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A STUDY OF FUNCTIONAL ABILITIES IN TRANSGENIC MOUSE MODELS OF ALZHEIMER'S DISEASE

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A STUDY OF FUNCTIONAL ABILITIES IN TRANSGENIC MOUSE MODELS OF ALZHEIMER'S DISEASE

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Abstract

The appropriateness of three animal models in mimicking pathophysiology and symptoms of Alzheimer's disease was assessed. Behavioural tasks provided measures of cognitive function, affective behaviour and locomotor ability. Adult and aged transgenic mice, expressing the C104 fragment of the human ß-Amyloid Precursor Protein, displayed increased anxiety by seven months of age in the Thatcher-Britton Novelty Conflict paradigm, memory impairments in the Forced Alternation T-Maze, the Porsolt Forced Swim test and the Recognition test, and decreased hippocampal acetylcholinesterase staining. BIBN99, a muscarinic M2-antagonist that increases release of acetylcholine, failed to ameliorate these changes. Apolipoprotein E deficient mice showed impairments of spatial memory in the Morris swim maze, but were unaffected by the administration of tacrine, an acetylcholinesterase inhibitor. Segmental trisomy 16 mice, a model of Down Syndrome and Alzheimer's disease, showed increases in hippocampal choline acetyl-transferase activity and mild memory impairments in the swim maze. BIBN99 had no effect on this deficit. These findings suggest that each of these models is beneficial in its own right for studying the functional deficits associated with Alzheimer's disease.

Résumé

L'exactitude avec laquelle trois modèles animal imitent la pathophysiologie et les symptômes de la démence de type Alzheimer a été evaluée. Des tâches comportementales ont fourni des measures de cognition, de comportement affectif et de locomotion. A partir de l'âge de sept mois, des souris mutantes adultes et âgées possédant le fragmant C104 de la protéine beta-amyloïde humaine ont démentrées une augmentation d'anxiété dans un paradigme de conflit dû à la nouveauté. A la fois, ces animaux ont démontrées des problèmes de mémoire dans un dédale d'alternation forcé et dans les tâches de nage forcé et de reconnaissance. De plus, les résultats ont révélés une réduction dans la concentration de l'acétylcholinesterase au niveau del'hippocampe accompagnant ces changements comportementaux, mais l'antagoniste de récepteur muscarinique M2, BIBN99, qui augmente le relâchement de l'acétylcholine, n'a pas amélioré le comportement des animaux. Les souris déficiantes pour la apolipoprotéin E ont montrées un déficit d'apprentissage spatial dans un labyrinthe aquatique, et l'administration de la tacrine, un inhibiteur de l'acétylcholinesterase, a eu aucun effect sur leur performance dans le dédale d'eau. Les souris trisomie 16 segmentales, un modèle de la trisomie 21 et de la démence de type Alzheimer, ont démontrées une augmentation dans l'activité de la choline acétyltransferase au niveau de l'hippocampe et un léger déficit sélectif d'apprentissage spatial. Ces résultats suggerent que chacun de ces modèles est utile pour étudier les déficits fontionnels associés à la démence de type Alzheimer.

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I. INTRODUCTION

I.A. Dementia and Alzheimer's Disease

I.A.1. Aging in Humans

There is undoubtedly great variability in human aging. Neuro-degenerative disorders aside, many elderly people develop a senescence, or mild alterations in their mental life (Cummings and Benson, 1992). Some of this decline may be artificial, in as much as it is caused by extracerebral factors of a physical or social nature (Rowe and Kahn, 1987). Senescence is an elusive term. The scientific literature on disease and aging is fraught with inconsistency, especially in defining what the demographics of an aged control population should be. As much as the scientific community is trying to determine what disposes a person to Alzheimer's disease (AD), successful aging is also possible (Rowe and Kahn, 1987), living free of intellectual deficits, and occurs in an as yet undetermined percentage of the population over 65.

Benign senescent forgetfulness more properly characterizes the slowed memory retrieval of aged subjects. While actual learning and retrieval are comparable to younger subjects, latencies to do so are longer (Craik, 1977) and tend to require semantic clues (Smith, 1977). Recall tends to be impaired more than recognition (Erber, 1974). Motor slowing (Welford, 1965; Birren, 1974), slowing of sensory processing (Birren, 1974; Salthaus, 1976), and slowing of central processing (Welford, 1965; Fozard, 1976) all appear to occur with advanced age. Language ability and verbal intelligence are well maintained throughout life, barring serious injury (Dopplet et al, 1955; Riegel, 1968), but difficulty in naming objects is a frequent complaint of elderly people, as is difficulty in finding words (Goodlass, 1980; Albert, 1981). Although many reasons have been posited for this, linguistic ability is not severely hampered with age, and some aspects of narration actually demonstrate increasing complexity (Obler, 1979).

Over 80% of the elderly have some chronic condition, such as arthritis, hypertension, heart problems, hearing and visual impairments, and diabetes (Jarvik and Perl, 1981). Despite this, relative health has increased over the years due to better medical and social support (Palmone, 1986), and chronic conditions do not necessarily

interfere with daily functioning. Muscle mass decreases (Grimby and Saltin, 1983), and postural sway and impaired balance occur often (Overstell et al, 1977). Changes in gait include flexion of the upper body, decreased arm swing, and short steps (Galasko et al, 1990). These changes might be related to the involvement of the extrapyramidal system (Barbeau, 1973) since mild loss of neurons in the substantia nigra occurs (Carlsson, 1983). In non-demented elderly subjects, mild neuronal loss in the nucleus basalis also occurs with impaired release of acetylcholine (ACh) and a decrease in choline acetyltransferase (ChAT) activity in the cortex and hippocampus (Muller et al, 1991).

Many previous attempts at defining age-related functional decline were compromised by problems inherent to cross-sectional studies (Schaie et al, 1973). One possible limitation of aging studies, which may indicate a greater proportion of centenarians with AD, is their lack of very old individuals, ages 90 to 99 (Perls, 1995). Men in their eighties with poor cognitive functioning have 20% higher mortality than comparably-aged women, resulting in a select group of nonagenarian men with higher cognitive functioning than men in their eighties and women in their nineties (Perls et al, 1993). Some reports indicate that people in their late nineties are healthier than those in their eighties and early nineties (Perls, 1993). Preliminary studies have indicated that the risk of AD decreases after age 90 (Perls, 1995; Lautenschlager et al, 1996), which is contrary to how earlier studies extrapolated a steady increase in the incidence of AD with age (Rocca et al, 1986; Evans et al, 1989). This effect is more pronounced in men than women, since the men susceptible to AD are believed to have died, leaving the healthier ones living. In some aspects it appears that age-related mortality peaks and has less effect at greater ages.

Cluster analysis of neuropsychological features of AD shows that the clinical features are not exaggerations of changes that occur with normal aging (Leibovici et al, 1995). Another indication that cognitive decline may not be an inevitable concomitant of aging, but may have more to do with predisposing factors, comes from the Nun Study. Snowdon et al (1996) have recently provided information from their study of a group of Catholic nuns, a highly homogeneous cohort. Poor cognitive

functioning (as measured by linguistic ability) in early twenties appeared to have been strongly related to cognitive ability and Alzheimer's disease more than sixty years later; the nuns with low linguistic ability had increased densities of neurofibrillary tangles (NFT) in hippocampal and cortical tissue. Snowdon et al (1996) also argue that this relation seems to have little to do with lifestyle or environmental factors.

I.A.2. Epidemiology

In Canada, dementia occurs with a prevalence of 80 per 1000 of those over the age of 65 years, and 345 per 1000 over the age of 85 years (Canadian Study of Health and Aging, 1994). Alzheimer's disease accounts for 64% of all dementia cases, and 350 000 people are estimated to have AD by the year 2001. Some estimate that social and medical care costs are 5 to 9 billion dollars annually in Canadian currency (Mohr et al, 1995).

The incidence and prevalence of AD increase dramatically with age (Rocca et al, 1986; Evans et al, 1989). Women appear to have a higher risk of developing AD than men, even after adjusting for the differential survival rates, but the age at onset is the same in both sexes (van Duijn et al, 1993; Rao et al, 1994). In Canada, female patients with AD outnumber males by 2 to 1 (Canadian Study of Health and Aging, 1994), and AD is diagnosed more often in women than men (Rocca et al, 1986).

Low levels of education and occupational attainment have been occasionally associated with an increased occurrence and onset of AD (Zhang et al, 1990; Stern et al, 1994), but other reports have failed to find this association (Beard et al, 1992). In the United States (U.S.), increasing years of education predicts an earlier age of onset, while outside of the U.S. the opposite occurred. No explanation has as yet been offered for this contradiction (Rao et al, 1995).

Early reports identified the occurrence of Down Syndrome in relations, advanced age of birth mother, and head injuries as risk factors (Rocca et al, 1986). Smoking has occasionally been implicated as a preventative measure for the onset of AD (Graves et al, 1991; Brenner et al, 1993; Lee, 1994), which would be in keeping with findings of an increase in hippocampal nicotinic binding sites and increases in

ChAT activity in smokers (Benwell et al, 1988; Perry et al, 1996), yet many reports have not found support for the association (Hebert et al, 1992; Riggs, 1993; Letemeur et al, 1994; Forster et al, 1995). In patients with a low genetic susceptibility to AD, heavy smoking and alcoholic drinking were associated with earlier onset, while in patients with high genetic susceptibility that were not heavy smokers, alcoholic beverages tended to delay onset by more than three years (Rao et al, 1995).

Many genetic and non-genetic factors likely interact to influence the age at onset of AD (Rao et al, 1995). The lifetime risk of AD in first degree relatives is 39% (Lautenschlager et al, 1996). If both parents have AD, by age 80 their siblings have a 54% cumulative risk of AD, which is nearly five times greater than siblings of normal parents (Lautenschlager et al, 1996). Earlier onset of the disorder is associated with a history of clinical depression and the absence of arthritis and high blood pressure (Rao et al, 1995). This is consistent with previous findings that indicated the use of anti-inflammatory drugs is associated with a later onset of AD and better cognitive performance (Breitner et al, 1994; Rich et al, 1995).

I.A.3. Genetics

The discovery of genetic loci associated with AD has important implications for future disease management. This not only applies to widespread screening and early detection, but also in the development of novel treatments (Scott, 1993).

Rao et al (1994), using segregation analysis, demonstrated that AD cannot be explained by a single Mendelian transmission model, and sporadic occurrence was also rejected as a hypothesis. Although the differences between early and late onset AD are not always clear, Rao et al (1994) believe there is heterogeneity between the early and late onset AD, and within late onset AD.

The first genetic links to AD were found to be associated with mutations in the ß-amyloid precursor protein (ßAPP) of chromosome 21. This locus was eventually shown to cause early onset AD in 2-3% of all familial cases (Goate et al, 1991). At least five missense mutations within or in the immediate vicinity of the amyloid ß-peptide (Aß) region of the ßAPP gene have been identified in these families with an

autosomal dominant pattern of inheritance (Chartier-Harlin et al, 1991; Goate et al, 1991; Murrell et al, 1991; Hendriks et al, 1992; Mullan et al, 1992).

The apolipoprotein E (apoE) gene of chromosome 19 was implicated as a risk factor for AD in sporadic (Poirier et al, 1993; Saunders et al, 1993), late onset familial (Corder et al, 1993), and early onset familial (Okuizumi et al, 1994; van Duijn et al, 1994) forms of AD. ApoE is believed to be involved in membrane synthesis and remyelination by mobilizing and transporting cholesterol after central nervous system (CNS) or peripheral nervous system (PNS) injury (Mahley et al, 1988; Poirier et al, 1996). ApoE alleles appear to modify the risk of developing AD by lowering the age at onset (Corder et al, 1993). The association between the $\epsilon 4$ allele and AD was more pronounced in women, and $\epsilon 4$ tended to be associated with an earlier onset of AD than did the $\epsilon 2$ and $\epsilon 3$ alleles (Poirier et al, 1993). While the $\epsilon 4$ allele is over-represented in 57% of AD cases as opposed to only 7% of controls, actual predictive ability of apoE genotyping is limited (Nalbantoglu et al, 1994). As many as 43% of AD cases do not carry an $\epsilon 4$ allele. So on an individual basis, apoE is not predictive of age at onset (van Gool et al, 1994; ACMG/ASHG, 1995). For those over the age of 65 years, being heterozygous for $\epsilon 4$ increases the lifetime risk of developing AD to 29%, while those without any copies of the allele have a lifetime risk of 9% (Seshadri et al, 1995).

ß-amyloid precursor protein mutations and apoE genotype may interact to form variable progressions of the disease. In a report by St. George-Hyslop (1994), families that carry the 717 mutation of ßAPP tended to have AD onset by 47.6 ± 3.0 years, yet all were heterozygous carriers of $\epsilon 4$; one exception was a $\epsilon 2/3$ carrier that also had the ßAPP mutation and was still cognitively intact at 54 years of age, well above the mean onset for the family. Families with the double 670/671 ßAPP mutation show the protective effect of $\epsilon 2$ and the deleterious effect of $\epsilon 4$ on age of onset (Alzheimer's Disease Collaborative Group, 1993).

The third genetic locus (AD3) that confers inherited susceptibility to Alzheimer's disease was recently cloned by Sherrington et al (1995). AD3 had previously been linked to chromosome 14q24.3 and may account for up to 70% of

cases of early-onset autosomal dominant AD (Schellenberg et al, 1992a; St. George-Hyslop et al, 1992; Van Broeckhoven et al, 1992), the most aggressive form of the disorder (onset 30 to 60 years). The AD3 product, termed S182, has been identified as a seven membrane spanning protein, resembling the integral membrane protein, spe-4 (Sherrington et al, 1995). Five missense mutations occur in the conserved domains of this gene and co-segregate with early-onset AD. During spermatogenesis, the spe-4 protein appears to be involved in the formation and stabilization of the fibrous body-membrane organelle complex, which in turn may be involved in the transport and storage of soluble and membrane-bound polypeptides (L'Hernault et al, 1992). Similar functions for S182 may be in the aberrant production of membrane-bound proteins such as ßAPP and abnormal interactions with cytoskeletal proteins such as Tau (Sherrington et al, 1995). Interactions of AD3 with apoE appear to be negligible (Van Broeckhoven et al, 1994; Locke et al, 1995).

AD patients of the Volga German kindreds (Cook et al, 1979; Bird et al, 1988) have been excluded of other known AD genetic loci (Schellenberg et al, 1988, 1991, 1992a, 1992b, 1993). This autosomal dominant early-onset form of AD (ranging from 50 to 70 years), common to the Volga Germans, has been linked to a gene locus on chromosome 1 and may account for the remaining 20% of early onset familial cases (Levy-Lahad et al, 1995a). A 112-base pair allele of D1S479 co-segregated with AD in many of the families, and results from a missense mutation of isoleucine substituting for asparagine. This gene, the second seven transmembrane gene associated with AD (STM2), is homologous to the chromosome 14 gene, S182 (Levy-Lahad et al, 1995b), and so is proposed to have similar functions.

I.A.4. Amyloid Theory

Amyloid ß-peptide and its larger precursor, the ßAPP, have been implicated in the pathophysiology of AD by two broad lines of evidence: one, that the ßAPP gene is located on chromosome 21 and is overexpressed in Down Syndrome (DS), and two, that diffuse amyloid deposits may precede all other lesions in DS and AD. Direct evidence was the discovery of at least five missense mutations of the ßAPP gene in

families with an autosomal dominant, early onset type of AD (Chartier-Harlin et al, 1991; Goate et al, 1991; Murrell et al, 1991; Hendriks et al, 1992; Mullan et al, 1992). The amyloid cascade theory of AD (Hardy et al, 1992) implicates abnormal processing of ßAPP as the first step toward increased Aß secretion and deposition in plaques, followed by NFT formation and neuronal death.

ßAPP is a large protein with a membrane spanning region near its carboxyl terminus (Kang et al, 1987). The 39- to 43-residue Aß is located 28 residues N-terminal to the transmembrane domain of the ßAPP, and extends 11-15 residues within. Several alternatively spliced isoforms of ßAPP exist (Joachim et al, 1992). The ßAPP695 transcript is highly expressed in neurons of the brain (Shivers et al, 1988), and ßAPP751 is widely expressed in several tissues including the brain (Tanzi et al, 1988). While the ßAPP751 (Ponte et al, 1988; Tanzi et al, 1988) and ßAPP770 (Kitaguchi et al, 1988) isoforms contain a sequence of unknown function that is approximately 50% homologous to the Kunitz family of serine protease inhibitors, the ßAPP695 isoform lacks this sequence. The 695 isoform of ßAPP is reduced in AD cortical tissue by as much as 65%, which results in a two fold increase in the neural ratio of 751 to 695 (Johnson et al, 1988). This shift in ratio might also support the theory of impaired proteolysis of ßAPP leading to abnormal Aß processing (Tanzi et al, 1988). The higher ßAPP 751/695 ratio is correlated with plaque density (Johnson et al, 1988, 1990; Neve et al, 1988), and while the ßAPP751-770 isoforms tend to dominate in Alzheimer's disease, the relative changes of 751-770 over 695 isoforms also similarly develop in aged controls (Koo et al, 1990)

ßAPP can undergo proteolytic cleavage by an unknown protease, called α-secretase (Sisodia et al, 1990), dividing the Aß sequence, and releasing the soluble, extracellular domain termed APP, (Weidemann et al, 1989). Other pathways were shown to exist that did not destroy the Aß sequence (Golde et al, 1992), and Haass et al (1992) demonstrated that ßAPP could be re-internalized and targeted to an endosomal-lysosomal pathway. Secretory sequences, however, were identified whose cleavage possibly allowed 28 residues or more of Aß to exist (Anderson et al, 1992; Seubert et al, 1992). It has been suggested that cells may routinely release a 40

residue Aß, which appears to be entirely soluble, as opposed to its insoluble aggregated, plaque forming condition (Haass et al, 1992; Seubert et al, 1992). As of yet, it is still unclear by which pathways Aß is processed, but Haass et al (1993) have suggested that Golgi modifications and acidic compartmentalization are necessary.

The sequence of ßAPP indicates that it may be a cell-surface protein, although no specific ligands have yet been identified (Kang et al, 1987), and it has been shown to promote hippocampal neurite outgrowth independent of the soluble effects of ßAPP or Aß (Qiu et al, 1995). ßAPP can, at least, associate *in vitro* with the GTP-binding protein G_o (Nishimoto et al, 1993). The overexpression of different forms of ßAPP leads to ***in vitro*** degeneration of neurons derived from a mouse embryonic cell line (Yoshikawa et al, 1992). Some reports indicate that Aß and C-100 terminal fragments of ßAPP are neurotoxic (Yanker, 1990a, 1990b; Kowall, 1991), while Aß has also been shown to have some neurite growth promoting activity in culture (Whitson et al, 1989; Koo et al, 1993). Aß is neurotrophic for undifferentiated hippocampal neurons and, at higher concentrations, neurotoxic to differentiated neurons (Yanker et al, 1990b).

I.A.5. Pathology

Brain atrophy is common in AD, especially atrophy of temporo-parietal and frontal regions (Terry et al, 1981), which results in marked reductions in brain weight and enlarged ventricles. Areas of early neuronal loss are the entorhinal cortex, subiculum, and CA1 hippocampal area (Hyman et al, 1984). While the dentate granule area does appear to have some rudimentary reinnervation abilities in normal brain and in a small proportion of AD brain (Geddes et al, 1985), the majority of AD subjects show marked reductions in hippocampal ChAT immunoreactivity (Ransmayer et al, 1989), proliferating hippocampal dendrites (Flood et al, 1986), dendritic spine density, and spine length (de Ruiter et al, 1987).

Pre-amyloid or diffuse plaques are amorphous and less dense, without surrounding neuritic components (Tagliavini et al, 1988; Yamaguchi et al, 1988). While they form the majority of amyloid staining plaques in AD, these deposits can

occur in cognitively normal individuals, but are not an inevitable side-effect of advanced age (Davies et al, 1988). Diffuse amyloid plaques have a different pattern of distribution than do neuritic plaques or NFT (Braak et al, 1989). Classic, neuritic plaques consist of a core of amyloid filaments surrounded by dystrophic axon terminals and dendrites, and possibly activated microglial cells and reactive fibrous astrocytes (Wisniewski et al, 1989). These plaques can be found in hippocampal, amygdalar, entorhinal, striatal, thalamic and association cerebral cortical tissue. They are areas of tissue degeneration accumulating remnants of neuronal processes and granular deposits. The outer plaque zone is comprised largely of degenerating neuritic processes, the middle area involves swollen axons and dendrites, and the inner core is formed of amyloid (Kidd, 1964; Terry, 1964). Decrease in synapse density occurs within the plaques (Krigman et al, 1965). Amyloid pathology was used early-on as a diagnostic tool in the postmortem AD brain. Khachaturian (1985) detailed that in postmortem tissue, AD pathology in patients under 50 years of age was defined as 2-5 plaques per field (x200 magnification), greater than 8 plaques for those under 65 years, greater than 10 plaques per field for those less than 75 years, and greater than 15 plaques per field for those older than 75 years.

Abnormally phosphorylated tau protein appears to be a component of the paired helical filaments that contribute to the formation of NFT (Grundke-Iqbal et al, 1986). The tangles can appear without amyloid deposits (Tabaton et al, 1989). NFT gradually fill the cell body and these neurons eventually die (Sumpter et al, 1986), accounting for some of the neuronal loss in AD (Bondareff et al, 1989; Mann, 1991). Neurofibrillary tangles are found in brains of cognitively normal individuals, independent of dementia status, and tend to develop in the anterior olfactory nucleus and the parahippocampal gyrus; the density of tangles increases in these same areas in older nondemented cases (aged 73 to 89 years) with additional formations in the hippocampal field CA1 (Price et al, 1991). Cases with mild dementia have a similar distribution but a much greater density of tangles, and only in severely demented cases are tangles located in the neocortex (Price et al, 1991). Neurofibrillary tangles also accumulate in the cytoplasm of pyramidal neurons in the neocortex, hippocampus, and

amygdala, and in the neurons of the raphe nuclei and locus ceruleus (Tomlinson, 1992). These results indicated that tangles appear normally with age, but are rare in the neocortex except with dementia (Price et al, 1991).

Diffuse amyloid deposits appear to precede the development of mature plaques. Neural degeneration, neuritic plaques, and NFTs are thought to be late-stage events of AD (see Higgins, 1994). Within the hippocampus, the CA1 subfield tends to accumulate the most amyloid deposits, neuritic plaques, and NFT (Braak and Braak, 1991). Diffuse deposits are common throughout the thalamic nuclei, while neuritic plaques and NFT only occasionally form there (Braak and Braak, 1991). Diffuse amyloid plaques and neuritic plaques show highly variable location patterns (Braak and Braak, 1991); however, NFT and neuropil threads tend to affect transentorhinal areas first, followed by involvement of limbic areas, such as hippocampus, thalamic nuclei, amygdala, and basal forebrain, and finally an increased involvement of the cerebral association area (Braak and Braak, 1991). Primary cortical areas are initially spared.

I.A.6. Clinical Phenotype

Dementia of the Alzheimer's type causes severe and progressive intellectual deterioration without exception in all patients (see Cummings and Benson, 1992). Although there is variability in the symptomatology throughout the disorder (Katzman et al, 1986; Rubin et al, 1987a; Petry et al, 1989), there appears to be no remission and there are few periods of stability. Changes of personality often occur early in the disease, and may even predate the cognitive impairments (Petry et al, 1988, 1989); the primacy of these changes and the lack of correlation with dementia severity on the Mini Mental State indicate that the changes cannot be entirely attributed to declining intellectual ability (Petry et al, 1988). While individual personality traits may, along the course of AD, show increased deterioration, stability, or remission, the overall pattern is one of progressive deterioration in social functioning and loss of integrity in personality (Petry et al, 1989).

The most common personality change is that of disengagement, in which the person has a loss of initiative and enthusiasm, a decrease in energy, and emotional blunting (Rubin et al, 1987a; Petry et al, 1988). Anxiety, agitation, and self-centred behaviours are reported in many patients suffering from AD (Rubin et al, 1987a, 1987b; Petry et al, 1988; Mendez et al, 1990), occurring early in the disorder and increasing in later stages. Passivity and agitation often manifest concurrently (Rubin, 1987a).

The classic symptoms of AD, however, are the impairments of cognition. AD patients have learning deficits, poor recall of recent and remote memory, and often poor abstraction and judgement (Cummings and Benson, 1986). Learning impairments are often the earliest symptoms noticed. The impairment lies in an inability to properly encode information (Miller, 1971, 1972). Several different mnemonic tasks do not appear to improve recall in AD patients at all (Butters, 1983; Davis and Mumford, 1984; Grober, 1988). In the late stages, there is a near complete loss of intellectual function. The ability to actually perform certain skills, however, is relatively spared and there is a clear dissociation of the different subsystems of memory effected by AD (Eslinger and Damasio, 1986).

The AD patients often find themselves lost and disoriented in familiar environments and this may be related to a compromise of visuospatial abilities (Sim et al, 1966). Patients have difficulty copying elementary and three-dimensional figures (Henderson et al, 1989), and they do poorly on subtests of the Wechsler Adult Intelligence Scales that rely on visuospatial skills (Perez, 1975).

Alzheimer's disease patients also suffer from impairments of naming, comprehension (Knesevich et al, 1986; Shuttleworth et al, 1988; Williams et al, 1989), and descriptive writing (Neils et al, 1989). Language impairments resemble a transcortical sensory aphasia (Cummings et al, 1985), in as much as they have fluent paraphasic output, impaired auditory comprehension, preserved ability to repeat, difficulty in completing common expressions, and poor reading comprehension. Dysarthria often develops in late stages of AD with further impairments resembling Wernicke's aphasia (Cummings et al, 1985).

In the absence of histopathological data collected postmortem, the provisional diagnosis of probable Alzheimer's disease relies on clinical neurological and neuropsychological testing. Some reports indicate that strict clinical testing can be sensitive to the earliest stages of the disease (Morris et al, 1988), but upon postmortem assessment AD is correctly diagnosed in upwards of only 81 to 88% of cases (Tierney et al, 1988; Burns et al, 1990; Becker et al, 1994; Gearing et al, 1995; Klatka et al, 1996). The National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) established several clinical criteria for the diagnosis of AD (McKhann et al, 1984). Probable AD relies on an established dementia with deficits of cognition and worsening memory, but no disturbances of consciousness and an absence of other systemic illnesses that might account for the dementia. Possible AD requires a second systemic disorder that is sufficient to produce dementia but is not considered the primary cause. Definite AD is a postmortem diagnosis upon histopathologic evidence.

There are many different dementias that can be misdiagnosed as, and confused for, AD. Diagnostic inventories have been produced to minimize these errors and operationalize the clinical criteria. Diagnosis requires the presence of intellectual impairments in patients lacking indications of tumour, stroke, or trauma, and having normal levels of B_{12}, folate, and thyroid functioning (Cummings and Benson, 1986). The patients must also exhibit nonspecific findings on electroencephalogram and computer axial tomography (CAT) scans of the head. Complete medical histories are required to distinguish Alzheimer's from several of the other dementias. The Dementia of the Alzheimer Type Inventory by Cummings and Benson (1986) requires the following: the presence of poor memory and cued recall, poor visuospatial abilities, impaired abstraction and mathematical abilities, apathy with or without irritability, fluent aphasia and decreased comprehension; and the absence of motor speech difficulties, psychomotor speed reductions, abnormal posture, abnormal gait, tremors, akinesia, rigidity and chorea. These criteria are, however, least useful in the early stages of the disease, when only mild deficits are evident, and in the late stages of the disease, when most dementia patients suffer from profound deterioration of

intellectual and motor abilities (Cummings and Benson, 1986).

Correlations of neuropathology and severity of antemortem dementia indicate that in the midfrontal cortex, synapse density was more strongly correlated than NFT counts, but that in the nucleus basalis of Meynert (nbM), the NFT counts have a higher correlation with dementia severity than synapse density (Samuel et al, 1994). The midfrontal synapse density was the strongest predictor of measures of higher cortical functions, and NFT counts in the nbM were the strongest predictors of memory ability (Samuel et al, 1994).

I.A.7. Down's Syndrome

Down's Syndrome is the most prominent cause of mental retardation, usually occurring because of trisomy for chromosome 21 as a result of a nondisjunction during meiosis. The majority of Down's children are moderately to severely retarded with IQ scores below 50 (Wong and Ciaranello, 1987). Common features of DS fall into three categories (see Epstein, 1986): physical, including dwarfed stature, small rounded ears, strabismus, oblique palpebral fissures, brachycephaly, small cerebellum and brainstem, hyperflexibility, flat nasal bridge, short neck, epicanthal folds, protruding tongue, folded ear, congenital heart disease, irregular positioning of ganglion cells in layer III of the cortex, pituitary abnormalities, hypogonadism and gastrointestinal malformations; functional, including mental subnormality, hypotonia, AD, altered immune response, increased frequency of leukaemia, and growth retardation; and cellular, including sensitivity to interferon, exaggerated fibroblast cAMP response to ß-adrenergic agonists, increased adhesiveness of fetal lung and heart fibroblasts, and sensitivity to viral transformation. It is still not possible to explain the morphological phenotype as an effect of a single gene or several genes. While it is likely that the overproduction of normal genes on chromosome 21 distort the balance of certain proteins whose role in biochemical pathways is to regulate proper development and function of organs (Epstein, 1988), the process cannot simply be stated as a non-specific chromosomal imbalance.

Any mechanism proposed to explain how trisomy for chromosome 21 causes

mental retardation must begin with extra copies of specific genes resulting in a 1.5 fold increase in product, and relate that increase to the initiation of a morphogenetic or functional abnormality (Epstein, 1986). Epstein (1986) has suggested that a small 50% increase in enzyme activity due to the trisomic state is unlikely to result in significant changes in phenotype. Receptors, cell surface adhesion molecules, growth factors, and regulatory molecules are more likely to effect significant changes. Examples exist where a 1.5 fold increase in the expression of a gene product can produce a several fold increase in the product's effects (Hoffman et al, 1983; Wright et al, 1984).

Perhaps 10-20 genes from the DS specific segment on chromosome 21 are responsible for the DS phenotype (Anneren and Edman, 1993). Korenberg et al (1990), having excluded ßAPP and superoxide dismutase type-1 (SOD-1), further defined the DS region as 21q22.2 and 21q22.3, with genes from each likely accounting for DS facial features, the congenital heart defect, dermatoglyphic changes, and components of mental retardation. Superoxide dismutase, located outside this DS-specific narrow band on chromosome 21, is not necessary to account for the DS phenotype (Korenberg et al, 1990), but increased levels of SOD-1 activity are associated with AD (Zemlan et al, 1989; Perrin et al, 1990; Vrakami et al, 1995), and so SOD-1 along with ßAPP may have a role in the development of AD in DS.

Amyloid deposition, in the form of neuritic plaques, occurs in DS patients several decades sooner than in a normal aging population, often by the age of 35 or 40, and it precedes NFT formation (Wisniewski et al, 1985; Rumble et al, 1989). Dementia is clinically detected several times more frequently in the DS population than in a non-DS population (Wisniewski et al, 1985). In a study of institutionalized DS patients (Lai et al, 1989), dementia occurred in 8% of those under 50 years of age, in 55% of those 50-59, and in 75% of those over 60. Common symptoms of the DS dementia are personality changes, apathy and loss of motivation, a decrease in activity of daily living skills, gait deterioration, and sphincteric incontinence (Lott et al, 1982).

CAT scans indicate progressive cortical atrophy. There is still much debate as to the inevitability of dementia in DS, possibly because of the difficulties in diagnosing AD with a severe pre-existing intellectual subnormality. Since only some older people with DS have a clinically identified dementia syndrome (Schapiro et al, 1989; Caltagirone et al, 1990; Johanson et al, 1991), apoE might also play a modulatory role in DS. Down's Syndrome patients with one copy of $\epsilon 4$ had earlier ages of death than those without, and $\epsilon 2$ disposed patients to greater longevity and higher cognitive functioning (Royston et al, 1996).

ß-amyloid precursor protein may be responsible for the AD phenotype of DS patients. Both increased gene dosage and overexpression of ßAPP may underlie the development of the diffuse plaques, which appear to be unassociated with dystrophic neurites, gliosis, or NFT, but are common in DS patients as early as their twenties (Giaccone et al, 1989).

I.B. Genetic Models of Alzheimer's Disease

I.B.1. Aging in Mice

Aging in mice, as in the case of humans, is a heterogeneous process (Gower et al, 1993). Little behavioural data or measures of cholinergic markers exist for aged hybrid C57BL/6J x C3H/HeJ (B6C3) mice, but the C57BL/6J inbred mouse strain has been well characterized and demonstrates parallel behaviour impairments to other mammalian species. Aged mice have impairments of prolonged retention (Strong et al, 1980; Dean et al, 1981; Kubanis et al, 1982) and spatial memory (Foldyce et al, 1993), with increased perseveration (Dean et al, 1981), while simple cued and discrimination learning are comparable to younger mice (Goodrick et al, 1975; Crady et al, 1989). Such age impairments are consistent across other strains (Lamberty et al, 1992, 1993; Gower et al, 1993).

Some studies show no changes in emotionality with aged C57BL/6J mice (Goodrick et al, 1975), while others demonstrate increased exploration and fewer anxiety behaviours (Ammassari-Teule et al, 1981). Consistent increased or decreased anxiety, then, is not found in several stains of senescent mice (Sarter, 1986;

Miyamoto, 1991; Lamberty et al, 1992)

Sensorimotor abilities, such as visual acuity, may be altered in aged mice, confounding behavioural task interpretations (Gower et al, 1993). In tasks assessing motor ability, more demanding tasks reveal the detrimental effects of aging (Crady et al, 1989), and while mice may perform similarly to younger mice, they take longer to complete tasks (Ammassari-Teule et al, 1981; Crady et al, 1989). Aged mice tend to be less active (Goodrick et al, 1975). Free wheel running, as an exercise, acts to increase memory retention of aged mice in passive avoidance tasks, indicating, much as in humans, that age-related impairments might be influenced by extracerebral factors (Samorajski et al, 1985).

Studies of ChAT activity in C57BL/6J mice are conflicting in that some show an age related decrease in cortical ChAT activity (Strong et al, 1980), while others show an increase in ChAT activity in the hippocampus and cortex without changes in the striatum (Sherman et al, 1990). Reductions of bound hippocampal protein kinase C (Foldyce et al, 1993) and decreased sodium dependent high affinity choline uptake in the hippocampus and cortex (Sherman et al, 1990) have been found in aged mice. Decreased muscarinic binding in the striatum (Sherman et al, 1990) and cortex (Strong et al, 1980) conflict with other findings of no change in muscarinic receptor binding (Kubanis et al, 1982). The findings tend to support the idea that aging is a compromise of presynaptic functioning with the preservation of the integrity of innervation (Sherman et al, 1990). Aged B6C3 mice, however, show gliosis with glial fibrillary-acidic protein (GFAP) in white matter (Bronson et al, 1993), possibly indicative of age-related neural lesions.

The hybrid B6C3 survival at 24 months of age is 80% (Cameron et al, 1985). C3HeB/FeJ male and female lifespans tend to be approximately 21.5 months (Storer et al, 1966). The females have a high incidence of liver or breast tumours, while the males develop hepatomas. C57BL/6J mice have a lifespan of approximately 29 months for males and 26 months for females (Kunstyr et al, 1975), but low incidence of age-related pathology (Storer et al, 1966).

I.B.2. Amyloid Precursor Protein Models

Attempts have been made to produce transgenic mice carrying excess copies of an isoform, fragment, or mutation of the ßAPP gene, but several models have since been retracted (Kawabata et al, 1991; Wirak et al, 1991). Amyloid plaques have been identified in aged non-human primates (Wisniewski et al, 1973), but not in mice or rats. Some have proposed that while ßAPP metabolism may be similar in humans and mice, the primary sequences of ßAPP differ by 22 amino acids, three of which are in the Aß region (Yamada et al, 1987), and this may serve as a protective factor against deposition; perhaps the disruption of endogenous amyloid is necessary prior to transfection with the human gene (Lannfelt et al, 1993). Animals with the same sequence have developed amyloid deposits with increasing age (Wisniewski et al, 1970; Selkoe et al, 1987).

Quon et al (1991) created a transgenic mouse construct with the ßAPP751 isoform under control of rat neural enolase promoter. Immunoreactive Aß deposits, both compact and diffuse, were identified in hippocampal and cortical regions of transgenic mouse brain. This may indicate a relationship between the ßAPP isoforms with a Kunitz inhibitor and amyloid deposition (Quon et al, 1991). The deposits closely resemble the pre-amyloid, diffuse deposits of early AD and young Down Syndrome brains (Higgins et al, 1994), and most likely originate from the human transgene and not from endogenous amyloid expression. Young (5-6 mo) and aged (9-12 mo) female mice with the ßAPP751 transgene were assessed on a series of behavioural tests. The young transgenics demonstrated slowed learning, while learning by the aged transgenic mice was abolished in the Morris Swim Maze. Aged transgenics also showed memory impairments in spontaneous alternation behaviour in a Y-maze (Moran et al, 1995). Although the transgenics did not have gross motor, physiological, or behavioural impairments, they had lower nocturnal activity than controls (Moran et al, 1995).

In a comparison of transgenic mice carrying either ßAPP695 or ßAPP751, using C57BL/6JxLT/Sv F1 mice with a metallothionine IIA promoter, the ßAPP751 mice had abnormal swimming abilities and did not appear to learn the Morris Swim

Maze, whereas no deficits appeared on a Passive Avoidance task (Perry et al,1995). The ßAPP695 deficits were inconclusive (Yamaguchi et al, 1991; Perry et al, 1995). Both had lower nocturnal activity levels than controls (Perry et al, 1995), which may be a possible confound in the interpretation of swim latencies and the cognitive deficits noted in the Morris Swim Maze. Up to 20 weeks of age, these ßAPP695 failed to demonstrate any amyloid neuropathology (Yamaguchi et al, 1991).

A transgenic model with a C-100 ßAPP fragment, under control of the JC viral early region promoter, expressed increased amyloid immunoreactivity in type 1 astrocytes, but no further pathology (Sandhu et al, 1991). Other C-100 ßAPP fragment models, such as that by Kammesheidt et al (1992) using the brain dystrophin promoter, showed intracellular amyloid immunoreactivity, but no behavioural work was published. The pathology is in keeping with other findings that amyloid deposition is not exclusively extracellular (Cataldo et al, 1990; Joachim et al, 1991; Golde et al, 1992)

A model recently created by Games et al (1995), placed the human ßAPP gene with the 717 mutation (Murrell et al, 1991), and introns 6, 7, and 8, under control of the platelet-derived growth factor-ß promoter. Young mice demonstrated little pathology, but aged mice began to exhibit distorted neurites. Diffuse, followed by dense, Aß deposits formed in the hippocampus, corpus callosum, and cerebral cortex. GFAP-positive astrocytes and active microglial cells surrounded the plaques in the cortices, and in the molecular layers of the dentate gyrus, synaptic and dendritic densities were reduced. While no NFTs or neuronal losses were evidenced, these mice appear to closely manifest the pathological features of AD. These results also strengthen the role of amyloid in the genesis of Alzheimer pathology, but no behavioural data has yet to be produced for this model.

In a study of FVB/N mice transgenic for murine ßAPP695, human ßAPP695 with the double 670/671 mutations, human ßAPP695 with the 717 mutation, or normal human ßAPP695, Hsiao et al (1995) found that the transgenic mice died earlier than control mice and become neophobic with spatial memory impairments at younger ages than the control mice. No extracellular amyloid pathology was evident in the

transgenic mice, possibly indicating that the deleterious properties of amyloid are not reliant on deposition. Young transgenic mice also developed reductions in corticolimbic glucose utilization, which was reduced in control mice at later ages (Hsiao et al, 1995).

Partially ßAPP deficient mice showed an increased incidence of missing a corpus callosum and severe memory impairments in the Morris Swim Maze (Muller et al, 1994). Reductions in locomotor activity and exploratory behaviour indicate that the loss of ßAPP may have influenced motivational systems.

I.B.3. Apolipoprotein E Deficient Model

Apolipoprotein E and the utilization of cholesterol appear to underlie an important pathway in the maintenance of integrity in the CNS (Poirier, 1996). For this reason, and upon realization that the different isoforms of apoE modify the age at onset of AD (Corder et al, 1993), further studies were needed to fully understand how apoE contributed to the dysfunctional and pathologic systems in AD. Mice homozygous deficient for ApoE were shown by Masliah et al (1995) to display significant synaptic loss and neuronal disruption in the hippocampus and neocortex (Masliah et al, 1995). Compensatory synaptogenesis following entorhinal cortex lesioning is absent, but these mice do show normal sciatic nerve regeneration (Popko et al, 1993), further indicating that the CNS is reliant on apoE for reinnervation and maintenance of the stability of the synapto-dendritic cytoskeleton, but that the PNS can make use of other apolipoproteins.

Young, two month old mice (C57BL/6J) were tested in the Morris swim maze to assess the effect of a total loss of apoE activity on cognitive performance (Poirier et al, 1996). The homozygous apoE-deficient (knockout) mice had longer latencies in finding the hidden platform on the first three of four testing days, indicating that the loss of apoE results in deficits in spatial learning and memory, and suggests a compromised cholinergic system.

I.B.4. Segmental Trisomy 16 Model

Mouse models of DS caused by trisomy 21 are possible because the distal end segment of mouse chromosome 16 has at least 13 loci that are homologous to genes on human chromosome 21 (Reeves et al, 1992). Previous models of full trisomy 16 in mice were limited in that the aneuploidy was lethal to the developing fetus at 16-20 days of gestation, likely due to the atrioventricular septal heart defect which terminates many DS human fetuses during their first trimester (Davisson et al, 1993). These whole chromosome trisomy 16 mice thus made it impossible to study postnatal development of the nervous system, and a further drawback was that mouse chromosome 16 has conserved segments from the human chromosomes 3, 16, and 22 (Davisson et al, 1993). Due to an incomplete homology between human chromosome 21 and mouse chromosome 16, pure trisomy 16 mice are not trisomic for some human chromosome 21 genes and have an overabundance of genes not on chromosome 21 (Reeves et al, 1995). It is possible that trisomy for that genetic material or simply the dosage imbalance of too much genetic material causes fatality.

In the segmental trisomy model, the small translocation chromosome that contains the mouse chromosome 16 segment homologous to human chromosome 21 is present in addition to the normal chromosomal complement (Davisson et al, 1993). The segmental trisomy 16 mice, Ts65(17^{16})Dn, are generated by irradiating the testes of DBA/2J mice and mating them with C57BL/6J mice (Davisson et al, 1990). The production of trisomy for the distal region of chromosome 16 occurs through the normal nondisjunction of chromosomes at meiotic anaphase I (Davisson et al, 1993). This failure of segregation results in gametes with a full haploid complement of chromosomes and the small translocation chromosome. Female mice heterozygous for the reciprocal translocation are mated to C57BL/6JxC3H/HeJ F1 hybrids. The homozygous Ts65Dn mice have a dosage imbalance for most of the genes conserved through evolution between mouse chromosome 16 and human chromosome 21 (Davisson et al, 1993).

While this model mimics the effects of trisomy for those genes on mouse chromosome 16 that are homologous to human chromosome 21, the features of DS

may also in part be due to human chromosome 21 genes conserved on other mouse chromosomes, namely 10 and 17 (Davisson et al, 1993).

Phenotypically, the segmental trisomy mice are somewhat consistent with features of DS. The features of the trisomy mice include smaller size prior to weaning, hyperactivity, early obesity, and muscular trembling, which possibly indicates neuropathology (Davisson et al, 1993). Mild hydrocephalus was observed as early as one month of age, possibly relating to the brachycephaly of DS patients. All homozygous Ts65Dn males are sterile, no sensorimotor impairments are evident, the mice do not display cardiac or skeletal abnormalities common in DS, and neither leukaemia nor AD pathology have been discovered in any of the mice up to 21 months of age (Escorihuela et al, 1995; Reeves et al, 1995). This lack of AD pathology might indicate that the AD phenotype of DS may result from the overexpression of genes not present on the 17^{16} chromosome (Reeves et al, 1995). The mice demonstrated increased nocturnal locomotor activity and were moderately impaired in the Morris Swim Maze (Escorihuela et al, 1995; Reeves et al, 1995). The Ts65Dn mice do demonstrate some stereotypical exploration patterns (Escorihuela et al, 1995).

I.C. Pharmacology of Cholinomimetics

Acetylcholine and the central cholinergic system have traditionally been implicated as the underlying neurotransmitter system of cognitive processes and memory (Bartus et al, 1982b). Pharmacotherapy has usually focused on enhancing cholinergic transmission for the maintenance of memory processes, especially in disorders such as AD that show profound loss of cholinergic innervation (Whitehouse et al, 1981, 1982). Acetylcholine precursors, postsynaptic agonists, inhibitors of acetylcholinesterase (AChE), and presynaptic M2 muscarinic receptor antagonists have been previously used to improve memory in impaired animals and in humans with AD (Bartus et al, 1982b; Kaye et al, 1982; Cherkin et al, 1985; Flood et al, 1985; Summers et al, 1986; Davis et al, 1992; Quirion et al, 1995). Although there are a number of cholinomimetic agents that exist, only the ones used in the present study

will be introduced here.

Tetrahydroaminoacridine (tacrine) is a potent, centrally active, AChE inhibitor, which acts to increase the concentration of ACh in the synaptic cleft. Tacrine has previously been found to offer improvements of memory in mice (Flood et al, 1985; Fitten et al, 1987) and AD patients (Summers et al, 1986; Davis et al, 1992). Recent evidence implicates the cholinergic system in ßAPP processing (Nitsch et al, 1992), and AChE inhibitor activity has been shown to increase the release of soluble ßAPP, thus indicating a role for ACh in activation of normal ßAPP processing (Mori et al, 1995). While inhibitors of AChE, such as tacrine, provide symptomatic relief for AD patients, they may also have a neuroprotective role in slowing the progress of the disease (Giacobini, 1994).

BIBN99 is a tricyclic muscarinic M2 antagonist (Doods et al, 1993a) that facilitates the release of ACh in cortex and hippocampus via its action on presynaptic autoreceptors (Hoss et al, 1990; Quirion et al, 1993). Early M2 receptor antagonists had little central nervous system activity, likely due to their inability to cross the blood-brain barrier (Doods et al, 1993b). As therapeutic agents, M2 antagonists should have good penetration of the blood-brain barrier and a high M2 versus M1 selectivity, so as not to counteract the presynaptic action by a postsynaptic M1 blockade (Doods et al, 1993a). BIBN99 has a 30-fold higher affinity for M2 than M1 receptors (Doods et al, 1993a), yet is not a potent peripheral antagonist of M2 receptors, likely because it is quickly cleared from plasma due to a high lipophilicity or rapid metabolism. As a result, M2 antagonists may be more advantageous than traditional muscarinic agonists, which supply a continuous, non-physiological postsynaptic stimulation, or traditional AChE inhibitors, which may induce presynaptic acetylcholine autoregulation (Doods et al, 1993). With the discovery that cholinergic agents also inhibit glutamate release via post-synaptic M2 receptors (Marchi et al, 1989; Raiteri et al, 1990; Mrzljak et al, 1993), BIBN99 might jointly relieve inhibition of ACh and glutamate release, facilitating cognitive processes.

I.D. Scope of Study

The studies in this thesis examine three new animal models that may increase understanding of the contributions of specific genes to the pathophysiology and intellectual decline associated with Alzheimer's disease. That much of the work focuses on behaviourally testing the models underlines the need for behavioural correlates with pathology in the current literature of AD models. Cholinergic enhancing pharmacological agents are used here to test their efficacy in mouse models of AD. The use of human-Aß expressing mice originated from the understanding that amyloid is intrinsically related to AD pathology. The use of these transgenic mice in experiments reported in section II allows closer study of the functional effects of the ßAPP gene in a model of AD. The efficacy of BIBN99 is assessed in this model given that it has been previously shown to increase the release of ACh in aged-impaired rats (Quirion et al, 1995) and ameliorate cognitive impairments assessed with a swim maze task in aged rats (Quirion et al, 1995) and in rats with traumatic brain injury (Pike et al, 1995). Using apoE deficient (knockout) mice, section III reports behavioural effects which result from the use of tacrine on an impaired apolipoprotein system, and may provide clues as to what effects tacrine will have on humans with an inheritance of hetero- or homo-zygosity for the ϵ4 allele of apoE. Finally, as Down Syndrome has been intimately linked with AD, both in eventual pathology and the clinical syndrome of dementia, the use of segmental trisomy 16 mice in section IV allows an investigation of the effectivness of the M2 muscarinic antagonist, BIBN99, in ameliorating previously reported spatial learning deficits (Escorihuela et al, 1995; Reeves et al, 1995).

II. ß-Amyloid Transgenic Model

These series of experiments were conducted to assess how the Aß-containing C-terminal fragment of ßAPP will affect behavioural outcomes in several paradigms at different stages of the natural lifespan of the C57 B6C3 hybrid mouse. The experiments include an assessment of the efficacy of an M2 muscarinic antagonist, BIBN99, to improve any memory deficits. Two general series of experiments were conducted: Series One tested adult transgenics against adult controls, while Series Two tested aged transgenics against aged controls and young controls.

II.A. MATERIALS AND METHODS

II.A.1. Animals

The age ranges provided below indicate initial age at beginning of testing to final age at the conclusion of testing. In Series One, adult ß-amyloid transgenic mice (7-8 mo, 6 females, 5 males) were developed by Dr. J. Nalbantoglu and colleagues at the Montreal Neurological Institute, and C57 B6C3 F2 adult controls (7-8 mo, 10 males) were obtained from the National Institute of Aging (Bethesda, MD). Nalbantoglu and colleagues constructed the transgenic colony by introducing, under transcriptional control of the human neurofilament NF-L promoter, a cDNA fragment of amino acids 591-695 of the Amyloid Precursor Protein into the mice against a C57 B6C3/H background. In Series Two, aged ß-amyloid transgenic mice (18-23 mo, 5 females, 3 males), aged littermate controls (5 females, 5 males), and young controls (2-7 mo, 3 females, 6 males) were of a C57 B6C3/H F2 hybrid strain, and were all selectively bred at the Montreal Neurological Institute. All mice were maintained on a 12 hour light-dark cycle (on at 8:00 am) in temperature (22° C) and humidity (45-70%) controlled rooms. Males and females were housed separately in groups of 3 to 6 in microisolated cages. All had *ad libitum* access to both standard laboratory mouse chow and tap water, except when indicated below.

For the sake of clarity, age classifications will be maintained regardless of eventual changes in age (for example, the young control mice were 2 months old upon commencing behavioural testing, but were sacrificed at 7 months old and should

justifiably be labelled adult mice; however, they are still referred to as young throughout this thesis).

The adult transgenic mice (47.4 ± 1.8 g) were significantly heavier than the adult controls (39.3 ± 1.4 g) toward the end of behavioural testing in Series One (unpaired t-test: t=3.379, df=19, p=0.0031). Part way through the Series Two experiments, young controls (34.7 ± 1.0 g) weighed significantly less than both aged controls (50.4 ± 2.2 g) and aged transgenic mice (46.0 ± 4.0 g) (F=6.236, df=2,23, p=0.0069; Newman-Keuls $p<0.01$ for young controls versus aged controls, and $p<0.05$ for young controls versus aged transgenics). Aged controls and aged transgenics did not differ in weight. Coat colour varied between black and brown, but colour was not indicative of a specific group.

During the period of testing, several male and female mice from each group died of natural causes; their test scores were included from tests completed in a healthy state prior to death. Mice were cared for according to McGill University and Canadian Council on Animal Care approved guidelines.

II.A.2. Drugs

BIBN99 was kindly supplied through Dr. Remi Quirion from K. Thomae GmbH, Biberach, Germany. The drug was stored in powder form in opaque containers at room temperature for the duration of testing, and was freshly prepared each morning prior to testing. A 1 mg/kg dose in 0.25 mg/ml of 0.9% saline solution was prepared and the pH adjusted to 6.5 using 0.1 N HCl.

II.A.3. Behavioural Testing

The following tests were administered according to the outline detailed in Table 1. Any time measurements were recorded by either a digital stop watch or with IBM compatible computers. The order of testing groups was always randomized, and all testing was completed blind to the genetic identity of the groups.

Table 1: CHRONOLOGY OF BEHAVIOURAL TESTING

Adult Transgenic Mice	
Days	Test
1	Elevated Plus Maze
3-5	Single Trial Passive Avoidance
8-11	Locomotor Activity
15-16	Porsolt Forced Swim Test
19	Thatcher-Britton Novelty Conflict
30	String Test
37-38	Single Trial Recognition Task
56	Animals Sacrificed

Aged Transgenic Mice	
Days	Test
1-3	Multiple Trial Step-Down Passive Avoidance
6-9	Locomotor Box Activity
14-15	Porsolt Forced Swim Test
22	Thatcher-Britton Novelty Conflict
35	Long Term Passive Avoidance
68-93	Forced Alternation T-Maze Training
113-116	T-Maze Testing
121-124	T-Maze Testing (1 mg/kg BIBN99)
181	Animals Sacrificed

II.A.3.a. Forced Alternation T-Maze

The T-maze is an enclosed wooden platform, shaped as a T, with a transparent top. The short arms each measured 15 x 10 cm, the long arm measured 55 x 10 cm, and the walls measured 16 cm high. Because this task uses a food reward throughout training and testing, body weights of the mice were, on average, maintained close to 80% of their original weights; indication of greater weight loss occurred in a few instances and was countered by transiently increasing food availability.

The first four days of the experiment were for habituation. Food reward (Froot Loops) was placed throughout the maze, and on each subsequent day the food was increasingly isolated into the end of both small arms of the T. For habituation, mice were allowed to explore for three minutes on the first day, two minutes on the second day, one minute on the third day, and on the fourth day the mice were allowed only one run down the length of the maze to where the food was isolated.

The protocol for the remaining days of testing was fixed. Mice were placed into the end of the long arm of the T (start box) and could immediately run down the maze. Food reward was always present in both left and right small arms of the T (choice arms). Mice were initially run on four trials a day and this was eventually increased to six trials. Each trial consisted of a forced run and a choice run. With the forced run, one of the choice arms was blocked off, forcing the mouse to turn into the other arm for its reward. The mouse was allowed two minutes on the forced run to enter an arm and eat one piece of food (¼ Froot Loop); it was then removed and placed back into the start box, without delay, while the block was being removed. On this, the choice run, the mouse was allowed one minute to run up the long arm of the maze and now choose an arm. To obtain food, the mouse had to choose the arm that had been previously blocked. Once the correct choice was made, the mouse was allowed to eat one piece of food before being removed. If it chose the same arm as it did on the forced run, it was immediately removed without reward. Omissions were scored if the mouse failed to move on either forced or choice run, or if the mouse failed to choose an arm on the choice run.

Inter-trial intervals (ITI) for individual mice varied depending on how long each interposed mouse required to run the maze. Toward the end of training, ITI was less than 4 minutes. The order of trials was randomly chosen each day from all possible left-right combinations, allowing for certain restrictions: the number of left and right choices were equal each day, three same-direction choices could not be tested consecutively (eg. LLLRRR) in a day, and a given schedule could not be used on three consecutive days.

Training continued for eighteen days, non-consecutively, followed by a three week interval during which no other behavioural tasks were administered. Four days of non-drug T-Maze testing, a five day interval, and four days of BIBN99 administration and testing completed the test. Administration consisted of 1.0 mg/kg of BIBN99 subcutaneous (s.c.) to each mouse 50 minutes prior to testing, with the six trials ending approximately 90 minutes after injection.

II.A.3.b. Single-Trial Recognition Task

In order to test working memory without any aspects of reference memory involvement, the following test was based on the differential exploration of familiar and novel objects (Ennaceur and Delacour, 1988). Mice were individually placed in a large shuttle box (47 x 26 x 20 cm) and allowed to explore for three minutes to habituate to the novel environment. In order to prevent object discrimination based on olfactory clues, the box was not cleaned over the two days of testing so as to saturate it with olfactory stimuli.

Twenty-four hours later two identical objects were placed at one end of the shuttle box, in opposite corners. A mouse was placed at the opposite side of the cage, facing toward the wall. Measurements were taken for time spent exploring the object (directing nose $\leq$ 2 cm from objects or nose touching objects). Each mouse was allowed 3 minutes to explore and was then removed from the cage for a five minute delay, which was followed by another three minute exploration of the cage. During the second exploration, both objects were replaced, one with a novel object and the other with an object identical to the first two (to avoid olfactory traces).

Measurements were again taken of exploration times. Left or right corner novel object replacement was randomized to avoid possible position biases in the mice.

II.A.3.c. Porsolt Forced Swim Test

Mice were placed into a one litre beaker (10 cm in diameter), filled to 800 ml with water (depth equal to 10 cm) maintained at 27° C; the water was frequently changed. Each mouse was placed into the beaker for six minutes on both the first (acquisition) day and on the second (retention) day. The duration of immobility was recorded, as were the number of transitions between mobility and immobility. Immobility was defined as the lack of escape behaviour and the relative inactivity of front and rear paws, such that only minimal movements were made to stay afloat.

II.A.3.d. Multiple Trial Step Down Passive Avoidance

The apparatus consisted of a small plexiglass case (23 x 23 x 20 cm) with a hinged roof, and a floor consisting of a steel grid connected to a shock generator (LaFayette Instruments, Model 80201). A wooden block (3 x 3.8 x 9 cm) was located on one side of the case. The first day of testing was the habituation day and the mice were allowed to explore the plexiglass case for 15 minutes each, without the risk of shock. The second day of testing was the acquisition day. Each mouse was placed on the wooden block and as it stepped down, placing at least three paws on the steel grid, a 1 second, 0.6 mA scrambled mild electric shock was triggered manually and the mouse was then removed. The mice were placed back into the plexiglass case until they remained on the wooden block for two consecutive trials of 120 seconds without stepping down. On this first day, testing continued until each mouse received at least one shock followed by its two consecutive 120 second periods on the block. Both the latency to step down for each trial and the number of trials necessary to achieve the learning criterion were recorded. The third day of testing was the retention day. Each mouse needed to remain on the block for the same testing criterion. Mice were allowed several minutes between trials.

One month following the last day of passive avoidance testing, the mice were

retested. Each mouse was repeatedly placed into the glass case until it remained on the wooden block for two consecutive trials of 120 seconds. Several minutes were interposed between trials. A shock was delivered if the mouse stepped off the wooden block with at least three paws. Once again, the latency to step down and the number of trials required to meet criterion were recorded.

II.A.3.e. Single Trial Step Down Passive Avoidance

The same apparatus used for the multiple trial task was used here. On the first day of testing, each mouse was placed on the wooden block and the latency to step down onto the grid floor was measured. When at least three paws touched the floor, a 1 second, 0.6 mA scrambled mild electric shock was administered and the mouse was immediately removed from the cage. Within one hour of receiving that shock the mouse was placed back in the cage, on the wooden block, and the latency to step down was again recorded. No shocks were administered at step down. Twenty-four hours and forty-eight hours later, the mice were again tested for their latency to step off the block without receiving a shock.

II.A.3.f. Elevated Plus Maze

The elevated plus maze consists of a four arm cross (each arm 30 x 5 cm), two of which are enclosed by walls (12 cm high). The maze is elevated 50 cm above floor level. Mice were placed at the cross intersection, facing a closed arm and were free to explore both closed arms and both open arms. Each mouse was allowed five minutes of exploration. The following measures were taken: time spent in open arms, time spent in closed arms, time spent in centre intersection, number of open arm entries, number of closed arm entries, and the latency to first enter an open arm.

II.A.3.g. Thatcher-Britton Novelty Conflict

All mice were food deprived 24 hours prior to commencing this test. The apparatus consisted of a black, wooden box, 57 x 57 x 50 cm, with an open top. Six pellets of standard laboratory chow were placed at its centre. Each mouse was placed

in one of the corners of the box and the latency to begin eating the food was recorded, as were the number of approaches where the mouse was in reach to eat, but did not. If after six minutes the mouse did not approach the food, it was removed and a latency of 360 seconds was scored.

II.A.3.h. Locomotor Activity Boxes

These boxes measured 20 x 30 x 40 cm and were equipped with two computer-monitored photocell detectors, 3.8 cm above the wire mesh floor. Mice were each placed into an activity box for one hour of their light cycle every day for four days. As the mice explored the box, they would interrupt either of the photocell beams, incrementing the computer counter. The testing room was sound attenuated and darkened.

II.A.3.i. String Test

Two poles were set 10 inches apart and a taut string was tied across from both poles, 10 inches high. Mice were individually placed with forepaws clasping the string. Depending on the animal's dexterity and strength, scores above zero were given to the highest level of achievement if the mouse stayed on the wire longer than ten seconds. Scoring criteria were as follows: 0, mouse fell off the string in under 10 seconds; 1, mouse hung with two paws; 2, hung with three or four paws; 3, mouse attempted some horizontal movement along string; and 4, mouse escaped off side of string onto either pole.

II.A.4. Analysis of Brain Tissue

II.A.4.a. Acetylcholinesterase Histochemistry

Following behavioural testing the young control (actually 7 months), aged control (23 months) and aged transgenic mice were killed by live decapitation. The brains were removed, immersed into isopentane on dry ice, and stored at -80°C until sliced on a microtome. Coronal sections, 20 µm thick, were mounted on poly-L-lysine coated microscope slides. AChE staining was performed using the direct

colorimetric method (Karnovsky, 1964). Each slide was incubated for four hours in a solution containing: 0.1 M NaH_2PO_4, 0.3 M sodium citrate, 30 mM $CuSO_4$, and 5 mM K-ferrycyanide. The slides were then incubated overnight in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), dehydrated with several concentrations of ethanol, and rinsed with xylene for four minutes. Staining was evaluated in the superficial, medium, and deep levels of neocortex, nucleus basalis magnocellularis (NBM), and the CA1, CA2 and CA3 subfields of the hippocampus of each animal; the use of an image analysis system (MCID System, St.Catharines, ON) provided measures of relative optical density (ROD). RODs for twelve samples of each cortical area from four sections per animal were recorded and averaged for each group. RODs for two samples of each area of hippocampus and NBM from two sections per animal were recorded and averaged for each group.

II.A.4.b. Choline Acetyltransferase

All materials for the ChAT assay were kindly supplied by Dr. Brian Collier (Department of Pharmacology, McGill University). ChAT activity was determined using the method described by Fonnum (1969) and Tucek (1978). Adult control and ß-Amyloid transgenic mice were sacrificed after behavioural testing by live decapitation. Their brains were removed, quickly frozen in isopentane, and stored at -80°C until time for the assay. Dr. J. Nalbantoglu supplied seven month and eighteen month old ß-amyloid transgenic mice that were sacrificed and perfused with phosphate buffered saline (PBS).

Tissue was thawed in saline and bilateral hippocampi and complete cortices were dissected out. The samples were homogenized in 100 mg/ml (w/v) buffer, consisting of: 200 mM NaCl, 40 mM sodium phosphate buffer (pH 7.4), 0.5% Triton X-100, and 100 mM $MgCl_2$. Homogenized samples of 35 µl were incubated at 38°C for 15 minutes in a 15 µl medium containing: 300 mM NaCl, 0.2 mM eserine, 0.5 mg/ml bovine serum albumin, H_2O, 12.5 mM choline, and unlabelled acetyl CoA and ^{3}H-acetyl CoA (for a final concentration of 0.25 mM acetyl CoA). The reaction was terminated by adding 10 mM phosphate buffer and 0.2 mM ACh on an ice block. The

addition of 10 mg/ml of tetraphenylboron (TPB)-heptanone extracted the ^{3}H-ACh but not the ^{3}H-acetyl CoA. Radioactivity of the top organic phase was measured by liquid scintillation spectrometry.

Protein levels were determined by the Pierce BCA method, using bovine serum albumin as a standard. Homogenized tissue samples were dissolved in BCA reagents at 37° C for 30 min, and absorbency readings were taken from the spectrophotometer at 562 nm.

II.B. RESULTS

All data are expressed as mean ± standard error of the mean (SEM). Data were analyzed using either Graphpad Instat V2.02 (1990) or Datasim V1.1 (1994). Parametric analysis of variance (ANOVA) or t-tests were routinely used, except where noted. No statistical differences based on gender occurred and the results of male and female performance were pooled.

II.B.1. Adult Mice (Series One)

II.B.1.a. Single Trial Recognition Task

General levels of exploration on the initial exposure to the similar objects were equivalent between controls and transgenics at 38.6 ± 5.2 seconds compared to 32.1 ± 2.5 seconds, respectively (unpaired t-test, t=1.089, df=19, p=0.2897; due to differences in variability, the data were re-analyzed with a non-parametric Mann-Whitney U and the results obtained were compared to those obtained from the raw data unpaired t-test, both of which indicated a lack of significant difference), nor was there a preference for either the left side or the right side of the box (left object exploration time subtracted from right object exploration time for controls 4.7 ± 2.5 seconds, transgenics 1.8 ± 2.4; unpaired t-test, t=0.7926, df=19, p=0.4378). For the second exposure, a Recognition Ratio was incorporated into calculations to account for possible differences between mice in overall exploratory time. The ratio was calculated as the difference between left and right exploration times over the sum of the left and right exploration times. Control mice spent more time exploring the novel object than the transgenic mice, whose scores failed to indicate a preference for the novel object over the familiar one (Figure 1: controls' ratio of recognition 0.3080 ± 0.1686, transgenic -0.1923 ± 0.1248; unpaired t-test, t=2.297, df=19, p=0.0332).

II.B.1.b. Porsolt Forced Swim Test

Figure 2 indicates the durations of immobility for the controls and transgenics on both testing days. A separate Group x Minutes ANOVA was performed for each day. Significant main effects of Group occurred such that the adult controls were significantly more immobile than the adult transgenics on day one (F=9.79, df=1,19,

p=0.0055) and day two (F=7.93, df=1,19, p=0.011). Significant effects of Minutes indicated that immobility increased across minutes on both day one (F=33.28, df=5,95, p<0.0001) and day two (F=7.57, df=5,95, p<0.0001). A Group x Minute interaction occurred on day one (F=6.10, df=5,95, p<0.0001); subsequent F tests for simple main effects indicated that on day one, the adult controls were significantly more immobile on minutes 4 (p=0.0005), 5 (p=0.0029), and 6 (p<0.0001). The number of transitions between mobility and immobility were not significantly different between groups on either day (day one: 12.6 ± 2.2 for controls and 11.9 ± 2.4 for transgenics; day two: 13.0 ± 2.0 for controls and 15.2 ± 3.2 for transgenics).

II.B.1.c. Single Trial Passive Avoidance

In the single trial Passive Avoidance paradigm, four female transgenic mice did not step down from the block on the first day of testing, and so these mice were removed from the test results since they did not receive a foot shock. One other female transgenic mouse was removed from the analysis because it lacked a shock response, such as a shriek or jump, making the certainty of shock delivery questionable. A Group x Time ANOVA resulted in a significant main Time effect, indicating that the stepdown latencies (Figure 3) for both the remaining control and transgenic mice increased significantly over the immediate, 24 hour, and 48 hour testing times (F=4.65, df=2,28 p=0.0181). The Group effect, comparing controls and transgenic mice, was not statistically significant (F=3.79, df=1,14, p=0.0719).

II.B.1.d. Elevated Plus Maze

On all measures in the Elevated Plus Maze (Table 2), no differences between controls and transgenic animals occurred. A maximum value of 300 seconds for latency to enter an open arm was given to one control and one transgenic mouse because they failed to enter an open arm all together. All comparisons were made with separate t-tests (df=19).

	TOpen	T-Close	T-Cen	Open#	Tot#	OpenLat
Control						
Mean	18.9	218.0	53.1	4.3	19.0	69.2
SEM	5.3	7	4.4	0.9	1.9	26.4
Transgenic						
Mean	19.3	214.1	66.6	3.4	17.9	57
SEM	9.4	14.5	6.9	1.1	1.9	25
t	0.03895	0.2402	0.4157	0.6842	0.4012	0.3186
P value	0.9693	0.8127	0.6823	0.5021	0.6928	0.7535

Table 2 - Results and Analysis of Elevated Plus Maze

All times are reported in seconds. Measures included time spent in open arms (T-Open), time spent in closed arms (T-Close), time spent in centre square (T-Cen), number of open arm entries (Open#), total number of arm entries (Tot#), and the latency to first enter an open arm (OpenLat).

II.B.1.e. Thatcher-Britton Novelty Conflict

Figure 4 shows that transgenic animals were significantly slower than controls in approaching the food at the centre of the testing box (unpaired t-test, t=4.864, df=19, p=0.0001). The number of approaches to the food was not statistically different between groups (Controls 5.0 ± 1.6 and Transgenic 6.3 ± 0.5).

II.B.1.f. Locomotor Activity

A Group x Time ANOVA was performed on the locomotor data, resulting in a significant Time effect (F=25.34, df=5,90, p<0.0001). Averaging across the four testing days, both control and transgenic mice had a significant decline in locomotor activity levels from the beginning to the end of the one hour of testing (Figure 5). There was no difference in activity level between groups.

II.B.1.g. String Test

Nearly all control mice escaped from the string task (Figure 6) to one of the side poles, scoring significantly higher scores than the transgenic animals (Control

3.50 ± 0.38, Transgenic 1.36 ± 0.45; Mann-Whitney Test: p=0.0052). The transgenic mice tended to either fall before the 10 second limit, or remain hanging with one or two paws, but could not usually lift their hind paws up to the string. Hanging times were not significantly different between the two groups (Control time 9.36 ± 0.61 seconds, Transgenic 6.97 ± 1.03; t=1.851, df=19, p=0.0798; due to differences in variability, the square-roots of the data were calculated and results from an unpaired t-test on the transformed data were compared to those obtained from the raw data unpaired t-test, both of which indicated a lack of significant difference).

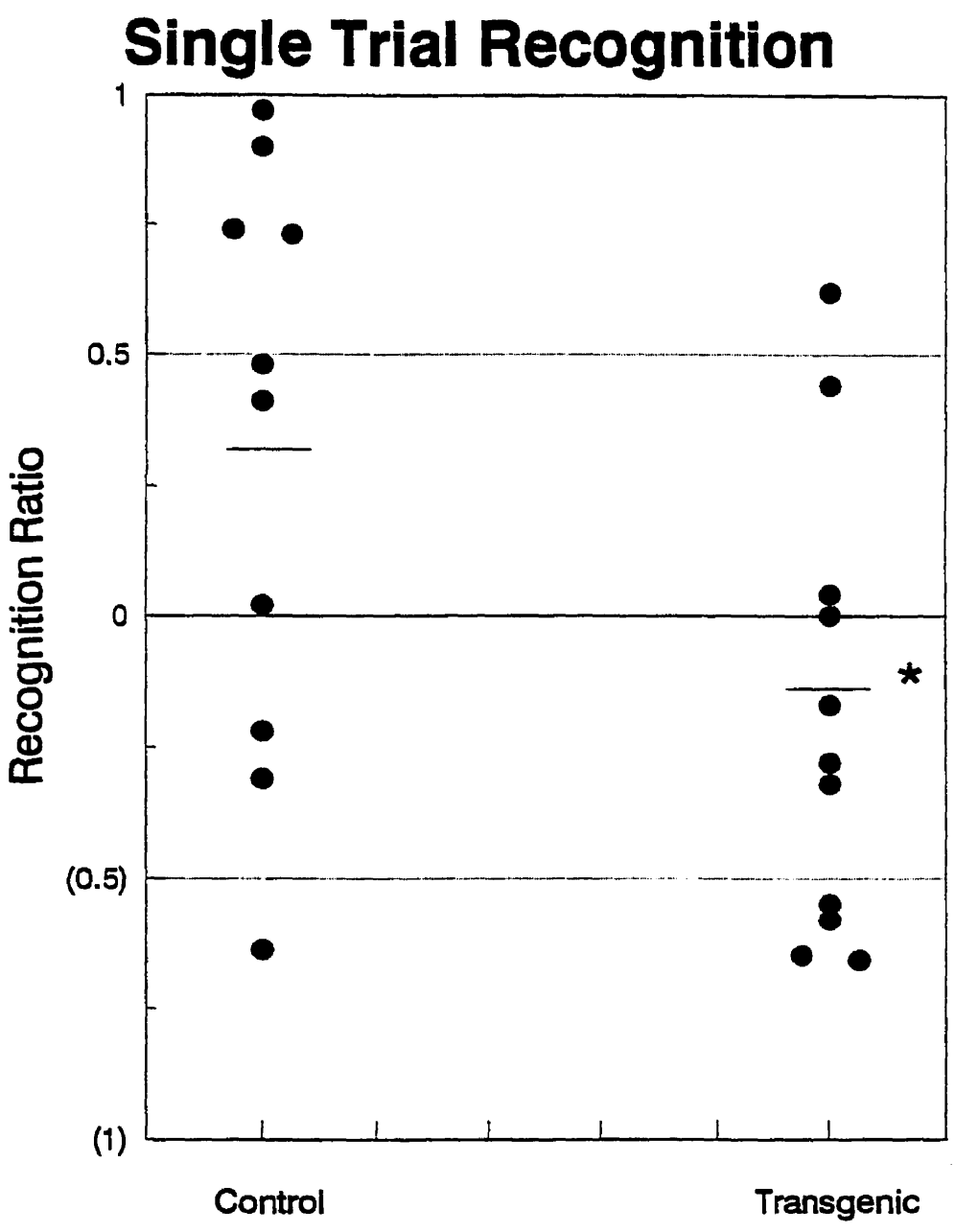

Figure 1. Single Trial Recognition Task. Each point represents an animal's ratio of recognition, calculated by the (difference of object exploration times) divided by the (sum of object exploration times), and defined as a 1 meaning total exploration of the novel object to the exclusion of the familiar one, and a -1 meaning the total exploration of the familiar object. Means are indicated by a horizontal line. Transgenic mice (n=11) spent significantly less time with the novel object than did controls (n=10) [t=2.297, df=19, p=0.0332].

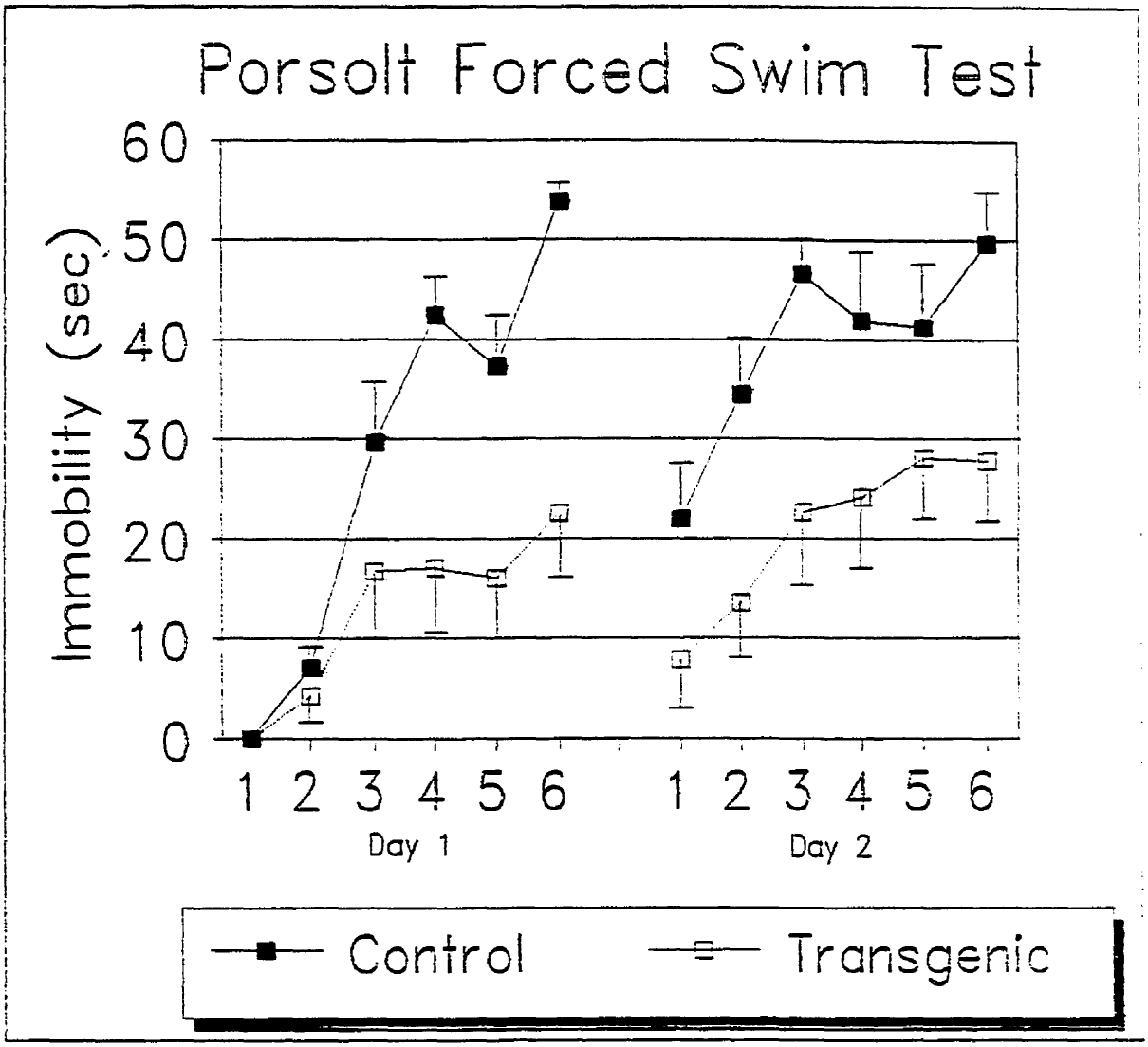

Figure 2. Porsolt Forced Swim Test. Curves indicate the average amounts of immobility for groups across six minutes of acquisition (day one) and retention (day two). Transgenic mice (n=11) were significantly less immobile than control mice (n=10) [day 1 (F=9.79, df=1,19, p=0.0055) and day two (F=7.93, df=1,19, p=0.011)]. Error bars indicate SEM.

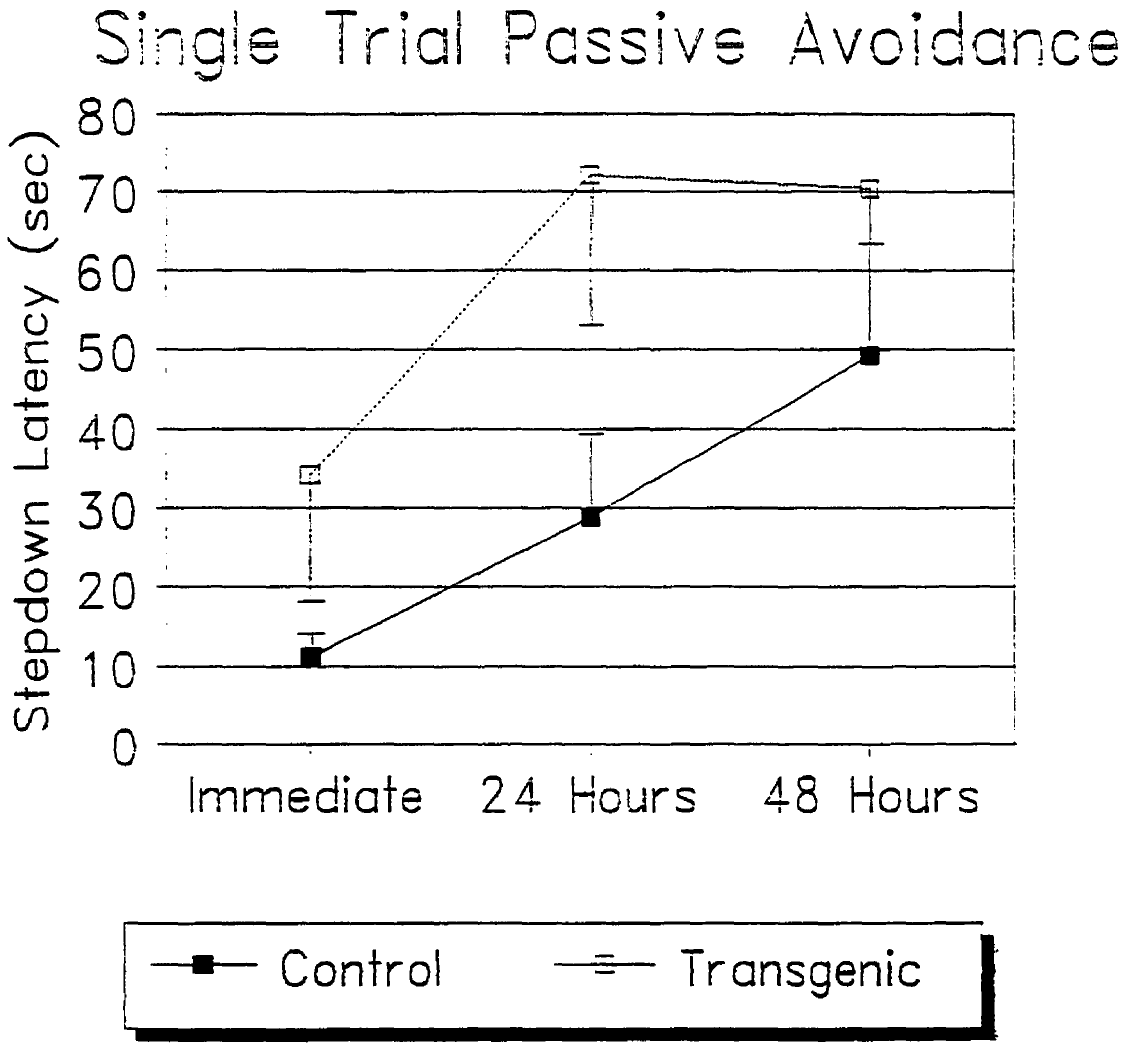

Figure 3. Single Trial Passive Avoidance. Data indicates the average latency for each group to step down on the immediate, 24 hour, and 48 hour testing trials, with the maximum allowable time being 120 seconds. Error bars represent SEM. No statistical differences between the transgenic mice (n=5) and the control mice (n=10) occurred.

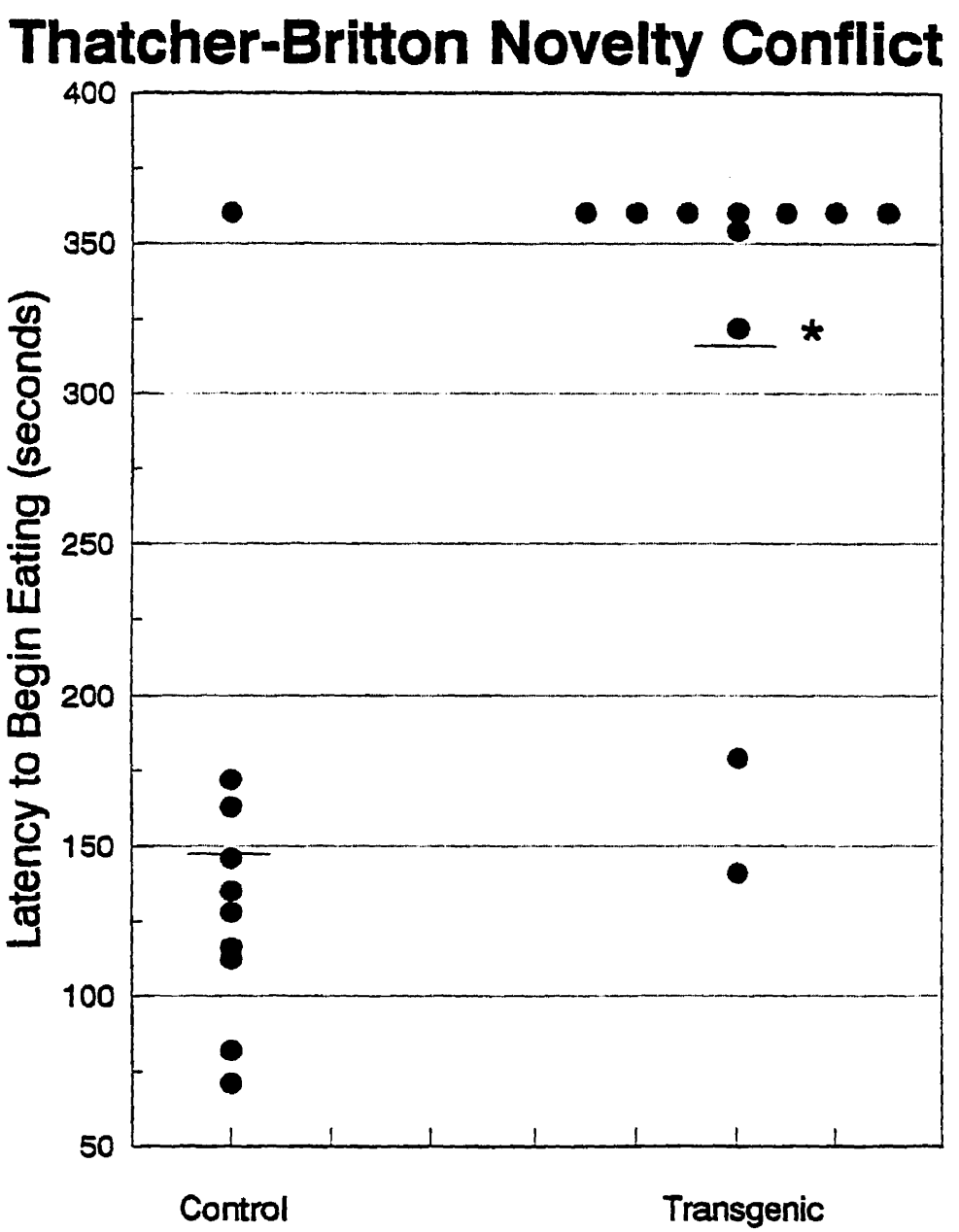

Figure 4. Latency for each animal to commence feeding in the Thatcher-Britton Novelty Conflict paradigm, with a maximum allowable time of 360 seconds. Means are represented by a horizontal line. Adult transgenic mice (n=11) had significantly longer latencies than adult controls (n=10) [t=4.864, df=19, p=0.0001].

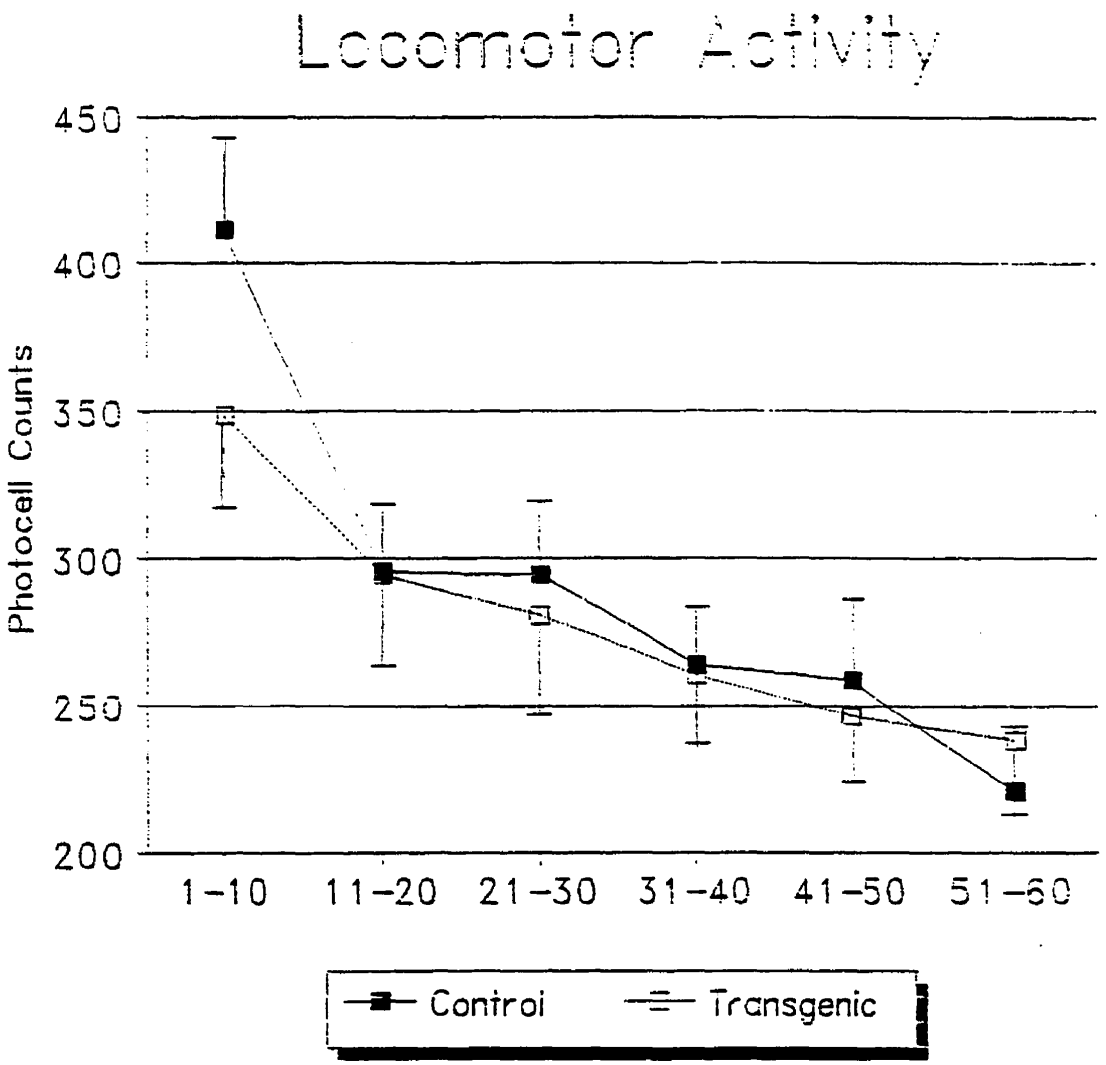

Figure 5. Average locomotor activity throughout a one hour period divided into 10 minute bins, as measured by photocell beam interruptions. Data is represented as the mean ± SEM, and is the average of groups across four consecutive days. No significant differences between transgenic (n=11) and control (n=10) groups occurred at any time.

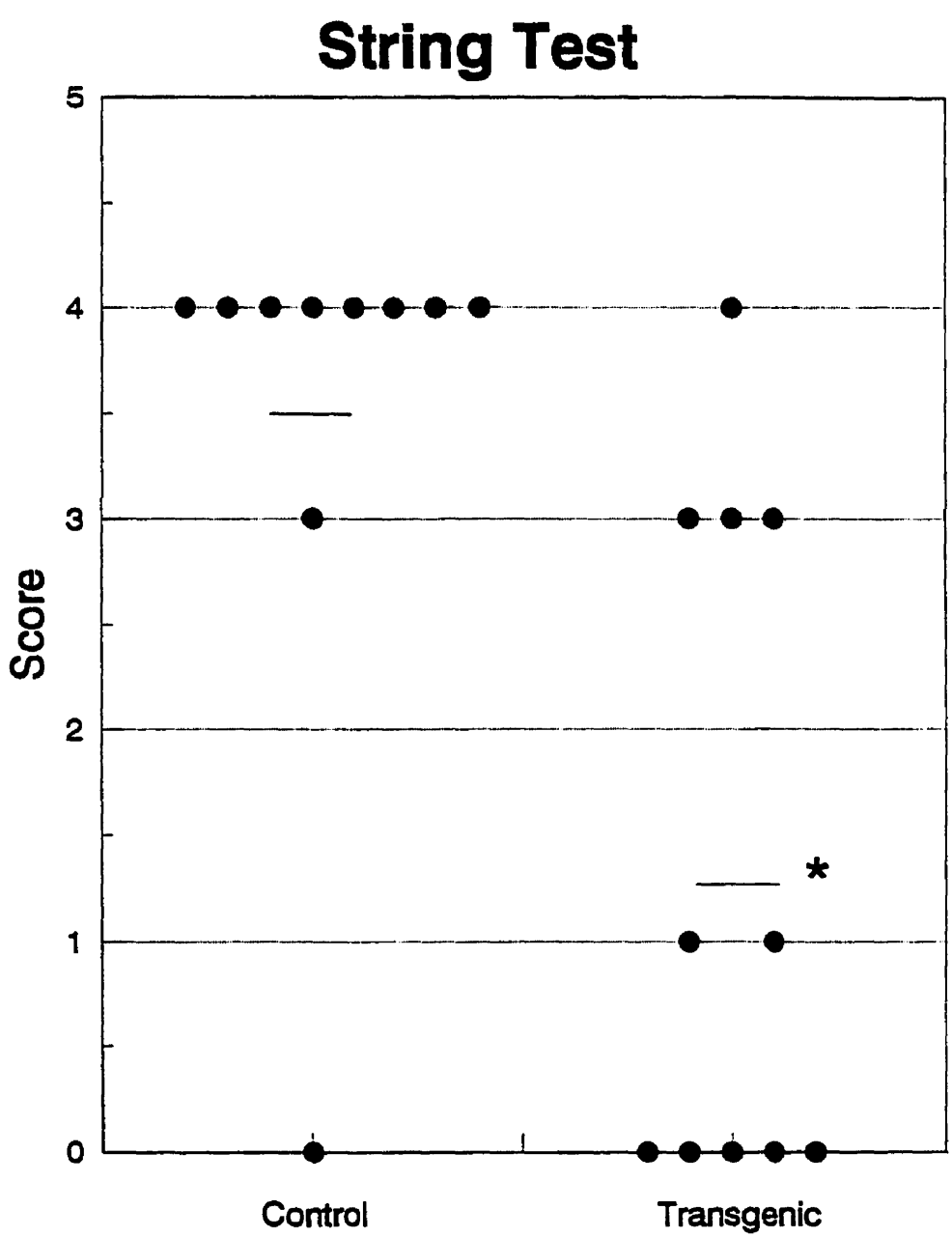

Figure 6. String Test. Each point indicates an animal's score in the task (see text for scoring criteria). Group means are represented by horizontal lines. Adult transgenic mice (n=11) performed significantly poorer than adult controls (n=10) [t=3.411, df=19, p=0.0029].

II.B.2. Aged Mice (Series Two)

II.B.2.a. Forced Alternation T-Maze

The most demanding cognitive test of those used in the present study was the Forced Alternation T-Maze. Aged transgenic and control mice and young control mice were trained for 18 days, not consecutively, and the number of trials omitted and numbers of correct and incorrect responses were recorded. All three groups were performing above an average 70% correct, based on the trials run on the last three days of this training (72.59% ± 4.81, 76.52% ± 3.78, and 71.1% ± 3.72 for young control, aged control, and aged transgenic mice, respectively; oneway ANOVA, F=0.3996, df=2,20, p=0.6758). The number of trials omitted were also analyzed in a oneway ANOVA, indicating a lack of a significant difference between groups (2.89 ± 0.68, 2.25 ± 1.45, and 6.33 ± 2.11 for groups of young control, aged control, and aged transgenic mice, respectively; F=2.253, df=2,20, p=0.1311). It should be noted that the number of trials omitted varied considerably from one animal to another, but that there was not a significant linear relationship between omissions and correct performance (Pearson product moment correlation analysis, r(23) = -0.3423, two-tailed p=0.1099).

Three weeks after training had ended, animals were tested over a four day period for retention of the task (Figure 7). A Group x Drug ANOVA resulted in a main Group effect (F=5.33, df=2,20, p=0.014). *Post hoc* comparisons indicated that, in the non-drug condition, aged transgenic mice performed poorly in comparison to aged controls (p=0.0336), but that the controls were not significantly different from each other (p=0.6449) and the young controls were not different than the aged transgenics (p=0.0746). With 1.0 mg/kg of BIBN99, the aged transgenics performed significantly poorer than the aged controls (p=0.0108) and the young controls (p=0.0250), but the controls did not differ (p=0.6510). No main Drug effect occurred (p=0.5213).

A Group x Drug ANOVA was also performed on the omission data during the testing period. Of the 24 trials run without drug administration, 0.33 ± 0.24, 3.0 ± 1.78, and 7.33 ± 2.29 were omitted by young control, aged control, and aged transgenic groups, respectively. Of the 24 trials run with BIBN99 administration, 0.33

± 0.24, 5.12 ± 2.69, and 9.50 ± 2.82 were omitted by young controls, aged controls, and aged transgenics, respectively. A significant Group effect occurred (F=5.49, df=2,20, p=0.0126). *Post hoc* comparisons indicated that in the non-drug condition only the omissions for the aged transgenics differed from the young controls (p=0.0132), while the aged controls did not differ from either the transgenics (p=0.1192) or the young controls (p=0.2802). With BIBN99 administration, aged transgenics differed from the young controls (p=0.0018) but not the aged controls (p=0.1159), and the controls did not differ from each other (p=0.0585). A significant Drug effect occurred, such that the groups omitted more trials during the BIBN99 test than when tested without drug (F=4.58, df=1,20, p=0.0449). Omissions and correct performance during the non-drug condition approached but did not reach a significant Pearson product moment correlation (r(23)= -0.4008, p=0.0581).

II.B.2.b. Porsolt Forced Swim Test

A separate Group x Minutes ANOVA was performed on data for each day (Figure 8). On day one, a significant main Minutes effect occurred (F=25.18, df=5,120, p<0.0001). No overall differences in time spent immobile occurred among any of the groups (F=1.07, df=2,24, p=0.3583). On day two, a significant main Minutes effect occurred (F=6.00, df=2,120, p<0.0001). Groups did not differ in immobility (F=0.18, df=2,24, p=0.8392). As well, an interaction of Group x Minutes occurred on day two (F=2.21, df=10,120, p=0.0212), such that only the transgenic mice showed a statistically significant difference across minutes (p<0.0001). Transitions between mobility and immobility did not differ on either acquisition (young controls 19.9 ± 2.9, aged controls 20.6 ± 1.9, and transgenics 25.9 ± 1.9) or retention days (young controls 24.6 ± 3.1, aged controls 23.9 ± 3.2, and transgenics 18.1 ± 3.0).

II.B.2.c. Multiple Trial Passive Avoidance

All three groups learned the Step-Down Passive Avoidance task in approximately the same number of trials (young controls in 4.0 ± 0.4 trials, aged

controls in 3.7 ± 0.2, and aged transgenics in 5.1 ± 1.3). The number of trials was calculated only from the first trial that the animal received a shock, and any previous trials were not included. Figure 9 reports the first retention trial of both the 24 hour retention day and the one month retention day. A Group x Day ANOVA on average step-down latencies indicated that there was no significant main Group effect ($F=1.53$, $df=2,23$, $p=0.2377$), and there was no significant main Day effect from 24 hour to one month time ($F=2.26$, $df=1,23$, $p=0.1464$). Since mice could meet the testing criterion on the 24 hour retention day without receiving a shock, a Fisher's Exact Test was used to indicate that receiving a shock on the 24 hour retention day did not effect retention at one month (two-sided $p=1.00$).

II.B.2.d. Thatcher-Britton Novelty Conflict

The aged transgenic mice demonstrated the longest average latency to commence eating in the Thatcher-Britton Novelty Conflict paradigm (Figure 10), and were significantly greater than the young control mice by analyzing data in a oneway ANOVA ($F=3.685$, $df=2,24$, $p=0.0402$; $p<0.05$ by subsequent Newman-Keuls test). There was no difference between the aged control and the aged transgenic mice, nor was there a difference between the aged control and young control mice. Approaches to the food were analyzed in a separate oneway ANOVA. The number of approaches were not statistically different among groups (young controls 4.1 ± 0.8, aged controls 5.7 ± 0.7, and transgenic mice 6.6 ± 1.1; $F=1.811$, $df=2,24$, $p=0.1860$). There was no linear association between scores of anxiety from the Novelty paradigm and scores of correct performance from the T-Maze for individual animals (Pearson product moment correlation $r(23)=-0.1823$, $p=0.4051$).

II.B.2.e. Locomotor Ability

The Group x Time ANOVA was performed on locomotor activity photocell counts, indicating a significant main Time effect ($F=44.47$, $df=5,125$, $p<0.0001$). Activity decreased in all groups as time progressed (Figure 11) for the average hour duration, but there was no significant Group effect ($F=0.42$, $df=2,25$, $p=0.6603$).

II.B.2.e. AChE Staining

Multiple AchE values of each brain region were averaged for each animal and then those means were averaged to obtain a value indicative of the group. Separate oneway ANOVAs were performed for each region. No lateralization for AChE staining occurred, so all left and right values were combined. No differences existed among the young controls, aged controls, and aged transgenics for all levels of cortex (figure 12). Significant differences occurred at the CA1 level ($F=6.140$, $df=2,8$, $p=0.0242$) and CA2 level ($F=11.676$, $df=2,8$, $p=0.0042$). Aged transgenics had significantly lower AChE levels in the CA2 region than young controls ($p<0.01$ by *post hoc* Newman-Keuls), and significantly lower AChE levels in the CA1 ($p<0.01$) and CA2 ($p<0.05$) regions than aged controls. No differences in the CA3 subfield or NBM were discerned.

II.B.2.f. ChAT Activity

Hippocampal and cortical ChAT measures were treated in separate oneway ANOVAs. There were no differences in results between methods of tissue preparation for the ChAT assay (quick freeze versus perfusion with PBS), so the seven month old transgenic groups were pooled. ChAT activity (Figure 13) of seven month old control, seven month old transgenic, and eighteen month old transgenic mice indicated no significant differences in either the cortex (39.57 ± 2.20 n moles Ach/mg protein/hr, 37.66 ± 1.82, and 37.93 ± 3.32, respectively; $F=0.2113$, $df=2,25$, $p=0.8109$) or hippocampus (25.29 ± 2.89, 25.61 ± 1.30, and 29.08 ± 1.14, respectively; $F=1.053$, $df=2,23$, $p=0.3650$).

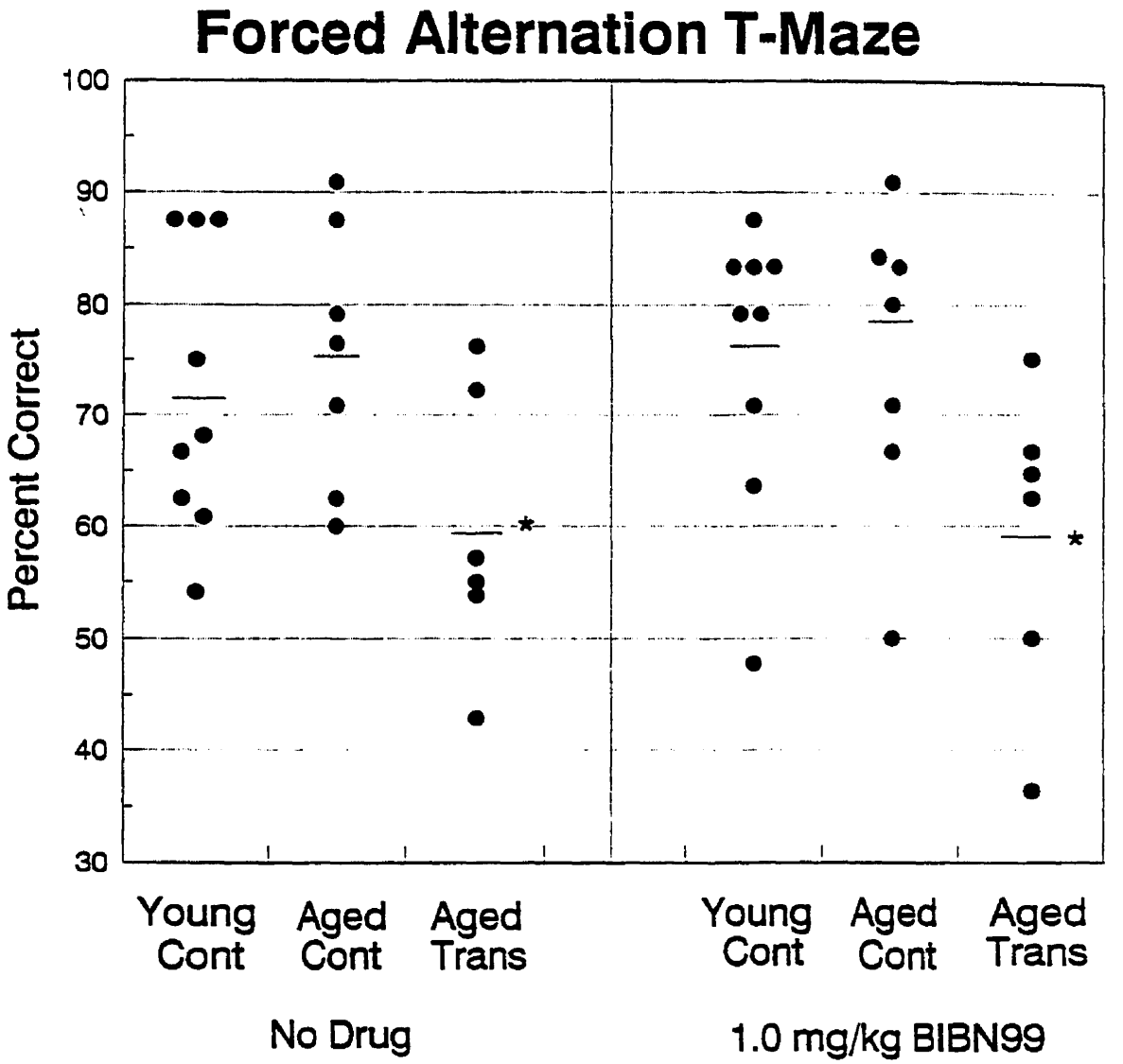

Figure 7. Performance on Forced Alternation T-Maze. Each point represents an animal's percentage of correct responding based on the trials run over four days of testing. Mean performance for each group is indicated by a horizontal line. Transgenic mice (n=6) were significantly inferior to aged controls (n=8) during non-drug testing [p=0.0336], and to both aged [p=0.0108] and young controls (n=9) [p=0.0250] during 1.0 mg/kg of BIBN99 administration.

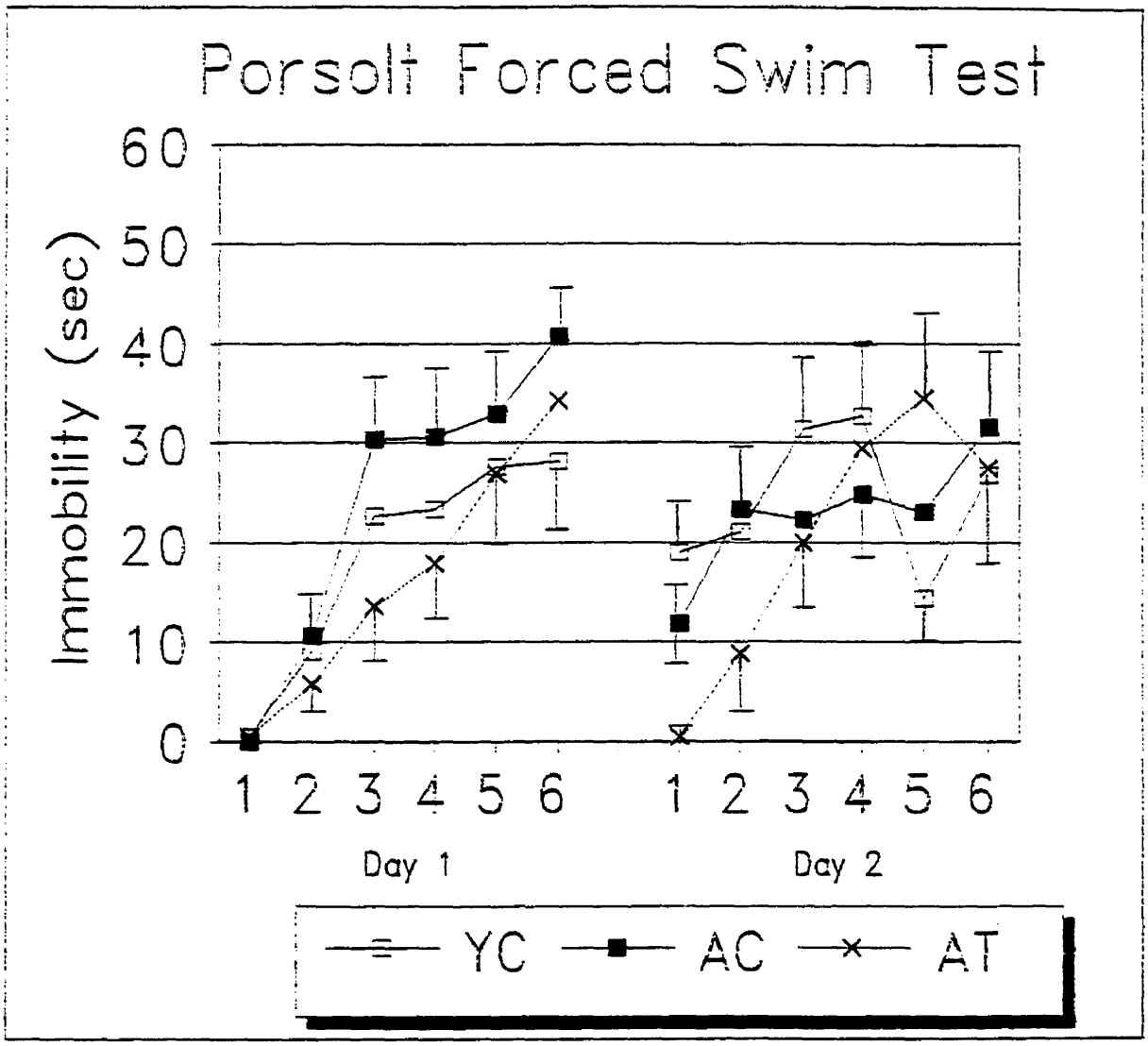

Figure 8. Porsolt Forced Swim Test. Data (mean ± SEM) indicates the average immobility for each group across the six minutes of day one (acquisition) and day two (retention). No differences in total immobility occurred among transgenic mice (n=8), young controls (n=9), and aged controls (n=10). Only the transgenic mice has a significant increase in immobility across minutes on day two [$p<0.0001$].

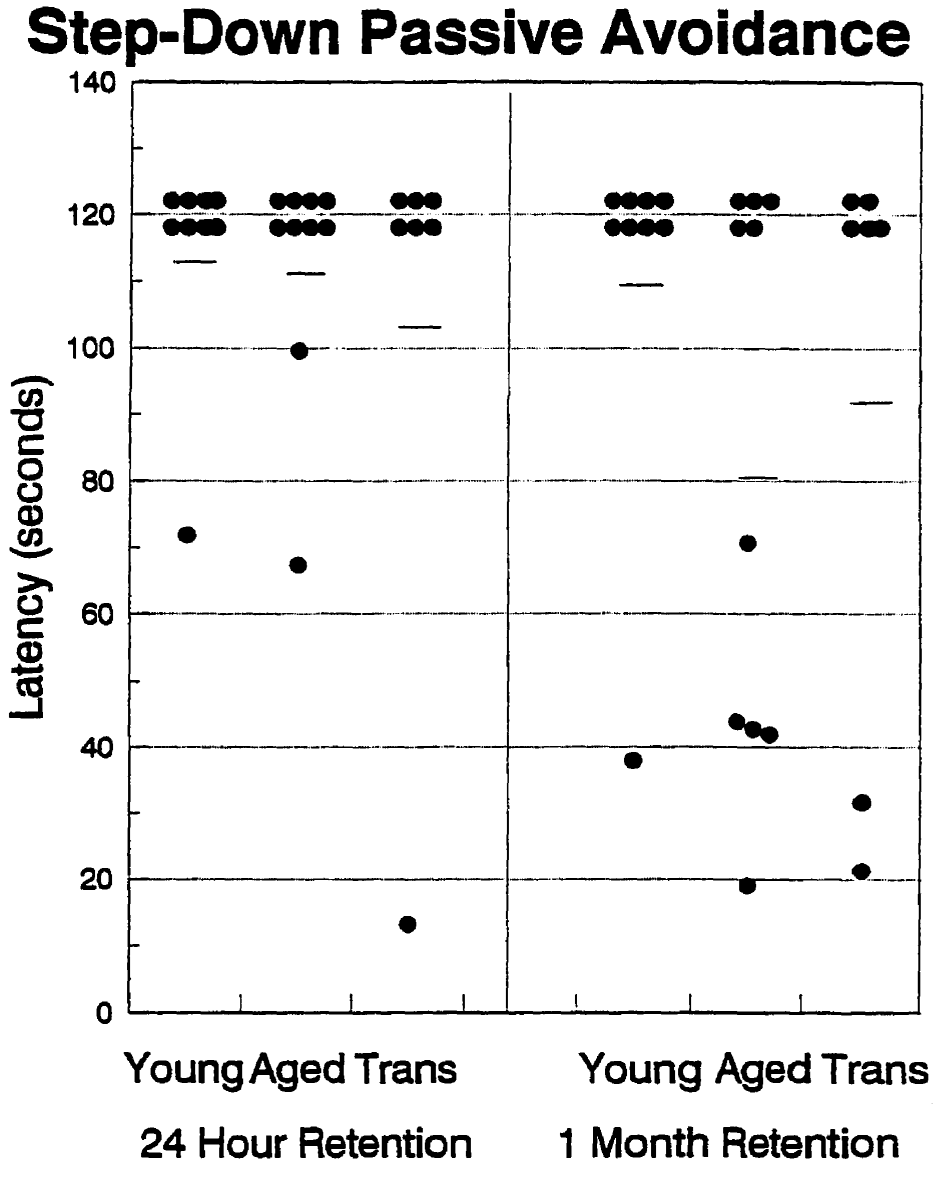

Figure 9. Task retention 24 hours and one month after initial acquisition of the Step-Down Passive Avoidance paradigm. Data indicates the latency for each animal to step down on the first trial of either testing day, the maximum time allowed being 120 seconds, and group means are indicated by a horizontal line. No significant differences among transgenic mice (n=8), young controls (n=9), and aged controls (n=10) were evident.

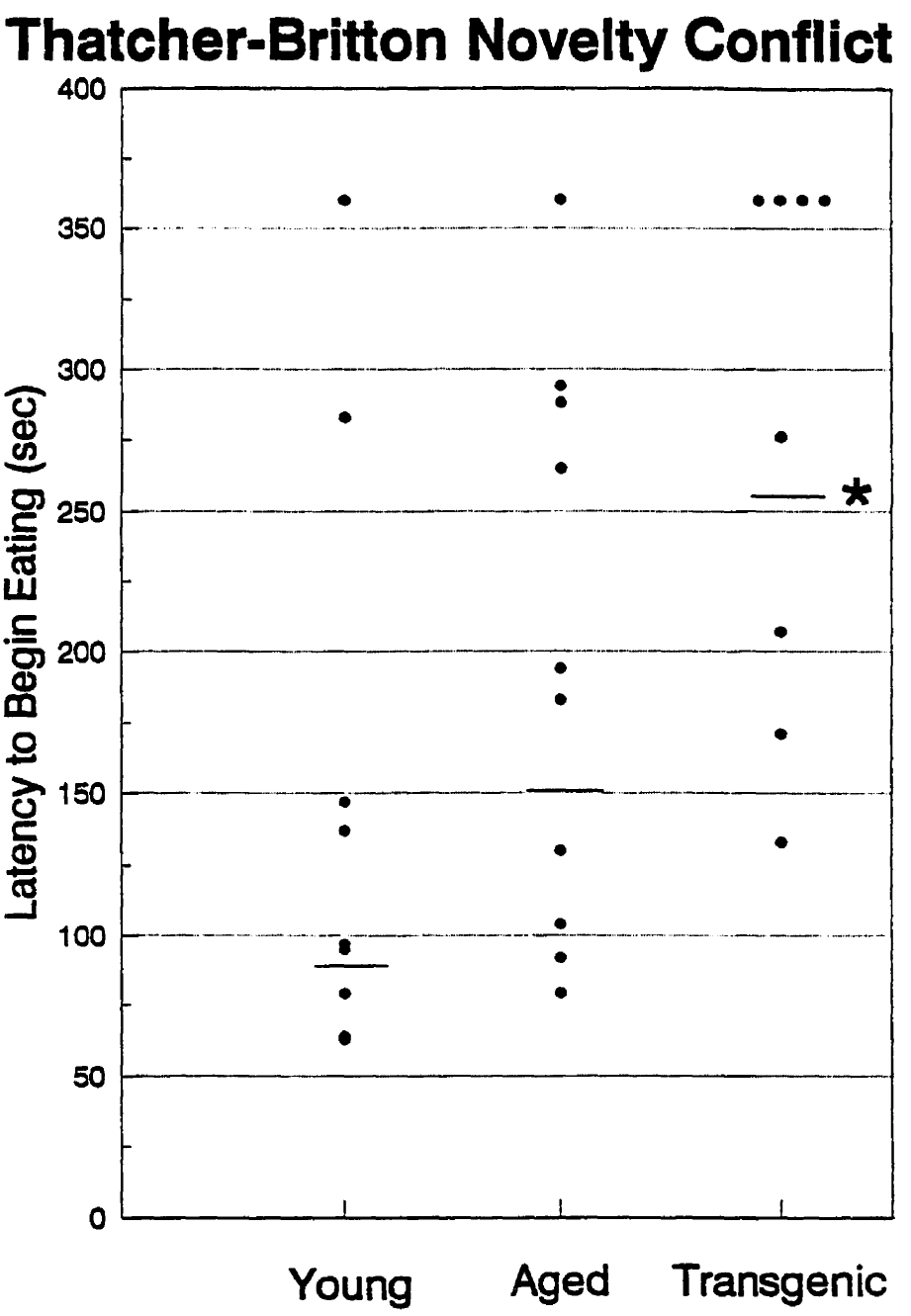

Figure 10. Latency for each animal to commence feeding in the Thatcher-Britton Novelty Conflict paradigm, with a maximum allowable time of 360 seconds. Means are represented by a horizontal line. Transgenic mice (n=8) took significantly more time than young controls (n=9), but not aged controls (n=10) [F=3.685, df=2,24, p=0.0402; p<0.05 by *post hoc* Newman-Keuls test]. There were no significant differences between control groups.

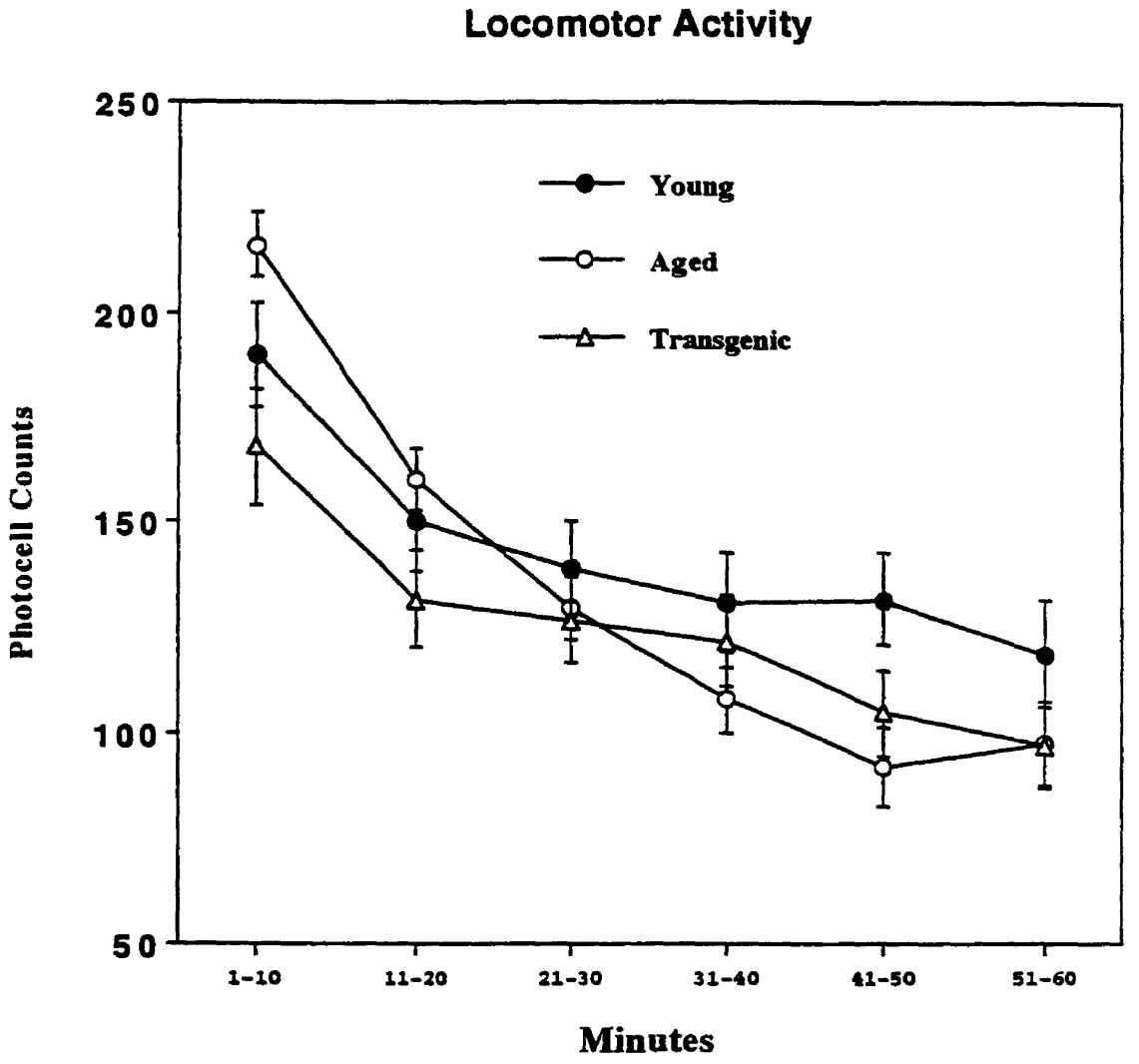

Figure 11. Average Locomotor activity throughout a one hour period divided into 10 minute bins, as measured by photocell beam interruptions. Data is represented as the mean ± SEM, and is the average of groups across four consecutive days. No significant differences among transgenic (n=8), young control (n=10), and aged control (n=10) groups occurred at any time; however, activity did significantly decrease with time for all groups [$F=44.47$, $df=5,125$, $p<0.0001$].

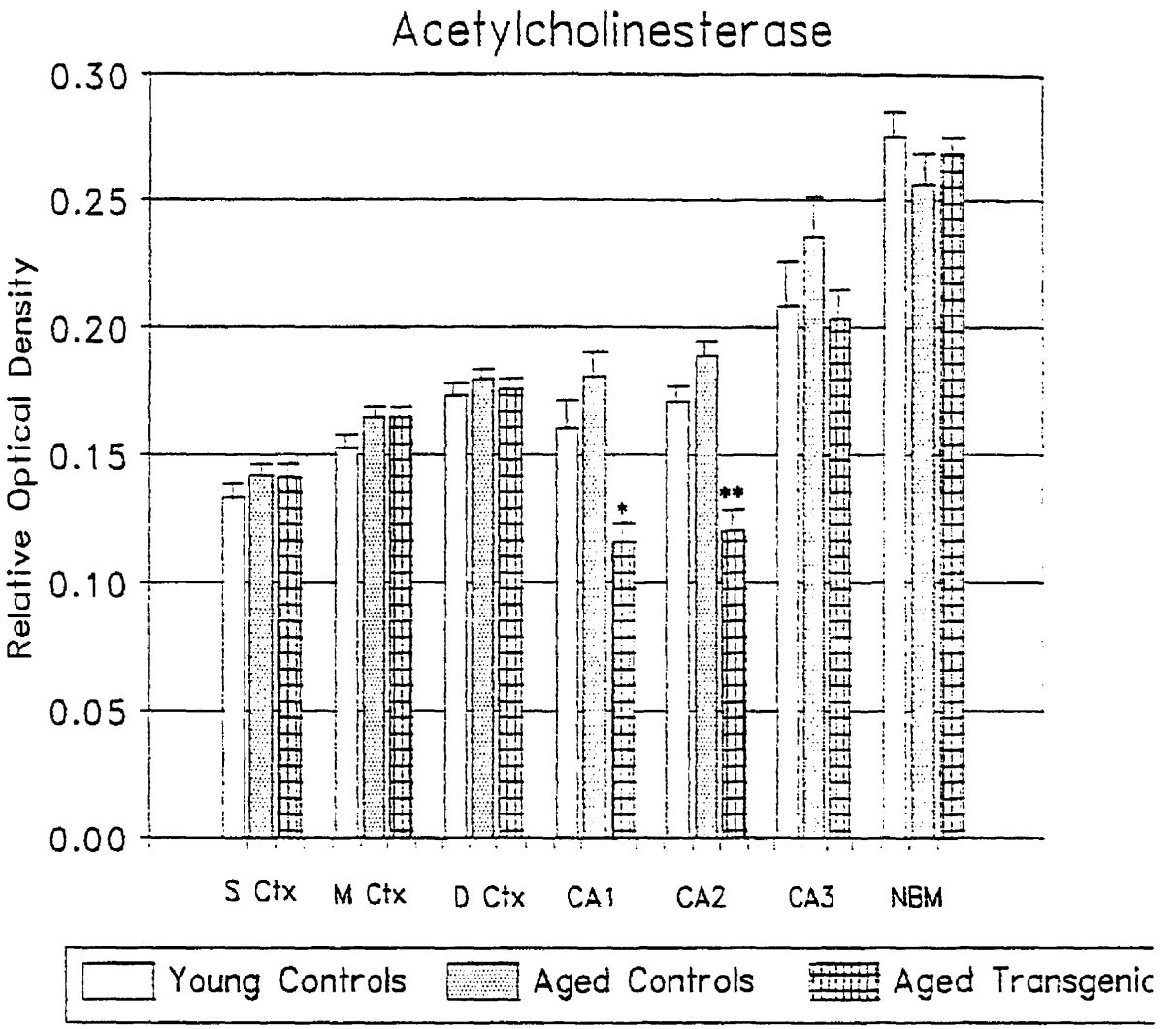

Figure 12. No differences in acetylcholinesterase levels were identified for superficial cortex (S Ctx), medial cortex (M Ctx), and deep cortex (D Ctx) in young controls, aged controls, and aged transgenics (n=9, 5, and 6 respectively). Nor were differences between groups found in the CA3 subfield of the hippocampus (n=8, 5, and 3) and in the NBM (n=8, 6, and 6). Aged transgenics (*) had significantly lower AChE levels in the CA1 subfield compared to aged controls (n=4, 5, 2; F=6.140, df=2,8, p=0.0242; p<0.05 by Newman-Keuls), and aged transgenics (**) had significantly lower AChE levels in the CA2 subfield compared to young and aged controls (n=4, 5, 2; F=11.676, df=2,8, p=0.0042; p<0.01 by Newman-Keuls).

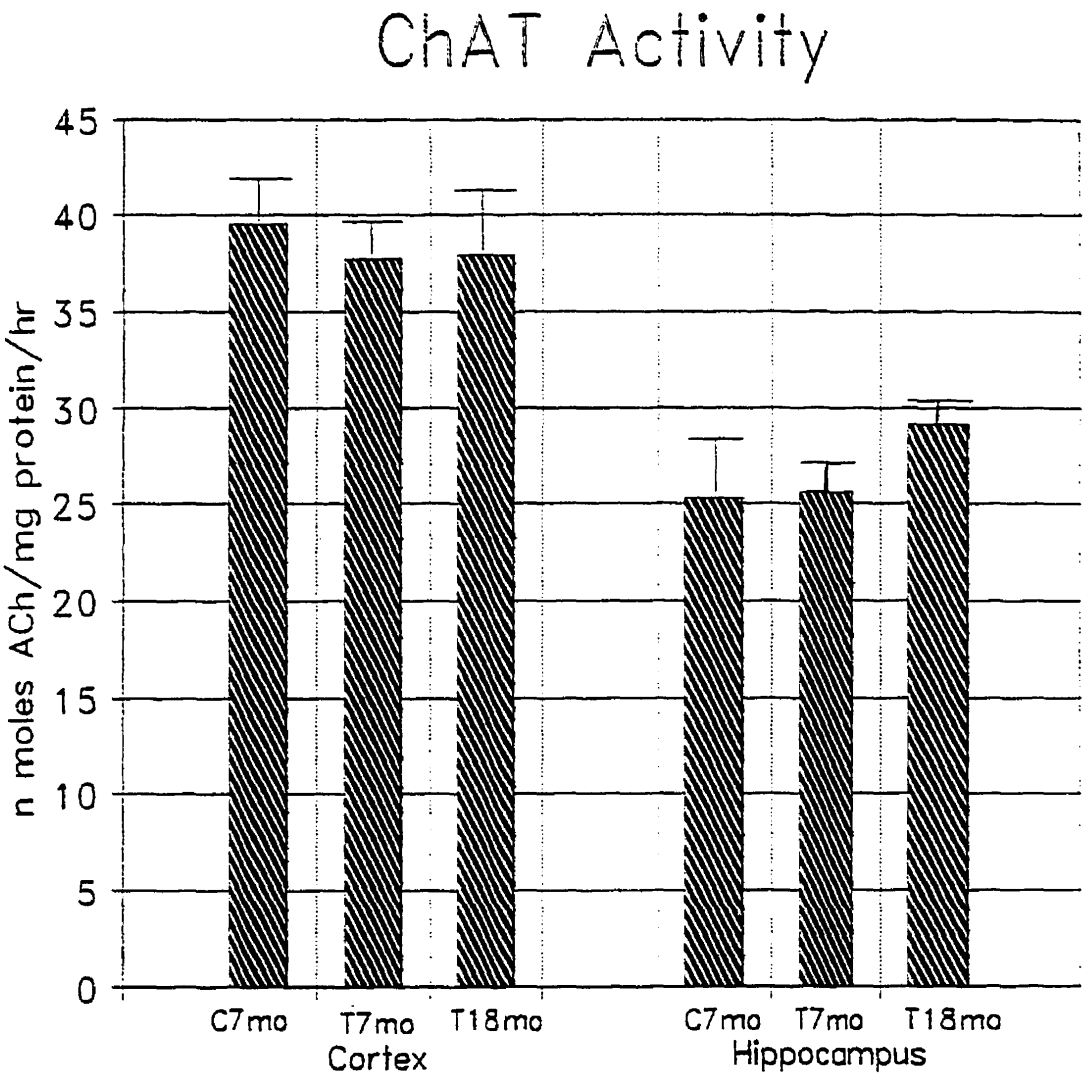

Figure 13. Choline Acetyltransferase Activity. Each column represents the mean (± SEM) moles of acetylcholine produced per milligram of protein per hour. No differences in bilateral cortical tissue ChAT activity occurred in seven month control (C7mo, n=10), seven month transgenic (T7mo, n=14), and eighteen month transgenic (T18mo, n=4) mice. No difference in bilateral (when available) hippocampal tissue ChAT activity occurred in seven month control (n=9), seven month transgenic (n=13), and eighteen month transgenic (n=4) mice.

II.C. DISCUSSION

The different tests used here provided measures of cognitive function and anxiety. The results indicated that the expression of the Aß containing C104 fragment of human ßAPP in transgenic mice may be detrimental to working memory or longterm retention. The mice performed poorly in the Forced Alternation T-Maze, the Porsolt Forced Swim test, and the Recognition Task. As well, transgenic mice appeared more anxious than control mice in the Thatcher-Britton paradigm. The reductions of AChE staining in the CA1 and CA2 subfields of the hippocampus are in keeping with the impairments of memory and the cell loss in CA1 described by Nalbantoglu et al (1996).

Although the aged transgenic mice did learn how to perform in the T-Maze, they had a retention deficit in spatial memory, in comparison to aged controls, when challenged with a long delay. This supports the spatial memory impairment reported by Nalbantoglu et al (1996) using the same mouse transgenic construct, and it is in keeping with the spatial impairments found in other amyloid transgenic models (Yamaguchi, 1991; Hsiao et al, 1995; Moran, 1995; Perry et al, 1995). One difficulty in this analysis is that, due to circumstance, each animal was not necessarily trained to a criterion of correct performance; rather, all groups after 18 days of training had an average correct performance of above 70%. This does not, however, affect the final results since the transgenic mice clearly demonstrated the largest average decline in performance.

Due to the limited animal availability, non-drug controls were not included in the drug designs. This limits the interpretation of the data in that the interval between the non-drug and drug tests cannot alone be ascertained for its effect on task performance. BIBN99 is a selective M2 antagonist (Doods et al, 1993a), which should increase cholinergic release in terminal fields such as cortex and hippocampus. Using slightly lower doses as the present study, BIBN99 has been successful in reversing spatial memory impairments in aged rats (Quirion et al, 1995). That the drug did not ameliorate cognitive performance in the aged transgenic group may be due to its inadequacy in compensating for the 20% cell loss in area CA1 of the hippocampus

(Nalbantoglu et al, 1996), and the loss of AChE in subfields CA1 and CA2, both of which are part of the intrinsic circuitry of the hippocampal formation. Four days of acute drug administration, however, may not be sufficient for BIBN99 to have a functional effect in the T-maze paradigm. Training the mice with chronic drug administration instead of acute doses at times of testing may be more efficacious in producing an ameliorative effect.

In the single trial recognition task, the adult transgenic mice appeared less able than controls to discriminate the novel object from the familiar object, indicating an impairment in working memory. Neophobia does not readily explain this behaviour since exploration of the blocks during the initial exposure was quantitatively and qualitatively similar between the control and transgenic mice. Neither group showed a left-right bias in either exploration or novel object preference. The recognition ratio is a pure measure of working memory, free of any reference memory components, such as rule learning (Ennaceur and Delacour, 1988). Working memory is considered a component of short term memory, accounting for the maintenance of transient information (Olton, 1983). The impairment of working memory in these adult transgenic mice may underlie the retention deficit of the aged transgenics.

The Porsolt, although originally intended to provide measures of affect, such as behavioural despair, is increasingly being interpreted as a test of cognition--the retention of acquired immobility (Hawkins, 1978, 1980; Jefferies et al, 1984, 1985; Velhuis et al, 1985; De Pablo et al, 1989). The adult transgenic mice were less immobile than the adult control mice, and although both groups showed steadily increasing levels of immobility across minutes of both days, the transgenic group never attained the level of immobility of the controls. Assuming that the Porsolt measures the acquisition of an adaptive strategy, either to conserve energy or simply upon realization of the maze being inescapable, these data indicate a possible learning impairment. Although this slowed learning may be accounted for as an increased general activity, no group differences in locomotor activity boxes were obtained.

The results indicate that overall immobility on both testing days was not statistically different among aged transgenic, aged control, and young control groups.

De Pablo et al (1989) have indicated that the first testing day may measure the acquisition of a strategy and the second day may measure the retention of that strategy. As is demonstrated in Figure 8, on the second day there is an almost complete lack of immobility for transgenics during the first minute. The aged transgenics were the only group to show a significant effect of increasing immobility across the six minutes of the second day of testing, likely due to their levels of immobility reverting back to the low durations of the first day -- in other words, these data may indicate an impairment of retention that occurred over the twenty-four hours between tests. This reinforces the conclusion that the transgenic mice have a specific memory impairment of retention, but that this deficit is not limited to spatial memory.

It is peculiar that the young controls, aged controls, and aged transgenics all attained levels of immobility comparable to the adult transgenics, but that the adult controls had much higher levels of immobility (compare Figure 8 to Figure 2). Although this might be explained by a hypoactivity of the adult control group, in which case the adult transgenic deficit in this task is merely an artifact, the lack of differences in activity levels (from locomotor activity boxes) between the adult groups does not bear this hypothesis out. Perhaps the differences in control results are due to an age factor or changes in test surroundings. Regardless, the adult transgenic deficits are extremely significant, and neither the data for the control nor the transgenic groups had large variances, indicating that the results are not skewed by some outliers.

The Passive Avoidance paradigm is designed to eliminate a response that occurs normally and frequently–in this case, stepping down from a wooden block to explore. In the single-trial paradigm, an acquisition deficit would result in poor performance immediately after the shock trial (implied by shorter latencies to step off the block since longer latencies are interpreted to mean an association has formed between stepping off the block and receiving a shock), while a retention deficit would manifest itself in a time-dependent decline of performance. The seven month old transgenic and control mice were tested in a single trial passive avoidance paradigm to see if the transgene would have an effect at this early age in a design that is often used to assess age-related decline (Dean, 1981; Kubanis, 1981). The obtained data are

interesting as they appear to run contrary to expectation. Both adult controls and transgenics remain on the wooden platform longer after 48 hours than immediately after receiving the footshock. A negative slope reflecting memory decay, as opposed to a positive slope reflecting consolidation, was expected. Possibly, the experiment was not carried on long enough, and in the subsequent days stepdown latencies would have dropped. Regardless, control and transgenic groups only approached a significant difference ($F=3.79$, $df=1,14$, $p=0.0719$). Perhaps both groups became conditioned to avoid, not the footshock, but rather their own removal immediately after stepping down onto the grid. While the shock was not present after its initial administration, removal may have been a conditioning stimulus at each test. In this way, both groups remained on the block longer after each test and provided a false indication of their memory of the aversive electric shock.

Using a multiple trial paradigm, aged transgenic mice and aged controls performed at young control levels in the Passive Avoidance task, and even remembered after a one month delay not to step down from the wooden block. This would appear to contradict the previously mentioned conclusion of an impairment in retention. While some studies have outlined that retention of single-trial passive avoidance task is the most clearly effected in aged mice (Dean, 1981), this study made use of multiple shock trials. The use of multiple trials as opposed to a single trial has been shown to occasionally negate any age effects found in the Passive Avoidance paradigm (Bartus, 1982a). This does not entirely explain the results, however, since the aged transgenic mice were able to learn the T-Maze task to criterion performance and the impairment manifested itself after the mice were challenged with a long delay. A possible explanation is that the Passive Avoidance task is not as cognitively challenging because the actual task is made easy by the salient reinforcement of the electric shock. However, since all the groups did achieve criterion performance, this highlights the fact that the impairment is not attributable to the acquisition or learning aspect of their deficits.

The Thatcher-Britton Novelty Conflict paradigm provides an assessment of anxiety, in which the motivation of hunger is in conflict with the exploratory

behaviour in a novel environment (Britton, 1981). The seven month transgenic mice were significantly more anxious than seven month old controls in the Thatcher-Britton. The aged transgenic mice were more anxious than the young controls but not the aged controls. This increased degree of anxiety, then, cannot be interpreted as an effect of the amyloid transgene alone, but may be the result of the interaction of the age factor and the expression of amyloid. Increased anxiety with age, however, is not consistently found in other animal models of senescence (Ammassari-Teule et al, 1981; Sarter, 1986; Miyamoto, 1991; Lamberty et al, 1992), some indicating a decrease and some an increase. The adult control latencies to begin feeding were comparable to the young control latencies. Although not statistically significant, the adult transgenic mice have slightly elevated levels of anxiety over the aged transgenic mice. The trend that emerges is one of low anxiety in younger controls, slightly higher anxiety in aged controls, followed by aged transgenics, and finally adult transgenics demonstrating the most anxious behaviour.

The elevated plus maze is an ethologically valid model of anxiety likely based on the fear of open spaces (Handley et al, 1993). Seven month old transgenic and control mice performed similarly in the elevated plus maze, indicating similar levels of anxiety. Each possible measure of anxiety, including the latency to step out onto an open arm and the time spent on the open arms, indicated no differences between the two groups, as opposed to the results of the Thatcher-Britton. These two tasks both purport to offer a measure of anxious behaviour, but differences in results may emphasize an underlying difference in the requirements of the tasks. It may be that the lack of a goal-oriented motivational factor in the elevated plus maze did not allow differences in the groups to become apparent. More sensitive measures, such as observation of "risk assessment behaviours", might have been utilized (Dawson et al, 1995). The difference in task requirements is further supported by the Recognition Task results, where again, the task is not appetitively motivated. Controls and transgenics did not differ in their exploration of objects in a novel environment. This emphasizes that anxiety did not necessarily disrupt performance in any of the cognitive tasks.

Overall mobility in the locomotor activity boxes was similar between aged controls and aged transgenics, thus providing an indication that gross locomotion is not impaired due to the cumulative effects of amyloid expression over time. No differences between control and transgenic groups occurred at any age during their light cycle in the locomotor boxes, and since all behavioural testing was completed during day hours, differences in motor-activity cannot explain the impairments obtained in the other behavioural tests; however, a lowered nocturnal activity was measured in other functional studies for mice transgenic for both the 695 and 751 ßAPP isoforms (Yamaguchi et al, 1991; Moran et al,1995; Perry et al, 1995). It is peculiar, though, that the adult groups had much higher activity levels than the aged and young groups (compare Figures 5 and 11). Although this is opposite to activity levels in the Porsolt, it is likely due to an age effect or differences in the test surroundings. The adult mice were additionally tested for fine motor control, such as limb strength and dexterity, and the adult transgenic mice showed deficits in relation to adult controls. This is not clearly due to intrinsic motor impairment, but may be due to the adult transgenic mice having a statistically greater body weight, which poses difficulties in a test requiring suspension from a string by the forepaws. The cause of this difference in body weight is likely attributed to the adult control mice being raised to seven months in a different environment and then shipped by air to our lab, and was not necessarily a factor of the transgene. If it were, the aged controls and aged transgenics, which were both raised in the same environment, would be expected to have differences in body weight, but none existed.

Inbred mice with a C3H background have been well documented to be carriers of an autosomal recessive mutation (rd) which causes selective degeneration of the photoreceptor layer of the retina (Sidman, 1961; Sidman et al, 1965). Blindness in mice is not easily detected by casual observation alone, as they have other well developed sensory resources. Drager et al (1978) found that young C3H mice had good eyesight while the older mice were practically blind, with a central scotoma developing by three weeks of age and the periphery remaining responsive for several months. Although visual acuity of our B6C3/F2 hybrids could be a contributing factor

to the impairments demonstrated in several of the tasks, Nalbantoglu et al (1996) demonstrated that the transgenic mice did improve on the visual platform portion of the swim maze, indicating that the memory impairments in their cohort of transgenic mice were not likely a product of poor visual ability. In our experiments, no direct tests of visual ability were conducted and this must be considered in the interpretation of the results; however, it is also not clear how much of the performance in the T-Maze relies on visual cues, because only two directional choices are possible and olfactory cues may be sufficient to locate the reward. Other tests, with the exception of the Recognition Task, appear not to rely on visual information.

It is noted that while an aged population of mice will show cognitive decline, there is heterogeneity in the degree of impairment (Gower et al, 1993). Ammassari-Teule et al (1994) suggest that reference memory, not working memory, is mildly impaired in aged C57BL/6 mice. Certainly, Nalbantoglu et al (1996) have demonstrated retarded learning of spatial relationships in these transgenic mice, and that one of the specific impairments found in the Morris Swim maze was age-dependent. While this is important to remember, our results did not clearly demonstrate any age-specific impairments in the cognitive tests. Experimentation with young transgenic mice is necessary to clarify any age-dependent deficits that interact with the expression of ß-amyloid.

Since the pathology of the transgenic mice did not include actual amyloid plaques, and yet clear behavioural impairments were measured, these results raise the question of whether the amyloid plaques themselves are necessary for the cognitive impairments in humans with AD. Other studies have found behavioural impairments without overt amyloid pathology (Yamaguchi et al, 1991; Hsiao et al, 1995). Our transgenic mice were found to have a 20% cell loss in the CA1 subfield of the hippocampus, extracellular amyloid immunoreactivity, gliosis identified with GFAP, and microglia identified MAC-1 (Nalbantoglu et al, 1996). Anoxic damage to CA1 has been previously linked to anterograde amnesia in human subjects (Zola-Morgan et al, 1986), and the memory impairments of the Aß transgenic mice may be well explained by the damage to CA1. Several studies have demonstrated that severity of

dementia does not correlate well with plaque density (Price et al, 1991; Samuel et al, 1994; Bierier et al, 1995). Although speculative, mature amyloid deposits may not be necessary to disrupt neuronal activity. Certainly, abnormal processing of the amyloid precursor protein, without mature amyloid deposits, may be sufficient to induce learning impairments in rats (Beeson, 1994). Actual ventricular and hippocampal injections of synthetic peptides homologous to amyloid ß-protein resulted in impaired retention in several different behavioural paradigms when the injections were made immediately post-training and not at a later time (Flood, 1991; McDonald, 1994).

In people with AD, reductions in the neocortex of ChAT, AChE, somatostatin, corticotrophin releasing factor, and serotonin have been measured, but ChAT activity was most tightly correlated with the severity of dementia (Bierier et al, 1995). This highlights the importance of the cholinergic system in cognition. In rats, the cholinergic system has been related to cognitive function, including attention, learning, and memory (Smith, 1988). Correlational studies, between behavioural impairments and cholinergic activity in aged mice, have demonstrated that decreased acetylcholine content in frontal cortex, hippocampus, and posterior cortex is related to impairments in spatial memory (Ikegami, 1994). Aged C57BL/6J mice show reduced AChE and ChAT activity in hippocampus (Vijayan et al, 1977), but neither appeared altered in our aged B6C3 mice. In order to address the possibility that a cognitive-related pathology involving acetylcholine may account for the impairments in the Aß transgenic mice, analysis of their central cholinergic system was necessary. ChAT is localized in nerve terminals and terminal end particles. Our measures were taken from complete cortices and hippocampi, both of which are terminal field projections stemming from the basal forebrain and other regions. Without measures of ChAT activity in NBM and septum, areas analogous to the human brain nbM and septum, both of which are areas of the basal forebrain that tend to be severely affected in AD patients (Whitehouse et al, 1981, 1982), discussion of the functional significance of the lack of change in ChAT levels in terminal fields is limited. Reductions of 40-90% of ChAT activity have been identified in the hippocampus, temporal cortex, and amygdala of AD subjects (Perry et al, 1978, 1986; Davies, 1979; Araujo et al, 1988;

Aubert et al, 1992), especially those homozygous for the apoE ϵ4 allele (Poirier, 1995). It is likely that the loss of AChE in CA1 and CA2 of our transgenic mice is indicative of related cell damage in those areas (Butcher and Marchand, 1978; Emson et al, 1979) and implies impaired hippocampal circuitry, which is in agreement with the findings of Nalbantoglu et al (1996), but that there was some compensatory ChAT expression. Although some studies have documented reductions in AChE in hippocampi of AD patients (Davies et al, 1979; Henke et al, 1983), increases of AChE activity have also been found in the dentate gyrus (Geddes et al, 1985; Hyman et al, 1987; Senut et al, 1991). The increase in AChE staining has traditionally been interpreted as evidence of compensatory proliferation of cholinergic septohippocampal fibres, but recent evidence casts doubt on this theory (Aubert et al, 1994). The stability of the neural structures in the cortex, basal forebrain, and areas other than CA1 of the hippocampus, and the status of M1 and M2 receptors, are not known for these transgenic mice. Profound cholinergic cell loss occurs in the basal forebrain (Whitehouse et al, 1981, 1982) and in layers II and III of the entorhinal cortex (Hyman et al, 1987) of AD subjects. Presynaptic M2 receptors also decrease in the neocortex, while postsynaptic M1 receptors are unaltered (Ladinsky et al, 1990). Severity of dementia in AD is correlated with altered presynaptic markers (Perry et al, 1978) and cortical neuronal loss (Neary et al, 1986). The loss of CA1 neurons and AChE in the Aß transgenic mice is a preliminary step toward finding some neuropathological explanation of the behavioural impairments. As well, Nalbantoglu et al (1996) have also demonstrated that the Aß transgenic mice show poor maintenance of hippocampal long-term potentiation (LTP), which has been implicated as a possible mechanism of spatial memory formation (Morris et al, 1986).

The lack of a control group with a different transgene necessitates mention of the possibility that any extra genomic DNA might disrupt cognitive ability, and it is not the expression of amyloid itself that caused the behavioural impairments. This is unlikely, however, since Nalbantoglu et al (1996) included a control group with a chloramphenicol acetyltransferase gene to replace the ßAPP fragment of the transgenic cohort, all under the control of the same NF-L promoter, and the amyloid transgenic

mice were spatially impaired relative to these controls.

We are aware of few functional studies using mice transgenic for ß-amyloid. Mice with a ßAPP695 construct were shown to have impairments of spatial learning in the Morris Swim Maze (Yamaguchi et al, 1991) and the Spontaneous Alternation Y-maze (Hsiao et al, 1995), although this may have been confounded by decreased motor activity in one study (Yamaguchi et al, 1991). Transgenic mice with a ßAPP751 construct were also found to be impaired in the Morris Swim Maze and Spontaneous Alternation Y-Maze, indicating specific deficits in the acquisition of tasks requiring spatial and working memory (Moran et al, 1995; Perry et al, 1995). Nalbantoglu et al (1996) found a spatial memory impairment with the Morris Swim Maze using the same construct of transgenic mice as in this present study. Taken together, although Moran et al (1995) also implicate abnormal tau protein, these results support a possible role for amyloid in the intellectual decline of AD patients, especially if preliminary evidence indicates the preferred accumulation of ß-amyloid in areas that are necessary for the functions most impaired in these transgenic mice.

We do not presume that these aged transgenic mice are manifesting symptoms that are qualitatively similar to AD, yet there are certain parallel features that these mice may be expressing. Impairments of memory, for example, have been well documented in amyloid expressing mice, possibly indicating that the neural substrate necessary for memory is being affected in these mice in ways similar to that in AD patients. These aged transgenic mice also omitted many more trials than young controls during T-maze testing, even though all groups were similarly food-deprived and maintained at the same percentage body weight. Further, the female adult transgenics remained on the passive avoidance platform, demonstrating little of the exploratory behaviour the other mice demonstrated prior to stepping off the block. Although it is peculiar that all were female, this may be in keeping with the increased omissions demonstrated by the aged transgenics in the T-Maze, and can tentatively be considered a lack of motivation. Whether this lack of motivation was due more to anxiety than passivity, is not entirely clear, but toward the end of training, the trials that the mice did run were run with a fairly high correct performance–which would be

unlikely if anxiety was interfering with acquisition of spatial memory. So the aged transgenic mice may be concurrently more anxious and less motivated than the young controls, paralleling the personality alterations seen in humans with advanced AD, albeit a possible yet unclear interaction between aging and amyloid expression in the transgenic mice. Conclusive evidence of this would require the administration of an anxiolytic to monitor its interaction with the anxiety in the transgenics.

Three general hypotheses can apparently account for the results of the present study. The first theory proposes that the outlined behavioural deficits are truly pure cognitive impairments. The second and third possibilities encompass the theory that motivational factors differ between the transgenic groups and their respective controls, and that the cognitive impairments are secondary. Large differences in anxiety could account for poor performance in most of the mazes. Alternatively, age related perseveration or passivity can lead to false interpretation of many of the tasks. Undoubtedly, the latter two possibilities have some role in the behaviour of the transgenic mice, and the results lend some credence to each theory. Overall, however, the experiments best fit a model that supports amyloid in a neurotoxic role that leads to cognitive decline.

Future work will endeavour to tease apart the roles of anxiety and passivity and cognition. Simple experiments to dissociate anxiety and passivity could be performed with anxiolytic drugs in the anxiety paradigms. Measures of defecation, freezing behaviour, and exploratory behaviour could be incorporated for greater sensitivity. Fully controlled simple cognitive tasks testing a more diverse age range of mice, without incorporating appetitive motivation, would clarify the role of cognition. Larger sample sizes are recommended for controlled pharmacological testing as well as for more complete analysis of pre- and post-synaptic neuronal cholinergic markers.

III. Apolipoprotein E Deficient Model

This experiment was conducted to assess if a deficiency of the apolipoprotein E cholesterol transporter would result in behavioural impairments at six months of age in the natural lifespan of a C57BL/6J mouse, and if these impairments could be ameliorated with administration of tacrine, an AChE inhibitor.

III.A. MATERIALS AND METHODS

III.A.1. Animals

C57BL/6J controls (10 males) and apoE deficient mice (10 males) at 7½ months of age were provided by Dr. J. Poirier (Douglas Hospital Research Centre). The apoE mice were created by injecting targeted clones into C57BL/6J host blastocytes, transferring back to surrogate mothers, and backcrossing to C57BL/6J to produce homogeneity (Plump et al, 1992).

Controls had a brown coat colour while apoE mice had pale beige coats, which required dyeing fur black to ensure enough contrast to be properly monitored by the Swim Maze video tracking equipment. All mice were maintained on a 12 hour light-dark cycle (on at 8:00 am) in temperature (22° C) and humidity (45-70%) controlled rooms. Mice were housed separately in groups of 5. All had *ad libitum* access to both standard laboratory mouse chow and drinking water (see section III.A.2).

III.A.2. Drugs

Tacrine (0.2 mg/ml) was delivered through the drinking water, to which 0.02% sodium saccharin was added. All groups were habituated to the saccharin solution for four days, following which drug groups had tacrine added. Non-drug groups were maintained on the saccharin solution alone for the test period.

III.A.3. Behavioural Testing

III.A.3.a. Morris Swim Maze

This task requires mice to locate a platform submerged 1 cm below the water surface in a 1.6 m diameter pool. The water (22° C) was made opaque by the addition

of milk powder. Tracking of the mice was accomplished by an RCA video camera hung above the pool and a VIDEOMEX-V (Columbus Instruments, Columbus, Ohio) video image analyzer. Four positions around the edge of the pool were designated as north, south, east, and west, thereby dividing the pool into four quadrants. The platform remained in the same north-east quadrant throughout testing. Each mouse received sixteen training trials over four days, being placed into the pool at the four major compass points in a randomized order each day. Trials lasted a maximum of 120 seconds, at which time if the mouse had not located the platform it was placed on it for 5-6 seconds. The animals were to solve the task using distal cues placed around the testing room. A test probe trial, without a platform and lasting 30 seconds from when the mice were placed at the west position, and a control visible platform trial, during which the platform was elevated 1 cm above water level, were given 24 hours following the last day of training.

III.B. RESULTS

Swim Maze data were analyzed in a Group x Day x Drug ANOVA. Irrespective of drug treatment with tacrine, a main Group effect occurred such that apoE transgenic mice were significantly slower than controls in locating the hidden platform across training days (Figure 14; $F=10.06$, $df=1,15$, $p=0.0063$). Tacrine did not alter performance in either controls or apoE mice ($F=0.90$, $df=1,15$, $p=0.3575$), but there was a significant decrease in latency to find the platform for all groups across the four training days ($F=21.08$, $df=3,45$, $p<0.0001$).

Twenty-four hours following the final training day, during the probe trial (Table 3), results of a Group ($F=2.35$, $df=1,15$, $p=0.1459$) x Quadrant ($F=1.40$, $df=3,45$, $p=0.2548$) x Drug ($F=2.35$, $df=1,15$, $p=0.1459$) ANOVA indicated that neither controls nor apoE deficient mice preferred the training quadrant over any of the other quadrants. Distance measures and visible platform data on the probe day were analyzed in separate Group x Drug ANOVAs. In distances swum on the probe trial, there were no significant effects of Group ($F=0.31$, $df=1,15$, $p=0.5844$) or Drug ($F=0.31$, $df=1,15$, $p=0.5859$). There were also no significant effects for Group

(F=3.43, df=1,15, p=0.0838) or Drug (F=2.98, df=1,15, p=0.1050) for latencies to locate the platform on the visible trial.

	NW (sec)	T (sec)	SW (sec)	SE (sec)	Vis (sec)	Probe (cm)
Con	7.76(1.28)	5.74(1.25)	9.42(2.24)	7.12(2.40)	18.4(6.33)	786.2(76.47)
ConT	7.32(2.19)	7.44(2.15)	10.9(3.75)	4.32(1.60)	11.6(1.80)	851.8(63.25)
Apo	8.20(3.28)	6.05(2.09)	8.35(3.29)	7.40(2.17)	34.2(4.72)	748.7(99.81)
ApoT	9.54(2.51)	3.82(1.06)	10.3(3.63)	6.32(0.90)	19.2(7.46)	786.0(88.66)

Table 3 - Results of Probe Trial

Times spent in quadrants northwest (NW), northeast or training (T), southwest (SW), and southeast (SE), and the time spent locating the platform on the visible trial (Vis), were recorded for controls without drug (Con), controls with tacrine (ConT), apoE deficient mice without drug (Apo), and apoE deficient mice with tacrine (apoT). Data listed in table are mean (± SEM) in seconds (sec) except for the probe trial distances (Probe), which are reported in centimetres (cm).

Swim Speeds on the probe trial were not statistically significant for group or drug effect; however, the apoE deficient mice were significantly slower on the first day of training. A Group x Drug ANOVA was performed on distances and latencies of the second and third trials of that day, and a main Group effect occurred (F=24.71, df=1,15, p=0.0002). Due to apparent exhaustion, four apoE mice were removed from the maze before completing the last trial of the first day and were given a maximum latency of 120 seconds.

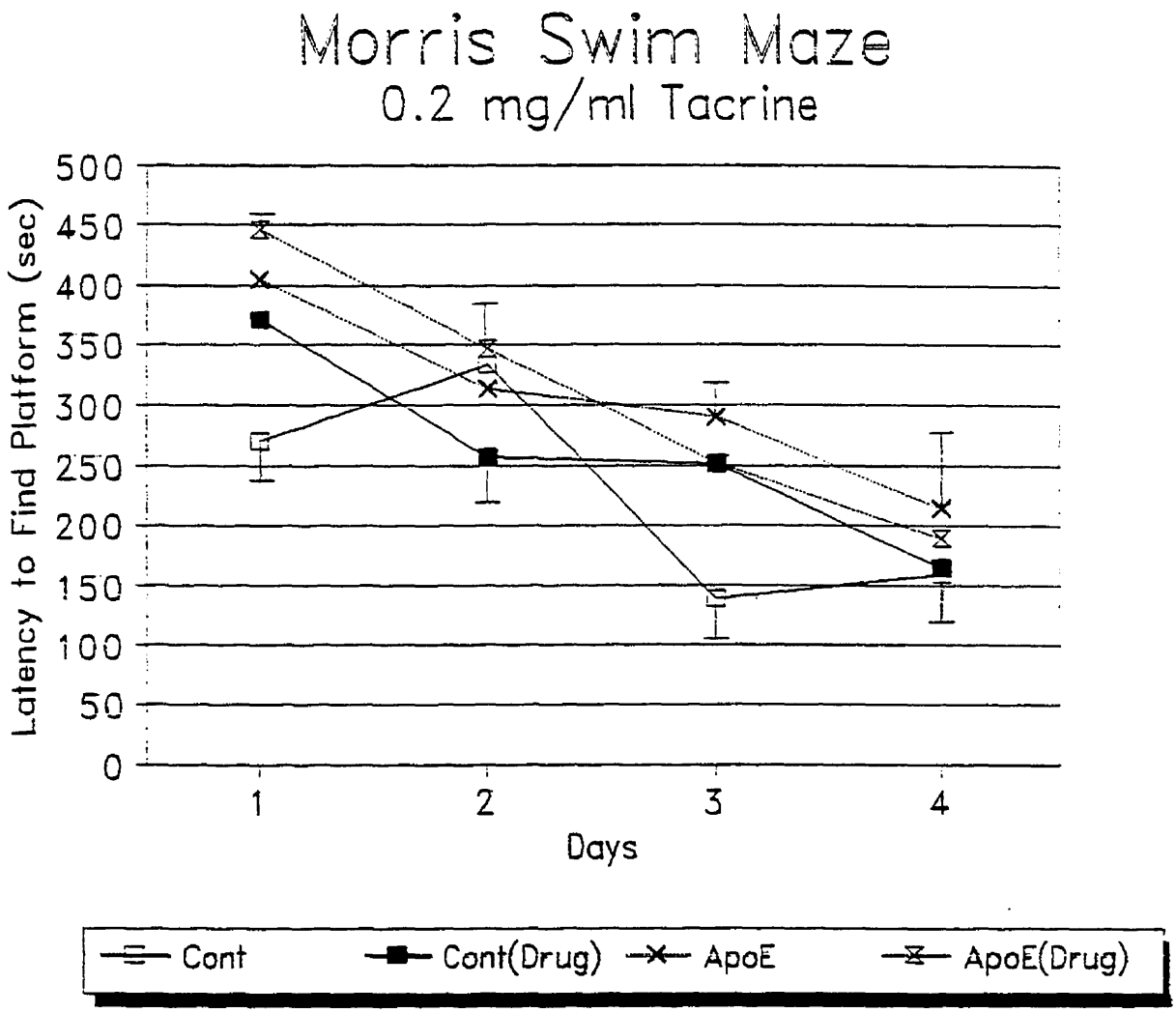

Figure 14. ApoE Mice in Morris Swim Maze. Data represents average latency of four trials per day (± SEM) to find hidden platform across four training days. Although controls (n=10) had significantly shorter latencies than did apoE deficient mice (n=10) [F=10.06, df=1,15, p=0.0063], tacrine failed to have any beneficial effects.

III.D. DISCUSSION

The Morris Swim Maze has been traditionally used to assess different animal models of aging (Gage et al, 1984). That both the control and apoE mice were much faster at locating the hidden platform on the final day of training as compared to the first, indicates acquisition of the task. Controls were consistently better at finding the platform, which is consistent with earlier results from two month old apoE mice (Poirier et al, 1996). Tacrine did not have an effect on performance for either group. While it would be unlikely to increase learning ability in unimpaired control mice, spatial memory might be ameliorated by a cholinergic enhancing drug in a system with impaired cholinergicity; however, Masliah et al (1995) have demonstrated that in apoE deficient mice there is a significant 15 to 40% loss of synapses and a disruption of the dendritic cytoskeleton of hippocampal and neocortical tissue in an age-dependent manner. These changes were widespread but most prominent in the CA1-CA2 pyramidal subfield of the hippocampus, in the molecular layer of the dentate gyrus, and in the fronto-parietal areas of the cortex (Masliah et al, 1995). Such changes to the synaptic and cytoskeletal system, effecting both axonal and dendritic processes, indicate a disruption of the central nervous system beyond a focal alteration of the cholinergic system. Masliah et al (1994) have also shown that there is reduced compensatory synaptogenesis in the hippocampal area of these knockout mice. Increasing the half-life of ACh in the synaptic cleft, via tacrine administration, may therefore not be sufficient to ameliorate behavioural deficits in the Morris Swim Maze and compensate for the wide synaptic disruption caused by apoE deficieny. This is also consistent with recent evidence of the decreased efficacy of tacrine in AD patients carrying the $\epsilon 4$ allele (Poirier et al, 1995).

Although the reduced platform finding latencies are often attributed to learning the spatial location of the hidden platform (Morris et al, 1985), neither the controls nor the apoE mice preferred the training quadrant during the probe trial. Perry et al (1995) have interpreted this as the adoption of a search strategy rather than spatial learning. The mice adopt a strategy of swimming close to the pool walls, but far enough out so as to happen upon the platform. This would explain the lack of preference for the training

quadrant without undermining the cognitive interpretation of this paradigm. Further measures, such as a cumulative proximity to platform, would have been helpful to differentiate true place learning, which obviously did not occur in these animals, from the adoption of some other swim strategy (Gallagher et al, 1993).

The initial poor swimming ability of the apoE mice is problematic in that it may account for some of the initial cognitive impairment, but toward the end of training the mice were swimming as well as controls. Aged animals appear to be more susceptible to cold water due to impaired thermoregulation (Lindner et al, 1991). Such systems in these knockout mice have not been examined, but two apoE mice appeared to shiver and not groom themselves between trials. Although the difference between groups is likely a verifiable cognitive impairment, which is consistent with previous Morris Swim Maze findings (Poirier et al, 1996), the dye may have exerted a possible toxic effect.

Future experiments would assess the apoE deficient mice in a wider battery of behavioural tasks, analyzing affect, locomotor activity, sensorimotor abilities, and aspects of memory other than spatial. While dose curves could not be tested in the present design, the doses used were not associated with toxic effects (Fitten et al, 1987), and so opting for higher concentrations of tacrine might be tolerable and beneficial to the impaired mice.

IV. Segmental Trisomy 16 Model

These experiments were conducted to assess the efficacy of a cholinergic M2-antagonist, BIBN99, for its ameliorative effects on spatial memory impairments of mice trisomic for several genes, among which are those from the distal end of human chromosome 21. Such a model of Trisomy 21 may be beneficial to understand the effects of these genes on children with Down Syndrome and the early onset of dementia in Down Syndrome.

IV.A. MATERIALS AND METHODS

IV.A.1. Animals

Ts65Dn (2-3 mos, 16 males) and controls (2-3 mos, 16 males) were obtained from Jackson Laboratories (Bar Harbor, Maine). The Ts65Dn mice were not inbred, but were generated by mating carriers of the 17^{16} chromosome to C57 B6C3H F1 hybrid mice, thereby producing trisomy on a variable genetic background (Reeves et al, 1995).

All mice were maintained on a 12 hour light-dark cycle (on at 8:00 am) in temperature (22°C) and humidity (45-70%) controlled rooms. Males and females were housed separately in groups of 1 to 4. All had *ad libitum* access to both standard laboratory mouse chow and tap water. The trisomy mice were of significantly lighter weight than the controls (26.9 ± 0.7 mg and 31.4 ± 0.5 mg, respectively; unpaired t-test, $t=5.192$, $df=32$, $p<0.0001$). Coat colour was not an indicator of trisomy background.

IV.A.2. Drugs

BIBN99 was freshly prepared each morning prior to testing. Depending on design, either a 0.5 mg/kg in 0.125 mg/ml 0.9% saline solution or a 1.0 mg/kg in 0.25 mg/ml 0.9% saline solution was prepared, and 0.1 N HCl was added to a pH of 6.5. Vehicle was saline and HCl at pH 6.5.

IV.A.3. Behavioural Testing

IV.A.3.a. Morris Swim Maze

This task requires mice to locate a platform submerged 1 cm below surface of water in a 1.6 m diameter pool. The water (22° C) was made opaque by the addition of milk powder. The mice were tracked using an RCA video camera hung above the pool and a VIDEOMEX-V (Columbus Instruments, Columbus, Ohio) video image analyzer. Four positions around the edge of the pool were designated as north, south, east, and west, thereby dividing the pool into four quadrants. The hidden platform remained in the same NE location throughout testing. Each mouse received twenty trials over five days, being placed into the pool at the four major compass points in a randomized order each day. Trials lasted a maximum of 120 seconds, at which time if the mouse had not located the platform it was placed on it for 5-6 seconds. The animals were to solve the task using distal cues placed around the testing room. A probe trial, without a platform and lasting 30 seconds, and a visible platform trial, during which the platform was elevated 1 cm above water level, were given 48 hours following the last day of testing.

A dose of 0.5 mg/kg BIBN99 was administered s.c. to half of the controls (n=4) and half of the Ts65Dn mice (n=4) 20 minutes prior to the first trial of each day. The following three trials were completed within 1 hour following administration. Saline was administered s.c. to the remaining controls (n=5) and Ts65Dn mice (n=4). All testing and drug administration were completed blind to the genetic identity of the mice. In a separate cohort of animals, 1.0 mg/kg BIBN99 and an equivalent volume of saline was administered in a similar fashion to the 0.5 mg/kg trial.

IV.A.3.b. Thatcher-Britton Novelty Conflict

The apparatus and methods used are as outlined in section II.A.3.g.

IV.A.4. Analysis of Brain Tissue

IV.A.4.a. Choline Acetyltransferase

Ts65Dn mice and control mice were sacrificed by live decapitation and brain tissue was quick frozen in isopentane. All tissue was prepared and assayed for ChAT activity as outlined in Section II.A.4.b.

IV.B. RESULTS

Swim Maze data were analyzed by a Group x Day x Drug ANOVA. In the 0.5 mg/kg BIBN99 trial (Figure 15), a Group x Drug interaction occurred ($F=6.66$, $df=1,13$, $p=0.0228$). The Ts65Dn mice without drug had significantly longer latencies in finding the platform throughout training than did the controls without drug ($p=0.0169$), but BIBN99 had no appreciable effect ($p=0.3788$). This effect was limited to the non-drug condition if only because the drug had opposite (but non-significant) effects on the two groups: BIBN99 tended to be detrimental to the controls and beneficial to the Ts65Dn mice. All groups showed significant changes in latency across days of training as indicated by a significant Day main effect ($F=7.23$, $df=4,52$, $p=0.0001$).

A Group x Quadrant x Drug ANOVA was used to analyze time spent in each quadrant on the probe trial (Table 4). There were no overall ANOVA effects of Group or Drug on the time spent in the training quadrant (NE or T). There was a significant Quadrant main effect, so that all the groups spent unexpectedly more time in the quadrant (SE) directly clockwise from the training quadrant ($F=3.18$, $df=3,39$, $p=0.0346$; using *post hoc* comparisons, $p=0.0205$ for NW versus SE; $p=0.0139$ for T versus SE; and $p=0.0135$ for SW versus SE)

	NW (sec)	T (sec)	SW (sec)	SE (sec)	Vis (sec)	Probe (cm)
Con	3.74(1.30)	10.1(1.48)	4.92(1.30)	11.3(1.88)	17.2(4.93)	917.2(89.3)
ConB	6.28(1.08)	5.45(1.66)	7.33(1.80)	11.0(1.41)	19.7(5.27)	960.0(63.3)
Ts	10.0(2.39)	6.88(1.52)	5.98(1.09)	7.13(1.30)	19.7(3.13)	807.2(132.8)
TsB	6.93(1.49)	3.60(0.93)	7.70(1.37)	11.8(2.51)	25.2(2.43)	859.7(47.78)

Table 4 - Results of Probe Trial with 0.5 mg/kg BIBN99

Times spent in quadrants northwest (NW), northeast or training (T), southwest (SW), and southeast (SE), and time to locate the platform in the visible trial (Vis), were recorded for controls without drug (Con), controls with BIBN99 (ConB), Ts65Dn mice without drug (Ts), and Ts65Dn mice with BIBN99 (TsB). Data listed in table are mean (± SEM) in seconds (sec) except for the probe trial (Probe) distances, which are reported in centimetres (cm).

In the 1.0 mg/kg BIBN99 trial (Figure 16), a Group x Day x Drug ANOVA was used to analyze the data. The control group approached but did not reach a statistically significant difference from the Ts65Dn group ($F=3.84$, $df=1,13$, $p=0.0717$). BIBN99 did not have a significant effect on performance ($F=3.00$, $df=1,13$, $p=0.1071$). All groups showed significant changes in latencies across training days ($F=5.75$, $df=4,52$, $p=0.0007$). The probe trials (Table 5), analyzed by a Group x Quadrant x Drug ANOVA, yielded similar results to the 0.5 mg/kg trials. No overall Drug or Group effects occurred, yet a significant Quadrant effect indicated that all the groups tended to spend the most time in the quadrant immediately clockwise from the training quadrant ($F=7.14$, $df=3,39$, $p=0.0006$; using *post hoc* comparisons, $p=0.0002$ for NW versus SE; $p=0.0004$ for T versus SE; and $p=0.0058$ for SW versus SE).

	NW (sec)	T (sec)	SW (sec)	SE (sec)	Vis (sec)	Probe (cm)
Con	3.65(1.18)	7.23(1.01)	6.10(2.40)	13.0(2.07)	21.0(2.89)	986.5(145.6)
ConB	3.92(1.15)	4.80(1.23)	11.0(1.55)	10.3(2.26)	17.0(4.36)	902.6(103.1)
Ts	6.10(1.06)	8.23(1.67)	3.50(1.04)	12.1(0.96)	12.7(1.29)	1004.0(84.06)
TsB	5.85(2.93)	1.13(0.97)	8.05(2.77)	15.0(4.56)	25.0(4.33)	680.75(132.6)

Table 5 - Results of Probe Trial with 1.0 mg/kg BIBN99

Times spent in quadrants northwest (NW), northeast or training (T), southwest (SW), and southeast (SE), and time to locate the platform in the visible trial (Vis), were recorded for controls without drug (Con), controls with BIBN99 (ConB), Ts65Dn mice without drug (Ts), and Ts65Dn mice with BIBN99 (TsB). Data listed in table are mean (± SEM) in seconds (sec) except for the probe trial (Probe) distances, which are reported in centimetres (cm).

Distance measures and visible platform data on the probe day were analyzed in separate Group x Drug ANOVAs. There were no significantly different latencies on the visible platform trials during the 0.5 mg/kg and the 1.0 mg/kg trials, nor were there differences in the distances swum on the probe trials between groups or drug treatments.

Novelty-Conflict data were analyzed by grouping the different drug dose

cohorts, to assess if, in general, previous drug or saline administration had any effect on Ts65Dn or control performance in a Group x Drug ANOVA. A Group effect occurred, such that mean latencies to approach the centrally placed food in the Thatcher-Britton task (Figure 17) were slightly but significantly greater in the controls compared to the Ts65Dn mice, irrespective of previous drug or saline administration ($F=4.26$, $df=1,27$, $p=0.0487$). The control mice also approached the food, prior to eating, more times than did Ts65Dn mice (Controls without and with drug, 5.3 ± 0.8 and 4.9 ± 1, respectively; Ts65Dn mice without and with drug, 2.8 ± 0.5 and 3.3 ± 0.6, respectively; Group effect in a twoway ANOVA; $F=5.37$, $df=1,27$, $p=0.0283$).

Hippocampal and cortical ChAT measures were treated in separate Group x Drug ANOVAs. ChAT activity (Figure 18) was not significantly different in any measures drawn from cortical tissue, regardless of group or drug administration, although an increase in ChAT activity due to BIBN99 approached significance ($F=3.59$, $df=1,22$, $p=0.0713$). The trisomy 16 mice, however, had a significant main Group effect of elevated levels of ChAT in hippocampal tissue in both the drug and non-drug conditions when compared to controls ($F=19.62$, $df=1,21$, $p=0.0002$). Moreover, administration of BIBN99 was related to a significant decrease in hippocampal ChAT activity for both control and trisomy mice (main Drug effect; $F=9.14$, $df=1,21$, $p=0.0065$).

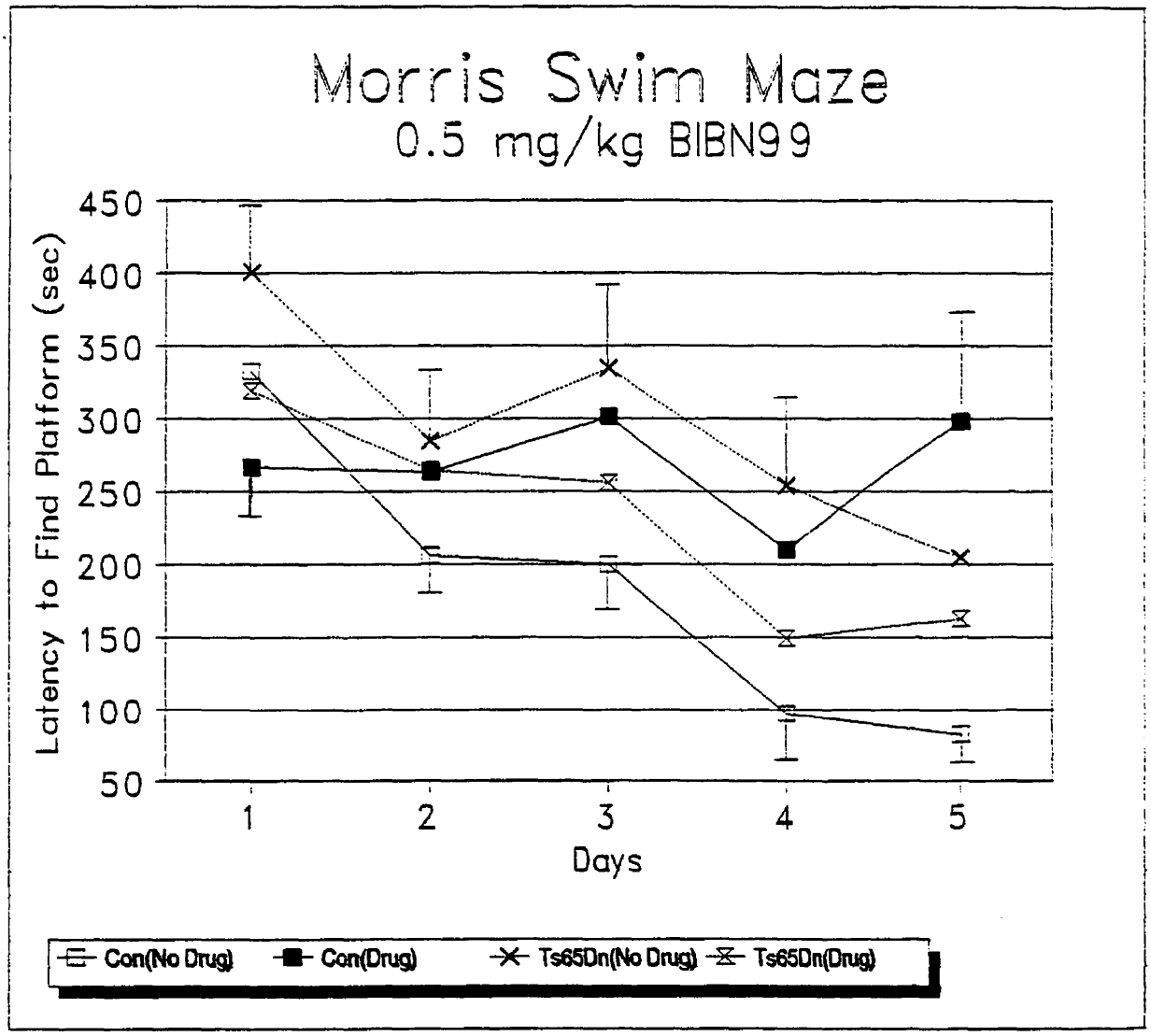

Figure 15. Ts65Dn mice in Morris Swim Maze. Data represents average latency (± SEM) to find the hidden platform across five training days. The trisomy mice without drug (n=4) were significantly slower in finding the platform than controls without drug (n=5) [F=6.66, df=1,13, p=0.0228]. 0.5 mg/kg BIBN99 had no effect on Ts65Dn (n=4) or controls (n=4).

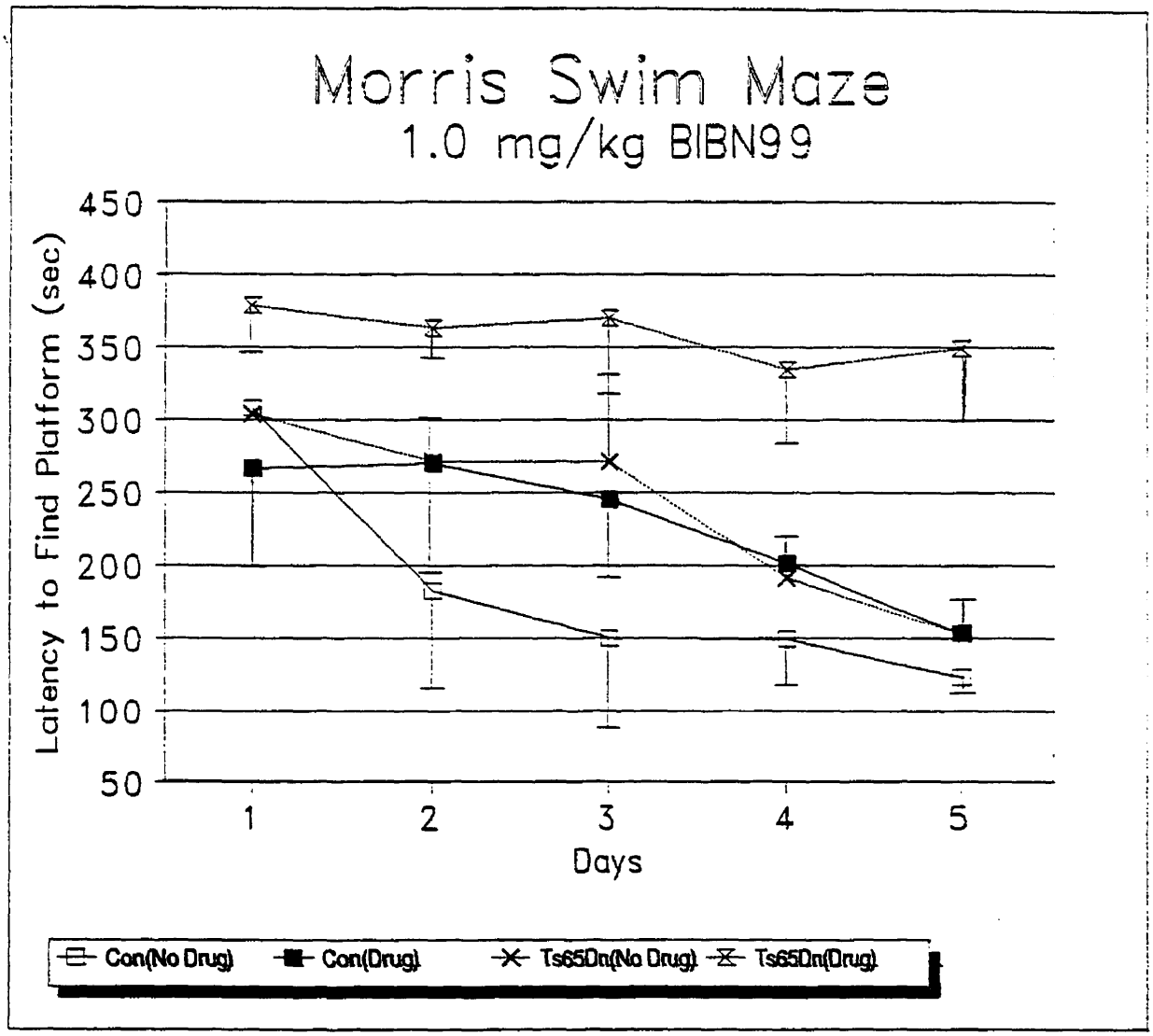

Figure 16. Ts65Dn mice in Morris Swim Maze. Data represents average latency (± SEM) to find hidden platform across five training days. The trisomy mice (non-drug n=4, 1.0 mg/kg BIBN99 n=4) and the control mice (non-drug n=4, 1.0 mg/kg BIBN99 n=5) did not have significantly different latencies.

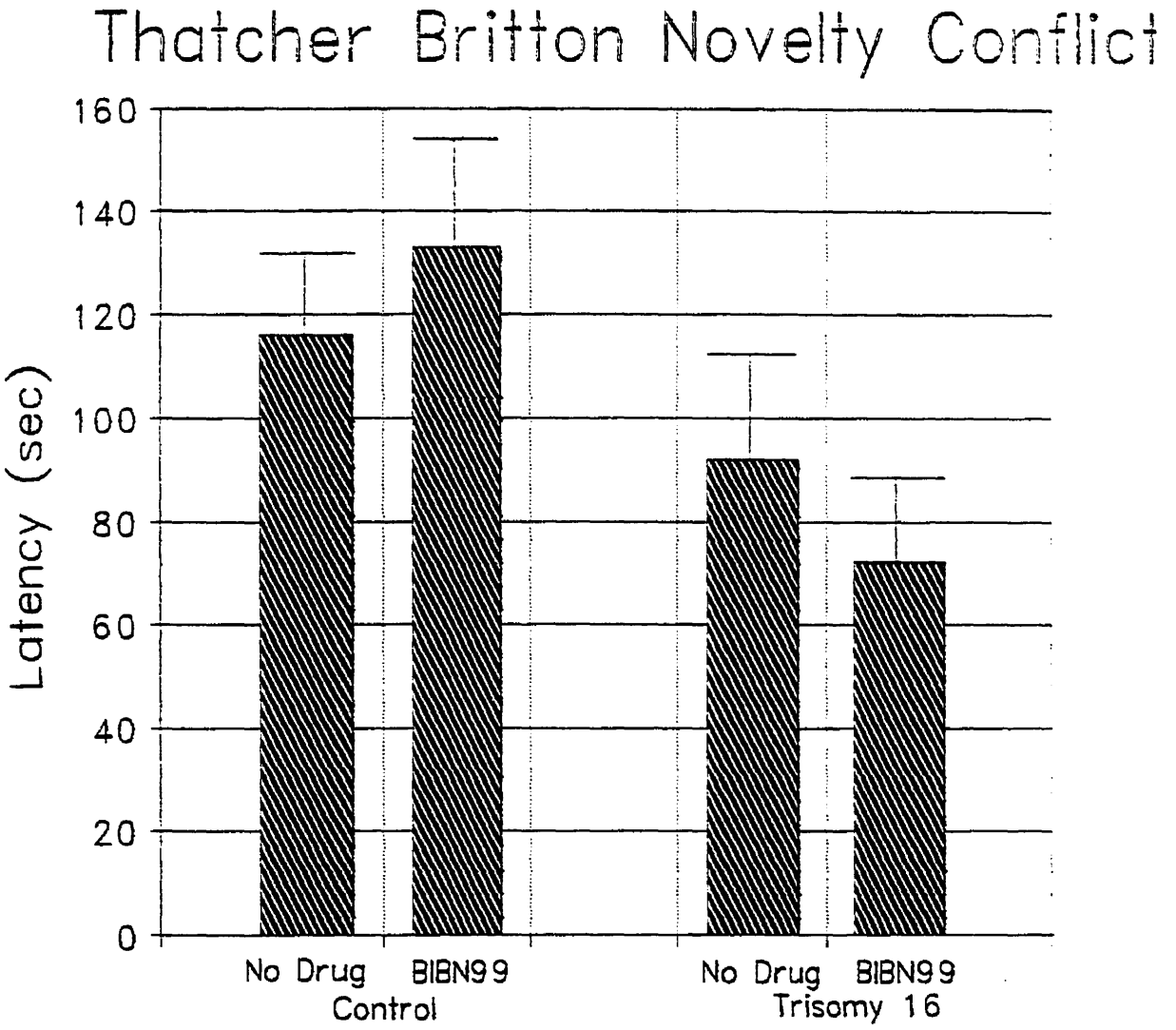

Figure 17. The average latency to commence feeding in the Thatcher Britton Novelty Conflict task (mean ± SEM). Controls (n=18) had significantly longer latencies than Ts65Dn mice (n=13) [F=4.26, df=1,27, p=.0487], but previous BIBN99 administration had no effect on feeding latency.

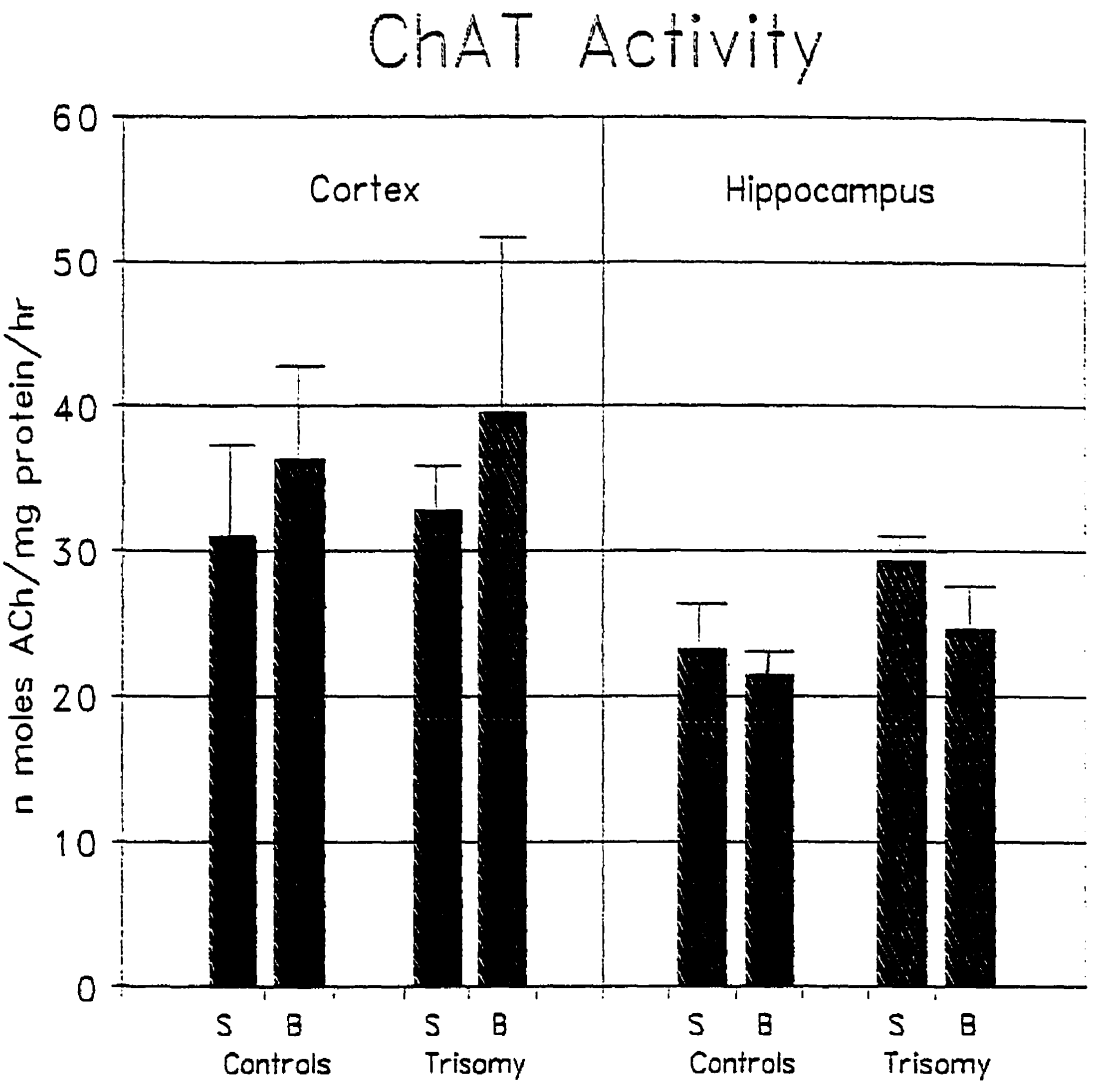

Figure 18. Choline Acetyltransferase Activity. Each column represents the mean (± SEM) moles of acetylcholine produced per milligram of protein per hour. No differences in bilateral cortical tissue ChAT activity occurred between controls (n=13) and Ts65Dn mice (n=13), whether administered BIBN99 (B) or saline (S). Irrespective of drug condition, Ts65Dn mice (n=14) had higher ChAT activity in bilateral hippocampal tissue than controls (n=13) [F=19.62, df=1,21, p=0.0002]. Previous BIBN99 administration reduced hippocampal ChAT activity in both control and trisomy mice [F=9.14, df=1,21, p=0.00065].

IV.C. DISCUSSION

Previous groups have demonstrated that the Ts65Dn mice are impaired in some aspects of spatial memory (Escorihuela et al, 1995; Reeves et al, 1995). While the 0.5 mg/kg drug trial reproduced this impairment, the controls and Ts65Dn mice performed similarly in the 1.0 mg/kg trial, despite a trend for the Ts65Dn mice having longer latencies to reach the platform. This inconsistency is likely due to the small group sizes used in the present study. Although BIBN99 has been shown to be effective in ameliorating impairments in aged impaired rats (Quirion et al, 1995), likely through its effects on the age-related increased density of putative M2 pre-synaptic autoreceptors, BIBN99 failed to change the performance of the impaired Ts65Dn mice. The status of several cholinergic markers in the brains of these Ts65Dn mice is unknown. Future receptor binding experiments and measures of cholinergic markers are needed to ascertain if the cholinergic system in these mice would be receptive to presynaptic receptor antagonists. The increased ChAT activity in the hippocampus region of the trisomy mice is peculiar in that similar alterations are not documented in DS cases (Yates et al, 1980, 1983) or in previous analyses of fully trisomic mice (Orzand et al, 1984), both of which show decreased activity of ChAT. More study of the cholinergic system is needed, although the significant difference in ChAT activity is a fairly robust result due to large sample sizes. Perhaps a unique interaction of the specific trisomic genes on mouse chromosome 16, as opposed to the full trisomy of human chromosome 21, produced an increased activity of ChAT.

While there are no published reports of the efficacy of BIBN99 on impairments in mice, the results of the present study do not support its cognitive enhancing effect at slightly higher doses to that used by Quirion et al (1995). Five days of acute drug administration, however, may not be sufficient for BIBN99 to have a functional effect. Maintaining the mice on chronic drug administration instead of acute doses at times of testing may be more efficacious in producing amelioration of impairments. Contrary to this, it would appear that BIBN99 did have a long lasting effect on ChAT activity, which was significantly decreased compared to non-drug groups.

The Ts65Dn mice appear to be less anxious than controls, which might be a

basis for the abnormal exploration patterns documented by Escorihuela et al (1995). It would be difficult to attribute changes in motivational systems to the increased expression of only the ßAPP gene, excluding all the others, and there is likely a complex interaction of several gene products occurring in the trisomy model. The amyloid being overexpressed in the trisomy mice is endogenous to that species. If overexpression of ßAPP results in changes in anxiety, the production of mice transgenic specifically for mouse ßAPP should produce similar behavioural consequences as in the Ts65Dn mice. Hsiao et al (1995) created mice transgenic for murine ßAPP695, which resulted in spatial memory impairments and neophobia. Different measures of anxiety, such as latency to begin eating in the Thatcher-Britton paradigm and corner sniffing as a neophobia index (Hsiao et al, 1995), might provide different indications of levels of anxiety. The Ts65Dn mice appear to be less anxious than controls, while the murine ßAPP695 transgenics are more neophobic than controls (Hsiao et al, 1995). While this apparent inconsistency might be due to the Ts65Dn having multiple gene interactions, another possibility is that the Ts65Dn mice have a more endogenous, natural amyloid production than the transgenic mice, thus dissociating the roles of overexpression of a gene product and of abnormal expression of that product. As well, the behavioural differences may be simply due to different mouse strain effects.

No AD pathology has yet been ascertained in the Ts65Dn mice (Reeves et al, 1995), which could be a contraindication to the role of amyloid in Alzheimer pathology, or it could be more proof positive of the detrimental role amyloid may have without blatant amyloid deposition.

These Ts65Dn are trisomic for several loci homologous to genes on human chromosome 21, such as SOD-1, myxovirus resistance-1, glutamate receptor subunit-5 (GluR5), E26 avian leukaemia oncogene-2, interferon receptor, and phophoribosyl glycinamide synthetase (Reeves et al, 1995). No behavioural analyses have to date been published on mice that are transgenic for each of these specific genes, but such models would be necessary to assess behavioural outcomes for each of their effects. Although S100ß may be implicated in the phenotype of DS, it is located on mouse

chromosome 10, and is not overexpressed in these segmental trisomy mice (MacDonald et al, 1991). SOD-1 activity in Cu,Zn-superoxide dismutase mice is increased by 1.6 to 6 fold in brain tissue (Epstein et al, 1987). Due to trisomy for the SOD-1 gene, the Ts65Dn mice would be expected to have a higher SOD activity and a possible secondary production of reactive oxygen species, which have been postulated to have a role in aging by way of molecular damage (Harmon et al, 1981). The deleterious role of SOD, however, is not entirely clear since it has been suggested to contribute to neuro-protection following cerebral ischemia (Chan, 1992). GluR5 maps between ßAPP and SOD-1 on human chromosome 21 and mouse chromosome 16 (Gregor et al, 1993). The association of GluR5 to glutamate suggests that this gene product has a possible role in excitotoxicity, and since GluR5 is highly expressed during embryogenesis in areas of differentiation and synaptogenesis (Bettler et al, 1990), it could have detrimental effects on the development of the CNS in a trisomic organism. Future experiments could beneficially make use of the aged Ts65Dn model to explore the role of ßAPP, SOD-1, and glutamate toxicity. Although this model was used here as a limited model of AD, to study what disposes Down Syndrome patients to early aging and dementia the effects of trisomy for genes on human chromosome 21 would have to be studied in aged trisomic mice. Aging our cohort of Ts65Dn mice was not possible for the present study, but such future experiments would be interesting, especially given the putative role of ßAPP and SOD-1 in aging.

V. CONCLUSION

The three models discussed in the previous sections may be used as tools to provide glimpses of different aspects of Alzheimer's disease. The Aß transgenic model suggests that the expression of a fragment of human ßAPP in these mice contributed to decreased AChE staining in the CA1 and CA2 subfields of the hippocampus, impairments of retention, increased anxiety, and increased passivity. Deficiency of apoE in mice was here shown to result in impairments of spatial learning. In the segmental trisomy 16 model, the overexpression of several genes homologous to human chromosome 21 genes, including murine ßAPP, resulted in mild impairments of spatial memory and increased hippocampal ChAT activity. The administration of BIBN99, a muscarinic M2 receptor antagonist, had no effect in improving the deficits of the Aß mice or Ts65Dn mice, but did appear to chronically decrease hippocampal ChAT activity in the Ts65Dn and control mice after only acute administration. Tacrine, an inhibitor of AChE, did not improve the cognitive impairment of apoE deficient mice.

It would be difficult to attribute changes of cognition or anxiety in the Ts65Dn model to the increased expression of only the ßAPP gene, excluding all the others, and while the Aß transgenic results can be attributed to increased expression of the C104 fragment, there is likely a complex interaction of several gene products occurring in the trisomy model. The amyloid being produced in the trisomy mice is endogenous to that species, while human amyloid was transfected into the Aß mice. The overexpression of some chromosome 16 genes would seem to dispose the mice to decreased anxiety, while anxiety is heightened in the transgenic mice expressing human ßAPP. The experimental design is not sufficient to address why these differences occur. Perhaps the differences in the primary sequence of murine and human amyloid account for the different effects on anxiety in the two models, but motivational effects may also be attributable to a number of gene products. A difference in ßAPP human and mouse sequences resulting in different behavioural effects is supported by a comparison of mice transgenic for either the human ßAPP695 or the mouse ßAPP695 (Hsiao et al, 1995). Human ßAPP695 disposed the transgenic

mice to a shorter lifespan and greater neophobia than did murine ßAPP695 (Hsiao et al, 1995). While the present results implicate human ßAPP in increasing anxiety, the mice expressing transgenic murine ßAPP695 in the study of Hsiao et al (1995) showed increased neophobia compared to controls but decreased neophobia compared to human ßAPP695 transgenic mice. The differences between the present results and those of Hsiao et al (1995) are likely attributed to differences in the regulation and expression of the gene product in specific strains of mice.

Apolipoprotein E and amyloid, and thus the apoE deficient model and Aß transgenic model, still remain the foci of somewhat competitive and exclusive theories until future research more fully uncovers the roles of the two gene products in the pathophysiology of AD. Based on the present data and other published behavioural and pathological reports, animal models of either gene products provide results that are parallel, if not consistent, with clinical and pathophysiological changes in AD. The Aß and apoE deficient models appear to possess multiple Alzheimer-related behavioural features; this, in combination with neuropathological findings, indicates that these models, and the Ts65Dn model pending future aging studies, could be advantageously used to assess novel treatments for Alzheimer's disease and dementia in Down's Syndrome.

VI. CONTRIBUTIONS TO ORIGINAL KNOWLEDGE

The present study furthers the behavioural characterization of the C104 ßAPP transgenic mice published by Nalbantoglu et al (1996). Not only are these transgenic mice impaired in spatial learning (Nalbantoglu et al, 1996), but behaviour is consistent with increased anxious behaviour and deficits of working memory and longterm retention. As well, chronic expression of the amyloidogenic ßAPP fragment appears to result in loss of acetylcholinesterase in the CA1 and CA2 subfields of the hippocampus. BIBN99, which was previously successful in improving age-related impairments in spatial learning (Quirion et al, 1995), was shown to be ineffectual at ameliorating behavioural impairments caused by pathological systems, such as those of the overexpression of the C104 fragment of ßAPP or the trisomic state of the segmental trisomy mice. Tacrine administration, as well, was unable to improve the deficits previously described in the apoE deficient mice (Poirier et al, 1996). Interestingly, trisomy for specific genes homologous to genes on the distal end of human chromosome 21 resulted in an increase in ChAT activity in the Ts65Dn mice, and acute BIBN99 administration appeared to chronically decrease that ChAT activity.

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