Alzheimer's disease: two experimental models

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ALZHEIMER'S DISEASE:

TWO EXPERIMENTAL MODELS.

by

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I declare that the work contained in this Thesis has not been previously submitted for any other degree.

ABSTRACT

Alzheimer's disease is the most common form of senile dementia, though its cause is unknown. Of the six principal hypothesised origins of the disease, two were examined experimentally.

The cholinergic hypothesis proposes that Alzheimer's disease is caused by neuropathological damage to the nucleus basalis of Meynert (nbM), causing a subsequent global reduction in brain acetylcholine levels, which results in the observed loss of recent memory and subsequent behavioural symptomatology. To test this hypothesis, rats were injected with either 0.01, 0.03 or 0.06 mg/kg scopolamine hydrobromide (a cholinergic muscarinic blocker) and tested on a non-spatial task of object recognition, nonmatching-to-sample. The highest dose of scopolamine produced a significant impairment on a retention interval of 60 sec on the task, but there was no evidence that the drug produced faster forgetting of the stimuli. The result suggests that the drug caused a general depression in performance which may or may not reflect amnesic properties. In contrast, simultaneous tests with the acetylcholinesterase, physostigmine, indicated that increasing available acetylcholine might attenuate the effects of the retention interval. A series of control tests revealed that scopolamine and physostigmine had no effect on the motor activity of the animals. A second series of control tests indicated that the rats relied on cues from a variety of sensory modalities in order to perform the nonmatching task. The notion that a cholinergic dysfunction is sufficient to produce the symptoms of Alzheimer's disease is questioned. It is proposed that structural damage (perhaps to the limbic system) may be a more likely cause of this disease, rather than a specific cholinergic abnormality.

The second part of this thesis examined the toxin hypothesis of Alzheimer's disease, which proposes that the disease is caused by the accumulation of aluminium in the brain. Accordingly, rats were administered 72 mg/kg aluminium hydroxide orally, for three months, and recent memory capacities were assessed over 20 and 40 sec retention intervals on the nonmatching-to-sample task. No effects of aluminium administration on memory were noted. Additionally, aluminium did not appear to have any effect on the motor activity of the rats, and no accumulations of aluminium were detected in the nbM, hippocampus, amygdala or neocortex of the animals. Various reasons for the failure to find either accumulations of aluminium in the brain or recent memory effects are outlined.

Finally, the AF64A-treated animal is considered as an alternative to the scopolamine-treated animal model, and other possible causes of Alzheimer's disease are discussed - notably the genetic hypothesis; and the possibility that a combination of accumulations of brain aluminium with decreases in CAT levels may be responsible for the disease.

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Glossary of Abbreviations.

ACh	:	Acetylcholine
AChE	:	Acetylcholinesterase
ALS	:	Amyotrophic Lateral Sclerosis
BBB	:	Blood-brain barrier
CAT	:	Choline acetyltransferase
Ch	:	Choline
CNS	:	Central Nervous System
CSF	:	Cerebro spinal fluid
HAChT	:	High affinity transport of choline
LAChT	:	Low affinity transport of choline
mAChr	:	Muscarinic receptor binding
nbM	:	Nucleus Basalis of Meynert
NFT	:	Neurofibrillary Tangle
PD	:	Parkinsonism Dementia
PHF	:	Paired Helical Filament
PTH	:	Parathyroid Hormone

Contents.

CHAPTER ONE. INTRODUCTION.

1.1.1. Alzheimer's Disease and its Diagnosis.

Alzheimer's disease is the most common form of senile dementia, with an estimated over one million affected individuals in the U.S.A. (Whitehouse et al., 1982). Three stages in the clinical course of this illness have been described (Pearce, 1984).

Stage 1 is characterised by amnesia, disturbances of spatial orientation and lack of spontaneity. This lasts for 2 - 4 years and then merges into the second stage.

Stage 2 is characterised by a progressive dementia involving many aspects of higher mental function, but is accompanied by focal features. These consist of agnosia, apraxia and dysphasic disturbances and may be accompanied by hypertonic and akinetic disturbances of motor activity.

Stage 3. In the final stage the patient shows complete dementia, often epileptic seizures, and may have features of the Kluver-Bucy syndrome. At this stage the patient is withdrawn, incontinent, and largely dependant on others in respect of even the simplest necessities of life.

This pattern of abnormalities is not, however, unique to Alzheimer's disease, and so cannot be used as the sole basis for diagnosing the illness. For instance, the clinical features of Pick's disease are usually impossible to differentiate from Alzheimer's disease. The difference between the two illnesses lies in their pathology: Alzheimer's disease is characterised by diffuse cerebral atrophy (see P.2), whereas in Pick's disease the atrophy is sharply demarcated and largely restricted to the anterior portions of the frontal and temporal lobes. Hence, Alzheimer's disease can only be distinguished from Pick's disease with certainty by pathological

examination, such as brain biopsy. Recent advances in radiological techniques such as the Computerised Tomography Scan and Positron Emission Tomography are encouraging, however, as these techniques yield high diagnostic accuracy in the assessment of (respectively) the degree and distribution of brain atrophy, and decreases in metabolic activity, in various forms of senile dementia. Thus it may soon be possible to diagnose Alzheimer's disease without having to rely on autopsy or biopsy data.

The duration of Alzheimer's disease is variable, commonly between 5 and 10 years, but in certain instances it may be considerably longer. The age of onset of the illness also varies considerably, and for many years two distinct types of the disease were distinguished; presenile dementia (classical Alzheimer's disease) in which the clinical symptoms could be evident from the age of 45, and senile dementia of the Alzheimer type, which applied to people first exhibiting the symptoms of the disease after the age of 65. It is now generally recognised that the two are functionally the same disease, though there is a tendency for them still to be treated as two separate entities in the literature.

Autopsy reveals highly characteristic pathological and neurochemical changes in the brain of individuals who have suffered from Alzheimer's disease. The pathological features consist of diffuse degeneration of the neocortex, this being most severe in the temporal, parietal and occipital lobes. The posterior cingulate gyrus, amygdala, hippocampus and hypothalamus are also involved, though the anterior cingulate gyrus, caudate nucleus, thalamus and mamillary bodies are affected only minimally. Neurofibrillary tangles (intracellular bundles of paired helical filaments that fill the perikaryal cytoplasm surrounding a normal nucleus) are found in degenerating neurons

in the neocortex, hippocampus, amygdala and brain stem. Senile plaques (extracellular clusters of abnormal cell processes in which a central mass of amyloid, a protein, is surrounded by unmyelinated neurites) are found predominantly in the grey matter of the cortex. Plaque formation in areas such as the hippocampus and amygdala occurs at an early stage in Alzheimer's disease, but is also extensive in non-demented elderly people. Senile plaques are sometimes found in the caudate nucleus, putamen, thalamus and mamillary bodies, and in the brain stem, cerebellum and diencephalon in advanced cases of the disease. In these regions, however, plaques never acquire the density seen in cortical regions. Since plaque and tangle formation is most extensive in the cortex, this region has received the most intensive investigation. At first sight it would seem reasonable to associate the brain atrophy, nerve cell degeneration and the plaques and tangles with the clinically observed dementia and focal signs of the disease, but although there is some evidence to support this (see P.17), the association is by no means established as fact.

The neurochemistry of Alzheimer-type dementia is related to the involvement of different neurotransmitter and neuropeptide systems which exist in the brain. The significant loss of neurons from regions at the base of the forebrain consequently reduces the amount of neurotransmitters, notably acetylcholine, normally released from the terminals of those neurons in higher brain centres. Consistent biochemical changes in postmortem tissue are also seen (but to a lesser extent) in the noradrenergic, serotonergic and somatostatin systems. The cholinergic abnormality in particular appears to be closely related to cognitive impairment, although the precise nature of this relationship has yet to be determined.

1.1.2. Prevalence.

Attempts to establish the prevalence of Alzheimer's disease are problematic for two reasons. Firstly, problems arising in the diagnosis of the disease (see 1.1.1.) mean that the figures regarding the occurence of the disease in total terms will be sparse, and at best only estimates. Secondly, the distinction between Alzheimer's disease and senile dementia of the Alzheimer type (see P. 2), while no longer widely accepted, is nevertheless reflected in the figures quoted for the prevalence of this illness.

For example, Gordon and Sim (1967) recorded 80 cases of presenile dementia. The final diagnosis in these 80 patients was Alzheimer's disease in 48 (60%) and Creutzfeldt-Jakob disease, Pick's disease and 'others' collectively represented in 32 (40%). Thus Alzheimer's disease is the likeliest diagnosis in a patient with presenile dementia.

A representative series of 68 cases described by Sourander and Sjogren (1970) showed the age of onset as follows:

Age 45 - 59 in 50% of cases

Age 60 - 64 in 30% of cases

Age 65 - 69 in 20% of cases

This indicates the distribution in the classic presenile dementia, but since senile dementia is almost certainly the same disorder, the figures do not represent the total picture of Alzheimer's disease and they underestimate the high incidence in patients over 70 years of age.

In Britain, extensive studies of the population over the age of 65 have been carried out in Newcastle Upon Tyne. Kay et al. (1964) found a total prevalence of about 5% for cases of severe mental deter-

ioration in Newcastle Upon Tyne, 5% for cases of mild mental deterioration, and a total prevalence of 10% for dementing illnesses of all degrees of severity.

Kay et al. (1970) continued the Newcastle work by examining a further sample of elderly subjects in that city. When the data were pooled with those from the previous investigation (Kay et al., 1964) they were able to estimate prevalence rates for "chronic brain syndromes" for different age groups within the senile range. These are shown in Table 1.1.. It can be seen that the proportion of the population judged to be demented increases with age, and that there is a dramatic increase in prevalence once the age of 80 is exceeded. The overall prevalence rate given by Kay et al. (1970) for the over 65's is 6.2%, lower than the 10% given in the earlier Newcastle study. This is probably due to the later report adopting a more stringent set of diagnostic criteria for dementia.

Finally, Larsson et al. (1963) estimated that the lifetime risk of contracting senile dementia is 1.8% in males and 2.1% in females. The higher risk in females is at least partly, if not entirely, due to their tendency to live to a greater age than males, when dementia becomes more prevalent.

It should be noted that there have been no new major surveys of dementia for some time, so the figures quoted are derived from a previous generation of elderly people. This means that there is a possibility of small changes in the prevalence of those judged to be suffering from dementia since the studies quoted were undertaken.

AGE (Years)	PREVALENCE (%)
65 - 69	2.3
70 - 74	2.8
75 - 79	5.5
80+	22.0
TOTAL	32.6

Table 1.1. Prevalence of "chronic brain syndrome" in Newcastle Upon Tyne.

(From Kay et al., 1970).

1.1.3. Why Alzheimer's Disease is a Problem.

Dementing illness inflicts a grave burden on patients, family and doctor. Dementia, with its associated physical ill health and loss of mobility, can help to produce social isolation. In some cases, isolation could augment mental deterioration by leading to depression which, in turn, can produce self-neglect and dietary inadequacies. Therefore afflicted individuals make great demands upon medical and social support services. In the later stages of the disease, patients are bedridden; unable to speak, or take care of themselves.

Kay et al. (1970) examined the load placed on various residential facilities by their sample of 449 elderly subjects. Of those with "chronic brain syndromes", 61% were admitted to an institution of some kind (hospital, local authority home, etc.) as compared with only 25% of those with functional psychiatric illnesses, and 19% of those considered to be psychiatrically normal. Not only were more patients with "chronic brain syndromes" admitted, but their average length of stay was much longer. However, there is no known treatment for Alzheimer's disease and until there is, the burden of dementia for the patient, family, medical, social and welfare services, is likely to increase with increasing longevity.

To transform Alzheimer's disease from a disease that can at present only be described to a disease that is understood and can be treated necessitates a thorough understanding of its aetiology. This is the fundamental problem of Alzheimer's disease - no-one knows what causes the disease or how its characteristic changes are brought about. From a vast array of experimental work, certain factors have however emerged which may contribute to the neuronal

degeneration which is an essential and probably a fundamental part of Alzheimer's disease. These factors are discussed in the following sections.

1.2.1. Aetiology of Alzheimer's Disease. Hypotheses.

Six hypotheses underlie most of the research on Alzheimer's disease and its causes. Although they are usually treated separately in the literature, they are not necessarily mutually exclusive. The Genetic, Viral, Abnormal Protein and Vascular hypotheses are described briefly below, while the Toxin and Acetylcholine hypotheses (since these form the basis of the experimental work of this thesis) are discussed in detail in Sections 1.3. and 1.4., and in Chapters two and three.

1.2.2. Viral Hypothesis.

The hypothesis that Alzheimer's disease may be caused by a virus arises from alleged similarities between this illness and Creutzfeldt-Jakob disease.

Creutzfeldt-Jakob disease is a rare brain disease (one new case is discovered per million of population per year, Wurtman, 1985), which usually affects people between 55 and 75 years of age. It causes progressive dementia, disturbances in posture, vision and the control of movement. In these respects, Creutzfeldt-Jakob disease is very similar to Alzheimer's disease, with the exception that in the former, death comes only about a year after the onset of the disease.

Neither disease causes brain inflammation or fever, yet it is known that Creutzfeldt-Jakob disease can be transmitted to chimpanzees by the injection into the brain of extracts of infected tissue (Gibbs et al., 1968). This indicates that it must be caused by an infectious agent, presumably a virus. In Creutzfeldt-Jakob disease,

a long incubation period (roughly one quarter of the affected individual's normal life span) may pass between exposure to the agent and the first appearance of clinical signs. The infectious agent has therefore been thought to be an unconventional, "slow" virus.

Gajdusek et al. (1976) have observed an abnormal structure made up of twisted filaments in the brainsof people with Creutzfeldt-Jakob disease. They have proposed that this filamentous structure may be the infectious agent causing this disorder, and also Alzheimer's disease. The structures are not present in Alzheimer's disease, but they do share some immunological properties with the amyloid fibrils of neurofibrillary tangles.

If Alzheimer's disease is caused by a virus, it should be transmissible, but attempts to transmit it to experimental animals have been unsuccessful. Infection by the agent of the disease may require a particular genetic make-up, a concurrent immune disorder, or prior exposure to an environmental toxin.

1.2.3. Vascular Hypothesis.

It has been recognised since the 1950's that there is a decrease in total hemisphere blood flow in dementia. For example, Ingvar (1968) found a reduction in hemisphere flow and in the relative amount of grey matter in senile and presenile dementia, the reduction being proportional to the degree of dementia. Simard et al. (1971) also observed reduced blood flow in both presenile and senile dementia. Scheibel and Scheibel (1975) found that small neurons controlling the dilation and contraction of brain arterioles tend to disappear with normal aging and are particularly deficient in the brain of demented patients. It was therefore proposed that the loss

of nerves controlling blood flow prevents the delivery of enough blood to neurons, and that this causes the extensive neuron death characteristic of Alzheimer's disease.

While it is accepted that Alzheimer's disease is associated with a reduction in the amount of blood delivered to the brain (and consequently in the amount of oxygen and glucose extracted from this blood, and in the energy generated from the oxygen and glucose), it is unclear whether reduced blood flow is in fact the cause, or the consequence, of the neuronal death. While reduced blood flow would cause neuronal damage, it is also true that a decrease in the number of cells consuming oxygen and glucose would be expected to diminish the brain's demand for blood. Until the sequence of these alternatives is determined, the causal contribution of inadequate blood flow in Alzheimer's disease remains unproven.

1.2.4. Abnormal Protein Hypothesis.

Alzheimer's disease is clearly associated with abnormal protein structures. Its three major pathological signs are neurofibrillary tangles within neurons, the amyloid that surrounds and invades cerebral blood vessels, and amyloid-rich plaques that replace degenerating nerve terminals. Each of these signs reflects an accumulation of proteins not normally found in the brain.

Glenner et al. (1973) have proposed a theory for the pathogenesis of Alzheimer's disease based on cerebrovascular amyloid. They suggest that "amyloidogenic" proteins in the blood of a patient may be converted, by an enzyme in cerebral blood vessels, into amyloid. The amyloid damages the vessels, causing the leakage into brain tissue of other blood proteins; these proteins are toxic to neurons

and activate an enzyme that converts neurofilaments into paired helical filaments, which further damage the neurons. Eventually the damaged neurons, with their neurofibrillary tangles, are engulfed in neuritic plaques. The number of plaques tends to correlate closely with the extent of dementia (Perry et al., 1978).

This theory is unconvincing for a number of reasons. For example, it does not say what causes the conversion process of amyloidogenic proteins into amyloid (perhaps the synthesis of abnormal proteins is directed by abnormal genes, or by an environmental toxin?). Also, the proteins may give rise to the observed loss of neurons not because thay are toxic to the neurons, but because they crowd the neurons physically. Furthermore, in addition to accumulating the three principal types of abnormal proteins (neurofibrillary tangles, amyloid, and senile plaques), the brain of Alzheimer's disease patients seems to synthesise abnormal quantities of proteins in general (Wurtman, 1985). How or why the brain of demented patients should do this, is not explained by Glenner et al.'s (1973) theory.

Finally, the question arises as to whether the protein accumulations cause the disease, or whether they could be unrelated to whatever process destroys neurons, and be present only as an indication that the destruction has taken place, i.e., as a consequence of the disease.

1.2.5. Genetic Hypothesis.

There are families in which the incidence of Alzheimer's disease is unusually high, and this constitutes strong evidence for the involvement of a heritable factor in at least some forms of the

disease.

Families have been identified in which 10 or more members, representing four or five generations, have developed a dementia of the Alzheimer type. In such cases it appears that an aberrant gene is transmitted as an autosomal dominant (a gene that affects both sexes equally and that need only be inherited from one parent to have full effect). A genetic component can be indicated by large-scale studies of family trees even in families in which the disease appears only sporadically. For example, Heston et al. (1981) either examined or obtained information about the parents, siblings and second-degree relatives of 125 patients, in different families, who died in Minnesota hospitals or nursing homes from 1952 to 1972, and showed adequate evidence at autopsy for a diagnosis of Alzheimer's disease. By 1981, 87 of the relatives had developed a dementing illness. In all autopsied cases that illness was diagnosed as Alzheimer's disease.

In another study, Folstein and Breitner (1981) examined the role of genetic factors in cases they classified as being either mild or severe. Analysis of their data led them to conclude that there are two distinct forms of Alzheimer's disease; a relatively mild non-familial form generally affecting very old people, and a genetically transmitted variant that accounts for about three-quarters of all cases. They calculate that for someone with an afflicted parent or sibling, the risk of developing the disease by the age of 80 is about 17%.

Besides the epidemiological evidence, the genetic model is supported by the fact that nearly all people with Down's Syndrome (Trisomy 21), a known genetic disorder, develop Alzheimer's disease by about the age of 40. The link between Alzheimer's disease and

Down's syndrome has recently been clarified by evidence that DNA markers on chromosome 21 are linked both to an abnormal protein gene, and to the autosomal dominant gene for Alzheimer's disease (Goldgaber et al., 1987; Tanzi et al., 1987; St George-Hyslop et al., 1987; Selkoe et al., 1987). This evidence will be discussed in some depth in Chapter four.

1.3. Cholinergic Hypothesis.

1.3.1. Biochemical Alterations and Mechanisms of Cholinergic Transmission.

Davis and Maloney (1976) and Bowen et al. (1976) reported the first clear biochemical abnormality associated with Alzheimer's disease. They found that the level of the enzyme choline acetyltransferase (CAT) can be reduced by as much as 90% in the hippocampus and cerebral cortex of Alzheimer disease patients. This enzyme catalyses the synthesis of the transmitter acetylcholine from its precursors, choline and acetyl coenzyme A. The loss of CAT activity reflects the loss of cholinergic, or acetylcholine releasing, nerve terminals in these two regions of the brain. The missing terminals are those of neurons whose cell bodies are in the basal forebrain or septum (see Diagram 1.1., which illustrates the major cholinergic pathways implicated in Alzheimer's disease).

The dramatic biochemical disturbance first identified by Davis and Maloney (1976) and Bowen et al. (1976) has been confirmed repeatedly by other investigators (e.g., Perry et al., 1977; Bowen and Davison, 1980; Carlsson et al., 1980; Sims et al., 1980), and seems to be the clue most likely eventually to point to the cause of Alzheimer's disease.

Although a few studies have reported decreases in CAT activity in brains from non-demented (normal) elderly people (McGeer and McGeer, 1975; Davies and Verth, 1978), many more have failed to find any changes over a range of disease-free age groups (e.g., Bowen et al., 1976; Carlsson et al., 1980; Spokes, 1979). This suggests that the severe and consistent decrease found in Alzheimer's patients may reflect a disease-specific disturbance.

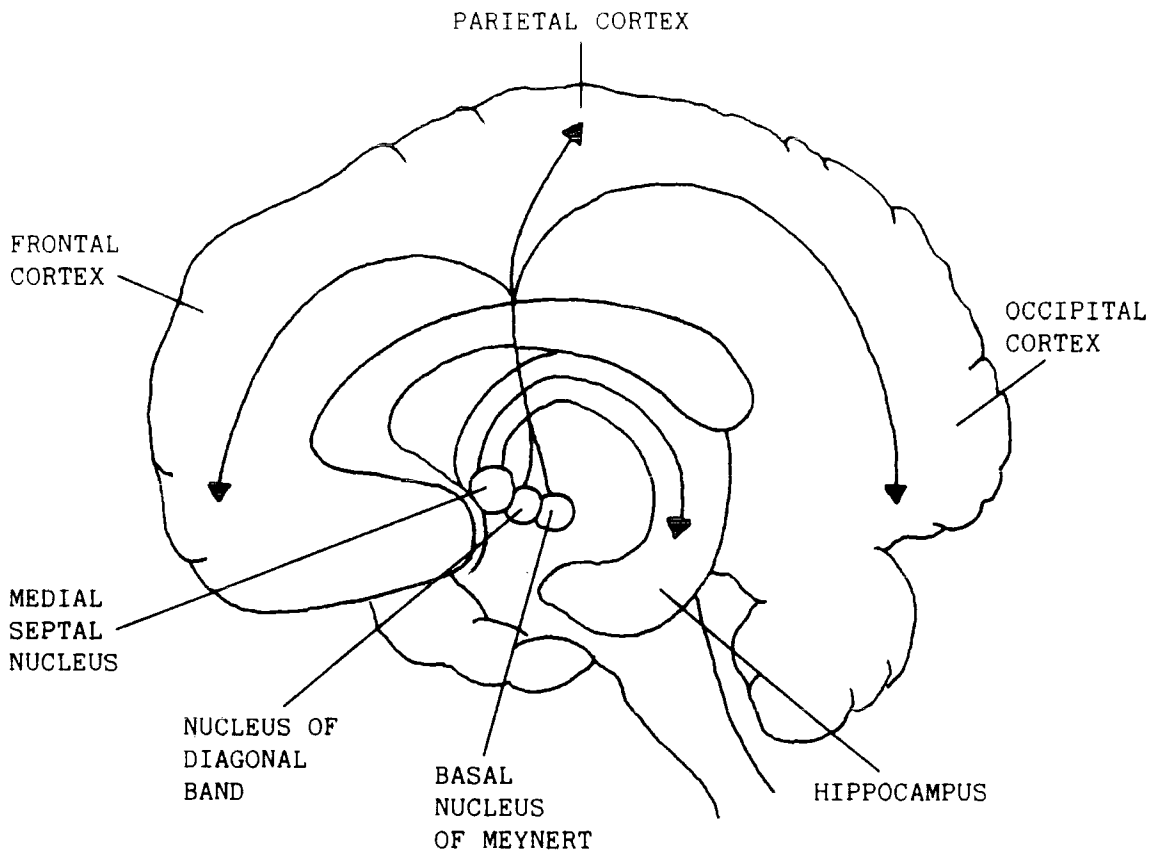

Figure 1.1. Cholinergic pathways innervating cortex.

The cholinergic neuronal cell bodies of the basal forebrain located in the nucleus basalis of Meynert, the diagonal band of Broca, and the medial septal nucleus send axons that innervate the entire cortex including the frontal, parietal and occipital cortex, as well as the hippocampal formation.

(From Coyle, Price and DeLong, 1983).

Many studies have also been conducted to assess changes in CAT activity in aged animals. Although some studies comparing brains from rodents of different ages report reliable decreases in CAT activity, these changes are typically small (15 to 25%). Further, many studies have failed to find similar decreases (Strong et al., 1980; Meek et al., 1977). Thus, most animal aging data agree with the general human literature, failing to demonstrate large or reliable decreases in the activity of CAT as a function of increased (normal) age.

In order to understand the mechanism of cholinergic transmission, and the importance of CAT in this process, a brief description of the metabolism and movement of an acetylcholine molecule follows. The transmitter is manufactured primarily in the terminals from which it is released. The first step in its synthesis is the uptake of choline from the synaptic cleft. The choline can be taken up into the terminal by either of two transport proteins in the pre-synaptic cell membrane. One of them is present not only on cholinergic neurons, but also on all cells, since choline is required for the synthesis of phosphatidycholine (or lecithin), a consituent of all cell membranes. The other transport protein is found only on cholinergic terminals, where its particularly high affinity for choline enables the terminal to take up almost all the choline available in the synaptic cleft.

The next step, catalysed by CAT, combines the choline with acetyl coenzyme A to make acetylcholine. Terminals have an excess of CAT and too little of the precursors choline and acetyl coenzyme A to keep the enzyme molecules fully occupied; the rate at which the transmitter is synthesised is therefore limited not by the amount of CAT enzyme but by the level of the two precursors (hence the proposal

that precursor loading may increase acetylcholine levels - see P. 27). Once synthesised, acetylcholine molecules are stored in the terminal until the arrival of a nerve impulse discharges some of into the synapse. Once it is in the synapse, an acetylcholine molecule can cross the cleft and interact with a receptor on the postsynaptic neuron, thereby transmitting the signal generated by the nerve impulse. It can be broken down by the enzyme acetylcholinesterase to generate choline, or it can interact with a receptor on the presynaptic membrane from where it came, thereby delivering a signal that modulates the subsequent release of acetylcholine from the presynaptic terminals. Diagram 1.2. summarises the metabolism and movement of an acetylcholine molecule.

The principal receptor which triggers the postsynaptic response to released acetylcholine - the muscarinic receptor - is apparently normal in Alzheimer's disease - at least as far as can be judged from the levels of antagonist binding membranes in vitro (Perry et al., 1977; Davies and Verth, 1978). So at the cellular level, the cholinergic defect of Alzheimer's disease appears to be related to changes on the presynaptic (rather than the postsynaptic) side of the cholinergic neurons, and most probably reflects degenerative changes in the cholinergic neuronal process (Pearce, 1984).

The principal biochemical findings (to-date) in relation to cholinergic activities in autopsy and biopsy brain tissue are summarised in Table 1.2.. That a large reduction in CAT is found, has already been mentioned. Additionally, in biopsy tissue, the rate of acetylcholine synthesis is markedly reduced (Sims et al., 1980), as is acetylcholinesterase (ACE). Anatomically, cholinergic abnormalities measured biochemically are generally most marked in the cerebral cortex, although extensive reductions are also found in the amygdaloid

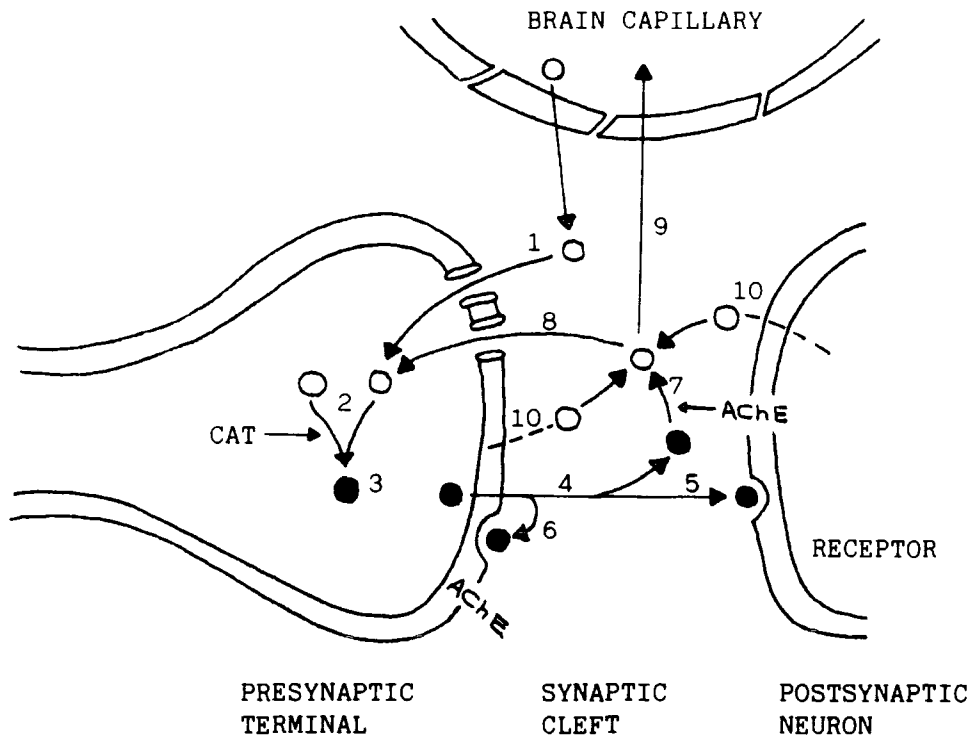

Figure 1.2. Trace of the metabolism and movement of an acetylcholine molecule.

Choline (1) delivered to the synaptic cleft by the blood enters a presynaptic cholinergic terminal, where CAT combines it with acetyl coenzyme A (2) to form acetylcholine (3). When the arrival of a nerve impulse depolarises the neuron, acetylcholine is released (4). It can cross the synapse to interact with a receptor on the post-synaptic neuron, thereby transmitting the signal generated by the nerve impulse (5). It can instead interact with a receptor on the presynaptic membrane (6), thereby modulating the further release of acetylcholine. Or it can be broken down by acetylcholinesterase (AChE) yielding choline (7), which is taken up by the terminal (8), or carried off in the blood (9). The break down of phosphatidylcholine (lecithin), a constituent of cell membranes, contributes to the choline supply (10).

(From Wurtman, 1985).

	ACTIVITY	REPORTED FINDINGS
Transmitter precursor or metabolite	Acetylcholine	Decreased level in autopsy tissue; decreased synthesis in biopsy tissue.
	Choline	Normal
	Choline acetyltransferase	Reduction (50-90%) related to clinical and pathological features.
Related enzymes	Acetylcholinesterase	Reductions in biochemical and histochemical activities; some plaques and tangles positive histochemically.
	Muscarinic receptor binding	Normal.
Receptors	Nicotinic receptor binding	Unclear - reports vary - normal, reduced or increased.

Table 1.2. Cholinergic activities in Alzheimer's Disease (Cerebral Cortex).

(From Pearce, 1984).

nucleus, hippocampus, and in the basal nucleus of Meynert (Perry et al., 1982).

1.3.2. Neurofibrillary Tangles and Senile Plaques.

The significance of the two major Alzheimer-type neuropathological abnormalities, neurofibrillary tangles and senile plaques, is not known precisely at present, though both appear to have a pathological involvement in the dementing process (Perry et al., 1978). In the amygdala and hippocampus, the number of tangles tends to increase as a function of age, and in the entorhinal cortex of the hippocampal formation the number and density of tangles is often used to distinguish normal, non-demented from demented (Alzheimer-type) cases. Deficits of CAT have been linked to the degree of neurofibrillary change in Alzheimer's disease by White et al. (1977).

Senile plaques are found predominantly in the grey matter of the cortex, and in the neocortex, hippocampus and amygdala. The relationship of plaques to dementia is thought to be quantitative, based on the concentration or density of plaques. In most demented individuals with Alzheimer's disease, plaque densities range from 15 to 40 plaques per mm^2, and in clinically severe, neuropathologically advanced cases, may reach densities of 50 to 60 plaques per mm^2 (Pearce, 1984).

The reduction in CAT levels has been shown to correlate with the number of senile plaques in the brains of patients with Alzheimer's disease (Perry et al., 1978). However, the precise relationship between the decrease in CAT levels from the cortex, hippocampus and amygdala and the increased occurrence of tangles and plaques in these same areas, has yet to be defined unequivocally.

1.3.3. Other Neurotransmitter Systems.

The cholinergic system is not the only transmitter system involved in Alzheimer's disease, and in particular, consistent changes relating to another classical transmitter (nor-adrenaline) and one of the neuropeptides (somatostatin) have recently emerged. What is not clear, however, is whether these other changes are as closely linked to the disease process as changes in acetylcholine levels seem to be, or whether they may occur later as secondary-type changes. In this respect it would be useful to have information available on the sequence of changes occuring in the various neuro-transmitter systems over the duration of the illness, although in any case the changes seen in the other systems are not as dramatic or as extensive as those seen in the cholinergic system. Research has focussed principally, therefore, on the possible link between Alzheimer's disease and deficits in the cholinergic system.

1.3.4. Nucleus Basalis of Meynert.

Comparisons between Alzheimer's patients and age matched controls reveal a severe loss of neurons in the basal nucleus of Meynert (nbM); a subcortical nucleus located within the substantia innominata. Because this brain area provides the primary cholinergic input to the cortical mantle these data offer the possibility that the decrease in cortical CAT in Alzheimer's patients may reflect a specific loss of cholinergic input to the cortex, with the nbM being the source of the damage. That this is the case is corroborated by Johnston et al. (1979), who demonstrated that lesions of the nbM in animals causes decreased cortical cholinergic enzyme activities.

Similarly, Aigner et al. (1987) used ibotenic acid to lesion the nbM in four monkeys. The percentage decrease in cortical CAT was as much as 60% when compared to control animals.

Anatomical investigations of the connectivity of the basalis neurons in non-human species have suggested that these cells receive afferents from a variety of sources, including the amygdala, hypothalamus, midbrain, and other brain stem nuclei (Jones et al., 1976; Aggleton et al., 1987) and that, in turn, nbM neurons project to the amygdala, thalamus, hypothalamus, brain stem, hippocampus, entorhinal cortex, and neocortex (Jones et al., 1976; Segal and Landis, 1974). Hence, the nbM is in a critical position to integrate information from a variety of sources and to directly influence the cerebral cortex. For example, abundant evidence suggests that cholinergic terminals in the hippocampus are critically important for memory formation (Olton, 1982) in man and animals. Damasio et al. (1985) examined five patients with damage to the basal forebrain, and found behavioural disturbances featuring a prominent amnesic syndrome. They proposed that the memory disorder can be explained by malfunctioning in the hippocampal system, secondary to damage in the basal forebrain structures, with which the hippocampus is strongly interconnected.

In a neuropathological investigation, Whitehouse et al. (1981) found a 90% loss of neurons in the nbM in a patient with a familial form of Alzheimer's disease. In 1982, Whitehouse et al. examined the brains from five demented patients who had had a history typical of Alzheimer's disease, and who exhibited the classical pathology of the disease, including neurofibrillary tangles and senile plaques. Each of the patients with Alzheimer's disease showed a severe loss of nbM neurons, with an average neuron loss of over 75% when compared

to control subjects. Additionally, some of the remaining neurons in the nbM of Alzheimer's patients showed neurofibrillary degeneration.

A key question in relation to these recent neuropathological findings is whether degeneration of cholinergic cells in the nbM is one of the most important, perhaps primary, features of Alzheimer's disease which may account for the clinical symptoms, or whether it is instead a later, secondary type of change. If it is, as Whitehouse et al. (1982) suggest "a critical subcortical lesion in Alzheimer's patients", a major loss of these neurons should be apparent in all established cases of the disease, and should occur in conjunction with the reductions in cortical cholinergic activities.

In contrast to the Whitehouse et al. study (1982), Perry et al. (1982) found only a 33% neuron loss in the nbM when cell counts were carried out objectively, and suggest that the loss of cholinergic neurons may be a secondary rather than a primary change in Alzheimer's disease, the key pathological change being a "down-regulation" of transmitter-specific enzyme production in cholinergic neurons. There is thus some dispute in the literature regarding the precise role of the nbM in cholinergic loss. This is currently an active area of research.

The ability to reproduce certain aspects of the neurochemical pathology of Alzheimer's disease in experimental animals, permits greater evaluation of the role of the nbM and the basal forebrain cholinergic pathways in the pathophysiology of the disease, than can be deduced from post mortem samples of human brains. Animal models of the dementia also allow more detailed analysis of the memory and cognitive deficits arising from nbM lesions than can be derived from Alzheimer's disease patients. Although the role of the nbM in behaviour and cognition has yet to be defined, as few animal studies

have actually been carried out in this area, psychopharmacological and experimental lesion studies to-date suggest that the basal forebrain cholinergic pathways, particularly those projecting to the hippocampal formation (Segal and Landis, 1974), play an important role in memory processes (Smith and Swash, 1978).

In a recent study, Hepler et al. (1985) used ibotenic acid to destroy the nbM in albino rats, and evaluated the functional contribution of the nbM to memory in four behavioural tasks: Post-operative acquisition of a win-stay spatial discrimination in a T-maze; a win-shift spatial discrimination on a radial arm maze; active avoidance in a shuttle box, and passive avoidance in a shuttle box. When compared to controls, the rats with nbM lesions had significantly impaired choice accuracy in the T-maze and radial maze tasks, required significantly more trials to reach criterion in the passive avoidance task, and were impaired on retention of the active avoidance task. The results of these studies suggest that the nbM is involved in memory processes. Specifically, Hepler et al. (1985) found that the pattern of behavioural changes seen after lesioning the nbM was the same as that seen on the above tasks following experimental damage to the hippocampus in rats (LoConte et al., 1982; Olton, 1982). This suggests that the cholinergic system, nbM and hippocampus are closely linked in a system mediating memory.

There are, however, certain differences between the effects of lesions of the nbM in experimental animals, and damage to this area in man. For example, Wenk and Olton (1984) injected ibotenic acid into the nbM in rats. Seven days after injection neocortical CAT levels had decreased by 60%, but after three months CAT levels had returned to normal. Wenk and Olton (1984) propose that this may be the result of a compensatory increase in CAT enzyme levels within

surviving cortical neuronal terminals. Thus the system in animals shows extensive plasticity and recovery of function with time. Similar recovery of function has not been demonstrated in Alzheimer's disease patients with lesions of the nbM.

Salamone et al. (1984) examined the results of nbM lesions in rats, on performance of an appetitive T-maze task involving spatial memory. The lesions were found to result in reduced acetylcholinesterase levels in the neocortex, reduced CAT levels (by approximately 30%) in frontal cortex, and the disruption of rewarded alternation procedure. The lesioned rats showed some improvement in performance over 8 subsequent test days, however, and this can be related to the report of Wenk and Olton (1984) that reduced CAT activity in cortex following nbM lesions recovers over a 12 week period. Furthermore, the results of the study by Salamone et al. (1984) do not unequivocally demonstrate that nbM lesions cause a memory deficit on the spatial T-maze task: The lesions could have led to a deficit in the processing of spatial information, or to non-specific sensory or motor biases, all of which would result in the observed choice inaccuracy. Similarly, a number of authors have demonstrated that lesions of the nbM in rats cause deficits in passive avoidance responding (Flicker et al., 1983; Friedman et al., 1983; LoConte et al., 1982), and while these results are consistent with a hypothesised memory dysfunction caused by lesions of the nbM, they do not provide conclusive evidence of that hypothesis.

In general, the evidence available so far demonstrates that destruction of neurons in the basal forebrain may be responsible for some of the cognitive disturbances occurring in those individuals with Alzheimer's disease, though a wider variety of test procedures needs to be used in order to delineate precisely the types of

memory function that are spared and impaired following damage to the nbM; to establish the reason for recovery of function in animals; and to eliminate the effects of confounding variables on performance of the tasks.

To summarise so far:

(1) It has been consistently demonstrated that CAT is markedly diminished in the cerebral cortex and hippocampal regions of patients with Alzheimer's disease when compared with healthy age-matched subjects, and with other psychiatric controls (Davis and Maloney, 1976; Perry et al., 1978; White et al., 1977).

(2) The number and density of neurofibrillary tangles and senile plaques increases in the hippocampus, cortex and amygdala of Alzheimer's patients compared with elderly, non-demented patients (White et al., 1977; Perry et al., 1978), and these authors have further shown that the reduced level of CAT correlates both with neurofibrillary change and with the number of senile plaques present in the brains of patients with the disease.

(3) Finally, Whitehouse et al. (1982) found that there is extensive loss of neurons in the nbM of patients with Alzheimer's disease. They propose that the loss of neurons from this region may be responsible for the decrease in cholinergic activity observed in the cortex, hippocampus and amygdala, since the nbM projects to these areas and is thought to provide the primary cholinergic input to the cortex.

1.3.5. The Cholinergic Hypothesis of Memory Loss and Scopolamine.

The principal cognitive deficit observed in patients with Alzheimer's disease is a severe loss of memory, and the finding that

cholinergic activity is markedly reduced in the brains of Alzheimer's disease patients, leads to the hypothesis that cholinergic loss is related to the dementia, and specifically, to the memory dysfunction. Indeed, Perry et al. (1978) have found that the reductions in CAT and ACE (see P.17) can be correlated to the clinical extent of the disease process, i.e., the lower the level of CAT, the more pronounced the memory loss. However, simply demonstrating that dementia-related changes in the cholinergic system occur, does not directly prove that these changes might be responsible for the dementia and its associated memory loss.

One method of gaining additional information about the extent to which changes in the cholinergic system contribute to the memory loss associated with age and dementia would be to pharmacologically impair function in this neurotransmitter system in non-demented subjects and compare the changes in memory ability with those occurring naturally in demented subjects. If age-related changes in the cholinergic system contribute to the memory loss observed in Alzheimer's patients, pharmacological disruption of that system should induce similar changes in the behaviour of normal subjects.

Cognitive deficits in demented patients typically occur in situations requiring recent events to be remembered, usually without the benefit of extensive rehearsal or practice (Bartus et al., 1978, 1980). The primary pharmacological data supporting an important cholinergic involvement in this memory deficit is that blockade of central muscarinic receptors by the drug scopolamine induces a similar deficit in recent memory in normal subjects. For example, Drachman et al. used a number of clinical measures to find that normal human subjects tested under a low dose of scopolamine exhibited memory (Drachman and Leavitt, 1974) and other cognitive

(Drachman, 1980) deficits similar to those found naturally in aged people with Alzheimer's disease who were tested on the same clinical battery. The tests which revealed the most severe deficits in both cases involved memory for recent events.

In animal studies, aged monkeys tested on a number of different behavioural tasks have been found to suffer a very consistent and severe deficit on tasks requiring memory for recent sensory events (Bartus et al., 1978, 1980), with the greatest memory deficits occuring under those conditions requiring the longest retention of recent information. Young monkeys injected with the central cholinergic receptor blocker scopolamine exhibit a deficit strikingly similar to that occuring naturally in the aged monkeys (Bartus and Johnson, 1976; Robustelli et al., 1969; Pontecorvo and Evans, 1985; Aigner and Mishkin, 1986), and this deficit of recent memory in young and aged monkeys shares many similarities with that suffered by demented humans.

In general, many human studies, e.g., Crow and Grove-White (1973), Sitarem, Weingartner and Gillian (1978), Richardson et al. (1984) etc., have reported disruptive effects on memory after the administration of scopolamine, and these studies all lend support to the hypothesis that a disorder of the cholinergic system might be related to the memory and cognitive deficits seen in Alzheimer's disease.

Although animal studies which have examined the effects of scopolamine on memory function in both monkeys and rodents seem to provide additional support for this hypothesis (Evans, 1975; Pontecorvo and Evans, 1985; Robustelli et al., 1969; Dunnett, 1985; Heise et al., 1976), a number of problems associated with the studies

make the results (as far as scopolamine's effects on memory are concerned) difficult to interpret. Some of these problems will be discussed in Section 1.3.7., and in more detail in Chapter two.

The human and non-human primate studies largely corroborate each other, and demonstrate that one of the most severe and consistent deficits observed with dementia occurs on tasks requiring memory for relatively recent events. Furthermore, of the classes of drugs tested on memory tasks, drugs having anticholinergic effects seem to produce deficits most closely (though perhaps only superficially) mimicking the natural, dementia-related memory impairments, which satisfies another logical pre-requisite for the cholinergic hypothesis. When the pharmacological data are considered with the correlative neurochemical and neurophysiological changes discussed earlier, the cholinergic hypothesis does seem to be the most likely theory that will eventually explain some of the clinical syndrome associated with Alzheimer's disease, and perhaps even pinpoint the cause of the disease. Furthermore, there is one other area of research which also lends support to the cholinergic hypothesis.

1.3.6. The Cholinergic Hypothesis of Memory Loss and Physostigmine.

The hypothesis that the cognitive impairments of Alzheimer's disease patients may be a reflection of decreased cholinergic activity is supported by studies using anticholinergic agents, such as scopolamine, in normal subjects. Conversely, it should be possible to improve memory and cognition in both normal subjects and Alzheimer's disease patients by increasing cholinergic activity.

In relation to the cholinergic neuronal system, the demonstration of selective abnormalities on the pre-synaptic side and apparent

normality of receptors on the post-synaptic side (see P. 16) have encouraged the view that some kind of 'replacement' therapy, aimed at increasing levels of acetylcholine in the brain, might be effective in at least partially restoring cholinergic functions. Various possible means of therapeutically rectifying a central cholinergic deficit are outlined in Table 1.3. (Pearce, 1984). Trials aimed at increasing acetylcholine synthesis in Alzheimer's disease via increased precursor (choline or lecithin) availability have not so far resulted in any consistent or long-lasting improvement. In contrast, trials of the acetylcholinesterase inhibitor, physostigmine, have been considerably more promising, though this drug seems to have a narrow effective dose range.

For example, Davis et al. (1976) administered physostigmine 3 mg/kg intravenously to human subjects, and found that at this dose the drug resulted in a decrement in all aspects of memory. In contrast, doses of 1 - 2 mg. produced a slight improvement in memory storage and the effect at 1 mg. was greater than for 2 mg. The dose of physostigmine used therefore seems to be crucial.

Davis et al. (unpublished data) infused 0.5 mg. physostigmine at a constant rate over a 30 minute period, and found a significant improvement in long-term memory in normal (human) subjects. Specifically, an improvement was seen in the process of consolidation. In a later study, Davis et al. (1978) administered 1.0 mg. of physostigmine or 1.0 mg. of saline by slow intravenous infusion to 19 normal male subjects, on two non-consecutive days, and found that physostigmine significantly enhanced the storage of information into long-term memory, as indicated by the results obtained on a verbal learning task. Retrieval of information from long-term memory was also improved, though short-term memory processes (as

THERAPEUTIC POSSIBILITY	PRACTICAL APPROACH	REPORTED OUTCOME IN ALZHEIMER'S DISEASE	COMMENTS
Increasing acetylcholine (ACh) synthesis via:			
Choline availability	Oral choline or lecithin	Little or no improvement	Unclear if ACh synthesis is normally increased by supplementary choline
	Choline plus piracetam	More encouraging results in aged animals and some Alzheimer-type patients	Suggests metabolic stimulation may be useful adjunct in cholinergic therapy
Acetyl-coenzyme A availability			Stimulating glucose catabolism might increase ACh synthesis since the reverse is true, with impaired synthesis accompanying mild metabolic impairment
Decreasing ACh breakdown	Intravenous physostigmine	Small but consistent improvements in Alzheimer's disease and aged animals	Optimal dose variable; short-acting drugs with unpleasant side-effects; need for other anticholinesterases
Cholinergic receptor stimulation	Intramuscular arecoline	Improvements in aged primates	Optimal dose more consistent compared with physostigmine; current need for new muscarinic and nicotinic agonists
Stimulating ACh release			Agents (e.g. neuropeptides) may directly stimulate ACh release mechanisms

Table 1.3. Theoretical therapeutic approaches to directly countering a cholinergic deficit.

(From Pearce, 1984).

tested by digit span and memory scanning tasks) were not significantly altered by physostigmine.

In a 1982 study, Davis and Mohs administered physostigmine (0.125 mg., 0.25 mg. or 0.5 mg.) or placebo intravenously to ten neuroleptic-free patients with Alzheimer's disease over a 30 minute period. All patients performed better on a recognition memory task while receiving physostigmine. When placebo or the dose of physostigmine previously associated with an improvement in memory was re-administered, physostigmine again enhanced performance on a recognition memory task. These studies indicate that the acute augmentation of cholinergic activity in some patients can partially reverse the memory deficit of that disorder, and one specific case report lends credibility to this suggestion, (Peters and Levin, 1977).

In this study (Peters and Levin, 1977), a 20 year old woman with a profound memory deficit secondary to an attack of herpes simplex encephalitis she had sustained two years earlier, was given varying doses of physostigmine and placebo in a double blind protocol. On three separate occasions following a dose of 0.8 mg. physostigmine given subcutaneously there was a marked enhancement in the woman's ability to store new information in long-term memory from short-term memory, as well as improvements in her retrieval from long-term memory. Both lower and higher doses of physostigmine did not produce this effect.

Beneficial responses to physostigmine have also been reported by a number of other authors, e.g., Christie et al., 1981; Peters and Levin, 1979; Sullivan et al., 1982; Kaye et al., 1982. All of these studies are consistent with the neurochemical investigations of Alzheimer's disease and with the scopolamine data.

Drachman (1977) gave scopolamine 1.0 mg. to normal subjects to

produce a memory impairment on two tasks; digit span, and free word recall. Half of the test subjects then received physostigmine (1.0 mg. or 2.0 mg.) and half d-amphetamine, 10 mg.. [d-amphetamine is a catecholamine agonist that potentiates the release and interferes with the re-uptake of dopamine and noradrenaline: Effects on acetyl-choline are indirect and minor (Cooper et al., 1974) though d-amphetamine is known to increase alertness (Estler, 1975). If d-amphetamine antagonised the scopolamine dementia more effectively than physostigmine, it would imply that disruption of memory/cognitive functions by scopolamine might be the result simply of behavioural lethargy (since scopolamine is known to produce lethargy, fatigue and sleepiness in humans), without clear dependence on specific memory-related properties of the cholinergic neuronal system]. Physostigmine markedly improved memory and cognitive function; d-amphetamine had no effect on memory, although alertness was improved. This comparison supports a specific role for cholinergic neurons in memory/cognitive processes.

In addition to physostigmine, the muscarinic agonist arecoline has been evaluated for effects on performance in memory tasks. After receiving a single injection of arecoline, young adult volunteers exhibited significant improvements in their ability to recall recently learned verbal material (Sitarem, Weingartner and Gillian, 1978). Short-term doses of arecoline have also been shown to enhance performance on a memory task in aged monkeys (Bartus, Dean and Beer, 1980), and Alzheimer's patients (Christie et al., 1981).

Hence, it is apparent that reliable improvements on tasks intended to measure memory can be obtained in the laboratory and clinic by pharmacologically manipulating the cholinergic system. The ability of physostigmine and arecoline to measurably improve

performance must be tempered, however, by the short half-life, narrow effective dose range and adverse side effects that are hallmarks of both of these drugs. Peripheral side effects typical of muscarinic agonists such as arecoline and anticholinesterases such as physostigmine include increased salivation, sweating, flushing, slight hypertension and nausea.

Wettstein and Spiegel (1984) attempted to reduce the adverse side effects by administering the post synaptic muscarinic agonist RS 86 (2-ethyl-8 methyl-2, 8-diazospiro-(4.5)-decane 1, 3-dione hydrobromide) to six patients with Alzheimer's disease, in a series of controlled clinical trials. Daily doses were up to 3.0 mg. orally for a maximum duration of six weeks. Positive clinical changes with regard to cognitive functions, mood and social behaviour were observed in four of the Alzheimer's disease patients. RS 86 produced typical peripheral effects, but appeared to be better tolerated than similar drugs such as arecoline or physostigmine. Nevertheless, physostigmine is still the drug most commonly used in order to study the congnitive improvements produced by increased cholinergic activity.

The studies quoted above support the hypothesis that central cholinergic neurotransmission may play a role in the processing of recent memories, and that abnormalities of this system may underlie some of the symptomatic manifestations of Alzheimer's disease. Furthermore, the studies indicate that reversal of the cognitive deficit may provide an approach to the treatment of the disorder.

1.3.7. Animal Studies.

Analysis of animal models of dementia not only allows for a more detailed characterisation of neurotransmitter related processes than is possible from post mortem samples of human brain tissue, but also allows the potential for analysis of the sequence of neurotransmitter changes that occurs as the dementia develops. For these reasons, animal models which share neuropathologic features of the human dementing disorders are of particular value. Consequently, there is a massive literature on the effects of cholinergic manipulation on animal memory.

In general, it has been shown that blockade of the cholinergic system by scopolamine impairs learning in tasks involving discrimination, passive avoidance responses, and the learning of 'fear' (conditioned suppression) responses (Berger and Stein, 1969; Chiapetta and Jarvik, 1969; Meyer, 1965; Whitehouse, 1966; Buresova et al., 1964). One exception occurs with avoidance tasks, where large doses of cholinergic blocking agents often produce 'improved' performance due to increased motor activity and more rapid escape (Renfro et al., 1972). Physostigmine may enhance learning of maze tasks (Whitehouse, 1966), while decreasing avoidance responses in an escape situation (Rech, 1968). Problems arise in the interpretation of these observations, however, because many factors other than memory and cognitive function enter into tasks of this nature. The cholinergic system affects many aspects of behaviour in addition to memory, and so many learning paradigms can be readily contaminated. For example, cholinergic agents influence appetitive behaviour and nociception, and thus interfere with reward or punishment motivation (Karczmar, 1976). Similarly, interference with the motor system can

influence the results on learning tasks without affecting the memory process. These studies are further complicated by differences in drug administration and dose.

The animal studies underline the necessity for future human experimentation to use relatively standardised cognitive tasks, drug dose and routes of administration if comparable data are to be gathered. With regard to the animal work currently in progress, it is believed that discrimination tasks are more likely to test cognitive and memory functions similar to human tests, than are avoidance tasks (Drachman, 1977).

1.3.8. Summary and Objective.

There is ample evidence from both human and animal studies that the drug scopolamine, which blocks cholinergic (muscarinic) receptors, can impair tasks of memory. Unfortunately, this same drug has pronounced effects upon motivation and arousal and it has proved difficult to dissociate these various effects.

The present study was designed specifically to measure whether scopolamine produces a general depression in the performance of a working memory task (a task of discrimination), or whether its effects are most evident when the memory demands of the task are increased. If the latter were true, this would lend support to the proposal that scopolamine can have a specific effect upon memory processes, which supports the hypothesis that decreased cholinergic activity may be responsible for some of the memory and cognitive impairments characteristic of Alzheimer's disease.

1.4. Toxin Hypothesis.

1.4.1. The Hypothesis.

The toxin hypothesis proposes that Alzheimer's disease is caused by the accumulation of aluminium salts in the brain, the aluminium producing neuronal degeneration in regions such as the neocortex, the limbic system, and the basal forebrain. Since aluminium salts are present in a wide range of consumables including drinking water, certain medicines and food stuffs, the proposed link between aluminium and dementia is receiving considerable attention. Evidence of an involvement of aluminium in at least some cases of Alzheimer's disease has come from a variety of sources, and this evidence will be reviewed in the following sections.

1.4.2. Sources of Aluminium.

Aluminium composes approximately 80% of the earth's crust, making it the third most common element and the most abundant metal. Because aluminium has a great affinity for oxygen, it occurs only in the oxidised form, mainly as alumina $Al_2 O_3$. Aluminium is found in almost 300 different minerals, especially as double silicates such as feldspars and micas and their weathering products, the clays. Aluminium is very versatile, and has many diverse uses. Hence aluminium production and manufacturing now ranks in third position behind iron and steel.

Aluminium is found in the air, mainly as aluminosilicates associated with dust particles. Water may also contain large quantities of aluminium, and there are numerous foods and non-prescription drugs that contain substantial amounts of added aluminium

(see Section 3.3. for further discussion of sources). Underwood (1977) recently estimated the typical daily (dietary) intake of aluminium to be 22 mg.. However, the innappropriate choice of foods and non-prescription drugs containing intentionally added aluminium salts can increase the daily dietary intake of aluminium 10 to 100 fold (Lione, 1985). There is no evidence that aluminium is a required nutrient (Underwood, 1977), and numerous reports describe the neurotoxicity of this metal. While more research is done to determine the complex processes controlling the absorption, distribution and excretion of this metal, it would appear prudent to restrict the intake of aluminium in both demented and non-demented (normal) people.

1.4.3. Relevant Properties of Aluminium.

Regardless of whether or not aluminium is the cause of Alzheimer's disease, this metal does have several properties which means that it can be neurotoxic to both man and animals if it is present in the body in sufficiently high quantities (i.e., as might occur due to excretory failure):

(1) The aluminium ion can strongly and uniquely affect the central nervous system because it is a powerful flocculent, and, as such, causes shrinkage of colloids (Bjorksten, 1982). The human brain contains large amounts of colloidal gels. Abnormal coagulation of these gels causes shrinkage, thereby severing interneuronal connections, a process similar to that occurring in dementia.

(2) Aluminium is a strong reactant, causing cross-linkage between large vital molecules. Deleterious effects of cross-linkage are:

(a) Formation of tangled molecular chains or nets which

progressively impede intracellular transport;

(b) loss of elasticity of all tissues, with increased susceptibility to rupture;

(c) conversion of essential molecules into inert aggregates;

(d) inactivity of vital molecules by creating steric hindrances, and;

(e) secondary effects caused by any of the above on the accuracy of mitotic processes, protein and other synthesis, or disturbance of enzymes.

(3) Whereas organisms have been forced to develop systems for handling other metals such as zinc, which is neurologically toxic at relatively low concentrations but which is needed in normal metabolism, aluminium has no known metabolic function. It cannot be reduced and can form extremely firm attachments that do not permit its removal by any biological means available to the organism.

Having established that aluminium is a neurotoxic agent, I shall now consider a neurological syndrome in which aluminium has been specifically implicated - dialysis dementia.

1.4.4. Aluminium and Dialysis Dementia.

Aluminium has been strongly implicated in human dialysis dementia. This syndrome is characterised by speech difficulties, motor abnormalities, personality changes, seizures, loss of memory and progressive dementia, terminating in convulsions and death. The clinical features of the syndrome indicate that it is some type of toxic or metabolic encephalopathy. Evidence of a slow virus is lacking in that Alfrey et al. (1972) injected extracts of the brain of one demented, dialysed patient into primates. These were still experiencing no

adverse effect over 3.5 years later.

There is no specific histopathology in dialysis dementia, although it has been shown that aluminium levels in plasma (Berlyne et al., 1970), bone (Parsons et al., 1971), and in particular, the grey matter of the brain (Alfrey et al., 1976), are considerably elevated in patients suffering from the dementia. Furthermore, the neurobehavioural syndrome described above is remarkably similar to that described in experimental animals which have been exposed to aluminium (see Section 1.4.12.). The proposed link between aluminium and dialysis dementia was reinforced by the reports of several researchers who demonstrated that the dementia is associated with high aluminium content in the water used to make up the dialyzate (Berlyne et al., 1970; Kaehny et al., 1977) and/or with intestinal absorption of aluminium-containing phosphate-binding gels (Clarkson et al., 1972; Alfrey et al., 1976). Brain grey matter aluminium levels were shown to vary directly with duration of dialysis by Alfrey et al. (1976), and a number of authors have reported that an elimination or reduction of dementia is observed if aluminium-rich dialyzate solutions are substituted with de-ionized water (Dunea et al., 1978; Rozas et al., 1978; Schreeder, 1979; Hagstram et al., 1980). These studies all support a primary role of aluminium in the dialysis dementia syndrome. It is now generally recommended that de-ionized water should be used in the hemodialysis procedure.

The reported mean brain aluminium content in patients with dialysis dementia ranges from 12.4 to 33.0 mg/kg in different studies (Alfrey, 1980; McDermott et al., 1978; Arieff et al., 1979; Mahoney and Arieff, 1982; Cartier et al., 1978). As would be expected with impaired excretory functioning, other patients on dialysis have died showing elevated brain aluminium, but not dementia. The aluminium

levels in such patients have not, however, been within the range of those with encephalopathy. While most normal brains have aluminium contents under 4 mg/kg the mean aluminium content for non-demented dialysis patients was from 3.8 to 8.5 mg/kg (Petit, 1985). Alfrey (1980) reported that non-demented dialysis patients may have brain aluminium contents of 8.5 ± 3.3 mg/kg. This level of aluminium in the brains of cats and rabbits is sufficient to induce an encephalopathy and death (see Section 1.4.12.) which suggests that the human is more resistant to the neurotoxicologic effects of aluminium than some other species: Instead of requiring 3 to 6 times the aluminium content of normal brains to reach an encephalopathy (as in cats and rabbits), neurologic signs only appear in the human when the tissue content of aluminium reaches or exceeds 10 to 20 times that of normal brains.

Elevated aluminium levels in dialysis dementia patients probably occur because aluminium passing from dialysis water directly into the bloodstream bypasses the body's two principal natural defences:
(1) Low absorption of aluminium by the gastro-intestinal system, and
(2) removal of aluminium by the kidneys.
It is unclear, however, by what mechanism the aluminium should accumulate preferentially in the grey matter of the brain (Alfrey et al., 1976). Wisniewski (1985) proposes that the dialysis dementia syndrome may result from an aluminium overload condition brought about by changes in the permeability of the blood-brain barrier (BBB) as a result of renal failure, but this has yet to be proved. (Evidence relating to possible malfunctioning of the BBB is presented in Section 1.4.8.).

1.4.5. Amyotrophic Lateral Sclerosis and Parkinsonism Dementia.

Amyotrophic Lateral Sclerosis (ALS) is a progressive disease of the central nervous system characterised by accumulations of fibrillary material in the neuronal perikaryon. The neurofibrillary tangles of ALS consist of tangles of neurofilaments and not of paired helical filaments. ALS, along with Parkinsonism dementia (PD) occurs in certain isolated populations with great frequency, such as the Chamorros of Guam; in the Kii Peninsular of Japan; and in southern West New Guinea, three areas in the Western Pacific region. Recent studies have shown an abnormal mineral distribution in the soil and drinking water of these areas consisting of a virtual lack of calcium and magnesium coupled with high levels of aluminium and manganese. Examination of neurons containing neurofibrillary tangles from brains of cases of ALS and PD by scanning electron microscopy with energy-dispersive X-ray spectrometry shows greater accumulations of aluminium in both the cytoplasm and the nucleus in comparison to non-tangle-bearing neurons from control cases (Perl et al., 1982). Similar comparisons have revealed somewhat higher levels of calcium in neurons with neurofibrillary changes, but no differences in magnesium, silicon, manganese and iron. In addition, in the PD cases and in the control cases with paired helical filaments, mineralisation of the blood vessel walls within the globus pallidus is observed in which the aluminium content is considerably greater than has previously been observed in other neuropathological conditions examined (Wisniewski, 1985). The tentative conclusion reached from these results is that the pathogenesis of ALS, PD, and premature occurence of paired helical filaments in neurologically normal people in this region may include environmental factors, especially the high

natural abundance of aluminium.

1.4.6. Bulk Brain Aluminium and Peripheral Markers of Aluminium in Alzheimer's Disease.

Crapper et al. (1976) and Trapp et al. (1978), using atomic absorption spectroscopy, described elevated aluminium levels in the brains of individuals with Alzheimer's disease. For example, Crapper et al. (1976) found that 28% of their Alzheimer's samples were 3 S.D. above the control mean of 4 μg/gm dry weight. These findings have not, however, been replicated. Markesbury et al. (1981) used instrumental neutron activation analysis procedures to determine the aluminium content of various brain regions in histologically verified Alzheimer's disease cases and in controls. No difference was found at the bulk sample level between Alzheimer's disease specimens and adult controls, corrected for age and sex, or when frontal, temporal and hippocampal specimens were compared. The observations of Markesbury et al. (1981) are in agreement with the findings of McDermott et al. (1979) who, using dry ashing and atomic absorption spectroscopy, detected no difference in brain aluminium concentrations between control and Alzheimer's disease specimens.

The reasons for the difference in the Markesbury et al. (1981) data and those cited for Crapper et al. (1976) are not clear, but it is possible that differences existed in the occupational and environmental exposure to aluminium for the individuals studied, i.e., many of the patients of Crapper et al. (1976) were from a region with a high proportion of aluminium sulphates in the metropolitan water supply, whereas the patients of Markesbury et al. (1981) were from an area of comparatively low water-aluminium content. Although

aluminium in the diet can come from many sources, there is general agreement that aluminium-treated water may make a substantial contribution to tissue levels of this element (Dunea et al., 1978; McDermott et al., 1978; and see Section 1.4.4.).

Furthermore, while the bulk brain data of Markesbury et al. (1981) and McDermott et al. (1979) do not support the concept that aluminium has a role in Alzheimer's disease, it seems that major differences exist in its concentration at the submicroscopic level of the disease, as suggested by the X-ray spectrometry studies of Perl and Brody (1980). These authors described the presence of aluminium in the nuclei of neurofibrillary tangle-bearing neurons from individuals with Alzheimer's disease, but not in normal neurons from these patients (see next Section). Hence aluminium appears to be involved in the disease at the submicroscopic level, rather than at the bulk sample level.

Given the failure to find clear changes in bulk brain levels of aluminium it is not surprising that attempts to find a measure of aluminium in a body compartment more accessible than the brain (for example, as a constituent of cerebrospinal fluid (CSF), urine, or circulating blood), have also met with little success. Although studies by Guard (1980) and Delaney (1979) suggested elevated aluminium levels in the CSF of Alzheimer's patients, problems of statistical analysis have shed doubt on Guard's data, whereas Delaney's study has been questioned on technical methodological grounds (Shore et al., 1980[1]).

Shore et al. (1980[1]) compared CSF aluminium levels in five Alzheimer's patients with that in 16 non-demented controls, but did not find any significant differences between the groups. Shore et al. (1980[2]) extended their investigation to include serum aluminium

levels. Again they found no significant differences between their 15 Alzheimer subjects and controls. In a further study, Shore et al. (1980[3]) investigated the status of circulating parathyroid hormone (PTH) in patients with Alzheimer's disease and age/sex matched controls. In animal studies, elevated PTH produces increased absorption of aluminium from the gastro-intestinal tract and elevations of aluminium in cerebral cortex. It has been proposed that PTH elevations may increase tissue aluminium loads in patients with Alzheimer's disease, but Shore et al. (1980[3]) found no significant differences in the levels of circulating PTH between their patient and control groups. When PTH elevations did occur, they seemed to be related to the degree of renal impairment rather than dementia.

Shore and Wyatt (1983) studied 10 out-patients in the early to middle stages of Alzheimer's disease, and measured aluminium concentrations in the hair of the patients and age-matched controls eating similar diets (spouses of the 10 patients). They found that the Alzheimer patients had no elevations of hair aluminium concentrations when compared with controls (mean age 62.2 years, n=10).

Table 1.4. summarises the results of the studies by Shore et al. (1980[1], 1980[2]), and Shore and Wyatt (1983). As yet no peripheral marker of aluminium in Alzheimer's disease has been found, and the inability of the various authors cited above to find evidence of a general overload of aluminium in the disease leads to the conclusion that othere factors must be responsible for the focal accumulations of aluminium in paired helical filament-containing neurons (see Section 1.4.9.).

	Hair Al in parts per million (mg/l)	Serum Al in parts per billion (µg/l)	CSF Al in parts per billion (µg/l)
A.D. patients			
$\bar{x}$	7.5	7.1	30.7
SD	4.1	5.9	7.3
n	10	15	5
Age-matched controls			
$\bar{x}$	6.2	5.9	35.3
SD	3.8	2.3	10.8
n	10	8	9

A.D. = Alzheimer's Disease CSF = Cerebro spinal fluid
Al = Aluminium

Table 1.4. Aluminium concentrations in Alzheimer's disease patients and Age-Matched (Non-demented) Controls.*

*There are no significant differences between Alzheimer's disease patients and controls in the aluminium content of hair, serum or spinal fluid.

(From Shore and Wyatt, 1983).

1.4.7. Aluminium in Neurofibrillary Tangles and Senile Plaques.

Perl and Brody (1980) studied the elemental content of neurons in the hippocampus by a combination of scanning electron microscopy and X-ray spectrometry, in autopsy-derived brain tissue from three cases of Alzheimer's disease and three non-demented elderly controls. Of neurons with tangles examined in the cases of Alzheimer's disease, 91.2% demonstrated a peak for aluminium within the nuclear region, whereas aluminium was essentially not detected in adjacent normal-appearing neurons. In the few neurofibrillary tangle-containing neurons from the three elderly non-demented individuals, aluminium peaks were identified within the nuclear region in 88% of cells but were rarely encountered in the normal-appearing neurons examined in these cases. Silicon was also detected within the tangle-bearing neurons of the Alzheimer's disease cases, but was rarely seen in the cells of non-demented individuals. These findings indicate an association of focal intranuclear accumulations of aluminium with the presence of neurofibrillary degeneration in the hippocampal neurons of cases of Alzheimer's disease. A similar association is suggested by the data on the occasional neurofibrillary tangle-containing neurons of the hippocampus of elderly non-demented individuals.

Edwardson et al. (1986) used energy-dispersive X-ray microprobe analysis of isolated cores and senile plaques in situ from patients with Alzheimer's disease. They found a co-localisation of high concentrations of aluminium (4 - 19%) and silicon (6 - 24%) at the centre of the cores. The presence of these elements as alumino-silicates was confirmed using solid-state aluminium nuclear magnetic resonance. These findings provide a link with the other major neuro-pathological feature of Alzheimer's disease, the neurofibrillary

tangle-bearing neurons, where high intracellular levels of aluminium and silicon have also been reported (see above).

Several factors point to the possibility that the deposition of alumino-silicates may be an essential stage in plaque formation. Their localisationat the centre of the core suggests an early involvement in plaque formation since secondary deposits would be more likely to be peripheral or homogenous throughout the core. Also, if the presence of aluminium and silicon simply reflects non-specific binding by pre-existing core material it seems likely that other elements such as calcium, iron, magnesium and potassium would be present to the same extent, but this is not the case (Edwardson et al., 1986; Candy et al., 1984, 1985, 1986). Even if the deposition of alumino-silicates is secondary, the space occupying effect of these bulky inorganic deposits makes it highly probable that they would contribute to the disruption of adjacent neuronal and glial processes.

The mechanism of alumino-silicate formation and deposition in the plaque core is at present unknown - this is also true of the deposition of aluminium and silicon in tangle-bearing neurons. While there is a general view that the involvement of aluminium in Alzheimer's disease is non-specific or may reflect an increased uptake by degenerating neurons, the studies of Perl and Brody (1980) and Edwardson et al. (1986) show that, irrespective of total brain load, plaque and tangle related aluminium is massively increased in Alzheimer's disease since these lesions are much rarer in normal subjects. Further research is needed to elucidate the mechanism whereby aluminium accumulates in tangles and plaques (and most importantly, whether this is a primary cause or secondary consequence of the disease) although at present the most favoured explanation involves a possible malfunction of the blood-brain barrier.

1.4.8. Blood-Brain Barrier.

In order to be absorbed by neurons, aluminium must first penetrate the blood-brain barrier (BBB) (Bardbury, 1984). Several lines of evidence suggest the competence of this barrier varies widely among mammalian species (Crapper McLachlan et al., 1985). Compared to cats or rabbits, rodents and primates are relatively resistant to the encephalopathic effects of either intravenous or intracranial injections of aluminium (King et al., 1975). It has been hypothesised that an abnormally functioning BBB of unknown cause allows endogenous, behaviourally active substances to gain access to the brain (Kastin et al., 1981; DeBoni et al., 1980). Banks and Kastin (1983) investigated the possible role of aluminium in the aetiology of this postulated BBB dysfunction. Their study showed that aluminium affects the permeability of the BBB of rats to small peptides. Intraperitoneal injections of aluminium chloride increased the permeability of the BBB to iodinated N-Tyr-delta-sleep-inducing peptide and B-endorphin by 60 - 70%. Thus, aluminium can affect the BBB in ways that might be involved in dementia. I-N-Tyr-DSIP and I-B-endorphin are representative of the small behaviourally active peptides that are present in both the brain and the periphery. An increase in the permeability of the BBB to these or other substances could lead to changes in brain biochemistry and behaviour that might ultimately result in dementia, e.g., Alzheimer's disease or dialysis dementia, or other expressions of central nervous system (CNS) disorder.

1.4.9. Factors which may be Responsible for the Focal Accumulations of Aluminium in Paired Helical Filament-Containing Neurons.

Aluminium could accumulate preferentially in paired helical filament (PHF) -containing neurons as a result of an increased vulnerability of neuronal DNA to aluminium (Crapper et al., 1980; Liss et al., 1976; Shore et al., 1980[2]), which in turn could result from genetic factors, viral or virus-like infection, or causes unknown: Down's syndrome (trisomy 21) and viral illnesses such as subacute sclerosing panencephalitis and postencephalitic Parkinsonism are associated with PHF-bearing neurons in the brain. Or it may be that certain neurons of Alzheimer's disease patients' brains are unable to detoxify the aluminium that is normally present in all body tissues. The resulting accumulations of aluminium on nuclear chromatin could have neurotoxic effects.

Another possibility is that aluminium accumulations may be a secondary, relatively non-specific 'marker' of neurons that are degenerating, rather than an etiological factor. But not all types of neuronal destruction lead to aluminium accumulation in the brain, as Creutzfeldt-Jakob disease patients have normal brain aluminium concentrations (Traub et al., 1981). Creutzfeldt-Jakob disease brains do not typically have neurofibrillary degeneration with PHFs, however, and aluminium accumulations are generally associated with PHF-bearing neurons in degenerative brain diseases (Perl and Brody, 1980; Perl et al., 1982). It is still possible that the neurofibrillary degenerative changes in the neurons of patients with PHF-related diseases allow aluminium to enter the nuclei of such neurons, without resulting in neurotoxic effects.

At present it is possible only to put forward tentative suggest-

-ions as to why aluminium should accumulate in tangle-bearing neurons in Alzheimer's disease. The issue of whether aluminium is a cause of the disease or merely a consequence, will be taken up in Chapter three. Certainly, if aluminium was a primary cause of Alzheimer's disease one might expect to find elevations of this metal in serum or cerebrospinal fluid, if not in bulk brain samples, but as discussed in Section 1.4.6., this is not the case.

1.4.10. Comparison of the Level and Distribution of Aluminium in Alzheimer's Disease and in Other Disease States.

As noted in Section 1.4.6., Crapper et al. (1976) found elevated bulk brain levels of aluminium in cases of Alzheimer's disease. Even though their data has not been replicated, a close examination of the figures indicates that the overall means for aluminium are still considerably lower than those found in dialysis dementia: All overall means are less than 5 mg/kg and the majority have ranges within those found in non-demented dialysis patients. Although the levels reported by Crapper et al. (1976) are clearly elevated above those for controls (and are within the encephalopathic range for cats and rabbits), they do not appear to be within the general encephalopathic range for humans. However, this observation must be made with caution due to a number of factors:

(1) The distribution reported is patchy, and elevated levels in certain areas may reach toxic levels;

(2) our knowledge of the encephalopathic levels for aluminium in humans is gained from diseased populations, and may not represent true toxicologic data;

(3) aluminium may exert its effects through different neurobiologic

mechanisms in the two diseases.

Some support may be found for the third possibility in the finding that the aluminium in the neurons of dialysis patients is located in the cytoplasm of cells, rather than being associated with the nucleus as is seen in Alzheimer's disease (Crapper and DeBoni, 1978). Although the aluminium concentration in dialysis dementia may be several times greater than that observed in Alzheimer's disease, there is only one report in the literature of neurofibrillary tangles and plaques in dialysis dementia brains (Brun and Dictor, 1981). In this study, tangles and plaques were found in three of five patients with dialysis dementia, but they were few in number, and the distribution differed markedly from that seen in Alzheimer's disease, being particularly sparse in the hippocampus and amygdala. One would expect that if aluminium plays a toxic role at the cellular level in both dialysis dementia and Alzheimer's disease, there would be more neurobiologic similarities between the two diseases.

A further difference between Alzheimer's disease and dialysis dementia is that high serum levels of aluminium are found in the latter (Alfrey et al., 1976; Berlyne et al., 1970), but not in the former (see Section 1.4.6.). The one major similarity between the two appears to be in the clinical syndrome of motor impairments, ataxia, and memory and learning impairments, which are apparent in both disorders.

Elevations of brain aluminium concentrations are present in a variety of human disease states associated with impairments of the the blood-brain barrier (e.g., metastatic carcinoma) which, like dialysis dementia, are not generally associated with Alzheimer-like degenerative changes in the brain (Arieff et al., 1979). On the

other hand, neurons containing neurofibrillary tangles are common in patients with the ALS-PD complex of Guam (see Section 1.4.5.) and are present in small numbers in non-demented elderly men and women. In such cases, as in Alzheimer's disease, aluminium is found in the nuclei of the tangle-bearing neurons (Perl and Brody, 1980; Perl et al., 1980), although the tangles seen in the ALS-PD complex do not consist of paired helical filaments.

Comparisons of aluminium in Alzheimer's disease and other disease states reveal a number of differences in both the level and distribution of this metal. However, sufficient similarities exist between the various disorders to merit further investigation into the toxin hypothesis of Alzheimer's disease. Furthermore, the results available to-date from animal studies also support a possible toxic role of aluminium in the disease, as will be seen in Section 1.4.12..

1.4.11. Other Metals in Alzheimer's Disease.

Besides aluminium, other metal ions have been studied for a pathogenic role in Alzheimer's disease. Crapper et al. (1978) examined cerebral tissue for concentrations of lead, iron, manganese, copper, zinc and cadmium. They found no abnormality in metal content in either whole tissue or nuclei of Alzheimer brains. A deleterious function for any of these heavy metal ions is accordingly considered unlikely.

1.4.12. Animal Studies.

Elevated levels of aluminium in the brains of adult rabbits and cats cause a syndrome characterised by behavioural, anatomic,

electrophysiological, and neurochemical alterations. Approximately seven to ten days following brain aluminium infusion, behavioural changes can be observed. Learning and memory impairments have been noted in a variety of testing situations by different investigators. Crapper and Dalton (1973[1+2]) found short-term memory deficits in cats on a delayed response procedure and acquisition deficits on a conditioned (one-way) active avoidance task. In adult rabbits, Petit et al. (1980) found both learning and retention deficits on a one-way active avoidance task following aluminium infusion, while Yokel (1983) found a classical-conditioning learning deficit following systematic aluminium administration.

In addition to the learning and memory deficits, subtle deficits in motor coordination are detectable within a week of infusion. These symptoms steadily increase as the animals enter a progressive encephalopathy. There is a deterioration in motor capacities, with the animals becoming rapidly incapacitated. In the final stages the animals become apathetic and frequently show focal or generalised seizures prior to death (Crapper and Dalton, 1973[1]; Petit et al., 1980; Klatzo et al., 1965; Crapper, 1976). Alterations are also noted in neural spontaneous firing rates, evoked potentials, and the EEG prior to and during the encephalopathic stage (Petit, 1983). The neurobehavioural syndrome is remarkably similar to that seen in dialysis dementia (see Section 1.4.4.).

When the brains of these animals are examined histologically, neurofibrillary tangles are observed. These appear in cat and rabbit brains with aluminium concentrations of 4 - 8 µg Al/g dry weight or greater (Crapper and Tomko, 1975). Neurofibrillary degeneration is not observed with whole cerebral cortical tissue concentrations of below 4 µg Al/g dry weight (Crapper and DeBoni, 1980).

It is important to note, however, that the aluminium-induced tangles are different from those found in Alzheimer's disease: Both tangles are composed of accumulations of 10 nm. neurofilaments, but the filaments in the Alzheimer tangle are twisted in a double helix while those in the aluminium tangle are not. Cultured cortical neurons taken from aborted human foetuses and exposed to aluminium salts develop neurofibrillary tangles made up of 10 nm. filaments indistinguishable from those found in aluminium treated animals, and not paired helical filaments as found in Alzheimer's disease (Crapper et al., 1978). These observations suggest that aluminium salts induce neurofibrillary changes with a common structure in human and animal nerve cells. Further differences between aluminium-induced neurofibrillary changes and PHF are found in the topography. In aluminium treated animals, neurofibrillary changes occur extensively throughout the spinal cord and in specific regions of the hippocampus (Wisniewski et al., 1965; Klatzo et al., 1965). In Alzheimer's disease, neurofibrillary changes are not found in the spinal cord (Hirano and Zimmerman, 1962).

Also, neurofibrillary changes in Alzheimer's disease are associated with the presence of senile plaques. Senile plaques are not found in aluminium treated animals. In this respect the aluminium induced encephalopathy is similar to the ALS-PD complex of Guam, in which many neurons in the cortex show neurofibrillary tangles without the presence of senile plaques. Finally, there has been some evidence presented that aluminium induced neurofibrillary degeneration in animals can disappear and that affected cells can recover (Troncoso et al., 1982; Wisniewski, Sturman and Shek, 1980), though the mechanism of this recovery is not known. There is no evidence, however, of recovery from neurofibrillary degeneration in patients suffering

from Alzheimer's disease. In spite of these differences there are sufficient similarities to warrant the use of aluminium induced neurofibrillary changes as a model for the changes found in Alzheimer's disease. For example, and perhaps most importantly, both Alzheimer and aluminium tangles are space occupying lesions which reduce the life supporting machinery of nerve cells.

The effects of aluminium exposure are developmentally dependent in animals, with younger animals being more resistant to the behavioural and encephalopathic effects (Wisniewski et al., 1980; Petit et al., 1985). Although neurofibrillary tangles are seen in the rabbit following perinatal infusions of approximately the equivalent (based on brain weight) adult dose of aluminium, behavioural and encephalopathic changes are not observed. At approximately twice the adult dosage, reductions in dendritic development are seen (dendritic development was not examined at lower doses) but no encephalopathic signs or alterations in learning and memory are observed. Overt neurologic signs are observed only following three times the relative adult dose, and 26.7% of these animals still showed no encephalopathic signs 17 days post-infusion (Petit et al., 1985). These findings suggest that the infant rabbit is a more appropriate animal model of aluminium neurotoxicity than the adult, the lack of encephalopathy making behavioural testing considerably easier.

Another important finding from the experimental animal research is the large species differences in reaction to aluminium. The characteristic neurobehavioural response to aluminium described above is readily produced in cats and rabbits. The rhesus macaque monkey also develops tangles, but only after a prolonged period, i.e., 380 days or more (DeBoni et al., 1976). However, rats and mice develop neither neurofibrillary tangles nor an encephalopathy at 10 times the

dose effective in cats and rabbits (King et al., 1975). The rat does, however, show behavioural alterations with elevated aluminium levels. For example, Bowdler et al. (1979) found that oral administration of high doses of aluminium to rats resulted in increased activity, and increased sensitivity to flicker. Thorne et al. (in press) report an inverse relationship between brain aluminium and open-field activity (i.e., as aluminium levels increase, activity decreases) and a correlation between high aluminium levels and poor performance on tasks of single-trial passive avoidance and visual discrimination with reversal. However, not all researchers have found such changes (King et al., 1975). It remains a possibility, therefore, that different species, like individuals at different developmental ages, may have either different neurobiologic responses to aluminium, or different thresholds for this response.

Before turning to a consideration of possible methods of therapy based on the toxin hypothesis, it should be pointed out that there are two principal problems with the animal studies cited above:

(1) The neurotoxic effects of aluminium typically limit the duration of study and necessitate that aluminium injected animals be tested within one to two weeks after injection. They also complicate the interpretation of the cause of the learning deficits observed in aluminium treated animals, because the deficits could arise not only from neurofibrillary changes, but also from seizures and/or motor impairments.

(2) To remedy these limitations, a new chronic rabbit model for aluminium induced neurofibrillary degeneration was developed by Wisniewski et al. (1980) which was based on the infant rabbit (see above). However, it has already been noted that there are species differences in response to aluminium (see above and P. 37 : Section

1.4.4.), with cats and rabbits showing rapid neurobehavioural changes following aluminium administration, and rats and mice, very little change. Since it has been demonstrated that humans are also naturally resistant to the effects of aluminium (see Section 1.4.4.), this implies that the cat or rabbit is not necessarily the best model of the human dementing syndrome. Rather, it could be more worthwhile to develop the rat or mouse model. This point will be taken up again in Section 1.4.14., and in Chapter three.

1.4.13. Therapy.

Several research groups have been studying possible ways to prevent aluminium from gaining access to the DNA in neuronal nuclei, in the hope of preventing neurotoxicity and clinical deterioration. There are a number of possible approaches to this problem. One can attempt to eliminate all sources of aluminium intake, but this is not really possible since aluminium is present in all foods, in air, water, dust, etc.. Another approach is to minimise the absorption of the aluminium which is ingested. A third possibility is to attempt to facilitate the excretion of aluminium, for example, by chelation with drugs like desferroxamine.

Desferroxamine (DFO) is a trivalent metal chelating agent that binds iron with high affinity and specificity. DFO has been widely employed to treat patients suffering from iron overload, and has been used to remove aluminium from patients with renal failure who were stabilised on standard hemodialysis (Ackrill et al., 1980). More recently, DFO has been employed in an attempt to remove aluminium from the brains of patients suffering from Alzheimer's disease

(McLachlan - report in progress).

A recent preliminary report by King et al. (1985) stated that a single subcutaneous administration of DFO (100 mg/kg) two hours after intracisternal injection of aluminium chloride (1 mg. intracranially) in rabbits, followed by 33 mg/kg of DFO at three-day intervals, did not generally prevent the aluminium induced encephalopathy in three of four animals. These workers concluded that "there is no evidence that systemic administration of desferroxamine can remove aluminium from the central nervous system". This conclusion is in contrast to the previously published observations on dialysis dementia in which DFO treatment resulted in reversal of the neurologic symptoms (Ackrill et al., 1980).

The observations of Crapper McLachlan et al. (1985) employing DFO treatment in both patients with Alzheimer's disease and aluminium overload indicate effective aluminium removal. In aged patients with Alzheimer's disease under long-term DFO treatment and who died of unrelated illness, neocortical brain analysis revealed near normal concentrations of aluminium. In aluminium overload conditions in the presence of normal renal function, DFO treatment (7 mg/kg bi-daily) resulted in an initial 50-fold increase in excretion of aluminium (Crapper McLachlan et al., 1985). During prolonged aluminium treatment, excretion was approximately three times the pretreatment level. These observations support continued clinical trials of DFO under controlled conditions for aluminium-associated diseases.

A related method of aluminium removal to the above is to administer an anion which complexes aluminium in the body, in the hope of lessening the ability of aluminium to complex with nuclear chromatin in the brain. Such an anion might also facilitate the excretion of aluminium from the body.

It has recently been reported that aluminium in man binds fluorine in the gastro-intestinal tract, and prevents the absorption of excessive dietary fluorine (Spencer et al., 1981). Shore et al. (1985) considered the possibility that the converse might be true - that complexation of fluorine with aluminium might diminish aluminium toxicity. Ondreicka et al. (1971) had previously studied nutritional aspects of aluminium and fluorine and reported that feeding rats large doses of aluminium caused elevated aluminium concentrations in a variety of tissues, including the brain. Feeding fluorine along with aluminium prevented these increased concentrations, and increased aluminium excretion. On the basis of this report (Ondreicka et al., 1971), Shore et al. (1985) conducted a study to determine whether increasing fluorine concentrations in man might provide a way to bind aluminium, reduce its cationic charge, and facilitate its excretion: In a series of rabbits, half were given fluorine in their water for several days, before all rabbits received intrathecal aluminium injections. Fluorine pretreatment was found to result in lower brain aluminium concentrations on sacrifice, indicating that fluorine may prevent aluminium induced neurotoxicity. Since this implies that fluorine administration may be a possible form of treatment for Alzheimer's disease, dialysis dementia, aluminium overload conditions, etc., further research in this area is certainly warranted.

Some may feel that clinical studies of anti-aluminium drugs are premature (Cole and Branconnier, 1978), as the association between aluminium and Alzheimer's disease is far from clear and consistent. However, it might be possible to demonstrate therapeutic effects of anti-aluminium drugs before the basic scientific questions are answered. This is not very different from the searches for drug

treatments for depression or schizophrenia, searches which have met with remarkable success.

Furthermore, if it can be demonstrated that therapy with DFO or fluorine is capable of both reducing aluminium levels and alleviating the clinical symptoms of Alzheimer's disease, this will provide very strong evidence in favour of a causal role of aluminium in this disease, and might also provide valuable information on the mechanism whereby aluminium accumulates on nuclear chromatin.

1.4.14. Summary and Objective.

Evidence for an involvement of aluminium in Alzheimer's disease has arisen from a variety of sources: Elevated concentrations of aluminium have been found in some brain regions of such patients and there is evidence that aluminium is present in a high percentage of neurons containing neurofibrillary tangles, while it seems to be absent in adjacent neurons that appear normal (Crapper and DeBoni, 1980; Crapper, 1976). Of particular interest is the recent finding that aluminium is also present in the core of senile plaques, most probably compounded as an alumino-silicate (Candy et al., 1986; Edwardson et al., 1986). Thus aluminium appears to be closely linked to those diagnostic neuropathological features of Alzheimer's disease. Aluminium has also been implicated as a toxic factor in dialysis encephalopathy, an irreversible dementia seen in some people who have undergone repeated kidney dialysis with aluminium-rich solutions (Alfrey et al., 1976). Lastly, it has been shown that there are prominent accumulations of aluminium in the cytoplasm of neurofibrillary tangle-bearing neurons in the hippocampus in a form of Parkinsonism associated with severe dementia seen in certain

native communities on the island of Guam in the Western Pacific (Perl et al., 1982).

These clinical data are supported by studies of the experimental encephalopathy induced by the intracranial injection of soluble aluminium into animals such as cats and rabbits (Crapper and DeBoni, 1980; Crapper, 1976). These animals may exhibit memory impairments, as evidenced by impaired acquisition and retention of avoidance responses and impaired learning of spatial tasks. Subsequently the animals become slower in their movements and develop ataxia. Finally general convulsions are observed, the animals usually dying within 10 - 28 days of the injection of aluminium salts. Post mortem analyses have shown that the animals' brains contain a significant percentage of neurons which have undergone neurofibrillary degeneration. These neurons, which contain dense parallel arrays of 10 nm. single filaments, bear general similarities to those observed in Alzheimer's disease.

Attempts to treat aluminium overload conditions and Alzheimer's disease by the administration of desferroxamine or fluorine (in order to facilitate the excretion of aluminium and prevent aluminium from accumulating in the brain) have yielded encouraging results (Shore et al., 1985; Crapper McLachlan et al., 1985), thus providing further support for an involvement of aluminium in the disease. Finally, there is no evidence of a similar involvement of any other metal in Alzheimer's disease (Crapper et al., 1978).

Although Alzheimer's disease can be linked with the occurrence of aluminium in the brain, the critical question is whether the presence of aluminium is a secondary consequence of the disease or whether aluminium itself is responsible for the disease. The apparent lack of elevated aluminium concentrations in bulk brain analysis,

serum or cerebrospinal fluid of Alzheimer disease patients (Markesbury et al., 1981; Shore et al., 1980[1], 1980[2]) suggests that aluminium alone is not a 'cause' of Alzheimer's disease. Aluminium may be just a 'marker' of paired helical filament-bearing neurons, or Alzheimer disease patients may be 'vulnerable' to aluminium neurotoxicity on the basis of some other neuronal dysfunction (e.g., malfunction of the blood-brain barrier), allowing aluminium to enter neuronal nuclei. The rate of deposition in tangles and plaques would, in addition, be determined by the extent of environmental exposure to aluminium.

The only direct way of investigating the precise role of aluminium in Alzheimer's disease is to determine whether conditions which lead to increased aluminium uptake produce dementia. Chapter three will examine this question experimentally by assessing the correlation between aluminium uptake and cognitive performance in laboratory animals. The behavioural tasks will concentrate on measures of recent memory as this is one of the first cognitive disorders to appear in Alzheimer's disease.

In order to assist aluminium uptake the animals will be given a diet which is high in aluminium salts, but lacking in calcium and magnesium. This novel manipulation follows from recent epidemiological evidence that a deficiency of these metals potentiates the uptake of aluminium (Perl et al., 1982), an innovation which provides the opportunity to challenge the toxin hypothesis and produce an animal model with direct value for the assessment of possible preventative treatments and therapies.

Rats will be used as the subjects of study since the available evidence suggests that these animals have a natural resistance to aluminium neurotoxicity in the same way as man (King et al., 1975; Petit, 1985), thus making them a more appropriate species on which

to develop an animal model of the toxin hypothesis of Alzheimer's disease than the cat or rabbit which have typically been used.

1.5. Overall Objectives.

The overall objectives of this study are:

(1) To examine two hypotheses of the cause of Alzheimer's disease; the cholinergic hypothesis, and the toxin hypothesis.

(2) Specifically, (since amnesia is one of the most severe symptoms of Alzheimer's disease) to assess the effect on memory of cholinergic manipulations (Chapter two) and the ingestion of a diet with a high aluminium content (Chapter three).

(3) To achieve (1) and (2) by developing an animal model of the human amnesic syndrome using the rat as the species of study.

(4) To base the experiments of Chapters two and three on a nonspatial task of working memory, (delayed) nonmatching-to-sample, which is comparable to tasks used to assess memory impairments in man, but which has not been used before to study learning and memory in rodents.

CHAPTER TWO. CHOLINERGIC STUDY.

2.1.1. Introduction.

There is substantial evidence from human studies that drugs such as scopolamine, which disrupt cholinergic transmission, can produce impairments in memory (Crow and Grove-White, 1973; Drachman and Leavitt, 1974; Sitarem et al., 1978) (see P.25). Similar findings have been reported in studies of experimental animals. Unfortunately scopolamine, a muscarinic blocker, appears to have multiple actions and it is uncertain whether the observed deficits in animals reflect a primary dysfunction in memory or whether they are a secondary consequence of changes in attention, perception, or motivation.

For example, Evans (1975) found that scopolamine impaired discrimination performance by monkeys in a way that mimicked alterations in stimulus luminance. Evans (1975) argued that a drug induced impairment of visual acuity, resulting in reduced stimulus sensitivity, was responsible for the marked decrement in choice accuracy on the task, independent of any effect scopolamine might have on memory processes. Warburton and Brown (1971) had previously used signal detection theory to reveal dose dependent decreases in stimulus sensitivity (d') with systemic injections of low doses of scopolamine (0.062 - 0.25 mg/kg) on a discrimination task with rats. They, as in the later Evans (1975) study, argued that scopolamine's effects were on sensory or attentional processes rather than on memory functioning.

One approach to the problem of defining the precise effects of scopolamine (i.e., whether its effects are on memory or on sensory or attentional processes) has been to assess whether scopolamine produces a depression in performance when the mnemonic demands of a task

are reduced to a minimum, or whether its effects are only pronounced when the mnemonic demands are high. This distinction has been examined by looking at the effects of scopolamine upon the slope of forgetting curves. Such studies, which have typically used tests of spatial working memory in order to generate forgetting curves have, however, produced inconsistent results. For example, Heise et al. (1976) trained rats on a spatial alternation task. The rats were then pretreated with scopolamine, and the interval between trials was varied. Drug treatment did not, however, interact with delay interval, and so it was concluded that pretreatment with scopolamine did not affect memory storage.

In contrast, Bartus and Johnson (1976) demonstrated a highly significant interaction between scopolamine pretreatment and delay interval using an automated, 9-choice delayed response task. They found that with a low scopolamine dose (10 - 15 μg/kg), performance on the task was unimpaired with zero delays, but fell to approximately 40 - 70% at 10 second delays when compared to saline controls. Doses higher than 20 - 30 μg/kg impaired accuracy at all intervals, including zero delay conditions. Bartus and Johnson (1976) argued that "...the disruption of short-term memory by interference with central cholinergic mechanisms essential to the process remains the most parsimonious interpretation of these observations". Yet other studies (Robustelli et al., 1969; Dunnett, 1985) are in agreement with the results of the Heise et al. (1976) study, failing to find an interaction between delay interval and pretreatment with scopolamine indicative of a true memory impairment.

In view of these inconsistent results, the present study re-examined the effect of scopolamine on memory, and more specifically, on the slope of a forgetting curve, by using a non-spatial test of

working memory, delayed nonmatching-to-sample. This test of object recognition has recently been shown to be sensitive to scopolamine in the monkey: Aigner and Mishkin (1986) examined the effects of scopolamine upon delayed nonmatching-to-sample, using a sample set of 1200 junk objects. They had no difficulty in training monkeys to non-match after seeing lists of up to 20 objects and sample-choice intervals of 5 minutes. Scopolamine (1.0 - 32.0 μg/kg) caused a dose dependant impairment of performance on the task, though in the absence of data concerning different delay intervals or list lengths, no specific conclusions about cholinergic involvement in recent memory could be drawn. Nevertheless, the delayed nonmatching-to-sample procedure with trial-unique stimuli is promising. In terms of comparability with the human data it would seem to be a particularly appropriate task to use with animals, since it is known that visual recognition abilities are considerably reduced in patients suffering from Alzheimer's disease, and these deficits are still apparent when the subject is tested using a delayed nonmatching-to-sample procedure which is deliberately modelled on those used with primates (Albert and Moss, 1984). Furthermore, and perhaps most importantly, nonmatching (or matching)-to-sample tasks can be non-spatial, and therefore are most similar to the tasks given to humans which are typically non-spatial (Drachman and Leavitt, 1974; Sitarem et al., 1978).

Just as drugs which disrupt cholinergic transmission can impair cognitive performance, so drugs such as physostigmine which increase available acetylcholine can improve performance on some tests of learning and memory. For example, Davis et al. (1978) found that physostigmine enhanced both the storage and retrieval of information in long-term memory in humans, and Dunnett (1985) demonstrated that

physostigmine can induce a mild but significant enhancement of performance in rats trained on a delayed matching-to-position task.

In view of recent evidence that physostigmine can improve delayed nonmatching-to-sample performance in the monkey (Aigner and Mishkin, 1986), the effects of this drug were tested upon an analogous task for rats.

Experiment One.

2.1.2. Introduction.

The first experiment examined whether scopolamine impaired performance on a test of object recognition, delayed nonmatching-to-sample. In this task the rat is rewarded for selecting between two goal boxes, one of which differs from the start box (Figure 2.1.). This task taxes working memory as both the start and goal boxes are changed after every trial.

The results of a pilot study suggested that the best dose range in which to look for a memory impairment (specifically, an interaction between drug and task difficulty) and to minimise the peripheral effects of the drug, should be not more than 0.05 mg/kg scopolamine hydrochloride, and this was taken into account in the doses used in the present study.

Experiment One also included concurrent sessions in which the rats received either scopolamine methylbromide, or the acetylcholinesterase inhibitor, physostigmine. Methylscopolamine has the same peripheral actions as scopolamine, but it does not cross the blood-brain barrier and hence does not mimic its central actions. The dose of 0.03 mg/kg scopolamine methylbromide was selected again on the basis of the pilot experiment, which indicated that this is the highest dose which can be used and not affect performance at the shortest retention interval on the task. The dose of physostigmine was determined from other studies which have reported that this drug can improve performance (Aigner and Mishkin, 1986; Dunnett, 1985; Hagan and Morris, 1987).

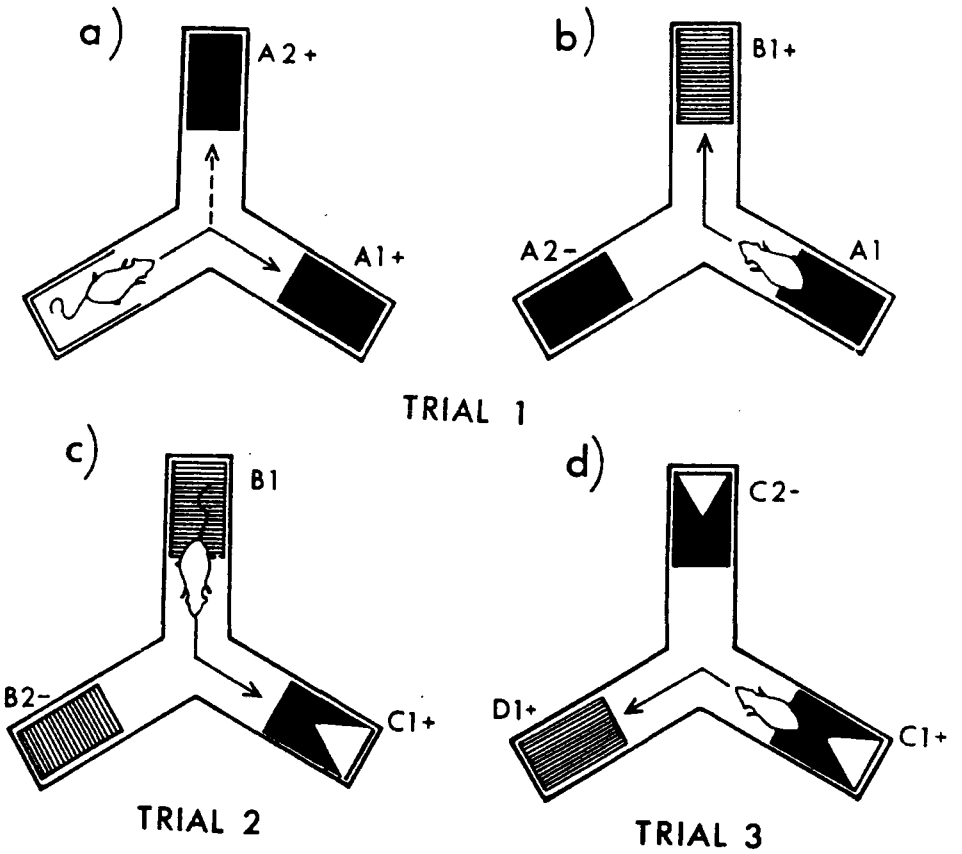

Figure 2.1.

Diagrammatic representation of the nonmatching-to-sample procedure in which the animal was rewarded for choosing the novel goal box. Arrows show the direction of correct choices.

2.1.3. Method.

Subjects.

The subjects were 8 male pigmented rats of the DA strain, supplied by Bantin and Kingman, Hull. The rats were housed individually and were maintained on approximately 18 g. of laboratory chow each day. The chow was always mixed with water to form a gruel - this minimised the effects of the drugs upon the animals' daily consumption of food. Water was freely available in the home cage. The rats were aged about 14 weeks at the start of the experiment.

Apparatus.

The animals were tested in an aluminium Y-maze (Figure 2.2.). Each maze arm was 12.7 cm. wide, 20 cm. high, and covered with a wire grid. Fifty pairs of hardboard boxes served as both start boxes and goal boxes. These boxes could be fitted into the end of each arm of the maze, forming an arm length of 26 cm.. The boxes in each pair were made as similar as possible, but each pair was distinctive. This was achieved by painting the walls and floors a variety of colours and patterns, and lining the floors with materials such as sandpaper, wooden strips, metal, perspex and cloth. In addition, each pair of boxes contained an identical object, such as a plastic cup, a metal bracket, or a wooden block, although no two pairs contained the same object. The boxes were 11.5 cm. wide, 9 cm. long and either 16 or 19 cm. high. The floors protruded an additional 9 cm. from the box, so that the floor of each box began 8 cm. from the centre of the Y-maze. An aluminium guillotine door was set in the centre of the maze, blocking the three arms. Metal tubes, which dispensed the reward pellets (45 mg., Campden Instruments Ltd.), ran

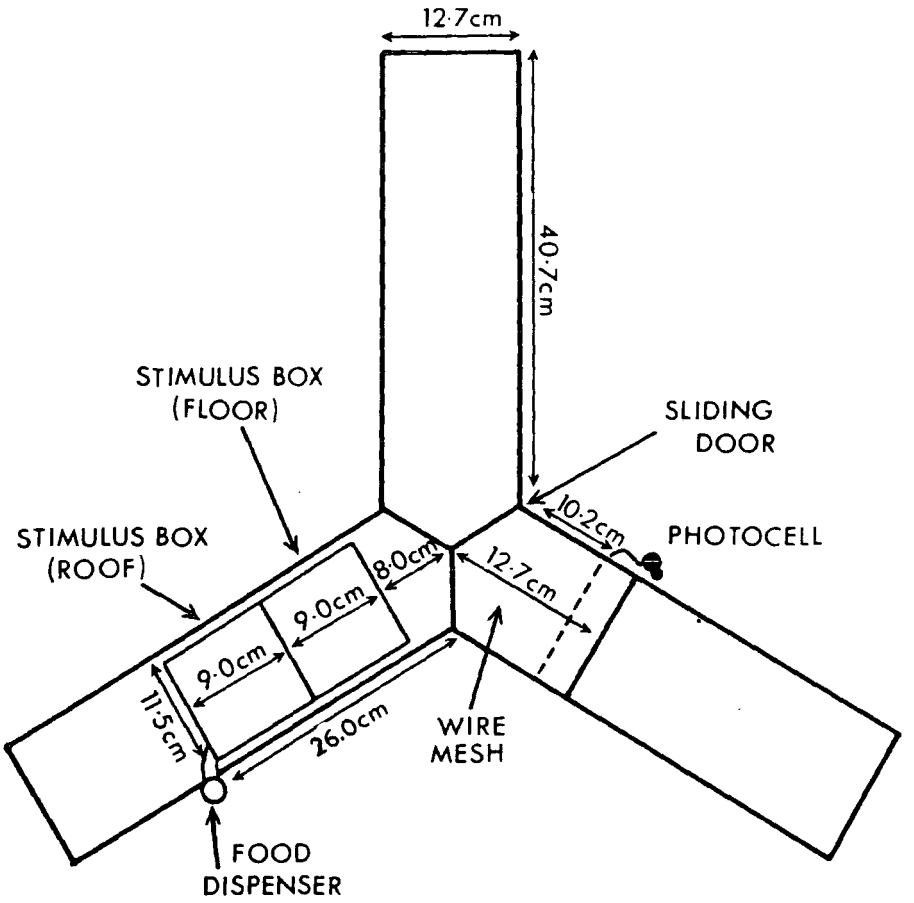

Figure 2.2.

Schematic representation of Y-maze, indicating maze dimensions.

to the back wall of each start box which had been cut away to allow the animals access to food. The rats were allowed constant access to dilute orange juice from fluid dispensers set behind each test box. This was to reduce the effect of the dry mouth caused by scopolamine. The Y-maze was illuminated by a fluorescent ceiling light 215 cm. above the apparatus.

Procedure.

Each test session consisted of 10 trials. To begin the first trial, the rat was placed in an arm with a blank start box. The central door was raised, and the animal was allowed to choose between two arms that contained a matching pair of goal boxes (A1, A2, Figure 2.1.). The rat was deemed to have made a choice when both hind feet had been placed upon the choice arm, whereupon the guillotine door was lowered. The animal was rewarded with three reward pellets, whichever box had been selected. The animal was confined to the goal box (A1) for 20 seconds, during which time the other two boxes were removed and replaced. The central door was then raised, simultaneously revealing a familiar box, A2, in one arm and a novel box, B1, in the other (Figure 2.1.). The animal was rewarded with three food pellets if it chose the novel box B1. These pellets were dispensed after the choice had been made. In this training condition the delay between the last experience of the sample stimulus, A1, and the choice between B1 and A2 was close to '0' seconds (Figure 2.1.). Following confinement in box B1 for 20 seconds, the second trial began. The central door was raised and the animal was allowed to choose between box B2 and a novel box C1 (Figure 2.1.). The novel box (C1) was positive. This sequence was repeated with new pairs of boxes for a total of 10 trials, during which selection of the novel

box was always rewarded. The position of the novel, and hence positive, box varied from right to left according to a pseudorandom schedule. The 50 pairs of test boxes ensured that any particular pair would be used on average every fifth day. The sequence of test boxes was revised after every 40 trials.

If the animal made an incorrect choice, i.e., chose the goal box that matched the start box, up to a maximum of four correction trials were run to encourage the animal to select the novel box. During these correction trials the goal boxes were arranged so that the novel, and hence positive, box was in the same arm, right or left, relative to the rat as in the test trial.

The animals were trained until they reached a criterion of 40 or more correct responses over five consecutive days (80% correct). They were then immediately transferred to the drug/delay regime. The rats received intraperitoneal injections of one of the following drugs dissolved in saline approximately 20 minutes before each session: 0.1 mg/kg physostigmine salicylate (Sigma Chemical Co.), 0.01 mg/kg, 0.03 mg/kg and 0.06 mg/kg scopolamine hydrobromide, 0.03 mg/kg scopolamine methylbromide (Sigma Chemical Co.), or saline. For all injections the dose was 1 ml/kg of rat. Unlike the pilot study, scopolamine hydrobromide was used instead of scopolamine hydrochloride. This form, which has a molecular weight approximately 6% heavier than the hydrochloride salt, provides a closer match with the scopolamine methylbromide control condition.

In the first phase of the experiment each drug was tested not only under the training condition ('0' seconds), but also with a 60 second retention delay imposed between the sample stimulus and the test. The delay was achieved by replacing the start box, after the animal had been in it for 20 seconds, by a blank, featureless box.

The animal was confined within this arm for 60 seconds before the central door was raised, revealing a novel goal box and one that matched the original start box. Thus in order to perform the task correctly the rat had to remember the start box for 60 seconds. Again the rat was rewarded for choosing the novel box, and incorrect choices were followed by up to a maximum of 4 correction trials.

In the first phase there were thus 12 conditions in all, 2 delays and 6 drugs. The animals received 3 days (30 trials) at each of the 12 conditions (36 sessions) in a balanced order, with no more than two 60 second delays, or two doses of scopolamine, occuring on consecutive days.

After completion of the 36 test sessions, testing with both 0.03 mg/kg scopolamine hydrobromide and 0.03 mg/kg scopolamine methylbromide stopped as it was evident that these drug conditions had no discernable effect upon performance (see next Section). Each of the remaining drug conditions received an additional 20 trials, again at both '0' and 60 seconds, following a series of activity measures and control tests (Experiments Two and Three). This second phase of the experiment began approximately three months after completion of the initial 36 sessions, and within two weeks of completion of Experiment Three.

2.1.4. Results and Discussion.

In the first phase of the experiment (Figure 2.3.) all drugs were tested over a total of 30 trials for each of the two delays ('0' seconds and 60 seconds). An analysis of variance revealed that there was no main effect of drug ($F=1.92$, $df=5, 35$), or delay ($F=2.96$, $df=1, 7$), although there was a significant interaction between drug

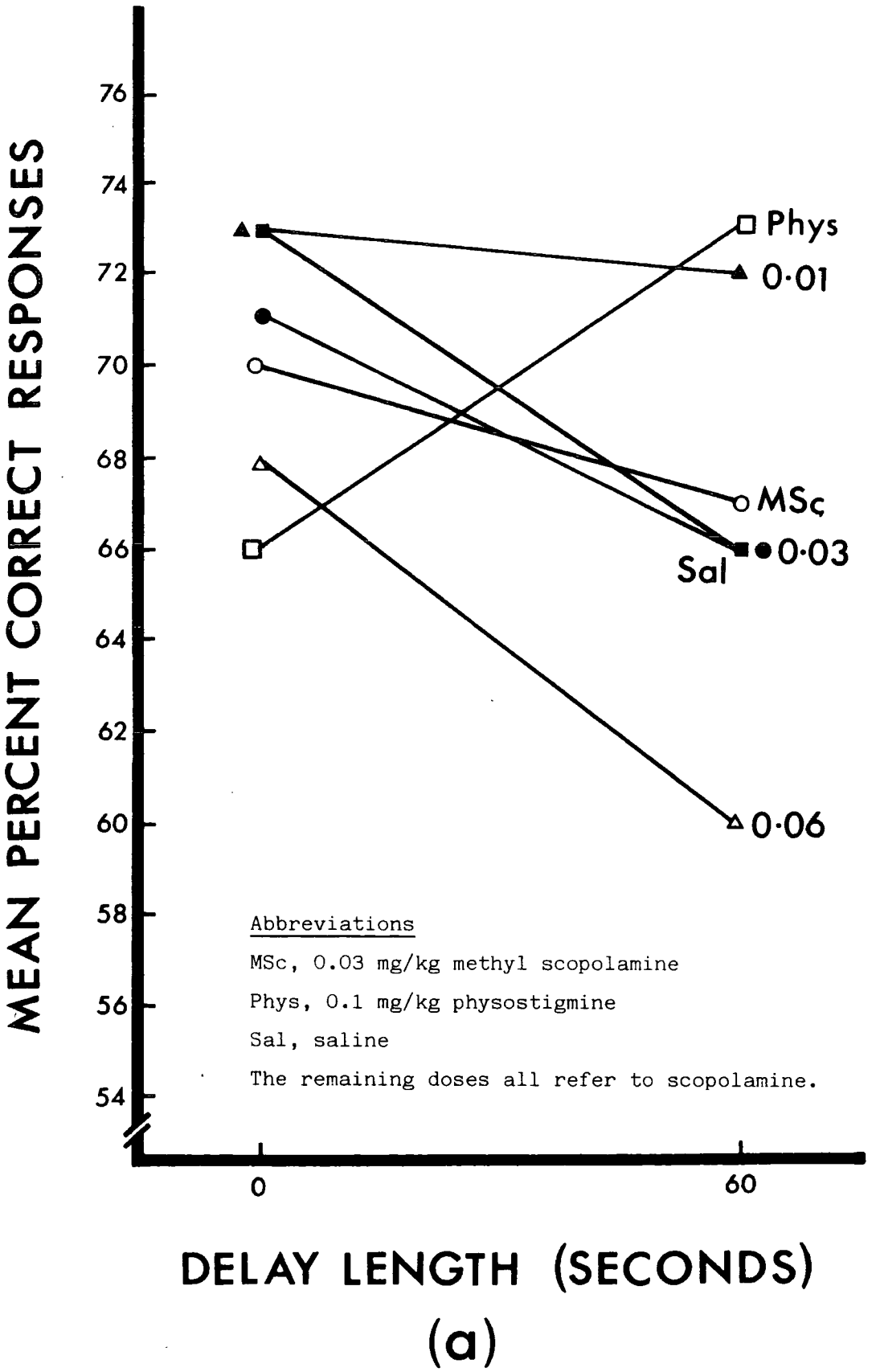

Figure 2.3.

Mean performance on the nonmatching-to-sample task following 30 trials at each of the five drug conditions and over the '0' and 60 sec. retention intervals - First phase.

and delay ($F=3.24$, $df=5, 35$, $p<0.05$). This interaction reflected the forgetting slope for the physostigmine condition (Figure 2.3.). It was evident that neither 0.03 mg/kg scopolamine or 0.03 mg/kg methylscopolamine had any effect upon performance and these drugs received no further testing.

The combined results from both phases of Experiment One are depicted in Figure 2.4. . An analysis of variance showed that both the drugs ($F=9.22$, $df=3, 21$, $p<0.01$) and the delays ($F=5.81$, $df=1, 7$, $p<0.01$) affected performance levels, and that there was a significant interaction between these factors ($F=5.95$, $df=3,21$, $p<0.01$). Further examination of the simple main effects revealed that there were no clear differences between the drug scores at 0 seconds ($F=2.16$, $df=3, 21$) but that significant differences were found after retention delays of 60 seconds ($F=10.72$, $df=3, 21$, $p<0.01$). Subsequent analysis with the Newman-Keuls test revealed that the scores following injections of 0.06 mg/kg scopolamine were significantly lower than those of all other conditions, including saline ($p<0.01$). It is, however, important to note that despite these differences there was no significant interaction between saline and the 0.06 mg/kg scopolamine condition (Figure 2.4.), and that the significant interaction in the overall analysis reflected the performance scores following physostigmine. This is borne out by the non-significant interaction when the saline scores were compared with the two scopolamine conditions (0.01 and 0.06 mg/kg), and the physostigmine scores excluded ($F=2.07$, $df=2, 14$). In contrast, a comparison between just the saline and physostigmine scores over the two delays retained the significant interaction ($F=5.57$, $df=1, 7$, $p<0.05$).

Although a higher dose of scopolamine was not used it was very evident from the pilot study that higher doses will impair performance

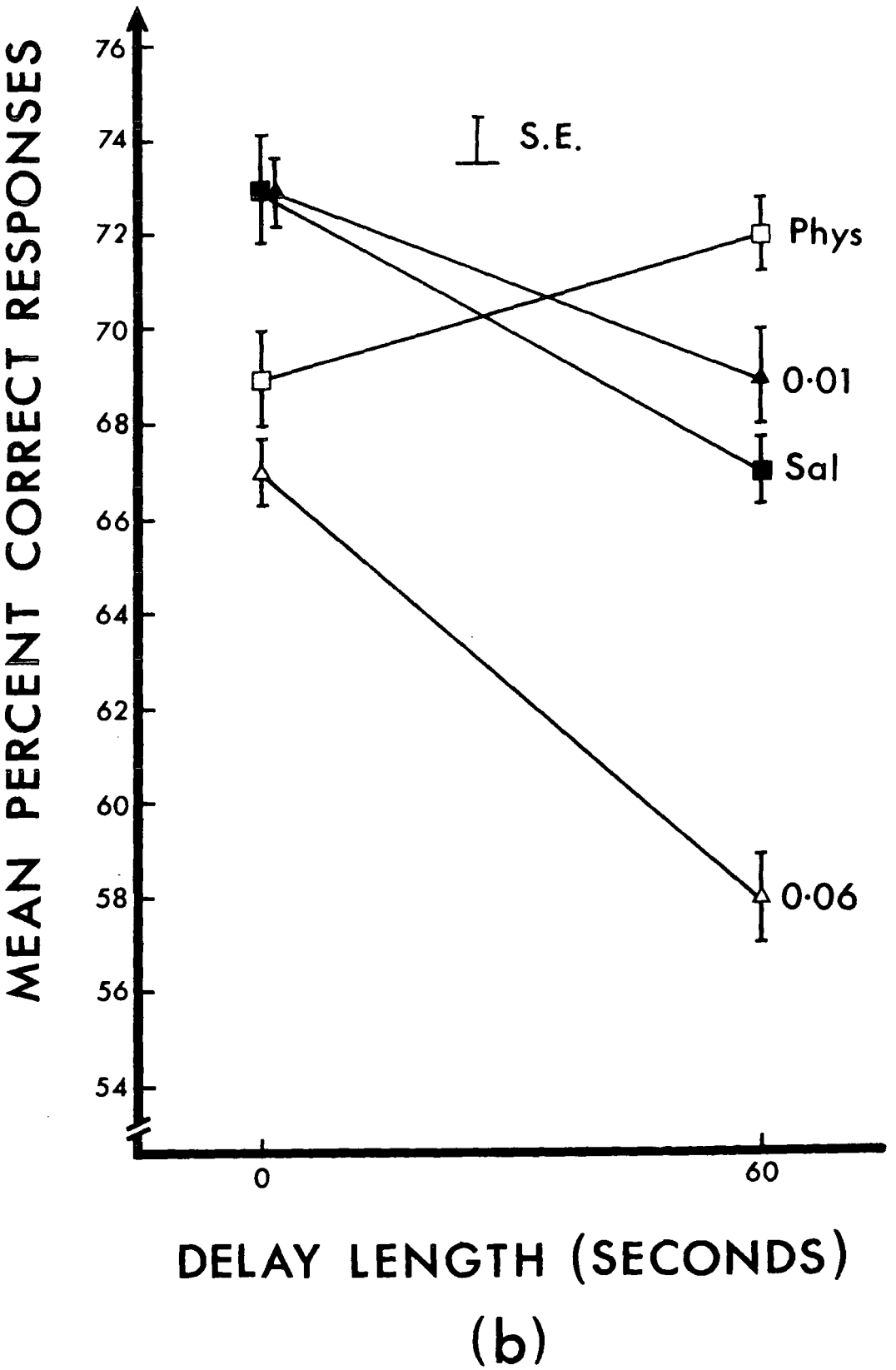

Figure 2.4.

Mean performance following 50 trials at each of three drug conditions and over the two retention intervals - Second phase.

Abbreviations: Phys, 0.1 mg/kg physostigmine; Sal, saline.

The remaining doses both refer to scopolamine.

at the '0' second condition and so invalidate direct comparisons between forgetting curves. Indeed, the dose of 0.06 mg/kg scopolamine hydrobromide did lower the mean score at '0' seconds (Figure 2.3.) and it was this fall, albeit non-significant, which ensured that there was no interaction between the 0.06 mg/kg scopolamine and saline conditions. This, combined with the lack of effect of 0.03 mg/kg scopolamine, indicates that there was no dose of scopolamine which would produce faster forgetting over the retention delay and not effect performance on the '0' second condition.

There was evidence that physostigmine improved performance at the 60 second delay (Figure 2.4.). This improvement was supported by the significant interaction between the forgetting slopes for saline and physostigmine ($F=5.57$, $df=1, 7$, $p<0.05$), although the physostigmine scores at 60 seconds did fail to differ significantly from the saline scores (Newman-Keuls $0.1>p>0.05$). It should be noted that although Figure 2.4. suggests that injections of physostigmine actually resulted in better performance on the more difficult 60 second condition, these scores did not differ significantly from those at '0' seconds. This may be contrasted with the clear fall in performance over the longer retention interval for the saline condition ($F=5.43$, $df=1, 14$, $p<0.05$).

Experiment Two.

2.2.1. Introduction.

Scopolamine methylbromide 0.03 mg/kg did not have a comparable effect on the object recognition task to any of the scopolamine doses used in Experiment One, and therefore did not act as an effective control measure for the peripheral effects of scopolamine on the animals' performance. In particular, the effects of scopolamine on motor activity cannot be discounted from the results of Experiment One, since signs of sluggishness were observed with doses of 0.06 mg/kg scopolamine hydrobromide.

It is possible that if an animal was less active in the Y maze after being injected with scopolamine (as was occasionally observed following the administration of 0.06 mg/kg scopolamine), then this would increase the total delay length at the choice point, and hence the memory requirement between seeing the start box and choosing the (novel) goal box, to over '0' or 60 seconds, respectively. Any reduction in performance on the task could therefore be accounted for as a function of increased delay length caused by decreased activity, rather than as an expression of a true memory decrement resulting from cholinergic blockade by scopolamine. In view of this, Experiment Two was designed to measure the effects on the overall activity of the animals of 0.01 mg/kg, 0.03 mg/kg and 0.06 mg/kg scopolamine hydrobromide. Physostigmine 0.1 mg/kg and scopolamine methylbromide 0.03 mg/kg were included for comparison purposes, with saline as the ultimate control condition.

2.2.2. Method.

Subjects.

The subjects were the same as for Experiment One.

Apparatus.

The activity boxes consisted of three cages similar in every respect to the animals' home cages. No food was provided in the activity boxes, although water was freely available.

Three 0.5 volt photo-electric cells were fitted at equal distances along the outer length of each box, and were designed to be as unobtrusive as possible when viewed from inside the boxes. Each time a photocell beam was broken the signal was passed via an interface to a BBC computer. The computer was programmed to record the number of beam breaks made by the rats over a period of one hour, analysed into five minute 'bins', and for each 'bin', to subdivide the total number of beam breaks into 'repetitive' breaks of just one beam, or of gross movement between two or more different beams.

Procedure.

This experiment began between 17 and 35 days after the completion of Experiment One. Prior to the commencement of the experiment, the rats were placed in the activity boxes (one rat per box) for one hour on each of two consecutive days, to allow them to become accustomed to the procedure. This was followed by two 'test free' days. On the second day, the rats which were to be tested were placed on the same deprivation diet as used in Experiment One. All animals were run on the activity schedule for six consecutive days, and at approximately the same time each day. Each rat was injected with one

of the following drugs - 0.01 mg/kg, 0.03 mg/kg or 0.06 mg/kg scopolamine hydrobromide; 0.03 mg/kg scopolamine methylbromide; 0.1 mg/kg physostigmine, or saline, and was placed in an activity box (one rat per box) 15 minutes after the injection. The computer then recorded the 'repetitive' and gross movements made by each of the rats for a period of one hour (see Apparatus section). The drugs were administered according to a pseudorandom order, with no two rats receiving the same drug in the same one hour session.

2.2.3. Results and Discussion.

The results of this experiment are illustrated graphically in Figure 2.5. . The total beam break figures are comprised of the sum of the scores for gross movement and 'repetitive' movements. Figure 2.5. indicates that the greatest level of activity occurred with 0.01 mg/kg scopolamine hydrobromide. An analysis of variance based on total beam break scores, however, revealed that there were no significant differences between the activity scores for any of the six drug conditions ($F<1$). In other words, there was no main effect of drug on activity.

These results indicate that scopolamine does not cause any greater inactivity than physostigmine, methylscopolamine, or saline. Decreased motor activity is not, however, the only effect of scopolamine. The decreased performance on the 60 second condition of Experiment One after injections of 0.06 mg/kg scopolamine may be due to any one (or more) of the drug's other actions (Hagan and Morris, 1987).

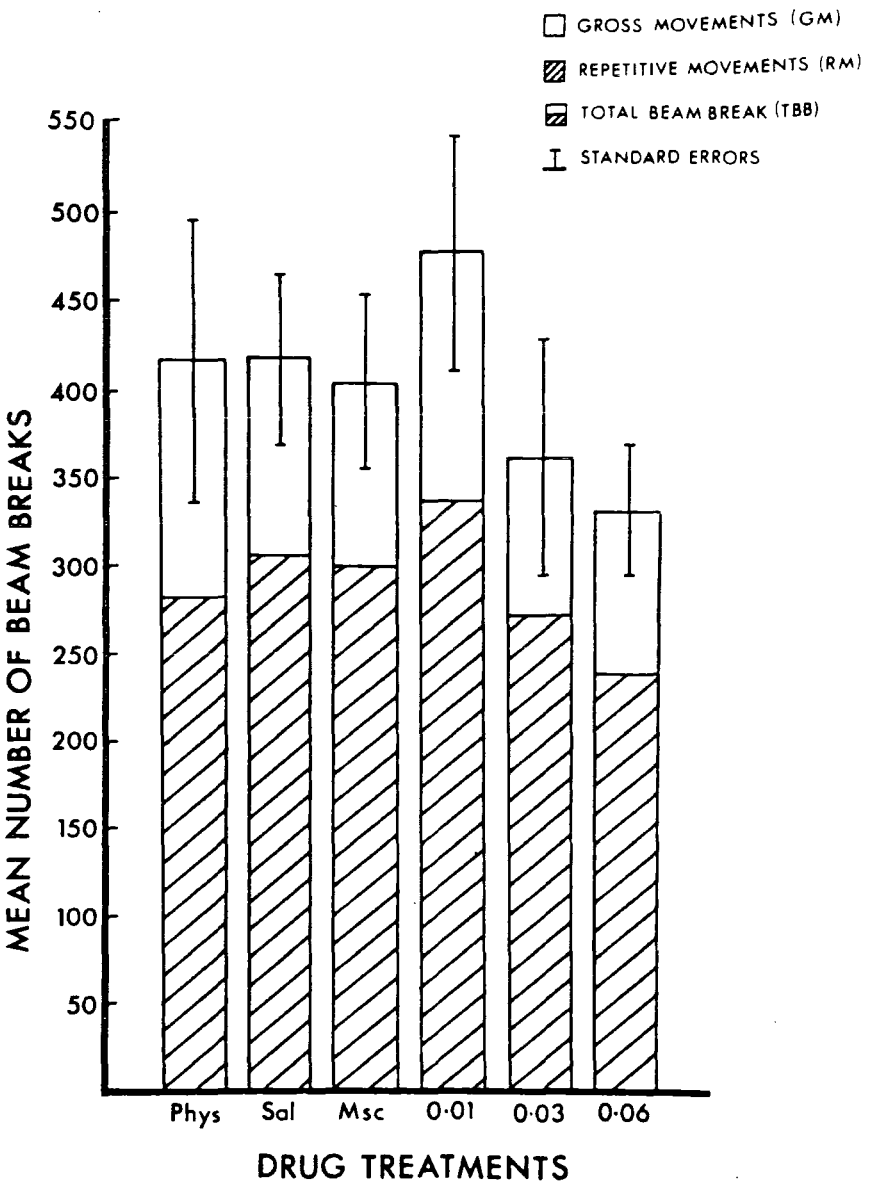

Figure 2.5.

Mean activity scores (gross movements, repetitive movements and total beam breaks) over a period of one hour.
Abbreviations: Phys, 0.1 mg/kg physostigmine; Sal, saline; Msc, 0.03 mg/kg methyl scopolamine. The remaining doses all refer to scopolamine.

Experiment Three.

2.3.1. Introduction.

This control experiment examined which sensory modality the rats used to solve the delayed nonmatching-to-sample task, i.e., whether they had used visual, olfactory or tactile cues. The importance of these modalities was assessed by systematically varying the salience of the cues from the different sensory modalities. While it has been assumed that the rodents primarily make use of vision, it is possible that other senses may alternatively, or additionally be used. If this is so, the task may not be strictly comparable to a test of object recognition given to either monkeys or man since in both of these cases, the task will be solved on the basis of visual cues only.

2.3.2. Method.

Subjects.

The subjects were the same as for Experiments One and Two.

Apparatus.

The apparatus was the same as that used in Experiment One, except that entirely new test boxes were used throughout. A control series (A) consisted of 21 new pairs of hardboard boxes of the same dimensions and specifications as Experiment One. Again, the boxes were in pairs which were made as similar as possible, but each pair was distinctive (Figure 2.6.). Series B consisted of 21 pairs of boxes of the same specifications and dimensions as Series A except that the floors protruded only 2 cm. (rather than 9 cm.) towards the

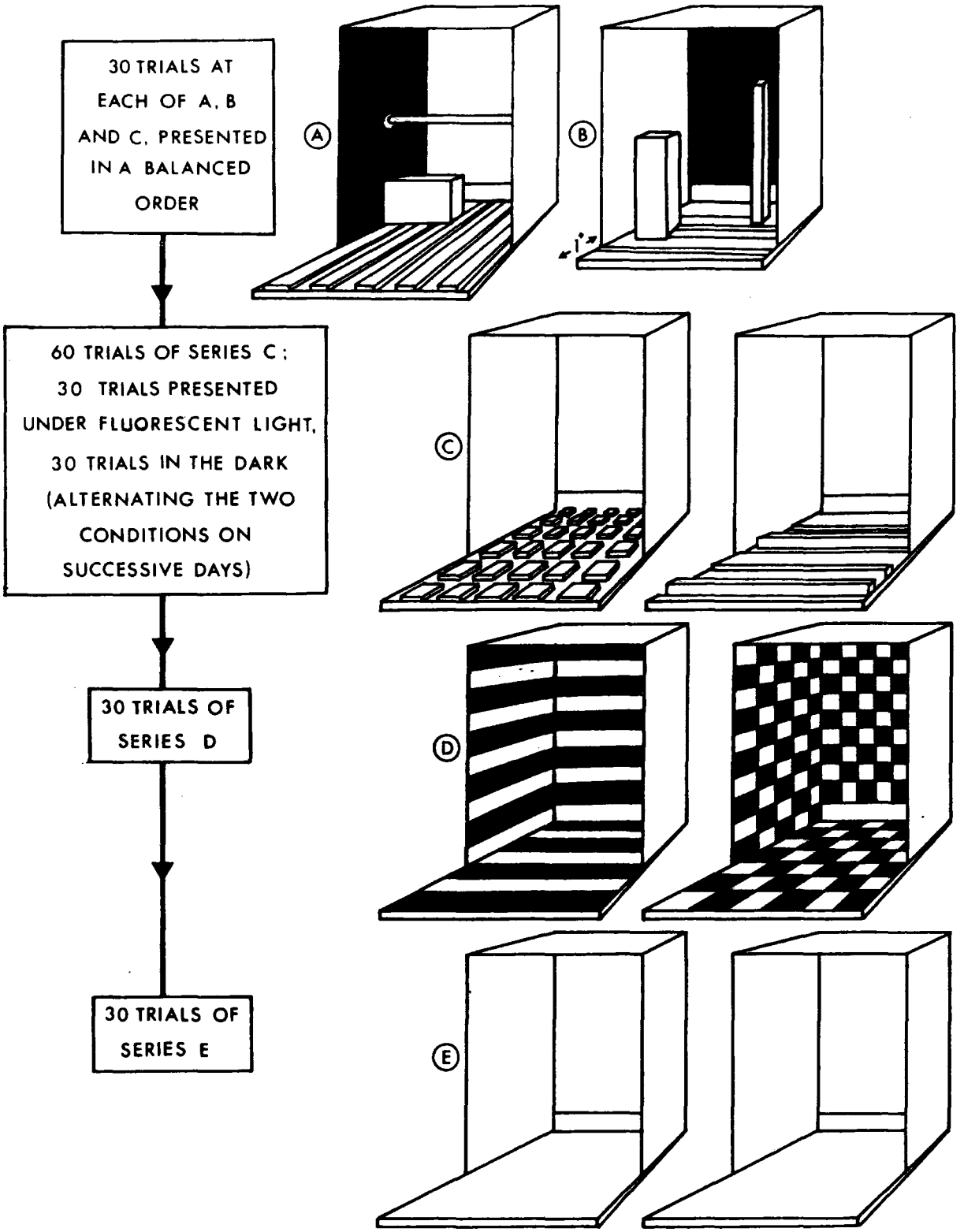

Figure 2.6.

Diagrammatic representation of the test sequence and stimuli used in Experiment Three.

centre of the maze. This reduction meant that the rats could not receive any tactile cues before making a choice in the Y-maze. Like Series A these boxes contained distinctive objects and had different floor and wall coverings. Series C consisted of 21 pairs of boxes with different floor coverings. No other embellishments were used, and the interior of every box was painted a uniform grey. As a consequence visual, and probably olfactory cues were reduced while tactile cues were intact. It should, however, be noted that any change in tactile surface must inevitably produce some change in visual appearance. Series D consisted of 21 pairs of boxes in which the interiors were covered in photographic prints of various grey, black and white patterns. This ensured that the boxes could only be discriminated visually. Series E consisted of three pairs of completely blank, hardboard boxes (Figure 2.6.).

Procedure.

Experiment Three began two months after the completion of the first 36 day drug session of Experiment One and between 33 and 58 days after the completion of Experiment Two. Due to the interval between this and Experiment One, the animals were given an additional 60 - 170 training trials until performance became stable.

All of the control trials were run with '0' second delays, correction trials, and a pseudorandom sequence of left/right responses. Experiment Three was divided into four distinct stages which followed immediately after each other. In Stage One the animals received 30 trials (three sessions) from each of series A, B or C in a mixed sequence. Only one series was used per session. In Stage Two the animals received six sessions with series C. During alternative sessions the task was run under very low lighting, the illumination

at the choice point in the centre of the Y-maze being 0.17 cd/mm (S.E.I. exposure photometer). In Stage Three the animals received 30 trials with series D, 10 trials on each of three consecutive days. Finally, the animals performed 30 trials with series E (Stage Four). The blank boxes, which served as both start and goal boxes for every trial, were re-arranged after every trial.

2.3.3. Results and Discussion.

Figure 2.7. illustrates the mean performance of the animals during the various stages of the experiment and also indicates those sensory cues which were present. In Stage One the animals averaged 24.0 correct responses (80%) on series A (the normal, control series), and 23.3 and 23.4 on series B and C respectively. All of these scores were significantly above the chance score of 15 (minimum $t=10.38$, $df=7$, $p<0.001$), and there was no evidence of a difference between the three series ($F<1$). Clearly overall performance was unaffected when tactile cues were completely removed (series B) or when visual and olfactory cues were presumably reduced (series C).

In Stage Two the animals averaged 23.0 correct responses in the light condition with series C, and 19.0 correct responses in the dark condition. Although both of these scores were significantly above chance (minimum $t=7.57$, $df=7$, $p<0.001$) the animals performed better in the light than in the dark ($t=2.22$, $p<0.05$). This difference in performance may reflect some partial use of visual cues in discriminating series C, although it is not possible to rule out the effects of a "generalisation decrement" (Hull, 1943; Mackintosh, 1974). Of equal interest was the finding that the scores in the dark were still above chance. Thus the animals would appear to be

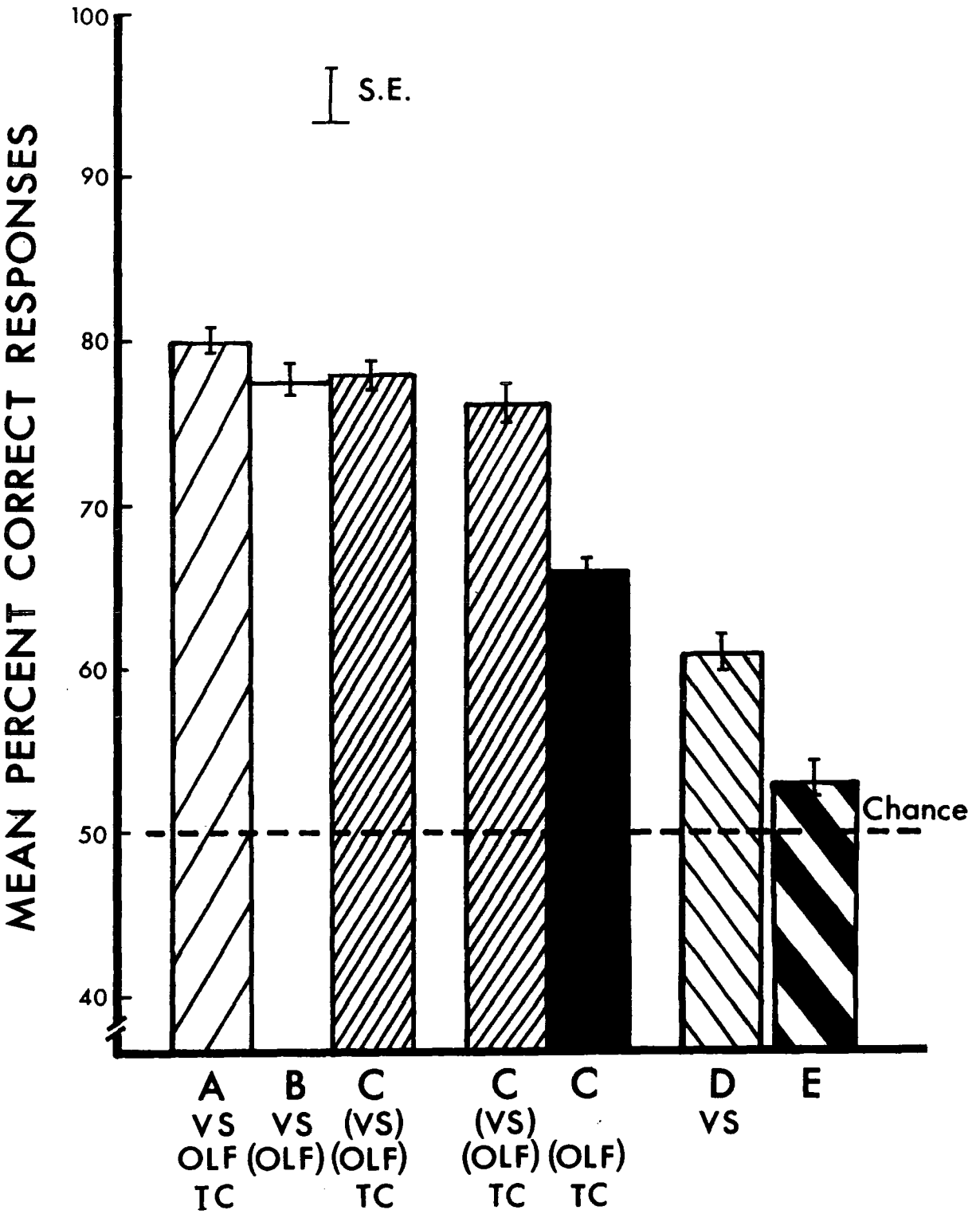

Figure 2.7. Mean percent correct on the control tests.

The subscripts show the test series (see Figure 2.6.) and the sensory cues (OLF, olfaction; TC, touch; VS, vision) that were available to the animals. Symbols in parentheses indicate those sensory modalities for which cues may be present but are probably less than in the standard condition (series A).

able to use olfactory or tactile cues.

When the stimuli were restricted to two-dimensional visual patterns (Stage Three) the mean score was 18.4. This score was still significantly above chance ($t=3.22$, $p<0.01$) and was significantly better than the blank box (series E) control condition ($t=1.95$, $p<0.05$). The scores on this final condition (series E) did not differ from chance (mean=16.0). These results showed that the animals could perform the task visually, even when the stimuli were only two-dimensional. The relatively poor performance on series D (Figure 2.7.) may partially reflect the use of stimuli which provide no depth cues, in contrast to the normal condition (series A). Support for this explanation comes from pilot experiments which have shown that rats can perform at up to 80% accuracy when the stimuli provide three-dimensional cues but no olfactory cues. This was achieved by making the boxes and the distinctive stimuli out of the same material.

In summary, it has been shown that the animals could perform the task when tactile (Stage One), visual (Stage Two) and finally tactile and olfactory (Stage Three) stimuli were removed. These findings strongly indicate that the rats were using cues from more than one sensory modality in order to perform the standard nonmatching task and hence could readily transfer when cues were systematically removed. It is not possible, however, to specify the relative value of these different cues in the standard condition from the results obtained in this experiment.

2.4. General Discussion.

Experiment One examined the way in which the muscarinic blocker, scopolamine, disrupted a non-spatial test of working memory (non-matching-to-sample). It was found that with a retention interval of 60 seconds, a dose of 0.06 mg/kg scopolamine hydrobromide was sufficient to reduce performance. Although the results of Experiment Two suggest that this reduced performance cannot be attributed to the effects of scopolamine on activity, there is no evidence either that at this dose the drug increases the rate of forgetting on the object recognition task since no interaction was found between the 0.06 mg/kg scopolamine and saline conditions (P. 70).

The present study is not the first to show that scopolamine can impair tests of recognition memory in experimental animals. It should be noted, however, that the large majority of previous studies have used stimuli, either spatial or non-spatial, that are shown repeatedly within a session (Bartus and Johnson, 1976; Dunnett, 1985; Heise and Hudson, 1985; Robustelli et al., 1969). As a consequence such tests measure recency judgements. The only previous study to have used a large pool of stimuli was that of Aigner and Mishkin (1986), who showed that scopolamine impaired delayed nonmatching-to-sample with trial unique stimuli in monkeys. The present study is in close accord with their findings. The control tests (Experiment Three), which sought to determine which sensory modality the rats were using to solve the recognition task, revealed that the rats were not relying exclusively on visual cues but were using information from a range of sensory modalities. This is therefore one respect in which the present task differs from similar tasks given to monkeys.

The present study failed to find evidence that scopolamine can

promote faster forgetting at doses which do not disrupt the simplest version of the task. This finding contributes to the growing body of studies that have examined the effect of this drug upon rates of forgetting in rats. These studies, which have almost exclusively used tests of spatial working memory, have consistently shown that scopolamine does not selectively disrupt performance over longer retention intervals (Robustelli et al., 1969; Ksir, 1974; Heise et al., 1976; Dunnett, 1985; Godding et al., 1982). The results from studies of primates are, however, less clear-cut, with some reports providing evidence of a selective interaction between drug and delay (Pontecorvo and Evans, 1985).

The failure of scopolamine to show clear evidence of a specific effect upon retention in rats does not rule out an amnestic action for this drug; the drug could act upon some other aspect of memory. There is, however, clear evidence that this drug has multiple effects and a more parsimonious explanation of the actions of scopolamine may not require an effect on memory processes per se. For example, Warburton and Brown (1971) have shown that in rats doses of 0.063 mg/kg scopolamine hydrobromide and above may affect attentional processes, while other studies have suggested an action upon sensory systems (Evans, 1975; Hagan and Morris, 1987; Spencer, Pontecorvo and Heise, 1985).

In addition to its central actions, scopolamine has peripheral effects which may also influence the ability of animals to perform behavioural tasks. The drug methylscopolamine, which does not cross the blood-brain barrier, was therefore used to test the possible contribution of these peripheral effects. The pilot study had previously shown that the peripheral actions of scopolamine are not sufficient to impair performance at a low dose level. Although in

Experiment One the highest dose of scopolamine was not matched by a comparable dose of methylscopolamine, there is evidence from other studies of working memory in rats that doses of up to 0.5 mg/kg methylscopolamine do not reduce performance accuracy. For example, Beatty and Bierley (1985) found that 0.5 mg/kg scopolamine methylbromide had no effect on either working memory or reference memory in a study of rats tested in a 12-arm radial maze. Dunnett (1985) and Heise and Hudson (1985) obtained similar results in tests of delayed matching-to-position and continuous delayed response, respectively. This suggests that only doses of methylscopolamine in excess of 0.5 mg/kg may have any effect on performance accuracy.

It has been shown that at certain doses physostigmine may improve performance on the delayed nonmatching-to-sample task in monkeys (Aigner and Mishkin, 1986). Although only one dose level was used in the present experiment there was evidence of an improvement at the more difficult, 60 second condition. This is in general agreement with other studies of working memory in rats which have shown that physostigmine may improve performance (Dunnett, 1985; Squire, 1969), although in the present study the main effect of physostigmine was to flatten the forgetting slope, rather than to produce a general improvement at all conditions (Dunnett, 1985). As a consequence, it would be of particular interest to examine the effects of this drug over even longer retention intervals.

Although the results of this chapter do not provide any stronger support for the use of physostigmine as a reliable therapeutic agent for dementia-related cognitive impairments than any other reports cited earlier, they do provide additional circumstantial evidence for a cholinergic role in such impairments. The results also contribute towards the belief that appropriate pharmacological manipulation of

the cholinergic system (perhaps in conjunction with other neurotransmitter systems) may eventually be developed to alleviate some of the cognitive declines associated with Alzheimer's disease.

Summary and Relevance to Alzheimer's Disease.

Human studies have shown that scopolamine can disrupt tasks of recent memory (Crow and Grove-White, 1973; Drachman and Leavitt, 1974; Sitarem et al., 1978), indicating that cholinergic disruption by muscarinic blockade can cause memory impairments which are comparable to those seen in Alzheimer's disease patients. The results of studies which have examined the effects of the cholinergic agonist physostigmine in man also lend support to the cholinergic hypothesis of memory dysfunction (Davis et al., 1978). However, much of the psychopharmacological data derived from animal experiments are confusing and contradictory, offering no clear support for the memory hypothesis. This is largely attributable to the effects scopolamine and physostigmine have on processes other than memory, such as attention, arousal and motivation: There is good evidence for cholinergic involvement in sensory and attentional mechanisms, thus, in many experiments which demonstrate changes in learning rate, a sensory/attentional hypothesis may provide a parsimonious and viable alternative to a learning/memory hypothesis. Also, cholinergic intervention can result in overt motor effects, including stereotypy. In some cases, motor effects may interfere with task performance independently, or in addition to, effects upon learning, memory or retrieval. (It should be noted, however, that the mere observation of effects on movement or other processes does not preclude the possibility that these are secondary to a cognitive impairment).

In general, the results from animal studies question the notion that a cholinergic dysfunction is sufficient to produce the symptoms of Alzheimer's disease. The reader will recall that the neuropathology of this disease consists of cell loss in the nbM, the neocortex, and in limbic structures - particularly the hippocampus and amygdala. The sequence of these neuropathological changes is not known. The behavioural symptoms consist of recent memory impairments; language disorders - typically aphasia and agnosia; emotional changes and finally, total dementia. In terms of the cholinergic hypothesis that damage to the nbM causes global cholinergic dysfunction, the fact that anterograde amnesia is the first behavioural symptom to emerge in cases of Alzheimer's disease suggests that:

(1) This function may be the most sensitive to the cholinergic abnormality. This is questionable, however, since decreasing CAT levels in experimental animals with drugs such as scopolamine does not cause a specific memory deficit. Furthermore, since loss of acetylcholine in the brain causes global damage rather than solely a deficit in memory, one would not necessarily expect to find a specific memory loss simply by blocking ACh with scopolamine.

(2) The first neuropathologic structure to be diseased (the nbM, neocortex, and/or limbic system) has an important role in memory, hence producing a memory impairment if damaged. It has been assumed that the nbM may be the first structure to be damaged and hence be responsible for the amnesia. Recent evidence, however, indicates that this is not the case: It is more likely that damage to the nbM causes a general dementia rather than a specific memory impairment (Aigner et al., 1987). A comparison of Alzheimer's disease with encephalitis supports this notion,

and also suggests that damage to the limbic system (which is heavily interlinked with the cortex) is the most likely cause of memory loss.

The same limbic and cortical structures are affected in both Alzheimer's disease and encephalitis; likewise emotional, language and memory deficits are evident in both disorders. Encephalitic patients, however, exhibit no evidence of dementia, and as far as is known, do not sustain either damage to the nbM or exhibit cholinergic dysfunctions. This implies that the recent memory impairments of encephalitic patients must be caused by damage to structures other than the nbM, and the same could also be true of the anterograde amnesia characteristic of Alzheimer's disease.

There is a wealth of information suggesting that the limbic system has an important role in memory processes (Olton, 1982; Mishkin et al., 1982; Scoville and Milner, 1957; Squire and Zola-Morgan, 1983). If this system was the first structure to be damaged in Alzheimer's disease, so causing recent memory impairments, this would be consistent with the finding that blocking ACh with scopolamine does not mimic the memory loss, as it is far more likely that damage to brain structures, rather than a cholinergic dysfunction, is responsible for the observed memory deficits.

There is a need for a longitudinal study of Alzheimer's disease to be conducted, correlating progressive behavioural deterioration with the onset and sequence of neuropathological damage. In theory, this would be the simplest way of firmly establishing the functional contribution of damage to the nbM, cortex and limbic system to the memory loss and dementia characteristic of Alzheimer's disease.

Despite the experimental disadvantages outlined above, the cholinergic hypothesis is still widely believed to be the most

viable explanation for the memory impairments characteristic of Alzheimer's disease, and so the psychopharmacological research continues. However, alternative procedures for the assessment of cholinergic involvement in the dementia and memory syndrome are beginning to emerge, for example, studies with the agent AF64A (ethylcholine aziridinium ion). This alternative will be discussed in Chapter Four.

CHAPTER THREE. ALUMINIUM STUDY.

3.1.1. Introduction.

While there is growing evidence linking the presence of aluminium with dementia it is necessary to determine whether this association is causal, or whether it is merely a secondary consequence of the disease. The aim of the experiments in this chapter was to examine the former alternative. It is already known that an intracranial injection of aluminium salts can, in certain species, induce an experimental encephalopathy in which evidence of neurofibrillary degeneration is accompanied by deficits in learning and memory (Crapper and DeBoni, 1980; Crapper, 1976). Although this model has directed attention to the metal, it has failed to resolve the vital question of whether aluminium is responsible for any of the symptoms of Alzheimer's disease.

This failure reflects serious shortcomings in the experimental model. For example, it has been pointed out that only single filaments are produced in this experimental encephalopathy while double filaments are found in man (Wisniewski et al., 1976). Other important shortcomings concern the limited period following the injection of aluminium salts during which behavioural experiments can be carried out, and the restricted number of susceptible species. These limitations have narrowed the range of behavioural tasks which can be used. For example, many of these studies have been concerned with avoidance behaviour (Crapper and Dalton, 1973[1], 1973[2]; Petit et al., 1980) despite the lack of evidence that this class of task is sensitive to human dementia. Furthermore, interpretation of these animal studies is often complicated by the imminent onset of non-cognitive neurological symptoms, such as ataxia, which may provide a purely physical limit to performance.

If the neuronal changes observed in Alzheimer's disease are due to the gradual accumulation of aluminium over many decades, then a more appropriate manipulation would be to examine the effects of chronic exposure to aluminium in animals. One simple method of doing this is to supplement the diet with aluminium salts. While the outcome of the few experiments which have attempted this have proved encouraging (Hinz and Dufort, 1983; Thorne et al., in press), a major difficulty is encountered in ensuring reliable uptake of aluminium to the brain. Recent epidemiological findings have, however, suggested a new way of solving this problem. The peculiar form of Parkinsonism dementia found in just a few sites (the island of Guam, the Kii Peninsular of Japan, and in southern West New Guinea) is restricted to regions containing high levels of aluminium in the water and soil, and prominent accumulations of aluminium have been found in the neurofibrillary tangle-bearing neurons of such patients. An additional common factor linking these three geographical regions is that they have unusually low levels of calcium and magnesium (Perl et al., 1982), and one interpretation is that it is this combination of high aluminium coupled with low calcium and magnesium which potentiates the uptake of aluminium and so produces the dementia. This possibility provides the conditions under which a chronic aluminium encephalopathy might be induced in animals. Such a model would have the clear advantage that the conditions under which aluminium enters the central nervous system match those in man. Furthermore, this technique may be amenable to a much wider range of species than the acute model, and the longer survival time provides the opportunity for much more extensive, sophisticated behavioural testing. This model also offers the opportunity to examine those variables which might effect aluminium uptake, e.g., age, calcium

balance, duration of exposure, form of aluminium, etc., and so help predict which members of the population are at most risk to this disease. It could also be used to test possible treatments designed to reduce levels of aluminium, e.g., chelating agents.

It is important to stress that behavioural testing is necessary in order to demonstrate whether increased levels of aluminium have any cognitive consequences and whether they are consistent with those observed in dementia. This is all the more important as the evidence from previous animal studies suggests that the pathological consequences of aluminium upon the brain may be similar, but not identical, to those observed in dementia, and the significance of this difference remains unknown.

So, the aim of the present study was to develop a chronic aluminium encephalopathy in animals by the administration of a diet high in aluminium salts but low, or absent, in calcium and magnesium. The rat was used for the study since it has become the species of choice for most neuroscientific experiments, and also because there is evidence that rats and man may have a comparable resistance to the neurotoxic effects of aluminium (see Sections 1.4.4. and 1.4.12.).

The experiments were carried out in collaboration with Dr. A. Sahgal at the M.R.C. Unit for Neuroendocrinology at Newcastle General Hospital. A large cohort of 80 infant P.V.G. rats was split between the M.R.C. Unit and Durham; 56 at Newcastle, 24 at Durham. Both centres administered an identical diet, the experimental animals receiving a high aluminium, low magnesium, calcium-free diet. There were, in addition, three control groups: One group was maintained on a low magnesium, calcium-free diet which was free from aluminium; one group was maintained on a normal diet; and the last group received a normal diet supplemented with aluminium.

The behavioural experiments concentrated on tests of memory, reflecting thecon sistent finding that memory deficits are the earliest and most frequent symptom of Alzheimer's disease. The animals were therefore tested on the non-spatial task of object recognition (nonmatching-to-sample) as used in Experiments One and Three of Chapter Two. As previously noted, one particular value of this task is that it closely matches tests of visual recognition which are known to be disrupted in Alzheimer's disease (Albert and Moss, 1984). An additional feature is that the task offers the opportunity to examine not only the rate of learning the task but also the rate of forgetting simuli used within a session (see Chapter Two).

Experiment One.

3.1.2. Introduction.

This experiment examined the effect of a diet high in aluminium (but low in magnesium and calcium-free) on performance of the delayed nonmatching-to-sample task in rats. The performance of the experimental animals was compared to that of controls subject to a variety of dietary manipulations (see below), in order to provide a correlation between cognitive performance and aluminium uptake. It was also hoped to assess the extent to which a lack of dietary calcium might contribute to aluminium uptake. The doses of aluminium, magnesium and calcium used were the same in both this experiment and in a nonmatching-to-position study conducted at the M.R.C. Unit in Newcastle.

It should be pointed out that this and the Newcastle experiment were not set out to be definitive studies with regard to the toxin hypothesis of Alzheimer's disease. Rather, they were conducted as pilot studies with the aim of assessing whether a chronic rodent model based on dietary manipulations of aluminium and calcium could be developed. If so, this model would then provide the basis of further experiments to assess the effect on aluminium uptake and memory loss of other variables e.g., form of aluminium, duration of exposure, precise calcium balance, treatment of aluminium neurotoxicity, etc.. Hence the present study should be regarded as no more than the first step towards an assessment of the precise role of aluminium in Alzheimer's disease.

3.1.3. Method.

Subjects.

The subjects were 24 male P.V.G. rats supplied by the M.R.C. Neuroendocrinology Unit at Newcastle General Hospital. The rats were housed in groups of three, and were each maintained on approximately 15 g. of one of Diets A, B, C, or D, each day (see below for diet details). Distilled, de-ionised water was freely available in the home cage. The rats were aged approximately 6 weeks old when they were started on the diets, and were approximately 4.5 months old at the start of the experiment. They were kept on the diets throughout the behavioural testing and so were maintained on these diets for a total of 9.5 months.

Apparatus.

Calcium and magnesium-free diet, in a powdered form, was obtained from Labsure. Chemicals were then added to this basic diet to make four different diets, as follows:

(1) Diet A consisted of basic diet; 1 g/kg magnesium trisilicate powder; 72 g/kg aluminium hydroxide powder; and 10 g/kg calcium chloride crystals. (+Aluminium, +Calcium).

(2) Diet B consisted of basic diet; 1 g/kg magnesium trisilicate powder; and 72 g/kg aluminium hydroxide powder. (+Aluminium, -Calcium).

(3) Diet C consisted of basic diet; 1 g/kg magnesium trisilicate powder; and 10 g/kg calcium chloride crystals. (-Aluminium, +Calcium).

(4) Diet D consisted of basic diet and 1 g/kg magnesium trisilicate powder. (-Aluminium, -Calcium).

The calcium chloride crystals used in Diets A and C were dissolved in distilled water before being added to the basic diet. The required daily amount of each diet was measured out into plastic containers and mixed into a paste with distilled water. The magnesium trisilicate powder, aluminium hydroxide powder and calcium chloride crystals were all supplied by B.D.H..

Approximately 3.5 months after the start of the experiment, it was necessary to feed the animals on a substitute base diet for five weeks before being returned to the original Labsure base. The substitute base was made up of the following: Human grade cornflour (a pure source of starch); sodium chloride AnalR (Riedel-de Haen); potassium chloride AnalR (Koch-Light Ltd.); multivitamins (Bimeda); and caseine (Special Diet Services). Magnesium, aluminium and calcium were added to this substitute diet in the same proportions as before (see above) to form a comparable series of the four diets A, B, C and D.

The maze apparatus was the same as that used in Experiment One, Chapter Two, except that 70 pairs of test boxes were used throughout. Water was not available in the maze, and the reward pellets used were 45 mg. calcium and magnesium deficient dustless pellets (Custom Biological Diets, Bioserv Inc.).

Procedure.

The rats were arbitrarily divided into four diet groups, comprising six rats per diet. The study was run blind, so that throughout testing, the experimenter did not know which diet label (A, B, C or D) corresponded to which particular diet constituents.

The rats were given 2 - 3 weeks pretraining on the maze to familiarise them with the apparatus. Thereafter the nonmatching-to-

sample procedure (including correction trials) was the same as that followed in Experiment One, Chapter Two, except that rather than being trained until they reached a criterion of 80% correct responses, all rats received a total of 400 acquisition trials on the nonmatching task, regardless of whether or not they actually reached the above criterion level within this time. The rats were tested in the same order each day, as follows; 3 each of Diets A, B, C, D, D, C, B, and A.

Immediately after the completion of the 400 acquisition trials, the rats were transferred to the delay regime, which lasted for 9 days. The delays of '0', 20 and 40 seconds were presented in a pseudorandom order, with 30 trials at each delay, and again, the procedure over this part of the experiment was the same as that followed in Experiment One, Chapter Two.

Between three and five weeks after the completion of Experiment One and following completion of Experiment Two (P. **96**), all of the animals were sacrificed. The rats were perfused intracardially with 5% formol saline. The brains were subsequently blocked, embedded in wax (Paraplast) and cut into 10 µm. coronal sections. Every 10th section was mounted and stained with cresyl violet.

3.1.4. Results and Discussion.

The results of the histological examination were negative: At least every third coronal section from all of the experimental animals was studied with light microscopy for evidence of cell loss and/or gliosis. Particular attention was given to the nbM, the hippocampal formation, the amygdala, and the neocortex. There was no evidence that any of the groups appeared unusual.

Figure 3.1. illustrates the acquisition scores obtained over the first 400 trials of Experiment One. An analysis of variance which compared performance between the groups over the full block of 400 trials revealed that there was a main effect of diet on the results ($F=4.36$, $df=3, 15$, $p<0.05$). Further examination of this main effect with the Newman-Keuls test identified that the scores obtained with Diet D (-Aluminium, -Calcium) were significantly higher than those of the other three conditions ($p<0.05$). In fact, it is interesting to note that five of the six rats on Diet D reached the criterion of 80% correct responses (the criterion used in Experiment One, Chapter Two), within the 400 acquisition trials, compared to only 2 of the Diet B animals, 3 of the Diet A and none of the Diet C animals. It should be stressed, however, that there were no differences between the acquisition scores for Diets A, B or C. The main effect of diet noted above probably results from the relatively small number of subjects used in the experiment (i.e. $n=6$ in each diet group).

The only other point to note with regard to the acquisition phase of Experiment One is that the P.V.G. rats appeared much slower to run round the Y-maze than the D.A. rats used in the experiments of Chapter Two. This could be a consequence of the dietary manipulations on the locomotor activity of the P.V.G.s, a point which will be

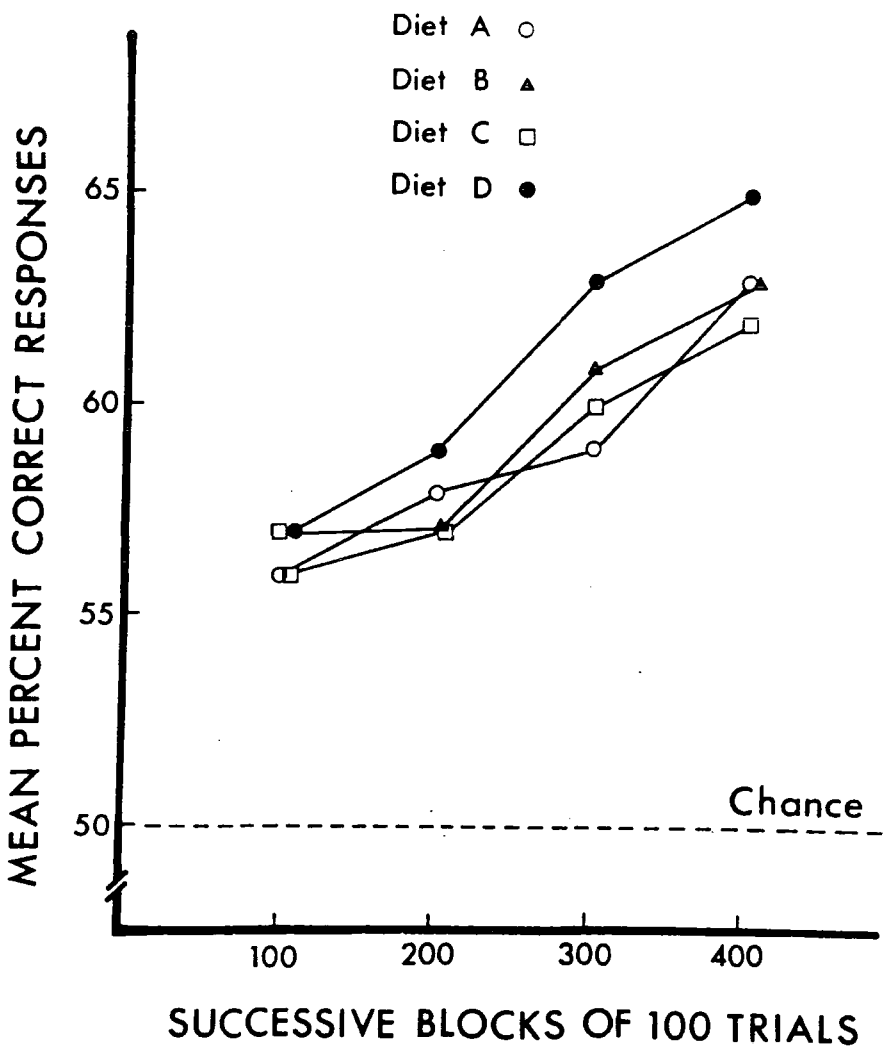

Figure 3.1.

Mean performance over the 400 acquisition trials of Experiment One.

Diet A: +Aluminium, +Calcium

Diet B: +Aluminium, -Calcium

Diet C: -Aluminium, +Calcium

Diet D: -Aluminium, -Calcium

discussed further in Section 3.2..

The results of the delay regime are illustrated graphically in Figure 3.2. . It should be noted that two rats, one from Diet B and one from Diet C, failed to complete all 9 days of the delay sequence and so their results are excluded. An analysis of variance revealed that there were no main effects of either diet ($F=2.85$, $df=3, 18$) or delay ($F=1.04$, $df=2, 36$), and that there was no significant interaction between diet and delay ($F<1$).

Previous studies using this paradigm have consistently shown that retention delays of 60 seconds produce reliable decrements in performance. The failure to find an effect of delay in the present study may reflect the shorter retention interval, but may also reflect the poor performance of individual animals on even the shortest delays.

The failure to find a deleterious effect of supplementing aluminium to the diet is highlighted when the results of the two groups which received additional aluminium (A and B) are compared with the two groups which received no aluminium (C and D). An analysis of variance revealed no significant differences between A+B and C+D ($F=3.63$, $0.05>p<0.1$).

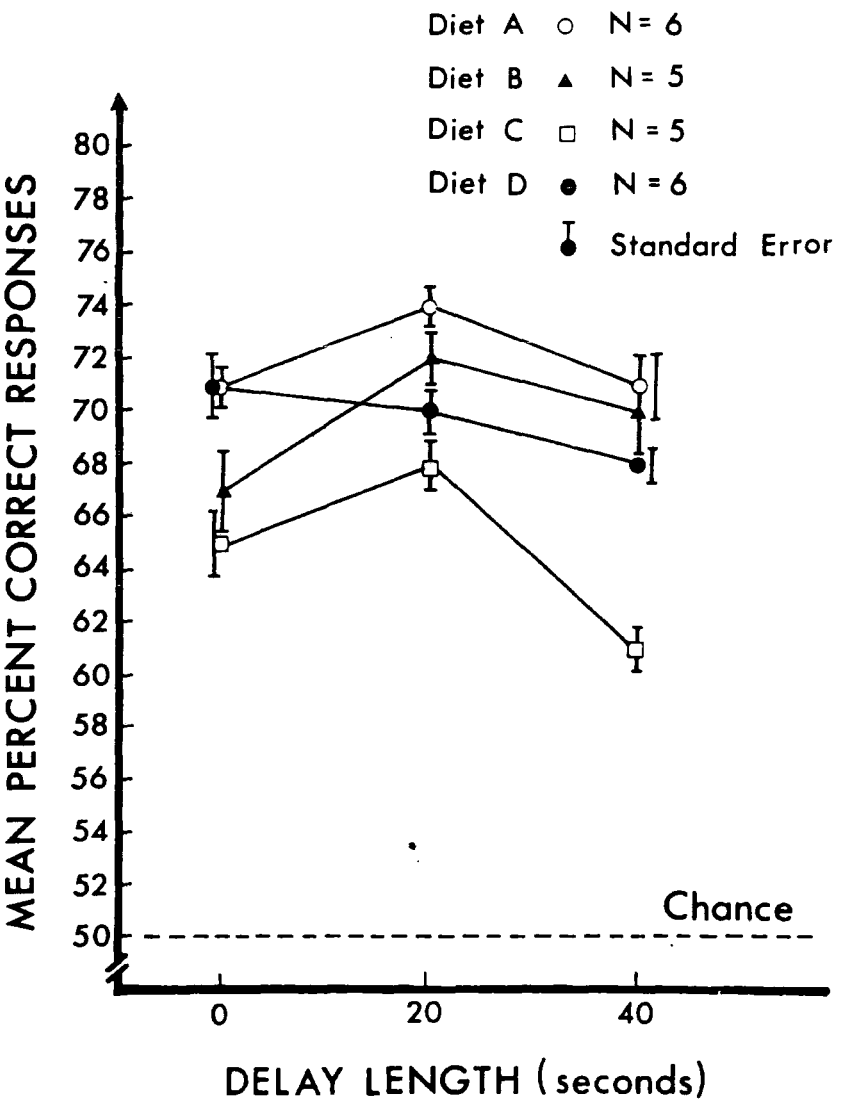

Figure 3.2.

Mean performance following 30 trials at each of the three retention intervals.

Diet A: +Aluminium, +Calcium

Diet B: +Aluminium, -Calcium

Diet C: -Aluminium, +Calcium

Diet D: -Aluminium, -Calcium

Experiment Two.

3.2.1. Introduction.

This experiment was undertaken as a control for Experiment One, to provide some gauge of the effects of the various dietary manipulations (outlined in 3.1.2.) on the motor activity of the animals. As previously mentioned (Section 3.1.4.), it was generally apparent that the P.V.G.s ran more slowly round the maze than the D.A.s. Although no immediately discernible differences in activity were noted between the four groups of P.V.G. rats in Experiment One, if there were differences or if one or two groups were particularly slow, this would make performance of the delayed nonmatching-to-sample task more difficult and consequently reduce the number of correct choices made on the task. Since this could account for the subtle performance differences noted on the acquisition phase of Experiment One (Section 3.1.4.), it was considered appropriate to assess specifically whether or not there were any differences in locomotor activity between the four groups.

It should be stressed that this experiment was strictly a control test, and was not intended to be a full assessment of the effects of aluminium on locomotion. Hence it was limited to only one test session per rat. It should also be pointed out that although examining rates of activity is a logical procedure to follow, the activity and reactions recorded in the activity boxes will be dissimilar to those occuring in the Y-maze since food motivation will affect activity in the maze.

3.2.2. Method.

Subjects.

The subjects were the same as for Experiment One, with the exception of two rats, both from the group maintained on Diet B (+Aluminium, -Calcium), which were excluded for health reasons.

Apparatus.

The apparatus was the same as that used in Experiment Two, Chapter Two, except that four boxes were used rather than three. Also, the computer was programmed to record the number of beam breaks occurring over a period of only half an hour (rather than a full hour), since it was apparent from a brief survey of the results of Experiment Two (Chapter Two) that the majority of the animals' movements occur within the first half-hour of being placed in the boxes.

Procedure.

This experiment began between one and three weeks after the completion of Experiment One. The rats were placed in the activity boxes (one rat per box) for 30 minutes each, and the computer recorded the gross and repetitive movements made by each of the rats over this period. The animals were tested in a pseudorandom order, according to diet, and were all tested once each over the course of a day.

3.2.3. Results and Discussion.

The results of this experiment are illustrated graphically in Figure 3.3. . An analysis of variance based on total beam break scores

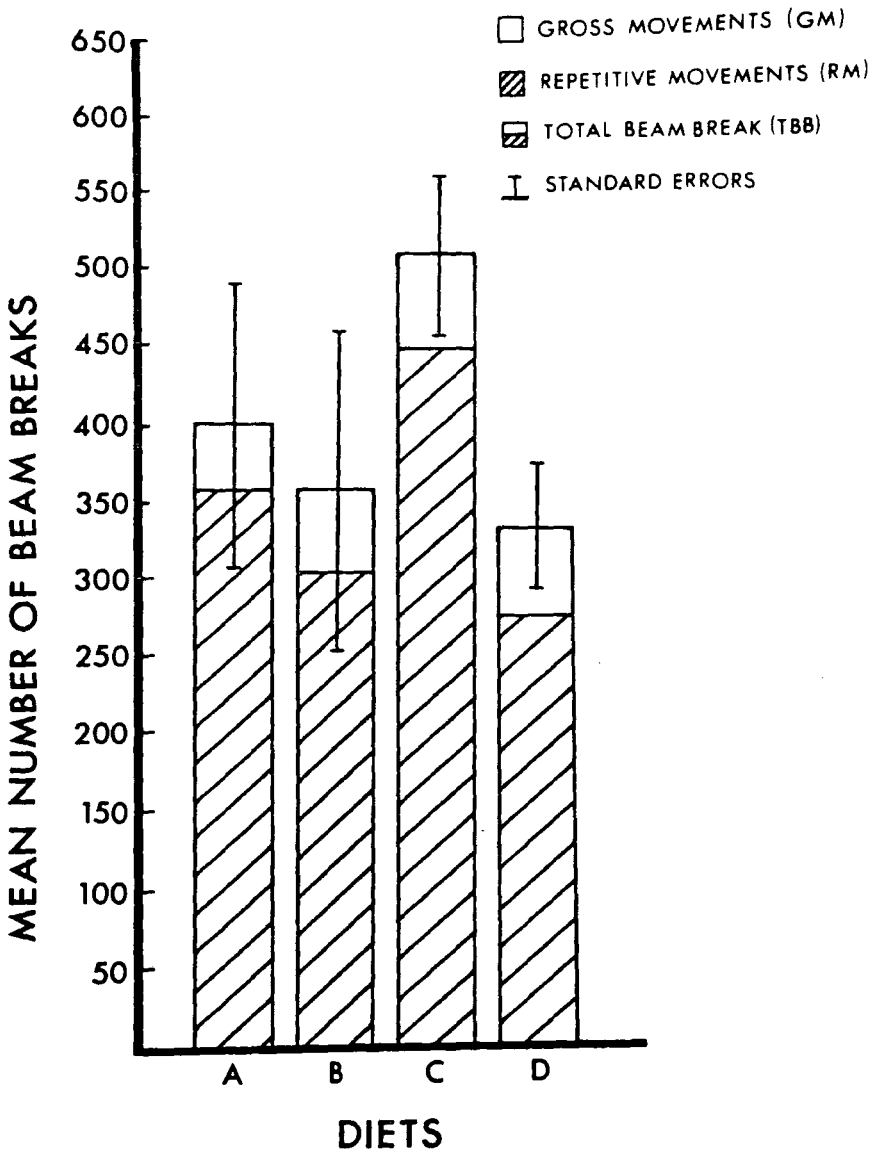

Figure 3.3.

Mean activity scores (gross movements, repetitive movements and total beam breaks) over a period of 30 minutes.

Diet A: +Aluminium, +Calcium

Diet B: +Aluminium, -Calcium

Diet C: -Aluminium, +Calcium

Diet D: -Aluminium, -Calcium

revealed that there were no significant differences between the activity scores for any of the four dietary groups. Thus, it is unlikely that changes in locomotor activity could account for the acquisition differences observed on the object recognition task.

3.3. General Discussion.

On the basis of the toxin hypothesis of Alzheimer's disease, this part of the study was designed to assess the extent to which a diet high in aluminium (but low in magnesium and calcium-free) might be taken up by the rodent brain, and consequently what effects (if any) this might have on memory processes. With regard to behavioural testing, particular attention was given to tasks known to be sensitive to the effects of damage to those regions which suffer the greatest pathology in cases of this disease, such as the hippocampus, amygdala, and basal nucleus of Meynert. Hence Durham tested rodents on a non-spatial task of object recognition, nonmatching-to-sample, and the M.R.C. Unit in Newcastle tested their rats on a spatial task of working memory, nonmatching-to-position, which was based on a task previously developed by Dunnett (1985).

Experiment One did not provide any evidence that dietary manipulations of aluminium and calcium have an effect upon memory for the object recognition task. The results of the nonmatching-to-position study conducted by the M.R.C. Unit similarly revealed that there were no significant differences between the various dietary groups tested on the task.

The rats at Newcastle were first autoshaped on the nonmatching-to-position task for 100 trials. The criterion was five consecutive, correct lever presses. There was no main effect of diet over this acquisition phase of the experiment. Four of Diet A, two of Diet C and one of the Diet D animals failed to learn the task, and the remaining animals were transferred to the delay regime: At '0' secs delay the criterion was 90% correct (or greater) on three consecutive days, followed by a regime consisting of delays of up to 32 secs.

No group differences were detected at any delay when all four groups were compared ($F<1$), although the analysis was based on final group sizes of only four Diet A, six Diet B, two Diet C and ten Diet D, since many animals failed to complete the delay sequence.

The brains from the experimental animals were subsequently analysed using a Perkin Elmer Atomic Absorption Graphite Furnace, but no group differences were found in tissue aluminium concentrations.

Hence, we were unable to find any evidence that a diet high in aluminium salts could affect cognitive processes on either a spatial or non-spatial task of working memory. Additionally, Experiment Two failed to find any significant differences in activity levels between the four dietary groups.

These negative results can best be explained on the basis of the histological data, which provided no evidence of a prominent accumulation of aluminium in the brains of those rats subjected to diets containing aluminium hydroxide (Diets A and B) in either Durham or Newcastle. Since the experimental methodology of this chapter was based on the hypothesis that accumulations of aluminium in the brain cause the learning and memory deficits associated with Alzheimer's disease (rather than the hypothesis that such accumulations are simply a secondary consequence of the dementia), it would be expected that memory impairments would not be in evidence if levels of aluminium in the brain were not elevated. On the other hand, the present study failed to find any positive proof in favour of the hypothesis that brain aluminium causes cognitive deficits of the type seen in Alzheimer's disease, so the reason for the presence of aluminium in the brains of patients suffering from this disease remains an open question.

It has been reported that at brain concentrations five or six

times that used in cats, aluminium injected rats do not show neurofibrillary degeneration or more than a slight depression in behavioural responses (King et al., 1975). In contrast, it has been demonstrated that the oral administration of aluminium to rats can result in behavioural change. For example, aluminium was found to be absorbed and deposited in the brain in a study in which it was administered in varying doses by intubation (Bowdler et al., 1979). High levels were associated with increased activity, decreased roto-rod performance, and increased sensitivity to flicker. Hinz and Dufort (1983) reported that aluminium administered to rats in the form of an aqueous solution of Amphojel (an antacid containing aluminium hydroxide) resulted in poor performance on an eight-arm radial maze relative both to the rats' pre-administration performance and to control behaviour. In a later study, Hinz (1983) both replicated and extended her earlier work, although unfortunately, Hinz and Dufort (1983) and Hinz (1983) were unable to perform an analysis of brain aluminium content. Without this analysis it is impossible to attribute the observed memory deficit to the effects of neural incorporation of aluminium. Thorne et al. (in press) attempted to remedy this defect: Adult Long-Evans rats were fed one of three doses of aluminium hydroxide for 30 days:- 1513 mg Al/kg of body weight (low aluminium); 2697 mg Al/kg (moderate aluminium); or 3617 mg Al/kg (high aluminium), and were subsequently tested for open-field activity, passive avoidance learning, and active avoidance learning with reversal. Tissue aluminium concentrations were determined by graphite atomic absorption spectroscopy.

Thorne et al. (in press) found no significant differences for whole brain comparisons of aluminium levels between the three experimental groups and controls, although the means were in the right

direction: Controls, 6.01 ppm; low aluminium, 5.85 ppm; moderate aluminium, 6.49 ppm; high aluminium, 9.49 ppm. Significant differences were found, however, in hippocampal aluminium content, the high aluminium group having greater aluminium levels in the hippocampus than either the controls or moderate aluminium group. Higher doses of aluminium were associated with decreased activity in the open-field tests, and in addition, elevated brain aluminium was correlated with relatively poor performance on the two avoidance tasks.

Hence, the experiments of Bowdler et al. (1979) and Thorne et al. (in press) both demonstrate that oral administration of aluminium to rats can result in aluminium uptake by the brain. These, and the studies by Hinz and Dufort (1983) and Hinz (1983) have also shown that oral administration is effective in altering a variety of behaviours.

In contrast to the above studies, the results of our experiments were rather disappointing. Although the exact reason for our negative results is not immediately discernible, there are a number of possible explanations for our lack of success in increasing aluminium uptake by the brain and producing an experimental encephalopathy.

(1) The P.V.G. rats may have been too young at the start of the experiments, since they were only six weeks old when they were first placed on the diets. The rats used by Thorne et al. (in press) were placed in their diets at 8 months of age, and tested when they were 9 months old - hence they were considerably older than our animals. It is postulated that infant rats (in comparison to adults) may have a resistance to the neurotoxic effects of aluminium in the same way as that reported in infant rabbits (Wisniewski et al., 1980; Petit et al., 1985). Also, given the late onset of Alzheimer's disease, it is a distinct possibility that old (or adult) rats may be more

sensitive to the effects of aluminium or may retain a higher proportion of aluminium in their diet. For this reason, cohorts of old D.A. rats from Durham are being maintained on a high aluminium, calcium-free diet at the M.R.C. Unit in Newcastle. The rats were 16.5 months old, on average, when they were first placed on the diet, and have currently been on this diet for 2.5 months. They will presently be subjected to behavioural testing of the sort described in this chapter (i.e., nonmatching-to-position) and the brains will be assayed for aluminium content.

(2) Thorne et al. (in press) postulate that pre-treatment exposure to aluminium (i.e., from birth to 8 months of age) could account for the lack of clear differentiation between their groups in terms of brain aluminium content. Contamination from extraneous sources is not, however, a particularly likely explanation as to why there were no group differences between the P.V.G. rats in the present experiment, since extreme care was taken (from weaning) to ensure that the rats could not come into contact with any easily controlled-for source of aluminium, e.g., contaminated food or reward pellets, tap water, aluminium-containing food or water dispensers. The only possible remaining source of aluminium contamination is room dust (Crapper et al., 1976), which would be unlikely to contribute greatly (on its own) to brain aluminium content. Nevertheless, contamination from an as yet unidentified source remains a possible explanation for our negative results in respect of brain aluminium content.

(3) Perhaps P.V.G. rats, as a strain, are particularly resistant to the effects of oral ingestion of aluminium. However, while there might be evidence of a species difference in resistance to the neurotoxic effects of aluminium (Petit, 1985) there is, as yet, no comparable evidence with regard to different strains of just one

species. Nevertheless, it might be interesting to repeat the experiments with D.A. rats (since they at least are able to learn the nonmatching-to-sample task far more quickly than the P.V.G.s) and/or with Long-Evans rats, since the latter have previously been shown to be sensitive to the oral ingestion of aluminium (Thorne et al., in press).

(4) It is possible that 72 mg/kg was not a high enough dose of aluminium. The doses used by Thorne et al. (in press) far exceeded ours (see P.101), but even so, failed to produce large differences between control animals and the highest dose group in either brain aluminium content or behavioural effect, many of the comparisons made being non-significant. Although the dose of 72 mg/kg is reasonable, i.e., not unreasonably high, it is a possibility that the experiments should be repeated following the administration of a diet with a much greater aluminium content. As mentioned in Section 1.4.4., humans may require 10 to 20 times the aluminium content of normal brains to exhibit an encephalopathy (see P.37). The same might also be true of rats - much greater levels of aluminium may need to be administered in order to produce any effect.

(5) The P.V.G. rats used in the experiments at Durham and Newcastle were maintained on the various diets (outlined in 3.1.3.) for a total of 9.5 months, but we were unable to demonstrate either increased levels of aluminium in the brain, or any behavioural effects of aluminium administration, by the end of this period. In contrast, Thorne et al. (in press) kept their rats on diets containing aluminium for only 30 days and yet were able to report both increased uptake of aluminium and behavioural deficits. Hence, while it might be tempting to assume that the duration of exposure to aluminium was too short in the present study, this is perhaps the most unlikely

explanation for our non-significant results.

(6) A combination of any two or more of the above might account for the lack of both elevated brain aluminium and behavioural deficits reported in this chapter.

Having considered various fairly specific reasons which could explain why the experimental regime of the present study failed to find any neurobehavioural effects of aluminium administration we are left with only the wider implications, i.e., that perhaps the ingestion of a diet high in aluminium but lacking in calcium is not an ideal model of aluminium encephalopathy. Perhaps the ALS-PD complex of Guam, the proposed origin of which provided the basis of the experimental methodology of this chapter, is not mediated solely by high environmental levels of aluminium with correspondingly low levels of calcium and magnesium. Perhaps certain individuals within this environment are susceptible to aluminium neurotoxicity on the basis of some other, more primary factor, e.g., a malfunction of the blood-brain barrier due to viral infection, genetic predisposition, or causes unknown. Or perhaps aluminium is merely a secondary marker of various disease states such as the ALS-PD complex, dialysis dementia, or Alzheimer's disease. There are many unanswered questions in this area of research, but in general, the results of previous studies investigating the oral ingestion of aluminium in rats are encouraging (Bowdler et al., 1979; Hinz, 1983; Hinz and Dufort, 1983; Thorne et al., in press), indicating that with perseverance, a rodent model of the learning and memeory deficits typical of Alzheimer's disease, and based on the toxin hypothesis, could be developed.

As previously mentioned, future experiments at Durham and Newcastle will examine the effects of a diet high in aluminium but deficient in calcium in adult rats (as opposed to infants) and the

dose of aluminium will be increased. It is clear that under some circumstances aluminium is a neurotoxic substance, and that we need to know more about its mechanism of action before we can determine with certainty its role in disease, and the conditions in which it may be hazardous.

Aluminium is certainly widespread in the environment. As mentioned in Chapter One (Section 1.4.2.), aluminium has a variety of sources, making it impossible to totally eliminate ingestion of this toxin. For instance, as far as food in concerned, some forms of sodium aluminium phosphate are used to release carbon dioxide gas from baking soda and leaven self-raising flours, cake mixes, pancake batters and frozen dough. At typical usage levels in these products, the final concentration of aluminium in each serving ranges between 5 and 15 mg.. Alkaline aluminium phosphates are used as additives in processed cheeses, where each slice of processed cheese may contain 50 mg. of aluminium (Ellinger, 1972). Aluminium salts such as sodium aluminium sulphate are used as firming agents in pickled vegetables and fruits, and aluminium silicates are commonly used as anticaking agents in salt, non-dairy creamers and other dry, powdered products where they may constitute up to 2% of the final powder mixture.

Aluminium (as aluminium hydroxide) is present in many non-prescription drugs such as the antacids and internal analgesics (buffered aspirin). These drugs are commonly consumed for the relief of minor gastric irritation or arthritic pain, and typical dosage levels can contain between 1 and 7 g. of aluminium per day. Gorsky et al. (1979) suggest that individuals ingesting a 1 g. dose of aluminium per day (as an aluminium hydroxide antacid) absorb 25% of the ingested aluminium into the body. Thus, the chronic consumption of aluminium in the form of drugs can account for a large cumulative

load of dietary aluminium.

Aluminium is also a constituent of phosphate binding gels administered to patients with renal failure, antidiarrhoeal products, and may constitute up to 50% of some haemorrhoidal medications (Lione, 1985).

The aluminium ion is a potent colloid-precipitating agent. With a certain precipitating power, for example 3000 ppm. sodium ion in an aqueous medium, this power can be doubled by adding only 22 ppm. calcium or 5 ppm. aluminium. These enormous changes in colloid stability can occur without noticeable change in specific conductance, a common parameter of water supplies (Riddick, 1968). For this reason, aluminium is being widely used to precipitate colloid turbidity in water purification. Water may, however, naturally contain large quantities of aluminium. It has been noted that aluminium is the most common metal on the surface of the earth, and is found in many clays and minerals. Surface water that has been acidified by acid rainfall dissolves substantial amounts of aluminium from the soil, which then accumulates in rivers, lakes, etc..

Underwood (1977) has suggested that a minimal daily intake of dietary aluminium may be about 22 mg.. In comparison to this value, data presented by Lione (1985) suggests that the use of aluminium cookware can contribute between 9 and 17% of the daily aluminium load in each serving of food. A report by Schlettwein-Gsell and Mommsen-Straub (1973) notes that the amount of aluminium in food cooked in aluminium pans is substantially elevated in acid dishes such as rhubarb or lemon. The use of aluminium pots to brew and reheat acidic beverages such as coffee is also likely to add significantly to daily aluminium intake.

While some restrictions were introduced in 1979 limiting perm-

issible levels of volatile aluminium compounds in air, there are no restrictions for soluble aluminium compounds in food or drugs (American Conference of Governmental Industrial Hygienists, 1979).

In view of the widespread presence and use of aluminium, and the fact that it can be neurotoxic, we need to know more precisely its role in dementia, and (by controlled studies) if reducing aluminium ingestion does serve to reduce or eliminate the aluminium encephalopathy.

CHAPTER FOUR. CONCLUSIONS AND POSSIBLE DIRECTIONS FOR FUTURE RESEARCH.

Alzheimer's disease is characterised by progressive abnormalities of memory, behaviour and cognition. The brains of these patients show neurofibrillary tangles, senile plaques, and loss of specific populations of nerve cells. Neurochemical studies indicate that presynaptic cholinergic markers are markedly reduced in the cerebral cortex and hippocampus of affected individuals. This cholinergic deficiency appears to be due principally to a loss of neurons in the nucleus basalis of Meynert, a basal forebrain cholinergic system which projects directly to the hippocampus and neocortex. Loss of this cell population has also been implicated in other types of dementias showing features in common with Alzheimer's disease, for example, Down's syndrome (Price et al., 1982); and Parkinson's dementia (Betz and Goldstein, 1972). Additionally, the plaques and tangles of Alzheimer's disease have been found to contain aluminium (Perl and Brody, 1982; Edwardson et al., 1986). The above findings have led to proposals that either (1) cholinergic dysfunctions or (2) accumulations of aluminium in the brain are responsible for both the morphological characteristics and behavioural deficits of Alzheimer's disease. In general, it is the cholinergic hypothesis which has given rise to most research into the disease and also to the most attempts at therapy, although it is important to realise that many gaps remain in establishing a primary, causative role of the cholinergic system in Alzheimer's disease.

A major problem in the basic research and drug development for Alzheimer's disease is the lack of adequate animal models that can mimic all aspects of this disease. Generally, an animal model should allow for a more detailed evaluation of the neurochemical, neuropathological, and behavioural sequelae of the primary cholinergic hypofunction suggested in this disorder. Such a model would be

instrumental in testing a wide variety of drugs, to determine whether they correct the cholinergic hypofunction and restore cognitive functions to normal. An animal model would obviously provide information in studies that cannot readily be performed in humans, e.g., an analysis of neurotransmitter levels and metabolism in brain areas in vivo. Finally, an animal model that reproduces the specific neuronal deficits and cognitive dysfunctions of Alzheimer's disease may offer some clue about the underlying deficits of this disease state.

The ideal model would be one that exhibits the same biochemical, behavioural, and histopathological abnormalities as the human disease state. However, there is at present no homologous animal model for Alzheimer's disease, since the aetiology of this disorder is still unknown. Partial success can therefore be achieved at this stage only with isomorphic models; that is, despite parallelism between the model and the human condition, the cause of the condition in the animal may be quite different from the cause in man.

A number of experimental approaches or paradigms have been used that mimic different aspects of this progressive neurological disorder. These include:

(1) Aged rodents and aged monkeys;

(2) Scopolamine/aluminium treated experimental animals;

(3) Excitotoxin lesioned rats or monkeys.

Aged rodents and aged monkeys.

Since Alzheimer's disease is a neurological disorder associated with aging, aged animals have been evaluated in many studies as possible animal models for age-related memory deficits and for Alzheimer's disease (Bartus et al., 1983). Aged rodents have been shown to suffer memory deficits on a variety of tasks (Bartus et al.,

1983), the deficits being conceptually similar to the memory loss reported in humans. Further, drugs known to produce subtle improvements in Alzheimer disease patients also improve the performance of aged rodents on tasks sensitive to age-related loss of memory, and similar findings have been reported for the aged monkey (Bartus et al., 1983). Also, neuritic plaques have been found in aged (23 - 31 years) rhesus monkeys (Struble et al., 1983). Hence both aged rodents and nonhuman primates are important models for testing hypotheses about human memory and cognitive impairments. The aged rodent or monkey model does, however, have several disadvantages. For example, since Alzheimer's disease is a neurologically progressive disease distinct from normal aging (Terry and Davies, 1983; Coyle et al., 1983[1]), aged rodents or monkeys are not ideal animal models for this disorder (Gibson and Peterson, 1983). Moreover, aged rodents mimic to a certain degree the neurochemical changes associated with mormal aging, but not those presynaptic cholinergic dysfunctions (e.g., decreased choline acetyltransferase activity) reported in Alzheimer's disease (Bartus et al., 1982). The high cost of old monkeys and even old rats in drug screening also severely restricts the practical use of these animals for research purposes. Finally, the poor health of aging animals and individual pharmacokinetic variabilities in drug absorption, metabolism and distribution, may sometimes yield statistically inconclusive data.

Scopolamine/aluminium treated experimental animals.

The literature pertaining to the use of scopolamine or aluminium treated experimental animals as potential models for Alzheimer's disease has been reviewed in Chapter One, and will not be repeated here. With regard to our work, the present study has not provided

any conclusive evidence in favour of either the cholinergic or the toxin hypothesis of Alzheimer's disease, although the experiments of Chapter Three (in particular) were only intended to be pilot studies on which to base more detailed research. On the other hand, several positive points can be made with regard to the experimental work of this Thesis:

Principally, it has been shown that rodents are capable of learning a test of object recognition, nonmatching-to-sample. This is important, as such a task provides greater comparability to the non-spatial tasks typically given to assess learning and memory capacities in primates (both human and nonhuman) than the more traditional tests usually given to rodents, e.g., passive/active avoidance. The object recognition task could now provide the behavioural basis for examining the effects on memory of lesions (e.g., to the nbM); or the consequences of combining a diet high in aluminium salts with cholinergic manipulations (see below); or for examining the validity of other animal models of Alzheimer's disease which have recently been proposed (e.g., the AF64A-treated animal - see P.114).

Secondly, the similar effects of scopolamine and physostigmine upon delayed nonmatching-to-sample performance in rats and monkeys (Aigner and Mishkin, 1986) coupled with the effects of these drugs upon recognition in man (Davis and Mohs, 1982) suggests that equivalent manipulations in primates and rodents affect memory (and other, sensory processes) in the same way. The demonstration of related behavioural deficits and pharmacological responses in human and non-human primates and rodents suggests that common etiologies may exist across a range of mammalian species, and supports the idea that rodent models for dementia-related memory loss can indeed be valuable.

In general, the scopolamine treated animal provides a fairly

good model for evaluating the cognitive effects of pharmacological disruption of cholinergic function, though it lacks certain features necessary for studying the pathophysiology of Alzheimer's disease: Alzheimer's disease is a progressive, irreversible neurological disease whereas the effects induced by scopolamine in animals are reversible. Also, in Alzheimer's disease the cholinergic system is irreversibly compromised as a result of presynaptic degeneration of cholinergic neurons, without a significant decrease in postsynaptic muscarinic receptor binding (Vickroy et al., 1985). Therefore scopolamine, which causes mainly reversible blockade of postsynaptic muscarinic receptor binding, mimics only some features of Alzheimer's disease (Ridley et al., 1984).

Aluminium treated experimental animal studies also raise a number of problems. For instance, the morphology of the neurofibrillary tangle induced by aluminium differs significantly from that of human Alzheimer-type tangles (Terry and Davies, 1983; Lee et al., 1984). Also, the brain areas affected by aluminium in animals are significantly different from those areas affected in Alzheimer's disease (Wisniewski et al., 1980). Hence it appears that the induction by aluminium of neurofibrillary tangles in experimental animals, while an intriguing phenomenon definitely worth further exploration, is not a perfectly matching animal model for Alzheimer's disease. Moreover, its relevance to the etiology of this disorder is still an unsettled issue.

Excitotoxin-lesioned rats and monkeys.

A number of excitotoxins, e.g., kainic acid, ibotenic acid, and quinolinic acid, have been used for lesioning the nucleus basalis of Meynert, in order to induce in experimental animals cortical cholin-

ergic lesions similar to those reported in Alzheimer's disease. A wealth of information regarding these types of lesions in the nucleus basalis has been accumulated in the last few years, including neurochemical, histochemical, behavioural, and pharmacological observations (see Section 1.3.4.). The data described in Section 1.3.4. suggests that excitotoxin lesions can be useful in the production of animal models of the cortical cholinergic deficiency in Alzheimer's disease. However, several deficiencies in these models should be pointed out. Specifically, the excitotoxin-induced lesions do not produce the histopathological features characteristic of Alzheimer's disease such as neuritic plaques and neurofibrillary tangles (Terry and Davies, 1983; Davies, 1979). Secondly, since these excitotoxins generally affect all neuronal perikarya regardless of neurotransmitter used, these lesions provide only limited information on the specific role of the cholinergic system in memory and learning deficits (Fisher and Hanin, 1986). Thirdly, excitotoxin-induced lesions of the nucleus basalis mimic only the cortical presynaptic cholinergic deficit of Alzheimer's disease; choline acetyltransferase activity in the hippocampus is not affected in excitotoxin-lesioned animals.

For the reasons outlined above, neither aged animals, nor scopolamine/aluminium treated animals, or excitotoxin lesioned animals, provide totally adequate models on which to base an investigation of Alzheimer's disease.

In spite of this, Fisher and Hanin (1986) have recently proposed the agent AF64A (ethylcholine aziridinium ion) as a potential tool in developing an animal model for Alzheimer's disease, and despite some caveats, data obtained to-date with the AF64A-treated animal are certainly encouraging. Cholinergic neurons possess a high affinity transport mechanism for choline, the HAChT, which is tightly linked

to ACh synthesis and appears to be highly concentrated on cholinergic neurons. AF64A is a chemical analog of choline which is selectively targeted toward the HAChT system because of its structural similarity to choline, but which, at the same time, is cytotoxic at the site of its accumulation in vivo. Hence AF64A induces a persistant central cholinergic hypofunction of presynaptic origin.

Extensive neurochemical, electrophysiological, behavioural and histochemical studies have been conducted to evaluate the AF64A-treated animal as a potential animal model for Alzheimer's disease. Most of these studies have been conducted with rats, and the following is a brief survey of the most pertinent results obtained to-date.

Mantione et al. (1983) injected 2 nmol. AF64A intracerebrally into the dorsal hippocampal area of rats, and found that ACh levels in this tissue were significantly lowered (-57%) within five days. This was paralleled by a significant decrease in the activity of HAChT (-77%) and ChAT (-58%), while choline levels and mAChr were unchanged in the same brain area. Moreover, serotonin uptake and noradrenaline levels were also unchanged. These results have been confirmed by other authors (Arst et al., 1983), though the consequences of AF64A administration into the nbM are equivocal: While some authors have reported decreased AChE staining in the nbM and a decrease in cortical activity of ChAT following administration of AF64A into the nbM (Arbogast and Kazlowski, 1984), others have reported appreciable non-selective damage in both the caudate putamen complex and nucleus basalis (McGurk and Butcher, 1985). The results obtained seem to depend on the dose of AF64A used, with higher doses causing correspondingly more non-selective damage. The optimal dose for administration into the nbM of rats has yet to be established.

Behaviourally, rodents treated intracerebroventricularly (I.C.V.)

with AF64A have been reported to be impaired in retention of a step through passive avoidance task, and in radial arm maze performance (Walsh et al., 1984). Neurochemical changes were also evaluated in the same rats, with the finding that AF64A caused a persistent decrease in ACh levels in selected brain areas - notably the hippocampus and frontal cortex (Walsh et al., 1984). Behvioural observations were also made with AF64A (3 and 5 nmol. I.C.V.) in rats using the one-trial passive avoidance test (Brandeis et al., 1986). Interestingly, the AF64A-induced memory impairment on this task could be reversed by intraperitoneal pretreatment of the animals with physostigmine (0.06 mg/kg), thus indicating the potential use of this animal model in evaluating new drug treatments for Alzheimer's disease (Brandeis et al., 1986).

The studies cited above demonstrate that AF64A mimics, at least qualitatively, the profound reduction of presynaptic cholinergic markers observed in most regions of the forebrain, and particularly the hippocampus and cortex, of Alzheimer disease patients (Terry and Davies, 1983). In addition, the persistent cholinergic deficiencies induced by AF64A in rats are paralleled by a long-term impairment of cognitive function in the affected animals. In Alzheimer's disease a decrease of presynaptic cholinergic markers is paralleled by chronic cognitive dysfunction (Terry and Davies, 1983), particularly anterograde amnesia. A similar behavioural pattern is observed in rats as a result of AF64A-induced cholinotoxicity (Walsh et al., 1984; Brandeis et al., 1986).

The AF64A animal model therefore has both neurochemical and behavioural similarities with Alzheimer's disease, and has two principal advantages over excitotoxin lesion studies. Firstly, AF64A effects the cholinergic system but leaves other neurotrans-

-mitter systems intact, and secondly, AF64A administration decreases ChAT activity in the hippocampus in addition to mimicking the pre-synaptic cholinergic deficit of Alzheimer's disease. The principal deficiency of this model (as in the excitotoxin lesion model) is that it does not mimic the histopathological characteristics (i.e., plaques and tangles) of the disease, or the accumulations of aluminium found in such features. Furthermore, the AF64A work is based on the cholinergic hypothesis that a cholinergic dysfunction (as a result of damage to the nbM) is responsible for the memory loss of Alzheimer's disease. As discussed in Section 2.4., it may be that nbM damage and/or the cholinergic dysfunction cause general dementia rather than a specific memory loss, and that damage to the limbic system is necessary to produce a recent memory impairment. The AF64A studies cited above are in accordance with this possibility:- they show that AF64A administration can cause a general dementia, but it is quest-ionable whether the studies demonstrate that such treatment causes a specific deficit of recent memory.

Nevertheless, from the literature to-date it can be deduced that AF64A, when used at appropriate concentrations, is a unique, selective and specific presynaptic cholinotoxin capable of inducing a long-term or even persistent cholinergic hypofunction in vivo. It could therefore prove to be a very useful model on which to base investigations into the cholinergic hypothesis of Alzheimer's disease, and for developing possible drug treatments for the disorder.

The three principal (and interlinked) aims of developing an animal model of Alzheimer's disease are:

(1) To establish the cause of the disorder;

(2) to establish objective diagnostic criteria for the disease;

(3) to develop an effective treatment for the disease.

Whilst much of the recent research into the neurochemical pathology of Alzheimer's disease has identified the involvement of particular neuronal systems which may ultimately improve the diagnosis and treatment of the disease, the original cause of the disease has not yet been identified. It is still not possible to attribute the cause of this disorder to either a malfunction of the cholinergic neurotransmitter system, or to exposure to an environmental toxin such as aluminium. However, the reader will recall that, in addition to the cholinergic and toxin hypotheses of Alzheimer's disease, which were examined in detail in the present study, four other hypotheses have been proposed to explain the cause of this disease. These are; the viral, vascular, abnormal protein and genetic hypotheses. While these hypotheses have, up to now, stimulated considerably less interest than the cholinergic or toxin hypotheses, they should not be discounted from a consideration of the cause of Alzheimer's disease. In particular, there is recent growing evidence that the disease may be caused by a genetic abnormality.

Preliminary evidence in favour of a genetic component has come from the discovery of certain families with an unusually high incidence of Alzheimer's disease (Sjogren et al., 1952; Heston et al., 1981), the pattern of inheritance being consistent with an autosomal dominant condition; and the higher concordance rate of the disorder in monozygotic twins than in dizygotic twins (Kallman and Sander, 1949). The importance of genetic factors in Alzheimer's disease was also supported by the apparent relationship between this disease and Down's syndrome (Trisomy 21). Individuals over the age of 35 with Down's syndrome almost invariably develop the neuropathologic features of Alzheimer's disease including senile plaques, neurofibrillary

tangles, and neuronal loss in the basal forebrain cholinergic system (Casanova et al., 1985; Ellis et al., 1974; Haberland, 1969). In addition, many of these individuals also develop a dementing syndrome superimposed in the already present mental retardation. Moreover, Heston et al. (1981) demonstrated an increased number of individuals affected with Down's syndrome in the pedigrees of patients with Alzheimer's disease. As mentioned in Section 1.2.5., the link between Down's syndrome and Alzheimer's disease has recently been made much more explicit, as follows:

Study of the proteins comprising the extracellular amyloid filaments deposited in the centres of senile plaques in Alzheimer disease brains has revealed that this disorder appears to involve a particular mutant protein that is processed and polymerised into amyloid filaments. Several laboratories have provided evidence that the principal protein component of plaque amyloid in Alzheimer's disease is a low molecular weight (4 - 5 kDa) hydrophobic protein (termed the β-protein), whose composition shows no close homology to any known proteins (Selkoe et al., 1986; Allsop et al., 1986). Various authors have reported the localisation of the β-protein gene to chromosome 21 (Goldgaber et al., 1987; Tanzi et al., 1987). These important findings provide for the first time a direct explanation for the development of Alzheimer disease histopathological changes, particularly amyloid-bearing neuritic plaques, in virtually all patients with trisomy 21 surviving into adulthood. St. George-Hyslop et al. (1987) identified two tightly linked anonymous DNA markers (designated D21S1/D21S11) on the proximal part of the long arm of chromosome 21, which show linkage to the gene responsible for familial (autosomal dominant) Alzheimer's disease in four families tested to-date. Close linkage between the β-protein gene and D21S1/D21S11 has

also been established (Tanzi et al., 1987). Confirmation of these results in further families would point to DNA alterations in the region of chromosome 21 containing the β-protein gene as the defect responsible for at least some inherited cases of Alzheimer's disease.

The results summarised above suggest that genes on the long arm of chromosome 21, including a gene encoding a major amyloid precursor protein, play an important, perhaps primary role in the pathogenesis of Alzheimer's disease. However, the possibility that other proteins contribute to the amyloid deposits cannot be excluded, since the quantity of the β-protein relative to other constituents in Alzheimer disease amyloid has not yet been determined. It is now important to determine the identification of other proteins associated with the β-protein and their possible role in amyloidogenesis.

Selkoe et al. (1987) suggest that the amyloidogenic proteins associated with neuritic degeneration in Alzheimer disease brains are closely related to those deposited in animal brains during aging. Such a conclusion is consistent with recent molecular studies showing evolutionary conservation of the β-protein gene (Goldgaber et al., 1987; Tanzi et al., 1987). From this point of view, the severe, widespread expression of amyloidosis observed in Alzheimer's disease and Down's syndrome brains could represent an acceleration of an involutional process that occurs normally in the aged mammalian nervous system. Whether amyloid deposition in Alzheimer's disease involves specific mutations or increased gene dosage of amyloid precursor protein found in normal brain aging, and whether such putative changes are the site of the familial Alzheimer disease gene, remains to be seen. Although senile plaque neurites and neuronal cell bodies in old animals do not contain Alzheimer-type PHF, aged non-human primates provide a biochemically relevant model for a

principal feature of Alzheimer's disease:- cerebrovascular amyloidosis and senile plaque formation.

Evidence for linkage of DNA markers on chromosome 21 to both the amyloid β-protein gene and the autosomal dominant Alzheimer gene in some families fuels the hypothesis that an abnormality of DNA encoding the amyloid precursor protein, or perhaps more likely controlling its expression, leads to greater amyloid deposition in patients with Alzheimer's disease and Down's syndrome than in normal aged humans. One of the many unanswered questions is whether the amyloid fibrils themselves can exert a neuritotoxic effect on the brain or whether other molecular changes associated with their formation or local processing are actually responsible for cortical damage.

Although neuritic cytoskeletal changes may account in part for memory failure and other intellectual deficits of Alzheimer disease patients, understanding the cellular and molecular mechanisms of amyloid deposition and neuritic plaque formation appears more likely to lead to an elucidation of the etiology of Alzheimer's disease, thus warranting further research in this area.

Whether indeed one solitary factor - genetic, cholinergic or environmental - is ultimately responsible for Alzheimer's disease, is not yet clear. It is possible that Alzheimer's disease may be caused by a combination of several factors rather than resulting from one discrete factor. Alternatively, the disease may simply have a number of different origins. Different investigators tend to see, or at least to focus on, six different sets of manifestations of Alzheimer's disease, and they collect six different sets of data. They then assume that Alzheimer's disease has its origin in genetics, protein accumulation, infection, a toxin, a neurochemical disturbance, or vascular insufficiency. Not many years ago different investigators

assumed that cancer is caused by substances in food, chemicals, environmental toxins, radiation or sunlight. All the investigators turned out to be correct - hence cancer can be the result of any one of the above individual causes. In time, it may emerge that the same is also true of Alzheimer's disease.

I believe that it is possible that the cause of Alzheimer's disease may lie in the relationship between accumulations of aluminium and decreases in cholineacetyltransferase (CAT), and that this relationship should be investigated in depth (this relationship, in turn, may be directly or indirectly linked to genetic factors). As detailed in Section 1.3., dramatic decreases in CAT activity in the cortex and hippocampus of Alzheimer disease patients have been reported (Davies, 1979), and other studies (Whitehouse et al., 1981; 1982) have indicated that the basal forebrain cholinergic neurons which innervate the cortex and hippocampus undergo a selective degeneration in Alzheimer's disease. The number of neurons in the basal nucleus of Meynert was decreased by 80% compared with age-matched non-demented controls, and some of the surviving neurons in this nucleus had neurofibrillary tangles (Whitehouse et al., 1982). In view of this, and the finding that aluminium accumulates in the nuclei of tangle-bearing neurons, (Perl and Brody, 1980), a number of authors have postulated that there may be a link between CAT reductions and aluminium accumulations in neurofibrillary tangles (King, 1984; Yates, 1979).

In animal studies, aluminium injections produced neurofibrillary tangles and reduced CAT activity in the spinal cord, "presumably due to the presence of tangles in the cholinergic motor neurons (Yates, 1979). Miller and Levine (1974) conducted a cell culture experiment in which murine neuroblastoma cells developed tangles of 10 nm. filaments after six days in a medium to which aluminium had been

added. CAT activity declined by 36% in the aluminium-treated cells, compared with cells that were not exposed to added aluminium and did not develop tangles. In contrast, however, Hetnarski et al. (1980) reported that the activity of CAT in the brain may not be decreased following aluminium injections, and Wisniewski et al. (1980) notes that aluminium cannot be directly responsible for the inhibition of CAT, since 550 ug of Al/g of tissue (wet weight) produces only a 20% inhibition of the enzyme activity. The brains of patients with Alzheimer's disease and animals with aluminium-induced tangles generally contain less than 5 ug Al/g wet weight.

Johnson and Jope (1987) examined the effects of aluminium administration on presynaptic cholinergic markers (CAT activity, high-affinity choline uptake and in vivo ACh and choline levels) in the hippocampus, cortex and striatum of rats. Aluminium was administered orally either as aluminium citrate (each rat consuming 5.6 mmol per day for one month), or as aluminium sulphate (each rat consuming 1.8 mmol per day for one month), or I.C.V. as aluminium citrate (1 umol per rat). CAT activity, high-affinity choline uptake and ACh levels were not significantly affected by any of the above aluminium treatments, though it is possible that the administration of aluminium in higher doses and/or for a longer period of time may alter these markers. However, dietary aluminium citrate reduced choline levels by approximately 30% in all three brain regions examined. Wurtman et al. (1985) suggest that reduced availability of choline may play an important role in the destruction of cholinergic nerve endings. Therefore, this effect of aluminium citrate warrants further investigation.

In general, it appears unlikely that the concentrations of aluminium present in the brains of patients with Alzheimer's dementia

cause the marked reduction (approximately 50%) in neocortical CAT activity via an acute, direct inhibition of enzyme activity. However, it is possible that prolonged exposure of neurons to aluminium may inhibit CAT activity indirectly via a reduction of choline levels.

Johnson and Jope (1987) found that the most significant effect of the oral administration of aluminium sulphate was a 60% increase in cortical cyclic AMP levels, clearly demonstrating that dietary aluminium can alter metabolic processes in the brain. Since it has been suggested that abnormally high levels of cyclic AMP may induce neurofilaments or proteins associated with microtubules, and lead to abnormal structural re-arrangements of proteins (Theurkauf and Vallee, 1983; Johnson and Jope, 1987), further studies are needed to determine if increased cyclic nucleotide levels induced by aluminium play a role in the development of dementia, senile plaques or neurofibrillary tangles.

The search for the cause of Alzheimer's disease will undoubtedly become a high priority for biomedical research. Although some therapeutic strategies can be pursued without a major concern addressed to etiology - for example, the development of neurotransmitter-specific therapies (Whitehouse et al., 1986) - progress toward the cure and prevention of Alzheimer's disease will require a greater understanding of its pathogenesis. In order to approach the goal of understanding the disease mechanism, certain steps need to be taken: (1) Clinical and pathologic descriptions of the disease and any subtypes that exist need to be as complete as possible. Prospective epidemiological studies need to be performed in which diagnosis is confirmed at autopsy. Several different pathologies occur in neurons (and perhaps non-CNS cells) of individuals afflicted with Alzheimer's disease. It is critical to identify which of these cellular alter-

ations are non-specific and which are primarily characteristic of the disorder.

(2) The structure of the proteins or other chemicals which are aggregated as insoluble fibrillary polymers in both amyloid and neurofibrillary tangles need to be described in more detail. Techniques for isolating these components from human brain tissue containing plaques and tangles have been greatly refined in the last few years (Selkoe, 1982). Knowledge of the chemical nature of these key pathological changes in Alzheimer's disease may well provide new insights into the nature and etiology of the disease.

(3) We need to know more about the relation between the cholinergic deficits, degeneration of component neurons in the nbM system, and the variation in the symptomatic manifestations of the disease. The degenerative process may result in the appearance of neuronal-specific markers in the cerebro-spinal fluid or blood (though not for aluminium) or the development of specific immune responses that could serve as direct diagnostic tests for the disorder. Longitudinal studies are required, to correlate the neuropathology and cholinergic deficits with the behavioural symptomatology of the disease, at different stages in the course of this degenerative illness.

(4) The significance of accumulations of aluminium salts in plaques and tangles needs to be determined - whether these accumulations are a cause or a consequence of neuronal loss has yet to be established.

(5) The neuropsychological significance of the relationship between the appearance of multiple plaques and tangles and the development of the mental decline which characterises the disease needs to be explored. Furthermore, whether these pathological changes are a consequence of neuronal loss in key subcortical nuclei supplying axons to the cortex or whether they, in turn, induce subcortical

neuronal loss, has still to be determined.

(6) The role of abnormal genes in Alzheimer's disease needs to be more fully defined. Specifically, the relationship between DNA markers on chromosome 21 to the amyloid β-protein gene and to the autosomal dominant Alzheimer gene, and the etiological implications of this relationship, needs further exploration.

(7) Comprehensive theories of the pathophysiology of the disease need to be constructed. Animal models should be developed to test aspects of these theories. Such theories need to consider how genes, the cholinergic system and the environment may interact to produce the disease, why certain populations of neurons are selectively affected, and why the disease occurs in the elderly.

It is clear that further exploration of the possible causes of Alzheimer's disease will lead not only to understanding neurologic diseases but to fundamental advances in our understanding of neurobiology. However, for the time being we are left with the fact that the majority of patients are victims of a primary degenerative process. It is difficult to foresee rational therapy for these patients, particularly the more elderly ones. But unless some remediable cause is found, the professions have to face the inescapable problems of very large numbers of dements for whom the only present management is care, support and protection. With the increasing number of dements, the social and governmental implications are obvious. The present provisions in long-term psychiatric and geriatric departments are totally inadequate for patients, families, and indeed the clinicians involved. It seems likely that international governments will be forced by sheer weight of numbers to confront this important issue and to consider new projects for the proper care and protection of these subjects outside the conventional

hospital environment.

Whatever the future holds it can be confidently stated that the last decade of research has opened up new vistas in our understanding of brain biochemistry in Alzheimer's disease. It is to be hoped that progress within the next decade will lead directly towards the ultimate goal of relieving the suffering imposed on individuals and society by the disease.

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