Nicotine induced improvements in cognition: A possible role for the α7 nicotinic acetylcholine receptor.

By

Jared W. Young B.Sc. (Hons)

Submitted in accordance with the requirements for the degree of

Doctor of Philosophy

University of Edinburgh

2004

Abstract

Cognitive dysfunction is evident in a wide variety of neurological disorders from schizophrenia to Alzheimer's disease (AD). Impaired attention has been recorded in each of these patient groups and has been hypothesised to directly impact on their general cognitive ability and symptomatology. To date there is a paucity of treatment options for this impairment. Administration of nicotine, the prototypical agonist of the nicotinic class of acetylcholine receptors (nAChR), improves attention and the overall symptomatology of various CNS disorders. Its use as a possible therapeutic agent is limited by its adverse side-effects profile which includes addictiveness and nausea. Identification of the receptors/pathways through which nicotine produces these beneficial effects is a prerequisite to the discovery of more selective agonists with minimal side-effects. Current interest has focussed on the homopentameric alpha 7 (α7) receptor (nAChR) due to its proposed role in attention and memory, and neuroprotection in AD and other neurodegenerative disorders. In the thesis, the role of the α7 nAChR in modulating nicotine-induced cognitive improvement has been studied using both pharmacological and genetic means.

Assessment of sustained attention in rodents can be performed using the 5-choice serial reaction-time (5-CSR) task; analogous to the continuous performance test used in man. A protocol was established which allowed the demonstration of nicotine-induced improvements in sustained attention in mice. In this task α7 nAChR knockout (KO) mice exhibited impaired acquisition and performance, providing additional evidence that this receptor may be a valid therapeutic target for cognitive enhancement. In order to investigate the role of nAChR manipulation on working memory, the odour span task, a test of olfactory working memory capacity, was established in mice. Nicotine administration did not improve performance of C57Bl/6J mice probably as a consequence of ceiling effects. Transgenic mice over-expressing human caspase-3 (hc-3) displayed a robust impairment in the task that was attenuated by nicotine administration. Moreover α7 nAChR KO mice exhibited impaired acquisition and performance in the task but in a different pattern to that of the hc-3 mice. This pattern may reflect an impaired ability to attend to the task as opposed to a working memory deficit. These demonstrations provide further support for a role of the α7 nAChR in cognition. Tg2576 mice represent the best well characterised transgenic mouse model of AD, however there remains a dearth of information on their attentional and olfactory capabilities. The mice exhibited a deficit in sustained attention as measured by the 5-CSR task as well as an age-related impairment in the odour span task.

In conclusion the development of the 5-CSR task for mice was used to identify a nicotine-induced improvement in normal mice and impaired performance in α7 KO and Tg2576 mice. The establishment of the odour span task in mice allowed the demonstration of impaired working memory performance in hc-3 mice (attenuated by nicotine administration), α7 KO mice and Tg2576 mice (age-related). In summary these data provide some evidence for a role of the α7 nAChR in nicotine-induced improvement in cognition, and the tasks developed provide new tools for the assessment of putative cognitive enhancing compounds.

Est. word count = 50,000

Declaration

I declare that the work submitted here is composed by this candidate and that appropriate acknowledgments has been given where reference is made to the work of others.

The radioligand binding studies described in chapter 3.3.8 were as appeared in the publication arising from this thesis and was performed by Miss Nicola Crawford MSc. The histopathological studies described in chapter 3.3.9 were performed by Geoffrey Carlson. Whilst the nicotine injections into the Tg2576 mice described in chapter 5.3.9 was performed by myself, the analysis of Aβ levels in these mice was performed by Dr. Jui-Lee Birse-Archbold and Mrs. Joyce McLuckie.

Signed:

Date:

03/06/05

"It's been said that the quest for truth is the noblest occupation of man, but there be dragons lurking in the dark forests of ignorance"

From 'The Hidden City' by David Eddings

Acknowledgements

Thanks to all of you whom loaned me an axe when I strayed into the dark forests. This includes all the staff of FINE, but in particular Drs. Keith Finlayson and John Sharkey. You were there when I needed you, and as that was pretty much all of the time, I thank you. Keith, without your massive support, patience and humour, I would not have gotten this far. John, your understanding of Weegie land made a lot of this possible. To my supervisors, Dr. Hugh Marston and Professor John Kelly, thank you for your time, patience and 'nudging' me in the right direction, even when I deserved a push. Thank you Hugh, for you invaluable advice and experience. John, thank you for opening my eyes to a world beyond behaviour, as scary as that is, and for the considerable help with this thesis. To Dr. Christopher Spratt, thank you. You were there for me always. Never would I have gotten this far without your help, humour, kindness and scathing sarcasm. To Dr. Ailsa McGregor and Mrs. Margaret Rennex, I give thanks for all the mothering and support a fledgling needed. I would also like to thanks Mrs. Nicola Logan, Mr. Geoff Carlson and Ms. Laraine Wells, for your help, your time, but most of all you patience. To Mr. Ian Heron, Mr. David MacDonald and Ms. Louise Dickson, thank you for all your support every step of the way, you helped me more than you know. To all others in FINE, you made it a pleasure to come in everyday, and it was a joy to work with you all.

I would also like to thank Fujisawa Pharmaceutical Co. Ltd and the Fujisawa Institute of Neuroscience in Edinburgh, Department of Neuroscience, for providing both the funding and resources to carry out this research.

Finally I would like to thank my family and friends for their support through these past years, especially Miss Barbra Milligan. You gave me the chance to be all that I can. Thank you.

Publications arising form this thesis

Paper

Young, JW., Finlayson, K., Spratt, C., Marston, HM., Crawford, N., Kelly, JS., and Sharkey, J., (2004). Nicotine improves sustained attention in mice: Evidence for the involvement of the alpha 7 nicotinic acetylcholine receptor. *Neuropsychopharmacology*, 29, 891-900.

Abstracts

Young, JW., Finlayson, K., Marston, HM., Kelly, JS., and Sharkey, J., (2004). Modification of the mouse 5-CSR task to assess effects of pharmacological and genetic manipulations on attention. 779.11. Abstract Viewer/Itinerary Planner. Washington, DC: *Society for Neuroscience*, Online.

Young, JW., Finlayson, K., Spratt, C., Marston, HM., Crawford, N., Kelly, JS., and Sharkey, J., (2004). Nicotinic Acetylcholine Receptor Involvement in Sustained Attention in Mice. J Psychopharm. Suppl. 18(3) TB37.

Kelly, JS., **Young, JW**., Finlayson, K., Spratt, C., Marston, HM., Crawford, N., and Sharkey, J., (2004). The Involvement of the alpha 7 nicotinic acetylcholine receptor in sustained attention in mice. Presented at the GRC on Ligand Recognition and Molecular Gating.

Young, JW., Spratt, C., Marston, HM., Finlayson, F., Kelly, JS., and Sharkey, J., (2003). Effect of nicotine on visuo-spatial sustained attention in mice as measured by the 5-choice serial reaction time task. J Psychopharm. Suppl. 17(3) pA64.

Young, JW., Amet, L., E., Asada, T., Spratt, C., Marston, HM., Kelly, JS., and Sharkey, J., (2002). Mice over-expressing human caspase-3 exhibit cognitive deficits in an olfactory working memory task. FENS Abstr. vol 1, A151.20.

Table of Contents **Page**

List of Figures and *Tables* **Page**

Chapter 1 General Introduction

1.1 Frontal Lobe Syndrome

Levin and colleagues (1991) described the history of the frontal lobes, citing Varolio as the first to discuss the frontal lobes in 1573. By 1807, Chaussier had proposed the names frontal, temporal and occipital for these three areas (cited in Levin *et al.*, 1991). The consequences of damage to the frontal lobes were not documented until the end of the 18th century when several groups detailed the increased awkwardness and irritability, plus reduced levels of socialisation, attention and intelligence, following bilateral lesions of the frontal lobes in a variety of species including dogs, monkeys and humans (Ferrier, 1876, cited in Levin *et al.*, 1991; Welt, 1888, cited in Niedermeyer, 1998). However, the group of symptoms that subsequently became known as the frontal lobe syndrome was first described by Harlow (1968), when describing the effect of an iron bar passing through the frontal lobes of one of his patients (cited in Andrés, 2003).

Welt (1888, cited in Niedermeyer, 1998) identified that it was specific damage to the orbitofrontal cortex that that resulted in the irritability and personality changes that partially characterised frontal syndrome. Feuchteanger's (1923, cited in Pantelis and Brewer, 1995) later demonstrated that lesions to other brain areas led to symptoms akin to those observed in frontal syndrome patients, making the simplistic view of a single frontal lobe syndrome untenable. However many researchers still used it as an umbrella term to categorise a variety of disorders that had similar behavioural

manifestations and which appeared to relate to levels of frontal lobe damage in the patients. More recent research supplementing the work detailed by Feuchtwanger (1923) has led to the term being referred to less often within the literature (Pantelis and Brewer, 1995).

The term now used to encompass these disorders is 'dysexecutive syndrome' (Baddeley, 1986; Pantelis and Brewer, 1995; Baddeley and Della Sala, 1996; Godefroy, 2003), with executive functions defined as the cognitive processing involved in planning, initiating and regulating behaviour (Lezak, 1983; cited in Parkin, 1999). This syndrome covers a broad spectrum of disorders, with Alzheimer's disease (Sultzer *et al.*, 1995; Chen *et al.*, 1998; Tekin *et al.*, 2001), and schizophrenia (Franzen *et al.*, 1975; Levin, 1984; Elliot and Sahakian, 1995) at opposite poles, and includes ADHD (Benson, 1991; Barkley *et al.*, 1992; Neidermeyer, 1998), autism (Gedye, 1991a; Hill, 2004), Tourette's syndrome (Gedye, 1991b) and traumatic brain damage patients (Mattson and Levin, 1990). A decline in frontal lobe function has also been reported in subjects with age-associated memory impairments (Hanninen, 1997), also known as mild cognitive impairments (MCI).

1.1.1 Schizophrenia and Dysexecutive syndrome

Kraepelin (1896) first described as *dementia praecox* the disorder now known as schizophrenia (cited in Hirsch and Weinberger, 1985). It carries a lifetime risk of 1% (Cannon and Jones, 1996), and has both a genetic and environmental aetiology

(lifetime risk for a dizygotic twin of a schizophrenic is ~40%, Kläning, 1999; van Os and McGuffin, 2003). Diagnosis for schizophrenia is based on a set of characterised positive and negative symptoms (Andreason, 1997; Pearlson, 2000). In 1996, the NHS spent a greater proportion of money treating schizophrenic patients (24%) than it did on any other mental disorders, except the treatment of dementia (26%), which includes Alzheimer's disease (NHS executive, 1996). In 1992/1993, the NHS spent £809 million on treating patients with schizophrenia (Patel and Knapp, 1998).

Whilst the behavioural phenotype of schizophrenic patients suggested that they suffer from dysexecutive syndrome, it was reports by Franzen and colleagues (1975) of reduced functional activity in their frontal lobes that provided confirmation. This diagnosis soon achieved general acceptance; with Shallice and colleagues (1991) even writing that "All schizophrenics have problems with processes tapped by 'frontal' tests". Support came from post-mortem (Shapiro, 1993) and imaging studies of the frontal lobes (Chua and McKenna, 1995; Hazlett and Buchsbaum, 2001; Weinberger *et al.*, 2001). Hypofrontality was also observed in neuroleptic naïve schizophrenics (Biver *et al.*, 1995; Taylor, 1996; Andreasen *et al.*, 1997), and those with childhood onset (Jacobsen *et al.*, 1997). The dysexecutive syndrome classification of schizophrenic patients also stems from neurodevelopmental model theories (Lillrank *et al.*, 1995; Chambers *et al.*, 1996; Laplante *et al.*, 2004), and neuropsychological evidence (Shallice *et al.*, 1991). Whilst both Kraepelin (1896) and Bleuler (1911; cited in Hirsch and Weinberger, 1985) characterised the primary symptom of schizophrenia to be that of impaired cognition, that viewpoint became neglected after the development of antipsychotics that treated positive symptoms

(Harrison, 1999). However there has recently been renewed interest in the cognitive deficits exhibited by schizophrenic patients.

1.1.2 Alzheimer's Disease and Dysexecutive Syndrome

Alois Alzheimer (1907) first described a patient with the disease that bears his name. Not only did he note the cognitive symptoms (memory loss and disorientation), but even then he identified a curious clumping and distortion of the cortical neurofibrils. Today it is a common form of dementia and although our knowledge of the disease is now far greater, no truly effective treatment has been developed (Nordberg, 2001). With an ever increasing elderly population, this problem will inevitably worsen with time, with 4.5 million sufferers in the U.S.A. in 2000 and with the expectation numbers rising to 13.2 million by 2050 (Hebert *et al.*, 2003). The percentage of the population with Alzheimer's disease is approximately 1% of those > 60 years, but 30% of those > 85 years (Yorm, 1991). The huge cost in treating these patients will progressively increase with the concomitant increase in the elderly population. Whilst £3,312 million was spent caring for English patients with Alzheimer's disease in 1998, this figure was expected to rise to £7,920 million by 2031 (Knapp *et al.*, 1998). Identifying sufferers remains challenging, with non-invasive tests being only between 65-90% accurate (Kukull *et al.*, 1990). Once diagnosed, life expectancy of the patient is estimated to be between 8 – 10 years (Walsh *et al.*, 1990)

Alzheimer's disease patients exhibit a range of behavioural abnormalities that have long led researches to suspect that it was also frontally mediated. These include

memory loss, impaired attention, personality changes and agitation. Sultzer and colleagues (2003), identified hypometabolism in the areas of the frontal lobes in the brains of Alzheimer's disease patients as measured by positron emission tomography (PET) scans. These findings were supported by Chen and colleagues (1998), and Tekin and colleagues (2001) who suggested that the agitation experienced by Alzheimer's disease patients may actually be a surrogate marker for dysexecutive syndrome. Pathology in orbitofrontal cortex has been observed in patients with Alzheimer's disease and, similarly to Welt's observations in 1888, has been linked to the irritability, agitation and personality changes exhibited by these patients (Chu *et al.*, 1997; Van Hoesen *et al.*, 2000; Tekin *et al.*, 2001).

1.2 Neuropathology

An understanding of the neural mechanisms underlying the cognitive impairments in schizophrenia and Alzheimer's disease patients may prove to be essential in developing treatment therapies. Despite both Alzheimer's disease and schizophrenia falling within the concept of dysexecutive syndrome, the pathologies that underlie each are quite disparate. Precisely identifying the sequence of pathogenic events underlying the aetiology of neurological disorders is difficult as once diagnosed, any developmental sequence is likely to be altered by pharmacotherapy (Selkoe, 2001). The recent advent of *in vivo* imaging techniques such as functional Magnetic Resonance Imaging (fMRI) and PET, has greatly increased our knowledge of the pathology of these two disorders.

1.2.1 Schizophrenia

As discussed above, schizophrenia was originally thought of as a 'brain disease' (Kraepelin, 1986). However, trying to identify consistencies in the autopsies of schizophrenic patients proved difficult with pharmacotherapy confounding disease pathology (Chakos *et al.*, 1994). With the recent advent of *in vivo* imaging techniques, greater consistency has been found, though ideally first-episode non-medicated patients should be studied where possible (Copolov *et al.*, 2000). There is now a consensus that patients with schizophrenia have enlarged ventricles (Johnstone *et al.*, 1976; Spence *et al.*, 1998), with loss of cortical brain tissue (Lawrie and Abukmeil, 1998) with no correlation between the two (Harrison, 1999).

Monozygotic twin and familial studies suggest that enlarged ventricle size may be a marker for a genetic liability to the disorder (Noga *et al.*, 1996; Sharma *et al.*, 1998). This is supported by the findings that subjects at high risk of developing schizophrenia also exhibit enlarged ventricles in addition to reduced medial temporal lobes. These findings therefore support a neurodevelopmental model for the disorder (Walker, 1994; Bunney *et al.*, 1995; Lawrie *et al.*, 1999). However, whether these pathologies remain static or progressively worsen is controversial. It is also difficult to come to any conclusion as after the first episode, medication, age, severity and symptomatology confounds are present (Vita *et al.*, 1997; Gur *et al.*, 1998).

Reduction in size of the superior temporal gyrus size appears to consistently correlate with severity of thought disorder and auditory hallucinations (Shenton *et al.*, 1992; Marsh *et al.*, 1997). The contradictions within the literature on the macroscopic features of schizophrenic brains viewed ante-or post-mortem, makes a meta-analysis of the existing data unrealistic (Harrison, 1999; Powers, 1999). Another challenge when analysing schizophrenic brain pathology is the high proportion of non-specific focal degenerative abnormalities present in the tissue (Harrison, *et al.*, 1999). Bogerts (1999) suggested that the limbic dysfunction in schizophrenia patients may lead to a dissociation between cognitive activities and basic emotional reactions. Hazlett and Buchsbaum (2001) identified hypofrontality in schizophrenic patients when compared to controls, when assessed using a simple attention to prepulse task. Moreover these authors identified that attending to the prepusle in a prepulse inhibition paradigm (where a non-startling stimulus, the prepulse, reduces the startle response to the subsequent startle stimulus) lead to an increase in frontal activation in

controls but not schizophrenics. They suggested that this reflects an impaired response of the frontal lobes to environmental stimuli.

Copolov and colleagues, (2000) suggest that the diverse neuropathologies observed for schizophrenic patients may reflect altered structural and functional connectivity between regions, with particular emphasis being placed on the functional relationship between the medial temporal lobe and the prefrontal cortex (Heckers *et al.*, 1998). Aberrant pathology of the medial temporal lobe has been suggested as a link to the positive symptoms of schizophrenia, suggesting aberrant memory functioning can cause inappropriate 'memory replays', such that the differences between external events and perceptual thought cannot be distinguished (Copolov and McKinnon, 1998 in Copolov *et al.*, 2000).

On measuring functional related changes in local cerebral blood flow, Heckers and colleagues (1998), suggested that reduced hippocampal activation in schizophrenic patients when compared to controls correlated with poor performance in a memory task. Nelson and colleagues (1998) performed a meta-analytic study of hippocampal volume (bilaterally) in schizophrenic patients, and suggested there was a consistent reduction in size, though they were not specific as to the phase of the illness. Velakoulis and colleagues (1999) assessed the hippocampal volumes in first episode patients and observed an even greater reduction, with left hippocampal volume being smaller than right, though they admit to there being confounding variables. For example marijuana use was prevalent amongst the patients they assessed and the main psychoactive component 9-tetrahydrocannabinol has been shown to be

neurotoxic to hippocampal neurones in low concentrations (Chan *et al.*, 1998; Velakoulis *et al.*, 1999).

The alterations in neurotransmitter systems that occur in the brains of schizophrenic patients may provide greater insight and potentially more viable therapeutic targets to treat the cognitive dysfunction observed in schizophrenia. The dopamine hypothesis of schizophrenia has been the most prominent hypothesis of schizophrenia in recent years and suggests that the symptoms of schizophrenia are a result of dopaminergic hyperactivity of the mesolimbic cortical system (Kapur and Mamo, 2003). This hypothesis originated from the findings that effective antipsychotics, such as haloperidol, were dopamine (D_2) antagonists. Further evidence arose from the discovery that psychomimmetic drugs, drugs that produce schizophrenia-like symptoms such as amphetamine, cocaine or phencyclidine, released dopamine. However, new studies assessing dopamine neurotransmitter abnormalities in schizophrenic patients are confounded by antipsychotic treatment. This was demonstrated when D_2 receptor densities were reported to be reduced in the brains of schizophrenic patients (Zakanis and Hansen, 1998), yet PET studies in drug naïve patients reported no differences to their control subjects (Nordstrom *et al.*, 1995). Perhaps the most compelling evidence for dopamine dysregulation is the increased sensitivity of schizophrenic patients to stimulation of dopaminergic neurons exacerbating the symptoms these patients exhibit (Breier *et al.*, 1997). However, such drugs also act on the serotonergic system, as do many atypical antipsychotics (Meltzer, 1989). Serotonergic receptor abnormalities have been identified in the brains of treatment naïve schizophrenic patients suggesting they may be as a result of

the disease progression (Burnet *et al.*, 1997; Harrison *et al.*, 1999; Roth *et al.*, 2004). Phencyclidine is principally however, a non-competitive antagonist to the NMDA subtype of glutamate receptor (Coyle, 1996), suggesting a possible link to the glutamatergic neurotransmitter system in the symptoms of schizophrenia. This has been supported with genetic linkage, post-mortem and psychopharmacological studies (Krystal *et al.*, 2003; Moghaddam, 2003).

However, increasing interest has beer placed on the cholinergic system in schizophrenia. Similarities between the cognitive deficits observed in schizophrenic to those in Alzheimer's disease patients (discussed below, section 1.3, pp. 20) have fuelled such speculation (Friedman, 2004). A correlation between the degree of cognitive functioning and the cortical levels of choline acetyltransferase activity (marker for cholinergic innervation) of schizophrenic patients has been observed (Powchik *et al.*, 1998). Conversely it has been hypothesised that the putative cognitive-enhancing effects of atypical antipsychotics are linked to their ability to increase acetylcholine levels in the medial prefrontal cortex (Ichikawa *et al.*, 2002). Thus, whilst the obvious neuropathology of the cholinergic system observed in Alzheimer's disease patients is absent in schizophrenic patients (el-Mallakh *et al.*, 1991), the latter do exhibit several cholinergic neurotransmitter abnormalities.

Acetylcholine predominantly acts via two classes of cholinergic receptors, muscarinic or nicotinic. Altered expression of muscarinic acetylcholine receptors (mAChR) have been observed in the brains of schizophrenic patients (Crook *et al.*, 2000; Dean *et al.*, 2002). Also scopolamine, the non-selective mAChR antagonist,

impairs cognitive performance of a variety of species including mice (Bontempi *et al.*, 2003), rats (Stolerman *et al.*, 2000; van Kampen *et al.*, 2004), monkeys (Terry *et al.*, 1993) and man (Wesnes and Warburton, 1984; Koller *et al.*, 2003). These impairments are reversible by the administration of nicotinic acetylcholine receptor (nAChR) agonists (discussed in greater detail below, sections 1.3.6, pp. 26 and 1.3.7, pp. 28).

Abnormalities in nAChR expression have been identified in the post-mortem brains of schizophrenic patients. Whilst reduced protein levels of α7 nAChR were reported in the frontal cortex of schizophrenic patients, no differences were observed for the α4 nAChR (Guan *et al.*, 1999). However reduced expression of α4β2 nAChRs was noted in the hippocampus, cortex, striatum and thalamus in schizophrenic patients by Breese and colleagues (2000). The identification of such nAChR specific changes may assist in identifying therapeutic targets for the disease. Both schizophrenic patients and their relatives suffer from abnormal sensory gating, suggestive of an underlying genetic cause (Adler *et al.*, 1992; 1993; see section 1.3.3, pp. 23). Further investigation has localised this genetic abnormality to chromosome 15, proximal to the α7 nAChR locus (Freedman *et al.*, 1997). It has been hypothesised that their inability to gate sensory information may cause and/or exacerbate many of a schizophrenic patients symptoms (Venables, 1992), thus treatments targeted at the α7 nAChR may prove efficacious at treating these patients. This has been postulated as a possible reason why such a high percentage of the schizophrenic population (80 – 90 %) smoke compared to the general population (25 - 30%; Dalack *et al.*, 1998;

Rippoll *et al.*, 2004). This hypothesis is supported by the discovery that nicotine enhances cognitive performance in these patients (discussed in section 1.3.6, pp. 26).

1.2.2 Alzheimer's Disease

As discussed previously (see section 1.1.2, pp. 4) Alois Alzheimer (1907) first described several of the neuropatholigical features of the disease that bears his name. He observed what are now referred to as β-amyloid (Aβ) plaque deposits and neurofibrillary tangles (NFTs) of abnormally phosphorylated tau protein. Other hallmark feature have now been recognised and include inflammation and cholinergic degeneration. The exact cause of the cognitive decline observed in Alzheimer's disease has been the subject of much debate, being dominated for years by the 'cholinergic hypothesis'. This hypothesis stemmed from work in a variety of fields. Drachman and Leavitt (1974) first showed that administration of an acetylcholine receptor antagonist (scopolamine) impaired cognitive performance in young humans. This finding was repeated with other acetylcholine receptor antagonists, including the non-selective nAChR antagonist mecamylamine, with comparisons made to the pattern of cognitive deficits observed in Alzheimer's disease patients (Bartus and Johnson, 1976; Smith and Swash, 1978; Newhouse *et al.*, 1992; 1994). Compounds increasing levels of acetylcholine, such as nAChR agonists (e.g. nicotine or ABT-418) or anticholinesterase inhibitors (e.g. physostigmine, tacrine and galantamine), reversed these antagonist induced deficits in cognition (Drachman, 1977; Potter *et al.*, 1999). Finally several groups discovered reduced cortical cholinergic activity in the post mortem brains of patients

with Alzheimer's disease, which correlated with the cognitive decline observed in these patients, latterly supported by PET scans (Bowen *et al.*, 1976; Davies and Maloney, 1976; Perry *et al.*, 1978a,b; Davies, 1979; Shinotoh *et al.*, 2000).

It has been advocated that cholinergic cell loss in the basal forebrain with consequent cholinergic dysfunction in other brain regions, correlates more consistently and strongly with the cognitive decline of Alzheimer's disease patients than the other two pathological hallmarks of Alzheimer's disease - (Aβ) plaques or NFTs (Perry *et al.*, 1978a; McGeer *et al.*, 1984; Roberson and Harrell, 1997). More specifically, following post mortems, the loss of nAChR in the cortex of patients with Alzheimer's disease correlates with the cognitive impairments observed during life (Perry *et* al., 1987; Nordberg *et al.*, 1988; 1991; 1997; Marutle *et al.*, 1999; Sihver *et al.*, 1999; Nordberg, 2001). A reduction in α7 nAChRs density are also observed in the brains of traumatic brain injury patients, a known risk factor for Alzheimer's disease (Verbois *et al.*, 2000). This decrease in α7 nAChRs has also been linked with the cognitive impairments observed in traumatic brain injury patients, and can also be ameliorated by chronic nicotine treatment (Verbois *et al.*, 2000; 2003).

Current symptomatic treatments for Alzheimer's include acetylcholinesterase inhibitors, and to date four have been approved for use in this clinical population. These are donepezil (E2020, Aricept, Pfizer Inc.), rivastigmine (SDZ ENA 713, Exelon, Novartis Pharmaceuticals), galantamine (Reminyl, Jansen-Cigal) and tacrine (9-amino-1,2,3,4-tetrahydroacridine, Cognex, Parke-Davis), although the latter was rarely used due to its hepatoxic effects in nearly half of those treated (Watkins *et al.*,

1994; Kelly, 1999). These inhibitors were developed to counter the reduced cholinergic activity observed in Alzheimer's disease patients, though it is recognised that they may work via a variety of additional mechanisms.

One such mechanism is the direct or indirect activation of nAChRs, and this is proposed to enhance the efficacy of galantamine (Maelicke *et al.*, 2000; Raskind *et al.*, 2000; Wilcock *et al.*, 2000). The potential benefits of stimulating nAChRs include modulating several neurotransmitter systems, enhancing neuronal survival and possibly antagonising the toxicity of Aβ (Wonnacott, 1997; Kaiser *et al.*, 2000; Allain *et al.*, 2003). Direct activation of the nAChRs via nicotine administration has been shown to improve cognitive function in Alzheimer's disease patients and healthy controls even after chronic treatment (Newhouse *et al.*, 1988; Sahakian *et al.*, 1989; Jones *et al.*, 1992; Levin *et al.*, 1999; White and Levin, 1999; Mumenthaler *et al.*, 2003; see chapters 1.3.6, pp. 26; 3.3.3, pp. 86; 3.3.4, pp. 88 and 4.3.5, pp. 142). In fact the direct activation of the nAChRs by nicotine administration has been shown to be more efficacious at enhancing performance of experienced pilots in a flight simulator task than donepezil (Mumenthaler *et al.*, 2003).

Another mechanism by which nicotine is potentially therapeutically beneficial in Alzheimer's disease is because of its reported neuroprotective effect. These observations were initially based on reports that smokers had a lower risk for Alzheimer's disease (van Duijn and Hofman, 1991; Brenner *et al.*, 1993). Subsequent studies have however failed to replicate these findings. Indeed some show an increased risk of Alzheimer's disease in smokers (Prince *et al.*, 1994; Ott *et*

al, 1998; Doll *et al.*, 2000). These contradictory findings could stem from the fact that tobacco smoking is clearly not equivalent to direct nicotine administration, and tobacco product ingredients vary from brand to brand (Sabbagh *et al.*, 2002). Whilst the clinical effects of smoking in human subjects appear inconsistent, there is a wealth of evidence supporting a neuroprotective action of nicotine *in vivo* and *in vitro*. Nicotine administration has been shown *in vitro* to inhibit Aβ aggregation and neurotoxicity and protect against NMDA-induced neurotoxicity, (Marin *et al.*, 1994; Zamani *et al.*, 1997; Kihara *et al.*, 1999). *In vivo* studies using the Tg2576 transgenic mouse model of Alzheimer's disease (see chapter 5, pp. 156) have demonstrated both long- (5.5 months) and short-term (10 days) nicotine administration can significantly reduce the levels of Aβ plaque burden in a variety of brain regions, though most predominantly in the olfactory system (Hsiao *et al.*, 1996; Norberg *et al.*, 2002; Hellström-Lindhal *et al.*, 2004). The mechanism of Aβ induced neurotoxicity and potential cholinergic blockade has yet to be definitively explained, however the evidence gathered to date has given extra credence to the amyloid hypothesis, which suggests that Alzheimer's disease progression is a result of abnormal amyloid precursor protein (APP) processing (Blessed *et al.*, 1968). Cummings (2004) recently discussed a possible pathway (Fig. 1.1) explaining the histopathological and clinical observations observed in Alzheimer's disease, and placed abnormal APP processing as the primary cause of Alzheimer's disease, which eventually leads to the development of NFTs and neurotransmitter abnormalities including cholinergic loss (Roberson and Harrell, 1997; Newhouse *et al.*, 2001; Hardy and Selkoe, 2002; Götz *et al.*, 2004).

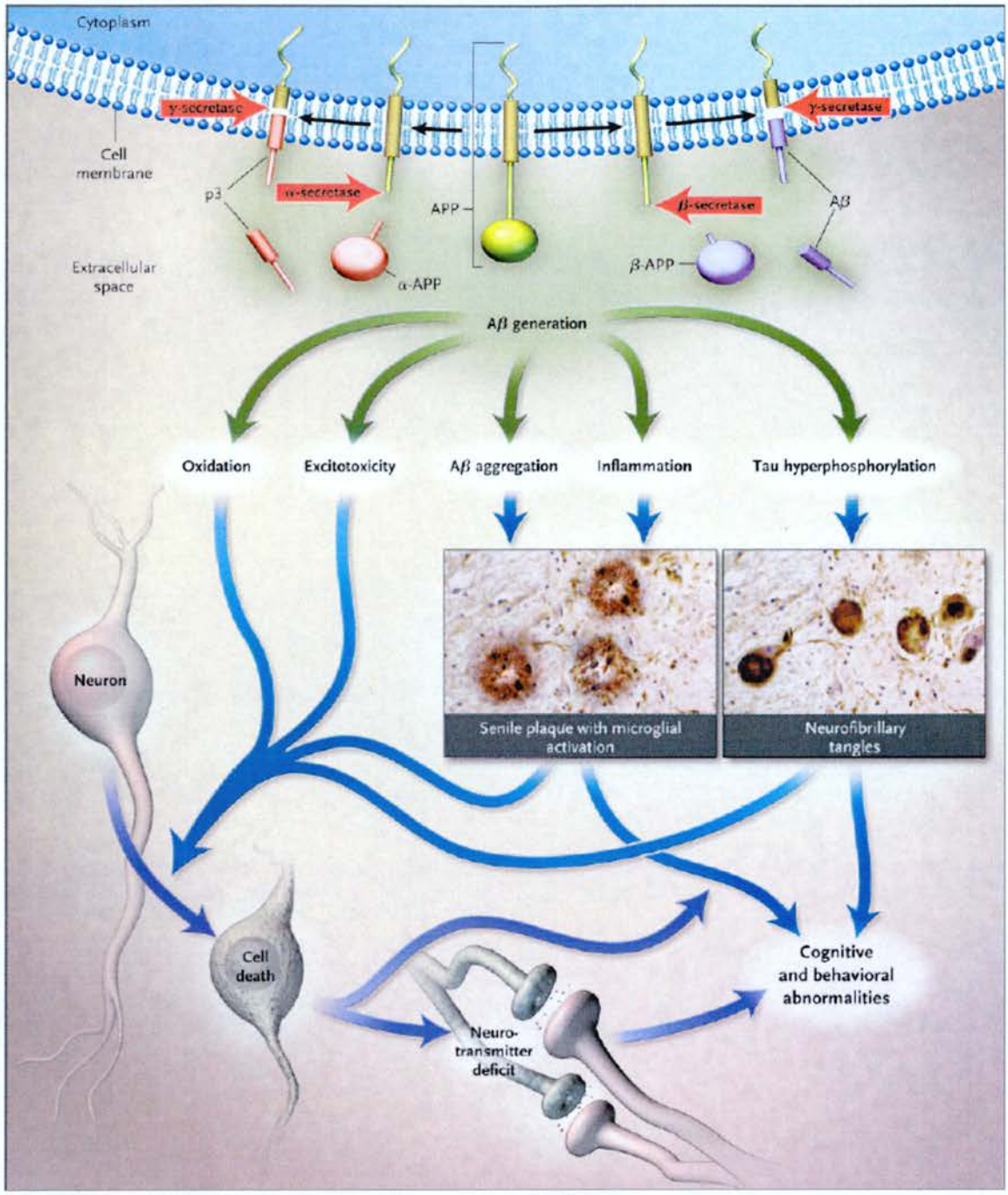


Fig. 1.1: Putative model of Amyloid cascade (from Cummings, 2004)

Hypothesis of the amyloid cascade charting its progression from the initial generation of the β-amyloid (Aβ) peptide via amyloid precursor protein (APP) cleavage by β and γ-secretases, to cell death, neurotransmitter deficits and cognitive and behavioural abnormalities.

APP exists in a variety of isoforms that vary from 651 to 770 amino acids long, including APP695, APP765 and APP770 (Oltersdorf *et al*, 1989). Abnormal processing of these APPs can underlie Aβ deposition in the brains of patients with Alzheimer's disease (Goate *et al.*, 1991; Mullan *et al.*, 1992). The term amyloid describes a heterogenous class of protein aggregates with a β-pleated sheet secondary structure that confers affinity to the histochemical dye congo red (Götz *et al.*, 2004). In normal processing APP is proteolytically cleaved either by α-sectretase or β-secretase (Fig. 1.1). Cleavage by α-secretase is non-amyloidogenic as it creates a fragment, α-APP, that is released from the cell surface leaving a C-terminal fragment of 83 amino acids (C83) still embedded to the membrane which when cleaved by γ-secretase produces the latter two thirds of Aβ (designated p3). However, when APP is cleaved by β-secretase the surface terminal fragment is 99 amino acids long (C99), which when cleaved by γ-secretase, produces Aβ. The C99 cleavage site by γ-secretase is critical as it determines the peptide length, producing either $A\beta_{(1-40)}$ (more common) or $A\beta_{(1-42)}$ (less common but the neurotoxic form.

Blessed and colleagues (1968) first proposed what is now referred to as the amyloid hypothesis when they suggested a correlation (+0.770) between the intensity of Aβ plaque formation and the degree of cognitive decline. Whilst some studies support these findings, others have found this correlation difficult to reproduce (Dekosky *et al.*, 1990; 1992; Terry *et al.*, 1991; Samuel *et al.*, 1994b; Cummings *et al.*, 1996). The presence of diffuse Aβ deposits could account for some of the inconsistencies observed (Hardy and Selkoe, 2002). Whilst it has been suggested that there is a better correlation between NFTs and cognitive decline, this link is confounded by the

presence of dementia in some Alzheimer's disease patients without NFTs (Samuel *et al.*, 1994b). There is increasing consensus in the proposition that the formation of NFTs in Alzheimer's disease represents a cytological response by neurons to the accumulation of Aβ and associated proteins (Götz *et al.*, 2001; Hardy and Selkoe, 2002; Oddo *et al.*, 2003; Selkoe *et al.*, 2004; Fig. 1.1).

Evidence supporting the amyloid hypothesis comes from numerous sources. For example, whenever Aβ pathology was identified tau pathology was also detected whereas the opposite is not true (Arriagada *et* al., 1992; Delacourte *et al.*, 2002). Also to date no mutations in the tau gene have been discovered in patients with Alzheimer's disease, whereas they are present in other patients with other neurodegenerative disorders such as frontotemporal dementia, in which Aβ deposition does not occur (Spillantini and Goedert 1998; Hutton *et al.*, 1998; Götz *et al.*, 2004; Selkoe, 2004). Numerous mutations in genes that contribute to Aβ plaque formation have been identified (see table 1.1).

The first mutation of the APP gene identified was in a British family (Goate *et al.*, 1991), where a valine was replaced by an isoleucine at codon 717 (V717I; the 'London mutation'). Mutations at codons 670 and 671 were also discovered in two Swedish families (Mullan *et al.*, 1992), whereby lysine and methionine were replaced by aspartic acid and leucine (K670D/M671L; 'Swedish mutation' APP_{swe}). Presenilin 1 and presenilin 2 mutations, linking to chromosomes 14 and 1 respectively, were later discovered and have since been identified in most cases of familial Alzheimer's disease (Van Broeckhoven *et al.*, 1992; Levy-Lahad *et al.*,

1995; Sherrington *et al.*, 1995; Götz *et al.*, 2004). However, the only definitive risk factor gene for sporadic Alzheimer's disease identified to date is the apolipoprotein E4 (ApoE4) gene that promotes Aβ deposition (see review by Rocchi *et al.*, 2003).

Chromosome	Gene Defect	Phenotype
21	Aβ precursor protein mutations	Increased production of all Aβ proteins or $A\beta_{(1-42)}$
19	Apolipoprotein E4 polymorphism	Increased density of Aβ plaques and vascular deposits + early onset
14	Presenelin 1 mutation	Increased production of $A\beta_{(1-42)}$ + very early onset
1	Presenelin 2 mutation	Increased production of $A\beta_{(1-42)}$ + very early onset

Table 1.1: Genetic Factors Predisposing to Alzheimer's disease: Relationships to the Aβ phenotype

1.3 Cognitive Consequences of the Syndrome

Some of the most common behavioural consequences of dysexecutive syndrome include attentional impairments, with increased distractibility, poor memory, perseveration, and agitation (Levin, 1984; Trimble, 1990; Godefroy *et al.*, 1996; Baddeley *et al.*, 1997; Adler *et al.*, 1998). Naturally there are also impairments of executive function. Unlike other cognitive domains such as attention and memory, there is no intuitive lay concept of executive function. Funahashi, (2001) defined it as 'a product of the co-ordinated operation of various processes to accomplish a particular goal in a flexible manner'. Greater importance is being placed on neuropsychological assessment of clinical patients, where identification of the exact cognitive impairments may be more relevant in the search for therapeutics (Evans *et al.*, 1997).

1.3.1 Impairments of Executive Function

The Wisconsin Card Sort Task (WCST; Grant and Berg, 1948) is the most widely used test for executive dysfunction. In this test, subjects are required to sort cards according to three perceptual dimensions (e.g. shape, colour and number). The cards change constantly, yet a correct choice is required each time. To do this task the subject learns to formulate a rule, such as always select the card with the red colouring. The rule is gauged to have been learned when six consecutively correct choices are made. The rule can then be altered, for example to select green colouring only, equating to an intra-dimensional shift as the new rule is within the same

dimension – colour. Six consecutively correct responses are required once again. This intra-dimensional shift forces the subject to abandon a learned rule in light of new evidence. An extra-dimensional shift occurs when a previously irrelevant dimension (shape) becomes the new sorting rule. It generally takes more attempts to shift attention to this new dimension, and patients with executive dysfunction take even longer. This is known as perseverative responding, and is interpreted as an impaired ability to shift an attentional set (Owen *et al.*, 1993).

Patients with schizophrenia perform poorly in the WCST (reviewed in Wienberger and Lipska, 1995) as they perseverate and do not readily shift attentional set, even in comparison to their monozygotic twins (Berman *et al.*, 1992). This has been linked to hypofrontality of the dorsolateral prefrontal cortex (Weinberger *et al.*, 1986; 1988; Taylor, 1996; Bunney and Bunney, 2000). However, it has recently been recognised that impaired performance in the WCST may be confounded by impairments in attention and working memory, reducing its suitability as a test solely selective for executive dysfunction (see Reitan and Wolfson, 1994 for a critical review of the WCST; Demakis, 2003). Similar confounds of attentional and working memory deficits may also mar the poor WCST performance in Alzheimer's disease patients (Michon *et al.*, 1994; Paolo *et al.*, 1996).

1.3.2 Attentional Impairment

One of the most consistent cognitive deficits in schizophrenia is attention (Cornblatt and Kelip, 1994). This attentional impairment has been proposed to underlie the

positive, negative and other cognitive symptoms of the disorder (Venables, 1992; Cullum *et al.*, 1993; Cornblatt and Kelip, 1994). Alzheimer's disease patients also exhibit attentional impairments that are suggested to be a core feature of the disease, contributing to the reduction in cognitive performance observed in these patients (Lawrence and Sahakian, 1995; Perry and Hodges, 1999, Rizzo *et al.*, 2000).

Attention can be split into separate yet inter-related subsystems, such as selective attention (ignoring irrelevant stimuli in the environment in order to attend to the relevant), divided attention (attending to two sets of stimuli simultaneously) and sustained attention (maintaining focus on a specific stimulus over a period of time). Whilst impairments in both selective and divided attention have been noted in schizophrenic patients (Jacobsen *et al.*, 2004), greater focus has been placed on sustained attention. One of the most widely used tests of sustained attention is the Continuous Performance Test (CPT; Rosvold *et al.*, 1956) which requires a subject to attend to visual stimuli over a sustained period of time, making a specific response when the target stimuli appear. Though there are many forms of this test, the performance of schizophrenic patients is consistently low (Katz *et al.*, 1996; Chen *et al.*, 1998; Cornblatt *et al.*, 1998; Liu *et al.*, 2000; Cornblatt and Malhorta, 2001). Hazlett and colleagues (2000) used PET to show that whereas normal subjects had increased cerebral blood flow in the frontal cortex during task performance, schizophrenics did not. Barch and colleagues (2001) more specifically claimed a dysfunction in the dorso-lateral prefrontal cortex in medication-free first-episode patients mediates this impaired attentional functioning.

Alzheimer's disease patients also exhibit impaired sustained attention (White and Levin, 1999). Uncertainty still abounds as to whether this impairment occurs prior to impaired episodic memory (Becker, 1988; Baddeley *et al.*, 1991; Becker *et al.*, 1992) or whether episodic memory impairments occur first, followed by attentional/executive, then visuo-spatial and language impairments (Binetti *et al.*, 1996; Reid *et al.*, 1996; Perry and Hodges, 2000). Though not all components of attention are affected at the same stages in Alzheimer's disease patients (Perry and Hodges, 1999), Simone and colleagues (1997) suggest that early attentional deficits in Alzheimer's disease patients may contribute to the performance reductions in other cognitive domains such as memory and executive functions. Indeed Lawrence and Sahakian (1995) suggest it may be a core feature of Alzheimer's disease. These proposals are supported by the findings that visual sustained attention and processing speed impairments in patients with mild Alzheimer's disease correlate with specific cognitive deficits such as memory and decision-making, and with overall cognitive ability (Rizzo *et al.*, 2000).

1.3.3 Memory Deficits

Schizophrenic patients exhibit a consistent impairment in working memory (Castner *et al.*, 2004). This is commonly assessed by the digit span task, whereby subjects are required to remember a list of random numbers and the poor performance of schizophrenic patients in this task suggests a reduced capacity of their 'online' memory (Baddeley, 1986; Byrne *et al.*, 1999; Appels *et al.*, 2003). The consistency of this impairment has recently been confirmed by a meta-analysis by Sitskoorn and

colleagues (2004). The first memory impairment to manifest in Alzheimer's disease is episodic memory, particularly the formation of new memory traces. Episodic memory is the long-term memories of events in your life, and an episodic buffer (Baddeley, 2000) has recently been incorporated into Baddeley's (1986) seminal construct of working memory. Unfortunately this area of cognition has proven to be especially difficult to model in rodents, and thus the development of drugs to alleviate this symptom has been hampered. However shorter-term memory trace impairments are also apparent in Alzheimer's disease. This has been assessed using a variety of methods such as the repetition test (Bayles, 2003) and spatial working memory task (Rahman *et al.*, 1999), the latter being a test based on the radial arm maze (RAM) test of spatial working memory for animals. Both tasks assess visuo-spatial working memory span capacity, which is severely reduced in patients with Alzheimer's disease. Reduced working memory capacity in Alzheimer's disease patients have also been identified using the digit span task (Jones *et al.*, 1992; Pasquier *et al.*, 2001).

1.3.4 Impaired Pre-attentional Processing

The sensory gating (pre-attentive) impairments exhibited by schizophrenic patients have also been well documented (Adler *et al.*, 1982; Freedman *et al.*, 1997; Kumari *et al.*, 2000; Freedman *et al.*, 2003). It has been suggested that these deficits might indirectly be the cause of impaired sustained attention (Nieoullon, 2002). Sensory gating refers to pre-attentive information processing at a subconscious level (Ellenbroek, 2004). This processing can be assessed in a variety of ways, but two of

the most widely used is Pre-Pulse Inhibition (PPI), the reducing effect of a weak, non-startling stimulus on a subsequent startle stimulus (Graham, 1975), and P50 gating, the reducing effect a pre-stimulus has on a subsequent stimulus. The former is measured by the startle amplitude of the subject, whilst the latter is assessed using electroencephalograph. Whilst results using the two techniques share similarities, there are differences that should be taken into consideration (see Ellenbroek, 2004). However, as no response is required of the subject, confounds that hamper other neuropsychological tests such as background and education level are absent.

Venables (1992) theorised that this sensory gating impairment in schizophrenic patients leads to information overload, irrelevant stimuli are not filtered out, and their symptoms (cognitive and positive) exacerbated. The reproducibility of this impairment has led it to be a focus of animal models of schizophrenia (Ellenbroek and Cools 1990; Swerdlow-Geyer, 1998; Sams-Dod, 1999; Geyer *et al.*, 2002). Uncertainty exists as to whether patients with Alzheimer's disease impaired sensory gating (Jessen, 2001; Hejl *et al.*, 2004), though this may reflect the stage in the progression of the disease at which the patients were tested.

1.3.5 Impairments of Olfactory Functioning

Interestingly olfactory capabilities (acuity, threshold and working memory) are impaired in patients with Alzheimer's disease and schizophrenia (Kopala *et al.*, 1993; Wu *et al.*, 1993; Moberg *et al.*, 1997; Larsson *et al.*, 1999; Devanand *et al.*, 2000; Kohler, 2001). These impairments appear to be related to disease progression, may be present prior to diagnosis, with no apparent relation to other cognitive

deficits (Moberg *et al.*, 1997; Devanand *et al.*, 2000). As such they have been proposed as possible early markers for neurological diseases and psychosis (Kwapil *et al.*, 1996; Martzke *et al.*, 1997). In support of these findings is the observation that MCI patients also have poor olfactory capabilities (Devanand *et al.*, 2000).

1.3.6 Nicotine therapy

In comparison to the general population, patients with schizophrenia exhibit higher rates of smoking (Dalack *et al.*, 1998) smoke more heavily (Leonard *et al.*, 2001) and extract more nicotine from cigarettes (Olincy *et al.*, 1997). This is thought to be a form of self-medication, whereby indirectly, nicotine ameliorates some of the cognitive impairments associated with the disease (George *et al.*, 2002), or antipsychotic medication (Levin *et al.*, 1996). Nicotine-induced cognitive enhancement has also been observed in patients with Alzheimer's disease (Sahakian *et al.*, 1989; Jones *et al.*, 1992; White and Levin, 1999) and even in normal control subjects (Levin *et al.*, 1998; Mumenthaler *et al.*, 2003). The use of smokers si known to confound the interpretation of studies and therefore not all studies have identified improved working memory post nicotine administration (Sahakian *et al.*, 1989; Heishman and Henningfield, 2000; Harris *et al.*, 2004).

It has also been proposed that nicotine-induced improvement in cognitive performance may only occur in tasks of high attentional demand or in subjects with impaired performance (Robbins *et al.*, 1988; Warburton and Rusted, 1993; Warburton and Arnall, 1994; Levin and Simon, 1998; White and Levin, 1999; Harris

et al., 2004; Newhouse *et al.*, 2004). Therefore the lack of effect of nicotine in some cognitive tasks could be due to a lack of attentional demand in the task. This was elegantly demonstrated by Bernard and colleagues (1991) who developed a memory task with a variable attentive load, and found that nicotine only improved performance in the task with the high attentional load. The attention-enhancing efficacy of nicotine was further demonstrated by Mumenthaler and colleagues (2003). These authors administered nicotine or the acetylcholinesterase inhibitor donepezil to experienced pilots performing on a flight simulator, a task requiring continuously high levels of sustained attention, and found that both improved performance to a similar degree. Of specific therapeutic interest was that this effect was observed in an acute, single dose of nicotine, whilst donepezil was administered over a month. Newhouse and colleagues (2004) explained the observed effects of nicotine in relation to the Yerkes-Dodson principal. That is to say, a person operating at near-optimal levels in a cognitive task, would exhibit a ceiling effect, making drug administration ineffective, or deleterious to performance as occurred consistently in normal healthy volunteers administered nicotine (Heishman and Heningfield, 2000; Sakurai and Kanazawa, 2002). Conversely, in patient groups where a cognitive deficit is present and performance sub-optimal, a window for improvement is present and nicotine administration could normalise their performance, (Newhouse *et al*, 1988; White and Levin, 1999; Davranche and Audiffren, 2002). However, improvements in cognition observed when nicotine is administered to normal subjects could also be explained in terms of the Yerkes-Dobson principal. Tasks of extreme difficulty could force sub-optimal performance in subjects, thus proffering an opportunity for drug-induced improvement.

Confirmation of this concept is provided when nicotine-induced improvements in attention were observed in healthy controls performing the CPT (Levin *et al.*, 1998), and pilots in a flight simulator (Mumenthaler *et al.*, 2003). The side-effects profile of nicotine is extensive and includes addiction, nausea, cardiovascular stress and weight loss, the costsof which are deemed to outweigh nicotine's potential benefits (Waldum *et al.*, 1996; Levin, 2002; White and Levin, 2004; Yildiz, 2004). Therefore the identification of the receptors/pathways through which nicotine produces its beneficial cognitive effects is a prerequisite to facilitate the discovery of more selective ligands with minimal side-effects (Levin, 2002).

1.3.7 Nicotinic Acetylcholine Receptors

Nicotine is the prototypical ligand to the nAChRs. The nAChRs are transmembrane proteins composed of distinct subunits (Arias *et al.*, 2000). To date twelve genes have been described that encode neuronal nAChRs subunits (α2-α10, β2-β4), forming both heteromeric and homomeric ligand gated ion channels. Heteromeric nAChRs are composed of both α (α2-α6) and β (β2-β4) subunits, whilst homomeric nAChRs consist of only α (α7-10) subunits. Every neuronal nAChR subunit has four transmembrane spanning domains (Fig. 1.2a) with the second (M2) transmembrane domain hypothesised to line the pore of the nAChR ion channel (Unwin, 1995, 1998; Arias, 2000; Fig. 1.2b). Several conserved amino acids in the M2 domain are critical for pore function, and such is the specificity that in humans, a missense mutation of a conserved serine ring to phenylalinine (S248F), or another conserved serine to leucine (S252L) in the α4 subunit has been identified in families

with a form of frontal lobe epilepsy (Steinlein *et al.*, 1995; 1997; Hirose *et al.*, 1999). The interface between an α subunit and its neighbouring subunit has been identified as being the agonist-binding domain of the nAChRs (Czajkowski & Karlin, 1995; Chiara & Cohen, 1997; Arias, 2000; Fig. 1.2c). Specific nAChRs are preferentially expressed in certain brain regions although the α7 and α4β2 nAChRs are highly expressed in the hippocampus, providing a hypothetical link to learning and memory (Levin, 2002). These receptors can modulate a variety of neurotransmitter systems in the brain by both pre-and post-synaptic mechanisms (Bednar *et al.*, 1998; Levin and Simon, 1998; Keiser *et al.*, 2000).

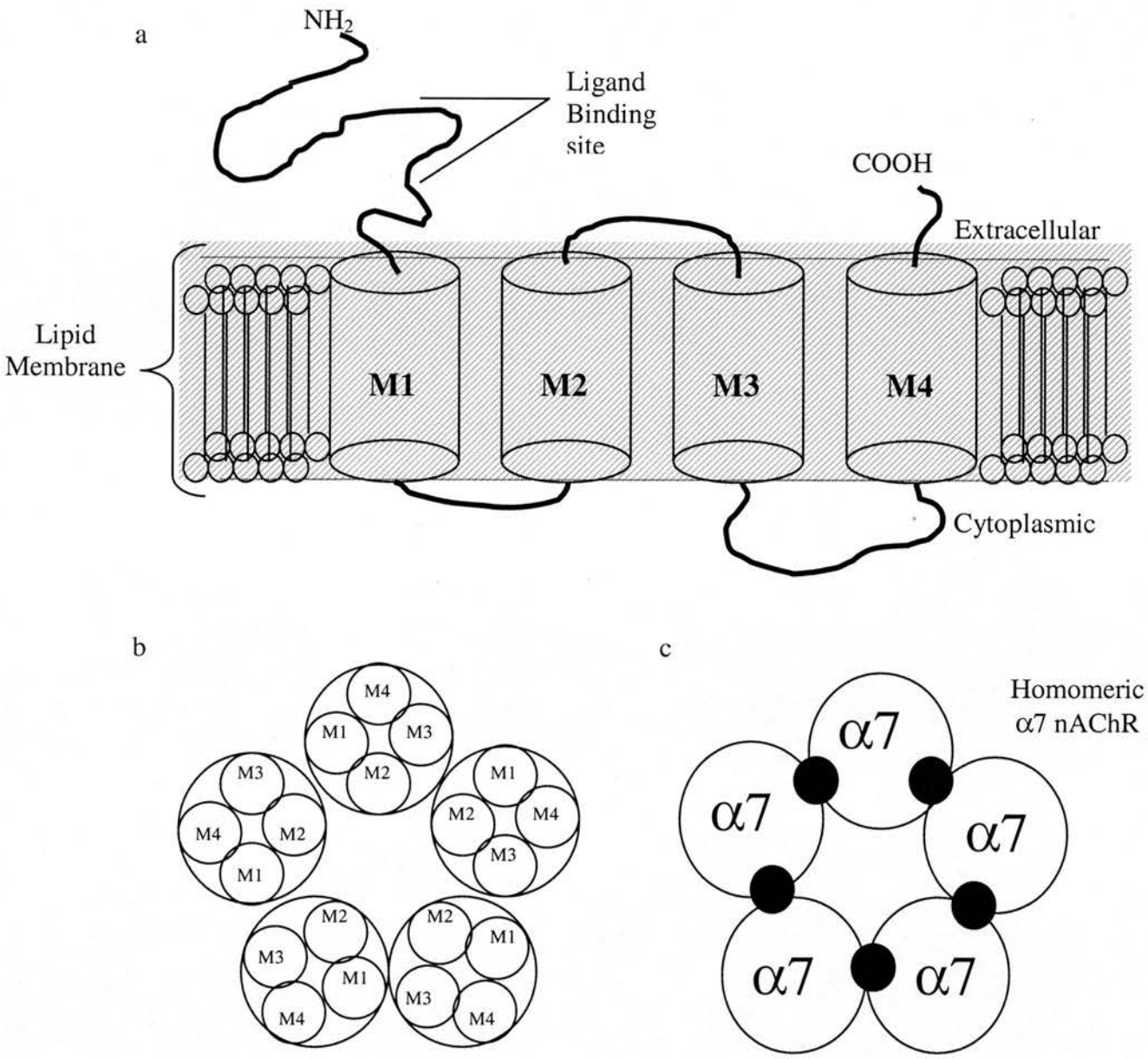


Fig. 1.2: Schematic representation of the nAChR.

Each nAChR consists of four transmembrane spanning domains (a). The M2 domain has been hypothesised to line the pore of the nAChR ion channel, with mutations in this domain capable of altering pore function. Finally the interface between an α subunit and its neighbouring subunit is the likely agonist-binding of the nAChRs (c).

Task	Primary cognitive domain assessed	Species	Pros	Cons	Motivation	References
5-choice serial reaction-time task	Sustained attention	Rats / mice	Automated with high face and predictive validity with CPT	Extensive training and specialist equipment required	Appetitive	Carli *et al.*, (1983); Young *et al.*, (2004)
Attentional set-shifting	Executive functioning	Rats / mice	Simple design with high homology between WCST	Not widely established, predictive validity unknown	Appetitive	Birrel & Brown, (2000); Colacicco *et al.*, (2002)
Pre pulse inhibition and P20N40 waveform	Pre-attention (sensory gating)	Rats / mice	Simple, + high face and predictive validity with human tests	Specialist equipment required + not reflect 'real world' attention	None	Stevens *et al.*, (1996); Sallinen *et al.*, (1998)
Morris water maze	Spatial learning and memory	Rats / mice	Simple, quick design, well characterised	Confound of stress and of behavioural responses to task	Aversive	Morris *et al.*, (1981); Chen *et al.*, (2000)
Delayed non-match to place	Spatial working memory	Rats	Fully automated, well characterised	Non-memory strategies used, high levels of PI	Appetitive	Dunnet and Martel, (1990)
T-Maze alternation	Spatial working memory	Rats / mice	Can be automated, simple design	Ceiling effects and PI confounds	Appetitive	Levin *et al.*, (1997)
Spontaneous alternation in T-Maze	Exploratory behaviour	Mice	Very simple and quick + can be automated	No clear results obtained in terms of cognition	Innate	Gerlai (1998)
Radial arm maze	Spatial working memory	Rats / mice	Can be automated, simple design, high predictive and face validity	Ceiling effects	Appetitive	Levin *et al.*, (1996); Sigurdsson *et al.*, 2004
Odour span task	Non-spatial working memory	Rats / mice	Simple design, no PI, ethologically relevant stimuli	Not widely established, predictive validity unknown	Appetitive	Dudchenko *et al.*, (2000); Young *et al.*, (*in press*)

Table 1.2: Summary of tasks discussed within this thesis.

Focus shall be placed on assessing attention, not only as deficits in attention are purported to being central to the cognitive deficits suffered by schizophrenic and Alzheimer's disease patients, but also due to the central role of attention in animal learning theories. Mackintosh (1975a) suggested that animals attend to stimuli in their environment that are more salient or provide greater validity for predicting an event, and that the saliency of some stimuli is innate. This theory therefore allows for selective association, whereby some stimulus/event associations are more readily learned than others. This is observed in Garcia and Koelling's (1966) classic experiment showing that rats associate a novel flavour cue with subsequent illness far more readily than an auditory or visual cue, whilst the opposite is true if the aversive event is an electric shock. This also offers an explanation as to why learning is faster when mice are required to dig to retrieve a food reward (the odour span task, see section 1.4.3, pp. 37), than when they are required to nose poke into a brightly lit hole for a reward that is presented in a separate area (5-choice serial reaction-time task, see section 1.4.1, pp. 34). Mackintosh (1975b) also claimed that more salient/valid stimuli in the environment will have greater associative strength (strength by which a stimulus is associated with an event) than other stimuli within that environment, and that when this is reinforced, its associative strength increases with a concomitant decrease in the associative strengths of other environmental stimuli, a phenomenon he termed as the inverse hypothesis. However, whilst the associative strengths of a stimuli may be great (e.g. 80% of attentional resources), attention is also given to other stimuli in the environment. The ability of rats to shift their attentional set from one dimension to another (Birrell and Brown, 2000) provides support for this theory (see section 1.4.2, pp. 36).

The theory of Pearce and Hall (1980) also places attention as core to their animal learning theory. However, whilst Mackintosh (1975a) proposed that greater attention would be given to stimuli that predict an event, Pearce and Hall (1980), claim the opposite. They suggested that the more predictable a stimulus is to an event, the more it will be processed automatically and thus less attention is paid to it. Only surprising stimuli or unexpected events will receive attention as they are yet to be learned about. Whilst this theory receives some experimental support (Hall and Pearce, 1982), it also has its shortcomings. For example, this theory cannot explain learned irrelevance, whereby the pairing of a particular stimulus to an event takes longer if said stimulus was previously randomly paired with said event (thus did not reliably predict that event), than if the stimuli and event were novel.

1.4.1 Sustained Attention

The 5-choice serial reaction-time (5-CSR) task is a very good example of an animal task with a high degree of face validity. The 5-CSR task is a rodent test of sustained attention, originally developed by Carli and colleagues over twenty years ago in 1983. The 5-CSR task was based on Leonard's 5 choice test of serial reaction, devised to assess the attentional performance of enlisted men (cited in Wilkinson, 1963), and has latterly became accepted as being analogous to the CPT (Jones and Higgins, 1995). Though it was primarily designed to assess sustained attention, it can be modified to examine other areas of attention (Parusaruman, 1998; Chudasama and Robbins, 2004).

The 5-CSR task is the most widely used test of attention in rodents, and though a variety of protocols and apparatus have been utilised (Muir *et al.*, 1996; Humby *et al.*, 1999; Grottick and Higgins, 2000; Hahn and Stolerman, 2000; Marston *et al.*, 2001) the task largely remains unchanged (Carli *et al*, 1983). The rodent is placed in an operant box with 9 apertures located at the rear of the box, with 4 occluded by metal caps (see Fig. 2.1, pp. 54). A magazine for trial initiation and reward delivery is located at the front of the box, with trials initiated by a nose-poke in the magazine, after which a predetermined delay begins (inter-trial interval; ITI). At the end of each ITI one of the 5 apertures at the rear of the box is illuminated for a brief time, and within that time the rodent must nose poke into that aperture to gain a reward. Thus during the ITI the rodent must vigilantly attend to the apertures (array) at the rear of the box, and divide its attention between the 5 apertures. This behaviour must be maintained for a predetermined number of trials (100-120), or session length (25-30 min). See chapter 2.2 (pp. 52) for a more detailed description of the task and the modifications available. Although the design is simple, training rodents to perform the task is time-consuming and labour intensive, taking 3 – 4 months, and producing approximately 5000 bits of data per animal for acquisition alone. However, the high degree of face validity between this task and the CPT makes it an invaluable test of attention in rodents.

In fact the predictive validity between the 5-CSR task and the CPT has recently been reinforced. As described previously nicotine improves sustained attention in man, including schizophrenic and Alzheimer's disease patients (Levin *et al* 1998; White and Levin, 1999; Yong *et al.*, 2002; Mumenthaler *et al.*, 2003, see chapter 1.3.6, pp.

26). More recently nicotine has been shown to improve rat performance of the 5-CSR task (Grottick and Higgins, 2000, Hahn and Stolerman, 2002; Grottick *et al.*, 2003), following considerable effort invested in attempts to consistently produce this effect (Mirza and Stolerman, 1998; Blondel *et al.*, 1999; Grottick and Higgins, 2000; Stolerman *et al.*, 2000; Mirza and Bright, 2001; Hahn *et al.*, 2002; Terry *et al.*, 2002; Grottick *et al.*, 2003; Hahn *et al.*, 2003a,b).

1.4.2 Executive Function

Birrell and Brown (2000) recently described a rodent analogue to the WCST, which they named the attention set-shifting task. In this test, instead of using colours and shapes to assess attentional set-shifting, the authors utilised different odours and digging mediums. This is appropriate as rodents exhibit preferential attendance to olfactory as opposed to visual cues (Jennings and Keefer, 1969). Hence an intra-dimensional shift in this task is represented by shifting the rule for a correct response to a different stimulus within the same dimension (e.g. for odour, shifting from parsley to sage). An extra-dimensional shift represented shifting the rule from one dimension to the other (e.g. from odour to digging medium). As with the WCST, 6 consecutively correct responses are required prior to 'shifting' the rule.

With this task Birrell and Brown (2000) showed that although the rodent medial prefrontal cortex is not anatomically analogous to the primate (including man) dorsolateral prefrontal cortex, it does appear functionally homologous. Evidence in support of this notion already existed with impaired performance of medial prefrontal

cortex lesioned rats in the 5-CSR task in response to altering the task protocol (Muir *et* al., 1996; Granon *et al.*, 1998). This attention set-shifting task has recently been adapted for use in mice (Colacicco *et al.*, 2002), and so provides a valuable tool for modelling executive functioning in transgenic animals. More recently, rats administered chronic phencyclidine, an animal model of schizophrenia (Cochran *et al.*, 2003), exhibited impaired performance in attention set-shifting task akin to that observed in schizophrenic patients (Reid *et al.*, 2004).

Thus the high degree of face (using intra- and extra-dimensional shifts) and predictive (lesions and animal models producing similar results to those observed in humans) validity between the attentional set-shifting task and the WCST gives credence to its continued use in modelling executive functioning in animals. However, this is a novel task and requires further validation. Moreover, the lack of studies examining the effects of nicotine in schizophrenic or Alzheimer's disease patients using the WCST, and the confounds of the WCST discussed above (see section 1.3.1, pp. 20), may limit the use of this task to identify cognitive enhancing compounds.

1.4.3 Sensory Gating

Schizophrenic, and more recently Alzheimer's disease patients, exhibit impairments in pre-pulse inhibition (PPI) and P50 suppression of the event related potential (ERP; Adler *et al.*, 1982; Freedman *et al.*, 1987; Kumari *et al.*, 2000; Jessen, 2001), two measures of sensory gating (pre-attentional processing; Braff and Light, 2004).

These two tasks have been modelled in animals, with PPI being a direct comparison, whilst the P50 suppression of ERP has been modelled using the P20-N40 hippocampal waveform in mice (Stevens *et al.*, 1996; Simosky *et al.*, 2001; Simosky *et al.*, 2003). PPI is measured by the ability of a subject (man or rodent) to inhibit their startle response to an auditory (or tactile – puff of air) stimulus when it is preceded by a similar but sub-startle threshold stimulus. The P50 suppression of the ERP test is conducted in a similar fashion, with a startle and pre-startle stimuli. However, its effects are measured directly as hippocampal waveforms of electric current.

The greater inhibition of startle response of schizophrenic patients in these tasks following nicotinic administration (Adler *et al.*, 1992) has also been modelled in rats (Acri *et al.*, 1994; Suemaru *et al.*, 2004) and mice (Semenova *et al.*, 2003). In fact work conducted on the P20-N40 waveform in different lab mouse strains found a positive correlation between impaired sensory gating performance and reduced levels of the α7 nAChR in the hippocampus (Stevens *et al.*, 1996). The following year the sensory gating impairment observed in schizophrenics was linked to SNIp or mutation in chromosome 15, proximal to the α7 locus (Freedman *et al.*, 1997), with P50 suppression being regulated by neural circuitry with a prominent role for hippocampal circuitry (Waldo *et al.*, 1994). Using the P20-N40 waverform and PPI tasks to model attentional processing in rodents has various benefits, the main being the high throughput that can be achieved when compared with the 5-CSR task, as no training is required. Both acoustic and tactile startle stimuli can be employed to for reliability. However, these tests do not involve conscious attentional processing,

therefore do not reflect the 'real world' attentional problems schizophrenic patients face. In fact PPI and P50 suppression can be altered by the level of effortful attention placed on detecting the startle stimulus (Heekeren *et al.*, 2004). Also, the incidental nature of performance in these tasks is reflected in the involvement of the hippocampus in mediating the response (Stevens *et al.*, 1996; Freedman *et al.*, 1997; Le Pen, *et al.*, 2003), whilst hippocampal ablation has no effect on the attentional performance of rats in the 5-CSR task (Kirkby and Higgins, 1998). The high throughput availability in the task still makes it a valuable tool for the psychopharmacologist, using it as a possible screen for attentional enhancing agents prior to testing them on the more laborious 5-CSR task.

1.4.4 Working Memory

1.4.4.1 Morris Water Maze

One of the most commonly used tool in behavioural neuroscience is the Morris Water Maze (MWM), which is in part due to its simple and elegant design. The MWM was first developed by Morris and colleagues (1981) to assess spatial learning and memory in rats, and involved placing the test animals in a pool of opaque water, in which traditionally they used spatial cues to locate a platform situated just under the water's surface (Fig. 1.3a). Their ability to learn the location of the platform was originally measured by their escape latency (length of time they took to reach the platform). Whilst many researchers still report this measure alone, increasingly more complex measures have been incorporated. Temporal working memory can also be

assessed by retesting the animal after a delay period. However, researchers are becoming increasingly aware of the influence of apparatus, training procedure, species, strain, gender and even bodyweight, on performance of the animals tested (D'Hooge and Deyn, 2001). Whilst both rats and mice have been tested using this apparatus, many innate differences exist between these two species affect performance. For example, floating and thigmotaxis (tendency to swim close to the pool wall) is more common in mice. Hence the recommendation that path length taken by the animal to reach the platform is the most appropriate measure of the task, as opposed to reporting escape latencies alone (Lindner, 1997). Lipp and Wolfer (1998) found that spatial working memory of C57Bl/6 to be comparable to that of Long-Evans hooded rats when assessed on a dry-land task, but was worse when assessed in the MWM.

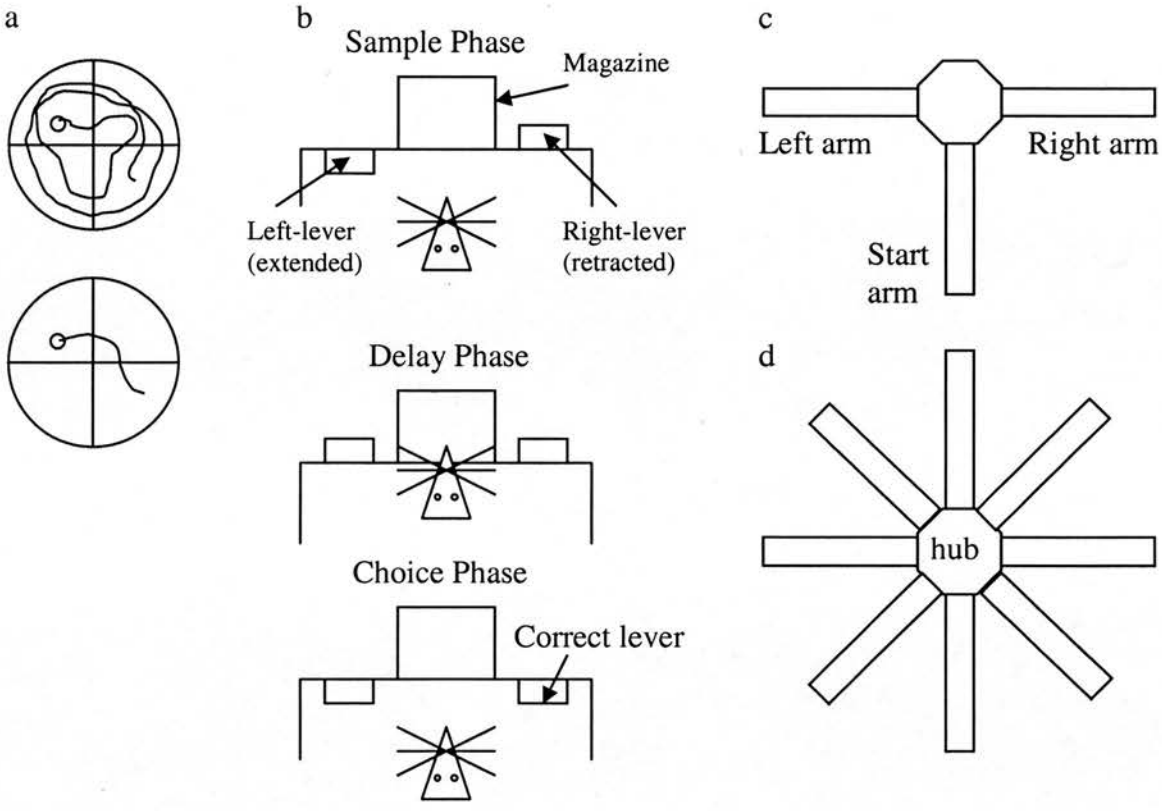


Fig. 1.3: Schematics of the various tasks discussed.

The Morris Water Maze apparatus (a) is a circular pool filled with opaque water, plus an escape platform located just under the water's surface in a predetermined position. The top panel gives an example of the path length taken to reach the platform on the first day of acquisition, with performance greatly improved after several sessions of training (bottom panel). The Delayed Non-Match To Place apparatus (b) is a two-lever operant box. Within each trial, the rodent will progress through the sequence shown, depressing the extended lever (left) in the sample phase, nose-poking in the magazine continuously during the choice phase, and being required to depress the opposite lever (right) in the choice phase. The T-maze (c) requires the rodent to leave the start arm and turn left or right. The rodent is then returned (or returns on own accord in continuous format) to start arm, and then must enter the alternate arm, either spontaneously or reinforced. Each new trial requires alternation to the opposite arm previously visited. The Radial Arm Maze apparatus (d) has 8 arms (though 16 is possible) and the rodent begins the task in the hub. Each arm is baited,

and the rodent visits each arm to retrieve the reward. Performance is measured on the number of arms entered prior to re-entry.

Even the temperature of the water has been shown to differentially affect performance (Seldon *et al.*, 1990). One of the major disadvantages of the task is that it requires the animal to escape from an aversive environment that is apparently devoid of escape routes (Block, 1999), therefore causing considerable stress in the test animal. Thus animals with greater anxiogenic reactivity perform worse in the task (Holscher, 1999). In fact anxiolytic drugs treatment alters the response of the animal being tested, as do other drug and lesions, by altering their search strategy (see Hodges, 1996 for a review). This may complicate the interpretation of results obtained during drug treatment by altering anxiety levels as opposed to spatial working memory. The innate behaviour of the test animals could also confound performance, even within a species. Galea and colleagues (1994) found that the performance of deer mice from an island population was significantly better than those from a mainland population.

Despite reports of the task being solved utilising spatial cues, rats may also use non-spatial cues, such as odour-trails, which can also confound the interpretation of the results (Lindner *et al.*, 1997). However, the MWM still remains a popular tool for assessing genetically modified mice and drug administration. The behavioural assessment of some of the first transgenic models of Alzheimer's disease was assessed in the MWM (Hsiao *et al.*, 1996; Chen *et al.*, 2000). It is quick to establish, easy to use, and does not require food deprivation, thus the MWM remains a popular and well characterised test of learning and memory in rodents.

1.4.4.2 Delayed Non-Match to Place

Another task capable of assessing temporal working memory is the delayed non-match/match to position (DNMTP/DMTP) task. This has traditionally been assessed in a two-lever operant conditioning box (Fig. 1.3b), and is predominantly performed by rats (Dunnett, 1985; Dunnett and Martel, 1990; Chudasama and Muir, 1997; Marston *et al.*, 1999), although other species have been tested including pigeons and monkeys (Kelly and Soetch, 2000; Sawaguchi and Yamane, 1999; Terry *et al.*, 2002). During the task, one lever is presented and remains extended until it is depressed by the animal (sample phase). A randomly selected delay period then ensues (traditionally between 0 and 30 s), after which the animal is required to nose-poke into the lit magazine. Thus, the animal is required to repeatedly nose-poke in the magazine until the magazine light is extinguished. After the light is extinguished, both levers are extended (choice phase) and the animal is required to depress the opposite (DNMTP), or matching (DMTP) lever from that extended during the sample phase. If completed correctly the animal receives a reward and the second trial begins. The high number of trials (commonly 100) and the fact that a computer controls the apparatus are two of the main perceived advantages of this task. Unfortunately rats have been known to use positioning strategies to complete the task. While the animal nose-pokes in the magazine, they angle their bodies to face the goal lever. Training mice to complete this task has also proven to be notoriously difficult (Dr. C Spratt, *personal communication*), and is reflected in a paucity of publications, meaning the task cannot be utilised to examine the behaviour of transgenic mice.

However, the main confound of this task in terms of identifying a therapeutic agent is the high level of pro-active interference (PI) in the task (Dunnett and Martel; 1990; Levin and Simon, 1998). PI is a phenomenon whereby previously learned information interferes with the learning and/or memory of recently presented material (Melton, 1963). Any compounds that strengthen memory traces will therefore make the previously learned information harder to forget, further increasing the proactive interference inherent in the task. Dunnett and Martel (1990) elegantly demonstrated the existence of this interference in the DNMTP and the resulting effects of nicotine. Thus performance of a given trial in the task was significantly worse if the correct response of the previous trial was in the opposite lever and hence nicotine was found to significantly impair DNMTP task performance (Dunnet and Martel, 1990). Though the PI effects can be limited by increasing the time between trials in the task to 15-30 s, the confound remains when assessing pharmacological manipulations.

1.4.4.3 T-maze

T-maze alternation is another such task that assesses the temporal capacity of spatial working memory. The apparatus of this task is in the shape of a T, with a start arm leading to a junction where a turn into the left or right arm can be made (Fig. 1.3c). During performance of the task, the animal is required to traverse along the start arm, and then turn in to one arm to receive a reward. It is then placed back in the start arm and a second trial begins. This time the animal must choose the opposite arm from its previous entry in order to gain a reward. Therefore to complete the task successfully the animal must remember which arm it had previously entered in order

to alternate and enter the opposite arm. Performance of rodents is excellent (>90% accuracy) when there is no delay between trials (inter-trial interval; ITI), and is only impaired when ITIs are inserted (performance drops to ~80% at a 40 s delay; Levin *et al.*, 1997). The apparatus can also be used to assess *spontaneous* alternation (see chapter 2.4, pp. 67), whereby no rewards are proffered and the innate, exploratory behaviour of the animal is assessed (Gerlai, 2001). Although Gerlai, (1998) suggests it is a measure of spatial working memory, this is just one facet that contributes towards performance in this task, and recently this task has been labelled as a test of exploratory behaviour (Lalonde *et al.*, 2002). Animal should naturally alternate when exploring an environment. If it no longer alternated after treatment, or did so at chance level, this could be due to varying levels of anxiety, age, altered exploratory behaviour, motoric impediments, or perseverative behaviour. Hence the reinforced T-maze task is more widely used. However, as in the DNMTP, nicotine actually impairs rat performance in this task (Levin *et al.*, 1997), whilst mecamylamine, a nAChR antagonist, improves performance (Moran, 1993; Levin *et al.*, 1997), likely to be due to the levels of PI inherent in this task (Levin and Simon, 1998).

1.4.4.4 Radial Arm Maze

The radial arm maze (RAM) is one of the oldest tests of spatial working memory in rodents (Olton and Samuelson, 1976). The RAM has been used to assess spatial working memory in a variety of species including rats (Levin *et al.*, 1996; Bettany and Levin, 2001, Rezvani and Levin, 2002; Levin *et al.*, 2003), mice (Pick and

Yanai, 1983; Jaffard *et al.*, 1989), rabbits and avians (Lipp *et al.*, 2001) and even man (Aadland *et al.*, 1985). This task traditionally uses an octagonal central chamber with eight attached arms (Fig. 1.3d). Each arm can be baited, and the animal is required to enter each arm and retrieve the reward therein. Thus to complete the task the animal must not re-enter a previously visited arm, adopting a win-shift foraging strategy. The spatial working memory of the animal is measured by the number of baited arms entered prior to re-entering a previously visited arm, thus the maximum number obtainable is 8. Unfortunately this low number limits its power to recognise group differences in basic spatial working memory (Lipp *et al.*, 2001), although 16 arm mazes have been developed in an attempt to overcome this difficulty (Levin *et al.*, 1997). Also, by never baiting certain arms during task acquisition, spatial reference memory can also be assessed. Individual search strategies are evident in the performance of this task, with the animal visiting the opposite arm after it re-enters the hub, or the adjacent arm, especially when the animal is hungrier (Hodges, 1996).

This task differs from both the MWM and DNMTP for although spatial working memory is measured, the RAM assesses spatial working memory span capacity (i.e. the number of items that can be held on-line in working memory) as opposed to its temporal capacity (i.e. the length of time one item can be remembered for). As the rodent is supposed to enter each arm only once, the task is not subject to the levels of PI inherent in the T-maze and the DNMTP, therefore any drug treatment effects are likely to reflect levels of spatial memory. Nicotine, at similar doses that caused deleterious effects on performance in the DNMTP task (Dunnett and Martel, 1990)

and the T-maze (Levin *et al*, 1996), improves spatial working memory capacity in rats performing the radial arm maze (RAM), an affect antagonised by co-administration of mecamylamine, a non-competitive nicotinic antagonist (Levin *et al.*, 1996; Bettany and Levin, 2001). In interpreting RAM data, caution has to be exercised to ensure that the drug does not alter the appetitive state or perception of reward for the animal (Hodges, 1996), though obviously this holds true for any appetitively motivated task. Performance in the RAM is consistent and reliable to such an extent that a human version of the RAM has since been added to the Cambridge Neuropsychological Test Automated Battery (CANTAB; Fray *et al.*, 1996), thus increasing the model's face validity.

1.4.4.5 Odour Span Task

Whilst the RAM assesses visuo-spatial working memory span capacity, another task has recently been developed to assess non-spatial olfactory working memory span capacity. The odour span task (OST) originally developed for rats (Dudchenko *et al.*, 2000) has recently been developed for use in mice (see chapter 4, pp. 118 and 5, pp. 156). In the OST (described in greater detail below, chapter 2.3, pp. 60) the rodent is placed on a table and presented with a bowl filled with a scented digging medium (sand/woodchip - rats/mice), in which a reward is buried. The animal must dig to retrieve the reward, following consumption, the animal is removed from the table, and a second bowl, filled with a digging medium and a different scent, is placed on the table. The novel scented bowl is the only one that is baited with a reward. The location of the old and novel odours on the table is randomly selected.

The animal must only dig in the bowl containing the novel odour, thus retrieving the reward and continuing in the task. A third novel scented (baited) bowl is placed on the table in a random location whilst bowls one and two (no longer baited) are moved to a new location. Again the animal must dig only in the novel scented bowl to gain the reward and continue. The task traditionally continues until 12 differently scented have been presented, though 24 and 22 bowls have been presented to rats and mice respectively in probe trials (Dudchenko *et al.*, 2000; see chapters 4.3.1, pp. 125, and 5.3.3 – 5.3.5, pp. 184 – 190). Thus to gain the highest score possible, the animal must only ever dig in the novel scented bowl, remembering the previously scented odours so as to avoid them.

To ensure the animal does not use any other strategies such as scent marking the previously presented bowls, or by smelling the bait through the digging medium, probe trials and counter-measures have been used in rats and mice to ensure neither strategy is employed. Task performance in rats was found not to rely on the hippocampus (Dudchenko *et al.*, 2000), whereas a recent version created for humans found poor performance in hippocampally lesioned patients (Levy *et al.*, 2003). This may however reflect the extent of damage in the human patients, or the strategy used by humans may have been different to that used by rats. Humans reported remembering the names of the presented odours, and whilst the strategy used by rats is unknown, they may simply remember the odour.

There is a paucity of tasks that assess the olfactory capabilities in mice, despite the plethora of evidence of olfactory impairments in patients with Alzheimer's disease (Larssen *et al.*, 1999; Devanand *et al.*, 2000) and schizophrenia (Kopala *et al.*, 1992; Wu *et al.*, 1993; Moberg *et al.*, 1997; Kohler, 2001). This is also despite the observation that these impairments may be an early marker for neurological diseases and psychosis (Kwapil *et al.*, 1996; Martzke *et al.*, 1997; Devanand *et al.*, 2000). Therefore the development of this task for use in mice allows greater phenotypic characterisation of transgenic animal models of CNS disorders. Transgenic mice may also be used in this task to aid in the identification of the neuro-circuitry responsible for olfactory impairments. That the entorhinal (olfactory) cortex connects via a segment of the thalamic mediodorsal nucleus to the orbitofrontal cortex (Powell, *et al.*, 1965), an area altered in schizophrenic and Alzheimer's disease patients (Chu *et al.*, 1997), supports the need to identify the possibly common neural substrate mediating these olfactory deficits (Moberg *et al.*, 1997). As discussed earlier (section 1.2.2, pp. 12) pathophysiological hallmarks of Alzheimer's disease are known to deposit in the entorhinal cortex early in disease progression (Braak and Braak, 1995). This effect is also observed in a transgenic mouse model of the disease over-expressing the amyloidogenic Swedish mutation of human APP_{695} (Mullan *et al.*, 1992; Hsiao *et al.*, 1996; Nordberg *et al.*, 2002). As the odours presented to the mice are not repeated within a session, PI cannot confound the results of drug administration as it does in the T-maze alternation and DNMTP tasks (Dunnet and Martel, 1990; Levin *et al.*, 1997; Levin and Simon, 1998).

1.5 Aims of the Thesis

As discussed above, patients with dysexecutive syndrome, including schizophrenic and Alzheimer's disease patients, exhibit a variety of cognitive deficits. Nicotine has proven effective in enhancing cognition in these patients and in 'normal' subjects. However due to its adverse side-effects profile, its use as a therapeutic target is severely limited. Thus identification of the receptor(s) that mediate this improvement may facilitate the production of similar cognitive enhancing compounds with fewer side-effects.

Attempts to identify the receptor(s) responsible have been made, predominantly using rats in the tasks described above. As yet mice have received limited use, despite the opportunity to combine both pharmacological and genetic techniques. To this end I wanted to model nicotine-induced improvement in cognition in mice. I also wanted to investigate a possible role of the α7 nAChR in this improvement using α7 nAChR transgenic mice.

As the predominant effect of nicotine appears to be in sustained attention, focus was placed on modelling this using the mouse 5-CSR task. The odour span task was also established in mice in order to investigate nicotine's effects on working memory. Finally, the suitability of the Tg2576 mice as a mouse model of Alzheimer's disease was also investigated.

Chapter 2 - General Methodologies

2.1 Animal Maintenance

All animals were group housed (where possible) in a temperature controlled room (22 ± 2 °C), with a 12 h light/dark cycle (lights on at 07:30 h) and were tested during the light phase of the cycle. Mice were maintained at 85% of their free-feeding weight and were permitted free access to water during training and testing. The animals were given *ad libitum* access to food approximately every 5 weeks in order to re-establish a free-feeding weight. Studies were performed under license by U.K. authorities (Scientific Animal Procedures Act 1986), approved by the Home Office and reviewed by the University of Edinburgh Ethics committee.

2.2 5-Choice Serial Reaction-time (5-CSR) task

The 5-CSR task training protocol for rats was first described in the literature by Carli and colleagues (1983). Several groups now train rodents in the 5-CSR, and therefore a variety of protocols are currently employed (Grottick and Higgins, 2000; Hahn and Stolerman, 2002). The standard protocol used in this thesis and how it differs from the original (Carli *et al.*, 1983) is detailed below. The schematic of the nine-hole operant chamber (25 x 25 x 25 cm, Cambridge Cognition, Cambridge, U.K.) utilised remains the same (Fig. 2.1), with one mechanical difference employed (note no reduction in box size was incorporated unlike that described by Humby *et al.*, 1999). Carli and colleagues (1983) utilised a hinged panel to block the magazine entrance, where pushing the panel activated a switch, alerting the computer to the rodent's entry into the magazine. We removed this panel and magazine entry was assessed using an infra-red beam. The mice therefore had no physical barriers to surmount to initiate a trial or retrieve their positive reinforcement as such barriers often cause difficulties in training mice (Drs. C Spratt and L Reid, *personal communication*). Positive (liquid) reinforcement (20 μl of strawberry milkshake; Yazoo®; U.K.) was delivered by a peristaltic pump to a spigot inside the magazine at the chamber front, opposite to the 9-hole array. The house light was set into the roof of the operant chamber, which was housed within a sound-attenuating box, containing a fan that provided ventilation and a constant low background noise. An infra-red camera was installed within each box allowing performance to be monitored. Each operant chamber was interfaced to an Acorn computer (RISC OS). The software required was programmed in house using the Arachnid extension to BBC Basic (Paul Fray

Ltd, Cambridge, U.K.). Several modifications were made to the protocols employed. Carli and colleagues (1983) utilised solid food reinforcers whereas we employed liquid, allowing the mice to consume the reward in less time. Whilst the original protocol had the house light constantly on and extinguished only when an error was made (time out, TO), in our protocol the house light remained off during a session, and errors were punished with 4 s of house light illumination. The final task length employed in this thesis was 25 min, or 120 trials, whichever was completed first (except where noted), in comparison to 30 min and 100 trials in the original paper. This was to increase the number of responses obtained in one session and because it was observed that mice readily completed 120 trials in less then 25 min. The inter-trial interval (ITI) employed in the original protocol was initially 2 s with a 2 s TO, followed by a 5 s ITI and TO. We employed a 2 s ITI with a 4 s time out (TO) phase during training and testing (except where noted) to ensure continuity between the two.

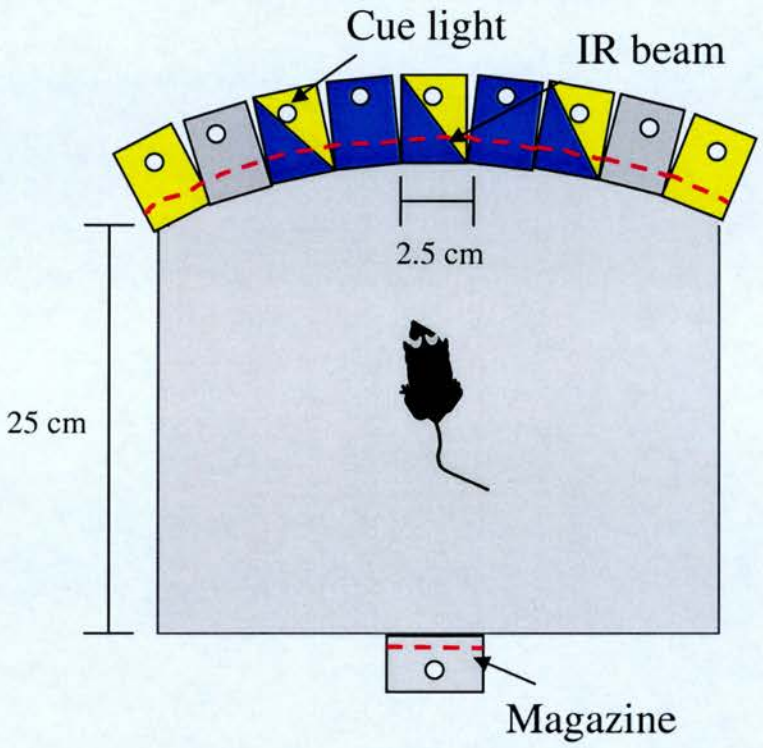


Figure 2.1 Schematic of the '9-hole box' apparatus used in the 5-CSR task

At the rear of the box there are a series of 9 holes, in each of which there is a cue light with the entrance monitored by a vertical infra-red beam. The entrance to every hole can be blocked, however traditionally four are blocked, thus creating the choice of five holes. Any four holes can be blocked with two possibilities shown above. The four holes on the outside can be blocked, creating a five hole narrow array which is shaded blue. Alternatively the four intervening holes can be blocked, creating a five hole wide array, shaded in yellow. The magazine where reinforcement is delivered is situated at the front of the chamber, and includes a cue light with its entrance monitored by a horizontal infra-red beam.

2.2.1 Training in the 5-CSR task

Mice were maintained at 85% of their free-feeding weight and permitted *ad libitum* access to water throughout the study. *Ad libitum* access to food was allowed approximately every 5 weeks in order to re-establish a free-feeding weight. Mice were introduced to the strawberry milkshake reinforcer in their homecage prior to training, reducing the risk of a neophobic response. During the task, mice were required to respond to visual stimuli (recessed into the holes), with a nose-poke. Responses were detected by the interruption of an infra-red beam crossing the entrance of the each hole.

2.2.2 Behavioural handling and procedures

All mice were handled for approximately 10 min per day for 3 days prior to training. On training days 1 and 2, mice were placed in the 9 hole boxes for 10 min, during which liquid reinforcement was dispensed every 15 s into the well of the magazine, whilst the magazine was lit. Entry into the magazine caused the light to be extinguished until the next reinforcement was delivered. At the end of this and subsequent sessions, the wells beneath the spigots were inspected to ensure no liquid was present. On day 3, in order to obtain reinforcement, mice were required to nose poke in any of the 5 lit holes at the chamber rear. This process was repeated every day until all mice were able to make at least 60 responses to the light cue within a 25 min session.

2.2.3 5-CSR task

At the beginning of each session the house light was extinguished and the magazine was lit. A nose poke in the magazine initiated the session (Fig. 2.2). An inter trial interval (ITI) of 2 s preceded one of the five response holes being illuminated. If the mouse nose poked in the lit hole within 12 s (10 s stimulus duration, SD, + 2 s limited hold (LH, time in which the mice can respond after the cue light is extinguished)), a *correct response* was recorded, the cue light extinguished, the magazine light illuminated, and a reinforcement dispensed. On entry to the magazine a 4 s reward interval (RI) was initiated. Failure to respond during the SD + LH resulted in an *omission error* being recorded and a 4 s time out (TO) initiated. During a TO the house light was on and all holes were unresponsive, then as the TO phase ended the house light was extinguished, and the magazine illuminated. The mouse could then begin a new trial by responding in the magazine. If, during the choice phase, the mouse responded in a hole other than the one that was lit, the response was registered as an incorrect response and a TO phase began. If the mouse responded during the ITI, an *anticipatory error* was recorded and a TO phase initiated. Each session lasted 25 min, or 120 trials if completed sooner. The SD was initially set at 10 s and was only reduced to 8 s following attainment of a mean correct latency half that of the SD and a minimum of 10 correct responses per session, maintained for over two consecutive sessions. The SD was further reduced to 4, 2 then 1 s based upon these response latency criteria. Successful acquisition of the task was defined as attainment of a 1 s SD, with proportion correct > 0.8, and omission levels of < 40 %. This required approximately 3 months of training.

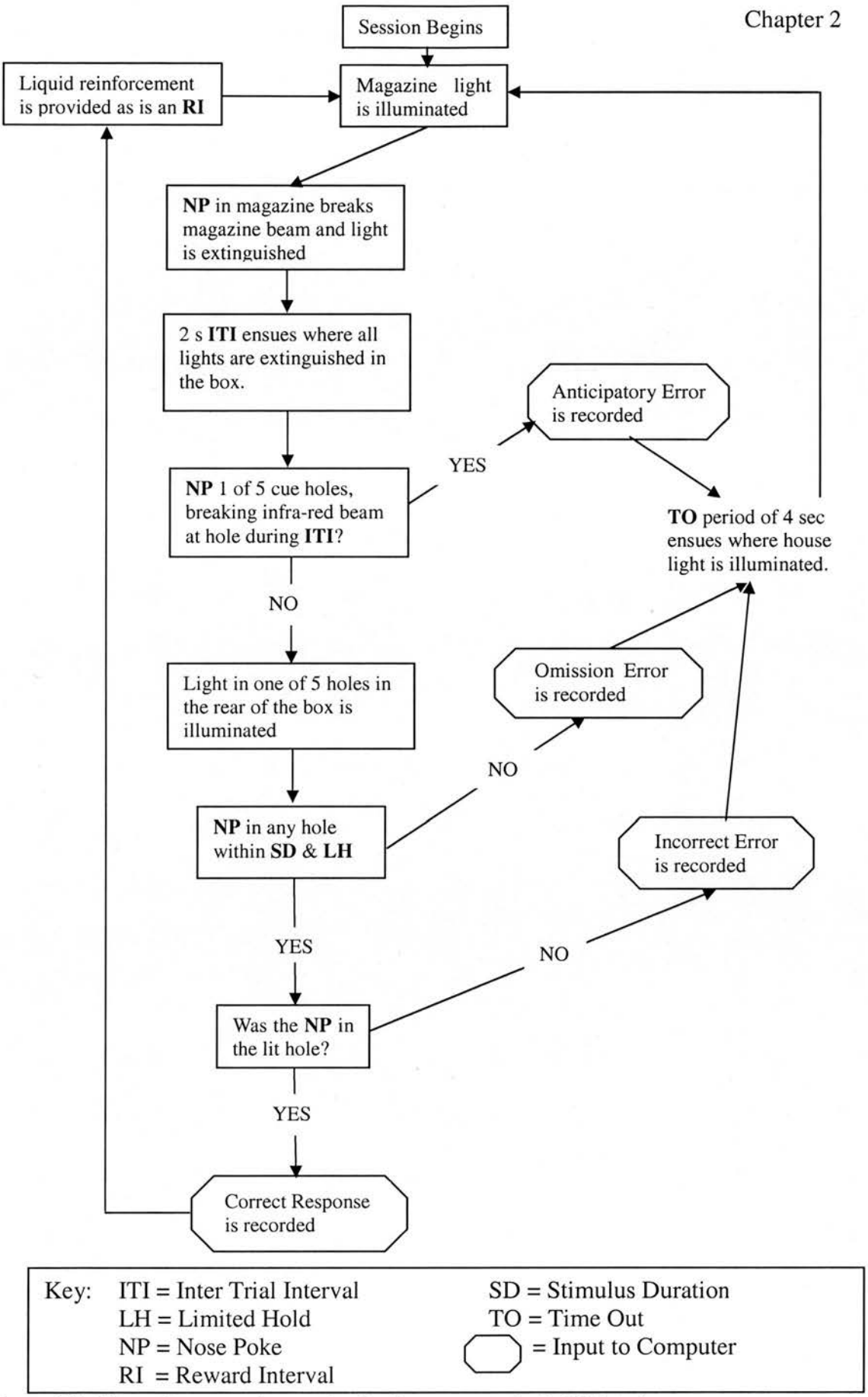


Figure 2.2: Flow diagram of a session in the asymptotic 5-CSR task.

2.2.4 Asymptotic performance

Asymptotic performance was assessed over a four-day period (Tuesday – Friday) once the mice had attained acquisition and a stable level of performance.

Measures of asymptotic performance include:
- Total trials
- Omissions
- Correct
- Incorrect
- Anticipatory responses
- Cumulative correct latency

% omissions	=	omissions / (correct + incorrect + omissions)
Proportion correct	=	correct / (correct + incorrect + anticipatory errors)
Mean correct latency	=	cumulative correct latency / number correct

2.2.5 Increased attentional load

This challenge increased the attentional load placed on the mice by increasing the session length from the standard 25 to 40 min. The maximum number of trials possible was increased from 120 to 1000. However, due to the minimum length of time taken to complete one trial (e.g. a latency to correctly respond (0.5 s) + ITI (2 s) + time to collect reward and start next trial (0.5 s), equates to 1 trial in every 3 s and 1000 trials in 50 min) the trial number was effectively unlimited and hence the session ended only once 40 min had elapsed. Extension of the session length increases the sustained attentional load placed upon the mice as they are required to maintain performance for a longer period, thereby increasing the likelihood of a vigilance decrement being observed (Parasuraman *et al.*, 1998; Grottick *et al.*, 2003). Analysis of mean reward latency (cumulative time taken to collect the reward / correct) was added to this program at a later date. This measure is reported to be a putative indicator of motivation (Robbins, 2002).

2.2.6 Noise distracter

This challenge assessed the ability of bursts of noise to distract mice during asymptotic performance of the 5-CSR task. Bursts of noise (0.5 s, 100 Hz – 20 k Hz) were presented at two possible intervals; 1) half way through the ITI (i.e. 1 s prior to onset of cue stimulus), 2) at the end of the ITI (i.e. coinciding with cue stimulus). No noise trials were also included, with the three possibilities were randomly assigned.

2.2.7 Statistical Analysis

The main dependent behavioural variables analysed, included % omissions, proportion correct, mean correct latency, total trials and mean reward latency. If proportion correct was not normally distributed, the data were arc-sine transformed. If mean correct latency or mean reward latency were not normally distributed, the data were logarithmically transformed. However, for all figures shown in the thesis the raw data are presented. Each variable in chapters 3.3.3 (pp. 86), and 3.3.4 (pp. 88) were compared to the mean scores obtained with saline using a 3-Way ANOVA (dose, day, and ITI time), with Tukey *post hoc* analysis. All other asymptotic performance data were analysed using a 2-Way repeated measures ANOVA. Acquisition performance was analyzed by assessing the increase in proportion correct across sessions per subject, and the data fitted using a 4-parameter logistic. The number of sessions required to attain 0.50 proportion correct (A_{50}) was calculated for each subject, and compared between the groups using a t-test. All statistics were performed using Sigma Stat, (v. 3.0) SPSS, U.S.A..

2.3 Odour Span task

One of the main challenges in this thesis was the development of a mouse version of the odour span task (OST). The OST was first described by Dudchenko and colleagues (2000) and was established using rats. To adopt the task for use in mice, the bowls utilised were reduced in size, as was the table the task was performed on. In addition woodchip was employed as the digging medium as opposed to sand as digging in sand appeared aversive to mice. To mask the scent of the reinforcement pellets (Noyes Precision Pellets, 45 gm, Lancaster, U.K.), 18 pellets were crushed and added to the mix of woodchip and scent when it was first created (see below).

2.3.1 Training in the Odour Span task

As described for the 5-CSR task, mice were maintained at 85% of their free-feeding weight and were permitted *ad libitum* access to water. *Ad libitum* access to food was provided every 5 weeks in order to re-establish a free-feeding weight. Mice were introduced to the pellet reinforcers in their homecage prior to training, to reduce the risk of a neophobic response.

2.3.2 Behavioural Apparatus

Training and testing took place on a platform (61 X 61 cm) raised 76 cm above the floor. The platform was numbered 1 – 24 around its perimeter. The odours used were as follows: ground cinnamon, nutmeg, coriander, allspice, fenugreek and

ginger; dried thyme, parsley sage, mint oregano and rosemary; plus caraway seed, onion powder, paprika, chinese five spice, celery salt, coffee powder, dill, lemon tea, cocoa and English breakfast tea (all odours purchased locally). Ground cumin and garlic were found to be aversive to mice and were not employed (see table 2.1). All odours were created as follows: 3 g of an odour was added to 100 g of sawdust, and 18 crushed pellets. Approximately 3 g of this scented mix was placed in white porcelain bowls (5.5 cm in diameter and 3.5 cm high; Fisher, Loughborough, UK), individually marked with a letter corresponding to each particular odour.

Odours utilised		
Dried	Ground	Miscellaneous
Mint	Allspice	Caraway seed
Oregano	Cinnamon	Celery Salt
Parsley	Coriander	Chinese five spice
Rosemary	Fenugreek	Cocoa
Sage	Ginger	Coffee Powder
Thyme	Nutmeg	Dill
		English breakfast tea
		Lemon tea
		Onion Powder

Table 2.1 Odours utilised in the OST

List of odours utilised in the OST. Ground cumin and garlic were found to be aversive to mice and were not used.

2.3.3 Behavioural Handling and Procedures

Initially, all mice were handled for approximately 10 min per day for 3 days prior to training. On day 1 of training mice were placed in an clear cage (44.5 X 22 X 19 cm) on the platform, which contained a bowl of unscented sawdust filled with 10 reward pellets, and were left in situ until every pellet was retrieved (approximately 15 min). On day 2, 18 crushed pellets were added to 100 g of sawdust, and approximately 3 g of this mixture was placed in two bowls, one of which was baited with 5 reward pellets. Both bowls were then placed in the cage with the mouse, until the mouse retrieved all 5 pellets after which the task was immediately repeated once. On day 3 a scented odour was chosen at random, 1 pellet was added and the bowl placed in the cage alongside a second bowl with unscented sawdust, after which the mouse was also placed in the cage. Once the pellet was retrieved, the mouse was removed to a second clear cage below the table, the locations of the bowls were randomly swapped, a pellet again placed in the scented bowl and the mouse returned to the first cage. This was repeated a further 8 times, with constant monitoring ensuring the pellet was retrieved each time. This process was repeated on days 4, 5 and 6 to ensure rapid retrieval (10 pellets in < 8 min.) of the pellet.

2.3.4 Odour span task

In the first span, a random odour and location were chosen using an in-house written program in Arachnid on-line control language (Paul Fray Ltd, Cambridge, UK) interfaced to an Acorn computer (RISC OS). A bowl was filled with the scented

mix, baited and placed on the platform at the appropriate randomly chosen location. A mouse was then placed on the platform, at which point the session and a timer began (Fig. 2.3, pp. 64). The mouse was then required to dig in the bowl for the reward, and remember the odour in that bowl. Upon consumption of the reward the mouse was then placed in the same clear cage positioned below the platform. A new odour and location was then randomly selected, the bowl baited, and placed on the platform, with the first odour (not baited) moved to a new location. The second span began when the mouse was placed back on the platform and the timer started. The mouse was required to dig only in the novel odour. The timer was stopped as soon as the mouse dug in an odour (bowl), and it was returned to the clear cage. If the correct odour was dug in, the mouse was given time to consume the reward prior to being returned to the cage and a third baited odour was then introduced. If an incorrect choice was made, the odours were randomly relocated, and span 2 repeated until a correct response was made. The next span (span 3) then included the 2 previously sampled odours (not baited) and 1 novel odour (baited) placed on randomly selected locations. This process continued until 12 (span 12) odours had been introduced, or the mouse had spent 10 min on the platform (Fig. 2.4, pp. 65). If at any time the mouse dug in an incorrect odour, it would be removed, and the timer stopped. The odours would then be randomly relocated, and the mouse reintroduced to that span, with the same baited odour as before. If a mouse made 10 consecutive incorrect responses, the task would be terminated. The number of odours the mouse successfully remembered prior to making an error was taken to be that subjects' span length for that session. Thus with 12 odours the maximum possible span length was 11, for at span 1 the mouse did not choose and hence had no odour to remember.

To ensure that the mice were not scent marking the bowls, a bowl that had previously been dug in was randomly selected on every third span and replaced with an identical bowl (filled with the identical odour), but one which the mouse had not previously sampled. If the subjects relied on this method, then performance would be severely disrupted at those spans. The table was wiped down with ethanol between sessions. Mice were deemed to have acquired the task when a span length (number of correct digs prior to an error) of 5 was achieved for 2 consecutive days. Mice were then continuously trained until a stable level of performance had been reached.

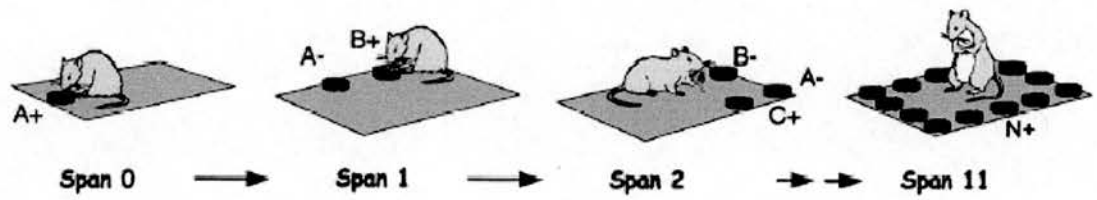


Figure 2.3: Schematic of the OST (from Dudchenko *et al.*, 2000)

Mice are first presented with a bowl of woodchip scented with a specific odour (e.g., parsley, A). After retrieving a buried reward, the mouse is removed from the table, and a second bowl of woodchip, scented with a different odour (e.g., Fenugreek, B) is added. The mouse must remember odour A in order to dig only in the novel odour (B). This continues until 12 odours are presented (representing a span of 11) or the mouse has spent 10 min on the table.

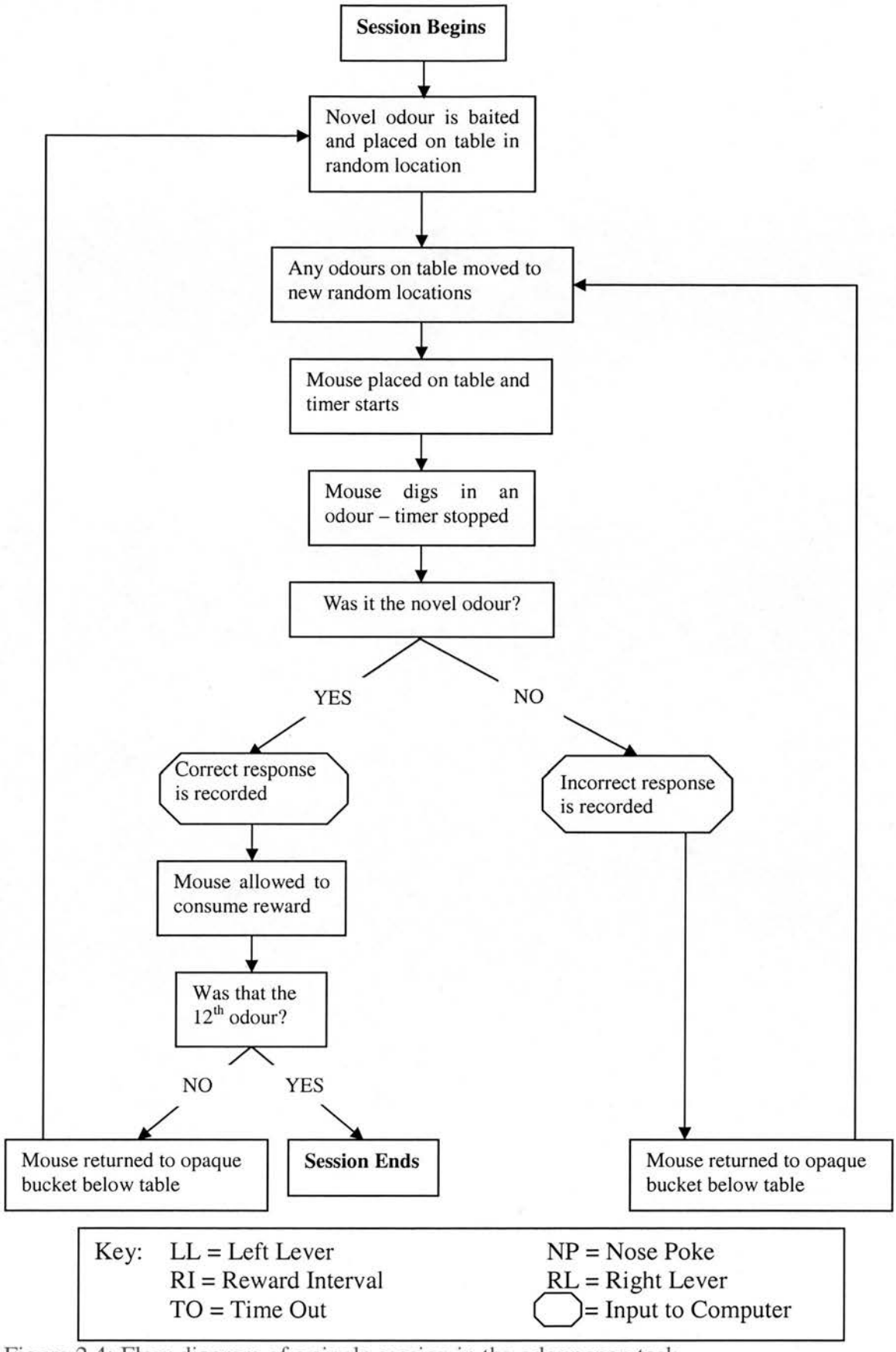


Figure 2.4: Flow diagram of a single session in the odour span task

2.3.5 Asymptotic performance

Asymptotic performance was assessed over a four-day period (Tuesday – Friday) once mice had attained acquisition and stable performance. Measures include:

Span length	Total number of spans completed
Errors	Simple discrimination (span 1)
Mean span latency	Time taken to engage in the task (i.e. dig in bowl 1)

2.3.6 Extended Version (22-OST)

To increase the working memory load placed on mice thus accommodating for potential ceiling effects, an extended version of this task which utilises 22 odours instead of 12 was developed. This allowed the mice to potentially complete 21 spans in each session within the 10 min time limit.

2.3.7 Statistical Analysis

Acquisition was assessed by comparing the total number of days each group took to attain criteria and analysed using a One-Way ANOVA. Span length, maximum spans, error numbers and simple 2-odour discrimination were compared using a Mann Whitney Rank Sum test between 2 groups, and a Kruskall Wallis Rank Sum Test between more than 2 groups (for example during drug administration; see chapters 4.3.1 - 4.3.2, pp. 125 - 128). Mean span latency and time taken for initial response was compared using a Two-Way Repeated Measures ANOVA with day and genotype as between subject factors. Due to the low group sizes utilised in this task, significant results may be as a result of sampling error and thus caution must be taken when drawing conclusions.

2.4 T-maze continuous alternation task (T-CAT)

Spontaneous alternation on the T-maze is recognised as a method with which to measure innate exploratory behaviour (Lalonde *et al.*, 2003). Traditionally, assessment requires handling, when the animal is moved from its chosen arm back to its start arm. However constant handling of the test subject may confound results as initial handling can produce an anxiogenic effect, whilst chronic handling can act as an anxiolytic (*unpublished observations*). The production of either can alter levels of spontaneous alternation. Gerlai (1998) developed the T-maze continuous alternation task (T-CAT) which allows the test animal to return to the start arm by its own volition. Thus the next trial can automatically begin and no handling is required.

2.4.2 T-maze apparatus and testing

Unlike the previous two tasks, the T-CAT requires no training, and performance is monitored on a single day, with the mice maintained on *ad libitum* feeding. Testing apparatus consists of an octagonal hub (14 cm across), with two arms (61 cm long) attached opposite each other and one (72 cm long) attached to the side of the hub between the first two arms (Fig. 2.5). Thus viewed from above the apparatus resembles a T (Fig. 1.3c, pp. 41). A door which can be either open or closed is positioned where each arm is attached to the central hub. In total there are 16 trials, with trials 1 and 2 being forced entries, whilst the remaining 3 – 16 are free-choices (Fig. 2.6). For trial 1 the mouse begins in the central arm with all three doors closed. The start-arm door and either the left or right door are raised, leaving one arm

inaccessible. The mouse must then enter the novel arm, progress at least halfway, and re-enter the start arm, after which all 3 doors are closed. After a 5 s period in the start arm, trial 2 begins which is also a forced entry trial. The start-arm door is opened as is the previously lowered door, and thus the mouse can only enter the previously inaccessible arm. Again after reaching halfway down the accessible arm, the mouse must return to the start arm, again after which all 3 doors are closed. The following 14 free-choice trials use the same following format. After a 5 s delay in the start-arm all 3 doors are opened. The mouse is free to explore either arm, but once halfway down the left or right arm, the choice and time is recorded and entry to the opposite arm is rescinded as its door is lowered. Once the mouse returns to the start-arm, the remaining two doors (start-arm and chosen arm doors) are closed. The mouse is given a 30 min time limit in which to complete all 16 trials. If < 8 free choice trials are completed within the 30 min period then the subject is removed from the analysis. Otherwise, the numbers of alternations (entering the opposite arm from the previously entered arm) are recorded as a percentage:

$$\frac{\text{Number of alternations X 100}}{\text{Total free choice arm entries}}$$

2.4.2 Statistical Analysis

Exploratory behaviour was measured by the percentage of spontaneous alternation. Any differences in the percentage of spontaneous alternation between groups were compared using a student's *t*-test. To assess whether any group were performing at better than chance levels (50%) a one-sample *t*-test was performed. Session latency data were compared using a one-way ANOVA.

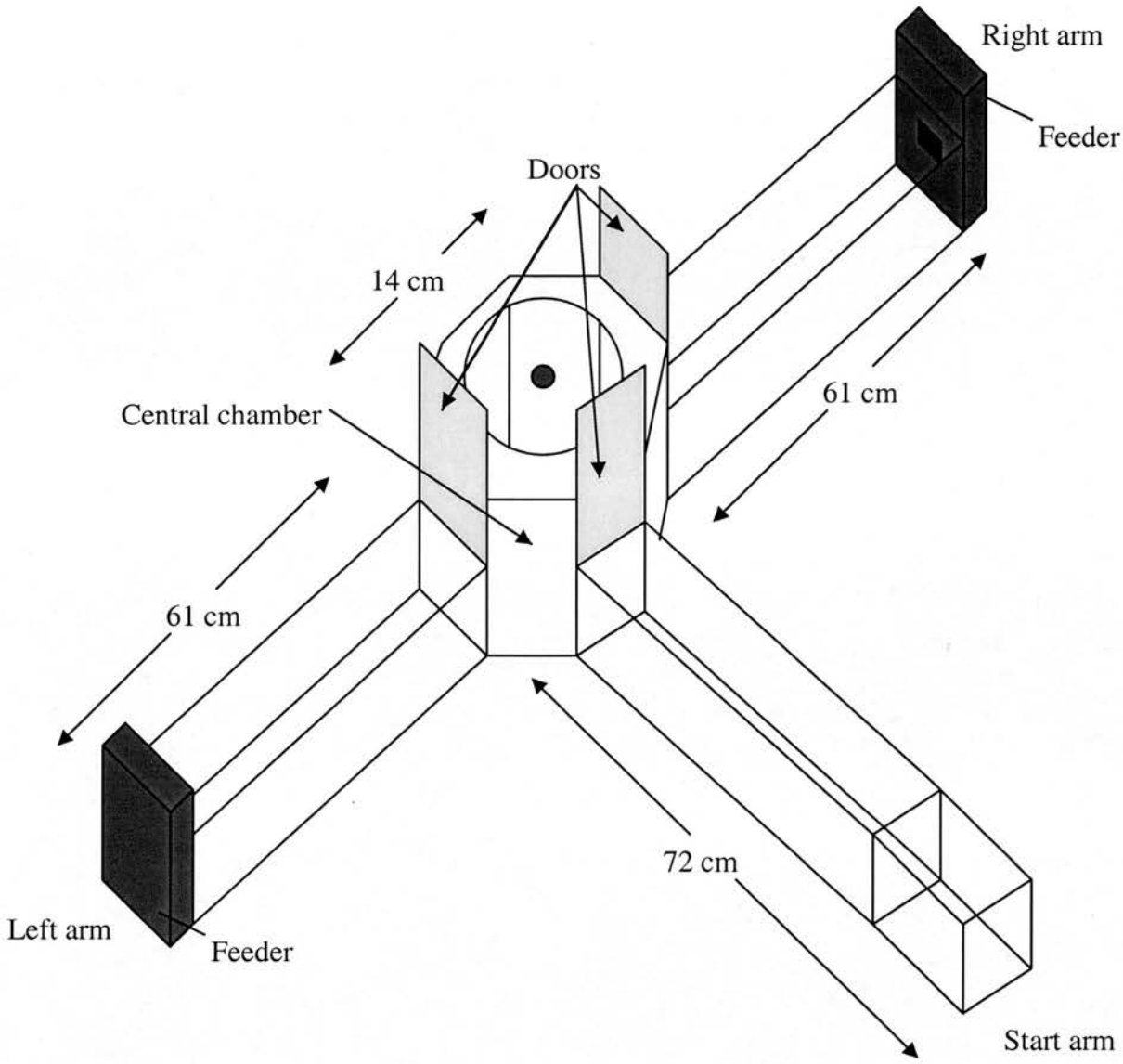
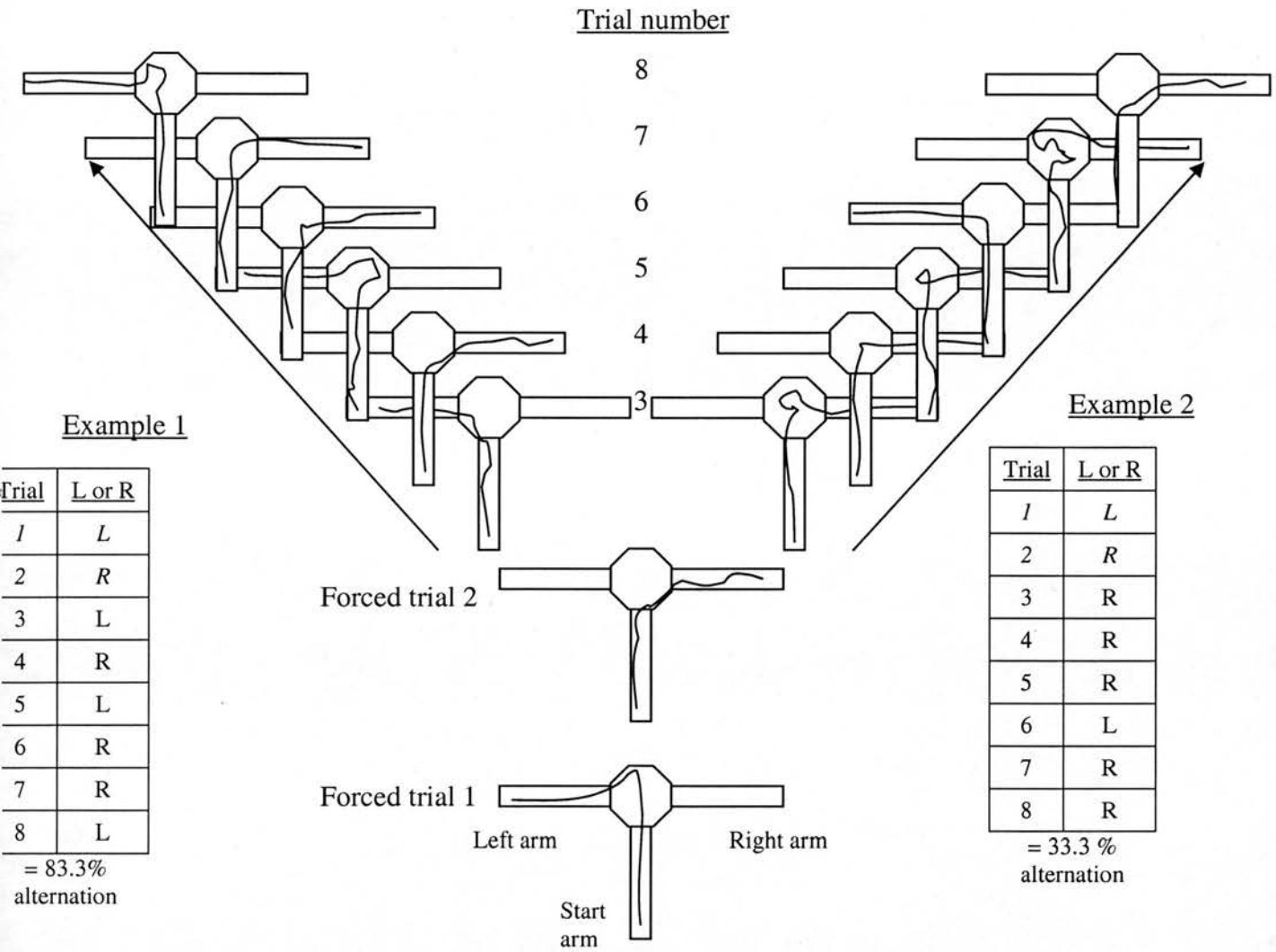


Figure 2.5: Schematic of the T-maze apparatus

The apparatus used is so-called as the start, left and right arms join to form a T. Entry to the arms is controlled by doors located at their joining to the central chamber. If reinforced alternation is being assessed, the animals can be fed by food being placed in the feeders located at either end of the left or right arms.



Trial	L or R
1	*L*
2	*R*
3	L
4	R
5	L
6	R
7	R
8	L

= 83.3% alternation

Trial	L or R
1	*L*
2	*R*
3	R
4	R
5	R
6	L
7	R
8	R

= 33.3 % alternation

Figure 2.6: Two examples of performance in the T-CAT

Spontaneous alternation on the T-maze continuous alternation (T-CAT) is measured in terms of % alternation. In both examples, trial 1 and 2 are forced entries, whereby on trial 1 one arm is closed (here the right arm) to ensure the mouse enters the opposite arm. In trial 2 the opposite arm is closed (here left arm), ensuring the mouse enters the right arm. Thus the mouse has experienced both arms prior to the 14 choice trials (though 3 – 8 are only given in the examples). A mouse with high spontaneous alternation (83%), and thus exploratory behaviour, would consistently alternate in their choice trials (example 1). Poor spontaneous alternation (33%) would be exemplified by a mouse consistently choosing the same arm to enter in a given choice trial (example 2).

2.5 Spontaneous Locomotor Activity

Individual mice were placed into locomotor cages (RS Biotech, Alva, Clackmananshire, UK; Fig. 2.7) for 40 min per day for 5 consecutive days. Two infrared photo-beams were positioned 4 cm from each end and 2.2 cm above the floor. One locomotor count was recorded by the computer (program written in-house) if the two beams were consecutively interrupted. Each cage was interfaced to an ACORN computer (RISC OS) and programmed using Arachnid on-line control language (Paul Fray Ltd, Cambridge, UK).

2.5.1 Statistical Analysis

Spontaneous locomotor activity was compared using a Two-Way Repeated Measures ANOVA, with genotype and day as between subject factors.

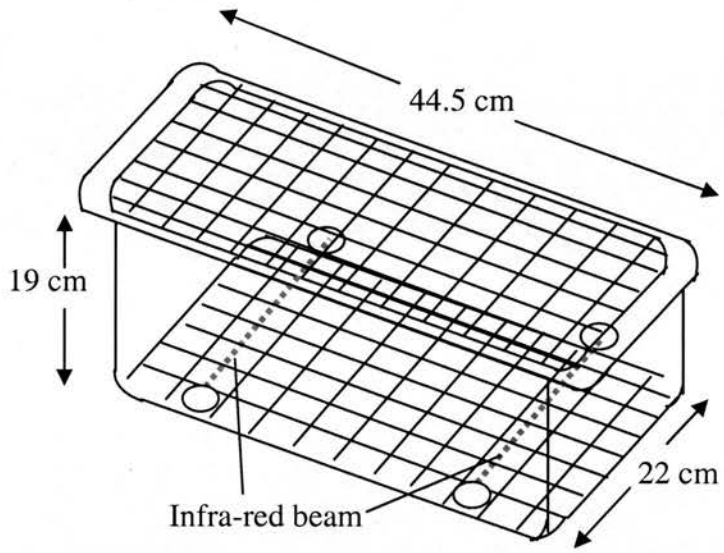


Figure 2.7: Schematic of a spontaneous locomotor activity cage

The spontaneous locomotor cages used are depicted above. The two infra-red beams are located 4 cm from either end of the cage, and 2.2 cm above the floor. One locomotor count was accrued if the mouse broke both infra-red beams in succession.

2.6 Genotyping

For confirmation of the genotype of the transgenic mice, animals were tail tipped under halothane/nitrous oxide anesthesia, and DNA obtained by proteinase K treatment of tail samples (Promega, Southampton, U.K.). α7 nAChR transgenic mice were genotyped as follows: Three primers were used, corresponding to both wild-type (forward primer 5'-CCT GGT CCT GCT GTG TTA AAC TGC TTC-3', reverse primer 5'-CTG CTG GGA AAT CCT AGG CAC ACT TGA G-3', producing 440 bp product) and disrupted (same forward primer as for wild-type, but reverse primer 5'GAC AAG ACC GGC TTC CAT CC-3', producing 750 bp product) alleles. Caspase-3 over-expressing mice were genotyped as follows: Tissue was heated at 95°C for 20 min in 25mM NaOH/0.2 mM EDTA then neutralised by the addition of an equal volume of 40mM Tris-HCl. Three primer pairs corresponding to both YAC ends (long arm: forward primer 5'-ATT GCT AAC GCA GTC AGG CAC C-3', reverse primer 5'-TAG TGG CTC CAA GTA GCG AAG C-3', producing 278 bp product; and short arm: forward primer 5'-TCT CCG AAC AGA AGG AAG AAC G-3', reverse primer 5'-TGT TAC TTC TGC CGC CTG C-3', producing 568 bp product) and human caspase-3 (3'-UTR: forward primer 5'-TGA TGA TGT GGA AGA ACT TAG G-3', reverse primer 5'-ACG GCT CCG CAC CTG CTG AGG C-3' producing 944 bp product) were used for PCR genotyping. Genotyping of the Tg2576 mice required two primers (forward primer 5'-CTG ACC ACT CGA CCA GGT TCT GGG T-3', and reverse primer 5'-GTG GAT AAC CCC TCC CCC AGC CTA GAC CA-3', producing no band for wild-type mice and 466 bp product for the TG2576 mice).

2.7 Binding

2.7.1 Tissue procurement and P_2 synaptosomal membrane preparation

For radioligand binding studies, P_2 synaptosomal membranes were prepared as described previously (Maemoto *et al,* 1997). Mice were sacrificed by cervical dislocation, the brains removed, and immediately placed in ice-cold saline (0.9% NaCl). The whole brain minus cerebellum (due to the low density of α7 nAChRs) was used for preparation of synaptosomal membranes. Brain tissue from each animal was treated independently in the α7 nAChR KO study. Tissue samples were homogenized in 15 volumes (15 vol.) of ice cold (4°C) 0.32 M sucrose using a glass/Teflon homogenizer, centrifuged at 1 000 g for 10 min (4°C), and the resulting supernatant centrifuged at 17 000 g (20 min, 4°C). The synaptosomal/mitochondrial P_2 pellet was lysed in 30 vol. of ice-cold (4°C) milliQ H_2O for 60 min and centrifuged at 50 000 g (10 min, 4°C). The membrane pellet was then resuspended in 30 vol. of ice cold (4°C) 50 mM potassium phosphate assay buffer (50 mM potassium phosphate, 1 mM EDTA and 0.01% sodium azide, pH 7.4), centrifuged at 50 000 g for 10 min (4°C) and resuspended in 5 vol. (original tissue weight) of assay buffer and stored at -20°C. On the day of use, frozen membranes were thawed, diluted to 30 vol. with ice cold (4°C) assay buffer and the suspension centrifuged at 50 000 g for 10 min at 4°C. The pellet was then resuspended in the appropriate volume of assay buffer and the protein content determined as described previously (Finlayson *et al,* 2001).

2.7.2 [^{3}H]-Methyllycaconitine ([^{3}H]-MLA) binding to the α7 nAChR

[^{3}H]-MLA (19.8 Ci/mmol; Tocris, Bristol, U.K.) binding to the α7 nAChR was carried out as previously described (Davis *et al,* 2000). Binding assays were conducted in a total volume of 250 µl; consisting of 50 or 100 µl (no drug present) of potassium phosphate assay buffer, 50 µl of test drug, 50 µl of [^{3}H]-MLA (final, 2 nM), and 100 µl of membrane suspension. Test compounds (methyllycaconitine and (±) epibatidine, Tocris, Bristol, U.K.; (-)nicotine bitartrate and d-tubocurarine chloride, Sigma, Poole, U.K.) were prepared by serial dilution in assay buffer. Non-specific binding was determined in the presence of 1 mM d-tubocurarine. Binding was initiated by the addition of membranes, and samples were incubated for 60 min at 25°C. Binding was terminated by filtration onto glass fiber filters (GF/B, Whatman; pre-soaked for 3 h in 0.3% polyethylenimine) using a Brandel cell harvester, followed by three rapid (1 ml) washes with ice cold phosphate buffered saline (20 mM Na_2HPO_4, 5 mM KH_2PO_4, 150 mM NaCl, pH 7.4). Filter disks were transferred to RT30 tubes (Sterling, UK) and radioactivity determined using a Packard 2500TR liquid scintillation counter.

2.8 Histopathology

Mice were killed by cervical dislocation, the brains removed, and immediately placed in isopentane (-42°C) for three min. Cryostat sections were taken at 20 µm and Nissl stained with thionin.

Chapter 3: Nicotinic modulation of sustained attention in mice

3.1 Introduction

Attentional impairments are common in patients with dysexecutive syndrome (Cornblatt and Kelip, 1994; White and Levin, 1999; see chapter 1.3.2, pp. 21). It has been proposed that these impairments may underlie the psychopathology of Schizophrenia (Cullum *et al.,* 1993; Cornblatt and Keilp, 1994). As described in chapter 1.3.2 (pp. 21) human attentional performance is commonly assessed using the continuous performance test (CPT), where subjects have to attend to visual stimuli over a sustained period of time (Levin *et al.,* 1998; White and Levin, 1999; Shytle *et al.,* 2002). Nicotine has been shown to enhance sustained attention in normal humans by reducing omission levels (Levin *et al.,* 1998). Moreover, it has been suggested that nicotine can lock the brain in to an attentional processing mode whereby there are fewer lapses in attention and therefore less omissions (Mancuso *et al.,* 1999). Critically, this beneficial effect of nicotine on attentional performance translates to every-day tasks in real life-scenarios (Rusted *et al.*, 2000). This property of nicotine may underlie its ability to enhance attention and improve symptomatology in various human diseases including schizophrenia (Yang *et al.,* 2002), Alzheimer's disease (White and Levin, 1999), attention deficit hyperactivity disorder (Shytle *et al.,* 2002), Parkinson's disease (Kelton *et al.,* 2000), and Tourette's syndrome (Sanberg *et al.,* 1997). Unfortunately the side-effect profile of nicotine includes addiction, nausea, and cardiovascular stress, all limiting its potential role as a therapeutic agent as these side-effects outweigh nicotine's

potential benefits (Waldum *et al.*, 1996; Levin, 2002; Shytle, 2002; White and Levin, 2004; Yildiz, 2004).

Thus identifying the nicotinic acetylcholine receptor (nAChR) subtype(s) that mediate the cognitive enhancing effects of nicotine may facilitate the generation of compounds that have a reduced side-effect profile (Levin, 2002). The roles these receptors play in rat sustained attention have been examined using the 5-choice serial reaction-time (5-CSR) task (Mirza and Stolerman, 1998; Blondel *et al.*, 1999; Grottick and Higgins, 2000; Stolerman *et al.*, 2000; Mirza and Bright, 2001; Hahn *et al.*, 2002; Terry *et al.*, 2002; Grottick *et al.*, 2003; Hahn *et al.*, 2003a,b; see chapter 1.4.1, pp. 34). However, no consistent nicotine-induced improvements in sustained attention have been reported in unimpaired rats (Mirza and Bright, 2001; Terry *et al.*, 2002). While nicotine-induced improvements in sustained attention have been reported in rats, these studies have required the additional complexities afforded by brain lesions, poorly performing subjects, or task challenges (Muir *et al.*, 1995; Grottick and Higgins, 2000; Hahn *et al.*, 2002). The improvements observed may therefore not reflect enhanced attention as nicotine has been shown to enhance learning (Faiman *et al.*, 1991), a confound introduced when utilising both task challenges or poor performers, whilst lesion studies are complicated in that an abnormal state has been introduced.

Clear identification of the receptor subtypes underlying the beneficial effects of nicotine is made more onerous by the difficulties associated with producing nAChR subtype selective drugs (Gotti *et al.*, 2000; Broad *et al.*, 2002; Shoaib *et al.*, 2002). It

has therefore been suggested that a combined approach of pharmacological interventions and transgenic animals may help delineate the nAChR subtypes involved (Gotti *et al.,* 2000, Chapman, 2002; Levin, 2002).

As noted on the previous page, since its first description by Carli *et al* in 1983, the 5-CSR task has been extensively used in rat studies. In contrast, the task has received only limited and comparatively recent attention in mice (Humby *et al.,* 1999; Marston *et al.,* 2001). Hitherto, no reports addressing the effects of nicotine on the performance of mice in the 5-CSR task have been published. Such information would be advantageous, as a variety of nAChR transgenic mice have been generated including α3, α4, α5, α7, β2 and β4 nAChR knockouts, and α4 and α7 nAChR knockins (hypersensitive to nicotine, Picciotto *et al.*, 1995; Orr-Urtreger *et al.*, 1997; Marubio *et al.*, 1999; Xu *et al.*, 1999; Zoli *et al.*, 1999; Rossi *et al.*, 2001; Lester *et al.*, 2003; Salas *et al.*, 2003). Therefore establishing mouse 5-CSR provides the opportunity to assess by both pharmacological and genetic manipulations, which nAChR may mediate the nicotine-induced improvement in sustained attention.

Therefore in this chapter I will describe the development and use of the 5-CSR task to examine the hypothesis that nicotine can improve sustained attention in mice. In addition, due to the lack of commercially available selective agonists, the role that the α7 nAChR plays in attention was examined by using α7 knockout (KO; B6.12957- $Chrna7^{tm1bay}$, Orr-Urtreger *et al.,* 1997; creation of which are detailed in Appendix I, pp. 256) mice in the task. These were examined due to the purported role of the α7 nAChR in preattention and it's possible link to sustained attention (see

chapter 1.4.3, pp. 37). If the α7 nAChR plays a role in sustained attention these mice should exhibit a deficit as identified as an increase in omission levels and a decrease in proportion correct. Attempts to combine the two approaches and ensure reproducibility of findings will be detailed.

3.2 Methodology

3.2.1 Animal maintenance and genotyping

C57Bl/6J male mice were used in sections 3.3.1 – 3.3.4 (sections 3.3.1 – 3.3.3, n = 16; and section 3.3.4, n = 25, Charles River, Margate, UK). The same group was used for sections 3.3.1, 3.3.2, and 3.3.3. The mice weighed between 22 and 26 g at the start of each study. Eight α7 nAChR KO (B6.12957 - $Chrna7^{tm1bay}$; Jackson Laboratories, Bar Habor, U.S.A.) and eight age-matched littermates (backcrossed onto a C57Bl6/J background 8 times), weighing between 22 and 27 g at the start of the study, were used in sections 3.3.5 and 3.3.6. The mice used in section 3.3.7 were comprised of a group of ten α7 nAChR KO, ten heterozygote, and twelve age-matched littermate mice (age range; 19 - 28 weeks; backcrossed onto a C57Bl6/J background 11 times), all weighing between 21 and 31 g at the start the study.

3.2.2 Behavioural apparatus and 5-CSR training

The behavioural apparatus used in all studies was as described in chapter 2.2 (pp. 52), though some modifications were introduced. In sections 3.3.1, 3.3.2 and 3.3.5, training and testing utilised the narrow array format, whereas the wide array format was used in all other studies (including section 3.3.2). In every study the mice were trained using a constant inter-trial interval (ITI). In sections 3.3.3, 3.3.4, and 3.3.6, when acquisition criteria had been attained, the mice were moved to a 3 – 7 then a 2 – 10 s variable ITI. Training then continued until a stable performance was attained.

3.3 Results

3.3.1 (-)Nicotine had no effect on sustained attention in mice in the standard 5-CSR task

Initially, mice (n = 16) were trained to perform the 5-CSR task using the narrow array (holes 3 – 7), and a constant 2 s ITI. All mice attained criteria and were administered saline or three different doses of (-)nicotine (3, 30, and 100 μg/kg) according to a Latin-square design and tested over a 10-week period (table 3.1). The mice were administered their allocated dose for four consecutive days (Tuesday – Friday), with each 4 day dosing period separated by a 10-day washout period, ensuring a baseline response to saline was re-established. Overall no significant effect of drug or test week was observed. A 2-Way ANOVA with nicotine dose, and day as between subject factors, yielded no significant main effects of drug (Fig. 3.1) or day. There was no effect of nicotine dose on % omissions ($F(3,192) = 0.668$, $p = 0.573$; Fig. 3.1a), proportion correct ($F(3,192) = 0.437$, $p = 0.727$; Fig. 3.1b) or mean correct latency ($F(3,192) = 2.257$, $p = 0.083$; Fig. 3.1c) was observed. Likewise no significant main effect of day was observed on % omissions ($F(15, 192) = 0.488$, $p = 0.945$), proportion correct ($F(15, 192) = 1.412$, $p = 0.145$) or mean correct latency ($F(15, 192) = 1.172$, $p = 0.297$). Also there was no significant interaction between the two factors on % omissions ($F(45, 192) = 0.845$, $p = 0.745$), proportion correct ($F(45, 192) = 1.095$, $p = 0.331$) or mean correct latency ($F(45, 192) = 0.810$, $p = 0.796$).

Week	Cohort A	Cohort B	Cohort C	Cohort D
1	Saline	Saline	Saline	Saline
2	Saline	3 μg/kg	30 μg/kg	100 μg/kg
3	Saline	Saline	Saline	Saline
4	3 μg/kg	100 μg/kg	Saline	30 μg/kg
5	Saline	Saline	Saline	Saline
6	100 μg/kg	30μg/kg	3 μg/kg	Saline
7	Saline	Saline	Saline	Saline
8	30 μg/kg	Saline	100 μg/kg	3 μg/kg
9	Saline	Saline	Saline	Saline
10	Saline	Saline	Saline	Saline

Table 3.1: Design of section 3.3.1

Mice (n = 16) were separated into four cohorts in a counterbalanced design. They were then administered saline subcutaneously (s.c.) or (-)nicotine (s.c., 3, 30 or 100 μg/kg) over four consecutive days (Tuesday – Friday) in the sequence illustrated. The intervening weeks of saline administration provided a 10 day washout period.

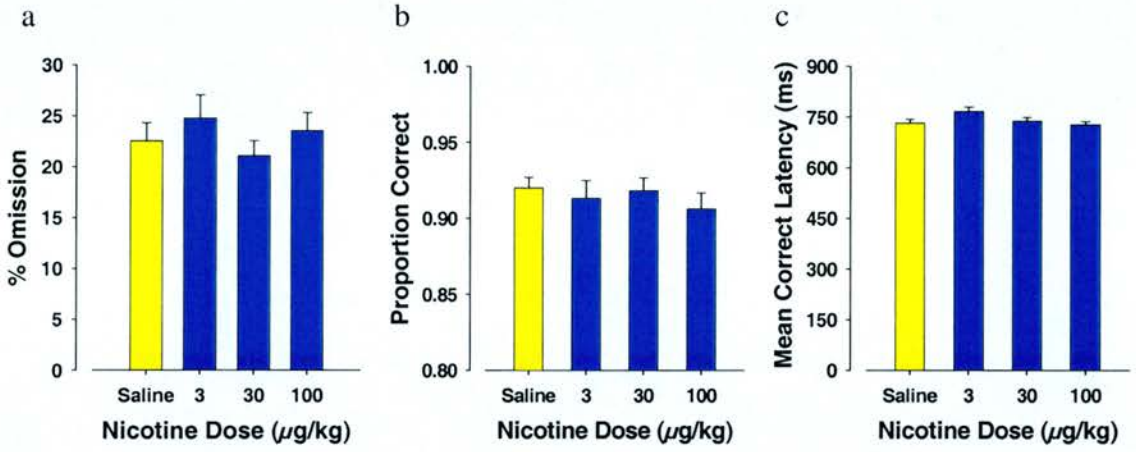


Figure 3.1: (-)Nicotine had no effect on mice performing the standard 5-CSR task

A group of C57Bl/6J mice (n=16) were trained to perform the standard 5-CSR task. They were then split into four groups in a counter-balanced design, and administered (-)nicotine (s.c.; 3, 30 or 100 μg/kg) their allocated dose over four consecutive days (Tuesday – Friday) in a Latin square design (table 3.1). No effect of drug was observed on any parameter measured. Parameters measured included % omissions (a), proportion correct (b) or mean correct latency (c). Data presented as mean + s.e.m..

3.3.2 Baseline performance of mice was lowered by modification of 5-CSR task

Examination of the literature and Fig 3.1 suggested that the 5-CSR task is subject to ceiling effects (Grottick and Higgins, 2000; Hahn and Stolerman, 2002). Therefore a nicotine-induced improvement in performance would be difficult to achieve in animals that had achieved asymptote. Newhouse and colleagues who work extensively with control and patient groups suggested in 1997 that improvements in performance following drug administration are more likely to be observed if baseline performance is already low. Indeed Bates and colleagues (1995) had previously widened the array that human subjects had to attend to in order to increase task difficulty, and had reduced baseline performance in an attentional task. Using this principal I subsequently modified the 5-CSR task by opening apertures 1, 3, 5, 7 and 9 (wide array) as opposed to apertures 3 – 7 (narrow array; Fig. 2.1, pp. 54) in an attempt to increase task difficulty. Performance in the task was assessed by comparing performance of one group of mice (n = 8) in the (standard) narrow array, with a second group (n = 8) in the (modified) wide array over a period of four consecutive days (Tuesday – Friday). As can be seen in Fig. 3.2, widening the array clearly decreased performance as measured by % omissions, proportion correct and mean correct latency. A 2-way Repeated Measures ANOVA, with array width and day as between subject factors revealed a significant main effect of array width on % omissions ($F(1,42) = 11.793$, $p < 0.005$; Fig. 3.2a), proportion correct ($F(1,42) = 6.38$, $p < 0.05$; Fig. 3.2b) and on mean correct latency ($F(1,42) = 14.585$, $p < 0.005$; Fig. 3.2c). Also there was no significant interaction between the two factors on %

omissions ($F(3,42) = 0.606$, $p = 0.615$), proportion correct ($F(3,42) = 0.832$, $p = 0.484$) or mean correct latency ($F(3,42) = 2.047$, $p = 0.122$).

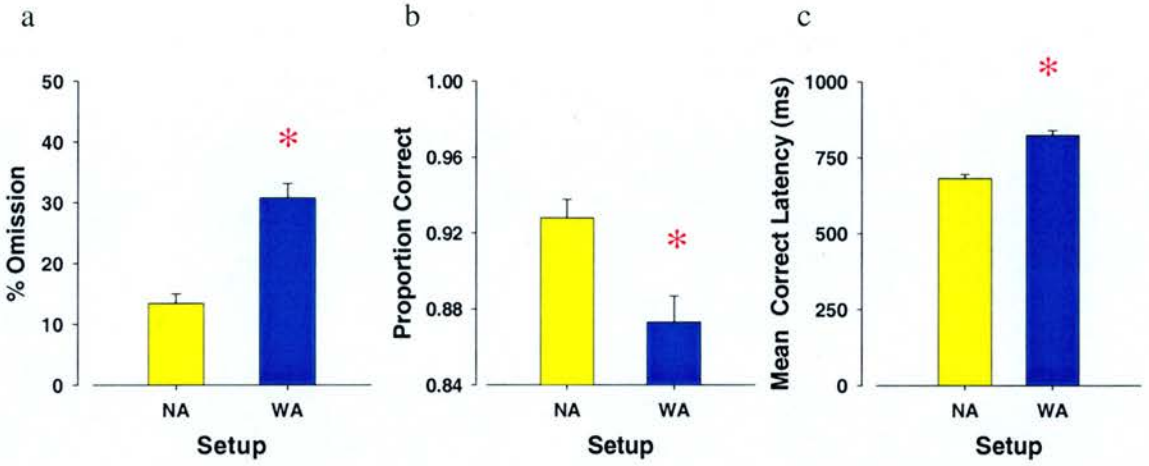


Figure 3.2: Modification of the mouse 5-CSR task lowered baseline performance

The mice (n = 16) trained in the previous study were split into two groups in a counter-balanced design. Over a period of four consecutive days (Tuesday – Friday), performance of the two groups was assessed in a narrow array (NA) and a wide array (WA). Increasing the width of the array had significant effects on every measure assessed resulting in higher % omissions (a), lower proportion correct (b) and a slower mean correct latency (c). Data presented as mean + s.e.m. (* denotes $p < 0.05$ when compared with the standard narrow array).

In addition to widening the array a variable ITI, akin to that used for acute challenges in the rat 5-CSR task (Hahn *et al.,* 2002), and that used as a standard protocol in many human measures of attention (Riccio *et al.*, 2002), was also introduced. Robbins, (2002) described that differences observed whilst using a variable ITI are 'more likely to reflect attentional than simple sensory functions'. These two modifications differ from the acute challenges utilised for rats as they are employed in both training and testing days, thus bearing greater resemblance to their use in the human literature and reducing any confounding effects of learning.

3.3.3 (-)Nicotine improved mouse performance in the modified 5-CSR task

Following a further 3-week washout period the mice (n = 16) used in sections 3.3.1 (pp. 81) and 3.3.2 (pp. 84) were again administered saline or (-)nicotine (3, 30 and 300 μg/kg) over four consecutive days (Tuesday – Friday). As can be seen in Fig. 3.3, the 3 μg/kg dose of nicotine produced a significant reduction in % omissions and an increase in proportion correct when compared to the control group. A 3-Way ANOVA with nicotine dose, ITI time, and day as between subject factors, yielded significant main effects of: nicotine dose on % omissions ($F(3,36) = 17.4$, $p < 0.001$; Fig. 3.3a), proportion correct ($F(3,36) = 8.83$, $p < 0.001$; Fig. 3.3b), and mean correct latency ($F(3, 36) = 5.27$, $p = 0.004$; Fig. 3.3c); ITI time on % omissions ($F(3,36) = 28.2$, $p < 0.001$), and on mean correct latency ($F(3,36) = 57.8$, $p < 0.001$); and day on proportion correct ($F(3,36) = 5.15$, $p = 0.005$). Tukey *post hoc* analysis on nicotine dose revealed a significant effect of 3 μg/kg of nicotine on % omissions ($F(3,36) = 17.4$, $p < 0.001$), and proportion correct ($F(3,36) = 8.83$, $p < 0.05$) compared to the

saline group. Nicotine at 300 μg/kg also increased proportion correct ($F(3,36) = 8.83$, $p = 0.001$), and decreased mean correct latency ($F(3,36) = 5.27$, $p < 0.005$), but had no effect on % omissions. No effect was observed at the intermediate nicotine dose of 30 μg/kg on any parameter examined.

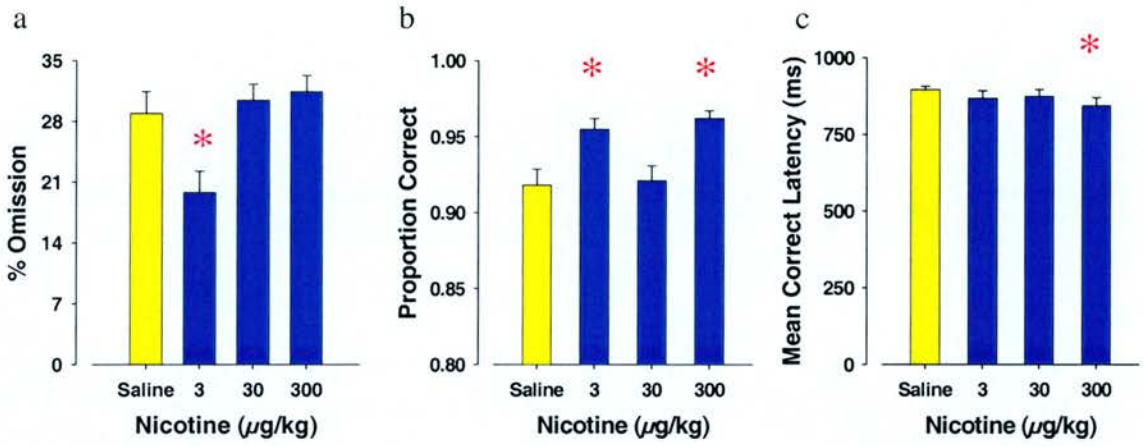


Figure 3.3: (-)Nicotine improved mouse performance in the modified 5-CSR task

Each mouse in this study had received extensive training and repeated nicotine (s.c.) injections 3 weeks prior to testing. The mice (n = 16) were separated into four groups in a counter-balanced design and administered saline or their allocated dose of (-)nicotine (3, 30, 300 μg/kg) for four consecutive days (Tuesday – Friday). The 3 μg/kg dose of (-)nicotine significantly reduced % omissions (a) and increased proportion correct (b) without affecting mean correct latency (c). In contrast, 300 μg/kg of (-)nicotine had no effect on omissions whilst increasing proportion correct (b), and reducing mean correct latency (c). The 30 μg/kg dose of (-)nicotine had no effect on any measure. Data presented as mean + s.e.m. (* denotes $p < 0.05$ when compared with saline).

3.3.4 (-)Nicotine improved drug-naïve mouse performance in the modified 5-CSR task

As the study conducted in section 3.3.3 was performed using mice that had been repeatedly exposed to nicotine, the significant improvements observed in sustained attention could have been confounded by factors such as receptor upregulation and/or desensitisation of multiple nAChRs (Paradiso and Steinbach, 2003; Maggi *et al.*, 2004). Therefore it was important to examine the cognitive effects of nicotine on drug-naïve in the modified 5-CSR task. As the 3 μg/kg dose of nicotine had produced the most robust improvement in performance the effect of nicotine was examined at 1, 10 and 100 μg/kg. A new group of C57Bl/6J mice (n = 25) were trained to perform the modified 5-CSR task as described previously. Nicotine significantly improved the performance of drug-naïve mice in the modified 5-CSR task (Fig. 3.4). A 3-Way ANOVA with nicotine dose, ITI time, and day as between subject factors, yielded significant main effects of: nicotine dose on % omissions ($F(3,36) = 211$, $p < 0.001$; Fig 3.4a), proportion correct ($F(3,36) = 3.285$, $p < 0.05$; Fig. 3.4b), and mean correct latency ($F(3,36) = 13.3$, $p < 0.001$; Fig 3.4c); ITI time on % omissions ($F(3,36) = 40.4$, $p < 0.001$), proportion correct ($F(3,36) = 3.512$, $p = 0.016$), and on mean correct latency ($F(3,36) = 9.83$, $p < 0.001$); day on % omissions ($F(3,36) = 6.58$, $p = 0.001$). Tukey *post hoc* analysis on nicotine dose revealed significant effects of 1, 10 and 100 μg/kg of nicotine on % omissions ($F(3,36) = 22.6$, $p < 0.001$). Moreover the 1 μg/kg dose of nicotine significantly increased proportion correct, whilst the 10 μg/kg dose significantly altered mean correct latency. There were significant main effects of the 1 μg/kg dose of nicotine on

proportion correct ($F(3,36) = 3.29$, $p = 0.05$), and the 10 μg/kg dose significantly increased mean correct latency ($F(3,36) = 13.3$, $p < 0.05$).

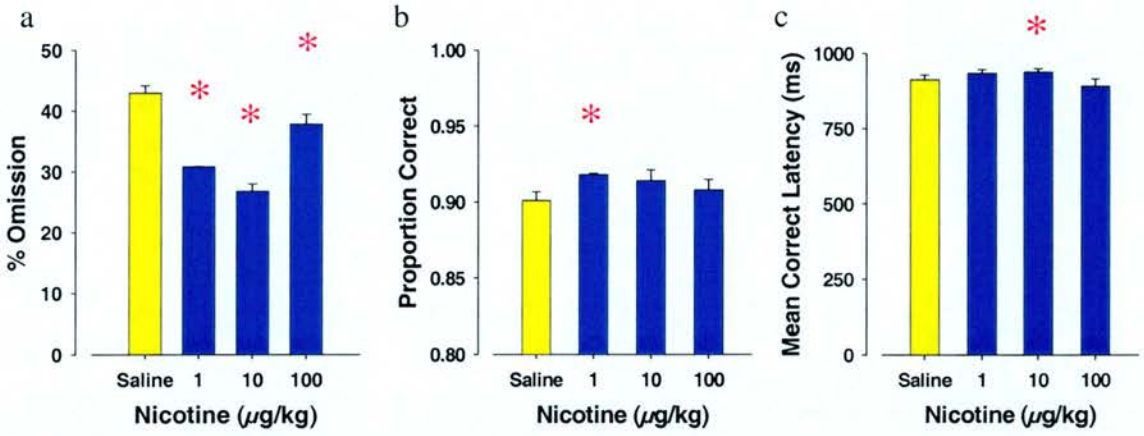


Figure 3.4: (-)Nicotine improved drug-naïve mouse performance in the modified 5-CSR task

Mice (n = 25) were trained specifically for this study and as such were nicotine naïve. The mice were separated into four groups in a counter-balanced design and administered saline subcutaneously (s.c.) or their allocated dose of (-)nicotine (0, 3, 30, 300 μg/kg) for four consecutive days (Tuesday – Friday). Every dose of (-)nicotine examined significantly reduced % omissions (a), with 1 μg/kg increasing proportion correct (b). The 10 μg/kg dose of (-)nicotine produced a significant increase in mean correct latency (c). Data presented as mean + s.e.m. (* denotes $p < 0.05$ when compared with saline).

3.3.5 α7 nAChR KO mice exhibit impaired acquisition and asymptotic performance of the standard 5-CSR task

The debate over which nicotinic receptor subtype is responsible for the cognitive enhancing effects of nicotine is far from resolved. As discussed in chapter 1.3.4 (pp. 24), there has been considerable interest in the influence of the α7 nAChR in attentional processing. Therefore I examined the effect of performance of α7 nAChR knockout (KO) mice in the standard 5-CSR task. The α7 nAChR KO (n = 8) mice and their age-matched littermates (WT, n = 8) were trained to perform the standard 5-CSR task (constant ITI and narrow array). As Fig. 3.5a shows, α7 KO mice took significantly longer to acquire the task when compared to their age-matched littermates (WT), with the time taken to reach 50% of asymptotic performance (A_{50}) approximately 5 days longer ($F(1,12) = 4.76$, $p = 0.05$; Fig. 3.5b). Once a stable baseline performance had been attained, the two groups were compared over four consecutive days (Tuesday – Friday). The α7 KO mice exhibited significantly higher levels of % omissions in comparison to the WT mice. A two factor ANOVA of repeated measures with genotype and day as between subject factors yielded significant main effects of genotype on % omissions ($F(1,36) = 7.67$, $p < 0.05$; Fig. 3.5c) and day on proportion correct ($F(1,36) = 4.50$, $p < 0.01$). There were no significant effects of genotype on proportion correct ($F(1,36) = 0.096$, $p = 0.762$; Fig. 3.5d), or mean correct latency ($F(1,36) = 0.354$, $p = 0.563$; Fig. 3.5e). Nor were there significant interactions between day and genotype in % omissions ($F(3,36) = 0.213$, $p = 0.887$), proportion correct ($F(3,36) = 0.409$, $p = 0.747$) or mean correct latency ($F(3,36) = 0.060$, $p = 0.980$).

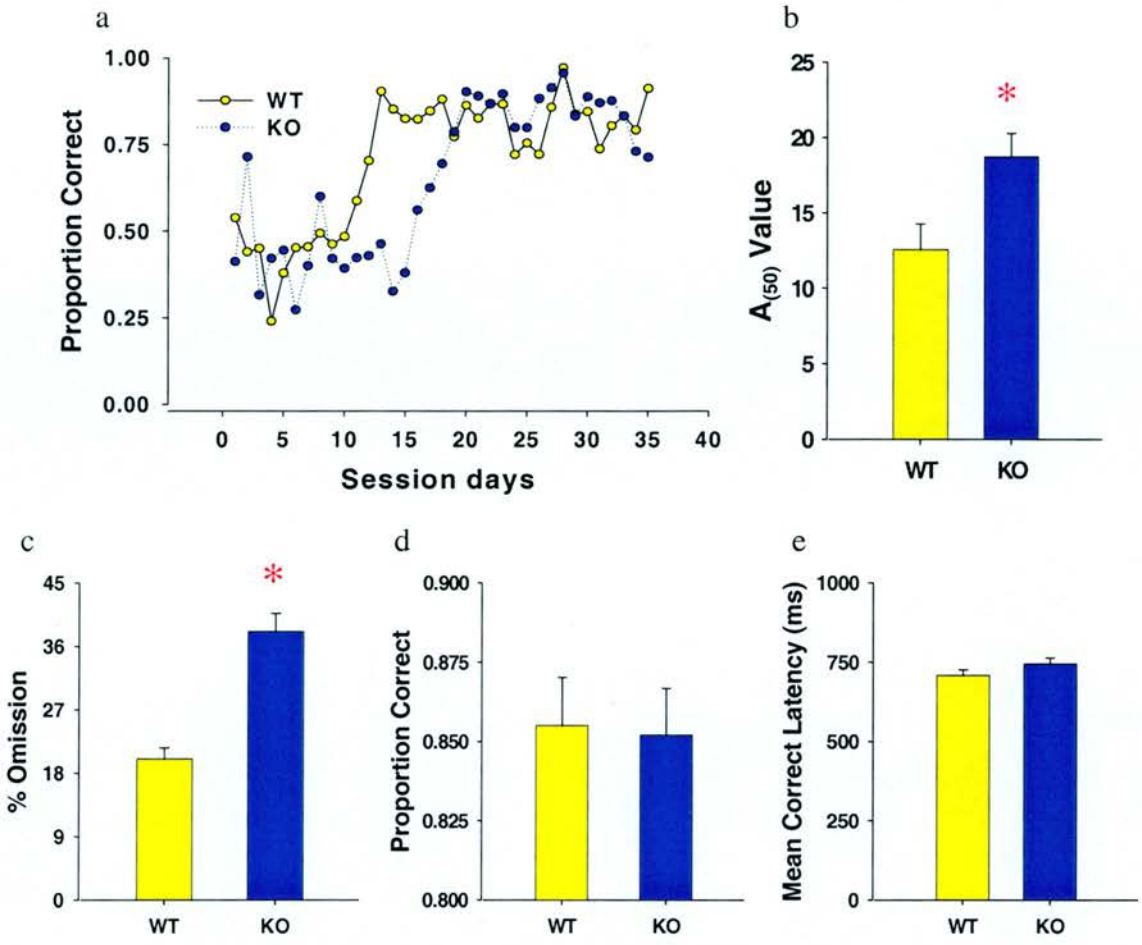


Figure 3.5: α7 nAChR KO mice exhibit impaired acquisition and asymptotic performance in the standard 5-CSR task

α7 nAChR KO mice (n = 8) and wildtype littermates (WT, n = 8) were trained in the narrow array in combination with the constant ITI. The α7 nAChR KO had impaired acquisition as shown in (a) with data shown as proportion correct across session days for one representative subject from each group, and (b) the mean time taken by each group to reach halfway to asymptote (A_{50}). α7 nAChR KO mice were also impaired in asymptotic performance of the task, as measured over four consecutive days (Tuesday – Friday). A significant effect of genotype was measured in % omissions (c). Proportion correct (d) and mean correct latency (e) are also shown but were unaffected by α7 nAChR KO in the standard task. Data presented as mean + s.e.m. (* denotes $p < 0.05$ when compared to WT mice).

3.3.6 (-)Nicotine does not improve performance of α7 nAChR KO mice in the modified 5-CSR task

The mice used in the previous study (3.3.5) were retrained (at ~ 18 months; WT n = 6, KO n = 7) in the modified 5-CSR task (2 – 10 s variable ITI and wide array). Such small group sizes prevented the possibility of utilising the same dosing protocol that was used in sections 3.3.3 and 3.3.4 (pp. 86 – 88). Both groups were administered saline on the Thursday, Friday and Monday prior to testing. In week 1 every subject received five consecutive days saline injections (Tuesday – Monday). In week 2, the mice were administered 1 μg/kg of (-)nicotine on following four days (Tuesday – Friday). After confirmation of a lack of day effect, the data were analysed using a 2-Way Repeated Measures ANOVA. No significant main effect of drug or genotype was identified for every measure tested. Whilst there appeared to be increased % omissions in the KO mice in both saline and nicotine treated weeks, this was not significant ($F(1,11) = 1.37$, $p = 0.267$; Fig. 3.6a). There was no genotypic effect on proportion correct ($F(1,11) = 0.003$, $p = 0.96$; Fig. 3.6b) or mean correct latency ($F(1,11) = 0.128$, $p = 0.727$; Fig. 3.6c). The trend towards lower % omissions and increased proportion correct only in WT mice was not significant (% omissions - $F(1,11) = 0.004$, $p = 0.948$; Fig. 3.6a; proportion correct; $F(1,11) = 1.16$, $p = 0.305$; Fig. 3.6b). Nor was there a significant effect on mean correct latency ($F(1,11) = 0.032$, $p = 0.86$; Fig. 3.6c). Finally no genotype by drug interaction was observed for any measure including % omissions ($F(1,11) = 0.226$, $p = 0.644$; Fig. 3.6a), proportion correct ($F(1,11) = 1.339$, $p = 0.272$; Fig. 3.6b) or mean correct latency ($F(1,11) = 0.213$, $p = 0.654$; Fig. 3.6c).

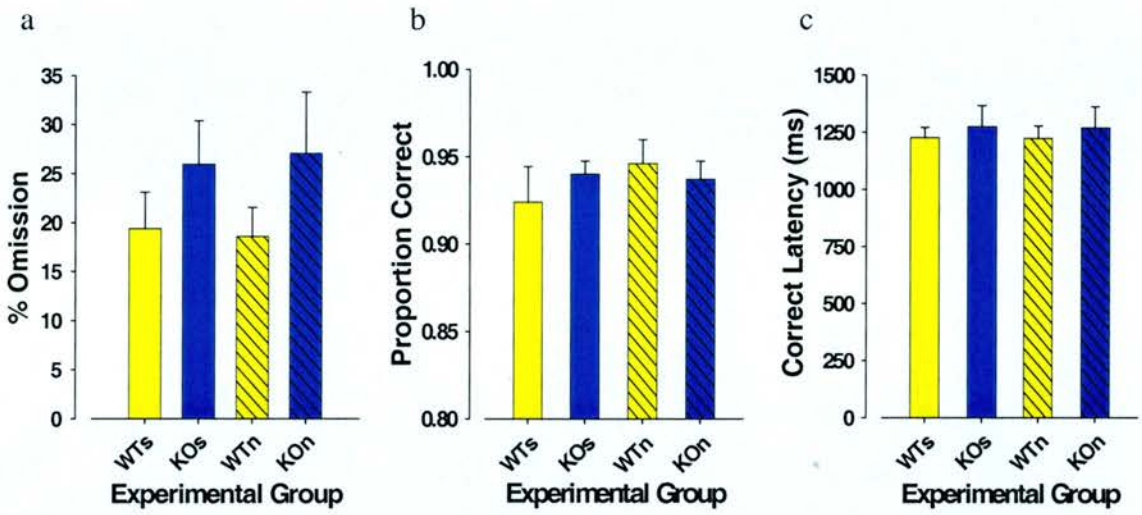


Figure 3.6: (-)Nicotine did not improve performance of the α7 nAChR KO mice in the modified 5-CSR task

α7 nAChR KO mice (n = 8) and wildtype littermates (WT, n = 8) were retrained in the modified (wide array and variable ITI) 5-CSR task. Training continued until asymptotic performance was attained. The mice were administered saline (s.c.) for four consecutive days (Tuesday – Friday, WTs and KOs – no pattern). The following week all the mice were injected with 1 μg/kg (-)nicotine (s.c.) for four consecutive days (Tuesday – Friday, WTn and KOn – diagonal bands). Measures of performance assessed included % omissions (a), proportion correct (b) and mean correct latency (c). No effect of nicotine or genotype was observed in any measure. Whilst the KO mice appeared to exhibit higher levels of % omissions than WT mice, this was not significant. No effect of nicotine administration was observed for any measure. Data presented as mean + s.e.m..

3.3.7 Reproduction of the deficit observed in α7 nAChR KO performing the 5-CSR task

A group of drug naïve α7 KO, heterozygote (HT) and WT mice (n = 10, 10 and 12 respectively) were trained to perform the 5-CSR task. They were trained with a constant 2 s ITI and wide array. This was designed to be a longitudinal study, where asymptotic performance, and the effect of introducing challenges would be measured at 6, 12, and 18 months. After 18 months the mice were to be administered nicotine in the same manner described in sections 3.3.3 and 3.3.4 (pp. 86 – 88). The following challenges were to be used on a Tuesday and Thursday, with training days on Monday, Wednesday and Friday:

1) Extended session; whilst the protocol remained the same, the session length was extended to 40 min and there was no limit to the number of trials.

2) Variable ITI; a 2 – 10 s ITI was introduced and was used as an acute challenge to assess the dependence of each strain to a temporal mediating strategy.

3) Stimulus duration reduction; the protocol was unchanged from training (wide array and constant 2 s ITI), but the SD was altered from 1 s to 0.85, 0.7, 0.55, 0.4, 0.25, 0.4, 0.55, 0.7, 0.85 then back to 1 s over the course of 10 consecutive days assessing their ability to maintain attention with increasing degrees of difficulty, and the speed with which their performance recovered.

Acquisition is shown for a representative mouse from each of the three groups (Fig. 3.7a), with proportion correct represented across time between 3 and 5 mth. No significant group difference was observed in the ability of each group to acquire the 5-CSR task as measured by the time latency to reach 50% of asymptotic performance (A_{50}, $F(2,25) = 1.586$, $p = 0.225$; Fig. 3.7b). This was in contrast to previous findings for α7 nAChR KO mice (section 3.3.5, pp. 90). Having attained acquisition criteria and a stable baseline, asymptotic performance (at 6 mth) was assessed over four consecutive days (Tuesday – Friday). No genotypic differences were observed in their performance in this task. A 2 way Repeated Measures ANOVA with genotype and day as between subject factors revealed no significant main effects of genotype or day for any measure. No significant differences were observed between the three groups in % omissions ($F(2,77) = 1.05$, $p = 0.355$; Fig. 3.7c) or levels of proportion correct ($F(2,77) = 0.775$, $p = 0.464$; Fig. 3.7d). Also mean correct latency did not differ significantly between the three groups ($F(2,77) = 1.627$, $p = 0.203$; Fig. 3.7e). Also there were no significant interactions between day and genotype as measured by % omissions ($F(4,77) = 1.022$, $p = 0.401$), proportion correct ($F(4,77) = 0.459$, $p = 0.766$) or mean correct latency ($F(4,77) = 0.540$, $p = 0.707$).

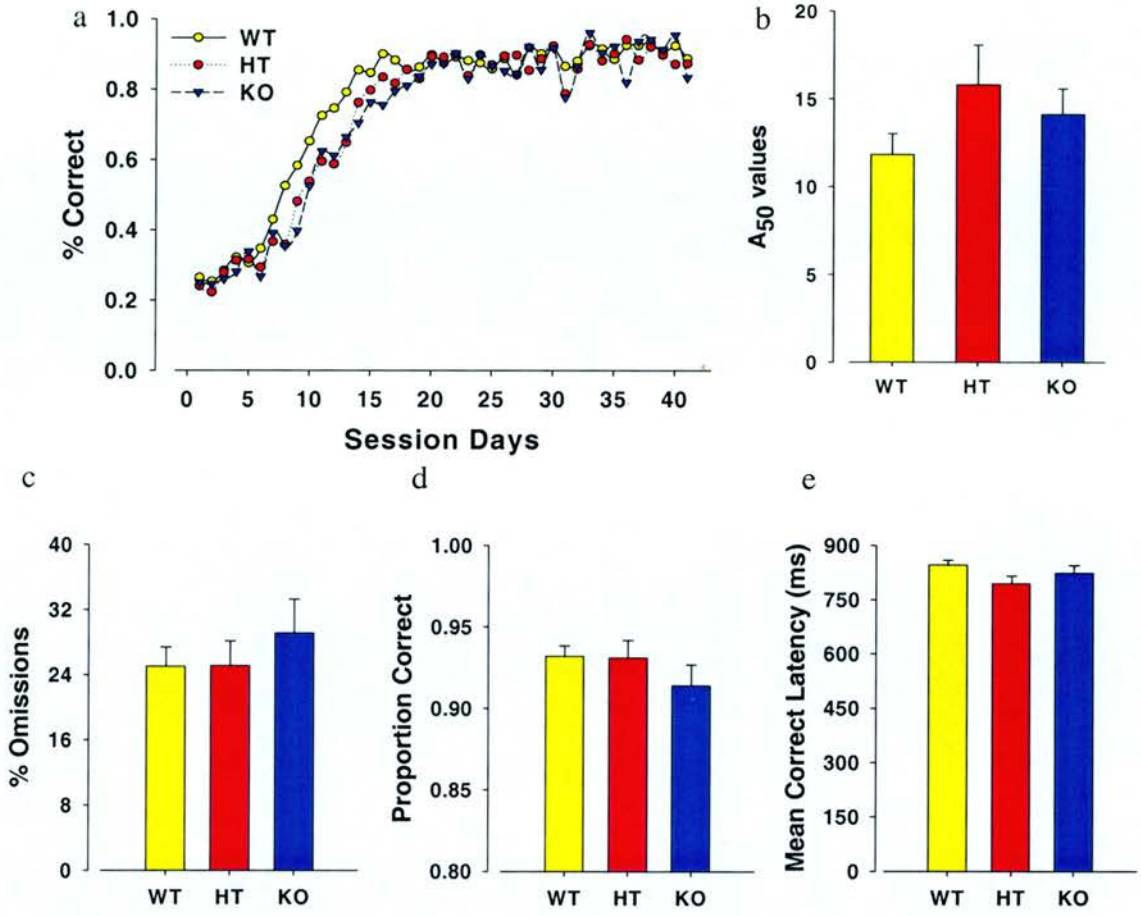


Figure 3.7: Normal acquisition and asymptotic performance of α7 nAChR mice in the 5-CSR task

A group of α7 nAChR knockout (KO, n = 10), heterozygote (HT, n = 10) and wild-type age-matched littermate (WT, n = 12) mice (3 – 5.5 mth) were trained in the 5-CSR task with wide array format. There was no difference in acquisition of the three groups as shown in (a) with data shown as proportion correct across session days (one representative subject from each group). In (b) the mean time taken by each group to reach halfway to asymptote (A_{50}) was not significantly different. Training continued after acquisition until a stable level of performance had been reached (asymptote, 6 mth) and the following measures were assessed over four consecutive days (Tuesday – Friday): % omissions (c), proportion correct (d) and mean correct latency (e). There were no significant differences in asymptotic performance between the three groups. Data plotted as mean + s.e.m..

Two weeks later the same mice were then assessed on an extended session probe. The session length was increased from 25 to 40 min. while the trial limit was increased from 120 to 1000 trials. The mice were assessed over a two-day period (Tuesday and Thursday) interspersed with training days (Monday, Wednesday and Friday). This schedule is commonly used in the literature (Hahn and Stolerman, 2002; Grottick *et al.*, 2003). A 2 way Repeated Measures ANOVA revealed no significant main effects on day of testing for any measure. However, a main effect of genotype was observed in % omissions ($F(2,55) = 3.201$, $p < 0.05$; Fig. 3.8a), with Dunnett's *post hoc* analysis suggesting the KO mice made significantly higher % omissions when compared to WT mice. The % omissions of the HT mice appeared to lie in between the two groups, although they did not differ significantly from either group. There was no significant main effect of genotype on levels of proportion correct ($F(2,55) = 0.446$, $p = 0.642$; Fig. 3.8b) or in mean correct latency ($F(2,55) = 1.756$, $p = 0.182$; Fig. 3.8c). Nor were there any significant interactions between the two factors as measured by % omissions ($F(2,55) = 0.365$, $p = 0.696$), proportion correct ($F(2,55) = 0.446$, $p = 0.642$) or mean correct latency ($F(2,55) = 0.321$, $p = 0.727$).

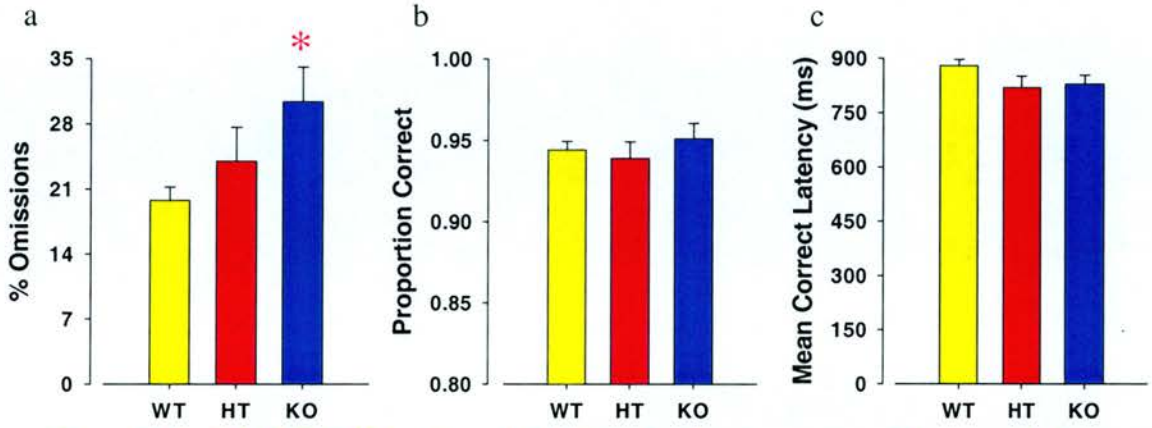


Figure 3.8: α7 nAChR KO mice exhibit impaired performance of the extended session 5-CSR task

The same group of α7 nAChR knockout (KO, n = 10), heterozygote (HT, n = 10) and wild-type age-matched littermate (WT, n = 12) mice were then assessed for their ability to perform the extended session 5-CSR task with wide array format. This task differed from the previous simply by increasing the length of the session by 15 min, and removing the 120 trial number limit. Performance was assessed over two days (Tuesday and Thursday) interspersed with training days (Monday, Wednesday and Friday). The KO mice exhibited significantly higher % omissions when compared with WT mice (a). No differences were observed in any other measure including proportion correct (b) and mean correct latency (c). Data plotted as mean + s.e.m. (* denotes $p < 0.05$ when compared with the WT mice).

At this stage the mice were currently 7 months old, and until this time these mice had exclusive use of the 5-CSR task apparatus. However after this time the apparatus was to be used in the afternoon for a different mouse 5-CSR task study. Whilst the mice in my study continued to be trained in the morning, the α7 KO, HT and WT mice appeared to be able to detect the presence of the second group of mice. On closer inspection it was clear that performance differed from that observed in the previous week, with fewer trials completed, an increase in the number of omissions, and a rise in mean correct latencies for every group. The mice appeared to spend more time exploring the operant box than previously, ultimately attending to the array less often (*personal observation*). However, training of the mice in the task continued until my mice habituated to the presence of the new group of mice and once more attended to the task; this took approximately 1 month. Unfortunately the performance exhibited by the mice after this month different to that previously measured. It appeared that the distraction caused by the exposure to a novel group of mice either impaired the performance of the WT mice or enhanced the performance of the α7 KO mice, though the former is more likely (data not shown).

The variable ITI and SD reduction challenges only served to further compound these effects. No significant effect of genotype on performance was observed in either challenge. The mice were then given 5 months of *ad lib* feeding and no training, then subsequently retrained and reassessed in the 5-CSR task. However, performance continued to be different to that observed at 6 months, prior to the introduction of the second group. The mice were therefore not retested at 18 months and nicotine was not administered as the study was abandoned.

3.3.8 Genotyping and characterisation of [^{3}H]-MLA binding sites in α7 nAChR KO and littermate mice

To confirm the behavioural deficits observed for the α7 KO mice, genotyping was performed and a radioligand binding assay using the putatively selective α7 nAChR antagonist [^{3}H]-MLA was established. Genotyping was performed with α7 nAChR specific primers that identified wild type (W) and disrupted (D) alleles (Fig. 3.9a) with representative subjects shown for clarity. For each subject, two lanes are presented, the first lane confirming the presence of the wild type allele, and the second lane showing the presence of the disrupted allele. Hence for WT mice, a band is visible in lane one, whereas for the KO mice, a band is visible in only lane two, showing disrupted alleles. As the α7 nAChR heterozygous (HT) mouse has both wild type and disrupted alleles, bands are clearly visible in both lanes. The positive (+ve) and negative (-ve) controls were included to ensure the primers were functioning accurately and that no cross-contamination had occurred. Verification of the absence of α7 nAChRs at the protein level was obtained by establishing a [^{3}H]-MLA binding assay. P_2 synaptosomal membranes were prepared from WT mice and a range of cholinergic drugs were examined for their ability to inhibit [^{3}H]-MLA binding. All four drugs examined inhibited [^{3}H]-MLA binding in a concentration dependent manner with the following rank order of potency; MLA > epibatidine > d-TC ≥ nicotine (Fig. 3.9b). The affinity of MLA was 1.31 ± 0.35 nM (nH = 0.81 ± 0.02; n = 43), with K_i values of 167 ± 106 nM (nH = 1.46 ± 0.14; n = 3) for epibatidine, 1.80 ± 0.07 μM (nH = 1.48 ± 0.44; n = 3) for d-Tc and 1.41 ± 0.82 μM for nicotine (nH = 1.06 ± 0.09; n = 3). For α7 KO mice and their WT littermates,

each brain was treated individually. As Fig. 3.9(c) clearly shows, in WT littermates the level of specific [^{3}H]-MLA binding was approximately 60% of total binding, whereas no specific binding was observed for the α7 KO mice.

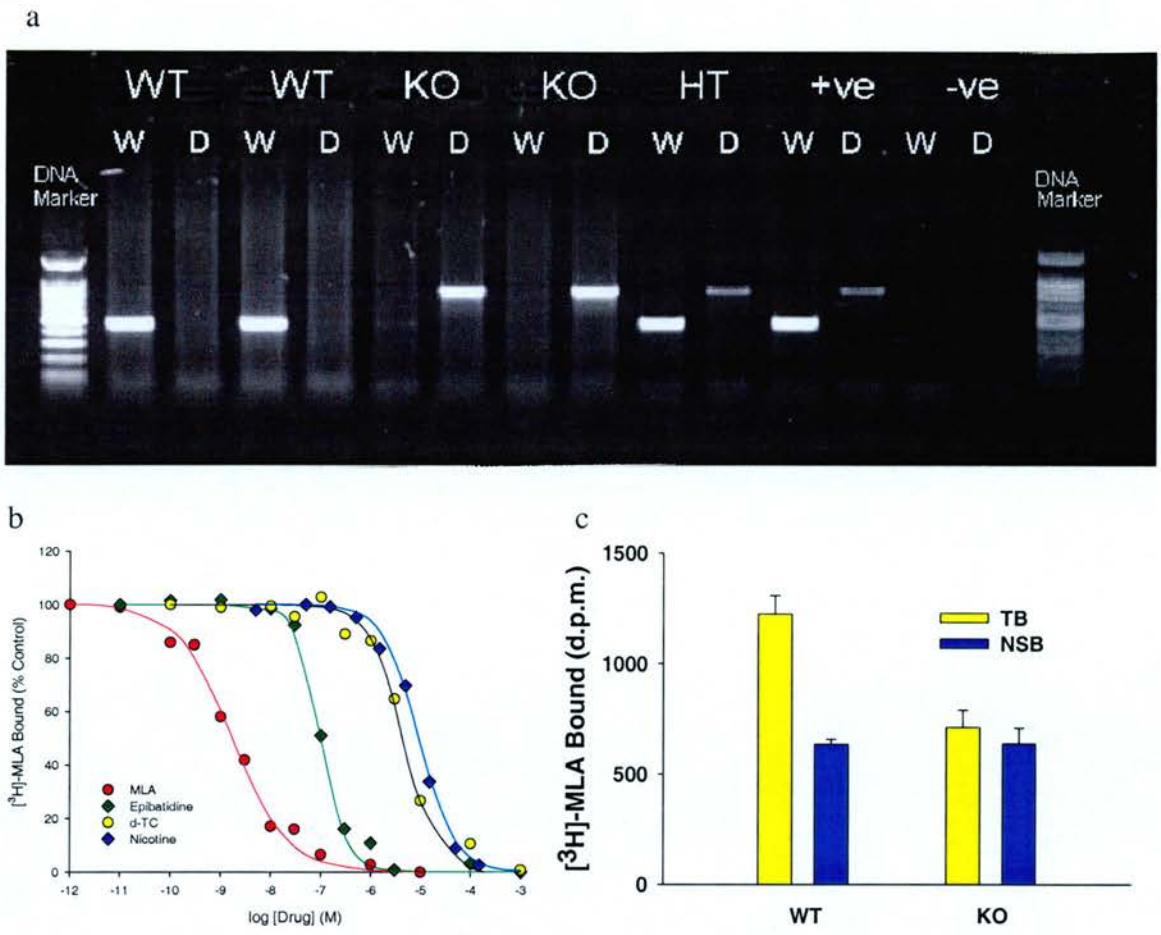


Figure 3.9: Genotyping [^{3}H]MLA binding of α7 nAChR transgenic mice

Confirmation of the genotypes of α7 nAChR knockout (KO) and wild-type littermate (WT) mice are shown in panel (a). Two lanes are shown for each mouse, the former exhibiting the presence of wild type (W) alleles (440 bp product), and the latter exhibiting the presence of disrupted (D) alleles (750 bp product). Hence WT mice have only W alleles, whilst KO mice exhibit only D alleles. An α7 nAChR heterozygous (HT) mouse is included for comparison, and contains both W and D alleles. Panel (b) shows the concentration dependent inhibition of [^{3}H]-MLA (2nM) binding in P2 synaptosomal brain membranes from WT mice, by a range of cholinergic drugs. In panel (c) the level of specific [^{3}H]-MLA binding in P_2 membranes prepared from individual control and transgenic mice (n = 4 per group) is shown. Clear specific [^{3}H]-MLA binding was observed for control animals, which is absent in α7 KO mice.

3.3.9 No overt differences were observed in the histopathology of α7 nAChR KO and WT mice

To show that ablation of the α7 nAChR introduced no gross CNS abnormalities the neuroanatomy of α7 nAChR KO and WT mice was assessed. Mice from section 3.3.6 (pp. 92) at 20 months were used for histopathalogical assessment. Mice were killed by cervical dislocation, the brains removed, and immediately placed in isopentane (-42°C) for three min. Cryostat sections were taken at 20 μm and Nissl stained with thionin. In general the histopathological analysis revealed no overt differences between α7 nAChR KO and WT mice. Whilst the highest expression of the α7 nAChR is in the hippocampus (Breese *et al.*, 1997), no hippocampal abnormalities were detected (Fig. 3.10a and b). Also the infralimbic cotex (Fig. 3.10c), orbital cortices (Fig. 3.10d) and pedunculopontine tegmental nucleus (Fig. 3.10e) all appeared ostensibly normal. Therefore the impaired attentional performance exhibited by the α7 nAChR KO does not appear to be as a result of gross abnormalities.

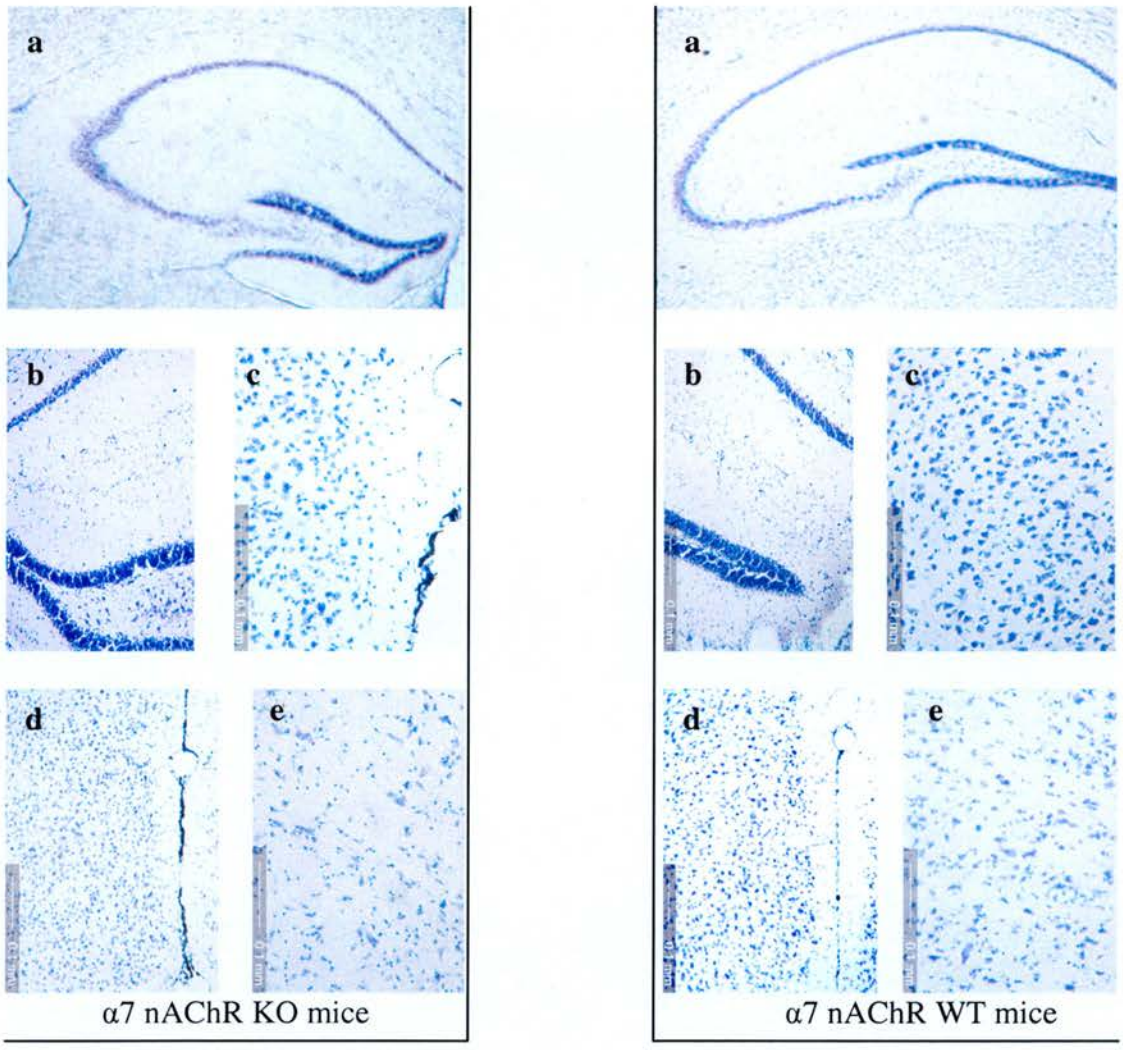


Figure 3.10: No overt differences were observed in the histopathology of the α7 nAChR mice

Histopathology of 20 mth old α7 nAChR knockout (KO, left panels) mice and their wild-type age-matched littermates (WT, right panels) was assessed. Cryostat sections of 20 μm were Nissl stained with thionin. No overt differences were observed in sections of: the hippocampus at magnification x 40 (a) and x 100 (b); the infralimbic cortex at magnification x 100 (c); the medial and lateral orbital cortices at magnification x 100 (d); and of the pedunculopontine tegmental nucleus at magnification x 100 (e).

3.4 Discussion

3.4.1 Nicotine improved sustained attention in C57Bl/6J mice after modification of the 5-CSR task

Initial attempts to mimic, in mice, the nicotine-induced improvement in sustained attention observed in normal humans proved challenging (Levin *et al.*, 1998; section 3.3.1, pp. 81). As discussed previously, attempts at producing this effect in rats have also proven difficult to attain (Mirza and Stolerman, 1998; Blondel *et al.*, 1999; Grottick and Higgins, 2000; Stolerman *et al.,* 2000; Mirza and Bright, 2001; Hahn *et al.,* 2002; Terry *et al.,* 2002), with improvement of baseline performance deemed improbable as a consequence of ceiling effects (Grottick and Higgins, 2000; Hahn and Stolerman, 2002). In contrast, ceiling effects were not a problem when assessing performance in normal humans (Levin *et al.*, 1998; Mumenthaler *et al.*, 2003). However whilst the 5-CSR task is regarded as analogous to the continuous performance test (CPT) used to assess sustained attention in humans (Jones and Higgins, 1995), it is not a precise mimic. In the human CPT factors such as a variable inter-trial interval (ITI), non-target stimuli, and an increase in the array width the subjects are required to attend to have been introduced in order to observe cognitive enhancing effects (Bates *et al.*, 1995; Riccio *et al.*, 2002). This increase in complexity of the CPT supports the assertion of Vorhees (1991) that more pronounced experimental effects would be observed with increased task complexity. Therefore nicotine would be more likely to improve attention if baseline performance was low at the outset (Newhouse *et al.*, 1997; 2004).

In mice, performance of the saline group in the 5-CSR task (section 3.3.1, pp. 81) was excellent following training (% omissions ~ 20%, proportion correct ~0.92; Fig. 3.2, pp. 85). It is therefore probable that their performance was also subject to the ceiling effects observed in both the rat and human literature (Bates *et al.*, 1995; Mirza and Stolerman, 1998; Blondel *et al.*, 1999; Grottick and Higgins, 2000; Stolerman *et al.,* 2000; Mirza and Bright, 2001; Hahn *et al.,* 2002; Terry *et al.,* 2002). Thus for experimental effects to be observed, it was obvious that task complexity needed to be increased in order to reduce baseline performance (Vorhees, 1991; Newhouse *et al.*, 1997; 2004). The 5-CSR task was made more complex by firstly increasing the width of the array (section 3.3.2, pp. 84), which led to a reduction in baseline performance (Fig. 3.2, pp. 85), and then introducing a variable ITI (minimising the possibility of mice using temporal mediating strategies). It has been suggested that results gleaned using a variable ITI are more likely to reflect attentional as opposed to sensory processing (Robins, 2002).

Following the modification of the 5-CSR task the same mice were retrained (for 2 mth) and then administered nicotine to assess its effect on performance (section 3.3.3, pp. 86). The drug treatment protocol was simplified with nicotine administered over a four day period rather than over 10-weeks (table 3.1, pp. 82). This adaptation is similar to dosing regimens described in the literature (Grottick and Higgins, 2000; Hahn and Stolerman, 2002) and limits a number of possible confounding variables (see section 3.4.2, pp. 112). The doses of nicotine examined were based upon previous rodent and human studies (Faiman *et al.*, 1991; Jones *et al.*, 1992; Acri *et al.*, 1994; Muir *et al.,* 1995; Grobe *et al.,* 1998, Stolerman *et al.,*

2000; Mirza and Bright, 2001). A significant improvement in sustained attention following nicotine administration was observed (Fig. 3.3, pp. 87). The 3 μg/kg dose of nicotine produced a clear improvement in performance, with a significant reduction in percent omissions and a concomitant increase in proportion correct. This outcome is consistent with the hypothesis of Mancuso and colleagues (1999) whereby the nicotine-induced improvement in attention is a consequence of nicotine acting to 'lock the brain into the attentional processing mode and so there are fewer lapses in attention' and therefore fewer errors of omission would be expected. As the 300 μg/kg dose of nicotine did not affect % omissions (Fig. 3.3a, pp. 87), it suggests that the improvement of proportion correct at this dose was not a true reflection of nicotine improving attention. Moreover as this dose also reduced mean correct latency (Fig. 3.3c, pp. 87), it suggests that the increase in accuracy may reflect psychomotor stimulation, an increased readiness to respond (Grottick and Higgins, 2000), which resulted in the mice consequently not attending to the cue array any more than control subjects. This would be consistent with nicotine being reported to produce motoric excitation in mice at doses of 125 and 250 μg/kg in mice (Nordberg and Bergh, 1985).

As pre-exposure to nicotine is known to alter nAChR density (Gentry and Lukas, 2002), confirmation that the observed improvements were not merely a consequence of alterations in the proportion or density of nAChRs necessitated that the effects of nicotine be examined in groups of drug-naive mice. Therefore, a new study (section 3.3.4, pp. 88) was conducted using a modified dose range that took cognisance of the improvements observed at the 3 μg/kg dose of nicotine. This study demonstrated a

significant enhancement in sustained attention in drug-naive mice at all three doses (1, 10 and 100 μg/kg) of nicotine examined (Fig. 3.4, pp. 89). All three doses significantly lowered % omissions (Fig. 3.4a), again consistent with the hypothesis of Mancuso and colleagues (1999). Moreover 1 μg/kg significantly increased levels of proportion correct (Fig. 3.4b) exhibiting a similar effect to that of the 3 μg/kg dose of nicotine used in the previous study (section 3.3.3, pp. 86).

The doses of nicotine used are consistent with those reported to improve attention in normal humans and pre-attention in normal rats (Acri *et al.*, 1994; Levin *et al.,* 1999; Heishman and Henningfield, 2000; Min *et al.,* 2001). In contrast, the doses of nicotine used in rat 5-CSR task studies are generally higher and the studies have required the inclusion of lesions, task challenges and poor performers (Muir *et al.,* 1995; Mirza and Stolerman, 1998; Stolerman *et al.,* 2000; Hahn *et al.,* 2002; Grottick *et al.,* 2003; Hahn *et al* 2003a,b). Moreover, whereas nicotine reduced % omission in normal mice in the current study, consistent with reports in normal human subjects (Levin *et al.,* 1998), in rats, the most consistent manifestation of an improvement in attention is an increase in accuracy (proportion correct; Stolerman *et al.,* 1998, Hahn and Stolerman, 2002). The reasons underlying the different effect on omissions between rat, mouse and human studies have yet to be clearly defined. One possible explanation is that in rats, the lack of effect of nicotine on % omission may be due to a floor effect as baseline % omissions are already < 10%. This is in contrast to mice where % omissions are higher at ~ 20% (Inglis *et al.,* 2001; Spratt *et al.,* 2001). This difference in % omissions cannot be attributed simply to the fact that mice were tested in the same size of apparatus commonly used for rats, for when the operant

box was scaled down, omission levels were still ~ 20% (Humby *et al.,* 1999). The reduction in % omissions observed in mice is therefore consistent with the nicotine-induced improvement in attention hypothesis proposed by Mancuso and colleagues (1999). Whilst it appears that the 1 and 3 μg/kg doses of nicotine enhance sustained attention, at higher doses the physiological effects become increasingly complex (Picciotto *et al.,* 2003; Fig. 3.11, pp. 111). Between 10 and 100 μg/kg the overall trend continued to be that of a reduction in omissions, but with no effect on proportion correct. In contrast, at 300 μg/kg nicotine increased proportion correct without altering % omissions. As this dose also reduced mean correct latency, this suggests the increase in accuracy may reflect psychomotor stimulation (Grottick and Higgins, 2000), with these mice consequently not attending to the cue array any more than control subjects. This would be consistent with nicotine being reported to produce motoric excitation in mice at 125 and 250 μg/kg (Nordberg and Bergh, 1985). Further increases in nicotine dose have lead to motoric inhibition and hypothermia (Marks *et al.,* 1983; Fig. 3.11, pp. 111). These physiological effects may have confounded the interpretation of studies which examined the effect of nicotine in mice. For example improvements in active avoidance learning have been observed in mice receiving doses of nicotine that cause hyperlocomotion (Castellano, 1976; Sasone *et al.*, 1994), whilst improved passive avoidance learning has been observed after doses of nicotine that induce hypothermia and thus hypolocomotion (Zarrindast *et al.*, 1996). However, Faiman and colleagues (1991) observed improved passive avoidance learning in mice, following administration of similar doses of nicotine to those used in the previous studies (1 – 30 μg/kg; 20 - 1500 fold

lower than the doses used by Zarrindast *et al.*, 1996) in which no confounding physiological effects have yet to be reported.

In conclusion I have modified the 5-CSR task so that nicotine-induced improvements in mice sustained attention has been observed. Moreover this improvement is consistent with that observed in humans. Therefore a protocol now exists with which to use both pharmacological and genetic manipulations in the attempt to identify the nAChR that mediates the cognitive enhancing effects of nicotine.

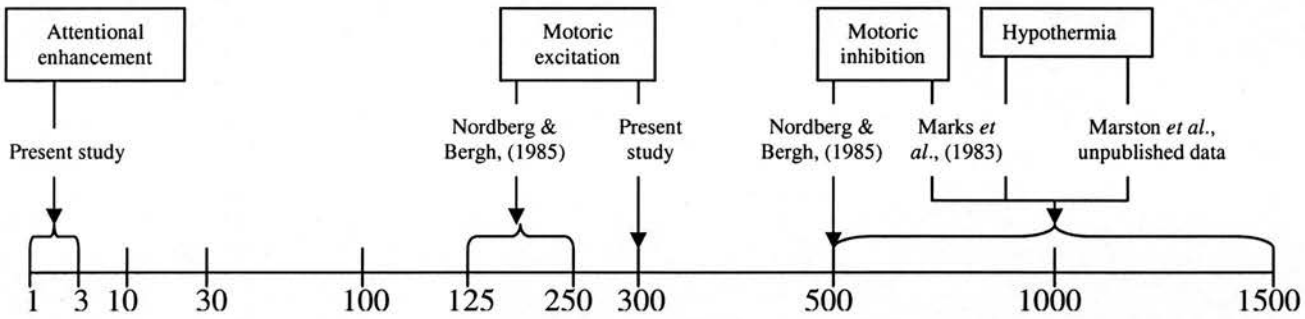


Figure 3.11: Schematic representation of nicotine-induced behavioural effects in mice

Nicotine exerts both cognitive and physiological effects that are largely dose dependent. The present studies (sections 3.3.3 and 3.3.4, pp. 86 – 88) suggest that low doses of nicotine (1 – 3 μg/kg) produce improvements in sustained attention. Higher doses appear to reduce mean correct latency, suggesting an enhancement of motoric capabilities, consistent with previous studies (Nordberg and Bergh, 1985). Doses of nicotine of 500 mg/kg and above inhibit motoric responses, with hypothermic effects observed. A further increase to 5 mg/kg has been shown to induce seizures in mice (Damaj *et al.*, 1999).

3.4.2 Impaired 5-CSR task performance of α7 nAChR knockout mice

α7 nAChR knockout (KO) mice were impaired in the acquisition of the 5-CSR task which suggests that this nAChR may indeed be required for either normal learning, or at least the learning of an attentional operant procedure (Fig. 3.5a and b, pp. 91). Moreover, as the impaired performance in the task was reflected by an increase in % omissions (Fig. 3.5c), this suggests that the α7 nAChR may also be required for sustaining attention in normal mice. Whilst the second group of α7 nAChR KO mice trained in the 5-CSR task did not exhibit a significant impairment in task acquisition, the age matched littermate (WT) mice did appear to acquire the task quicker than both the heterozygote (HT) and KO mice (Fig. 3.7a and b, pp. 96). However, whilst no difference in baseline performance was observed for the three groups in the task (3.7c, d and e), as observed in section 3.3.5 (Fig. 3.5c, pp. 91), the α7 nAChR KO mice were significantly impaired in the more attentionally demanding extended session version of the task, and again this deficit was reflected by an increase in % omissions (Fig. 3.8a, pp. 98). Interestingly, HT mice which had 50% reduction of [^{3}H]-MLA binding sites, exhibited % omissions halfway between that of WT and KO mice, though were not significantly different from either group. Thus it appears that task performance as measured by % omissions are robust and that it appears to be related to the binding site density of α7 nAChRs.

It appears unlikely that the deficiencies observed for the α7 nAChR KO and HT mice are attributable to developmental problems that are a consequence of the genetic manipulation, as these animals have an ostensibly normal appearance; with standard

growth, survival, gait, anatomy, and no apparent nervous system abnormalities (Orr-Urtreger *et al.,* 1997), sections were taken from all three groups of mice (though KO and WT mice shown only) and as Fig. 3.10 (pp. 104) shows, we confirmed there were no gross abnormalities in areas deemed important to learning and 5-CSR task performance. In addition, Paylor *et al.,* (1998) reported no differences in the behavioural phenotype of α7 nAChR KO mice and their WT littermates when subjected to a battery of behavioural tests, including contextual and auditory fear conditioning, spatial learning in the Morris water maze, and anxiety tests. However, unexpectedly it was noted that these mice did not exhibit a sensory gating deficit in the PPI paradigm (Paylor *et al.,* 1998), although these results may have been confounded by the use of female mice in the tests. As the deficit in sustained attention observed for the α7 nAChR KO mice was in the same parameter by which nicotine improved attention (i.e. % omissions), this suggests the α7 nAChR may be central to the nicotine-induced improvement in sustained attention. Conversely if nicotine administration had improved attentional performance in the α7 nAChR KO mice this would imply that the α7 nAChR is not the only nAChR involved in attention in mice. The data in section 3.3.6 (pp. 92) show that nicotine administration did not improve performance in the α7 nAChR KO mice which is consistent with a key role for this receptor in sustained attention (Fig. 3.6, pp. 93). However, nicotine did not significantly improve the performance in the WT mice either, despite a reduction in % omissions (Fig. 3.6a) and increase in proportion correct (Fig. 3.6b). Overall, the studies do however suggest that the α7 nAChR may play a role in mediating the cognitive enhancing effects observed with nicotine.

One unexpected finding was that these mice performed at a slower rate than traditionally observed (section 3.3.5, Fig. 3.5, pp. 91). Whilst this could reflect age and task complexity (use of a wide array and variable ITI as opposed to narrow array and constant ITI in section 3.3.5, pp. 90), in the two studies, other old mice (17.5 month) have been assessed in the wide array 5-CSR task, and they did not exhibit such slow latencies (see section 5.3.2, Figs 5.4 and 5.5, pp. 175 and 177). The use of the variable ITI appeared to slow performance (Fig. 3.3 and 3.4, pp. 87 and 89) but again not to the extent observed in Fig. 3.6 (pp. 93). The relative slowing of response to the extent observed here has only been noted when noise distracters were employed (section 5.3.2, Figs. 5.4 and 5.5).

One possibility that needs to be considered is that unfortunately at the time this study, major building work was being conducted on the floors below. Whilst the mice were assessed in sound attenuating boxes during testing and training, these are not 'sound-*proof*' boxes, and the resulting noise which varied in intensity and duration from day to day was likely to confound performance. Noise has been used as a distracter in attentional tasks in man (Wolach and Pratt, 2001), monkeys (Terry *et al.*, 2002), rats (Robbins 2002) and mice (Humby *et al.*, 1999; see section 5.3.2.3 and 5.3.2.4, pp. 178 – 180) with the effects dependent on the background strain (Humby *et al.*, 1999; see section 5.3.2; Fig. 5.4 and 5.5, pp. 175 and 177). In addition, drilling occurred at intermittent times during testing which caused considerable vibration in the buildings structure. This also caused the 9-hole boxes to vibrate, an effect which was likely to distract the mice. Such tactile distracters have been used to induce startle responses in mice and the response can vary

depending on the background strains (Logue *et al.*, 1997). During this period there was a notable reduction in litter numbers throughout the mouse holding room during building work (Dr. L. E. Kerr *et al.*, *personal communication*). In conclusion, the noise and vibrations that occurred at unpredictable times may unfortunately have added an uncontrollable confound to the latter part of this study and made it difficult to observe a significant effect of nicotine on the performance of WT mice. The second α7 nAChR transgenic study (WT, HT and KO mice; section 3.3.7, pp. 94) was designed to be a longitudinal study with nicotine administered to drug-naïve mice at 18 months. However, the baseline performance of these mice changed following procedural difficulties and repeated testing, such that a deficit was no longer observed in the α7 nAChR KO mice (see section 3.3.7, pp. 94). In conclusion however the data supports the α7 nAChR as essential for maintenance of sustained attention in mice. Furthermore the protocols I have developed in this thesis can further assist in addressing its validity as a therapeutic target for the nicotine-induced improvement in cognition.

There is a plethora of evidence demonstrating nicotine-induced improvements in attention in different species. However there has been no definitive identification of which nAChR subtype(s) underlie this effect. Several studies indicate that the α7 nAChR appears crucial in maintaining pre-attention (sensory gating; Stevens *et al.*, 1996; 1998; Simosky *et al.*, 2001). Schizophrenics have poor sensory gating with a reduction in the P50 auditory evoked potential (Waldo *et al.*, 1995). This deficiency has been linked to a dinucleotide polymorphism on chromosome 15q13-14, proximal to the locus of the α7 nAChR gene CHRNA7 (Freedman *et al.*, 1997; Stassen *et al.*,

2000). Moreover, the DBA/2 mouse strain, which has a natural reduction in α7 nAChR density in the hippocampus, show sensory gating deficits (Stevens *et al.,* 1996), that can be ameliorated by treatment with the α7 partial agonist DMXBA (GTS-21; Stevens *et al.,* 1998; Simosky *et al.,* 2001) and atypical anti-psychotics (Simosky *et al.,* 2003). DMXBA also attenuated the sensory gating deficits observed in rats that were reared in isolation (O'Neill *et al.,* 2003), an animal neuro-developmental model of schizophrenia (Geyer *et al.,* 1993). Furthermore, both DMXBA (Kem, 2000) and the full α7 nAChR agonist AR-R17779 (Mullen *et al.,* 2000) are able to replicate the beneficial effects observed with nicotine on working memory (Felix & Levin, 1997; Levin & Simon, 1998). In contrast, and perhaps somewhat surprisingly, recent studies in rats suggested that AR-R17779 had no effect on attention in the 5-CSR task (Grottick and Higgins, 2000; Grottick *et al.,* 2003; Hahn *et al.,* 2003). Van Kampen and colleagues (2004) recently suggested that these findings be re-examined. To date, independent evaluation of these agents remains impossible as these compounds are not commercially available.

3.5 Conclusion

In conclusion, I established a modified version of the 5-CSR task for mice that lowered baseline performance, facilitating the demonstration of an enhancement in sustained attention following administration of low doses of nicotine. Moreover, I have also shown that α7 nAChR KO mice have a profound and reproducible deficit in attention. Interestingly heterozygote mice with a 50% reduction in α7 nAChR density showed an intermediate performance level. The sensitivity of environmental confounds and repeated testing on mouse performance in the 5-CSR task were also highlighted. In summary, these data support the conclusion that α7 nAChRs play a role in sustained attention in mice.

Chapter 4 - Nicotinic modulation of working memory

4.1 Introduction

Nicotine has been shown to improve working memory performance in patients suffering from dysexecutive syndrome (see chapter 1.3.6, pp. 26), including Alzheimer's disease (Newhouse *et al.*, 1988; Sahakian *et al.*, 1989; Parks *et al.*, 1996) and schizophrenia (Levin *et al.*, 1996; Smith *et al.*, 2002). However, not all studies report a positive effect of nicotine (Sahakian *et al.*, 1989; Wilson *et al.*, 1995; Heishman and Henningfield, 2000; Harris *et al.*, 2004). It has been proposed that nicotine-induced improvements in working memory are more likely to be observed when there is a high attentional load placed upon the subject during task performance (Warburton and Rusted, 1993; Phillips and Fox, 1998; Newhouse *et al.*, 2004; Fig. 4.1).

Studies attempting to identify the nAChR involved in nicotine's beneficial effects on working memory have largely utilised rats and the radial arm maze (RAM; Levin *et* al., 1993; Kim and Levin, 1996; Felix and Levin, 1997; Levin *et al*, 1999; Bancroft and Levin, 2000; Bettany and Levin, 2001; Levin *et al.*, 2002, Addy *et al.*, 2003; see chapter 1.4.4.4, pp. 45). This task is not confounded by proactive interference, as each session does not involve repetition of similar trials. As performance becomes more difficult after every correct response, there is high attentional component to the task.

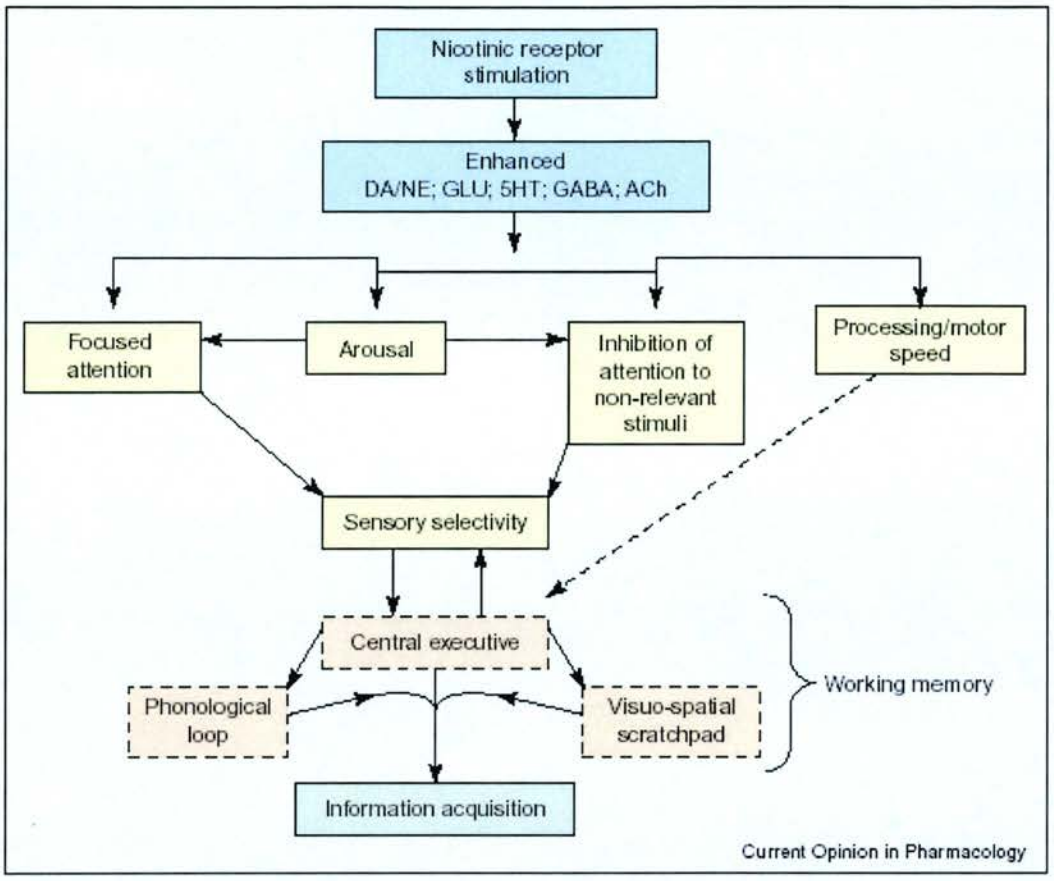


Figure 4.1: Proposed model for nAChR modulation of cognitive function

The model proposed by Newhouse and colleagues (2004), relates to the effects of nAChR stimulation on neurotransmitter regulation and attentional function. In their model, nAChR stimulation is presumed to lead to enhanced neurotransmitter release in particular brain areas that are relevant to arousal, sustained attention, inhibitional processes and processing/motor speed. Sensory selectivity is conceptualised as being secondary to improved attentional performance. There is then an effect on the central executive component of Baddeley's model (1986; equivalent to the supervisory attentional system by Norman and Shallice, 1980) for working memory, resulting in improved working memory and acquisition of information.

The odour span task (OST), originally developed for rats (Dudchenko *et al.*, 2000; see section 1.4.4.5, pp. 47), is similar to the RAM as it is not subject to the proactive interference confounds. Likewise, as a session continues, the cognitive demand placed upon the animal increases, thereby increasing the attentional load. However, the OST assesses non-spatial olfactory working memory by utilising randomly located odours, whereas the RAM assesses spatial working memory by utilising visual cues to identify location. The use of olfactory cues in the OST is potentially advantageous as rodents exhibit preferential attendance to olfactory as opposed to visual cues (Jennings and Keefer, 1969). Assessing olfactory memory may also provide ethologically relevant stimuli, an importance stressed by Gerlai and Clayton (1999) and Slotnick (2001), when phenotyping transgenic mice.

Therefore the task may prove suitable for behaviourally phenotyping the various transgenic mouse models of Alzheimer's disease that have been created (see table 5.1, pp. 158). Moreover, several clinical groups suffer form impaired olfactory capabilities, including Alzheimer's disease and schizophrenic patients (Kopala *et al.*, 1992; Wu *et al.*, 1993; Moberg *et al.*, 1997; Larssen *et al.*, 1999; Devanand *et al.*, 2000; Kohler, 2001), which have been postulated as a possible early marker for neurological diseases and psychosis (Kwapil *et al.*, 1996; Martzke *et al.*, 1997; Devanand *et al.*, 2000). As such the OST may provide greater phenotypic characterisation of the transgenic mouse models of these disorders, and assess the validity of olfactory impairments being an early marker for the disorders. The OST could also assist in the identification of the nAChR that mediates the nicotine-induced improvement in working memory/cognitive function, as both

pharmacological and genetic manipulations could be utilised. However, no reports have been published on the use of the OST in mice. Therefore during my thesis I tried to establish the OST for use in mice. The OST was validated in caspase-3 over-expressing mice (Kerr *et al.*, 2004, the creation of which is detailed in Appendix I, pp. 256) as the expression of caspase-3, the main proapoptotic effector caspase in the CNS, is highest within the olfactory system (de Bilbao et al, 1999; Cowan et al, 2001), an area thought to mediate a rodent's ability to remember odours (Staubli *et al.*, 1986; Slotnick and Thanos, 1997), particularly projections to the entorhinal cortex (Fig. 4.2). It was hypothesised that the over-expression of caspase-3 may affect the apoptotic processes within these structures with a detrimental effect on learning and memory, thus providing a suitable model for validation of this task. Therefore the performance of caspase-3 over-expressing mice was assessed in this task, and as these mice may exhibit enhanced neurodegeneration, longitudinal performance was also measured. Finally the ability of nicotine to rescue any deficit these mice exhibited was assessed. The effects of nicotine on the performance of normal mice in the OST were also assessed in a group of C57Bl/6J mice trained in the task. Preliminary studies indicated that the performance of C57Bl/6 mice was excellent in the task and ceiling effects would be expected in the standard 12 odour OST. Thus task difficulty was immediately increased to 22 odours in order to try to observe a nicotine-induced improvement in standard performance. Finally, as the α7 nAChR mice showed a deficit in the 5-CSR task it was hypothesised that they would also exhibit impaired performance in this task due to its high attentional load.

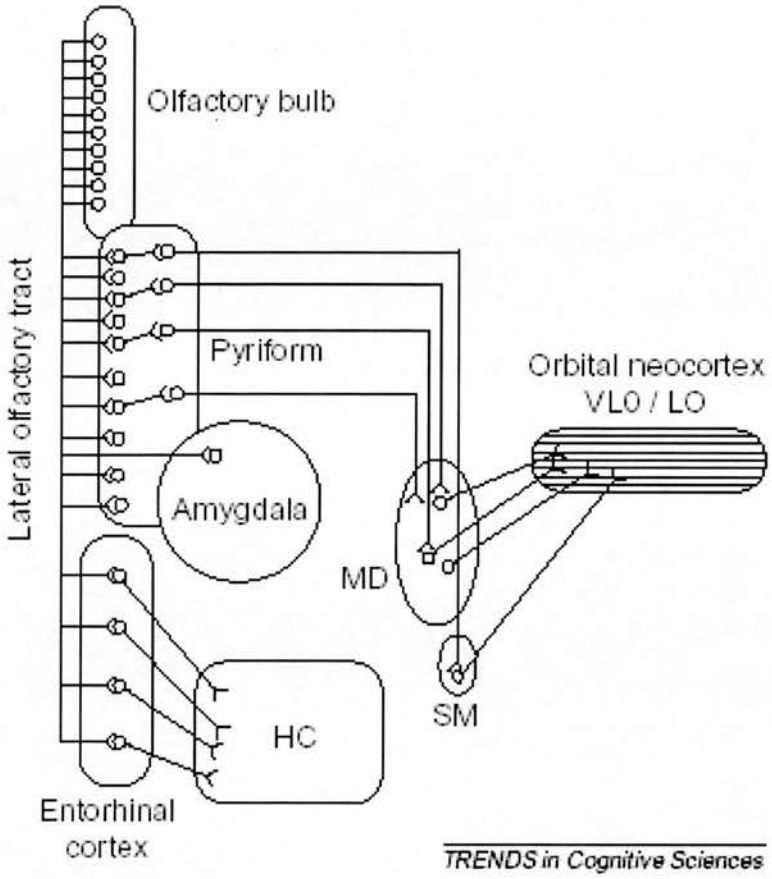


Figure 4.2: Schematic diagram of the central olfactory projections from the main olfactory bulb

The olfactory bulb projects directly to the amygdala, hypothesised to mediate emotions and hence the ease at which odours can elicit an emotional response. The olfactory bulb also projects to the piriform and entorhinal cortices. Whilst the former projects to the mediodorsal (MD) and submedial (SM) nuclei, then ventro-lateral orbital (VLO) and lateral orbital (LO) cortices, the latter projects to the hippocampus (HC). Alzheimer's disease pathology occurs early in the entorhinal cortex, perhaps mediating the olfactory deficits observed and representing an early marker of the disease (adapted from Slotnick, 2001).

4.2 Methodology

4.2.1 Animal maintenance and genotyping

Three separate groups of human caspase-3 transgenic mice (Tg; C57BL/6J-Tg(*CASP*3)F18Fine, Kerr *et al.*, 2004) and wild type littermates (WT) were used in the final three sections (section 4.3.1, WT n = 6, Tg n = 5, backcrossed onto a C57Bl6/J background for 6 generations; section 4.3.2, WT n = 5, Tg = 5, backcrossed onto a C57Bl6/J background for 8 generations; section 4.3.3, WT n = 5, Tg n = 9, backcrossed onto a C57Bl6/J background for 10 generations). The average age of mice in section 4.3.1 was 9 mth (range 8 to 10.5 mth, weight range 25.7 – 32.9 g) at start of training and 10.5 mth when tested. The average age of mice in section 4.3.2 was 4.5 mth (range 3 to 5 mth, weight range 23.7 – 30.7 g) at start of training and asymptotic performance was assessed at 6, 12 (weight range 27.5 – 35.4 g) and 18 (weight range 27.9 – 39.3 g) mth. The average age of mice in section 4.3.3 was 3.5 mth (range 3 – 4 mth, weight range 20.2 and 26.8 g). All studies are listed in table 4.1. The mice were all trained in the OST in accordance with section 2.3 (pp. 60). C57Bl/6J male mice were used in section 4.3.4 (n = 12; Charles River, Margate, UK). The mice weighed between 25.2 and 29.6 g at the start of the study. The mice in section 4.3.5 comprised of a group of 5 α7 nAChR KO (B6.12957 - $Chrna7^{tm1bay}$; Jackson Laboratories, Bar Habor, U.S.A.), and 5 age-matched littermates (subset from section 3.3.7, pp. 94; age range 17 – 18 mth; backcrossed onto a C57Bl6/J background 11 times), weighing between 33.2 – 43.6 g at the beginning the study.

Mice	Section number	Study	Age (mth)	Group sizes	
				WT	Tg
Human caspase-3 over-expressers	*4.3.1*	Acquisition	9 – 10	6	5
		Asymptote	10.5	6	5
	4.3.2	Acquisition	4 – 5.5	5	5
		Asymptote	6	5	5
		Asymptote	12	5	5
		Asymptote	18	5	5
	4.3.3	Asymptote	5	5	9
		Nicotine administration	5.5	5	9
C57Bl6/J	*4.3.4*	Nicotine administration in the 22-OST	N/A	12	
α7 nAChR KO and WT	*4.3.5*	Acquisition	17 – 18.5	5	5
		Asymptote	19	5	5

Table 4.1: List of behavioural studies

The studies performed in this chapter are listed above. The standard 12 odour OST was used unless otherwise stated.

4.3 Results

4.3.1 Human caspase-3 over-expressing mice exhibit impaired working memory

The first study was conducted to assess whether mice could be trained in the olfactory working memory task. Human caspase-3 over-expressing mice (Tg, n = 6) and their age-matched littermates (WT, n = 5) were trained to a stable baseline performance and the span of odours remembered (span length, number of correct spans prior to the first error) by Tg mice was significantly shorter than the WT (T=554.5; $p < 0.05$; Fig. 4.3a). Both groups were however perfect in their ability to distinguish between only two odours (T=490; $p = 0.992$). Furthermore both the WT and Tg mice completed nearly all 11 spans, with no significant difference between groups (T=517; $p = 0.115$; Fig. 4.3b). Thus whilst the Tg mice had the opportunity to record a similar span length to the WT mice, they made their first error earlier. The Tg mice erred significantly more often than the WT mice (T=36; $p < 0.05$; Fig. 4.3c), with more than one incorrect dig per session, as opposed to the WT mice erring once every three sessions. Whilst the speed of response (mean span latency) of the Tg mice appeared slower than that of the WT mice, it was not significant ($F(1,27) = 0.33$, $p = 0.58$; Fig. 4.3d), and nor was there an interaction between the two factors as measured by mean span latency ($F(3,27) = 0.431$, $p = 0.733$). To address any possibly confounding effects such as impoverished locomotor capabilities, the spontaneous locomotor activity counts of the two groups were assessed and compared. Whilst the counts for the Tg were lower than that of the WT mice, this was not significant ($F(1,38)=0.391$, $p=0.536$; Fig. 4.3e).

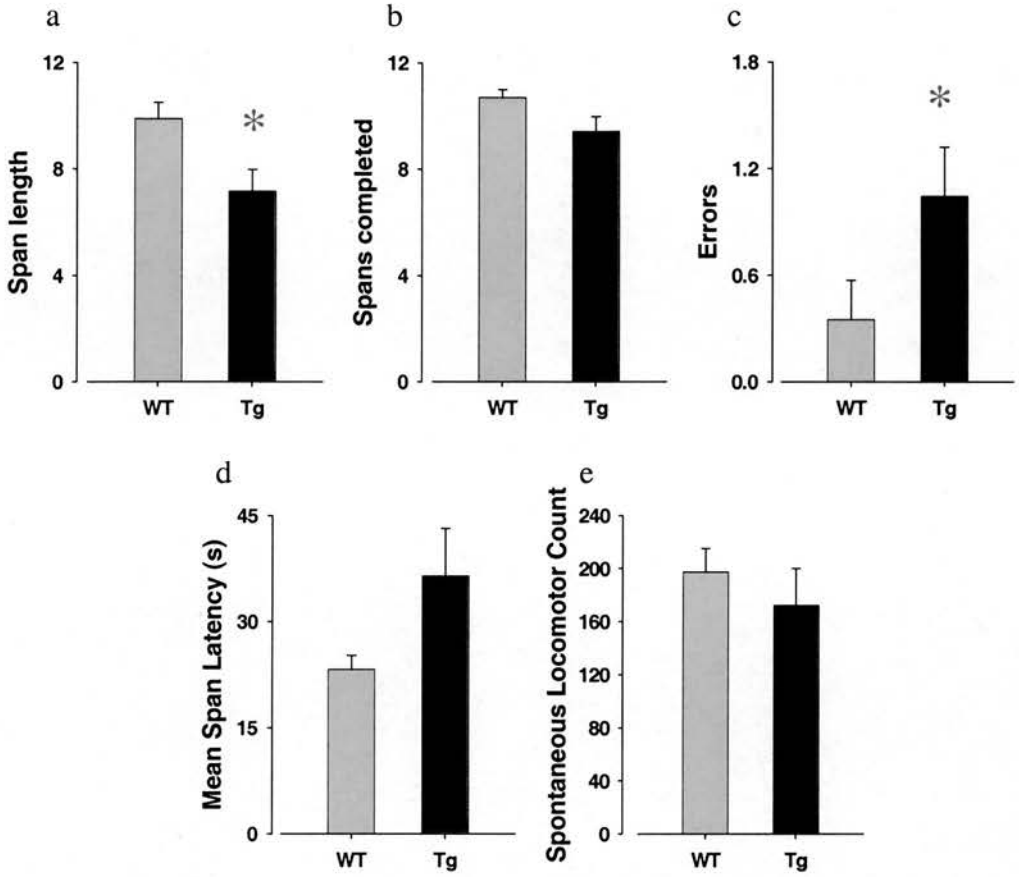


Figure 4.3: Impaired OST performance of human caspase-3 over-expressing mice (10.5 mth)

Performance of a group of 8 – 10.5 month old caspase-3 transgenic mice (Tg, n = 5) and their age-matched wild type littermates (WT, n = 6) was assessed in the odour span task. The mice were trained until a stable baseline performance had been attained. Their performance over four days (Tuesday – Friday) was compared first by day (no effect) then by genotype. Performance was measured by span length (a), spans completed (b), errors per session (c), and mean span latency (d). Tg mice exhibited a reduced span length and a significant increase in errors compared to the WT mice. There was no difference in spans completed or mean span latency. Neither was there a difference in spontaneous locomotor count (e). Data presented as mean + s.e.m. (* denotes $p < 0.05$ when compared to WT controls).

4.3.2 Human caspase-3 over-expressing mice exhibit impaired acquisition and longitudinal performance of the OST

A second group of human caspase-3 over-expressing mice (Tg, n = 5) and their age-matched littermates (WT, n = 5) mice were used to assess longitudinal task performance. Mice were trained at 4 mth and performance assessed at 6, 12, and 18 months. The acquisition of the two groups is shown in Fig. 4.4a with the mean span length of each group increasing as the session numbers increased. However when the number of days to attain criteria (span length ≥ 5 for two consecutive sessions) by each mouse was grouped by genotype and compared, it was discovered that the Tg mice took significantly longer than WT mice ($F(1,9) = 7.827$, $p < 0.05$; Fig. 4.4b). In agreement with data obtained in the previous section, the span of odours attained by the Tg mice was significantly lower than that of the WT at 6 ($T=557.5$; $p < 0.05$, Fig. 4.5a), 12 ($T=562$ $p < 0.05$, Fig. 4.5b) and 18 mth ($T=307$; $p < 0.005$, Fig. 4.5c), with no age-related impairment in span length evident in either group (Fig. 4.5d). There were no significant differences in the performance of the two groups when required to discriminate between two odours at 6 ($T=450$; $p = 0.99$), 12 ($T=410$; $p = 0.989$) or 18 mth ($T=240$; $p = 0.768$). Despite the Tg mice appearing to complete significantly fewer spans than the WT mice at every age tested, there were no significant differences of genotype, at 6 ($T=410$; $p = 0.989$; Fig. 4.6a), 12 ($T=451$; $p = 0.27$; Fig. 4.6b) or 18 mth ($T=479.5$; $p = 0.06$; Fig. 4.6c). At 6 mth the increased number of errors per session of the Tg mice in comparison to the WT mice was not significant ($T=380.5$; $p = 0.103$; Fig. 4.7a). However by 12 mth there was a significant genotypic difference, with the Tg mice making more errors per session

than the WT mice (T=320; $p < 0.05$; Fig. 4.7b). This difference was still apparent at 18 mth, with the Tg mice producing significantly more errors than WT mice (T=177; $p < 0.05$; Fig. 4.7c). Analysis of mean time taken to complete a span (mean span latency) found no significant difference between the two genotypes at any age ($F(1,16) = 0.272$; $p = 0.616$; Fig. 4.8). In contrast, a significant effect of age was detected ($F(1,16) = 9.171$; $p < 0.005$; Fig. 4.8), with *post hoc* analysis revealing that mean span latency at 12 and 18 mth was significantly slower than at 6 mth ($p < 0.05$). Although time taken to engage in the task appeared longer when assessed at 12 and 18 mth when compared to performance at 6 mth, this apparent difference was not significant ($F(2,16) = 0.513$, $p = 0.608$). Similarly no significant genotypic effects were found when time taken to engage in the task was compared ($F(1,16) = 0.509$, $p = 0.496$; Fig. 4.9). Nor was there any significant interactions between day and genotype in mean span latency ($F(2,16) = 0.826$, $p = 0.456$) or in the time taken to engage in the task ($F(2,16) = 0.513$, $p = 0.608$).

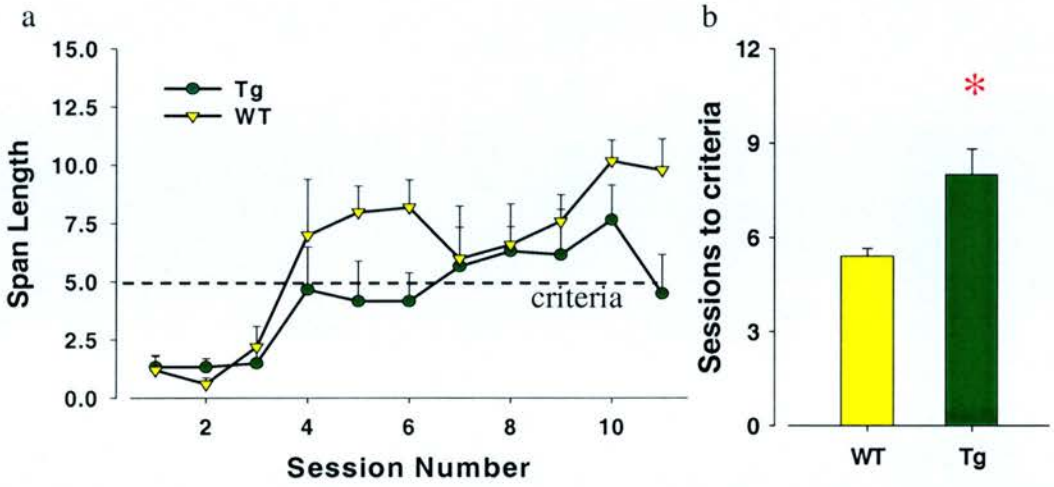


Figure 4.4: Impaired OST acquisition of human caspase-3 over-expressing mice (4 – 5.5 mth)

Acquisition of the OST by a group of caspase-3 transgenic mice (Tg, n =5) and their age-matched wild type littermates (WT, n = 5; mean age during acquisition = 4 – 5.5 mth). Acquisition of the task was set at a span length (number of correct spans prior to error) of 5 for two consecutive days. The acquisition curve of each group is shown in (a) as span length by session number. The Tg mice appeared to take longer to reach a span length of 5 on two consecutive days in comparison with the WT mice. This difference was statistically significant as with the Tg mice taking longer to attain criteria than the WT mice (b). Data presented as mean + s.e.m. (* denotes $p < 0.05$ when compared with WT mice).

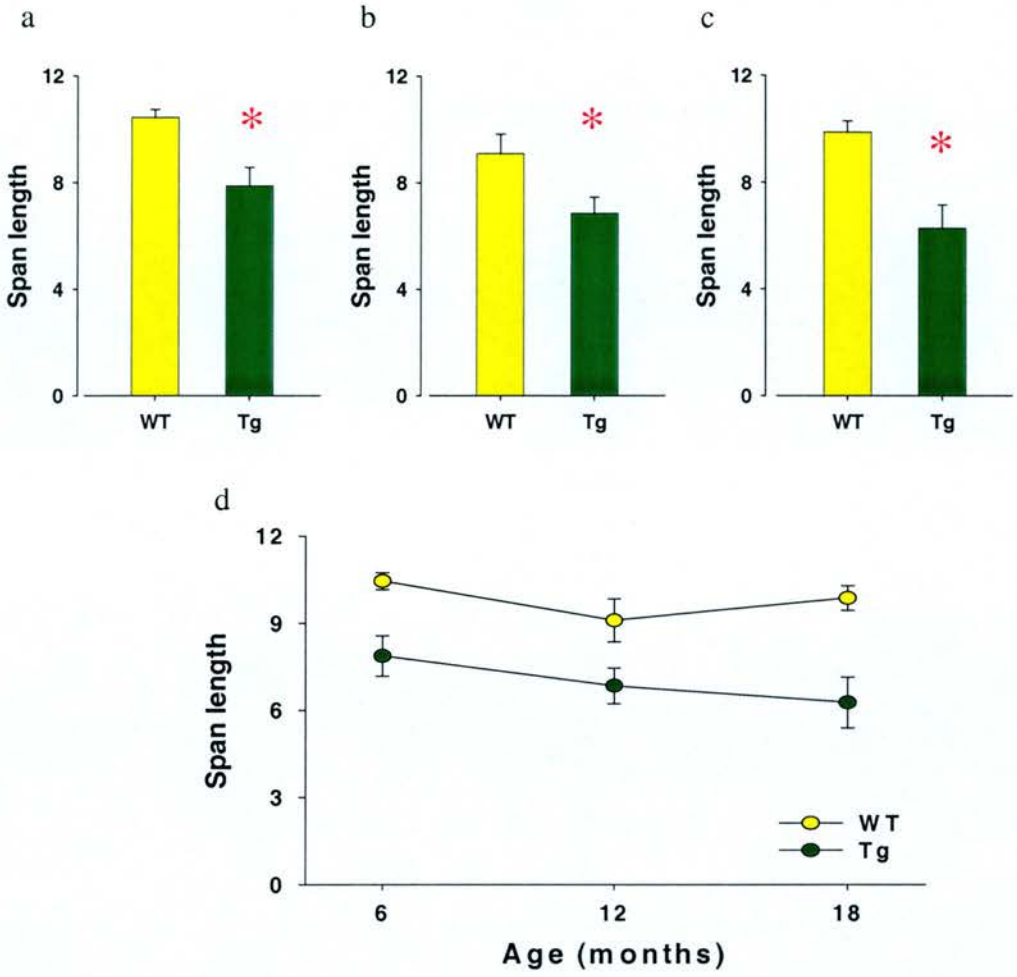


Figure 4.5: Span length measures from mice in study 2 in standard and extended odour span task.

Longitudinal performance (mean ages 6, 12, and 18 months) of a group of caspase-3 transgenic mice (Tg, n = 5) and their age-matched littermates (WT n = 5) in the odour span task as measured by span length. At each age, performance was measured over four consecutive days (Tuesday – Friday). Span length was measured at 6 (a), 12 (b) and at 18 months (c). Performance in the standard task at each age is presented together (d). The Tg mice exhibited a significantly reduced span length when compared with the WT mice at every age tested. Data presented as mean ± s.e.m. (* denotes $p < 0.05$ when compared to WT controls).

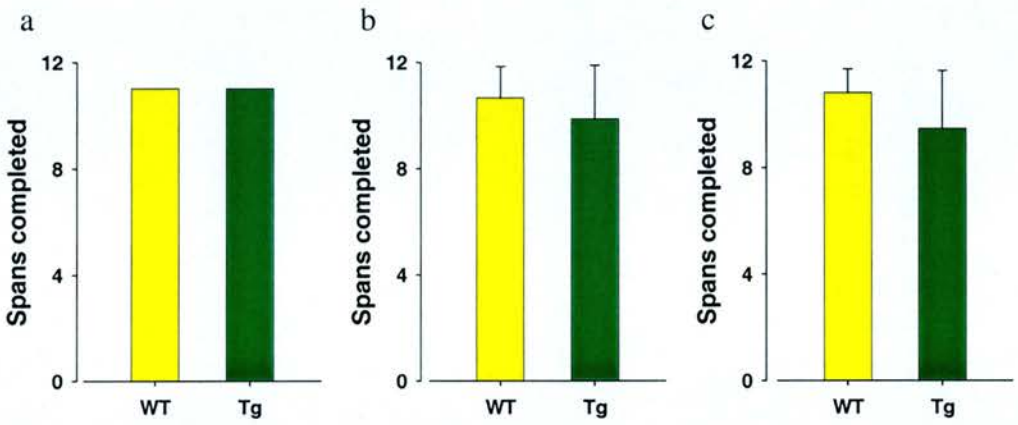


Figure 4.6: Spans completed data from mice in study 2 in the standard odour span task

Longitudinal performance of a group of caspase-3 transgenic mice (Tg, n = 5) and their age-matched wild type littermates (WT, n = 5) in the odour span task as measured by spans completed. Performance was measured over four consecutive days (Tuesday – Friday) at 6 (a), 12 (b), and 18 months (c). No effects of genotype were observed at any age tested. Data presented as mean + s.e.m..

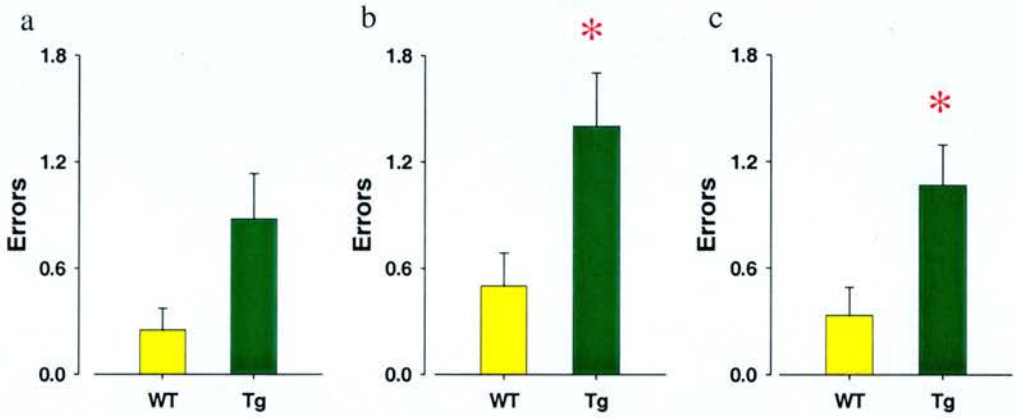


Figure 4.7: Errors per session data from mice in study 2 in standard odour span task

Longitudinal performance of a group of caspase-3 transgenic mice (Tg, n = 5) and their age-matched wild type littermates (WT, n = 5) in the odour span task as measured by errors made per session. Performance was measured over four consecutive days (Tuesday – Friday) at 6 (a), 12 (b) and 18 months (c). At 6 months of age there was no significant difference in errors made. However when reassessed at 12 and 18 months the Tg mice made significantly more errors per session than did the WT mice. Data presented as mean + s.e.m. (* denotes $p < 0.05$ when compared to WT controls).

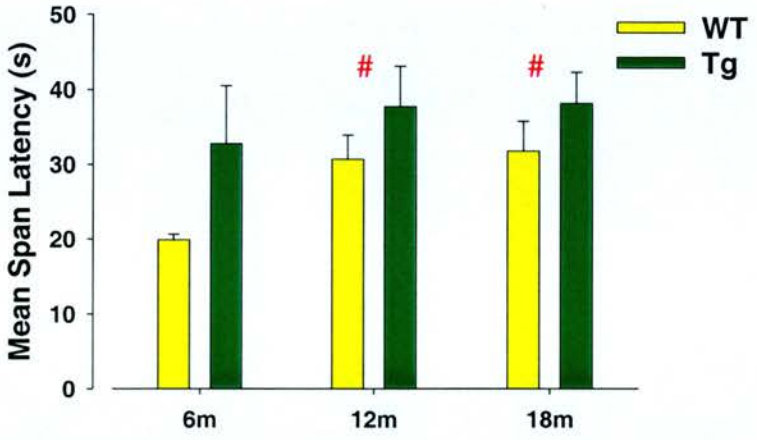


Figure 4.8: Mean correct latency from longitudinal odour span task study.

Longitudinal performance (mean ages 6, 12, and 18 months) of caspase-3 transgenic mice (Tg, n = 5) and their age-matched wild type littermates (WT, n = 5) in mean span latency. At each age tested, performance was measured over four consecutive days (Tuesday – Friday). While no effect of genotype was observed, there was a significant effect of age with latency at 12 and 18 months significantly slower than at 6 months. Data presented as mean + s.e.m. (# denotes $p < 0.05$ when compared to 6 months).

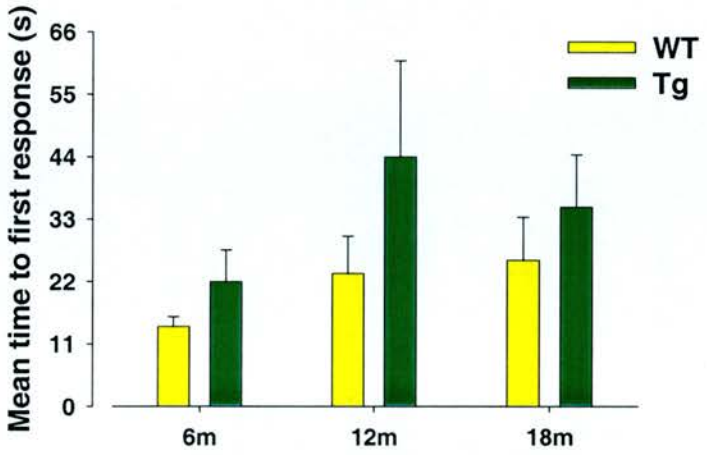


Figure 4.9: Time taken to engage in the task from longitudinal odour span task study. Longitudinal performance (mean ages 6, 12, and 18 months) of caspase-3 transgenic mice (Tg, n = 5) and their age-matched wild type littermates (WT, n = 5) in mean time taken to first response. At each age tested, performance was measured over four consecutive days (Tuesday – Friday). No effect of genotype or age was observed. Data presented as mean + s.e.m..

4.3.3 (-)Nicotine improves OST performance of human caspase-3 over-expressing mice

The previous studies identified a consistent OST deficit as measured by a reduced span length in mice over-expressing human caspase-3. A group of human caspase-3 over-expressing mice (Tg, n = 9) and their age-matched littermates (WT, n = 5) were trained to perform the OST. The performance of the two groups was compared over a four day period (Tuesday – Friday) once the two groups had attained a stable level of performance. As had previously been observed, the Tg mice exhibited a significantly lower span length than the WT mice (T=566, $p < 0.05$; Fig. 4.10a), with no significant differences in the number of spans the two groups completed (T=459, $p = 0.875$; Fig. 4.10b). There was no significant difference in the ability of the Tg mice when compared to the WT mice in their ability to discriminate between two odours (T=592, $p = 771$). The Tg mice again erred significantly more per session than the WT mice (T=4475, $p < 0.05$; Fig. 4.10c). As observed previously when the two groups were assessed on their performance speed, no significant differences were observed in mean span latency ($F(1,35) = 2.235$, $p = 0.153$; Fig. 4.10d). Interestingly the time taken by the Tg mice to engage in the task was significantly less than the WT mice ($F(1,35) = 18.039$, $p < 0.01$; Fig. 4.10e). This however appeared to reflect three mice in the WT group taking longer than the other WT mice. Once these mice engaged in the task, they performed similarly to the other WT mice. No reason for their increased time was discovered.

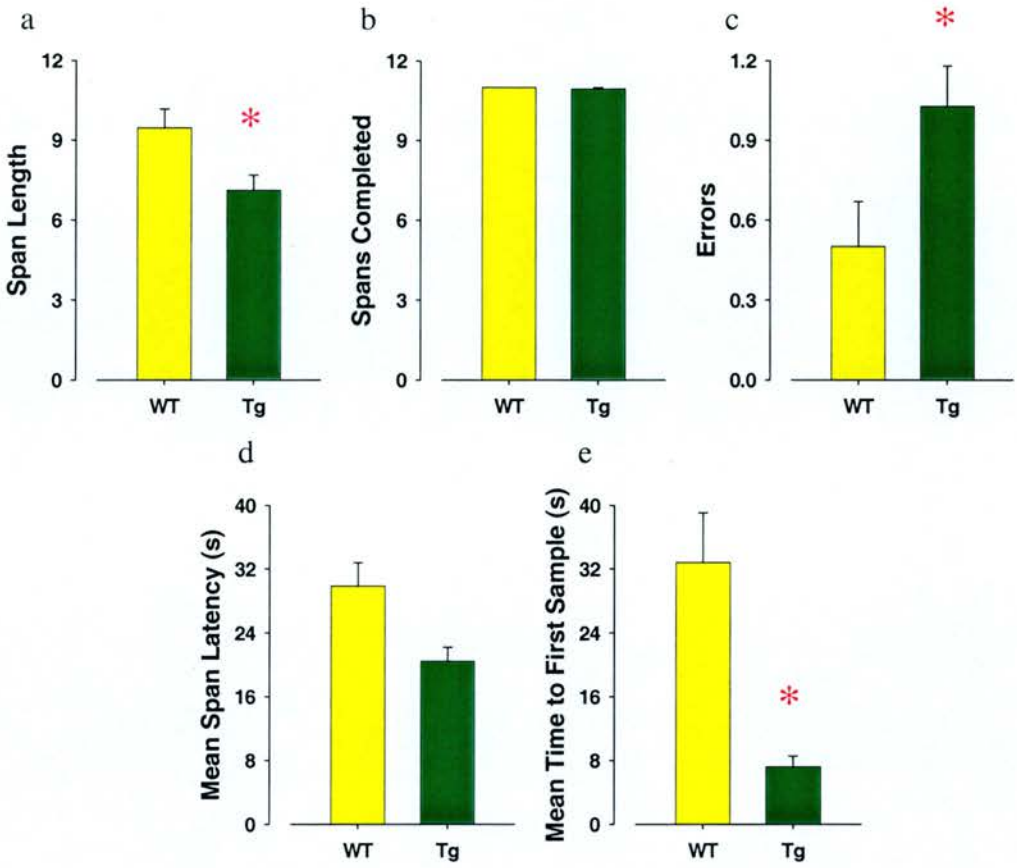


Figure 4.10: Human caspase-3 over-expressing mice exhibit a deficit in performance in the OST

A group of human caspase-3 over-expressing mice (Tg, n = 9) and their age-matched littermates (WT, n = 5) were trained to perform the OST. Once at asymptote the performance of the two groups was compared over four consecutive days (Tuesday – Friday). As observed previously, the Tg mice exhibited a significant impairment in the task as measured by span length (a). No effect of genotype was observed in spans completed (b). The Tg group did err significantly more often than the WT mice (c). No significant differences in mean span latency (d) were observed, but the Tg mice took significantly less time to engage in the task than the WT mice (e). Data presented as mean + s.e.m. (* denotes $p < 0.05$ when compared to WT mice).

The ability of nicotine to normalise the working memory performance of the Tg mice was then assessed. The following week every mouse was injected (s.c.) with saline for three days (Thursday, Friday, and Monday). The Tg mice were then split into 2 groups in a counter-balanced design. One group were administered saline (s.c.), as were the WT mice, whilst the other Tg group was administered (s.c.) (-)nicotine (3 μg/kg) on the following four days (Tuesday – Friday). The impaired performance of mice over-expressing human caspase-3 was again observed in this study. A significant difference of working memory capacity (span length) was observed between the three groups ($H = 15.9$; $p < 0.001$; Fig. 4.11a), and a multiple comparison procedure (Dunn's Method) was used to isolate the groups that differed. The Tg mice administered saline exhibited a significantly lower span length than the control (WT + saline) mice ($p < 0.05$). However, the Tg mice administered (-)nicotine (3 μg/kg) did not significantly differ from the control mice ($p > 0.05$). There were no differences in spans completed as all three groups completed all 11 spans within the time limit ($H = 0$, $p = 1.0$; Fig. 4.11b). The Tg mice administered saline made significantly more errors than both the WT mice and the Tg mice administered (-)nicotine ($H = 23.2$, $p < 0.001$; Fig. 4.11c). No significant differences were observed in mean span latency ($F(2,33) = 3.338$; $p = 0.074$; Fig. 4.11d) and despite both Tg groups apparently starting the task faster than the WT mice, there was no significant difference in time taken to first sample ($F(2,33) = 1.346$; $p = 0.3$; Fig. 4.11e). Nor were there significant interactions between day and drug for mean span latency ($F(2,16) = 1.594$, $p = 0.158$) or mean time to engage in the task ($F(3,35) = 0.844$, $p = 0.479$).

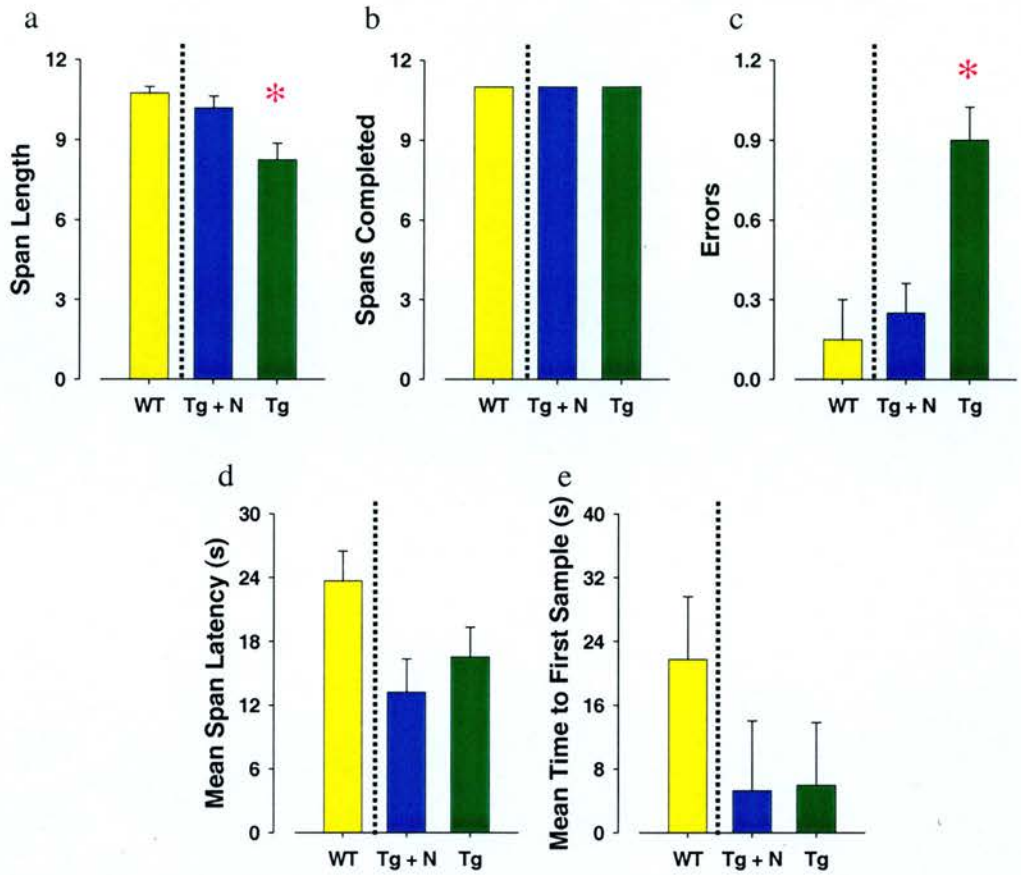


Figure 4.11: (-)Nicotine improves performance of human caspase-3 over-expressing mice in the OST

A group of human caspase-3 over-expressing mice (Tg, n = 9) and their age-matched littermates (WT, n = 5) were trained to perform the OST. Once at asymptote the mice were split into three groups, both the WT and one Tg group received saline, whilst the other Tg group received nicotine (3 μg/kg; Tg + N) in a counter-balanced design. Mice were administered saline (s.c.) for three consecutive days (Thursday, Friday and Monday), then their allocated dose for four consecutive days (Tuesday – Friday). As observed previously, the Tg mice exhibited a significant impairment in the task as measured by span length (a). However the Tg + N mice did not differ significantly from the WT mice. No effect of nicotine or genotype was observed in spans completed (b). The Tg group did err significantly more often than the WT mice, whereas the Tg + N mice did not (c). No significant differences in performance were observed in any other measure including mean span latency (d) or mean time taken to engage in the task (e). Data presented as mean + s.e.m. (* denotes $p < 0.05$ when compared to WT mice).

4.3.4 (-)Nicotine altered mouse performance in the OST

A group of C57Bl/6J mice (n = 12) were trained to perform the odour span task (OST). They advanced through training rapidly and very quickly attained criteria of a span length of 5 for two consecutive days. Training continued until a stable level of performance was reached and the mice were split into three groups in a counter-balanced design. All three groups were then administered saline (s.c.) for three days (Thursday, Friday and Monday). Despite relatively slow response times in comparison to other studies (see section 4.3.1, 4.3.2, 4.3.3), the majority of the mice completed a full session within the time limit, with their span length performance near perfect (8.7 ± 0.7) and spans completed almost maximal (10.0 ± 0.4). Taking cognisance of the ceiling effects observed during testing in the 5-CSR task, the difficulty of the task was increased by increasing the odour number from 12 to 22 for the following four testing days (Tuesday – Friday) during saline or (-)nicotine (3 or 300 μg/kg) administration. Analysis of span length over this period revealed no significant effect of drug dose on span length ($H=2.573$, $p = 0.276$; Fig. 4.12a), despite an apparent increased span length of mice given 3 μg/kg (-)nicotine when compared to saline. There was however a significant main effect of drug on spans completed ($H=12.634$, $p < 0.05$; Fig. 4.12b). Dunn's method for *post hoc* comparison identified that both the 3 and 300 μg/kg dose of nicotine increased the total number of spans completed in comparison to the saline group. There appeared to be a differential effect of the two doses of nicotine on the number of errors made, with the 3 μg/kg dose reducing, and the 300 μg/kg increasing the number of errors in comparison to the saline group. However this effect was not significant ($H=2.496$, p

= 0.287; Fig. 4.12c). The ability of the three groups to discriminate between two odours also did not differ (H=2, p = 0.368). The two groups (3 and 300 μg/kg) receiving (-)nicotine were faster per span than the saline group, although this drug effect was not significant (F(2,17) = 3.878, p = 0.082; Fig. 4.12d). The increase in speed of performance was also apparent with the 3 μg/kg group, but not the 300 μg/kg group, compared to saline, when the time taken to engage in the task was assessed, but again this was not significant (F(2,17) = 0.828, p = 0.481; Fig. 4.12e). Nor was there a significant interaction between the two factors in mean span latency (F(6,17) = 0.724, p = 0.636) or in time taken to engage in the task (F(6,17) = 0.605, p = 0.723).

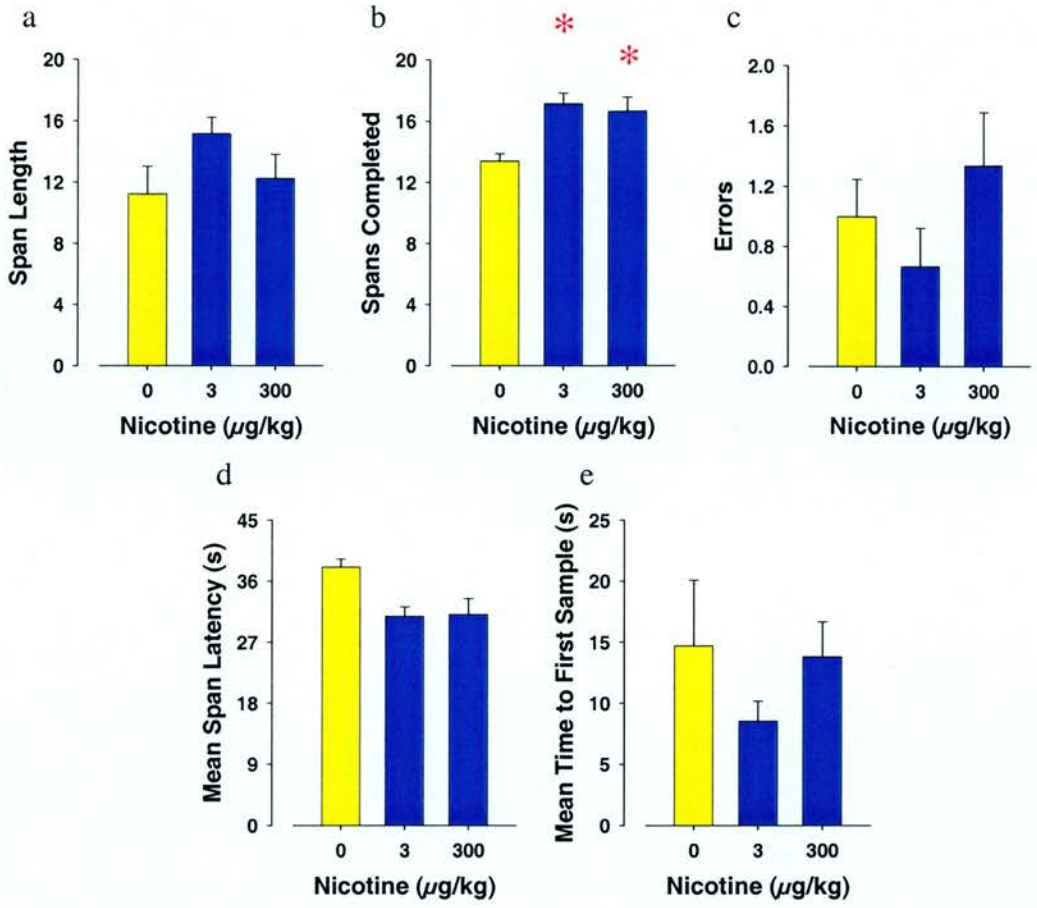


Figure 4.12: (-)Nicotine altered the performance of C7Bl/6J mice in the 22-OST

A group of C57Bl/6J mice (n = 12) were trained in the OST until a stable level of performance was attained. The mice were split into three groups, saline and (-)nicotine (3 and 300 μg/kg) in a counter-balanced design. Saline was administered three days (Thursday, Friday and Monday) prior to testing, after which the allocated dose was administered for four consecutive days (Tuesday – Friday). Neither dose of nicotine had any effect on span length (a). However, both the 3 and 300 μg/kg dose of (-)nicotine increased the number of spans completed. (b). No effect of either dose was observed in any other measure including mean number of errors per session (c), mean span latency (d) or mean time to first sample (e). Data presented as mean + s.e.m. (* denotes $p < 0.05$ when compared to saline group).

4.3.5 α7 nAChR knockout mice exhibit poor working memory

A group of α7 nAChR knockout (KO) mice and their age-matched littermates (WT) were trained to perform the OST (mean age = 17 mth). Their acquisition (number of days to criteria) and asymptotic performance (assessed over four consecutive days; Tuesday – Friday) in the task was compared. α7 nAChR KO mice exhibited a lower span length across session days of training when compared to the WT mice (Fig. 4.13a). The number of days the individual mice took to reach criteria (span length ≥ 5 for two consecutive days) was grouped according to genotype and compared and the number of days taken by the α7 nAChR KO mice was significantly longer than the WT mice ($t = 2.571$, $p < 0.05$; Fig. 4.13b). During asymptotic performance (19 mth) the α7 nAChR KO mice exhibited a significantly lower span length ($T=540.5$, $p < 0.001$; Fig. 4.14a) and number of spans completed ($T=556.5$, $p < 0.001$; Fig. 4.14b) than the WT mice. The number of errors made did not differ significantly between the two groups ($T=432.5$, $p = 0.55$; Fig. 4.14c), nor did their ability to discriminate between two odours ($T=410$, $P = 0.989$). The α7 nAChR KO mice exhibited a significantly longer mean span latency ($F(1,24) = 5.989$; $p < 0.05$, Fig. 4.14d), and whilst this difference in latency was apparently reflected in the time they took to engage in the task, this was not significant ($F(1,24) = 4.105$; $p = 0.077$; Fig. 4.14e). There was also no significant interactions between the two factors as measured by mean span latency ($F(3,24) = 2.935$, $p = 0.054$) or time taken to engage in the task ($F(3,24) = 1.163$, $p = 0.344$).

Hence the α7 nAChR KO mice appeared simply to be taking longer to complete the task than the WT mice. They may have been less motivated to perform the task than the WT mice, although they did not exhibit differences in motivation (as measured by mean reward latency) whilst performing the 5-CSR task (data not shown). Also, when assessed on their latency to retrieve 10 pellets in a simpler environment (in a clear cage on the table; training days 3 - 6; see chapter 2.3.3, pp. 62), the time taken by the α7 nAChR KO mice to retrieve all 10 pellets did not differ from the WT mice ($T=19$, $p = 0.095$; Fig 4.14f). Whilst it would have been beneficial to assess nicotine's effects on performance of the α7 nAChR KO and WT mice, it was deemed that the group sizes were too small and as such the study would have been statistically underpowered. The mice were therefore killed as a part of the histopathological study (section 3.3.9, pp. 103).

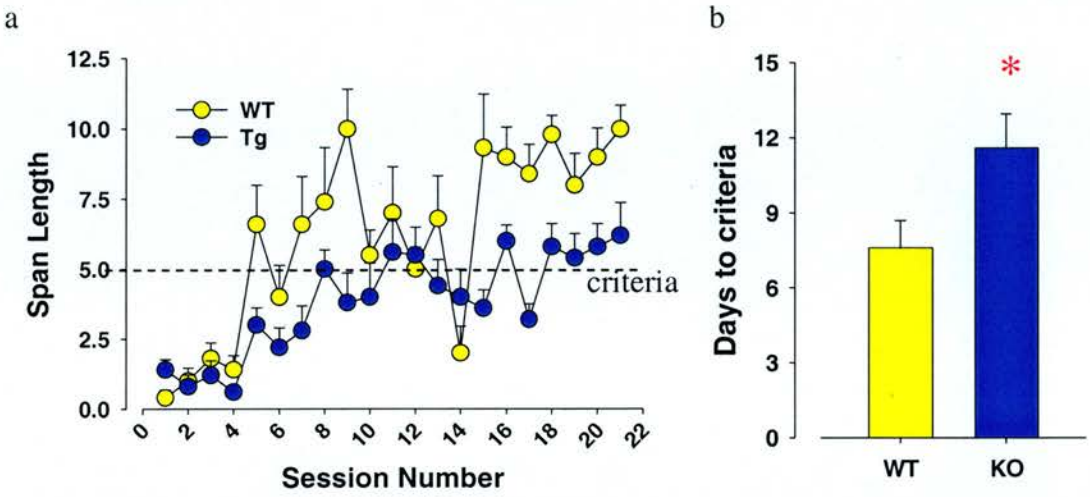


Figure 4.13: α7 nAChR knockout mice exhibit impaired acquisition of the OST

A group of α7 nAChR knockout (KO, n = 5) and age-matched littermate (WT, n = 5) mice were trained to perform the OST. Shown in (a) is the mean span length of each group across the session days they were trained. As can be seen by the graph the KO mice appeared to take longer to learn the task. The number of days taken by the KO mice to attain criteria (span length $\geq$ 5 for 2 consecutive days; dotted line) was significantly more than the WT mice (b). Data presented as mean +sem (* denotes $p < 0.05$ in days taken to attain criteria when compared to WT mice).

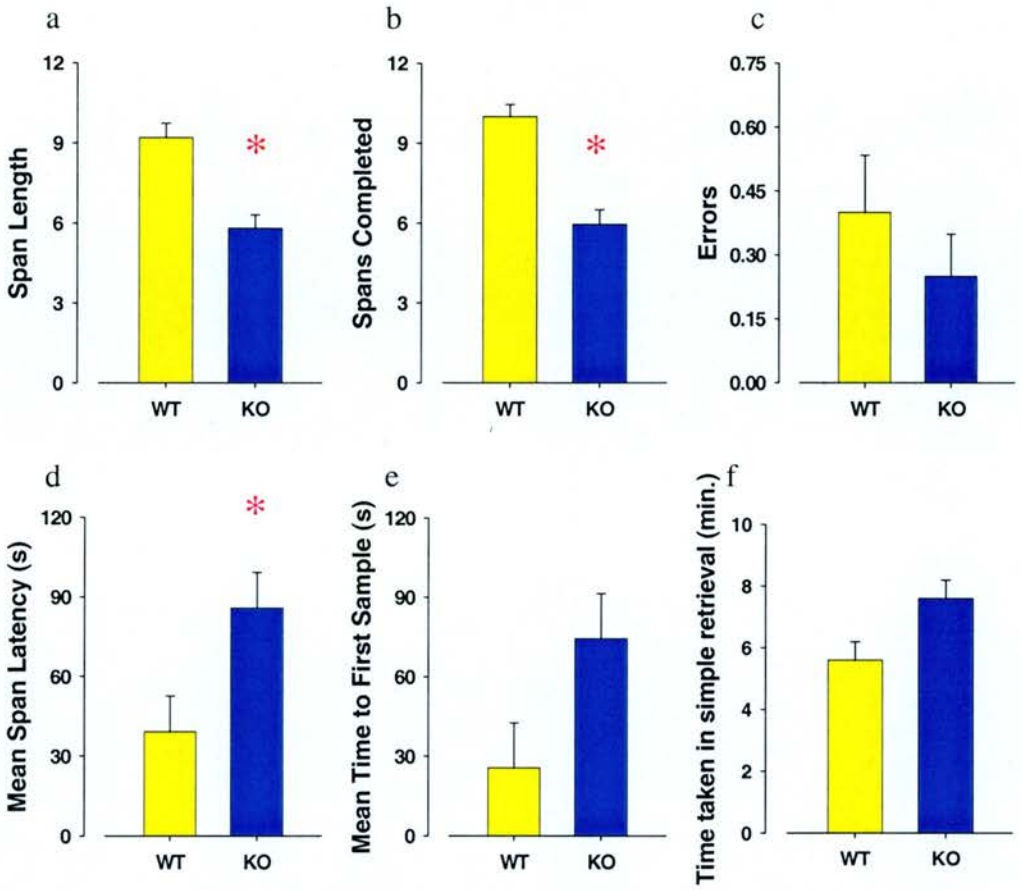


Figure 4.14: α7 nAChR KO mice exhibit impaired working memory

Once the α7 nAChR knockout (KO, n = 5) mice and their age-matched littermates (WT, n = 5) had attained asymptote, their performance over four consecutive days (Tuesday to Friday) was compared. The KO mice exhibited a significantly reduced span length when compared with the WT mice (a) and they also completed significantly fewer spans in total (b). However the KO mice did not make significantly more errors per session than the WT mice (c). There was a significant increase in mean span latency in the KO mice when compared to the WT mice (d). The time taken by the KO mice to engage in the task to obtain the first pellet reward did not differ from the WT mice (e), nor did the length of time they took to retrieve ten pellets in a simplified environment (f). Data presented as mean + s.e.m. (* denotes $p < 0.05$ when compared to WT mice).

4.4 Discussion

Mice can acquire this adapted odour span task (OST). Furthermore the data show that the task can be used to assess both pharmacological and genetic manipulation. Moreover the results also demonstrate the synergy between working memory and attentional performance in mice, and provide additional evidence that an awareness of the attentional load employed in a task is essential.

The olfactory working memory span observed for mice was comparable to that of rats (Dudchenko *et al.*, 2000). In the original publication, the possibility that the rats could smell the reinforcement through the digging medium (sand) was discounted when tested in a non-baited probe session. Hence the use of sand appeared to be successful in masking the rewards' scent. Initial attempts to train the mice to dig in sand were unsuccessful. However, although not systematically examined, similar observations in non-baited probes for mice suggested the use of woodchip (with crushed pellet rewards) was a viable alternative to sand as a digging medium for mice, sufficiently masking the smell of the reinforcement. Also randomly removing every third bowl on the table ensured that the mice could not scent mark the bowls they had already visited. The excellent level of performance of rodents (mice and rats; Dudchenko *et al.*, 2000) in this task was expected as rodents exhibit preferential attendance to olfactory, as opposed to visual cues (Jennings and Keefer, 1969). Also, previous research demonstrates that rats trained to olfactory stimuli acquire a learning set as rapidly as primates in response to visual stimuli (Slotnick and Katz, 1974; Lu *et al.*, 1993).

4.4.1 Nicotine and working memory in human caspase-3 over-expressing mice

Whilst it is evident that mice can readily acquire the odour span task, the over-expression of human caspase-3 appears to be detrimental to performance in this task. In the initial study (section 4.3.1, pp. 132) the littermate mice exhibited a span length (number of correct spans prior to an error) of ~ 10, whilst the human caspsase-3 over-expressing mice could only reach ~ 7 (Fig. 4.3a, pp. 126). This was not simply attributable to the human caspsase-3 over-expressing mice completing significantly fewer spans than their littermates due to time constraints, as there were no differences in the spans completed (~ 11 vs. ~ 10 respectively; Fig. 4.3b, pp. 126). Such a genotypic difference in span length could therefore only be accounted for by the human caspsase-3 over-expressing mice erring earlier in a session than their littermates. In accordance, the human caspsase-3 over-expressing mice also erred significantly more often (Fig. 4.3c, pp. 126). The difference observed in mean span latency (Fig. 4.3d, pp. 126) was not significant, thus combined with the lack of locomotor count differences between the two groups (Fig. 4.3e, pp. 126) suggest that any differences observed between the two groups are not due to motoric confounds. Finally, despite the preferential expression of caspase-3 in the olfactory system (Cowan *et al.*, 2001) and its importance for cell turnover which has been shown to be important for odour discrimination (Gheusi et al, 2000), the over-expressing mice could discriminate between two odours as readily as their age-matched littermates, suggesting that the results were not due to simple olfactory discriminatory differences.

The results from section 4.3.2 (pp. 134) support the findings that human caspase-3 over-expressing mice can discriminate between two odours as readily as their littermates. They also provide support that the performance deficit observed in the human caspase-3 over-expressing mice is reproducible and apparently age-unrelated (Fig. 4.5, pp. 130). Whilst the biology of this impairment is still being established, this deficit can be attenuated by nicotine administration at doses that enhance sustained attention (3 μg/kg, chapter 3.3.3, pp. 86). The human caspase-3 over-expressing mice given nicotine exhibited fewer errors and an improved span length equivalent to that of their age-matched littermates administered saline (Fig. 4.11, pp. 138). This clearly shows that nicotine can improve working memory deficit observed in human capsease-3 over-expressing mice who have no known cholinergic dysfunction, therefore providing a new tool for assessment of putative cognitive enhancers. Nicotine-induced improvements in working memory have been observed in a variety of species including mice (Maviel and Durkin, 2003), rats (Levin *et al.*, 1993), monkeys (Buccafusco *et al.*, 2003) and man (Phillips and Fox, 1998). Observing consistent improvements in humans has proven arduous. Whilst beneficial effects on working memory have been observed in some studies (Newhouse *et al.*, 1988; Sahakian *et al.*, 1989; Levin *et al.*, 1996; Philips and Fox, 1998; Smith *et al.*, 2002) others report no effect, (Heishman and Henningfield, 2000; Ernst *et al.*, 2001; Min *et al.*, 2001), or performance deficits (Park *et al.*, 2000). Procedural differences have been cited as possible confounding effects when comparing studies (Phillips and Fox, 1998; Park *et al.*, 2000), with impairments generally reflecting the degree of proactive interference in the task (Dunnett and Martel, 1990; Levin *et al.*, 1997; Park *et al.*, 2000) and improvements only occurring

if the task had a sufficiently high degree of attentional load placed upon the subjects (Phillips and Fox, 1998). Therefore there is considerable evidence that supports the hypothesis that the main cognitive effect of nicotine is to enhance attention (Rusted and Eaton-Williams, 1991; Mancuso *et al.*, 1999; Newhouse *et al.*, 2004; Fig. 4.1, pp. 118). Levin and colleagues that the main cognitive effect of nicotine in humans is that of enhanced attention in spite of their excellent reports on the effects of nicotine on rat working memory performance in the RAM (see reviews Levin and Simon, 1998; Rezvani and Levin, 2001; Levin 2002). However the high attentional load employed in the RAM may provide evidence that suggests that nicotine-induced improvement in attention in rats. The OST places a high attentional component on the subjects performing the task. Thus the improvement seen with nicotine in the human caspase-3 over-expressing mice may reflect an enhanced ability of these mice to attend to the task, for the duration of the session. Interestingly human caspase-3 over-expressing mice did not exhibit impaired sustained attention in the 5-CSR task (Kerr *et al.*, 2004). However, they were not subject to any task challenges (Dr. C. Spratt *personal communication*). Therefore their apparent lack of impaired performance in the 5-CSR task may have been a consequence of ceiling effects (see chapter 3.3.1, pp. 81). Hence, an attentional component cannot yet be ruled out in the impaired performance of mice over-expressing human caspase-3 in the OST and in the improvements observed with nicotine. Human caspase-3 over-expressing mice in the OST could therefore be used to assess nAChR selective compounds, as and when they become available, to identify the nAChR(s) underlying the beneficial effects observed.

4.4.2 Nicotinic modulation of working memory

In cognisance of the ceiling effects of the C57Bl/6J mice discussed and observed previously (Vorhees, 1991; chapter 3.3.1 to 3.3.4, pp. 81 to 88) the difficulty of the task was increased during testing. Nicotine (3 and 300 μg/kg) significantly enhanced the number of spans completed when compared to the control mice, suggesting the low spans completed was not as a result of using the same time limit as that in the 12-OST. Nor was it due to an increased number of errors, as those observed (~ 1) were comparable to other studies (~ 1, chapter 5.3.3, pp. 184). The increased number of spans completed could have been as a result of the trend for nicotine at both doses to reduce mean span latency (p = 0.082). Whilst hyperlocomotion has been observed at higher doses of nicotine (125 and 250 μg/kg, Nordberg and Bergh, 1985) no effect has been observed other than that of enhanced sustained attention (see chapter 3.3.3, pp. 86). The 300 μg/kg dose of nicotine may have increased speed, facilitating an increase in spans completed. The 3 μg/kg dose appears to have exerted a different effect as there was a trend for increased span length, reduced errors, and a faster time to engage in the task. This could reflect enhanced attentiveness to the task and thus the more rapid progression through the task when compared to mice administered saline. If so it would provide further evidence that nicotine might enhance cognitive performance by enhancing attentive processing (Mancuso *et al.*, 1999; Newhouse *et al.*, 2004; Fig. 4.1, pp. 118).

The performance of α7 nAChR KO mice in the OST appear to provide further support for this hypothesis, as the pattern of deficit does not appear to simply reflect

an impairment in working memory capacity. There appeared to be a general slowing of response, resulting in a working memory span deficiency, possibly as a result of task cessation due to the time limit imposed upon the subjects. Their impairment in sustained attention (chapters 3.3.6 and 3.3.7, pp. 92 and 94) could be responsible for the increased mean span latency and thus reduced span length and total spans completed exhibited. Overall the α7 nAChR KO mice appeared as active as their littermates, but simply did not select a bowl to dig in as readily as the WT mice (*personal observations*). Verification of the foraging paths taken by the α7 nAChR KO mice would possibly corroborate such an hypothesis. Etho-Vision equipment that monitors the paths taken by rodents performing the Morris Water Maze should perhaps be included in any future OST studies. Future studies could include a longitudinal assessment of OST performance in these mice, corroborating the deficit observed here. Ideally larger group sizes would be preferred, for nicotine could be administered to half of the α7 nAChR KO group to assess whether it could improve performance in mice lacking the α7 nAChR. This was not possible in the present study due to low group sizes and mice being required for histopathological analysis. α7 nAChR transgenic mice exhibit low fertility rates (Orr-Urtreger *et al.*, 1997) and this combined with the detrimental effects of building work severely affected the breeding program, curtailing the possibility of retraining a new group of α7 nAChR transgenic mice.

No weight differences between the KO and WT mice were observed (data not shown), nor were there any differences in their latencies to collect the reward in the 5-CSR task (data not shown), a putative index of motivation (Robbins, 2002).

Moreover, the time taken by the two groups to retrieve 10 pellets in a simplified version of the task (Fig. 4.14f, pp. 145) did not differ significantly, suggesting that in a simpler environment the α7 nAChR KO mice do not take longer to complete a similar task. However it would also be interesting to assess the motivation of these mice to collect a reward using a fixed ratio breakpoint study, which can be established in the 5-CSR task equipment. This would provide further information on whether the slowing of response and thus impaired performance observed was due to different motivational levels

Impairment in attention does however fit the model of nicotinic receptor stimulation proposed by Newhouse and colleagues (2004), whereby a baseline loss of stimulation may impair attention, filtering down to impaired working memory (Fig. 4.1, pp. 124). This would also be consistent with the nAChR loss observed in patients with Alzheimer's disease (see chapter 1.2.2, pp. 13) whom also exhibit a reduced working memory span when the attentional load in the task is increased (Bayles, 2003). The concomitant improvements observed following nicotine administration may therefore reflect enhancement of that attention (Phillips and Fox, 1998; Newhouse *et al.*, 2004).

Levin and colleagues have utilised the radial arm maze (RAM – a task with a high attentional load, see chapter 1.4.4.4, pp. 45) plus a variety of nAChR agonist/antagonist and lesion studies in an attempt to delineate the nAChR that mediates nicotine-induced improvement in cognition (Levin *et al.*, 1993; Kim and Levin, 1996; Felix and Levin, 1997; Levin *et al.*, 1999; Bancroft and Levin, 2000;

Bettany and Levin, 2001; Levin *et al.*, 2002, Addy *et al.*, 2003). Ceiling effects complicating rat performance in the RAM requiring baseline performance to be artificially impaired (Levin, 2002). Levin's group have shown that blockade of both the α7 and α4β2 nAChR (MLA and DHβE, respectively) produces working memory impairment in the RAM (Felix and Levin, 1997; Bancroft and Levin, 2000; Bettany and Levin, 2001). Furthermore, in the presence of MLA, nicotine does not improve working memory, but in the presence of DHβE, nicotine did improve working memory in the RAM (Bancroft and Levin, 2000; Bettany and Levin, 2001). Thus suggesting that it is the α7 nAChR that mediates the nicotine-induced performance observed in this task. However, as mentioned previously (see chapter 3.1, pp. 76), the administration of cholinergic drugs may be confounded by the lack of receptor subtype selectivity, as MLA also inhibits the $5HT_3$ and $P2X_3$ receptors (Papke *et al.*, 2004; Lalo *et al.*, 2004).

Agonist studies also provide support for the α7 nAChR in nicotine-induced improvements in working memory. Kitagawa and colleagues (2003) administered 3-(2,3-dimethoxybenzylidine)anabaseine (DMXBA or GTS-21), an α7 nAChR partial agonist (also an α4β2 nAChR antagonist), to healthy human male volunteers and found it improved working memory, attention and episodic memory. Previously, Arendash and colleagues (1995a) found that whilst DMXBA did not improve working memory in aged (22 months) rats in a 17-arm RAM due to ceiling effects (~90% correct), it did improve learning and reference memory. These results were similar to the effects observed with chronic nicotine administration (Arendash *et al.*, 1995a,b). Administration of AR-R17779, a full α7 nAChR agonist (Mullen *et al.*,

1995), was shown to improve learning in the 8-arm RAM (Levin *et al.*, 1999). Similarly to nicotine in the 8-arm RAM, AR-R17779 administration also reversed the working memory deficit induced by fimbra-fornix lesion (Levin *et* al., 1993; Levin *et al.*, 1999) and enhanced long-term social recognition memory (van Kampen *et al.*, 2004).

4.5 Conclusion

In conclusion, the OST, a test of non-spatial olfactory working memory capacity was established in mice and can therefore be used to assess both pharmacological and genetic manipulations in mice. Nicotine administration improved working memory performance in human caspase-3 over-expressing mice, who exhibit a deficit in this task, and in C57Bl6/J mice. The dose (3 μg/kg) used to improve working memory performance was identical to a dose that enhanced sustained attention (chapter 3.3.3, pp. 86). The impaired performance working memory performance of α7 nAChR KO mice in the OST, may indicate poor sustained attention during the task as opposed to simply a reduced olfactory working memory capacity. The findings therefore provide support for the hypothesis that the effects of nAChR manipulation on working memory may be mediated primarily through their manipulation of attention. Thus these studies again provide data in support of the hypothesis, that the α7 nAChR may have a role in producing the beneficial effects of nicotine.

Chapter 5 – Behavioural Phenotyping Tg2576 model of Alzheimer's disease

5.1 Introduction

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder that manifests as a premature cognitive decline in the elderly (Cummings, 2004). Amyloid-β (Aβ) plaque deposition, neurofibrillary tangles (NFTs) and cholinergic degeneration are the central hallmarks of this disease. Whether the cognitive decline observed is a result of cholinergic dysfunction, NFTs, Aβ deposition, or a combination of any three is still the subject of much debate. There is now some evidence to suggest that impaired processing of the amyloid precursor protein (APP) may underlie Aβ plaque deposition, cholinergic dysfunction and NFTs, resulting in cognitive decline (Hardy and Selkoe, 2002; Cummings, 2004; Selkoe, 2004; chapter 1.2.2 and Fig. 1.1, pp. 16). Support for the 'amyloid hypothesis' of AD stems in some part from genetic analysis of patients with familial AD (which accounts for 5 – 10% of all cases of AD; Tanzi, 1999). Mutations in APP, preselinin and apolipoprotein E (ApoE) genes have been discovered and are all thought to contribute to Aβ plaque (Goate *et al.*, 1991; Mullan *et al.*, 1992; Van Broeckhoven *et al.*, 1992; Levy-Lahad *et al.*, 1995; Sherrington *et al.*, 1995; Selkoe and Hardy, 2002; Rocchi *et al.*, 2003; Götz *et al.*, 2004; Selkoe, 2004; see chapter 1.2.2, table 1.1, pp. 19).

The identification of genetic mutations that produce familial AD has led to the creation of several transgenic models of the disease, in a variety of species. These include the fruit fly *Drosophila melanogaster* (Shulman *et al.*, 2003), the nematode *Caenorhabditis elegans* (Anderton, 1999) and mice *Mus musculus* (table 5.1). Whilst creating transgenic models in mice is the most challenging, they do however offer the greatest construct and face validity (Götz *et al.*, 2004). A large variety of different AD transgenic mouse models have been created, with each varying in the degree to which they resemble the cause of the disease (construct validity) and the appearance of the disease (face validity). The mouse lines generated have been based upon both the mutations observed in familial AD, such as the London and Swedish mutations (Goate *et al.*, 1991; Mullan *et al.*, 1992), but also on common risk factors observed in idiopathic AD such as the ApoE4 gene, and on mutations in tau processing (see table 5.1).

Generic Name	Mutation	References	Pathological and behavioural manifestations
NSE:β-APP751	APP751 isoform	Quon *et al.*, (1991); Moran *et al.*, (1995)	Express Aβ; age related impairments in Y-maze spontaneous alternation and learning in the MWM.
PDAPP	V717F: APP695 751 and 770 isoforms	Games *et al.*, (1995); Chen *et al.*, (2000)	Express Aβ @ 7 mth; presence of age related and unrelated impairments in spatial memory and learning as measured by MWM; no neuronal loss.
Tg2576	Swedish: APP695 isoform	Hsiao *et al.*, (1996); Chapman *et al.*, 1999) King *et al.*, (1999)	Express Aβ @ 6-8 mth; presence of age related and unrelated impairments in spatial memory; no neuronal loss; Impaired LTP in CA1 and dentate gyrus regions of hippocampus.
APP23	Swedish APP751	Sturchler-Pierat *et al.*, (1997); Boincrisstiano *et al.*, (2002)	Express Aβ @ 6 mth; plaques immunoreactive for hyperphosphorylated tau, reminiscent of an early tau pathology; exhibit cell loss in CA1 region of the hippocampus.
TgCRND8	Swedish + V717F	Janus *et al.*, (2000); Chishti *et al.*, (2001)	Impaired spatial reference learning and memory at 3 mth.
R406W	Tau mutant	Tatebayashi *et al.*, (2002)	Development of forebrain NFTs by 18 mth with poor associative learning by 16 – 23 m; little cognitive testing performed; do not develop Aβ deposition.
P301S	Tau mutant	Allen *et al.*, (2002)	Develop NFTs in the spinal cord by 5-6 mth, and exhibit severe paraparesis, thus no cognitive testing performed; do not develop Aβ deposition.
P301L	Tau mutant	Lewis *et al.*, (2000); Arendash *et al.*, (2004)	Develop widespread NFTs (spinal cord, brainstem & forebrain) by 6.5 mth leading to progressive motoric impairments and death, thus no cognitive testing performed; do not develop Aβ deposition.
V337M	Tau mutant	Tanemura *et al.*, (2002)	Development of NFTs within the hippocampus by 10 months; no Aβ deposition or cognitive impairment.
TAPP	Tg2756 X P301L cross	Lewis *et al.*, (2001)	Expresses Aβ and NFT; Aβ similarly to Tg2576; NFT intensified in comparison to P301L; exhibit retinal degeneration severely limiting possibilities for cognitive assessment.

Table 5.1: Common transgenic mouse models of Alzheimer's disease

It was only a little over ten years ago that Quon and colleagues (1991), developed the first APP transgenic mice (NSE:β-APP751) that exhibited Aβ deposition. In 1995, Games and colleagues successfully expressed high levels of the V717F mutant form of APP, under control of the platelet derived growth factor (PDGF) mini-promoter. These 'PDAPP' mice exhibited a number of the characteristics of AD including Aβ deposition in the hippocampus that spread into cortical areas. Impairment in spatial learning in the MWM appeared to be age-independent, occurring in both young (pre-Aβ deposition) and older (post-Aβ deposition) mice (Games *et al.*, 1995). Subsequent modifications of the task that increased subtlety, identified an age-related impairment (Chen *et al.*, 2000). Hsiao *et al.*, (1996) developed mice that expressed the Swedish mutation (Mullan *et al.*, 1992) by inserting it into a hamster prion protein cosmid vector. The resulting Tg2576 line exhibited Aβ plaque deposition, with age-dependent impairments in spatial learning and memory, assessed in the Y-maze and the MWM which corresponded to a 14-fold increase in Aβ plaque deposition (Hsiao *et al.*, 1996). However, no tau pathology or neuronal loss is evident in either PDAPP or Tg2576 mice. In 1997 Sturchler-Pierat and colleagues, created APP23 mice that had a seven-fold increase in APP expression with plaques appearing at 6 months of age. These plaques were immunoreactive for hyperphosphorylated tau, reminiscent of an early tau pathology (Boincrisstiano *et al.*, 2002). Unlike the Tg2576 and PDAPP lines, the APP23 mice exhibit neuronal loss, with a 14% reduction in CA1 pyramidal neurons (Dickson, 2004). Schmitz and colleagues (2004) developed an APP/PS1 double-transgenic mouse line (Swedish and London mutations crossed with PS1 transgenics), which have Aβ deposition and neuronal loss, although they have yet to be behaviourally phenotyped. Other

transgenic mice with altered APP processing also include such as the TGCRND8 and J20 mice (Janus *et al.*, 2000; Mucke *et al.*, 2000). Initial attempts to produce preselinin 1 (PS1) knockout mice resulted in lethality that could be rescued by the expression of the mutated PS1 A246E (Shen *et al.*, 1997). Preselinin 2 (PS2) KO mice were viable however there are minor complications such as mild pulmonary fibrosis (Herreman *et al.*, 1999). Although combined mutations of APP and preselinin genes have not yet been identified in any Alzheimer's disease patients, APP and PS1 bigenic mice have been created by crossing Tg2576 mice with the PS1 mutant A264E, resulting in elevated Aβ deposition levels and a more rapid deposition rate (Borchelt *et al.*, 1996; 1997). Expression of mutant APP on an ApoE4 KO background did not effect the age of Aβ deposition onset, but did reduce the number of diffuse Aβ plaques (Bales *et al.*, 1997). APP V717F mutant mice on a null ApoE background developed Aβ plaque deposits by 9 months, but at lower levels (Holtzman *et al.*, 1999). On a human ApoE3 and ApoE4 background, these mice did not deposit Aβ until 15 months, with a 10 fold greater deposition level was observed in the ApoE4 mice (Holtzman *et al.*, 1999).

When ApoE4 was expressed in neurons of transgenic mice they exhibited tau hyperphosphorylation but unfortunately had motor problems, muscle loss and premature death (Tesseur *et al.*, 2000). Tau transgenics that express NFTs have also been created such as the P301L, P301S and R406W mutants and exhibit varying degrees of cognitive and motoric impairment but no Aβ plaque deposition (Lewis *et al.*, 2000; Allen *et al.*, 2002; Tatebayashi *et al.*, 2002). Crossing the Tg2576 APP mutant mice with the P301L tau mutant mice created a line (TAPP mice) that

exhibited both Aβ plaque deposition and neurofibrillary tangles (Lewis *et al.*, 2001). However, these mice show reduce viability and exhibit both retinal and motoric degeneration making cognitive assessment extremely complex (Lewis *et al.*, 2001; Arendash *et al.*, 2004).

It is clear that all these mice have different limitations, however the transgenic line used will be governed by the research goals. Any attempt to identify a vaccine that will modify the histopathological hallmarks of AD, requires the use of a mouse transgenic line that most closely mimic those hallmarks, such as the TAPP line (Lewis *et al.*, 2001). However, the physiological impairments suffered by these mice make them extremely difficult to behaviourally phenotype. Using the TAPP mice to model the cognitive aspects of AD in the search for a cognitive enhancing compound could prove fruitless. In contrast the Tg2576 transgenic mouse line developed by Hsiao and colleagues (1996), has been extensively behaviourally phenotyped and histopathologically assessed. However, cognitive testing has largely been within the domain of spatial learning and memory (Hsiao *et al.*, 1996; Chapman *et al.*, 1999; King *et al.*, 1999; Arendash *et al.*, 2001; King and Arendash, 2002). Whilst Alzheimer's disease patients do exhibit cognitive impairments within these domains (spatial learning and memory), the predominant and earliest impairments observed appear to be in either episodic memory (memory for events in life) or attentional function (Baddeley *et al.*, 1991; Becker *et al.*, 1992; Binetti *et al.*, 1996a,b; Reid *et al.*, 1996; Perry and Hodges, 2000; see chapter 1.3, pp. 20). Though not all components of attention are affected at the same stages in AD, it has been proposed that the early attentional and processing speed deficits in AD patients may contribute

to performance reductions in other cognitive domains such as memory and executive function (Simone *et al.*, 1997; Perry and Hodges, 1999; Perry *et al.*, 2000; Rizzo *et al.*, 2000). Lawrence and Sahakian (1995), suggest it may be a core feature of AD.

It has also been suggested that olfactory capabilities and memory impairments may represent an early marker for AD (Devanand *et al.*, 2000). This is supported by the findings that the earliest pathological hallmarks in AD occur in the entorhinal cortex, part of the main olfactory system (Fig. 4.2, pp. 122), and an area highly sensitive to Aβ deposition induced loss of nAChRs (Braak and Braak, 1995; Perry *et al.*, 1995; Mufson *et al.*, 1999). Aβ deposition is also high in the entorhinal cortex of patients with mild cognitive impairment (MCI), and, if accompanied by olfactory impairments, these patients have a high risk of being diagnosed with AD later in life (Mufson *et al.*, 1999; Devanand *et al.*, 2000). Likewise the highest region of Aβ deposition in the Tg2576 mouse model of AD is in the olfactory tract (Nordberg *et al.*, 2002).

However to my knowledge the Tg2576 mice have not been assessed in any of these cognitive and olfactory domains. Assessing episodic memory in rodents has proven problematic due to the difficulty in rodents relaying information regarding previous events. A recent behavioural paradigm developed locally by Professor Morris' group in the University of Edinburgh to assess episodic memory in rats shows some promise (Day *et al.*, 2003). However an analogous task for use in mice has not yet been developed. Assessing attentional and olfactory memory capabilities in mice is however possible (see chapters 3.3 and 4.3, pp. 81 and 125). Knowledge of the

cognitive abilities of the Tg2576 mice within these domains (attention, olfaction and memory) would prove advantageous when assessing the possible efficacy of a novel nicotinic-based cognitive enhancing compound to treat AD patients.

Therefore the Tg2576 mice (the creation of which is detailed in Appendix I, pp. 156) were trained and their performance assessed in sustained attention (5-CSR task) and olfactory working memory (OST). It was hypothesised that these mice would exhibit both age-related attentional and olfactory working memory impairments that may be related to Aβ deposition. More interestingly these models may detect a deficit in attention or olfaction prior to Aβ deposition, and allow us to examine the effect of drugs at an earlier stage (MCI) in AD progression. An assessment of their exploratory behaviour was also conducted using spontaneous alternation on the T-maze, as exploratory behaviour might decrease with increasing AD pathology as curiosity levels are reported to decrease in AD patients. Wang and colleagues (2001), postulated that the α7 nAChR may be pivotal to the deposition of Aβ. Therefore we established a breeding programme to generate mice over-expressing Aβ on an α7 nAChR null background, with an aim to biochemically and behaviourally phenotype these animals for comparison with both Tg2576 and α7 nAChR KO mice. Moreover the ability of nicotine to reduce Aβ accumulation in aged Tg2576 mice with a high level of Aβ deposition was assessed.

5.2 Methodology

5.2.1 Animal maintenance and genotyping

Mice over-expressing the Swedish amyloid precursor protein (APP) mutation, Tg(HuAPP695.K70N-M671L)2576 and their age-matched littermates were purchased from Taconic (New York, U.S.A.). The mice were quarantined and initially group housed according to vetinary guidelines, but due to high levels of aggression observed (Moechars *et al.*, 1996; 1998), they were subsequently individually housed. The mice were purchased for the following studies. Longitudinal assessment of performance in sustained attention, olfactory working memory and exploratory behaviour at 6, 12, and 18 mth in the 5-CSR task, 7, 13, and 19 mth in the OST, and at 6.5, 12.5 and 18.5 mth in the T-maze. At 6 and 12 mth, 2 mice from each group were to be removed for Aβ measurements (ELISA) and plaque deposition, with the remaining mice assessed at 19 mth.

Unfortunately due to a reduced survival rate in the Tg2576 (Tg) mice; consistent with previous observations (Lalonde *et al.*, 2003) and other unexpected difficulties (section 5.2.2, pp. 169), no mice were removed for assessment of Aβ levels and the ages of training and testing could not correspond with original plans. Hence a second group of mice were brought in for inclusion in the studies. Training and testing took place as follows: 5-CSR task at ~ 10 (section 5.3.1) and 16 (section 5.3.2) months; OST at ~ 4 (section 5.3.3), ~ 12 (section 5.3.4) and ~ 18 (section 5.3.5) months; T-CAT at 4.5 (section 5.3.6) and 16.5 (section 5.3.7) months

(summarised in table 5.2). The variations in the group numbers shown were due to mice dying, not acquiring the task, or losing stability during testing (see below).

A breeding programme was established to generate Tg2576 mice on an α7 nAChR null background. Initially Tg2576 male mice were bred with α7 nAChR knockout (KO) females from the in-house α7 nAChR colony. The resulting F1 progeny would therefore express either the Tg2576 gene (APP+) and be an α7 nAChR heterozygote (HT), or not express the Tg2576 gene (APP-) and be an α7 nAChR HT. The former mice in the F1 progeny would then be bred with α7 nAChR HT mice from the in-house α7 nAChR colony. The resulting F2 progeny would then be one of six different genotypes: APP+ X α7 nAChR KO (desired genotype); APP+ X α7 nAChR HT; APP+ X α7 nAChR wildtype (WT; desired control genotype); APP- X α7 nAChR KO; APP- X α7 nAChR HT; APP- X α7 nAChR WT. Due to Mendelian genetics, there would be a 1 in 4 chance of the F2 progeny being of the desired genotype. However as only males were desired for behavioural assessment, the chance of the F2 progeny being the desired genotype and gender was 1 in 8 (summarised in Fig. 5.1).

Group	Section number	Cognitive Task	Performance Measured	Age (m)	Group sizes	
					WT	Tg
1	*5.3.1*	5-CSR task	Acquisition	4.5 – 10	9	5
			Asymptote	10.5	9	5
1	*5.3.2*	5-CSR task	Asymptote	16	5	4
			Extended session	16	5	4
			Noise distraction (100dB)	16.5	5	4
			Noise distraction (110dB)	17	5	4
2	*5.3.3*	OST	Acquisition	3 – 4	6	8
			Asymptote	4.5	6	8
			22 odour challenge	4.5	6	8
1	*5.3.4*	OST	Acquisition	10.5-11.5	4	5
			Asymptote	12	4	5
			22 odour challenge	12	4	5
1	*5.3.5*	OST	Asymptote	19.5	3	4
2	*5.3.6*	T-CAT	Spontaneous exploration	4.5	6	6
1	*5.3.7*	T-CAT	Spontaneous exploration	18	7	9
1	*5.3.8*	None	Aβ plaques post nicotine	20	10	8
Both	*5.3.8*	None	Weight differences observed	3 – 20	3 – 10	4 – 10

Table 5.2: Studies performed using the Tg2576 mice

The studies performed using the Tg2576 (Tg) mice and their age-matched littermates (WT). Due to behavioural difficulties some studies originally planned (group 1) had to be performed by a second group of mice (group 2). Extended session and noise distraction challenges for the 5-CSR task were not performed at 10.5 months as these protocols were not in place at that time. Assessment of whether 10-day nicotine administration could reduce Aβ plaque levels (section 5.3.8, pp. 204) was performed on the remaining mice from group 1. Weight comparisons (section 5.3.9, pp. 207) were made during each study for both groups.

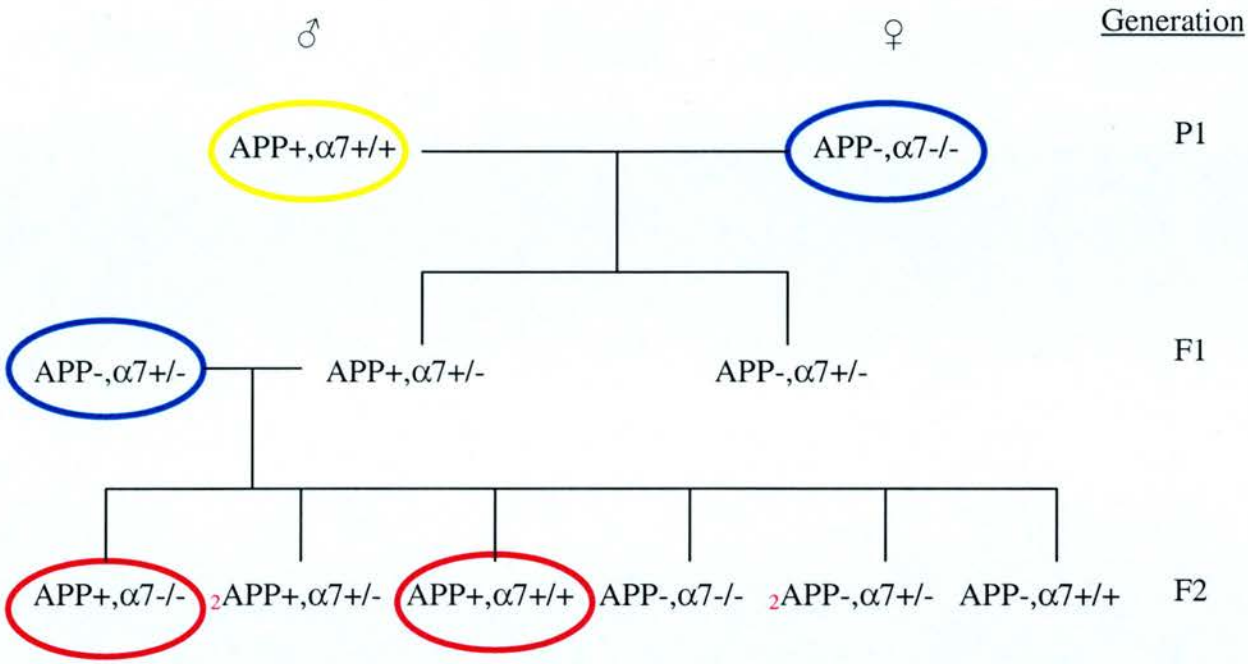


Figure 5.1: Breeding programme to assess the effects of Tg2576 expression on an α7 nAChR null background

The following breeding programme was designed to generate mice that were Tg2756 positive (APP+) and α7 nAChR knockouts (-/-), and compare their levels of Aβ deposition to APP+ and α7 wildtype (+/+) age-matched littermates. Male (♂) Tg2576 mice from the first group brought in (represented by ⬭), bred with a female (♀) α7-/- from the in-house colony (colony mice represented by ⬭). The resulting APP+,α7+/- F1 progeny were then crossed with an α7 heterozygote (+/-) from the in-house colony. Any progeny that did not express the Aβ deposition genotype (APP-) were not used for further breeding. There were six possible genotypic progeny from the F1 cross. The desired genotypes in the F2 generation were APP+,α7-/- and APP-,α7+/+ (represented by ⬭). Therefore there was a 1 in 4 chance of obtaining a ♂ or ♀ with a desired genotype in the F2 progeny.

Finally, the effects of nicotine on amyloid processing/deposition were assessed in aged Tg2576 mice when Aβ plaque levels were high (20 months old; WT n = 8; Tg n = 10). The mice were subdivided into four groups receiving either saline or nicotine (WT nicotine = 4, WT saline = 4, Tg nicotine = 5, Tg saline = 5) in a counter-balanced design according genotype, age and the level of previous behavioural training (OST and 5-CSR task; section 5.3.8). Previous behavioural training was considered, as it has been reported that increased cognitive activity can delay the onset of Alzheimer's disease in humans, and also that rats performing the 5-CSR task exhibit increased levels of endogenous acetylcholine in the prefrontal cortex, which may in itself be neuroprotective (Passsetti *et al.*, 2000; 2001). The mice were administered (s.c.) their allotted drug (either saline or (-)nicotine) twice a day (09:30 and 15:30 hrs), over a 10-day period exactly as described by Court and colleagues (2004). The mice receiving (-)nicotine were administered 0.25 mg/kg on day 1, 0.35 mg/kg on day 2, then 0.45 mg/kg for the following 8 days. The brains of each mouse was removed and levels of soluble and insoluble Aβ (1-40 and 1-42) in each group were compared. This study was performed in conjunction with Dr. Finlayson's group within F.I.N.E..

5.2.2 Behavioural problems encountered in the Tg2576 mice

The 48 mice were purchased (3 mth, n = 24 per group). Several difficulties were encountered prior to the initiation of training in the 5-CSR task. Initial group sizes were to be 14 from each group. After assessment in the 5-CSR task at 6 and 12 mth, 2 mice from each group were to be removed for Aβ measurements (ELISA) and plaque deposition, with remaining mice assessed at 19 mth. As training in the 5-CSR task usually requires 3 months, training was to begin at 3 mth. However training was delayed as two Tg mice were still under 20 g in weight and as such could not be food restricted. Another two Tg mice also required Dermisol treatment, twice daily, for a skin infection. Training commenced when the mice were 4.5 mth old. Several mice also exhibited signs of stereotypy behaviour, complicating the selection of 14 mice from each group. The stereotypy behaviour including spinning round in a circle and jumping against a side-wall, which has been observed in other transgenic mouse models of AD and likened to myoclonus observed in AD patients (Caviness, 2003; Lalonde *et al.*, 2003). Further complications arose after training began when genotyping of the mice became possible and was discovered that Taconic had incorrectly labelled three of the mice. Therefore final group sizes were 13 Tg and 15 WT mice. Training these two groups proved more arduous than any other in this thesis. On several occasions upon attaining criteria, performance would deteriorate, requiring that subject to be moved one or several stage/s back. Also 7 Tg mice died during training, now recognised as common in this transgenic line (King *et al.*, 1999; King and Arendash, 2002; Nordberg *et al.*, 2002; Fig. 5.2).

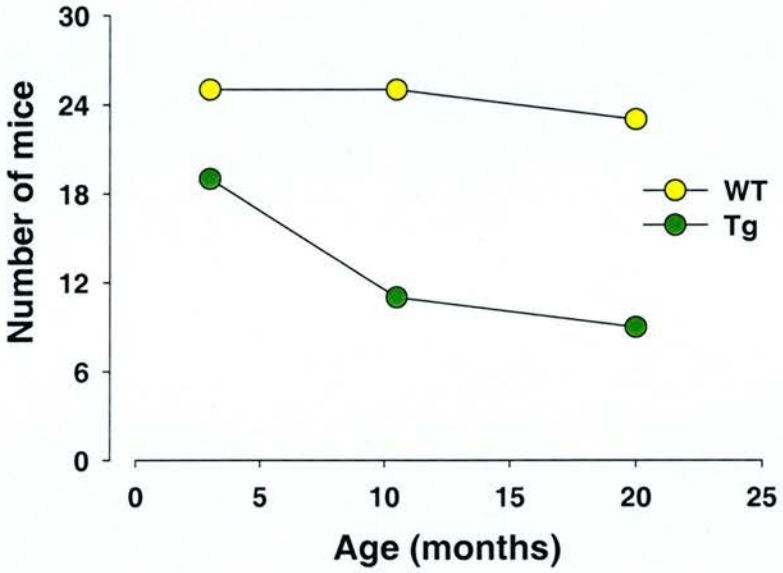


Figure 5.2: Survivability plot of the Tg2576 mice

Tg2576 (Tg) mice have a reduced survival rate when compared to their age-matched littermates. In 2002, King and Arendash identified the survival rate of the Tg mice as significantly worse than the WT mice. Data plotted as total numbers.

5.3 Results

5.3.1 Normal performance of Tg2576 mice (4.5 – 10.5 m) in the 5-CSR task

Of the 48 mice imported to the MFAA quarantine facilities at FINE, 4 were dead on arrival, 1 died within 5 days, then 7 more died during 5-CSR task training. All deaths were in the Tg2576 (Tg) group (final numbers = 9 WT and 5 Tg). Other problems occurred during 5-CSR task training, including large weight alterations in the Tg mice (to maintain 85% of free-feeding weight, two were fed within their cage and one only consumed powdered diet). This weight variation may have contributed to the variability in performance during training of the Tg mice, requiring some mice moved back stages. Hence training took longer than any other study. Final group numbers eventually consisted of 9 WT and 5 Tg mice.

Despite the difficulties, mice exhibited stable acquisition curves of proportion correct over increasing session days (Fig. 5.3a). Each subject's A_{50} (score indicating half way to acquisition) was calculated (as per chapter 3.3.5, pp. 90) and the values of the two groups compared using a one-way ANOVA. No significant differences between groups were observed ($F(1,12) = 0.0362$, $p = 0.852$; see Fig. 5.3b). Training continued until a stable level of performance had been attained (10.5 mth) then assessed over a four day period (Tuesday – Friday) using a 2-Way Repeated Measures ANOVA, with genotype and day as between subject factors and subject identity as the within subject factor. No significant main effects were observed for any measure (table 5.3, pp. 182). There was no significant main effect of genotype on % omissions ($F(1,32) = 0.0003$, $p = 0.985$; Fig. 5.3c), or proportion correct

($F(1,32) = 2.63$, $p = 0.131$; Fig. 5.3d), nor was there an effect on mean correct latency ($F(1,32) = 0.13$, $p = 0.725$; Fig. 5.3e). Nor was there any significant interactions between day and genotype on % omissions ($F(3,32) = 0.033$, $p = 0.992$), proportion correct ($F(3,32) = 0.857$, $p = 0.474$), or mean correct latency ($F(3,32) = 1.366$, $p = 0.271$).

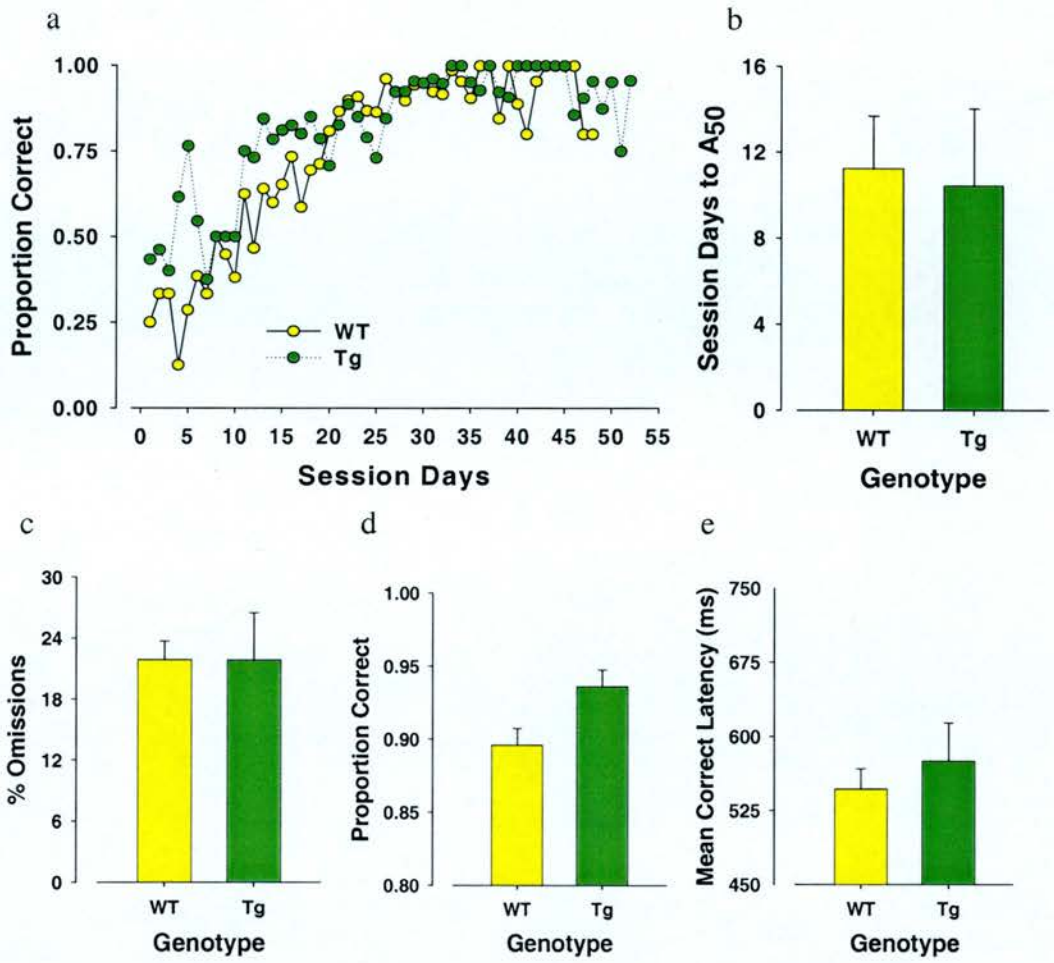


Figure 5.3: Normal acquisition and asymptotic performance of Tg2576 mice (4.5 – 10.5 mth) in the 5-CSR task

Tg2576 mice (Tg, n = 5) and their age-matched littermates (WT, n = 9) were trained (4.5 – 9.5 mth) to perform the 5-CSR task (wide array format). The acquisition of the 5-CSR task for one representative subject from each group with data shown as proportion correct across session days (a). The Tg mice acquired the task as readily as the WT mice as measured by the mean time taken by each group to reach halfway to asymptote (A_{50}; b). The asymptotic performance of the mice (10.5 mth) was measured over four consecutive days (Tuesday – Friday), with no significant differences observed between the two groups for every measure, including % omissions (c), proportion correct (d) and mean correct latency (e). Data presented as mean + s.e.m..

5.3.2 Tg2576 mouse (16 – 17 m) performance of the 5-CSR task

The mice used in the previous study (section 5.3.1) were retrained in the 5-CSR task. The stable baseline performance of the Tg and WT mice was compared as were their responses to several challenges, including extending the session length (see chapter 3.3.7, pp. 94) and noise distracters at 100 and 110 dB.

5.3.2.1 Tg2576 mouse (16 m) performance at asymptote

Analysis of asymptotic performance in the 5-CSR task of Tg (n = 4) and WT (n = 5) mice (16 mth) again revealed no significant genotypic effects at any measure tested. The two groups did not differ significantly in levels of % omissions ($F(1,20) = 0.0004$, $p = 0.985$; Fig. 5.4a), proportion correct ($F(1,20) = 0.005$, $p = 0.943$; Fig. 5.4b), or mean correct latency ($F(1,20) = 0.7$, $p = 0.443$; Fig. 5.4c). Nor was there any significant interactions between the two factors, as measured by % omissions ($F(3,20) = 1.561$, $p = 0.230$), proportion correct ($F(3,20) = 0.658$, $p = 0.587$), or mean correct latency ($F(3,20) = 1.366$, $p = 0.271$).

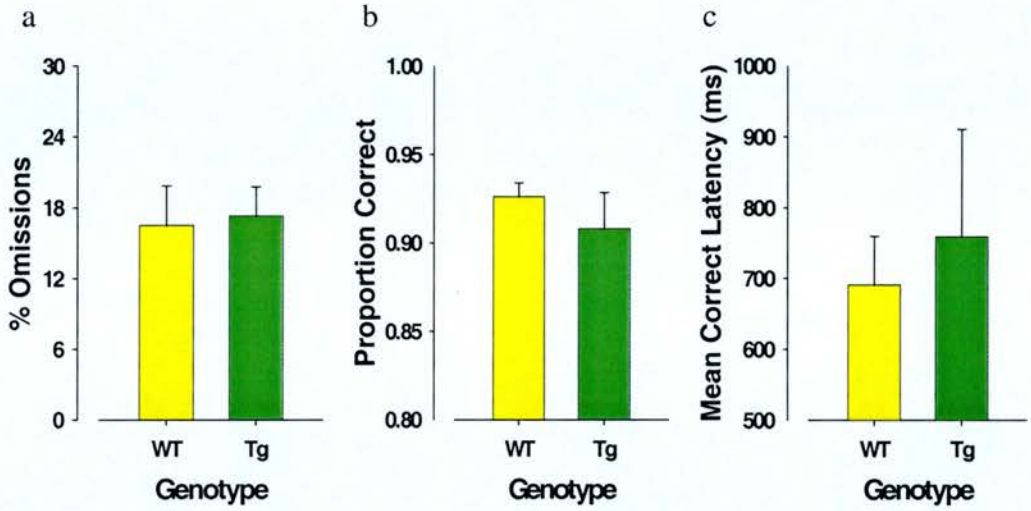


Figure 5.4: Normal asymptotic performance of Tg2576 mice (16 m) in the 5-CSR task

Tg2576 mice (Tg, n = 4) and their age-matched littermates (WT, n = 5) were trained (16 mth) were assessed in their asymptotic performance of the 5-CSR task (wide array format). The Tg and WT mice from section 5.3.1 were retrained in the 5-CSR task. Stable baseline was measured over 4 days (Tuesday – Friday). As at 10 months of age, the asymptotic performance of the Tg mice did not differ from that of the WT mice as measured by % omissions (a), proportion correct (b) and mean correct latency (c). Data presented as mean + s.e.m..

5.3.2.2 Normal 5-CSR task performance of Tg2576 mice (16 mth) when session length was extended

After assessment of baseline performance, the ability of these mice to sustain attention for a longer period of time was examined (40 min, with an unlimited trial number compared to the standard 25 min, and 120 trials; see chapters 2.2.5 and 3.3.7, pp. 58 and 94). The performance of the mice was assessed over a two-day period (Tuesday and Thursday) interspersed with training days (Monday, Wednesday and Friday), a schedule commonly used in the literature (Hahn and Stolerman, 2002; Grottick *et al.*, 2003). Performance was assessed using a 2 Way Repeated Measures ANOVA with day and genotype as between subject factors and mouse identity as the within subjects factor. Again no significant effect of day or genotype was found on any measure assessed, including proportion correct ($F(1,6) = 0.314$, $p = 0.596$; Fig. 5.5a), % omissions ($F(1,6) = 1.088$, $p = 0.337$; Fig. 5.5b), mean correct latency ($F(1,6) = 0.0322$, $p = 0.863$; Fig. 5.5c), mean reward latency ($F(1,6) = 0.245$, $p = 0.638$; Fig. 5.5d) and total trials ($F(1,6) = 0.903$, $p = 0.379$; Fig. 5.5e). No significant effects of genotype were observed for any other measure of raw data examined (see table 5.3, pp. 182). Nor were there any significant interactions between day and genotype observed as measured by % omissions ($F(1,6) = 1.155$, $p = 0.324$), proportion correct ($F(1,6) = 0.00001$, $p = 0.997$) or mean correct latency ($F(1,6) = 3.945$, $p = 0.094$).

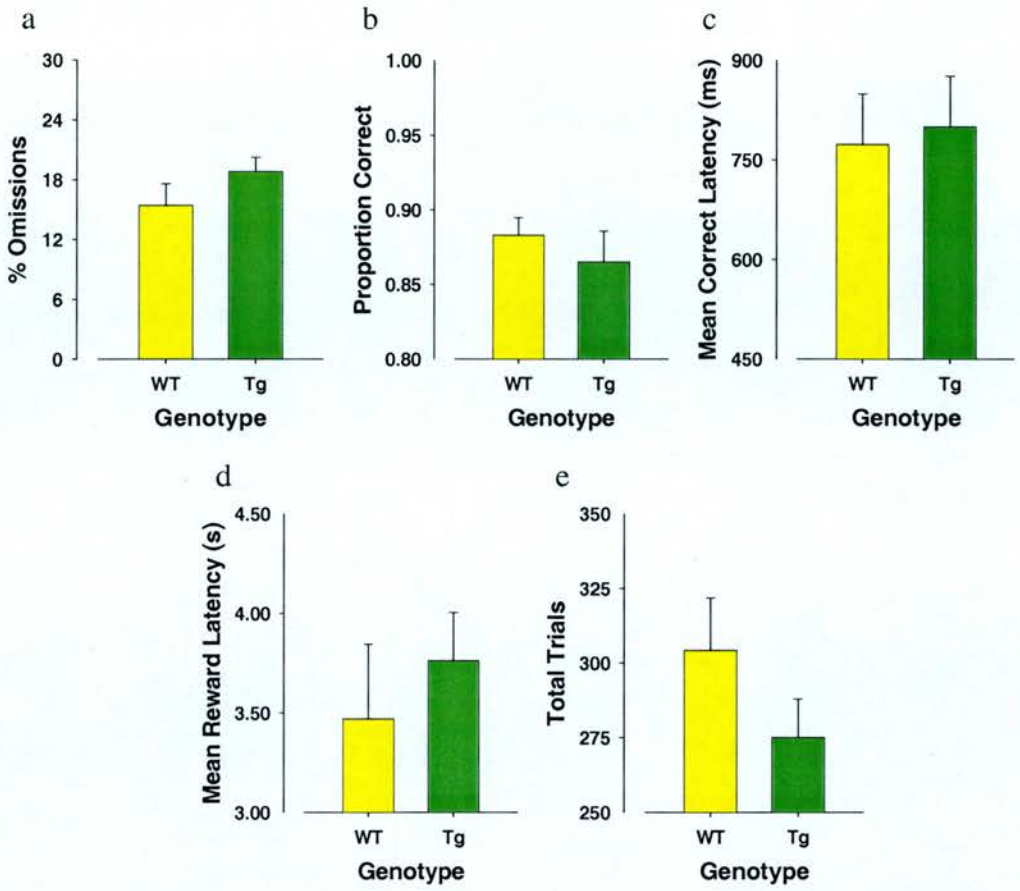


Figure 5.5: Normal performance of Tg2576 mice in the extended session 5-CSR task (16 mth)

The performance of the Tg2576 (Tg, n = 4) mice and their age-matched littermates (WT, n = 5) (from Fig. 5.4) were then assessed in their ability to perform the extended session 5-CSR task (wide array format). This task differed in that the session length was increased by 15 min, and the trial limit removed. Performance was assessed over two days, interspersed with training days. As before the Tg mice did not differ from that of the WT mice on any measure, including % omissions (a), proportion correct (b), mean correct latency (c), mean reward latency (d) and total trials completed (e). Data presented as mean + s.e.m..

5.3.2.3 Slowed response of Tg2576 mice (16.5 mth) performing the 5-CSR task during 100 dB noise distracters

After a short interlude (0.5 mth), the mice were trained in the standard 5-CSR task until performance was stable, and then their ability to sustain attention in the presence of auditory distracters was assessed. Initially attempts to distract the mice (16.5 mth) utilised 0.5 s 100 dB noise bursts, occurring either at the same time as the cue stimulus, 1 s prior to cue stimulus onset (halfway through the ITI), or not at all, in a randomised manner. No significant main effects of genotype was observed on % omissions ($F(1,16) = 1.255$, $p = 0.295$; Fig. 5.6a) or proportion correct ($F(1,16) = 0.151$, $p = 0.707$; Fig. 5.6b). However, there was a significant genotypic effect on mean correct latency ($F(1,16) = 6.152$, $p < 0.05$; Fig. 5.6c). No significant effects of genotype were observed for any measure of raw data (see table 5.3, pp. 182). No significant effect of noise onset was observed for any measure, including % omissions, proportion correct, mean correct latency, total number of correct responses, and total number of omission errors (data not shown). Nor was there a significant interaction between noise onset and genotype for % omissions ($F(2,16) = 1.292$, $p = 0.302$), proportion correct ($F(2,16) = 0.469$, $p = 0.634$) or mean correct latency ($F(2,16) = 0.429$, $p = 0.0659$).

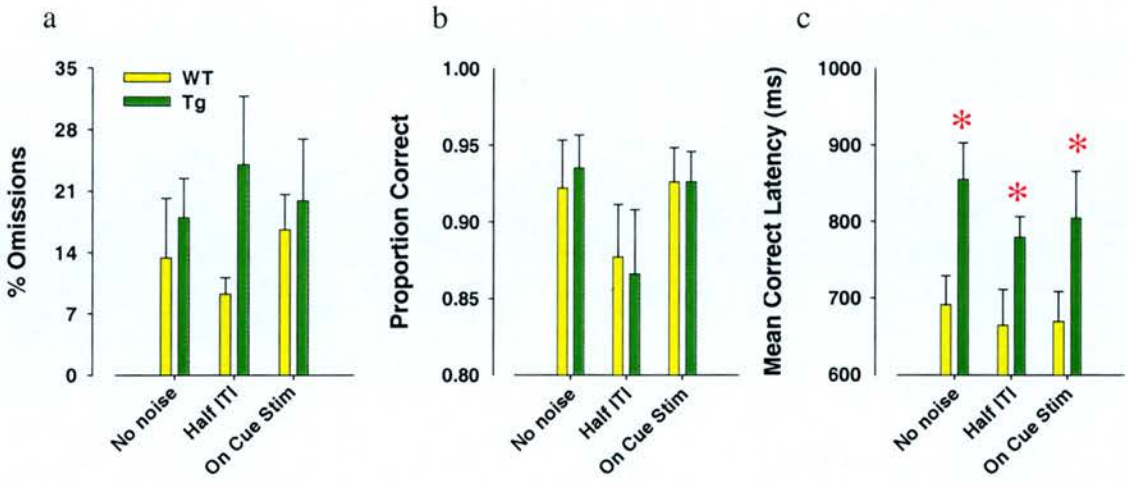


Figure 5.6: Slowed response of Tg2576 mice in the 5-CSR task during auditory distracters (16.5 m)

The distractibility of the Tg2576 (Tg, n = 4) and their age-matched littermates (WT, n = 5) was assessed by interpolating 100dB bursts (0.5 s) of noise during performance of the standard 5-CSR task. The presence and timing of the noise distraction was in one of three randomly selected states. Either no noise was present during a trial (No noise), the noise would begin 1 s prior to the cue stimulus (Half ITI), or the noise would begin at the same time as the presentation of the cue stimulus (On Cue Stim). The performance of the Tg and WT mice was compared. Performance was measured over two days (Tuesday and Thursday) which were interspersed with training days (Monday, Wednesday and Friday). Whilst some trends were apparent there was no significant main effect of genotype on % omissions (a) or proportion correct (b). However, there was a significant main effect of genotype on mean correct latency, with the Tg mice performing slower in every distraction state when compared with the WT mice (c). Data presented as mean +sem (* denotes $p < 0.05$ in genotypic comparison).

5.3.2.4 Increased effect of 110 dB noise distracters slowing response of Tg2576 mice (17 mth) performing the 5-CSR task

After a short interlude (0.5 mth), 5-CSR task baseline performance was re-established, the response of the Tg mice to louder (110 dB) noise distracters was compared to WT mice. One Tg was removed as performance had become erratic. The louder noise was used as a transgenic mouse model of AD (TgCRND8 mice, Janus *et al.*, 2000, see table 5.1, pp. 158) exhibited a startle response at levels greater than 105 dB (McCool *et al.*, 2003). No effect on % omissions ($F(1,14) = 0.466$, $p = 0.517$; Fig. 5.7a), or proportion correct ($F(1,14) = 0.192$, $p = 0.674$; Fig. 5.7b) was observed. Again however, a significant genotypic effect on mean correct latency was present ($F(1,14) = 9.941$, $p < 0.05$; Fig. 5.7c). Although no significant genotypic effect was observed for the raw data (see table 5.3, pp. 182), there were significant main effects of time of noise onset (at cue stimulus, halfway through ITI, or not at all). A significant effect of noise onset on % omisisons ($F(1,2) = 4.650$, $p < 0.05$; Fig. 5.7a) was observed with Tukey *post hoc* test revealing % omissions at noise halfway through the ITI significantly higher than no noise ($p < 0.05$). There was also a significant effect of noise onset on total correct responses ($F(1,2) = 9.651$, $p < 0.005$; Fig. 5.7e), with Tukey *post hoc* analysis revealing significantly fewer correct responses at both noise onsets compared to no noise ($p < 0.05$). There was also a significant genotypic/noise onset interaction for total correct responses ($F(1,2) = 4.077$, $p < 0.05$). Whilst there was a trend towards an effect of noise onset on the total number of omission errors, it was not significant ($F(1,2) = 2.841$, $p = 0.092$). No, effect of noise onset was observed in total trials ($F(1,2) = 0.616$, $p = 0.514$), or incorrect responses ($F(1,2) = 1.586$, $p = 0.239$).

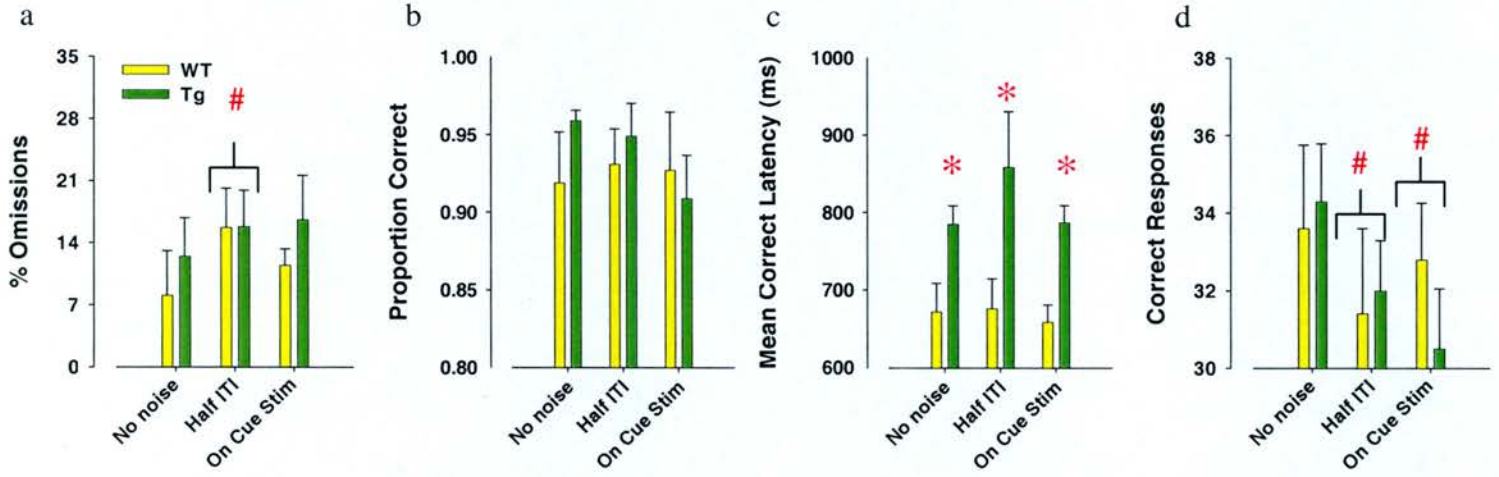


Figure 5.7: Slowed response of Tg2576 mice in the 5-CSR task during distracters (17 mth)

The distractibility of the Tg2576 (Tg, n = 4) and their age-matched littermates (WT, n = 5) was further assessed by interpolating 110 dB bursts (0.5 s) of noise during performance of the standard 5-CSR task. The presence and timing of the noise distraction was in one of three randomly selected states. Either no noise was present during a trial (No noise), the noise would begin 1 s prior to the cue stimulus (Half ITI), or the noise would begin at the same time as the presentation of the cue stimulus (On Cue Stim). The performance of the Tg and WT mice was compared. Performance was measured over two days (Tuesday and Thursday) which were interspersed with training days (Monday, Wednesday and Friday). No significant differences between the two groups were observed in % omissions (a) or proportion correct (b). There was again a significant main effect of genotype on mean correct latency, with the Tg mice performing slower at every distraction state when compared with the WT mice (c). An effect of noise onset was observed in % omissions (a). There was both a significant genotypic/noise onset interaction and noise onset effect on correct responses (e). Data presented as mean + s.e.m. (* denotes $p < 0.05$ in genotypic comparison, # denotes $p < 0.05$ when compared to no noise state).

Age (m)	5-CSR task performance assessed	Genotype	Raw data measured			
			Total Trials	Omissions	Correct	Anticipatory Responses
10.5	Asymptote	WT	120 ± 0	24 ± 1	88 ± 6	1.5 ± 0.4
		Tg	120 ± 0	18 ± 2	95 ± 12	0.8 ± 0.5
16	Asymptote	WT	115 ± 3	20 ± 4	88 ± 6	1.0 ± 0.3
		Tg	120 ± 0	21 ± 3	91 ± 4	0.9 ± 0.4
16	Extended Session	WT	304 ± 18	45 ± 6	251 ± 18	8.6 ± 1.7
		Tg	275 ± 13	50 ± 2	199 ± 17	6.6 ± 2.3
16.5	100 dB noise distracter	WT	120 ± 0	15 ± 4	92 ± 6	3.8 ± 2.2
		Tg	119 ± 1	24 ± 7	88 ± 7	0.6 ± 0.4
17	110 dB noise distracter	WT	120 ± 0	14 ± 4	98 ± 6	1.8 ± 1.2
		Tg	120 ± 0	18 ± 6	97 ± 5	1.0 ± 0.1

Table 5.3: Raw data figures for Tg2576 mice in the 5-CSR task

Raw data from the performances of the Tg2576 (Tg) mice and their age-matched littermates (WT) in the 5-CSR task (sections 5.3.1 and 5.3.2, pp. 171 - 1180). No significant effect was observed for any measure. Data presented as mean ± s.e.m..

In summary, whilst there was no main effect of genotype on asymptotic performance of the Tg2576 mice in the 5-CSR task at 10.5 or 16.5 months of age (sections 5.3.1 and 5.3.2.1; Fig. 5.3 and 5.4, pp. 173 and 175). Nor was there a genotypic effect when the task was extended to 40 min, although there was a trend towards the Tg2576 mice exhibiting higher % omissions and a lower proportion correct (section 5.3.2.2; Fig. 5.5a and b, pp. 177). There was a significant genotypic effect on mean correct latency when the mice were subjected to both 100 and 110 dB noise distracters, with the Tg2576 mice exhibiting significantly slower responses than their age-matched littermates (sections 5.3.2.3 and 5.3.2.4; Fig. 5.6c and 5.7c, pp. 179 and 181). Also, whilst no effect of time of noise onset was observed for any measure at 100 dB, when 110 dB was employed (at a level known to induce a startle response in TgCRND8 mice, McCool *et al.*, 2003) the performance of both groups was significantly worse during trials with auditory distracters as measured by % omissions and correct responses (section 5.3.2.4; Fig. 5.7, pp. 181). Also there was a significant genotypic/noise onset interaction on the number of correct responses, although the source of significance was unidentifiable. However, it appears that whilst the littermate mice maintained a high level of correct responses at each time of noise onset, the Tg2576 mice exhibited fewer correct responses as the time of noise onset drew closer to the cue stimulus (Fig. 5.7e, pp. 181).

5.3.3 Tg2576 mice (3 – 4.5 mth) exhibit normal acquisition and asymptotic performance of the 12, but not 22-OST

To date, in the literature there are numerous papers assessing the effects APP over-expression in mice on spatial working memory (section 5.1, pp. 156). However, there is little/no information available on the performance of these mice in a non-spatial working memory task. Olfactory working memory is a form of non-spatial working memory, and is clearly impaired in patients with AD. It has been suggested that this impairment may be an early marker with which to diagnose patients with mild cognitive impairments who are likely to progress to fully blown AD (Devanand *et al.*, 2000). This hypothesis is supported by the findings that Aβ deposition, and AD pathology occurs first in the entorhinal cortex (Braak and Braak, 1995; Nordberg *et al.*, 2002). Therefore the performance of a second group of Tg2576 mice and their age-matched littermates were examined in the OST. A group of young (3 – 4 mth) Tg2576 (Tg) mice were trained to perform the OST. Fig 5.8a represents the span length performance of the two groups over the period of task acquisition. A one-way ANOVA of the number of days taken by each group to attain criteria (span length $\geq$ 5 for two consecutive days) revealed no significant genotypic effect ($F(1,11) = 0.0589$, $p = 0.813$; Fig. 5.8b). Once a stable baseline performance had been reached, the performance of the two groups (at 4.5 mth) was assessed over four consecutive days (Tuesday – Friday). There were no significant differences between the two genotypes on every measure examined. The Tg mice did not differ from the WT mice in span length ($T=608$, $p = 0.144$; Fig. 5.9a), spans completed ($T=530$, $p = 0.992$; Fig. 5.9b), or in the total number of errors they made ($T=460$, $p = 0.190$; Fig.

5.9c). A 2-Way Repeated Measures ANOVA with genotype and day as between subject factors, and mouse identity as the within subject factor, revealed no significant main effects of day or genotype on mean span latency ($F(1,33) = 2.521$, $p = 0.141$; Fig. 5.9d) or time taken to first sample ($F(1,33) = 0.378$, $p = 0.551$; Fig. 5.9e) or ability to discriminate between two odours ($T=530$, $p = 0.992$). There was no significant interaction between day and genotype in mean span latency ($F(3,33) = 0.330$, $p = 0.804$) or time taken to engage in the task ($F(3,33) = 0.755$, $p = 0.728$). There did however appear to be a trend towards poorer performance in the Tg mice, that may have been being masked by ceiling effects in the task. Therefore the two groups of mice were then assessed in their ability to perform the 22-OST, which places increased demand on the olfactory working memory of the mice. The performance of the mice (at 4.5 mth) over two days (Tuesday and Thursday), with normal training sessions on the intervening days (Monday, Wednesday and Friday), was grouped and assessed for a genotypic effect. The observation that ceiling effects appeared to mask the differences in performance between the two groups proved correct. When assessed in the 22-OST there were significant effects of genotype on performance of the task. The Tg mice exhibited a significantly shorter span length than the WT mice ($T=181$, $p < 0.05$; Fig. 5.10a). The Tg mice also completed significantly fewer spans ($T=175$, $p < 0.05$; Fig. 5.10b) and committed more errors per session ($T=102.5$, $p < 0.05$; Fig. 5.10c). A 2-Way Repeated Measures ANOVA with genotype and day as between subject factors, and mouse identity as the within subject factor, revealed a significant main effect of genotype on mean span latency ($F(1,11) = 7.347$; $p < 0.05$; Fig. 5.10d), but not on time taken to first sample ($F(1,11) = 0.156$, $p = 0.701$; Fig. 5.10e). The differences appear to lie in the time taken to

complete individual spans. The Tg group appear slower to complete each individual span, and the difference was exaggerated when the span number is increased from 12 to 22 (Fig. 5.11). There was no significant interaction between the two actors as measured by mean span latency ($F(1,11) = 4.842$, $p = 0.054$) or time taken to engage in the task ($F(1,11) = 0.005$, $p = 0.943$).

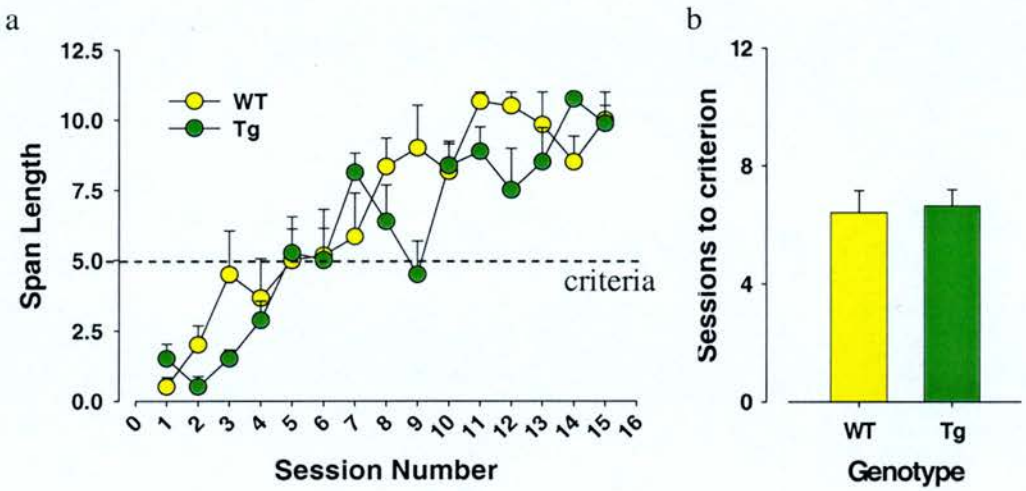


Figure 5.8: Normal OST acquisition of Tg2576 mice (3 – 4 mth)

The acquisition of young (3 – 4 mth) Tg2576 (Tg, n = 8) mice were compared with their age-matched littermates (WT, n = 6) in the standard (12 odour) OST. The mean span length across session days of each group is shown in (a). The average number of sessions each mouse took to acquire the task was grouped by genotype an compared. As the two groups attained a span length of 5 on two consecutive days at relatively the same time, the mean number of days to attain criteria taken by two groups was not statistically different (b). Data presented as mean + s.e.m..

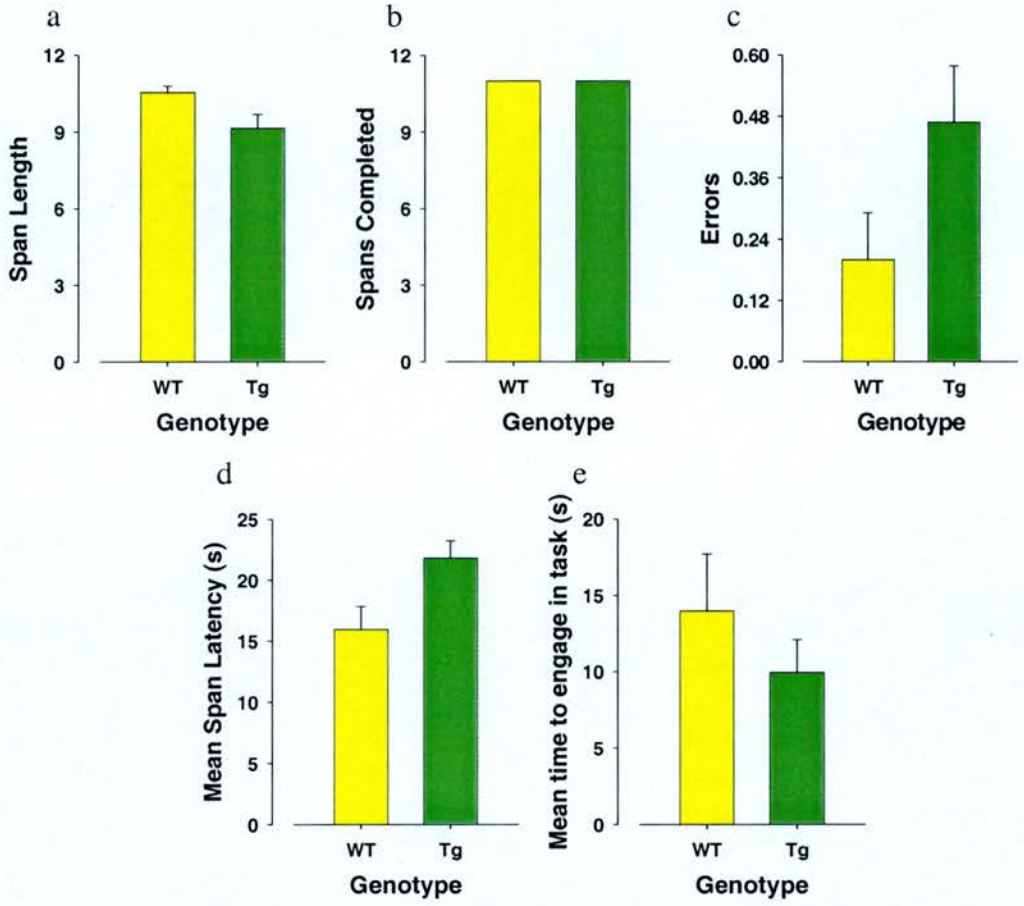


Figure 5.9: Normal asymptotic performance of Tg2576 mice (4.5 mth) in the OST

The asymptotic performance of young (4.5 mth) Tg2576 (Tg, n = 8) mice were compared with their age-matched littermates (WT, n = 6) in the standard (12 odour) OST. The performance of the two groups did not differ on any measure tested. There was no significant difference in span length (a), spans completed (b), errors per session (c), mean span latency (d) or time taken to engage in the task (e). Data presented as mean + s.e.m..

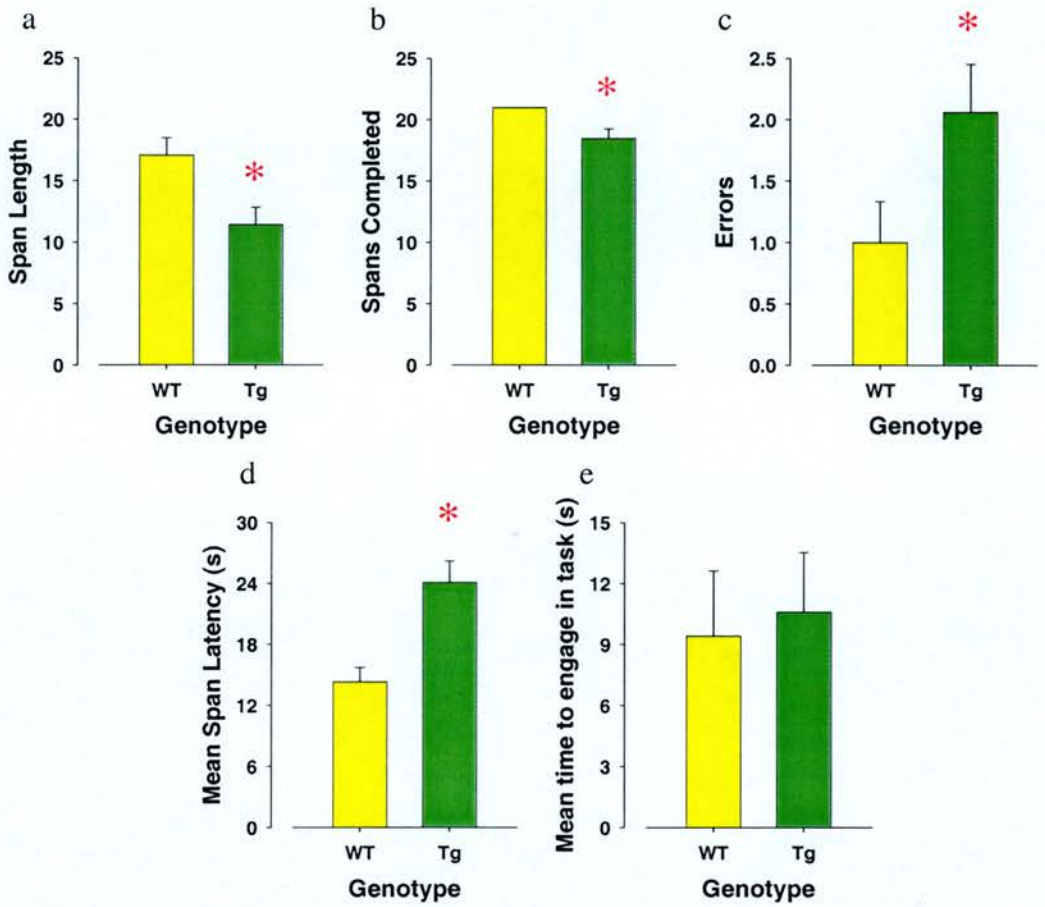


Figure 5.10: Impaired performance of Tg2576 mice in the 22-OST (4.5 mth)

The performance of young (4.5 mth) Tg2576 (Tg, n = 8) mice was compared with their age-matched littermates (WT, n = 6) in the 22 odour OST. Performance was assessed over two days (Tuesday and Thursday), with normal training on the intervening days (Monday, Wednesday and Friday). The Tg mice exhibited a significantly lower span length (a), and total number of spans completed per session (b) when compared with the WT mice. The Tg mice also committed more errors per session (c), and had a significantly higher mean span latency (e) than the WT mice. However, as with the 12 odour task, no significant difference was observed in the mean time taken by each group to engage in the task (e). Data presented as mean + s.e.m. (* denotes $p < 0.05$).

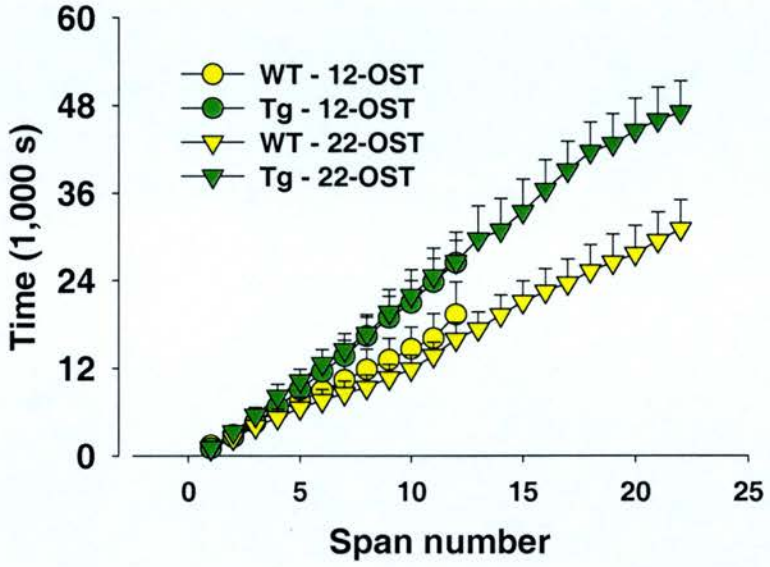


Figure 5.11: Slowing response of Tg2576 mice (4.5 mth) in the OST when difficulty increased from 12 to 22 odours

The performance of young (4.5 mth) Tg2576 (Tg, n = 8) mice was compared with their age-matched littermates (WT, n = 6) in time taken to complete the individual spans of a session. Their performance in both the12 and 22 OST was examined. The data revealed a significant difference in mean span latency in the 22 but not 12 OST (Fig. 5.9d vs. Fig. 5.10d). The figure above demonstrates the difference between the 12 and 22 OST in time taken to complete the spans. Once beyond the standard 12 spans, the two groups increasingly differ in their time taken to complete each span. Data presented as mean + s.e.m..

5.3.4 Impaired acquisition and asymptotic performance of Tg2576 mice (10.5-12 mth) in the OST

After initial assessment of baseline performance in the 5-CSR task the Tg2576 (Tg) mice and their age-matched littermates (WT) from section 5.3.1 (pp. 171) were trained to perform the 12 span OST. One Tg mouse was removed from the study as it had lost its lower teeth, and thus prevented consumption of the pellet rewards. Final group sizes were, WT n = 6, Tg n = 5. The acquisition of the OST by these mice is shown in Fig. 5.12a with the mean span length performance of each group shown across session days. The number of days each mouse took to attain criteria was grouped by genotype and compared using a one-way ANOVA. The Tg mice took significantly longer than the WT mice to attain the set criteria ($F(1,7) = 7.658$, $p < 0.05$; Fig. 5.12b). Training continued until a stable level of performance was attained (12 mth old). Their performance over four consecutive days (Tuesday – Friday), were grouped and analysed. The Tg mice were found to have a significantly lower span length than the WT mice ($T=416$, $p < 0.001$; Fig. 5.13a). However, the two groups did not differ in total spans completed ($T=296$, $p = 0.987$; Fig. 5.13b), or in their ability to discriminate between two odours ($T=304$, $p = 0.809$). The Tg mice erred significantly more per session than the WT mice ($T=185.5$, $p < 0.001$; Fig. 5.12c). A 2-Way Repeated Measures ANOVA with genotype and day as between subject factors with mouse identity as the within subjects factor revealed no significant main effects of day or genotype on mean span latency ($F(1,21) = 3.514$, $p = 0.103$; Fig. 5.13d), or mean time to first response ($F(1,21) = 1.728$, $p = 0.23$; Fig. 5.13e), with no significant interactions between the two factors on either measure ($F(3,21) = 2.139$, $p = 0.126$ and $F(3,21) = 1.194$, $p = 0.336$ respectively).

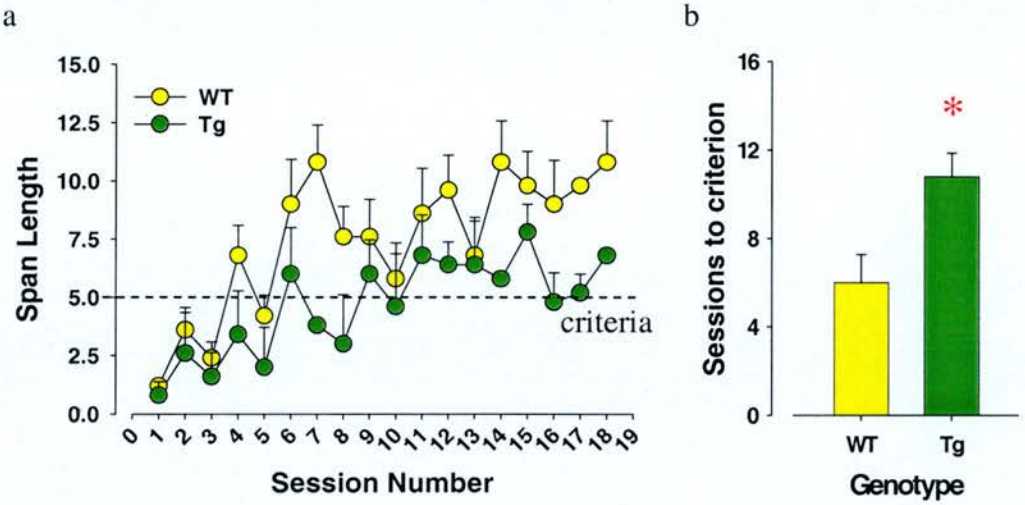


Figure 5.12: Impaired OST acquisition of Tg2576 mice (10.5 – 11.5 mth)

The acquisition performance of Tg2576 (Tg, n = 5) mice in the OST was compared with their age-matched littermates (WT, n = 4; 10.5 – 11.5 mth). The acquisition curve of each group is shown in (a) as mean span length across session number. The Tg mice appeared to take longer to reach a span length of 5 on two consecutive days in comparison with the WT mice. Analysis of the number of days the mice took to attain criteria grouped by genotype revealed that the Tg mice took significantly longer than the WT mice (b). Data presented as mean + s.e.m. (* denotes $p < 0.05$ when compared with WT mice).

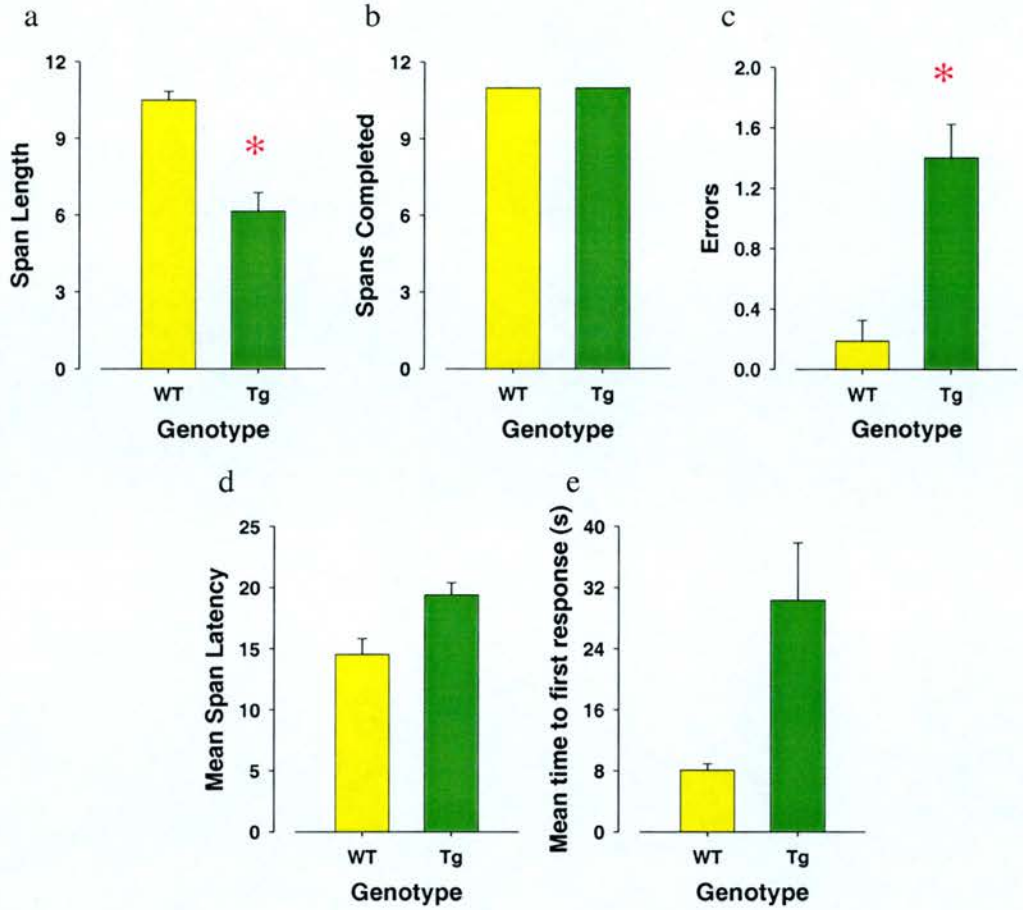


Figure 5.13: Impaired asymptotic performance of Tg2576 mice (12 mth) in the OST

Tg2576 (Tg, n = 5) mice and their age-matched littermates (WT, n = 4; 12 mth) were compared in their performance of the standard (12 odour) OST. Stable performance was assessed over four consecutive days (Tuesday – Friday). Unlike data at 4.5 mth, there was a difference in asymptotic performance of the Tg and WT mice. The Tg mice exhibited a significantly lower span length (a) than the WT mice. No differences between the two groups were observed in the total number of spans completed per session (b) but the Tg group did err significantly more often per session than the WT mice (c). No differences were observed in the mean span latency (d) or the mean time taken by each group to engage in the task (e) between each group. Data presented as mean + s.e.m. (* denotes $p < 0.05$).

Although it is unlikely that the differences observed were confounded by ceiling effects, the number of odours in the task was increased from 12 to 22 on two test days (Tuesday and Thursday) interspersed with three training (12 odour) days (Monday, Wednesday and Thursday). No effect of day was observed for any measure. Again the Tg mice exhibited a significantly lower span length when compared to the WT mice ($T=101.5$, $p < 0.05$; Fig. 5.14a). The total number of spans completed by the two groups did not differ ($T=84$, $p = 0.497$; Fig. 5.14b), nor did their ability to discriminate between two odours ($T=76$, $p = 0.964$). The Tg mice again erred significantly more times per session than the WT mice ($T=49$, $p < 0.05$; Fig. 5.14c). No effect of day, or genotype on mean span latency ($F(1,7) = 0.54$, $p = 0.486$; Fig. 5.14d), or time to first response ($F(1,7) = 1.281$, $p = 0.295$; Fig. 5.14e) was observed. Nor was there a significant interaction between the two factors as measured by mean span latency ($F(1,7) = 0.016$, $p = 0.903$) or time taken for the first response ($F(1,7) = 4.143$, $p = 0.081$).

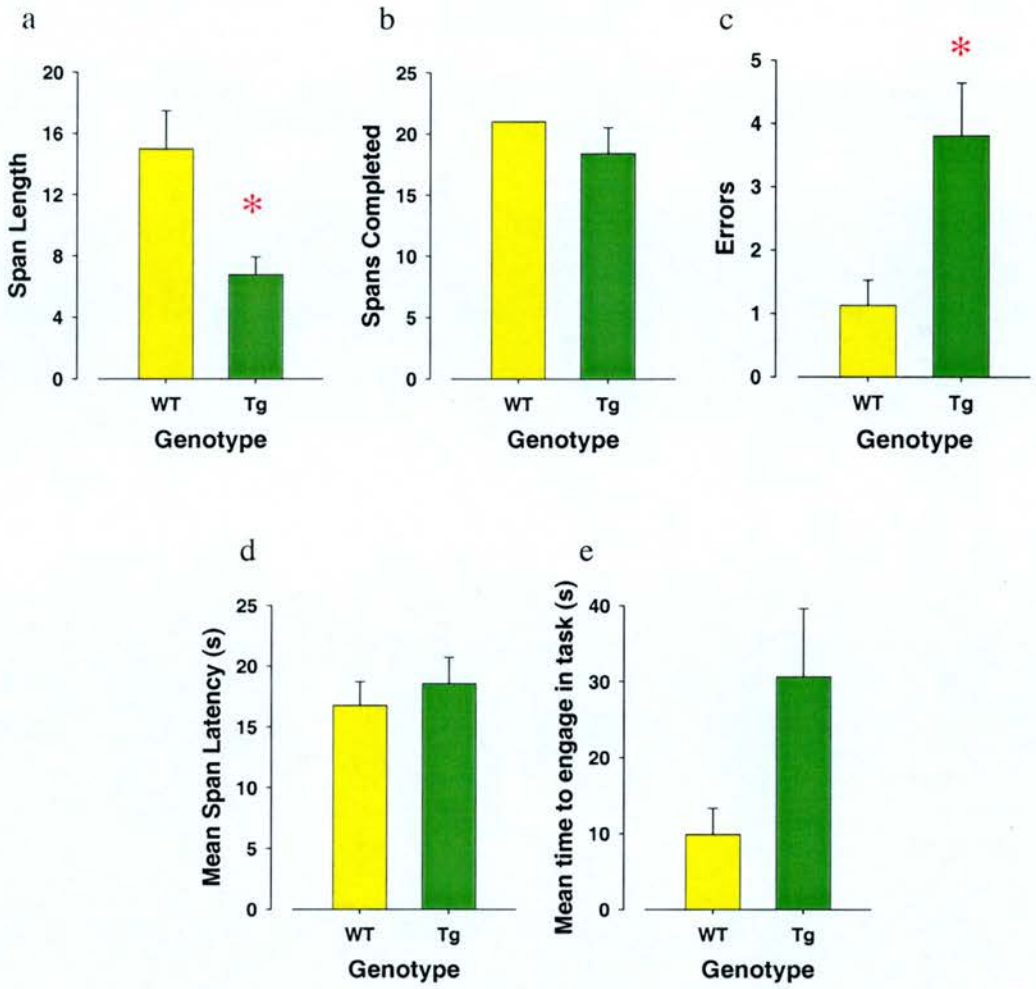


Figure 5.14: Impaired performance of Tg2576 mice in the 22-OST (12 mth)

The performance of Tg2576 (Tg, n = 5) mice was compared with their age-matched littermates (WT, n = 4; 12 mth) in the increased difficulty (22 odour) OST. Performance was assessed over two days (Tuesday and Thursday), with normal OST training on the intervening days (Monday, Wednesday and Friday). The Tg mice exhibited a significantly lower span length when compared with the WT mice (a). However the total number of spans completed by the two groups did not differ (b). The Tg mice made significantly more errors per session than the WT mice (c). However, no significant differences were observed in mean time taken to complete a span (d) or mean time taken time to begin the task (e). Data presented as mean + s.e.m. (* denotes $p < 0.05$ when compared to WT mice).

5.3.5 Impaired performance of Tg2576 mice (19.5 mth) in the OST

After exploratory behaviour was assessed on the T-maze (section 5.3.7, pp. 202), the Tg2576 (Tg) mice and their age-matched littermates (WT; 19.5 mth) from section 5.3.4 (pp. 190) were retrained to a stable baseline performance in the OST. In the interim period two WT mice had died and one Tg was removed due to diarrhoea (WT, n = 3 and Tg, n = 4). Their performance over four consecutive days (Tuesday – Friday) was grouped and analysed. The Tg mice were again found to have a significantly lower span length than the WT mice (T=223, $p < 0.01$; Fig. 5.15a), but did not differ in total spans completed (T=174, $p = 0.981$; Fig. 5.15b), or in their ability to discriminate between two odours (T=186, $p = 0.809$). The number of errors made by the Tg mice was again significantly greater than the WT mice (T=112.5, $p < 0.01$; Fig. 5.15c). A 2-Way Repeated Measures ANOVA with genotype and day as between subject factors, and mouse identity as the within subjects factor, revealed no significant main effects of genotype on mean span latency ($F(1,15) = 1.867$, $p = 0.358$; Fig. 5.15d) or mean time to initial response ($F(1,15) = 1.023$, $p = 0.23$; Fig. 5.15e).

However, a significant effect of day was observed for both mean span latency ($F(1,15) = 5.737$, $p < 0.05$), and mean time to first response ($F(1,15) = 4.998$, $p < 0.05$). Tukey *post hoc* comparison indicated that mean span latency and time taken to engage in the task performance on day 2 (Wednesday) differed from days 1 and 3 (Tuesday and Thursday respectively). This was the first time a day effect was observed. Attempts to identify the cause proved inconclusive. Analysis assessing

the effect of day on the remaining measures using a Kruskal Wallis ANOVA on Ranks identified no significant main effects on any other measure. Thus the span length ($H=3.661$, $p = 0.3$), spans completed ($H=0$, $p = 1.0$) and errors per session ($H=0.954$, $p = 0.812$) did not significantly differ between days. Finally there was no significant interaction between day and genotype on mean span latency ($F(3,15) = 1.339$, $p = 0.299$) or time taken for the first response ($F(3,15) = 0.206$, $p = 0.891$).

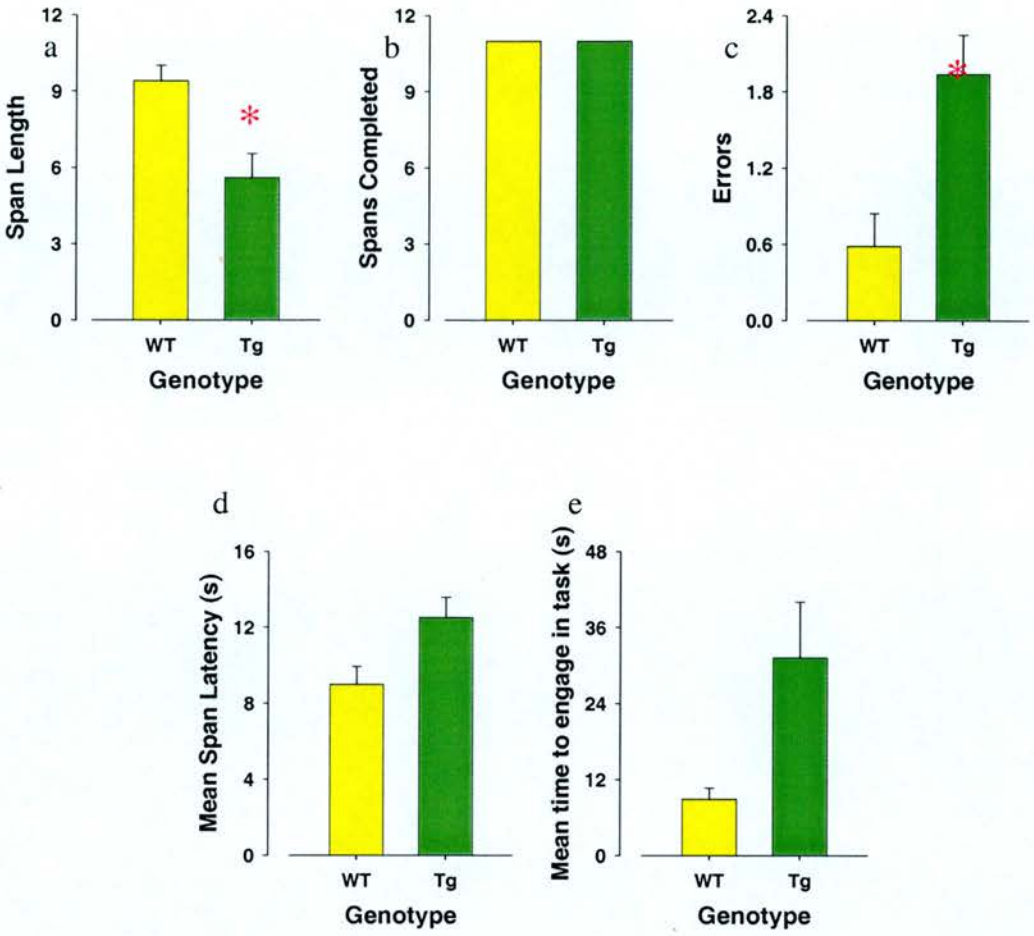


Figure 5.15: Impaired asymptotic performance of Tg2576 mice in the OST (19.5 mth)

The performance of Tg2576 (Tg, n = 4) mice was compared with their age-matched littermates (WT, n = 3; 19.5 mth) in the standard (12 odour) OST. The mice from section 5.3.5 (pp. 195) were retrained in the task. Asymptotic performance was assessed over four consecutive days (Tuesday – Friday). The pattern of performance was akin to that when tested at 12 months. Tg mice again exhibited a significantly lower span length (a) than the WT mice, but did not differ from the WT mice in the total number of spans completed per session (b). The Tg group did err significantly more often per session than the WT mice (c) though no genotypic differences were observed in the mean span latency (d) or the mean time taken by each group to engage in the task (e). Data presented as mean + s.e.m. (* denotes $p < 0.05$).

In summary, the Tg mice exhibited normal learning and asymptotic performance in the 12-OST at 3 – 4.5 months of age. However, when assessed in the 22-OST, their performance was significantly worse than the WT mice. Whilst the mean span latency of the Tg mice was significantly longer than the WT mice, resulting in their completion of fewer spans, there was a possible speed/accuracy trade-off, whereby they performed more slowly to remain as accurate as possible. However, the Tg mice also erred significantly more often than the WT mice. The lack of olfactory discriminatory deficit provides support for an impaired olfactory working memory span in this more arduous task 22 span OST. When assessed at 12 months, the Tg mice exhibited both impaired acquisition and asymptotic performance in the standard 12-OST. There was a significant age-related effect of acquisition, as older Tg mice (10.5 – 11.5 mth) took significantly longer to attain criteria than young Tg mice (3 – 4 mth, $F(3, 18) = 5.966$, $p < 0.005$). There was also a significant age-related effect of OST performance in the standard task as measured by span length ($H=41.261$; $p < 0.001$; Fig. 5.16), with Dunn's pairwise comparison identifying that the Tg mice at 4.5 months performed significantly better than when assessed at both 12 and 19.5 months ($p < 0.05$). Thus with increased age, the olfactory acquisition and working memory impairment observed in these mice became even more apparent. Moreover, when performance was reassessed at 19.5 months, the Tg mice remained significantly worse than the WT mice.

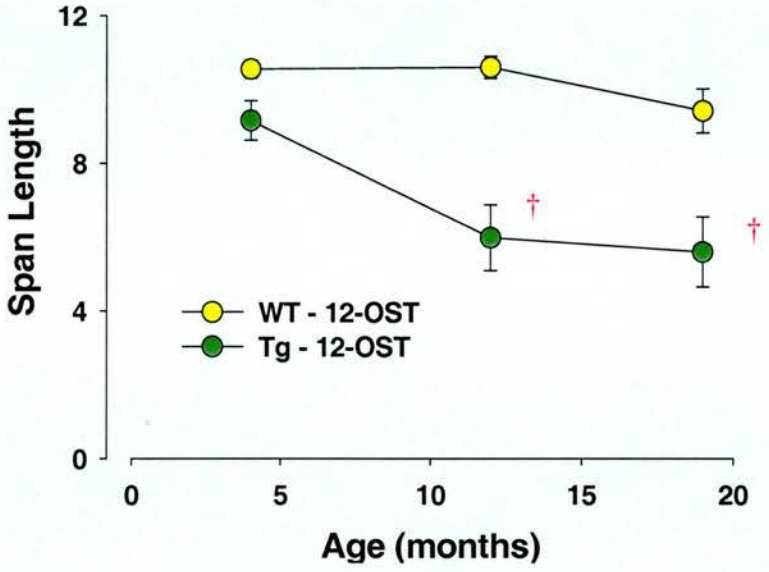


Figure 5.16: Age-related impairment of Tg2576 mice in the OST

The performance of Tg and WT mice in 12-OST is represented across the three ages tested (4.5, 12 and 19.5 mth). An effect of age was observed with the Tg mice exhibiting a higher span length at 4.5 mth when compared to 12 and 19.5 mth. Data presented as mean ± s.e.m. († denotes $p < 0.05$ when compared to Tg at 4.5 mth).

5.3.6 Normal Tg2576 mouse performance in the T-maze (4.5 mth)

After assessment of performance in the 22-OST, the mice from section 5.3.3 (pp. 184) were permitted *ad libitum* access to food for several days. The exploratory behaviour of the two groups (WT = 6, Tg = 6; 4.5 mth) was then evaluated by assessing their rates of spontaneous alternation in the T-maze continuous alternation task (T-CAT) on a single day. The rate of spontaneous alternation of each group was compared using a t-test. Despite the WT mice having a higher rate of spontaneous alternation than the Tg mice, it was not significantly different ($t=1.728$, $p = 0.115$; Fig. 5.17a). Caution is recommended when interpreting the result however as the study was underpowered due to the low group sizes. A one-sample t-test comparing the % spontaneous alternation of the WT group with expected chance levels (50%) suggested that despite alternation rates of ~ 60%, they did not significantly differ from chance levels ($t=1.6592$, $p = 0.158$). Neither did the spontaneous alternation rate of the Tg (~ 45 %) differ significantly from chance levels ($t=0.7251$, $p = 0.5009$). A one-way ANOVA revealed no significant effect of genotype on length of time taken (s) to complete the task ($F(1,10) = 0.523$, $p = 0.486$; Fig. 5.17b).

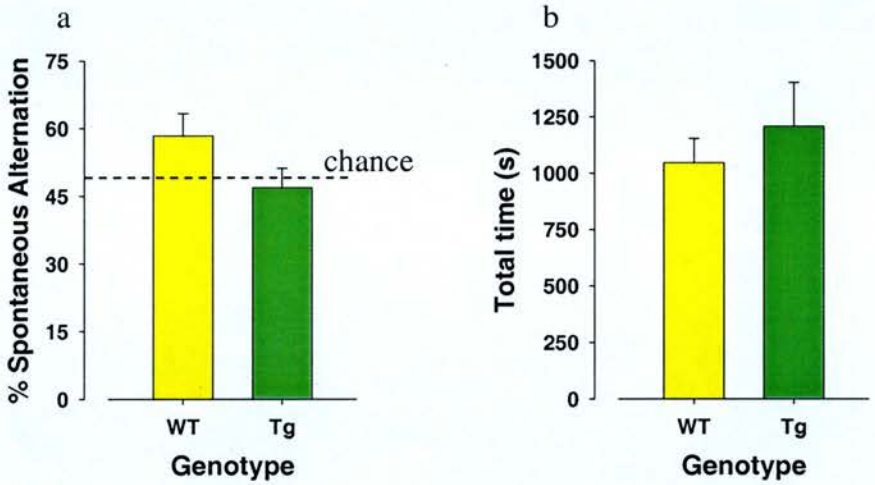


Figure 5.17: Normal spontaneous alternation of Tg2576 mice (4.5 mth) in the T-CAT

Spontaneous alternation rates of young (4.5 mth) Tg2576 (Tg, n = 6) mice were compared with their age-matched littermates (WT, n = 6) using the T-maze continuous alternation task (T-CAT) over one day. The two groups did not differ in % alternation (a), nor did either group differ significantly from chance (broken line). Likewise no differences in total time taken by each group to complete the session were observed (b). Data presented as mean + s.e.m..

5.3.7 Normal spontaneous alternation of Tg2576 mice in the T-maze (18 mth)

After assessment of performance in the 5-CSR task, the mice from section 5.3.2 (pp. 174) were permitted *ad libitum* access to food for one week. Their exploratory behaviour was then assessed (WT = 6, Tg = 6, at 18 months of age) by examining their rates of spontaneous alternation in the T-CAT. Spontaneous alternation rates of each group were compared using a t-test. The results were similar to those observed in the 4.5 month old mice. Despite the apparent increase in spontaneous alternation in the WT mice when compared to the Tg mice, this was not significant ($t=2.037$, $p = 0.061$; Fig. 5.18a). However as before caution is recommended in interpreting a negative result as the test was underpowered due to low group sizes. Again, despite alternation rates of ~ 60%, a one-sample t-test comparing the % spontaneous alternation of the WT group with expected chance levels (50%) revealed a non-significant trend toward above chance level performance ($t=2.1213$, $p = 0.0781$). The Tg group (45%) did not differ significantly from chance ($t=0.7833$, $p = 0.456$). A One-way ANOVA with genotype as the between subjects, factor revealed no significant difference between the Tg and WT mice in time taken to complete the session ($F(1,14) = 0.0264$, $p = 0.873$; Fig. 5.18b).

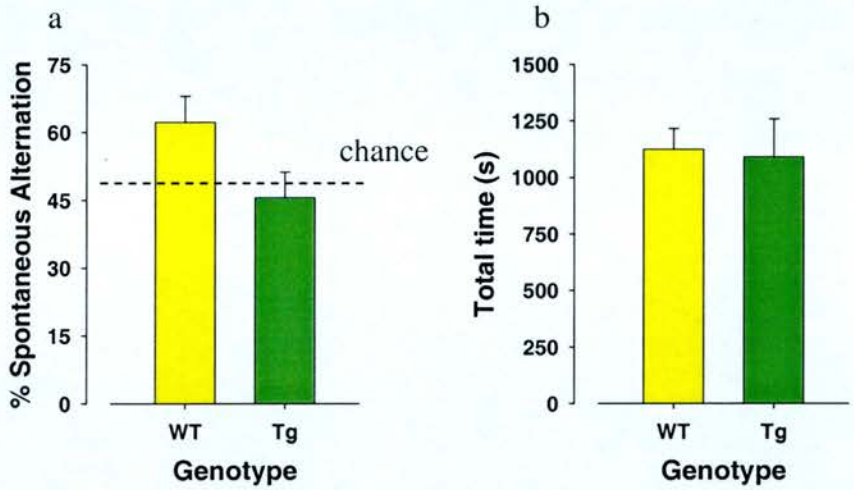


Figure 5.18: Normal spontaneous alternation of Tg2576 mice (18 mth) in the T-CAT

Spontaneous alternation rates of Tg2576 (Tg, n = 9) mice were compared with their age-matched littermates (WT, n = 7; 18 mth) using the T-maze continuous alternation task (T-CAT) over one day. The two groups did not differ significantly in their levels of % alternation (a). Nor did each group differ significantly from chance levels (broken line). Likewise no differences in the mean time taken by each group to complete the session were observed (b). Data presented as mean + s.e.m..

5.3.8 Difficulties in breeding the Tg2576 mice on an α7 nAChR null background

The breeding programme was designed such that the behaviour of Tg2576 and α7 nAChR KO mice could be compared with α7 nAChR KO, T2576 and age matched littermate mice. It was hypothesised that if the α7 nAChR was essential for Aβ deposition, and if the behaviour of the Tg2576 mice was because of their levels of Aβ deposition, then the mice would behave similarly to α7 nAChR KO mice, with impaired performance in the 5-CSR task when young, and impaired OST due to their attentional deficits when older. Otherwise, they would behave similarly to both groups. The programme began by mating four of the first group of Tg2576 mice (not used in any behavioural study) with α7 nAChR KO females (see Fig. 5.1, pp. 167). However, due to the low fertility of α7 nAChR KO mice (Orr-Urtreger *et al.*, 1997), and the high levels of aggression in the Tg2576 mice (discussed section 5.2.1, pp. 164), it was some time (2.5 mth) before litters were produced that survived beyond weaning. Thus in the F1 mating it was ensured that only female Tg2576 mice were utilised, further slowing the programme.

Further problems arose with the F1 generation, as several mice died shortly after weaning, and genetic analysis identified that it was predominantly the mice required that died (Tg2576 gene and α7 nAChR HT). Of the 22 mice born with this genotype, only 27.3% (6 out of 22) survived beyond 2 mth. Moreover, significant weight differences were apparent when these mice were first weighed at approximately 2 mth. The weights were compared using a one-way ANOVA, with genotype (no Tg2576 gene, Tg2576 gene with at least one disrupted α7 nAChR allele, i.e. HT or

KO, or Tg2576 gene with wildtype α7 nAChr alleles; n = 8, 7, 3 for males and 10, 7, 2 for females, respectively) as the between subject factor. Male and female mice were compared separately. A significant main effect of genotype was observed in the male mice ($F(2,15) = 28.551$, $p < 0.001$, Fig. 5.19a), with Holm-Sidak pairwise comparison identifying that the two groups of mice with the Tg2576 genotype were significantly lighter than mice with no Tg2576 gene ($p < 0.001$). When the weights of the female mice were compared, again a significant main effect of genotype was observed ($F(2,16) = 8.535$, $p < 0.01$; Fig. 5.19b), with Holm-Sidak pairwise comparison identifying that the mice with the Tg2576 gene and disrupted α7 nAChR alleles were significantly lighter than mice with no Tg2576 gene ($p < 0.001$), although the mice with the Tg2576 gene and wildtype α7 nAChR alleles was nearly significantly different to the mice with no Tg2576 gene ($p = 0.058$), and lack of difference was likely to reflect group sizes. These weight differences continued throughout the life-span of the mice, with many not reaching 20 g in weight even at 3.5 months (10 out of 15 males). Therefore, due to their low weights making early behavioural assessment impossible, and the difficulties breeding these mice, no behavioural or histological results have been obtained, and the breeding programme terminated.

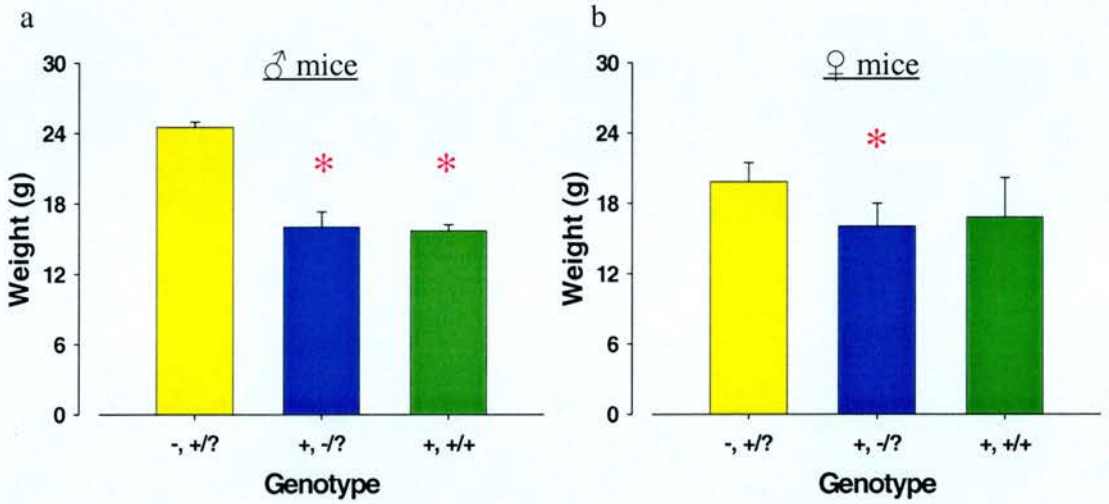


Figure 5.19: Reduced weights of young mice with the Tg2576 gene

Tg2576 mice were bred onto an α7 nAChR null background. The weights of mice without the Tg2576 gene on a α7 nAChR HT or WT background (-, +/?) were compared with mice with the Tg2576 gene and at least one disrupted α7 nAChR allele (+, -/?) and mice with the Tg2576 gene with only wildtype α7 nAChR alleles (+, +/+). Weights were taken at approximately 2 mth old. It was discovered that male mice with the Tg2576 gene were significantly lighter than those without the gene (a). Whereas female mice with the Tg2576 gene only differed from those without the gene if they also expressed disrupted α7 nAChR alleles (b). Data presented as mean + s.e.m. (* denotes $p < 0.05$ when compared to -, +/? control mice).

5.3.9 (-)Nicotine administration had no effect on Aβ levels in Tg2576 mice (20 mth)

Recent studies suggest that nicotine administration can reduce the level of soluble and insoluble Aβ in the brains of Tg2576 mice (Nordberg *et al.*, 2002; Hellström-Lindahl *et al.* 2004). These studies utilised a 5.5 mth ingestion protocol (Aβ levels assessed at 14.5 mth) and a 10-day injection protocol (Aβ levels assessed at 9 m) respectively. As our Tg2576 mice from group 1 were 20 mth of age we followed the 10-day nicotine injection protocol (20mth, WT n = 8, Tg n = 10). Whilst the Tg mcie exhibited significantly higher levels of soluble $A\beta_{(1\text{-}40)}$ ($F(,1,14) = 16.279$, $p < 0.001$; Fig. 5.20a), insoluble $A\beta_{(1\text{-}40)}$ ($F(1,14) = 17.274$, $p < 0.001$; Fig. 5.20a) and insoluble $A\beta_{(1\text{-}42)}$ ($F(1,14) = 68.277$, $p < 0.001$; Fig. 5.20a), when compared to WT mice, no effect of drug was observed on any measure. Thus nicotine administration had no effect on levels of soluble $A\beta_{(1\text{-}40)}$ ($F(,1,14) = 0.143$, $p = 711$; Fig. 5.20b) insoluble $A\beta_{(1\text{-}40)}$ ($F(1,14) = 0.192$, $p = 0.668$; Fig. 5.20b) or insoluble $A\beta_{(1\text{-}42)}$ ($F(1,14) = 0.289$, $p = 0.599$; Fig. 5.20b).

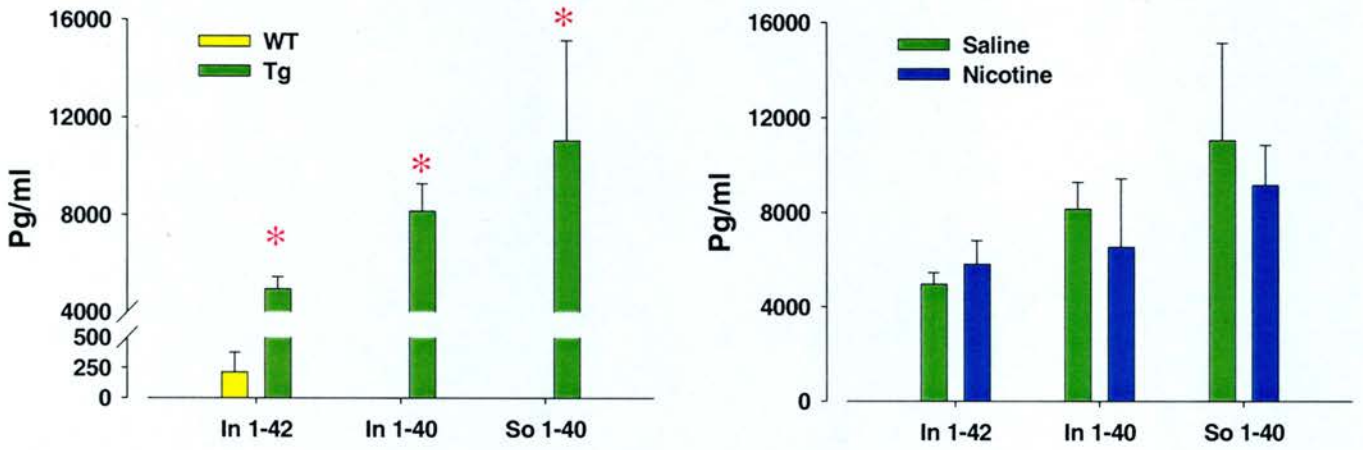


Figure 5.20: Tg2576 mice (20 mth) exhibited higher levels of Aβ than WT mice, but (-)nicotine had no effect on these levels

The effects of a 10-day (-)nicotine administration on levels of insoluble $A\beta_{(1-42)}$ (In 1-42), insoluble $A\beta_{(1-40)}$ (In 1-40), and soluble $A\beta_{(1-40)}$ (So 1-40) was assessed in Tg2576 (Tg, n = 8) mice and their age-matched littermates (WT, n = 10; 20 months) by ELISA. Soluble levels of $A\beta_{(1-42)}$ were below the level of detection. The Tg mice exhibited significantly higher levels in each measure when compared with the WT mice (a). However, there was no effect of nicotine administration on any measure in the Tg mice (b). Data presented as mean + s.e.m. (* denotes $p < 0.05$ when compared to WT).

5.3.10 Weight differences of Tg2576 mice in the above sections

As the behavioural tasks used for assessment of cognitive function in the Tg2576 mice utilised food as their motivation, weight differences at the time of testing may account for the performance differences observed in the tasks. The weights of the mice in section 5.3.1 (pp. 171) were measured when placed on *ad libitum* feeding during 5-CSR acquisition were compared using a 2-Way Repeated Measures ANOVA with genotype and age as between subject factors, and identity as the within subject factor. A significant main effect of genotype and age was identified, with Tg mice significantly lighter than WT mice ($F(1,87) = 60.318$, $p < 0.001$). A significant main effect of age was identified also ($F(3,87) = 10.213$, $p < 0.001$), with Dunnett's *post hoc* analysis revealing that mice weights at 7 and 8.5 mth were significantly heavier than at 4 mth ($p < 0.05$). When tested for the second time in the 5-CSR task (section 5.3.2, pp. 174), the weights of the mice when placed on *ad libitum* feeding were again compared using a 2-Way Repeated Measures ANOVA with genotype and age as between subject factors, and mouse identity as the within subject factor. Again no significant main effect of age was observed ($F(2,16) = 1.019$, $p = 0.383$), but there was a significant main effect of genotype ($F(1,16) = 10.860$, $p < 0.01$) with Tg mice again being lighter than WT mice.

Weights taken at the start of each OST study (sections 5.3.3 – 5.3.5, pp. 184 – 198) were compared for genotypic effects. In the first iteration (3 mth), a Student's t-test revealed that the Tg mice were significantly lighter than the WT mice ($t=-2.294$, $p < 0.05$). The Tg mice at 12 mth was again significantly lower than the WT mice (t=-

3.111, $p < 0.05$). In the final iteration of the task at the oldest age examined (19.5 mth), again the Tg mice were significantly lighter than the WT mice (t=-3.325, $p < 0.05$).

The weights of young mice (4.5 mth) performing the T-maze (section 5.3.6, pp. 200) were also compared after the group had finished testing in the OST, having been on *ad libitum* for one week. A student's t-test revealed a significant difference between the two groups (t=-3.363, $p < 0.01$), with the Tg mice being lighter than the WT mice. This difference in weights was also observed in the older (17.5 mth) mice performing the T-maze (section 5.3.7, pp. 202). These mice had just finished testing in the 5-CSR task and were on *ad libitum* feeding for one week. Again the Tg mice were significantly lighter than the WT mice as revealed by a student's t-test (t=-3.983, $p < 0.01$).

Therefore significant differences were observed in the weights of the Tg mice compared to the WT mice. However, as these differences occurred in every task, yet the performance of the Tg2576 mice was normal on several of these tasks (see sections 5.3.1, pp. 171, 5.3.2.1, pp. 174, 5.3.2.2, pp. 176, 5.3.3, pp. 184) it is unlikely that the impairments in performance observed was due to weight differences. Graphs for a comparison of the weights of the WT and Tg mice in each task and at each age tested are located in Appendix II, pp. 257).

5.4 Discussion

In this chapter I have systematically and comprehensively examined the behavioural phenotype of the Tg2576 mouse line, the most commonly used model in AD research (Hsiao *et al.*, 1996). Identification of their impaired sustained attention and poor olfactory capabilities strengthens their use in AD research. The difficulties in crossbreeding two different transgenic mouse lines have also been highlighted. Also the identification that nicotine administration does not reduce levels of Aβ deposition in old mice (20 months of age), guides future research to administering the compound early in the disease progression.

5.4.1 Sustained attention in Tg2576 mice

Initial attempts to identify impaired sustained attention in the Tg2576 mice proved inconclusive. Acquisition and asymptotic performance (4.5 – 10.5 months; Fig. 5.3, pp. 173) clearly showed normal performance of the Tg2576 mice. The asymptotic performance of the Tg2576 mice was normal again at 16 months of age (Fig. 5.4, pp. 175). Although the numbers in each study were limited due to the high mortality of the Tg2576 mice (King and Arendash, 2002; Fig. 5.2, pp. 170), which is a disadvantage when cognitive testing and may mask experimental effects, I do not believe the group sizes affected the results of the studies. Significant effects of drugs and genotype have been observed in rodents performing the 5-CSR task with small group sizes (Hahn and Stolerman, 2002; see chapters 3.3.3, 3.3.4, pp. 86 and 88).

Modelling impaired sustained attention observed in AD patients in this mouse model would be advantageous (White and Levin, 1999; Perry and Hodges, 2000). Whilst the lack of effect observed was unlikely to reflect low group sizes, aforementioned ceiling effects may mask any group differences (see chapters 3.3.1 and 3.3.2, pp. 81 and 84). Increasing the difficulty of the task could be achieved by extending session length, a challenge not altering the task protocol, and therefore does not introduce a learning confound. This challenge simply increased the load placed on the mice according to theories on taxing sustained attention, and has been utilised in assessing sustained attention in AD patients (Nebes and Brady, 1993; Brazzelli *et al.*, 1994; Parasuraman *et al.*, 1998; Grottick *et al.*, 2003; see chapter 3.3.7, pp. 94). Whilst there was a trend towards a higher % omissions and lower proportion correct in the Tg2576 mice in this challenge (Fig. 5.5, pp. 177), this was not significant. This could possibly reflect the low group sizes utilised in these studies. A further increase in session length (50 or 60 min) may have proven effective in differentiating the performance of the two groups without needing to increase group numbers, as increasing session length from 15 to 45 min in a sustained attention task sufficiently taxed AD patients and lead to a reduction in accuracy (proportion correct; Brazzelli *et al.*, 1994). However, a further increase was not introduced in order to limit the possibility of shifts in baseline performance, as observed previously following repeated challenges (see chapter 3.3.7, pp. 94). The most consistent effect of increasing session length in Alzheimer's disease patients performing sustained attention tasks was increased response latencies, suggesting a shift in response strategy (Nebes and Brady, 1993; Brazzelli *et al.*, 1994), in order to remain as accurate as possible – known as a speed/accuracy trade-off. This suggests they could

not sustain attention as equally as their age-matched controls (Busemeyer and Townsend, 1993).

AD patients are easily distracted when performing a task (Vecera and Rizzo, 2003). Thus the Tg2576 mice were assessed for their ability to perform the 5-CSR task whilst subject to noise distractions. Various levels of noise (dB) have been used in the literature in the 5-CSR task, with 100 dB the more common (Humby *et al.*, 1999; Hahn and Stolerman, 2002). Initial levels were therefore set at 100 dB and whilst the noise slowed the performance of the Tg2756 mice in comparison to their age-matched littermates, there was no effects on % omissions or proportion correct (Fig. 5.4, pp. 175). This slowing of response, yet maintenance of proportion correct, was akin to the speed/accuracy trade-off observed in Alzheimer's disease patients (Nebes and Brady, 1993; Brazzelli *et al.*, 1994). To investigate whether noise at a dB level that elicits a startle response from TgCRND8 mice (McCool *et al.*, 2003) could separate performance Tg2576 and WT mice any further, noise distractions at 110 dB were used. Again a speed/accuracy trade-off strategy was observed by the Tg2756 mice (Fig. 5.5c, pp. 177). At this dB level there was a significant effect of the timing of the noise onset, with the performance of both groups significantly worse during trials with distracters as measured by % omissions and correct responses (section 5.3.2.4; Fig. 5.5d and e). There was also a significant genotypic/noise onset interaction, although the source of significance was not clear. However, it appears that whilst the WT mice maintained a high level of correct at each time of noise onset, the Tg2576 mice exhibited fewer correct responses the closer the time of noise onset came to the cue stimulus (Fig. 5.5e). As observed in AD patients, the

speed/accuracy trade-off could reflect an impoverished ability to sustain attention, thus switching response strategies to maintain a high degree of accuracy. The medial and antero-dorsal prefrontal cortices have been implicated in the maintenance of attention during distracters, and the shifting of response strategies (Muir *et* al., 1996; Zaborszky *et al.*, 1997; Gill *et al.*, 1999; Birrell and Brown, 2000; Sarter *et al.*, 2001). Thus the Tg2576 mice may exhibit pathology in these areas at least at 17 months old.

Whilst it could have been invaluable to assess the effects of noise distractions similarly at 10 months of age, and therefore identifying whether this impaired ability was apparent at a younger age, unfortunately I had not developed the programs and equipment at that time. Hence, whilst the results provide further evidence in support of the Tg2756 mice as a valid animal model of AD in terms of sustained attention performance, greater information on the progression of this impairment is still required. As the 5-CSR task has both selective and divided attentional components in it (Robbins, 2002), it would also be beneficial to analyse the longitudinal performance of these mice in tasks specifically assessing selective and divided attention, possibly providing further face validity for the Tg2576 transgenic mouse model of AD.

5.4.2 Working memory in Tg2576 mice

In the literature, the assessment of the attentional performance of Tg2576 mice is limited. However, the visuo-spatial working memory ability of the mice has been extensively tested. In the first publication of the cognitive performance of these

mice, Hsiao and colleagues (1996) identified that Tg2756 mice exhibited age-related (post Aβ deposition) impairment of visuo-spatial working memory as assessed by the MWM (see chapter 1.4.4.1, pp. 39). This effect was reproduced by Chapman and colleagues (1999), who also identified an age-related impairment in long-term potentiation in the dentate gyrus and CA1 regions of the hippocampus, therefore identifying a neuroanatomical basis for impaired learning and memory. In contrast, other research groups have had difficulty in reproducing these visuo-spatial working memory deficits, with King and colleagues (1999) observing impaired performance in mice as young as 3 months, whereas Westerman and colleagues (2002) did not observe any impairments until 15 months, 5 months later than the impairments observed by Hsiao and colleagues (1996). When visuo-spatial learning and working memory was assessed in a dry land version of the MWM, the Barnes circular platform test, King and colleagues (1999) observed impaired performance at 3, but not 9 months, whilst the same group later found no differences at any age tested (5 mth to 8.5 mth; Arendash *et al.*, 2004). These apparently conflicting reports on the memory capabilities of the Tg2576 mice also occur within other transgenic mouse models of Alzheimer's disease, such as the PDAPP mice (Games *et al.*, 1995; Chen *et al.*, 2000). This has generated some debate on the possible source(s) of these differences. Whilst procedural differences between laboratories could be a factor, some have suggested that the mice suffer from both age-related and age-unrelated cognitive impairments, whilst others suggest the tasks used are too insensitive to detect subtle cognitive deficiencies (Hsiao *et al.*, 2001; Westerman *et al.*, 2002; Arendash *et al.*, 2004).

Gerlai and Clayton (1999), and Slonick (2001), both recommend the use of ethologically relevant stimuli when attempting to identify differences between groups of rodents, especially in transgenic mice when differences in perofrmance may be subtle. The OST represents a simple, easy to run task that assesses working memory capacity by utilising ethologically relevant stimuli. I have already shown that the task is sensitive enough to detect differences as following both pharmacological and genetic manipulations (chapters 4.3.2 – 4.3.5, pp. 128 – 142). Also as the task utilises olfactory stimuli, it might be capable of detecting the effects of Aβ deposition before many other tasks, as the histopathological changes in both humans and mice begin to occur first in the entorhinal cortex, part of the main olfactory cortex (Fig. 4.2, pp. 122), the area of greatest $A\beta_{(1-42)}$ plaque burden in Tg2576 mice (Braak and Braak, 1995; Slotnick, 2001; Nordberg *et al.*, 2002). This hypothesis is supported by the present data. The Tg2576 mice exhibited an age-related learning impairment in the OST. Young (3 - 4 month old) Tg2576 mice acquired the task as readily as their age-matched littermates, whilst older (10.5 – 11.5 month) Tg2576 mice took significantly longer to acquire the task compared to both their age-matched littermates and the young Tg2576 mice (Figs. 5.8, pp. 186 and 5.12, pp. 191). Such age-related learning impairments have been observed elsewhere in tasks using spatial cues (Hsiao *et al.*, 1996; Chapman *et al.*, 1999). The Tg2576 mice also exhibited age-related performance deficits of the standard 12-OST. Young (4.5 months) Tg2576 mice performed as well as their age-matched littermates, and significantly better than the older Tg2576 counterparts (12 and 19.5 months), whom performed worse than their age-matched littermates (Fig. 5.16, pp. 199).

Closer inspection of the Tg2576 mice and their age-matched littermates at 4 months revealed that there appeared to be a trend towards a reduced span length and an increased error number for Tg2576 mice in the 12-OST. As both groups of mice were performing at near optimal levels, the lack of any differences may have been due to ceiling effects. When the 'ceiling' was raised by increasing the maximum number of spans to 21, the Tg2576 mice exhibited a significantly reduced span length and spans completed, increased error number and significantly longer mean span length than their age-matched littermates (Fig. 5.10, pp. 188). Further analysis of the time taken to complete individual spans in both tasks identified that whilst the mean span latency of both groups remained similar at the beginning of the tasks, by span 11 the mean span latency for each group had begun to separate, and by span 21 the differences were exacerbated, with the Tg2756 mice clearly taking longer to complete each span than their age-matched littermates (Fig. 5.11, pp. 189). This slowing with increased task length may be as a result of a speed/accuracy trade-off, or fatigue due to task length. The performance of the Tg2576 mice in the 5-CSR task would suggest the former as when the 5-CSR task length was increased to 40 min (double the time the mice spend performing the OST; see section 5.3.2.2, 173), no significant differences in latencies or total number of trials completed were observed (Fig. 5.5, pp. 177). The Tg2576 mice may therefore have been performing more slowly as the difficulty increased, in an attempt to ensure accuracy of responding. The search strategy of the two groups did not appear to differ (*personal observations* – verification could be obtained through the use of an Etho-Vision tracker as discussed earlier, chapter 4.3.2, pp. 128), but the Tg2576 mice would often bypass the correct odour and thus spend more time circling the odours.

As the Tg2576 mice also made significantly more errors than their age-matched littermates, they spent more time repeating spans, thus further limiting the time they appeared on the table. This could account for the lower number of spans completed by the Tg2576 mice as the time limit of 10 min spent on the table remained in place for the 22-OST. Although increasing the time limit might allow the Tg2576 mice to complete the same number of spans as their littermates, the Tg2576 mice also erred significantly more often. This not only suggests a cognitive component to their impaired performance, but also that even an increase in the task time limitwould probably still result in a reduced span length. These differences in performance between the two groups were also evident when assessed at 12 months. Again the Tg2576 mice exhibited a reduced span length and an increased error number. No differences were observed in mean span latency (Fig. 5.15, pp. 197) which could reflect the general slowing of performance in the task with increased age observed elsewhere (see chapter 4.3.4, pp. 134). The differences observed in olfactory working memory performance is unlikely to be due to impoverished olfactory discriminatory abilities as the performance of both groups when required to choose the novel odour from a choice of two (span 1) was always near perfect. Whilst the mice could discriminate between two odours, their ability to identify a specific odour among many (when the signal to noise ratio is lower, i.e. there are an increasing number of non-target stimuli) may have been impaired (Marston, 1996). Requiring them to consistently pick one specific odour out of twelve could have assessed this concern.

The consistently reduced weight of the Tg2576 mice was unlikely to have affected motivational levels (collect a food reward) in the 5-CSR task and OST. No differences between the two groups were observed in mean reward latency, a putative measure of motivation (Robbins, 2002), or total trials, during performance of the 40 min 5-CSR task (Fig. 5.5, pp. 177). However a definitive measure of motivation to collect a food reward (e.g. a progressive ratio breakpoint study) could be assessed using the 5-CSR task apparatus. Progressive ratio breakpoint studies evaluate the willingness of a subject to retrieve a reward by increasing the workload required to gain that reward after every trial. Assessing the trial at which the mice will not respond a sufficient number of times to collect the reward identifies their breakpoint and hence their level of motivation to attain the reward. Identification of the breakpoint of the Tg2576 mice and their age-matched littermates would be advantageous in order to ensure altered motivational levels have not confounded the data obtained (Samson *et al.*, 1998; Grottick *et al.*, 2000).

5.4.3 Exploratory behaviour of Tg2576 mice

In their original work, Hsiao and colleagues (1996) identified an age-related (post Aβ deposition) loss of spontaneous alternation of the Tg2576 mice in the T-maze. This has been suggested to reflect a diminished desire to explore a novel situation and has been likened to the loss of curiosity observed in Alzheimer's disease patients (Daffner *et al.*, 1992; Frisoni *et al.*, 1999; Chung and Cummings, 2000; Lalonde *et al.*, 2003). Whilst the current studies did not support these findings, there was a trend for increased spontaneous alternation in the age-matched littermates but not

Tg2576 mice (Figs 5.17; pp. 201 and 5.18; pp. 203). This however was neither significant nor age-related, although it may reflect the low number of animals used in this study compared to the number used in the original T-CAT study (n = ~10 per group vs. n = ~30 per group respectively; Gerlai, 1998). More recent studies have however used fewer mice (Spowart-Manning and van der Staay, 2004). The T-CAT was chosen as opposed to the procedure used in the original study (where the mice were picked up from their chosen arm and replaced in the start arm) in order to limit the possible confounding effect of altering anxiety levels, which are known to effect spontaneous alternation behaviour (Quintero *et al.*, 1985; Belotti *et al.*, 1998; Lalonde *et al.*, 2003). That the spontaneous alternation rate of the littermate mice was not significantly above chance levels suggests that there was a lack of statistical power due to low group sizes, as both age-related and age un-related spontaneous alternation in these mice have previously been recorded (Chapman *et al.*, 1999; King *et al.*, 1999; Lalonde *et al.*, 2003).

5.4.4 Nicotinic modulation of Aβ deposition in Tg2576 mice

As discussed in section 5.2.1 (pp. 164), Aβ deposition (Fig. 5.21) was to be measured in a subset of animals in parallel to the behavioural studies. Due to the high death rate of the Tg2576, te number of animals trained in the 5-CSR task was lower than preferred and prohibited the possibility of removing mice for analysis of Aβ deposition at each stage of cognitive assessment. However, Aβ deposition in the Tg2756 transgenic mouse model of Alzheimer's disease is well characterised (Hsiao *et al.*, 1996; Kawarabayashi *et al.*, 2001). In their extensive study, Kawarabayashi

and colleagues (2001) confirmed the observations of Hsiao and colleagues (1996) with Aβ deposition levels increasing from ~ 6 months, with a considerable increase between 9 (10 pmols/g) and 12 months (500 pmols/g; see Fig. 5.22). This would suggest that the age-related worsening of performance of the Tg2576 mice in the odour span task (Fig. 5.16, pp. 199) was not as a result of Aβ deposition as impaired 22-odour performance was observed at 4 months of age (Fig. 5.10, pp. 188). However, as Aβ levels have been shown to be highest in the main olfactory system in Alzheimer's disease patients and Tg2576 mice (Braak and Braak, 1995; Nordberg *et al.*, 2002), subtle changes occurring prior to the formation of plaques may underlie the deficit in olfactory working memory at 4 months, and that the OST can pick up such subtle changes. It has been proposed that Aβ first accumulates intracellularly, which eventually undergoes lysis, resulting in the local dispersal of their cytoplasmic contents including Aβ (Wang *et al.*, 2000; D'Andrea *et al.*, 2001). Intracellular Aβ has been observed in the CA1 region of the hippocampus in a mouse model of AD at 4 months of age (Shie *et al.*, 2003). Therefore intracellular accumulation would likely have occurred within the olfactory system as well, disrupting olfactory working memory performance prior to extracellular Aβ deposition (Devanand *et* al., 2000; Nagelle *et al.*, 2002; Oddo *et al.*, 2003). Also ultrastructural changes such as dystrophic neuritis and lipid deposits have been shown to occur in the TAS10 transgenic mouse model of Alzheimer's disease prior to $A\beta_{(1-42)}$ plaque deposition (Richardson *et al.*, 2003). Surprisingly no alterations in choline acetyltransferase and anticholinesterase activity akin to that observed in Alzheimer's disease patients have been reported in the Tg2576 mice (Davies and Maloney, 1976; Perry *et al.*, 1978; Davies, 1979; Shinotoh *et al.*, 2000; Apelt *et al.*, 2002), though they do exhibit

Impaired synaptic plasticity (Chapman *et al.*, 1999). This may represent a mouse's natural resistance to $A\beta_{(1-42)}$ plaque-induced toxicity and why no neuronal loss has been observed in Tg2576 mice (Ashe, 2001). However, the cause(s) of the reduced survival rate of the Tg2576 mice have yet to be determined (King *et al.*, 1999; King and Arendash, 2002; Nordberg *et al.*, 2002; Fig. 5.2, pp. 170).

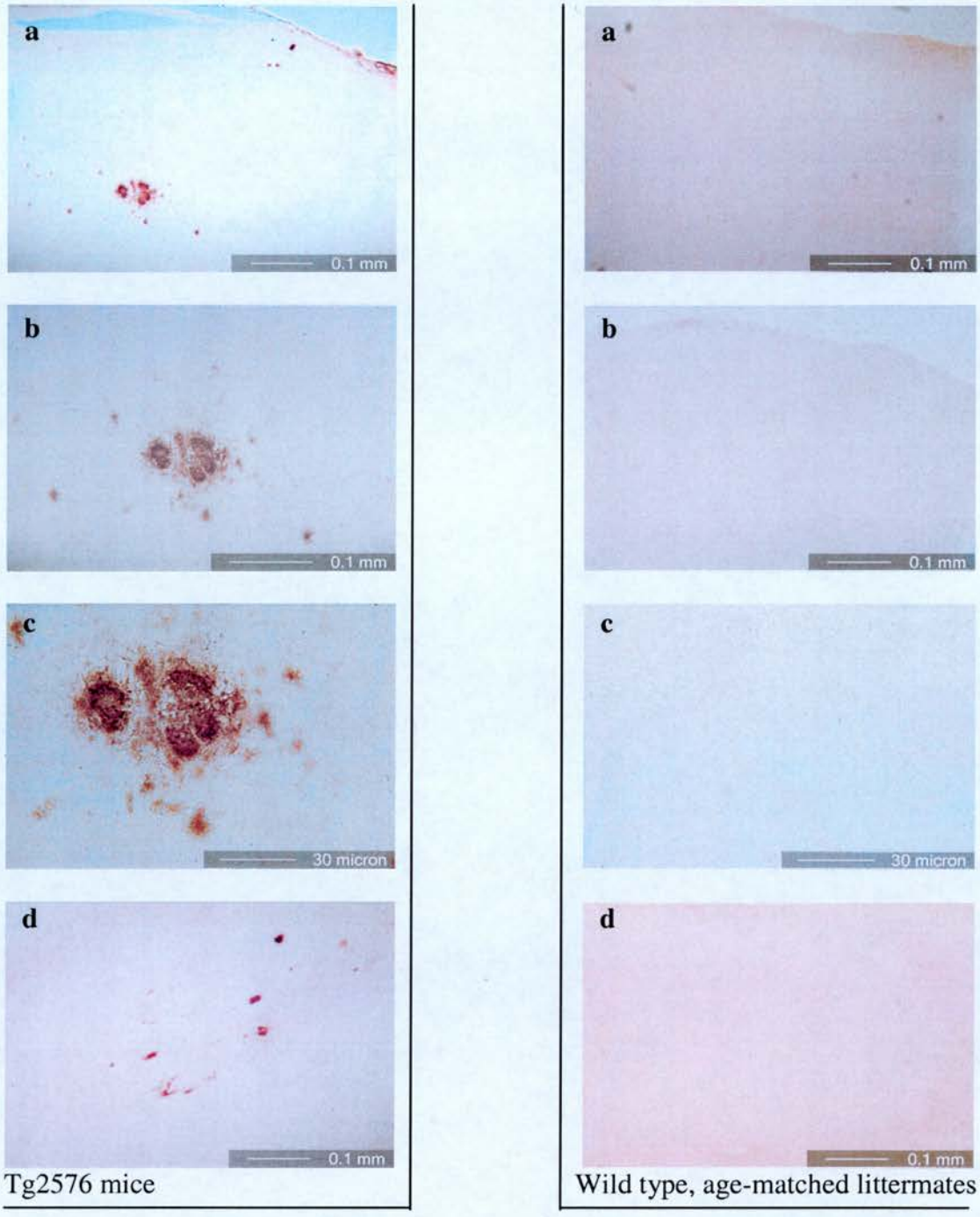


Figure 5.21: Histopathological differences of Tg2576 mice (12 mth) compared to their age-matched littermates

Histopathology of 12 mth old Tg2576 mice (panels on left) and their age-matched littermates (panels on right) was assessed. Unlike the littermate mice, Tg2576 mice exhibited Aβ deposition in the striatum at magnification x 10 (a), magnification x 20 (c), magnification x 40 (c) and in the hippocampus at magnification x 20 (d).

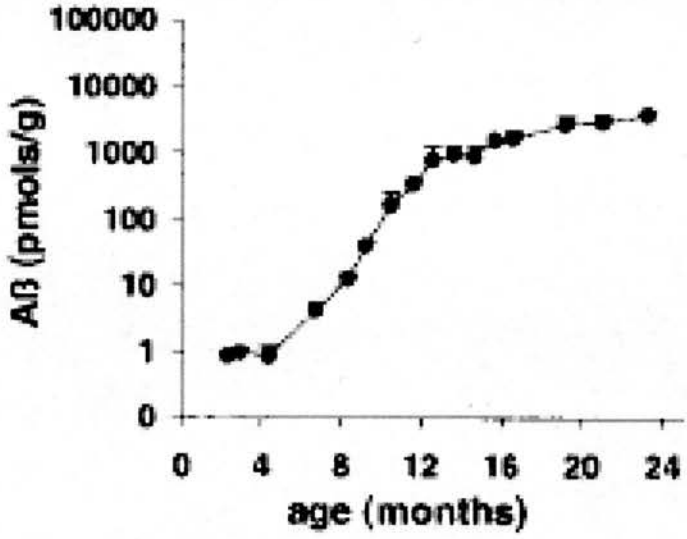


Figure 5.22: $A\beta_{(1-42)}$ plaque levels in Tg2576 mice (adapted from Kawarabayashi *et al.*, 2001)

The level of $A\beta_{(1-42)}$ plaque levels in ageing Tg2576 mouse brain is depicted. There was no detectable Aβ in mice at 2-5 months old. Deposition does not begin to occur until 6 months, after which the $A\beta_{(1-42)}$ plaque levels increase steadily.

The α7 nAChR plays a pivotal role in Wang and colleagues' (2000) hypothesis that $A\beta_{(1-42)}$ first accumulates intracellularly. To investigate whether the α7 nAChR plays a central role in Aβ deposition and/or the mediation of the effects of nicotine on Aβ reduction, attempts were made in creating Tg2576 mice on an α7 nAChR null background (Hellström-Lidahl *et al.* 2004; see section 5.3.8, pp. 204). Difficulties were encountered due to the high levels of aggression on the Tg2576 males (Moechers *et al.*, 1996; 1998), low weights (see Appendix II, pp. 257) and reduced fertility of α7 nAChR null mice (Orr-Urtreger *et al.*, 1997). This study was subsequently abandoned.

If Tg2576 mice on an α7 nAChR knockout background were created and did deposit Aβ, they could have been assessed for the ability of nicotine to reduce Aβ levels. As yet we have not shown nicotine to reduce levels of Aβ in Tg2576 mice, as has been observed previously (Nordberg *et al.*, 2002; Hellström-Lindahl *et al.* 2004; section 5.3.9, pp. 207). The lack of effect in these studies could however reflect the age differences employed in these studies. Hellström-Lindahl and colleagues (2004) examined Aβ levels in 9 month old mice after 10-day nicotine administration, observing a 77 – 85% reduction. Nordberg and colleagues (2002) examined Aβ levels in 15.5 month old mice after chronic nicotine administration, observing a 37 – 56 % reduction. In section 5.3.9 we did not observe any effects of 10-day nicotine administration on the Aβ levels of 20 month old mice (Fig. 5.20b, pp. 208).

It has been proposed that $A\beta_{(1-42)}$ binds to the α7 nAChR with picomolar affinity, preventable in the presence of more selective ligands (Wang *et al.* 2000a). Thus

nicotine administration may prevent the $A\beta_{(1-42)}$ from binding to these receptors and activating the Janus kinase 2 pathway (Shaw *et al.*, 2002). Whilst the exact mechanism is unknown, whether advanced age can block the neuroprotective effects of nicotine can be investigated by completing a similar study to section 5.3.8, but with mice at 9, 12, 15, and 18 months old. Furthermore, the co-administration of nAChR selective antagonists to a cohort in this study may establish the nAChR that mediates the nicotine-induced neuroprotection.

5.5 Conclusion

In conclusion, Tg2576 mice exhibit impaired attention in the 5-CSR task when the attentional load is increased by utilising using distracters. Moreover an age-related deficit in olfactory working memory was observed in the Tg2576 mice when compared with their age-matched littermates. The OST has therefore proven to be a valuable tool in assessing the age-related cognitive decline in Tg2576 mouse model of Alzheimer's disease and could be used to assess the effects of drug administration designed at improving cognitive functioning in Alzheimer's disease patients.

Chapter 6 - General Discussion

6.1 The α7 nAChR and nicotine-induced improvements in cognition

This thesis provides further evidence that nicotine can improve cognitive performance in a variety of domains. Improved sustained attention and olfactory working memory post nicotine administration have not previously been observed in mice, and emphasise the ability of nicotine to improve cognition across a variety of species. The results also provide evidence to support the α7 nAChR as a valid therapeutic target for the treatment of cognitive impairment, as mice lacking this receptor exhibited sustained attention and olfactory working memory deficits. Since the pattern of working memory impairment observed in the α7 nAChR KO mice could be interpreted as the result of a deficit in sustained attention, the hypothesis that modulation of attention via nAChRs can impact on all areas of cognition is in agreement with Newhouse and colleagues (2004; Fig. 4.1, pp. 118). As both schizophrenic and Alzheimer's disease patients suffer from a wide range of cognitive deficits, a compound that globally alleviates these deficits would be highly beneficial (see chapter 1.3, pp. 20). Whilst nicotine has been shown to improve cognition and symptomatology in a variety of diseases including schizophrenia and Alzheimer's disease, its side-effect profile discussed previously (chapter 1.3.6 pp. 26) limits its potential role as a therapeutic agent (Waldum *et al.*, 1996; Levin, 2002; White and Levin, 2004; Yildiz, 2004). Thus the identification of the pathway/receptor(s) nicotine acts through to improve cognition, should enhance the possibility of generating more selective agonists with a limited side-effect profile (Levin, 2002).

The α7 nAChR has received a great deal of interest as a possible target underlying the nicotine-induced improvement in cognition, and has led to several companies developing agonists for this receptor. These compounds include an (E)-N-methyl-5 (2-pyridinal)-4-peten-2-amine at Targacept, a 1,4-diaza-bicyclo[3.2.2]nonane-4-carboxylic acid 4-pyridin-2-yl-phenyl ester at Pfizer, and substituted-heteroaryl-7-aza[2.2.1]bicycloheptanes at Pharmacia & Upjohn. AR-R17779, a full agonist of the α7 nAChR, 3-(2,4-dimethoxybenzylidene)-anabaseine (GTS-21 or DMXBA), a partial agonist of the α7 nAChR (though a weak antagonist of the α4 β2 nAChR) and ABT-418, an agonist of the α4β2 and α7 nAChR (though affinity is lower in the latter) have also been developed (Briggs *et al.*, 1995; De Fiebre *et al.*, 1995; Kem *et al.*, 1996; Gordon *et al.*, 1998; Mullen *et al.*, 2000). The therapeutic interest in this receptor has arisen for a variety of reasons. Several lines of investigation implicate the α7 nAChR in the deficiency of schizophrenic patients to 'gate' sensory information (Adler *et al.*, 1985; Boutros *et al.*, 1991; Cullum *et al.*, 1993; Clementz *et al.*, 1997). Further to Broadbent's (1958) influential filter theory of attention, Venables (1992) suggested that this inability to cope with incoming sensory input might affect schizophrenia patients' perception of the world around them, leading to the variety of cognitive and perceptual disorders that manifest in this condition. Certainly this impairment has been associated with poor attention in these patients (Cullum *et al.*, 1993; Yee *et al.*, 1998). Administration of nicotine to these patients attenuates their impaired sensory gating, possibly reflecting why such a large percentage of schizophrenics smoke compared to the general population (Adler *et al.*, 1992; 1993; Dalack *et al.*, 1998). The levels of smoking in these patients are hypothesised to be high enough to activate the α7 nAChR (Adler *et al.*, 1993).

The α7 nAChR link to this impairment was supported by the discovery that mecamylamine administration, a non-selective nicotinic antagonist, did not block the nicotine-induced enhancement of sensory gating in these patients (Freedman *et al.*, 1994). It was surmised that the doses administered only blocked high affinity nAChRs such as α4β2 and not the low affinity α7 nAChRs, suggesting the nicotine-induced improvement was acting via α7 nAChRs (Freedman *et al.*, 1994). Further evidence of an α7 nAChR involvement in sensory gating appeared in other species. It was discovered that neither mecamylamine nor scopolamine administration (a non-selective muscarinic acetylcholine receptor antagonist) affected the ability of rats to gate sensory information (measured as the P20-N40 field potential), whereas administration of α-Bungarotoxin (α-BgTx), a selective α7 nAChR antagonist, impaired rat sensory gating (Luntz-Leybman *et al.*, 1992). Also, when Stevens and colleagues (1996; 1997) were investigating the ability of several strains of mice to sensory gate using the P20-N40 paradigm, they found that sensory gating performance negatively correlated with levels of α7 nAChRs in the CA3 region of their hippocampus. Thus DBA/2 mice exhibited the lowest levels of hippocampal α7 nAChRs and the worst ability to gate sensory information. The sensory gating of these mice could be improved by both nicotine-administration and treatment with the α7 nAChR partial agonist DMXBA, both subcutaneously or orally (Stevens *et al.*, 1998; Simosky *et al.*, 2003). The fact that relatives of schizophrenic patients exhibited deficient sensory gating suggested a genetic basis (Waldo *et al.*, 1991). The impairment has since been linked to chromosome 15q14 proximal to the locus of the α7 nAChR gene (Freedman *et al.*, 1997; Freedman *et al.*, 2001; Liu *et al.*, 2001; Tsuang *et al.*, 2001).

Clozapine, an atypical antipsychotic used for the treatment of schizophrenia, is the most effective treatment for refractory schizophrenia, and unlike other antipsychotics, has shown efficacy in attenuating the sensory gating deficit of schizophrenic patients, with paralleled clinical improvement (Kane *et al.*, 1988; 1998; Griffith *et al.*, 1995; Nagamoto *et al.*, 1999; Light *et al.*, 2000). The ability of clozapine to improve sensory gating has also been demonstrated in DBA/2 mice, an effect antagonised by α-BgTx co-administration, suggesting clozapine may attenuate the sensory gating impairment via the α7 nAChRs (Simosky *et al.*, 2003). However recent evidence suggests that clozapine blocks the open channel of nAChRs at the neuromuscular junction (Nguyen and Miledi (2002). Galanthamine is an acetylcholinesterase inhibitor used in the treatment of Alzheimer's disease (see chapter 1.2.2, pp. 12). It's ability to interact directly with nAChRs (Dr. K. Finlayson, *personal communication*), including the α7 nAChR, is now being recognised and touted as the reason why its clinical efficacy is at least equivalent to that of donepezil and rivastigmine. This is at doses which are unlikely to reach its IC_{50} value for cholinesterase inhibition in the human brain (Raskind *et al.*, 2000; Wilcock *et al.*, 2000; Samchocki *et al.*, 2003). In accordance with these observations, direct activation of nAChRs by nicotine proved to be more efficacious in improving cognition than the use of the cholinesterase inhibitor donepezil (Mumenthaler *et al.*, 2003).

Studies in other cognitive domains provide further support for the α7 nAChR in mediating the cognitive enhancing effects of nicotine. Kitagawa and colleagues (2003) recently administered DMXBA to healthy human male volunteers and found

it improved working memory, attention and episodic memory above that of baseline performance. This is akin to the effects observed with nicotine (Levin *et al.*, 1998). Arendash and colleagues (1995a), found that whilst DMXBA did not improve working memory in aged rats (22 months old), in a 17-arm RAM due to ceiling effects (~90% correct), it did improve learning and reference memory, akin to that observed with chronic nicotine administration (Arendash *et al.*, 1995a,b). DMXBA also improved eye blink conditioning in rabbits and delayed match to sample performance in rhesus macaque monkeys (Woodruff-pak *et al.*, 1994; Briggs *et al.*, 1997). Furthermore DMXBA and AR-R17779 have proven efficacious in enhancing learning in rats (Meyer *et al.*, 1997; Levin *et al.*, 1999). AR-R17779, like nicotine, enhanced long-term social recognition memory and reversed the working memory deficit induced by fimbra-fornix lesion in the RAM (Levin *et al.*, 1999; van Kampen *et al.*, 2004). Further support for the α7 nAChR in mediating the nicotine-induced improvement in RAM performance has also come from Levin and colleagues. Initially they identified that blockade of both the α7 and the α4β2 nAChR (by MLA and DHβE administration respectively) led to a working memory impairment in the RAM (Felix and Levin, 1997; Bancroft and Levin, 2000; Bettany and Levin, 2001). However, whilst nicotine co-administration could reverse the α4β2 nAChR antagonist induced impairment, it could not reverse the α7 nAChR antagonist induced impairment (Bancroft and Levin, 2000; Bettany and Levin, 2001). Hence their conclusion that α7 nAChRs mediate the nicotine-induced performance observed in this task (Bettany and Levin, 2001; Levin and Rezvani, 2002, Levin, 2002).

As yet however, improvements with nicotine or a nAChR agonist have yet to be demonstrated in the RAM without the use of a lesion or corresponding antagonist. The studies conducted within this thesis have identified mice (human caspase-3 over-expressers) that exhibit a deficit in olfactory working memory (OST performance) that is reversible upon nicotine administration, providing a model with which to assess the role of nAChRs, without the use of a surgical lesion or addition of antagonists (see chapter 4.3.5, pp. 142). This can be investigated by assessing whether selective nAChR agonists mimic the effects of nicotine, or whether selective nAChR antagonists can block the effects of nicotine.

As discussed in chapter 4.1 (pp. 118) and 4.4 (pp. 146), nAChRs appear to modulate cognition by modulating attentional processes (Newhouse *et al.*, 2004; Fig. 4.1, pp. 118). The pattern of working memory impairment observed in α7 nAChR knockout mice provides further support for this theory. However, a longitudinal measure of performance in the OST is desirable to ensure reproducibility, and to examine possible age-related effects. This theory receives support from data in this thesis. Chapter 3.3.3 (pp. 86) demonstrates the ability of 3 μg/kg dose if bicotine to improve attention by increasing accuracy (proportion correct) and reducing errors (% omissions), whilst 300 μg/kg dose enhanced accuracy but had no effect of errors. This patter of the effects of nicotine was also observed in mice performing a working memory task (OST; chapter 4.3.1, pp. 125), where the 3 μg/kg dose of nicotine appeared to enhance accuracy and reduce errors, whilst the 300 μg/kg dose of nicotine only appeared to affect responses without effecting errors. This nAChR modulation of cognition via attention is also observed in the literature as nicotine

only appears to exert cognitive enhancing effects above baseline performance in normal humans, when the task utilised is attentionally demanding. This includes the rapid visual information-processing task, Conner's continuous performance test, n-back test, or performance in a flight simulator (Wesnes and Warburton, 1984; Foulds *et al.*, 1996; Levin *et al.,* 1998; Phillips and Fox, 1998; Mumenthaler *et al.*, 2003).

Thus the main effect of nicotine appears an ability to enhance sustained attention (see chapters 3.3.3, pp. 86 and 3.3.4, pp. 88). Whilst there is a large body of support for the α7 nAChR mediating the cognitive enhancing effects of nicotine (see above), this support is limited within the cognitive domain of sustained attention. DMXBA administration to schizophrenic patients and normal healthy control volunteers results in improved cognitive functioning in a variety of domains including sustained attention (Kitagwa *et al.*, 1998; Kem, 2000; Kitagwa *et al.*, 2003). However the α4β2 nAChR agonist ABT-418 has also been reported to improve the cognitive performance of Alzheimer's disease patients, although not in healthy aged humans (Potter *et al.*, 1998). ABT-418 has a similar affinity to nicotine for nAChRs, both exhibiting a 500-fold greater selectivity for the α4β2 nAChR compared to the α7 nAChR (Damaj *et al.*, 1995). Also as with nicotine, ABT-418 exhibits neuroprotective properties (Donnelly-Roberts *et al.*, 1996; see chapter 5.4, pp. 211). However, it was blockade of α7 nAChRs, not α4β2 nAChRs that antagonised the neuroprotective effect of ABT-418, suggesting ABT-418 may also act via the α7 nAChR, despite its low affinity for this receptor (Donnelly-Roberts *et al.*, 1996).

Further uncertainty over the nAChR that mediates the effects of nicotine on sustained attention comes from investigation of the performance of rats in the 5-CSR task (see chapter 3.4, pp. 105). Grottick and Higgins, (2000), first described the action of selective nicotinic agonists and antagonists on rat 5-CSR task performance. They showed that AR-R17779, unlike the α4β2 agonist SIB 1765F, did not mimic the nicotine-induced improvement in performance of rats who had not reached task criteria. However, Grottick and Higgins (2002), also discussed the difficulty they experienced in producing nicotine-induced improvement in rats that had acquired the 5-CSR task and have consistently been unable to reproduce the ability of nicotine to improve standard baseline performance, as is observed in humans (Wesnes and Warburton, 1984; Foulds *et al.*, 1996; Levin *et al.*, 1998; Mumenthaler *et al.*, 2003). Therefore the effect observed with nicotine in rats might not reflect that of sustained attention. As the effect was observed in poor performers, the apparently α4β2-nAChR mediated improvements in sustained attention could reflect improved learning. However, their use of only one dose of AR-R17779 (20 mg/kg) has led others to question their conclusions, as van Kampen and colleagues (2004), recently showed that AR-R17779 to be effective at 1 mg/kg in a social memory task. After establishing a replicable nicotine-induced delay of the vigilance decrement in aged (24 months) rats tested in the 5-CSR task for 40 min instead of the standard 30 (as also used in this thesis, see chapters 3.3.7, pp. 94 and 5.3.2.3, pp. 178), Grottick and colleagues (2003) again only administered one dose of AR-R17779 (20 mg/kg). Hahn and colleagues (2003) also showed that AR-R17779 was unable to mimic the nicotine-induced improvement in performance of rats in the 5-CSR task (Hahn and Stolerman, 2002; see chapter 3.4, pp. 105). The protocol they developed in order to

consistently observe nicotine-induced improvements in rat 5-CSR task performance, involved altering the task protocol during drug administration by increasing the inter-trial interval (ITI) during that session, with a concomitant increase in the session length (Hahn *et al.*, 2002). Whilst this challenge is based on the attentional theory of reduced event rate, the rats are still required to modify their performance in order to respond accurately (Parasuraman *et al.*, 1998). This may confound results whereby an enhanced adaptability to the new protocol would represent improved performance, thus any beneficial effect seen with nicotine may occur via its abilities to enhance learning (Levin *et al.*, 1999; Brown *et al.*, 2000). Differing effects in response to task challenges as opposed to standard performance has also been observed in lesion studies, whereby quinolinic acid lesions to the antero-dorsal prefrontal cortex does not impair baseline performance, but does impair responses to subsequent challenges (Muir *et al.*, 1996). Finally, the only agonist both groups have used is AR-R17779 (Grottick and Higgins, 2000; Hahn *et al.*, 2003; Grottick *et al.*, 2003). Despite both AR-R17779 and DMXBA being selective for the α7 nAChR, DMXBA improved sensory gating in both DBA/2 mice and Sprague dawley rats, whereas AR-R17779 did not (Stevens *et al.*, 1998; Schreiber *et al.*, 2002; Simosky *et al.*, 2003). Also the chemical stability of AR-R17779 has recently been questioned (Dr. K Finlayson, *personal communication*). This has also been reflected in the literature whereby Hahn and colleagues (2003a) reported a four-fold lower affinity of AR-R17779 to the α7 nAChR than its original publication (Mullen *et* al., 2000).

The protocol developed in this thesis (see chapter 3.3.3, pp. 86 and 3.3.4, pp. 88), for nicotine-induced improvement in baseline mouse performance of the 5-CSR task,

may offer greater predictive validity than those developed in the rat, as it more closely mimics the nicotine-induced improvement observed in human sustained attention tasks (Wesnes and Warburton, 1984; Foulds *et al.*, 1996; Levin *et al.,* 1998; Kitagawa *et al.*, 2003; Mumenthaler *et al.*, 2003). Its development also allows the use of both pharmacological and genetic manipulations concurrently in attempting to identify the nAChR that mediates the improvements observed with nicotine (Chapman, 2000; Levin, 2002; Ohno *et al.*, 2002). However, using this new protocol in combined pharmacological and genetic studies may first require increased knowledge of the dose range of nicotine, to ensure the optimal dose is used as a positive control. Identifying the optimal dose would simply require a large group of mice to be trained in the task and administered nicotine over a lower dose range e.g. 0.1, 0.3, 0.5, 1, 3, 5, and 10 μg/kg. The optimal dose identified could be used in a repeat of the experiment described in chapter 3.3.6 (pp. 92), to assess whether nicotine can improve performance of the α7 nAChR knockout mice as well as their age-matched controls. Larger groups would again be required for this study to ensure the administration protocol follows that used in the positive nicotine studies, and hence both knockout and littermate mice could be split evenly into two groups receiving both saline and nicotine (chapter 3.3.3, pp. 86 and 3.3.4, pp. 88). Considering the sensory gating deficit of DBA/2 mice and its attenuation by nicotine, training and testing these mice in this established 5-CSR task protocol, and other mouse strains assessed by Stevens and colleagues (1996; 1997; St/b, C3H, C57/Bl, BALB, BUB/Ibg, DBA/1J and BUB/J mice), may give further insight into how well sensory gating and sustained attention performance correlate with one another. Other nAChR transgenic mice could also be trained and tested, providing more

information as to the identity of the nAChR(s) that mediate the beneficial effects of nicotine. Whilst testing β2 nAChR subunit knockout mice would prove problematic due to deficits in the development of their visual system, α4 nAChR subunit knockout mice could be tested, although again care would need to be taken in interpreting any results due to their increased anxiety levels, which can impact on attentional performance (Ross *et al.*, 2000).

While the α4β2 nAChR cannot be discounted in mediating the beneficial effects of nicotine, it would be therapeutically preferable if it were mediated by the α7 nAChR. There is evidence suggesting that α4β2 nAChRs, not α7 nAChRs, mediate many of the unwanted side-effects of nicotine. For example β2 nAChR subunit knockout mice do not self-administer nicotine as often as their littermate controls, whereas α7 knockout mice do, suggesting the β2 but not the α7 subunit is required for the rewarding effects of nicotine (Picciotto *et al.*, 1998; Lena and Changeux, 1999; Stolerman *et al.*, 2004). Antagonist ligand studies in mice (DHβE and MLA), and agonist ligand studies in rats (SIB 1765F and AR-R17779) also support a role for the α4β2, but not the α7 nAChR, in the rewarding properties of nicotine (Gommans *et al.*, 2000; Grottick *et al.*, 2000). Van Haaren and colleagues (1999) found that DMXBA did not substitute for the nicotine cue, providing further support using a different α7 nAChR ligand that the α7 nAChR does not contribute to the nicotine cue or self-administration (Briggs *et al.*, 1997). Also, it has been suggested that DMXBA administration did not substitute for, nor did MLA antagonise, the analgesic and anxiolytic effects of nicotine, suggesting that these responses are not mediated by the α7 nAChR (Decker *et al.*, 1995; Rao *et al.*, 1996). Finally DMXBA

administration does not appear to worsen cardiovascular function (Van Haaren *et al.*, 1999). Therefore any α4β2 nAChR agonists developed would likely share many of the adverse side-effects of nicotine that my research is attempting to avoid, whereas it appears an α7 nAChR agonist would not. The protocols developed in this thesis provide an opportunity both to identify the nAChR responsible for the beneficial effects of nicotine, but also to assess putative cognitive enhancing compounds. The data produced provides further support for the α7 nAChR as a valid therapeutic target for the production of cognitive enhancing compounds.

6.2 Neuro-anatomical pathway of nicotinic-induced improvement

As previously discussed, it has been proposed that nicotine exerts its beneficial effect on cognitive processing via enhancement of attention (Mancuso *et al.*, 1999; Lawrence *et al.*, 2002; Newhouse *et al.*, 2004; see chapters 3.1, pp. 76, 4.1, pp. 118 and Fig. 4.1, pp. 119). Mancuso and colleagues (1999) hypothesised that nicotine improves cognitive function by 'locking the brain into the attentional processing mode so there are fewer lapses in attention'. The locus and pathway of the 'attentional processing mode' Mancuso and colleagues (1999) speak of has been the subject of much debate. The neuropsychological model of attention proposed by Posner and Petersen (1990; Fig. 6.1) has received considerable interest. It has gained support from various research domains including single-unit recordings in monkeys, functional imaging, neuropsychological testing of brain lesioned patients, and lesioned animal performing attentional tasks (Posner *et al.*, 1984; Bickford and Wear, 1995; Muir *et al.*, 1995; Coull *et al.*, 1996; McGaughy *et al.*, 1996; Muir *et al.*, 1996; Everritt and Robbins, 1997; Nobre *et al.*, 1997; Cabeza and Nyberg, 2000; Inglis *et al.*, 2001).

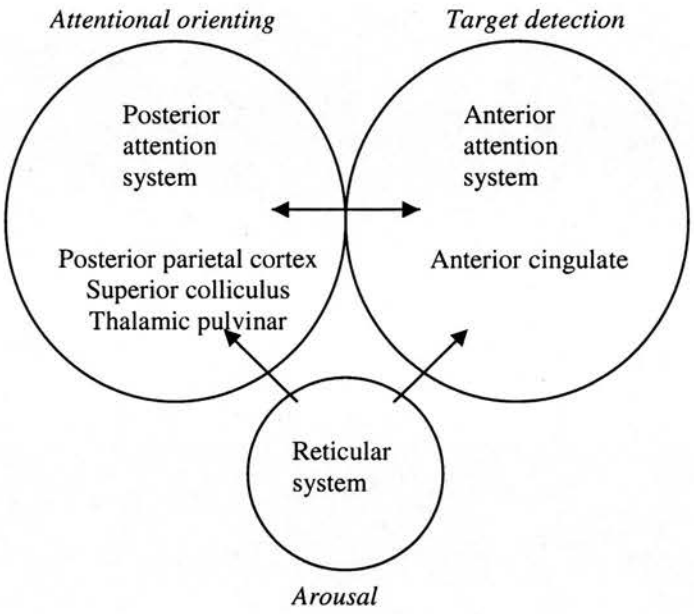


Figure 6.1: Neuropsychological model of attention

Posner and Petersen (1990) proposed the attentional network could be divided into two subsystems. The posterior attentional system is involved in the detection of target stimuli driven by the salient features of the stimulus (bottom-up processing) such as hearing your name mentioned elsewhere whilst engaged in a separate conversation. The anterior attentional system is involved in the orientation to visual locations via knowledge driven mechanisms (top-down processing) such as finding Waldo in a 'where's Waldo?' cartoon. Arousal state can affect both these systems.

There is considerable evidence that the cholinergic system influences this model. For example the ascending cholinergic system (Ch4 in Fig. 6.2) from the basal forebrain that projects to most of the cortex, has been shown to be essential for sustaining attention (Muir *et al.*, 1995; McGaughy *et al.*, 1996; Breese *et al.*, 1997; Everitt and Robbins, 1997). Lesions to the pedunculopontine tegmental nucleus (cholinergic pathway Ch5 in Fig. 6.2) cause deficits in baseline sustained attention (Inglis *et al.*, 2001; see chapter 3.3.9, pp. 103). Further evidence for the involvement of acetylcholine in maintaining attention is the increased endogenous levels observed in the cortex during performance of a sustained attention task (Himmelherber *et al.*, 2000; Passetti *et al.*, 2000; Dalley *et al.*, 2000), where distracting stimuli cause further increases in levels (Himmelherber *et al.*, 2000).

The role of acetylcholine and the cholinergic pathways in the maintenance of attention is now widely accepted (Baxter and Chiba, 1999; Sarter *et al.*, 2001; Lucas-Meunier *et al.*, 2003). Nicotine-induced improvements in attention are proposed to act on one or several of these pathways. However, these pathways can also be influenced by other neurotransmitter systems. For example glutamatergic projections on the medial prefrontal cortex have been implicated in the modulation of the basal forebrain cholinergic projections, possibly acting as a filter for distracters, changes in task protocols and shifts in attentional sets (Muir *et al.*, 1996; Miner *et al.*, 1997; Birrell and Brown, 2000; Sarter *et al.*, 2001). This role of the medial prefrontal cortex in response to a changing environment and rules, also implicates it in supporting the central executive of Baddeley's (1986) model of working memory (see chapter 4.1, Fig. 4.1; pp. 119).

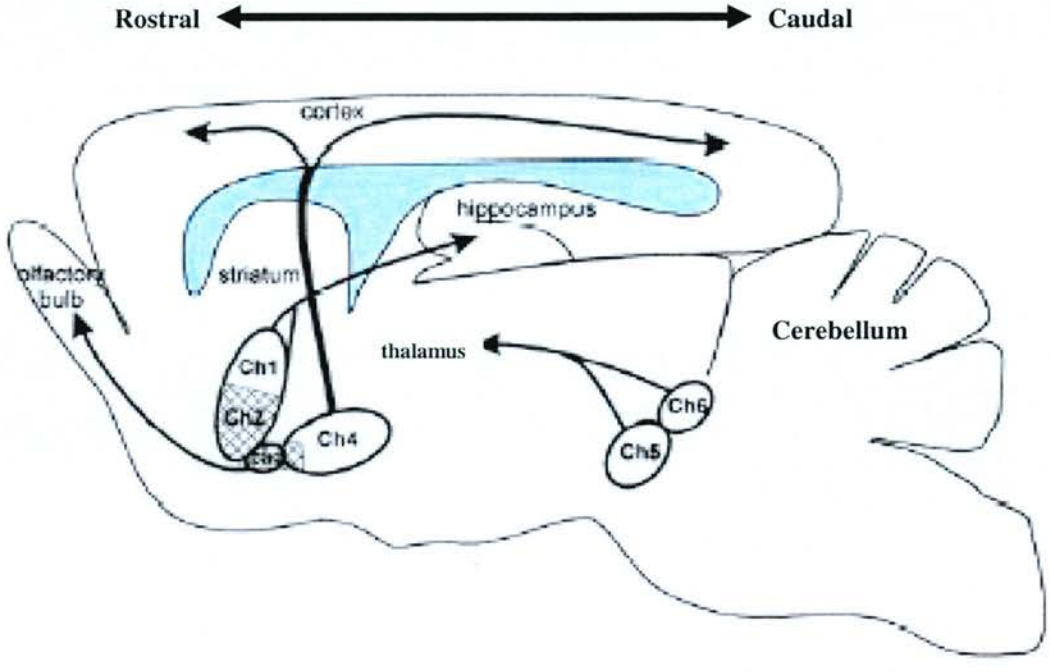


Figure 6.2: Rat cholinergic pathways (from Lucas-Meunier *et al.*, 2003)

Cholinergic nuclei from the septum (Ch1) and the vertical limb of the diagonal band (Ch2) project to the hippocampus. Those from the horizontal limb of the diagonal band (Ch3) project to the olfactory bulb. Cholinergic cortical innervation is mediated via the rodent homologue of the human nucleus basalis of Meynert (Ch4), whilst cholinergic nuclei from the pedunculopontine tegmental nucleus (Ch5) and laterodorsal tegmental nucleus (Ch6) project on the thalamus.

Nicotine administration produces a long lasting and near instantaneous enhancement of glutamatergic transmission, suggesting an alternative route for the improved performance observed in the rat 5-CSR task discussed above (Radcliffe and Dani, 1998; Turchi and Sarter, 2001; Hahn and Stolerman, 2002). However, a role for GABAergic transmission in the basal forebrain, in the maintenance of visual signal detection and inhibition of responses to non-signals (part of the top-down processing sub-system of Posner and Peterson's (1990), model of attention; see Fig. 6.1) has been also been supported (Holley *et al.*, 1995; Moore *et al.*, 1995). The activation of α7 nAChRs has been shown to enhance the release of GABA from GABAergic interneurones, via a pre-synaptic calcium dependent mechanism, and may therefore modulate the role of GABA in top down processing (Albuquerque *et al.*, 1998; Frazier *et al.*, 1998). As discussed above however, the state of arousal can alter the level of both top-down and bottom-up attentional processing (Fig. 6.1), and it has been proposed that nicotine may also exert its effect on attention via this arousal mechanism. Sarter and colleagues (2001) have addressed the arousal state in the model of Posner and Peterson (1990; Fig. 6.1). They suggest arousal state affects attention via the ascending noradrenergic (NA) projection from the locus coeruleus (Aston-Jones *et al.*, 1991; Smith and Nutt, 1986; Sarter *et al.*, 2001; Fig 6.3). Nicotine-induced improvements in attention were antagonised by clonidine administration, a NA agonist that reduces NA transmission (Coull *et al.*, 1997). Thus nicotine may enhance sustained attention and thus cognition by increasing cortical arousal via the ascending acetylcholine and NA projections (Lawrence *et al.*, 2002).

Evidence of the pathway by which nicotine enhances cognition has also been gleaned from studies in other cognitive domains. For example, lesion to the fimbra-fornix in rats, disrupting the septohippocampal cholinergic pathway (Ch1 and diagonal band of Ch2, see Fig. 6.2), an area of high α7 nAChR density leads to sensory gating and RAM deficits, that can be attenuated by nicotine and AR-R1779 administration (Levin *et al.*, 1993; Bickford and Wear, 1995; Beerse *et al.*, 1997; Levin *et al.*, 1999). Therefore whilst the septohippocampal cholinergic pathway is required for baseline performance in these two tasks, it is unlikely to mediate the nicotine-induced improvement in cognition. Also DMXBA improved passive avoidance learning in rats with lesions of the nucleus basalis magnocellular (cholinergic pathway Ch4, see Fig. 6.2), suggesting this structure is not part of the pathway by which nAChR agonists improve performance (Meyer *et al.*, 1994). Whilst Levin and colleagues demonstrated that the α7 nAChR mediated the nicotine-induced improvement in RAM performance (discussed above), they also spent time attempting to identify the pathway responsible for this improvement. While septohippocampal lesions impaired RAM performance, they can be attenuated by nicotine and AR-R17779 administration, suggesting the septohippocampal pathway may not the critical site of action for nicotinic mediated improvement in performance (Levin *et al.*, 1993; 1999). Local infusion of DHβE and MLA into the ventral hippocampus also impaired RAM performance, and whilst the former was attenuated by nicotine administration, the latter was not (Bancroft and Levin, 2000; Bettany and Levin, 2001). Therefore both ventral hippocampal α4β2 and α7 nAChRs are important for baseline spatial working memory function, but it is the α7 nAChRs that are critical for nicotine-induced improvements (Levin, 2002).

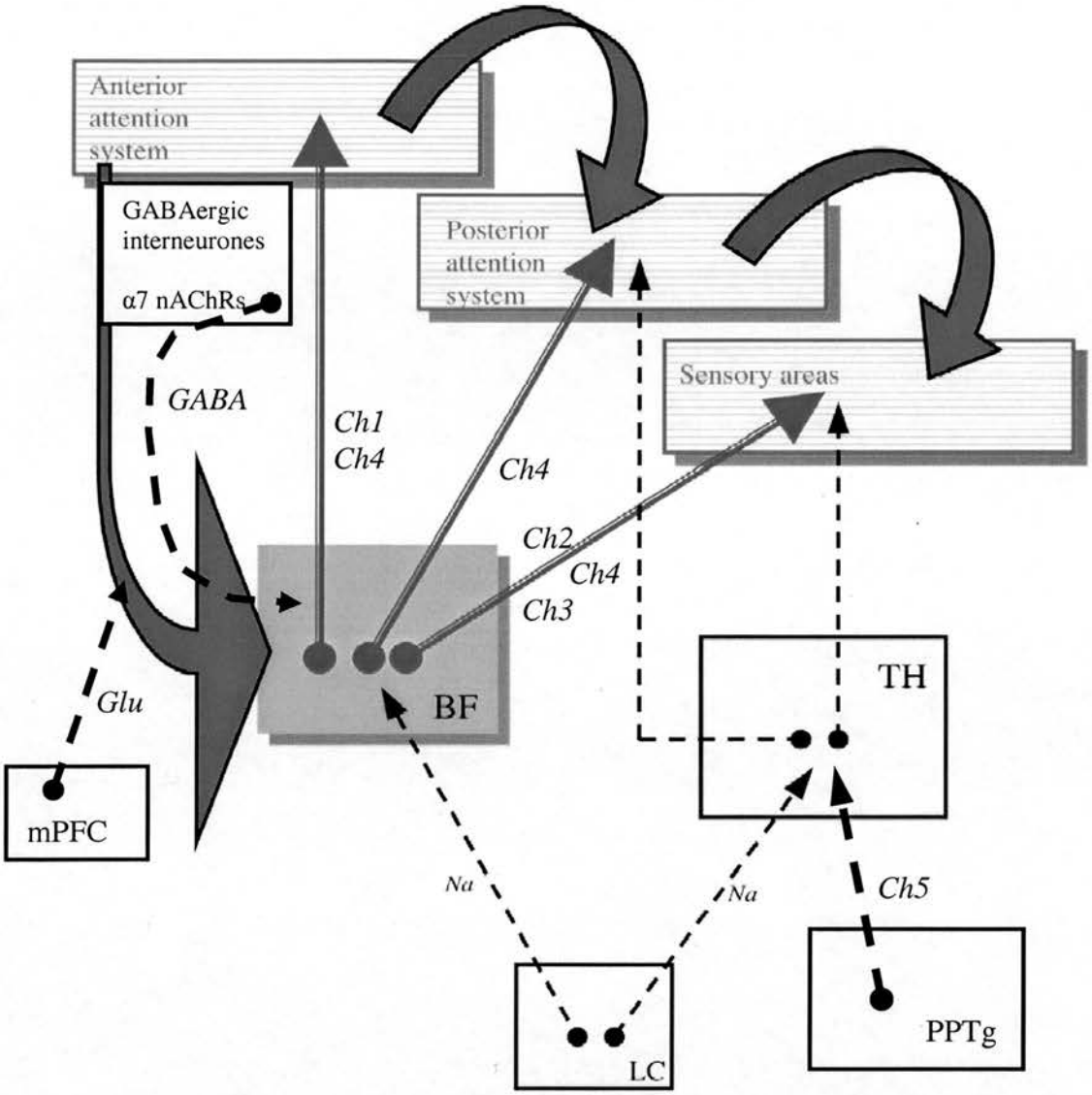


Figure 6.3: Schematic of transmitter systems influencing Posner and Petersons (1990) network model of attention (adapted from Sarter *et al.*, 2001)

The schematic represents a merger of ideas, placing the cholinergic pathways as central to Posner and Peterson's model of attention. The basal forebrain cholinergic neurones (BF) are proposed to act on the anterior attentional system using the cholinergic pathways (Ch1 and Ch4), which modulates top-down the functions of posterior cortical areas, enhancing and biasing posterior attentional system, sensory areas and the BF through sensory-associational regions (red curved arrows). Furthermore, cholinergic inputs (Ch4) also mediate the facilitation of bottom-up sensory information processing. The ability of arousal-inducing stimuli to trigger attentional processing is mediated bottom-up largely via noradrenergic (Na) projections originating in the locus coeruleus (LC) and terminating in the thalamus (TH) and the BF, although thalamic stimulation from the pedunculopontinus nucleus (PPTg) via Ch5 is also possible. Medial prefronto-cotical (mPFC) modulation of the BF via glutamate (Glu) transmission is thought to affect filtering of distracters, or modifying performance to new protocols. α7 nAChR release of GABA from GABAergic internuerones acts on the BF to maintain visual signal detection.

6.3 Use of animal models to assess putative cognitive enhancing compounds

Even with the identification of a therapeutic target (α7 nAChR), coupled with a known pathway and an observed improvement, preclinical assessment of novel compounds are still required. Thus the efficacy of any putative therapeutic compound requires testing in a suitable animal model of the disease. However, before the compound can be tested, steps must be taken to ensure that the animal model chosen is suitable. As discussed in chapter 5.1 (pp. 156) the model chosen is dependant upon the research direction, as treatment directed at the pathology of a disease clearly requires a model exhibiting that pathology, i.e. **face validity**. However, treatments directed at the symptomatology of the disease, such as cognitive impairment, would be first required to exhibit equivalent symptomatology, indicative of pathological face validity. The ability of current treatments of the disease to antagonise the pathology or symptomatology of the animal model would represent that model's **predictive validity**. The **construct validity** of a model is indicative of the degree with which the origin of the model is responsible for the disease in man.

The Tg2576 transgenic mouse model of Alzheimer's disease represents the best characterised model of Alzheimer's disease, offering construct and face validity (Hsiao *et al.*, 1996; see chapters 5.1, pp. 156 and 5.4, pp. 211). To the authors knowledge the predictive validity via treatment with acetylcholinesterase inhibitors, of these and other transgenic models of Alzheimer's disease, have yet to be published. Whilst the cause of sporadic Alzheimer's disease is currently unknown,

some forms of familial Alzheimer's disease is represented by models such as the Tg2576 mice, thus exhibiting construct validity. Face validity is represented at both pathological and symptomatological levels, with Aβ plaque deposition and age-related cognitive impairments. Thus the value of the Tg2576 model lies in the high degree with which both face and construct validity have been established (Hsiao *et al.*, 1996; Chapman *et al.*, 1999; King *et al.*, 1999; Kawerabayashi *et al.*, 2001; King and Arendash, 2002; Nordberg *et al.*, 2002; see chapter 5.1, pp. 156).

Despite the Tg2576 model being the most established to date however, cognitive assessment has previously only been within the domains of spatial learning and memory, and have been plagued by different testing procedures and the use of cognitive tests that may not subtle enough to delineate differences (Hsiao *et al.*, 1996; Chapman *et al.*, 1999; King *et al.*, 1999; Hsiao-Ashe, 2001, see chapter 5.1, pp. 156). However, prior to the present studies, there has been little to no published studies of experiments in non-spatial working meory, attention, olfactory capabilities and episodic memory, despite the dominance of these cognitive impairments in Alzheimer's disease patients (discussed in chapters 1.3, pp. 20 and 5.1, pp. 156). Whilst episodic memory has still not been assessed in these mice owing to testing difficulties, their attentional, olfactory and non-spatial working memory performance was assessed.

Despite the initial difficulties in testing these mice, due to health problems, stereotypic behaviour and reduced survival rate (see chapter 5.2.2, pp. 169), attentional deficits were observed in mice at 17 months of age (chapter 5.3.2, pp.

174). Unfortunately noise distracters were not available when the attentional performance of the mice was first assessed at 10 months, and thus longitudinal attentional performance has not been addressed. However, now that a greater phenotypic understanding of these mice is available, many studies could be designed that would include large group sizes and the assessment of a longitudinal profile of their attentional capabilities.

The performance of the Tg2576 mice in the odour span task provides further support for their use in assessing putative therapeutic compounds (see chapters 5.3.3 – 5.3.5, pp. 184 – 195). The acquisition and performance deficits observed in this task were age-related (Fig. 5.14, pp. 194). The ethological relevance of the task (utilising olfactory cues and requiring the mice to dig for their reward), supports claims that such relevance is required to observe differences in the cognitive performance of transgenic mice and their littermates, when such differences may be subtle and so difficult to detect (Gerlai and Clayton, 2000; Hsiao-Ashe, 2001). Further longitudinal analysis of the youngest group of Tg2576 mice tested in the OST (chapter 5.3.3, pp. 184) is still possible as they are only presently 7 months old (Oct '04). Assessment of OST performance of these mice at 8, 12, 16 and 20 months would test the reproducibility of the age-related decline in performance, and allow further characterisation of their longitudinal performance in this task. Reversing the attentional and/or olfactory working memory deficits of these mice with an acetylcholinesterase inhibitor would provide further evidence in support of the predictive validity of this model. Attempts to attenuate the deficits observed with nicotine or a putative cognitive enhancing compound (such as an α7 nAChR agonist)

could also be made, further establishing this model as one of the best characterised transgenic mouse models of Alzheimer's disease.

As discussed in chapter 5.4 (pp. 211), the odour span task has a two-fold advantage when assessing the performance of a transgenic mouse model of Alzheimer's disease. Firstly it characterises the non-spatial working memory capabilities of the mice using a task that is ethologically relevant, increasing the possibility of observing obscure differences in transgenic animals and their littermates (Gerlai and Clayton, 2000; Hsiao-Ashe, 2001; Slotnick, 2001). Also olfactory impairments have been postulated as a possible early marker for Alzheimer's disease (Devanand *et al.*, 2000). Patients that have been diagnosed with mild cognitive impairment (MCI, or age-associated memory impairment) are at a greater risk of developing Alzheimer's disease (Celsis, 2000; Devanand *et al.*, 2000). Those suffering from olfactory impairments have an association with the apolipoprotein E4 genotype, and are even more likely to be diagnosed with Alzheimer's disease later in life (Devanand *et al.*, 2000; Wang *et al.*, 2002). This is consistent with the observation that Alzheimer's disease pathology is present first in the entorhinal cortex, part of the main olfactory system (Braak and Braak, 1995; Slotnick, 2001; Nordberg *et al.*, 2002; see chapter 5.1, pp. 156, Fig. 4.2, pp. 122). The fact that nicotine attenuated the cognitive impairments in MCI patients, gives further weight to the work in this thesis that examined the effects of nicotine in olfactory working memory, combined with behaviourally phenotyping of the Tg2576 transgenic mouse model of Alzheimer's disease (White and Levin, 2004; see chapters 3.3, pp. 81, 4.3, pp. 125, 5.3, pp. 171).

The choice of animal model used in research is mainly determined by the aims of that research, as discussed above and in chapter 5.1 (pp. 156). The use of an animal model exhibiting both Aβ deposition and neurofibrillary tangles, such as TAPP mice (Tg2576 mice crossed with P301L mutants; Lewis *et al.*, 2001; see table 5.1, pp. 158), is possibly more representative of the pathology pf Alzheimer's disease and therefore may be more effective when assessing ways to modify that pathology. However, the physiological problems that these mice exhibit make behavioural phenotyping extremely challenging, and as such translating the results and possible side effects into human testing possibly more awkward. Unforseen side effects have already prematurely ended the clinical trials of possible immunotherapy drugs (ELAN compound AN-1792 – synthetic Aβ) against Aβ plaque deposition (Schenk *et al.*, 1999; Check, 2002). Other trials have used non-steroidal anti-inflammatories (in conjunction with COX-2 inhibitors), lithium, β-secretase inhibitors and adeno-associated viruses (Dominguez *et al.*, 2001; Hoozemans *et al.*, 2003; Phiel *et al.*, 2003; Zhang *et al.*, 2003; Coruzzi *et al.*, 2004). The ELAN immunotherapy trial was designed to reduce Aβ deposition levels in the brains of Alzheimer's disease patients (Hardy and Selkoe, 2002). In fact although the trial was prematurely terminated, it did appear that the first patients examined had reduced Aβ levels (Nichol *et al.*, 2003). The reduction of Aβ levels in transgenic mice led to a concomitant increase in cognitive performance (Janus *et al.*, 2000; Morgan *et al.*, 2000). In mice, nicotine administration over a 5.5 month or 10 day period has been shown to reduce Aβ in the Tg2576 mice and the nicotinic component confirmed as the effect was antagonised by mecamylamine (Hsiao *et al.*, 1996; Nordberg *et al.*, 2002; Hellström-Lindahl *et al.* 2004). The reduction in Aβ levels was greater when nicotine was administered

acutely for 10 days when compared to chronic administration over 5.5 months (Nordberg *et al.*, 2002; Hellström-Lindahl *et al.* 2004). This however could be due to Aβ levels being assessed at 9 and 14.5 months respectively, where the Aβ concentration is around 200 times greater in the older animals. When in the present study the assessment was carried out in 20 month old Tg2576 mice, a 10-day acute administration of nicotine had no effect on Aβ levels (chapter 5.3.8; Fig. 5.17b, pp. 201). Hence the mechanism by which nicotine-induced a reduction in Aβ levels in younger mice, but not in old mice, is yet to be determined (Hellström-Lindahl *et al.* 2004). Whilst γ secretase levels are increased in Tg2576 mice, nicotine administration had no effect on this enzyme (Hellström-Lindahl *et al.* 2004). Despite the increased levels of β secretase in the brains of Alzheimer's disease patients, the Tg2576 mice do not exhibit increased β secretase levels, and nicotine administration did not alter its expression in any way (Izzary *et al.*, 2001; Rossner *et al.*, 2001, Funkomoto *et al.*, 2002; Tyler *et al.*, 2002; Hellström-Lindahl *et al.* 2004). Galanthamine exhibits neuroprotective effects, and physostigmine administration reduces Aβ plaque levels in the neocortex of guinea pigs (Birch *et al.*, 2001). As galanthamine, physostigmine and nicotine increase levels of acetylcholine, perhaps there is a common pathway by which they reduce Aβ deposition. This effect may be mediated by acetylcholine competitively binding to nAChRs and thus inhibiting the binding of $A\beta_{(1-42)}$ (De Fiebre *et al.*, 1995; Donnelly-Roberts *et al.*, 1996; Kem, 2000).

In addition, there is a large body of evidence showing that the α7 nAChR maybe important in nicotine-induced neuroprotection. The α7 nAChR whilst widely

expressed throughout the nervous system is highly expressed in the basal forebrain cholinergic neurons that project to the hippocampus and the cortex, which is similar to the expression pattern of $A\beta_{(1-42)}$ plaque deposition in brains of Alzheimer's disease patients (Breese *et al.*, 1997; Wevers *et al.*, 1999; Guan *et al.*, 2000). It has been shown that $A\beta_{(1-42)}$ can directly modulate nAChR channel activity and block the response of the α7 nAChR in rat hippocampal slices and neurones (Liu *et al.*, 2001; Pettit *et al.*, 2001). This blockade of cholinergic neurotransmission has been presented as the possible 'missing link' between $A\beta_{(1-42)}$ plaque deposition and impaired cognition, i.e. the 'cholinergic' and 'amyloid' hypotheses (Auld *et al.*, 1998). Wang and colleagues (2000a), showed that $A\beta_{(1-42)}$ inhibited binding to α7 nAChRs. Thus a competitive α7 nAChR selective agonist may inhibit the interaction of $A\beta_{(1-42)}$ with this receptor as well as improve cognition. Nicotine and ABT-418 inhibited $A\beta_{(1-42)}$ induced toxicity *in vitro*, an effect that was blocked by an α7, but not an α4β2 nAChR antagonist (De Fiebre *et al.*, 1995; Donnelly-Roberts *et al.*, 1996; Kem, 2000). The neuroprotective effect observed following galantamine administration is also likely to be mediated via its action on α7 nAChRs (Arias *et al.*, 2004). In addition, DMXBA is neuroprotective *in vitro*, against a variety of neurotoxic insults and $A\beta_{(1-42)}$ toxicity (Akaike *et al.*, 1994; Kihara *et al.*, 1997; Shimohama *et al.*, 1997; Meyer *et al.*, 1998; Nanri *et al.*, 1998; Li *et al.*, 1999).

Rather than $A\beta_{(1-42)}$ directly interacting with the α7 nAChR, Shaw and colleagues (2002), presented another pathway that may mediate α7 nAChR induced neuroprotection. They proposed that ligands act on the α7 nAChR, to activate Janus kinase 2, which in turn inhibits the $A\beta_{(1-42)}$ induced apoptotic cascade, and thus is

neuroprotective. In the present study, the generation of Tg2576 mice on an α7 nAChR null background may have assisted in delineating which pathway was responsible. Unfortunately the breeding programme was terminated due to low fertility yet high mortality rates, high levels of aggression, and low body weights (see chapter 5.3.8, pp. 204). Examination of the effects of selective agonists and antagonists of the α7 nAChR on amyloid deposition in Tg2576 mice at various ages would help in elucidating the roles of this receptor in APP processing and neuroprotection.

6.4 Summary

In conclusion, the development of the modified 5-CSR task was central in showing a nicotine-induced improvement in sustained attention in normal mice and impairment in performance in α7 nAChR knockout and Tg2576 mice. Also, the establishment of the odour span task in mice identified impaired working memory performance in human caspase-3 over-expressing mice (attenuated by nicotine administration), α7 nAChR knockout mice and Tg2576 mice (age-related). In summary, these studies provide newly developed tasks with which to assess putative cognitive enhancing compounds and the data provides further evidence in support of a role for the α7 nAChR in nicotine-induced improvements in cognition.

Appendix I

Creation of Transgenic Lines

Alpha 7 nicotinic acetylcholine receptor (nAChR) knockout (KO) mice

The deletion mutation was introduced into the ABI 2.1 embryonic stem (ES) cell line and transmitted to the germline as described in Bullard *et al.*, (1996). Chimeric mice were backcrossed onto the C57Bl/6J strain of mice.

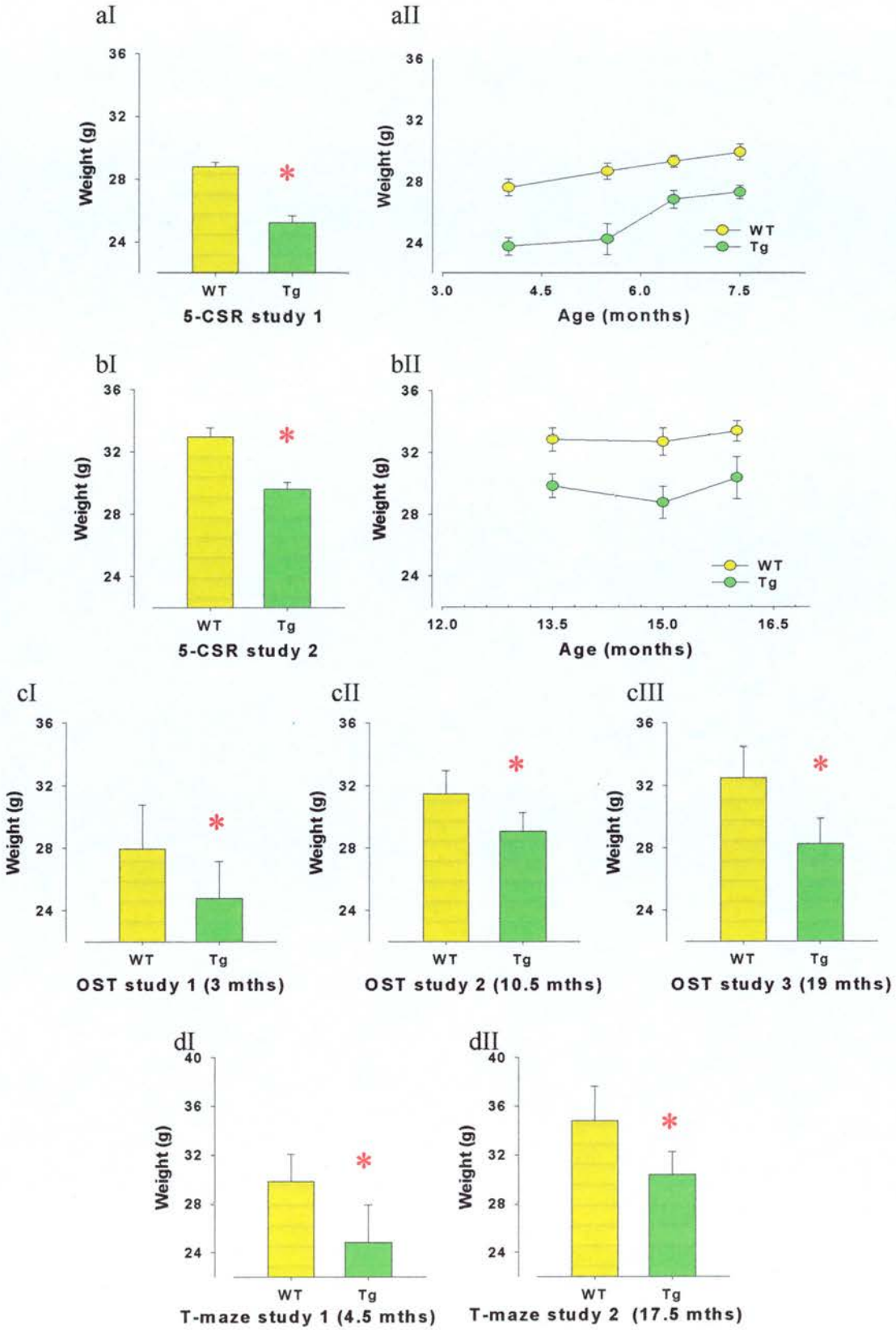
Human Caspase-3 over-expressing mice

YAC 35EB2 containing the human caspase-3 gene was isolated from transformed yeast as described in Bellis *et al.*, 1989). A total of 840 F1 C57Bl/6J X CBA fertilised eggs were microinjected with a preparation of highly purified YAC DNA (2ng/μl) yielding 89 live births (10%) of which 10 were found to be transgenic (11%). These mice were backcrossed onto the C57Bl/6J strain of mice.

Tg2576 mice

First created in 1996 by Hsiao and colleagues and based on the double mutation found in a large Swedish family with early-onset Alzheimer's disease. These mice were created by inserting Human APP695 containing the double mutation Lys670 → Asn, Met671 → Leu (K670N,M671L; APP770 numbering) into a hamster prion protein (PrP) cosmid vector in which the PrP open reading frame (ORF) was replaced with the variant APP ORF, creating Tg(HuAPP695.K670N-M671L)2576 mice. The resultant transgenic mice were bred to C57BL/6 mice. The colony was maintained by mating hemizygous males to B6SJL F1 females.

Appendix II


aI
aII
Weight (g)
36
32
28
24
WT
Tg
5-CSR study 1
3.0
4.5
6.0
7.5
Age (months)
bI
bII
5-CSR study 2
12.0
13.5
15.0
16.5
cI
cII
cIII
OST study 1 (3 mths)
OST study 2 (10.5 mths)
OST study 3 (19 mths)
dI
dII
40
T-maze study 1 (4.5 mths)
T-maze study 2 (17.5 mths)

Effects of over-expressing human APP mutation on *ad libitum weights*

The effects of over-expressing the human APPswe mutation (Tg) on weight were compared with their age-matched littermates (WT), during *ad libitum* feeding prior to and during testing. The weights were compared during 5-CSR task performance (a,b), with the overall weight difference depicted (I) as well as the weights when on periods of *ad libitum* feeding during training (II). The weights prior to the OST studies were also depicted (b) at each age tested (I – III). The weights were also compared prior to assessment in the T-CAT (c) at both young (I) and old age (II). The Tg mice were significantly lighter than the WT mice at every age and in every test, although the two groups increased in weight at a similar rate. Data presented as mean + s.e.m. (* denotes $p < 0.05$ when compared to WT).

References

Aadland,J., Beatty,W.W. & Maki,R.H. (1985) Spatial memory of children and adults assessed in the radial maze. *Dev.Psychobiol.*, **18**, 163-172.

Acri,J.B., Morse,D.E., Popke,E.J. & Grunberg,N.E. (1994) Nicotine increases sensory gating measured as inhibition of the acoustic startle reflex in rats. *Psychopharmacology (Berl)*, **114**, 369-374.

Adams,C.E., Stevens,K.E., Kem,W.R. & Freedman,R. (2000) Inhibition of nitric oxide synthase prevents alpha 7 nicotinic receptor-mediated restoration of inhibitory auditory gating in rat hippocampus. *Brain Res.*, **877**, 235-244.

Addy,N.A., Nakijama,A. & Levin,E.D. (2003) Nicotinic mechanisms of memory: effects of acute local DHbetaE and MLA infusions in the basolateral amygdala. *Brain Res.Cogn Brain Res.*, **16**, 51-57.

Adler,L.E., Waldo,M.C. & Freedman,R. (1985) Neurophysiologic studies of sensory gating in schizophrenia: comparison of auditory and visual responses. *Biol.Psychiatry*, **20**, 1284-1296.

Adler,L.E., Hoffer,L.J., Griffith,J., Waldo,M.C. & Freedman,R. (1992) Normalization by nicotine of deficient auditory sensory gating in the relatives of schizophrenics. *Biol.Psychiatry*, **32**, 607-616.

Adler,L.E., Hoffer,L.D., Wiser,A. & Freedman,R. (1993) Normalization of auditory physiology by cigarette smoking in schizophrenic patients. *Am.J.Psychiatry*, **150**, 1856-1861.

Adler,L.E., Olincy,A., Waldo,M., Harris,J.G., Griffith,J., Stevens,K., Flach,K., Nagamoto,H., Bickford,P., Leonard,S. & Freedman,R. (1998) Schizophrenia, sensory gating, and nicotinic receptors. *Schizophr.Bull.*, **24**, 189-202.

Akaike,A., Tamura,Y., Yokota,T., Shimohama,S. & Kimura,J. (1994) Nicotine-induced protection of cultured cortical neurons against N-methyl-D-aspartate receptor-mediated glutamate cytotoxicity. *Brain Res.*, **644**, 181-187.

Albuquerque,E.X., Pereira,E.F., Braga,M.F. & Alkondon,M. (1998) Contribution of nicotinic receptors to the function of synapses in the central nervous system: the action of choline as a selective agonist of alpha 7 receptors. *J.Physiol Paris*, **92**, 309-316.

Allain,H., Bentue-Ferrer,D., Tribut,O., Gauthier,S., Michel,B.F. & Drieu-La Rochelle,C. (2003) Alzheimer's disease: the pharmacological pathway. *Fundam.Clin.Pharmacol.*, **17**, 419-428.

Allen,B., Ingram,E., Takao,M., Smith,M.J., Jakes,R., Virdee,K., Yoshida,H., Holzer,M., Craxton,M., Emson,P.C., Atzori,C., Migheli,A., Crowther,R.A., Ghetti,B., Spillantini,M.G. & Goedert,M. (2002) Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein. *J.Neurosci.*, **22**, 9340-9351.

Alzheimer,A. (1907) *Algg. Z. Psychiat.*, **64**, 146-148: cited in McMenemy, W.H., (1970). Alois Alzheimer and his disease. In Alzheimer's disease, Wolstenhome, G.E.W., & O'Connor,M., (eds.).London, J & A Churchill, U.K.

Anderson,N.D., Iidaka,T., Cabeza,R., Kapur,S., McIntosh,A.R. & Craik,F.I. (2000) The effects of divided attention on encoding- and retrieval-related brain activity: A PET study of younger and older adults. *J.Cogn Neurosci.*, **12**, 775-792.

Anderton,B.H. (1999) Alzheimer's disease: clues from flies and worms. *Curr.Biol.*, **9**, R106-R109.

Andreasen,N.C., Flaum,M., Schultz,S., Duzyurek,S. & Miller,D. (1997) Diagnosis, methodology and subtypes of schizophrenia. *Neuropsychobiology*, **35**, 61-63.

Andreasen,N.C. (1997) The evolving concept of schizophrenia: from Kraepelin to the present and future. *Schizophr.Res.*, **28**, 105-109.

Apelt,J., Kumar,A. & Schliebs,R. (2002) Impairment of cholinergic neurotransmission in adult and aged transgenic Tg2576 mouse brain expressing the Swedish mutation of human beta-amyloid precursor protein. *Brain Res.*, **953**, 17-30.

Arendash,G.W., Sanberg,P.R. & Sengstock,G.J. (1995) Nicotine enhances the learning and memory of aged rats. *Pharmacol.Biochem.Behav.*, **52**, 517-523.

Arendash,G.W., Sengstock,G.J., Sanberg,P.R. & Kem,W.R. (1995) Improved learning and memory in aged rats with chronic administration of the nicotinic receptor agonist GTS-21. *Brain Res.*, **674**, 252-259.

Arendash,G.W., Gordon,M.N., Diamond,D.M., Austin,L.A., Hatcher,J.M., Jantzen,P., DiCarlo,G., Wilcock,D. & Morgan,D. (2001) Behavioral assessment of Alzheimer's transgenic mice following long-term Abeta vaccination: task specificity and correlations between Abeta deposition and spatial memory. *DNA Cell Biol.*, **20**, 737-744.

Arendash,G.W., Lewis,J., Leighty,R.E., McGowan,E., Cracchiolo,J.R., Hutton,M. & Garcia,M.F. (2004) Multi-metric behavioral comparison of APPsw and P301L models for Alzheimer's disease: linkage of poorer cognitive performance to tau pathology in forebrain. *Brain Res.*, **1012**, 29-41.

Arias,E., Ales,E., Gabilan,N.H., Cano-Abad,M.F., Villarroya,M., Garcia,A.G. & Lopez,M.G. (2004) Galantamine prevents apoptosis induced by beta-amyloid and

thapsigargin: involvement of nicotinic acetylcholine receptors. *Neuropharmacology*, **46**, 103-114.

Arias,H.R. (2000) Localization of agonist and competitive antagonist binding sites on nicotinic acetylcholine receptors. *Neurochem.Int.*, **36**, 595-645.

Ashe,K.H. (2001) Learning and memory in transgenic mice modeling Alzheimer's disease. *Learn.Mem.*, **8**, 301-308.

Aston-Jones,G., Chiang,C. & Alexinsky,T. (1991) Discharge of noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance. *Prog.Brain Res.*, **88**, 501-520.

Auld,D.S., Kar,S. & Quirion,R. (1998) Beta-amyloid peptides as direct cholinergic neuromodulators: a missing link? *Trends Neurosci.*, **21**, 43-49.

Baddeley, A., (1986) Working Memory. Oxford: Oxford Press, U.K.

Baddeley,A. & Della,S.S. (1996) Working memory and executive control. *Philos.Trans.R.Soc.Lond B Biol.Sci.*, **351**, 1397-1403.

Baddeley,A., Della,S.S., Papagno,C. & Spinnler,H. (1997) Dual-task performance in dysexecutive and nondysexecutive patients with a frontal lesion. *Neuropsychology.*, **11**, 187-194.

Baddeley,A. (2000) The episodic buffer: a new component of working memory? *Trends Cogn Sci.*, **4**, 417-423.

Baddeley,A.D., Bressi,S., Della,S.S., Logie,R. & Spinnler,H. (1991) The decline of working memory in Alzheimer's disease. A longitudinal study. *Brain*, **114 (Pt 6)**, 2521-2542.

Bales,K.R., Verina,T., Dodel,R.C., Du,Y., Altstiel,L., Bender,M., Hyslop,P., Johnstone,E.M., Little,S.P., Cummins,D.J., Piccardo,P., Ghetti,B. & Paul,S.M. (1997) Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nat.Genet.*, **17**, 263-264.

Bancroft,A. & Levin,E.D. (2000) Ventral hippocampal alpha4beta2 nicotinic receptors and chronic nicotine effects on memory. *Neuropharmacology*, **39**, 2770-2778.

Barch,D.M., Carter,C.S., Braver,T.S., Sabb,F.W., MacDonald,A., III, Noll,D.C. & Cohen,J.D. (2001) Selective deficits in prefrontal cortex function in medication-naive patients with schizophrenia. *Arch.Gen.Psychiatry*, **58**, 280-288.

Bartus,R.T. & Johnson,H.R. (1976) Short-term memory in the rhesus monkey: disruption from the anti-cholinergic scopolamine. *Pharmacol.Biochem.Behav.*, **5**, 39-46.

Bates,T., Mangan,G., Stough,C. & Corballis,P. (1995) Smoking, processing speed and attention in a choice reaction time task. *Psychopharmacology (Berl)*, **120**, 209-212.

Baxter,M.G. & Chiba,A.A. (1999) Cognitive functions of the basal forebrain. *Curr.Opin.Neurobiol.*, **9**, 178-183.

Bayles,K.A. (2003) Effects of working memory deficits on the communicative functioning of Alzheimer's dementia patients. *J.Commun.Disord.*, **36**, 209-219.

Becker,J.T., Huff,F.J., Nebes,R.D., Holland,A. & Boller,F. (1988) Neuropsychological function in Alzheimer's disease. Pattern of impairment and rates of progression. *Arch.Neurol.*, **45**, 263-268.

Becker,J.T., Lopez,O.L. & Wess,J. (1992) Material-specific memory loss in probable Alzheimer's disease. *J.Neurol.Neurosurg.Psychiatry*, **55**, 1177-1181.

Bednar,I., Zhang,X., Dastranj-Sedghi,R. & Nordberg,A. (1998) Differential changes of nicotinic receptors in the rat brain following ibotenic acid and 192-IgG saporin lesions of the nucleus basalis magnocellularis. *Int.J.Dev.Neurosci.*, **16**, 661-668.

Belotti,M., Cagniard,B., Mano,M.P. & Galey,D. (1998) Modulation of spatial alternation and anxiety by septal scopolamine systemic diazepam in mice. *Pharmacol.Biochem.Behav.*, **60**, 733-738.

Benson,D.F. (1991) The role of frontal dysfunction in attention deficit hyperactivity disorder. *J.Child Neurol.*, **6 Suppl**, S9-12.

Berman,K.F., Torrey,E.F., Daniel,D.G. & Weinberger,D.R. (1992) Regional cerebral blood flow in monozygotic twins discordant and concordant for schizophrenia. *Arch.Gen.Psychiatry*, **49**, 927-934.

Bettany,J.H. & Levin,E.D. (2001) Ventral hippocampal alpha 7 nicotinic receptor blockade and chronic nicotine effects on memory performance in the radial-arm maze. *Pharmacol.Biochem.Behav.*, **70**, 467-474.

Binetti,G., Cappa,S.F., Magni,E., Padovani,A., Bianchetti,A. & Trabucchi,M. (1996) Disorders of visual and spatial perception in the early stage of Alzheimer's disease. *Ann.N.Y.Acad.Sci.*, **777**, 221-225.

Binetti,G., Magni,E., Padovani,A., Cappa,S.F., Bianchetti,A. & Trabucchi,M. (1996) Executive dysfunction in early Alzheimer's disease. *J.Neurol.Neurosurg.Psychiatry*, **60**, 91-93.

Birrell,J.M. & Brown,V.J. (2000) Medial frontal cortex mediates perceptual attentional set shifting in the rat. *J.Neurosci.*, **20**, 4320-4324.

Biver,F., Goldman,S., Luxen,A., Delvenne,V., De,M., V, De La,F.J., Mendlewicz,J. & Lotstra,F. (1995) Altered frontostriatal relationship in unmedicated schizophrenic patients. *Psychiatry Res.*, **61**, 161-171.

Blessed,G., TomlinsoB.E., & Roth,M. (1968) Association between the quantitative measures of dementia and senile change in the cerebral grey matter of elderly subjects. *Brit. J. Psychiatr.*, 114, 797.

Bleuler, E. (1911) Dementia Praecox or the group of schizophrenias. International Universities Press, New York. Translated by Zinkin, J. (1950) from Dementia Praecox oder der gruppe der schizophrenien. In Aschaffenburg,G., (ed) Handbuch der Geisteskrankheiten. Deuticke, Leipzig: Cited in Wing, J.K. (1995) Concepts of schizophrenia., in Hirsch,S.E., & Weinberger,D.R. (eds) Schizophrenia. Cambridge University Press, Cambridge, U.K.

Block,F. (1999) Global ischemia and behavioural deficits. *Prog.Neurobiol.*, **58**, 279-295.

Blondel,A., Simon,H., Sanger,D.J. & Moser,P. (1999) The effect of repeated nicotine administration on the performance of drug-naive rats in a five-choice serial reaction time task. *Behav.Pharmacol.*, **10**, 665-673.

Bogerts,B. (1999) The neuropathology of schizophrenic diseases: historical aspects and present knowledge. *Eur.Arch.Psychiatry Clin.Neurosci.*, **249 Suppl 4**, 2-13.

Boncristiano,S., Calhoun,M.E., Kelly,P.H., Pfeifer,M., Bondolfi,L., Stalder,M., Phinney,A.L., Abramowski,D., Sturchler-Pierrat,C., Enz,A., Sommer,B., Staufenbiel,M. & Jucker,M. (2002) Cholinergic changes in the APP23 transgenic mouse model of cerebral amyloidosis. *J.Neurosci.*, **22**, 3234-3243.

Borchelt,D.R., Thinakaran,G., Eckman,C.B., Lee,M.K., Davenport,F., Ratovitsky,T., Prada,C.M., Kim,G., Seekins,S., Yager,D., Slunt,H.H., Wang,R., Seeger,M., Levey,A.I., Gandy,S.E., Copeland,N.G., Jenkins,N.A., Price,D.L., Younkin,S.G. & Sisodia,S.S. (1996) Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio in vitro and in vivo. *Neuron*, **17**, 1005-1013.

Borchelt,D.R., Ratovitski,T., van Lare,J., Lee,M.K., Gonzales,V., Jenkins,N.A., Copeland,N.G., Price,D.L. & Sisodia,S.S. (1997) Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. *Neuron*, **19**, 939-945.

Boutros,N.N., Zouridakis,G. & Overall,J. (1991) Replication and extension of P50 findings in schizophrenia. *Clin.Electroencephalogr.*, **22**, 40-45.

Bowen,D.M., Smith,C.B., White,P. & Davison,A.N. (1976) Neurotransmitter-related enzymes and indices of hypoxia in senile dementia and other abiotrophies. *Brain*, **99**, 459-496.

Braak,H. & Braak,E. (1995) Staging of Alzheimer's disease-related neurofibrillary changes. *Neurobiol.Aging*, **16**, 271-278.

Braff,D.L. & Light,G.A. (2004) Preattentional and attentional cognitive deficits as targets for treating schizophrenia. *Psychopharmacology (Berl)*, **174**, 75-85.

Brazzelli,M., Cocchini,G., Della,S.S. & Spinnler,H. (1994) Alzheimer patients show a sensitivity decrement over time on a tonic alertness task. *J.Clin.Exp.Neuropsychol.*, **16**, 851-860.

Breese,C.R., Adams,C., Logel,J., Drebing,C., Rollins,Y., Barnhart,M., Sullivan,B., Demasters,B.K., Freedman,R. & Leonard,S. (1997) Comparison of the regional expression of nicotinic acetylcholine receptor alpha7 mRNA and [125I]-alpha-bungarotoxin binding in human postmortem brain. *J.Comp Neurol.*, **387**, 385-398.

Breese,C.R., Lee,M.J., Adams,C.E., Sullivan,B., Logel,J., Gillen,K.M., Marks,M.J., Collins,A.C. & Leonard,S. (2000) Abnormal regulation of high affinity nicotinic receptors in subjects with schizophrenia. *Neuropsychopharmacology*, **23**, 351-364.

Breier,A., Su,T.P., Saunders,R., Carson,R.E., Kolachana,B.S., de Bartolomeis,A., Weinberger,D.R., Weisenfeld,N., Malhotra,A.K., Eckelman,W.C. & Pickar,D. (1997) Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. *Proc.Natl.Acad.Sci.U.S.A*, **94**, 2569-2574.

Brenner,D.E., Kukull,W.A., van Belle,G., Bowen,J.D., McCormick,W.C., Teri,L. & Larson,E.B. (1993) Relationship between cigarette smoking and Alzheimer's disease in a population-based case-control study. *Neurology*, **43**, 293-300.

Briggs,C.A., McKenna,D.G. & Piattoni-Kaplan,M. (1995) Human alpha 7 nicotinic acetylcholine receptor responses to novel ligands. *Neuropharmacology*, **34**, 583-590.

Briggs,C.A., Anderson,D.J., Brioni,J.D., Buccafusco,J.J., Buckley,M.J., Campbell,J.E., Decker,M.W., Donnelly-Roberts,D., Elliott,R.L., Gopalakrishnan,M., Holladay,M.W., Hui,Y.H., Jackson,W.J., Kim,D.J., Marsh,K.C., O'Neill,A., Prendergast,M.A., Ryther,K.B., Sullivan,J.P. & Arneric,S.P. (1997) Functional characterization of the novel neuronal nicotinic acetylcholine receptor ligand GTS-21 in vitro and in vivo. *Pharmacol.Biochem.Behav.*, **57**, 231-241.

Broad,L.M., Felthouse,C., Zwart,R., McPhie,G.I., Pearson,K.H., Craig,P.J., Wallace,L., Broadmore,R.J., Boot,J.R., Keenan,M., Baker,S.R. & Sher,E. (2002) PSAB-OFP, a selective alpha 7 nicotinic receptor agonist, is also a potent agonist of the 5-HT3 receptor. *Eur.J.Pharmacol.*, **452**, 137-144.

Brown,R.W., Gonzalez,C.L. & Kolb,B. (2000) Nicotine improves Morris water task performance in rats given medial frontal cortex lesions. *Pharmacol.Biochem.Behav.*, **67**, 473-478.

Buccafusco,J.J., Jackson,W.J., Jonnala,R.R. & Terry,A.V., Jr. (1999) Differential improvement in memory-related task performance with nicotine by aged male and female rhesus monkeys. *Behav.Pharmacol.*, **10**, 681-690.

Bunney,B.G., Potkin,S.G. & Bunney,W.E., Jr. (1995) New morphological and neuropathological findings in schizophrenia: a neurodevelopmental perspective. *Clin.Neurosci.*, **3**, 81-88.

Bunney,W.E. & Bunney,B.G. (2000) Evidence for a compromised dorsolateral prefrontal cortical parallel circuit in schizophrenia. *Brain Res.Brain Res.Rev.*, **31**, 138-146.

Burnet,P.W., Eastwood,S.L. & Harrison,P.J. (1997) [3H]WAY-100635 for 5-HT1A receptor autoradiography in human brain: a comparison with [3H]8-OH-DPAT and demonstration of increased binding in the frontal cortex in schizophrenia. *Neurochem.Int.*, **30**, 565-574.

Busemeyer,J.R. & Townsend,J.T. (1993) Decision field theory: a dynamic-cognitive approach to decision making in an uncertain environment. *Psychol.Rev.*, **100**, 432-459.

Cannon,T.D. (1996) Abnormalities of brain structure and function in schizophrenia: implications for aetiology and pathophysiology. *Ann.Med.*, **28**, 533-539.

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

Castellano,C. (1976) Effects of nicotine on discrimination learning, consolidation and learned behaviour in two inbred strains of mice. *Psychopharmacology (Berl)*, **48**, 37-43.

Caviness,J.N. (2003) Myoclonus and neurodegenerative disease--what's in a name? *Parkinsonism.Relat Disord.*, **9**, 185-192.

Celsis,P. (2000) Age-related cognitive decline, mild cognitive impairment or preclinical Alzheimer's disease? *Ann.Med.*, **32**, 6-14.

Chakos,M.H., Lieberman,J.A., Bilder,R.M., Borenstein,M., Lerner,G., Bogerts,B., Wu,H., Kinon,B. & Ashtari,M. (1994) Increase in caudate nuclei volumes of first-episode schizophrenic patients taking antipsychotic drugs. *Am.J.Psychiatry*, **151**, 1430-1436.

Chambers,R.A., Moore,J., McEvoy,J.P. & Levin,E.D. (1996) Cognitive effects of neonatal hippocampal lesions in a rat model of schizophrenia. *Neuropsychopharmacology*, **15**, 587-594.

Chan,G.C., Hinds,T.R., Impey,S. & Storm,D.R. (1998) Hippocampal neurotoxicity of Delta9-tetrahydrocannabinol. *J.Neurosci.*, **18**, 5322-5332.

Chapman,P.F., White,G.L., Jones,M.W., Cooper-Blacketer,D., Marshall,V.J., Irizarry,M., Younkin,L., Good,M.A., Bliss,T.V., Hyman,B.T., Younkin,S.G. & Hsiao,K.K. (1999) Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nat.Neurosci.*, **2**, 271-276.

Chapman,P.F. (2002) Giving drugs to knockout mice: can they do that? *Trends Neurosci.*, **25**, 277-279.

Chaussier,F. (1807) Exposition Sommaire de la Structure et des Differentes Parties de l'Encephale ou Cerveau. Paris: Theophile Barrois: cited in Levin,H.S., Eisenberg,H.M., & Benton,A.L. (1991) Frontal Lobe Function and Dysfunction. Oxford University Press, New York, U.S.A..

Check,E. (2002) Nerve inflammation halts trial for Alzheimer's drug. *Nature*, **415**, 462.

Chen,G., Chen,K.S., Knox,J., Inglis,J., Bernard,A., Martin,S.J., Justice,A., McConlogue,L., Games,D., Freedman,S.B. & Morris,R.G. (2000) A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Nature*, **408**, 975-979.

Chen,S.T., Sultzer,D.L., Hinkin,C.H., Mahler,M.E. & Cummings,J.L. (1998) Executive dysfunction in Alzheimer's disease: association with neuropsychiatric symptoms and functional impairment. *J.Neuropsychiatry Clin.Neurosci.*, **10**, 426-432.

Chiara,D.C. & Cohen,J.B. (1997) Identification of amino acids contributing to high and low affinity d-tubocurarine sites in the Torpedo nicotinic acetylcholine receptor. *J.Biol.Chem.*, **272**, 32940-32950.

Chishti,M.A., Yang,D.S., Janus,C., Phinney,A.L., Horne,P., Pearson,J., Strome,R., Zuker,N., Loukides,J., French,J., Turner,S., Lozza,G., Grilli,M., Kunicki,S., Morissette,C., Paquette,J., Gervais,F., Bergeron,C., Fraser,P.E., Carlson,G.A., George-Hyslop,P.S. & Westaway,D. (2001) Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. *J.Biol.Chem.*, **276**, 21562-21570.

Chu,C.C., Tranel,D., Damasio,A.R. & Van Hoesen,G.W. (1997) The autonomic-related cortex: pathology in Alzheimer's disease. *Cereb.Cortex*, **7**, 86-95.

Chua,S.E. & McKenna,P.J. (1995) Schizophrenia--a brain disease? A critical review of structural and functional cerebral abnormality in the disorder. *Br.J.Psychiatry*, **166**, 563-582.

Chudasama,Y. & Muir,J.L. (1997) A behavioural analysis of the delayed non-matching to position task: the effects of scopolamine, lesions of the fornix and of the prelimbic region on mediating behaviours by rats. *Psychopharmacology (Berl)*, **134**, 73-82.

Chudasama,Y. & Robbins,T.W. (2004) Psychopharmacological approaches to modulating attention in the five-choice serial reaction time task: implications for schizophrenia. *Psychopharmacology (Berl)*, **174**, 86-98.

Chung,J.A. & Cummings,J.L. (2000) Neurobehavioral and neuropsychiatric symptoms in Alzheimer's disease: characteristics and treatment. *Neurol.Clin.*, **18**, 829-846.

Clementz,B.A., Geyer,M.A. & Braff,D.L. (1997) P50 suppression among schizophrenia and normal comparison subjects: a methodological analysis. *Biol.Psychiatry*, **41**, 1035-1044.

Cochran,S.M., Kennedy,M., McKerchar,C.E., Steward,L.J., Pratt,J.A. & Morris,B.J. (2003) Induction of metabolic hypofunction and neurochemical deficits after chronic intermittent exposure to phencyclidine: differential modulation by antipsychotic drugs. *Neuropsychopharmacology*, **28**, 265-275.

Colacicco,G., Welzl,H., Lipp,H.P. & Wurbel,H. (2002) Attentional set-shifting in mice: modification of a rat paradigm, and evidence for strain-dependent variation. *Behav.Brain Res.*, **132**, 95-102.

Copolov,D., & McKinnon,A. (1998) The symptomatological complexity of schizophrenia, 5th Australasian Schizophrneia conference, Hobart, Abstract: cited in Copolov,D., Velakoulis,D., McGorry,P., Carina,M., Yung,A., Rees,S., Jackson,G., Rehn,A., Brewer,W. & Pantelis,C. (2000) Neurobiological findings in early phase schizophrenia. *Brain Res.Brain Res.Rev.*, **31**, 157-165.

Copolov,D., Velakoulis,D., McGorry,P., Carina,M., Yung,A., Rees,S., Jackson,G., Rehn,A., Brewer,W. & Pantelis,C. (2000) Neurobiological findings in early phase schizophrenia. *Brain Res.Brain Res.Rev.*, **31**, 157-165.

Cornblatt,B., Obuchowski,M., Schnur,D. & O'Brien,J.D. (1998) Hillside study of risk and early detection in schizophrenia. *Br.J.Psychiatry Suppl*, **172**, 26-32.

Cornblatt,B.A. & Keilp,J.G. (1994) Impaired attention, genetics, and the pathophysiology of schizophrenia. *Schizophr.Bull.*, **20**, 31-46.

Cornblatt,B.A. & Malhotra,A.K. (2001) Impaired attention as an endophenotype for molecular genetic studies of schizophrenia. *Am.J.Med.Genet.*, **105**, 11-15.

Coull,J.T., Frith,C.D., Frackowiak,R.S. & Grasby,P.M. (1996) A fronto-parietal network for rapid visual information processing: a PET study of sustained attention and working memory. *Neuropsychologia*, **34**, 1085-1095.

Cowan,C.M., Thai,J., Krajewski,S., Reed,J.C., Nicholson,D.W., Kaufmann,S.H. & Roskams,A.J. (2001) Caspases 3 and 9 send a pro-apoptotic signal from synapse to cell body in olfactory receptor neurons. *J.Neurosci.*, **21**, 7099-7109.

Coyle,J.T. (1996) The glutamatergic dysfunction hypothesis for schizophrenia. *Harv.Rev.Psychiatry*, **3**, 241-253.

Cullum,C.M., Harris,J.G., Waldo,M.C., Smernoff,E., Madison,A., Nagamoto,H.T., Griffith,J., Adler,L.E. & Freedman,R. (1993) Neurophysiological and neuropsychological evidence for attentional dysfunction in schizophrenia. *Schizophr.Res.*, **10**, 131-141.

Cummings,B.J., Pike,C.J., Shankle,R. & Cotman,C.W. (1996) Beta-amyloid deposition and other measures of neuropathology predict cognitive status in Alzheimer's disease. *Neurobiol.Aging*, **17**, 921-933.

Cummings,J.L. (2004) Alzheimer's disease. *N.Engl.J.Med.*, **351**, 56-67.

Czajkowski,C. & Karlin,A. (1995) Structure of the nicotinic receptor acetylcholine-binding site. Identification of acidic residues in the delta subunit within 0.9 nm of the 5 alpha subunit-binding. *J.Biol.Chem.*, **270**, 3160-3164.

D'Hooge,R. & De Deyn,P.P. (2001) Applications of the Morris water maze in the study of learning and memory. *Brain Res.Brain Res.Rev.*, **36**, 60-90.

Daffner,K.R., Scinto,L.F., Weintraub,S., Guinessey,J.E. & Mesulam,M.M. (1992) Diminished curiosity in patients with probable Alzheimer's disease as measured by exploratory eye movements. *Neurology*, **42**, 320-328.

Dalack,G.W., Healy,D.J. & Meador-Woodruff,J.H. (1998) Nicotine dependence in schizophrenia: clinical phenomena and laboratory findings. *Am.J.Psychiatry*, **155**, 1490-1501.

Dalley,J.W., McGaughy,J., O'Connell,M.T., CardinalmR,N., Levita,L., & Robbins,T.W., 2001) Distinct changes in cortical acetylcholine and noradrenaline efflux during contingent and noncontingent performance of a visual attention task. *J.Neurosci.*, **21**, 4908-4914.

Damaj,M.I., Creasy,K.R., Welch,S.P., Rosecrans,J.A., Aceto,M.D. & Martin,B.R. (1995) Comparative pharmacology of nicotine and ABT-418, a new nicotinic agonist. *Psychopharmacology (Berl)*, **120**, 483-490.

Damaj,M.I., Glassco,W., Dukat,M. & Martin,B.R. (1999) Pharmacological characterization of nicotine-induced seizures in mice. *J.Pharmacol.Exp.Ther.*, **291**, 1284-1291.

Davies,P. & Maloney,A.J. (1976) Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet*, **2**, 1403.

Davies,P. (1979) Biochemical changes in Alzheimer's disease-senile dementia: neurotransmitters in senile dementia of the Alzheimer's type. *Res.Publ.Assoc.Res.Nerv.Ment.Dis.*, **57**, 153-166.

Davis,D.H.J, Lukas,R.J., Peng,J.H., Kelly,J.S., & Finlayson,K. (2000) Characterisation of [^{3}H]methyllcaconitine binding sites in rat brain tissue and cells stably expressing the human alpha-7 nicotinic receptor. *Eur.J.Neurosci.*, **12,** 374.

Davranche,K. & Audiffren,M. (2002) Effects of a low dose of transdermal nicotine on information processing. *Nicotine.Tob.Res.*, **4**, 275-285.

Day,M., Langston,R. & Morris,R.G. (2003) Glutamate-receptor-mediated encoding and retrieval of paired-associate learning. *Nature*, **424**, 205-209.

de Fiebre,C.M., Meyer,E.M., Henry,J.C., Muraskin,S.I., Kem,W.R. & Papke,R.L. (1995) Characterization of a series of anabaseine-derived compounds reveals that the 3-(4)-dimethylaminocinnamylidine derivative is a selective agonist at neuronal nicotinic alpha 7/125I-alpha-bungarotoxin receptor subtypes. *Mol.Pharmacol.*, **47**, 164-171.

Decker,M.W., Brioni,J.D., Bannon,A.W. & Arneric,S.P. (1995) Diversity of neuronal nicotinic acetylcholine receptors: lessons from behavior and implications for CNS therapeutics. *Life Sci.*, **56**, 545-570.

Dekosky,S.T. & Scheff,S.W. (1990) Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Ann.Neurol.*, **27**, 457-464.

Dekosky,S.T., Harbaugh,R.E., Schmitt,F.A., Bakay,R.A., Chui,H.C., Knopman,D.S., Reeder,T.M., Shetter,A.G., Senter,H.J. & Markesbery,W.R. (1992) Cortical biopsy in Alzheimer's disease: diagnostic accuracy and neurochemical, neuropathological, and cognitive correlations. Intraventricular Bethanecol Study Group. *Ann.Neurol.*, **32**, 625-632.

Delacourte,A., Sergeant,N., Champain,D., Wattez,A., Maurage,C.A., Lebert,F., Pasquier,F. & David,J.P. (2002) Nonoverlapping but synergetic tau and APP pathologies in sporadic Alzheimer's disease. *Neurology*, **59**, 398-407.

Demakis,G.J. (2003) A meta-analytic review of the sensitivity of the Wisconsin Card Sorting Test to frontal and lateralized frontal brain damage. *Neuropsychology.*, **17**, 255-264.

Devanand,D.P., Michaels-Marston,K.S., Liu,X., Pelton,G.H., Padilla,M., Marder,K., Bell,K., Stern,Y. & Mayeux,R. (2000) Olfactory deficits in patients with mild cognitive impairment predict Alzheimer's disease at follow-up. *Am.J.Psychiatry*, **157**, 1399-1405.

Dickson,D.W. (2004) Building a more perfect beast: APP transgenic mice with neuronal loss. *Am.J.Pathol.*, **164**, 1143-1146.

Doll,R., Peto,R., Boreham,J. & Sutherland,I. (2000) Smoking and dementia in male British doctors: prospective study. *BMJ*, **320**, 1097-1102.

Dominguez,D.I., De Strooper,B. & Annaert,W. (2001) Secretases as therapeutic targets for the treatment of Alzheimer's disease. *Amyloid.*, **8**, 124-142.

Drachman,D.A. & Leavitt,J. (1974) Human memory and the cholinergic system. A relationship to aging? *Arch.Neurol.*, **30**, 113-121.

Drachman,D.A. (1977) Memory and cognitive function in man: does the cholinergic system have a specific role? *Neurology*, **27**, 783-790.

Dudchenko,P.A., Wood,E.R. & Eichenbaum,H. (2000) Neurotoxic hippocampal lesions have no effect on odor span and little effect on odor recognition memory but produce significant impairments on spatial span, recognition, and alternation. *J.Neurosci.*, **20**, 2964-2977.

Dunnett,S.B. (1985) Comparative effects of cholinergic drugs and lesions of nucleus basalis or fimbria-fornix on delayed matching in rats. *Psychopharmacology (Berl)*, **87**, 357-363.

Dunnett,S.B. & Martel,F.L. (1990) Proactive interference effects on short-term memory in rats: I. Basic parameters and drug effects. *Behav.Neurosci.*, **104**, 655-665.

el Mallakh,R.S., Kirch,D.G., Shelton,R., Fan,K.J., Pezeshkpour,G., Kanhouwa,S., Wyatt,R.J. & Kleinman,J.E. (1991) The nucleus basalis of Meynert, senile plaques, and intellectual impairment in schizophrenia. *J.Neuropsychiatry Clin.Neurosci.*, **3**, 383-386.

Ellenbroek,B.A. & Cools,A.R. (1990) Animal models with construct validity for schizophrenia. *Behav.Pharmacol.*, **1**, 469-490.

Ellenbroek,B.A. (2004) Pre-attentive processing and schizophrenia: animal studies. *Psychopharmacology (Berl)*, **174**, 65-74.

Elliott,R. & Sahakian,B.J. (1995) The neuropsychology of schizophrenia: relations with clinical and neurobiological dimensions. *Psychol.Med.*, **25**, 581-594.

Ernst,M., Matochik,J.A., Heishman,S.J., Van Horn,J.D., Jons,P.H., Henningfield,J.E. & London,E.D. (2001) Effect of nicotine on brain activation during performance of a working memory task. *Proc.Natl.Acad.Sci.U.S.A*, **98**, 4728-4733.

Evans,J.J., Chua,S.E., McKenna,P.J. & Wilson,B.A. (1997) Assessment of the dysexecutive syndrome in schizophrenia. *Psychol.Med.*, **27**, 635-646.

Everitt,B.J., & Robbins,T.W. (1997) Central cholinergic systems and cognition. *Annual Rev. Psychol.*, **48**, 649-684.

Faiman,C.P., de Erausquin,G.A. & Baratti,C.M. (1991) The enhancement of retention induced by vasopressin in mice may be mediated by an activation of central nicotinic cholinergic mechanisms. *Behav.Neural Biol.*, **56**, 183-199.

Felix,R. & Levin,E.D. (1997) Nicotinic antagonist administration into the ventral hippocampus and spatial working memory in rats. *Neuroscience*, **81**, 1009-1017.

Ferrier,D. (1876) The functions of the brain. London: Smith, Elder, 2nd ed. 1886: cited in Levin,H.S., Eisenberg,H.M., & Benton,A.L. (1991) Frontal Lobe Function and Dysfunction. Oxford University Press, New York, U.S.A..

Feuchteanger,E., (1923) Die Funktionen des Stirnhirns., Berlin: Springer: cited in Levin,H.S., Eisenberg,H.M., & Benton,A.L. (1991) Frontal Lobe Function and Dysfunction. Oxford University Press, New York, U.S.A..

Finlayson,K., McLuckie,J., Hern,J., Aramori,I., Olverman,H.J. & Kelly,J.S. (2001) Characterisation of [(125)I]-apamin binding sites in rat brain membranes with HE293 cells transfected with SK channel subtypes. *Neuropharmacology*, **41**, 341-350.

Foulds,J., Stapleton,J., Swettenham,J., Bell,N., McSorley,K. & Russell,M.A. (1996) Cognitive performance effects of subcutaneous nicotine in smokers and never-smokers. *Psychopharmacology (Berl)*, **127**, 31-38.

Franzen,G. & Ingvar,D.H. (1975) Absence of activation in frontal structures during psychological testing of chronic schizophrenics. *J.Neurol.Neurosurg.Psychiatry*, **38**, 1027-1032.

Fray,P.J. & Robbins,T.W. (1996) CANTAB battery: proposed utility in neurotoxicology. *Neurotoxicol.Teratol.*, **18**, 499-504.

Freedman,R., Adler,L.E., Bickford,P., Byerley,W., Coon,H., Cullum,C.M., Griffith,J.M., Harris,J.G., Leonard,S. & Miller,C. (1994) Schizophrenia and nicotinic receptors. *Harv.Rev.Psychiatry*, **2**, 179-192.

Freedman,R., Coon,H., Myles-Worsley,M., Orr-Urtreger,A., Olincy,A., Davis,A., Polymeropoulos,M., Holik,J., Hopkins,J., Hoff,M., Rosenthal,J., Waldo,M.C., Reimherr,F., Wender,P., Yaw,J., Young,D.A., Breese,C.R., Adams,C., Patterson,D., Adler,L.E., Kruglyak,L., Leonard,S. & Byerley,W. (1997) Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. *Proc.Natl.Acad.Sci.U.S.A*, **94**, 587-592.

Freedman,R., Leonard,S., Gault,J.M., Hopkins,J., Cloninger,C.R., Kaufmann,C.A., Tsuang,M.T., Farone,S.V., Malaspina,D., Svrakic,D.M., Sanders,A. & Gejman,P. (2001) Linkage disequilibrium for schizophrenia at the chromosome 15q13-14 locus of the alpha7-nicotinic acetylcholine receptor subunit gene (CHRNA7). *Am.J.Med.Genet.*, **105**, 20-22.

Freedman,R., Olincy,A., Ross,R.G., Waldo,M.C., Stevens,K.E., Adler,L.E. & Leonard,S. (2003) The genetics of sensory gating deficits in schizophrenia. *Curr.Psychiatry Rep.*, **5**, 155-161.

Friedman,J.I. (2004) Cholinergic targets for cognitive enhancement in schizophrenia: focus on cholinesterase inhibitors and muscarinic agonists. *Psychopharmacology (Berl)*.

Frisoni,G.B., Rozzini,L., Gozzetti,A., Binetti,G., Zanetti,O., Bianchetti,A., Trabucchi,M. & Cummings,J.L. (1999) Behavioral syndromes in Alzheimer's disease: description and correlates. *Dement.Geriatr.Cogn Disord.*, **10**, 130-138.

Funahashi,S. (2001) Neuronal mechanisms of executive control by the prefrontal cortex. *Neurosci.Res.*, **39**, 147-165.

Galea,L.A., Kavaliers,M., Ossenkopp,K.P., Innes,D. & Hargreaves,E.L. (1994) Sexually dimorphic spatial learning varies seasonally in two populations of deer mice. *Brain Res.*, **635** , 18-26.

Games,D., Adams,D., Alessandrini,R., Barbour,R., Berthelette,P., Blackwell,C., Carr,T., Clemens,J., Donaldson,T. & Gillespie,F. (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature*, **373**, 523-527.

Garcia,J., & Koelling,R.A. (1966) Relation of cue to consequence in avoidance learning, *Psychonomic Science*, **4**, 123-124, Cited in: Pearce,J.M. (2004) Animal Learning and cognition: An introduction, 2nd edition. Psychology Press Ltd., Hove, U.K..

Gedye,A. (1991a) Frontal lobe seizures in autism. *Med.Hypotheses*, **34**, 174-182.

Gedye,A. (1991b) Tourette syndrome attributed to frontal lobe dysfunction: numerous etiologies involved. *J.Clin.Psychol.*, **47**, 233-252.

Gentry,C.L. & Lukas,R.J. (2002) Regulation of nicotinic acetylcholine receptor numbers and function by chronic nicotine exposure. *Curr.Drug Targets.CNS.Neurol.Disord.*, **1**, 359-385.

George,T.P., Vessicchio,J.C., Termine,A., Sahady,D.M., Head,C.A., Pepper,W.T., Kosten,T.R. & Wexler,B.E. (2002) Effects of smoking abstinence on visuospatial working memory function in schizophrenia. *Neuropsychopharmacology*, **26**, 75-85.

Gerlai,R. (1998) A new continuous alternation task in T-maze detects hippocampal dysfunction in mice. A strain comparison and lesion study. *Behav.Brain Res.*, **95**, 91-101.

Gerlai,R. & Clayton,N.S. (1999) Analysing hippocampal function in transgenic mice: an ethological perspective. *Trends Neurosci.*, **22**, 47-51.

Gerlai,R. (2001) Behavioral tests of hippocampal function: simple paradigms complex problems. *Behav.Brain Res.*, **125**, 269-277.

Geyer,M.A., Wilkinson,L.S., Humby,T. & Robbins,T.W. (1993) Isolation rearing of rats produces a deficit in prepulse inhibition of acoustic startle similar to that in schizophrenia. *Biol.Psychiatry*, **34**, 361-372.

Geyer,M.A., McIlwain,K.L. & Paylor,R. (2002) Mouse genetic models for prepulse inhibition: an early review. *Mol.Psychiatry*, **7**, 1039-1053.

Gheusi,G., Cremer,H., McLean,H., Chazal,G., Vincent,J.D. & Lledo,P.M. (2000) Importance of newly generated neurons in the adult olfactory bulb for odor discrimination. *Proc.Natl.Acad.Sci.U.S.A*, **97**, 1823-1828.

Goate,A., Chartier-Harlin,M.C., Mullan,M., Brown,J., Crawford,F., Fidani,L., Giuffra,L., Haynes,A., Irving,N. & James,L. (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature*, **349**, 704-706.

Godefroy,O., Lhullier,C. & Rousseaux,M. (1996) Non-spatial attention disorders in patients with frontal or posterior brain damage. *Brain*, **119 (Pt 1)**, 191-202.

Godefroy,O. (2003) Frontal syndrome and disorders of executive functions. *J.Neurol.*, **250**, 1-6.

Gommans,J., Stolerman,I.P. & Shoaib,M. (2000) Antagonism of the discriminative and aversive stimulus properties of nicotine in C57BL/6J mice. *Neuropharmacology*, **39**, 2840-2847.

Gotti,C., Carbonnelle,E., Moretti,M., Zwart,R. & Clementi,F. (2000) Drugs selective for nicotinic receptor subtypes: a real possibility or a dream? *Behav.Brain Res.*, **113**, 183-192.

Gotz,J., Chen,F., van Dorpe,J. & Nitsch,R.M. (2001) Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils. *Science*, **293**, 1491-1495.

Gotz,J., Streffer,J.R., David,D., Schild,A., Hoerndli,F., Pennanen,L., Kurosinski,P. & Chen,F. (2004) Transgenic animal models of Alzheimer's disease and related disorders: histopathology, behavior and therapy. *Mol.Psychiatry*, **9**, 664-683.

Graham,F.K. (1975) Presidential Address, 1974. The more or less startling effects of weak prestimulation. *Psychophysiology*, **12**, 238-248.

Granon,S., Hardouin,J., Courtier,A. & Poucet,B. (1998) Evidence for the involvement of the rat prefrontal cortex in sustained attention. *Q.J.Exp.Psychol.B*, **51**, 219-233.

Grant,D.A., & Berg,E.A. (1948) A behavioural analysis of degree of reinforcement and ease of shifting to new responses in a Weigl-type card-sorting problem. *J. Exp. Psychol*, **38**, 404-411.

Grobe,J.E., Perkins,K.A., Goettler-Good,J. & Wilson,A. (1998) Importance of environmental distractors in the effects of nicotine on short-term memory. *Exp.Clin.Psychopharmacol.*, **6**, 209-216.

Grottick,A.J. & Higgins,G.A. (2000) Effect of subtype selective nicotinic compounds on attention as assessed by the five-choice serial reaction time task. *Behav.Brain Res.*, **117**, 197-208.

Grottick,A.J., Trube,G., Corrigall,W.A., Huwyler,J., Malherbe,P., Wyler,R. & Higgins,G.A. (2000) Evidence that nicotinic alpha(7) receptors are not involved in the hyperlocomotor and rewarding effects of nicotine. *J.Pharmacol.Exp.Ther.*, **294**, 1112-1119.

Grottick,A.J., Haman,M., Wyler,R. & Higgins,G.A. (2003) Reversal of a vigilance decrement in the aged rat by subtype-selective nicotinic ligands. *Neuropsychopharmacology*, **28**, 880-887.

Guan,Z.Z., Zhang,X., Blennow,K. & Nordberg,A. (1999) Decreased protein level of nicotinic receptor alpha7 subunit in the frontal cortex from schizophrenic brain. *Neuroreport*, **10**, 1779-1782.

Guan,Z.Z., Zhang,X., Ravid,R. & Nordberg,A. (2000) Decreased protein levels of nicotinic receptor subunits in the hippocampus and temporal cortex of patients with Alzheimer's disease. *J.Neurochem.*, **74**, 237-243.

Gur,R.E., Cowell,P., Turetsky,B.I., Gallacher,F., Cannon,T., Bilker,W. & Gur,R.C. (1998) A follow-up magnetic resonance imaging study of schizophrenia. Relationship of neuroanatomical changes to clinical and neurobehavioral measures. *Arch.Gen.Psychiatry*, **55**, 145-152.

Hahn,B., Shoaib,M. & Stolerman,I.P. (2002) Nicotine-induced enhancement of attention in the five-choice serial reaction time task: the influence of task demands. *Psychopharmacology (Berl)*, **162**, 129-137.

Hahn,B., Sharples,C.G., Wonnacott,S., Shoaib,M. & Stolerman,I.P. (2003) Attentional effects of nicotinic agonists in rats. *Neuropharmacology*, **44**, 1054-1067.

Hahn,B., Shoaib,M. & Stolerman,I.P. (2003) Involvement of the prefrontal cortex but not the dorsal hippocampus in the attention-enhancing effects of nicotine in rats. *Psychopharmacology (Berl)*, **168**, 271-279.

Hall,G., & Pearce,J.M. (1982) Restoring the associability of a preexposed CS by a surprising event. *Q. J. Exp. Psychol.*, **34B**, 127-140.

Hanninen,T., Hallikainen,M., Koivisto,K., Partanen,K., Laakso,M.P., Riekkinen,P.J., Sr. & Soininen,H. (1997) Decline of frontal lobe functions in subjects with age-associated memory impairment. *Neurology*, **48**, 148-153.

Hardy,J. & Selkoe,D.J. (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*, **297**, 353-356.

Harlow,J.M. (1868) Recovery from the passage of an iron bar through the head. Boston Medical and Surgical Journal, 39: 389-393, cited in Andres,P. (2003) Frontal cortex as the central executive of working memory: Time to revise our view. *Cortex*, **39**, 871-895.

Harris,J.G., Kongs,S., Allensworth,D., Martin,L., Tregellas,J., Sullivan,B., Zerbe,G. & Freedman,R. (2004) Effects of nicotine on cognitive deficits in schizophrenia. *Neuropsychopharmacology*, **29**, 1378-1385.

Harrison,P.J. (1999) The neuropathology of schizophrenia. A critical review of the data and their interpretation. *Brain*, **122 (Pt 4)**, 593-624.

Hazlett,E.A., Buchsbaum,M.S., Jeu,L.A., Nenadic,I., Fleischman,M.B., Shihabuddin,L., Haznedar,M.M. & Harvey,P.D. (2000) Hypofrontality in unmedicated schizophrenia patients studied with PET during performance of a serial verbal learning task. *Schizophr.Res.*, **43**, 33-46.

Hazlett,E.A. & Buchsbaum,M.S. (2001) Sensorimotor gating deficits and hypofrontality in schizophrenia. *Front Biosci.*, **6**, D1069-D1072.

Heckers,S., Rauch,S.L., Goff,D., Savage,C.R., Schacter,D.L., Fischman,A.J. & Alpert,N.M. (1998) Impaired recruitment of the hippocampus during conscious recollection in schizophrenia. *Nat.Neurosci.*, **1**, 318-323.

Heekeren,K., Meincke,U., Geyer,M.A. & Gouzoulis-Mayfrank,E. (2004) Attentional modulation of prepulse inhibition: a new startle paradigm. *Neuropsychobiology*, **49**, 88-93.

Heishman,S.J. & Henningfield,J.E. (2000) Tolerance to repeated nicotine administration on performance, subjective, and physiological responses in nonsmokers. *Psychopharmacology (Berl)*, **152**, 321-333.

Hejl,A.M., Glenthoj,B., Mackeprang,T., Hemmingsen,R. & Waldemar,G. (2004) Prepulse inhibition in patients with Alzheimer's disease. *Neurobiol.Aging*, **25**, 1045-1050.

Hellstrom-Lindahl,E., Court,J., Keverne,J., Svedberg,M., Lee,M., Marutle,A., Thomas,A., Perry,E., Bednar,I. & Nordberg,A. (2004) Nicotine reduces A beta in the brain and cerebral vessels of APPsw mice. *Eur.J.Neurosci.*, **19**, 2703-2710.

Herreman,A., Hartmann,D., Annaert,W., Saftig,P., Craessaerts,K., Serneels,L., Umans,L., Schrijvers,V., Checler,F., Vanderstichele,H., Baekelandt,V., Dressel,R., Cupers,P., Huylebroeck,D., Zwijsen,A., Van Leuven,F. & De Strooper,B. (1999) Presenilin 2 deficiency causes a mild pulmonary phenotype and no changes in amyloid precursor protein processing but enhances the embryonic lethal phenotype of presenilin 1 deficiency. *Proc.Natl.Acad.Sci.U.S.A*, **96**, 11872-11877.

Hill,E.L. (2004) Executive dysfunction in autism. *Trends Cogn Sci.*, **8**, 26-32.

Himmelheber,A.M., Sarter,M. & Bruno,J.P. (1997) Operant performance and cortical acetylcholine release: role of response rate, reward density, and non-contingent stimuli. *Brain Res.Cogn Brain Res.*, **6**, 23-36.

Himmelheber,A.M., Sarter,M. & Bruno,J.P. (2000) Increases in cortical acetylcholine release during sustained attention performance in rats. *Brain Res.Cogn Brain Res.*, **9**, 313-325.

Hirose,S., Iwata,H., Akiyoshi,H., Kobayashi,K., Ito,M., Wada,K., Kaneko,S. & Mitsudome,A. (1999) A novel mutation of CHRNA4 responsible for autosomal dominant nocturnal frontal lobe epilepsy. *Neurology*, **53**, 1749-1753.

Hodges,H. (1996) Maze procedures: the radial-arm and water maze compared. *Brain Res.Cogn Brain Res.*, **3**, 167-181.

Holcomb,L.A., Gordon,M.N., Jantzen,P., Hsiao,K., Duff,K. & Morgan,D. (1999) Behavioral changes in transgenic mice expressing both amyloid precursor protein and

presenilin-1 mutations: lack of association with amyloid deposits. *Behav.Genet.*, **29**, 177-185.

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

Holscher,C. (1999) Stress impairs performance in spatial water maze learning tasks. *Behav.Brain Res.*, **100**, 225-235.

Holtzman,D.M., Bales,K.R., Wu,S., Bhat,P., Parsadanian,M., Fagan,A.M., Chang,L.K., Sun,Y. & Paul,S.M. (1999) Expression of human apolipoprotein E reduces amyloid-beta deposition in a mouse model of Alzheimer's disease. *J.Clin.Invest*, **103**, R15-R21.

Hoozemans,J.J., Veerhuis,R., Rozemuller,A.J. & Eikelenboom,P. (2002) The pathological cascade of Alzheimer's disease: the role of inflammation and its therapeutic implications. *Drugs Today (Barc.)*, **38**, 429-443.

Hsiao,K., Chapman,P., Nilsen,S., Eckman,C., Harigaya,Y., Younkin,S., Yang,F. & Cole,G. (1996) Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science*, **274**, 99-102.

Humby,T., Laird,F.M., Davies,W. & Wilkinson,L.S. (1999) Visuospatial attentional functioning in mice: interactions between cholinergic manipulations and genotype. *Eur.J.Neurosci.*, **11**, 2813-2823.

Hutton,M., Lendon,C.L., Rizzu,P., Baker,M., Froelich,S., Houlden,H., Pickering-Brown,S., Chakraverty,S., Isaacs,A., Grover,A., Hackett,J., Adamson,J., Lincoln,S., Dickson,D., Davies,P., Petersen,R.C., Stevens,M., de Graaff,E., Wauters,E., van Baren,J., Hillebrand,M., Joosse,M., Kwon,J.M., Nowotny,P. & Heutink,P. (1998) Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature*, **393**, 702-705.

Ichikawa,J., Dai,J., O'Laughlin,I.A., Fowler,W.L. & Meltzer,H.Y. (2002) Atypical, but not typical, antipsychotic drugs increase cortical acetylcholine release without an effect in the nucleus accumbens or striatum. *Neuropsychopharmacology*, **26**, 325-339.

Inglis,W.L., Olmstead,M.C. & Robbins,T.W. (2001) Selective deficits in attentional performance on the 5-choice serial reaction time task following pedunculopontine tegmental nucleus lesions. *Behav.Brain Res.*, **123**, 117-131.

Jacobsen,L.K., Hamburger,S.D., Van Horn,J.D., Vaituzis,A.C., McKenna,K., Frazier,J.A., Gordon,C.T., Lenane,M.C., Rapoport,J.L. & Zametkin,A.J. (1997) Cerebral glucose metabolism in childhood onset schizophrenia. *Psychiatry Res.*, **75**, 131-144.

Jacobsen,L.K., D'Souza,D.C., Mencl,W.E., Pugh,K.R., Skudlarski,P. & Krystal,J.H. (2004) Nicotine effects on brain function and functional connectivity in schizophrenia. *Biol.Psychiatry*, **55**, 850-858.

Jaffard,R., Durkin,T., Toumane,A., Marighetto,A. & Lebrun,C. (1989) Experimental dissociation of memory systems in mice: behavioral and neurochemical aspects. *Arch.Gerontol.Geriatr.Suppl*, **1**, 55-70.

Janus,C., Pearson,J., McLaurin,J., Mathews,P.M., Jiang,Y., Schmidt,S.D., Chishti,M.A., Horne,P., Heslin,D., French,J., Mount,H.T., Nixon,R.A., Mercken,M., Bergeron,C., Fraser,P.E., George-Hyslop,P. & Westaway,D. (2000) A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature*, **408**, 979-982.

Jennings,J.W., & Keefer,L.H. (1969) Olfactory learning set in two varieties of domestic rat. *Psychol. Report.* **24**: 3-15: cited in Slotnick,B. (2001) Animal cognition and the rat olfactory system. *Trends Cogn Sci.,* **5**, 216-222.

Jessen,F., Kucharski,C., Fries,T., Papassotiropoulos,A., Hoenig,K., Maier,W. & Heun,R. (2001) Sensory gating deficit expressed by a disturbed suppression of the P50 event-related potential in patients with Alzheimer's disease. *Am.J.Psychiatry*, **158**, 1319-1321.

Johnstone,E.C., Crow,T.J., Frith,C.D., Husband,J. & Kreel,L. (1976) Cerebral ventricular size and cognitive impairment in chronic schizophrenia. *Lancet*, **2**, 924-926.

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

Jones,G.M., Sahakian,B.J., Levy,R., Warburton,D.M. & Gray,J.A. (1992) Effects of acute subcutaneous nicotine on attention, information processing and short-term memory in Alzheimer's disease. *Psychopharmacology (Berl)*, **108**, 485-494.

Kaiser,S. & Wonnacott,S. (2000) alpha-bungarotoxin-sensitive nicotinic receptors indirectly modulate [(3)H]dopamine release in rat striatal slices via glutamate release. *Mol.Pharmacol.*, **58**, 312-318.

Kane,J., Honigfeld,G., Singer,J. & Meltzer,H. (1988) Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. *Arch.Gen.Psychiatry*, **45**, 789-796.

Katz,M., Buchsbaum,M.S., Siegel,B.V., Jr., Wu,J., Haier,R.J. & Bunney,W.E., Jr. (1996) Correlational patterns of cerebral glucose metabolism in never-medicated schizophrenics. *Neuropsychobiology*, **33**, 1-11.

Kelly,J.S. (1999) Alzheimer's disease: the tacrine legacy. *Trends Pharmacol.Sci.*, **20**, 127-129.

Kelly,R. & Spetch,M.L. (2000) Choice biases in delayed matching-to-sample duration with pigeons: Manipulations of ITI and delay illumination. *Q.J.Exp.Psychol.B*, **53**, 309-323.

Kelton,M.C., Kahn,H.J., Conrath,C.L. & Newhouse,P.A. (2000) The effects of nicotine on Parkinson's disease. *Brain Cogn*, **43**, 274-282.

Kem,W.R. (2000) The brain alpha7 nicotinic receptor may be an important therapeutic target for the treatment of Alzheimer's disease: studies with DMXBA (GTS-21). *Behav.Brain Res.*, **113**, 169-181.

Kerr,L.E., McGregor,A.L., Amet,L.E., Asada,T., Spratt,C., Allsopp,T.E., Harmar,A.J., Shen,S., Carlson,G., Logan,N., Kelly,J.S. & Sharkey,J. (2004) Mice overexpressing human caspase 3 appear phenotypically normal but exhibit increased apoptosis and larger lesion volumes in response to transient focal cerebral ischaemia. *Cell Death.Differ.*, **11**, 1102-1111.

Kihara,T., Shimohama,S., Sawada,H., Kimura,J., Kume,T., Kochiyama,H., Maeda,T. & Akaike,A. (1997) Nicotinic receptor stimulation protects neurons against beta-amyloid toxicity. *Ann.Neurol.*, **42**, 159-163.

Kihara,T., Shimohama,S. & Akaike,A. (1999) Effects of nicotinic receptor agonists on beta-amyloid beta-sheet formation. *Jpn.J.Pharmacol.*, **79**, 393-396.

Kim,J.S. & Levin,E.D. (1996) Nicotinic, muscarinic and dopaminergic actions in the ventral hippocampus and the nucleus accumbens: effects on spatial working memory in rats. *Brain Res.*, **725**, 231-240.

King,D.L., Arendash,G.W., Crawford,F., Sterk,T., Menendez,J. & Mullan,M.J. (1999) Progressive and gender-dependent cognitive impairment in the APP(SW) transgenic mouse model for Alzheimer's disease. *Behav.Brain Res.*, **103**, 145-162.

King,D.L. & Arendash,G.W. (2002) Behavioral characterization of the Tg2576 transgenic model of Alzheimer's disease through 19 months. *Physiol Behav.*, **75**, 627-642.

Kirkby,D.L. & Higgins,G.A. (1998) Characterization of perforant path lesions in rodent models of memory and attention. *Eur.J.Neurosci.*, **10**, 823-838.

Kitagawa H, Takenouchi T, Wesnes K, Kramer W, Clody DE. (1998) Phase 1 studies of GTS-21 to assess the safety, tolerability, PK and effects on measures of cognitive function in normal volunteers. *Neurobiol. Aging*. **19**:S182.

Kitagawa,H., Takenouchi,T., Azuma,R., Wesnes,K.A., Kramer,W.G., Clody,D.E. & Burnett,A.L. (2003) Safety, pharmacokinetics, and effects on cognitive function of

multiple doses of GTS-21 in healthy, male volunteers. *Neuropsychopharmacology*, **28**, 542-551.

Klaning,U. (1999) Greater occurrence of schizophrenia in dizygotic but not monozygotic twins. Register-based study. *Br.J.Psychiatry*, **175**, 407-409.

Knapp,M., Wilkinson,D., & Wigglesworth,R. (1998) The economic consequences of Alzheimer's disease in the context of new drug developments. *Int J Geriatr Psychiatry*, **13**, 531-543.

Kohler,C.G., Moberg,P.J., Gur,R.E., O'Connor,M.J., Sperling,M.R. & Doty,R.L. (2001) Olfactory dysfunction in schizophrenia and temporal lobe epilepsy. *Neuropsychiatry Neuropsychol.Behav.Neurol.*, **14**, 83-88.

Koller,G., Satzger,W., Adam,M., Wagner,M., Kathmann,N., Soyka,M., & Engel,R. (2003) Effects of scopolamine on matching to sample paradigm and related tests in human subjects. *Neuropsychobiology*, **48**, 87-94.

Kopala,L.C., Clark,C. & Hurwitz,T. (1993) Olfactory deficits in neuroleptic naive patients with schizophrenia. *Schizophr.Res.*, **8**, 245-250.

Kraepelin,E. (1986) Dementia praecox. In Cutting,J., & Shepherd,M. (eds) (1987) The clinical roots of schizophrenia Concept pp. 15-24. Cambridge University Press, Cambridge, U.K. Cited in Wing,J.K. Concepts of schizophrenia., in Hirsch,S.R., & Weinberger,D.R. (eds.) (1995) Schizophrenia. Cambridge University Press, Cambridge, U.K.

Krystal,J.H., D'Souza,D.C., Mathalon,D., Perry,E., Belger,A. & Hoffman,R. (2003) NMDA receptor antagonist effects, cortical glutamatergic function, and schizophrenia: toward a paradigm shift in medication development. *Psychopharmacology (Berl)*, **169**, 215-233.

Kukull,W.A., Larson,E.B., Reifler,B.V., Lampe,T.H., Yerby,M. & Hughes,J. (1990) Interrater reliability of Alzheimer's disease diagnosis. *Neurology*, **40**, 257-260.

Kumari,V., Soni,W., Mathew,V.M. & Sharma,T. (2000) Prepulse inhibition of the startle response in men with schizophrenia: effects of age of onset of illness, symptoms, and medication. *Arch.Gen.Psychiatry*, **57**, 609-614.

Kwapil,T.R., Chapman,J.P., Chapman,L.J. & Miller,M.B. (1996) Deviant olfactory experiences as indicators of risk for psychosis. *Schizophr.Bull.*, **22**, 371-382.

Lalo,U., Pankratov,Y., Krishtal,O. & North,R.A. (2004) Methyllycaconitine, alpha-bungarotoxin and (+)-tubocurarine block fast ATP-gated currents in rat dorsal root ganglion cells. *Br.J.Pharmacol.*, **142**, 1227-1232.

Lalonde,R. (2002) The neurobiological basis of spontaneous alternation. *Neurosci.Biobehav.Rev.*, **26** , 91-104.

Lalonde,R., Qian,S. & Strazielle,C. (2003) Transgenic mice expressing the PS1-A246E mutation: effects on spatial learning, exploration, anxiety, and motor coordination. *Behav.Brain Res.*, **138**, 71-79.

Larsson,M., Semb,H., Winblad,B., Amberla,K., Wahlund,L.O. & Backman,L. (1999) Odor identification in normal aging and early Alzheimer's disease: effects of retrieval support. *Neuropsychology.*, **13**, 47-53.

Lawrence,A.D. & Sahakian,B.J. (1995) Alzheimer disease, attention, and the cholinergic system. *Alzheimer Dis.Assoc.Disord.*, **9 Suppl 2**, 43-49.

Lawrence,N.S., Ross,T.J. & Stein,E.A. (2002) Cognitive mechanisms of nicotine on visual attention. *Neuron*, **36**, 539-548.

Lawrie,S.M. & Abukmeil,S.S. (1998) Brain abnormality in schizophrenia. A systematic and quantitative review of volumetric magnetic resonance imaging studies. *Br.J.Psychiatry*, **172** , 110-120.

Lawrie,S.M., Whalley,H., Kestelman,J.N., Abukmeil,S.S., Byrne,M., Hodges,A., Rimmington,J.E., Best,J.J., Owens,D.G. & Johnstone,E.C. (1999) Magnetic resonance imaging of brain in people at high risk of developing schizophrenia. *Lancet*, **353**, 30-33.

Le Pen,G., Grottick,A.J., Higgins,G.A. & Moreau,J.L. (2003) Phencyclidine exacerbates attentional deficits in a neurodevelopmental rat model of schizophrenia. *Neuropsychopharmacology*, **28**, 1799-1809.

Lena,C. & Changeux,J.P. (1999) The role of beta 2-subunit-containing nicotinic acetylcholine receptors in the brain explored with a mutant mouse. *Ann.N.Y.Acad.Sci.*, **868**, 611-616.

Leonard,S., Adler,L.E., Benhammou,K., Berger,R., Breese,C.R., Drebing,C., Gault,J., Lee,M.J., Logel,J., Olincy,A., Ross,R.G., Stevens,K., Sullivan,B., Vianzon,R., Virnich,D.E., Waldo,M., Walton,K. & Freedman,R. (2001) Smoking and mental illness. *Pharmacol.Biochem.Behav.*, **70**, 561-570.

Lester,H.A., Fonck,C., Tapper,A.R., McKinney,S., Damaj,M.I., Balogh,S., Owens,J., Wehner,J.M., Collins,A.C. & Labarca,C. (2003) Hypersensitive knockin mouse strains identify receptors and pathways for nicotine action. *Curr.Opin.Drug Discov.Devel.*, **6**, 633-639.

Levin,E.D., Christopher,N.C., Briggs,S.J. & Rose,J.E. (1993) Chronic nicotine reverses working memory deficits caused by lesions of the fimbria or medial basalocortical projection. *Brain Res.Cogn Brain Res.*, **1**, 137-143.

Levin,E.D., Wilson,W., Rose,J.E. & McEvoy,J. (1996) Nicotine-haloperidol interactions and cognitive performance in schizophrenics. *Neuropsychopharmacology*, **15**, 429-436.

Levin,E.D. & Torry,D. (1996) Acute and chronic nicotine effects on working memory in aged rats. *Psychopharmacology (Berl)*, **123**, 88-97.

Levin,E.D., Christopher,N.C. & Briggs,S.J. (1997) Chronic nicotinic agonist and antagonist effects on T-maze alternation. *Physiol Behav.*, **61**, 863-866.

Levin,E.D., Kaplan,S. & Boardman,A. (1997) Acute nicotine interactions with nicotinic and muscarinic antagonists: working and reference memory effects in the 16-arm radial maze. *Behav.Pharmacol.*, **8**, 236-242.

Levin,E.D., Conners,C.K., Silva,D., Hinton,S.C., Meck,W.H., March,J. & Rose,J.E. (1998) Transdermal nicotine effects on attention. *Psychopharmacology (Berl)*, **140**, 135-141.

Levin,E.D. & Simon,B.B. (1998) Nicotinic acetylcholine involvement in cognitive function in animals. *Psychopharmacology (Berl)*, **138**, 217-230.

Levin,E.D., Christopher,N.C., Weaver,T., Moore,J. & Brucato,F. (1999) Ventral hippocampal ibotenic acid lesions block chronic nicotine-induced spatial working memory improvement in rats. *Brain Res.Cogn Brain Res.*, **7**, 405-410.

Levin,E.D. (2002) Nicotinic receptor subtypes and cognitive function. *J.Neurobiol.*, **53**, 633-640.

Levin,E.D. & Christopher,C.N. (2003) Lobeline-induced learning improvement of rats in the radial-arm maze. *Pharmacol.Biochem.Behav.*, **76**, 133-139.

Levin,H.S., Eisenberg,H.M., & Benton,A.L. (1991) Frontal Lobe Function and Dysfunction. Oxford University Press, New York, U.S.A..

Levin,S. (1984) Frontal lobe dysfunctions in schizophrenia--II. Impairments of psychological and brain functions. *J.Psychiatr.Res.*, **18**, 57-72.

Levy-Lahad,E., Wijsman,E.M., Nemens,E., Anderson,L., Goddard,K.A., Weber,J.L., Bird,T.D. & Schellenberg,G.D. (1995) A familial Alzheimer's disease locus on chromosome 1. *Science*, **269**, 970-973.

Levy,D.A., Manns,J.R., Hopkins,R.O., Gold,J.J., Broadbent,N.J. & Squire,L.R. (2003) Impaired visual and odor recognition memory span in patients with hippocampal lesions. *Learn.Mem.*, **10**, 531-536.

Lewis,J., McGowan,E., Rockwood,J., Melrose,H., Nacharaju,P., Van Slegtenhorst,M., Gwinn-Hardy,K., Paul,M.M., Baker,M., Yu,X., Duff,K., Hardy,J., Corral,A., Lin,W.L.,

Yen,S.H., Dickson,D.W., Davies,P. & Hutton,M. (2000) Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. *Nat.Genet.*, **25**, 402-405.

Lewis,J., Dickson,D.W., Lin,W.L., Chisholm,L., Corral,A., Jones,G., Yen,S.H., Sahara,N., Skipper,L., Yager,D., Eckman,C., Hardy,J., Hutton,M. & McGowan,E. (2001) Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science*, **293**, 1487-1491.

Lezak,M.D. (1983) Neuropsychological Assessment, 2nd Edition. Oxford, Oxford University Press: Cited in Parkin,A.J. (1999) Memory: Phenomenon, Experiment and Theory. Psychology Press, Cornwall, U.K..

Li,Y., King,M.A., Grimes,J., Smith,N., de Fiebre,C.M. & Meyer,E.M. (1999) Alpha7 nicotinic receptor mediated protection against ethanol-induced cytotoxicity in PC12 cells. *Brain Res.*, **816**, 225-228.

Light,G.A., Geyer,M.A., Clementz,B.A., Cadenhead,K.S. & Braff,D.L. (2000) Normal P50 suppression in schizophrenia patients treated with atypical antipsychotic medications. *Am.J.Psychiatry*, **157**, 767-771.

Lillrank,S.M., Lipska,B.K. & Weinberger,D.R. (1995) Neurodevelopmental animal models of schizophrenia. *Clin.Neurosci.*, **3**, 98-104.

Lindner,M.D. (1997) Reliability, distribution, and validity of age-related cognitive deficits in the Morris water maze. *Neurobiol.Learn.Mem.*, **68**, 203-220.

Lindner,M.D., Plone,M.A., Schallert,T. & Emerich,D.F. (1997) Blind rats are not profoundly impaired in the reference memory Morris water maze and cannot be clearly discriminated from rats with cognitive deficits in the cued platform task. *Brain Res.Cogn Brain Res.*, **5**, 329-333.

Lipp,H.P. & Wolfer,D.P. (1998) Genetically modified mice and cognition. *Curr.Opin.Neurobiol.*, **8**, 272-280.

Lipp,H.P., Pleskacheva,M.G., Gossweiler,H., Ricceri,L., Smirnova,A.A., Garin,N.N., Perepiolkina,O.P., Voronkov,D.N., Kuptsov,P.A. & Dell'Omo,G. (2001) A large outdoor radial maze for comparative studies in birds and mammals. *Neurosci.Biobehav.Rev.*, **25**, 83-99.

Liu,Q., Kawai,H. & Berg,D.K. (2001) beta -Amyloid peptide blocks the response of alpha 7-containing nicotinic receptors on hippocampal neurons. *Proc.Natl.Acad.Sci.U.S.A*, **98**, 4734-4739.

Liu,S.K., Chen,W.J., Chang,C.J. & Lin,H.N. (2000) Effects of atypical neuroleptics on sustained attention deficits in schizophrenia: a trial of risperidone versus haloperidol. *Neuropsychopharmacology*, **22**, 311-319.

Logue,S.F., Owen,E.H., Rasmussen,D.L. & Wehner,J.M. (1997) Assessment of locomotor activity, acoustic and tactile startle, and prepulse inhibition of startle in inbred mouse strains and F1 hybrids: implications of genetic background for single gene and quantitative trait loci analyses. *Neuroscience*, **80**, 1075-1086.

Lu,X.C., Slotnick,B.M. & Silberberg,A.M. (1993) Odor matching and odor memory in the rat. *Physiol Behav.*, **53**, 795-804.

Lucas-Meunier,E., Fossier,P., Baux,G. & Amar,M. (2003) Cholinergic modulation of the cortical neuronal network. *Pflugers Arch.*, **446**, 17-29.

Luntz-Leybman,V., Bickford,P.C. & Freedman,R. (1992) Cholinergic gating of response to auditory stimuli in rat hippocampus. *Brain Res.*, **587**, 130-136.

Mackintosh,N.J. (1975a) A theory of attention: Cariations in the associability of stimuli with reinforcement. *Psychol. Review*, 82, 276-298.

Mackintosh,N.J. (1975b) The Psychology of animal learning. Academic Press, London, U.K..

Maelicke,A., Schrattenholz,A., Samochocki,M., Radina,M. & Albuquerque,E.X. (2000) Allosterically potentiating ligands of nicotinic receptors as a treatment strategy for Alzheimer's disease. *Behav.Brain Res.*, **113**, 199-206.

Maemoto,T., Finlayson,K., Olverman,H.J., Akahane,A., Horton,R.W. & Butcher,S.P. (1997) Species differences in brain adenosine A1 receptor pharmacology revealed by use of xanthine and pyrazolopyridine based antagonists. *Br.J.Pharmacol.*, **122**, 1202-1208.

Maggi,L., Sola,E., Minneci,F., Le Magueresse,C., Changeux,J.P. & Cherubini,E. (2004) Persistent decrease in synaptic efficacy induced by nicotine at Schaffer collateral-CA1 synapses in the immature rat hippocampus. *J.Physiol*, **559**, 863-874.

Mancuso,G., Warburton,D.M., Melen,M., Sherwood,N. & Tirelli,E. (1999) Selective effects of nicotine on attentional processes. *Psychopharmacology (Berl)*, **146**, 199-204.

Marin,P., Maus,M., Desagher,S., Glowinski,J. & Premont,J. (1994) Nicotine protects cultured striatal neurones against N-methyl-D-aspartate receptor-mediated neurotoxicity. *Neuroreport*, **5**, 1977-1980.

Marks,M.J., Burch,J.B. & Collins,A.C. (1983) Genetics of nicotine response in four inbred strains of mice. *J.Pharmacol.Exp.Ther.*, **226**, 291-302.

Marsh,L., Harris,D., Lim,K.O., Beal,M., Hoff,A.L., Minn,K., Csernansky,J.G., DeMent,S., Faustman,W.O., Sullivan,E.V. & Pfefferbaum,A. (1997) Structural magnetic resonance imaging abnormalities in men with severe chronic schizophrenia and an early age at clinical onset. *Arch.Gen.Psychiatry*, **54**, 1104-1112.

Marston,H.M. (1996) Analysis of cognitive function in animals, the value of SDT. *Brain Res.Cogn Brain Res.*, **3**, 269-277.

Marston,H.M., Reid,M.E., Lawrence,J.A., Olverman,H.J. & Butcher,S.P. (1999) Behavioural analysis of the acute and chronic effects of MDMA treatment in the rat. *Psychopharmacology (Berl)*, **144**, 67-76.

Marston,H.M., Spratt,C. & Kelly,J.S. (2001) Phenotyping complex behaviours: assessment of circadian control and 5-choice serial reaction learning in the mouse. *Behav.Brain Res.*, **125**, 189-193.

Martzke,J.S., Kopala,L.C. & Good,K.P. (1997) Olfactory dysfunction in neuropsychiatric disorders: review and methodological considerations. *Biol.Psychiatry*, **42**, 721-732.

Marubio,L.M., Mar Arroyo-Jimenez,M., Cordero-Erausquin,M., Lena,C., Le Novere,N., de Kerchove,d.A., Huchet,M., Damaj,M.I. & Changeux,J.P. (1999) Reduced antinociception in mice lacking neuronal nicotinic receptor subunits. *Nature*, **398**, 805-810.

Marutle,A., Warpman,U., Bogdanovic,N., Lannfelt,L. & Nordberg,A. (1999) Neuronal nicotinic receptor deficits in Alzheimer patients with the Swedish amyloid precursor protein 670/671 mutation. *J.Neurochem.* , **72**, 1161-1169.

Mattson,A.J. & Levin,H.S. (1990) Frontal lobe dysfunction following closed head injury. A review of the literature. *J.Nerv.Ment.Dis.*, **178**, 282-291.

Maviel,T. & Durkin,T.P. (2003) Role of central cholinergic receptor sub-types in spatial working memory: a five-arm maze task in mice provides evidence for a functional role of nicotinic receptors in mediating trace access processes. *Neuroscience*, **120**, 1049-1059.

McCool,M.F., Varty,G.B., Del Vecchio,R.A., Kazdoba,T.M., Parker,E.M., Hunter,J.C. & Hyde,L.A. (2003) Increased auditory startle response and reduced prepulse inhibition of startle in transgenic mice expressing a double mutant form of amyloid precursor protein. *Brain Res.*, **994**, 99-106.

McGaughy,J., Kaiser,T. & Sarter,M. (1996) Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: selectivity of the behavioral impairment and relation to cortical AChE-positive fiber density. *Behav.Neurosci.*, **110**, 247-265.

McGeer,P.L., McGeer,E.G., Suzuki,J., Dolman,C.E. & Nagai,T. (1984) Aging, Alzheimer's disease, and the cholinergic system of the basal forebrain. *Neurology*, **34**, 741-745.

Meltzer,H.Y. (1989) Clinical studies on the mechanism of action of clozapine: the dopamine-serotonin hypothesis of schizophrenia. *Psychopharmacology (Berl)*, **99 Suppl**, S18-S27.

Meyer,E.M., Tay,E.T., Papke,R.L., Meyers,C., Huang,G.L. & de Fiebre,C.M. (1997) 3-[2,4-Dimethoxybenzylidene]anabaseine (DMXB) selectively activates rat alpha7 receptors and improves memory-related behaviors in a mecamylamine-sensitive manner. *Brain Res.*, **768** , 49-56.

Meyer,E.M., King,M.A. & Meyers,C. (1998) Neuroprotective effects of 2,4-dimethoxybenzylidene anabaseine (DMXB) and tetrahydroaminoacridine (THA) in neocortices of nucleus basalis lesioned rats. *Brain Res.* , **786**, 252-254.

Meyer,E.M., Tay,E.T., Zoltewicz,J.A., Meyers,C., King,M.A., Papke,R.L. & de Fiebre,C.M. (1998) Neuroprotective and memory-related actions of novel alpha-7 nicotinic agents with different mixed agonist/antagonist properties. *J.Pharmacol.Exp.Ther.*, **284**, 1026-1032.

Michon,A., Deweer,B., Pillon,B., Agid,Y. & Dubois,B. (1994) Relation of anosognosia to frontal lobe dysfunction in Alzheimer's disease. *J.Neurol.Neurosurg.Psychiatry*, **57**, 805-809.

Min,S.K., Moon,I.W., Ko,R.W. & Shin,H.S. (2001) Effects of transdermal nicotine on attention and memory in healthy elderly non-smokers. *Psychopharmacology (Berl)*, **159**, 83-88.

Miner,L.A., Ostrander,M., & Sarter,M. (1997) Effects of ibotenic acid-induced loss of neurons in the medial prefrontal cortex of rats on behavioral vigilance: evidence for executive dysfunction. *J. Psychopharmacol.*, **11**, 169-78.

Mirza,N.R. & Stolerman,I.P. (1998) Nicotine enhances sustained attention in the rat under specific task conditions. *Psychopharmacology (Berl)*, **138**, 266-274.

Mirza,N.R. & Bright,J.L. (2001) Nicotine-induced enhancements in the five-choice serial reaction time task in rats are strain-dependent. *Psychopharmacology (Berl)*, **154**, 8-12.

Moberg,P.J., Doty,R.L., Mahr,R.N., Mesholam,R.I., Arnold,S.E., Turetsky,B.I. & Gur,R.E. (1997) Olfactory identification in elderly schizophrenia and Alzheimer's disease. *Neurobiol.Aging*, **18**, 163-167.

Moechars,D., Lorent,K., De Strooper,B., Dewachter,I., & Van Leuven,F. (1996) Expression in brain of amyloid precursor protein mutated in the alpha-secretase site causes disturbed behavior, neuronal degeneration and premature death in transgenic mice. *EMBO J,* **15,** 1265-1274.

Moechars,D., Gilis,M., Kuiperi,C., Laenen,I., & Van Leuven,F. (1998) Aggressive behaviour in transgenic mice expressing APP is alleviated by serotonergic drugs. *Neuroreport*, **9**, 3561-3564.

Moghaddam,B. (2003) Bringing order to the glutamate chaos in schizophrenia. *Neuron*, **40**, 881-884.

Moore,H., Sarter,M. & Bruno,J.P. (1995) Bidirectional modulation of cortical acetylcholine efflux by infusion of benzodiazepine receptor ligands into the basal forebrain. *Neurosci.Lett.*, **189**, 31-34.

Moran,P.M. (1993) Differential effects of scopolamine and mecamylamine on working and reference memory in the rat. *Pharmacol.Biochem.Behav.*, **45**, 533-538.

Moran,P.M., Higgins,L.S., Cordell,B. & Moser,P.C. (1995) Age-related learning deficits in transgenic mice expressing the 751-amino acid isoform of human beta-amyloid precursor protein. *Proc.Natl.Acad.Sci.U.S.A*, **92**, 5341-5345.

Morris,R.G.M. (1981) Spatial localization does not require the presence of local cues. *Learn. Motiv.*, **12**: 239.

Mucke,L., Yu,G.Q., McConlogue,L., Rockenstein,E.M., Abraham,C.R. & Masliah,E. (2000) Astroglial expression of human alpha(1)-antichymotrypsin enhances alzheimer-like pathology in amyloid protein precursor transgenic mice. *Am.J.Pathol.*, **157**, 2003-2010.

Mufson,E.J., Chen,E.Y., Cochran,E.J., Beckett,L.A., Bennett,D.A. & Kordower,J.H. (1999) Entorhinal cortex beta-amyloid load in individuals with mild cognitive impairment. *Exp.Neurol.*, **158**, 469-490.

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

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

Mullan,M., Houlden,H., Windelspecht,M., Fidani,L., Lombardi,C., Diaz,P., Rossor,M., Crook,R., Hardy,J. & Duff,K. (1992) A locus for familial early-onset Alzheimer's disease on the long arm of chromosome 14, proximal to the alpha 1-antichymotrypsin gene. *Nat.Genet.*, **2**, 340-342.

Mullen,G., Napier,J., Balestra,M., DeCory,T., Hale,G., Macor,J., Mack,R., Loch,J., III, Wu,E., Kover,A., Verhoest,P., Sampognaro,A., Phillips,E., Zhu,Y., Murray,R., Griffith,R., Blosser,J., Gurley,D., Machulskis,A., Zongrone,J., Rosen,A. & Gordon,J. (2000) (-)-Spiro[1-azabicyclo[2.2.2]octane-3,5'-oxazolidin-2'-one], a conformationally restricted analogue of acetylcholine, is a highly selective full agonist at the alpha 7 nicotinic acetylcholine receptor. *J.Med.Chem.*, **43**, 4045-4050.

Mumenthaler,M.S., Yesavage,J.A., Taylor,J.L., O'Hara,R., Friedman,L., Lee,H. & Kraemer,H.C. (2003) Psychoactive drugs and pilot performance: a comparison of nicotine, donepezil, and alcohol effects. *Neuropsychopharmacology*, **28**, 1366-1373.

Nagamoto,H.T., Adler,L.E., McRae,K.A., Huettl,P., Cawthra,E., Gerhardt,G., Hea,R. & Griffith,J. (1999) Auditory P50 in schizophrenics on clozapine: improved gating parallels clinical improvement and changes in plasma 3-methoxy-4-hydroxyphenylglycol. *Neuropsychobiology*, **39**, 10-17.

Nagele,R.G., D'Andrea,M.R., Anderson,W.J. & Wang,H.Y. (2002) Intracellular accumulation of beta-amyloid(1-42) in neurons is facilitated by the alpha 7 nicotinic acetylcholine receptor in Alzheimer's disease. *Neuroscience*, **110**, 199-211.

Nanri,M., Miyake,H., Murakami,Y., Matsumoto,K. & Watanabe,H. (1998) GTS-21, a nicotinic agonist, attenuates multiple infarctions and cognitive deficit caused by permanent occlusion of bilateral common carotid arteries in rats. *Jpn.J.Pharmacol.*, **78**, 463-469.

Nebes,R.D. & Brady,C.B. (1993) Phasic and tonic alertness in Alzheimer's disease. *Cortex*, **29**, 77-90.

Nelson,M.D., Saykin,A.J., Flashman,L.A. & Riordan,H.J. (1998) Hippocampal volume reduction in schizophrenia as assessed by magnetic resonance imaging: a meta-analytic study. *Arch.Gen.Psychiatry*, **55**, 433-440.

Newhouse,P.A., Sunderland,T., Tariot,P.N., Blumhardt,C.L., Weingartner,H., Mellow,A. & Murphy,D.L. (1988) Intravenous nicotine in Alzheimer's disease: a pilot study. *Psychopharmacology (Berl)*, **95**, 171-175.

Newhouse,P.A., Potter,A., Corwin,J. & Lenox,R. (1992) Acute nicotinic blockade produces cognitive impairment in normal humans. *Psychopharmacology (Berl)*, **108**, 480-484.

Newhouse,P.A., Potter,A., Corwin,J. & Lenox,R. (1994) Age-related effects of the nicotinic antagonist mecamylamine on cognition and behavior. *Neuropsychopharmacology*, **10**, 93-107.

Newhouse,P.A., Potter,A. & Levin,E.D. (1997) Nicotinic system involvement in Alzheimer's and Parkinson's diseases. Implications for therapeutics. *Drugs Aging*, **11**, 206-228.

Newhouse,P.A., Potter,A., Kelton,M. & Corwin,J. (2001) Nicotinic treatment of Alzheimer's disease. *Biol.Psychiatry*, **49**, 268-278.

Newhouse,P.A., Potter,A., & Singh, A. (2004) Effects of nicotinic stimulation on cognitive performance. *Curr Opin Pharmacol.*, **4,** 36-46.

Nguyen,Q.T. & Miledi,R. (2002) Inhibition of skeletal muscle nicotinic receptors by the atypical antipsychotic clozapine. *Neuropharmacology*, **42**, 662-669.

NHS Executive (1996) Burdens of disease in personal social services research unit and centre for the economics of mental health, 5 may 1998, *Mental Health Review*. No. 5, University of Kent at Canterbury.

Nicoll,J.A., Wilkinson,D., Holmes,C., Steart,P., Markham,H., & Weller.R.O. (2003). Neuropathology of human Alzheimer's disease after immunization with amyloid-beta peptide: a case report. *Nat. Med.*, **9**, 448-452.

Niedermeyer,E. (1998) Frontal lobe functions and dysfunctions. *Clin.Electroencephalogr.*, **29**, 79-90.

Nieoullon,A. (2002) Dopamine and the regulation of cognition and attention. *Prog.Neurobiol.*, **67**, 53-83.

Nobre,A.C., Sebestyen,G.N., Gitelman,D.R., Mesulam,M.M., Frackowiak,R.S. & Frith,C.D. (1997) Functional localization of the system for visuospatial attention using positron emission tomography. *Brain*, **120 (Pt 3)**, 515-533.

Noga,J.T., Bartley,A.J., Jones,D.W., Torrey,E.F. & Weinberger,D.R. (1996) Cortical gyral anatomy and gross brain dimensions in monozygotic twins discordant for schizophrenia. *Schizophr.Res.*, **22**, 27-40.

Nordberg,A. & Bergh,C. (1985) Effect of nicotine on passive avoidance behaviour and motoric activity in mice. *Acta Pharmacol.Toxicol.(Copenh)*, **56**, 337-341.

Nordberg,A., Adem,A., Hardy,J. & Winblad,B. (1988) Change in nicotinic receptor subtypes in temporal cortex of Alzheimer brains. *Neurosci.Lett.*, **86**, 317-321.

Nordberg,A., Hartvig,P., Lilja,A., Viitanen,M., Amberla,K., Lundqvist,H., Andersson,J., Nyback,H., Ulin,J. & Anderson,Y. (1991) Nicotine receptors in the brain of patients with Alzheimer's disease. Studies with 11C-nicotine and positron emission tomography. *Acta Radiol.Suppl*, **376**, 165-166.

Nordberg,A., Lundqvist,H., Hartvig,P., Andersson,J., Johansson,M., Hellstrom-Lindahi,E. & Langstrom,B. (1997) Imaging of nicotinic and muscarinic receptors in Alzheimer's disease: effect of tacrine treatment. *Dement.Geriatr.Cogn Disord.*, **8**, 78-84.

Nordberg,A. (2001) Nicotinic receptor abnormalities of Alzheimer's disease: therapeutic implications. *Biol.Psychiatry*, **49**, 200-210.

Nordberg,A., Hellstrom-Lindahl,E., Lee,M., Johnson,M., Mousavi,M., Hall,R., Perry,E., Bednar,I. & Court,J. (2002) Chronic nicotine treatment reduces beta-amyloidosis in the brain of a mouse model of Alzheimer's disease (APPsw). *J.Neurochem.*, **81**, 655-658.

Nordstrom,A.L., Farde,L., Eriksson,L. & Halldin,C. (1995) No elevated D2 dopamine receptors in neuroleptic-naive schizophrenic patients revealed by positron emission tomography and [11C]N-methylspiperone. *Psychiatry Res.*, **61**, 67-83.

Norman,D.A., & Shallice,T. (1980) Attention to action. Willed and automatic control of behaviour. *University of California San Diego* CHIP Report 99: cited in Baddeley,A. (1986) Working Memory. Oxford, Oxford University Press, U.K.

O'Neill,H.C., Rieger,K., Kem,W.R. & Stevens,K.E. (2003) DMXB, an alpha7 nicotinic agonist, normalizes auditory gating in isolation-reared rats. *Psychopharmacology (Berl)*, **169**, 332-339.

Oddo,S., Caccamo,A., Kitazawa,M., Tseng,B.P. & LaFerla,F.M. (2003) Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. *Neurobiol.Aging*, **24**, 1063-1070.

Olincy,A., Young,D.A. & Freedman,R. (1997) Increased levels of the nicotine metabolite cotinine in schizophrenic smokers compared to other smokers. *Biol.Psychiatry*, **42**, 1-5.

Oltersdorf,T., Fritz,L.C., Schenk,D.B., Lieberburg,I., Johnson-Wood,K.L., Beattie,E.C., Ward,P.J., Blacher,R.W., Dovey,H.F. & Sinha,S. (1989) The secreted form of the Alzheimer's amyloid precursor protein with the Kunitz domain is protease nexin-II. *Nature*, **341**, 144-147.

Orr-Urtreger,A., Goldner,F.M., Saeki,M., Lorenzo,I., Goldberg,L., De Biasi,M., Dani,J.A., Patrick,J.W. & Beaudet,A.L. (1997) Mice deficient in the alpha7 neuronal nicotinic acetylcholine receptor lack alpha-bungarotoxin binding sites and hippocampal fast nicotinic currents. *J.Neurosci.*, **17**, 9165-9171.

Ott,A., Slooter,A.J., Hofman,A., van Harskamp,F., Witteman,J.C., Van Broeckhoven,C., van Duijn,C.M. & Breteler,M.M. (1998) Smoking and risk of dementia and Alzheimer's disease in a population-based cohort study: the Rotterdam Study. *Lancet*, **351**, 1840-1843.

Owen,A.M., Roberts,A.C., Hodges,J.R., Summers,B.A., Polkey,C.E. & Robbins,T.W. (1993) Contrasting mechanisms of impaired attentional set-shifting in patients with frontal lobe damage or Parkinson's disease. *Brain*, **116 (Pt 5)**, 1159-1175.

Pantelis,C. & Brewer,W. (1995) Neuropsychological and olfactory dysfunction in schizophrenia: relationship of frontal syndromes to syndromes of schizophrenia. *Schizophr.Res.*, **17**, 35-45.

Paolo,A.M., Axelrod,B.N., Troster,A.I., Blackwell,K.T. & Koller,W.C. (1996) Utility of a Wisconsin Card Sorting Test short form in persons with Alzheimer's and Parkinson's disease. *J.Clin.Exp.Neuropsychol.*, **18**, 892-897.

Papke,R.L., Porter Papke,J.K. & Rose,G.M. (2004) Activity of alpha7-selective agonists at nicotinic and serotonin 5HT3 receptors expressed in Xenopus oocytes. *Bioorg.Med.Chem.Lett.*, **14**, 1849-1853.

Paradiso,K.G. & Steinbach,J.H. (2003) Nicotine is highly effective at producing desensitization of rat alpha4beta2 neuronal nicotinic receptors. *J.Physiol*, **553**, 857-871.

Park,S., Knopick,C., McGurk,S. & Meltzer,H.Y. (2000) Nicotine impairs spatial working memory while leaving spatial attention intact. *Neuropsychopharmacology*, **22**, 200-209.

Parkin,A.J. (1999) Memory: Phenomenon, Experiment and Theory. Psychology Press, Cornwall, U.K.

Parks,R.W., Becker,R.E., Rippey,R.F., Gilbert,D.G., Matthews,J.R., Kabatay,E., Young,C.S., Vohs,C., Danz,V., Keim,P., Collins,G.T., Zigler,S.S. & Urycki,P.G. (1996) Increased regional cerebral glucose metabolism and semantic memory performance in Alzheimer's disease: a pilot double blind transdermal nicotine positron emission tomography study. *Neuropsychol.Rev.*, **6**, 61-79.

Pasquier,F., Grymonprez,L., Lebert,F. & Van der,L.M. (2001) Memory impairment differs in frontotemporal dementia and Alzheimer's disease. *Neurocase.*, **7**, 161-171.

Passetti,F., Dalley,J.W., O'Connell,M.T., Everitt,B.J. & Robbins,T.W. (2000) Increased acetylcholine release in the rat medial prefrontal cortex during performance of a visual attentional task. *Eur.J.Neurosci.*, **12**, 3051-3058.

Patel,A. Knapp,M. (1998) op cit, cited by http://mind.org.uk/Mind/Templates/Content%20(RelatedTopics).aspx?NRMODE=Publi

shed&NRORIGINALURL=%2fInformation%2fFactsheets%2fStatistics%2fStatistics%2b5%2ehtm&NRNODEGUID=%7b35340F7C-03E4-4EF4-98DF-8B9D350D50FF%7d&NRCHACHEHINT=NomodifyGuest#_ftn3

Paylor,R., Nguyen,M., Crawley,J.N., Patrick,J., Beaudet,A. & Orr-Urtreger,A. (1998) Alpha7 nicotinic receptor subunits are not necessary for hippocampal-dependent learning or sensorimotor gating: a behavioral characterization of Acra7-deficient mice. *Learn.Mem.*, **5**, 302-316.

Pearce,J.M. (2004) Animal Learning and cognition: An introduction, 2nd edition. Psychology Press Ltd., Hove, U.K..

Pearce,J.M., & Hall, G. (1980) A model for Pavlovian learning: Variations in the effectiveness of conditioned but not unconditioned stimuli. *Psychol. Review*, **87**, 532-552.

Pearlson,G.D. (2000) Neurobiology of schizophrenia. *Ann.Neurol.*, **48**, 556-566.

Perry,E.K., Tomlinson,B.E., Blessed,G., Bergmann,K., Gibson,P.H. & Perry,R.H. (1978a) Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Br.Med.J.*, **2**, 1457-1459.

Perry,E.K., Perry,R.H., Blessed,G. & Tomlinson,B.E. (1978b) Changes in brain cholinesterases in senile dementia of Alzheimer type. *Neuropathol.Appl.Neurobiol.*, **4**, 273-277.

Perry,E.K., Perry,R.H., Smith,C.J., Dick,D.J., Candy,J.M., Edwardson,J.A., Fairbairn,A. & Blessed,G. (1987) Nicotinic receptor abnormalities in Alzheimer's and Parkinson's diseases. *J.Neurol.Neurosurg.Psychiatry*, **50**, 806-809.

Perry,E.K., Morris,C.M., Court JA, Cheng,A., Fairbairn,A.F., McKeith,I.G., Irving,D., Brown,A. & Perry,R.H. (1995) Alteration in nicotine binding sites in Parkinson's disease, Lewy body dementia and Alzheimer's disease: possible index of early neuropathology. *Neuroscience*, **64**, 385-395.

Perry,R.J. & Hodges,J.R. (1999) Attention and executive deficits in Alzheimer's disease. A critical review. *Brain*, **122 (Pt 3)**, 383-404.

Perry,R.J. & Hodges,J.R. (2000) Fate of patients with questionable (very mild) Alzheimer's disease: longitudinal profiles of individual subjects' decline. *Dement.Geriatr.Cogn Disord.*, **11**, 342-349.

Pettit,D.L., Shao,Z. & Yakel,J.L. (2001) beta-Amyloid(1-42) peptide directly modulates nicotinic receptors in the rat hippocampal slice. *J.Neurosci.*, **21**, RC120.

Phiel,C.J., Wilson,C.A., Lee,V.M. & Klein,P.S. (2003) GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides. *Nature*, **423**, 435-439.

Phillips,S. & Fox,P. (1998) An investigation into the effects of nicotine gum on short-term memory. *Psychopharmacology (Berl)*, **140**, 429-433.

Picciotto,M.R., Zoli,M., Lena,C., Bessis,A., Lallemand,Y., Le Novere,N., Vincent,P., Pich,E.M., Brulet,P. & Changeux,J.P. (1995) Abnormal avoidance learning in mice lacking functional high-affinity nicotine receptor in the brain. *Nature*, **374**, 65-67.

Picciotto,M.R., Zoli,M., Rimondini,R., Lena,C., Marubio,L.M., Pich,E.M., Fuxe,K. & Changeux,J.P. (1998) Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature*, **391**, 173-177.

Picciotto,M.R. (2003) Nicotine as a modulator of behavior: beyond the inverted U. *Trends Pharmacol.Sci.*, **24**, 493-499.

Pick,C.G. & Yanai,J. (1983) Eight arm maze for mice. *Int.J.Neurosci.*, **21**, 63-66.

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

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

Potter,A., Corwin,J., Lang,J., Piasecki,M., Lenox,R. & Newhouse,P.A. (1999) Acute effects of the selective cholinergic channel activator (nicotinic agonist) ABT-418 in Alzheimer's disease. *Psychopharmacology (Berl)*, **142**, 334-342.

Powchik,P., Davidson,M., Haroutunian,V., Gabriel,S.M., Purohit,D.P., Perl,D.P., Harvey,P.D. & Davis,K.L. (1998) Postmortem studies in schizophrenia. *Schizophr.Bull.*, **24**, 325-341.

Powers,R.E. (1999) The neuropathology of schizophrenia. *J.Neuropathol.Exp.Neurol.*, **58**, 679-690.

Prince,M., Cullen,M. & Mann,A. (1994) Risk factors for Alzheimer's disease and dementia: a case-control study based on the MRC elderly hypertension trial. *Neurology*, **44**, 97-104.

Quintero,S., Buckland,C., Gray,J.A., McNaughton,N. & Mellanby,J. (1985) The effects of compounds related to gamma-aminobutyrate and benzodiazepine receptors on behavioural responses to anxiogenic stimuli in the rat: choice behaviour in the T-maze. *Psychopharmacology (Berl)*, **86**, 328-333.

Quon,D., Wang,Y., Catalano,R., Scardina,J.M., Murakami,K. & Cordell,B. (1991) Formation of beta-amyloid protein deposits in brains of transgenic mice. *Nature*, **352**, 239-241.

Radcliffe,K.A., Fisher,J.L., Gray,R. & Dani,J.A. (1999) Nicotinic modulation of glutamate and GABA synaptic transmission of hippocampal neurons. *Ann.N.Y.Acad.Sci.*, **868**, 591-610.

Rahman,S., Sahakian,B.J., Hodges,J.R., Rogers,R.D. & Robbins,T.W. (1999) Specific cognitive deficits in mild frontal variant frontotemporal dementia. *Brain*, **122 (Pt 8)**, 1469-1493.

Rao,T.S., Correa,L.D., Reid,R.T. & Lloyd,G.K. (1996) Evaluation of anti-nociceptive effects of neuronal nicotinic acetylcholine receptor (NAChR) ligands in the rat tail-flick assay. *Neuropharmacology*, **35**, 393-405.

Raskind,M.A., Peskind,E.R., Wessel,T. & Yuan,W. (2000) Galantamine in AD: A 6-month randomized, placebo-controlled trial with a 6-month extension. The Galantamine USA-1 Study Group. *Neurology*, **54**, 2261-2268.

Reid,W., Broe,G., Creasey,H., Grayson,D., McCusker,E., Bennett,H., Longley,W. & Sulway,M.R. (1996) Age at onset and pattern of neuropsychological impairment in mild early-stage Alzheimer disease. A study of a community-based population. *Arch.Neurol.*, **53**, 1056-1061.

Reitan,R.M. & Wolfson,D. (1994) A selective and critical review of neuropsychological deficits and the frontal lobes. *Neuropsychol.Rev.*, **4**, 161-198.

Rezvani,A.H. & Levin,E.D. (2002) Nicotine-alcohol interactions and cognitive function in rats. *Pharmacol.Biochem.Behav.*, **72**, 865-872.

Riccio,C.A., Reynolds,C.R., Lowe,P. & Moore,J.J. (2002) The continuous performance test: a window on the neural substrates for attention? *Arch.Clin.Neuropsychol.*, **17**, 235-272.

Ripoll,N., Bronnec,M. & Bourin,M. (2004) Nicotinic receptors and schizophrenia. *Curr.Med.Res.Opin.*, **20**, 1057-1074.

Rizzo,M., Anderson,S.W., Dawson,J., Myers,R. & Ball,K. (2000) Visual attention impairments in Alzheimer's disease. *Neurology* , **54**, 1954-1959.

Robbins,T.W. (1988) Alzheimer's disease. Arresting memory decline. *Nature*, **336**, 207-208.

Robbins,T.W. (1997) Arousal systems and attentional processes. *Biol.Psychol.*, **45**, 57-71.

Robbins,T.W. (2002) The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. *Psychopharmacology (Berl)*, **163**, 362-380.

Roberson,M.R. & Harrell,L.E. (1997) Cholinergic activity and amyloid precursor protein metabolism. *Brain Res.Brain Res.Rev.*, **25**, 50-69.

Roberts,A.C. (1996) Comparison of cognitive function in human and non-human primates. *Brain Res.Cogn Brain Res.*, **3**, 319-327.

Rocchi,A., Pellegrini,S., Siciliano,G. & Murri,L. (2003) Causative and susceptibility genes for Alzheimer's disease: a review. *Brain Res.Bull.*, **61**, 1-24.

Rossi,F.M., Pizzorusso,T., Porciatti,V., Marubio,L.M., Maffei,L. & Changeux,J.P. (2001) Requirement of the nicotinic acetylcholine receptor beta 2 subunit for the anatomical and functional development of the visual system. *Proc.Natl.Acad.Sci.U.S.A*, **98**, 6453-6458.

Rossner,S., Apelt,J., Schliebs,R., Perez-Polo,J.R. & Bigl,V. (2001) Neuronal and glial beta-secretase (BACE) protein expression in transgenic Tg2576 mice with amyloid plaque pathology. *J.Neurosci.Res.*, **64**, 437-446.

Rosvold,H., Mirsky,A., Sarason,I., Bransome,E.D.Jr., & Beck,L.H. (1956) A continuous performance test of brain damage. *J Consulting Psych*, **20**, 343–350: cited in Riccio,C.A., Reynolds,C.R., Lowe,P. & Moore,J.J. (2002) The continuous performance test: a window on the neural substrates for attention? *Arch.Clin.Neuropsychol.*, **17**, 235-272.

Roth,R.M., Flashman,L.A., Saykin,A.J., McAllister,T.W. & Vidaver,R. (2004) Apathy in schizophrenia: reduced frontal lobe volume and neuropsychological deficits. *Am.J.Psychiatry*, **161**, 157-159.

Rusted,J. & Eaton-Williams,P. (1991) Distinguishing between attentional and amnestic effects in information processing: the separate and combined effects of scopolamine and nicotine on verbal free recall. *Psychopharmacology (Berl)*, **104**, 363-366.

Rusted,J.M., Caulfield,D., King,L. & Goode,A. (2000) Moving out of the laboratory: does nicotine improve everyday attention? *Behav.Pharmacol.*, **11**, 621-629.

Sabbagh,M.N., Lukas,R.J., Sparks,D.L. & Reid,R.T. (2002) The nicotinic acetylcholine receptor, smoking, and Alzheimer's disease. *J.Alzheimers.Dis.*, **4**, 317-325.

Sahakian,B., Jones,G., Levy,R., Gray,J. & Warburton,D. (1989) The effects of nicotine on attention, information processing, and short-term memory in patients with dementia of the Alzheimer type. *Br.J.Psychiatry*, **154**, 797-800.

Salas,R., Pieri,F., Fung,B., Dani,J.A. & De Biasi,M. (2003) Altered anxiety-related responses in mutant mice lacking the beta4 subunit of the nicotinic receptor. *J.Neurosci.*, **23**, 6255-6263.

Sams-Dodd,F. (1999) Phencyclidine in the social interaction test: an animal model of schizophrenia with face and predictive validity. *Rev.Neurosci.*, **10**, 59-90.

Samson,H.H., Slawecki,C.J., Sharpe,A.L. & Chappell,A. (1998) Appetitive and consummatory behaviors in the control of ethanol consumption: a measure of ethanol seeking behavior. *Alcohol Clin.Exp.Res.*, **22**, 1783-1787.

Samuel,W., Masliah,E., Hill,L.R., Butters,N. & Terry,R. (1994a) Hippocampal connectivity and Alzheimer's dementia: effects of synapse loss and tangle frequency in a two-component model. *Neurology*, **44**, 2081-2088.

Samuel,W., Terry,R.D., DeTeresa,R., Butters,N. & Masliah,E. (1994b) Clinical correlates of cortical and nucleus basalis pathology in Alzheimer dementia. *Arch.Neurol.*, **51**, 772-778.

Sanberg,P.R., Silver,A.A., Shytle,R.D., Philipp,M.K., Cahill,D.W., Fogelson,H.M. & McConville,B.J. (1997) Nicotine for the treatment of Tourette's syndrome. *Pharmacol.Ther.*, **74**, 21-25.

Sansone,M., Battaglia,M. & Castellano,C. (1994) Effect of caffeine and nicotine on avoidance learning in mice: lack of interaction. *J.Pharm.Pharmacol.*, **46**, 765-767.

Sarter,M., Givens,B. & Bruno,J.P. (2001) The cognitive neuroscience of sustained attention: where top-down meets bottom-up. *Brain Res.Brain Res.Rev.*, **35**, 146-160.

Sawaguchi,T. & Yamane,I. (1999) Properties of delay-period neuronal activity in the monkey dorsolateral prefrontal cortex during a spatial delayed matching-to-sample task. *J.Neurophysiol.*, **82**, 2070-2080.

Schenk,D., Barbour,R., Dunn,W., Gordon,G., Grajeda,H., Guido,T., Hu,K., Huang,J., Johnson-Wood,K., Khan,K., Kholodenko,D., Lee,M., Liao,Z., Lieberburg,I., Motter,R., Mutter,L., Soriano,F., Shopp,G., Vasquez,N., Vandevert,C., Walker,S., Wogulis,M., Yednock,T., Games,D. & Seubert,P. (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature*, **400**, 173-177.

Schmitz,C., Rutten,B.P., Pielen,A., Schafer,S., Wirths,O., Tremp,G., Czech,C., Blanchard,V., Multhaup,G., Rezaie,P., Korr,H., Steinbusch,H.W., Pradier,L. & Bayer,T.A. (2004) Hippocampal neuron loss exceeds amyloid plaque load in a transgenic mouse model of Alzheimer's disease. *Am.J.Pathol.*, **164**, 1495-1502.

Selkoe,D.J. (2001) Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev.*, **81**, 741-766.

Selkoe,D.J. (2004) Alzheimer disease: mechanistic understanding predicts novel therapies. *Ann.Intern.Med.*, **140**, 627-638.

Semenova,S., Bespalov,A. & Markou,A. (2003) Decreased prepulse inhibition during nicotine withdrawal in DBA/2J mice is reversed by nicotine self-administration. *Eur.J.Pharmacol.*, **472**, 99-110.

Shallice,T., Burgess,P.W. & Frith,C.D. (1991) Can the neuropsychological case-study approach be applied to schizophrenia? *Psychol.Med.*, **21**, 661-673.

Shapiro,R.M. (1993) Regional neuropathology in schizophrenia: where are we? Where are we going? *Schizophr.Res.*, **10**, 187-239.

Sharma,T., Lancaster,E., Lee,D., Lewis,S., Sigmundsson,T., Takei,N., Gurling,H., Barta,P., Pearlson,G. & Murray,R. (1998) Brain changes in schizophrenia. Volumetric MRI study of families multiply affected with schizophrenia--the Maudsley Family Study 5. *Br.J.Psychiatry*, **173**, 132-138.

Shaw,S., Bencherif,M. & Marrero,M.B. (2002) Janus kinase 2, an early target of alpha 7 nicotinic acetylcholine receptor-mediated neuroprotection against Abeta-(1-42) amyloid. *J.Biol.Chem.*, **277**, 44920-44924.

Shen,J., Bronson,R.T., Chen,D.F., Xia,W., Selkoe,D.J. & Tonegawa,S. (1997) Skeletal and CNS defects in Presenilin-1-deficient mice. *Cell*, **89**, 629-639.

Shenton,M.E., Kikinis,R., Jolesz,F.A., Pollak,S.D., LeMay,M., Wible,C.G., Hokama,H., Martin,J., Metcalf,D. & Coleman,M. (1992) Abnormalities of the left temporal lobe and thought disorder in schizophrenia. A quantitative magnetic resonance imaging study. *N.Engl.J.Med.*, **327**, 604-612.

Sherrington,R., Rogaev,E.I., Liang,Y., Rogaeva,E.A., Levesque,G., Ikeda,M., Chi,H., Lin,C., Li,G. & Holman,K. (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature*, **375**, 754-760.

Shinotoh,H., Namba,H., Fukushi,K., Nagatsuka,S., Tanaka,N., Aotsuka,A., Ota,T., Tanada,S. & Irie,T. (2000) Progressive loss of cortical acetylcholinesterase activity in association with cognitive decline in Alzheimer's disease: a positron emission tomography study. *Ann.Neurol.*, **48**, 194-200.

Shoaib,M., Gommans,J., Morley,A., Stolerman,I.P., Grailhe,R. & Changeux,J.P. (2002) The role of nicotinic receptor beta-2 subunits in nicotine discrimination and conditioned taste aversion. *Neuropharmacology*, **42**, 530-539.

Shulman,J.M., Shulman,L.M., Weiner,W.J. & Feany,M.B. (2003) From fruit fly to bedside: translating lessons from Drosophila models of neurodegenerative disease. *Curr.Opin.Neurol.*, **16**, 443-449.

Shytle,R.D., Silver,A.A., Wilkinson,B.J. & Sanberg,P.R. (2002) A pilot controlled trial of transdermal nicotine in the treatment of attention deficit hyperactivity disorder. *World J.Biol.Psychiatry*, **3**, 150-155.

Sigurdsson,E.M., Knudsen,E., Asuni,A., Fitzer-Attas,C., Sage,D., Quartermain,D., Goni,F., Frangione,B. & Wisniewski,T. (2004) An attenuated immune response is sufficient to enhance cognition in an Alzheimer's disease mouse model immunized with amyloid-beta derivatives. *J.Neurosci.*, **24**, 6277-6282.

Sihver,W., Gillberg,P.G., Svensson,A.L. & Nordberg,A. (1999) Autoradiographic comparison of [3H](-)nicotine, [3H]cytisine and [3H]epibatidine binding in relation to vesicular acetylcholine transport sites in the temporal cortex in Alzheimer's disease. *Neuroscience*, **94**, 685-696.

Simone,P.M. & Baylis,G.C. (1997) Selective attention in a reaching task: effect of normal aging and Alzheimer's disease. *J.Exp.Psychol.Hum.Percept.Perform.*, **23**, 595-608.

Simosky,J.K., Stevens,K.E., Kem,W.R. & Freedman,R. (2001) Intragastric DMXB-A, an alpha7 nicotinic agonist, improves deficient sensory inhibition in DBA/2 mice. *Biol.Psychiatry*, **50**, 493-500.

Simosky,J.K., Stevens,K.E., Adler,L.E. & Freedman,R. (2003) Clozapine improves deficient inhibitory auditory processing in DBA/2 mice, via a nicotinic cholinergic mechanism. *Psychopharmacology (Berl)*, **165**, 386-396.

Sitskoorn,M.M., Aleman,A., Ebisch,S.J., Appels,M.C., & Kahn,R.S. (2004) Cognitive deficits in relatives of patients with schizophrenia: a meta-analysis. *Schizophr Res*, **71**, 285-295

Slotnick,B. (2001) Animal cognition and the rat olfactory system. *Trends Cogn Sci.*, **5**, 216-222.

Smith,A. & Nutt,D. (1996) Noradrenaline and attention lapses. *Nature*, **380**, 291.

Smith,C.M. & Swash,M. (1978) Possible biochemical basis of memory disorder in Alzheimer disease. *Ann.Neurol.*, **3**, 471-473.

Smith,R.C., Singh,A., Infante,M., Khandat,A. & Kloos,A. (2002) Effects of cigarette smoking and nicotine nasal spray on psychiatric symptoms and cognition in schizophrenia. *Neuropsychopharmacology*, **27**, 479-497.

Spence,S.A., Hirsch,S.R., Brooks,D.J. & Grasby,P.M. (1998) Prefrontal cortex activity in people with schizophrenia and control subjects. Evidence from positron emission tomography for remission of 'hypofrontality' with recovery from acute schizophrenia. *Br.J.Psychiatry*, **172**, 316-323.

Spillantini,M.G. & Goedert,M. (1998) Tau protein pathology in neurodegenerative diseases. *Trends Neurosci.*, **21**, 428-433.

Spillantini,M.G. & Goedert,M. (2002) Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P310S tau protein. *J.Neurosci.*, **22**, 9340-9351.

Spowart-Manning,L. & van der Staay,F.J. (2004) The T-maze continuous alternation task for assessing the effects of putative cognition enhancers in the mouse. *Behav.Brain Res.*, **151**, 37-46.

Stassen,H.H., Bridler,R., Hagele,S., Hergersberg,M., Mehmann,B., Schinzel,A., Weisbrod,M. & Scharfetter,C. (2000) Schizophrenia and smoking: evidence for a common neurobiological basis? *Am.J.Med.Genet.*, **96**, 173-177.

Staubli,U., Fraser,D., Kessler,M. & Lynch,G. (1986) Studies on retrograde and anterograde amnesia of olfactory memory after denervation of the hippocampus by entorhinal cortex lesions. *Behav.Neural Biol.*, **46**, 432-444.

Steinlein,O.K., Mulley,J.C., Propping,P., Wallace,R.H., Phillips,H.A., Sutherland,G.R., Scheffer,I.E. & Berkovic,S.F. (1995) A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nat.Genet.*, **11**, 201-203.

Steinlein,O.K., Magnusson,A., Stoodt,J., Bertrand,S., Weiland,S., Berkovic,S.F., Nakken,K.O., Propping,P. & Bertrand,D. (1997) An insertion mutation of the CHRNA4 gene in a family with autosomal dominant nocturnal frontal lobe epilepsy. *Hum.Mol.Genet.*, **6**, 943-947.

Stevens,K.E., Freedman,R., Collins,A.C., Hall,M., Leonard,S., Marks,M.J. & Rose,G.M. (1996) Genetic correlation of inhibitory gating of hippocampal auditory evoked response and alpha-bungarotoxin-binding nicotinic cholinergic receptors in inbred mouse strains. *Neuropsychopharmacology* , **15**, 152-162.

Stevens,K.E., Kem,W.R., Mahnir,V.M. & Freedman,R. (1998) Selective alpha7-nicotinic agonists normalize inhibition of auditory response in DBA mice. *Psychopharmacology (Berl)*, **136** , 320-327.

Stolerman,I.P., Mirza,N.R., Hahn,B. & Shoaib,M. (2000) Nicotine in an animal model of attention. *Eur.J.Pharmacol.*, **393**, 147-154.

Stolerman,I.P., Chamberlain,S., Bizarro,L., Fernandes,C. & Schalkwyk,L. (2004) The role of nicotinic receptor alpha 7 subunits in nicotine discrimination. *Neuropharmacology*, **46**, 363-371.

Sturchler-Pierrat,C., Abramowski,D., Duke,M., Wiederhold,K.H., Mistl,C., Rothacher,S., Ledermann,B., Burki,K., Frey,P., Paganetti,P.A., Waridel,C., Calhoun,M.E., Jucker,M., Probst,A., Staufenbiel,M. & Sommer,B. (1997) Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proc.Natl.Acad.Sci.U.S.A*, **94**, 13287-13292.

Sultzer,D.L., Mahler,M.E., Mandelkern,M.A., Cummings,J.L., Van Gorp,W.G., Hinkin,C.H. & Berisford,M.A. (1995) The relationship between psychiatric symptoms and regional cortical metabolism in Alzheimer's disease. *J.Neuropsychiatry Clin.Neurosci.*, **7**, 476-484.

Sultzer,D.L., Brown,C.V., Mandelkern,M.A., Mahler,M.E., Mendez,M.F., Chen,S.T. & Cummings,J.L. (2003) Delusional thoughts and regional frontal/temporal cortex metabolism in Alzheimer's disease. *Am.J.Psychiatry*, **160**, 341-349.

Swerdlow,N.R. & Geyer,M.A. (1998) Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophr.Bull.*, **24**, 285-301.

Tanemura,K., Murayama,M., Akagi,T., Hashikawa,T., Tominaga,T., Ichikawa,M., Yamaguchi,H. & Takashima,A. (2002) Neurodegeneration with tau accumulation in a transgenic mouse expressing V337M human tau. *J.Neurosci.*, **22**, 133-141.

Tanzi,R.E. (1999) A genetic dichotomy model for the inheritance of Alzheimer's disease and common age-related disorders. *J.Clin.Invest*, **104**, 1175-1179.

Tatebayashi,Y., Miyasaka,T., Chui,D.H., Akagi,T., Mishima,K., Iwasaki,K., Fujiwara,M., Tanemura,K., Murayama,M., Ishiguro,K., Planel,E., Sato,S., Hashikawa,T. & Takashima,A. (2002) Tau filament formation and associative memory deficit in aged mice expressing mutant (R406W) human tau. *Proc.Natl.Acad.Sci.U.S.A*, **99**, 13896-13901.

Taylor,S.F. (1996) Cerebral blood flow activation and functional lesions in schizophrenia. *Schizophr.Res.*, **19**, 129-140.

Tekin,S., Fairbanks,L.A., O'Connor,S., Rosenberg,S. & Cummings,J.L. (2001) Activities of daily living in Alzheimer's disease: neuropsychiatric, cognitive, and medical illness influences. *Am.J.Geriatr.Psychiatry*, **9**, 81-86.

Terry,A.V., Jr., Buccafusco,J.J. & Jackson,W.J. (1993) Scopolamine reversal of nicotine enhanced delayed matching-to-sample performance in monkeys. *Pharmacol.Biochem.Behav.*, **45**, 925-929.

Terry,A.V., Jr., Risbrough,V.B., Buccafusco,J.J. & Menzaghi,F. (2002) Effects of (+/-)-4-[[2-(1-methyl-2-pyrrolidinyl)ethyl]thio]phenol hydrochloride (SIB-1553A), a selective ligand for nicotinic acetylcholine receptors, in tests of visual attention and distractibility in rats and monkeys. *J.Pharmacol.Exp.Ther.*, **301**, 284-292.

Terry,R.D., Masliah,E., Salmon,D.P., Butters,N., DeTeresa,R., Hill,R., Hansen,L.A. & Katzman,R. (1991) Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann.Neurol.*, **30**, 572-580.

Tesseur,I., van Dorpe,J., Bruynseels,K., Bronfman,F., Sciot,R., Van Lommel,A. & Van Leuven,F. (2000) Prominent axonopathy and disruption of axonal transport in transgenic mice expressing human apolipoprotein E4 in neurons of brain and spinal cord. *Am.J.Pathol.*, **157**, 1495-1510.

Thanos,P.K. & Slotnick,B.M. (1997) Short-term odor memory: effects of posterior transection of the lateral olfactory tract in the rat. *Physiol Behav.*, **61**, 903-906.

Trimble,M.R. (1990) Psychopathology of frontal lobe syndromes. *Semin.Neurol.*, **10**, 287-294.

Turchi,J. & Sarter,M. (2001) Bidirectional modulation of basal forebrain N-methyl-D-aspartate receptor function differentially affects visual attention but not visual discrimination performance. *Neuroscience*, **104**, 407-417.

Tyler,S.J., Dawbarn,D., Wilcock,G.K. & Allen,S.J. (2002) alpha- and beta-secretase: profound changes in Alzheimer's disease. *Biochem.Biophys.Res.Commun.*, **299**, 373-376.

Unwin,N. (1995) Acetylcholine receptor channel imaged in the open state. *Nature*, **373**, 37-43.

Unwin,N. (1998) The nicotinic acetylcholine receptor of the Torpedo electric ray. *J.Struct.Biol.*, **121**, 181-190.

Van Broeckhoven,C., Backhovens,H., Cruts,M., De Winter,G., Bruyland,M., Cras,P. & Martin,J.J. (1992) Mapping of a gene predisposing to early-onset Alzheimer's disease to chromosome 14q24.3. *Nat.Genet.*, **2**, 335-339.

van Duijn,C.M. & Hofman,A. (1991) Relation between nicotine intake and Alzheimer's disease. *BMJ*, **302**, 1491-1494.

van Haaren,F., Anderson,K.G., Haworth,S.C. & Kem,W.R. (1999) GTS-21, a mixed nicotinic receptor agonist/antagonist, does not affect the nicotine cue. *Pharmacol.Biochem.Behav.*, **64** , 439-444.

Van Hoesen,G.W., Parvizi,J. & Chu,C.C. (2000) Orbitofrontal cortex pathology in Alzheimer's disease. *Cereb.Cortex*, **10**, 243-251.

Van Kampen,M., Selbach,K., Schneider,R., Schiegel,E., Boess,F. & Schreiber,R. (2004) AR-R 17779 improves social recognition in rats by activation of nicotinic alpha7 receptors. *Psychopharmacology (Berl)*, **172**, 375-383.

van Os,J., Hanssen,M., Bak,M., Bijl,R.V. & Vollebergh,W. (2003) Do urbanicity and familial liability coparticipate in causing psychosis? *Am.J.Psychiatry*, **160**, 477-482.

Van Os,J., & McGuffin,P. (2003) Can the social environment cause schizophrenia? *Br J Psychiatry*, **182,** 291-292.

Varolio,C. (1573) De Nervis Opticis. Padua: Meitti: cited in Levin,H.S., Eisenberg,H.M., & Benton,A.L. (1991) Frontal Lobe Function and Dysfunction. Oxford University Press, New York, U.S.A..

Vecera,S.P. & Rizzo,M. (2003) Spatial attention: normal processes and their breakdown. *Neurol.Clin.*, **21**, 575-607.

Velakoulis,D., Pantelis,C., McGorry,P.D., Dudgeon,P., Brewer,W., Cook,M., Desmond,P., Bridle,N., Tierney,P., Murrie,V., Singh,B. & Copolov,D. (1999) Hippocampal volume in first-episode psychoses and chronic schizophrenia: a high-resolution magnetic resonance imaging study. *Arch.Gen.Psychiatry*, **56**, 133-141.

Venables,P.H. (1992) Hippocampal function and schizophrenia. Experimental psychological evidence. *Ann.N.Y.Acad.Sci.*, **658**, 111-127.

Verbois,S.L., Sullivan,P.G., Scheff,S.W. & Pauly,J.R. (2000) Traumatic brain injury reduces hippocampal alpha7 nicotinic cholinergic receptor binding. *J.Neurotrauma*, **17**, 1001-1011.

Verbois,S.L., Hopkins,D.M., Scheff,S.W. & Pauly,J.R. (2003) Chronic intermittent nicotine administration attenuates traumatic brain injury-induced cognitive dysfunction. *Neuroscience*, **119**, 1199-1208.

Vita,A., Dieci,M., Giobbio,G.M., Tenconi,F. & Invernizzi,G. (1997) Time course of cerebral ventricular enlargement in schizophrenia supports the hypothesis of its neurodevelopmental nature. *Schizophr.Res.* , **23**, 25-30.

Vorhees,C.V., Weisenburger,W.P., Acuff-Smith,K.D. & Minck,D.R. (1991) An analysis of factors influencing complex water maze learning in rats: effects of task complexity, path order and escape assistance on performance following prenatal exposure to phenytoin. *Neurotoxicol.Teratol.*, **13**, 213-222.

Waldo,M., Myles-Worsley,M., Madison,A., Byerley,W. & Freedman,R. (1995) Sensory gating deficits in parents of schizophrenics. *Am.J.Med.Genet.*, **60**, 506-511.

Waldo,M.C., Carey,G., Myles-Worsley,M., Cawthra,E., Adler,L.E., Nagamoto,H.T., Wender,P., Byerley,W., Plaetke,R. & Freedman,R. (1991) Codistribution of a sensory gating deficit and schizophrenia in multi-affected families. *Psychiatry Res.*, **39**, 257-268.

Waldo,M.C., Cawthra,E., Adler,L.E., Dubester,S., Staunton,M., Nagamoto,H., Baker,N., Madison,A., Simon,J. & Scherzinger,A. (1994) Auditory sensory gating, hippocampal volume, and catecholamine metabolism in schizophrenics and their siblings. *Schizophr.Res.*, **12**, 93-106.

Waldum,H.L., Nilsen,O.G., Nilsen,T., Rorvik,H., Syversen,V., Sanvik,A.K., Haugen,O.A., Torp,S.H. & Brenna,E. (1996) Long-term effects of inhaled nicotine. *Life Sci.*, **58**, 1339-1346.

Walker,E.F. (1994) Developmentally moderated expressions of the neuropathology underlying schizophrenia. *Schizophr.Bull.*, **20**, 453-480.

Walsh,J.S., Welch,H.G., & Larson,E.B. (1990) Survival of outpatients with Alzheimer-type dementia. *Ann Intern Med*, **113,** 429-434.

Wang,H.Y., Lee,D.H., D'Andrea,M.R., Peterson,P.A., Shank,R.P. & Reitz,A.B. (2000) beta-Amyloid(1-42) binds to alpha7 nicotinic acetylcholine receptor with high affinity. Implications for Alzheimer's disease pathology. *J.Biol.Chem.*, **275**, 5626-5632.

Warburton,D.M. & Rusted,J.M. (1993) Cholinergic control of cognitive resources. *Neuropsychobiology*, **28**, 43-46.

Warburton,D.M. & Arnall,C. (1994) Improvements in performance without nicotine withdrawal. *Psychopharmacology (Berl)*, **115**, 539-542.

Watkins,P.B., Zimmerman,H.J., Knapp,M.J., Gracon,S.I. & Lewis,K.W. (1994) Hepatotoxic effects of tacrine administration in patients with Alzheimer's disease. *JAMA*, **271**, 992-998.

Weinberger,D.R., Berman,K.F. & Zec,R.F. (1986) Physiologic dysfunction of dorsolateral prefrontal cortex in schizophrenia. I. Regional cerebral blood flow evidence. *Arch.Gen.Psychiatry*, **43**, 114-124.

Weinberger,D.R. (1988) Schizophrenia and the frontal lobe. *Trends Neurosci.*, **11**, 367-370.

Weinberger,D.R. & Lipska,B.K. (1995) Cortical maldevelopment, anti-psychotic drugs, and schizophrenia: a search for common ground. *Schizophr.Res.*, **16**, 87-110.

Weinberger,D.R., Egan,M.F., Bertolino,A., Callicott,J.H., Mattay,V.S., Lipska,B.K., Berman,K.F. & Goldberg,T.E. (2001) Prefrontal neurons and the genetics of schizophrenia. *Biol.Psychiatry*, **50**, 825-844.

Welt,L. (1988) Uber Charakterveranderungen des Menschen infogle von Lasionen des Stiirnhirns. *Deutsche Archiv fur Klinische Medizin*. **42**: 339-390: cited in Niedermeyer,E. (1998) Frontal lobe functions and dysfunctions. *Clin.Electroencephalogr.*, **29**, 79-90.

Wesnes,K. & Warburton,D.M. (1984) Effects of scopolamine and nicotine on human rapid information processing performance. *Psychopharmacology (Berl)*, **82**, 147-150.

Westerman,M.A., Cooper-Blacketer,D., Mariash,A., Kotilinek,L., Kawarabayashi,T., Younkin,L.H., Carlson,G.A., Younkin,S.G. & Ashe,K.H. (2002) The relationship between Abeta and memory in the Tg2576 mouse model of Alzheimer's disease. *J.Neurosci.*, **22**, 1858-1867.

Wevers,A., Monteggia,L., Nowacki,S., Bloch,W., Schutz,U., Lindstrom,J., Pereira,E.F., Eisenberg,H., Giacobini,E., de Vos,R.A., Steur,E.N., Maelicke,A., Albuquerque,E.X. & Schroder,H. (1999) Expression of nicotinic acetylcholine receptor subunits in the cerebral cortex in Alzheimer's disease: histotopographical correlation with amyloid plaques and hyperphosphorylated-tau protein. *Eur.J.Neurosci.*, **11**, 2551-2565.

White,H.K. & Levin,E.D. (1999) Four-week nicotine skin patch treatment effects on cognitive performance in Alzheimer's disease. *Psychopharmacology (Berl)*, **143**, 158-165.

White,H.K. & Levin,E.D. (2004) Chronic transdermal nicotine patch treatment effects on cognitive performance in age-associated memory impairment. *Psychopharmacology (Berl)*, **171**, 465-471.

Wilcock,G.K., Lilienfeld,S. & Gaens,E. (2000) Efficacy and safety of galantamine in patients with mild to moderate Alzheimer's disease: multicentre randomised controlled trial. Galantamine International-1 Study Group. *BMJ*, **321**, 1445-1449.

Wilson,A.L., Langley,L.K., Monley,J., Bauer,T., Rottunda,S., McFalls,E., Kovera,C. & McCarten,J.R. (1995) Nicotine patches in Alzheimer's disease: pilot study on learning, memory, and safety. *Pharmacol.Biochem.Behav.*, **51**, 509-514.

Wolach,I. & Pratt,H. (2001) The mode of short-term memory encoding as indicated by event-related potentials in a memory scanning task with distractions. *Clin.Neurophysiol.*, **112**, 186-197.

Wonnacott,S. (1997) Presynaptic nicotinic ACh receptors. *Trends Neurosci.*, **20**, 92-98.

Woodruff-Pak,D.S., Li,Y.T. & Kem,W.R. (1994) A nicotinic agonist (GTS-21), eyeblink classical conditioning, and nicotinic receptor binding in rabbit brain. *Brain Res.*, **645**, 309-317.

Wu,J., Buchsbaum,M.S., Moy,K., Denlea,N., Kesslak,P., Tseng,H., Plosnaj,D., Hetu,M., Potkin,S. & Bracha,S. (1993) Olfactory memory in unmedicated schizophrenics. *Schizophr.Res.*, **9**, 41-47.

Xu,W., Gelber,S., Orr-Urtreger,A., Armstrong,D., Lewis,R.A., Ou,C.N., Patrick,J., Role,L., De Biasi,M. & Beaudet,A.L. (1999) Megacystis, mydriasis, and ion channel defect in mice lacking the alpha3 neuronal nicotinic acetylcholine receptor. *Proc.Natl.Acad.Sci.U.S.A*, **96**, 5746-5751.

Yang,Y.K., Nelson,L., Kamaraju,L., Wilson,W. & McEvoy,J.P. (2002) Nicotine decreases bradykinesia-rigidity in haloperidol-treated patients with schizophrenia. *Neuropsychopharmacology*, **27**, 684-686.

Yee,C.M., Nuechterlein,K.H., Morris,S.E. & White,P.M. (1998) P50 suppression in recent-onset schizophrenia: clinical correlates and risperidone effects. *J.Abnorm.Psychol.*, **107**, 691-698.

Yildiz,D. (2004) Nicotine, its metabolism and an overview of its biological effects. *Toxicon*, **43**, 619-632.

Zakzanis,K.K. & Hansen,K.T. (1998) Dopamine D2 densities and the schizophrenic brain. *Schizophr.Res.*, **32**, 201-206.

Zamani,M.R., Allen,Y.S., Owen,G.P. & Gray,J.A. (1997) Nicotine modulates the neurotoxic effect of beta-amyloid protein(25-35)) in hippocampal cultures. *Neuroreport*, **8**, 513-517.

Zarrindast,M.R., Sadegh,M. & Shafaghi,B. (1996) Effects of nicotine on memory retrieval in mice. *Eur.J.Pharmacol.*, **295**, 1-6.

Zhang,J., Wu,X., Qin,C., Qi,J., Ma,S., Zhang,H., Kong,Q., Chen,D., Ba,D. & He,W. (2003) A novel recombinant adeno-associated virus vaccine reduces behavioral impairment and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Neurobiol.Dis.*, **14**, 365-379.

Zoli,M., Picciotto,M.R., Ferrari,R., Cocchi,D. & Changeux,J.P. (1999) Increased neurodegeneration during ageing in mice lacking high-affinity nicotine receptors. *EMBO J.*, **18**, 1235-1244.

Neuropsychopharmacology (2004), 1–10

www.neuropsychopharmacology.org

Nicotine Improves Sustained Attention in Mice: Evidence for Involvement of the α7 Nicotinic Acetylcholine Receptor

Jared W Young[1], Keith Finlayson*[,1], Christopher Spratt[1], Hugh M Marston[1,2], Nicola Crawford[1], John S Kelly[1] and John Sharkey[1]

[1]*Fujisawa Institute of Neuroscience, Division of Neuroscience, University of Edinburgh, Edinburgh, UK*

In humans, nicotine has been shown to improve attention in both normal and impaired individuals. Observations in rats reflect some, but not all aspects of the nicotine-induced improvements in humans. To date these findings have not been replicated in mice. To examine the effect of nicotine on sustained attention in mice, we have established a version of the 5-choice serial reaction-time (5-CSR) task with graded levels of difficulty, based upon spatial displacement and a variable intertrial interval. Using this paradigm, microgram doses of nicotine produced a consistent reduction in the level of omissions and an improvement in proportion correct in normal mice. This improvement in sustained attention was made irrespectively of whether mice had previously received nicotine. In an attempt to elucidate which nicotinic acetylcholine receptor (nAChR) subtype(s) mediate this effect, we examined the performance of α7 nAChR knockout (KO) mice in the 5-CSR task. α7 nAChR KO mice not only acquired the task more slowly than their wild-type littermates, but on attaining asymptotic performance, they exhibited a higher level of omissions. In conclusion, by increasing the level of task difficulty, the performance of mice was maintained at sufficiently low levels to allow a demonstrable improvement in performance upon nicotine administration. Furthermore, as α7 KO mice are clearly impaired in the acquisition and asymptotic performance of this task, the α7 nAChR may be involved in mediating these effects of nicotine.

Neuropsychopharmacology advance online publication, 18 February 2004; doi:10.1038/sj.npp.1300393

Keywords: nicotine; mice; attention; receptors; nicotinic (α7); knockout

INTRODUCTION

It has been proposed that attentional dysfunction may underlie the psychopathology of schizophrenia (Cullum *et al*, 1993; Cornblatt and Keilp, 1994). In humans, attentional performance is generally assessed using the continuous performance test (CPT), where subjects have to attend to visual stimuli over a sustained period of time (Levin *et al*, 1998; White and Levin, 1999; Shytle *et al*, 2002). Nicotine, the predominant psychoactive compound in tobacco smoke, has been shown to enhance sustained attention in normal humans by reducing omission levels (Levin *et al*, 1998). Moreover, it has been suggested that nicotine can lock the brain into an attentional processing mode whereby there are fewer lapses in attention and therefore less omissions (Mancuso *et al*, 1999). These observations may underlie the ability of nicotine to enhance attention and improve the symptomatology of various human diseases including schizophrenia (Yang *et al*, 2002), Alzheimer's disease (White and Levin, 1999), attention deficit hyperactivity disorder (Shytle *et al*, 2002), Parkinson's disease (O'Neill *et al*, 2002), and Tourette's syndrome (Sanberg *et al*, 1997).

The identity of the nicotinic acetylcholine receptor (nAChR) subtype(s) mediating the beneficial effects of nicotine on cognition has yet to be elucidated. Currently, nine genes have been described that encode neuronal nAChR subunits in mammals (α2–α7, β2–β4), with α9 and α10 subunits also found in mammalian cochlea. The majority of subunits appear capable of forming heteromeric channels, with the number of combinations identified in tissue continually increasing (Le Novere *et al*, 2002). In rodent brains, the two most predominant nAChRs appear to be the heteromeric α4β2 nAChR, and the homomeric α7 nAChR, accounting for 85 and 10% of neuronal nAChRs respectively (Clarke *et al*, 1985). In rats, the roles that these receptors play in sustained attention is examined using the 5-choice serial reaction-time (5-CSR) task (Mirza and Stolerman, 1998; Grottick and Higgins, 2000; Stolerman *et al*, 2000; Mirza and Bright, 2001; Hahn *et al*, 2002,

*Correspondence: Dr K Finlayson, Fujisawa Institute of Neuroscience, Division of Neuroscience, University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ UK, Tel: +44 131 650 8491, Fax: +44 131 667 9381, E-mail: Keith.Finlayson@ed.ac.uk

[2]Present address: Organon Laboratories, Newhouse, Lanarkshire, ML1 5SH UK.

Received 20 October 2003; revised 15 December 2003; accepted 18 December 2003

Online publication: 23 December 2003 at http://www.acnp.org/citations/Npp12230303480/default.pdf

2003a, b; Terry *et al*, 2002; Grottick *et al*, 2003). While nicotine-induced improvements in sustained attention have been reported in the rat literature, these studies have generally required the additional complexities afforded by brain lesions, poorly performing subjects, or task challenges (Muir *et al*, 1995; Grottick and Higgins, 2000; Hahn *et al*, 2002). Indeed, no consistent improvements have been reported in unimpaired rats (Mirza and Bright, 2001; Terry *et al*, 2002). Clear identification of the receptor subtypes underlying the beneficial effects of nicotine is made more onerous (Shoaib *et al*, 2002) by the current lack of truly selective compounds and the difficulties associated with producing nAChR subtype selective drugs (Gotti *et al*, 2000; Broad *et al*, 2002). It has therefore been suggested that a combined approach of pharmacological interventions and transgenic animals may help delineate the nAChR subtypes involved (Gotti *et al*, 2000; Chapman, 2002).

Since its first description by Carli *et al* (1983), the 5-CSR task has been extensively used in rat studies. In contrast, the task has received only limited, and comparatively recent attention in mice (Humby *et al*, 1999; Marston *et al*, 2001). Hitherto, no reports addressing the effects of nicotine on the performance of mice in the 5-CSR task have been published. In the present study, we describe the development and use of a version of the 5-CSR task to examine the hypothesis that nicotine can improve attentional function in mice by primarily reducing omission levels and secondarily by increasing proportion correct. In addition, the role that the α7 nAChR plays in attention was examined by using α7 knockout (KO; B6.12957-Chrna7^{tm1bay}, Orr-Urtreger *et al*, 1997) mice in the task, who should conversely exhibit a deficit as measured by increased omission levels and a lower proportion correct score. Some of the current findings have previously been presented in abstract form (Young *et al*, 2003).

MATERIALS AND METHODS

Animal Maintenance and Genotyping

Two groups of C57 Bl/6J male mice (study 1, $n = 16$; study 2, $n = 25$, Charles River, Margate, UK), weighing between 22 and 26 g at the start of the studies, were used in the behavioral paradigms described. Eight α7 nAChR KO (B6.12957-Chrna7^{tm1bay}; The Jackson Laboratory, Bar Habor, USA) and eight age-matched wild-type littermates (WT; N8F1 and F2), weighing between 22 and 27 g at the inception of the study, were used in study 3. For confirmation of genotype, transgenic animals were tail tipped under halothane/nitrous oxide anesthesia, and DNA obtained by proteinase K treatment of tail samples (Promega, Southampton, UK; Sambrook and Russell, 2001). The PCR protocol used was as described on the Jackson Laboratory website (www.jax.org). All animals were group housed (where possible) in a temperature controlled room (21 ± 1°C), with a 12 h light/dark cycle (lights on at 0730) and were tested during the light phase of the cycle. Mice were maintained at 85% of their free-feeding weight and were permitted free access to water during training and testing. The animals were given *ad libitum* access to food approximately every 5 weeks in order to re-establish a free-feeding weight. Studies were performed under license by UK authorities (Scientific Animal Procedures Act, 1986, http://www.homeoffice.gov.uk), and in accordance with the Guide for the Care and Use of Laboratory Animals as adapted and promulgated by the National Institute of Health.

Tissue Procurement and P_2 Synaptosomal Membrane Preparation

For radioligand binding studies, P_2 synaptosomal membranes were prepared as described previously (Maemoto *et al*, 1997). Mice were killed by cervical dislocation, the brains removed, and immediately placed in ice-cold saline (0.9% NaCl). The whole brain minus cerebellum (due to the low density of α7 nAChRs) was used for preparation of synaptosomal membranes. Brain tissue from each animal was treated independently in the α7 nAChR KO study. Tissue samples were homogenized in 15 volumes (15 vol) of ice-cold (4°C) 0.32 M sucrose using a glass/Teflon homogenizer, centrifuged at 1000 *g* for 10 min (4°C), and the resulting supernatant centrifuged at 17 000 *g* (20 min, 4°C). The synaptosomal/mitochondrial P_2 pellet was lysed in 30 vol of ice-cold (4°C) milliQ H_2O for 60 min and centrifuged at 50 000 *g* (10 min, 4°C). The membrane pellet was then resuspended in 30 vol of ice-cold (4°C) 50 mM potassium phosphate assay buffer (50 mM potassium phosphate, 1 mM EDTA, and 0.01% sodium azide, pH 7.4), centrifuged at 50 000 *g* for 10 min (4°C) and resuspended in 5 vol (original tissue weight) of assay buffer and stored at −20°C. On the day of use, frozen membranes were thawed, diluted to 30 vol with ice-cold (4°C) assay buffer and the suspension centrifuged at 50 000 *g* for 10 min at 4°C. The pellet was then resuspended in the appropriate volume of assay buffer and the protein content determined as described previously (Finlayson *et al*, 2001).

Behavioral Apparatus

Training and testing took place in 'nine-hole' operant chambers (25 × 25 × 25 cm, Cambridge Cognition, Cambridge, UK). The response holes were used in two configurations; 'narrow' with holes 3–7 open and 1, 2, 8, and 9 occluded, or 'wide' with holes 1, 3, 5, 7, and 9 open and 2, 4, 6, and 8 closed. The mice were required to respond to a visual stimulus recessed into the holes, with a nose poke. A response was detected by an infrared beam crossing the entrance of each hole. Liquid reinforcement in the form of strawberry milkshake (Yazoo®; UK; 20 μl) was delivered by a peristaltic pump to a spigot located within the magazine at the chamber front, on the wall opposite to the nine-hole array. Entry into the magazine was monitored by an infrared beam. The house light was set into the roof of the operant chamber, which was housed within a sound-attenuating box containing a fan, which provided ventilation and a constant low background noise. An infrared camera was installed within each box allowing performance to be monitored during testing. Each operant chamber was interfaced to an Acorn computer (RISC OS). The software required was programmed in house using the Arachnid extension to BBC Basic (Paul Fray Ltd, Cambridge, UK).

Behavioral Handling and Procedures

For the 3 days prior to training, all mice were handled for approximately 10 min per day. On the day before initiation of training mice were introduced to the liquid reinforcer. On training days 1 and 2, mice were placed in the nine-hole boxes for 10 min, during which liquid reinforcement was dispensed every 15 s into the well of the magazine, while the magazine was lit. Entry into the magazine caused the light to be extinguished until the next reinforcement was delivered. At the end of this and subsequent sessions, the wells beneath the spigots were inspected to ensure no liquid was present. On day 3, in order to obtain reinforcement, mice were required to nose poke in any of the 5 lit holes at the rear of the chamber. This process was repeated every day until all mice were able to make at least 60 responses to the light cue within a 25 min session.

5-CSR Task

At the beginning of each session the house light was extinguished and the magazine was lit. A nose poke in the magazine initiated the trial sequence. An intertrial interval (ITI) of 2 s preceded one of the five response holes being illuminated. To record a correct response the mouse had to respond within a stimulus duration (SD) period of 10 s, or during the following 2 s limited hold (LH) when the light was extinguished. The magazine light was then illuminated, a reinforcement dispensed, with entry in to the magazine initiating a 4 s reward interval (RI). Failure to respond during the SD + LH resulted in an 'omission' error being recorded and a 4 s time out (TO) initiated. During a TO the house light was on and all holes were unresponsive, then as the TO phase ended the house light was extinguished, and the magazine illuminated. The mouse could then begin a new trial by responding in the magazine. If, during the choice phase, the mouse responded in a hole other than the one that was lit, the response was registered as an incorrect response and a TO phase began. If the mouse responded during the ITI, an anticipatory error was recorded and a TO phase initiated. Each session lasted 25 min, or 120 trials if completed sooner. The SD was initially set at 10 s and was only reduced to 8 s following attainment of a mean correct latency half that of the SD and a minimum of 10 correct responses per session, maintained for over two consecutive sessions. The SD was further reduced to 4, 2, and then 1 s based upon these response latency criteria. Successful acquisition of the task was defined as attainment of a 1 s SD, a proportion correct score (correct/correct + incorrect + anticipatory errors) of >0.8, and with % omissions (omissions/correct + incorrect + omissions) of $<40\%$. In study 3, once every mouse had attained acquisition criteria, the mice were trained continuously until asymptotic performance had been established. In studies 1 and 2, once every mouse had attained acquisition criteria, a variable (2–10 s) ITI was introduced, and mice were trained continuously until asymptotic performance had been established. The mice were allocated to a drug group in a counter-balanced design. Each mouse received saline for the three training sessions prior to testing, and then given their allocated nicotine dose everyday in the four subsequent test sessions. The effects of subcutaneous (−)nicotine hydrogen tartrate (Sigma, Poole, UK) on performance (study 1; 3, 30, and 300 μg/kg; study 2; 1, 10, and 100 μg/kg) were assessed 10 min after drug injection, with nicotine prepared freshly everyday.

[^{3}H]Methyllycaconitine ([^{3}H]MLA) Binding to the α7 nAChR

[^{3}H]MLA (19.8 Ci/mmol; Tocris, Bristol, UK) binding to the α7 nAChR was carried out as previously described (Davis *et al*, 2000). Binding assays were conducted in a total volume of 250 μl; consisting of 50 or 100 μl (no drug present) of potassium phosphate assay buffer, 50 μl of test drug, 50 μl of [^{3}H]MLA (final concentration approximately 2 nM), and 100 μl of membrane suspension. Test compounds (methyllycaconitine and (±) epibatidine, Tocris, Bristol, UK; (−)nicotine hydrogen tartrate and d-tubocurarine chloride, Sigma, Poole, UK) were prepared by serial dilution in assay buffer. Nonspecific binding was determined in the presence of 1 mM d-tubocurarine. Binding was initiated by the addition of membranes, and samples were incubated for 60 min at 25°C. Binding was terminated by filtration onto glass fiber filters (GF/B, Whatman; presoaked for 3 h in 0.3% polyethylenimine) using a Brandel cell harvester, followed by three rapid (1 ml) washes with ice-cold phosphate-buffered saline (20 mM Na_2HPO_4, 5 mM KH_2PO_4, 150 mM NaCl, pH 7.4). Filter disks were transferred to RT30 tubes (Sterling, UK) and radioactivity determined using a Packard 2500TR liquid scintillation counter.

Data Analysis

The main dependent behavioral variables selected for analysis were: (a) % omissions, (b) proportion correct, and (c) mean correct latency (cumulative correct latency/correct). As the proportion correct in studies 1 and 3 was not normally distributed, the data were arcsine transformed. However, in Figures 1b and 3d raw data are presented. Each variable in studies 1 and 2 were compared to the mean scores obtained with saline using a three-way ANOVA (dose, day, and ITI time), with Tukey *post hoc* analysis. Acquisition performance in study 3 was analyzed by assessing the increase in proportion correct across sessions per subject, and the data fitted using a four-parameter logistic. The number of sessions required to attain 0.50 proportion correct (A_{50}) was calculated for each subject, and compared between the groups using a *t*-test. Asymptotic performance in study 3 was compared using a two-way repeated measures ANOVA (genetic make-up and day). All statistics were performed using Sigma Stat (v. 2.03, SPSS, USA). For the [^{3}H]MLA binding studies, data were analyzed using Sigma Plot 8.0 (Jandel, USA) and ligand affinities (K_D/K_i) were calculated as described previously (Finlayson *et al*, 2001).

RESULTS

Study 1—The Effect of Nicotine (3, 30, and 300 μg/kg) on Mouse 5-CSR Task Performance

Initially, a pilot study was conducted in which mice ($n = 16$) were trained to perform the 5-CSR task using a less

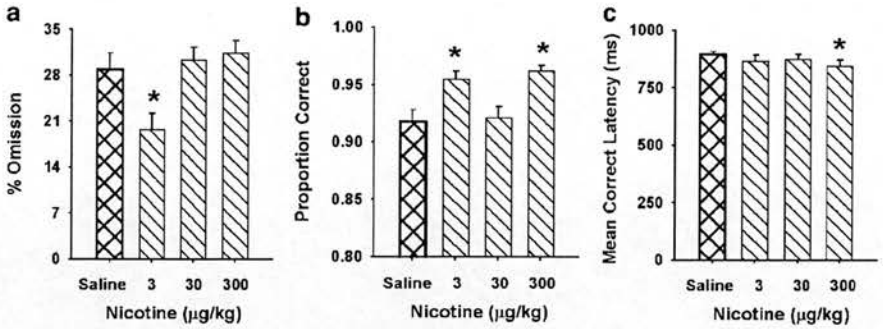


Figure 1 Effects of nicotine on the performance of mice in the modified 5-CSR task. Each mouse in this study had undergone extensive training and had received nicotine (s.c.) injections 3 weeks prior to testing. The mice were separated into four groups in a counter-balanced design and given saline for three prior training sessions (Thursday, Friday, and Monday), followed by their allocated dose for 4 consecutive days (Tuesday–Friday). The 3 μg/kg dose of (−)nicotine significantly reduced % omissions (a) and increased proportion correct (b) without altering mean correct latency (c). In contrast, 300 μg/kg of (−)nicotine reduced mean correct latency (c) and increased proportion correct (b), without affecting % omissions (a). The 30 μg/kg dose of (−)nicotine had no effect on any measure. Doses of nicotine that produced significant effects compared to saline on these measures are marked (*$p<0.05$), with data shown as mean ± SEM.

demanding narrow array (holes 3–7), with animals subsequently administered saline or three different doses of nicotine (3, 30, and 300 μg/kg). The mice were tested according to a Latin-square design over a 10-week period. Each 4-day dosing period was separated by a 10-day washout period, during which a baseline response to saline was re-established. No overall statistical difference was seen with any dose of nicotine examined (data not shown). However, as the mice had performed at near optimum levels (proportion correct approximately equal to 0.96), any clear enhancement in performance would be difficult to detect. The task was subsequently modified to increase the level of difficulty by using a wide array (holes 1, 3, 5, 7, and 9) and by varying the ITI (2–10 s instead of a constant 2 s). Such modifications have previously been used to increase task difficulty in human sustained attention tasks (Bates *et al*, 1995). These modifications differ from the acute challenges utilized for rats as they are employed in both training and testing days (Hahn *et al*, 2002). Following a 3-week washout period mice were again administered saline or nicotine (3, 30, and 300 μg/kg). As can be seen in Figure 1a, the 3 μg/kg dose of nicotine produced a significant reduction in % omissions and in Figure 1b, an increase in proportion correct when compared to the control group. A three-way ANOVA with nicotine dose, ITI time, and day as between-subject factors, yielded significant main effects of: nicotine dose on % omissions ($F(3,36)=17.4$, $p<0.001$), proportion correct ($F(3,36)=8.83$, $p<0.001$), and mean correct latency ($F(3,36)=5.27$, $p=0.004$); ITI time on % omissions ($F(3,36)=28.2$, $p<0.001$), and on mean correct latency ($F(3,36)=57.8$, $p<0.001$); day on proportion correct ($F(3,36)=5.15$, $p=0.005$). Tukey *post hoc* analysis on nicotine dose revealed a significant effect of 3 μg/kg nicotine on % omissions (Figure 1a) ($F(3,36)=17.4$, $p<0.001$), and on proportion correct (Figure 1b) ($F(3,36)=8.83$, $p<0.05$). Nicotine at 300 μg/kg also increased proportion correct (Figure 1b) ($F(3,36)=8.83$, $p=0.001$), and decreased mean correct latency (Figure 1c) ($F(3,36)=5.27$, $p<0.005$), but had no significant main effect on % omissions (Figure 1a). However, no effect was observed at the intermediate nicotine dose of 30 μg/kg on any of the parameters examined.

Study 2—The Effect of Nicotine (1, 10, and 100 μg/kg) on 5-CSR Task Performance in Drug-Naive Mice

As study 1 was performed in mice that had previously been exposed to nicotine on a repeated basis, the significant improvements observed in performance could have been confounded by factors such as receptor upregulation. Indeed, our studies suggest that a 1-week washout period with saline following nicotine administration is not always sufficient to ensure a return to baseline performance (data not shown). We therefore examined the cognitive effects of nicotine using the modified 5-CSR task and drug-naive mice. As the 3 μg/kg dose of nicotine had produced the most robust improvement in performance we examined the effect of nicotine at 1, 10, and 100 μg/kg. All three doses of nicotine produced a reduction in the levels of % omissions when compared to the control group (Figure 2a). A three-way ANOVA with nicotine dose, ITI time, and day as between-subject factors, yielded significant main effects of: nicotine dose on % omissions ($F(3,36)=212$, $p<0.001$), proportion correct ($F(3,36)=3.285$, $p=0.032$), and mean correct latency ($F(3,36)=13.3$, $p<0.001$); ITI time on % omissions ($F(3,36)=40.4$, $p<0.001$), proportion correct ($F(3,36)=3.512$, $p=0.016$), and on mean correct latency ($F(3,36)=9.83$, $p<0.001$); day on % omissions ($F(3,36)=6.58$, $p=0.001$). Tukey *post hoc* analysis on nicotine dose revealed significant effects of 1, 10, and 100 μg/kg nicotine on % omissions ($F(3,36)=22.6$, $p<0.001$ for each dose Figure 2a). Moreover the 1 μg/kg dose of nicotine significantly increased proportion correct (Figure 2b) and the 10 μg/kg dose significantly altered mean correct latency (Figure 2c). There were significant main effects of the 1 μg/kg dose of nicotine on proportion correct ($F(3,36)=3.29$, $p=0.05$), and the 10 μg/kg dose significantly increased mean correct latency ($F(3,36)=13.3$, $p<0.05$).

Study 3—The Effect of α7 nAChR KO on Mouse Performance in the 5-CSR Task

The debate over which nicotinic receptor subtypes are responsible for the cognitive effects of nicotine is far from

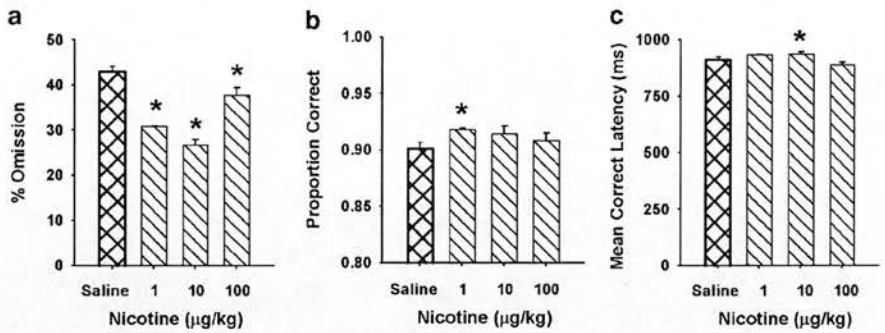


Figure 2 Effects of nicotine on the performance of drug-naive mice in the modified 5-CSR task. Mice were trained specifically for this study and as such were nicotine naive. The mice were separated into four groups in a counter-balanced design and given saline for three prior training sessions (Thursday, Friday, and Monday), followed by their allocated dose for 4 consecutive days (Tuesday–Friday). Every dose of (−)nicotine examined significantly reduced % omissions (a), with 1 μg/kg increasing proportion correct (b). The 10 μg/kg dose of (−)nicotine produced a significant increase in mean correct latency (c). Doses of nicotine that produced significant effects compared to saline on these measures are marked (*$p<0.05$), with data shown as mean ± SEM.

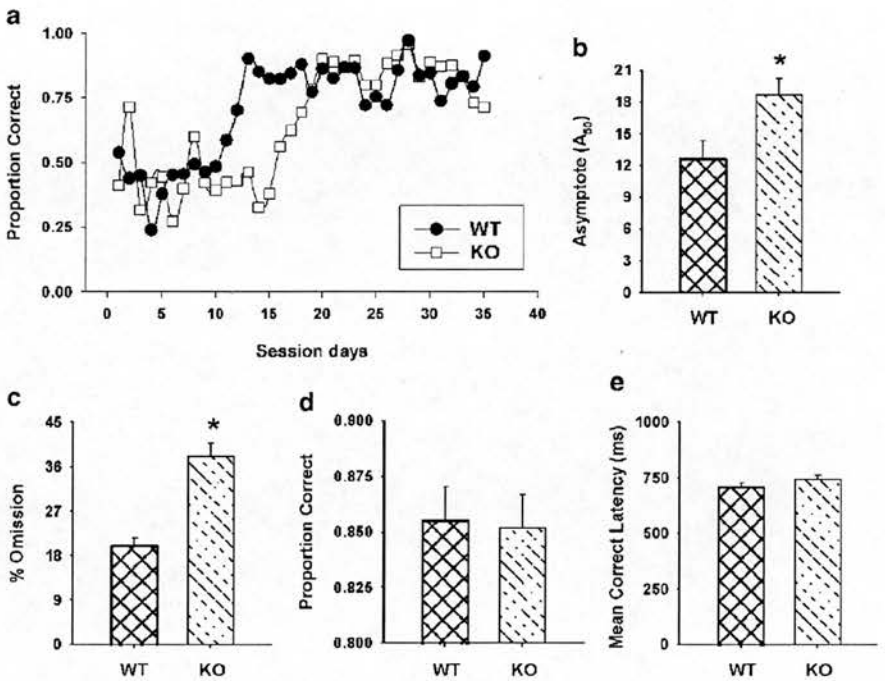


Figure 3 Effects of α7 nAChR KO on mouse performance of the 5-CSR task. The α7 nAChR KO mice had impaired acquisition as shown in (a), with data shown as proportion correct across session days for one representative subject from each group, and (b) the mean time taken by each group to reach halfway to asymptote (A_{50}). α7 nAChR KO mice were also impaired in asymptotic performance of the task, as measured by % omissions (c). Proportion correct (d), and mean correct latency (e) are also shown but were unaffected by removal of this receptor in the standard task. *Significant difference to that of the controls ($p<0.05$), with data shown as mean ± SEM.

resolved. As there are no commercially available α7 nAChR selective agonists or totally suitable antagonists we examined whether ablation of the α7 nAChR would have any detrimental effects on the performance of mice in the 5-CSR task. As Figure 3a shows, α7 KO mice took significantly longer to acquire the task than their age-matched WT littermates, with the time taken to reach 50% of asymptotic performance (A_{50}) being approximately 5 days longer (Figure 3b) ($F(1,12)=4.763$, $p=0.05$). After attaining acquisition criteria and asymptotic performance, the α7 KO mice exhibited significantly higher levels of % omissions (Figure 3c). A two-factor ANOVA for repeated measures with genotype and day as between-subject factors yielded significant main effects of genotype on % omissions ($F(1,36)=7.67$, $p<0.05$) and day on proportion correct ($F(1,36)=4.501$, $p=0.009$). There were no significant effects of genotype on proportion correct (Figure 3d) or mean correct latency (Figure 3e).

α7 nAChR Genotyping and [^{3}H]MLA Binding

To validate the behavioral deficits observed for the α7 KO mice, genotyping was performed, and a radioligand binding

assay established using the putatively α7 nAChR selective antagonist [^{3}H]MLA. Genotyping was conducted with α7 nAChR-specific primers that identified WT and disrupted alleles. These studies confirmed that the mice used were of the appropriate genotype (data not shown). Verification of the absence of α7 nAChRs at the protein level was obtained by establishing a [^{3}H]MLA binding assay. Using P_2 synaptosomal membranes prepared from normal mice and a range of cholinergic drugs, there was a concentration-dependent inhibition of [^{3}H]MLA binding, with the following rank order of potency; MLA > epibatidine > d-tubocurarine = nicotine (Figure 4a). The affinity (K_D) of MLA was 1.31 ± 0.35 nM (nH = 0.81 ± 0.02; $n = 4$), with K_i values of 167 ± 106 nM (nH = 1.46 ± 0.14; $n = 3$) for epibatidine, 1.80 ± 0.07 μM (nH = 1.48 ± 0.44; $n = 3$) for d-tubocurarine, and 1.41 ± 0.82 μM (nH = 1.06 ± 0.09; $n = 3$) for nicotine. For α7 KO mice and their WT littermates, each brain was treated individually. As Figure 4b clearly shows, in WT littermates' specific [^{3}H]MLA binding was approximately 60% of total binding, whereas no specific binding was observed for the α7 KO mice.

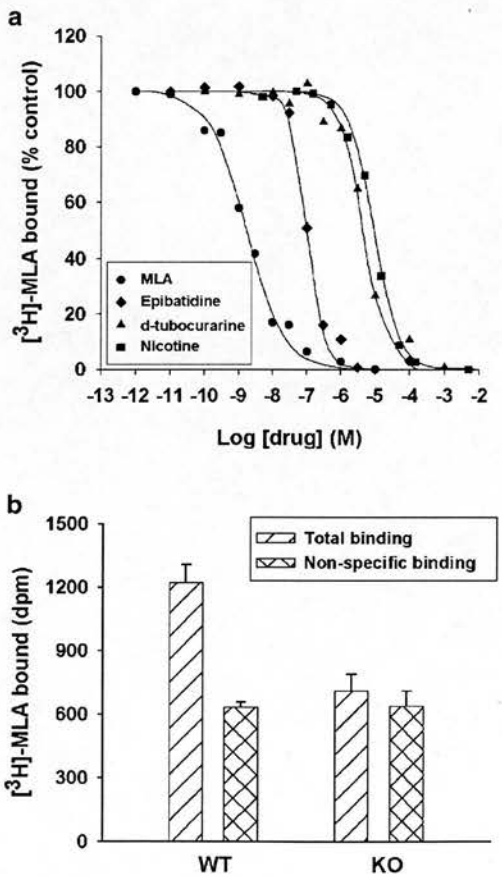


Figure 4 [^{3}H]MLA binding in P_2 synaptosomal brain membranes from WT and α7 KO mice. (a) Concentration-dependent inhibition of [^{3}H]MLA (2 nM) binding to P_2 synaptosomal brain membranes from WT animals by a range of cholinergic drugs. (b) The level of specific [^{3}H]MLA binding in P_2 membranes prepared from individual WT control and transgenic mice ($n = 4$ per group) is shown. Clear specific [^{3}H]MLA binding was observed for WT animals, which is absent in α7 KO mice.

DISCUSSION

This is the first study in normal mice to demonstrate nicotine-induced improvements in sustained attention. These results support the hypothesis which suggests that the nicotine-induced improvement in attention in normal humans is characterized by a reduction in omission levels, and by an increase in proportion correct. Moreover, our demonstration that α7 nAChR KO mice exhibit higher omission levels in a less demanding version of the task implicates the α7 nAChR in attentional function.

In humans, the CPT has been routinely used to evaluate attentional performance (White and Levin, 1999; Shytle *et al*, 2002). In normal human subjects, nicotine administration has consistently been shown to enhance attention by reducing omissions (Levin *et al*, 1998). This is consistent with the hypothesis of Mancuso *et al* (1999), whereby the nicotine-induced improvement in attention is a consequence of nicotine acting to 'lock the brain into the attentional processing mode and so there are fewer lapses in attention', hence fewer errors of omission might be expected. However, an improvement in accuracy (proportion correct) following nicotine administration has also been observed in subjects displaying impaired attentional function, associated with schizophrenia, Alzheimer's disease, and attention deficit hyperactivity disorder (White and Levin, 1999; Shytle *et al*, 2002; Yang *et al*, 2002). The 5-CSR task was developed to examine sustained attention in rodents (Carli *et al*, 1983) and is regarded as being analogous to the CPT (Jones and Higgins, 1995). This task has been used extensively with rats, with nicotine generally showing an improvement in attention, but only when lesions or specific task challenges have been introduced to impair performance (Mirza and Stolerman, 1998; Grottick and Higgins, 2000; Stolerman *et al*, 2000; Mirza and Bright, 2001; Hahn *et al*, 2002, 2003a, b; Grottick *et al*, 2003). A consistent demonstration of a nicotine-induced facilitation of attention in unimpaired rats, unlike observations in humans (Levin *et al*, 1998), has proven challenging (Mirza and Bright, 2001; Terry *et al*, 2002). While widely used with rats, the 5-CSR task was only comparatively recently modified for use in mice (Humby *et al*, 1999), with no reports to date, on the effects of nicotine. Our initial studies indicated that the lack of effect of nicotine in mice may have been due to a 'ceiling effect' (data not shown), a feature commonly observed for rats (Grottick and Higgins, 2000; Hahn *et al*, 2002). Modification of the task by introducing the wide array and a variable ITI (this latter alteration minimizes the possibility of mice using any temporal mediating strategies), resulted in an increase in % omissions and a reduction in proportion correct. These data clearly show that the performance of mice is not impaired when tested in the larger rat nine-hole operant chambers and so a reduction in box size, as used by Humby *et al* (1999), is not a prerequisite. Four groups of mice, previously exposed to different doses of nicotine, were given a 3-week washout period with saline to ensure a return to prestudy baseline performance. These mice were then tested in the modified task and given different doses of nicotine (Figure 1), based upon previous rodent and human studies (Muir *et al*, 1995; Grobe *et al*, 1998; Stolerman *et al*, 2000; Mirza and Bright, 2001). The 3 μg/kg dose of nicotine

produced a clear improvement in performance, with a significant reduction in percent omissions and a concomitant increase in proportion correct. As nicotine administration is known to alter nAChR density (Gentry and Lukas, 2002), confirmation that the observed improvements in sustained attention were not merely a consequence of increased receptor number, required nicotine to be examined in drug-naive mice. Therefore, a new study was conducted using a modified dose range that took cognizance of the improvements seen at 3 μg/kg of nicotine. This study revealed a significant enhancement in the performance of drug-naive mice that were administered a 1 μg/kg dose of nicotine. The doses of nicotine that improved attention in mice in the present studies are consistent with those reported to improve attention in normal humans (Levin *et al*, 1998; Heishman and Henningfield, 2000; Min *et al*, 2001). In contrast, studies in rats have generally required higher doses of nicotine and the inclusion of lesions or task challenges (Muir *et al*, 1995; Mirza and Stolerman, 1998; Stolerman *et al*, 2000; Hahn *et al*, 2002, 2003a, b; Grottick *et al*, 2003). In addition, whereas nicotine reduced omission levels in ostensibly normal mice in the current study, and previously in normal human subjects (Levin *et al*, 1998), in rats, the most consistent manifestation of an improvement in attention was an increase in accuracy (Mirza and Stolerman, 1998; Hahn *et al*, 2002). The reasons underlying these different effects of nicotine on omissions in rodents have yet to be clearly defined. One possible explanation is that in rats, the lack of effect of nicotine on omissions may be due to a floor effect as baseline omissions are already $<10\%$, whereas in mice they are approximately 20% (Inglis *et al*, 2001; Spratt *et al*, 2001). This difference in omission levels cannot be attributed simply to the fact that mice were tested in apparatus commonly used for rats, as when the apparatus was scaled down, omission levels were still about 20% (Humby *et al*, 1999). Therefore the reduction observed in omissions in mice is consistent with the nicotine-induced improvement in attention hypothesis proposed by Mancuso *et al* (1999).

While it appears that the 1 and 3 μg/kg doses of nicotine enhance sustained attention, at higher doses the physiological effects become increasingly complex (Picciotto, 2003; see Figure 5 for schematic representation). Between 10 and 100 μg/kg the overall trend continues to be that of a reduction in omissions, but with no effect on proportion correct. In contrast, at 300 μg/kg, nicotine increased proportion correct without altering % omissions. As this dose also reduced mean correct latency, this suggests the increase in accuracy may reflect psychomotor stimulation (Grottick and Higgins, 2000), with these mice consequently not attending to the cue array any more than control subjects. This would be consistent with nicotine being reported to produce motoric excitation at 125 and 250 μg/kg in mice (Nordberg and Bergh, 1985). Further increases in nicotine dose have been shown to lead to motoric inhibition and hypothermia (Marks *et al*, 1983; Figure 5).

Despite a plethora of evidence demonstrating nicotine-induced improvements in attention in different species, there has been no definitive identification of which nAChR subtype(s) are responsible. However, several studies indicate that the α7 nAChR appears crucial in maintaining one aspect of attentional function, namely sensory gating (Stevens *et al*, 1996, 1998; Simosky *et al*, 2001). Schizophrenics have poor sensory gating with a reduction in the P50 auditory evoked potential (Waldo *et al*, 1995). This deficiency has been linked to chromosome 15, in a region proximal to the α7 locus (Freedman *et al*, 1997). Moreover, the DBA/2 mouse strain, which has a natural reduction in α7 nAChR density, show sensory gating deficits (Stevens *et al*, 1996), that can be ameliorated by treatment with the α7 partial agonist DMXBA (GTS-21; Stevens *et al*, 1998; Simosky *et al*, 2001) and atypical antipsychotics (Simosky *et al*, 2003). DMXBA also attenuated the sensory gating deficits observed in rats that were reared in isolation (O'Neill *et al*, 2003), an animal neuro-developmental model of schizophrenia (Geyer *et al*, 1993). Furthermore, both DMXBA (Kem, 2000), and the full α7 nAChR agonist AR-R17779 (Mullen *et al*, 2000), are able to replicate the beneficial effects observed with nicotine on working memory (Felix and Levin, 1997; Levin and Simon, 1998). In contrast, and perhaps somewhat surprisingly, recent studies in rats showed that AR-R17779 had no effect on attention in the 5-CSR task (Grottick and Higgins, 2000; Grottick *et al*, 2003; Hahn *et al*, 2003a). To date, independent evaluation of these agents is not possible as these compounds are not commercially available. Similarly, investigation of the α7 nAChR subtype using the selective antagonists α-bungarotoxin and methyllycaconitine has proven difficult as the former is a large peptide which does not cross the blood–brain barrier, while there is now some debate over the selectivity of the latter (Mogg *et al*, 2002). Therefore, we chose to investigate the role of the α7 nAChR

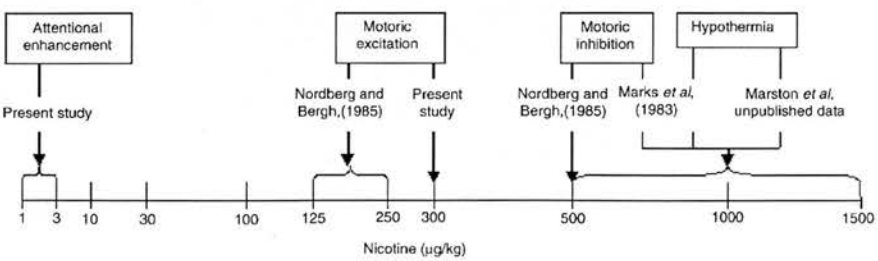


Figure 5 Schematic representation of nicotine-induced behavioral effects in mice. Nicotine exerts both cognitive and physiological effects that are largely dose dependent. The present study suggests that low doses of nicotine (1–3 μg/kg) produce improvements in attention. Higher doses appear to reduce mean correct latency, suggesting an enhancement of motoric capabilities, consistent with previous studies (Nordberg and Bergh, 1985). Doses of nicotine of 500 μg/kg and above inhibit motoric responses, with hypothermic effects observed. A further increase to 5 mg/kg has been shown to induce seizures in mice (Damaj *et al*, 1999).

in attention in mice by studying α7 KO animals (Orr-Urtreger *et al*, 1997) in the 5-CSR task. Even in the simple version of the 5-CSR task, the α7 KO mice had a clear deficit in attention, with omission levels significantly increased when compared to WT littermates. The absence of the α7 nAChR in the KO mice was verified by genotyping and radioligand binding studies using [^{3}H]MLA. For WT mice, the K_D/K_i values of the drugs examined in the [^{3}H]MLA binding assay were consistent with previous studies (Whiteaker *et al*, 1999). It is possible that the deficiencies observed are attributable to developmental problems that are a consequence of α7 nAChR KO, although these animals do have an ostensibly normal appearance; with standard growth, survival, gait, anatomy, and no nervous system abnormalities (Orr-Urtreger *et al*, 1997). In addition, Paylor *et al* (1998) reported no differences in the behavioral phenotype of α7 nAChR KO mice and their WT littermates when subjected to a battery of behavioral tests, including contextual and auditory fear conditioning, spatial learning in the Morris water maze, and anxiety tests. However, unexpectedly it was noted that the mice did not exhibit sensory gating deficits in the PPI paradigm (Paylor *et al*, 1998), although this result could have been confounded by the use female mice. It is conceivable that compensatory mechanisms, such as alterations in nAChR density, distribution, and/or receptor subtype, may result in gene KO studies producing fundamentally different results from that observed in traditional pharmacological studies. However, Orr-Urtreger *et al* (1997) did not detect differences in high affinity nicotinic binding sites in these α7 nAChR KO mice. The development of the modified 5-CSR task should allow confirmation of the α7 nAChR KO data, if and when, α7 selective agonists become available and we can then address whether species differences exist between rats and mice. In addition, future studies will address whether nicotine can improve the performance of α7 nAChR KO mice in this modified 5-CSR task and/or whether a similar effect is observed in α7 nAChR heterozygous mice.

In conclusion, modifying the 5-CSR task for mice impaired baseline performance and enabled the demonstration of an enhancement in sustained attention following nicotine administration. Moreover, we have also shown that α7 nAChR KO mice have a profound deficit in attention that was observed without increasing task difficulty. Therefore, it is conceivable that the α7 nAChR plays a role in nicotine-induced improvements in sustained attention.

ACKNOWLEDGEMENTS

We thank Dr LE Kerr, Mrs N Logan, and Mrs J McLuckie for their assistance and helpful comments during these studies and in the preparation of this manuscript. This work was funded by a research grant from the Fujisawa Pharmaceutical Company Limited (Osaka, Japan).

REFERENCES

Bates T, Mangan G, Stough C, Corballis P (1995). Smoking, processing speed and attention in a choice reaction time task. *Psychopharmacology* **120**: 209–212.

Broad LM, Felthouse C, Zwart R, McPhie GI, Pearson KH, Craig PJ *et al* (2002). PSAB-OFP, a selective alpha 7 nicotinic receptor agonist, is also a potent agonist of the 5-HT3 receptor. *Eur J Pharmacol* **452**: 137–144.

Carli M, Robbins TW, Evenden JL, Everitt BJ (1983). Effects of lesions to ascending noradrenergic neurones on performance of a 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. *Behav Brain Res* **9**: 361–380.

Chapman PF (2002). Giving drugs to knockout mice: can they do that? *Trends Neurosci* **25**: 277–279.

Clarke PB, Schwartz RD, Paul SM, Pert CB, Pert A (1985). Nicotinic binding in rat brain: autoradiographic comparison of [^{3}H]acetylcholine, [^{3}H]nicotine, and [^{125}I]-alpha-bungarotoxin. *J Neurosci* **5**: 1307–1315.

Cornblatt BA, Keilp JG (1994). Impaired attention, genetics, and the pathophysiology of schizophrenia. *Schizophr Bull* **20**: 31–46.

Cullum CM, Harris JG, Waldo MC, Smernoff E, Madison A, Nagamoto HT *et al* (1993). Neurophysiological and neuropsychological evidence for attentional dysfunction in schizophrenia. *Schizophr Res* **10**: 131–141.

Damaj MI, Glassco W, Dukat M, Martin BR (1999). Pharmacological characterization of nicotine-induced seizures in mice. *J Pharmacol Exp Ther* **291**: 1284–1291.

Davis DHJ, Lukas RJ, Peng JH, Kelly JS, Finlayson K (2000). Characterisation of [^{3}H]-methyllycaconitine binding sites in rat brain tissue and cells stably expressing the human alpha-7 nicotinic receptor. *Eur J Neurosci* **12**: 374.

Felix R, Levin ED (1997). Nicotinic antagonist administration into the ventral hippocampus and spatial working memory in rats. *Neuroscience* **81**: 1009–1017.

Finlayson K, McLuckie J, Hern J, Aramori I, Olverman HJ, Kelly JS (2001). Characterisation of [^{125}I]-apamin binding sites in rat brain membranes and HE293 cells transfected with SK channel subtypes. *Neuropharmacology* **41**: 341–350.

Freedman R, Coon H, Myles-Worsley M, Orr-Urtreger A, Olincy A, Davis A *et al* (1997). Linkage of a neuropsychological deficit in schizophrenia to a chromosome 15 locus. *Proc Natl Acad Sci USA* **94**: 587–592.

Gentry CL, Lukas RJ (2002). Regulation of nicotinic acetylcholine receptor numbers and function by chronic nicotine exposure. *Curr Drug Target CNS Neurol Disord* **1**: 359–385.

Geyer MA, Wilkinson LS, Humby T, Robbins TW (1993). Isolation rearing of rats produces a deficit in prepulse inhibition of acoustic startle similar to that in schizophrenia. *Biol Psychiatry* **34**: 361–372.

Gotti C, Carbonnelle E, Moretti M, Zwart R, Clementi F (2000). Drugs selective for nicotinic receptor subtypes: a real possibility or a dream? *Behav Brain Res* **113**: 183–192.

Grobe JE, Perkins KA, Goettler-Good J, Wilson A (1998). Importance of environmental distractors in the effects of nicotine on short-term memory. *Exp Clin Psychopharmacol* **6**: 209–216.

Grottick AJ, Haman M, Wyler R, Higgins GA (2003). Reversal of a vigilance decrement in the aged rat by subtype-selective nicotinic ligands. *Neuropsychopharmacology* **28**: 880–887.

Grottick AJ, Higgins GA (2000). Effect of subtype selective nicotinic compounds on attention as assessed by the five-choice serial reaction time task. *Behav Brain Res* **117**: 197–208.

Hahn B, Sharples CG, Wonnacott S, Shoaib M, Stolerman IP (2003a). Attentional effects of nicotinic agonists in rats. *Neuropharmacology* **44**: 1054–1067.

Hahn B, Shoaib M, Stolerman IP (2002). Nicotine-induced enhancement of attention in the five-choice serial reaction time task: the influence of task demands. *Psychopharmacology* **162**: 129–137.

Hahn B, Shoaib M, Stolerman IP (2003b). Involvement of the prefrontal cortex but not the dorsal hippocampus in the

attention-enhancing effects of nicotine in rats. *Psychopharmacology* **168**: 271–279.

Heishman SJ, Henningfield JE (2000). Tolerance to repeated nicotine administration, subjective and physiological responses in nonsmokers. *Psychopharmacology* **152**: 321–333.

Humby T, Laird FM, Davies W, Wilkinson LS (1999). Visuospatial attentional functioning in mice: interactions between cholinergic manipulations and genotype. *Eur J Neurosci* **11**: 2813–2823.

Inglis WL, Olmstead MC, Robbins TW (2001). Selective deficits in attentional performance on the 5-choice serial reaction time task following pedunculopontine tegmental nucleus lesions. *Behav Brain Res* **123**: 117–131.

Jones DN, Higgins GA (1995). Effect of scopolamine on visual attention in rats. *Psychopharmacology* **120**: 142–149.

Kem WR (2000). The brain alpha7 nicotinic receptor may be an important therapeutic target for the treatment of Alzheimer's disease: studies with DMXBA (GTS-21). *Behav Brain Res* **113**: 169–181.

Le Novere N, Corringer PJ, Changeux JP (2002). The diversity of subunit composition in nAChRs: evolutionary origins, physiologic and pharmacologic consequences. *J Neurobiol* **53**: 447–456.

Levin ED, Conners CK, Silva D, Hinton SC, Meck WH, March J *et al* (1998). Transdermal nicotine effects on attention. *Psychopharmacology* **140**: 135–141.

Levin ED, Simon BB (1998). Nicotinic acetylcholine involvement in cognitive function in animals. *Psychopharmacology* **138**: 217–230.

Maemoto T, Finlayson K, Olverman HJ, Akahane A, Horton RW, Butcher SP (1997). Species differences in brain adenosine A_1 receptor pharmacology revealed by the use of xanthine and pyrazolopyridine based antagonists. *Br J Pharmacol* **122**: 1202–1208.

Mancuso G, Warburton DM, Melen M, Sherwood N, Tirelli E (1999). Selective effects of nicotine on attentional processes. *Psychopharmacology* **146**: 199–204.

Marks MJ, Burch JB, Collins AC (1983). Genetics of nicotine response in four inbred strains of mice. *J Pharmacol Exp Ther* **226**: 291–302.

Marston HM, Spratt C, Kelly JS (2001). Phenotyping complex behaviours: assessment of circadian control and 5-choice serial reaction learning in the mouse. *Behav Brain Res* **125**: 189–193.

Min SK, Moon IW, Ko RW, Shain HS (2001). Effects of transdermal nicotine on attention and memory in healthy elderly nonsmokers. *Psychopharmacology* **159**: 83–88.

Mirza NR, Bright JL (2001). Nicotine-induced enhancements in the five-choice serial reaction time task in rats are strain-dependent. *Psychopharmacology* **154**: 8–12.

Mirza NR, Stolerman IP (1998). Nicotine enhances sustained attention in the rat under specific task conditions. *Psychopharmacology* **138**: 266–274.

Mogg AJ, Whiteaker P, McIntosh JM, Marks M, Collins AC, Wonnacott S (2002). Methyllycaconitine is a potent antagonist of alpha-conotoxin-MII-sensitive presynaptic nicotinic acetylcholine receptors in rat striatum. *J Pharmacol Exp Ther* **302**: 197–204.

Muir JL, Everitt BJ, Robbins TW (1995). Reversal of visual attentional dysfunction following lesions of the cholinergic basal forebrain by physostigmine and nicotine but not by the 5-HT3 receptor antagonist, ondansetron. *Psychopharmacology* **118**: 82–92.

Mullen G, Napier J, Balestra M, DeCory T, Hale G, Macor J *et al* (2000). (−)-Spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin-2′-one], a conformationally restricted analogue of acetylcholine, is a highly selective full agonist at the alpha 7 nicotinic acetylcholine receptor. *J Med Chem* **43**: 4045–4050.

Nordberg A, Bergh C (1985). Effect of nicotine on passive avoidance behaviour and motoric activity in mice. *Acta Pharmacol Toxicol* **56**: 337–341.

O'Neill HC, Rieger K, Kem WR, Stevens KE (2003). DMXB, an alpha(7) nicotinic agonist, normalizes auditory gating in isolation-reared rats. *Psychopharmacology* **169**: 332–339.

O'Neill MJ, Murray TK, Lakics V, Visanji NP, Duty S (2002). The role of neuronal nicotinic acetylcholine receptors in acute and chronic neurodegeneration. *Curr Drug Target CNS Neurol Disord* **1**: 399–411.

Orr-Urtreger A, Goldner FM, Saeki M, Lorenzo I, Goldberg L, De Biasi M *et al* (1997). Mice deficient in the alpha7 neuronal nicotinic acetylcholine receptor lack alpha-bungarotoxin binding sites and hippocampal fast nicotinic currents. *J Neurosci* **17**: 9165–9171.

Paylor R, Nguyen M, Crawley JN, Patrick J, Beaudet A, Orr-Urtreger A (1998). Alpha7 nicotinic receptor subunits are not necessary for hippocampal-dependent learning or sensorimotor gating: a behavioral characterization of Acra7-deficient mice. *Learn Mem* **5**: 302–316.

Picciotto MR (2003). Nicotine as a modulator of behavior: beyond the inverted U. *Trends Pharmacol Sci* **24**: 493–499.

Sambrook J, Russell DW (2001). *Molecular Cloning: A Laboratory Manual*, 3rd edn. New York: Cold Spring Harbor Laboratory Press.

Sanberg PR, Silver AA, Shytle RD, Philipp MK, Cahill DW, Fogelson HM *et al* (1997). Nicotine for the treatment of Tourette's syndrome. *Pharmacol Ther* **74**: 21–25.

Shoaib M, Gommans J, Morley A, Stolerman IP, Grailhe R, Changeux JP (2002). The role of nicotinic receptor beta-2 subunits in nicotine discrimination and conditioned taste aversion. *Neuropharmacology* **42**: 530–539.

Shytle RD, Silver AA, Wilkinson BJ, Sanberg PR (2002). A pilot controlled trial of transdermal nicotine in the treatment of attention deficit hyperactivity disorder. *World J Biol Psychiatry* **3**: 150–155.

Simosky JK, Stevens KE, Adler LE, Freedman R (2003). Clozapine improves deficient inhibitory auditory processing in DBA/2 mice, via a nicotinic cholinergic mechanism. *Psychopharmacology* **165**: 386–396.

Simosky JK, Stevens KE, Kem WR, Freedman R (2001). Intragastric DMXB-A, an alpha7 nicotinic agonist, improves deficient sensory inhibition in DBA/2 mice. *Biol Psychiatry* **50**: 493–500.

Spratt C, McQuatt NE, Sharkey J, Kelly JS, Marston HM (2001). Comparison of rats and mice in a serial reaction task. *Br Neurosci Assoc Abstr* **16**: P490.3.

Stevens KE, Freedman R, Collins AC, Hall M, Leonard S, Marks MJ *et al* (1996). Genetic correlation of inhibitory gating of hippocampal auditory evoked response and alpha-bungarotoxin-binding nicotinic cholinergic receptors in inbred mouse strains. *Neuropsychopharmacology* **15**: 152–162.

Stevens KE, Kem WR, Mahnir VM, Freedman R (1998). Selective alpha7-nicotinic agonists normalize inhibition of auditory response in DBA mice. *Psychopharmacology* **136**: 320–327.

Stolerman IP, Mirza NR, Hahn B, Shoaib M (2000). Nicotine in an animal model of attention. *Eur J Pharmacol* **393**: 147–154.

Terry AV, Risbrough VB, Buccafusco JJ, Menzaghi F (2002). Effects of (+/−)-4-[[2-(1-methyl-2-pyrrolidinyl)ethyl]thio]phenol hydrochloride (SIB-1553A), a selective ligand for nicotinic acetylcholine receptors, in tests of visual attention and distractibility in rats and monkeys. *J Pharmacol Exp Ther* **301**: 284–292.

Waldo M, Myles-Worsley M, Madison A, Byerley W, Freedman R (1995). Sensory gating deficits in parents of schizophrenics. *Am J Med Genet* **60**: 506–511.

White HK, Levin ED (1999). Four-week nicotine skin patch treatment effects on cognitive performance in Alzheimer's disease. *Psychopharmacology* **143**: 158–165.

Whiteaker P, Davies ARL, Marks MJ, Blagbrough IS, Potter BVL, Wolstenholme AJ *et al* (1999). An autoradiographic study of the distribution of binding sites for the novel α7-selective nicotinic radioligand [^{3}H]-methyllycaconitine in the mouse brain. *Eur J Neurosci* **11**: 2689–2696.

Yang YK, Nelson L, Kamaraju L, Wilson W, McEvoy JP (2002). Nicotine decreases bradykinesia-rigidity in haloperidol-treated patients with schizophrenia. *Neuropsychopharmacology* **27**: 684–686.

Young JW, Spratt C, Marston HM, Finlayson K, Kelly JS, Sharkey J (2003). Effect of nicotine on visuo-spatial sustained attention in mice as measured by the 5-choice serial reaction time task. *J Psychopharmacol* **17**(Suppl): pA64.