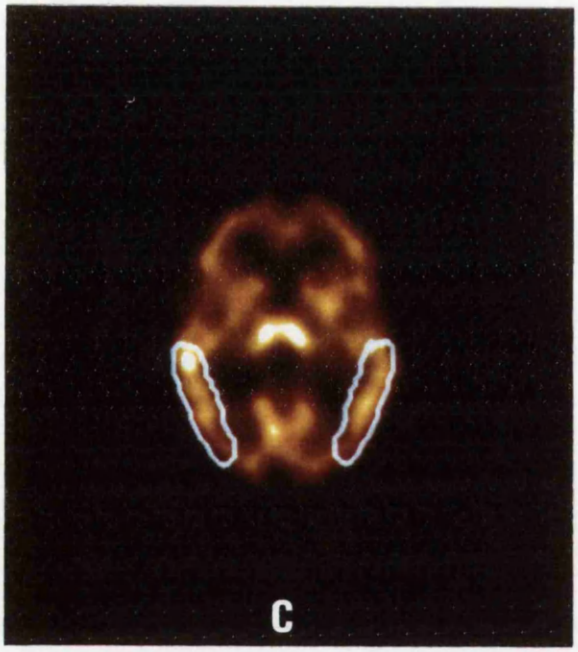
Two slices were used to define particular regions of interest. The lower of the two slices (the 'low' slice), usually at +30 to 40mm above the OM line was identified by the presence of putamen, thalamus and occipital cortex and the absence of cerebellum. The second (or 'high') slice of interest (usually at OM +70mm), was positioned immediately above the corpus callosum. Using the computing system of the Novo 810 a total of 14 regions of interest (ROI), were defined by drawing on the image with a light pen. In this way 5 pairs of

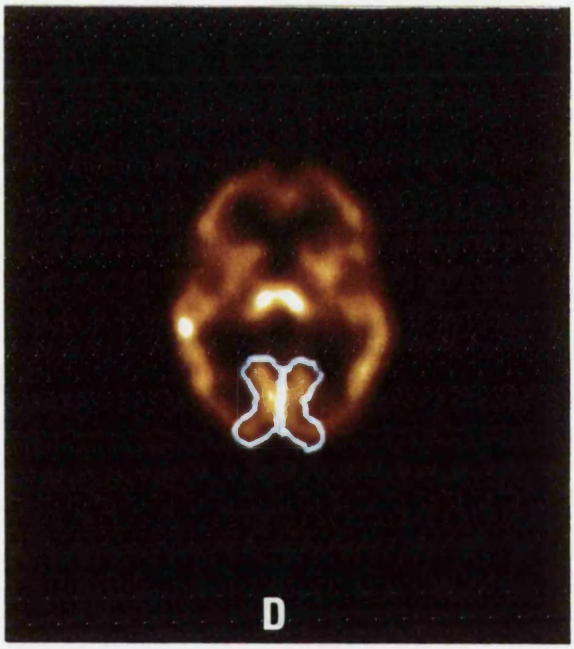
Figure 3.7 SPECT regions of interest

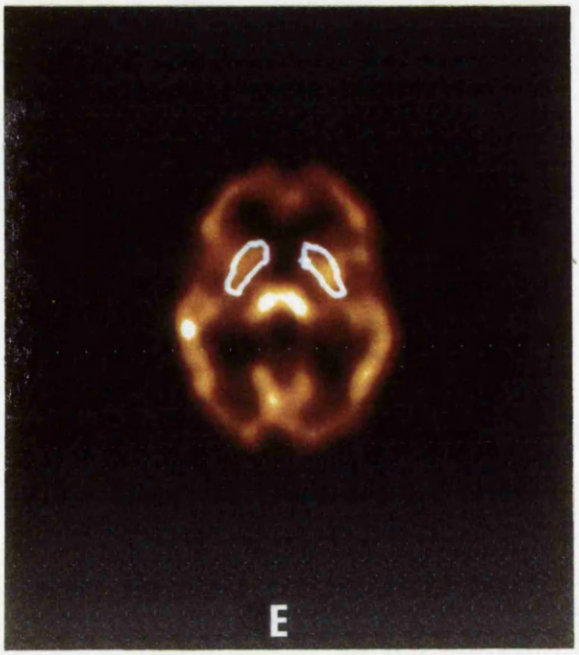
Transverse cross-sectional SPECT images at +30mm superior to the orbito-meatal (OM) line ('low' slices, A to E) showing how the regions of interest were defined on left and right sides for quantification, showing frontal (A), temporal (B), posterior temporal (C), occipital (D), and basal ganglia (E) regions. A transverse sectional image in the same plane at OM +70mm ('high' slice, F) is also illustrated and shows high frontal and parietal regions of interest on left and right sides.

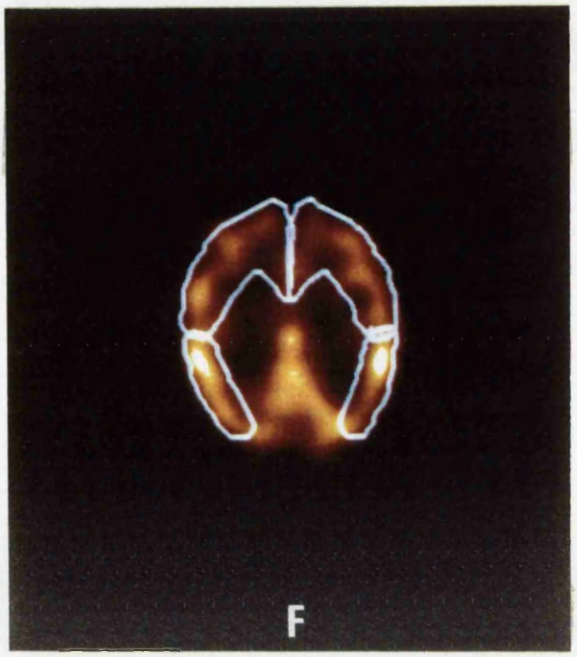

A


B


C


D


E


F

left and right ROIs in the low slice, and 2 pairs of left and right ROIs in the high slice were demarcated. Corresponding right and left regions were symmetrical. In the low slice the following symmetrical regions of interest (ROI) were defined (Figure 3.7): right and left frontal (RF, LF), right and left temporal (RT, LT), right and left posterior temporal (RPT, LPT), right and left occipital (RO, LO), and right and left basal ganglia (RBG, LBG). From the high slice at 70mm above the OM line symmetrical right and left high frontal (RHF, LHF), and right and left parietal (RP, LP) ROIs were also defined (Figure 3.7).

Neuroimages can show substantial variability in global metabolic activity, reflecting unknown combinations of various measurement problems including ROI identification, head movement, head positioning and reconstruction algorithm. As a crude adjustment for these effects some for of normalisation is sometimes useful. Normalisation usually takes the form of the division of the regional values by a measure of global activity or by the activity in a specific region. It is important that interpretation and statistical analyses should be in terms of the 'new' normalised variables and not in terms of the unadjusted measures. However the normalisation process itself can induce spurious patterns in the data and this must also be borne in mind (Ford, 1986: Ford et al 1991).

Right and left occipital regions were used as reference areas (see below) as these regions are relatively spared in Alzheimer's disease (Rogers & Morrison, 1985; Pearson et al 1985). In other studies the cerebellum has been used as a reference region (eg Burns et al 1989), but this was not used in the present work for two main reasons. Firstly, with a dedicated brain section imager, such as the Novo 810, a single slice image can be obtained in only 3 minutes. This facility for rapid data collection helps ensure a high level of patient cooperation. As measurement of uptake in cerebellum would require a different set of slices, and consequently longer periods of imaging time, with perhaps reduced patient cooperation, it appeared unjustifiable.

The second reason for not adopting the cerebellum for normalisation is that cerebellar blood flow may be affected by crossed cerebellar diaschisis resulting from damage to the cortico-ponto-cerebellar system (Baron et al 1980).

The analysis of sectional images to define ROIs was undertaken according to standardised criteria by the same medical physics technician who was blind to the diagnostic category. Raw data for each ROI was background corrected, typically by subtracting 15%. For each ROI and transverse sectional image, the area (in pixels) and background corrected uptake of ^{99m}Tc-HMPAO (in counts per pixel) were determined, and from these, mean uptake for each ROI relative to the occipital region was calculated as follows:

RELATIVE MEAN UPTAKE FOR ROI =

MEAN UPTAKE OF ^{99m}Tc-HMPAO IN ROI / MEAN UPTAKE OF ^{99m}Tc-HMPAO IN OCCIPITAL REGIONS.

For each region this measure of relative rCBF was calculated and used in the analysis of results.

3.7 Statistical analysis

The main data analysis was carried out using the Statistical Package for the Social Sciences (SPSS-X) available via the EMAS (Edinburgh Multi Access System) mainframe computer of the Edinburgh University Computing Service. Smaller data analyses were carried out using the Stat View 512+ package for the Apple Macintosh computer.

In order to avoid assumptions about the distribution of MTT, blood flow and neuropsychological measures in the population being sampled, non-

parametric tests were preferred to parametric tests. Although, in general, non-parametric tests are not as powerful as parametric tests, they are often more appropriate and valid than parametric equivalents when sample sizes are not large. Where it was thought statistically appropriate parametric tests were occasionally used, as in chapter 6. The statistical methods used are described more fully by Cohen and Holliday, 1982.

In Chapters 4 and 5 the median was used as a measure of central tendency and the range as a measure of variability. One point should be clarified; the median values in Chapter 4 for each group were calculated from the raw data values of net *mean* transit times for each subject.

Kruskal-Wallis one-way analysis of variance (K-W ANOVA) was used to test the differences in blood flow parameters (either MTT or relative mean uptake of ^{99m}Tc-HMPAO) and the differences in psychological parameters between the three subject groups, and post hoc Mann-Whitney U tests were used to locate significant differences between pairs of groups. The relationship between blood flow parameters and neuropsychological scores was examined using Spearman's rank correlation coefficients with an adjusted significance level of $p < 0.01$ to take account of multiple comparisons.

In the physostigmine study described in section 6.1 and the reproducibility studies described in section 6.2, measures of relative rCBF were approximately normally distributed, and therefore Student's paired t-test (two-tailed) was used to test the significance of differences between pairs of values.

CHAPTER 4

CEREBRAL VASCULAR TRANSIT TIME IN ALZHEIMER'S DISEASE AND KORSAKOFF'S PSYCHOSIS AND ITS RELATION TO COGNITIVE FUNCTION

4.1 Introduction

The rationale for interest in cerebral haemodynamics in dementia is that under resting circumstances CBF and cerebral metabolism are coupled (section 2.1). Gustafson and Risberg (1974) used the ^{133} Xenon intra-carotid injection technique to measure rCBF in patients with pre-senile dementia, and concluded that there was an approximate relationship between cognitive impairment and decreased perfusion, especially in grey matter regions. Abnormalities of regional perfusion were associated with specific cognitive functions in a way that was also found after focal brain lesions in hemispheric perfusion. However, the intra-carotid injection method was of limited clinical applicability, especially in routine diagnosis and follow-up of patients suffering from dementia. Intravenous (Lassen & Ingvar, 1972) or inhalational techniques (Mallett & Veall, 1965) for administration of inert gaseous tracers were more acceptable and have been used to investigate dementia (Ingvar, 1982). However, these approaches still require the head to be kept immobile for a considerable period of time which, in demented patients or others with psychiatric illness, can present difficulties. More recently PET has provided regional 3-dimensional CBF measurements but it is not widely available in the United Kingdom.

For routine clinical studies there is a need for simpler methods of evaluating cerebral haemodynamics in dementia and other brain diseases. One such approach is based on the measurement of vascular transit time in cortex. Transit time methods were originally developed by cardiologists to measure cardiac output (Thomson et al 1964) but can, in principle, be applied to any determination of tracer dynamics (Davenport, 1983; Merrick, 1984). Recent work using PET has suggested that a useful indicator of the extent to which CBF is maintained by vasodilatation is the ratio of CBF to cerebral blood

volume. This is the reciprocal of mean cerebral transit time (Gibbs et al 1984). The theoretical basis and practical details of the use of transit time methods to study cerebral vascular reserves (or the ratio of rCBF to volume) has been described by Merrick et al 1991. Naylor et al (1991) using the same methodology as that employed in the present study, have shown that within the range of end-tidal $C0_2$ which could be achieved in conscious subjects breathing spontaneously, hemisperic net MTT showed a linear relationship with end-tidal $C0_2$. Furthermore at a standardised end-tidal $C0_2$ good reproducibility of hemispheric net MTT was demonstrated (coefficient of variation 5.7%). In the following study cerebral haemodynamics in demented patients were investigated using the net MTT method. As net MTT and end-tidal $C0_2$ values are inversely related (Naylor et al 1991), interpretation of net MTT must be made in the context of the dynamic equilibrium between cerebral blood flow and blood volume.

4.2 Aims

The aims of the present study were as follows:

1) To measure cortical mean transit times of pertechnetate in three distinct clinical groups: pre-senile AD patients who have a predominantly cortical dementia, KOR patients where the significant pathology is reputed to be sub-cortical and healthy controls.

2) To consider whether transit times provide clinically useful information in these disorders, where perfusion abnormalities are known to exist.

3) To investigate the relationship between transit time measurements and cognitive impairment in such patients.

TABLE 4.1

Description of subjects studied using transit time method

		Sex		AGE (Years)	
	n	M	F	Mean	Range
AD	17	3	14	59	52-66
KOR	9	6	3	62	58-73
CON	10	1	9	61	50-76

The numbers (n) of patients in the study are shown as totals and males (M) and females (F). Groups were matched for age, the means and ranges for which are also shown.

TABLE 4.2

Net transit time in regions of interest for patients and controls

	CONTROL		AD		KORSAKOFF'S		Kruskal-Wallis
	Median	Range	Median (% increase of control)	Range	Median (% increase of control)	Range	one-way ANOVA
Left Cx	4.6	3.1 - 6.1	6.9(+50)***	5.7 - 14.2	7.1(+54)*	4.4 - 11.3	< 0.0005
Right Cx	4.6	2.9 - 5.7	6.6(+43)***	5.3 - 14.0	6.9(+50)*	4.6 - 11.2	< 0.0005
Left A	4.2	2.6 - 6.5	6.2(+48)**	3.7 - 14.9	6.0(+43)*	4.1 - 12.0	< 0.005
Right A	4.2	2.2 - 6.0	6.5(+55)**	3.5 - 14.6	6.3(+50)*	3.6 - 10.7	< 0.005
Left B	4.0	2.4 - 5.0	5.8(+45)**	3.9 - 13.3	6.0(+50)*	3.9 - 10.4	< 0.001
Right B	4.1	2.3 - 5.1	5.5(+34)*	3.7 - 13.2	5.7(+40)*	3.5 - 9.3	< 0.005
Left C	4.4	2.8 - 5.9	6.8(+55)***	5.1 - 15.8	6.6(+50)*	4.6 - 10.9	< 0.0005
Right C	4.5	2.7 - 5.5	6.9(+53)***	5.7 - 14.6	6.0(+33)*	4.6 - 10.7	< 0.0005
Left D	5.4	3.3 - 7.8	8.7(+61)***	6.5 - 16.6	8.4(+56)*	4.9 - 12.1	< 0.0005
Right D	5.4	3.8 - 7.1	8.7(+61)***	6.8 - 17.5	8.6(+59)*	5.8 - 14.3	< 0.0005
IF	6.3	3.6 - 8.5	6.5(+3)	5.2 - 9.1	7.7(+22)	5.7 - 11.4	NS

Results expressed as median and range (seconds).

IF is Input Function or the MTT from arm to aortic arch (see text).

All post-hoc comparisons were made between patient groups and controls using Mann-Whitney U tests;

*$p < 0.005$; **$p < 0.001$; ***$p < 0.001$.

Figure 4.1

The net mean transit times for left and right sides of the cortical image, and for regions of interest A, B, and C on left and right sides in all subjects studied: controls (●), Alzheimer patients(Δ), and Korsakoff patients(■).

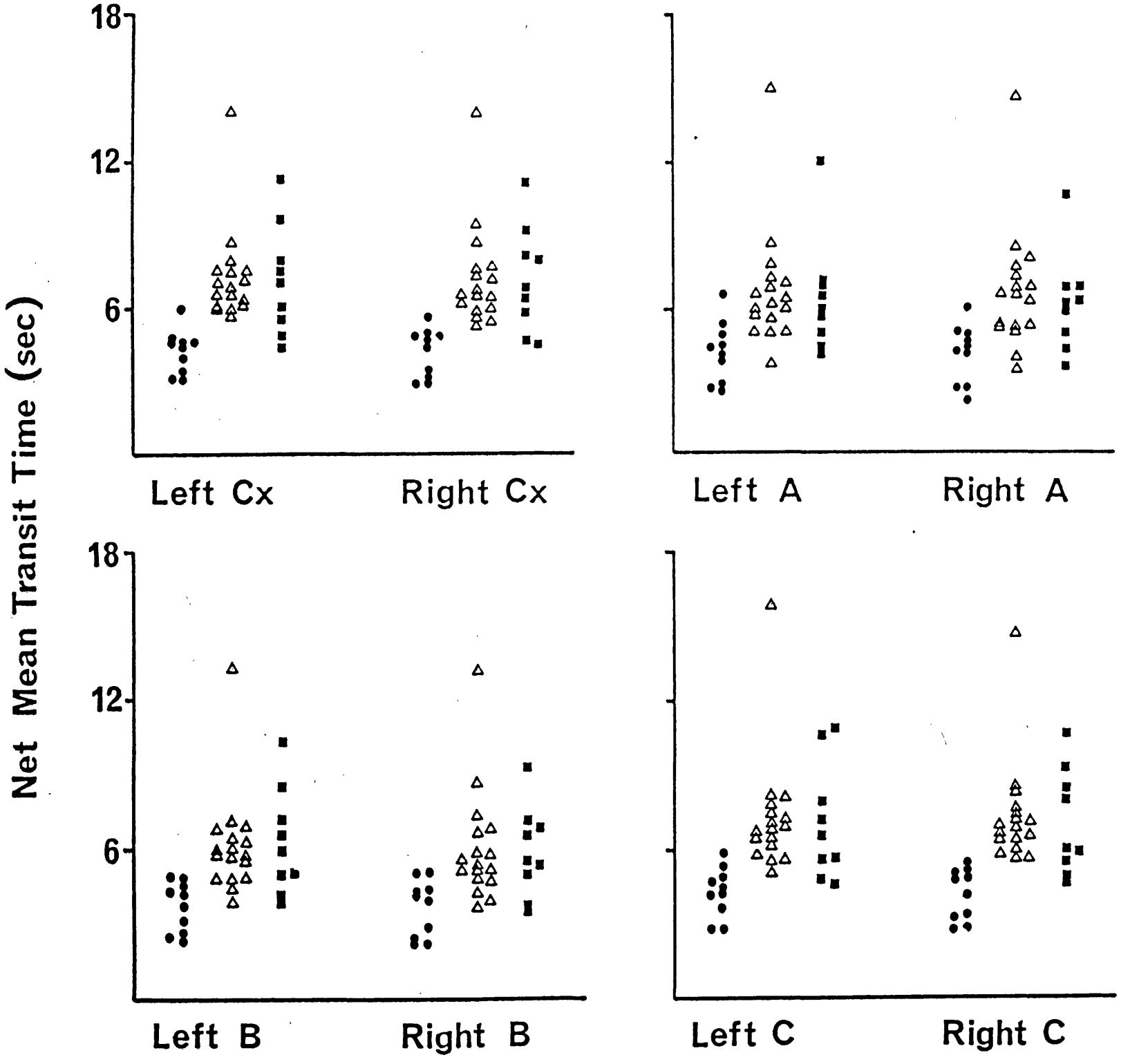
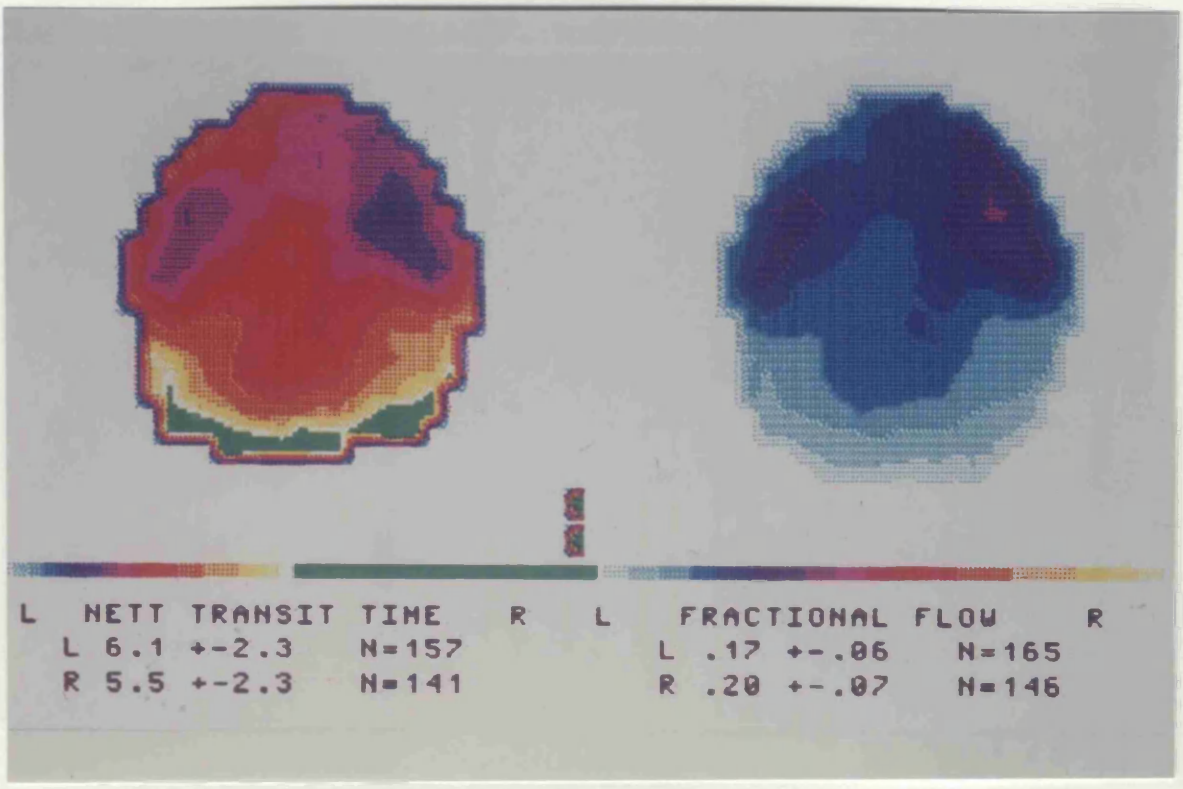

Net Mean Transit Time (sec)
18
12
6
Left Cx
Right Cx
Left A
Right A
Left B
Right B
Left C
Right C

Figure 4.2 Net MTT: Alzheimer's disease

Representative planar images of net MTT and fractional turnover rate (1/net MTT or cerebral vascular reserve) in cortex for two patients with presenile Alzheimer's disease. Values given are mean +/- standard deviation for left and right sides of the image.


L NETT TRANSIT TIME R L FRACTIONAL FLOW R
L 6.1 +-2.3 N=157
R 5.5 +-2.3 N=141
L .17 +-.06 N=165
R .20 +-.07 N=146

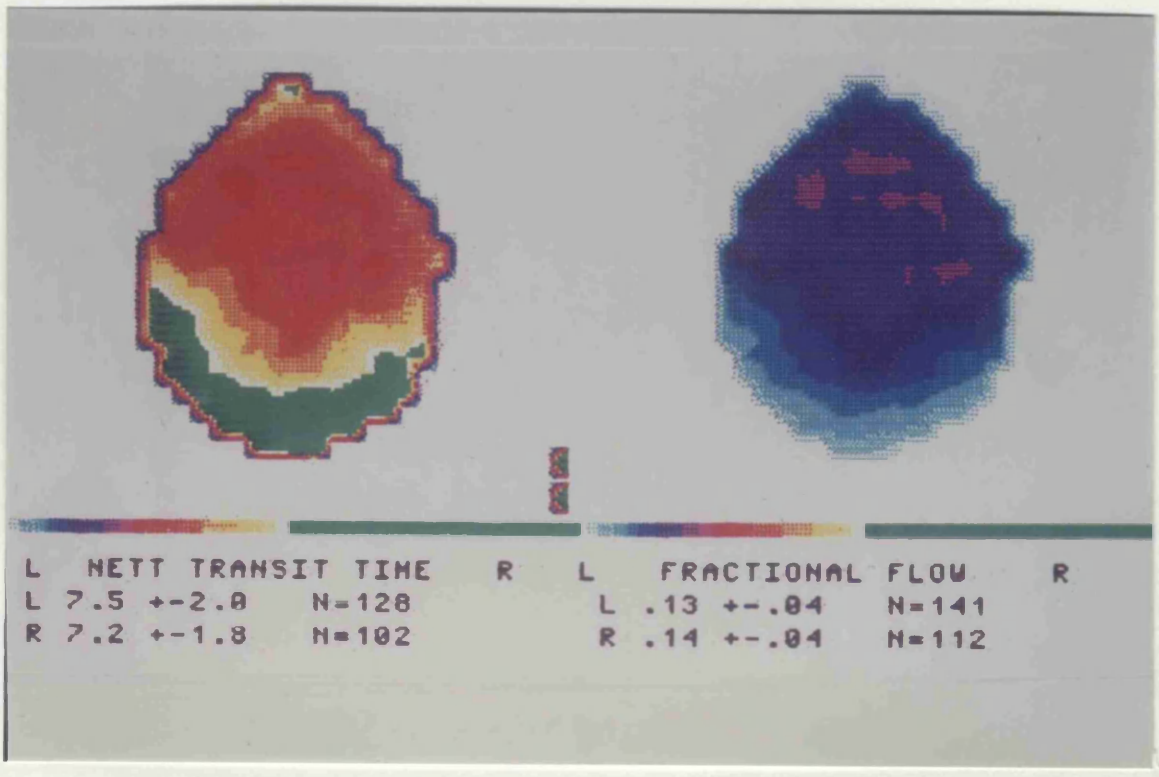
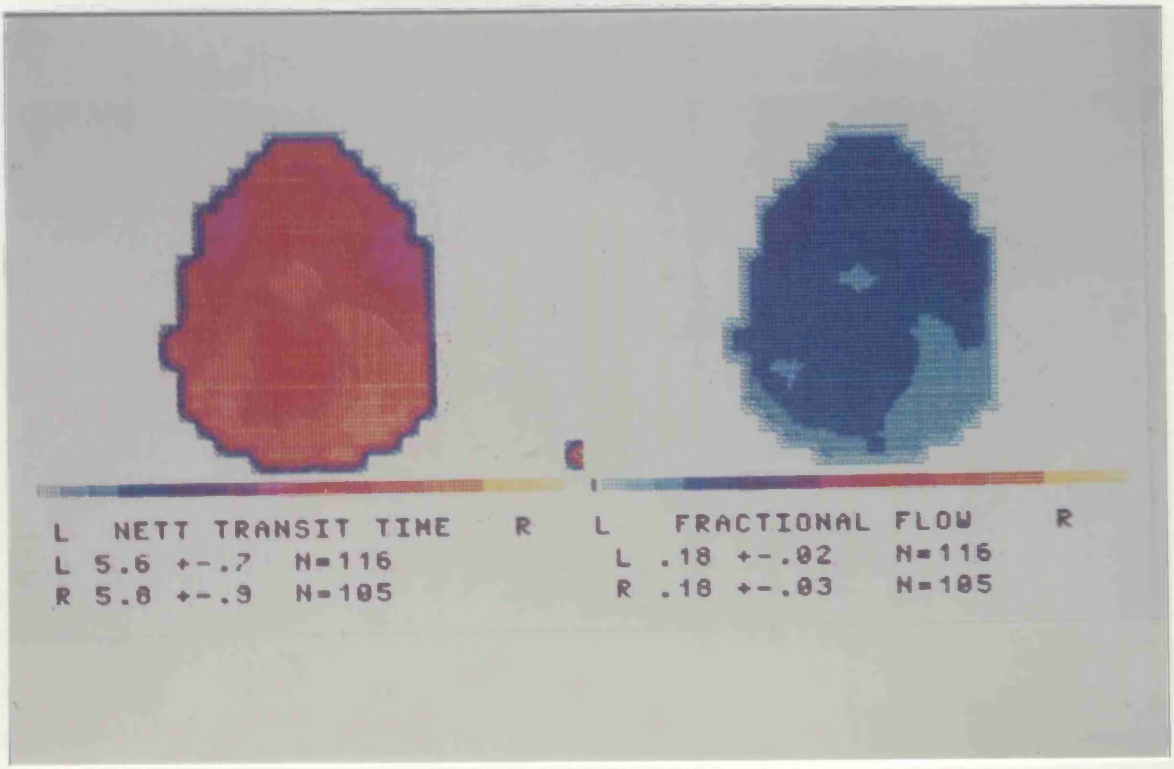

L NETT TRANSIT TIME R L FRACTIONAL FLOW R
L 7.5 +-2.8 N=128
R 7.2 +-1.8 N=102
L .13 +-.04 N=141
R .14 +-.04 N=112

Figure 4.3 Net MTT: Korsakoff's psychosis

Representative planar images of net MTT and fractional turnover rate (1/net MTT or cerebral vascular reserve) in cortex for two patients with Korsakoff's psychosis. Values given are mean +/- standard deviation for left and right sides of the image.


L NETT TRANSIT TIME R
L 5.6 +-.7 N=116
R 5.8 +-.9 N=105
L FRACTIONAL FLOW R
L .18 +-.02 N=116
R .18 +-.03 N=105

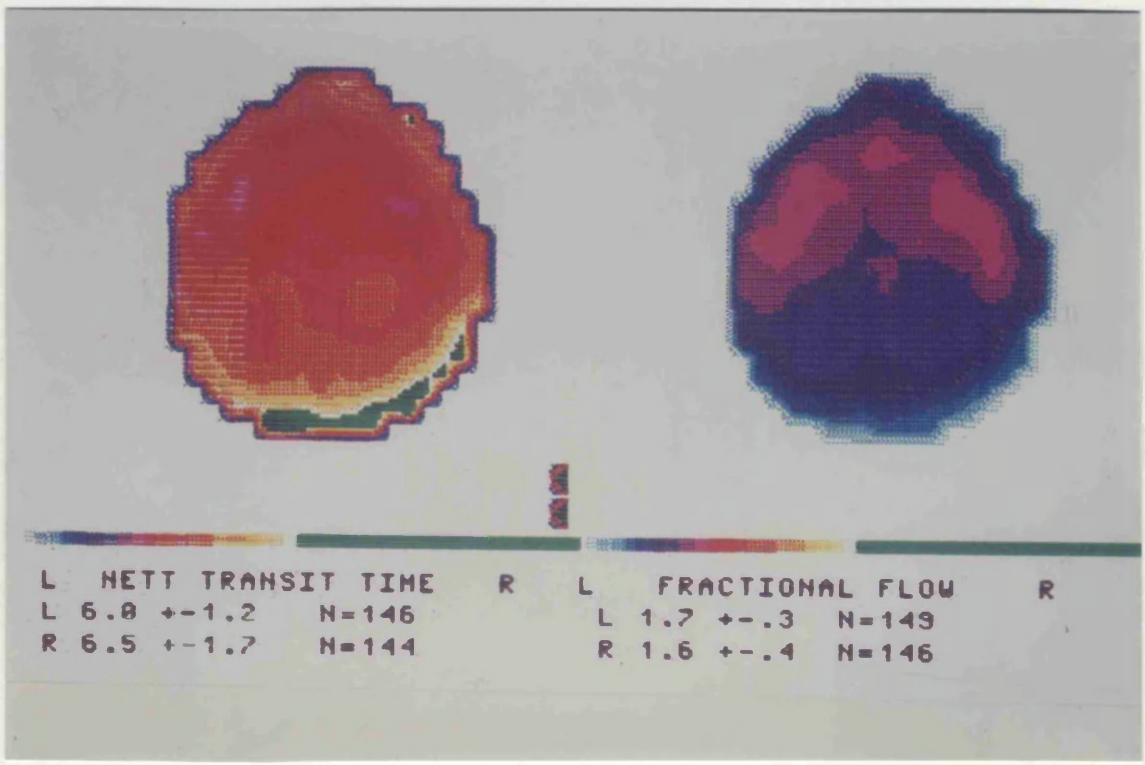
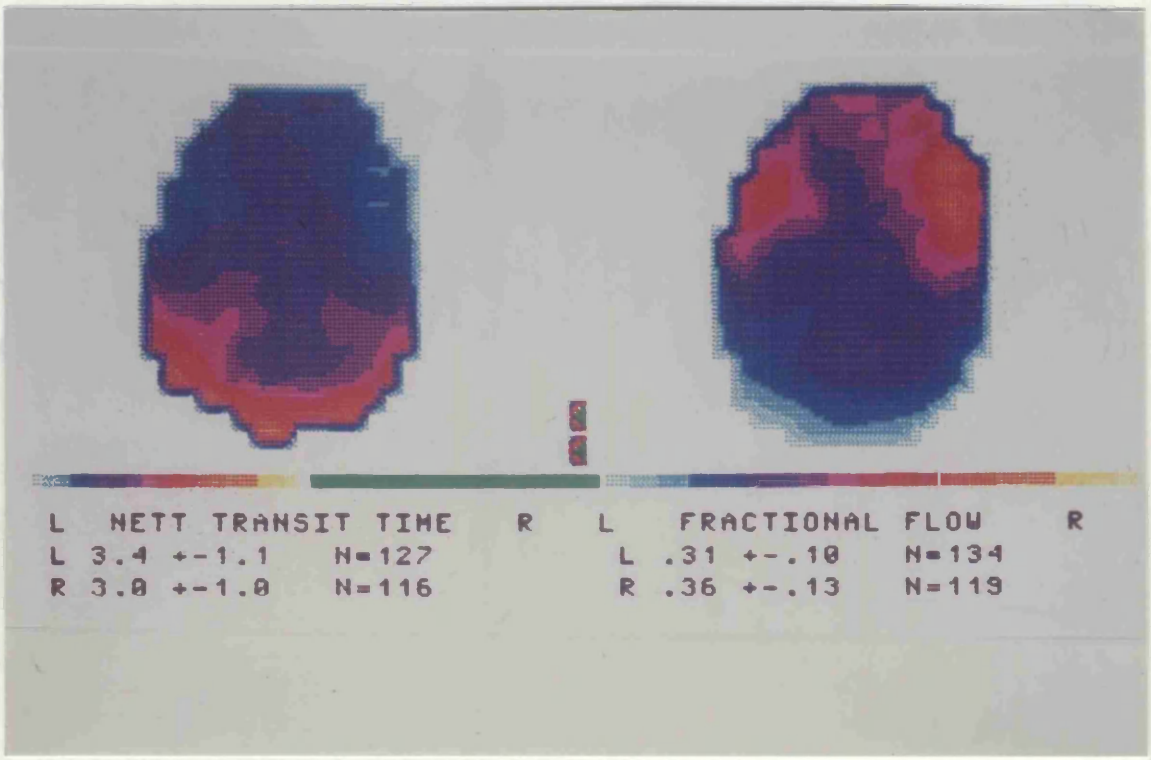
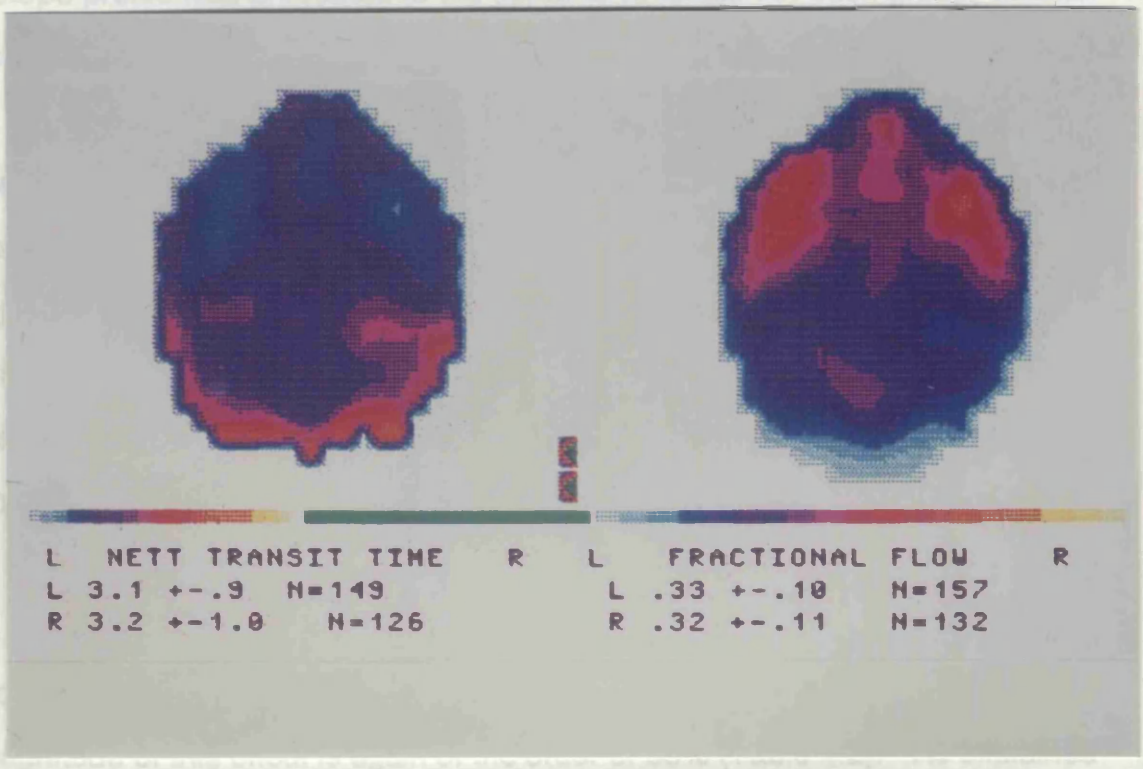

L NETT TRANSIT TIME R
L 6.0 +-1.2 N=146
R 6.5 +-1.7 N=144
L FRACTIONAL FLOW R
L 1.7 +-.3 N=149
R 1.6 +-.4 N=146

Figure 4.4 Net MTT: healthy control subjects

Representative planar images of net MTT and fractional turnover rate (1/net MTT or cerebral vascular reserve) in cortex for two healthy control subjects. Values given are mean +/- standard deviation for left and right sides of the image.


L NETT TRANSIT TIME R L FRACTIONAL FLOW R
L 3.4 +-1.1 N=127
R 3.0 +-1.0 N=116
L .31 +-.10 N=134
R .36 +-.13 N=119


L NETT TRANSIT TIME R L FRACTIONAL FLOW R
L 3.1 +-.9 N=149
R 3.2 +-1.0 N=126
L .33 +-.10 N=157
R .32 +-.11 N=132

4.3 Methods

Seventeen patients with probable pre-senile AD, nine patients with KOR and ten healthy controls were studied. All fulfilled the selection criteria outlined in section 3.2 and the study population is described in Table 4.1. The CAMDEX schedule was used to assess all subjects (see section 3.4) and mean vascular transit time (MTT) was measured at the Department of Nuclear Medicine, Western General Hospital, Edinburgh as described in section 3.5.

4.4 Results

4.4.1 Net mean transit times

The mean transit time of the input bolus from the arm to aortic arch (MTTs-a Input or Function (IF) of the radioactivity, which is thought to be principally a function of cardiac output, was similar in all three groups (Table 4.2). All patients and controls had comparable cardiac function; thus a similar isotope profile was presented to the cerebral circulation in each group, at least as measured at the level of the aorta where the input function was determined.

Values for net mean transit times (net MTT) for each subject in each of the three groups over each cerebral hemisphere (left and right cortex) is shown in figure 4.1 . Both AD and KOR patients showed net MTT in left and right cortex that were prolonged by about 50% (Table 4.2). Thus, if the assumption that transit time and flow are inversely related (section 2.2.4), is justified, then flow in left and right cortex in the patient groups appears to be appreciably reduced compared to normal controls (Table 4.2).

Individual values of net MTT in the individual cortical regions of interest A, B, and C, showed symmetrical prolongation of net MTT on left and right sides in AD and KOR groups compared to normal controls(Figure 4.1). The magnitude of this effect is again of the order of 50% (Table 4.2). As explained

in section 3.5, the distribution of net MTT (or 1/MTT, so-called fractional turnover rate or cerebral vascular reserve) for all cortical pixels, can be used to construct a cortical image. Representative examples of cortical images for AD, KOR and an age-matched healthy control are shown in Figures 4.2, 4.3, and 4.4 respectively.

4.4.2 Positive predictive value of net MTT measurements

The ideal clinical test is one in which there is no overlap in the range of results among subjects with and without the disease in question. As can be seen in figure 4.1 of net MTT measurements for individual patients, there is some overlap between healthy normal subjects and those with AD or KOR. In determining the clinical utility of a test, it is customary to define a 'normal range' as two standard deviations from the mean value of the normal group (Griner et al 1981). Such a range encompasses approximately 95% of the results of subjects without the disease.

Using this method cut-off points were calculated, above which net MTT values were defined as abnormal. Table 4.6 shows the upper cut-off point of normal for each ROI and the number of AD and KOR patients with MTT measurements above this 'normal' cut-off. Positive predictive values (Griner et al 1981), namely the probability that disease is present (either AD or KOR) when the test result is positive (net MTT above the cut-off), are shown in Table 4.6 for each ROI of the two patient groups.

4.4.3 CAMCOG neuropsychological scores and net MTT

Orientation and total memory scores were impaired in both AD and KOR patients compared with control subjects (Table 4.3). Language function was also reduced in AD patients compared with controls; KOR patients were less impaired. Scores for praxis were clearly reduced in AD patients but not

TABLE 4.3

Neuropsychological scores for patients and controls studied using transit time method

	CONTROL n = 10	AD n = 17	KOR n = 9	KRUSKAL-WALLIS one-way ANOVA
ORIENTATION	10 (10)	4 (0-9)**	5 (1-9)**	$p < 0.0005$
LANGUAGE	29 (28-30)	20 (0-27)**	26 (23-29)*	$p < 0.0005$
MEMORY	24 (23-26)	7 (0-15)**	12 (4-24)**	$p < 0.0005$
ATTENTION	7 (5-7)	1 (0-3)**	5 (3-7)	$p < 0.0005$
PRAXIS	12 (12)	7 (0-11)**	11 (7-12)	$P < 0.0005$
CAMTOTAL	102 (100-105)	51 (0-82)**	75 (56-99)	$p < 0.0005$
MMSE	30 (29-30)	12 (0-24)**	20 (11-29)	$p < 0.0005$

Results expressed as median and range. All post-hoc comparisons were made between patient groups and control subjects using Mann-Whitney U test; *$p < 0.005$; **$p < 0.001$.

TABLE 4.4

Relationship between net mean transit time and CAMCOG in Alzheimer patients

	LCx	RCx	LA	RA	LB	RB	LC	RC	LD	RD	IF
CAMTOTAL	-0.33	-0.60	-0.33	-0.57	-0.11	-0.46	-0.32	-0.42	-0.47	-0.46	-0.01
ORIENTATION	-0.38	-0.68*	-0.48	-0.68*	-0.14	-0.57	-0.33	-0.58	-0.51	-0.56	-0.04
LANGUAGE	-0.53	-0.66	-0.63	-0.54	-0.43	-0.57	-0.45	-0.25	-0.35	-0.38	+0.08
MEMORY	-0.38	-0.58	-0.35	-0.41	-0.18	-0.35	-0.21	-0.23	-0.35	-0.48	+0.16
ATTENTION	+0.04	-0.18	-0.27	-0.26	-0.08	-0.18	-0.02	-0.16	-0.19	-0.10	+0.09
PRAXIS	-0.15	-0.36	-0.34	-0.38	-0.06	-0.28	-0.12	-0.22	-0.34	-0.30	-0.007

Spearman's rank correlation coefficients; *$p < 0.01$. IF is the input function or MTT from arm to aortic arch (see text).

TABLE 4.5

Relationship between net mean transit time and CAMCOG in Korsakoff patients

	LCx	RCx	LA	RA	LB	RB	LC	RC	LD	RD	IF
CAMTOTAL	+0.07	+0.07	+0.07	+0.17	+0.07	+0.07	+0.06	+0.02	+0.02	+0.07	-0.07
ORIENTATION	-0.44	-0.44	-0.44	-0.39	-0.44	-0.44	-0.47	-0.38	-0.50	-0.44	-0.90*
LANGUAGE	+0.10	-0.09	-0.10	-0.22	+0.09	-0.10	-0.08	+0.02	-0.01	+0.10	+0.02
MEMORY	-0.31	-0.31	-0.31	-0.15	-0.31	-0.31	-0.32	-0.32	-0.33	-0.31	+0.02
ATTENTION	+0.82*	+0.82*	+0.82*	+0.67	+0.82*	+0.82*	+0.84*	+0.78	+0.75	+0.82	+0.49
PRAXIS	+0.05	+0.05	+0.05	+0.17	+0.05	+0.05	-0.04	+0.05	+0.09	+0.05	+0.05

Spearman's rank correlation coefficients; *$p < 0.01$. IF is the input function or MTT from the arm to the aortic arch (see text).

TABLE 4.6

Positive predictive value of net MTT in regions of interest for AD and KOR patients

	Left Cx	Right Cx	Left A	Right A	Left B	Right B	Left C	Right C
Upper limit of normal net MTT value (seconds)	6.2	6.2	6.7	6.4	5.8	5.9	6.3	6.3
Number of AD patients above normal cut-off value	11	13	7	9	11	7	12	13
Positive predictive value for AD (%)	65	76	41	53	65	41	71	76
Number of KOR patients above normal cut-off value	5	6	3	3	5	4	5	6
Positive predictive value for KOR (%)	56	67	33	33	56	44	56	67

Total number of AD patients studied was 17 and KOR patients 9.
Positive predictive value (%) is number of patients with net MTT value above cut-off value divided by total number of patients.
Abbreviations explained in text.

in KOR patients (Table 4.3). Measures of attention were markedly reduced in AD, but much less impaired in the KOR group. Overall cognitive function, as measured by total CAMCOG score (CAMTOTAL), or MMSE was lowest in the AD group compared with controls, but also significantly reduced in the KOR patients (Table 4.3).

The correlation matrix of net MTT and cognitive function scores is shown for AD subjects in Table 4.4. Negative association at the $p < 0.01$ level was detected only between orientation scores and right frontal cortex, although there is a trend for low cognitive scores to be associated with prolonged net MTT. This relationship is most apparent between hemispheric net MTT (left and right cortex) and CAMTOTAL, orientation, language, and to a lesser extent, memory function. By comparison, no association is observed between cognitive function scores and the input function.

The association between net MTT and psychological measures in KOR patients is shown in Table 4.5. No association was detected in the KOR group between net MTT and CAMTOTAL, language and praxis. As in the AD group there was an apparent trend for memory and orientation subscores to show a negative association with net MTT values in both sides of cortex. None of these correlation coefficients however was statistically significant. A positive association between net MTT and attention was detected in both sides of cortex in KOR patients (Table 4.5)

4.5 Discussion

Intravenously injected radioactive tracers allowing external monitoring of the transit over the head have been used for some years to study cerebral circulation. Although it might appear that transit times give information about local blood flow, without an estimate of local blood volume, MTT values cannot

be interpreted physiologically. Thus the trend in this study for net MTTs to be prolonged in AD and KOR patient groups, compared with healthy controls, might reflect reduced cortical blood flow, but without estimates of blood volume, no such inference can be made. At any given flow rate, MTT will depend on the volume into which the tracer diffuses - the volume of distribution. Vascular MTTs therefore require a tracer that is confined to the vascular compartment such as labelled human serum albumin. In normal brain pertechnetate appears to remain almost wholly intravascular during the first pass (Bartolini et al 1983) and is therefore acceptable as a blood marker. However in pathological states, where the blood brain barrier is substantially disrupted, e.g. in major head trauma, pertechnetate tends to diffuse out of the vascular bed into a larger volume of distribution, resulting in MTT prolongation. Although there is evidence that the integrity of the blood brain barrier is damaged in AD (e.g. Selkoe 1989), it is unlikely that, during the short time interval that transit time is measured, pertechnetate would be extravasated to any significant extent. Although MTT cannot be used as an index of blood flow without information on blood volume, transit time may however provide an index of cerebral haemodynamics or 'brain function' in situations where volume of distribution varies much less than blood flow and as such could have clinical utility. The normal range of values for net MTT observed in this study are not significantly different from those reported with PET (Gibbs et al 1984; Powers et al 1984) obtained by separately measuring blood flow and volume or following intra-arterial injection (Nylin et al 1960). It has been shown that there is a linear relationship between net MTT and end-tidal $C0_2$ (Naylor et al 1991). In comparing different groups of subjects it has been assumed that end-tidal $C0_2$ values are not significantly different between the groups. It is also assumed that during the net MTT examination, end-tidal $C0_2$ remains constant as the examination time of 1 minute is relatively short.

There was a trend for transit times to be prolonged in both AD and KOR groups, and on a group basis, significant differences in net MTT were detected between these groups and healthy controls, but not between AD and KOR groups. However there is overlap of individual net MTT values for all three groups in all regions of interest (individual MTT values shown in Figure 4.1). In order to determine accurately the clinical utility of test data such as net MTT, both sensitivity (the true positive rate) and specificity (the true negative rate) are required. It is not possible to calculate sensitivity and specificity from the net MTT data in this study because in the study design only patients satisfying clinical criteria for AD or KOR were recruited. In order to calculate sensitivity and specificity a different epidemiological study design would be necessary, with measurements of net MTT being made in a group of patients with dementia, not carefully selected AD or KOR patient groups. The present design does however allow positive predictive values to be calculated - i.e. the likelihood of the disease being present when net MTT is abnormal (Griner et al 1981).

In the AD group the positive predictive values (PPV) varied between regions of interest, with lowest values in frontal regions (Left A and Right A) and highest in more posterior regions (Left C and Right C). This is consistent with neuropathological and imaging evidence (see section 2.2) that posterior cortical areas are more often affected in AD. The PPV of greater than 70% for net MTT in posterior regions of AD patients suggests that further study with larger numbers of subjects may be worthwhile in establishing the clinical utility of net MTT. One reason why PPV of the transit time method may not be higher is that the clinical criteria used to select AD cases are themselves imperfect. When similar clinical criteria for AD case selections were compared with neuropathological diagnosis, estimates of their PPV vary from 46% (Kellet et al 1988), to 84% (Burns et al 1990 a). This issue is further

complicated by differences between laboratories of pathological diagnostic criteria for AD (Wisniewski et al 1988).

As might be expected from examination of individual net MTT data points in figure 4.1, positive predictive values of net MTT for the KOR group are less satisfactory. This may partly reflect the small group size, but is also likely to be attributable to difficulties in clinically defining cases of KOR (discussed in section 3.2.2).

Neuropathological studies of patients with moderate to severe AD show temporal, parietal and frontal lobes affected by neurofibrillary tangles, plaques and neuronal degeneration (Pearson et al 1985). These lesions result in widespread impairment of cerebral metabolism with consequent focal and global cognitive deficits. In this study net MTT was prolonged in all cortical areas in AD patients. There was also a trend for cognitive scores to be inversely associated with net MTT, as would be expected if transit time reflected function. However in both AD and KOR groups the association of net MTT and cognitive scores was not marked and rarely reached significance. This lack of association casts doubt on the usefulness of net MTT as an index of brain function, as does the paradoxically significant association between increased net MTT and improved attention scores in KOR patients.

In the KOR group, transit times were prolonged compared with healthy controls, but similar to those of AD patients. The finding of prolonged net MTT in stable abstinent subjects is unexpected as KOR patients are generally believed to have a relatively localised macroscopic neuropathological lesion in the diencephalon or hippocampal formation and an associated discrete impairment of memory without necessarily more global cognitive impairment (Victor et al 1971; Blackburn & Tyrer 1985). This is consistent with the neuropsychological data from this study, which separated the KOR patients from the more globally impaired AD group, especially with respect to

language, attention, praxis and total score, and suggests that the KOR group were not globally demented. However there is evidence that the brain dysfunction in KOR is more extensive than former neuropathological and clinical findings have suggested, even in reasonably well defined KOR populations. Magnetic resonance imaging has shown that a significant proportion of KOR patients, who do not appear to have a generalised alcoholic dementia have high proton spin-lattice relaxation time (T1 values) for both frontal and parietal white and grey matter (Christie et al 1988). Hata et al (1987) have shown that KOR patients have widespread reduction in rCBF throughout cortical and subcortical structures. Using ^{133}Xenon contrast computed tomography they have demonstrated reduced rCBF in hippocampus, nucleus basalis of Meynert and frontal white matter, as well as throughout cortical grey matter. Similar abnormalities in KOR patients include reduced mean hemispheric grey matter CBF using ^{133}Xe inhalation (Rogers et al 1983), and reduced whole brain CBF, $CMRO_2$ and CMR glc (Kruger et al 1980) using the Kety-Schmidt technique.

Lishman (1986) has recently suggested a unifying hypothesis to account for the symptoms common to AD and KOR patients. He suggests that in Korsakoff's psychosis patients, alcohol exerts toxic effects on the basal forebrain nuclei, causing damage to cholinergic projections to cortex with resultant cognitive impairment. Such a pathological process might be expected to result in a diffuse and variable reduction in cortical metabolism and blood flow.

4.6 Conclusion

This study was undertaken because transit time methods appeared to offer a number of attractions for the investigation of cerebral haemodynamics in dementia. Although measurement of net MTT is rapid, non-invasive and

inexpensive, the present investigation illustrates a number of serious shortcomings with the method. Lack of definition of tracer volume of distribution results in difficulties in the interpretation of MTT prolongation. Without distribution volume information it is not possible to make any interpretation about the relationship of MTT to blood flow. This limitation could perhaps be accepted if net MTT provided some clinical utility. Despite the overlap in individual net MTT values in regions of interest for the three patient groups, calculation of positive predictive values suggest that in comparing AD and healthy controls, posterior cerebral regions may have some clinical utility. These results suggest that it would be worthwhile embarking on an assessment of diagnostic accuracy of net MTT in posterior cortex in order to determine the sensitivity and specificity of the method in separating AD patients from controls. In KOR patients positive predictive values were lower, but the prolongation of net MTT in cortical regions supports other evidence for cortical dysfunction in this condition previously considered subcortical. There was no clear association between net MTT and neuropsychological test scores, although there was a trend towards negative correlation in AD and KOR, as might be expected if net MTT provides an index of cortical function in dementia.

CHAPTER 5

STUDIES OF BASAL BRAIN FUNCTION USING SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY

5.1 Basal patterns of rCBF investigated by single photon emission computed tomography with ^{99m}Tc-HMPAO in presenile Alzheimer's Disease and Korsakoff's Psychosis

5.1.1 Introduction

Techniques for the tomographic reconstruction of signals from radioactive tracers in different brain regions may change our understanding of brain function. Positron emission tomography (PET) has attracted attention but in practice, it is operationally complex and a limited resource. Alternative tomographic imaging techniques such as SPECT may allow advances from PET to be translated into more general clinical use and may push studies of brain function into new clinical areas where access to PET is and may remain limited. Single photon emission computed tomography (SPECT) with administration of intravenous radioligands promises to be an important technique to complement PET in this way.

Initial applications of PET have centered on estimation of regional cerebral metabolism and cerebral blood flow. The results suggest that cerebral blood flow is, under most circumstances, tightly coupled to local neuronal demand for glucose (section 2.1). Interest in SPECT proceeds in the first instance from the wish to investigate patterns of regional blood flow in the neocortex as a potential measure of neuronal function.

Radionuclides based on ^{99m}Tc are optimal for clinical SPECT imaging because the radio-isotope is inexpensive and has favourable properties for clinical use such as stability and relatively pure emission of 140KeV γ-radiation. The recent introduction of hexamethyl propyleneamine oxime (Exametazime or d,1 HMPAO, Amersham Intl. plc) thus offers considerable potential for imaging of brain perfusion patterns using SPECT. It complexes readily with ^{99m}Tc and its distribution following intravenous injection conforms

closely to that of an ideal 'first pass', high clearance, marker of brain blood flow (Neirinckx et al 1987; Costa et al 1986; Sharp et al 1986b).

The **aims** of this study were to use ^{99m}Tc-HMPAO SPECT to investigate the pattern of regional cerebral blood flow (rCBF) in patients with probable presenile Alzheimer's disease and Korsakoff's psychosis and to determine its relation to neuropsychological function in such patients. The central questions were:

(a) What is the pattern of perfusion deficits using SPECT in patients with presenile Alzheimer's disease and Korsakoff psychosis?

(b) Is there evidence of cortical dysfunction using SPECT in Korsakoff patients?

(c) Can we demonstrate meaningful relationships between rCBF patterns as measured by SPECT, and neuropsychological performance?

5.1.2 Methods

5.1.2.1 Patients and control subjects

Twelve patients with pre-senile Alzheimer's disease (AD) were studied and are described in Table 5.1. All met the agreed consensus criteria for probable Alzheimer's disease described in section 3.2.1.

Ten patients with Alcoholic Korsakoff Syndrome (KOR) were also studied and are described in Table 5.1. All KOR patients were resident in a psychiatric hospital ward or supervised hostel accommodation and all met the operational criteria for KOR described in section 3.2.2.

Twelve control subjects (Table 5.1) were recruited from local voluntary groups and the spouses of patients. All volunteers were in good physical and psychiatric health as described in section 3.2.3.

All patients and healthy volunteers gave informed consent to take part in the study (section 3.2.7) and only patients and subjects who met criteria for

right-handedness were admitted to the study (section 3.2.4). The study was approved by the Ethics of Medical Research Sub-Committee for Psychiatry and Psychology of the Lothian Health Board and the use of ^{99m}Tc-HMPAO approved by the Administration of Radioactive Substances Advisory Committee of the Department of Health (ARSAC).

5.1.2.2 Psychological assessment

The Cambridge Mental Disorders of the Elderly Examination (CAMDEX) was used for diagnosis and assessment of dementia (Roth et al 1986) and was completed for all subjects in the study. The CAMCOG protocol (of CAMDEX) and MMSE (Folstein et al 1975) were used to assess cognitive function (see section 3.3) in patients and to screen healthy control subjects. All control subjects obtained maximal or near maximal scores, providing evidence of normal cognitive function.

5.1.2.3 Measurement of regional cerebral blood flow

Regional CBF was measured at the Institute of Neurological Sciences, Glasgow using single photon emission tomography (SPECT) and the tracer ^{99m}Tc-hexamethyl propyleneamine oxime (^{99m}Tc-HMPAO), as described in section 3.6.

5.1.2.4 Statistical analysis

Measures of relative rCBF and CAMCOG scores are presented as median and range. Kruskal-Wallis one-way analysis of variance (K-W ANOVA) was used to test the differences in blood flow between the three groups and post-hoc Mann-Whitney U tests were used to locate significant differences. The relationship between relative mean uptake of ^{99m}Tc-HMPAO and cognitive scores was examined using Spearman's rank correlation

TABLE 5.1

Description of subjects studied using SPECT under basal conditions

	n	SEX M	F	AGE (YEARS) MEAN	RANGE
CON	12	3	9	64	55-76
AD	12	3	9	60	53-68
KOR	10	8	2	61	44-73

The numbers of patients in the study are shown as totals for each group (n) and broken down into males (M) and females (F). Alzheimer (AD) and Control (CON) groups were matched for age and sex.

TABLE 5.2

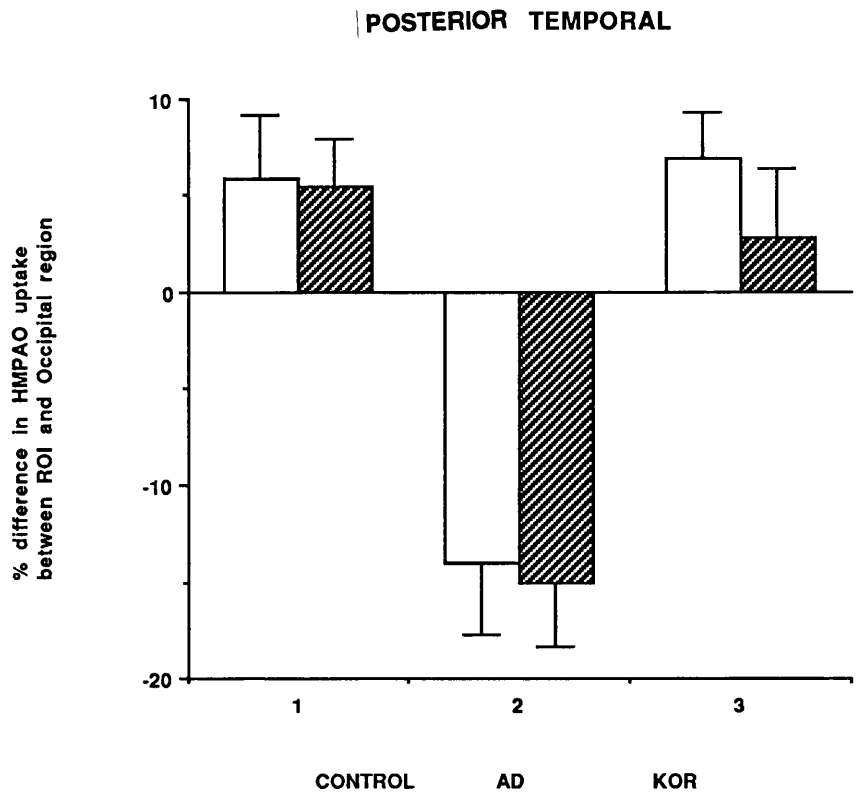
HMPAO uptake in control, Alzheimer and Korsakoff subjects by regions of interest

	CONTROL		AD		KOR		K-W
	Median	Range	Median	Range	Median	Range	ANOVA
RF	0.97	0.86-1.28	0.89	0.75-1.04	0.85	0.75-1.5	
LF	0.95	0.82-1.31	0.87	0.70-1.17	0.84	0.73-1.4	
RT	1.04	0.93-1.37	0.95	0.75-1.05	0.96	0.89-1.39	
LT	1.04	0.86-1.37	0.92*	0.39-1.18	0.97	0.78-1.23	$p < 0.05$
RPT	1.06	0.92-1.35	0.84**	0.70-1.11	1.07+	0.98-1.25	$p < 0.005$
LPT	1.05	0.95-1.22	0.83***	0.69-1.05	1.01+	0.89-1.31	$p < 0.001$
RBG	1.07	0.98-1.29	1.13	0.62-1.24	1.03	0.75-1.98	
LBG	1.04	0.99-1.36	1.12	0.85-1.30	1.02	0.57-2.06	
RHF	1.00	0.88-1.40	0.91	0.69-1.07	0.91	0.78-1.26	
LHF	1.00	0.91-1.46	0.90	0.63-1.05	0.91	0.74-1.22	
RP	1.04	0.85-1.33	0.86*	0.75-1.06	1.01	0.84-1.08	$p < 0.05$
LP	1.03	0.89-1.34	0.87*	0.69-1.13	0.98	0.75-1.03	$p < 0.01$

Results are presented as median and range; Post-hoc comparisons using Mann-Whitney U tests; AD v CONTROL and KOR v CONTROL: *$p < 0.01$, ** $p < 0.005$, ***$p < 0.001$. AD v KOR: +$p < 0.005$. Comparison of above median cortical values using sign test; AD v CONTROL: $p < 0.005$, KOR v CONTROL: $p < 0.05$.

Figure 5.1

Uptake of ^{99m}Tc-HMPAO in posterior temporal and parietal regions is shown for the three subject groups, (AD, Alzheimer's disease; KOR, Korsakoff's psychosis; CONTROL, healthy controls) as the mean percentage difference in uptake between the region of interest (ROI) and the reference area (occipital region). Vertical bars show SEM. Right sided regions are shown as white, and left sided regions are hatched in dark.


POSTERIOR TEMPORAL
% difference in HMPAO uptake between ROI and Occipital region
10
0
-10
-20
1
2
3
CONTROL
AD
KOR

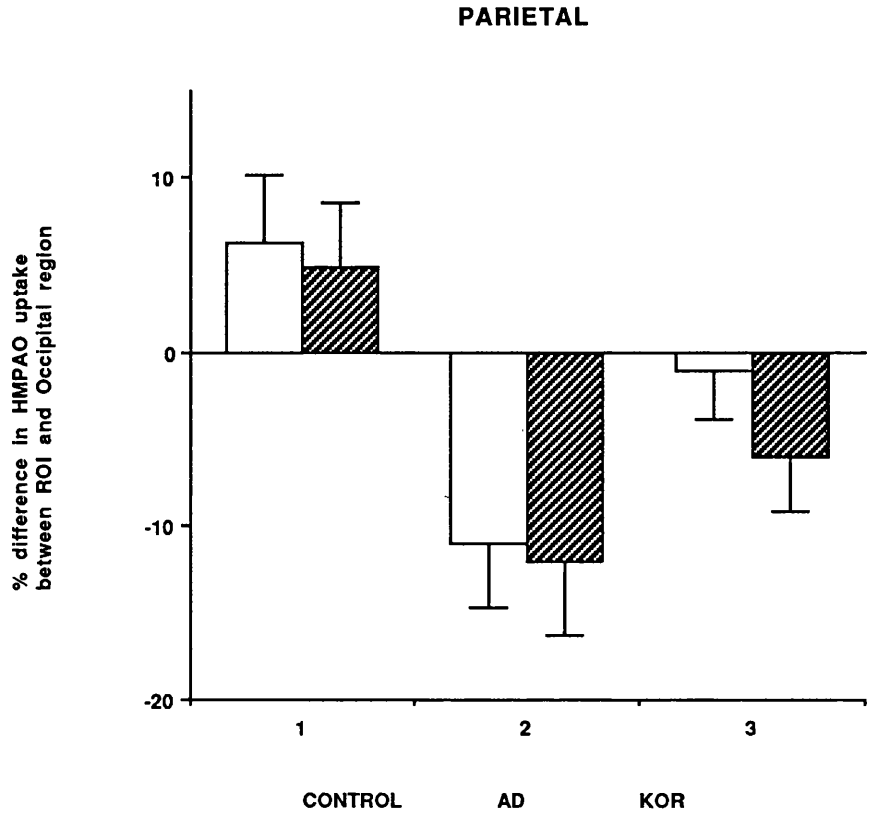

PARIETAL
% difference in HMPAO uptake between ROI and Occipital region
10
0
-10
-20
1
2
3
CONTROL
AD
KOR

Figure 5.4 SPECT: healthy control subject

Representative transverse sectional SPECT images at +30mm (lower image) and +70mm (top image) superior to the orbito-meatal line for a normal control subject. Orientation of images: frontal region to top of page; right side of image to left of page.

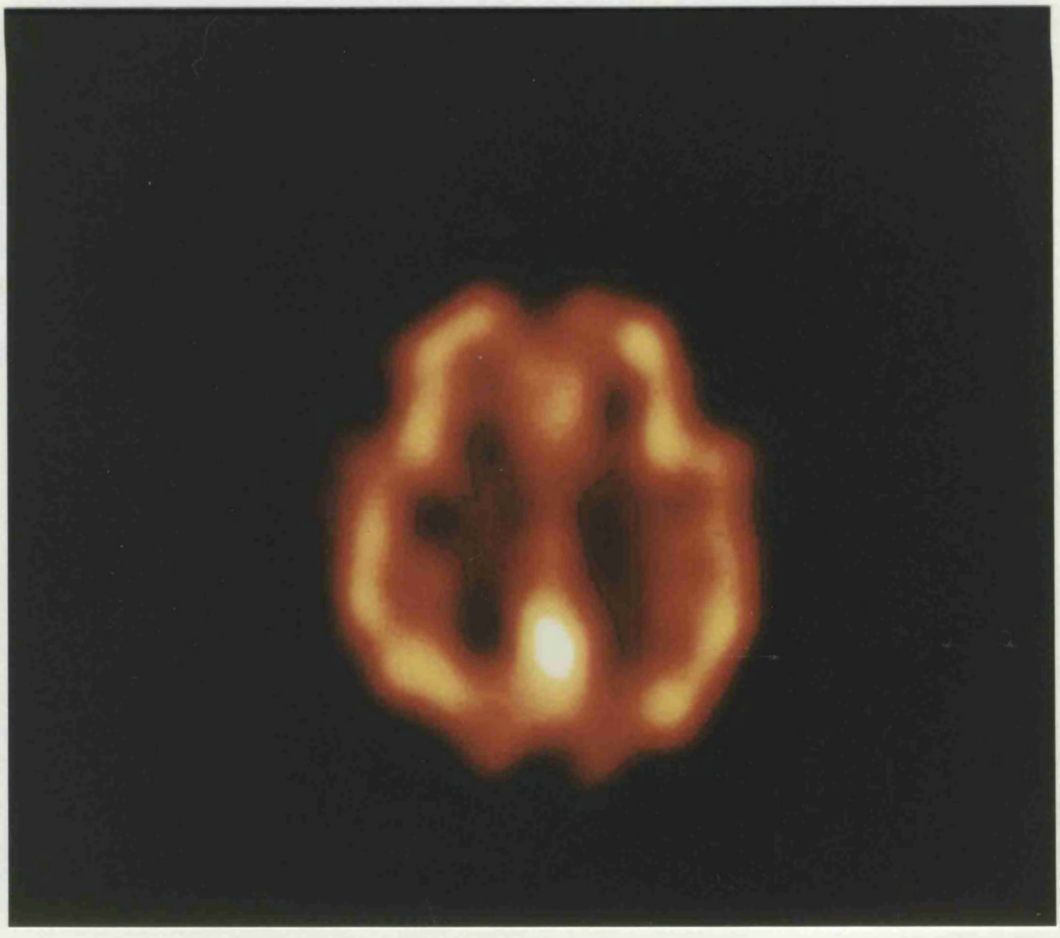
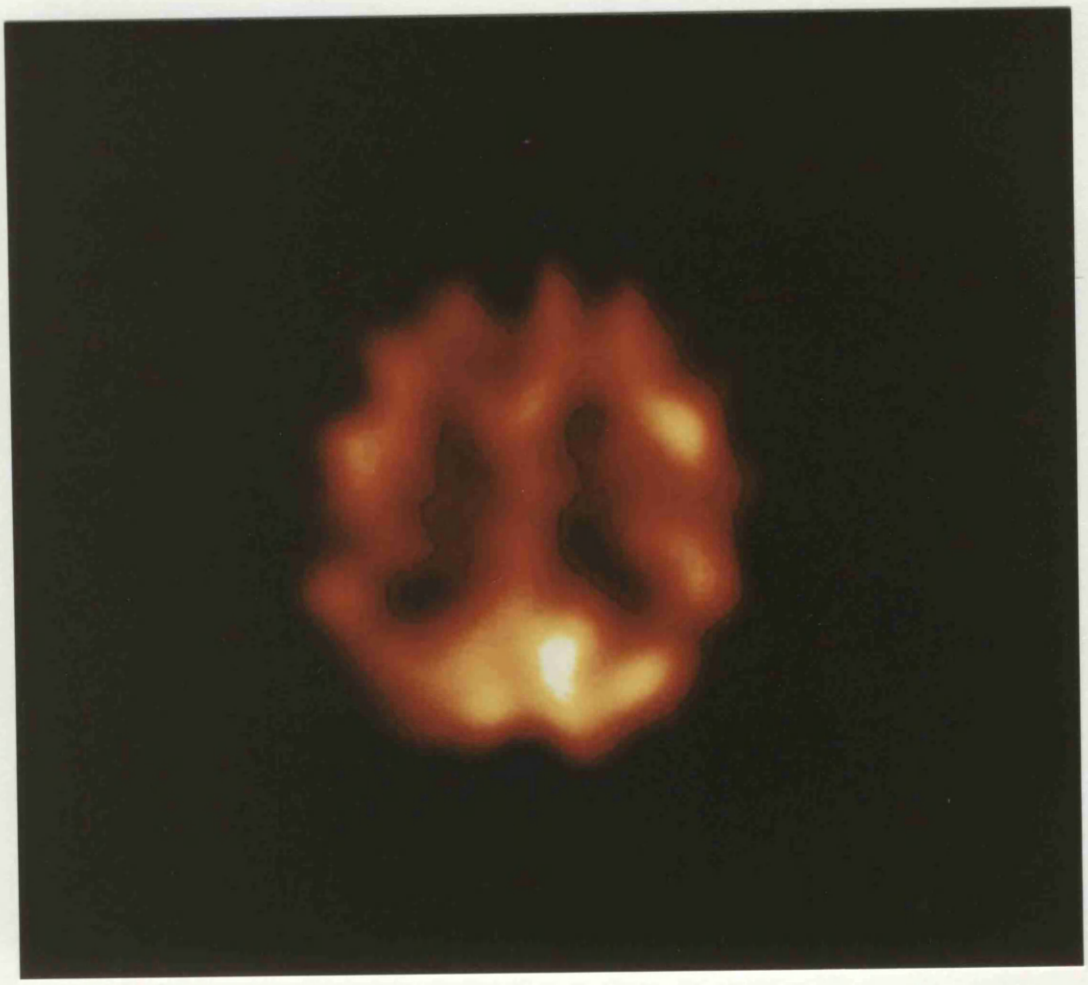
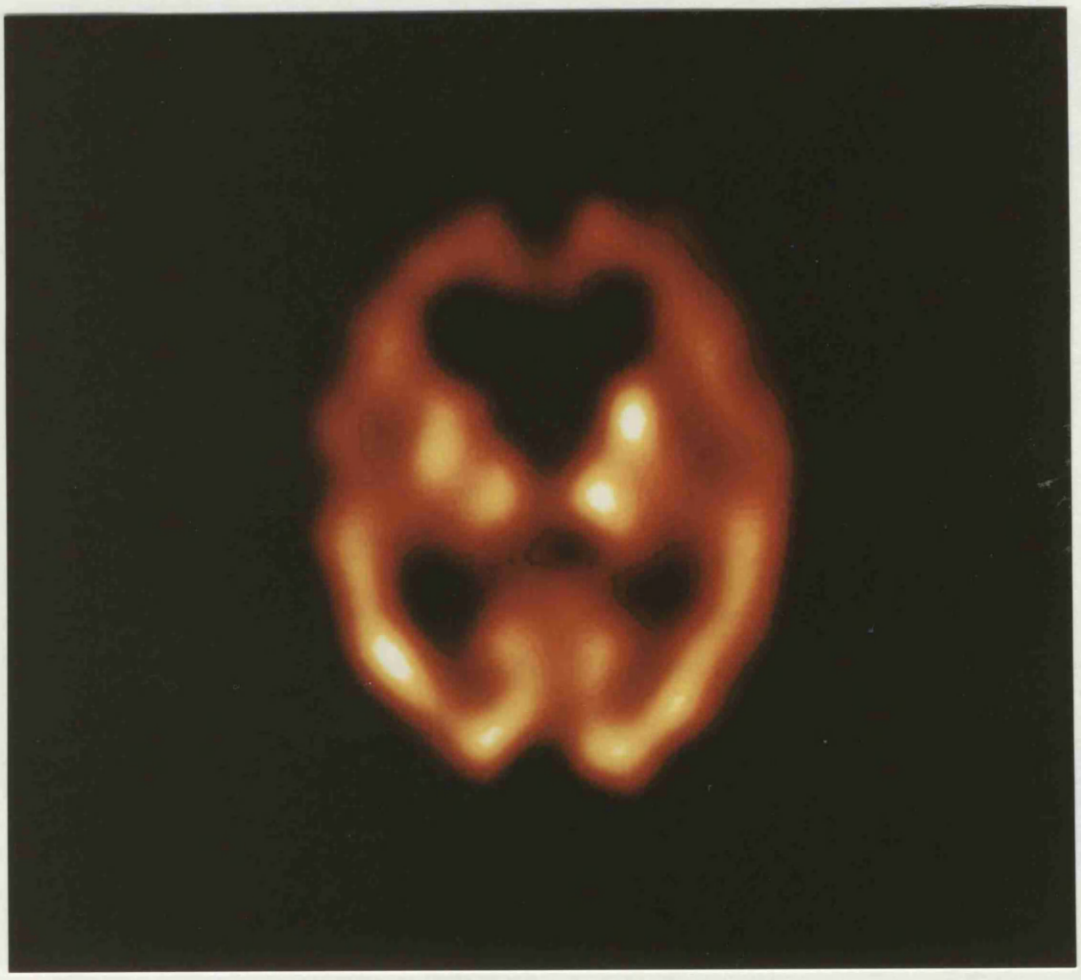


Figure 5.5 SPECT: Alzheimer's disease

Representative transverse sectional SPECT images at +30mm (lower image) and +70mm (top image) superior to the orbito-meatal line for a patient with Alzheimer's disease. Orientation of images: frontal region to top of page; right side of image to left of page.





in section 3.5, the distribution of net MTT (or 1/MTT, so-called fractional turnover rate or cerebral vascular reserve) for all cortical pixels, can be used to construct a cortical image. Representative examples of cortical images for AD, KOR and an age-matched healthy control are shown in Figures 4.2, 4.3, and 4.4 respectively.

4.4.2 Positive predictive value of net MTT measurements

The ideal clinical test is one in which there is no overlap in the range of results among subjects with and without the disease in question. As can be seen in figure 4.1 of net MTT measurements for individual patients, there is some overlap between healthy normal subjects and those with AD or KOR. In determining the clinical utility of a test, it is customary to define a 'normal range' as two standard deviations from the mean value of the normal group (Griner et al 1981). Such a range encompasses approximately 95% of the results of subjects without the disease.

Using this method cut-off points were calculated, above which net MTT values were defined as abnormal. Table 4.6 shows the upper cut-off point of normal for each ROI and the number of AD and KOR patients with MTT measurements above this 'normal' cut-off. Positive predictive values (Griner et al 1981), namely the probability that disease is present (either AD or KOR) when the test result is positive (net MTT above the cut-off), are shown in Table 4.6 for each ROI of the two patient groups.

4.4.3 CAMCOG neuropsychological scores and net MTT

Orientation and total memory scores were impaired in both AD and KOR patients compared with control subjects (Table 4.3). Language function was also reduced in AD patients compared with controls; KOR patients were less impaired. Scores for praxis were clearly reduced in AD patients but not

TABLE 4.3

Neuropsychological scores for patients and controls studied using transit time method

	CONTROL n = 10	AD n = 17	KOR n = 9	KRUSKAL-WALLIS one-way ANOVA
ORIENTATION	10 (10)	4 (0-9)**	5 (1-9)**	$p < 0.0005$
LANGUAGE	29 (28-30)	20 (0-27)**	26 (23-29)*	$p < 0.0005$
MEMORY	24 (23-26)	7 (0-15)**	12 (4-24)**	$p < 0.0005$
ATTENTION	7 (5-7)	1 (0-3)**	5 (3-7)	$p < 0.0005$
PRAXIS	12 (12)	7 (0-11)**	11 (7-12)	$P < 0.0005$
CAMTOTAL	102 (100-105)	51 (0-82)**	75 (56-99)	$p < 0.0005$
MMSE	30 (29-30)	12 (0-24)**	20 (11-29)	$p < 0.0005$

Results expressed as median and range. All post-hoc comparisons were made between patient groups and control subjects using Mann-Whitney U test; *$p < 0.005$; **$p < 0.001$.

TABLE 4.4

Relationship between net mean transit time and CAMCOG in Alzheimer patients

	LCx	RCx	LA	RA	LB	RB	LC	RC	LD	RD	IF
CAMTOTAL	-0.33	-0.60	-0.33	-0.57	-0.11	-0.46	-0.32	-0.42	-0.47	-0.46	-0.01
ORIENTATION	-0.38	-0.68*	-0.48	-0.68*	-0.14	-0.57	-0.33	-0.58	-0.51	-0.56	-0.04
LANGUAGE	-0.53	-0.66	-0.63	-0.54	-0.43	-0.57	-0.45	-0.25	-0.35	-0.38	+0.08
MEMORY	-0.38	-0.58	-0.35	-0.41	-0.18	-0.35	-0.21	-0.23	-0.35	-0.48	+0.16
ATTENTION	+0.04	-0.18	-0.27	-0.26	-0.08	-0.18	-0.02	-0.16	-0.19	-0.10	+0.09
PRAXIS	-0.15	-0.36	-0.34	-0.38	-0.06	-0.28	-0.12	-0.22	-0.34	-0.30	-0.007

Spearman's rank correlation coefficients; *$p < 0.01$. IF is the input function or MTT from arm to aortic arch (see text).

TABLE 4.5

Relationship between net mean transit time and CAMCOG in Korsakoff patients

	LCx	RCx	LA	RA	LB	RB	LC	RC	LD	RD	IF
CAMTOTAL	+0.07	+0.07	+0.07	+0.17	+0.07	+0.07	+0.06	+0.02	+0.02	+0.07	-0.07
ORIENTATION	-0.44	-0.44	-0.44	-0.39	-0.44	-0.44	-0.47	-0.38	-0.50	-0.44	-0.90*
LANGUAGE	+0.10	-0.09	-0.10	-0.22	+0.09	-0.10	-0.08	+0.02	-0.01	+0.10	+0.02
MEMORY	-0.31	-0.31	-0.31	-0.15	-0.31	-0.31	-0.32	-0.32	-0.33	-0.31	+0.02
ATTENTION	+0.82*	+0.82*	+0.82*	+0.67	+0.82*	+0.82*	+0.84*	+0.78	+0.75	+0.82	+0.49
PRAXIS	+0.05	+0.05	+0.05	+0.17	+0.05	+0.05	-0.04	+0.05	+0.09	+0.05	+0.05

Spearman's rank correlation coefficients; *$p < 0.01$. IF is the input function or MTT from the arm to the aortic arch (see text).

TABLE 4.6

Positive predictive value of net MTT in regions of interest for AD and KOR patients

	Left Cx	Right Cx	Left A	Right A	Left B	Right B	Left C	Right C
Upper limit of normal net MTT value (seconds)	6.2	6.2	6.7	6.4	5.8	5.9	6.3	6.3
Number of AD patients above normal cut-off value	11	13	7	9	11	7	12	13
Positive predictive value for AD (%)	65	76	41	53	65	41	71	76
Number of KOR patients above normal cut-off value	5	6	3	3	5	4	5	6
Positive predictive value for KOR (%)	56	67	33	33	56	44	56	67

Total number of AD patients studied was 17 and KOR patients 9.
Positive predictive value (%) is number of patients with net MTT value above cut-off value divided by total number of patients.
Abbreviations explained in text.

in KOR patients (Table 4.3). Measures of attention were markedly reduced in AD, but much less impaired in the KOR group. Overall cognitive function, as measured by total CAMCOG score (CAMTOTAL), or MMSE was lowest in the AD group compared with controls, but also significantly reduced in the KOR patients (Table 4.3).

The correlation matrix of net MTT and cognitive function scores is shown for AD subjects in Table 4.4. Negative association at the $p < 0.01$ level was detected only between orientation scores and right frontal cortex, although there is a trend for low cognitive scores to be associated with prolonged net MTT. This relationship is most apparent between hemispheric net MTT (left and right cortex) and CAMTOTAL, orientation, language, and to a lesser extent, memory function. By comparison, no association is observed between cognitive function scores and the input function.

The association between net MTT and psychological measures in KOR patients is shown in Table 4.5. No association was detected in the KOR group between net MTT and CAMTOTAL, language and praxis. As in the AD group there was an apparent trend for memory and orientation subscores to show a negative association with net MTT values in both sides of cortex. None of these correlation coefficients however was statistically significant. A positive association between net MTT and attention was detected in both sides of cortex in KOR patients (Table 4.5)

4.5 Discussion

Intravenously injected radioactive tracers allowing external monitoring of the transit over the head have been used for some years to study cerebral circulation. Although it might appear that transit times give information about local blood flow, without an estimate of local blood volume, MTT values cannot

be interpreted physiologically. Thus the trend in this study for net MTTs to be prolonged in AD and KOR patient groups, compared with healthy controls, might reflect reduced cortical blood flow, but without estimates of blood volume, no such inference can be made. At any given flow rate, MTT will depend on the volume into which the tracer diffuses - the volume of distribution. Vascular MTTs therefore require a tracer that is confined to the vascular compartment such as labelled human serum albumin. In normal brain pertechnetate appears to remain almost wholly intravascular during the first pass (Bartolini et al 1983) and is therefore acceptable as a blood marker. However in pathological states, where the blood brain barrier is substantially disrupted, e.g. in major head trauma, pertechnetate tends to diffuse out of the vascular bed into a larger volume of distribution, resulting in MTT prolongation. Although there is evidence that the integrity of the blood brain barrier is damaged in AD (e.g. Selkoe 1989), it is unlikely that, during the short time interval that transit time is measured, pertechnetate would be extravasated to any significant extent. Although MTT cannot be used as an index of blood flow without information on blood volume, transit time may however provide an index of cerebral haemodynamics or 'brain function' in situations where volume of distribution varies much less than blood flow and as such could have clinical utility. The normal range of values for net MTT observed in this study are not significantly different from those reported with PET (Gibbs et al 1984; Powers et al 1984) obtained by separately measuring blood flow and volume or following intra-arterial injection (Nylin et al 1960). It has been shown that there is a linear relationship between net MTT and end-tidal $C0_2$ (Naylor et al 1991). In comparing different groups of subjects it has been assumed that end-tidal $C0_2$ values are not significantly different between the groups. It is also assumed that during the net MTT examination, end-tidal $C0_2$ remains constant as the examination time of 1 minute is relatively short.

There was a trend for transit times to be prolonged in both AD and KOR groups, and on a group basis, significant differences in net MTT were detected between these groups and healthy controls, but not between AD and KOR groups. However there is overlap of individual net MTT values for all three groups in all regions of interest (individual MTT values shown in Figure 4.1). In order to determine accurately the clinical utility of test data such as net MTT, both sensitivity (the true positive rate) and specificity (the true negative rate) are required. It is not possible to calculate sensitivity and specificity from the net MTT data in this study because in the study design only patients satisfying clinical criteria for AD or KOR were recruited. In order to calculate sensitivity and specificity a different epidemiological study design would be necessary, with measurements of net MTT being made in a group of patients with dementia, not carefully selected AD or KOR patient groups. The present design does however allow positive predictive values to be calculated - i.e. the likelihood of the disease being present when net MTT is abnormal (Griner et al 1981).

In the AD group the positive predictive values (PPV) varied between regions of interest, with lowest values in frontal regions (Left A and Right A) and highest in more posterior regions (Left C and Right C). This is consistent with neuropathological and imaging evidence (see section 2.2) that posterior cortical areas are more often affected in AD. The PPV of greater than 70% for net MTT in posterior regions of AD patients suggests that further study with larger numbers of subjects may be worthwhile in establishing the clinical utility of net MTT. One reason why PPV of the transit time method may not be higher is that the clinical criteria used to select AD cases are themselves imperfect. When similar clinical criteria for AD case selections were compared with neuropathological diagnosis, estimates of their PPV vary from 46% (Kellet et al 1988), to 84% (Burns et al 1990 a). This issue is further

complicated by differences between laboratories of pathological diagnostic criteria for AD (Wisniewski et al 1988).

As might be expected from examination of individual net MTT data points in figure 4.1, positive predictive values of net MTT for the KOR group are less satisfactory. This may partly reflect the small group size, but is also likely to be attributable to difficulties in clinically defining cases of KOR (discussed in section 3.2.2).

Neuropathological studies of patients with moderate to severe AD show temporal, parietal and frontal lobes affected by neurofibrillary tangles, plaques and neuronal degeneration (Pearson et al 1985). These lesions result in widespread impairment of cerebral metabolism with consequent focal and global cognitive deficits. In this study net MTT was prolonged in all cortical areas in AD patients. There was also a trend for cognitive scores to be inversely associated with net MTT, as would be expected if transit time reflected function. However in both AD and KOR groups the association of net MTT and cognitive scores was not marked and rarely reached significance. This lack of association casts doubt on the usefulness of net MTT as an index of brain function, as does the paradoxically significant association between increased net MTT and improved attention scores in KOR patients.

In the KOR group, transit times were prolonged compared with healthy controls, but similar to those of AD patients. The finding of prolonged net MTT in stable abstinent subjects is unexpected as KOR patients are generally believed to have a relatively localised macroscopic neuropathological lesion in the diencephalon or hippocampal formation and an associated discrete impairment of memory without necessarily more global cognitive impairment (Victor et al 1971; Blackburn & Tyrer 1985). This is consistent with the neuropsychological data from this study, which separated the KOR patients from the more globally impaired AD group, especially with respect to

language, attention, praxis and total score, and suggests that the KOR group were not globally demented. However there is evidence that the brain dysfunction in KOR is more extensive than former neuropathological and clinical findings have suggested, even in reasonably well defined KOR populations. Magnetic resonance imaging has shown that a significant proportion of KOR patients, who do not appear to have a generalised alcoholic dementia have high proton spin-lattice relaxation time (T1 values) for both frontal and parietal white and grey matter (Christie et al 1988). Hata et al (1987) have shown that KOR patients have widespread reduction in rCBF throughout cortical and subcortical structures. Using ^{133}Xenon contrast computed tomography they have demonstrated reduced rCBF in hippocampus, nucleus basalis of Meynert and frontal white matter, as well as throughout cortical grey matter. Similar abnormalities in KOR patients include reduced mean hemispheric grey matter CBF using ^{133}Xe inhalation (Rogers et al 1983), and reduced whole brain CBF, $CMRO_2$ and CMR glc (Kruger et al 1980) using the Kety-Schmidt technique.

Lishman (1986) has recently suggested a unifying hypothesis to account for the symptoms common to AD and KOR patients. He suggests that in Korsakoff's psychosis patients, alcohol exerts toxic effects on the basal forebrain nuclei, causing damage to cholinergic projections to cortex with resultant cognitive impairment. Such a pathological process might be expected to result in a diffuse and variable reduction in cortical metabolism and blood flow.

4.6 Conclusion

This study was undertaken because transit time methods appeared to offer a number of attractions for the investigation of cerebral haemodynamics in dementia. Although measurement of net MTT is rapid, non-invasive and

inexpensive, the present investigation illustrates a number of serious shortcomings with the method. Lack of definition of tracer volume of distribution results in difficulties in the interpretation of MTT prolongation. Without distribution volume information it is not possible to make any interpretation about the relationship of MTT to blood flow. This limitation could perhaps be accepted if net MTT provided some clinical utility. Despite the overlap in individual net MTT values in regions of interest for the three patient groups, calculation of positive predictive values suggest that in comparing AD and healthy controls, posterior cerebral regions may have some clinical utility. These results suggest that it would be worthwhile embarking on an assessment of diagnostic accuracy of net MTT in posterior cortex in order to determine the sensitivity and specificity of the method in separating AD patients from controls. In KOR patients positive predictive values were lower, but the prolongation of net MTT in cortical regions supports other evidence for cortical dysfunction in this condition previously considered subcortical. There was no clear association between net MTT and neuropsychological test scores, although there was a trend towards negative correlation in AD and KOR, as might be expected if net MTT provides an index of cortical function in dementia.

CHAPTER 5

STUDIES OF BASAL BRAIN FUNCTION USING SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY

5.1 Basal patterns of rCBF investigated by single photon emission computed tomography with ^{99m}Tc-HMPAO in presenile Alzheimer's Disease and Korsakoff's Psychosis

5.1.1 Introduction

Techniques for the tomographic reconstruction of signals from radioactive tracers in different brain regions may change our understanding of brain function. Positron emission tomography (PET) has attracted attention but in practice, it is operationally complex and a limited resource. Alternative tomographic imaging techniques such as SPECT may allow advances from PET to be translated into more general clinical use and may push studies of brain function into new clinical areas where access to PET is and may remain limited. Single photon emission computed tomography (SPECT) with administration of intravenous radioligands promises to be an important technique to complement PET in this way.

Initial applications of PET have centered on estimation of regional cerebral metabolism and cerebral blood flow. The results suggest that cerebral blood flow is, under most circumstances, tightly coupled to local neuronal demand for glucose (section 2.1). Interest in SPECT proceeds in the first instance from the wish to investigate patterns of regional blood flow in the neocortex as a potential measure of neuronal function.

Radionuclides based on ^{99m}Tc are optimal for clinical SPECT imaging because the radio-isotope is inexpensive and has favourable properties for clinical use such as stability and relatively pure emission of 140KeV γ-radiation. The recent introduction of hexamethyl propyleneamine oxime (Exametazime or d,1 HMPAO, Amersham Intl. plc) thus offers considerable potential for imaging of brain perfusion patterns using SPECT. It complexes readily with ^{99m}Tc and its distribution following intravenous injection conforms

closely to that of an ideal 'first pass', high clearance, marker of brain blood flow (Neirinckx et al 1987; Costa et al 1986; Sharp et al 1986b).

The **aims** of this study were to use ^{99m}Tc-HMPAO SPECT to investigate the pattern of regional cerebral blood flow (rCBF) in patients with probable presenile Alzheimer's disease and Korsakoff's psychosis and to determine its relation to neuropsychological function in such patients. The central questions were:

(a) What is the pattern of perfusion deficits using SPECT in patients with presenile Alzheimer's disease and Korsakoff psychosis?

(b) Is there evidence of cortical dysfunction using SPECT in Korsakoff patients?

(c) Can we demonstrate meaningful relationships between rCBF patterns as measured by SPECT, and neuropsychological performance?

5.1.2 Methods

5.1.2.1 Patients and control subjects

Twelve patients with pre-senile Alzheimer's disease (AD) were studied and are described in Table 5.1. All met the agreed consensus criteria for probable Alzheimer's disease described in section 3.2.1.

Ten patients with Alcoholic Korsakoff Syndrome (KOR) were also studied and are described in Table 5.1. All KOR patients were resident in a psychiatric hospital ward or supervised hostel accommodation and all met the operational criteria for KOR described in section 3.2.2.

Twelve control subjects (Table 5.1) were recruited from local voluntary groups and the spouses of patients. All volunteers were in good physical and psychiatric health as described in section 3.2.3.

All patients and healthy volunteers gave informed consent to take part in the study (section 3.2.7) and only patients and subjects who met criteria for

right-handedness were admitted to the study (section 3.2.4). The study was approved by the Ethics of Medical Research Sub-Committee for Psychiatry and Psychology of the Lothian Health Board and the use of ^{99m}Tc-HMPAO approved by the Administration of Radioactive Substances Advisory Committee of the Department of Health (ARSAC).

5.1.2.2 Psychological assessment

The Cambridge Mental Disorders of the Elderly Examination (CAMDEX) was used for diagnosis and assessment of dementia (Roth et al 1986) and was completed for all subjects in the study. The CAMCOG protocol (of CAMDEX) and MMSE (Folstein et al 1975) were used to assess cognitive function (see section 3.3) in patients and to screen healthy control subjects. All control subjects obtained maximal or near maximal scores, providing evidence of normal cognitive function.

5.1.2.3 Measurement of regional cerebral blood flow

Regional CBF was measured at the Institute of Neurological Sciences, Glasgow using single photon emission tomography (SPECT) and the tracer ^{99m}Tc-hexamethyl propyleneamine oxime (^{99m}Tc-HMPAO), as described in section 3.6.

5.1.2.4 Statistical analysis

Measures of relative rCBF and CAMCOG scores are presented as median and range. Kruskal-Wallis one-way analysis of variance (K-W ANOVA) was used to test the differences in blood flow between the three groups and post-hoc Mann-Whitney U tests were used to locate significant differences. The relationship between relative mean uptake of ^{99m}Tc-HMPAO and cognitive scores was examined using Spearman's rank correlation

TABLE 5.1

Description of subjects studied using SPECT under basal conditions

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The numbers of patients in the study are shown as totals for each group (n) and broken down into males (M) and females (F). Alzheimer (AD) and Control (CON) groups were matched for age and sex.

TABLE 5.2

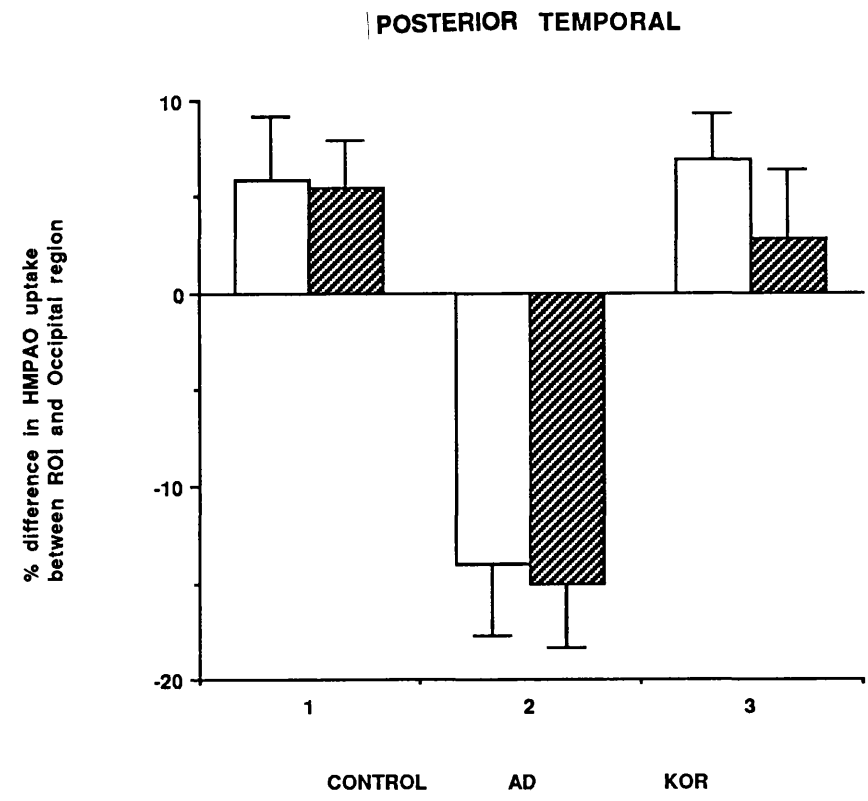
HMPAO uptake in control, Alzheimer and Korsakoff subjects by regions of interest

	CONTROL		AD		KOR		K-W
	Median	Range	Median	Range	Median	Range	ANOVA
RF	0.97	0.86-1.28	0.89	0.75-1.04	0.85	0.75-1.5	
LF	0.95	0.82-1.31	0.87	0.70-1.17	0.84	0.73-1.4	
RT	1.04	0.93-1.37	0.95	0.75-1.05	0.96	0.89-1.39	
LT	1.04	0.86-1.37	0.92*	0.39-1.18	0.97	0.78-1.23	$p < 0.05$
RPT	1.06	0.92-1.35	0.84**	0.70-1.11	1.07+	0.98-1.25	$p < 0.005$
LPT	1.05	0.95-1.22	0.83***	0.69-1.05	1.01+	0.89-1.31	$p < 0.001$
RBG	1.07	0.98-1.29	1.13	0.62-1.24	1.03	0.75-1.98	
LBG	1.04	0.99-1.36	1.12	0.85-1.30	1.02	0.57-2.06	
RHF	1.00	0.88-1.40	0.91	0.69-1.07	0.91	0.78-1.26	
LHF	1.00	0.91-1.46	0.90	0.63-1.05	0.91	0.74-1.22	
RP	1.04	0.85-1.33	0.86*	0.75-1.06	1.01	0.84-1.08	$p < 0.05$
LP	1.03	0.89-1.34	0.87*	0.69-1.13	0.98	0.75-1.03	$p < 0.01$

Results are presented as median and range; Post-hoc comparisons using Mann-Whitney U tests; AD v CONTROL and KOR v CONTROL: *$p < 0.01$, ** $p < 0.005$, ***$p < 0.001$. AD v KOR: +$p < 0.005$. Comparison of above median cortical values using sign test; AD v CONTROL: $p < 0.005$, KOR v CONTROL: $p < 0.05$.

Figure 5.1

Uptake of ^{99m}Tc-HMPAO in posterior temporal and parietal regions is shown for the three subject groups, (AD, Alzheimer's disease; KOR, Korsakoff's psychosis; CONTROL, healthy controls) as the mean percentage difference in uptake between the region of interest (ROI) and the reference area (occipital region). Vertical bars show SEM. Right sided regions are shown as white, and left sided regions are hatched in dark.


POSTERIOR TEMPORAL
% difference in HMPAO uptake between ROI and Occipital region
10
0
-10
-20
1
2
3
CONTROL
AD
KOR

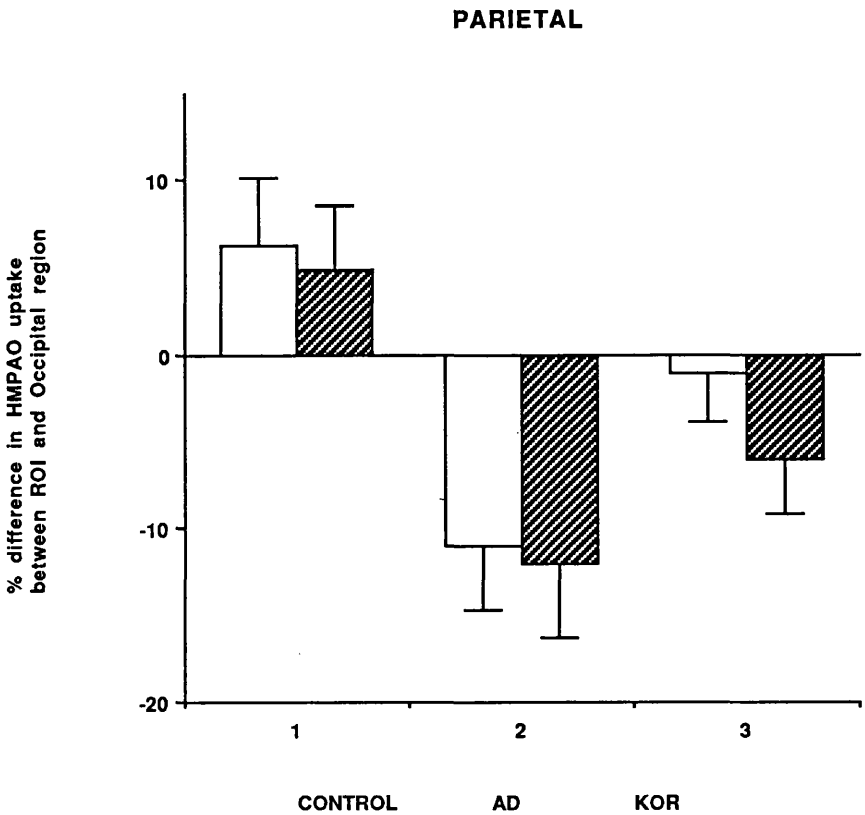
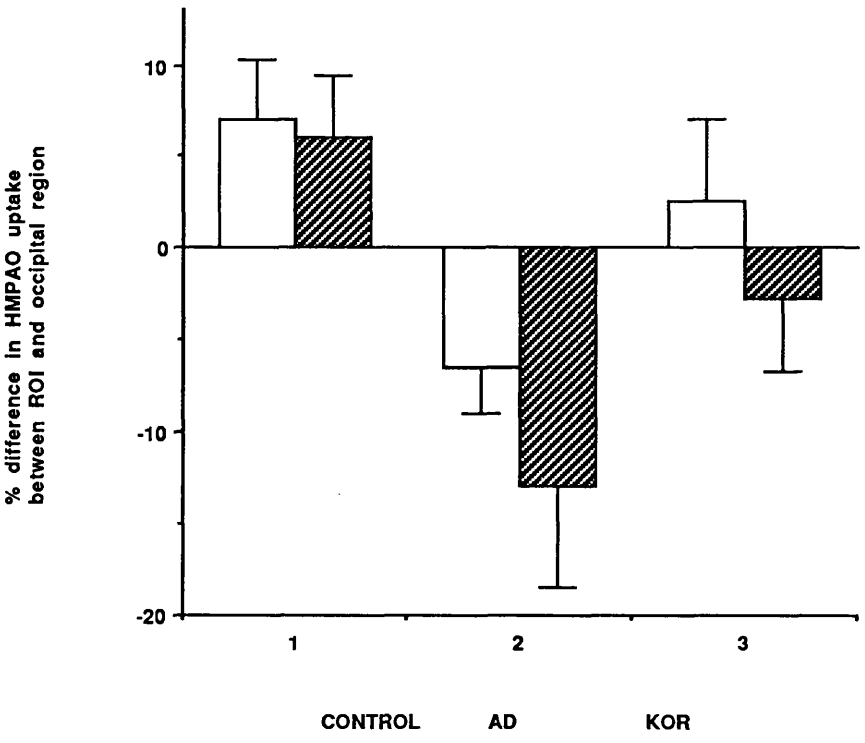

PARIETAL
% difference in HMPAO uptake between ROI and Occipital region
10
0
-10
-20
1
2
3
CONTROL
AD
KOR

Figure 5.2

Uptake of ^{99m}Tc-HMPAO in temporal and basal ganglia regions is shown for the three subject groups, (AD, Alzheimer's disease; KOR, Korsakoff's psychosis; CONTROL, healthy controls) as the mean percentage difference in uptake between the region of interest (ROI) and the reference area (occipital region). Vertical bars show SEM. Right sided regions are shown as white, and left sided regions are hatched in dark.

TEMPORAL



BASAL GANGLIA

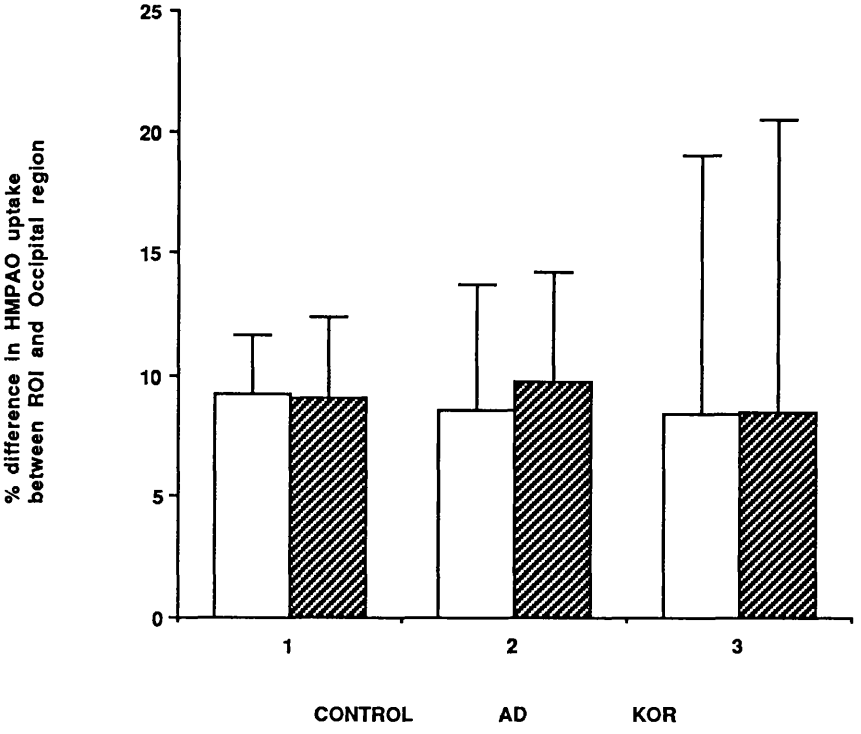
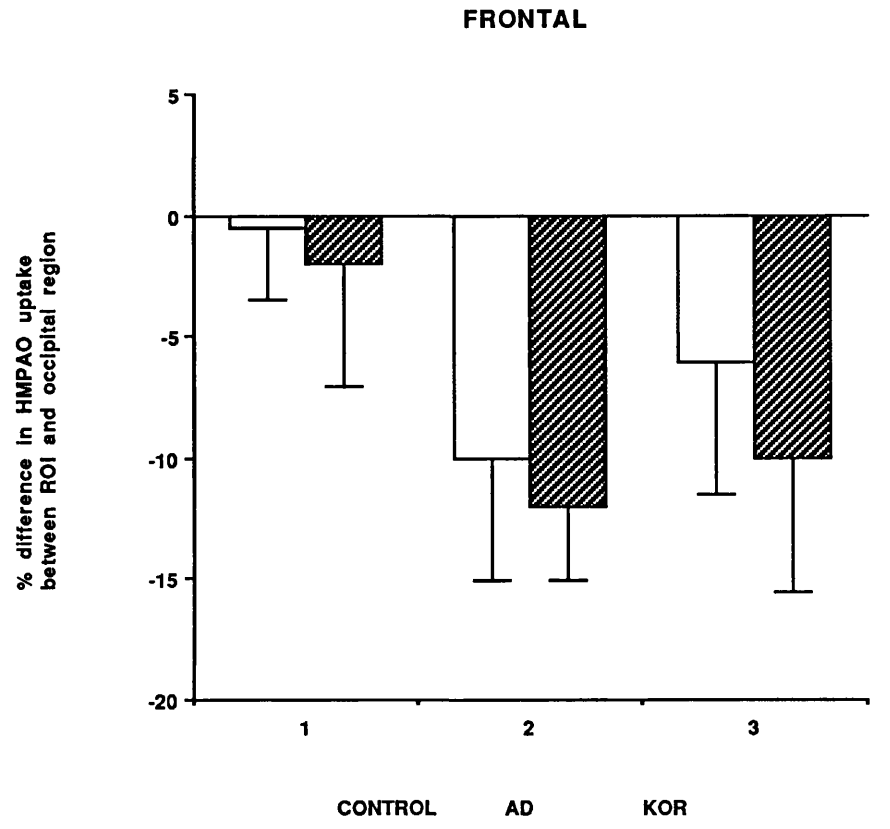


Figure 5.3

Uptake of ^{99m}Tc-HMPAO in frontal and high frontal regions is shown for the three subject groups, (AD, Alzheimer's disease; KOR, Korsakoff's psychosis; CONTROL, healthy controls) as the mean percentage difference in uptake between the region of interest (ROI) and the reference area (occipital region). Vertical bars show SEM. Right sided regions are shown as white, and left sided regions are hatched in dark.


FRONTAL
% difference in HMPAO uptake between ROI and occipital region
5
0
-5
-10
-15
-20
1
2
3
CONTROL
AD
KOR

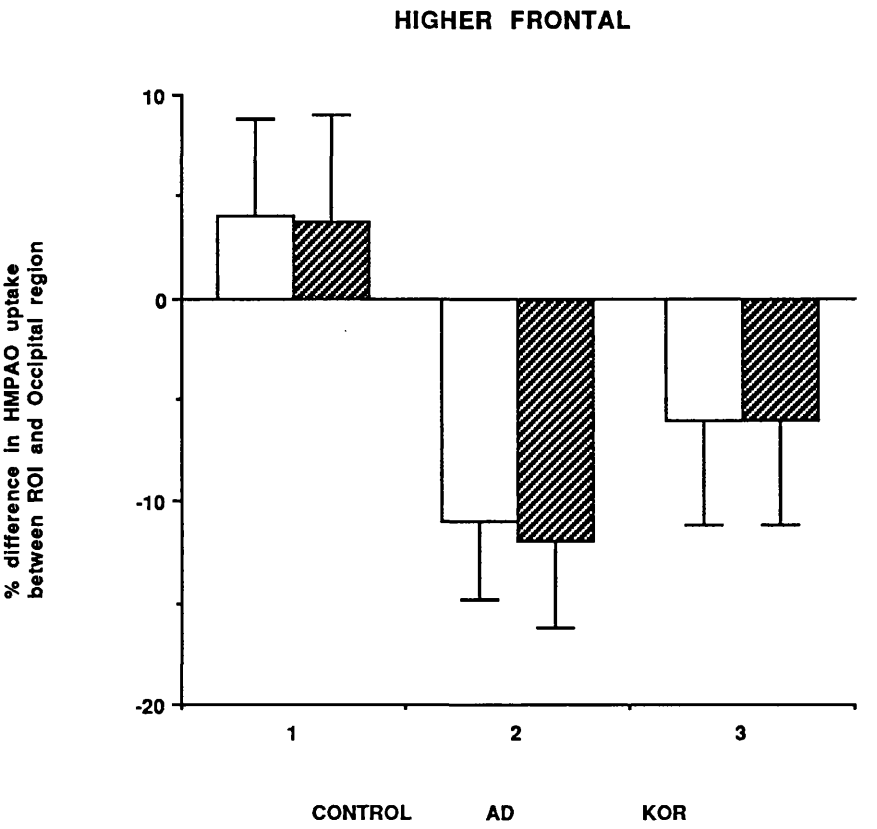

HIGHER FRONTAL
% difference in HMPAO uptake between ROI and Occipital region
10
0
-10
-20
1
2
3
CONTROL
AD
KOR

Figure 5.4 SPECT: healthy control subject

Representative transverse sectional SPECT images at +30mm (lower image) and +70mm (top image) superior to the orbito-meatal line for a normal control subject. Orientation of images: frontal region to top of page; right side of image to left of page.

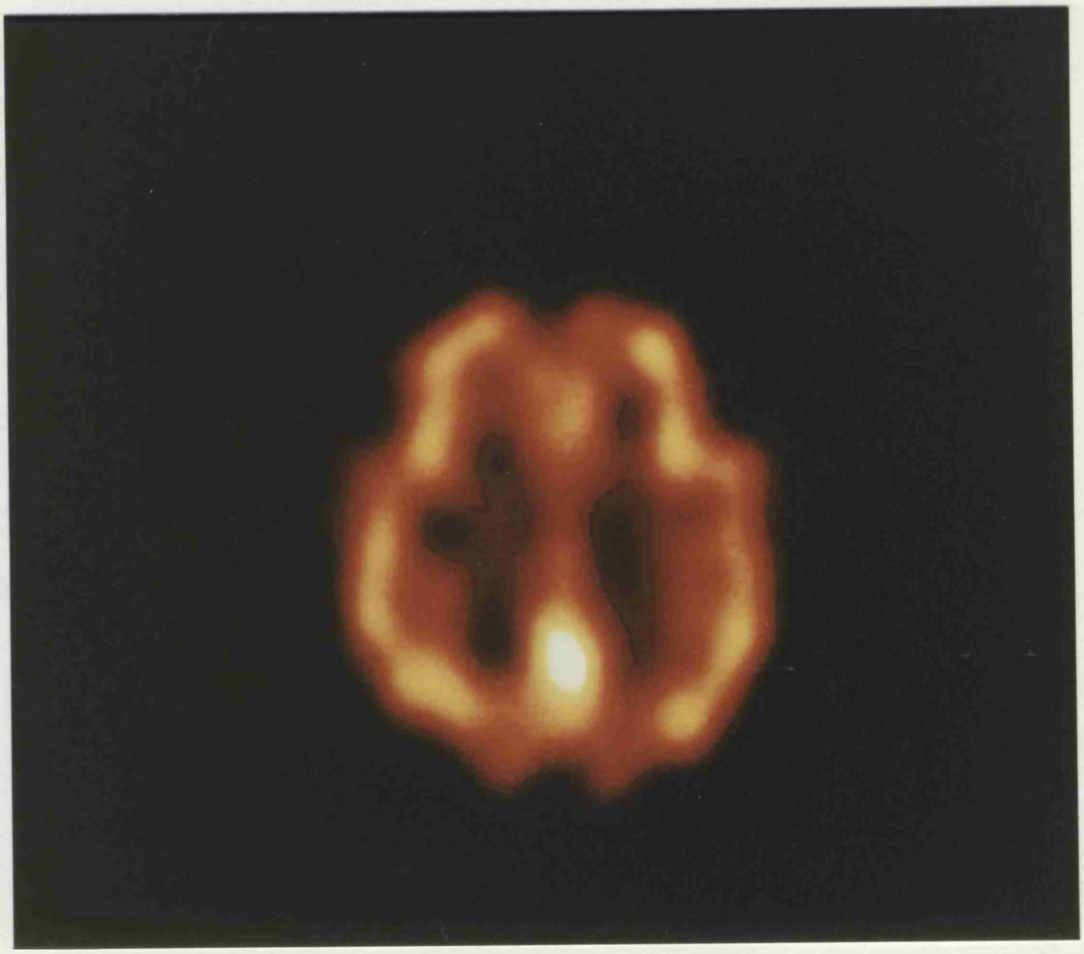
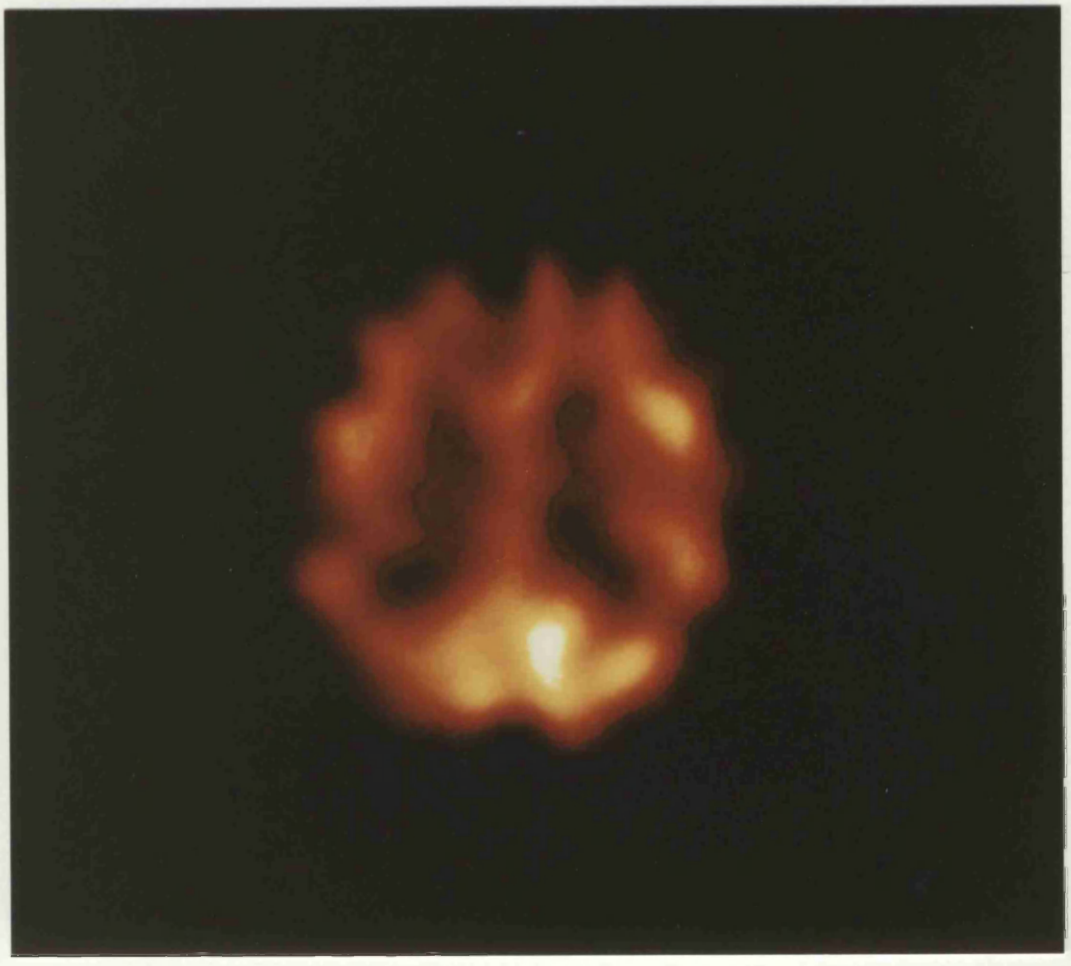


Figure 5.5 SPECT: Alzheimer's disease

Representative transverse sectional SPECT images at +30mm (lower image) and +70mm (top image) superior to the orbito-meatal line for a patient with Alzheimer's disease. Orientation of images: frontal region to top of page; right side of image to left of page.



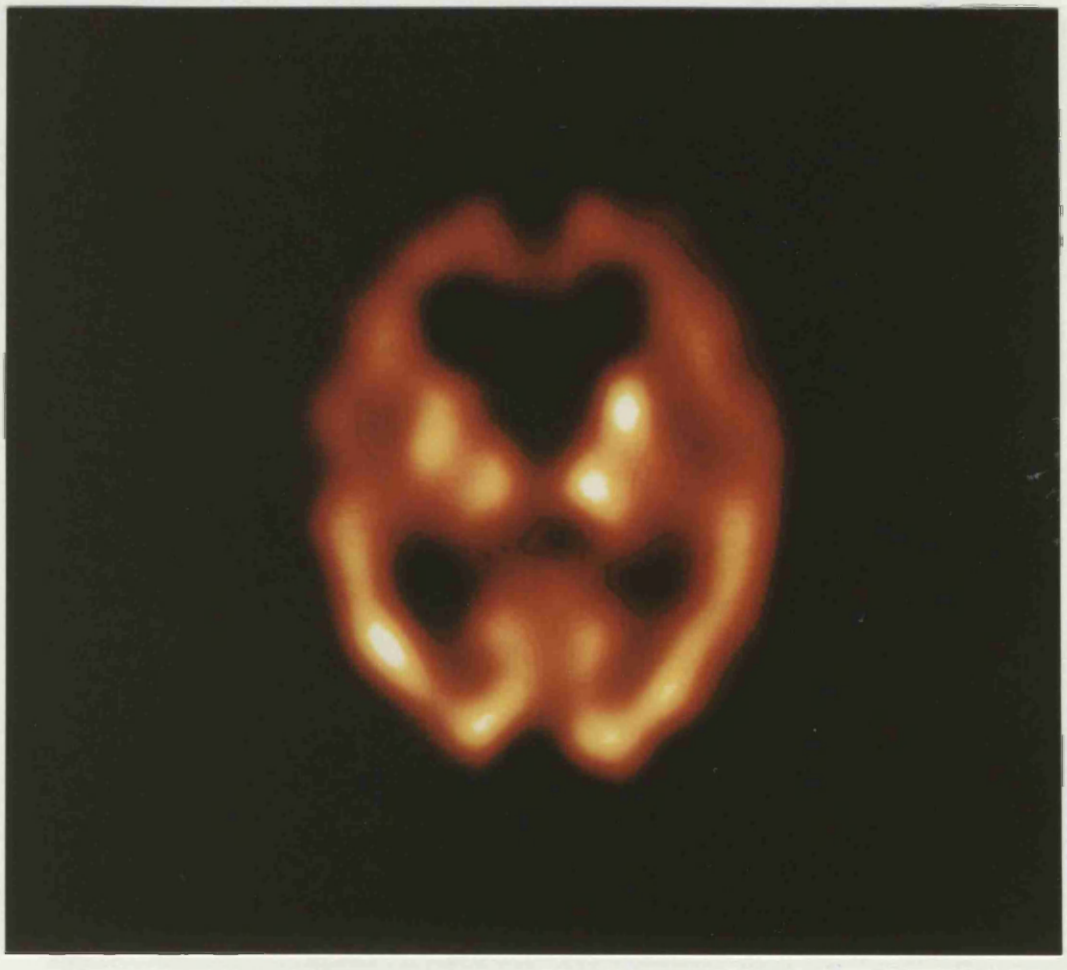
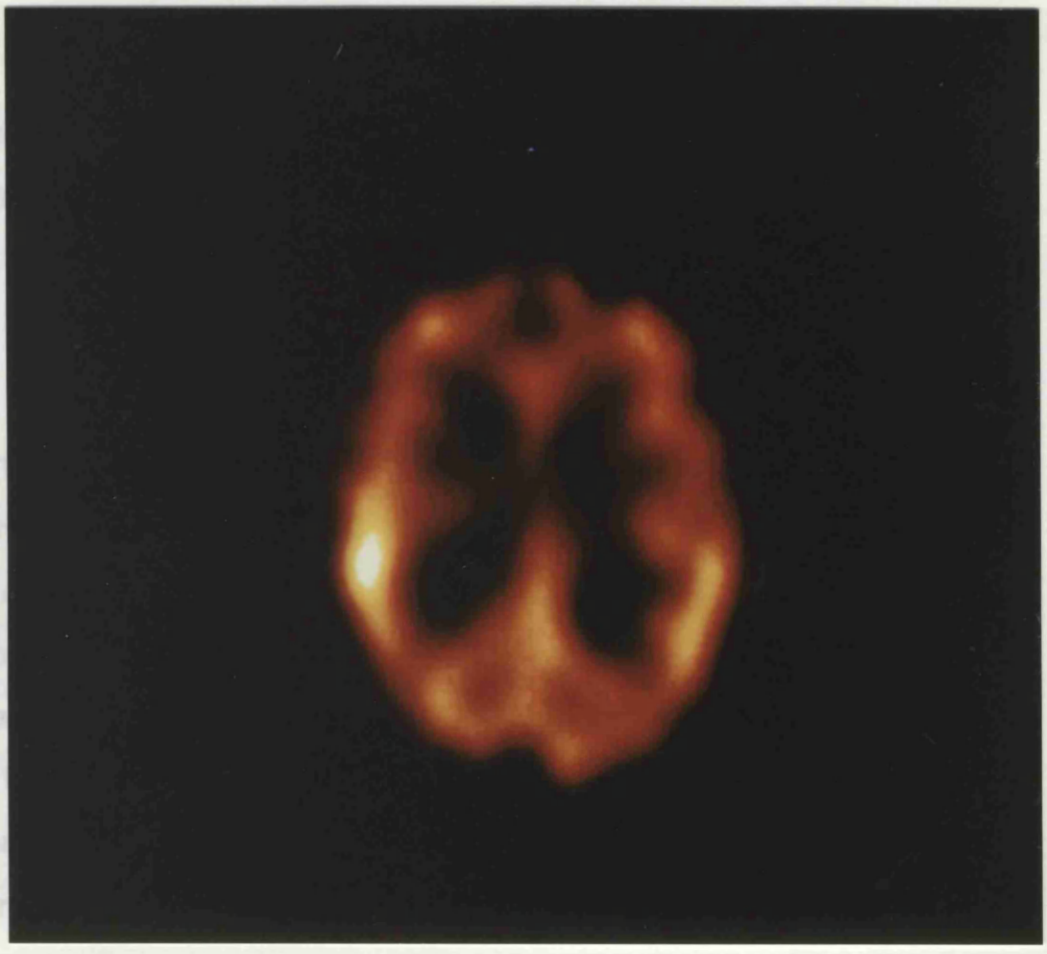
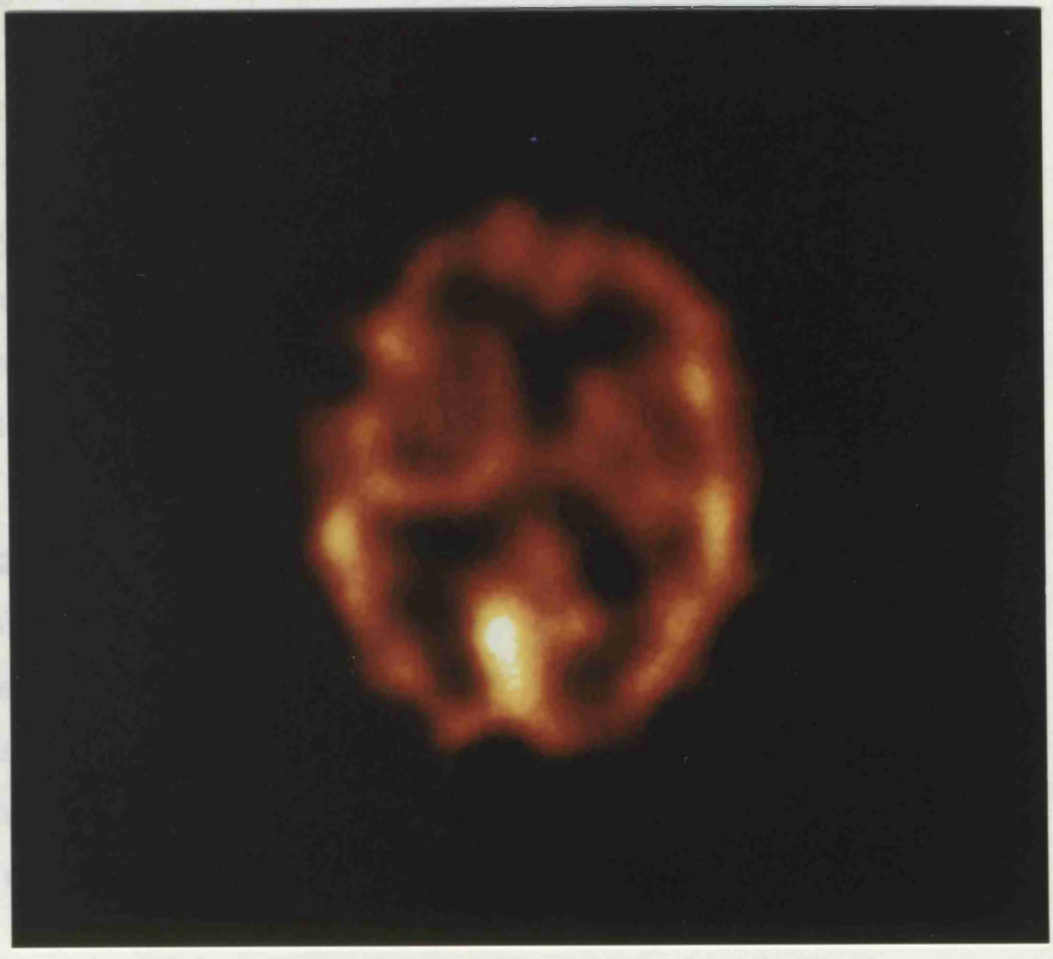


Figure 5.6 SPECT: Korsakoff's psychosis

Representative transverse sectional SPECT images at +30mm (lower image) and +70mm (top image) superior to the orbito-meatal line for a patient with Korsakoff's psychosis. Orientation of images: frontal region to top of page; right side of image to left of page.





coefficients. Significance levels were adjusted to take account of multiple comparisons as described previously.

5.1.3 Results

5.1.3.1 Regional CBF measurements

In patients with AD, relative rCBF was reduced in posterior temporal areas compared with controls; posterior temporal rCBF was maintained in KOR patients (Figure 5.1, Table 5.2). In AD, rCBF was also reduced in parietal (Figure 5.1, Table 5.2) and left temporal regions (Figure 5.2, Table 5.2). In other individual cortical areas for AD, and in all areas for KOR, differences between patient groups and normal controls were not statistically discernible (Table 5.2). However, there was a tendency for median values for all cortical areas to be lower in both the patient groups (Figures 5.1, 5.2, 5.3 and legend to Table 5.2). In the representative subcortical area studied (basal ganglia), rCBF was the same in all groups (Figure 5.2).

Representative examples of SPECT images at 'high' and 'low' levels, (OM +70mm and OM +30mm), obtained using the technique are shown for a healthy control subject (Figure 5.4), Alzheimer patient (Figure 5.5) and Korsakoff patient (Figure 5.6). In the Alzheimer patient, reduction in relative flow can be seen in fronto-temporal and parietal areas, while in the Korsakoff patient flow is reduced in frontal regions.

5.1.3.2 Positive predictive value of relative rCBF measurements

As explained in section 4.4.2, in order to determine the clinical utility of an investigative test, it is useful to define a 'normal range' of values. This can be established by defining 'normal' cut-off values as two standard deviations from the mean value of the healthy subjects; if normally distributed, such a range will encompass 95% of the individual subjects without disease. Using

this method lower 'normal' cut-off values were calculated for each ROI below which relative rCBF values were defined as abnormal. Table 5.6 shows the lower cut-off value of relative rCBF for each ROI and the number of AD and KOR patients below this cut-off. The extent of overlap of individual results between the AD group and healthy controls is reflected in the small numbers of AD patients below the cut-off. Only in the right and left posterior temporal groups are there 50% or more patients with values outwith the normal range. For the KOR group, most patients have relative rCBF values within the range of normal values for every ROI.

Positive predictive values (Griner et al 1981), or the probability that the disease is present (viz AD or KOR) when the relative rCBF value is below the cut-off are shown in Table 5.6 for each ROI in AD and KOR. Due to the degree of overlap of individual patient values with those of the 'normal range', the positive predictive values are only greater than 50% in posterior temporal regions of AD patients.

5.1.3.3 Neuropsychological scores

Total CAMCOG and MMSE scores were reduced in both AD and KOR patient groups (Table 5.3), although the pattern of subtests affected was different in AD and KOR, reflecting the more limited cognitive deficit associated with KOR, compared to AD. In all areas of cognitive function, CAMCOG subscores were reduced in AD patients compared with controls, reflecting their more global impairment (Table 5.3). Korsakoff patients also had impaired scores in many cognitive subtests in particular, orientation, recent memory and memory for newly learned information ('memory-learned'), but had better preserved function in many areas, including comprehension, expression, attention, praxis and calculation where scores were similar to controls (Table 5.3).

TABLE 5.3

Neuropsychological scores for patients and controls in basal SPECT study

	CONTROL		AD		KOR		K-W
	Median	Range	Median	Range	Median	Range	ANOVA
Orientation	10	10-10	4.5***	0-9	4.5**	1-10	**
Comprehension	9	9-9	6.5**	0-9	9	8-9	**
Expression	20	19-21	13***	0-19	17	16-20	**
Language	29	28-30	19***	0-27	26*	24-29	**
Memory (Remote)	6	5-6	1.5***	0-5	4*	2-6	**
Memory (Recent)	4	4-4	3***	0-3	2.5**	1-4	**
Memory (Learned)	14.5	13-17	3***	0-7	6**	0-14	**
Total Memory	24.5	23-26	7.5***	0-15	13**	4-24	**
Attention	7	5-7	1.0***	0-4	6.5	3-7	**
Praxis	12	12-12	7.5***	0-11	11	7-12	**
Calculation	2	2-2	1**	0-2	2	2-2	**
Abstraction	8	6-8	5.5**	0-8	5*	3-8	*
Perception	11	9-11	7.5**	0-11	9**	4-10	*
CAMTOTAL	102.5	99-105	51***	0-81	75**	56-99	**
MMSE	30	29-30	12.5***	0-24	20.5**	11-29	**

Results are presented as median and range; Kruskal-Wallis one way ANOVA, *$p < 0.001$; **$p < 0.0005$. All post-hoc comparisons between controls and patients were made using Mann-Whitney U tests, *$p < 0.005$; **$p < 0.001$; ***$p < 0.0001$.

TABLE 5.4

Relationship between HMPAO uptake and neuropsychological scores in Alzheimer patients

	RF	LF	RHF	LHF	RT	LT	RPT	LPT	RP	LP	RBG	LBG
Orientation	0.15	-0.03	0.63	0.35	0.29	0.02	0.72*	0.54	0.63	0.60	-0.02	0.05
Comprehension	-0.15	-0.12	0.18	0.28	0.15	0.11	0.58	0.76**	0.38	0.94***	-0.06	0.03
Expression	0.11	0.16	0 .13	0.13	0.43	0.18	0.23	0.44	0.70	0.85**	0.33	0.49
Language	0.08	0.17	0.12	0.15	0.42	0.27	0.29	0.51	0.62	0.97***	0.29	0.38
Memory (Remote)	0.09	0.06	0.14	0.14	0.24	0.20	0.46	0.83***	0.55	0.86**	0.03	0.38
Memory (Recent)	0.12	0.07	0.73	0.64	0.30	0.02	0.21	0.09	0.59	0.46	0.30	0.16
Memory (Learned)	0.39	0.51	0.57	0.61	0.31	0.56	0.08	0.49	0.37	0.68	0.36	0.32
Total Memory	0.27	0.27	0.47	0.43	0.36	0.36	0.33	0.51	0.60	0.80**	0.24	0.28
Attention	-0.08	-0.18	-0.04	-0.10	0.22	-0.05	0.66	0.85***	0.65	0.87**	-0.17	0.27
Praxis	-0.09	-0.12	0.19	0.17	0.29	0.06	0.64	0.85***	0.72	0.95***	-0.04	0.26
Calculation	0.08	0.05	0.11	0.05	0.17	0.34	0.45	0.72*	0.47	0.79*	-0.08	0.10
Abstraction	0.12	0.30	0.48	0.69	0.40	0.22	0.04	0.25	0.32	0.64	0.55	0.39
Perception	-0.08	-0.18	0.09	-0.09	0.24	-0.12	0.58	0.48	0.55	0.67	-0.12	0.08
CAMTOTAL	-0.06	-0.09	0.20	0.15	0.31	0.03	0.64	0.67	0.68	0.95***	0.01	0.18
MMSE	0.04	-0.08	0.54	0.43	0.30	-0.04	0.72*	0.58	0.68	0.74	0.07	0.15

Spearman's Rank Correlation Coefficients: *$p < 0.01$; **$p < 0.005$; ***$p < 0.001$. See text for meaning of abbreviations.

TABLE 5.5

Relationship between HMPAO uptake and neuropsychological scores in Korsakoff patients

	RF	LF	RHF	LHF	RT	LT	RPT	LPT	RP	LP	RBG	LBG
Orientation	0.59	0.69	0.78*	0.78*	0.68	0.59	0.39	0.46	0.14	0.43	0.33	0.04
Comprehension	-0.52	-0.43	0	0	-0.17	-0.43	-0.09	-0.17	0.41	0.41	-0.35	-0.43
Expression	0.31	0.20	0 40	0.25	0.36	0.06	0.19	-0.03	0.72	0.60	0.55	0.29
Language	0.06	-0.01	0.37	0.24	0.20	-0.12	0.06	-0.14	0.73	0.61	0.31	0.06
Memory (Remote)	0.40	0.40	0.57	0.53	0.48	0.21	0.17	0.36	0.59	0.78*	0.50	0.22
Memory (Recent)	0.36	0.27	0.82*	0.73	0.40	0.23	-0.09	0.09	0.38	0.67	0.32	0.11
Memory (Learned)	0.24	0.29	0.76	0.76	0.48	0.32	-0.21	0.36	0.12	0.59	0.24	0.15
Total Memory	0.33	0.32	0.80*	0.75	0.48	0.30	-0.15	0.31	0.30	0.70	0.34	0.20
Attention	-0.01	-0.12	-0.02	-0.10	0.29	0.14	0.32	-0.26	0.10	-0.15	0.09	-0.09
Praxis	-0.16	-0.04	0.17	0.15	0.31	-0.22	0.25	-0.32	0.22	0.27	-0.06	-0.40
Abstraction	0.36	0.30	0.14	0.02	0.34	-0.26	0.46	0.20	0.91**	0.70	0.67	0.41
Perception	-0.30	-0.16	0.76	-0.62	0.02	0.05	-0.22	-0.09	0.42	0.64	0.16	0.05
CAMTOTAL	-0.26	-0.25	0.71	0.64	0.47	-0.21	0.02	0.14	0.40	0.63	0.34	0.12
MMSE	0.47	-0.46	0.76	0.69	0.55	0.43	0.24	0.24	0.27	0.45	0.30	0.05

Spearman's Rank Correlation Coefficients: *$p < 0.01$; **$p < 0.005$; ***$p < 0.001$. See text for meaning of abbreviations.

TABLE 5.6

Positive predictive value of relative rCBF in regions of interest for AD and KOR patients

	RF	LF	RT	LT	RPT	LPT	RBG	LBG	RHF	LHF	RP	LP
Lower limit of normal relative rCBF value	0.74	0.69	0.80	0.81	0.83	0.87	0.93	0.86	0.74	0.71	0.81	0.82
Number of AD patients below normal cut-off value	0	0	1	3	6	8	2	1	2	1	2	3
Positive predictive value for AD (%)	0	0	8.3	25	50	67	17	8.3	17	8.3	17	25
Number of KOR patients below normal cut-off value	1	0	0	1	0	0	2	1	0	0	0	1
Positive predictive value for KOR (%)	8.3	0	0	8.3	0	0	17	8.3	0	0	0	8.3

Total number of healthy controls studied was 12, AD patients 12 and KOR patients 10.
Positive predictive value (%) is number of patients with relative rCBF value below cut-off value divided by total number of patients.
Abbreviations explained in text.

5.1.3.4 Association of HMPAO uptake and cognitive function

The correlation matrix of relative ^{99m}Tc-HMPAO uptake and cognitive scores for AD is shown in Table 5.4. Because of the large number of multiple comparisons, only correlations where $p < 0.01$ are highlighted. There are sixteen correlations at $p < 0.01$ where only two would be expected by chance. However, in addition, positive correlations showed patterning to particular cortical areas. For example clustering of positive correlations occurred for tracer uptake into left parietal (LP) cortex, where all values of the correlation coefficient, r, were positive (Table 5.4).

There were also significant associations between reduced LP flow and language function (including expression and comprehension), remote and total memory scores, attention and praxis. Association with right parietal tracer uptake, although showing positive correlates, were much less striking (Table 5.4).

Left posterior temporal cortex showed a similar pattern of associations to LP, confirming a general tendency for impairment of left lateral hemispheric function to be associated with reduced cognitive performance. Uptake in frontal areas (RF, LF, RHF, LHF) and basal ganglia did not show statistically significant correlations with performance for AD patients.

In the correlation matrix of relative uptake and cognitive scores for KOR patients there were six correlations at $p < 0.01$ (Table 5.5). Again, there were apparent patterns of positive correlation. In common with AD, uptake of tracer in left parietal cortex again tended to be correlated with scores for language and memory function. There was, however, no clear discrepancy between LP and RP. Furthermore, but again unlike AD, there was a cluster of r values apparently associating reduced flow in high frontal areas with impaired memory and orientation. Other areas showed little evidence of association between performance and rCBF.

5.1.4 Discussion

This study presents quantitative estimates of relative rCBF in presenile AD, Korsakoff's psychosis and controls using the intravenous ligand ^{99m}Tc-HMPAO. In summary the following conclusions can be drawn from this work; first, relative rCBF was bilaterally reduced in posterior temporal and parietal cortical areas in presenile Alzheimer's disease but not in Korsakoff's psychosis; second, apparently meaningful correlations exist between cognitive function in AD and relative rCBF in the cortical areas most affected; third, in KOR, the prominent impairments of orientation and memory that characterise the disease were correlated with impaired relative rCBF in frontal regions.

The aim of the present investigation was not to determine the accuracy of the diagnostic usefulness of SPECT imaging. To do this a different study design would be required where all patients with dementia were investigated, not just two carefully selected groups, and from which sensitivity and specificity could be calculated. Although the present study design does not allow sensitivity or specificity to be calculated, it does yield positive predictive values (PPV) - i.e. the likelihood of the disease (AD or KOR) being present when relative rCBF is low. As table 5.6 shows, PPVs are low for all brain regions studied with the exception of the posterior temporal regions in AD. The numbers of subjects investigated in this study were small and results need to be treated with caution. In a larger study the 'normal range' could be defined with greater accuracy and PPVs confirmed. Nevertheless the overlap of individual test results between groups was marked casting considerable doubt over the diagnositc utility of SPECT in dementia. This does not mean that SPECT may have no clinical utility. There may be value in knowing the pattern of perfusion deficit in individual patients, not with a view to diagnosis, but in providing information on which brain areas are likely to be dysfunctional. This is supported by the association of specific cognitive impairments and particular brain regions (discussed below).

In conclusion the findings suggest that SPECT may have limited potential in the clinical assessment of dementia. However they do support the view that SPECT can provide measures of brain function that may allow its further experimental application in organic brain disease and possibly also in the so-called 'functional' psychiatric disorders.

5.1.4.1 Alzheimer's Disease

There have been previous reports of non-quantitative application of SPECT in AD (see section 2.2.5.2), but not in KOR. Intravenous ligands including N-isopropyl-p-iodoamphetamine (IMP) labelled with ^{123}I (Jagust et al 1987; Johnson et al 1987) and ^{99m}Tc-HMPAO (Gemmell et al 1987; Neary et al 1987) have shown qualitative defects in posterior temporal blood flow. In a SPECT study of senile AD patients using ^{99m}Tc-HMPAO, significant reductions in rCBF were found in left and right posterior temporal regions, and left temporal and left parietal regions (Montaldi et al 1990). As in the present study of younger AD patients, these authors noted that in senile AD patients, rCBF was often also reduced in frontal regions. These results are consistent with earlier studies and suggest that Alzheimer's disease pathology may affect frontal lobes, although some authors have preferred to consider 'frontal dementias' as a separate nosological entity from AD (Neary et al 1988). Prospective studies in which the progression of the brain dysfunction in probable AD patients is followed using SPECT or PET, with neuropathological examination after death is required to resolve these difficulties.

Quantitative PET studies have shown reduced regional cerebral metabolic rates for glucose (rCMRglc), oxygen ($rCMRO_2$) and rCBF in temporo-parietal cortex in AD of mild-moderate severity (eg Frackowiak et al 1981; Duara et al 1986). In more severe cases, frontal regions were also significantly affected (Friedland et al 1983; Duara et al 1986). The percentage changes in regional metabolism revealed by PET in such studies

are of the same order as the relative changes documented in the present study using SPECT. Neuropathological studies of patients with moderate to severe AD (section 1.6) show that temporal, parietal and frontal lobes are the areas of neocortex most severely affected by neurofibrillary tangles, plaques and neuronal degeneration (Pearson et al 1985). This corresponds with the effects on CBF seen here and in previous studies.

5.1.4.2 Korsakoff's Psychosis

There have been few imaging studies of Korsakoff's psychosis. This is, to the knowledge of the author, the first employing SPECT and ^{99m}Tc-HMPAO. The patients in the present study were selected on the basis of operationally defined psychopathological features and abstinence from alcohol. There are difficulties in ensuring a reasonably homogeneous KOR group (see section 3.2.2), yet few other studies have attempted to address this problem.

The results of this study show that, unlike in the AD group, relative rCBF in posterior temporal areas was maintained in the KOR group. There was also little reduction in rCBF in parietal and temporal regions compared with healthy controls, and normal flow in basal ganglia. However there was a tendency for other areas to show reduced blood flow compared with controls with the most marked local trend in higher frontal areas. The probable functional significance of reduction in relative flow in this region may be supported by its apparent correlation with impairment of cognitive function. Victor et al (1971) has reported, that the microscopic pathological lesions in KOR are subcortical, with the amnesic syndrome particularly associated with lesions of the medial dorsal nucleus and pulvinar of thalamus and mamillary bodies. However, in the Victor study some generalised cortical atrophy was also noted in 25% of cases and recent studies using x-ray CT imaging also suggest that cortical atrophy occurs in KOR (Cala & Mastaglia, 1980; Carlen et al 1981). As will be discussed below, cortical atrophy can confuse the

interpretation of rCBF measures especially if atrophy is regionally selective. In one autopsy study of alcoholic patients, selective frontal atrophy was present in 33% of cases (Harper, 1983). The same author subsequently reported marked reduction in frontal white matter and ventricular enlargement of approximately 50% (Harper et al 1985), a finding consistent with that from the CT studies. Using quantitative counting techniques, Harper et al 1987, has also shown a 22% reduction in the number of neurones in the superior frontal cortex of alcoholics. In confirmation of these findings, magnetic resonance imaging (MRI) studies have demonstrated increased proton spin-lattice relaxation time (T_1 values) for frontal and parietal white and grey matter in KOR patients (Christie et al 1988), a finding which may reflect increased tissue water. One hypothesis that links the subcortical and cortical lesions of KOR patients is that deafferentation may occur secondary to the destruction of sub-cortical projection nuclei. Thus, it is possible that white matter changes and/or cortical atrophy, may account for the regional frontal impairment of CBF detected in the KOR patients. The ability to align accurately corresponding CT or MR images with SPECT should help to resolve the issue of atrophy.

Several studies suggest that cortical rCBF is globally reduced in patients who have taken excessive quantities of alcohol over long periods (eg Berglund, 1981), and Xe contrast computed tomography (Hata et al 1987) has suggested that alcoholic patients have widespread reduction in rCBF throughout cortical and subcortical structures. Such flow changes, may in part, reflect metabolism, for in a small preliminary study of KOR patients using PET, absolute rates of regional cerebral glucose metabolism were reduced in cortical and subcortical regions (Kessler et al 1984).

The findings from this, and the other imaging studies described above suggest strongly that in KOR, there is not only subcortical damage, but frontal pathology also. This is not unexpected when clinical information is considered; Korsakoff patients show not only memory impairment but usually

also changes in behaviour (e.g. apathy, inertia, loss of insight) indicative of frontal dysfunction. The frontal changes may also contribute to the amnesic syndrome (Moscovitch, 1982), as well as to other cognitive changes. In cognitive tests sensitive to frontal lobe lesions, such as verbal fluency and category sorting tasks, patients with KOR performed less well than other alcoholics and this was related to radiological measures of frontal atrophy (Jacobson, 1989).

5.1.4.3 Relationship between patterns of rCBF and neurpsychological function

The biological measurement, relative rCBF, (similarly net MTT in chapter 4) is confounded by counting variability, head positioning, head movement, ROI identification and the reconstruction algorithm used. Similarly cognitive subtest scores are influenced by patient and tester variability on the day when the test was administered. Although CAMCOG subtests are designed to assess particular areas of cognitive function, it should be recognised that any single subtest performance is also influenced by other cognitive functions. This is a complication of most neuropsychological test batteries and is not unique to CAMCOG. Therefore as both the biological and psychological measures are imprecise, it was not thought meaningful to test specific relationship hypotheses *a priori*. Examination of *general* relationships between biological function in particular brain areas and cognition offers a more cautious approach. This approach to the analysis, together with an adjusted significance level to account for multiple comparisons, may generate more specific hypotheses to test in future studies.

In both AD and KOR, correlations between neuropsychological function and relative rCBF could be demonstrated. In AD, left parietal and left posterior temporal regions were correlated with scores on a range of sub-tests of CAMCOG. These effects may reflect language dysfunction due to reduced

rCBF in dominant hemisphere association areas. While previous PET studies have shown an association between regional cortical metabolism and neuropsychological performance in AD patients, in many studies only Alzheimer patients with specific cognitive deficits were selected for inclusion (Foster et al 1983, 1986; Chase et al 1987; Martin et al 1986). This approach is quite different from that employed in this study, where unselected presenile probable Alzheimer patients fulfilling strict clinical diagnostic criteria were studied. In other PET studies the association between cognition and cerebral blood flow has been examined; eg Frackowiak et al (1981) found an association between severity of dementia and flow but did not report regional association between neuropsychological function and perfusion. Similarly, Ferris et al (1983) also found an association between severity and rCMRglc. In the present study, associations between specific aspects of cognitive performance and rCBF patterns in unselected cases of presenile AD were detected. Previous work with PET in presenile patients has produced similar evidence of association. Friedland et al (1983) reported that the ratio between right frontal and right temporo-parietal glucose uptake in 10 Alzheimer-type dementia patients was negatively correlated with performance subtests on the WAIS. However, this, and their other significant correlations were quoted in isolation without the number of correlations performed. In this study no left sided associations with language impairment were reported. Haxby et al (1985) used right/left asymmetry ratios for 10 early probable AD patients and demonstrated poorer language performance in patients with relatively decreased left hemisphere function. Preliminary data has also appeared on the association between parietal hypometabolism and visual memory deficits (Riege et al 1985). It should be noted that PET studies with ^{18}F-2DG require a much longer duration of cooperation between subjects and experimenter than do SPECT studies of the type described here. Thus, patients must remain in a constant basal state for up to 45 minutes while the

label accumulates in the brain, and then remain still for a further variable interval while the data continues to be acquired by the camera. Such requirements may be difficult to attain in many, if not most, demented patients. The findings support the earlier work of Gustafson and Risberg (1974), who showed, using intracarotid ^{133}Xe injection, that in dementia organic symptoms correlated with estimates of cerebral perfusion in the dominant hemisphere.

5.1.4.4 Effects of atrophy

The finding of regional reductions in tracer uptake could mean that normal tissue mass has reduced neuronal activity or that reduced tissue mass has normal levels of activity or some mixture of the two. This difficulty arises both for absolute estimates of tracer uptake and, as here, for relative estimates also. It is by no means established that efforts to measure cortical atrophy independently with x-ray CT or MRI are satisfactory; however, when such estimates have been made to correct for lobar atrophy, PET studies still showed significant impairment of rCBF in temporal lobes in AD (Herscovitch et al 1986; Chawluk et al 1987). Nevertheless, it is likely that reduced rCBF in, for example, posterior temporal cortex does reflect reduced structure as well as reduced function at least at the microscopic level of selective cell loss. In KOR, as already described, the evidence suggests that both frontal white and grey matter are affected by chronic alcoholism. Therefore, the finding of reduced tracer uptake using SPECT in frontal regions could reflect structural and/or functional impairment. However, the correlation between impaired cognitive performance and tracer uptake into frontal cortex in KOR may be more likely to reflect impaired function than simply reduced tissue mass. There is a need for studies using structural and functional imaging techniques to study the contribution of atrophy to functional image impairment. There are technical difficulties however in ensuring that scan images from the two

imaging types are comparable and represent the same brain region or same anatomical plane.

5.1.4.5 Conclusion

Although there is no support from this study for the diagnostic utility of SPECT, the present results indicate the potential for detecting and following the course of differential regional cerebral involvement in AD and KOR, and this may have clinical, as well as, experimental utility. The associations demonstrated between tracer uptake and cognitive function suggest the prospect of investigation of cerebral function in other major psychiatric disorders. The potential of SPECT may have to await the development of selective ligands either to label receptors or to allow more flexible dynamic studies of blood flow and metabolism. The present findings set the scene for more dynamic studies of the relationship between performance and neuronal activity in the dementias using SPECT or similar techniques. In Chapter 6 this approach is further discussed and a number of experiments described that demonstrate the potential of ^{99m}Tc-HMPAO SPECT for imaging the effects of perturbation of basal brain function.

5.2 Regional cerebral hypofunction in the presence of 'normal' structure in Creutzfeldt-Jacob Disease

5.2.1 Introduction

It is crucial to progress in the investigation of brain disease that changes in brain function can be distinguished from changes in brain mass. Estimation of functional activity in the brain can be continuously made by measuring regional brain metabolism (Sokoloff, 1981). Furthermore, recent work has proved that a close relationship exists between regional glucose metabolism and regional cortical blood flow (rCBF) under both resting and stimulated conditions in the human brain (section 2.1). In other words, rCBF is function related and patterns of rCBF can be estimated economically with SPECT using the ligand ^{99m}Tc-HMPAO (Neirinckx et al 1987). However, SPECT, like PET, is subject to partial volume artefacts such that loss of brain tissue may result in reduced apparent rCBF or metabolism in affected brain regions. This problem may be marked for changes measured in Alzheimer's disease (AD) and has cast doubt on the reliability of metabolic or rCBF measurements for estimating regional brain function (Herscovitch et al 1986; Chawluk et al 1987).

Creutzfeldt-Jacob disease (CJD) is a rare transmissable dementia that can be diagnosed with certainty only at post mortem by histological identification of characteristic spongiform degeneration of brain. Investigations, and in particular the electroencephalogram (EEG) and x-ray computed tomography (CT) are often normal until late in the course of the disease (Kuritzky et al 1980; Galvez & Cartier, 1984; Keshaven et al 1987). This structural preservation alongside gross functional impairment suggests that imaging of rCBF with SPECT in CJD may offer an unusual opportunity to highlight the utility of SPECT to distinguish between loss of structure and loss of function in the brain.

5.2.2 Case history

5.2.2.1 Clinical findings

A 66 years old retired electronics worker was referred by his GP for investigation of increasing forgetfulness which had become noticeable over six months. When first seen in December 1986, he complained of episodes of memory loss when he would forget what he was talking about. He was fully orientated but unable to recall the name of the Prime Minister or US President. He could not give his date of birth or the dates of World War 2, but did remember that he had served in the Army Dental Corps where he had worked as a dental mechanic. He showed moderate nominal dysphasia and poor memory for recent events. There was no left-right disorientation and he satisfactorily drew a complex geometric shape. He scored 21 out of a possible score of 30 in the Mini-Mental State Examination (MMSE) (Folstein et al 1975).

He had no other complaints and was leading a fairly normal retired life. Two years previously he had undergone prostatectomy for benign prostatic hypertrophy and recovered satisfactorily. He had been treated by his GP for mild angina pectoris for ten years, but there was no history of myocardial infarction or cerebrovascular disease. His only medication was propranolol 20mg and isosorbide dinitrate 30mg per day.

Physical examination was normal and in particular there were no focal neurological signs or evidence of hypertension or other cardiovascular abnormality. The following investigations were all normal: full blood count, ESR, serum electrolytes, renal, thyroid and liver function tests, blood glucose, syphillis serology, serum B12 and folate. Electrocardiogram, chest and skull radiology and EEG were within normal limits for his age. X-ray computed tomography (CT) in February 1987 showed that the ventricular system was normal with no evidence of cerebral atrophy (Figure 5.7). The most likely

diagnosis on this evidence was dementia of presenile onset probably of Alzheimer type.

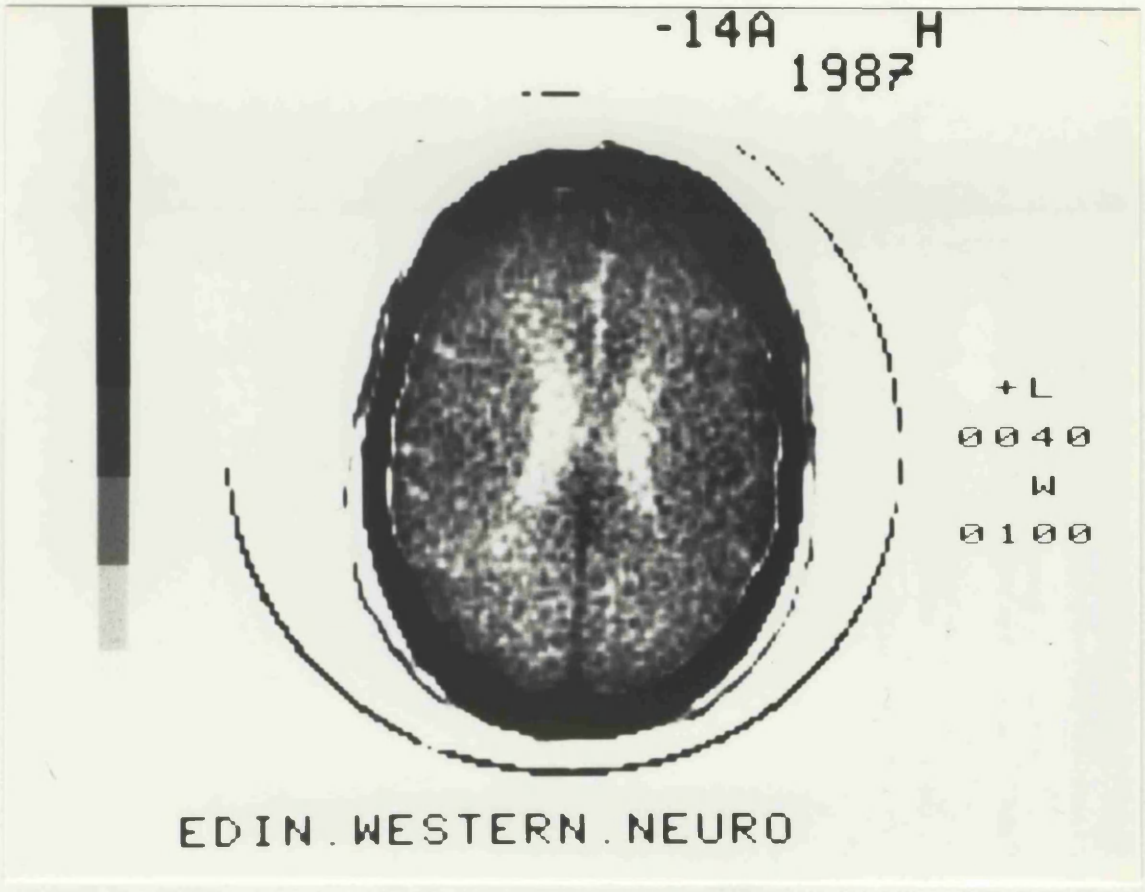
He was reviewed in March 1987 when further significant deterioration was noted; he was now perseverative in answering questions, had great difficulty in remembering his home address and could not name simple objects or describe their use. There was evidence of right-left disorientation and he was unable to draw simple objects, such as a house. MMSE score was 9/30. One month later he was assessed at home where he needed continual help with bathing, toileting, feeding and dressing. Although still fully mobile he was noted to have a shuffling gait and difficulty climbing stairs.

In May 1987 he was admitted to the Brain Metabolism Unit for further investigation. He was agitated and unable to cooperate with even simple forms of psychological testing. It was difficult to follow his speech as he now had marked expressive and receptive dysphasia. His behavioural disability in the ward was assessed daily using the Behavioural Rating Scale of the Clifton Assessment Procedures for the Elderly (CAPE) (Pattie & Gilleard, 1979). Whereas an independent, fit elderly person would be expected to score 0-3, our patient had daily scores ranging from 17-23 consistent with severe impairment and maximum dependence.

Single photon emission computed tomography (SPECT) was carried out using ^{99m}Tc-HMPAO in early May 1987, and showed marked reduction in the tracer uptake throughout the grey matter of the cerebral cortex especially in frontal regions (Figure 5.8). This was qualitatively obvious but was also confirmed quantitatively by determining count density relative to occipital cortex. Quantitative estimates of relative flow in frontal regions were calculated and compared to that of age matched controls (eg see Table 5.2), showing marked reduction: right high frontal 0.55 (normal range 0.88-1.40); left high frontal 0.51 (normal range 0.91-1.46). These images of function are in striking contrast to the normal x-ray CT images (Figure 5.7). Over the next

Figure 5.7 CT images of patient with Creutzfeldt-Jacob disease

X-ray computed tomography scans, in a plane +40mm (lower image), and +80mm (upper image) above the orbito-meatal line, showing details of brain structure in patient with Creutzfeldt-Jacob disease.


-14A H
1987
+L
0040
W
0100
EDIN.WESTERN.NEURO

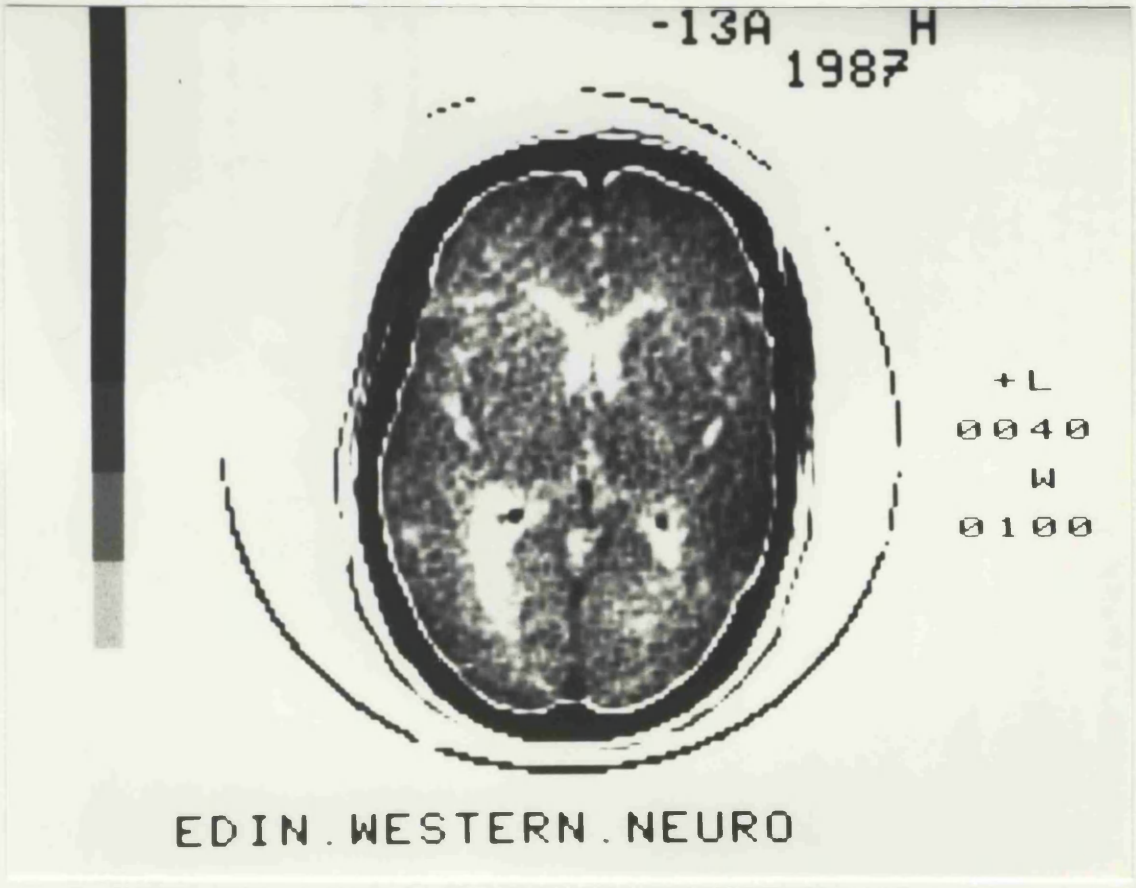
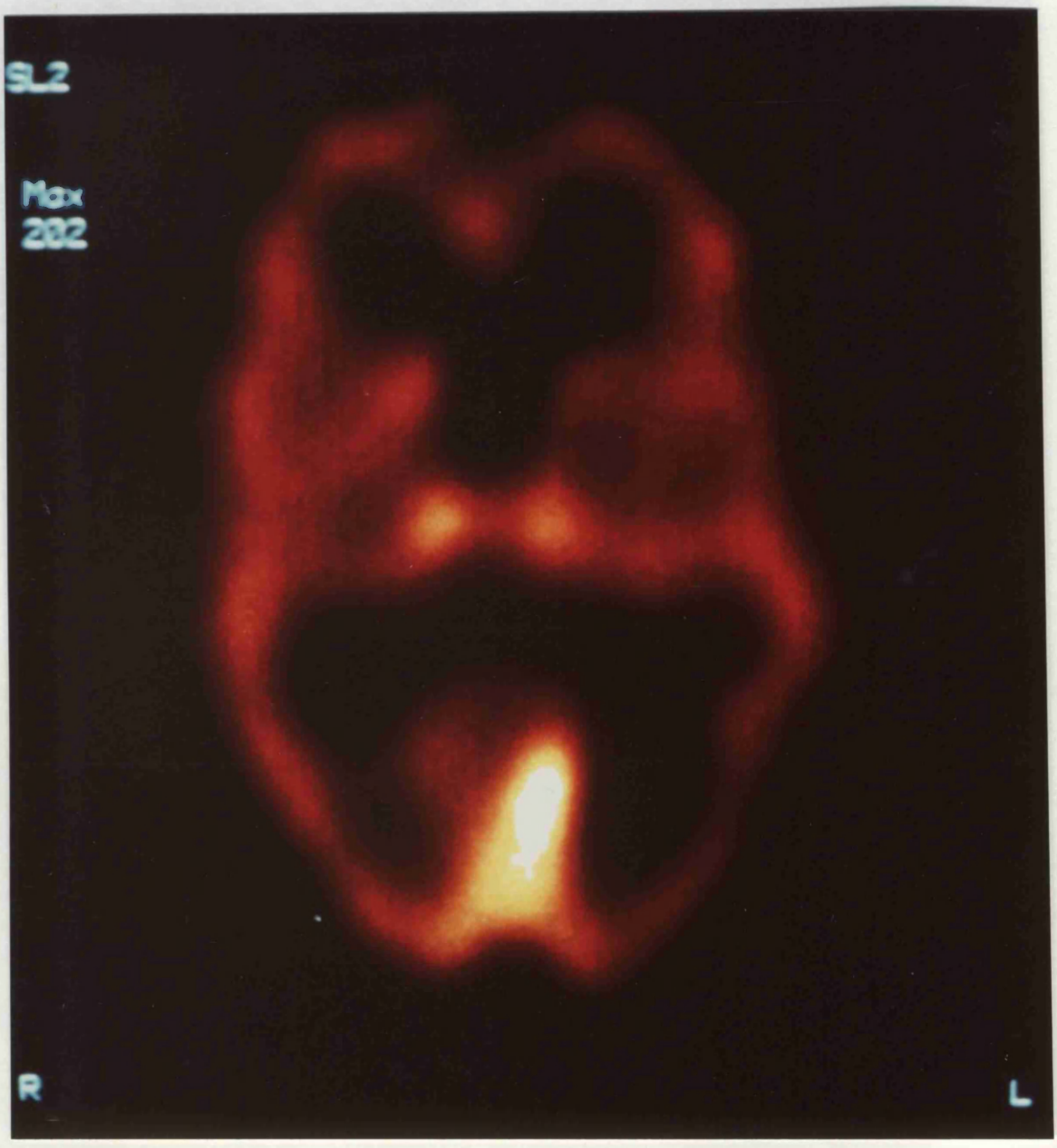
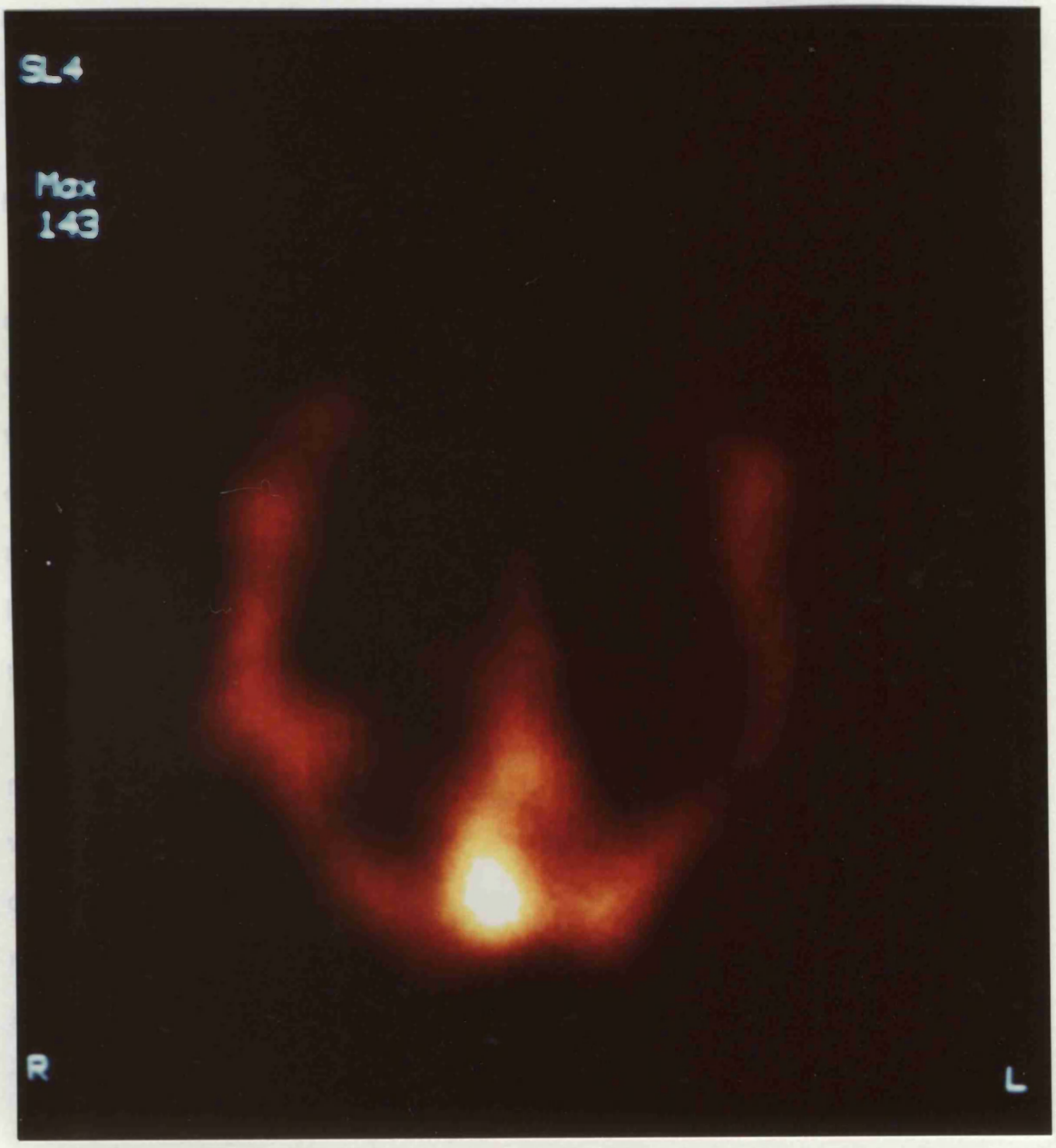

-13A H
1987
+L
0040
W
0100
EDIN.WESTERN.NEURO

Figure 5.8 SPECT images of patient with Creutzfeldt-Jacob disease

Transverse sectional SPECT images at +30mm (first image) and +70mm (second image) superior to the orbito-meatal line for patient with Creutzfeldt-Jacob disease


SL2
Max
282
R
L


SL4
Max
143
R
L

few months the patient deteriorated further and he died at home in October 1987, ten months after initial presentation. As the course of the illness had been rapid, a diagnosis other than AD was anticipated and post mortem brain examination was performed.

5.2.2.2 Neuropathological findings at necropsy

The brain weighed 1320 grams at macroscopic examination, and was remarkably normal in appearance. No abnormality of dura or leptomeninges was noted and the superior longitudinal sinus was patent. The arterial system was also normal apart from patchy atheroma. The cerebral hemispheres externally and on coronal sectioning showed only mild generalised atrophy and the lateral ventricles slight dilatation. There were no focal abnormalities in cortex, white matter or basal ganglia and brain stem, cerebellum and spinal cord were all normal in appearance.

After fixation, blocks from every brain region were examined microscopically. Widespread, severe neuronal depletion and vacuolation with rod cell infiltration and astrocytic gliosis was noted throughout the cerebral cortex. Spongiform degeneration was most striking in the supra-medial border of the frontal lobes bilaterally. The basal ganglia, particularly putamen and caudate, were also affected but the hypothalamus was relatively normal. The cortico-spinal tracts were also severely affected but mid-brain showed no evidence of involvement.

In summary, the neuropathological findings were consistent with a diagnosis of Creutzfeldt-Jacob Disease and excluded other causes of dementia.

5.2.3 Discussion

Since most investigations in this patient were normal, the rapidity of deterioration was the only real clinical clue to diagnosis. Even in advanced CJD, x-ray CT imaging is often normal and poorly correlated with clinical condition (Galvez & Cartier, 1984). In this case the CT appearances were entirely consistent with the normal macroscopic appearance of the brain at post mortem. On the other hand, the cross-sectional SPECT images, at comparable levels, were grossly abnormal and quite atypical of other dementias such as Alzheimer's or Korsakoff's disease (eg Figures 5.5 and 5.6). Unlike the EEG or CT findings, SPECT imaging suggested a severe and widespread disorder of brain function.

The reduced rCBF in frontal cortex detected by SPECT in this patient is likely to reflect reduced neuronal metabolism because the histological findings at post mortem demonstrated widespread microscopic evidence of CJD pathology, especially in frontal lobes, but there was no important change in tissue mass. It has been suggested that functional imaging techniques such as SPECT and PET merely reflect cortical atrophy (Herscovitch et al 1986), and at least in Alzheimer's disease, reduced function and atrophy may often be co-localised. However, this case illustrates that estimation of regional brain function with SPECT can provide additional information when structural brain imaging is apparently normal or at least not grossly abnormal and macroscopic brain structure apparently preserved.

CHAPTER 6

USING SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY TO STUDY THE EFFECTS OF INTERVENTION ON CEREBRAL FUNCTION

6.1 Cerebral pharmacodynamics of physostigmine in Alzheimer's Disease investigated using ^{99m}Tc-HMPAO SPECT imaging

6.1.1 Introduction

There is compelling evidence from many different studies (Perry, 1986; Rossor et al 1984; Sims et al 1983) linking cholinergic neuronal function and memory. Thus, the cholinergic hypothesis of dementia attributes cognitive impairment to defects in the synthesis of acetylcholine (ACh) in central cholinergic neurones (Davies & Maloney, 1976; Davies, 1979). Support for this hypothesis comes from post-mortem neurochemical studies of Alzheimer's disease (AD) which have shown a marked reduction in choline acetyl transferase (ChAT), the rate limiting enzymic step for the synthesis of ACh (Bowen et al 1983), and also confirmed the reduced production of ACh in such patients (Richter et al 1980). Accordingly there has been considerable interest in pharmacological treatment strategies aimed at facilitating cholinergic function. Although three types of strategy are possible, (precursor-loading, inhibition of ACh catabolism, and the use of cholinergic receptor agonists), the more successful results have been obtained using cholinesterase inhibitors to enhance central cholinergic function, and there have been several reports of improved memory/cognition following administration of the anticholinesterase physostigmine (Christie et al 1981; Davis & Mohs, 1982). Another anticholinesterase drug, tetrahydroaminoacridine (THA), which may act in a similar way to physostigmine, also appears to improve memory/cognition in Alzheimer's disease, (Summers et al 1986; Byrne & Arie, 1989) and has renewed interest in the use of pharmacological strategies to improve cognitive function in Alzheimer patients (Whalley, 1989).

Unfortunately, psychological assessment of improvement in demented patients is difficult because exhaustive batteries take so long to administer while short tests, for example of memory function, may show prominent floor or ceiling effects. Hence there can be many reasons for the failure of such tests to show an improvement, apart from simply lack of drug efficacy. In part, this must contribute to the inconsistent results reported from drug studies (Stern et al 1987). There is, therefore, a need for methods of assessing drug action which are more biologically based and hence less susceptible to the difficulties associated with current psychological test batteries.

Single photon emission tomography using the blood flow marker, HMPAO, permits regional patterns of cerebral blood flow (rCBF) to be measured in vivo. In chapters 2 and 5 evidence is presented suggesting that the reductions in rCBF in dementia are quantitatively related to deficits in neuropsychological function, therefore SPECT, by evaluating functional effects in brain, may provide a useful quantitative adjunctive measure of drug action. The aim of this study was to use SPECT imaging to examine the effects of administration of physostigmine in demented patients. In particular five specific hypotheses were investigated in the study:

1) As rCBF usually reflects underlying neuronal metabolism, the effect of physostigmine will be to increase cerebral uptake of 99mTc-HMPAO.
2) As the pattern of functional impairment in AD is focal, the effects of physostigmine may be regional.
3) Furthermore as cognitive functions tend to be lateralised, physostigmine may change left / right asymmetry patterns.
4) Physostigmine requires an intact cholinergic system, pre and post-synaptic for drug action; accordingly there will be a consistent relationship between change in relative ^{99m}Tc-HMPAO uptake after physostigmine and basal uptake patterns.

5) An association may exist between cognitive impairment and the pattern of relative uptake after physostigmine, such that less impaired patients and/or those with short illness duration will show larger increases in relative uptake.

6.1.2 Methods

6.1.2.1 Study design

The study had two complementary phases:

(1) Preliminary Dose-Finding Phase.

In the first phase, patients' cognitive performance was assessed double blind on three separate test occasions, with a different physostigmine dose (0.5mg, 0.375mg and placebo) on each occasion. Before and after each test infusion a short battery of tests, designed for assessing the psychological effects of drugs, was administered to each subject (Wesnes et al 1987). To avoid practice effects parallel forms of this test battery were used. Each patient had one practice session and was then tested before and after each infusion. Using this phase of the study, that dose which produced the maximum increase in cognitive performance was determined and used in the second phase. Where no change could be detected the higher dose (0.5mg) of the drug was employed.

(2) Single photon emission tomography after drug infusion

In the second phase which was single-blind, patients had SPECT brain imaging on two separate occasions, once after a saline infusion containing that dose of physostigmine chosen from preliminary testing, and on a different occasion (one week interval), with a saline infusion containing no drug. In the test retest SPECT reliability study, described in section 6.2 (see Table 6.7), no significant change in ^{99m}Tc-HMPAO uptake was found when patients had a repeat scan after one week. Apart from the presence or absence of active drug, test conditions were identical on each of the two SPECT test occasions.

The order in which patients received physostigmine and placebo infusions was randomised between patients. On both test days, patients were pre-treated with methylscopolamine to prevent the peripheral actions of the anticholinesterase.

6.1.2.2 Patient selection

Seven patients with a diagnosis of probable pre-senile Alzheimer's disease were recruited into the study and are described in the Table 6.1. All patients met agreed criteria for probable Alzheimer's disease as laid down by National Institute of Neurological and Communicative Disorders Stroke (NINCDS) and Alzheimer's Disease and Related Disorders Association (ADRDA) (section 3.2.1). All patients met criteria for right handedness (section 3.2.4). The Cambridge Mental Disorders of the Elderly Examination (CAMDEX) was completed for all patients in the study (section 3.4).

6.1.2.3 Single photon emission computed tomography

All subjects were studied at the Institute of Neurological Sciences, Southern General Hospital, Glasgow using single photon emission tomography (SPECT) and the tracer ^{99m}Tc-HMPAO. This agent shows high fractional uptake into brain and its distribution reflects regional cerebral blood flow (section 2.2.5.1; Neirinckx et al 1987). Its retention in brain without apparent redistribution for many hours, allows a 'snap-shot' image of cerebral blood flow pattern to be determined at a particular interval in time such as during drug infusion.

HMPAO was used as described in section 3.6. Injection of HMPAO was administered 20 minutes after the start of drug infusion, in a quiet room with subjects resting, eyes closed and ears unplugged. Imaging was carried out within 30-60 minutes using the Novo 810 brain SPECT system. Internal landmarks were used in the final definition of the 'low' (usually at +30mm

TABLE 6.1

Description of Alzheimer patients imaged after physostigmine

Patient No.	Age	Sex	Duration	CAMTOTAL (years)	Physostigmine Dose (mg)
1	65	F	6.0	39	0.375
2	57	M	1.5	66	0.375
3	55	F	2.5	58	0.375
4	62	F	6.0	40	0.500
5	58	M	6.5	40	0.375
6	60	F	1.5	77	0.375
7	58	F	3.0	55	0.500

above the OM line) and 'high' (usually at +70mm above OM line) slices. Symmetrical paired regions of interest (ROIs) were defined, representing frontal, temporal, posterior temporal, occipital and basal ganglia on the low slice, and high frontal and parietal on the high slice. These are illustrated in Figure 3.7.

6.1.2.4 Comparison of amount of HMPAO within ROIs

The technique of SPECT imaging with HMPAO provides measurements of the pattern of CBF distribution and not absolute values of CBF. Data normalisation is therefore necessary when making comparisons within groups. Two methods were used in this study: 1) adopting white matter (WM) as a control region and calculating the ratio of mean counts within cortical regions to mean counts within the WM region and 2) using an asymmetry index (AI), which is defined as the ratio of counts in each left sided ROI divided by the counts in the corresponding right sided ROI.

Method 1 The cortical/WM ratios

The amount of ^{99m}Tc-HMPAO in grey matter (GM) and white matter (WM) ROIs was determined as described above in 6.1.2.3. A special colour scale was developed to highlight contrast in low count regions, such as WM, and this was used consistently throughout the study. The WM ROI was outlined medial to high frontal cortex. A fronto-parietal GM ROI was also examined that encompassed both the high frontal and parietal ROIs of the high slice, as shown in Figure 3.7. The ROIs were outlined blind as to whether placebo or physostigmine images were being analysed and the order of analysis of images was random. The mean count density within the WM ROI was measured and then corrected using the equation,

$$W' = W - C/3$$

where W' = true count density of HMPAO in WM

W = measured count density of HMPAO in WM (which is affected by scatter)

and C = measured count density of HMPAO in adjacent high frontal cortex.

This equation was derived empirically using a physiologically realistic phantom which was filled with known concentrations of radioisotope (Wyper et al 1988). This procedure was used in order to give more accurate cortical/WM ratios, but the data was also analysed with no correction factor applied and the significance levels for change in this ratio during placebo and physostigmine conditions remained valid. Cortical/WM ratios during placebo and physostigmine infusions were approximately normally distributed and were therefore compared using Student's paired t-test (two-tailed). Hypotheses were also investigated using Pearson's product moment correlational method.

Method 2 The asymmetry index (AI)

The AI was used because it is a robust measure of asymmetrical change in pattern, and therefore suited for the detection of such changes after physostigmine. In order to ensure that both left and right ROIs had the same area for analysis of counts, the ROI drawn on one side of the brain image was automatically mirrored on to the other side. The AI was defined as the ratio of counts in each left sided ROI to the counts in the corresponding right ROI. Asymmetry indices during placebo and physostigmine infusions were approximately normally distributed and were compared using Student's paired t-test (two-tailed).

6.1.2.5 Physostigmine Infusion

All patients were given 0.2mg of methylscopolamine subcutaneously 20 minutes before the start of the infusion in order to antagonise peripheral muscarinic receptors, as methylscopolamine does not cross the blood brain barrier. The order in which patients received physostigmine and placebo, was randomised between subjects. Both the placebo and physostigmine infusions were administered intravenously via a forearm cannula and the rate of flow controlled using an IVAC volumetric control pump to ensure delivery of the 100ml volume over 30 minutes. The dose of physostigmine (Table 6.1) was administered in a volume of 100ml of normal saline, and prepared by the Pharmacy Department of the Royal Infirmary of Edinburgh such that the investigator was blind to whether physostigmine had been added to or omitted from the infusion on each of the two test occasions. Electrocardiographic and blood pressure measurements were monitored throughout the test procedures.

6.1.3 Results

Physostigmine infusions were well tolerated by all patients. The only detected side effect was dry mouth, which probably reflects effective peripheral muscarinic blockade by methylscopolamine. Measurements of heart rate and blood pressure remained constant throughout infusions of saline or physostigmine and for all patients recordings were similar in both conditions.

Table 6.1 shows the age and sex characteristics of the group. Also shown in Table 6.1 is the estimated duration of dementia for each patient (mean duration 3.9, range 1.5 to 6.5 years) and total CAMCOG (CAMTOTAL) score (mean 54, range 39 to 77).

Table 6.2 shows the mean uptake of ^{99m}Tc-HMPAO in bilateral frontal, temporal, posterior temporal, high frontal, parietal, occipital and basal ganglia regions expressed relative to uptake in white matter, for placebo and physostigmine conditions. There were significant increases in relative HMPAO uptake in the physostigmine condition compared with placebo in left frontal ($t = -3.76$, $P < 0.01$), left temporal ($t = -3.86$, $P = 0.01$), left high frontal ($t = 3.85$, $P < 0.01$) and left basal ganglia regions ($t = -3.72$, $P = 0.01$). Although in all other ROI's there was a consistent trend for more uptake in the physostigmine condition, this did not reach significance at the $P = 0.01$ level.

Table 6.3 compares the left / right ratio of HMPAO uptake (asymmetry index, AI) in placebo and physostigmine conditions in frontal, temporal, posterior temporal, basal ganglia, high frontal, parietal and occipital regions. Significant increases in AI occurred in frontal ($t = -4.95$, $P < 0.004$), high frontal ($t = -4.29$, $P = 0.005$) and basal ganglia ($t = -4.12$, $P = 0.006$). Increases in AI may represent increased HMPAO uptake on left side or decreased uptake on the right side. However the increase in cortical / white matter uptake ratios after physostigmine (Table 6.2) suggests that increases in AI are due to increased perfusion in left sided regions.

The hypothesis that change in relative HMPAO uptake after physostigmine may be a function of basal relative uptake was tested using Pearson's correlation coefficients; the correlations are shown in Table 6.4. There were no significant associations at $P < 0.01$. In addition the absence of a clear correlation trend provides little support for the hypothesis. CAMTOTAL score and illness duration were significantly negatively associated (Pearson's $r = -0.952$, $P < 0.001$). There were no significant associations between change in relative HMPAO uptake after physostigmine for any ROI, and CAMTOTAL score or duration of illness (Table 6.5). Similarly there were no significant associations between AI in either placebo or physostigmine conditions and CAMTOTAL or ilness duration (Table 6.6)

TABLE 6.2

Comparison of relative mean 99mTc-HMPAO uptake in different ROIs in placebo and physostigmine conditions.

ROI	PLACEBO	PHYSOSTIGMINE	% CHANGE FROM PLACEBO	SIGNIFICANCE
RF	8.20 +/- 0.49	8.49 +/- 0.64	2.95 +/- 2.53	NS
LF	8.43 +/- 0.56	9.04 +/- 0.68	6.81 +/- 1.80	$p < 0.01$
RT	6.90 +/- 0.66	7.10 +/- 0.52	4.61 +/- 3.90	NS
LT	7.07 +/- 0.82	7.54 +/- 0.75	8.00 +/- 2.50	$p < 0.01$
RPT	5.61 +/- 0.44	5.86 +/- 0.33	5.87 +/- 4.5	NS
LPT	5.72 +/- 0.59	6.27 +/- 0.54	10.82 +/- 4.5	NS
RBG	1.90 +/- 0.23	1.97 +/- 0.18	7.00 +/- 4.74	NS
LBG	1.93 +/- 0.20	2.19 +/- 0.18	16.14 +/- 5.20	$p = 0.01$
RHF	11.89 +/- 1.33	12.21 +/- 1.47	2.04 +/- 2.20	NS
LHF	11.92 +/- 1.41	13.01 +/- 1.50	9.18 +/- 2.68	$p < 0.01$
RP	6.25 +/- 0.34	6.52 +/- 0.34	4.80 +/- 3.90	NS
LP	6.38 +/- 0.43	6.88 +/- 0.45	8.28 +/- 4.19	NS
RO	6.91 +/- 0.68	7.29 +/- 0.60	7.60 +/- 5.88	NS
LO	7.23 +/- 0.62	7.51 +/- 0.57	5.03 +/- 4.30	NS

Data presented as mean values +/- SEM relative to white matter; % change from placebo calculated from individual values taking account of change direction; comparisons between two conditions by student's paired t-test; in order to adjust for multiple comparisons only $p < 0.01$ was deemed significant.

TABLE 6.3

Comparison of Asymmetry Index (AI) for all ROIs in both placebo and physostigmine conditions.

ROI	Placebo	Physostigmine	Significance
Frontal	1.03 +/- 0.04	1.07 +/- 0.04	$p < 0.005$
Temporal	1.01 +/- 0.03	1.05 +/- 0.04	NS
Posterior Temporal	1.01 +/- 0.03	1.07 +/- 0.06	NS
Basal Ganglia	1.03 +/- 0.03	1.12 +/- 0.02	$p < 0.01$
High Frontal	1.00 +/- 0.03	1.07 +/- 0.04	$p < 0.005$
Parietal	1.02 +/- 0.04	1.06 +/- 0.06	NS
Occipital	1.06 +/- 0.03	1.04 +/- 0.02	NS

Abbreviations and calculation of AI explained in text.
Results expressed as mean +/- S.E.M
Comparisons between two conditions by Students paired t-test. To adjust for multiple comparisons only $p < 0.01$ was deemed significant.

TABLE 6.4

Pearson r correlations between basal relative uptake in 99mTc-HMPAO and change in relative uptake after physostigmine in left and right ROIs.

RF	LF	RT	LT	RPT	LPT	RBG	LBG	RHF	LHF	RP	LP	RO	LO
-661	+569	+775	+774	+617	+429	-130	-070	-418	+024	+358	+209	+617	+532

Abbreviations explained in text.
Pearson correlations multiplied by 10^3.
None of the above correlations significant at $p < 0.01$.

TABLE 6.5

Relationship between CAMTOTAL, duration of illness and change in relative 99mTc-HMPAO uptake after physostigmine in left and right ROIs.

	CHANGE IN UPTAKE AFTER PHYSOSTIGMINE													
	RF	LF	RT	LT	RPT	LPT	RBG	LBG	RHF	LHF	RP	LP	RO	LO
CAMTOTAL	+024	+006	-370	-449	-420	-060	+290	-080	-143	-137	-348	-298	-152	-159
ILLNESS DURATION	+219	+241	+569	+667	+602	+203	+455	+314	+329	+383	+575	+561	+328	+306

Abbreviations explained in text.
Pearson correlations multiplied by 10^3. At the $p < 0.01$ level all correlations are NS.

TABLE 6.6

Relationship between CAMTOTAL, duration of illness and asymmetry index (AI) for different brain ROIs in placebo (-) and physostigmine (+) conditions.

	ASYMMETRY INDEX													
	F		T		PT		BG		HF		P		O	
	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)
CAMTOTAL	-236	-206	-447	-467	-245	-381	+521	+168	-242	-222	-056	-087	+266	+312
ILLNESS DURATION	+121	+137	+425	+509	+182	+362	-677	-410	+206	+129	+051	+052	-486	-477

Abbreviations explained in text.
Pearson correlations multiplied by 10^3. At the $p < 0.01$ level all correlations are NS.

6.1.4 Discussion

The results of this study suggest that changes in brain function, produced using specific cholinergic stimulation can be localised *in vivo* using SPECT functional imaging. However as the number of patients studied in this experiment was small, results should be interpreted with caution.

6.1.4.1 Effects of physostigmine

It is unlikely that the changes in rCBF pattern observed in this study were simply due to non-specific vasodilation by physostigmine for, in a study in which CO_2 was used to produce vasodilation of central vessels, blood flow to cortex *and* white matter increased (Davis et al 1983). As Table 6.2 shows, cortical/white matter ratios increased after physostigmine, significantly in left frontal/temporal regions, supporting the view that the observed increases in flow were due to a specific effect in grey matter.

There was no support from the results for the hypothesis that change in HMPAO uptake after physostigmine may be a function of basal uptake patterns (Table 6.4). In order to have a pharmacodynamic effect, physostigmine requires that acetylcholine is synthesised and released from the presynaptic terminal and has available postsynaptic receptor and catabolism system. Histochemical studies suggest that the presynaptic synthesis and release of acetylcholine is reduced in AD (Richter et al 1980; Bowen et al 1983). In such conditions where the intact metabolic substrate does not exist, it is perhaps not too surprising that no clear association exists between basal function and physostigmine-related function. However the number of subjects studied was small and larger numbers are needed to confirm this finding.

A related hypothesis that an association would exist between degree of cognitive impairment, as indicated by CAMTOTAL or duration of illness, and

HMPAO uptake after physostigmine was also investigated. Consequent upon the assumption that an intact metabolic substrate is required, it might be expected that less cognitively impaired patients, with higher CAMTOTAL scores or shorter durations of illness might show relatively greater increases in uptake. However no such association was detected for either CAMTOTAL or duration (Table 6.5), although there was a trend for longer illness durations to be associated with greater increases in HMPAO uptake after physostigmine. Support for this finding comes from a similar study where physostigmine had no effect on HMPAO uptake in brain regions in eight healthy controls (Geaney et al 1990). In this study there was also a trend for the more cognitively impaired patients to show the greatest increase after physostigmine. These findings challenge the hypothesis that AD patients who are less cognitively impaired may show greater relative rCBF changes after physostigmine. However as the numbers in the present study (n = 7) and the Geaney et al study (n = 8) are small, all such conclusions must be tentative. Nevertheless the results suggest that a larger study of rCBF responsiveness to physostigmine may usefully clarify its relationship with cognitive function.

This method of analysis using a WM reference area, should be considered only with imaging devices of relatively good spatial resolution (preferably less than 10mm), such as the Novo 810. The two principal sources of error were a) the difficulty in outlining a region which contains only white matter and b) the effect of scattered radiation emanating from surrounding cortical regions where the radioactivity concentration is much higher, on measured count density within a low count ROI. These errors were minimised using empirically derived procedures and the correction factor described in the methods section, although even before correction for errors in this way, similar significant increases in fronto-parietal cortical/WM were also detected. As patients were tested on two separate occasions, a week apart, it

is possible that matching of slices or regions may not always have been optimally achieved. Matching of slices and selection of regions was done by a technician experienced in SPECT imaging and blind to study conditions. The effect of mismatching of regions between conditions would be to increase variance and reduce the detection of differences in HMPAO uptake. It was to improve matching of regions between different conditions that the 'split-dose' approach of section 6.2 was developed.

It has also been shown in animals that rCBF increases after physostigmine, and that this is paralleled by corresponding increases in regional cerebral oxygen consumption ($rCMRO_2$) (Hoffman et al 1986). This suggests that the coupling relationship between flow and metabolism is maintained after physostigmine. Other animal studies show that after physostigmine there are similar increases in local cerebral glucose utilization (Nelson et al 1978; Friedland & Meibach, 1981; Dam & London, 1983). The nature of the coupling of rCBF and metabolism is complex and poorly understood, but there is evidence that cholinergic mechanisms may have an important role (Sokoloff, 1959; Scremin et al 1973). The abundant cholinergic innervation of the cerebrovasculature, could provide a mechanism whereby changes in cholinergic function are reflected in rCBF changes.

In humans, studies using PET show that rCBF and cerebral metabolism are intimately coupled, under both resting (Frackowiak et al 1981) and activated conditions, and that rCBF closely reflects regional glucose metabolism (Fox et al 1988). Therefore, the changes in rCBF detected after physostigmine in this study probably reflect changes in metabolic function. However little is known about the effect of cholinomimetics, such as physostigmine, on brain metabolism as only recently have techniques, such as PET and SPECT become available to undertake such studies. Using the ^{133}xenon inhalation planar imaging technique for examining cortical flow (section 2.2.3.3), Gustafson et al (1987) showed that small increases in

temporo-parietal rCBF occurred after physostigmine. Although limited in being non-tomographic and measuring only surface cortical activity, the results are consistent with the present SPECT study.

Post-mortem studies suggest that there are no consistent or substantial changes in post-synaptic muscarinic receptor number in AD (Palacios, 1982; Davies & Verth, 1978), and this does not appear to be an artefact related to the agonal state or changes after death. SPECT imaging in AD patients using ^{123}Iodo-QNB, a ligand for muscarinic receptors, has suggested that increased numbers of receptors may occur in non-atrophied areas of cortex but reduced numbers in areas of severe atrophy (Weinberger et al 1989). In relation to the use of drugs that potentiate cholinergic function, such as physostigmine, a key question for future investigations is do muscarinic receptors function normally in AD? Unfortunately, *in vitro* measurement of receptor function in autopsy specimens is technically difficult, and results have been unsatisfactory (Perry, 1986). This emphasises the potential advantage of the experimental approach of using SPECT to image functional changes *in vivo* after physostigmine.

6.1.4.2 Functional asymmetry after physostigmine

Alzheimer's disease is usually described as a disorder associated with diffuse atrophy of the cerebral cortex (Tomlinson & Corsellis, 1984). Post-mortem and biopsy studies of Alzheimer patients show that there are marked cholinergic deficits in frontal and parieto-temporal cortex (Davies & Maloney, 1976; Bowen et al 1983; Sims et al 1983), but there appears to be no consistent neuropathological or neurochemical involvement of any one hemisphere (Rossor et al 1984). However only a few post-mortem studies have compared left and right sides in AD, as traditionally one side of brain is examined neurochemically, and the other morphologically. The few studies that have compared left and right sides, have reported left-right differences in

density of plaques and tangles (Moossy et al 1985), and neurochemical deficits (Rossor et al 1982). Such pathological evidence is consistent with neuropsychological findings, that show asymmetrical patterns of cognitive dysfunction in AD, especially in the earlier stages (Haxby et al 1985, 1986; Huff et al 1987; Becker et al 1988). Similarly, although a symmetrical pattern of regional deficit in blood flow has been observed under resting, non-stimulated conditions with PET (Frackowiak et al 1981) and SPECT (Chapter 5) imaging, there is evidence of lateral asymmetry in cerebral glucose utilization in AD patients with either marked language or visuospatial cognitive deficits (Haxby et al 1985, 1986). It has also been reported that the left hemisphere may be more affected in AD (Rossor et al 1982; Friedland et al 1985; Haxby et al 1985, 1986). This was not confirmed in a recent post-mortem study of AD, although subtle asymmetries of plaque and neurofibrillary tangle distribution and cholinergic enzyme activity did occur (Moossy et al 1989). In normal brain, ChAT activity is reportedly higher in left temporal cortex than right, although it was not possible to decide whether this was due to structural or functional differences between the two sides (Amaducci et al 1981). The asymmetric activation seen in the present study (Table 6.3) may reflect a functional, rather than structural change, as left/right ratios of CBF changed only after physostigmine, and not during basal conditions. Two other studies have examined the effect of physostigmine on rCBF patterns in AD (Gustafson et al 1987; Geaney et al 1990). Interestingly both have also demonstrated greater apparent increases in left hemispheric CBF compared with right. In the present study, left/right ratios of HMPAO uptake (asymmetry index, AI) were not significantly correlated with CAMTOTAL or illness duration, basal or physostigmine conditions (Table 6.6). Further studies with larger numbers of patients are needed to confirm this apparent increase in AI after cholinergic stimulation. Such studies should also investigate whether the putative CBF asymmetry reflects an 'asymmetric' or focal change in cognition.

In presenile Alzheimer patients there is marked association of rCBF in left temporo-parietal cortex and attention/language function (chapter 5) and this suggests that the left sided cortical activation produced by physostigmine in the present study may involve similar left brain cognitive processes. However in the present study, patients were scanned under basal conditions, and therefore attention and arousal may be the more likely psychological functions involved. EEG brain mapping using DUP 996, a phenylindolinone that enhances the release of ACh in brain nerve terminals, shows attenuation of slow activity and increased alpha activity, normally associated with increased vigilance (Saletu et al 1989). Further evidence for the role of the cholinergic system in arousal is that physostigmine antagonises decreases in rCBF and $rCMRO_2$ caused by the sedative benzodiazepine, midazolam, probably by a central stimulatory action rather than a competitive effect at the benzodiazepine receptor (Hoffman et al 1986). It is therefore possible that, in the present study, physostigmine may have facilitated increased cortical vigilance by increasing brain ACh levels and the functional consequence of this was increased blood flow in left cortex relative to right.

Although there was a trend for an increase in left/right asymmetry in all regions, the magnitude of this effect reached significance only in frontal and high frontal cortical areas (Table 6.3). While the effect of physostigmine could be more specific in frontal areas, an alternative possibility is that after physostigmine, asymmetry increased in many brain regions (Table 6.3), but the measurement of this change only reached significance in frontal regions due to better reproducibility of head positioning in that region. This is supported by evidence from the reproducibility studies (section 6.2.5.2), in which Alzheimer patients who were scanned on two occasions a week apart under identical conditions, showed less variance in frontal areas compared with other brain regions.

6.1.4.3 Evaluation of anti-dementia drugs

One of the difficulties in evaluating putative 'anti-dementia' compounds, such as physostigmine, is the lack of objective biological measures of effect in brain. Until recently few valid measures apart from clinical ratings were available. Although clinical indicators are clearly important, there are several disadvantages in their use. For instance, not all patients with AD have the same pattern of functional disabilities; it is this which may partly determine how well a patient is able to perform a given task, and not simply the global severity of Alzheimer pathology. This is perhaps most apparent in rating demented patients with communication or praxic difficulties. Impairment of concentration also affects how well patients perform a test, and consequently the manner in which the test is presented to a patient, for example as a whole or in sections, together with the consistency of the rater's testing technique in this regard, will influence the results. Another source of error in the use of rating scales for assessing demented patients is the large component of subjective clinical judgement involved which may vary between raters. These difficulties are perhaps even more important when rating scales are used to assess clinical change resulting from therapeutic intervention, as the multiple application of a test to the same patient requires parallel test forms with increased chances of compounding errors. Finally, patients who are severely affected by the disease and difficult to test may be excluded from clinical studies and thus it is possible that studies biased towards 'testable' patients may be misleading.

It follows that there may be important advantages in using biological measures of psychotropic drug action in humans to complement clinical rating measures. Regional CBF, with its intimate relationship to cerebral metabolism, may provide an important biological measure of pharmacodynamic effect, and one which has been used very successfully in animal models (Sokoloff 1981). This study using SPECT imaging with the

tracer ^{99m}Tc-HMPAO to measure relative rCBF demonstrates that such an approach in patients is feasible, and may provide a useful way of evaluating the central actions of drugs, such as physostigmine, reputed to have beneficial effects in demented patients.

6.2 A SPECT technique to measure changes in rCBF produced by short term intervention using a 'split-dose' of ^{99m}Tc-HMPAO: Test retest reliability studies

6.2.1 Introduction

This purpose of this section is to describe a method that allows SPECT imaging to be used to investigate basal and stimulated patterns of rCBF during a single imaging session, using a 'split-dose' of the flow tracer ^{99m}Tc-HMPAO. In addition to assessing the reliability of this split-dose method, a series of test retest reliability experiments are also described that examine the components contributing to the overal variance: statistical noise, positioning of regions of interest (ROIs) during analysis, positioning of patients, patient movement, and physiological changes between measurements which are not associated with deliberate challenge.

The reproducibility of the split dose method was also compared with serial SPECT scanning at an interval of one week, as used in section 6.1 to examine the effect of physostigmine.

Technetium-99m labelled hexamethyl propyleneamine oxime (^{99m}Tc- HMPAO) has been developed as an agent for imaging cerebral blood flow using SPECT (Neirinckx et al 1987). The characteristics of the molecule that allow it to achieve this aim have been discussed in section 2.2.5.1. ^{99m}Tc-HMPAO has been validated against a number of other cerebral blood flow techniques including ^{133}Xenon (Andersen et al 1988b), positron

emission tomography (Yonekura et al 1988; Inugami et al 1988) and microspheres (Pupi et al 1989) in humans and ^{14}carbon labelled iodo-antipyrine autoradiography (Bacciottini et al 1989; Bullock et al 1989) in animals. The images obtained with ^{99m}Tc-HMPAO demonstrate cerebral blood flow both in normal and in pathological states (Bullock et al 1989). The exact relationship between ^{99m}Tc-HMPAO and CBF is non-linear (Lassen et al 1988) because of the partial washout of the tracer, which becomes of increasing importance at higher flows. In theory, above a certain flow level, net uptake may fail to increase as the flow increases but this has not, so far, been demonstrated in humans, even in hyperperfused areas. At low and moderate flows, as occur in AD or KOR, an essentially linear relationship can be assumed (Lassen et al 1988).

SPECT can be carried out using a dedicated SPECT neuroimager or a rotating gamma camera. There are several types of dedicated imager, such as the Novo 810 used in these studies, but the important common feature of these instruments for the technique described below, is that they are able to acquire data for single slice reconstruction (or multiple slices in the case of multi-planar instruments) in a short interval of time (of the order of 5 minutes).

6.2.2 Advantages and limitations of ^{99m}Tc-HMPAO-SPECT

The trapping model for ^{99m}Tc-HMPAO uptake allows scanning to take place over an extended time period after administration of the radiopharmaceutical. This is a considerable advantage of the method, for it enables brief states such as ictal episodes to be studied (Duncan et al 1990). However the advantage of the trapping procedure is also a limitation in situations where we wish to study changes in rCBF after some form of intervention. As a result the technique has largely been limited to imaging basal states (eg chapter 5) or to capture relatively brief phenomena. Repeat studies require a time interval of at least 24h for isotope decay and clearance,

and it is therefore technically very difficult to use the radiotracer in situations where change is occurring over short time periods. This is a serious limitation of ^{99m}Tc-HMPAO as response to challenge, either physiological (eg CO_2 inhalation), pharmacological, physical (eg digit movement), or cognitive (eg mental testing), is often of greater interest than resting flow patterns.

6.2.3 ^{99m}Tc-HMPAO SPECT imaging of changes in rCBF: rationale and outline of split-dose method

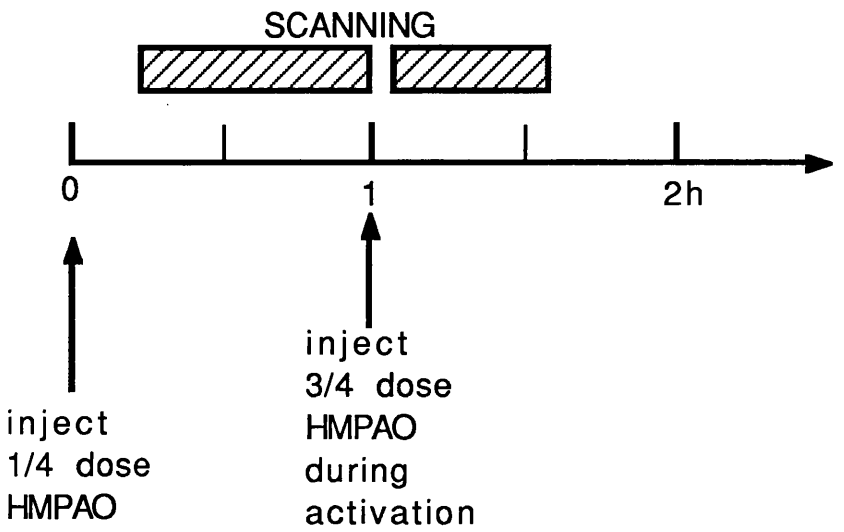
In order to overcome these limitations a different protocol for ^{99m}Tc-HMPAO use has been developed, which extends its application for use in rapidly changing situations. The technique is conceptually simple, but demands careful attention to detail in practice. The general approach used is to administer ^{99m}Tc-HMPAO in two doses which in total amount to the normal dose of tracer and radiation (500 MBq), hence the term 'split-dose' technique. An initial 25% dose of ^{99m}Tc-HMPAO is administered, before the challenge, in order to image basal CBF, while during the challenge the remaining 75% dose of ^{99m}Tc-HMPAO is given to image CBF during the challenge. The experimental paradigms using this approach are shown in Figures 6.1a and 6.1b. It follows from the trapping model that the initial scans reflect the distribution of ^{99m}Tc-HMPAO in the minute or so following intravenous injection and not the distribution at the time of scanning. Similarly, the second scan reflects the distribution of tracer at the time of 75% dose injection and it is therefore essential that the second injection is given at the time of maximum effect produced by the intervention. This can be illustrated by considering the approach used to image the effects on rCBF of administration of some pharmacological agent (see Figure 6.1b). The initial injection of 25% tracer establishes CBF patterns at that time. The drug challenge can then be administered but baseline imaging need not be undertaken until just before

Figure 6.1 Split-dose HMPAO SPECT challenge paradigm

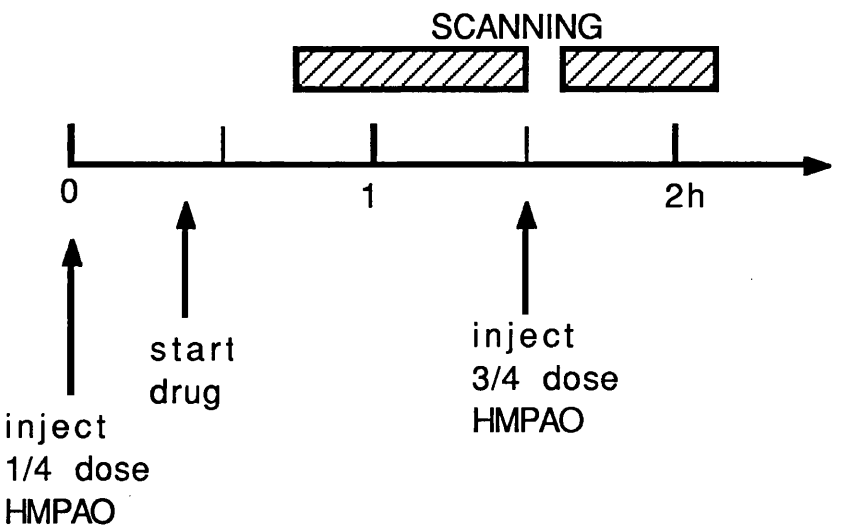
The general form of the paradigm is illustrated in Figure 6.1a, showing the 1/4 dose of ^{99m}Tc-HMPAO administered for basal imaging, and the 3/4 dose ^{99m}Tc-HMPAO given during the particular form of activation. In Figure 6.1b, the use of the paradigm to image the effect of drug action on brain function is shown. In the example illustrated, the maximal drug effect is expected to occur at 1.5 hours, and therefore the second dose of ^{99m}Tc-HMPAO is given at this time.

FIG 6.1 SPET SPLIT DOSE CHALLENGE PARADIGM

a. HMPAO Scanning with challenge



b. HMPAO Scanning with pharmacological challenge



the drug effect is expected. It has been shown by Andersen et. al. (1988b) that the change in HMPAO distribution which takes place between 10 minutes and 5 hours after injection is less than 4%. Immediately following the basal scan the patient will be given the remaining 75% dose of ^{99m}Tc-HMPAO in order to image the distribution of CBF at maximal drug effect. Although the drug may have been administered an hour or so previously, the baseline scan represents the distribution of ^{99m}Tc-HMPAO before drug treatment began. The initial 25% dose ^{99m}Tc-HMPAO scans typically require 15 minutes to acquire sufficient data for good SPECT reconstruction. As section scanners have to be used, head positioning is of paramount importance therefore each 15 minute scan was preceded with a rapid series of 2 minute positional scans which are sufficiently clear to enable topographic landmarks such as the caudate nucleus to be identified. Where possible, the two imaging sessions, namely the basal and challenge, should be close together so that the patient can lie in the same position on the scanning couch to ensure optimum realignment of basal and challenge scans.

In order to obtain data corresponding to the challenge condition, the first set of images (basal) are subtracted from the second set of images after correcting for length of acquisition time and isotope decay. Alternatively, counts within corresponding ROIs can be subtracted.

6.2.4 Aims of study

The aims of the present study were:

1) To develop and describe a method that may allow ^{99m}Tc-HMPAO SPECT neuroimaging to be used to investigate the effects of intervention on patterns of rCBF during a single imaging session, using a 'split-dose' of the tracer.

2) To determine the relative contributions of various sources of error to repeat SPECT measurements in the absence of challenge. The effects of data acquisition and image reconstruction, patient movement during the scan and patient realignment were examined.

3) To compare the reproducibility of the split-dose method with a 'two-dose' method.

6.2.5 Methods

6.2.5.1 Split-dose reliability study

(1) Data acquisition

To measure the variability associated with the split-dose paradigm, a control group of 10 patients of different pathologies had split-dose ^{99m}Tc-HMPAO scans with no challenge between scans. The radio-pharmaceutical ^{99m}Tc labelled HMPAO (^{99m}Tc-HMPAO) was prepared by adding ^{99m}Tc as pertechnetate in 5ml saline to the freeze dried mixture of HMPAO ligand. Each patient received an initial dose of 125MBq (25% of usual activity), injected intravenously while the patient lay on the imaging couch with eyes closed and ears unplugged. The initial scanning session lasted on average 45 mins and during this time pilot positional scans and two transverse slice images, each 12mm thick, were obtained in a plane parallel to the orbito-meatal (OM) line at +30 and +70 millimetres superior to this line. Acquisition time for each slice was 15 minutes during which between 1.5 and 2 million counts were acquired.

After acquisition of the initial scans, and with the patient still on the imaging couch in the same resting position, a further 375MBq dose of ^{99m}Tc-HMPAO was injected. After 5 minutes image acquisition was repeated, but on this occasion a shorter scan time of 5 minutes could be used as the

administered activity was higher. The imager used was a Novo 810 imager employing the Harvard multidetector scanning system with 12 sets of scintillation detectors and focussing collimators as described in section 3.6.

(2) Data analysis

A brain atlas was used to define brain regions. Using the low slice at +30mm above the OM line the following symmetrical regions of interest (ROI) were defined: right and left frontal (RF, LF), right and left temporal (RT, LT), right and left posterior temporal (RPT, LPT), right and left occipital (RO, LO), and right and left basal ganglia (RBG, LBG; Figure 3.7). From the high slice at 70mm above the OM line symmetrical right and left high frontal (RHF, LHF), and right and left parietal (RP, LP) ROIs were also defined (Figure 3.7). Mean uptake for each ROI relative to total uptake in the slice, was calculated as follows:

Relative mean uptake in each ROI for 1st scans = $C_{r(1)} / C_{t(1)}$

Relative mean uptake in each ROI for 2nd scans =

$C_{r(2)} - C_{r(1)} / C_{t(2)} - C_{t(1)}$

where C_r is the number of counts in region of interest r,

C_t is the total number of counts in the slice,

and the imaging session is given in brackets (1st or 2nd).

Appropriate corrections for isotope decay were also applied.

6.2.5.2 Factors affecting reproducibility

In order to measure the size of the components contributing to variability in repeat scanning, the following potential sources of error were also investigated:

Test A: the effect of data acquisition and image reconstruction

Data acquisition (eg statistical fluctuation) and image reconstruction errors were measured using an anatomical phantom constructed of perspex with compartments designed to simulate cortical structures (Wyper et al 1988).

These spaces were filled with a known quantity of 99mTc designed to simulate the typical count rate in normal HMPAO studies. The phantom was consecutively scanned six times without altering its position within the scanner.

Test B: the effect of head movement during a scan

It is not possible to rigidly immobilise the head during scanning, and this would clearly be very difficult to achieve in disturbed or cognitively impaired patients, such as those with Alzheimer's disease. Therefore, it is inevitable that some degree of head movement, voluntary and involuntary, may occur during scans. In order to examine the effect of such movement artefact during scans, the same transverse brain sections from the same patient were scanned twice. Eight patients with Alzheimer's disease who conformed to diagnostic and selection criteria described in section 3.2.1, were studied. Using this approach, the effect of involuntary movement or tilting of the head by the patient during scanning was determined. In this experiment scanning sessions were approximately 25 mins long.

Test C: the effect of re-aligning the patient

To assess the errors introduced by realigning the patient in the scanner, the above eight Alzheimer patients had repeat scans after they had been removed from the scanner and then repositioned in the scanner as accurately as possible using external landmarks and the scanner's optical alignment system as a guide.

Test D: serial scanning after one week

Although attempts should be made to ensure that environmental factors are unchanged when repeat scans are performed, namely the same quiet room, the patient positioned as before with eyes closed listening to the same relaxing conversation from the investigator administering the radiopharmaceutical, this is clearly very difficult to achieve in practice. For example, it would be difficult to control for differences in anxiety level and its

presumed corresponding effect on brain blood flow patterns. In order to investigate the variability of imaging data when patients are rescanned after a short interval, a test retest experiment was carried out in which the entire scanning procedure, including administration of the radiopharmaceutical, was repeated after one week. In the study, 10 patients with AD, who satisfied the selection criteria described in section 3.2.1, had repeat SPECT scans after an interval of 7 days. In this experiment it is reasonable to assume that differences observed between corresponding scans are unlikely to be caused by changes in disease process and probably reflect errors introduced in the procedures of rescanning or changes in the mental state of the patient between the two scanning sessions.

6.2.6 Results

In tests B,C, and D, the same measure of relative rCBF, as described in section 3.5.2, was calculated for each ROI on each of the two imaging occasions and used in the analysis of results. Relative rCBF in the split-dose experiment was calculated as described above in section 6.2.5.1. In the phantom experiments, raw counts were used for analysis. Measures of 'precision' at $P < 0.05$ level were computed as twice the standard deviation of the difference between corresponding first and second ROI measurements, expressed as a percentage. It follows that the lower the precision value the better the reliability of the measurement and vice versa. In columns A to E of Table 6.7, average precision measures for left and right ROIs are shown for the split-dose and other reproducibility experiments for eight brain regions. The split-dose technique appears to have better precision than repeat scans after one week.

The relative rCBF values were approximately normally distributed, and therefore Student's paired t-test (two-tailed) was used to test the significance of differences between values for corresponding ROIs in pairs of scans. In all

TABLE 6.7

Precision measurements of SPECT scanning in different test conditions

Region of Interest	Typical area of ROI (cm^2)	Test A Phantom	Test B Patient with no couch movement	Test C Patient with realignment	Test D Patient rescanned after 1 week	Test E Patient imaged using split dose
High frontal	ND	ND	ND	ND	9.7	3.6
Parietal	ND	ND	ND	ND	11.7	8.3
Frontal	10	2.7	4.6	6.9	11.1	5.7
Temporal	10	3.3	5.3	5.5	16.0	12.2
Posterior temporal	10	4.2	5.7	5.1	10.7	8.3
Occipital	7	3.5	7.0	10.8	12.7	8.1
Basal ganglia	3	9.3	9.2	11.5	14.3	15.9
n		6	8	8	10	9

The precision is measured as 2 x standard deviation of the difference between measurements expressed as a percentage. The values are averaged over the two hemispheres. ND indicates no data available.

the above reproducibility experiments, there was no significant difference between relative rCBF values in any ROI in corresponding pairs of scans.

6.2.7 Discussion

The importance of this study is that it suggests how SPECT imaging using ^{99m}Tc-HMPAO might be extended to investigate the effect of challenge on patterns of rCBF. Although challenge studies have been described using PET, the present technique may allow wider use of challenge paradigms by centres which have SPECT, but not PET. This may extend functional imaging to a wider range of clinical groups for investigation of physiological, psychological and pharmacological intervention studies. Up to one hour of cooperation from subjects is required, but this is comparable with other tomographic imaging methods.

As the two imaging sessions are contiguous (see Figure 6.1), the subject's head remains in a constant orientation throughout both the first (basal) and second (interventional) scans, facilitating efficient alignment of brain regions under investigation (see Table 6.7). The patients in this study were all cooperative and therefore efficient realignment of slice position was straightforward. With patients less able to cooperate, it may be necessary to adopt other means to ensure accurate realignment of position during data acquisition, such as the use of external markers attached to the subject's head. The problem of slice positioning is perhaps less important when gamma cameras are used for SPECT scanning or when instruments such as the Novo 810 are used with the facility to reconstruct data in any plane. This facility should represent a significant improvement for studies of this nature.

Not surprisingly, the poorest reproducibility is in the basal ganglia region, which, as well as being the smallest region with the lowest number of counts, is also subject to changes in the partial volume confinement caused by

slight alterations in slice position. This is also consistent with the large standard errors detected for the basal ganglia ROI in all three groups in chapter 5 (see Figure 5.2). The reproducibility in the temporal region is also relatively poor. This may be due to the variability at the medial aspect of the ROI (Figure 3.6). To obtain better reproducibility, this ROI might be better redefined to exclude all medial structures.

In the other regions, the reproducibility is better than 9% varying from 8.3% in the parietal and posterior temporal regions to 3.6% in the superior frontal region. This is better than for a test retest at 1 week (Table 6.7, column D), presumably because patients are not removed from the scanning couch between the two scanning sessions. The better precision found in the frontal regions may result from the shape of these structures making the measurement insensitive to slight axial misalignment.

As the activity of radiopharmaceutical administered to each patient is no greater than that used in a standard clinical brain SPECT scan, namely 500MBq, the technique has dosimetry advantages . Matsuda et al 1988 have reported using a double dose technique, as opposed to the split-dose paradigm described here, in which two doses of 740MBq of ^{99m}Tc-HMPAO were given in order to study changes in flow during a Matas test. This differs from the technique reported here in two important ways. Firstly, a very high dose of radiation is delivered to the patient from 1480 MBq of ^{99m}Tc. Secondly, keeping the count rate for the first scans as low as practically possible, results in a lower statistical error in the subtraction image compared to that obtained with a 50:50 split of the same dose. Electing to use 25% of the usual 500MBq dose for the first scan enabled an image to be obtained in 15 minutes, which was considered to be the maximum period patients could tolerate for single slice data acquisition.

It should be emphasised again that ^{99m}Tc-HMPAO measures the pattern of cerebral blood flow and not absolute values and the split-dose

technique therefore measures changes in pattern. The data for physiological state 2 is obtained by subtracting the first set of scans from the second after suitable correction for scan time, background activity and decay. This was done on a region of interest basis but small focal variations can be missed with this approach and pixel by pixel subtraction is better if accurate co-registration of the two data-sets can be achieved (Friston et al 1989).

These results show the extent to which various factors contribute to the reproducibility of the techniques (see columns A to D of Table 6.7). Precision measurements for phantom ROIs were consistently below 4.2%, apart from simulated basal ganglia (9.2%), for apparently similar reasons as discussed above. This suggests that the errors involved in data acquisition and reconstruction are small. Similarly when the same slice image is rescanned without moving the subject using the couch, precision is still low, but comparison with phantom precision indicates the effect of unwanted movement during the scan. Removing the patient from the scanner and realigning as carefully as possible in the same position results in further detriment to reproducibility precision, though not as much as when the complete imaging protocol is repeated after one week (Test D). Thus in investigations where two sets of imaging data are to be compared, eg basal and activational scans, the split-dose paradigm seems to offer advantages for accuracy of reproducibility, over other paradigms in which two scanning sessions occur on different days, such as the study described in 6.1 .

This study has shown that changes greater than 5.7% in frontal cortex or 8.3% in parietal cortex may be detected by the split-dose method with a confidence limit of 95% in a single case. Thus the technique may be capable of detecting the effects of visual stimulation which can increase rCBF in occipital cortex by as much as 38% (Woods et al 1991) or motor tasks which can increase rCBF in parietal cortex by 9.4% (George et al 1991). Cognitive challenges, however tend to produce a much smaller change in rCBF (eg

Ginsberg et al 1987). Parks et al (1988) found that a verbal fluency task produced a change of around 5% in regional cerebral metabolic rate for glucose in frontal cortex (with data normalised to occipital cortex) and George et al (1991) found an insignificant change of less than 5% in rCBF frontal cortex in two subjects performing the Stroop task. The split-dose technique as described here would be unlikely to detect the effects on rCBF of these cognitive challenges on individual subjects. However, significant differences between groups have been shown using the split-dose paradigm to examine the effect of verbal fluency on rCBF patterns in elderly normal females (Shedlack et al 1991).

In conclusion, two-dose techniques allow ^{99m}Tc-HMPAO SPECT imaging to be extended from its current use in basal conditions, to the wider application of imaging changes in brain function. In particular, the split-dose paradigm described above might be used to study the effects of pharmacological, physiological and psychological challenge to cerebral function. The method has the advantage of reducing the radiation dose given to subjects, as well as ensuring better head positioning reproducibility. In chapter 7 the role of the split-dose paradigm in future SPECT studies of the effects of activation will be considered.

CHAPTER 7

CONCLUSIONS

7.1 Investigation of brain function in Alzheimer's disease

It has been increasingly recognised in the last decade that younger and older AD patients are probably afflicted by the same neuropathological disease process. There is still considerable controversy, however, as to whether AD can be divided into two distinct subtypes on the basis of age (the so-called AD-1 and AD-2) or whether all patients with AD qualitatively show the same changes irrespective of age (eg Bondareff et al 1987 and Mann et al 1988b). Investigation of patterns of neuropathological involvement during life, would help to resolve this question, but there are few methods available to address this question. Brain biopsy is not considered ethically justified in most centres, and in any case, provides only very limited quantities of tissue for examination, usually from restricted brain areas. Patterns of functional deficit can be assessed from detailed clinical and neuropsychological description of cases, but some means of determining functional neuroanatomy is clearly essential. Structural neuroimaging methods, such as x-ray computed tomography (CT) or magnetic resonance (MR) imaging, can be used to determine degree and topography of brain involvement. For instance CT imaging shows that there does appear to be a close relationship between cognitive impairment and ventricular enlargement in demented patients (de Leon et al 1979). MR imaging, at least at present, is like CT, essentially a structural technique. The improved tissue resolution obtained with MR allows grey and white matter to be differentiated clearly and this makes MR particularly well suited for detailed anatomical examination. Apart from the paucity of available MR imagers, the major limitation of CT and MR imaging is their inability to measure functional processes in brain, and inferences about brain function drawn from structural images can be problematic as the case history described in chapter 5 clearly showed.

Improved nursing and medical care of patients with AD means that patients are living longer with two important consequences for investigation. Firstly, this provides an important opportunity for study of the natural course of the illness from early stages to death, and allows the different clinical courses of AD to be described. Secondly, the improved longevity of AD patients will result in proportionately more post mortem examinations being carried out in the later stages of AD. Neuropathological description after death provides useful information on the integrity of brain tissue at that end point, but relatively little information about the temporal sequence of involvement of different brain regions in the condition. Furthermore study of the relationships between mental function ante mortem and neuropathological involvement post mortem, are logistically difficult.

Techniques, such as PET and SPECT have the advantage that they provide information on regional brain function *in vivo* and can be employed repeatedly throughout the course of a patient's illness to measure the relationship between brain activity (eg as measured using rCBF) and mental performance. The development of *functional* brain imaging also provides a potentially powerful way of measuring how brain function responds to challenge, such as pharmacological or neuropsychological intervention.

7.2 Summary of findings

In many circumstances, including dementia, cerebral blood flow appears to be a useful index of brain metabolism. Therefore imaging techniques, such SPECT, which estimate CBF, or at least some function of CBF, provide a means of studying brain function *in vivo*, in both basal and activated conditions. In this thesis, two different imaging techniques, MTT and SPECT, were used to study the pattern of brain function in Alzheimer's disease and Korsakoff's psychosis, and its relationship to neuropsychological performance.

The mean transit time technique has the advantage of requiring only short periods of cooperation from patients. In many patients, such as those with major psychiatric illness, there are obvious advantages in techniques that require only minimal patient compliance. In both presenile AD and KOR, estimates of net MTT were considerably prolonged compared to those of healthy controls. Although these results are consistent with results from other functional imaging techniques, no inference can be made about CBF without cerebral volume of distribution estimates. Estimates of net MTT provided by the method, although able to differentiate, on a group basis, demented patients from healthy controls, were not helpful for distinguishing AD and KOR groups. Furthermore, examination of individual net MTT estimates indicates some overlap between net MTT values for all three groups studied. As this was not an epidemiological study and patient groups of AD and KOR carefully recruited, estimates of sensitivity and specificity cannot be calculated. However estimates of positive predictive value viz. the probability that the disease is present when the test result is positive, were calculated for net MTT. Positive predictive values for transit times in bilateral posterior cortical regions of AD patients were greater than 70%. The positive predictive value estimates for net MTT in AD suggest that the method may have some clinical utility. However in order to accurately assess clinical utility, an epidemiological study to calculate sensitivity and specificity would be required. There are difficulties in this approach also as pathological diagnostic criteria for dementing illnesses are by no means agreed. In both patient groups, the prolongation of net MTTs occurred in all cortical regions studied. In AD, this may reflect the well recognised patterns of cortical dysfunction. The cortical involvement, suggested by the MTT estimates, was perhaps more surprising in KOR patients, who traditionally have been viewed as having predominantly subcortical pathology. Indeed this was an important

consideration in electing to study KOR patients, as it was thought that their subcortical pattern of involvement might contrast with the cortical involvement in AD, and thus provide a suitable control group. The potential significance of this finding with respect to cortical function in KOR patients will be discussed further when considering the SPECT findings. The finding of consistently negative correlations between neuropsychological test scores and net MTT in both AD and KOR groups tends to support the view that net MTT may provide a meaningful estimate of cortical function in dementia. Modern techniques for CBF imaging are expensive and require highly specialised equipment. The attraction of transit time measurement when this study was initiated was its low cost and potential availability in any department of nuclear medicine using a gamma camera. The main limitation of MTT is not that it is non-tomographic and limited to planar cortical imaging, but the difficulties in interpreting its physiological significance. Without information about volumes of tracer distribution it is not possible to determine the relationship of net MTT to CBF.

Single photon emission tomography using the flow tracer ^{99m}Tc-HMPAO has several appealing characteristics for imaging CBF in dementia and also other psychiatric conditions. The relatively stable trapping of the radiotracer in brain allows the investigator reasonable control over which particular features of the patient's mental state will be imaged. For example, in chapter 5, injections of ^{99m}Tc-HMPAO were given to AD and KOR patients who were in a relaxed state with their eyes closed, whereas in chapter 6 the trapping property of the tracer was utilised in order to 'capture' the flow pattern during the effect of physostigmine on brain metabolism. The same advantages of the technique can also be utilized for functional imaging of other transient mental phenomena, such as hallucinosis, or mood disturbance, and have been used for investigation of temporal lobe seizure activity (Duncan et al 1990). The time

taken to inject ^{99m}Tc-HMPAO is very brief, and is similar to that of the entire MTT method, thus both measure 'function' in a very short time interval. Unlike MTT however, ^{99m}Tc-HMPAO has several other advantages. In particular, the relative stability of ^{99m}Tc-HMPAO distribution in brain, allows adequate time for tomographic imaging. In this study a dedicated brain sectional scanner, the Novo 810, able to acquire data for single slice reconstruction in a short interval of time (of the order of 5 minutes) was used. SPECT can, of course, also be carried out using a rotating gamma camera, but with this type of instrument, a longer imaging session (about 45 minutes) is needed to acquire the whole data set, and interruptions to the scanning session eg due to patient movement, may result in loss of all data. The ability of the Novo 810 to acquire sectional images was utilised in the 'split-dose' paradigm described in chapter 6.

Patterns of rCBF in AD and KOR patients at rest with eyes closed, were studied using ^{99m}Tc-HMPAO SPECT. In AD marked reduction in rCBF occurred in posterior temporal and parietal areas, although there was also a trend for relative flow to be reduced in frontal and temporal regions. Cortical flow reductions in resting AD patients were correlated with impairments of cognitive function; in particular, rCBF in left posterior temporal and left parietal regions were strongly correlated with most aspects of cognitive function, but notably language functions, memory scores, attention and praxis.

Measurements of ^{99m}Tc-HMPAO SPECT were undertaken to examine the patterns of perfusion deficits in AD and KOR patients, not to assess SPECT as a diagnostic technique. As explained above for net MTT, assessment of diagnostic utility would have required a very different study design from that used. In order to measure sensitivity and specificity all patients presenting with dementia would need to have been investigated with SPECT not, as in the present study only two carefully selected groups of patients (AD

and KOR). Positive predictive values were not impressive and only in posterior temporal regions of AD patients did they exceed 50%. As an aid to understanding the pattern of function-related perfusion in individual patients SPECT may have a place.

Unlike in the AD group, flow in KOR patients was preserved at normal or near normal levels in temporal, posterior temporal and parietal regions, but tended to be reduced in frontal regions. Furthermore, flow deficits in frontal regions in KOR patients were correlated with impaired performance on tests of memory and orientation. This work suggests that in KOR, there is frontal lobe dysfunction as well as the more recognised subcortical lesions of thalamus and mamillary bodies. There has been relatively little interest in cortical dysfunction in KOR, and very few studies using functional imaging techniques such as SPECT. While the subcortical lesions have been traditionally linked to the amnesic features, there is increasing evidence for the role of frontal regions in memory function (Moscovitch, 1982). Frontal lobe dysfunction in KOR is not unexpected in view of the well recognised clinical features of apathy, inertia, loss of initiative and rather bland disposition that often characterise the condition.

The use of two doses of ^{99m}Tc-HMPAO described in chapter 6 extends ^{99m}Tc-HMPAO SPECT to activational states as well as basal conditions. Using this technique, the effects on brain metabolism, as reflected in rCBF distribution, of both pharmacological or neuropsychological intervention can be determined and compared to basal rCBF patterns. Thus the technique offers a novel way of assessing the effects of drugs on brain function and provides a means of understanding the relationship between neurotransmitter system dysfunction and brain metabolism. Two dose ^{99m}Tc-HMPAO SPECT was used to measure the effect of cholinergic potentiation on rCBF patterns in

AD patients using the anti-cholinesterase physostigmine. After physostigmine there appeared to be an increase in left sided ^{99m}Tc-HMPAO uptake relative to that in the right side, with the most significant changes in frontal regions. These results, although preliminary, illustrate the potential of this approach, in which the functional consequences of manipulation of specific transmitter systems can be detected using SPECT and related to resultant changes in cognition.

7.3 Problems associated with functional imaging

A comprehensive review of the methodological problems associated with isotope brain imaging is outwith the scope of the present work, but some discussion of the more important difficulties is required.

The power of physiological imaging techniques will depend in part on the availability of standardized, reproducible and accurate methods of data analysis. PET, and potentially SPECT, have the ability to measure a wide range of biochemical, physiological and pharmacological processes in brain. As discussed in chapter 2, the tracer kinetic methods for measuring such processes vary in temporal resolution, statistical image quality (owing to the constraints imposed on radioisotope counting), and degree of structural information incorporated into the functional image. Imaging instruments vary widely in spatial resolution, both within the plane of the section, and in the axial direction. These variables contribute to measurement errors stemming from the techniques of functional brain imaging themselves. Additional variability is also contributed by differences in brain structure between individuals. For instance left-right hemispheric differences have been described at macroscopic (Galaburda et al 1978) and ultrastructural (Amaducci et al 1981) levels. Added to this, pathological processes may also introduce further variability as has been

noted in AD (eg chapter 6). Such variability in brain structure will have important consequences for functional imaging, and may result in global changes in function eg due to atrophy or more localised effects, as in some cases of AD.

Functional brain images can be analysed in three main ways: 1) quantitative values of CBF from particular brain regions can be determined; 2) semi-quantitative or relative measures of CBF can be determined, and 3) images can be qualitatively inspected by experienced observers. At present true quantitative SPECT imaging is limited to dynamic ^{133}Xe rCBF imaging (see section 2.2.5.2), but with ^{99m}Tc-HMPAO useful measures of rCBF can be obtained relative to some other reference brain area, such as occipital cortex, as in this thesis, and by others using cerebellum (Burns et al 1989). It should be recognised that the use of reference areas in this way is not peculiar to SPECT, but has also been widely used in PET. Qualitative image analysis has been employed by several groups (eg Neary et al 1987; Gemmell et al 1987) and should be viewed, not as an alternative to other methods but as a complementary method of analysis. Indeed pattern recognition by visual inspection has been employed in nuclear medicine and radiology for some time, and is a proven way of analysing complex data sets, such as images. Normal SPECT scans tend to be symmetrical and image asymmetry can be detected more readily by the eye, than using available quantitative protocols. The subjectivity of visual image recognition can be reduced by using experienced raters, blind to conditions or diagnosis. Such an approach was used to assign ROIs to SPECT images in the studies reported in chapters 5 and 6.

Thus potential sources of error in functional image analysis are considerable, but other sources of information can usefully contribute to image interpretation. In the SPECT studies in this thesis external anatomical

landmarks, eg the orbito-meatal line and brain atlas information, were used to assist in the assignment of structural localization to the functional images. Ancillary information on brain structure can also be obtained from skull radiographs (eg Fox et al 1985), x-ray CT (eg de Leon et al 1983b) and MR imaging (eg Evans et al 1988). However access to structural imaging facilities, particularly for research purposes, is likely to be very limited and/or logistically complex. In addition, reliable positioning between the different imaging modalities must be employed. The matching of MRI and PET/SPECT images is a major computational problem that, despite the development of fitting algorithms, requires to be solved and validated.

Another important potential source of variance is the subject's mental activity at the time of the measurement. No standard definition of 'resting' or 'basal' mental set exists and different groups have used different definitions of basal conditions. In this work, subjects were examined at rest, with eyes closed and ears unplugged, in a quiet room with average illumination. In many ways this is very much a compromise, as others have defined the resting state as one of relative sensory deprivation, while others examine patients with eyes open and ears unplugged, arguing that this 'background' stimulation reflects the cognitive state commonly encountered in everyday circumstances with a continuous baseline of cognitive activity. It can been argued that inter-subject variability may be reduced by attempting to standardize mental set during imaging. This might be accomplished by asking subjects to undertake some standardized cognitive task during imaging.

The development of functional imaging of brain is still at an early stage, but the potential of the approach appears considerable. SPECT imaging is potentially widely available, for although high resolution instruments, such as the dedicated section imager used in the present work, are available at

present in only a few UK centres, rotating gamma camera systems able to provide moderate resolution are available in most nuclear medicine departments. The introduction of the radiopharmaceutical ^{99m}Tc-HMPAO has enabled high resolution SPECT images of rCBF to be obtained quickly and with minimum radiation exposure to patients. Reservations about the non-linearity of the relationship between absolute estimates of flow and ^{99m}Tc-HMPAO uptake at high flow values, have led to development of correction algorithms (eg Lassen et al 1988). However conditions of high flow (eg encephalitis) are probably quite unusual in psychiatric illness, and there is a good correlation between absolute measures of rCBF measured using PET and ^{99m}Tc-HMPAO uptake at normal and low flow values, as occur in AD and KOR patients (Inugami et al 1988). Further work is required in order to verify the kinetic models used and overcome the disadvantage of lack of true quantification.

7.4 Future studies

Initial SPECT (and MTT) studies examined basal patterns of rCBF (chapters 4 and 5). Longitudinal studies in AD are essential in order to determine the change in patterns of rCBF and cognition occurring as the disease progresses. Such studies would also allow relationships between cerebral function and cognition to be monitored over time. There is some evidence that clinical course and neurochemical impairment differs in subjects developing AD at an older age (AD-1) compared to those developing the illness at a younger age (AD-2). In this thesis only AD-2 patients were studied, but clearly SPECT provides a suitable technique with which to examine cerebral function in the two putative 'types' of AD.

In chronic KOR syndrome neuropsychological function reputedly

remains relatively stable over time compared with AD (Butters & Granholm, 1987). Clearly longitudinal studies of cerebral function measured by SPECT, and neuropsychological performance would be necessary in order to test the validity of this hypothesis. There are indications that cortical pathology may be instrumental in determining some features of the KOR syndrome and that attention has focussed too closely on the basal brain changes in the disorder (Lishman et al 1987). This is supported by the MTT and SPECT studies described in this thesis, and consistent with the clinical signs of KOR syndrome. In particular the frontal deficit in function demonstrated by SPECT deserves further study with a larger series of patients.

The more restricted nature of the cognitive deficits in KOR, suggest that it might provide a better 'model' system than AD in which to determine the relationships between regional brain function and mental impairment. Such information might help to target studies in AD. For example the cholinergic deficit in AD may be paralleled by a similar deficit in KOR that could also be critically involved in memory impairment (Lishman, 1986; Butters & Granholm, 1987). Therefore investigation of putative cholinergic deficits, using SPECT to assess the cerebral effects of pharmacological challenge, might be better executed in KOR, rather than AD patient groups. The radiopharmaceutical ^{99m}Tc-HMPAO has clear advantages for imaging the functional consequences of pharmacological manipulation of particular neurotransmitter systems, using putative receptor agonists or antagonists in both healthy controls and psychiatric patients. In analogous studies, the 2-deoxyglucose technique (eg Sokoloff 1959;1982, chapter 2) has been successfully employed to map dynamic functional events in animals.

A similar approach is also feasible using ^{99m}Tc-HMPAO SPECT to investigate cerebral activity in human subjects during neuropsychological

challenge. The following example is described to illustrate the approach (Shedlack, Hunter et al 1991). The split-dose ^{99m}Tc-HMPAO SPECT imaging paradigm described in chapter 6 was used to compare the effects of the verbal fluency task (Borowski et al 1967), usually regarded as a test of the integrity of left frontal cortex, with simple verbalization (counting) in twenty elderly healthy female subjects (mean age 67 years, range 59-78 years). Each subject was studied under basal conditions after injection of 125MBq ^{99m}Tc-HMPAO. Without moving the head of the subject, they were scanned again after injection of 375MBq ^{99m}Tc -HMPAO. The second injection was made in 10 subjects during the verbal fluency task and in the other 10 subjects during counting. Verbal fluency was associated with reduced uptake bilaterally in the region of the basal ganglia and in left temporal (peri-sylvian) cortex when compared with an occipital unstimulated reference region. By contrast, counting produced relative activation in frontal and parietal areas. Thus, using the split-dose paradigm, clinically relevant neuropsychological tests might be characterised functionally in AD and other neurodegenerative disorders, such as KOR, by pattern of regional brain activity, localization of which might not be predicted from classical studies of brain lesions. Such an approach complements that of neuropharmacological challenge described above. The split-dose tracer technique allows radiation dose to be minimised, and permits multiple, anatomically identical but metabolically distinct scans in individual subjects, thus allowing the use of an intravenous SPECT ligand for the dynamic investigation of cerebral metabolic demand during normal cognitive processes.

The relationship between cognitive function and resting or stimulated rCBF values suggests a useful future for SPECT measurements in psychiatry. The split-dose approach allows both stimulated and basal cerebral function to be measured, but it is likely that data obtained under challenge conditions will

be more informative than that obtained at rest. Probably the method of choice for investigating the effects on brain function of cognitive or other challenge will remain H_2O^{15} PET (Lammerstma et al 1990). However SPECT challenge paradigms such as the split-dose technique may allow findings from PET to be investigated further in clinical settings.

Further impetus for SPECT investigation of AD and KOR will result from the development of labelled receptor ligands. Table 7.1 shows some of the neuroreceptor ligands that have been used for SPECT imaging. The muscarinic cholinergic receptor ligand, ^{123}I-3-quinuclidinyl 4-iodobenzilate (^{123}I-QNB), can be used for SPECT imaging of muscarinic receptors *in vivo* (Eckelman et al 1984). Preliminary findings with ^{123}I-QNB SPECT in AD suggest that in non-atrophic areas, muscarinic receptor upregulation may occur (Weinberger et al 1989). Much more work will be needed to confirm such findings, but they demonstrate that potentially novel information may result from this approach.

TABLE 7.1

Neuroimaging and SPECT neuroreceptor ligands

Receptor	Ligand
M1, M2	^{123}I - QNB
	^{123}I - Dexetimide
D2	^{123}I - IBZM
	^{123}I - 2 - Spiperone
	^{123}I - iodolisuride
D1	^{123}I - SCH 23892
	^{123}I - SCH 23390
BZ/GABA	^{123}I - Fumazenil
Serotonin S_2	^{123}I - Ketanserin
Opioid	^{123}I - Diprenorphine
Amines	^{123}I - IMP

Appendix. Some of the results presented in this thesis have been published as follows:

1. Christie J E, Goodwin G M, Hunter R & Merrick M V (1987) Measurement of regional cerebral blood flow in Alzheimer-type dementia using a novel approach. *Journal of Physiology* **392**, 78P.

2. Hunter R, Gordon A, McLuskie R, Wyper D, Patterson J, Christie, J E, Fink G, & Goodwin G M (1989) Gross regional cerebral hypofunction in the presence of normal X-ray CT in a case of Creutzfeldt-Jacob disease. *Lancet* **I**, 214-215.

3. Hunter R, Merrick M V, Ferrington C, Notghi A, McLuskie R, Christie J E & Goodwin G M (1989) Cerebral vascular transit time in Alzheimer's disease and Korsakoff's psychosis and its relation to cognitive function. *British Journal of Psychiatry* **154**, 790-796.

4. Hunter R, McLuskie R, Wyper D, Patterson J, Christie J E, Brooks D N, McCulloch J, Fink G & Goodwin G M (1989) The pattern of function-related regional cerebral blood flow investigated by single photon emission tomography with ^{99m}Tc-HMPAO in patients with presenile Alzheimer's disease and Korsakoff's psychosis. *Psychological Medicine* **19**: 847-855.

5. Hunter R, Merrick M V, Ferrington C, Notghi A, Christie J E & Goodwin G M (1989). Vascular transit time in dementia and its relation to neuropsychological function. *Journal of Cerebral Blood Flow and Metabolism* **9**, Suppl. 1, 5519.

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7. Shedlack K.J, Hunter R, Wyper D, McLuskie R, Fink G, Goodwin G.M. The pattern of cerebral activity underlying verbal fluency shown by split-dose single photon emission tomography (SPET or SPECT) in normal volunteers.
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