

Acetylcholine and Posttraumatic Stress Disorder

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Signed Statement

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Dedication

To my four daughters,
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who were all born during the conduct of this research.

Abstract

Posttraumatic Stress Disorder (PTSD) is a psychiatric condition that can develop following exposure to a traumatic event involving actual or threatened death or serious injury. Responses include intense fear, helplessness or horror. Symptoms are characterised into clusters, described as re-experiencing, avoidance, and arousal. These symptoms, which are also evident in other conditions, have been associated with dysfunctions in the central acetylcholinergic system. Benefits from administering acetylcholinesterase inhibitors (AChEI) to people suffering these symptoms have been demonstrated. Donepezil hydrochloride, a reversible inhibitor of the enzyme acetylcholinesterase, is used in the treatment of conditions with difficulties in cognitive function, but has not been used in PTSD.

The aim of this thesis was to determine (1) whether there was a difference in the ACh system in people with PTSD and (2) whether administration of an AChEI would change the symptomatology.

IDEX (I^{123} iododexetimide) has been useful in imaging muscarinic-ACh receptors using Single Photon Emission Computerised Tomography (SPECT) and was utilised to investigate whether cholinergic activity in PTSD is altered. One hundred and sixty eight potential subjects were screened and eleven PTSD subjects were enrolled in the IDEX SPECT study. Three healthy non-PTSD control subjects also completed the study. Due to technical complications only the data obtained from eight PTSD and two control subjects was available for analysis. Imaging data for 2 further healthy non-PTSD control subjects were obtained from another study. Sixteen subjects were enrolled in the donepezil open label study (assessed at baseline, Week 2, 6 and 10). Nine PTSD subjects completed the 10-week trial and seven withdrew prematurely (at or after Week 2) due to side effects or a worsening of

PTSD symptoms.

For the IDEX SPECT study, a voxel-by-voxel statistical analysis of the PTSD subject group versus the control group showed both areas of reduced and increased IDEX uptake. Significant clusters in the PTSD group with a **reduced** IDEX uptake centred around the bilateral hippocampus, left insula and right precuneus, while **increased** IDEX uptake appeared in the caudate head.

For the donepezil study, in the per-protocol analysis (including only the 9 subjects that completed the protocol), all psychological assessments revealed a difference between the totals obtained at the Week 10 visit compared to those at the Baseline visit and the improvement was in the order of 51%. The intention-to-treat analysis (including all 16 subjects), a repeated measures Analysis of Variance (ANOVA) with a mixed models approach showed that all psychological measures demonstrated statistically significant benefits of the treatment. All subjects who completed the protocol recounted considerable improvement in their overall PTSD symptom profile, which covered symptoms in each of the three clusters.

The results of the IDEX SPECT study suggest that alterations in ACh binding in PTSD are evident and may begin to explain a part of the altered cognitive symptomatology apparent in this condition. The pilot open label donepezil trial provided some preliminary evidence that treatment with an AChEI can lessen the intrusions and distress associated with traumatic memories in people with PTSD.

List of Abbreviations

5-HT	Serotonin
^{99m}Tc -HMPAO	99m -technetium-hexamethylpropyleneamineoxime
ACh	Acetylcholine
AChE	Acetylcholinesterase
AChEI	Acetylcholinesterase Inhibitor
AChE-E	Acetylcholinesterase - Erythrocytic
AChE-R	Acetylcholinesterase - Readthrough
AChE-S	Acetylcholinesterase - Synaptic
AD	Alzheimers Disease
ADAS-Cog	The Alzheimers Disease Assessment Scale - Cognitive Subscale
AINSE	Australian Institute of Nuclear Science and Engineering
ANOVA	Analysis of Variance
ANSTO	Australian Nuclear Science and Technology Organisation
CAPS	Clinician-Administered PTSD Scale for DSM-IV
CBT	Cognitive Behavioural Therapy
ChAT	Choline Acetyltransferase
CIDI	Composite International Diagnostic Interview
CNS	Central Nervous System
CTN	Clinical Trial Notification

dbB	Diagonal Band of Broca
DLB	Lewy Body Dementia
DSM-III-R	Diagnostic and Statistical Manual of Mental Disorders - Third Edition revised
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders - Fourth Edition
E2020	Donepezil - an AChEI
EMDR	Eye Movement Desensitisation and Reprogramming
ENA-713	Rivastigmine - an AChEI
ERP	Event Related Potential
FBE	Full Blood Examination
FDG	Fluorodeoxyglucose
fMRI	Functional Magnetic Resonance Imaging
FWHM	Full Width, Half Maximum
GABA	Gamma-Aminobutyric Acid
GHQ	General Health Questionnaire
HDB	Horizontal Limb of the Diagonal Band of Broca
HPA-axis	Hypothalamic - Pituitary - Adrenocortical Axis
ICD-10	International Classification of Diseases
IDEX	I ¹²³ Iododexetemide
IES	The Impact of Events Scale
IQ	Intelligence Quotient
K+	Potassium ions
LC	Locus Ceruleus
m-AChR	Muscarinic ACh Receptors
MBq	mega-Becquerel
MG	Myasthenia Gravis
MMSE	Mini-Mental State Examination

MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
MS	Medial Septal Nucleus
Na+	Sodium ions
n-AChRs	Nicotinic Acetylcholine Receptors
NART	The National Adult Reading Test - Second Edition
nbm	Nucleus Basalis of Meynert
NE	Noradrenaline
NWAHS	North West Adelaide Health Service
PCL-C	Posttraumatic Stress Disorder Check List - Civilian
PCL-M	Posttraumatic Stress Disorder Check List - Military
PD	Parkinson's Disease
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
PTSD	Posttraumatic Stress Disorder
rCBF	Regional Cerebral Blood Flow
SD	Standard Deviation
SNRI	Serotonin-Norepinephrine Reuptake Inhibitor
SNS	Sympathetic Nervous System
SPECT	Single Photon Emission Computerised Tomography
SPM	Statistical Parametrical Map
SSRI	Selective Serotonin Reuptake Inhibitor
TGA	Therapeutic Goods Administration
TQEH	The Queen Elizabeth Hospital
VChAT	Vesicular Choline Acetyltransferase
VDB	Vertical Limb of the Diagonal Band of Broca
WHO	World Health Organization

Chapter 1

Introduction

Early in the twentieth century, a scientist by the name of Otto Loewi dreamed an experiment that would demonstrate the existence of chemical transmission. When he woke up, he designed and successfully performed the experiment, and when this first neurotransmitter was eventually isolated and identified in 1926, it came to be known as Acetylcholine (ACh) (Van der Zee & Luiten 1999, Gazzaniga et al. 2002).

Historically it was believed that acetylcholine would be to cognitive deficits and memory disorders such as Alzheimers Disease, as L-DOPA is to Parkinson's disease: that regulating the significant neurotransmitter would lead to the deficits in all other neurotransmitter systems essentially recalibrating. Davies (1985) believed that "if there is a key transmitter for memory, and perhaps for other aspects of cognitive function, the best bet for that transmitter would be acetylcholine". Although research to date has not proven quite as clear-cut a relationship as this, studies within the fields of neurobiology, psychophysiology and pharmacology have shown that certain impairments in learning, memory and cognition are associated with anomalies of cholinergic neuro-transmission, and for these reasons it becomes a neurotransmitter of interest when studying any condition defined by symptomatology in these areas (Grutzendler & Morris 2001).

1.1 Acetylcholine

1.1.1 The Acetylcholine Pathway

By the catalytic action of the cytosolic enzyme choline-acetyltransferase (ChAT), acetyl-CoA (a product of cellular oxidative metabolism) and choline (broken down from food consumption) combine to form acetylcholine (ACh). This occurs within presynaptic neurons and ACh is stored in vesicles in the axon terminals. When a nerve impulse arrives, the synaptic vesicles fuse with the synaptic membrane and release the ACh into the synaptic cleft. Once in the synaptic cleft, the ACh binds almost immediately to the post-synaptic receptors, increasing the membrane's permeability to Na⁺ and K⁺ ions. The influx of Na⁺ ions depolarises the postsynaptic membrane resulting in the generation of a nerve impulse. ACh is inactivated by the enzyme acetylcholinesterase (AChE), converting it to acetate and choline, the latter being recycled into the pre-synaptic neurons to be resynthesised into ACh (Mathews & van Hold 1990, Van der Zee & Luiten 1999, Grutzendler & Morris 2001). See Fig. 1.1.1 for a schematic representation of the ACh synthesis and degradation pathway.

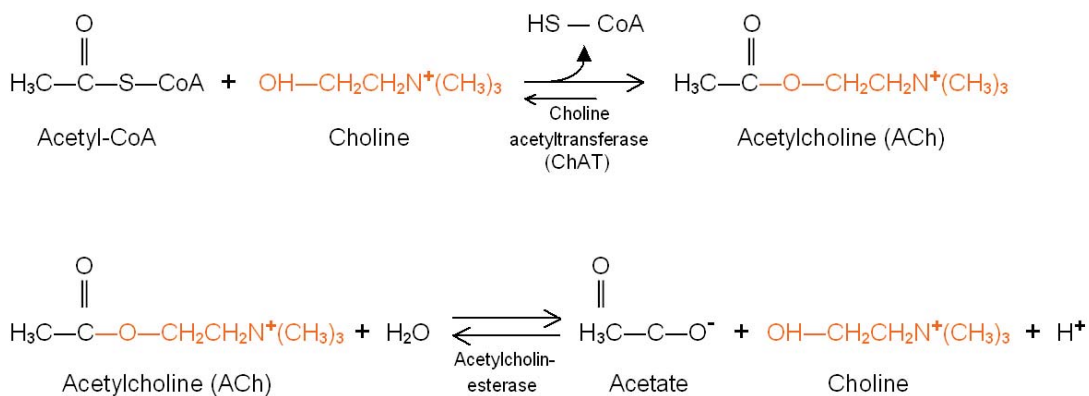


Figure 1.1.1: The synthesis and degradation of acetylcholine

1.1.2 Acetylcholine Receptors

Receptors are nerve terminals that respond to neurotransmitter stimulation by transmitting impulses. There are 2 families of receptors that specifically bind ACh. They are the nicotinic ACh receptors, which are antagonised by curare, and the muscarinic ACh receptors, which are antagonised by atropine. As pharmacological, biochemical, electrophysiological, immunological and molecular research techniques have improved dramatically over the last 3 decades, the receptors have been further categorised into subtypes (Van der Zee & Luiten 1999).

Nicotinic acetylcholine receptors (nAChRs) consist of α - and β - subunits, the combination of which determines the functional properties of the receptor complex. Several of these subunits have been cloned, including 9 neuronal α -subunits ($\alpha 2$ - $\alpha 10$) and 3 neuronal β -subunits ($\beta 2$ - $\beta 4$) (Buisson & Bertrand 2002).

The muscarinic acetylcholine receptors (mAChRs) activate signal transduction pathways through G-protein interactions, and have been characterised as M_1 - M_5 , with different genes responsible for the production each. Most recent research has primarily addressed the M_1 and M_2 m-AChR subtypes, the exception being a broader range of experiments in knockout mice (van Koppen & Kaiser 2003). The 5 receptor subtypes may be summarized as follows:

M_1 : located postsynaptically, they increase dopaminergic transmission in the striatum, increasing locomotor activity and mediate ACh-induced MAP kinase activation, essential for memory (blockade of M_1 mAChRs is associated with memory impairment and increased stimulation can lessen the cognitive decline associated with Alzheimers Disease).

M_2 : located predominantly presynaptically, they serve as autoreceptors to regulate the release of ACh, contribute to m-AChR-dependent bradycardia and also in a minor way to stomach, bladder and trachea contraction.

M_3 : have a key role in salivary secretion, pupillary constriction and regulation in food intake and appetite.

M_4 : modulate central dopaminergic responses.

M_5 : facilitate dopamine release in striatum, modulate morphine reward and withdrawal processes, and have a part to play in the dilatation of cerebral arteries and arterioles (Carey et al. 2001, van Koppen & Kaiser 2003).

Therefore, there is considerable heterogeneity, both of functional effects and of location of the various subtypes, with cerebral cortical receptors being apparently of M_1 type.

1.1.3 The Cholinergic System

There are two major groups of cholinergic neurons. The first group contain the medial septal nucleus (MS), the diagonal band of Broca (dbB) and the nucleus basalis of Meynert (nbm). This group of neurons is often referred to as the basal forebrain cholinergic system, but there is controversy as to whether they are a homogeneous group activated under common circumstances and having a unitary function or separable groups with separable functions determined and constrained by their cortical projection targets. They innervate neocortical (neocortex), juxtallocortical (cingulate cortex) and allocortical sites (hippocampus, basolateral amygdala and olfactory bulb). Moreover, the neurons of the nbm and the diagonal band supply about the 80% of the cholinergic innervation to the cortex and hippocampus (Everitt & Robbins 1997). See Fig. 1.1.2 for a summary of the basal forebrain cholinergic projections.

The second major group of cholinergic neurons are found in the brainstem (excluding the neurons found in cranial nerve motor nuclei) in the region of the pedunculo-pontine tegmental nucleus and the laterodorsal pontine tegmentum and are responsible for the cholinergic innervation of the thalamus (Everitt & Robbins 1997).

Mesulam et al. (1983) categorised these cholinergic neurons according to their size, shape, location and projection and were able to define the percentage of cholinergic activity that is attributable to each (See Table 1.1.1 for more detail).

In the human brain, the structure with the greatest percentage of cholinergic activity is the nucleus basalis (magnocellularus) of Meynert (nbm). This structure is an aggregation

Table 1.1.1: Classification of Cholinergic Neurons (Mesulam et al. 1983). Ch1 pertains to 'cholinergic cell group 1' etc. and Hce1 pertains to 'hypothalamic cholinesterase-rich cell group 1' etc.

Name	Location	%	Project to
Ch1	Medial Septal area (MS)	10%	Hippocampus
	70% are not ACh related. Referred to as the septohippocampal cholinergic projection.		
Ch2	Vertical limb of the Diagonal Band of Broca (VDB)	70%	Hippocampus, Hypothalamus, Cingulate cortex, Olfactory bulb, Subcallosal gyrus
	30% contain very low levels or no ACh. Long axes run parallel to the Diagonal Band of Broca and their dendrites overlap.		
Ch3	Horizontal limb of the Diagonal Band of Broca (HDB)	1%	Olfactory bulb Hypothalamus
	These neurons extend between the septal-preoptic region medially and the amygdaloid region laterally with the most diffuse boundaries. Referred to as the diagonal band-cingulate projection (Everitt & Robbins 1997).		
Ch4	Nucleus basalis (magnocellularis) of Meynert (NBM) - Anteromedial group	90%	Hippocampus, Amygdala, Ventrolateral orbital, middle insular, periarculate, peristriate and parahippocampal regions.
am	Projects to the ventral part of the somatosensory cortex, midprincipalis cortex and to several frontal, parietal and cingulate regions situated along the medial parts of the hemisphere.		
Ch4	Anteriolateral group	90%	Amygdala, Olfactory bulb, Medial frontal pole, Parahippocampal area and insula.
al	Projects to a number of frontoparietal opercular regions as well as the dorsomedial motor cortex, ventrolateral orbital cortex and inferiotemporal area.		
Ch4 id	Intermediodorsal and intermedioventral groups	90%	Parahippocampal areas, insular, periarculate and the peristriate
and iv	Projects to the orbitoinsular, prefrontal, posterior parietal and inferotemporal areas.		
Ch4	Posterior group	90%	See below
p	Projects to the auditory association areas in the superior temporal gyrus, to the temporal pole and to adjacent inferotemporal and posterior insular regions.		
Ch5	Pedunculopontine nucleus, cunifiform and laterodorsal tegmental nucleus	?	Reticular thalamic nuclei and the intralaminar thalamic nuclei.
and Ch6	Projections are hypothesised to be important in the maintenance of consciousness and arousal but are not affected by immunotoxins and do not show substantial degeneration in neuropsychiatric disorders (McGaughy et al. 2000).		
Hce1	Dorsomedial hypothalamic area	?	Posterior hypothalamic nucleus and the medial and dorsal perimammillary region.
and	Lateral hypothalamic region		Extends to the lateral perimammillary region.
Hce2	Approx. 50% of the large neurons in these areas show AChE activity but not ChAT immunoreactivity making them cholinoreceptive but not cholinergic (Mesulam et al. 1983).		

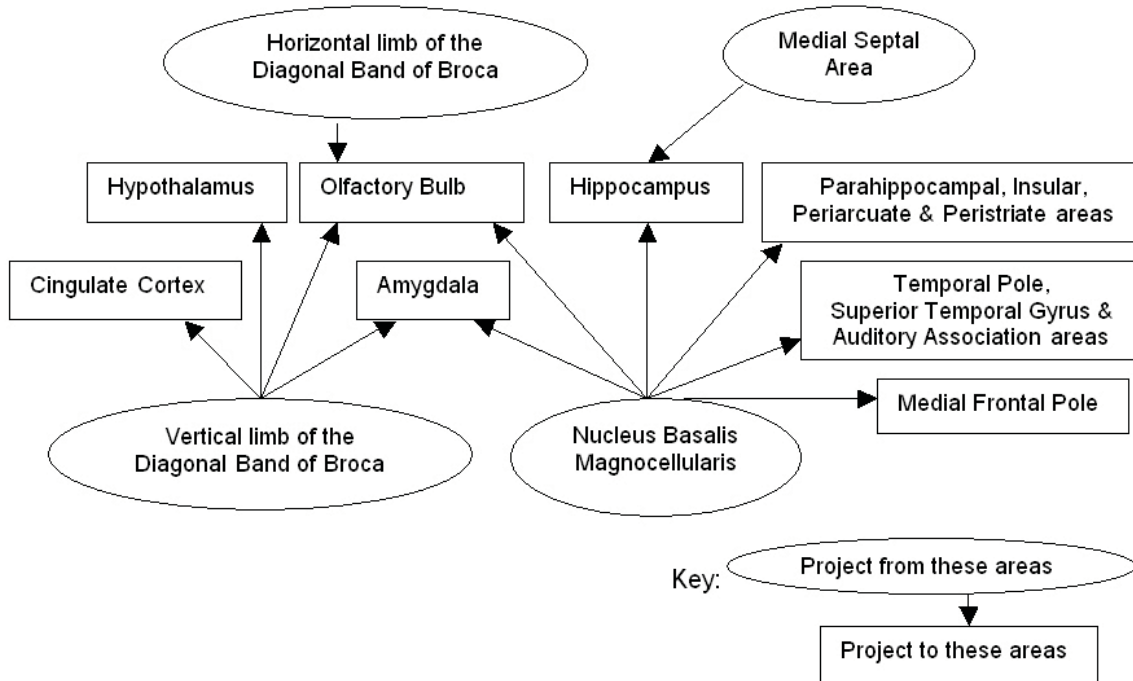


Figure 1.1.2: Summary of Cholinergic Projections: Medial Septal Area = Ch1, 10% cholinergic, Vertical limb of the Diagonal Band of Broca = Ch2, 70% cholinergic, Horizontal limb of the Diagonal Band of Broca = Ch3, 1% cholinergic and Nuclear Basalis (magnocellularis) of Meynert = Ch4, 90% cholinergic.

of neurons extending ventrally to the putamen and globus pallidus from the level of the mammillary body (corpora mammillaria) into the septum, and from the olfactory tubercle to the lateral geniculate nucleus. It is in intimate association with the fornix, diagonal band, medial forebrain bundle, anterior commissure, the globus pallidus, the stria terminalis, the inferior thalamic peduncle, the ansa lenticularis and the internal capsule (Bigl & Arendt 1991). In humans, the packing density of the neurons in the nbm is greater than that found in other animals and there are fewer dispersions of Ch4 cell islands into adjacent structures: differentiation of this cell group therefore appears to correlate with ascending phylogenetic development and the degree of cerebralisation, i.e. with the development of the neocortex in an animal species. This illustrates the importance of cholinergic neurotransmission in neocortical processes (Van der Zee & Luiten 1999).

The activity of the Ch4 subgroups of neurons is particularly responsive to motivational states. The nucleus basalis receives input from the amygdala, medial frontopolar cortex, ventromedial nucleus of the hypothalamus, caudal orbitofrontal cortex, the magnocellular component of the dorsomedial thalamic nucleus and the peripeduncular nucleus. This region also receives afferents from more than 70 regions and nuclei of the brain (Bigl & Arendt 1991). Mesulam et al. (1983) state that “this cell group is therefore in a unique position to act as a cholinergic relay between limbic areas and the entire content of neocortex in a fashion that may influence all aspects of complex behaviour according to the prevailing emotional and motivational state”.

1.1.4 Functional Correlates of Acetylcholine

Within the basal forebrain cholinergic system, the nucleus basalis-neocortical projection contributes to visual attentional function. These connections have a role in the retention of affective conditioning. The septohippocampal projection is involved in the modulation of short-term spatial (working) memory processes, perhaps by prolonging the neural representation of external stimuli within the hippocampus. The diagonal band-cingulate cortex cholinergic projection impacts on the ability to utilise response rules through conditional discrimination. The brainstem cholinergic neurons innervate the thalamus and affect basic arousal processes (e.g. sleep-wake cycle) (Everitt & Robbins 1997).

The cholinergic pathways that project from the basal forebrain to the cerebral cortex and hippocampus are known to be involved in learning and memory. Specific memory processes affected include consolidation, registration and retrieval (Erickson 1990). Winkler et al. (1995) suggest that acetylcholine is not only necessary for learning and memory but that its presence within the neocortex is sufficient to restore memory following damage to associated structures.

The prefrontal cortex (PFC) plays an important role in planning, guiding and organising behaviour through working memory. Lesions of the PFC can result in disinhibited behaviour,

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of the print copy of the thesis held in
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Figure 1.1.3: Schematic representation (Everitt & Robbins 1997) of the functional effects of lesions to various ACh cell groups within the basal forebrain. The shading represents the area providing cholinergic innervation (see Key-shading) while the boxes represent the cortical substrates of a variety of psychological processes (see Key-boxes).

increased motor activity, impaired attention and diminished ability to inhibit distracting stimuli (Southwick et al. 1999). Other research includes regional ablation or pharmacological blockade of ACh receptors, which alters attention and memory function in both experimental animals and humans, thereby demonstrating that the cholinergic system has an important role in cognitive function (Everitt & Robbins 1997, Grutzendler & Morris 2001).

“Lesioning” studies have been instrumental in widening our knowledge regarding the function of the basal forebrain cholinergic system (see Fig. 1.1.3 for a summary of the basal forebrain cholinergic system functions as revealed through lesion studies).

1.1.4.1 Learning and Memory

Three basic operations govern memory function: registration, storage, and retrieval. Registration refers to the encoding or acquisition of information and generally requires intact attentional resources and conscious effort. Retention (storage or consolidation) refers to the ability to store information over time in an intact form. Retrieval (decoding, recall) processes involve the ability to retrieve stored information. As with registration, retrieval generally requires conscious (volitional) processes (Erickson 1990).

There are several expressions in the literature that describe different forms of memory. Please refer to Table 1.1.2 for a comprehensive list of types and definitions.

Memory function is mediated by hippocampal activity in conjunction with the activity of the other limbic brain regions. These areas are also associated with fear-related behaviours and the stress response, including the emotional aspects of behaviour related to survival (Bremner et al. 1995). Short-term spatial (working) memory processes are modulated by the hippocampus which is innervated by cholinergic neurons (morphologically categorised as Ch1 in Mesulam’s classification, see Table 1.1.1) originating in the medial septal area i.e. the septohippocampal cholinergic projection. A greater impairment in spatial working memory is noted if medial septal cholinergic projections to the hippocampus are disrupted rather than the projections from the VDB or nbm (Everitt & Robbins 1997).

Declarative memory is supported by a complex of structures involving the medial temporal lobe and hippocampus. During encoding and retrieval these structures interact with

Table 1.1.2: The terms and definitions often used to describe different forms of memory, and tasks used to measure different aspects of the memory process (Erickson 1990).

Term	Description
Working memory	Recent information updated actively by the current experience.
Reference memory	Recent and remote information gained from previous experience.
Episodic memory	Information about events that occur at a specific place and time.
Generic memory	Items of knowledge independent of the particular occasion on which one had acquired them, containing external facts, principles, associations and rules about the world.
Semantic memory	The meanings of words and concepts.
Declarative memory (knowing that)	Pertains to facts about the world and past personal events that must be consciously retrieved to be remembered (secondary memory process).
Procedural memory (knowing how)	Learning and retaining a skill or procedure; such abilities become automatic and do not require conscious direction (secondary memory process).
Long-term potentiation	Associative learning
Explicit memory tasks	Require conscious, volitional processes. Used to measure free recall, cued recall and recognition.
Implicit memory tasks	More automatic processes are involved (a more robust measure of storage). Used to measure priming, word-stem completion, and word fragment completion.

the prefrontal cortex, an area variously thought to be the site of executive control, a working self system, or the capacity to be aware of one's own protracted existence across subjective time. As applied to memory, one function of executive control is to inhibit the entry of unwanted or irrelevant material into consciousness, with the help of mechanisms such as retrieval inhibition and post-retrieval decision processes. Such inhibitory processes are typically regarded as an essential element in effective cognitive functioning, to require attentional resources, and to have the potential to be disrupted by competing tasks, emotions or goals (Brewin 2001). In order for appropriate contextual cues to be attended to instead of other weaker contextual cues, the hippocampus needs to be inhibited. When the cholinergic afferents are disrupted, the hippocampus becomes disinhibited allowing an increase of noise or contextual signals. The hippocampal formation functions in part to keep the selected cues in the foreground of a context (Everitt & Robbins 1997).

Disruption of passive avoidance learning and retention may be due to cholinergic denervation of the amygdala, a structure known to be important in this form of aversive conditioning (Everitt & Robbins 1997).

1.1.4.2 Attention

It has been hypothesised that the development and expression of psychotic cognition is mediated by irregularities in cortical cholinergic transmission. Attentional dysfunctions include impairments in the ability to detect, select, and discriminate stimuli and associations for extended processing and to allocate the appropriate processing resources to these functions (Sarter & Bruno 1997). Hyperattentional dysfunctions include the breakdown of filtering functions, the over-processing of irrelevant stimuli and the resulting exhaustion of processing capacity.

Attentional functions can also be affected differentially by pharmacological manipulations of the Ch4 corticoparietal projections. For example, infusion of muscinol in the nbm of animals increased correct response latencies and decreased response accuracy. These impairments were attenuated by the administration of a cholinesterase inhibitor (physostigmine) (McGaughy et al. 2000). In human studies, selective cholinergic receptor agonists and

antagonists have also been shown to modulate inspection time, a measure of speed of early information processing, and that this function alone may serve as a physiological index of the integrity of the cholinergic system (Hutchison et al. 2001).

1.1.4.3 Cognition

A reduction in levels of choline acetyltransferase, specifically in the neocortex and the hippocampus, as well as a diminished number of cholinergic neurons which originated in the basal forebrain correlate with poor cognitive performance (Coyle et al. 1983). The majority of this research has been performed on a patient population experiencing symptomatology associated with dementia of the Alzheimer's type where degeneration of the acetylcholine-releasing neurons has been widely reported (Grutzendler & Morris 2001). However, cognitive impairments of other neuropsychiatric patients have also been shown to correlate with cholinergic dysfunction (McGaughy et al. 2000).

Nicotinic receptor antagonists do not appear to impair cognitive function, but nicotine itself has been shown to improve aspects of cognitive performance, including attentional function in human beings (Buisson & Bertrand 2002).

1.1.5 Neurotransmitter Interactions Involving the Cholinergic System

The view that the cholinergic system is solely responsible for alterations in memory is a naive one. Several neurotransmitter interactions determine behaviour and cognition through parallel synergistic roles (contribute through additive processes), serial interactive roles (modulate to determine a particular cognitive faculty) or a combination of parallel and serial processes (Cassel & Jeltsch 1995).

Due to the high proportion of ACh receptors in the cortex, stimulation of these receptors has been shown to influence the release of several neurotransmitters, including dopamine, glutamate and noradrenaline (NE), as well as ACh (Parikh et al. 2008). For example, in "lesion" studies it was found that following a multitransmitter hippocampal denervation

(i.e. fimbria-fornix lesions remove a part of the cholinergic, noradrenergic, GABAergic and serotonergic afferents of the hippocampus), the combination of cholinergic and serotonergic grafts produced the best functional (cognitive) recovery, and that individually, neither graft was able to produce such an improvement (Cassel & Jeltsch 1995).

1.1.5.1 Serotonin

The regions of the brain that receive both a serotonergic and a cholinergic innervation are the hippocampus, the cortex and the striatum. Although there is no direct link between serotonergic and cholinergic terminals in the cortex or the hippocampus, there is an interaction between the serotonergic and cholinergic systems, and it has been postulated that, due to this, serotonergic mechanisms might be involved in “muscarinic plasticity” (Cassel & Jeltsch 1995). The cholinergic system primarily alters accuracy in cognitive tasks, whereas serotonergic transmission modulates behaviour by altering bias (motivation, motor processes). Attention, stimulus processing and/or arousal can be influenced by both cholinergic and serotonergic systems independently of each other. Serotonergic-cholinergic interactions could also be of importance in the mediation of learning processes and trial-by-trial working memory (Steckler & Sahgal 1995).

The rationale for the ‘lesioning’ approach is that if a serotonergic/cholinergic interaction has cognitive relevance, the combined depletions should induce behavioural effects that are different (generally more marked) from those induced by each depletion performed separately. The cognitive effects of cholinergic denervation of the hippocampus (septal lesions) or the neocortex (nucleus basalis lesions) can be exacerbated by concomitant serotonin (5-HT) depletion. Serotonin depletion alone did not produce detrimental effects on spatial and non-spatial working or reference memory processes. When effects on memory were observed after 5-HT depletion, they consisted of an improvement of mnemonic performance in some tests (Cassel & Jeltsch 1995).

Systemic administration of 5-HT agonists increased the acetylcholine content in the rat striatum, suggesting that 5-HT exerts an inhibitory modulation of cholinergic function in the striatum. A serotonergic depletion may decrease the efficacy of pharmacological

agents enhancing cholinergic transmission in reversing the cognitive consequences of systemic cholinergic blockade. It has also been suggested that a serotonergic depletion may exacerbate the sensitivity of animals towards the cognitive consequences of anticholinergic drugs given systemically. In addition to the effects of 5-HT on cholinergic function, the possibility that 5-HT has its own cognitive actions needs consideration, for instance modulating functions that may be essential for mnemonic processes to occur efficiently (e.g., anxiety, arousal, attention) (Cassel & Jeltsch 1995).

1.1.5.2 Catecholamines

The prefrontal cortex (PFC) is rich in noradrenergic terminal fields from the locus ceruleus (LC). These play an important role in learning, planning, guiding and organising behaviour through working memory, anxiety, psychosis, in the sleep-wake system and in reward and reinforcement. The detection of a ‘threatening’ stimulus will increase firing rate of these noradrenergic nerves but a ‘novel’ stimulus will not. Lesions of the PFC can result in disinhibited behaviour, increased motor activity, impaired attention and diminished ability to inhibit distracting stimuli (Southwick et al. 1999).

Noradrenergic projections from the LC modulate PFC functioning through post-synaptic α_1 and α_2 receptors. Furthermore, α_{2A} receptor stimulation inhibits irrelevant and distracting sensory processing through effects on pyramidal cells that project to sensory association cortices. It is believed that noradrenaline (NE) acts to reduce distractibility by strengthening PFC function (Friedman et al. 1999), which is similar to the effect of ACh in the precuneus (parietal cortex). Alpha 2-agonists improve PFC functioning when NE is low (ie inc. NE to normal basal levels and PFC function increases), and produces behavioural calming (without sedation) and less agitation and disinhibition (Southwick et al. 1999), which becomes very important when considering methods of attenuating psychiatric symptomatology.

Scheiderer et al. (2008) found that ACh and NE can activate the same signalling pathways to cause synaptic depression in the hippocampus, and that a decrease in either neurotransmitter can, to some extent, be compensated by the other. In the hippocampus (*in vivo*), inhibiting NE uptake increases ACh release. Pharmacologically preventing the break-

down of ACh, once released, can also increase the release of NE. Both of these manipulations improve subsequent performance on memory tasks (Scheiderer et al. 2008).

Dopamine is the neurotransmitter activated when engaging in experiences that may be rewarding or experienced as pleasurable, as well as being involved in motor control and planning (Gazzaniga & Heatherton 2003). There is extensive crosstalk between G protein-coupled receptors and ligand-gated ion channels in dopaminergic nerve terminals. The D(2) autoreceptor adjusts the efficacy of non- α_7 nACh receptor-mediated modulation of dopamine release, specifically within the striatum (Quarta et al. 2007).

1.1.5.3 GABA

GABA (gamma-aminobutyric acid) is the primary inhibitory neurotransmitter in the nervous system responsible for hyperpolarising postsynaptic membranes (Gazzaniga & Heatherton 2003). In the hippocampus, neocortex and amygdala (and numerous other CNS regions), cholinergic innervation frequently terminates on GABAergic cells (Van der Zee & Luiten 1999). Wanaverbecq et al. (2007) found that hippocampal information processing functions are intimately affected when ACh release modulates GABA receptors.

1.1.5.4 Glutamate

Glutamate is the primary excitatory neurotransmitter in the nervous system, involved in fast-acting neuronal transmission throughout the brain (Gazzaniga & Heatherton 2003). Release of glutamate appears to function to potentiate many of the effects of cholinergic stimulation within the PFC (Parikh et al. 2008).

1.1.6 Conditions with Altered Cerebral Cholinergic Function

Many conditions can claim an alteration of memory, motivation and/or mood as part of their symptomatology and in most of these conditions it has also been shown that they are associated with dysfunctions in the central ACh system. The significance of cortical cholinergic inputs in mediating fundamental aspects of cognition and awareness becomes

even more apparent when these conditions and their symptom profiles are described.

1.1.6.1 Alzheimer's Disease

Alzheimer's Disease (AD) is a neurodegenerative disease characterised anatomically by the presence of neuritic plaques (abnormal axon terminals with an extracellular amyloid core) and neurofibrillary tangles (bundles of paired helical filaments which accumulate within the cell bodies) and is evident through the expression of a number of cognitive and neuropsychiatric symptoms. The cognitive abnormalities include disturbances in memory, language ability, visuospatial performance and executive functions. The loss of these cognitive abilities is often accompanied by psychiatric symptoms such as irritability, emotional instability, apathy, disinhibition, delusions and hallucinations (Coyle et al. 1983, Grutzendler & Morris 2001).

The degeneration that occurs in patients with AD has been shown in several areas including the frontal, parietal and temporal cortices, hippocampus, nucleus basalis of Meynert (posterior) and the Medial Septal area. When assessed shortly before death, the degree of cognitive impairment in AD patients was found to correlate significantly with the extent of loss of cholinergic markers in the basal forebrain and cortex at autopsy (McGaughy et al. 2000). A caudal to rostral pattern, hypothesised to correlate with the chronology of specific cognitive dysfunctions, coincides with initial mnemonic impairments, followed by disruptions of attention function. ACh is diminished in the cortex of patients with AD. McGaughy et al. (2000) observed that the ACh levels within the nbm were also markedly depleted. As the nbm projects ACh terminals to the cerebral cortex, this would account for the observed decrease in cortical ACh.

The benefits obtained from administering acetylcholinesterase inhibitors (AChEI) to people with AD have been demonstrated in several studies (Shintani & Uchida 1997, Hutchison et al. 2001, Inglis 2002). There are some modest improvements in memory and cognition, as well as noticeable changes in mood and behaviour (Weiner et al. 2000, Grutzendler & Morris 2001). In one study, five behaviour modalities (delusions, agitation, anxiety, disinhibition and irritability) showed a significant dose dependent improvement from baseline

values (Mega et al. 1999). **It is the anecdotal accounts of the positive effects on mood and other psychiatric symptoms that were elicited by the administration of AChEIs in people suffering from this condition that has generated interest in using this medication to combat the adverse behaviours and moods evident in other conditions.**

It must be noted however, that other neurotransmitters are also decreased in AD, so alterations in ACh levels cannot explain all of the degenerative effects. NE is diminished in the cerebral cortex, which probably correlates with the loss of NE containing neurons which project from the locus ceruleus of the pons, and somatostatin and Substance P are also decreased (Friedman et al. 1999).

1.1.6.2 Lewy Body Dementia

Lewy Body Dementia (DLB) is a condition that accounts for up to 25% of all elderly dementia cases, that is characterised by fluctuating cognitive impairment, attention deficits, recurrent visual and auditory hallucinations, delusions, depression, sleep disturbances and Parkinsonism. Studies into this condition have shown deficits in cholinergic transmission, which are more severe than those demonstrated in AD. Moreover, the presence of visual hallucinations and the severity of cognitive performance correlated with the reduction in cholinergic activity (McKeith et al. 2000).

Perry & Perry (1995) state that up to 50% of people suffering with DLB specifically show signs of hallucinations, delusions and fluctuations in cognitive performance. The same investigators also reported amelioration of symptoms with cholinergic agonist therapy, raising the possibility that this reflects a fundamental aspect of the pathophysiology of DLB.

1.1.6.3 Schizophrenia

Schizophrenia is a psychotic disorder characterised by motor, cognitive, behavioural and perceptual abnormalities which exert a large impact on the person's ability to interact socially, vocationally or personally. There are two categories of symptoms associated with schizophrenia: positive (excesses) which include delusions and hallucinations, and negative

(deficits) which include apathy, isolation, avoidance, reduction in speech, movement and social participation. The positive symptoms have been shown to respond to antipsychotic medications that act on neurotransmitter systems so they are deemed due to neurotransmitter dysfunction (Gazzaniga & Heatherton 2003).

Regarding the positive symptoms of schizophrenia, the hyperattentional dysfunctions that contribute to the evolution of hallucinations and delusions have been attributed to cortical cholinergic hyperactivity (Sarter & Bruno 1998). Due to cholinergic involvement, it also follows that in patients with schizophrenia, cognitive impairment is apparent in several domains. In one study, the administration of an AChEI led to significant improvements in several cognitive measures and increased activation of prefrontal cortex and basal ganglia on functional MRI. There was also a reduction of depressive symptoms and overall there was a significant improvement noted in functional abilities and general quality of life (Risch et al. 2001).

1.1.6.4 Parkinson's Disease

Parkinson's Disease (PD) is a neurological disorder characterised by muscular rigidity, tremors and difficulty initiating voluntary action, and at later stages, people suffer from cognitive and mood disturbances (Gazzaniga & Heatherton 2003). In PD, melanin containing dopaminergic neurons in the pars compacta of the substantia nigra degenerate and their projections to the basal ganglia are lost, with resultant reduction in striatal dopamine content, which is involved in controlling voluntary muscle movements. Other melanin containing dopaminergic neurons are lost in the locus ceruleus. These neurons project to the cerebral cortex and contain NE. The dementia observed in some PD patients could be caused by the loss of these NE terminals in the cortex, or the loss of the cholinergic neurons in the nucleus basalis. However, the imbalance between the concentrations of dopamine and acetylcholine in the striatum due to the loss of the nigrostriatal dopaminergic pathway is known to play a major role in the symptomatology (Pisani et al. 2001).

1.1.6.5 Excesses of ACh

Myasthenia gravis (MG) is an autoimmune disorder resulting from the production of antibodies against ACh receptors leading to the destruction of the postsynaptic membrane at the neuromuscular junction. With low rates of motor nerve stimulation (25 Hz), the immediate stores of ACh at the neuromuscular junction is depleted. Further reduction of ACh causes some endplate potentials to fail to reach depolarization threshold which results in a failure to elicit muscle fiber action potentials. With a reduced number of individual muscle fiber action potentials, the compound muscle action potential becomes reduced in both amplitude and area with a resulting decremental response. This condition primarily affects the peripheral nervous system and is most often treated with corticosteroids used as the initial immunotherapy and with peripherally acting AChEIs. Plasma exchange (with albumin or fresh frozen plasma that has the AChR antibodies removed) is also used in MG to achieve rapid, temporary improvement in strength (Juel & Massey 2007).

Cholinergic crisis in MG may develop with excessive dosing of AChEIs in patients with more severe MG. In cholinergic crises, depolarization blockade at diseased neuromuscular junctions results in increased weakness, and increased muscarinic activity generates copious oropharyngeal and bronchial secretions that may obstruct the airway or be aspirated. Signs of cholinergic crisis include weakness indistinguishable from myasthenic weakness, muscle fasciculations, and symptoms of increased muscarinic activity including bradycardia (Juel & Massey 2007).

Chemical warfare nerve agents also produce hyperactivity in cholinergically innervated end organs and induce an acute, life threatening cholinergic crisis within seconds to minutes. Initial inhalation of the vapor directly inhibits AChE in pupillary muscle, producing meiosis, and in the upper respiratory glands, producing rhinorrhea and salivation. The resultant hypersecretion of respiratory glands in bronchioles causes clinical bronchorrhea, and hypercontraction of smooth respiratory muscle leads to bronchospasm. Nerve agent vapor is well distributed by the blood, immediately inhibiting circulating cholinesterases. This will affect the gastrointestinal tract (cramping, abdominal pain, nausea, vomiting),

cardiac function (hyperstimulation of sympathetic and parasympathetic (vagal) input, affecting blood pressure and pulse rate), neuromuscular function (overstimulation of peripheral neuromuscular synapses causing fasciculation to twitching to flaccid paralysis), respiratory function (paralysis of the diaphragm and intercostal muscles), and cerebral activity (loss of consciousness, seizures, and inhibition of the medullary respiratory center, with central apnea) (Newmark 2004).

1.1.7 Therapeutic Enhancement of Central Cholinergic Function

Research into the conditions described in Section 1.1.6 has led to ‘the cholinergic hypothesis’: that cognitive function may be preserved if ACh levels are maintained. In order to increase cholinergic effects within the cholinergic system there are four options:

1. Administering precursors to ACh: however, studies focussing on this have not been successful.
2. Stimulating activity of synthetic enzymes (i.e. ChAT): no studies of this type have been reported to date.
3. Cholinergic agonists targeting specific receptors: to date these have had a lack of efficacy or high rates of adverse effects.
4. Acetylcholinesterase Inhibitors (AChEIs): these have shown significant differences in memory and cognition. Anecdotal evidence from clinicians also suggests that AChEIs may have a therapeutic affect on behavioural and psychiatric disturbances such as mood and perception (Grutzendler & Morris 2001).

ACh precursors, ACh release enhancers, AChEIs and receptor agonists have all been developed in an attempt to increase cholinergic neurotransmission. Although AChEIs appear to be the most promising, they have significant tolerability problems. Thus, AChEIs with improved side-effect profiles were developed and a greater range are now available (Grutzendler & Morris 2001).

1.1.7.1 Acetylcholinesterase Inhibitors

Cholinesterase inhibitors (or AChEIs) increase the availability of acetylcholine in central synapses. In addition to their effect on cognition, AChEIs have a positive effect on mood and behaviour. This class of medication is thought to facilitate cholinergic transmission by slowing the degradation of acetylcholine released by functionally intact cholinergic neurons (Grutzendler & Morris 2001). AChEIs are grouped into three structural classes: organophosphates, carbamates and tertiary and quaternary amines. The organophosphates cause irreversible inhibition by forming a stable covalent bond with the enzyme. Carbamate inhibitors cause the enzyme to be hydrolysed significantly slower by forming a carbamoylated complex instead of an acylated complex with the enzyme. Finally, the action of the amine compounds is more complicated, comprising of both competitive and noncompetitive inhibition. Some interact with both AChE and butyrylcholinesterase and all are rapidly metabolised by the hepatic cytochrome system (Enz & Floersheim 1996). The following AChEIs have been researched extensively in animal studies and clinical trials and are therefore described in more detail:

- i Tacrine, the first AChEI (a tertiary amine) to be registered and approved for the treatment of AD in the United States, has a broad side effect profile relating to peripheral effects and hepatic toxicity. More recently developed AChEIs have a more potent activity and a longer-lasting effect, therefore this medication is now rarely used clinically (Enz & Floersheim 1996, Kosasa et al. 1999).
- ii Metrifonate, although not an AChEI itself, is metabolised into an organophosphate AChEI that specifically inhibits AChE activity in the red blood cells for more than 6 hours. The study performed to evaluate the efficacy and safety of this medication was the first to demonstrate positive results in patients with AD in a prospective double-blind trial (Morris et al. 1998). Adverse effects include cramps and muscle weakness (Enz & Floersheim 1996, Grutzendler & Morris 2001).
- iii Pyridostigmine is a reversible carbamate AChEI used as a prophylactic for chemical war-

fare (such as Soman, which is an irreversible inhibitor) because of its ability to protect against the organo-phosphorus compounds. The activity of Pyridostigmine administered under non-stressful conditions was predominantly limited to the peripheral nervous system, with symptoms including abdominal pain, diarrhoea, frequent urination, increased salivation, rhinorrhoea and excess sweating. These symptoms are all associated with parasympathetic activation. However, stress exposure allows the drug to pass through the blood brain barrier (BBB) due to stress induced changes in permeability. Because of this, when Pyridostigmine was administered under conditions of war, the dominant symptom profile reverted to those relating to central nervous function, such as headaches, insomnia, drowsiness, nervousness, difficulties in focussing attention and impaired calculation capacities (Friedman et al. 1996).

- iv Physostigmine is a reversible tertiary amine AChEI that has a short biologic half-life and unpredictable bioavailability after oral administration. In tolerability studies higher doses produced centrally acting side effects (nausea, vomiting, headache and dizziness) but not peripheral cholinergic signs (sweating and salivation was absent or minimal). Because the risk-benefit ratio is deemed to be too high, Physostigmine is not an approved medication for AD therapy (Enz & Floersheim 1996).
- v Rivastigmine (ENA-713) is a brain-selective, non-competitive, long-acting, reversible, carbamate type inhibitor of the enzymes AChE and butyrylcholinesterase (pseudo-cholinesterase – an enzyme that is widely distributed in plasma and peripheral tissues). It bonds covalently to temporarily inactivate the enzyme. Animal studies have shown that it selectively increases the availability of acetylcholine in the cortex and hippocampus. Peak plasma concentrations occur around 1 hour after administration, and it crosses the BBB achieving CSF peak concentrations in approx 1.5 to 2.5 hours. Because of this relatively quick action, it must be administered frequently. Rivastigmine is metabolised to a pharmacologically inactive product by a process that is independent of the hepatic cytochrome P450 system. Gastro-intestinal adverse reactions include nausea, vomiting, anorexia and weight loss (McKeith et al. 2000, Grutzendler & Morris 2001).

- vi Galantamine is a competitive, reversible, phenanthrene alkaloid AChEI, which has been studied since the late 1960's and was initially used in the treatment of myasthenia gravis. Thirty years later it was shown to reduce cognitive deterioration and behavioural symptoms in patients with AD. This medication is currently approved for use in the treatment of AD (Grutzendler & Morris 2001).
- vii Donepezil (E2020) is a (piperidine-based) synthetic, noncovalent, reversible AChEI which exhibits a relatively high degree of selectivity for neuronal AChE (Shintani & Uchida 1997, Kosasa et al. 1999). Currently, Donepezil is the most widely used AChEI worldwide because it has the most favourable adverse event profile and proven efficacy (Grutzendler & Morris 2001). Specifically due to its popularity and proven record in AD, Donepezil was chosen as the medication of interest for use when endeavouring to maintain neuronal ACh in other conditions, and therefore this medication will be described more completely in Section 3.1.1 Donepezil.

1.2 Posttraumatic Stress Disorder

1.2.1 Historical Aspects

Before 1980, symptom profiles that were commonly elicited after exposure to traumatic events were given several names. The most common were associated with war, for example 'shell shock' and 'concentration-camp syndrome'. In 1968, the literature began to recognise it more specifically as Traumatic neurosis, and in 1975, a set of soldiers suffering "anxiety hysteria, accompanied by the nightmares" were described as experiencing posttraumatic reactions (Biran & Wertheimer 1975).

As this condition became a defense for compensation cases a wider interest in researching the causes of these reactions developed. In 1977, Braverman specifically ruled out the possibility that the litigation or compensation process actually caused the posttraumatic psychiatric reaction. However, as it became apparent that this condition would be increasingly utilised in law, it had to be clearly understood from a pathophysiological as well as

clinical point of view. Therefore, in 1980, the new diagnosis of Posttraumatic Stress Disorder was first defined in the Third Edition of the Diagnostic and Statistical Manual of Mental Disorders by the American Psychiatric Association.

1.2.2 Epidemiology

PTSD is potentially a highly significant area of clinical and social demand for better treatments. The National Comorbidity Study conducted in the USA and published in 1995 demonstrated that PTSD was the most common anxiety disorder in women with a 30 day prevalence of 2.4% and a life-time prevalence of 6.5%, making it the most common anxiety disorder in women (Kessler et al. 1995). The National Comorbidity Study also examined the issue of the impact of PTSD on work (Kessler et al. 1997). PTSD had the highest rates of workdays lost per month (2.8 days of work cut back a month) and was also associated with significantly increased uses of medical, human services and self help groups.

This study has been replicated in Australia. Here a population of 10,600 people over the age of 18 was examined. PTSD had a twelve-month prevalence of 1.3% using the DSM-IV criteria (Creamer et al. 2001). In another Australian study, the cost of PTSD as a diagnosis in veterans was examined by Marshall et al. (1998), who combined an epidemiological sample of 641 veterans with their health records. PTSD added \$79.00 in health care costs (other than psychiatric care) per fortnight, in contrast to a random physical diagnosis that added \$28.00 in cost. These data strongly argue for the importance of PTSD as a major public health issue and for the potential benefits that can be reaped.

1.2.3 Definition and Diagnosis

Posttraumatic Stress Disorder is now defined as a psychiatric condition that is distinguished from other disorders in that there is a known etiological component: an *extremely traumatic event or stressor* that involves threatened death, the threat of serious injury or other threat to physical integrity. This can be a direct personal threat, witnessing the threat of another person or discovering about the threat to a loved one or other close associate. The traumatic

events that can be experienced include war situations (military combat, terrorist attack, incarceration as a prisoner of war), taken hostage or torture, personal assault (physical, sexual, robbery), severe automotive accidents, natural or man made disasters and being diagnosed with a life-threatening condition. Events that can be equally traumatic include witnessing or learning about the events listed above occurring to another person, including the death, dead body or body parts of another person, or learning of the sudden death of a loved one. The individual's response to this traumatic event must involve intense fear, helplessness, or horror.

A range of symptoms can result from exposure to such a traumatic event and they are categorised to include re-experiencing, avoidance and arousal. When re-experiencing a traumatic event, an individual can have recurrent and intrusive memories, dreams or flashbacks where specific details of the event is replayed. Also, an individual may experience a dissociative state during which he believes he is reliving the event, and behaviours, sensory input (sights, sounds, smells) and distress are elicited as though the individual was actually within the traumatic moment once again. Intense psychological and physiological reactivity also occurs during exposure to triggers that remind the individual of the traumatic event.

Because of the distress involved when reminded of the traumatic event, triggers and other stimuli associated with the trauma are diligently avoided. A person with PTSD consistently and intentionally avoids thoughts, feelings, conversations, people, places, situations and activities that relate to or stimulate memories of the traumatic event. Specific examples associated with this category of symptoms include alterations in memory function, whether amnesia for precise details that occurred during the traumatic event or generalised memory impairments, anhedonia i.e. an inability to feel pleasure for things or activities that they previously thought of as pleasurable, a reduction of affect or lack of interest or involvement in activities, feeling detached or estranged from people and a reduced ability to feel emotions (deficits noted in tenderness, intimacy and sexuality). The individual also has a sense that his future will be cut short and he cannot imagine having a career, marriage, family or normal life-span.

An individual with PTSD would also constantly experience an *increased level of anxiety*

and arousal. This manifests in sleep difficulties which include difficulty falling or staying asleep and/or early waking (often due to persistent nightmares), hypervigilance and an increased startle response. Some also experience an increase in irritability, angry outbursts and a generalised difficulty in concentrating or completing tasks.

On top of this constellation of symptoms, there is a set of “specifiers” within this diagnosis that is defined by the onset and duration of the symptoms experienced. Foremost, a diagnosis of PTSD cannot be made unless the symptoms have lasted longer than one month. If the symptoms occur and last less than one month after the traumatic event happened a diagnosis of Acute Stress Syndrome is given. If the symptom duration is more than one month but less than three months, PTSD is said to be in the acute phase and it is described as chronic if it persists longer than three months. PTSD can also be described as delayed onset if the set of symptoms do not present until 6 months after the traumatic experience.

The condition as a whole causes a significant disruption to psychosocial performance, affecting social, occupational, and other important areas of functioning (American Psychiatric Association 1994). Thus, PTSD is conventionally defined on the basis of stringent criteria for severe emotional trauma, with an all-or-none definition and no conventional grading of severity of the resultant disorder. This approach is useful in avoiding dubious diagnoses but may result in arbitrary exclusion of “borderline” cases. From the point of view of this thesis, however, the inclusion of only clear-cut cases should be emphasised.

1.2.3.1 Psychological Assessments

The gold standard psychological tool utilised by clinicians to diagnose PTSD is the Clinician-Administered PTSD Scale (CAPS) (Blake et al. 1995). This is a long, comprehensive structured interview that closely corresponds to the criteria defined by the Diagnostic and Statistical Manual of Mental Disorders - Fourth Edition (DSM-IV) (American Psychiatric Association 1994). The Composite International Diagnostic Interview (CIDI), which encompasses several other psychiatric conditions is also very comprehensive regarding the diagnosis of PTSD (Blouin et al. 1988). Other less intense and less time consuming psychological tools can also be used, namely the the PTSD Check List (PCL) which is a self-administered tool

(Weathers et al. 1993) and the Impact of Events Scale - Revised (IES-R) which assesses the impact of a serious life event on individuals over periods of time (Horowitz et al. 1979). Refer to Section 2.2.2.2, Psychological Assessments for a more detailed description of these psychometric tools.

1.2.4 Currently Available Treatment Modalities

An evidence-based analysis of the literature concluded that there were high levels of evidence to support the use of Cognitive Behavioural Therapy (CBT), Eye Movement Desensitisation and Reprogramming (EMDR) and Selective Serotonin Reuptake Inhibitors (SSRIs) in the treatment of PTSD (Foa 2000). Whilst the SSRIs may be a treatment of choice for PTSD, many patients are left with significant residual symptoms such as the exaggerated startle response (Friedman 1997, Kozaric-Kovacic 2008). Other than their antidepressant and anti-anxiety effects on the symptoms of PTSD, these drugs therefore did not have a major theoretically-driven rationale for their use.

Although a number of other medications have been trialed in PTSD, including anti-convulsants, serotonin-norepinephrine reuptake inhibitor (SNRIs) and novel antipsychotics, regulatory approval has not been obtained for any medications other than the SSRIs. Hence there is an imperative to explore what medications, from a theoretical perspective, could contribute to the treatment of PTSD. Medications that enhance cholinergic fluctuation represent a novel and untried treatment option for PTSD.

1.2.5 The Pathogenesis of PTSD

By definition, PTSD is caused by exposure to a traumatic stressor. Because of this, the physiological response of the body to stress plays an important part in our understanding of the pathogenesis of PTSD. The central issue, therefore, is definition of the neuropathological mechanisms mediating the clinical aspects of the disorder.

1.2.5.1 Anatomical

The hippocampus is an important centrepiece for integrating cognitive, neurohormonal, and neurochemical responses which is profoundly influenced by responses to stress. The hippocampus has a rich concentration of glucocorticoid receptors that are regulated during the high release of glucocorticoids during acute stress. It also modulates glucocorticoid release through inhibitory effects on the hypothalamus-pituitary-adrenal axis. High levels of cortisol released at the time of a stressor result in damage to cells in the CA3 region of the hippocampus, which can persist for many years after the original trauma, leading to reductions in hippocampal volume, as measured with MRI. Repeated stress has been shown to cause shortening and debranching of dendrites in the CA3 region of the hippocampus and suppresses neurogenesis of dentate gyrus granule neurons (Bremner et al. 1999).

Zubieta et al. (1999) performed Single Photon Emission Computerised Tomography (SPECT) on twelve male veterans diagnosed with PTSD and found that there were significant increases in the regional cerebral blood flow (rCBF) responses to a combat stress-related auditory stimulus in the medial prefrontal cortex (PFC). This supports the involvement of the medial PFC in the pathophysiology of PTSD, possibly mediating some of its symptoms. When exposed to stress there is an increase in release of catecholamines in the PFC and it has been shown that NE can impair PFC function potentially leading to the inability to inhibit intrusive memories (Elzinga & Bremner 2002).

The hypervigilance seen in PTSD may be associated with the visuospatial aspects of memory function required to plan responses to potentially threatening stimuli. This information is processed by the PFC and mediated by the posterior cingulate, parietal and motor cortex, therefore the function of each of these structures may be implicated (Bremner et al. 1999).

The amygdala is responsible for fear conditioning. In fMRI, activity in the amygdala increased on fear conditioning tasks, electrical stimulation of the amygdala increases the acoustic startle response and when the amygdala is lesioned the fear potentiated startle is blocked. The increase in physiological response to triggers and the elevated startle response

seen in people with PTSD may be due to increased fear conditioning caused by amygdala dysfunction (Elzinga & Bremner 2002).

1.2.5.2 Neurochemical

The effect of stress on different areas of the brain and different neurotransmitter systems is complicated because all systems interact with and influence the functioning of the others. Dopamine has been implicated as it affects acetylcholine and both have been implicated in the stress response (Imperato et al. 1991). The activation of the sympathetic nervous system (SNS) and its associated effects of noradrenaline release have been researched in PTSD, showing a SNS hyperresponsivity and continuously elevated adrenergic function (Southwick et al. 1999, Schnurr et al. 2002).

Additionally, an increase in serotonin turnover in the hippocampus and the limbic fore-brain, including the nucleus accumbens was observed in rats that were exposed to psychological stress. The serotonergic system in the hippocampus might be selectively regulated by adrenal steroids in response to stress and imply the existence of negative feedback mechanisms via a hippocampal serotonergic system in the memory enhancement associated with corticosterone and psychological stress (Liu et al. 1999).

PTSD research has demonstrated a dysregulation of hypothalamic - pituitary - adrenocortical (HPA) function, however, there is some conjecture as to the specific abnormality identified in different studies (Schnurr et al. 2002). de Kloet et al. (2006) reviewed 26 challenge studies, using different methods to assess HPA-axis functioning. The key findings were enhanced salivary cortisol levels in response to cognitive challenge, and enhanced plasma cortisol suppression after administration of 0.5 mg of dexamethasone. All other results of pharmacological and cognitive challenge paradigms remained inconclusive.

Yehuda et al. (1998) found elevated levels of corticotropin-releasing factor and glucocorticoid receptor levels in PTSD. Glucocorticoids exert their effect through disruption of cellular metabolism and by increasing the vulnerability of hippocampal neurons to a variety of insults, including endogenously released excitatory amino acids. Glucocorticoids have also been shown to augment extracellular glutamate accumulation. Furthermore, reduction

of glucocorticoid exposure prevents the hippocampal cell loss associated with chronic stress (Bremner et al. 1999).

1.2.6 Functional Changes in PTSD

Individual suffering with PTSD experience both a tonic and phasic state. These two states, whilst obviously inter-related, may have relatively distinct underlying neurological mechanisms.

The phasic state is when the individual is preoccupied by the recurring and intrusive images of the triggering of the traumatic event. However, this generally only represents a small proportion of a persons waking life. The intrusive memories are associated with the psychological hyperarousal that has been well characterised in neuroimaging studies (Pitman et al. 2001). The role of temporal structures and dorsolateral prefrontal cortex in these symptoms has also been demonstrated.

In the interim periods, the other symptoms associated with the tonic state of disorder, namely, the numbing, interpersonal withdrawal, anhedonia and the hyperarousal indicated by the hypervigilance, irritability, exaggerated startle response and difficulties with concentration, dominate the clinical picture (Friedman 1997).

1.2.6.1 Learning and Memory

Alterations in memory form an important part of the clinical presentation with stress related psychopathology as the three symptom types expressed (re-experiencing, avoidance and hyperarousal) all relate in varying degrees to changes in the normal mechanism of memory processes. Patients with PTSD demonstrate a variety of memory problems including deficits in declarative memory (remembering facts or lists) and fragmentation of memories (both autobiographical and trauma-related). PTSD is also associated with alterations in non-declarative memory (types of memory that cannot be willfully brought up into the conscious mind). These types of non-declarative memories include conditioned responses and abnormal reliving of traumatic memories following exposure to situationally appro-

priate cues (Bremner et al. 1999). With this in mind it is not difficult to conceptualize PTSD as a disorder of memory, where the normal adaptive processes have malfunctioned (Brewin 2001, Schnurr et al. 2002).

Exposure to the stress of an unfamiliar environment resulted in deficits of working memory indicative of hippocampal dysfunction. High levels of glucocorticoids seen with stress were associated with deficits in new learning, the magnitude of which correlated with the extent of the damage to the hippocampus (Bremner et al. 1999).

Stress hormones released during emotionally arousing experiences activate noradrenergic mechanisms in the basolateral nucleus of the amygdala, resulting in enhanced memory for those events. Individuals with PTSD are physiologically more responsive to the recollection and imagery of traumatic events (Shin et al. 1999) and the PTSD avoidance method of thought suppression can actually lead to intrusive thoughts and memories (Schnurr et al. 2002).

1.2.6.2 Cognition

There are several information processing abnormalities evident in people with PTSD that range from perception of a feared situation, loss of stimulus discrimination, interpretation of the physiological and behavioural responses and representation of the information to the subsequent processing (van der Kolk 1997, Schnurr et al. 2002). These cognitive problems include selective attention, priority given to trauma-related information and excessive arousal levels impeding the integration of specific information (Elzinga & Bremner 2002), and the subjective complaints of cognitive problems correlate with poor performance (Schnurr et al. 2002).

Cognitive function is also specifically affected by stress and it has been shown that increases in glucocorticoids due to stressful experiences produce reversible deficits in episodic and spatial memory in the short-term and cognitive dysfunction after repeated exposure to elevated levels (McEwen 2000). Although specific PTSD research in this area remains inconclusive, it is important to note the similarities in PTSD symptomatology and glucocorticoid increases.

1.2.6.3 Attention

When attentional processes are functioning normally, an individual has the resources available to complete a task requiring executive control, including detection, selection and discrimination of stimuli. The attentional dysfunctions exhibited in PTSD include the breakdown of filtering functions, the over-processing of irrelevant stimuli, the allocation of attentional resources toward potentially threatening stimuli (attentional bias) and the resulting exhaustion of processing capacity which leads to loss of concentration (Sarter & Bruno 1997, Buckley 2000).

1.2.6.4 Mood/Behavioural Issues and Secondary Effects

PTSD causes a significant disruption to psychosocial performance, effecting social, occupational, and other important areas of functioning. People with PTSD find they experience a reduced affect and are no longer interested in activities or experience pleasure and other positive emotions. They find it difficult to connect with people and will avoid social situations.

The sleep deprivation caused by increased anxiety and arousal keeps people “on edge”, exacerbated by the persistent intrusive memories and nightmares. The hypervigilance and increased startle response will also add people experiencing an increase in irritability punctuated by angry outbursts. Often a sense of impending doom prevails (American Psychiatric Association 1994).

In the Australian National Survey, Creamer et al. (2001) found that PTSD is also associated with high rates of anxiety, depression and substance abuse, and it is also well documented that alcohol consumption significantly increases (Marshall et al. 1998). All of these issues cause a great level of discomfort to the sufferers, their loved-ones, and in a broader sense to the community at large.

1.2.7 The Cholinergic Neurotransmitter System in PTSD

1.2.7.1 ACh and Stress

In the healthy brain, with well-functioning muscarinic neurotransmission, both the administration of AChEIs and acute psychological stress elicit a transient increase in ACh release, ACh survival at the synapse and a phase of enhanced neuronal excitability. This intense cholinergic activation initiates a feedback pathway that suppresses cholinergic neurotransmission through the modulation of protein synthesis, by activating the M1 excitatory post-synaptic receptors and the M2 inhibitory pre-synaptic receptors. The M2 receptors block subsequent release of ACh into the synaptic cleft, while the increase in ACh at the M1 receptors triggers rapid induction of the gene encoding the transcription factor c-Fos (elevated c-Fos levels are a marker of neuronal hyperexcitation), which mediates selective regulatory effects leading to changes in the expression of genes regulating ACh metabolism. The combined effects of these changes should reduce the bioavailability of ACh by increasing AChE activity and reducing ChAT and VAcHT activity (Kaufer et al. 1998, Salmon et al. 2005). Kaufer et al. show that the administration of an AChEI can mimic the effects of acute stress on gene expression, in that c-Fos levels are increased, followed by an increase in AChE mRNA and a reduction in ChAT and VAcHT mRNA levels.

Apart from its catalytic function, AChE affects cell proliferation, differentiation and responses to various insults, including stress. These responses are at least in part specific to the three C-terminal variants of AChE which are produced by alternative splicing of the single AChE gene. Under normal conditions the AChE mRNA primarily produces “Synaptic” AChE-S, which is the principal multimeric enzyme in brain and muscle. The increase in AChE induced by stress is caused by rapid, yet long-lasting transcriptional AChE activation accompanied by a transcript-splicing shift, from the AChE-S variant to the normally rare, soluble, monomeric “readthrough” AChE-R mRNA. AChE-R appears in embryonic and tumor cells and is induced under psychological, chemical and physical stress. The third is a glypiated dimer of erythrocytic AChE-E which is associated with red blood cell membranes. These processes are at least in part induced by glucocorticoids (Grisaru

et al. 1999, Salmon et al. 2005).

The "read-through" variant of acetylcholinesterase (AChE-R) accumulates in the mammalian brain under acute stress, but it has been shown that excess AChE-R has a neuroprotective effect while AChE-S intensifies neurodeterioration. Acute stress increases the risk for neurodegeneration, but AChE-R serves as a modulator that may play a role in preventing the shift from transient, acute stress to progressive neurological disease (Sternfeld et al. 2000). However, chronic excess of AChE-S, as a result of a failure to induce the shifted splicing reaction, leads to progressive deterioration of learning and memory, neuromuscular malfunction, and cumulative neuronal stress markers (Salmon et al. 2005).

Curiously, inhibitors of the acetylcholine-hydrolysing enzyme acetylcholinesterase may induce psychopathologies that are reminiscent of PTSD (Kaufer et al. 1998).

1.2.7.2 Previous PTSD Studies - ACh and PTSD

PTSD in general has been extensively studied including topics such as details about development or emergence of the disorder (natural disasters, consequence of medically induced trauma, effects of war), clinical features (cognitive dysfunction, sleep disorders, behavioural issues), pathophysiological findings (neurophysiological and morphological manifestations, functional neuroanatomy, psychobiology) and treatments (CBT, pharmacotherapy).

There have, however, been few studies involving PTSD that have focussed on ACh. There has been some recent interest in the involvement of the nicotinic receptor using the radiotracer I¹²³5-IA-85380 and SPECT, correlating tracer binding with PTSD symptom clusters (Czermak et al. 2008), but mostly the attention has centred around the effects that stress has on the cholinergic system, namely the alternative splicing of the AChE mRNA and the cumulative effects of these changes (Kaufer et al. 1998, Grisaru et al. 1999, Kaufer et al. 1999, Sternfeld et al. 2000, Salmon et al. 2005).

1.2.7.3 Basis for Considering a Potential Role for ACh Neurotransmission

The role of the cholinergic pathways has been unexamined to date in PTSD. This is of particular interest for several reasons. Firstly, as indicated above, acetylcholine is one of the

neurotransmitters shown to play a role in working memory function (Beninger, Wirsching et al. 1989; Levin, Torry et al. 1997). Secondly, the flashbacks commonly reported in PTSD appear to be similar phenomenologically to the hallucinations reported in Lewy-body dementia. This form of dementia involves abnormalities of the cholinergic system even more profound than that for AD and responds well to cholinesterase inhibitors (McKeith et al. 2000).

A third reason derives from studies of soldiers in the context of “Gulf War Syndrome” and its relation to PTSD. AChEI exposure is associated with long-term effects reminiscent of posttraumatic stress disorder. This suggested that exposure to anti-ChEs leads to persistent changes in brain proteins (Kaufer et al. 1998). It has been found that soldiers pre-treated with the cholinesterase inhibitor, Pyridostigmine bromide, as a preventative measure for nerve gas attack, now complain of major cognitive and memory problems similar to those seen in PTSD (Sapolsky 1998).

From a theoretical perspective, improving cholinergic transmission in PTSD may prevent its tonic symptoms by helping the individual engage more actively with salient environmental stimuli. By normalising attention in this way, there may also be decrease in involuntary exposure to traumatic memories. It is hypothesised, therefore, that pharmacological agents such as donepezil that enhance cerebral ACh and improve memory and attention, will have a generalised effect in decreasing the symptoms of PTSD.

In summary, there are a series of observations that suggest the relevance of acetylcholine to neurocognitive disturbance in PTSD:

1. Anatomically, areas in the brain that have been shown to be altered structurally and functionally in PTSD have a high density of acetylcholine and its receptors.
2. In PTSD, selective attention and working memory are abnormal and acetylcholine is a candidate neurotransmitter for the mediation of such effects.
3. Acetylcholine plays a role in reversal of learning to aversive stimuli and this is likely to be relevant to the conditioning of PTSD sufferers to the traumatic event.

1.2.8 Cerebral Imaging and Other Activity Investigations of PTSD

There are various ways to measure brain activation including such techniques as Single Photon Emission Computerised Tomography (SPECT), Positron Emission Tomography (PET - fluorodeoxyglucose (FDG) and O_2), and functional Magnetic Resonance Imaging (fMRI). These three methods derive brain function indirectly from physiological measures such as regional cerebral blood flow (rCBF), blood oxygen levels, and glucose consumption based on the assumption that areas of high radioactivity (uptake of the radiolabel attached) are associated with brain activity. Glucose metabolism and blood flow, among other parameters, alter when certain brain areas become activated or inhibited, and an increase or decrease in metabolic demands of neurons leads to an increased or decreased blood flow to these areas (Sestini 2007).

Several different methods have now been utilised to image the brains of patients with PTSD in order to better understand the pathogenesis and pathophysiology of PTSD. MRI has been used in both structural and functional capacities. Structural abnormalities in PTSD found with MRI include nonspecific white matter lesions and decreased hippocampal volume. Patients with combat-related PTSD had smaller right hippocampal volume, increased incidence of small clefts in the callosal-septal interface and decreased neuronal density of the right medial temporal structures on MRI (Bremner et al. 1999, Sachinvala 2000). Quantitative volumetric MRI showed both left and right hippocampi were significantly smaller in PTSD subjects.

Functional MRI research has shown the amygdala to be hyper-responsive to fear-related stimuli in this disorder (Pitman et al. 2001). Functional neuroimaging symptom provocation and cognitive activation paradigms using O_2 PET have also revealed greater activation of the amygdala and anterior paralimbic structures (which are known to be involved in processing negative emotions such as fear), greater deactivation of Broca's region (motor speech) and other nonlimbic cortical regions, and failure of activation of the cingulate cortex (which possibly plays an inhibitory role) in response to trauma-related stimuli in individuals with PTSD (Bremner et al. 1999, Pitman et al. 2001).

Decreased glucose metabolism measured with FDG-PET (as an index of impaired neuronal activity) was found at baseline in temporal and prefrontal cortex in combat-related PTSD and in the parietal cortex in patients with PTSD from other traumatic events (Bremner et al. 1999). A number of other PET studies focussing on rCBF have implicated medial prefrontal cortex in stress and emotion. It also has inhibitory connections to the amygdala that play a role in extinction of fear responding therefore a dysfunction in this area may cause a failure of extinction to fear responding in PTSD. One study using a word-stem completion task to study rCBF in 16 firefighters found that the limbic and paralimbic regions of interest in individuals with PTSD include the amygdala, anterior cingulate gyrus, anterior temporal pole, insular cortex, and orbitofrontal cortex (Shin et al. 1999). These regions are involved in the processing of emotional stimuli and in the modulation of heart rate, blood pressure, and respiration through projections to autonomic centres of the brain stem, each of which would be activated during emotional and psychophysiological responses to the recollection of traumatic events.

Patients with PTSD have also been shown to have important dysfunctions of working memory. This underlies the difficulties with concentration and memory which have been well studied in this condition (Clark et al. 2000). An attentional bias underlies much of this phenomenology. Previous event related potential (ERP) work suggests that these people have abnormalities, both in short term storage and an executive processing component of working memory (Galletly et al. 2001). This research group has been investigating these abnormalities originally with ERP and more recently with Positron Emission Tomography (PET), and functional Magnetic Resonance Imaging (fMRI) in combination with ERP (Shaw et al. 2002). This body of research highlights the significant attentional dysfunction in PTSD and abnormalities of working memory in both verbal and auditory domains. The PET data highlight the disruption of the cortical neural networks involved in working memory, particularly the left dorsolateral prefrontal cortex (Clark et al. 2000).

There is an increasing amount of research performed in PTSD patients using SPECT to measure rCBF. Using the blood flow tracer 99m -technetium-hexamethylpropyleneamineoxime ($^{99m}\text{Tc-HMPAO}$), Zubieta and colleagues demonstrated significant increases in the blood

flow to the medial prefrontal cortex in PTSD patients (Zubieta et al. 1999). Pagani et al. (2007) also utilised ^{99m}Tc -HMPAO SPECT to determine the differences between patients with PTSD and controls, and their statistical parametric mapping (SPM) analysis showed significant uptake in the orbitofrontal cortex (Brodmann 11) and the temporal pole (Brodmann 38), the uncus (Brodmann 36) and the lateral temporal lobe (Brodmann 21). Increased activity was also observed in the parietal lobes, left hippocampus, thalamus and left prefrontal cortex during traumatic memory retrieval (Peres et al. 2007). An earlier study, however, only found that rCBF was significantly more heterogeneous in patients suffering from PTSD (from severe psychological trauma induced by torture) than in healthy controls (Mirzaei et al. 2001).

To date, there has only been one publication on cerebral cholinergic function in PTSD. Czermak et al. (2008) used $[^{123}\text{I}]5\text{-IA}$ to evaluate nicotinic ACh receptor binding in PTSD patients and found significantly higher binding in the mesiotemporal cortex and thalamus which potentially corresponded to the re-experiencing symptom cluster. These findings therefore are conspicuously at odds with the current understanding of cerebral ACh-mediated physiology.

1.2.9 Scope of the Present Study

The data summarized in Section 1.1.4: Functional Correlates of Acetylcholine, 1.2.6: Functional Changes in PTSD and 1.2.7: The Cholinergic Neurotransmitter System in PTSD provide a basis for the hypothesis that many of the symptoms associated with PTSD may be engendered in whole or in part by regional deficiencies in cerebral cholinergic function. The principal aim of the experiments outlined in this thesis was to test that hypothesis.

It was considered necessary to first determine whether the number of ACh receptors, or receptor activity in subjects with PTSD was reduced when compared to a normal control population. This study utilised I^{123} Iododexetimide (IDEX) SPECT to establish the existence of differences in ACh receptor activity in subjects with PTSD (refer to Chapter 2, The I^{123} Iododexetimide (IDEX) SPECT Study).

The separate clinical study (using some PTSD subjects from the IDEX SPECT Study and some new PTSD subjects) incorporated the administration of an acetylcholinesterase inhibitor (donepezil) to manipulate the cortical levels of ACh in people with PTSD, in order to investigate the effects on overall PTSD symptomatology (refer to Chapter 3, The Donepezil Clinical Trial).

Chapter 2

An I¹²³ Iododexetimide (IDEX) SPECT Study of Acetylcholine Neuro-receptors in Posttraumatic Stress Disorder

2.1 INTRODUCTION

One field of research occurring in the late part of the 20th century sought to shed light on the changes in the regional distribution of muscarinic ACh receptors (m-AChR) that had been demonstrated at autopsy in some neurodegenerative disorders. The method of choice was nuclear imaging, but at the time, the only radiotracers available had low yields and short half-lives, reducing the economic feasibility of conducting such research. Therefore, to increase noninvasive imaging of m-AChR in human studies by single photon emission computed tomography (SPECT), a new high specific activity radiotracer had to be developed.

Wilson et al. (1989) evaluated dexetimide ((S)-(+)- 3-phenyl- 3-[91-phenyl-methyl)- 4-piperidiny]- 2,6-piperidinedione), which was a known potent m-AChR antagonist. The binding of dexetimide had been previously demonstrated to be saturable, displaceable and

stereospecific, which is characteristic of receptor-ligand interactions. When labelled with radioactive Iodine (I^{123}), it became known as I^{123} iododexetimide (IDEX). Animal studies showed that the distribution of IDEX uptake corresponded to the known distribution of m-AChR in the human brain. Wilson et al. (1989) determined that, of all the radiohalogens, I^{123} was the best for SPECT imaging purposes on the basis of its availability, half-life (13 h), *in vivo* receptor saturability and stereospecificity, and excellent target to nontarget ratios. The high brain uptake obtained over several hours, together with its convenient preparation, suggested that IDEX would be useful in imaging and quantifying levels of m-AChR in the living human brain using SPECT (Wilson et al. 1989, Muller-Gartner et al. 1992, Wilson et al. 1991, Boundy et al. 1995).

IDEX has since been used to study the m-AChR activity in temporal lobe epilepsy (Boundy et al. 1996, Weckesser et al. 1997, Rowe et al. 1998), mild, moderate and severe Alzheimer's disease (Boundy et al. 1997, Boundy et al. 2005), and in psychiatric disorders such as schizophrenia (Lavalaye et al. 2001). As PTSD is characterized by intrusive trauma-related memories and deficits in everyday memory and attention (Veltmeyer et al. 2006, Brewin 2001), it was postulated that IDEX could be used to investigate whether the cholinergic activity in PTSD has been compromised as this could explain a part of the altered cognitive symptomatology apparent in this condition.

The cognitive symptomatology of PTSD appears to parallel, or even herald the onset, of certain dementias, in particular Lewy-body Dementia (DLB)(Johnston 2000). Patients with DLB experience fluctuating cognitive impairment and attention deficits in conjunction with visual and auditory hallucinations, delusions, depressed mood, apathy, anxiety and sleep disturbances. A reduction in cholinergic activity correlates with the global severity of cognitive impairment and the presence of visual hallucinations. Hallucinations (particularly visual), which occur in 70% of DLB cases but only 5-30% of AD cases, have been demonstrated to relate to the extent of the cortical cholinergic deficit (Perry & Perry 1995). Patients with DLB taking a cholinesterase inhibitor have been shown to suffer significantly less apathy and anxiety, and had fewer delusions and hallucinations. Other neuropsychological tests demonstrated that these patients were also significantly faster and better on

tasks with a substantial attentional component (McKeith et al. 2000).

Although the relationship between stress and acetylcholine has been an area of research for some time (Kaufer et al. 1998), the role of the cholinergic pathways specifically in PTSD has been relatively unexamined to date. However, this system is of particular interest for several reasons. Firstly, acetylcholine is one of the neurotransmitters that has a crucial role in working memory (Beninger et al. 1989, Furey et al. 2000). Secondly, flashbacks and other perceptual distortion symptoms seen in PTSD that have much in common with hallucinations, are also observed in DLB. This form of dementia has more profound abnormalities of the cholinergic system than Alzheimers Disease and these systems respond well to cholinesterase inhibitors (McKeith et al. 2000).

In DLB the cholinergic deficit is mostly neurochemical with the loss of choline acetyltransferase which is the enzyme synthesizing acetylcholine. In AD, the deficit relates more to neuroanatomical and neuro-receptor issues (eg within the hippocampus)(Perry & Perry 1995). Therefore, from a theoretical perspective, to experience the symptoms influenced by cholinergic activity, people with PTSD could possess either a reduction of acetylcholine neuro-receptors or a reduction in the acetylcholine available to bind with the receptors. Although the latter would show a greater improvement, it is possible that these people could be treated with cholinesterase inhibitors in either case. An increase in acetylcholine receptor activity would improve working memory in PTSD, and may in turn improve the hyperarousal symptoms by ensuring the individual is more engaged with their environment. Optimising the individuals involvement with current salient stimuli may have a secondary effect of decreasing intrusive traumatic memories and reducing the associated anxiety.

In the current study, it was deemed important to first determine whether the number of receptors, or receptor activity in people with PTSD was enhanced or reduced when compared to a normal control population. This could lead to a study that incorporated the administration of AChEs to manipulate the cortical levels of ACh in these patients.

2.1.1 Aim

To determine, in people with PTSD compared with a control group, whether there are differences in ACh receptor activity which can be detected with IDEX SPECT.

2.2 METHOD

2.2.1 Recruitment

Subjects were recruited via the media in the form of news articles in the visual (TV: Channel 7), audio (Radio 5UV and ABC) and written (Sunday Mail and Messenger) press. Interested people contacted the researcher (EAG) by telephone (see Fig.2.2.1 for a breakdown of the recruitment process). Every potential subject was screened by telephone (either at the time of first contact or at a subsequent predetermined appointment time). All callers were questioned to elicit information relating to the criteria for a diagnosis of PTSD as recorded in the American Psychiatric Association (1994) Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) which are reproduced in full for the reader's convenience:

A *The person has been exposed to a traumatic event in which both of the following were present:*

- 1. the person experienced, witnessed, or was actually confronted with an event or events that involved actual or threatened death or serious injury, or a threat to the physical integrity of self or others*
- 2. the person's response involved intense fear, helplessness, or horror.*

B *The traumatic event is persistently reexperienced in one (or more) of the following ways:*

- 1. recurrent and intrusive distressing recollections of the event, including images, thoughts, or perceptions.*
- 2. recurrent distressing dreams of the event.*

3. *acting or feeling as if the traumatic event were recurring (includes a sense of reliving the experience, illusions, hallucinations, and dissociative flashback episodes, including those that occur on awakening or when intoxicated).*
4. *intense psychological distress at exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event.*
5. *physiological reactivity on exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event.*

C *Persistent avoidance of stimuli associated with the trauma and numbing of general responsiveness (not present before the trauma), as indicated by three or more of the following:*

1. *efforts to avoid thoughts, feelings, or conversations associated with the trauma*
2. *efforts to avoid activities, places, or people that arouse recollections of the trauma*
3. *inability to recall an important aspect of the trauma*
4. *markedly diminished interest or participation in significant activities*
5. *feeling of detachment or estrangement from others*
6. *restricted range of affect (e.g., unable to have loving feelings)*
7. *sense of a foreshortened future (e.g., does not expect to have a career, marriage, children, or a normal life span)*

D *Persistent symptoms of increased arousal (not present before the trauma), as indicated by two (or more) of the following:*

1. *difficulty falling or staying asleep*
2. *irritability or outbursts of anger*
3. *difficulty concentrating*
4. *hypervigilance*
5. *exaggerated startle response*

E *Duration of the disturbance (symptoms in Criteria B, C, and D) is more than 1 month.*

F *The disturbance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.*

2.2.1.1 Inclusion Criteria

Inclusion criteria required that all subjects were right-handed and fluent in English, aged between 18 and 65 years, and all satisfied the criteria for a diagnosis of PTSD on the PCL (Civilian or Military) (Norris & Hamblen 2004) due to different traumatic events. All assessments were conducted by one researcher (EAG). Comorbidity (eg. depression) was only allowed if it was judged as a secondary disorder to the initiating PTSD.

Ideally, Control subjects were to be matched for age, gender, premorbid intelligence, and not suffer from any medical conditions that affect the central nervous system (CNS). Control subjects were recruited by word of mouth from amongst interested relatives and friends of PTSD subjects, work colleagues or recruited for another study on the same gamma camera (Boundy, private communication).

2.2.1.2 Exclusion Criteria

Subjects were excluded if they were on prescribed medications that may influence regional cerebral blood flow or would interfere with a cholinesterase inhibitor (see Appendix A, (Pharmacy Information: Medications with Cholinergic Properties)), suffered from Epilepsy or a serious medical illness, had experienced a head injury with greater than ten minutes post-traumatic amnesia or were currently engaged in substance abuse. See Fig.2.2.1 for a flow chart detailing the complete breakdown of the recruitment process.

2.2.1.3 Funding and Approval

This study was made possible by a grant supporting the supply of the radiotracer, IDEX provided by the Australian Institute of Nuclear Science and Engineering (AINSE) in conjunction with the Australian Nuclear Science and Technology Organisation (ANSTO) and

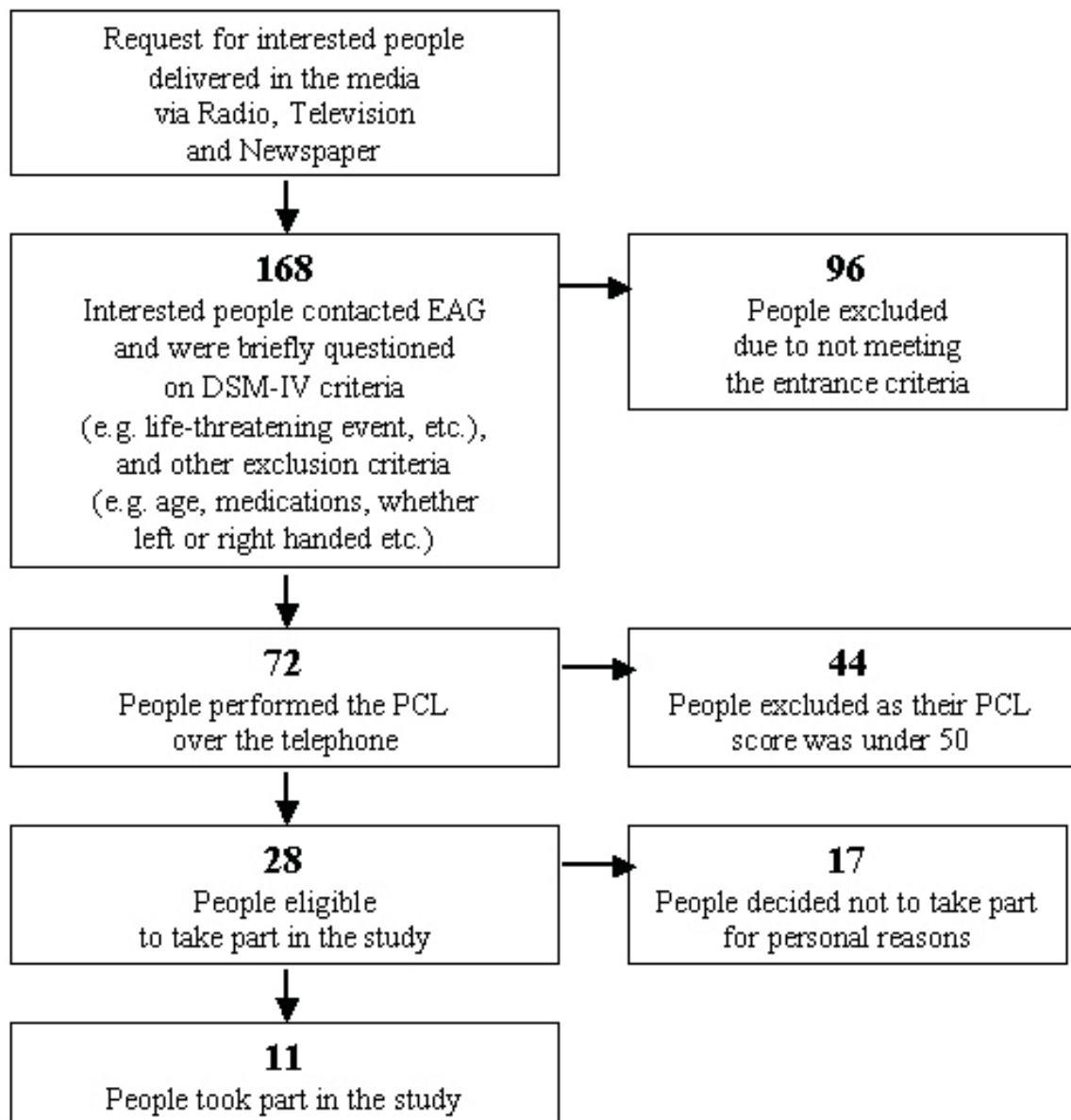


Figure 2.2.1: Flow chart of the stages of recruitment, demonstrating where the interested people were excluded from taking part in the study.

approved by the Ethics of Human Research Committee at the North Western Adelaide Health Service (The Queen Elizabeth Hospital). (See Appendix B, (IDEX Study Ethics Approval Letter) and Appendix C, (IDEX Study Patient Information and Informed Consent Form)). Written informed consent was obtained from all participants.

2.2.2 Procedure

2.2.2.1 Patient Preparation and Administration

Subjects arrived at TQEH Nuclear Medicine Department on the morning of their study appointment and were given one 120mg Potassium Iodide tablet to minimise thyroid uptake of unlabelled radioactive Iodide (Robbins 1983). An intravenous canula was inserted to enable infusion of the IDEX.

Each subject was instructed to lie down on the scan bed and relax with eyes closed. The IDEX was then administered to the subject by way of an injection through the intravenous canula into an antecubital vein while they kept their eyes closed (to both standardise the procedure and minimise the regional cerebral blood flow (rCBF) in the brain from optical stimulation as much as possible). The first SPECT was commenced within 5 minutes of the IDEX injection.

After the completion of the first scan, each subject had the intravenous canula removed before moving to the therapy room to complete the series of psychometric instruments or questionnaires. (See Section 2.2.2.2, Psychological Assessments)

The subjects were then taken to the Department of Radiology to undergo a structural MRI. All MRIs were acquired on a Philips Intera 1.5T MR Imaging System, using Software Release 8.0 (MDA 02 007). Safety Questionnaires were completed to determine if anything specifically required removal before the scan (eg. hearing aid or dentures) or whether another precautionary scan was required (eg. an ocular X-ray to rule out a previous penetrating metal injury to the eye). The MRI was expected to take 30mins and involve the subject lying still in the camera.

The subjects were then free to leave the Department until their second SPECT scan,

which took place between 4-6 hours post-injection of IDEX. (Refer to Fig.2.2.2 for a breakdown of the recruitment process.)

The order of scans and psychometric assessments was determined by the necessity of performing the Uptake phase SPECT scan within the first 5 minutes post-injection and the final SPECT scan 4-6 hours post-injection of IDEX. As the subjects were already in the Nuclear Medicine department, it was most prudent to complete the psychometric assessments immediately following the first scan. This was also desirable in light of the claustrophobic nature of the MRI scan to follow, which may have influenced the psychometric results had it been performed first.

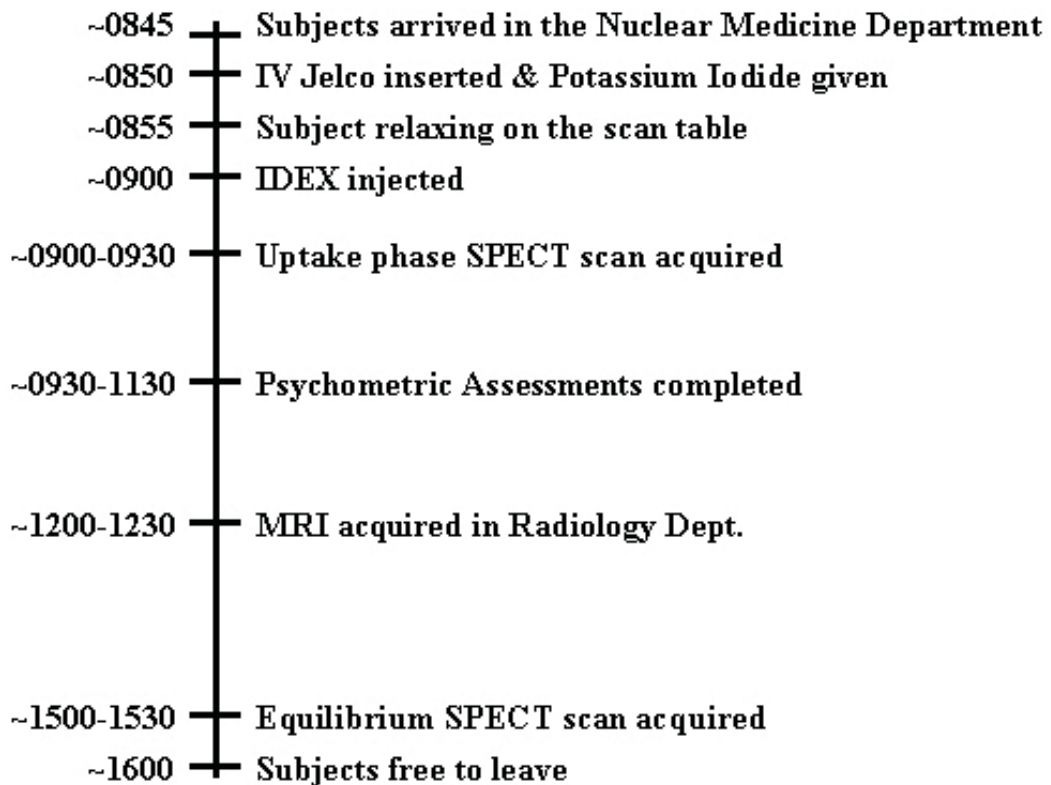


Figure 2.2.2: Flow chart of the approximate timeline used to achieve all subject study requirements.

2.2.2.2 Psychological Assessments

The following questionnaires were administered in the study:

PTSD Subjects -

The PTSD Check List (PCL)

Clinician-Administered PTSD Scale for DSM-IV (CAPS)

Composite International Diagnostic Interview (CIDI)

The Impact of Events Scale - Revised (IES-R)

The Alzheimer's Disease Assessment Scale - Cognitive Subscale (ADAS - Cog)

The National Adult Reading Test - Second Edition (NART)

Control Subjects -

PCL

CIDI

ADAS-Cog

NART

General Health Questionnaire (GHQ)

The PTSD Check List (PCL)

This is a self-administered tool designed to assess the 17 symptoms for PTSD outlined in the DSM-IV. The subjects are required to rate how much they were "bothered by that problem in the past month" (Weathers et al. 1993). Individual items are rated on a 5 point scale: 1 "not at all", 2 "a little bit", 3 "moderately", 4 "quite a bit" and 5 "extremely", and the total score can range from 17 to 85 (e.g. a subject that is not at all bothered by any symptom measured by the 17 item scale would obtain a score of 17). There are 3 versions of the PCL; PCL-M (Military) which was the first to be developed and tested using Vietnam veterans as a subject population, PCL-C (Civilian) where the questions are worded more generically to refer to "stressful experiences in the past" and the PCL-S (Specific) which is designed to describe reactions to a specific event (Norris & Hamblen 2004).

In the current study, the PCL was administered initially as a telephone screen, and

utilised again on those not excluded, for the IDEX Trial. It was also used to demonstrate that there was an absence of a PTSD diagnosis in the Control subjects.

Clinician-Administered PTSD Scale for DSM-IV (CAPS)

This diagnostic structured clinical interview is designed to assess the 17 symptoms for PTSD outlined in the DSM-IV, and is generally regarded as the gold standard in PTSD assessment.

The CAPS provides a means to evaluate: a) the frequency and intensity dimensions of each symptom; b) the impact of the symptoms on the patient's social and occupational functioning; c) the overall severity of the symptom complex; d) the patient's global improvement since baseline; and e) the validity of ratings obtained. Although all individual ratings are based on the patient's report, the final score is determined by combining the patient's report, the interviewer's confidence in that report, and the patient's behaviour during the interview process. To make a current or lifetime diagnosis, the time frame that patients were asked to consider when rating each item in the interview was the previous month.

Each frequency item is given a score ranging from 0 "Never" to 4 "Daily or almost every day", and the symptom intensity scores range from 0 "None" to 4 "Extreme, incapacitating distress, cannot dismiss memories, unable to continue activities". A total score of 50 or greater is considered to be diagnostic of PTSD. (American Psychiatric Association 1994, Blake et al. 1995).

Composite International Diagnostic Interview (CIDI)

The CIDI is a thorough, fully structured diagnostic interview for the assessment of mental disorders developed by the World Health Organization (WHO) and the former United States Alcohol, Drug Abuse and Mental Health Administration. The computerised version known as CIDI-Auto (utilised in this study), can be self-administered by the respondent, or administered by a technician interviewer who reads the questions as they appear on the screen (identical to those of the original interview). This tool provides both lifetime and current diagnoses according to the accepted definitions of the International Classification of Diseases (ICD-10) (World Health Organization 1993*b*) and DSM-III-R (American Psychiatric Association 1980) by means of computerised algorithms. The interview is modular

and presently covers somatoform disorders, anxiety disorders, depressive disorders, mania, schizophrenia, eating disorders, cognitive impairment, and substance use disorders (Blouin et al. 1988, World Health Organization 1993*a*).

In the current study, the interviewer utilised a subset of relevant sections to elicit information on specific areas. The sections selected for the study were A (Demographics), B (Tobacco), D (Anxiety Disorders), E (Depression), J (Alcohol) and K (OCD and PTSD). In the Control subjects, this interview was used to demonstrate the absence of other influencing mental illness.

The Impact of Events Scale - Revised (IES-R)

The IES, designed before PTSD was fully defined in the DSM-III-R, assessed the impact of a serious life event on individuals over periods of time. It was a 15-item measure and although it neglected to take into account the hyperarousal symptoms included in the criteria for the diagnosis of PTSD in the DSM-IV, its measures of intrusion and avoidance correlated well with the CAPS scores (Sundin & Horowitz 2002, Horowitz et al. 1979). The IES-R, which was used in this study, is a self-administered, 22-item measure of subjective stress, was developed in 1997 to include the hyperarousal symptoms. Each item is given a score ranging from 0 “Not at all” to 4 “Extremely”, giving a possible total score range of 0-88 (Weiss & Marmar 1997).

The Alzheimer’s Disease Assessment Scale - Cognitive Subscale (ADAS - Cog)

The fundamental scale (ADAS) was primarily designed to measure the severity of the most important symptoms of Alzheimer’s disease (AD). The 11-item memory assessment subscale, ADAS-Cog is the best validated cognitive testing instrument used in clinical trials of nootropics and is used to determine the presence or absence of a cognitive impairment. The 11 tasks measure disturbances of memory, language, praxis, attention and other cognitive abilities that are often referred to as the core symptoms of AD. Achievable scores range from 0 to 25 indicating normal cognitive ability (0-10), mild cognitive impairment (11-14) and mild to moderate dementia (15-25). Although essentially only an AD rating, was used in this study to exclude subjects with significant cognitive impairment (Rosen

et al. 1984, Doraiswamy et al. 1997)).

The National Adult Reading Test - Second Edition (NART)

The NART is a 50-word test that has become a widely accepted method for estimating premorbid levels of intelligence in neuropsychological research. Prediction of IQs using equations based on the revised NART score was more accurate than prediction with demographic variable equations (Blair & Spreen 1989). Also, scores achieved on the NART/NARTR demonstrated higher correlations with current WAIS/WAISR IQ (the gold standard of IQ testing) in controls and patients providing confidence in the continued use of NART as a valid estimate of premorbid intelligence in a number of conditions (Bright et al. 2002).

In this study the NART was used to enable the Control subjects to be IQ-matched to subjects with PTSD.

General Health Questionnaire (GHQ)

The GHQ was developed at the Institute of Psychiatry in London for use as a screening instrument in community settings, primary care and medical out-patients. It is a set of 60 items that attempts to focus on breaks in normal functioning, rather than lifelong traits or psychiatric disturbances. Respondents are asked to consider whether they have experienced symptoms over the last few weeks, and answers range from “Not at all” to “Much more than usual” or from “Better than usual” to “Much worse than usual” (Goldberg 1992).

2.2.2.3 IDEX SPECT Image Acquisition and Processing

All subjects were required to undertake 2 cerebral SPECT scanning procedures:

1. The Uptake phase scan: acquisition commenced at 5 minutes post injection of between 185-200 MBq IDEX (made up in 5ml saline) which has been shown to be the most effective dose equivalent (Boundy et al. 1995). Three scans of 10 mins each were acquired for this combined SPECT. The residual activity in syringe was measured so that the actual dose of IDEX could be determined.
2. The Equilibrium phase scan: acquisition commenced at 4-6 hours after injection,

previously determined to be the time at which the highest uptake of IDEX reflects the known distribution of mAChR (Boundy et al. 1995).

The IDEX was produced by the National Medical Cyclotron, (Sydney, Australia) in conjunction with the Radiopharmaceuticals Division of ANSTO (Wilson et al. 1989), and delivered to TQEH on the morning of the subjects' appointment.

SPECT was acquired using an IRIX triple-headed gamma camera system (Philips Medical Systems) with high-resolution parallel hole collimators. The acquisition used a 20% window centred on 159 keV. Projections were acquired at 120 angles over 360° into 128 x 128 matrices with 3.50 mm square pixels. The SPECT system resolution in air was 8mm full width at half maximum (FWHM). After acquisition, the SPECT data was transferred to a back-up system for storage. The SPECT projections were reconstructed with an iterative algorithm that incorporated uniform attenuation correction (coefficient 0.15/cm). The algorithm was OSEM (ordered subset expectation maximisation) and the body edge profile of the attenuation map was computed from the projection edges (Barnden et al. 2006). The resulting sections were converted to ANALYZE format for subsequent analysis.

2.2.2.4 MRI Acquisition and Processing

Structural MRI imaging was to be utilised as an adjunct technique to increase the accuracy of determining the anatomical locations of the receptor activity shown in the SPECT. The MRI images were archived using a DICOM compatible PACS (Picture Archive Communications System) /Teleradiology system developed by Central Data Networks (CDN).

2.2.3 SPECT Data Analysis

The SPM2 software package from the Wellcome Department of Cognitive Neurology, London, UK was utilised to perform spatial normalization to a standard anatomical space, voxel-by-voxel statistical analysis and estimation of statistical inference for clusters of voxels in the SPECT images. The following image processing steps were undertaken:

2.2.3.1 SPM Pre-processing

Preprocessing of the SPECT scans to prepare them for SPM voxel-by-voxel statistical analysis was performed using a protocol optimised within the Nuclear Medicine Department of T.Q.E.H. (Barnden & Behin Ain 2006). The tomographic images were first reoriented to be compatible with SPM (frontal lobe at top of transaxial and left on sagittal; crown at top of coronal and sagittal) and laterally inverted to appear in the ‘right-handed’ or ‘neurological convention’. Figure 2.2.3 illustrates the subsequent image processing steps. Images were then smoothed with a 10mm FWHM Gaussian filter ready for spatial normalization.

All spatial normalisation was performed by deforming the images to a custom IDEX template created within the Department, (Boundy et al. 2005). The first step was a 12 parameter affine transformation. Two spatial normalization steps were performed.

Scalp and facial activity were masked by multiplying the result by SPM’s brain mask that is unity in the brain and falls smoothly to zero at it’s edge. The second non-linear spatial normalisation was performed with a 25mm cutoff and 16 non-linear iterations. Two image sequences were generated by the processing. One sequence (right hand side Fig 2.2.3) used smoothed images to optimise the spatial normalisations. The other (left hand side Fig 2.2.3) applied the resulting transformations to the unsmoothed, unmasked images.

2.2.3.2 Global Scaling

Before the spatially normalised scans were subjected to voxel-based statistical analysis in SPM, variations between individual subjects in global intensity levels were corrected. These variations arise due to different levels of: 1. injected activity, 2. uptake and washout, and 3. competing uptake by other organs. The correction (scaling) was performed by multiplying each voxel value by a constant value chosen to render the mean value of a reference brain structure to be the same for all subjects. Two reference volumes were used: the whole brain and the basal ganglia. The whole brain scaling was achieved using SPM’s “proportional scaling” that is based on the mean value of all voxels above a threshold ($0.8 \times$ mean of non-zero voxel values). Global scaling relative to the basal ganglia was achieved by pre-scaling

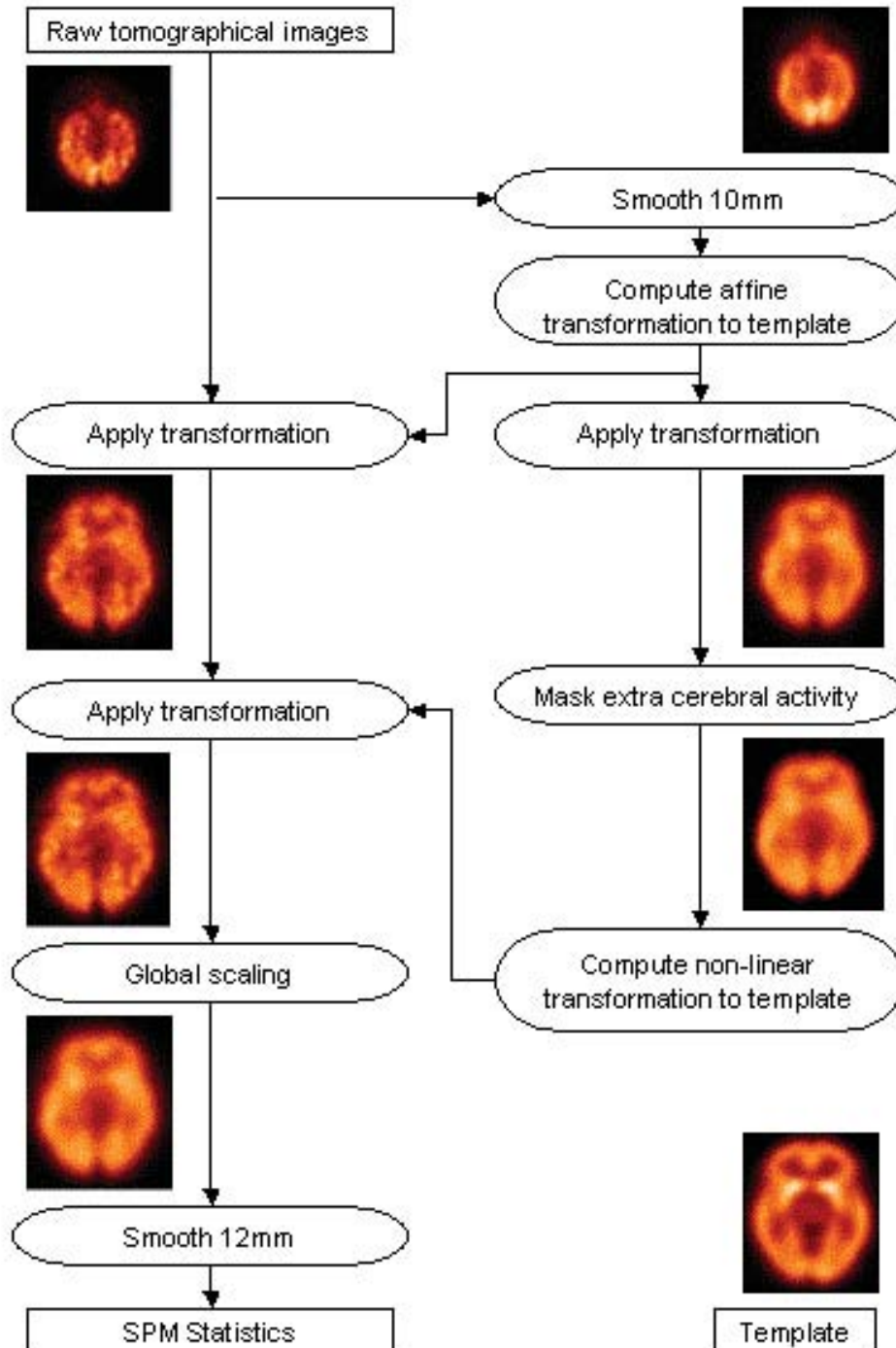


Figure 2.2.3: Flow chart of the SPM pre-processing sequence. Spatial normalisation (deformation/warping) steps follow the column on the right with the computed deformations applied to the unsmoothed images in the column on the left.

to the mean value in a reference basal ganglia region - see Fig 2.2.4. The basal ganglia were originally chosen as a reference in an earlier IDEX study of AD because they are rarely involved in early AD. We retained it for our PTSD study because it is a central area with appreciable uptake that is not specifically involved in ACh production. We used the SPM2 'Masks' toolbox of L. Barnden (<http://www.anzsnm.org.au>) for this purpose.

The scans were then smoothed using a 12mm FWHM Gaussian filter ready for SPM statistical analysis.

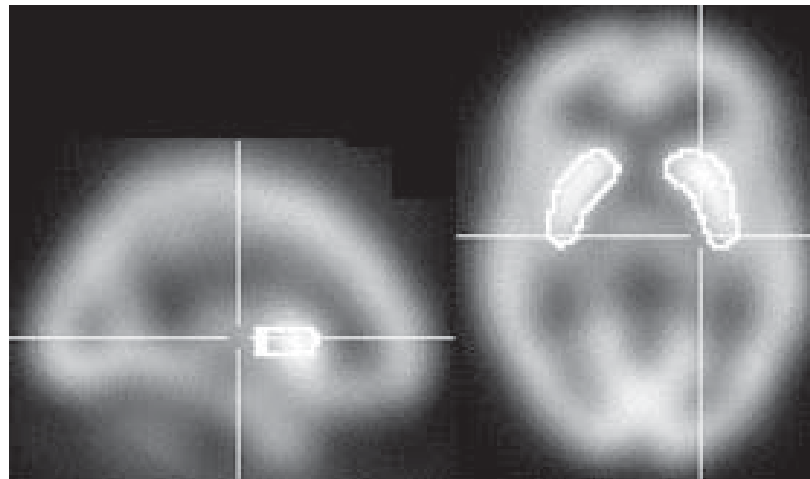
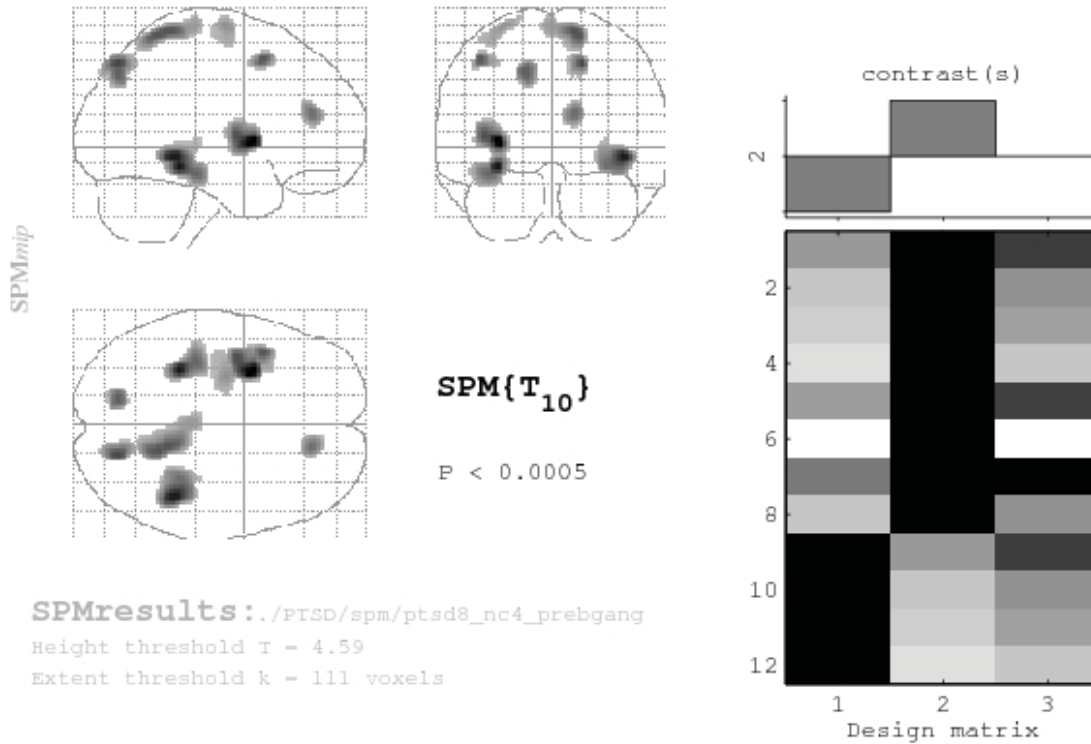


Figure 2.2.4: Template for scaling to the Basal ganglia

2.2.3.3 SPM Statistics

The statistical analyses of functional mapping experiments proceeds at the voxel level. It culminates in the formation and assessment of a Statistical Parametric Map (SPM) compiled by computing at each voxel a statistical parameter to characterise the significance of the difference between the means for the PTSD subjects and the normal controls. The statistical parameter used was the T statistic.

Voxel-by-voxel statistical analysis of the PTSD subject group versus the control group



Statistics: p-values adjusted for search volume

set-level		cluster-level			voxel-level					x, y, z (mm)		
P	c	$P_{corrected}$	k	$P_{uncorrected}$	$P_{FWE-corr}$	$P_{FDR-corr}$	T	(Z_{α})	$P_{uncorrected}$			
0.000	9	0.000	501	0.000	0.060	0.013	9.93	4.79	0.000	-32	6	4
					0.271	0.013	7.58	4.28	0.000	-40	-4	6
		0.001	450	0.000	0.100	0.013	9.10	4.63	0.000	-32	-38	-12
					0.398	0.013	6.97	4.12	0.000	-36	-28	-16
		0.000	644	0.000	0.105	0.013	9.03	4.61	0.000	42	-40	-6
		0.032	195	0.008	0.244	0.013	7.74	4.32	0.000	18	-74	50
		0.000	521	0.000	0.295	0.013	7.45	4.24	0.000	16	-50	66
					0.830	0.017	5.49	3.65	0.000	0	-32	70
		0.079	141	0.020	0.320	0.013	7.32	4.21	0.000	-42	12	52
		0.051	167	0.013	0.365	0.013	7.11	4.16	0.000	-16	-74	44
		0.075	144	0.019	0.446	0.013	6.78	4.06	0.000	14	40	20
		0.071	147	0.018	0.754	0.015	5.76	3.74	0.000	-28	-10	72

Figure 2.2.5: SPM statistical print out, including a Maximum Intensity Projection (MIP) of the t statistic (which only shows t above a threshold, corresponding to an uncorrected voxel $P < a$ threshold - in this case 0.0005), the design matrix and the table listing the p-values adjusted for the search volumes at cluster-level and voxel-level.

was undertaken utilising SPM2 to yield statistical parametric maps. SPM operates with no a priori assumptions to screen for regions of statistical difference. Each map is a three-dimensional projection showing voxels where there is a statistical difference between the PTSD and control populations. The value of each voxel in the map is the statistical parameter t , which is related to the probability value p for that voxel (uncorrected for multiple comparisons). p values are also computed for clusters of significant voxels. p values corrected for multiple comparisons are also generated and these are used in the assessment of significance. Fig 2.2.5 is an example of the format in which the information is provided by SPM for each statistic run. It includes the Maximum Intensity Projection (MIP) of the t statistic, the design matrix and the table listing the p -values.

2.2.3.4 SnPM Statistics (non-parametric)

SPM uses the conventional parametric statistics approach, which makes several assumptions and approximations. It assumes the data, at each voxel are normally distributed and, across voxels, are derived from continuous random fields with a stationary covariance (normality, independence and the basics of Gaussian random field theory). For a statistical comparison of two groups, when there are only small data sets available for analysis (less than 10 for example), the accuracy of the computed means that are used to compute the T statistic decreases, i.e. the standard error of the mean increases.

A non-parametric ‘toolbox’ for SPM exists (Holmes et al. 1996, Nichols & Holmes 2003) for when the assumptions of the parametric approach become weak. In this situation, the non-parametric approach becomes more powerful, and the validity is enhanced due to the minimal number of assumptions necessary, using only ‘exchangeability’. In certain circumstances, such as low subject numbers, the permutation method can outperform the parametric approach.

The non-parametric approach assumes that if the null hypothesis were correct and there were no difference between two experimental groups, then the labels of group membership

attributed to each condition could be swapped in a random fashion and the analysis would still give the same result. This technique generates a permutation distribution list and derives the appropriate significance as an F statistic for every possible variation of relabelling of the data. If the 'correct' permutation is not in the top 5%, then the null hypothesis is correct.

2.3 RESULTS

2.3.1 Demographic Characteristics

168 interested people contacted the researcher (EAG) by telephone and were screened (see Section 2.2.1 Recruitment). Although the recruitment articles were only intended for a South Australian audience, they were broadcast nationally and an enquiry was received from interstate. Although interested in the study, this individual was excluded due to travel issues. Eleven subjects met the PTSD inclusion criteria and were enrolled in this study. Three were females (27.3%) and seven were males (72.7%), with an age range of 22-57 years (mean age 42 years, SD 12.6). PTSD precipitating life-threatening events included weapon related incidents (6/11, 54.5%), motor vehicle accidents (5/11, 45.5%) and physical/emotional abuse related incidents eg. rape, assault (2/11, 18.2%). PTSD subjects often experienced more than one life-threatening event, thus accounting for the overlapping percentages.

The normal controls were relatives of PTSD subjects, interested parties who telephoned during the recruitment phase or were recruited from within TQEH. Due to the low number recruited, the IDEX SPECT images obtained using the same gamma camera from two other normal control subjects were used. These subjects were older as they were originally recruited for a parallel AD study. Refer to the lower panel of Table 2.3.1 for the demographic characteristics available for the normal controls.

The Therapeutic Goods Administration (TGA) gave the Clinical Trials Notification (CTN) for the IDEX trial approval to commence in July 2002 (Trial No. 2002/331, Protocol No. 156/2001). However, due to several unforeseen circumstances, the IDEX was only

available for subject administration for a six-week period between the 22nd October and 28th November 2002. IDEX has not been available for trial use since this time. See Appendix D, (Issues Influencing IDEX Supply).

2.3.2 Psychological Assessment Results

The results obtained from the questionnaires are shown in Table 2.3.1.

The score/result obtained from the CAPS for Subject 1001 demonstrated that, over the period of three months between the initial screening and the study appointment, this subject's PTSD had improved to the point that they no longer met the entrance criteria (CAPS of 50 or over), and as such was removed from further analysis. The other subjects CAPS scores showed that the majority of the subjects experienced quite moderate to severe PTSD symptoms as the mean total score was 91.5 (SD 19.3) with a range from 57-115.

The PCL results confirmed the PTSD diagnosis with a mean total score of 67 (SD 4.7) but the range of scores was less extreme (varied) being 58-72. However as this psychometric tool is more general than the CAPS, this is not unexpected as the depth of questioning cannot elicit as much detail to vary the answers.

The IES total score mean was 64 (SD 7.2) ranging from 50-71. With a highest possible score of 88, this demonstrated that the events that have led to the development of PTSD in this subject population have had a long lasting influence over their day-to-day life and activities.

The CIDI was performed on all PTSD subjects and shown that depression and anxiety were the two most common co-morbid disorders, along with re-confirming the diagnosis of PTSD.

IQ scores were determined directly from converting the NART totals. The mean IQ for the PTSD subject set was 100 (SD 8.2) with a range of scores from 96-121. Using the Wechsler IQ scale (Wechsler 1997), all PTSD subjects were of 'Average' intelligence apart from subjects 1009 and 1011, who achieved a 'Superior' intelligence score (Average = 90-

Table 2.3.1: The IDEX psychological scale results acquired for all subjects. ND indicates that the assessment was not completed. * identifies all subjects who were not included in the final SPM analysis. † denotes subjects who were recruited for another study.

PTSD Subjects			Psychometrics					
ID	Gender	Age	CAPS	PCL	IES	CIDI	IQ	ADAS-Cog
1001*	M	31	34	35	17	Y	98	10.1
1002	F	22	80	61	57	Y	100	9
1003	M	52	76	67	51	Y	98	10.6
1004	M	31	94	72	63	Y	97	11
1005	M	31	90	58	50	Y	98	6
1006	M	39	115	72	66	Y	98	4.5
1007	M	53	81	70	71	Y	96	9
1008*	M	56	57	67	66	Y	100	10
1009*	M	57	93	62	64	Y	113	9.5
1010	F	36	115	67	64	Y	108	6.8
1011	F	54	115	65	69	Y	121	8

Controls			Psychometrics					
ID	Gender	Age	GHQ	PCL	IES	NART	IQ	ADAS-Cog
0001	M	29	ND	18	0	17	110	3
0002*	M	56	2	ND	ND	26	98	18
0003	F	55	0	ND	ND	18	113	9.3
†	M	72	ND	ND	ND	ND	ND	ND
†	F	78	ND	ND	ND	ND	ND	ND

110, Superior = 110-120, Very Superior = 120-140). The mean IQ of the control subjects who took the NART was higher (mean = 110, SD 7.9), with a range of 98 to 113, but as there were so few control subjects it was impossible to match them to PTSD subjects.

When regarding the ADAS-Cog, the control subject 0002 obtained a score of 18, which deems the cognitive functioning of this individual as ‘moderately impaired’ or exhibiting ‘mild to moderate dementia’. As such this subject was also excluded from the SPM analysis. When combined, the mean ADAS-Cog total score was 9 (SD 2.1) with a range of 3-10.6. Apart from PTSD subject 1003, who’s total score was the highest and placed him in the “mild cognitive impairment” category, all other subjects were shown to have normal cognitive ability.

In the control group, the GHQ totals showed that subjects were healthy with no alterations in normal functioning.

2.3.3 Procedural Results

The average amount of IDEX delivered was 194.2 MBq IDEX (ranging from 170 to 211.9 MBq).

After acquisition, the SPECT data was transferred to a back-up system for storage. Due to technical complications relating to the back-up procedure on one study day, the scan information for 2 subjects (1008 & 1009) could not be retrieved, leaving the data obtained from eight PTSD subjects and two control subjects available for analysis.

The uptake phase SPECT data was not analysed.

The MRIs were also not able to be utilised any further. The images obtained from the subjects recruited for this trial were some of the first acquired on TQEH’s newly purchased MRI machine, and as such, the Radiology protocols were only in the preliminary formation stage. When the time came to retrieve the information, the MRI images were unable to be located on the Radiology database, and as such there was no longer any record of parameters used in the acquisition. Finally, when the images were located on the Central Data Networks’ company database and were decompressed by the company, they could not be opened using

current software as the files had been corrupted during the archiving process.

Table 2.3.2: The summary of the statistical results for clusters detected by performing the SPM analysis using alternate regions for scaling and non-sphericity correction (nsc). Corrected cluster P and cluster volume (k_E) in voxels for uncorrected voxel (uvP) < 0.001 (uvP for * < 0.0005).

SPM Analysis		L parahippocampus -32 -38 -12		L insula -32 6 4		R parahippocampus 42 -40 -6		R precuneus 16 -50 66	
Scaled to	nsc	p_{corr}	k_E	p_{corr}	k_E	p_{corr}	k_E	p_{corr}	k_E
whole brain	N	0.015	242	0.1	142				
whole brain	Y	0.019	234	0.118	136				
basal ganglia	N	0.07	241	0.007	459	0.022	342	0.023	340
basal ganglia	Y	0.002	170	0.0003	236	0.00015	266	0.004	138
basal ganglia*	Y	0.001	450	0.0005	501	0.00009	644	0.0004	521

2.3.4 SPM Statistical Results

The voxel-by-voxel statistical analysis of the PTSD subject group versus the control group were performed in two directions: (1) to determine whether there were statistically significant voxels or clusters where the PTSD subject group exhibited a lower level of ACh receptor activity, and (2) to establish whether there were brain regions in the collective PTSD group that demonstrated an increased level of ACh receptor activity when compared to the control group. Initially the analysis was performed with proportional scaling to the whole brain mean, but this showed weak results (Table 2.3.2) so analysis was performed again, pre-scaling to the Basal Ganglia (see Fig 2.2.4 for the template used to scale to the Basal Ganglia) and correcting for non-sphericity.

Table 2.3.3: The results for SPM analysis for differences in ACh activity: The left and right parahippocampus, left insula and right precuneus show a reduction in ACh activity, while the caudate head shows an increase in ACh receptor activity. Percentages indicate a difference in ACh receptor activity of the PTSD subject group from the control group. The brain regions were identified using the SPM reference anatomical MRI. Analysis was performed with proportional scaling to the basal ganglia mean with correction for non-sphericity.

Location		Cluster Level		% difference
label	x,y,z (mm)	p_{corr}	k_E	
L parahippocampus	-32 -38 -12	0.001	450	-27
L insula	-32 6 4	0.0005	501	-14
R parahippocampus	42 -40 -6	0.00009	644	-15
R precuneus	16 -50 66	0.0004	521	-18
Caudate head	14 0 12	0.041	278	19

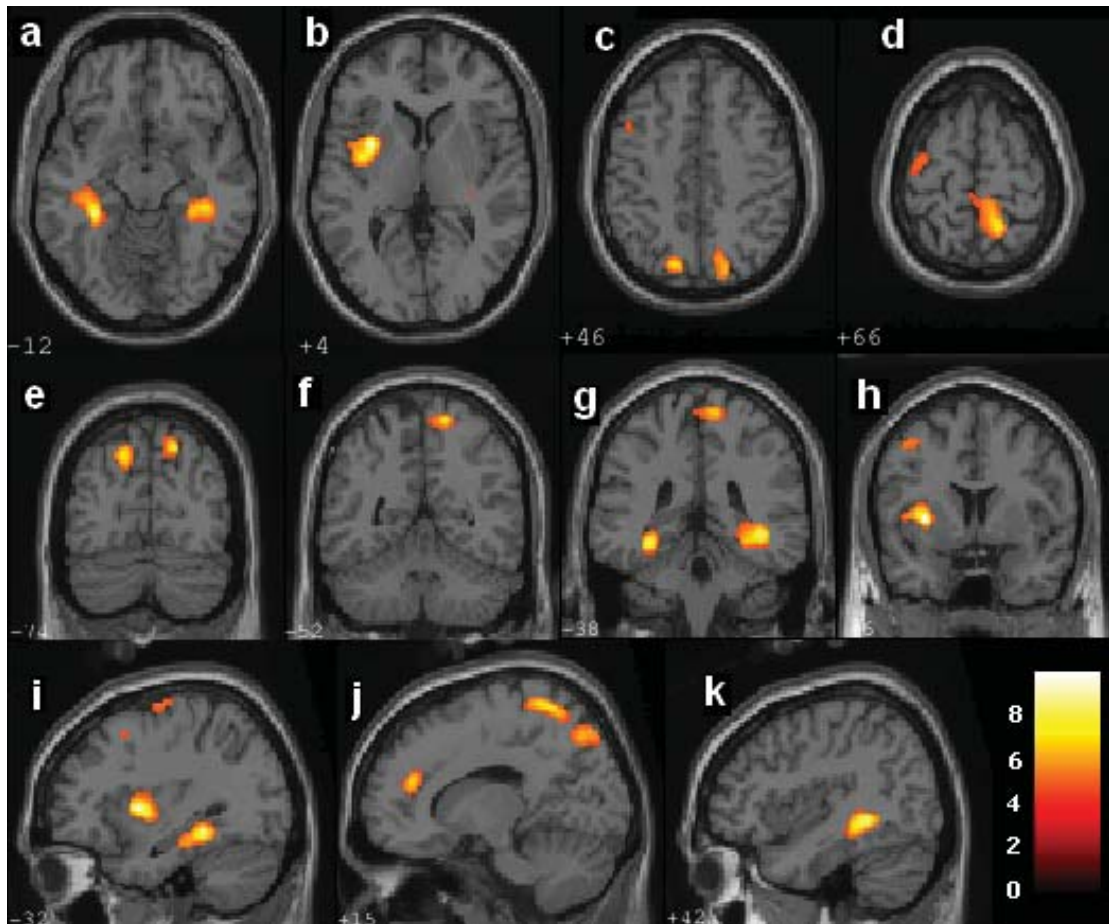


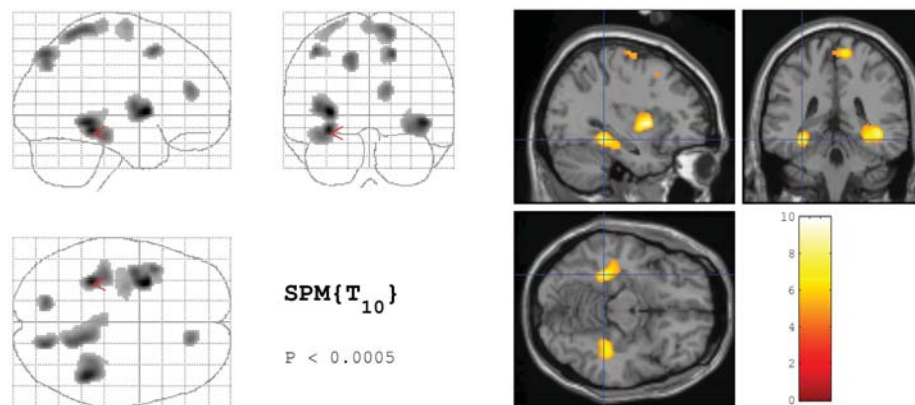
Figure 2.3.1: Slices of MRI colour overlays where significant deficits in IDEX uptake were detected; a,g, and i show deficits in the left parahippocampus; b, h, and i in the left insula; a and k in the right parahippocampus; d, e, f and j in the right precuneus. The numbers at the lower left of each image indicate the distance in mm of the section from the origin (anterior commissure). The colour bar at the lower right indicates the T statistic associated with the colours. The background MR image is SPM’s “single-subject” reference image.

volume summary (labels and percentages per cluster)

<i>x, y, z</i> mm	label	%
-32 6 4	Putamen_R	42.51
	Insula_R	38.52
	OUTSIDE	12.18
-32 -38 -12	Rolandic_Oper_R	6.79
	Fusiform_R	33.78
	ParaHippocampal_R	33.11
	Hippocampus_R	19.33
42 -40 -6	OUTSIDE	12.67
	OUTSIDE	62.58
	Hippocampus_L	15.06
	ParaHippocampal_L	11.65
	Temporal_Inf_L	5.59
	Fusiform_L	2.64
	Lingual_L	1.86
	Parietal_Sup_L	77.95
18 -74 50	Precuneus_L	15.90
	Occipital_Sup_L	6.15
	Precuneus_L	52.78
16 -50 66	Paracentral_Lobule_L	20.35
	Paracentral_Lobule_R	13.05
	Parietal_Sup_L	8.64
	OUTSIDE	3.45
	Postcentral_L	1.73
	Frontal_Mid_R	86.52
	Precentral_R	13.48
-16 -74 44	Precuneus_R	58.68
	Cuneus_R	37.72
	Parietal_Sup_R	2.40
	Occipital_Sup_R	1.20
	Frontal_Sup_Medial_L	36.11
14 40 20	OUTSIDE	35.42
	Cingulum_Ant_L	27.78
	Precentral_R	53.74
-28 -10 72	Frontal_Sup_R	44.90
	OUTSIDE	1.36

Figure 2.3.2: Cluster Volume Summary: including the regions involved in the cluster, the percentage of each region's involvement and the location of the most significant voxel within each cluster.

The statistical parametric map for the *deficits* in IDEX uptake in the PTSD group relative to the control group is shown in Fig 2.3.3 for voxels with uncorrected p values < 0.0005 and Fig 2.2.5 shows the tabulated statistics. Fig 2.3.3 shows clusters superimposed on a reference MRI at the slice containing the most significant voxel in the cluster in SPM2 anatomical space. Table 2.3.3 summarises the significant results. Fig. 2.3.1 shows the locations of all significant clusters (labeled a to k). These clusters centre around the right hippocampus and parahippocampal gyrus, left putamen and insula, left fusiform and parahippocampal gyrus, and left and right precuneus.

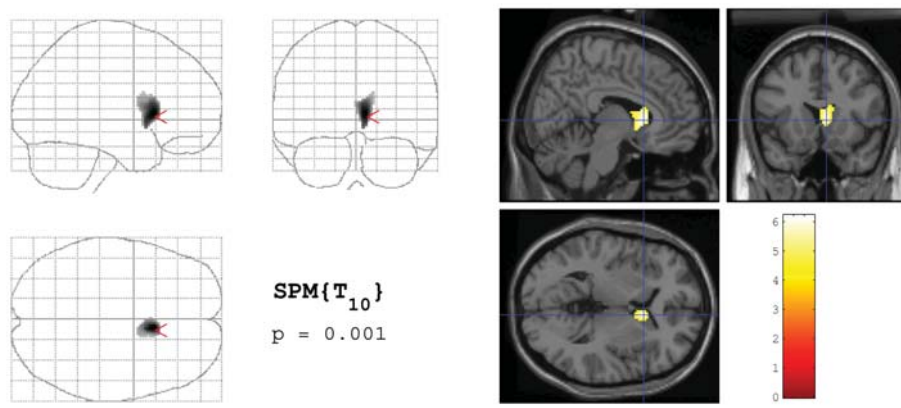


(a) SPM Maximum Intensity Projection (b) SPM results superimposed on an MRI

Figure 2.3.3: Statistical Parametric Map of reduced ACh receptor activity in the PTSD group. a. SPM Map of p-value, b. SPM results superimposed on an MRI

Most individual voxels were statistically insignificant, however, when corrected for multiple comparisons, several clusters retained a $p < 0.05$. The $[x,y,z]$ coordinates for the most significant voxel (in the right parahippocampus) are $[42, -40, -6]$ where the coordinates are in millimeters in Montreal Neurological Institute (MNI) space (SPM2's standard anatomical space), which approximates Talairach space (Talairach & Tournoux 1988). Fig 2.3.2 describes the significant clusters by stating the regions involved in the cluster, the percentage of that region's involvement and the location of the most significant voxel in that cluster.

The statistical parametric map for *increased* levels of IDEX uptake in the PTSD group relative to the control group is shown in Fig 2.3.4 for voxels with uncorrected p values < 0.001 . When superimposed on the SPM2 reference MRI, the main cluster appears in the Caudate head. The corrected cluster $p = 0.041$. After corrections for multiple comparisons, no individual voxels were statistically significant.



(a) SPM Maximum Intensity Projection (b) SPM results superimposed on an MRI

Figure 2.3.4: Statistical Parametric Map of increased ACh receptor activity in the PTSD group. a. SPM Map of p-value, b. SPM results superimposed on an MRI

An SPM regression analysis of the PTSD group versus PTSD severity (individual CAPS scores) did not show any significance.

An SPM regression analysis against age in the combined PTSD group and normal controls did not elicit any significance. Although the age distribution for the controls was unsatisfactory, the absence of any age dependence led us to omit it from the analysis. Over the whole cohort, comparison of the PTSD group and the normal controls with and without age adjustment yielded only slightly less significant results, providing further support for omitting it in the final analysis.

The non-parametric analysis did not yield any statistical significance between the PTSD group and the control group.

2.4 DISCUSSION

The current study shows a **decrease** in ACh receptor binding in the right precuneus, left insula and bilateral parahippocampi. Whether this is due to a reduction of ACh receptors or a decrease in the amount of ACh available cannot be determined with the current techniques, but in any case such deficits will have important consequences. Similarly, the **increase** in ACh receptor binding demonstrated in the caudate head can have important consequences. These are detailed below.

It needs to be noted that the majority of nuclear medicine studies performed in PTSD have measured glucose consumption to determine activity levels of individual brain regions. In the current study, the level of ACh binding to the available receptors was measured, and, as such, only conclusions relating to increases or reductions in ACh in individual areas can be made.

2.4.1 Regions of Interest and Clinical Relevance of Alterations in ACh

2.4.1.1 Precuneus

In the current study, the subjects were specifically instructed to lie on the scan table and relax with their eyes shut. In order to study the neuronal activity that occurs during this resting state (lying quietly with eyes closed), Raichle et al. (2001) used PET (with an oxygen tracer) to measure localised deviations in oxygen extraction fraction (O_2 delivered to O_2 used)(OEF). OEF is intended to represent the physiological basis of signals of changes in neuronal activity based on the assumption that areas of high radioactivity are associated with brain activity. Using quantitative metabolic and circulatory measurements from PET to obtain the OEF regionally throughout the brain, they were able report a high metabolic activity in the precuneus during the 'default' mode of brain function.

In another study using PET with fluorodeoxyglucose (FDG), Gusnard & Raichle (2001) demonstrated that when people are in a normal resting state of consciousness, an increased

level of precuneus activation is evident with up to a 35% increase in glucose consumption more than any other area of the cerebral cortex. When the same group performed a visual task, the activation of the precuneus was reduced or attenuated. A relaxed state, however, is not merely the absence of complex and demanding cognitive tasks requiring a large proportion of the individual's central executive processing resources, but can be characterised by the generation of spontaneous thought processes or an increase in the flow of inner mental events (Christoff et al. 2004).

There is a decrease in precuneate activity in the PTSD subjects in the current study while they are theoretically relaxed with their eyes closed. Spontaneous thought processes in a person with PTSD are often negative and distressful, and are often actively avoided. The process of preventing spontaneous thought may be utilising other cognitive mechanisms and therefore using more executive processing resources and this could indicate that the minds of people with PTSD are never actually relaxed, or that they cannot achieve a fully relaxed state. Buckley (2000) states that "fear networks are thought to arise in partially primed states and that this may be responsible for the re-experiencing symptoms of PTSD if a person's level of activation is pushed above the threshold of conscious awareness." The partially primed states of arousal that occur in people with PTSD could account for the precuneus being less activated. This, in turn, would add weight to the argument that PTSD patients are unable to enter a state of true relaxation.

The reduction in ACh receptor activity in the precuneus could account for the symptomatology relating to attentional problems and the filtering of irrelevant information (Sarter et al. 2005). Furthermore, a reduction of ACh in the basal forebrain projections to the cortex may account for the the attention deficits in people with PTSD caused by an inability to amplify the processing of attention-demanding signals. As people with PTSD are hyper-alert, most information would be regarded as attention-demanding, and would therefore overload the system (Sarter et al. 2005).

Finally, the precuneus has also been implicated in the active analysis of spatial information (Cavanna & Trimble 2006). A PET blood flow study has shown that hyperperfusion of the precuneus can turn to hypoperfusion when attention is directed from a peripheral point

to the centre of a visual display (Uecker et al. 1997). Again, the hyper-alert state experienced by people with PTSD could account for the lack of peripherally directed attention, leading to a hypoperfusion which in turn would influence the concentration of ACh available for binding to receptors in the precuneus.

2.4.1.2 Insula

Previous studies involving people with PTSD that have included the insula in their analysis have shown mixed results. Some of the symptoms studied include negative anticipation (fMRI) (Simmons et al. 2008), non-conscious fear (fMRI) (Felmingham et al. 2008), re-experiencing severity (fMRI and PET) (Hopper et al. 2007, Osuch et al. 2001), items encoded in emotional contexts (fMRI) (Whalley et al. 2008), and evaluation of threat and stimulus deviance under threat (Event Related Potential Brain Mapping) (Cornwell et al. 2007). The majority of these studies (14 out of 18) show an *increase* in activation when compared to controls, specifically when utilising fMRI techniques.

Only 4 studies showed a decrease in the insula, and the focus of these studies was the reduction of grey-matter density or volume as determined by voxel-based morphometry analysis rather than neurotransmitter activity. These studies demonstrated structural abnormalities of the hippocampus, anterior cingulate cortex and the insular cortex in patients with PTSD (Chen et al. 2006, Corbo et al. 2005). It may, however, follow that if there is a reduction of grey matter volume in the insula then there would be an associated reduction in ACh receptors in the area, which could account for the reduction in ACh receptor binding in the left insula found in the current study.

When a normal study population was required to make a decision and select a response based on limited processing of automatic information (requiring minimal cognitive resources), an increase in PET activity was shown in the insula, when compared to activity produced while engaging in a ‘no-decision’ task (Uecker et al. 1997). Increases occurred only on the right when an object was new and ‘possible’ (could exist in the real world), and only on the left when the decision related to an old object (seen before) that was ‘impossible’ (could not be constructed in the real world). The increased arousal symptoms of PTSD

which include difficulty concentrating (which can lead to indecision), could explain a reduction in an area responsible for being able to decide that an object, event, or occurrence is improbable.

2.4.1.3 Parahippocampus

Under normal conditions, the parahippocampus has been linked to functions including the processing of spatial information, navigation, recognition of landmarks, and deducing distances to boundaries, and its level of activation can be modulated by attention (Burgess et al. 2002). A PET study showed greater increases in activity when associated with recognising whether objects could exist in real space, as opposed to whether they were impossible, which activated the hippocampus (Uecker et al. 1997). The parahippocampus also has a role to play in memory-related processing such as influencing which items are preferentially associated with self-referential information in memory, as evidenced by activity in the parahippocampal region relating to memory retrieval of self-referential words in an fMRI study (Touryan et al. 2007).

In people with PTSD, other fMRI studies have found a reduced level of activation in the left parahippocampal gyrus during the retrieval condition of an associative learning paradigm (Werner et al. 2009) and retrieval of trauma-neutral paired associates (Geuze et al. 2008), but an enhanced left parahippocampal response while performing the symptom provocation paradigm (Hou et al. 2007) or when presented with masked traumatic stimuli (Sakamoto et al. 2005). Using PET, symptom severity has also been associated with an increase in rCBF in the parahippocampal gyrus (Shin et al. 2004).

Abnormalities have also been found in the parahippocampal area in ^{99m}Tc -HMPAO SPECT studies of other psychiatric disorders, such as Panic Disorder, with decrease in blood flow (Sachinvala 2000).

In the current receptor SPECT study, a significant decrease in ACh receptor activity was demonstrated. As there was no specific emotional component to be processed using this method, it could be assumed that the current result can be aligned with the decrease in blood flow or activation demonstrated in the parahippocampus during the non-provoking

fMRI studies, which could potentially relate to a reduction of ACh receptor binding in the area.

2.4.1.4 Caudate

In a PET study using recognition memory tasks, making a decision on whether you remember an object or not was associated with blood flow increases in the region of the caudate nucleus (Uecker et al. 1997). In her ^{99m}Tc -HMPAO SPECT study Sachinvala (2000) also found a significant increase in bilateral caudate perfusion in her PTSD population when compared to controls under neutral conditions. However, another SPECT study showed that PTSD syndrome severity correlated significantly negatively with the left and right caudate rCBF (Lucey et al. 1997). The current IDEX SPECT study demonstrated an increase in ACh receptor binding in the caudate, appearing analogous to the perfusion studies. Although the current study found no correlation of ACh activity with severity, it may be reasonable to infer that people who have more severe cases of PTSD, experiencing a consistently greater emotional input in their cognitive processing, could have an associated reduction in caudate activity in response to a greater limbic involvement (similar but opposite to the parahippocampus).

Under normal conditions, the caudate has a dense local innervation provided by ACh interneurons (Zhou et al. 2002). Generally in caudate neurons, acetylcholine (ACh) inhibited orthodromically activated firing and therefore neuronal activity (Akaike 1992). Therefore a increase in ACh receptor activity may actually reduce neuronal activity within the caudate. In accounting for PTSD symptomology, this effect could exacerbate the potential effect on indecision, and therefore contribute to the increased arousal symptoms seen in this condition.

One study of patients with schizophrenia revealed a 33% reduction of muscarinic receptors in the caudate-putamen and that ratings of 'positive' schizophrenia symptoms were significantly correlated with the reduction in muscarinic receptor availability in this area (Raedler et al. 2003). This is important because several of these 'positive' schizophrenia symptoms are analogous to the intrusive, re-experiencing symptoms that are characteristic of PTSD. Cholinergic function has been associated with several aspects of neuropsychiatric

disorders including memory, motivation, and mood, and although they may be mediated by different neurocircuits, there must be some overlap in the functional neuro-anatomy to account for the similarity in some symptom sets.

2.4.1.5 Combination or Network Effects

The basalforebrain cholinergic system includes the neuronal cell bodies in the medial septum and the diagonal band of Broca (dbB) which innervate the hippocampal formation and occipital cortex, and nerve cells in the nucleus basalis of Meynert (nbm) which project primarily to the frontal, pre-frontal, and parietal cortex (Coyle et al. 1983). Specifically the Ch4 subgroups of cholinergic projections described by Mesulam et al. (1983) are responsible for innervating the parahippocampus, insula, and precuneus.

In their study of spontaneous thought processes at rest, Christoff et al. (2004) described the random memory processes as “freely wandering past recollections, future plans, and other personal thoughts and experiences that appear to be loosely linked”, and revealed that under normal circumstances, the list of regions activated at rest included the bilateral parahippocampi, left insula and right inferior parietal cortex (which would include the precuneus). As ACh receptor binding in all of these regions was reduced in the current population, it is possible that this can account for certain diagnostic symptoms experienced by people with PTSD, such as actively avoiding past recollections and the absence of future plans to think about (Criteria C: Persistent avoidance), and indecision, difficulty concentrating, and persistent partial priming effects leading to hypervigilance (Criteria D: Increased arousal) (refer to Section 2.2.1, Recruitment for the diagnostic criteria for PTSD).

Such alterations in cholinergic function in people with PTSD as demonstrated in this study, whether due to receptor density, ACh concentration or AChE activity, warrant further assessment of cholinergic neuronal integrity.

2.4.2 Study Limitations

The patient sample size was substantially smaller than the 20 patients and 20 normal controls originally planned for this study. Recruitment of appropriate patients was particularly difficult due to the exclusion criteria of potential subjects taking prescribed medications that may influence the cholinergic system, and also other substance abuse, specifically alcohol, reducing the 168 interested people to only 11 taking part. This was the first study investigating ACh receptors with IDEX and it was always intended to be a pilot, exploratory study. The small sample size restricts statistical power, and, as such, a positive finding could be a Type 1 (Alpha) error.

Furthermore, too few control subjects were scanned. This was primarily due to an unforeseen lack in availability of IDEX. With ANSTO experiencing several administrative and precursor acquisition problems, the trial commencement date was months behind the expected schedule. IDEX was only available for a 6-week period before production was terminated. During the productive 6-week period, the priority was to process as many patients as possible, leaving the controls to a later date. It was anticipated that production of IDEX would recommence. However this did not occur and is the main reason that so few controls were eventually recruited.

Due to the lack of controls obtained, it was necessary to increase the power of the study by including normal controls that had been recruited for another study. It has been shown previously that, even after optimising the SPECT processing parameters, regional differences between normal databases are not able to be eliminated if images are acquired on different cameras (Barnden et al. 2004). (The optimisation processing incorporated into this paper was necessarily performed immediately prior to the conduct of this PTSD IDEX SPECT research, therefore a complete copy of this article is provided as Appendix E.) The images for these two extra normal control subjects (from the AD trial) were acquired using the same camera used for all images in the IDEX SPECT study, therefore no additional variance was introduced.

Although MRIs were acquired on all subjects, they were not able to be utilised any further as there was no record of parameters used, the images were unable to be located on the Radiology database, and the CDN were unable to return the original MRI data obtained in a form appropriate for analysis. All of these issues arose because the Radiology protocols were only in the preliminary formation stage; a situation which was out of the researcher's control.

2.5 CONCLUSION

Although the relationship between stress and ACh has been an area of research for some time (Kaufer et al. 1998), the role of the cholinergic pathways specifically in PTSD has been relatively unexamined to date. The symptomatology of PTSD, however, appears to be analogous to certain dementias (DLB and AD) and psychiatric conditions (schizophrenia) in which ACh has been implicated. Fluctuating cognitive impairment and attention deficits in conjunction with hallucinations, delusions, depressed mood, apathy, anxiety and sleep disturbances that are experienced with these conditions have been correlated with a reduction in cholinergic activity and the severity of these symptoms has been shown to directly relate to the extent of the cortical cholinergic deficit (Perry & Perry 1995). Patients with DLB taking a cholinesterase inhibitor have shown improvement by suffering significantly less apathy and anxiety, and experiencing fewer delusions and hallucinations. Other neuropsychological tests demonstrated that these patients were also significantly faster and better on tasks with a substantial attentional component (McKeith et al. 2000).

In some disorders that exhibit similar neuropsychiatric symptoms, changes in the regional distribution of muscarinic ACh receptors (m-AChR) have been demonstrated. As IDEX uptake corresponds to the known distribution of m-AChR, it was postulated that IDEX could be used to investigate whether the cholinergic activity in PTSD has been compromised.

In the PTSD group, deficits in IDEX binding were found in the right precuneus, left insula, and bilateral parahippocampi and an increase was demonstrated in the caudate head. The results of this study suggest that alterations in ACh binding in PTSD are evident and may begin to explain a part of the altered cognitive symptomatology apparent in this condition.

Further research is required to determine whether these alterations in ACh receptor binding can be influenced by manipulating the cholinergic pathway, and whether this would reduce the overall severity of PTSD symptomatology.

Chapter 3

A Clinical Trial Of An Acetylcholinesterase Inhibitor (Donepezil) In The Treatment Of Posttraumatic Stress Disorder

3.1 INTRODUCTION

Posttraumatic Stress Disorder (PTSD) is characterised by a heterogeneous symptom profile that is differentiated into three distinct symptom clusters: reexperiencing (cluster B), avoidance/numbing (cluster C) and hyperarousal (cluster D) (American Psychiatric Association 1994). The main goals of treatment are to reduce the symptom severity, decrease functional deficits and to improve quality of life.

The preferred treatment regimen consists of a combination of psychotherapy and pharmacological interventions (Hamner et al. 2004). The first line of pharmacotherapeutic treatment for PTSD is still considered to be the Selective Serotonin Reuptake Inhibitors (SSRIs), however, many patients do not satisfactorily respond and are left with significant residual symptoms, or do not tolerate the medications well and experience considerable side effects

(Berger et al. 2009).

A number of other medications have been trialed in PTSD, including serotonin - norepinephrine reuptake inhibitors (SNRIs), antidepressants (tricyclics and monoamine oxidase inhibitors), antipsychotics (acting on the serotonergic and dopaminergic systems), anticonvulsants (enhancing GABAergic and serotonergic transmission or inhibiting glutamatergic transmission), adrenergic-inhibiting agents (reducing noradrenergic function) and opioid antagonists. Research continues into the potential benefits of these medication groups, however, they are often found to improve only one or two of the clusters of symptoms, such as hyperarousal, producing limited improvements in sleep difficulties, hypervigilance or irritability (Berger et al. 2009).

Medications that enhance cholinergic function represent a novel and untried treatment option for PTSD and yet the cholinergic system has been implicated in eliciting several of the symptoms characteristic of this disorder (see Section 1.2.7.3 Basis for Considering a Potential Role for ACh Neurotransmission). To reiterate, PTSD has been shown to involve a critical breakdown in working memory function that underlies the difficulties with concentration and memory (Clark et al. 2000). The cholinergic neurotransmitter system plays an important role in memory and other cognitive functions. Alterations in the cholinergic system will influence performance on cognitive tasks involving memory function, and therefore also affect the symptomatology observed in the conditions for which impaired cognitive performance is a defining clinical characteristic.

3.1.1 Donepezil

Donepezil hydrochloride (Aricept®) is a reversible inhibitor of the enzyme acetylcholinesterase, known chemically as (\pm) -2,3-dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1*H*-inden-1-one hydrochloride, but is commonly referred to as E2020. It has an empirical formula of $C_{24}H_{29}NO_3HCl$, a molecular weight of 415.96, and it forms a white crystalline powder (Aricept®/Donepezil Hydrochloride Tablets) 2006).

From the results of animal studies, it has been shown that oral donepezil exhibits

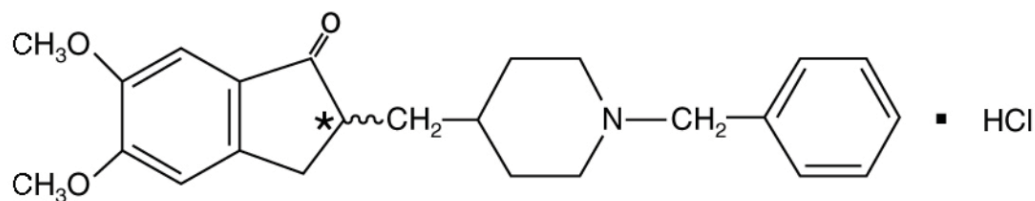


Figure 3.1.1: The structure of donepezil hydrochloride

tissue selectivity, because the drug inhibits AChE in the brain at doses that exert little effect on intestinal or cardiac AChE (Shintani & Uchida 1997, Grutzendler & Morris 2001). Donepezil also appears to inhibit AChE in red blood cells to the same degree as in the cortex. At relevant clinical doses, donepezil has weak inhibitory effects on butyrylcholinesterase (Grutzendler & Morris 2001).

Donepezil has a long terminal disposition half-life of approx 70 hours (60 hours in young adults and up to 104 hours in elderly patients) which supports once-daily administration. There is a fourfold to sevenfold accumulation that occurs with repetitive dosing and a steady state is achieved within approximately 15 days. Bioavailability approaches 100% and in healthy males the peak plasma concentration is reached within 4 hours (dose dependant). At this time the inhibition of AChE in red blood cells correlates with the plasma concentration. Donepezil is then excreted unchanged in the urine (< 20%), oxidised by cytochrome P-450 isoenzymes CYP3A4 and CYP2D6, or conjugated with glucuronic acid (Shintani & Uchida 1997). One metabolite, the 6-O-desmethyl derivative, is as potent as donepezil in inhibiting AChE and may be present at about 20% of the donepezil concentration.

Drug interactions appear to be minimal with no effects found when donepezil is simultaneously administered with theophylline, cimetidine, warfarin, or digoxin. Ketoconazole and quinidine inhibit the metabolism of donepezil in vitro, but does not actually decrease the clearance of donepezil (Shintani & Uchida 1997). However, there is the possibility of significant interactions with other drugs that are metabolised by the hepatic cytochrome system would need to be used conservatively in patients with many concomitant illnesses

(Inglis 2002).

Side effects of Donepezil use can include central nervous system events, extrapyramidal symptoms, sleep disturbances and cardiorespiratory events, associated with cholinergic activity in the cortex, caudate nucleus, brainstem and medulla, respectively, and muscle cramps and weakness, cardiorespiratory events and urinary incontinence, associated with peripheral cholinergic activity (Inglis 2002). The most common adverse reactions, based on donepezil's cholinomimetic effects, include nausea or vomiting (10% on donepezil (d), 5% on placebo (p)), diarrhoea (10% d, 3% p.), constipation (8% d, 3% p.), gastric upset (8% d, 3% p.), anorexia, sleep disturbances, muscle cramps, and fatigue (Shintani & Uchida 1997, Grutzendler & Morris 2001). Central effects can be elicited at high doses, during the transition period of dose increases and in patients with a low body weight. These effects can include insomnia, nightmares and agitation or a panic-like state (Grutzendler & Morris 2001).

Donepezil was given FDA approval for use in AD due to its clinical efficacy record. Significant improvements were found in the scores obtained by patients with AD on the Alzheimers Disease Assessment Scale - Cognitive Subscale (ADAS-Cog) and the Clinical Interview-Based Impression of Change (Shintani & Uchida 1997).

Selective cholinergic receptor agonists and antagonists have been shown to modulate inspection time, a measure of speed of early information processing. The decrease or improvement of inspection time following donepezil administration is consistent with the role of the cholinergic system in modulating speed of information processing, and adds evidence that inspection time may serve as a physiological index of the integrity of the cholinergic system (Hutchison et al. 2001).

Donepezil has been used in the treatment of other conditions that suffer difficulties with cognitive function. Preliminary findings suggest that it may improve some aspects of memory and behaviour in people with chronic traumatic brain injury (Masanic et al. 2001). For example, a schizoaffective disorder patient was noted to show significant improvements in several cognitive measures (Risch et al. 2001). Delirium, REM sleep behaviour disorder, hallucinations and emotional/behavioural symptoms in AD have all been influenced due to

the administration of donepezil (Ringman & Simmons 2000, Weiner et al. 2000).

The psychotropic properties of donepezil in people with AD are clear. In a study performed by Mega et al. (1999), agitation, anxiety and irritability were all reduced, along with the occurrence of delusions and episodes of disinhibited behaviour. By improving mood and behaviour, symptoms that most disturbed people with AD, as well as their carers, were significantly improved. In other studies, anecdotal accounts of happier, calmer, more content people who are “more comfortable in their own skin” have been very encouraging. It was these accounts that led to the generation of the hypothesis that this medication could be of use in other conditions where similar moods and behaviours comprise a large portion of the overall symptomatology.

In order to observe a case study, we treated a patient with PTSD in The Queen Elizabeth Hospital Psychiatry Department with donepezil for a period of one month and preliminary observations were extremely positive. Although the Mini-Mental State Examination (MMSE) values obtained did not substantially alter over the four-week period, a 24% improvement (12-point decrease) from baseline value on the Military version of the PTSD Check List (PCL-M) was achieved. This finding provided preliminary evidence that donepezil may be efficacious in the treatment of PTSD. Therefore, the primary objective of this project was to examine the efficacy of donepezil as a treatment for PTSD.

3.1.2 Aim

To examine the effect of the administration of an AChEI on the symptomatology of PTSD. The clinical trial involved the prescription of an AChEI (donepezil) that is hypothesised to lead to a general decrease in post-traumatic symptoms.

3.2 METHOD

3.2.1 Study Design

This study was a 10-week, open-label trial whereby 5mg of oral donepezil was self-administered once daily, in the morning for the whole 10 week period. See Table 3.2.1 for the schedule of visits and assessments.

Table 3.2.1: The schedule of visits and assessments for the donepezil trial

	Screening	Baseline	Week 2	Week 6	Week 10
Inclusion / Exclusion Criteria	X				
Demographics	X				
Medical History	X				
Informed Consent	X				
Psychiatrist Evaluation		X	X	X	X
Blood Collection		X		X	
Vital Signs		X	X	X	X
CAPS		X			X
CIDI		X			X
HAMD		X			X
PCL	X	X	X	X	X
TOP8		X	X	X	X
IES		X	X	X	X
BDI		X	X	X	X
Compliance			X	X	X
Adverse Events			X	X	X

3.2.2 Recruitment

Subjects were recruited using the same method described in Section 2.2.1 Recruitment.

3.2.2.1 Inclusion Criteria

As for the previous study, all subjects were right-handed and fluent in English, aged between 18 and 65 years, and all satisfied the criteria for a diagnosis of PTSD on the PCL (Civilian or Military version depending on whether the subject had military experience or not) due to different traumatic events. Comorbid disorders were assessed with the CIDI and only allowed if they were secondary disorders to the initiating PTSD.

No control subjects were recruited for this study. The baseline value recorded for each subject served as the control measure.

3.2.2.2 Exclusion Criteria

Subjects were excluded if they were on prescribed medications that would interfere with the cholinesterase inhibitor (see Appendix A (Pharmacy Information: Medications with Cholinergic Properties)), suffered from epilepsy or a serious medical illness, had experienced a head injury with greater than ten minutes post-traumatic amnesia or engaged in substance abuse (by self report). Any subject who developed any exclusion criterion during the trial was also excluded, along with those who refused to participate or were unable to consent.

No concomitant therapy was allowed. The length of any washout period required was determined by the nature of the agent prescribed and under the advice and direction of the affiliated psychiatrist.

3.2.2.3 Funding and Approval

This study was made possible by a grant from Pfizer Pharmaceuticals Pty. Ltd. supporting the supply of the AChEI donepezil, blood collection and associated research consumable costs. The study was insured and indemnified by the University of Adelaide and The Queen Elizabeth Hospital and approved by the Ethics of Human Research Committee at

the North Western Adelaide Health Service (The Queen Elizabeth Hospital). (See Appendix F, (Donepezil Study Ethics Approval Letter) and Appendix G, (Donepezil Study Patient Information and Informed Consent Form)). Written informed consent was obtained from all participants.

3.2.3 Procedure

The subjects were screened over the telephone using the PCL, and prior to the baseline visit each subject underwent a detailed interview to re-establish eligibility for the study, elicit information regarding demographics and medical history and to ensure that an informed consent was obtained.

At baseline, data on the basic physiological parameters (vital signs - blood pressure, pulse, respirations) were recorded and a blood sample was collected. All psychological assessments were completed (conducted by one researcher (EAG)) and each subject was seen by the affiliated psychiatrist for an initial evaluation. The medication required until the ensuing visit was dispensed and the subject was free to leave.

For each intermediate visit (Week 2 and Week 6) the subjects discussed their symptoms over the previous study period, described their experience with the medication including any adverse events experienced and returned any remaining tablets, the number of which were recorded as a measure of compliance. The vital signs were then recorded before the subjects completed the self-administered psychological assessments (PCL, TOP8, IES and BDI) and were monitored by a psychiatrist to determine if any adverse outcome warranted withdrawal from the trial. Finally, the necessary medication was dispensed and the subject was free to leave.

The concluding visit (Week 10) was the same as the intermediate visits, but also included the clinician-administered psychological assessments (CAPS, CIDI, HAMD) without dispensing any further medication.

If the subjects experienced disturbing side-effects inbetween the baseline and Week 2 visits, they were encouraged to contact the researcher and, after discussion with the psychi-

atrist, usually advised to reduce the dose of donepezil from 5mg to 2.5mg until they were better able to tolerate the medication. If side-effects persisted further into the schedule of visits, the subject could chose to withdraw from the study or the psychiatrist would determine whether withdrawal was necessary.

Blood samples were obtained at Baseline and Week 6. Kidney and liver function were monitored to determine whether there were any side effects of the medication and to detect potential problems in early stages of treatment; enzyme levels reflected chemical cellular activity and proteins levels were an index of overall health. Values outside of the normal ranges were evaluated by a psychiatrist.

The blood tests performed included:

Full blood examination (FBE): haemoglobin, red blood cells and indices, white blood cells and differentials.

Biochemical Analysis: electrolytes, creatinine, urea, calcium, phosphate, urate, liver function tests and cholesterol.

Red Blood Cell Cholinesterase.

3.2.3.1 Study Medication

Donepezil is indicated for the treatment for mild to moderately severe dementia of the Alzheimers type (AD). When prescribed for AD, the 5mg tablet of donepezil has achieved regulatory status in 22 countries including Australia, United States and the United Kingdom (See Appendix H (Donepezil Approved Product Information)).

3.2.3.2 Psychological Assessments

The following psychological instruments were administered in the study:

The PTSD Check List (PCL): used as a telephone screen, and on those not excluded, administered at the Baseline visit and Weeks 2, 6 and 10.

Clinician-Administered PTSD Scale for DSM-IV (CAPS): administered at the Baseline visit and Week 10.

Composite International Diagnostic Interview (CIDI): Sections A (Demographics), B (Tobacco), D (Anxiety Disorders), E (Depression), J (Alcohol) and K (OCD and PTSD) were used at the Baseline visit and Week 10.

Hamilton Depression Scale (HAMD): administered at the Baseline visit and Week 10.

Treatment Outcome PTSD Scale (TOP8): conducted at the Baseline visit, Weeks 2, 6 and 10.

The Beck Depression Inventory (BDI-II): self-administered at the Baseline visit and at Weeks 2, 6 and 10.

The Impact of Events Scale - Revised (IES-R): self-administered at the Baseline visit and Weeks 2, 6 and 10.

For a full description of the PCL, CAPS, CIDI and IES-R, refer to Section 2.2.2.2 Psychological Assessments. The scales that have not previously been described (HAMD, TOP8 and BDI) are represented as follows:

Hamilton Depression Scale (HAMD)

This 23 item clinician-administered assessment is used to rate the severity of a subject's major depression, including symptoms such as low mood, insomnia, agitation, anxiety and weight loss. The health professional administering this scale both interviews the subject and observes his/her behaviour to elicit information to assist in selecting the most applicable response. Each of the 23 questions has 3 - 5 possible responses which increase in severity, usually rated as 0 - 2 or 0 - 4. The total score can range from 2 - 75 (Hamilton 1960).

Treatment Outcome PTSD Scale (TOP8)

This is an 8 item assessment drawn from the three symptom clusters for PTSD that is used to assess a subject's response to treatment. The brief, usually clinician-administered scale (extrapolated from the Structured Interview for PTSD (SIP)) shows an improved ability to detect drug versus placebo differences in comparison with the original scale. The subjects are required to consider how much each symptom has troubled them over the past week.

Each of the 8 questions have 5 possible answers rated from 0-4, increasing in severity: e.g. 0 “not at all”, 1 “mild”, 2 “moderate”, 3 “severe” and 4 “extremely severe”. The total score can range from 0-32 (Davidson & Colket 1997).

The Beck Depression Inventory (BDI-II)

This is a 21-item, self-report instrument designed to assess the severity of depression in adolescents and adults. This version is composed of items relating to depression symptoms such as hopelessness and irritability, cognitions such as self-criticalness or feelings of being punished, as well as physical symptoms such as loss of energy and changes in appetite. Each question has a symptom title such as “1. Sadness” and subjects are required to circle the number corresponding to the statement that most applies to the way they have felt over the previous two week period: 0 “I do not feel sad.”, 1 “I feel sad much of the time.”, 2 “I am sad all the time.” or 3 “I am so sad or unhappy that I can’t stand it.” All items have a 0-3 rating scale apart from Item 16 “Changes in Sleeping Pattern” and Item 18 “Changes in Appetite” in which the “more” or “less” extremes for each option are denoted with an a or b. The total score can range from 0 to 63 (Beck et al. 1996).

3.2.3.3 Statistical Analysis

The data collected for this study has been presented using 3 methods:

1. Change in symptom severity was assessed by the decrease in the subjects’ scores from the Baseline calculated as a percentage improvement. Improvement was determined at the Week 2 visit to demonstrate initial treatment effects and upon completing the study at the Week 10 visit. This was performed in a “Per-protocol analysis” which includes only those subjects that completed all of the protocol requirements (9 subjects). The difference in the means was analysed using a paired samples t-test.
2. The change from baseline to endpoint scores during the 10-week treatment period was also analysed using a repeated measures ANOVA, utilising SAS software. In this intention-to-treat analysis, in order to use all available data including that of subjects

where the data was missing, a multilevel model with visit as the only predictor was used (Bryk & Raudenbush 1992). The main effect of visit was examined and for variables with more than two measurement occasions, planned comparisons were conducted amongst all time points.

3. Anecdotal accounts of subjects' experiences of their symptomatology while undergoing treatment on donepezil have been collated and are presented grouped in the 3 definitive PTSD symptom clusters (Criteria B, C and D).

3.3 RESULTS

3.3.1 Demographic Characteristics

A total of 16 subjects met the PTSD inclusion criteria and were enrolled in this study. Six of these subjects had previously taken part in the IDEX trial and all took part in an associated electro-physiological study. Nine were females (56.25%) and seven were males (43.75%), with an age range of 22-56 years (mean age 42 years, SD 11.0). PTSD precipitating life-threatening events included weapon related incidents (8/16, 50%), motor vehicle accidents (7/16, 43.5%) and physical/emotional abuse related incidents eg. rape, torture (4/16, 25%). PTSD subjects often experienced more than one precipitating life-threatening event, thus accounting for the overlapping percentages. The majority of subjects in this trial were not treatment naïve and had previously had a range of unsuccessful pharmacological and psychotherapeutic interventions prompting their willingness to consent for this trial.

Of the 16 subjects that took part, nine completed the 10-week trial and seven subjects prematurely withdrew (2 at the Week 2 visit and 5 at the Week 6 visit), due to side effects or a worsening of PTSD symptoms (refer to Table 3.3.1 for a complete summary of all side effects experienced). Within 2-3 days on donepezil, several subjects experienced side effects such as being "jittery" or having "more pent up energy", nausea and diarrhoea and hay fever-like symptoms with a slight headache. These effects usually subsided over the next 4-5 day period and after this time more benefits were noted.

Table 3.3.1: A summary of all the side effects experienced by subjects. Body system and percentage of subjects with any adverse event (n=16).

Body System and Side Effect	%	Body System and Side Effect	%
Subjects who experienced Side Effects	94	Psychological Symptoms	
		Depression/tearfulness	37.5
		Increase in intrusive thoughts	6.3
Gastrointestinal Symptoms		Nightmares	12.5
Abdominal pain/bloating	12.5	Panic attacks	6.3
Constipation	12.5	CNS / Nervous Symptoms	
Diarrhoea	37.5	Agitation/jittery	18.8
Nausea/Vomiting	25	Hostility/irritability	18.8
Dyspepsia/reflux	6.3	Fatigue/lethargy	12.5
Sore throat	6.3	Increased sleep disruptions	37.5
Dry mouth	6.3	Somnolence/drowsiness	6.3
Increased saliva	6.3	Sweating	12.5
Decreased Appetite	6.3	Headache	12.5
Weight gain	12.5	Dizziness	6.3
Genitourinary Symptoms		Blurred Vision	6.3
Urinary Incontinence	6.3	Hayfever/nasal congestion	12.5

Only one of the nine subjects who completed the 10-week protocol reported that she experienced no side effects nor noticed a change in symptom profile, and by the Week 10 visit, this subject (1116) showed deterioration in scores for 3 out of 6 psychological scales (-36% - -38% for 2 PTSD scales and 1 depression scale) and a marginal improvement on the other 3 scales (11% - 12% for 2 PTSD scales and 1 depression scale).

3.3.2 Psychological Assessment Results

The CIDI was performed on all PTSD subjects at the baseline visit and showed that depression and anxiety were the two most common co-morbid disorders, along with re-confirming the diagnosis of PTSD.

Of the 16 subjects who were recruited into the trial, only 9 completed the 10-week course due to adverse events. The following 'Per-protocol' results were calculated using only the values obtained for the 9 subjects who fully completed the 10-week protocol. The 'Intention-to-treat' analysis incorporated all data from all subjects, including those who withdrew. Furthermore, the results of the subjects who withdrew prematurely are also presented.

3.3.2.1 Per-protocol Analysis

The percentage improvement for the per-protocol analysis was obtained by dividing the difference between the baseline and Week 2 or 10 scores by the baseline score. For the psychological scales that did not range from 0 (lowest obtainable score for PCL is 17 and HAMD is 2), the lowest obtainable score was subtracted from both initial and final scores before calculating the percentages. Improvement was determined at the Week 2 visit to demonstrate initial treatment effects and upon completing the study at the Week 10 visit.

Table 3.3.2: The Per-protocol summary for the psychological score differences over each visit timepoint. (ND = Not done).

	Baseline			Week 2			Week 6			Week 10			Improve
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	
CAPS	9	98.0	18.2	0	ND	ND	0	ND	ND	9	49.7	29.2	49%
HAMD	9	28.7	7.9	0	ND	ND	0	ND	ND	9	14.7	11.0	54%
PCL	9	63.1	12.1	9	43.3	15.1	9	37.9	15.6	9	36.2	11.2	56%
TOP8	9	21.6	5.3	9	13.2	6.6	9	13.0	6.2	9	11.6	6.0	41%
IES	9	58.4	14.9	9	32.2	18.6	9	27.3	17.0	9	27.2	17.9	49%
BDI	9	39.2	10.7	9	21.0	10.8	9	18.4	12.6	9	15.1	10.3	58%

As shown in Table 3.3.2, all psychological assessments revealed a statistically significant difference between the totals obtained at the Week 10 visit when compared to those at the Baseline visit. The improvement shown was in the order of 51% for all rating scales.

The PTSD Check List (PCL) Results

The screening PCL results confirmed the PTSD diagnosis with a mean total score of 65 (SD 6.3) with a range of scores of 52-73. The screening values were not appreciably different to the baseline values and the diagnosis of PTSD was re-confirmed at this time. The mean total score for the Baseline PCL was 64 (SD 10.3) with scores ranging between 49 and 81. The time difference between the screening PCL and the Baseline PCL was between 2 weeks and 12 months (the later occurred for subjects who also took part in the IDEX study), during which time the subjects did not have treatment for their PTSD.

After 2 weeks of treatment with donepezil, the PCL mean score dropped to 51 (SD 16.4) which equated to an average improvement of 43%. Over the remainder of the trial the subjects continued to show improvement in their PCL scores until at the Week 10 visit their mean PCL score was 36 (SD 11.2) with a range of 17 to 47. The difference in the means at Week 10 constitutes an overall improvement from Baseline values of 56%. As the diagnostic cutoff point for this psychological tool is 50, none of the subjects tested would still fit the diagnostic criteria for PTSD after 10 weeks of treatment. See Table 3.3.3 for the subjects' individual PCL scores and improvement percentages.

Table 3.3.3: The PTSD Check List (PCL) results acquired for the nine subjects who completed the 10 week trial. Improvement is calculated for the first 2 weeks of treatment and the change in scores from Baseline to Week 10. N.B. the lowest score obtainable for the PCL is 17.

Subject	Screen	Baseline	Week 2	Improve.	Week 6	Week 10	Improve.
1010	66	69	17	100%	17	17	100%
1002	64	49	29	63%	31	36	41%
1003	56	64	60	9%	28	43	45%
1004	65	50	44	18%	26	21	88%
1115	67	64	52	26%	40	40	51%
1116	63	51	44	21%	55	47	12%
1117	73	81	31	78%	29	30	80%
1118	73	80	62	29%	64	46	54%
1119	62	60	51	21%	51	46	33%
Mean	65.4	63.1	43.3	41%	37.8	36.2	56%

The Clinician-Administered PTSD Scale (CAPS) Results

The Baseline CAPS results re-confirmed the PTSD diagnosis with a mean total score of 98 (SD 18.2) and a range of scores of 76-119. After 10 weeks of treatment with donepezil, the CAPS mean score dropped to 50 (SD 29.2) with a range of 9-90 which equated to an average improvement of 49%. See Table 3.3.4 for the subjects' individual CAPS scores and improvement percentages.

Table 3.3.4: The Clinician-Administered PTSD Scale for DSM-IV (CAPS) results acquired for the nine subjects who completed the 10 week trial.

Subject	Baseline	Week 10	Improvement
1010	113	12	89%
1002	76	25	67%
1003	86	64	26%
1004	79	9	89%
1115	114	64	44%
1116	81	71	12%
1117	119	39	67%
1118	119	90	24%
1119	95	73	23%
Mean	98.0	49.7	49%

The Treatment Outcome PTSD Scale (TOP8) Results

The mean total score for the Baseline TOP8 was 22 (SD 5.3) with scores ranging between 14 and 29. After 2 weeks of treatment, the TOP8 mean score dropped to 13 (SD 6.6) which equated to an average improvement of 35%. Over the remainder of the trial most of the subjects continued to show improvement until at the Week 10 visit their mean TOP8 score was 12 (SD 6.0) with a range of 1 to 19. This constitutes an overall improvement from Baseline values of 41%. See Table 3.3.5 for the subjects' individual TOP8 scores and improvement percentages.

Table 3.3.5: The Treatment Outcome PTSD Scale (TOP8) results acquired for the nine subjects who completed the 10 week trial. Improvement is calculated for the first 2 weeks of treatment and the change in scores from Baseline to Week 10.

Subject	Baseline	Week 2	Improvement	Week 6	Week 10	Improvement
1010	25	0	100%	1	1	96%
1002	20	9	55%	10	9	55%
1003	15	19	-27%	15	13	13%
1004	18	9	50%	9	6	67%
1115	23	19	17%	16	17	26%
1116	14	12	14%	20	19	-36%
1117	28	13	54%	9	8	71%
1118	29	20	31%	18	13	55%
1119	22	18	18%	19	18	18%
Mean	22	13	35%	13	12	41%

The Impact of Events Scale (IES) Results

The Baseline IES mean total score was 58 (SD 14.9) with scores ranging between 33 and 78. After 2 weeks of treatment, the mean IES score dropped to 32 (SD 18.6) which equated to an average improvement of 42%. All subjects other than 1116 continued to show modest improvement at the Week 6 visit but only Subject 1118 showed considerable improvement over the following 4 week period. At the Week 10 visit the mean IES score was 27 (SD 17.9) with a range of 0 to 46. This still constitutes an overall improvement from Baseline values of 41%. See Table 3.3.6 for the subjects' individual IES scores and improvement percentages.

Table 3.3.6: The Impact of Events Scale (IES) results acquired for the nine subjects who completed the 10 week trial. Improvement is calculated for the first 2 weeks of treatment and the change in scores from Baseline to Week 10.

Subject	Baseline	Week 2	Improvement	Week 6	Week 10	Improvement
1010	66	0	100%	1	0	96%
1002	41	24	42%	24	19	55%
1003	64	47	27%	29	34	13%
1004	46	14	70%	4	3	67%
1115	66	51	23%	42	46	26%
1116	33	36	-9%	45	46	-36%
1117	78	20	74%	17	19	71%
1118	69	46	33%	43	33	55%
1119	63	52	18%	41	45	18%
Mean	58	32	42%	27	27	49%

The Beck Depression Index (BDI) Results

The mean total score for the BDI at the Baseline visit was 39 (SD 10.7) with scores ranging between 18 and 51. After 2 weeks on donepezil, the IES mean score dropped to 21 (SD 10.8) which equated to an average improvement of 42%. Over the remainder of the trial there was a steady decrease in mean score until at the Week 10 visit their mean BDI score was 15 (SD 10.3) with a range of 1 to 34. This constitutes an overall improvement from Baseline values of 58%. See Table 3.3.7 for the subjects' individual BDI scores and improvement percentages.

Table 3.3.7: The Beck Depression Index (BDI) results acquired for the nine subjects who completed the 10 week trial. Improvement is calculated for the first 2 weeks of treatment and the change in scores from Baseline to Week 10.

Subject	Baseline	Week 2	Improvement	Week 6	Week 10	Improvement
1010	50	2	96%	2	1	98%
1002	18	10	44%	8	6	67%
1003	31	26	16%	15	21	32%
1004	44	19	57%	13	8	82%
1115	37	21	43%	19	16	57%
1116	38	36	5%	40	34	11%
1117	51	20	61%	9	10	80%
1118	49	20	59%	33	15	69%
1119	35	35	0%	27	25	29%
Mean	39	21	42%	18	15	58%

The Hamilton Depression Scale (HAMD) Results The Baseline HAMD mean total score was 29 (SD 18.2) with a range of scores of 18-42. After 10 weeks of treatment with donepezil, the HAMD mean score dropped to 15 (SD 11.0) with a range of 2-35 which equated to an average improvement of 54%. See Table 3.3.8 for the subjects' individual HAMD scores and improvement percentages.

Table 3.3.8: The Hamilton Depression Scale (HAMD) results acquired for the nine subjects who completed the 10 week trial. N.B. the lowest score obtainable for the HAMD is 2.

Subject	Baseline	Week 10	Improvement
1010	18	2	100%
1002	26	6	83%
1003	27	12	60%
1004	22	2	100%
1115	24	20	18%
1116	26	35	-38%
1117	42	14	70%
1118	37	26	31%
1119	36	15	62%
Mean	29	15	54%

3.3.2.2 Intention-to-treat Analysis

Table 3.3.9: The Intention-to-treat statistics summary for the psychological score differences over each visit timepoint. (ND = Not done, a = Weeks 2, 6 and 10 significantly lower than Baseline ($p < 0.05$), b = Week 10 significantly lower than Week 2 ($p < 0.05$).)

	Baseline			Week 2			Week 6			Week 10		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
CAPS	16	99.06	17.46	0	ND	ND	0	ND	ND	11	56.36	30.27
HAMD	16	29	8.56	0	ND	ND	0	ND	ND	11	18.82	13.67
PCL ^{a,b}	16	46.69	10.29	16	34.25	16.58	14	28.5	17	9	19.22	11.22
Top8 ^a	16	22.69	5.22	16	16.56	7.11	14	15.14	6.38	9	11.56	6.04
IES ^a	16	59.44	12.35	16	41.69	19.54	14	36.07	21.48	9	27.33	17.73
BDI ^{a,b}	16	37.38	11.42	16	26.5	13.99	14	22.79	13.5	9	15.11	10.3

A repeated measures ANOVA with a mixed models approach was used to analyse the data, due to missing data issues. All cases, including those with missing observations, were included. Repeated observations were nested within an individual and a compound symmetric covariances structure was assumed. The main effect of visit was examined to analyse the change from baseline to endpoint scores during the 10-week treatment period (Bryk & Raudenbush 1992). Results for the effect of time are presented in Table 3.3.10. For Top8, IES, PCL and BDI, pairwise differences among the observations were obtained between the Baseline, Week 2, Week 6 and Week 10 visits and are presented in Table 3.3.9.

This study showed that the specific psychological measures for PTSD (CAPS, the PCL, IES and the TOP 8), all demonstrated statistically significant benefits of the treatment. As shown in Table 3.3.10, there is a significant change over time in the CAPS ($F = 25.3$, $p < 0.05$). Specifically, the measurement at week 10 was significantly lower than the measurement at baseline ($b = -42.49$, $p < 0.05$). For the PCL, Top 8 and BDI there was a significant change over time ($F = 11.58$, $p < 0.05$; $F = 11.00$, $p < 0.05$; and $F = 9.66$, $p < 0.05$ respectively). The measurements at weeks 2, 6, and 10 were all significantly lower

Table 3.3.10: The Intention-to-treat statistics summary of the effect of time on the psychological scales total scores means.

Rating Scales	F value	df_n	df_d	p value
PCL	11.58	3	36	<0.0001
CAPS	25.3	1	10	<0.0005
TOP8	11.00	3	36	<0.0001
IES	11.40	3	36	<0.0001
BDI	9.66	3	36	<0.0001
HAMD	7.87	1	10	0.0186

than the baseline measurement, and in the PCL the Week 10 values obtained were also significantly lower than those obtained at the Week 2 visit (see Table 3.3.9).

The rating scales for depression also demonstrated significant changes over time with the HAMD measurement at week 10 being significantly lower than the measurement at baseline ($F = 7.87$, $p < 0.05$), and the BDI showing significant differences from baseline at each time point, including a significant difference between values obtained at the Week 2 visit and the Week 10 visit.

3.3.2.3 Subjects Who Withdrew

Of the 16 subjects who commenced this clinical trial, 94% experienced side effects and this was the main contributing factor leading to 44% of all subjects withdrawing. Such a large number of non-completers beg the question why. There is no hard evidence to suggest that differences in ACh expression is the cause, although when considering the “Gulf War Syndrome” where AChEI exposure was linked with the development of PTSD-like symptoms, variations in ACh could be considered a contributing factor (Sapolsky 1998). The main difference observed in the side effect profile between completers and non-completers was that those subjects who withdrew experienced an increase in their distressing PTSD symptoms, including intrusive thoughts, panic attacks, worsening of sleep disruptions and

depression. There does not appear to be any obvious reason why some subjects experienced a worsening of their PTSD symptoms while others did not. Original severity of PTSD is not likely to be a factor as the baseline CAPS range of total scores is not appreciably different for the two groups (Completers = 76-119 vs. Non-completers = 70-114). This issue requires more analysis than was possible in such a small subject set, however a breakdown of the baseline CAPS score was feasible.

Table 3.3.11: Comparison of the means, standard deviations and ranges of the three symptom clusters and total score as measured on the baseline CAPS for the subjects who completed the study versus the subjects who withdrew.

Symptom Cluster	Completers			Non-completers		
	Mean	SD	Range	Mean	SD	Range
B. Re-experiencing	26.9	6.2	18-34	26.6	7.3	16-32
C. Avoidance/numbing	41.6	7.9	31-51	41.2	9.6	28-49
D. Hyperarousal	29.9	6.5	22-38	27.2	8.3	17-36
Baseline CAPS Total	98	18.2	76-119	100	17.8	70-114

In order to determine whether those subjects who could not tolerate the drug had a particular pattern or preponderance of cluster B, C or D symptoms which might affect cholinergic distribution, the baseline CAPS score was further divided into its composite scores for each of the symptom clusters. The division of the genders was the only noticeable difference, with the completers having a 33%/66% male/female ratio while the non-completer gender separation was 57%/43% male/female. Other than this, the symptom spread and intensity do not appear to be appreciably different. See Table 3.3.11 for a breakdown of the mean, standard deviation and range of scores obtained by the completers and non-completers for each symptom cluster in the baseline CAPS.

In the subjects who withdrew early, the common intolerable symptoms were generally gastrointestinal or psychological. Subject 1114 felt an increase in depression, helplessness and hopelessness. Subject 1112 (withdrew at Week 6 visit) experienced an increase in his

gastrointestinal symptoms that he referred to as irritable bowel syndrome, and by Week 6 his intrusive negative thoughts were increasing, along with irritability, angry outbursts and suicidal thoughts. Subject 1011 was also withdrawn at the Week 6 visit due to gastrointestinal and urinary issues, headaches, lethargy, worsening of sleep disruptions, tearfulness and increased number of intrusive thoughts. The only exception to this deterioration profile exhibited by the subjects who withdrew was Subject 1113. He was improving on the medication over the first 6 weeks and then was un-contactable and was eventually deemed lost to follow up, although he had experienced gastrointestinal discomfort with bowel patterns alternating between constipation and diarrhoea earlier in the study.

Table 3.3.12: The summary of the psychological score differences over each visit time point for the subjects who withdrew from the study. Two subjects withdrew at the Week 2 visit and 5 withdrew at the Week 6 visit. Although they were generally only completed at the baseline and Week 10 visits, the CAPS and HAMD were completed for 2 of the subjects who withdrew at Week 6. (ND = Not done).

	Baseline				Week 2				Week 6			
	n	Mean	SD	Range	n	Mean	SD	Range	n	Mean	SD	Range
CAPS	7	100.4	17.8	70-114	0	ND	ND	ND	2	86.5	10.6	79-94
HAMD	7	29.6	9.9	11-41	0	ND	ND	ND	2	37.5	6.4	33-42
PCL	7	64.4	8.3	55-77	7	61.4	12.6	38-76	5	57.2	14.5	38-71
TOP8	7	24.1	5.1	16-31	7	20.9	5.5	10-26	5	19.0	5.2	13-24
IES	7	60.7	9.1	50-72	7	53.6	13.9	32-74	5	51.2	20.4	16-65
BDI	7	34.6	13.2	13-48	7	33.7	14.8	13-55	5	30.2	13.4	14-44

Despite the increased incidence and perceived intensity of side effects experienced, the subjects who prematurely withdrew from the study still demonstrated a mild improvement in their psychological measures (PCL **5%**, TOP8 **14%**, IES **12%** and BDI **58%**). As all of these subjects withdrew after the Week 2 visit, an improvement percentage was calculated only for the change from baseline scores to Week 2. See Table 3.3.12 for a summary of the psychological scores obtained from the subjects who prematurely withdrew from the study.

3.3.3 Anecdotal Accounts

All subjects who completed the protocol recounted considerable improvement in their overall PTSD symptom profile, and unlike previously trialed medications, improvements were noted in each of the three PTSD symptom clusters. The subjects' perceptions of the reduction of symptom severity are therefore summarised within these symptom sets.

The **re-experiencing** symptom set encompasses recurrent and intrusive distressing recollections including images, hallucinations, thoughts, dreams, and dissociative flashback episodes involving intense psychological distress and/or physiological reactivity at exposure to internal or external cues. A reduction in the frequency and intensity of negative thoughts (“mind chatter”), emotional responses and the associated anxiety was experienced by most subjects almost immediately, in some cases they were eliminated altogether.

The **avoidance/numbing** symptom set includes efforts to avoid thoughts, feelings, activities, places, or people that arouse recollections of the trauma, a diminished interest or participation in significant activities, feelings of detachment or estrangement from others and a restricted range of affect. Subject comments regarding general levels of functioning showed considerable improvement: work situations were less stressful and social situations were less daunting. Motivation and mood were enhanced, fear and active avoidance reduced, and interpersonal relationships with significant others had improved. Subjects noted that they were able to feel optimistic and they reported an improved quality of life. Substance abuse is a common numbing technique amongst people suffering from PTSD and an unelicited, self-reported reduction in daily alcohol consumption was specifically noted by 2 subjects.

The **hyperarousal** cluster of symptoms incorporates difficulty falling or staying asleep, irritability or outbursts of anger, difficulty concentrating, hypervigilance and an exaggerated startle response. By the Week 2 visit, several subjects were noticing better sleep patterns, improved concentration, less frustration and irritation. They were more controlled in their behaviour (observed less swearing) and they noted an increase in overall level of happiness.

3.4 DISCUSSION

The purpose of this study was to assess the effects of donepezil on the symptomatology of PTSD using objective self-report and clinician-administered psychological rating scales over a 10-week treatment period. These included 4 PTSD scales and 2 depression scales. Treatment effects were calculated from the percentage improvement obtained by the subjects who completed the protocol, by a post hoc full main effect analysis including data from all subjects enrolled in this study and also noted from the subjects' perspective.

Specifically based on the intention-to-treat data, all psychological scale scores showed a significant improvement over time while subjects underwent treatment with donepezil, with the overall improvement being in the order of 51%. This improvement combines the treatment effect and the placebo effect that essentially occurs in an open-labelled trial. Using the tools that we have, it is impossible to separate out this placebo effect. However it is unlikely that the placebo effect could account for such a large change from baseline scores, therefore, although a real effect exists it remains advisable to view the improvement percentages with caution.

The main treatment effect or improvement in this study population occurred rapidly, within the first two week period. The greatest number of side-effects were experienced within this time frame (94% of subjects experienced some initial side effects within the first week of treatment with donepezil) and the greatest change in subjects' perceptions of the benefits experienced also occurred within this period. The intention-to-treat analysis showed that there was only a slight further improvement after the Week 2 visit.

Hamner et al. (2004) describe a positive clinical response in PTSD drug trials as a $\geq 30\%$ reduction in CAPS scores and a complete remission is considered to be a CAPS score of ≥ 20). For the SSRIs, considered to be the first line of treatment for PTSD, they state a general positive response rate rarely exceeding 60% and a remission rate of 20-30% (Berger et al. 2009). In the current study, the positive response rate was 56% (5/9) and 22% of completers achieved complete remission (2/9), which is in line with previous SSRI research.

There is some confusion in the literature to date regarding whether AChEIs improve or

worsen PTSD symptoms. The majority of authors focussing on Gulf War syndrome have reported that the administration of AChEIs is implicated or even completely responsible for eliciting PTSD-like symptoms (Friedman et al. 1996, Sapolsky 1998, McLay & Ho 2007). It was also noted in this study that a large proportion of subjects (44%) did experience an increase in intensity of their PTSD symptoms and were withdrawn as a result. The number of subjects who took part in this study was not large enough to be able show a difference between responders and non-responders in order to determine in future who would be more likely to benefit from the administration of an AChEI and for whom it may be detrimental. Further research is required to address this issue.

One of the most difficult aspects associated with treating people with PTSD is that the symptoms experienced are heterogeneous. The medications that are available ameliorate the different symptom sets to different extents and often only have an effect on one or two of the symptom clusters, usually leaving the avoidance/numbing set of symptoms (which are the most functionally debilitating) less responsive (Berger et al. 2009). The advantage found in this AChEI study was that improvement in responders was experienced in all three symptom clusters, which decreases functional impairment and improves quality of life (Ursano et al. 2004).

Previous studies have commented that Veterans in particular are specifically refractory to conventional treatment and that results obtained from these studies may be less applicable to a general civilian PTSD population (Berger et al. 2009). The subjects recruited for this study were also known to be treatment-resistant, but the range of precipitating traumatic events experienced by this population encompassed the majority of potential possibilities and therefore the data obtained are more likely to be reflective of the greater population of patients with PTSD.

The profile of side-effects experienced was similar to that described by the drug company Product Information (*Aricept*® (*Donepezil Hydrochloride Tablets*) 2006), however the percentages of subjects experiencing each side effect were much larger. This was most likely due to the small number of subjects recruited for this study (n=16) compared to the numbers of patients enrolled in placebo-controlled trials from which the profile was obtained (n=747)

in that a side effect seen in 1 subject in the former study constitutes a 6% expression rate while in the latter study it constitutes a 0.1% expression rate. In spite of this, there was a very large percentage of subjects who experienced side effects (94% as opposed to 74% in the drug company studies). This may be reflective of the altered state of ACh in people suffering with PTSD, with some areas in the brain showing less ACh receptor uptake and others exhibiting an increase (see Section 2.3.4, SPM Statistical Results). The increased variability of ACh within the brain may make this study population more susceptible to adverse effects. As the areas that have a reduced level of ACh normalise, the caudate head may experience a temporary hyperproduction of ACh before the negative feedback loop attempts to restore equilibrium. Much more specifically targeted research is required to unravel the complexity of this ACh regulatory system.

Donepezil hydrochloride is postulated to exert its therapeutic effect by enhancing cholinergic function by increasing the concentration of ACh through reversible inhibition of its hydrolysis by AChE (*Aricept*® (*Donepezil Hydrochloride Tablets*) 2006). Despite the side effects, which were generally short-lived, enhancing cholinergic function in this population of people with PTSD has been demonstrated to be beneficial. This novel treatment option for PTSD focussed on the cholinergic system in an attempt to influence the symptoms in which ACh have been implicated. These include memory function including registration, storage, and retrieval (Erickson 1990), fear-related behaviours and the stress response (Bremner et al. 1995), executive control and the inhibition of unwanted or irrelevant material into consciousness leading to hyperattentional dysfunctions (Brewin 2001, Sarter & Bruno 1997) and cognitive function (Coyle et al. 1983).

PTSD has been shown to involve a critical breakdown in working memory function that underlies the difficulties with concentration and memory (hyperarousal cluster symptoms) and the cholinergic system plays an important role in these and other cognitive functions (Clark et al. 2000). Flashbacks and intrusive thoughts commonly reported in PTSD (re-experiencing cluster symptoms) appear to be similar phenomenologically to the hallucinations reported in Lewy-body dementia which involves abnormalities of the cholinergic system even more profound than that for AD and responds well to cholinesterase inhibitors

(McKeith et al. 2000). The general alterations in mood experienced by people with PTSD, including numbing, interpersonal withdrawal and anhedonia (avoidance/numbing cluster symptoms) are also deemed due to neurotransmitter dysfunction (Friedman 1997, Gazzaniga & Heatherton 2003). The administration of donepezil as a PTSD treatment in this study, specifically increased the availability of ACh in the brain of people with PTSD which was shown to increase concentration, reduce intrusive thoughts, and increase social and occupational functioning thereby reducing the negative symptomatology of PTSD over all symptom clusters.

3.4.1 Study Limitations

Because of the nature of this research, certain limitations need to be emphasized. This study was unblinded, with no placebo arm and no facility to control for practice effects. The subjects could have been influenced by the placebo effect of treatment with donepezil and it is impossible to extract the extent of the main effect from the combined effect, therefore the true value of the main treatment effect remains unknown. Also, the assessment instruments used were completely subjective (both the self report measures and the clinician administered measures rely on opinion only). An objective evaluation of cognitive processing would increase the validity of the results obtained and in the initial planning of this research more objective measures were included, such as functional MRI or brain mapping with evoked potentials. However, funding for novel research of this nature is limited and this was considered an exploratory study only, to be used as a pilot to stimulate further research.

A large proportion of the subjects withdrew due to side effects that call into question the tolerability of donepezil in this patient population. There were no obvious differences in overall initial symptom severity or a pattern of symptom expression originating from a specific cluster, therefore the answer to this question remains obscure. An improvement in the study would be to collect more complete information pertaining to other variables such as medical and medication history, which may increase the ability to predict whether donepezil would be tolerated by new patients with PTSD.

For subjects who were able to tolerate donepezil the results obtained in this study are dramatic, however the baseline CAPS totals indicate that the initial symptom scores were in the severe range. It may be that subjects with lower baseline CAPS scores may show a less marked level of improvement. The majority of subjects in this trial were not treatment naïve and had previously had a range of unsuccessful pharmacological and psychotherapeutic interventions prompting their willingness to consent for this trial. Hence a more thorough examination of the potential benefit of this medication in the treatment in PTSD is warranted.

Of the 16 subjects who were recruited into the trial, only 9 completed the 10-week course. The small sample size increased the possibility that the null hypothesis was rejected due to discerning a rare event, leading to a false positive (i.e., a Type I error). Also, the psychological rating scales selected for this trial generally measured the same information, which could lead to multiple comparison errors. The more tests performed on a set of data, the more likely the null hypothesis is rejected when it is true. Furthermore, the larger the number of tests, the easier it is to find rare events and therefore the easier it is to declare an effect when there is none. A common method to protect from Type I error is to correct the alpha level by making it smaller when performing multiple tests. In the current study, the p values obtained for all of the psychological rating scales (other than the HAMD) were very small (less than 0.0005), making them more likely to be detecting real effects.

3.5 CONCLUSION

PTSD and major depression represent the two most substantial causes of burden of disease by psychological disorders (Kessler et al. 1997), highlighting the importance of improving treatment outcomes. Although a number of medications have been trialed in PTSD, including anticonvulsants, serotonin-norepinephrine reuptake inhibitors (SNRIs) and novel antipsychotics, regulatory approval has not been obtained for any medications other than the SSRIs. Hence there is an imperative to explore what other medications could contribute

to the treatment of PTSD.

In this trial, significant improvement was demonstrated on all measures. The specific rating scales for PTSD (the CAPS, PCL, IES and TOP 8) all demonstrated statistically significant benefits of treatment. In the intention to treat analysis, the effect sizes are all of a magnitude that warrants further investigation of the potential of donepezil as a possible treatment for PTSD. This would need to be substantiated in a placebo-controlled trial with an increased sample size.

The small sample size and the fact that 7 subjects did not complete the trial indicate that limited conclusions can be drawn from this trial. However these were patients who had tried other forms of treatment that had not provided substantial therapeutic benefit. This pilot open labelled trial therefore provides some preliminary evidence that treatment with an AChEI can lessen the intrusions and distress associated with traumatic memories.

Chapter 4

Summary

4.1 Posttraumatic Stress Disorder and Acetylcholine

Posttraumatic Stress Disorder is a psychiatric condition that can develop following exposure to a traumatic event that involved actual or threatened death or serious injury, where the response involved intense fear, helplessness, or horror. A range of symptoms can result from exposure to such a traumatic event and they are categorised into three clusters which include re-experiencing, avoidance/numbing and arousal. The re-experiencing symptom cluster encompasses recurrent and intrusive distressing recollections including images, hallucinations, thoughts, dreams, and dissociative flashback episodes involving intense psychological distress and/or physiological reactivity at exposure to internal or external cues. The avoidance/numbing symptom cluster includes efforts to avoid thoughts, feelings, activities, places, or people that arouse recollections of the trauma, a diminished interest or participation in significant activities, feelings of detachment or estrangement from others and a restricted range of affect. The hyperarousal cluster of symptoms incorporates difficulty falling or staying asleep, irritability or outbursts of anger, difficulty concentrating, hypervigilance and an exaggerated startle response.

Alterations in memory, motivation and mood such as these have been associated with dysfunctions in the central acetylcholinergic system. The cholinergic system:

- contributes to visual attentional function with a role in the retention of affective conditioning, modulates short-term spatial (working) memory processes (Everitt & Robbins 1997)
- is involved in learning and memory including consolidation, registration and retrieval (Erickson 1990)
- impacts on the ability to utilise response rules through conditional discrimination and affects basic arousal processes (e.g. sleep-wake cycle) (Everitt & Robbins 1997)
- plays an important role in planning, guiding and organising behaviour which affects disinhibited behaviour, impaired attention and diminished ability to inhibit distracting stimuli (Southwick et al. 1999)
- is associated with fear-related behaviours and the stress response, including the emotional aspects of behaviour related to survival (Bremner et al. 1995)
- and influences most aspects of cognitive function (Everitt & Robbins 1997, Grutzendler & Morris 2001)

There are known deficits in cholinergic function evident in an number of neurological and psychiatric conditions which account for the expression of a number of cognitive and neuropsychiatric symptoms. In conditions such as AD, DLB and schizophrenia, cognitive abnormalities include disturbances in memory, language ability, visuospatial performance and executive functions, and the loss of these cognitive abilities is often accompanied by psychiatric symptoms such as irritability, emotional instability, apathy, disinhibition, delusions and hallucinations (Coyle et al. 1983, Grutzendler & Morris 2001). The benefits obtained from administering acetylcholinesterase inhibitors (AChEI) to people with AD have been demonstrated in several studies (Shintani & Uchida 1997, Hutchison et al. 2001, Inglis 2002). There are some modest improvements in memory and cognition, as well as noticeable changes in mood and behaviour (Weiner et al. 2000, Grutzendler & Morris 2001). In one study, five behaviour modalities (delusions, agitation, anxiety, disinhibition and irritability) showed a

significant dose dependent improvement from baseline values (Mega et al. 1999). It is the positive effects on mood and other psychiatric symptoms that were elicited by the administration of AChEIs in people suffering from this condition that generated interest in using this medication to combat the altered cognitions, behaviours and moods evident in PTSD.

Alterations in the ACh system have not been studied in great depth in people with PTSD. There has been some recent interest in the involvement of the nicotinic receptor using the radiotracer I^{123} -IA-85380 and SPECT, correlating tracer binding with PTSD symptom clusters (Czermak et al. 2008), but mostly the attention has centred around the effects that stress has on the cholinergic system, namely the alternative splicing of the AChE mRNA and the cumulative effects of these changes (Kaufer et al. 1998, Kaufer et al. 1999, Grisaru et al. 1999, Sternfeld et al. 2000, Salmon et al. 2005). It was, therefore, considered important to determine whether there was a difference in the ACh system in people who suffer with PTSD and whether altering the cortical concentrations of ACh would make an appreciable difference in the lives of those in which this disturbance causes distress or impairment in social, occupational, or other important areas of functioning.

4.2 The IDEX Study

IDEX (I^{123} iododexetimide) is the known potent m-AChR antagonist dexetimide labelled with radioactive Iodine. Animal studies show that the distribution of IDEX uptake corresponds to the known distribution of m-AChR in the human brain and it was therefore suggested that IDEX would be useful in imaging and quantifying levels of m-AChR in the living human brain using SPECT (Wilson et al. 1989, Muller-Gartner et al. 1992, Wilson et al. 1991, Boundy et al. 1995).

IDEX has since been used to study the m-AChR activity in temporal lobe epilepsy (Boundy et al. 1996, Weckesser et al. 1997, Rowe et al. 1998), mild, moderate and severe Alzheimers disease (Boundy et al. 1997, Boundy et al. 2005), and in psychiatric disorders such as schizophrenia (Lavalaye et al. 2001). As PTSD is characterized by intrusive trauma-related memories and deficits in everyday memory and attention (Veltmeyer

et al. 2006, Brewin 2001), it was postulated that IDEX could be used to investigate whether the cholinergic activity in PTSD has been compromised as this could explain a part of the altered cognitive symptomatology apparent in this condition.

168 potential subjects were screened by telephone (either at the time of first contact or at a subsequent predetermined appointment time). All callers were questioned to elicit information relating to the criteria for a diagnosis of PTSD as recorded in the American Psychiatric Association (1994) Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) and to determine eligibility according to the inclusion and exclusion criteria. Eleven subjects were enrolled in this study. 3 control subjects also completed the study and because of the lack of access to IDEX, the imaging data for 2 further control subjects were obtained from another study.

The psychological measures completed by the PTSD subjects demonstrated that the majority of the subjects experienced moderate to severe PTSD symptoms. The events that led to the development of PTSD in this subject population had a long lasting influence over their day-to-day life and activities. Depression and anxiety were the two most common co-morbid disorders and all PTSD subjects were of ‘average’ or ‘superior’ intelligence.

A voxel-by-voxel statistical analysis of the PTSD subject group versus the control group were performed to determine both whether there were statistically significant voxels or clusters where the PTSD subject group exhibited a lower level of ACh receptor activity, and also to establish whether there were brain regions in the collective PTSD group that would demonstrate an increased level of ACh receptor activity when compared to the control group.

Areas of both reduced and increased IDEX uptake in the PTSD group relative to the control group was shown. The locations of the significant clusters with a reduced IDEX uptake centred around the right hippocampus and parahippocampal gyrus, left putamen and insula, left fusiform and parahippocampal gyrus, and left and right precuneus. The location of the significant cluster with an increased IDEX uptake appeared in the caudate head. The results of this study provide support that alterations in ACh binding in PTSD are evident and may begin to explain a part of the altered cognitive symptomatology apparent

in this condition.

While there were limitations in this study which restricted statistical power and potentially introduced Type 1 (Alpha) error, this was the first study investigating ACh receptors with IDEX in people with PTSD. Taking into consideration the exploratory nature of the study, the results are suggestive that alterations in ACh binding in PTSD are evident and may begin to explain a part of the altered cognitive symptomatology apparent in this condition.

4.3 The Donepezil Study

The preferred treatment regimen for PTSD consists of a combination of psychotherapy and pharmacological interventions (Hamner et al. 2004). The first line of pharmacotherapeutic treatment for PTSD is still considered to be the Selective Serotonin Reuptake Inhibitors (SSRIs), however, many patients do not satisfactorily respond to these drugs and are left with either significant residual symptoms, or do not tolerate the medications well and experience considerable side effects (Berger et al. 2009). A number of other medications have been trialed in PTSD, however, they are often found to improve only one or two of the clusters of symptoms, such as hyperarousal, producing limited improvements in sleep difficulties, hypervigilance or irritability (Berger et al. 2009). Medications that enhance cholinergic function represent a novel and untried treatment option for PTSD and yet the cholinergic system has been implicated in eliciting several of the symptoms characteristic of this disorder.

Donepezil hydrochloride (Aricept®) is a reversible inhibitor of the enzyme acetylcholinesterase, and has been used in the treatment of conditions that are associated with difficulties in cognitive function. In people with AD, REM sleep problems, hallucinations and emotional/behavioural symptoms such as agitation, anxiety and irritability were all reduced, along with the occurrence of delusions and episodes of disinhibited behaviour after the administration of donepezil (Mega et al. 1999, Ringman & Simmons 2000, Weiner et al. 2000). The symptoms that most disturbed people with AD, as well as their carers found

most disturbing, were significantly improved while taking donepezil. Other studies report anecdotal accounts of happier, calmer, more content people who are “more comfortable in their own skin”. This led to the generation of the hypothesis that donepezil could be of use in other conditions where similar moods and behaviours comprise a large portion of the overall symptomatology.

A total of 16 subjects met the PTSD inclusion criteria and were enrolled in this study, six of whom had previously taken part in the IDEX study. Nine subjects completed the 10-week trial and seven subjects prematurely withdrew due to side effects or a worsening of PTSD symptoms. In the per-protocol analysis (including only the 9 subjects that completed the protocol), all psychological assessments revealed a difference between the totals obtained at the Week 10 visit when compared to those at the baseline visit and the improvement was in the order of 51% for all rating scales.

When incorporating all subjects results, including those with missing observations, a repeated measures ANOVA with a mixed models approach was used to analyse the data. This showed that the specific psychological measures for PTSD (CAPS, the PCL, IES and the TOP 8), all demonstrated statistically significant benefits of the treatment. The rating scales for depression also demonstrated significant changes over time with the HAMD measurement at week 10 being significantly lower than the measurement at baseline and the BDI showing significant differences from baseline at each time point, including a significant difference between values obtained at the Week 2 visit and the Week 10 visit.

All subjects who completed the protocol recounted considerable improvement in their overall PTSD symptom profile, and unlike previously trialled medications, improvements were noted in each of the three PTSD symptom clusters. Within the re-experiencing cluster, a reduction in the frequency and intensity of negative thoughts, emotional responses and the associated anxiety was experienced by most subjects almost immediately, in some cases they were eliminated altogether. In the avoidance/numbing cluster, general levels of functioning showed considerable improvement: work situations were less stressful and social situations were less daunting. Motivation, optimism, mood and general quality of life were enhanced, fear and active avoidance reduced and interpersonal relationships with significant others

had improved. The hyperarousal cluster was also shown to improve as several subjects were noticing better sleep patterns, improved concentration, less frustration and irritation and they noted an increase in overall level of happiness. This pilot open labelled trial therefore provides some preliminary evidence that treatment with an AChEI can lessen the intrusions and distress associated with traumatic memories in people with PTSD.

The limitations of this study include the fact that it was unblinded, with no placebo arm and no facility to control for practice effects. The subjects could have been influenced by the placebo effect of treatment with donepezil and it is impossible to extract the extent of the main effect from the combined effect. The assessment instruments used were completely subjective (both the self report measures and the clinician administered measures rely on opinion only).

4.4 Future Directions

Clearly the study limitations of an open-labelled medication trial are considerable. Ideally, any further research using this medication to treat this condition would be superior if performed using a double blind, placebo-controlled cross-over study design with a greater number of subjects to greatly enhance statistical validity.

Another inclusion necessary to improve the study design would be to complete an objective measure over all time points. It could be argued that any such study should include neuro-imaging: IDEX for studying muscarinic ACh receptors with SPECT and perhaps 18F-A85380 to study nicotinic ACh receptors with PET. Evoked potential studies, functional MRI, or even a SPECT study performed in conjunction with the medication trial would add greater confidence to the conclusions.

The range of symptoms experienced by people with PTSD are heterogeneous and as such are categorised into three clusters described as re-experiencing, avoidance/numbing and arousal and this constitutes one of the most difficult aspects associated with treating people with PTSD. The medications that are currently available ameliorate the different symptom sets to different extents and often only have an effect on one or two of the symptom clusters,

usually leaving the avoidance/numbing set of symptoms less responsive (Berger et al. 2009). As the current study effected an improvement in the symptomatology of PTSD over all three symptom clusters, any future research into treatment options for PTSD should focus on breaking down all psychological testing into the component cluster subsets for more detailed analysis. For example, of particular interest might be the impact of donepezil on more specific symptoms such as selective attention. Neuropsychological assessments that are symptom or function specific rather than condition specific may elicit more detailed information on the neuropsychiatric implications of this neuro-chemical intervention.

The number of non-responders to treatment is a concern. In the current study, preliminary review of the differences between subjects who responded to the treatment and those who could not tolerate the medication did not elicit any predictors of the subjects who would have been more likely to experience a beneficial effect from donepezil. In a study with larger subject numbers, perhaps potential responders would become easier to identify, and those who are recognised as less likely to benefit could be initially placed on a lower dose and titrated to the standard dose over time.

Finally, the benefits of using a cholinesterase inhibitor did not necessarily imply that there is an abnormality of cholinergic transmission in PTSD. However, the evidence that AChE gene expression can be modified by stress exposure suggests that this is area that may warrant further exploration.

Appendix A

Pharmacy Information: Medications with Cholinergic Properties

DRUG INFORMATION CENTRE

Doan Ngo, Drug Information Pharmacist

(B.Pharm)

The Queen Elizabeth Hospital Campus, 28 Woodville Road, Woodville South, South Australia 5011

Telephone (08) 8222 6777 Facsimile (08) 8222 6019 Email: Doan.Ngo@nwahs.sa.gov.au

Elizabeth Goble
Department of Psychiatry
The Queen Elizabeth Hospital

27 May 2002

Dear Elizabeth,

RE: Medications with cholinergic properties

In reply to your enquiry, please find the following list regarding medications with cholinergic properties.

As discussed, there has been no studies documented to show whether there is an interference between the medications listed below and IDEX, or with post traumatic stress disorder. Therefore, whether these medications will have any effect on the trial is unknown.

Degree of binding or effects these medications have on cholinergic transmission has not been looked at. Details such as half-life of the medication, withdrawal symptoms, and worsening of medical condition, should be considered if a decision to stop the medications for patient inclusion in the trial is necessary.

This list should only be used as a guide to provide a rough indication of medications that could potentially interfere with the trial, and all details of the interference has not been determined. The list is not comprehensive, and any medications with unclear mode of actions should be checked with pharmacy. It is advisable to check the list with all investigators of the trial.

Medications such as anticholinergics, cholinergic agonists, anticholinesterases, acetylcholinesterase inhibitors, some antipsychotics and antidepressants have direct activity on cholinergic transmissions. Other medications listed do not necessarily interact directly with the cholinergic receptors. However, their inclusion in the list is because they have been documented (in the references below) to exhibit anticholinergic signs and symptoms

The cholinergic properties used as a guide to assess the cholinergic involvement for these medications include: dry mouth, dry skin, thirst, tachycardia, palpitation, dilated pupils, blurred vision, urinary retention, constipation etc.

Once again, information provided has not been studied regarding their possible interference with the trial discussed. Information given should only be used as a rough

guide, and the medication list provided should be considered carefully prior to its inclusion to the trial.

I hope this information is useful and will be of some assistance to you in your study.

Yours faithfully

Doan Ngo

Reference:

1. *Australian Medicines Handbook (AMH) 2002*, Australian Medicines Handbook Pty Ltd, Australia
2. Dukes M @ Aronson J K (Editors), 2000, *Meyler's Side Effects of Drugs*, 14th edition, Elsevier Science BV, The Netherlands
3. Hardman J G & Limbird L E (Editors in Chief), 1996, *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th edition, McGraw Hill, USA
4. Parfitt K (Editor), 1999, *Martindale: The Complete Drug Reference*, 32nd edition, Pharmaceutical Press, USA
5. *BNF (British National Formulary) 2001*, Edition 42, British Medical Association, Royal Pharmaceutical Society of Great Britain
6. Mintzer J et al. Anticholinergic side-effects of drugs in elderly people. *J R Soc Med* 2000; 93(9):457-462

MEDICATIONS WITH CHOLINERGIC PROPERTIES

Anticholinergic Agents

- Atropine
- Benztropine
- Benzhexol (trihexyphenidyl)
- Biperiden
- Cyclopentolate
- Dicyclomine
- Glycopyrolate
- Homatropine
- Hyoscine (hyoscine butylbromide, hyoscine hydrobromide)
- Hyoscyamine
- Ipratropium
- Orphenadrine
- Oxybutynin
- Propantheline
- Tropicamide

Cholinergic Agents

- Bethanecol
- Carbachol
- Pilocarpine

Antidepressants

Tricyclic Antidepressants

- Amitriptyline
- Clomipramine
- Dothiepin
- Doxepin
- Imipramine
- Nortriptyline
- Trimipramine

SSRIs

- Citalopram
- Fluoxetine
- Fluvoxamine
- Paroxetine
- Sertraline

MAOIs (Monoamine oxidase inhibitors)

- Phenelzine
- Tranylcypromine
- Moclobemide (MAO-A Inhibitor)

Miscellaneous

- Mianserin

Anticholinesterases

- Neostigmine
- Pyridostigmine
- Edrophonium

Neuromuscular blocking agents

- Atracurium
- Pancuronium
- Rocuronium

Acetylcholinesterase inhibitors

- Donepezil
- Rivastigmine
- Tacrine
- Galantamine

Antihistamines

- Azatadine
- Chlorpheniramine
- Cyprohepatadine
- Dexchlorpheniramine
- Dimenhydrinate
- Diphenhydramine
- Methdilazine
- Pheniramine
- Promethazine
- Trimeprazine

Less Sedating

Antihistamines (potential for interference)

- Cetirizine
- Fexofenadine
- Loratadine

Antipsychotic Agents

- Chlorpromazine
- Clozapine
- Droperidol
- Flupenthixol
- Fluphenazine
- Haloperidol
- Olanzapine
- Pericyazine
- Pimozide
- Quetiapine
- Risperidone
- Thioridazine

- Thiothixene
- Trifluoperazine
- Zuclopenthixol (acetate, decanoate, dihydrochloride)

Antiarrhythmic Agents

- Disopyramide
- Procainamide
- Quinidine

Dopaminergic Agents

- Amantadine
- Bromocriptine
- Pergolide

Appendix B

IDEX Study Ethics Approval Letter

NORTH WESTERN ADELAIDE HEALTH SERVICE

Ethics of Human Research Committee

21 March 2002

Ms E Goble
Nuclear Medicine
The Queen Elizabeth Hospital

Dear Ms Goble

Application Number 156/2001

The Ethics of Human Research Committee at the last meeting considered your protocol entitled:

" An I-123 Iododexetimide (IDEX) SPECT study of Acetylcholine Neuroreceptors in the brain of patients with Post-Traumatic stress disorder

Approval status FINAL

*Where conditions require documents to be changed or submitted, final approval will not be given until sighting by the Chairman.

Protocols are approved for up to twelve months only and a report is required at the end of the study or 12 month period. Extensions will not be granted without a report to the Committee.

The Ethics of Human Research Committee must be notified should there be significant changes to a protocol.

Yours sincerely



—Dr M Hoby
Chairman
Ethics of Human Research Committee

Appendix C

IDEX Study Patient Information and Informed Consent Form

PATIENT INFORMATION SHEET

An I-123 Iododexetimide (IDEX) SPECT Study of Acetylcholine Neuroreceptors in the Brain of patients with Post-Traumatic Stress Disorder.

Simplified Title: *A comparison between normal subjects and patients with Post-Traumatic Stress Disorder (PTSD) of brain receptors for acetylcholine, a chemical important for memory and cognitive function, using a nuclear medicine scan called IDEX SPECT.*

INVITATION TO PARTICIPATE

We invite you to participate in a research project which we believe is of potential importance. However, before you decide whether or not you wish to participate, we need to be sure that you understand **why we are doing it**, and **what it would involve if you agreed**. We are therefore providing you with the following information. Please read it carefully and be sure to ask any questions you have.

The Investigator conducting the research will be happy to discuss it with you and answer any questions that you may have. You are also free to discuss it with outsiders if you wish (le family, friends and / or your local Doctor).

You do not have to make an immediate decision.

Your participation is purely voluntary, and you are under no pressure to participate. Should you agree to enter the trial, you may change your mind and withdraw at any stage.

What is the study about?

We are able to attach a small amount of short-lived radioactivity (iodine-123) to a drug called dexetimide. This way, we can measure brain receptors for a chemical called acetylcholine with a gamma camera. Acetylcholine is used by memory cells to talk to each other. Because this can be altered in PTSD patients, we believe these cells may be important in this condition. We hope to find a difference in the amount of acetylcholine receptors between normal subjects and patients with PTSD so that we can develop another treatment option.

What happens during the study?

In this study you will undergo an I-123 IDEX scan in the Department of Nuclear Medicine at the Queen Elizabeth Hospital. You do not need to fast. You will be given a potassium iodide tablet to take. This stops any IDEX uptake in the thyroid gland. You will then be given a small injection into a vein in the arm. The injection will have no effect on you as the amount of radiation given is tiny. This will happen while you are next to the scanner so that the first scan can take place within 5 minutes. The scan will take about 25 minutes during which time you will need to lie still in the camera while it moves around you taking pictures. After this, you will be asked to complete 6 quick questionnaires and then you are free to go until it is time for your second scan around 6 hours later when you need to return to the Department of Nuclear Medicine. You should drink plenty of fluids and empty your bladder every hour or two over this time to help wash out IDEX from the kidneys and bladder.

Within one month of the IDEX scan, you will need to attend the Department of Radiology at the Queen Elizabeth Hospital for another type of scan. This does not involve any injection. Again the scan will take about 30 minutes during which time you will need to lie still in the camera.

Possible risks, discomforts, and side-effects.

Over 80 persons have undergone I-123 IDEX scans at TQEH without any side-effects. You will not notice any effect from this scan. Allergic reactions to nuclear medicine scans are extremely rare due to the very small amount of compound administered (less than one thousandth of a milligram). Rarely, the intravenous injection of I-123 IDEX may leave a bruise or irritate the vein causing local redness and discomfort. Infection at the injection site is possible but extremely unlikely.

You will receive a small dose of radiation from the short-lived radioisotope iodine-123. The calculated exposure is 4.65 milliSievert which is below the limit recommended by the National Health and Medical Research Council (NH&MRC) for research subjects. It is similar to the amount received from X-ray examinations such as a chest CT scan or a barium enema. There is no direct evidence that low level radiation exposure of this degree has harmful effects on health.

The results of this study will be published in an international medical journal. Your involvement is confidential and your medical history and test results will not be published so as to reveal your identity.

What happens if I say no?

Before deciding whether or not to take part in this trial, you may wish to discuss the matter with a relative, friend or your local doctor. You should feel free to do this. It is important that you understand that your participation in this trial is voluntary, as is the case with all research projects in the hospital. If you do not wish to take part you are under no obligation to do so. If you decide to take part but later change your mind, you are free to withdraw from the project at any stage. Your decision to take part, not to take part, or to withdraw, will not affect your routine medical treatment or your relationship with those treating you or your relationship with the hospital.

Compensation in case of injury.

The Queen Elizabeth Hospital normal insurance conditions apply. You will not lose any normal legal rights by participating in this trial.

Funding

This research is being funded by a grant from the Australian Institute of Nuclear Science and Engineering.

What if I have a question about the study?

If you have any questions before, during or after the study you may contact the Principle Investigator, Elizabeth Goble on 8222 6944, or Dr. Rey Casse on 8222 6431.

This study has been approved by the North Western Adelaide Health Service Ethics of Human Research Committee. Should you wish to speak to a person not directly involved, in particular in relation to matters concerning policies, information about the conduct of the study or your rights as a participant, or should you wish to make a confidential complaint, you may contact the Executive Officer of this Committee Mr Paul Miller on 08 8222 6841.

**CONSENT FORM FOR INVOLVEMENT OF SUBJECTS IN MEDICAL RESEARCH
NORTH WESTERN ADELAIDE HEALTH SERVICE**

Title: **An I-123 Iododexetimide (IDEX) SPECT Study of Acetylcholine Neuroreceptors in the Brain of patients with Post-Traumatic Stress Disorder.**

I, the undersigned

.....

hereby consent to my involvement in the research project entitles: An I-123 Iododexetimide (IDEX) SPECT Study of Acetylcholine Neuroreceptors in the Brain of patients with Post-Traumatic Stress Disorder (Ethics of Human Research Committee approval number 156/2001).

My agreement is based on the understanding that:

1. My involvement entails: Taking a potassium iodide tablet, receiving an intravenous injection of 5mCi of I-123 IDEX, having the first scan immediately, then the second scan around 6 hours later, lying still on a scanning table while several pictures are taken.
2. The following risks, inconvenience and discomforts have been explained to me:
 - a) Allergic reactions to nuclear medicine scans are possible but are extremely rare due to the very small amount of compound administered (less than one thousandth of a milligram).
 - b) Rarely, the intravenous injection of I-123 IDEX may leave a bruise or irritate the vein causing local redness and discomfort.
 - c) Infection at the injection site is possible but extremely unlikely.
 - d) You will receive a small dose of radiation from the short-lived radioisotope iodine-123. The calculated exposure is 5 milliSievert which is well below the limit recommended by the National Health and Medical Research Council (NH&MRC) for research subjects. There is no direct evidence that low level radiation exposure of this degree has harmful effects on health.
3. Within 1 month I need to undergo an MRI scan at the Dept. of Radiology at the Queen Elizabeth Hospital
4. I have read the information sheet, and the nature, purpose and likely effects of the project so far as it affects me, have been fully explained to my satisfaction. My consent is given voluntarily.
5. I understand that the purpose of this study is to improve medical care and that it may not be of direct benefit to me.
6. My involvement is confidential and my medical history and test results will not be published so as to reveal my identity.
7. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.
8. My involvement in the project will not affect my relationship with my medical advisers. I understand I am free to withdraw from the project at any stage without having to give any reasons, and that if I do withdraw from the project it will not affect my treatment at this hospital in the future.

Signed:.....

Date:.....

Witness Name:.....

Witness Signature:.....

Appendix D

Issues Influencing IDEX Supply

The Therapeutic Goods Administration (TGA) gave the Clinical Trials Notification (CTN) for the IDEX trial approval to commence in July 2002 (Trial No. 2002/331, Protocol No. 156/2001). However, also at this time, ANSTO experienced an international shortage of Iodine, with old stores producing very low yields, requiring them to remake the precursor materials. The National Medical Cyclotron used to produce these materials also suffered damages and was shut down for maintenance at this time.

There were also difficulties experienced in August and September of 2002 ranging from issues arising from the AINSE Grant approved when there weren't actually the funds available to commit, and other internal problems.

Finally, the IDEX was available, and subsequently subjects commenced the trial on 22nd October 2002.

By the 18th of November, the National Medical Cyclotron was again experiencing difficulties and was unable to provide the Iodine, and by 2nd December 2002, ANSTO was audited by the TGA and the production of Iodine was shut down completely. As the entire internal environment of the ANSTO laboratories was to be rebuilt, it was determined that no more IDEX would be available for the remainder of 2002 and beginning of 2003.

In February 2003, the radiopharmaceutical division of ANSTO was still unable to give me a recommencement date, and although there were periods of optimism during the year, the IDEX did not actually materialise.

December 2003, ANSTO had attempted to restart their clinical trials (believing they were free to do so) although the TGA had not officially signed off, there were still some minor irregularities in their paperwork procedures and they had not followed the correct procedure in restarting. Therefore the TGA closed them down again.

To the best of my knowledge, IDEX has not been available for trial use since this time.

Appendix E

Optimisation Paper

Barnden, L.R., Hatton, R.L., Behin Ain, S., Hutton, B.F. and Goble, E.A. (2004),
'Optimisation of brain SPET and portability of normal databases',
European Journal of Nuclear Medicine and Molecular Imaging **31**(3), 378-387.

Barnden, L.R., Hatton, R.L., Behin Ain, S., Hutton, B.F. and Goble, E.A. (2004)
Optimisation of brain SPET and portability of normal databases.
European Journal of Nuclear Medicine and Molecular Imaging v. **31**(3), pp. 378-387,
March 2004.

NOTE: This publication is included in the print copy of the thesis
held in the University of Adelaide Library.

It is also available online to authorised users at:

<http://dx.doi.org/10.1007/s00259-003-1362-3>

Abstract Use of a normal database in quantitative regional analysis of brain single-photon emission tomography (SPET) facilitates the detection of functional defects in individual or group studies by accounting for inter-subject variability. Different reconstruction methods and suboptimal attenuation and scatter correction methods can introduce additional variance that will adversely affect such analysis. Similarly, processing differences across different instruments and/or institutions may invalidate the use of external normal databases. The object of this study was to minimise additional variance by comparing reconstructions of a physical phantom with its numerical template so as to optimise processing parameters. Age- and gender-matched normal scans acquired on two different systems were compared using SPM99 after processing with both standard and optimised parameters. For three SPET systems we have optimised parameters for attenuation correction, lower window scatter subtraction, reconstructed pixel size and fanbeam focal length for both filtered back-projection (FBP) and iterative (OSEM) reconstruction. Both attenuation and scatter correction improved accuracy for all systems. For single-iteration Chang attenuation correction the optimum attenuation coefficient (μ) was 0.45–0.85 of the narrow beam value ($N\mu$) before, and 0.75–0.85 $N\mu$ after, scatter subtraction. For accurately modelled OSEM attenuation correction, optimum μ was 0.6–0.9 $N\mu$ before and 0.9–1.1 $N\mu$ after scatter subtraction. FBP appeared to change in-plane voxel dimensions by about 2% and this was confirmed by line phantom measurements. Improvement in accuracy with scatter subtraction was most marked for the highest spatial resolution system. Optimised processing reduced but did not remove highly significant regional differences between normal databases acquired on two different SPET systems.

Appendix F

Donepezil Study Ethics Approval Letter

Ms Elizabeth Goble
Department of Psychiatry/Nuclear Medicine
Ext: 26944

Dr Hoby
Chairman of the Ethics Committee
NWAHS

Wednesday, 9th October, 2002

Dear Dr Hoby,

Re: Reference Number 157/2001

Acetylcholine function in Post-traumatic Stress Disorder - a controlled clinical trial of acetylcholinesterase inhibitors (donepezil) in treatment of memory difficulties in posttraumatic stress disorder.

In response to your letter dated 14th January 2002, please find the requested information below.

1. *Is Donepezil approved for use in the indication proposed?*

No. Donepezil was developed as a treatment for Dementia and to our knowledge has not been used as a treatment option for Post-traumatic Stress Disorder. The Therapeutic Goods Administration is currently processing the CTN form and the approval documentation will be forwarded to the NWAHS Ethics of Human Research Committee as soon as possible.

2. *What will be the formulation of the placebo and who will make it?*

Due to the initial rejection for funding, this protocol was amended and the amended NWAHS Ethics Committee Application Form, Patient Information Sheet and Consent form is attached for your perusal. The changes revolve around removing the 'placebo' arm of the trial and reducing the time line from the randomized, cross-over placebo controlled 20-week frame work to 10 weeks of active medication. The funding for the amended study has now been approved.

Please contact me if you require any further information.

Kind regards,

Elizabeth Goble

I acknowledge receipt of the above document,

Signature _____ Date: _

Name:

Paul F Miller Executive Officer

Appendix G

Donepezil Study Patient Information and Informed Consent Form

PATIENT INFORMATION SHEET

Acetylcholine function in Post-traumatic Stress Disorder - a clinical trial of acetylcholinesterase inhibitors (donepezil) in the treatment of memory difficulties in posttraumatic stress disorder.

Simplified Title: *Adjusting levels of acetylcholine, a chemical important for memory and cognitive function, in patients with Post-Traumatic Stress Disorder (PTSD)*

INVITATION TO PARTICIPATE

We invite you to participate in a research project which we believe is of potential importance. However, before you decide whether or not you wish to participate, we need to be sure that you understand **why we are doing it**, and **what it would involve if you agreed**. We are therefore providing you with the following information. Please read it carefully and be sure to ask any questions you have.

The Investigator conducting the research will be happy to discuss it with you and answer any questions that you may have. You are also free to discuss it with outsiders if you wish (ie family, friends and / or your local Doctor).

You do not have to make an immediate decision.

Your participation is purely voluntary, and you are under no pressure to participate. Should you agree to enter the trial, you may change your mind and withdraw at any stage.

What is the study about?

Acetylcholine is used by memory cells to talk to each other. Because memory and concentration can be altered in patients with PTSD, we believe these cells may be important in this condition. We are able to give you a medication (donepezil) which adjusts the amount of acetylcholine that you have in the brain. We can then measure your memory to see if this adjustment improves your ability. We hope to find an improvement in your memory so that we can develop another treatment option for posttraumatic stress disorder.

What happens during the study?

In this study you will need to be able to come to the Department of Psychiatry at Queen Elizabeth Hospital for 4 visits over a 10-week period.

At each visit you will be asked to complete a number of questionnaires. Your blood pressure and pulse will be taken before you are asked to give a small amount of blood, taken from a vein in your arm (on 2 visits only).

At the first visit you will receive tablets containing 5mgs of donepezil. You will be taking one tablet every morning for the 10 weeks that you are in the study.

During the first visit, and at week 10, you will have a type of brain scan. Please remember to wash your hair on the morning of this scan, but do not use conditioner. The scan involves wearing a cap with 32 electrodes. Each electrode will have a small amount of gel put through it before wires are clipped in place on the cap. The covered wires are then connected to a box that is attached to a computer.

When everything is ready, you will be asked to sit on a chair in front of a computer screen. You will be asked to perform a number of memory, concentration and reaction time tests. The computer will pick up and record the electrical activity that your brain produces while completing the tests.

Possible risks, discomforts, and side-effects.

Donepezil has a few side effects to look out for. In other clinical trials, the most common side effects were nausea, stomach disturbance, headache and insomnia, and these effects only occurred mildly in a small number of patients. Please inform the Investigator if you experience anything unusual.

Rarely, a blood test may leave a bruise or irritate the vein causing local redness and discomfort. Infection at the injection site is possible but extremely unlikely.

During the Brain Scan (called an Event Related Potential or ERP scan) the electrode cap can be a little uncomfortable if it is tight. The gel is inserted in the electrodes after the cap has been placed on your head. This is done using a blunt, rounded needle that will softly scrape the skin to move your hair aside.

What happens to the results?

The results of this study will be published in an international medical journal. Your involvement is confidential and your medical history and test results will not be published so as to reveal your identity.

What happens if I say no?

Before deciding whether or not to take part in this trial, you may wish to discuss the matter with a relative, friend or your local doctor. You should feel free to do this. It is important that you understand that your participation in this trial is voluntary, as is the case with all research projects in the hospital. If you do not wish to take part you are under no obligation to do so. If you decide to take part but later change your mind, you are free to withdraw from the project at any stage. Your decision to take part, not to take part, or to withdraw, will not affect your routine medical treatment or your relationship with those treating you or your relationship with the hospital.

Compensation in case of injury.

The Queen Elizabeth Hospital normal insurance conditions apply. You will not lose any normal legal rights by participating in this trial.

Funding

This research is being funded by a grant from Pfizer Pty. Ltd.

What if I have a question about the study?

If you have any questions before, during or after the study you may contact the Principle Investigator, **Elizabeth Goble on 8222 6944**, or Prof. A. C. McFarlane on 8222 6515.

The North Western Adelaide Health Service Ethics of Human Research Committee has approved this study. Should you wish to speak to a person not directly involved, in particular in relation to matters concerning policies, information about the conduct of the study or your rights as a participant, or should you wish to make a confidential complaint, you may contact the Executive Officer of this Committee Mr Paul Miller on 08 8222 6841.

**CONSENT FORM FOR INVOLVEMENT OF SUBJECTS IN MEDICAL RESEARCH
NORTH WESTERN ADELAIDE HEALTH SERVICE**

Title: Acetylcholine function in Post-traumatic Stress Disorder - a clinical trial of acetylcholinesterase inhibitors (donepezil) in the treatment of memory difficulties in posttraumatic stress disorder.

I, the undersigned

(Please print your name).....

hereby consent to my involvement in the research project entitles: Acetylcholine function in Post-traumatic Stress Disorder - a clinical trial of acetylcholinesterase inhibitors (donepezil) in the treatment of memory difficulties in posttraumatic stress disorder (Ethics of Human Research Committee approval number 157/2001).

My agreement is based on the understanding that:

1. My involvement entails: Taking one tablet every morning (of donepezil 5mg) for the duration of my active involvement in the study, completing several questionnaires and giving a blood on 2 visits, and having an ERP scan performed on three occasions.
2. The following risks, inconvenience and discomforts have been explained to me:
 - a) An allergic reaction to donepezil is possible but rare.
 - b) Rarely, the intravenous access made to take blood may leave a bruise or irritate the vein causing local redness and discomfort.
 - c) Infection at the injection site is possible but extremely unlikely.
3. I have read the information sheet, and the nature, purpose and likely effects of the project so far as it affects me, have been fully explained to my satisfaction. My consent is given voluntarily.
4. I understand that the purpose of this study is to improve medical care and that it may not be of direct benefit to me.
5. My involvement is confidential and my medical history and test results will not be published so as to reveal my identity.
6. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.
7. My involvement in the project will not affect my relationship with my medical advisers. I understand I am free to withdraw from the project at any stage without having to give any reasons, and that if I do withdraw from the project it will not affect my treatment at this hospital in the future.

Signed:.....

Date:.....

Witness Signature:.....

Witness Name:.....

Appendix H

Donepezil Approved Product Information

NOTE:

Appendix H is included in the print copy of the thesis held in the University of Adelaide Library.

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