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Cognitive and regional cerebral blood flow consequences of cholinergic antagonism in healthy human adults

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COGNITIVE AND REGIONAL CEREBRAL BLOOD FLOW CONSEQUENCES OF CHOLINERGIC ANTAGONISM IN HEALTHY HUMAN ADULTS

by

GWENN SMITH

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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Abstract

COGNITIVE AND REGIONAL CEREBRAL BLOOD FLOW CONSEQUENCES OF CHOLINERGIC ANTAGONISM IN HEALTHY HUMAN ADULTS

by

Gwenn Smith

Advisor: Professor Richard J. Bodnar

The interrelation of neurochemical function, cerebral metabolism and behavior has been largely unexplored in the human. The purpose of this investigation was to elucidate such interactions within the cholinergic system, a system implicated in the cognitive deficits of such neurodegenerative disorders as Alzheimer's disease. Neurochemical manipulation was performed by administration of scopolamine, a muscarinic cholinergic receptor antagonist. The effects of cholinergic receptor blockade on cerebral perfusion were assessed by measurements of regional cerebral blood flow (rCBF), using the ^{133}Xenon inhalation technique, with 16 detectors over each cerebral hemisphere. Neuropsychological assessment was conducted to evaluate scopolamine's effects on attention and memory.

To establish the dose respose and time course of scopolamine's effects, subjects were administered either low dose (6.1μg/kg,IV) or high dose (7.3μg/kg,IV) scopolamine.

The high dose group manifested significant declines in global grey matter perfusion, maximal at 25 minutes post-infusion, which were not observed in the low dose group. The greatest regional decline was observed in anterior cortex. Deficits in memory, but not attention were found in both groups. The aspect of memory most impaired was the consistent retrieval of words from long term memory.

The efficacy of physostigmine and neostigmine (centrally and peripherally acting cholinesterase inhibitors, respectively) in reversing scopolamine's effects was examined, to assess the specificity of scopolamine's effects. Physostigmine (0.02mg/kg) or neostigmine (0.01mg/kg or 0.007mg/kg) was administered to coincide with the maximal scopolamine effect, 25 minutes post-infusion.

Physostigmine antagonized the further global and anterior reduction in rCBF from 5 to 25 minutes post-scopolamine administration. Global and anterior CBF continued to decline after neostigmine administration. The memory impairment persisted after administration of both agents and again, consistent retrieval was most affected.

Cholinergic antagonism exerts widespread influence on cerebral perfusion and mnestic function and may influence the central nervous system by interacting with other neurotransmitter systems.

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INTRODUCTION

The interrelation of neurochemical function, cerebral metabolism and behavior has been largely unexplored in the human. The purpose of the studies described herein was to elucidate such interactions within the cholinergic system, a system implicated in the cognitive deficits of such neurodegenerative disorders as Alzheimer's disease. Neurochemical manipulation was performed by administration of the muscarinic receptor antagonist, scopolamine. The effects of cholinergic receptor blockade were assessed by measuring regional cerebral blood flow (rCBF) using the ^{133}Xenon inhalation technique, to provide an index of brain metabolism. A battery of neuropsychological tests was administered to evaluate effects on memory and attention. Finally, the efficacy of the cholinesterase inhibitors, physostigmine and neostigmine, in reversing the scopolamine induced rCBF and neuropsychological deficits was assessed. While there is an extensive literature concerning the behavioral effects of cholinergic antagonism, these studies represent the initial attempt to investigate simultaneously the neurometabolic and neuropsychological consequences of such perturbation.

In providing the background for these studies several independent lines of investigation are reviewed. The first sections describe the effects of cholinergic manipulation on behavior in rodents, nonhuman primates and humans. To provide background for a discussion of the rCBF technique,

the anatomy of the cerebral vasculature is described, followed by a discussion of neurotransmitter innervation of the vasculature, the CBF/metabolism coupling and putative intrinsic regulators of CBF and metabolism. Finally, the anatomy of the cholinergic system is reviewed to evaluate the topography of scopolamine's effects relative to cholinergic projections.

THE EFFECTS OF SCOPOLAMINE ON BEHAVIOR IN RODENTS AND NON-HUMAN PRIMATES

There is a large body of literature examining the behavioral sequelae of scopolamine administration. The comparison of scopolamine administration to other methods of producing cholinergic hypofunction has been described previously (see Smith, 1988, for a review). The design of the studies referred to in this section are described in Table 1. Before addressing the cognitive consequences of cholinergic manipulation, It is important to consider potential non-cognitive effects, since such perturbations could affect task performance. For example, Bammer (1984) identifies several factors which influence performance of the passive avoidance task. Changes in shock threshold, locomotor activity, exploratory behavior and habituation, light sensitivity and the biochemical response to stress may produce impairment in the passive avoidance task, which might confound interpretation of a retention deficit.

Table 1: Studies concerning the effects of cholinergic manipulation on behavior in non-human primates and rodents

First Author, Year	*Subjects, Age	Agent, Dosage, Route of Administration, Time of Testing	Dependent Measures
		Behavioral studies	
Bartus 1978	Rhesus Monkey 4-7years	titrated doses: scopolamine 0.015-0.02mg/kg, IM, 30 min post-drug methylphenidate 0.0125mg/kg physostigmine 0.02-0.03mg/kg	Food-reinforced delayed response test
Godding 1982	Albino 3 months	scopolamine 1.0-5.0mg/kg, IP 0 or 2 hours post-trial 4	Radial Arm Maze
Loullis 1983	Fischer 344 7 months	physostigmine 1mg/kg/day, SC 24 hours post-day 15 scopolamine 7.5mg/kg, per day (SC) for 15 days	OTPA

Table 1-Continued

Hiraga 1984	Wistar 3 months	scopolamine 0.25,0.5mg/kg 30 min pre chlorpromazine 1.0,2.0mg/kg propranalol 10,20mg/kg, IP	Radial Arm Maze
Wirsching 1984	Wistar 200-270g	scopolamine 0.1-0.8mg/kg,IP 30 min pre-training	Radial Arm Maze
Spangler 1986	Fisher-344 3 months	scopolamine 0.1-3.0mg/kg,IP 30 min post-drug n-methylscopolamine 1.0mg/kg or saline	One-way active avoidance 14-unit T-maze
Buresova 1986	Hooded 4 months	scopolamine 0.1,0.2mg/kg,IP or saline 20,60 min post-drug	Morris Water Maze, Radial Arm Water Maze

Table 1-Continued

Aigner 1986	Rhesus Monkey 3-3.5kg	scopolamine 1.0-32µg/kg, physostigmine 0.32-56.0µg/kg, IM or saline	Delayed non- match to sample test

Reversal of Deficit Studies

Cumin 1982	Rats 100-120g Albino SPF mice 21-25g	scopolamine 0.5mg/kg, IP aniracetam 30-300mg, PO piracetam 30-1000mg, PO immediately post-acquisition	OTPA
Flood 1986	CD-1 Mice 6 weeks	scopolamine 0.01, 0.1, 1.0 mg/kg 1 hour pre/2 min post arecholine 1.25mg tacrine 2.0mg D-amphetamine 2.5mg strychnine 1.0mg clonidine 3.0mg	T-Maze Task

Table 1-Continued

		fluoxetine 10.0mg picrotoxin 1.0mg piribedil 1.0mg ACTH $^{4-10}$ 1.0mg (all SC)	
Rush 1986	NMRI Mice 24-30g	scopolamine, 1,3 mg/kg naloxone 0.3,1,3,10 mg/kg scop-5 min pre-training Morphine 10mg/kg morphine, naloxone, 20 min pre-training	OTPA
DeNoble 1986	Sprague Dawley 175-225g	scopolamine 2.0mg/kg,SC 15min pre- training Vinpocetine 1-300mg/kg,SC vincamine 1-200mg/kg aniracetam 1-200mg/kg pemoline 1-100mg/kg hydergene 0.1-10mg/kg 60 min pre-scopolamine	OTPA

Table 1-Continued

*Subjects are rats, unless otherwise specified.

SC=subcutaneous, IP=intraperitoneal, IM=intramuscular,

PO=per oral, OTPA=one trial passive avoidance, min=minutes

However, the majority of studies fail to assess such non-cognitive functions.

The effects of scopolamine on learning and memory have been demonstrated in several behavioral paradigms. On a delayed response task, Bartus (1978) observed progressive impairments with increasing retention intervals (from 0 to 60 seconds). Aigner and Mishkin (1986) have shown dose dependent enhancements and impairments with physostigmine and scopolamine, respectively, on a delayed non-matched to sample test, a measure of recognition memory. In the one trial passive avoidance paradigm, deficits in 24 hour retention have been noted with 1.0,2.0 and 3.0 mg/kg dosages when scopolamine is administered 5,15 and 30 minutes pre-acquisition (DeNoble et al.,1986; Flood and Cherkin, 1986; Rush, 1986; Vogelsang and Piercy, 1986). Scopolamine administered after acquisition produces a retrograde amnesia for four hours after training in a multi-trial avoidance paradigm (Spangler et al.,1986).

The radial arm maze paradigm allows for an evaluation of working (short term, trial unique) and reference (long term, trial unchanging) memory. Working memory is impaired when scopolamine is administered before the fourth trial in the "four out of eight baiting procedure", a task which involves the correct identification of baited arms in the fewest number of trials. This deficit has been observed in dosages ranging from 0.2mg to 0.8mg, administered 20-30 minutes prior to training (Buresova et al.,1986; Wirsching

et al.,1984) and is not seen with administration of benzodiazepines and monoaminergic antagonists (Hiraga and Iwasaki, 1984). The working memory deficit is observed when scopolamine is administered after the fourth trial in dosages of 0.2 to 5.0 mg, with retention intervals of 5,10,20,40 minutes and 5 hours (Buresova et al.,1986; Godding et al.,1982). Reference memory has been shown to be unaffected by scopolamine (Buresova et al.,1986; Wirsching et al.,1984). In the Morris water maze, a task which involves spatial localization and motoric abilities, acquisition is impaired when scopolamine is administered 20 minutes prior to training. When the response is overtrained, a relatively higher dosage (1.0mg/kg) is needed to disrupt performance. This dosage impaired retention at 40 minutes in the working memory manipulation of this task (Buresova et al.,1986).

Therefore, scopolamine has been shown to produce deficits in acquisition, immediate and delayed retention and working memory in several paradigms.

Reversal of Deficits

The reversal of scopolamine induced deficits has been investigated in several studies with different agents and learning paradigms. Flood and Cherkin (1986) demonstrated that a variety of agents (including arecholine,clonidine, d-amphetamine, ACTH $^{4-10}$, picrotoxin) produced 70-90% reversal when scopolamine was administered after, but not before

acquisition. This study contradicts the findings of Bartus (1978) and Drachman (1974; 1977), who showed exacerbation of deficits with amphetamine and reversal of deficits with physostigmine, but there are differences in specie examined and relative task demands across these studies. Naloxone reversed 24 hour retention deficits on the passive avoidance task when administered both prior to and following scopolamine treatment. These effects are antagonized with morphine administration (Rush, 1986). Nootropic agents (cerebral metabolic enhancers, eg. Piracetam, Aniracetam), which have been shown to reverse deficits due to hypoxia and electroconvulsive shock, have been studied in relation to scopolamine induced deficits. Piracetam, administered 60 minutes prior to scopolamine treatment, prevents the 24 hour retention deficit in passive avoidance response (Vogelsang and Piercy, 1986). In a multi-trial acquisition procedure, piracetam has no effect on retention at two hours after training, but improves retention at a 100mg dosage, when scopolamine is administered after acquisition. Reversal is obtained with aniracetam in the 50-100mg dose range (Cumin et al.,1986). Vinpocetine, hydergine (vasodilators), and aniracetam (a nootropic) improved 24 hour passive avoidance response retention when scopolamine was administered prior to acquisition. In contrast, apovincaminic acid, pemoline, nicotine and mecamylamine were ineffective in improving retention.

A variety of agents appear to reverse scopolamine induced deficits. The mechanism by which reversal is produced is unclear and it is not known whether these effects are due to peripheral or central activation.

THE EFFECTS OF SCOPOLAMINE ON COGNITIVE FUNCTION IN HUMANS

In evaluating the effects of cholinergic manipulation on cognitive function, there are several methodological differences which hinder generalization across studies. Such factors include differences in drug dosages, routes of administration, the interval between drug administration and beginning of testing, use of a variety of neuropsychological tests and type of control condition used (saline versus peripherally acting anticholinergics). This section will summarize the literature concerning cognitive effects of cholinergic manipulation by addressing specific functions which have been assessed. The experimental features and designs of each study to be reviewed are summarized in Table 2 and will be referred to in addressing potential discrepancies across studies.

Autonomic and Affective Side Effects

In the studies reviewed, scopolamine was observed to produce dry mouth, mydriasis, blurred vision, dizziness, increased heart rate, and a decline in subjective alertness (Hammond et al.,1987, Parrot, 1986) Subjective mental and

Table 2: Studies concerning the effects of scopolamine on cognitive function in the human

First Author, Date	Subjects, Age	Drug dosage, route of administration, design of study	Dependent measures
Studies in young normals			
Safer 1971	N=11 18-26 yrs	scop 10µg/kg, IV ws design	Digit recall, Addition task, paired associates, Auditory and Visual vigilance task
Ketchum 1973	N=158 mean age 23 yrs	scop 5-24µg/kg, IV or IM bs design atropine 175µg/kg and neo or physo 60µg/kg, IM 2 hr post	Number facility test, General information test, Calculation, Proverb Interpretation. Similarities, Time Perception
Frumin 1976	N=20 per group 15-60 yrs	diaz 10mg, IV scop 0.5mg, IV or both combined bs design	Picture retention test

Table 2-Continued

Peterson 1977	N=24 per group mean age=23 yrs	scop 5,8, 10µg/kg,IV or saline 2.0ml Set 1 adm 30min pre tested 30min post Set 2 adm 1hr post tested 2hr post bs design	Immediate and delayed recall of low and high frequency words.
Peterson 1979	N=28 mean age 20.9	scop 5µm/kg,IV saline 2ml,IV tested 45-120min later bs design (groups= dd, dp, pd, pp)	Context cued recall,Free recall,Category retrieval without cues
Crow 1979	N=12 19-23 yrs	scop 0.4mg atrop 0.6mg or saline 1.0ml ws design	Word list recall test, Visual scanning test, Symbol-digit test
	N=8 19-23 yrs	scop 0.4mg or cpz 12.5 or 25.5mg	

Table 2-Continued

Richardson 1984	N=18 age= 20-52	scop 5.7µg/kg diaz 0.107 mg/kg or saline ,IV testing 25min post-scop 10min pre-diaz, ws design	Word recognition test
Frith 1984	N=9	scop 5.7µg/kg,IV diaz 0.107 mg/kg,IV or saline 1ml,IM testing 10-15min post ws design	Word list recall test
Caine 1981	N=9 mean age 20.8 yrs	scop 0.8mg,IM m-scop 0.5mg,IM or saline 1ml,IM randomized tested 1hr later ws design	Brown-Peterson task with delay), Selective Reminding test, Self-generated paired associates, word list recall test
	N=7	scop 0.8mg,IM or 0.6mg,IM	Category naming, Verbal fluency test (CFL), Tone discrimination, Pattern recall test
Wesnes 1984	N=12	scop 0.6 or 1.2mg PO, m-scop 1.2mg PO or placebo	Continuous performance test (detection of digit triads)

Table 2-Continued

	N=12	nicotine 0.5,1.0,1.5mg, PO or placebo testing 0-30min post bs design	
Wesnes 1984	N=12 age= 18-21 yrs	scop 1.2mg,PO nicotine 1.5mg PO, or placebo second drug adm 60 min post, testing 0-20min post drugs adm for a second time at 50min post ws design	Continuous Performance test, (detection of digit triads),Stroop test
Parrot 1985	N=38 age= 18-40 yrs	scop 0.5mg,TD or placebo testing 24hr post ws design	Letter cancellation test, Choice reaction time, Symbol-digit task
Parrot 1985	N=12 mean age=24 yrs	scop 0.15,0.3, PO scop, TD (dose unspecified) or placebo ws design	Letter cancellation test, Choice reaction time, Symbol-digit task

Table 2-Continued

Callaway 1985	N=12, age= 19-23 yrs	scop 0.6, 1.2mg or placebo, PO ws design testing 45min post scop	Span of Apprehension test,Motor sequencing test, evoked response during Sternberg memory scanning test, Sine wave grating discrimination test
Dunne 1986	N=12 mean age=27 yrs	scop 0.9mg,PO or placebo, testing 80min post ws design	Continuous Performance test (letter target detection
Beatty 1986	N=6 mean age=26 yrs	scop 0.5mg, glycopyrrolate or saline 1.3ml testing 45,75 105min post, ws design	Verbal fluency (CFL), Selective Reminding test, Paired associates

Table 2-Continued

Nissen 1987	N=24 age= 19-35 yrs	scop 0.43mg,SC or 0.5mg,saline testing 45min post bs design	Word fragment completion test Serial reaction time, digit span, Generation of city names and surnames, Verbal free recall, Boston Naming Test
<u>Studies in elderly normals and patients</u>			
Dubois 1987	N=34 PD, mean age= 63 yrs, N=34 normals, mean age=56yrs	scop 0.25mg,SC or saline testing 90min post, ws design	Digit span, Picture recall, Nonsense shape recognition test
Sunderland 1985, 1987	N=10 mean age= 61 yrs N=10 AD Mean age =58 yrs	scop 0.1,0.25, 0.5mg,IV or placebo, testing 90min post-drug, ws design	Selective reminding test category naming, continuous performance test, Word vigilance test, Short Portable Mental Status Questionaire

Table 2-Continued

Studies examining reversal of deficits

Drachman	drug	scop 1.0mg,SC	Digit span, Supraspan
1974	N=20	m-scop 1.0mg,SC	digit storage, Verbal
	mean	physo 1.0mg or	free recall, Category
	age=	2.0mg, SC+ m-scop	naming, WAIS
	23yrs	1.0mg,SC,	
	Control N=23	bs Design	
	mean age=	testing begun within	
	23yrs	30min of drug adm.	
	aged controls		
	mean age=67		
Davis	N=13	m-scop 1.0,SC	Digit span, Paired
1976	age=	physo 3.0mg,IV	associates, Story
	21-22	or saline 2.5mg	Recall, Trails Test
		adm over 30min	
		ws design	
Davis	*N=19	physo or saline	Digit Span (WAIS),
1978	age=	1.0mg, IV,	Sternberg Memory
	18-35	infused over	Scanning Test Selective
	yrs	60 min	Reminding Test
		m-scop infused 20min prior	
		testing 8-80 min post	
		beginning of infusion , ws design	

*(subjects excluded if performed at ceiling levels on memory test)

Table 2-Continued

Drachman, 1977	N=20, 30,10 per group	scop 1.0mg,SC physo 4.0mg,SC d-ampht 10.0mg,PO bs design	Digit span, Supraspan digit storage, Category naming, Free word recall WAIS
Ghoneim 1977	N=70 mean age=23 yrs	scop 8.0µg/kg,IM diaz 0.3mg/kg,IV physo 16 or 32µg/kg,IM m-scop 8 or 16µg/kg,IM physo or placebo adm 70min post diaz or scop testing 30-55min after first drug, 10-100min after second drug ws, latin square design	Word List recall and recognition
Sitaram 1978	N=14 mean age=22 yrs	scop 0.25 or 0.5mg m-scop 0.3mg,IM saline or arech 4 or 6mg,SC adm 105min post testing 10min post arech or saline ws design	Category Serial Learning Test

Table 2-Continued

Mewaldt 1979	N=70 mean age=22 yrs	scop 8µg/kg,IM physo 32µg/kg,IM ampht 0.2, 0.3mg/kg, IM or saline Second drug adm 75 min after first, testing 30-65 min after first drug, 95-140 min after second drug. ws design	Serial digit recall, Immediate and delayed word list recall
Mohs 1985	N=10 18-30 yrs	scop 0.35mg,SC or saline and choline 14.0gm,PO pre-scop ws design	Word Span,Verbal list recall
Hammond, 1987	N=3, 33-38 yrs	scop 0.4mg, IM physo 1.0mg, IM testing 1.5-2.5 hours post scop ws design	Auditory P300 and visual evoked potential, Digit Span , Selective Reminding Test

Table 2-Continued

SC=subcutaneous, IV=intravenous, IM=intramuscular, TD=transdermal, ws=within subjects, bs=between subjects, scop=scopolamine, cpz=chlorpromazine, diaz=diazepam, physo=physostigmine, neo=neostigmine, arech-arecholine, ampht=amphetamine, m-scop=n-methylscopolamine, WAIS-Wechsler Adult Intelligence Scale, adm=administration, hrs=hours, min=minutes, yrs=years, EC=elderly controls, AD=Alzheimer's disease, PD=Parkinson's disease

physical sedation were reported, comparable to that produced by diazepam. Physostigmine reversed these impairments after scopolamine administration, but exacerbated this condition after diazepam administration (Ghoneim and Mewaldt, 1977). Euphoria, visual and auditory hallucinations, disorientation, motor incoordination and elevated body temperature have been reported after administration of relatively large doses of scopolamine (10-24µg/kg, Safer and Allen, 1971; Ketchum et al., 1973). It is important to note that this is above the dose range used in the studies reviewed. At a dose of 5µg/kg, IV (intravenous) scopolamine produced a 20 beat per minute decline in heart rate 1-7 hours after administration (Ketchum et al.,1973). Davis et al.,(1976) report no overall change in affective state after administration of physostigmine, contrary to reports in patients with major affective disorders. Although subjects were pre-treated with n-methylscopolamine, they reported nausea, "fuzziness" of thought and manifest decreased spontaneous movement, paucity of speech, lethargy and decreased facial expression. Ketchum et al.,(1973) demonstrated that after administration of atropine, physostigmine and neostigmine produced a decline in heart rate relative to a saline reversal condition. The differences in magnitude of reduction were apparent one hour after administration, in that the effects of neostigmine were greater than that of physostigmine. Therefore, at longer time intervals, neostigmine manifests more potent

peripheral effects relative to an identical dose of physostigmine.

Primary Sensory Function

Caine et al.,(1981) demonstrated that tone discrimination was unimpaired after scopolamine administration. Visual evoked potentials and tasks requiring rapid processing of visual information are also unimpaired, despite reports of blurred vision after scopolamine (Hammond et al.,1987; Parrot, 1986; Crow, 1979). Based upon the evidence from these few studies, primary sensory function does not appear to be disrupted after administration of moderate doses of scopolamine.

Attention

Attentional function has been measured utilizing a variety of methods, requiring that the subject retain information over a relatively short span of time. The digit span test has been shown to be unaffected by scopolamine in young and elderly normals and Parkinson's patients (Drachman et al.,1974,1977; DuBois et al.,1987). Slight but significant impairments have been noted in other studies (Nissen et al.,1987) and deficits have been reported in Alzheimer disease patients and normal elderly controls after scopolamine administration (Sunderland et al.,1985; 1987). Relatively high doses of physostigmine produce deficits in both the digit span and the trails test (Davis et al.,1976).

In a continuous performance test paradigm, digit triad detection was impaired by relatively higher doses of scopolamine, but enhanced by nicotine (Wesnes, 1984; Wesnes and Warburton 1984). Dose dependent impairments in elderly controls and Alzheimer's disease patients have been observed (Sunderland et al.,1985, 1987). Reaction time performance is unchanged at lower doses of scopolamine (Hammond et al.,1987; Parrot, 1985), but significant slowing is noted in performance of a tone discrimination task (0.8mg,IM dose, Caine et al.,1981) and visual stimulus discrimination, irrespective of stimulus complexity (1.2mg, PO dose, Callaway et al.,1985). The Sternberg paradigm is unaffected by scopolamine or low dose physostigmine (Callaway et al.,1985; Davis et al.,1978). On a similar visual scanning test, Crow (1979) has demonstrated impairments after chlorpromazine, but not scopolamine. Therefore, response slowing is noted on tasks with greater information-processing demands.

Performance on the Stroop test was found to be significantly slower, accompanied by a non-significant increase in number of errors (Wesnes and Revell, 1984). Finally, both slowed P300 latency and elimination of the P300 response have been reported with scopolamine and restoration after physostigmine administration (Callaway et al.,1985; Hammond et al.,1987). Deficits are not observed in the visual evoked potential, nor in EEG recordings

indicating that the deficit is specific to functions involving information processing.

Attentional deficits are observed after higher doses of scopolamine (1.0mg and above) and on tasks with greater information-processing demands (Wesnes,1984,). Reaction time is slowed on several tasks, without concommittant increase in number of errors (Parrot, 1985).

Memory

Performance on list learning tasks (eg. selective reminding test, category serial learning test) is consistently disrupted by scopolamine in total number of words recalled (Caine et al.,1981; Beatty et al.,1986; Peterson, 1977). Analysis of the serial position curve shows maximal deficits in the early and middle portions (Frith et al.,1984; Crow, 1983). However, in the Serial Digit test global impairment is observed with scopolamine, with the shape of curve remaining the same (Mewaldt and Ghoneim, 1979). In the Brown-Peterson task, a procedure which prevents rehearsal of words presented, there is an increase in omission errors, not in intrusions of previously presented material, generally observed in the degenerative dementias (Caine et al.,1981; Beatty et al.,1986). Recognition deficits are observed in several paradigms, including list learning, word completion and word recognition tasks. This further indicates the disruption of storage, rather than retrieval processes (Peterson, 1977;

Ghoneim and Mewaldt, 1977, Richardson et al.,1984; Nissen et al.,1987).

On list learning tasks, deficits are observed for both low and high imagery words and subjects fail to employ a specific encoding strategy spontaneously (Caine et al.,1981). However, Frith et al.,(1984) showed an increase in recall of semantically related words. Paired associate recall is consistently unaffected (Safer and Allen 1971; Caine et al.,1981). Semantic cues provided to the subject appear to enhance recall, whereas the level of imagery has no effect.

In tests of retrieval, verbal fluency and category naming measures are impaired by scopolamine (Caine et al.,1981; Beatty et al.,1986). Deficits are also observed in Alzheimer's disease patients and elderly normals after scopolamine administration (Sunderland et al.,1985; 1987). Performance on the Boston Naming test (a measure of confrontation naming) is unimpaired (Nissen et al.,1987). Remote memory (general information test, Ketchum et al.,1973) and generation of city names and surnames is unaffected (Nissen et al.,1987). In a repeating sequences test, Nissen et al.,(1987) observed a non-significant reduction of response latency, which is thought to reflect procedural learning.

State dependency is not found in tasks with retrieval cues (context cued recall or category recall with cues), but for tasks without retrieval cues (free recall and category

recall without cues). Performance decrements were observed when the drug state was changed and no cues provided, relative to the conditions in which one type of cue (same drug state or task cue) was provided. In tests of non-verbal memory, pictures presented pre-drug and tested post-drug show worse recognition deficits with diazepam (64%) than scopolamine (14%, Frumin et al.,1976). Tests of pattern recall show profound deficits after scopolamine (Caine et al.,1981), indicating that both non-verbal and verbal memory is impaired.

Concerning reversal of memory deficits, physostigmine and arecholine reverse recall deficits in list learning and supraspan digit storage test, whereas d-amphetamine either exacerbates such deficits or shows no change (Sitaram et al.,1978; Drachman, 1977; Ghoneim and Mewaldt, 1977; Mewaldt and Ghoneim, 1979). This may indicate that the deficits observed are specific to the cholinergic system and are not mediated by a general arousal impairments.

Other Cognitive Functions

Deficits in arithmetic processing and tasks involving abstract reasoning, such as proverb interpretation and the Similarities subtest from the WAIS have been reported after high doses of scopolamine (greater than 10µg/kg, IV, Safer and Allen, 1971; Ketchum et al.,1973). Fuld (1984) has reanalyzed the Wechsler Adult Intelligence Scale data from Drachman (1974; 1977) and demonstrated greater deficits in

Digit Symbol, Block Design and Object Assembly subtests, relative to Information, Vocabulary, Similarities and Digit Span subtests. Therefore, at a lower dose of scopolamine, impairments in abstract reasoning, remote memory and semantic retrieval deficits are not observed, rather deficits are seen in timed tests with a visuo-constructive element, emphasizing motor output.

Summary

The mnestic deficits produced by scopolamine and reversed by cholinomimetic agents were first demonstrated in rodent and primate studies and have been replicated in the human. Scopolamine induced deficits in memory are not mediated by effects on attention. Attentional deficit are observed after administration of relatively large doses of scopolamine or with attentional tasks that have greater information-processing demands. Primary or short term memory is spared, whereas secondary or long term memory is consistently impaired. Recall is impaired, if scopolamine is administered before learning, but if administered after learning, recall is preserved. This implies acquisition or storage deficits, rather than retention or retrieval deficits (Ghoneim and Mewaldt, 1975; Mewaldt and Ghoneim, 1979; Peterson, 1977). The lack of retrieval deficits in verbal fluency, category naming and confrontation naming tasks and the observation of recognition impairments, further suggest relative deficits in storage or acquisition.

Greater retrieval deficits are observed in free recall paradigms using unrelated word stimuli (eg. Beatty et al.,1986) which suggests that subjects have difficulty in generating strategies that are efficacious.

CHOLINERGIC NEUROANATOMY

Since the discovery of significant loss of cortical choline acetyltransferase in Alzheimer's disease (Davies and Maloney, 1976) there has been a renewed interest in the characterization of the muscarinic cholinergic system in the cerebral cortex. New techniques for pathway tracing, enzyme and receptor localization have been refined and applied to elucidate cholinergic pathways in the central nervous system. These experimental approaches have been guided by neuropathological observations of significant cell loss in the Nucleus basalis of Meynert (Whitehouse et al.,1983) and the subsequent findings that selective neurotoxic lesion of this structure in rodent and primate, produces neurochemical and cognitive deficits which resemble Alzheimer's disease (Flicker et al.,1983; Bartus et al.,1985). Although much progress has been made in characterizing this system, there are many controversial issues and the relation of the neuropathology of Alzheimer's disease to deficits in the cholinergic system is unclear. In this section, the current literature is reviewed, concerning the characterization of muscarinic cholinergic pathways and distribution of cortical cholinergic markers in the nonhuman primate and human.

Studies in the rodent will not be included due to significant inter-species differences, which will be referred to in the next section.

Theoretical and Methodological Considerations

There are several issues, specific to the study of the cortical cholinergic system, which influence generalization across research findings. There is tremendous controversy over the localization of and the nomenclature applied to the Nucleus Basalis of Meynert (NBM), the nuclear group which has been shown to be responsible for the extrinsic cholinergic input to cerebral cortex. The confusion arises from the fact that the structure has been identified by either neurochemical or neuroanatomical criteria. The majority of recent investigators combine techniques in order to characterize both aspects of the structure. In reviewing the existing literature, it appears that the term Substantia Innominata refers to an anatomically defined region which contains among other elements, the large acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) positive cells which represent the NBM. Terms such as NBM or Ch4 refer specifically to these cholinergic neurons. Other aspects of this structure hinder exact localization, both across species and within individuals. For example, the NBM becomes increasingly differentiated across the phylogenetic scale and the structures is diffusely organized and overlaps with major fiber pathways. Inter-individual

differences have been reported with respect to enzyme localization in primate brain and correspondence between the topography in the primate brain and the human brain is controversial (Mesulam et al.,1986; Struble et al.,1984). Such variability is not observed to the same extent in the rodent (Rye et al.,1984; Struble et al.,1984) and may be influenced by methodological variability, as well.The history of the controversy surrounding NBM localization will not be reviewed here, however, the particular definition used in studies cited will be described. NBM localization across studies is especially important to note, because this influences interpretation of data concerning efferent and afferent projections.

Another issue that must be considered is the criteria used to identify of a cholinergic neuron. More specifically, is the demonstration of presence of AChE or ChAT enough to demonstrate the identity of a cholinergic neuron and which techniques are most reliable and valid for such determinations. The specificity of the enzyme AChE to cholinergic neurons has been questioned in light of evidence that AChE immunoreactivity is seen within such areas as the Substantia Nigra and ventral tegmental area, which are known to contain primarily catecholaminergic neurons (Butcher et al.,1978). It has been demonstrated that AChE significantly correlates with ChAT activity in primate cortex (r=0.89-0.97, Mesulam et al.,1986). Although these findings account for 79-94% of the variability, it appears that AChE staining

alone is not entirely conclusive. It is difficult to evaluate the significance of hypothalamic projections to cortex, for example, which are AChE, but not ChAT positive. ChAT is found specifically in cholinergic neurons and believed to be a more specific marker of cholinergic activity. Within the ChAT literature, there is a discrepancy between studies using radiochemical and immunocytochemical procedures for cortical localization. ChAT immunoreactivity has not been found in primate cortex, but has been observed in rodent cortex (Mesulam et al.,1986; Struble et al.,1984; Rye et al.,1984). Radiochemical assays have found ChAT in primate cortex, but in relatively small amounts (5-35% of that seen in NBM). Therefore, the presence of cholinergic interneurons in cortex and the cholinergic nature of cortical afferents to NBM is controversial. In a recent study, comparing both methodologies in the rodent amygdala, Hellendall et al.(1986) have concluded that radiochemical procedures show better regional discrimination and are more reliable for quantification than immunohistochemical procedures. These inconsistencies argue for the combined use of enzyme localization with pathway tracing techniques. such as retrograde HRP transport, to specifically elucidate cholinergic projections.

Knowledge about cholinergic projections in the central nervous system is limited by the problems inherent in available techniques for acetylcholine localization. The

following is a review of the literature considering these constraints.

Organization and Cytology of the Basal Forebrain Cholinergic System

Studies in the Nonhuman Primate

Based upon neuropathological data and morphological observations from the non-human primate, Mesulam et al.(1983;1984a,b;1986a) performed the most extensive mapping of cholinergic projections from the basal forebrain. Studies were conducted in a series of forty adolescent-juvenile male macaques and one twenty-five year old human male. The tracer horseradish peroxidase (HRP) was injected into all brains (to olfactory bulb, hippocampus and cerebral cortex) and processed for either: (1) HRP histochemistry with either benzidine dihydrochloride or tetramethyl benzidine as the chromagen, (2) acetylcholinesterase histochemistry with acetylthiocholine as the substrate and ethopropazine as an inhibitor of non-acetyl cholinesterases, (3) AChE histochemistry with thionin counter-staining, (4) concurrent HRP-AChE visualization and (5) cresyl violet stain for Nissl substance. Finally, one series of sections was processed for ChAT immunohistochemistry using a rodent antibovine monoclonal antibody to rodent ChAT and another series for concurrent ChAT and AChE.

The data generated from this study indicated that the basal forebrain cholinergic system could be divided into five major groups, based upon connectivity patterns, cytochemistry and cytoarchitectonics. Since the four groups contained neurons with high levels of ChAT and AChE, the designation Ch1-4 was applied. The staining patterns noted included: ChAT positive/ AChE positive, ChAT positive/AChE lightly stained, ChAT positive/AChE negative, ChAT negative/AChE positive, ChAT negative/AChE lightly stained. Combined ChAT-AChE staining yielded fewer AChE positive perikarya relative to AChE staining alone.

The morphology of neurons within the four subdivisions were elucidated as follows. The Ch1 group, consisted of small (25-30 x 25-30μm) ovoid cells located along the fibers of the precommissural fornix, which contain a relatively small cholinergic component (10% of cells reactive to ChAT and AChE) and correspond to the medial septal nucleus. The Ch2 group consisted of cells which were slightly larger (20-25 x 30 x 40μm), "fusiform to oval " in shape, mostly cholinergic (70% were AChE rich, 95-99% of these were also ChAT positive) and correspond to the nucleus of the vertical limb of the diagonal band, embedded within the diagonal band fibers. The nucleus of the horizontal limb of the diagonal band, or the Ch3 group, extended from the septal- preoptic region to the amygdaloid region and contained fusiform, small to medium sized (15-20 x 40-50μm), hypochromic cells, 1% of which were either AChE rich or ChAT positive. The Ch4

group corresponds to the NBM. The cells are primarily large (40-50 x 60-70μm), with a hyperchromic perikarya and a prominent nucleolus. Smaller, less hyperchromic cells are also found. The cell types include fusiform, pyramidal, globoid and multipolar neurons, which are intermingled in this region. The dendritic arborization was characterized by overlap across adjacent neurons, without a specific pattern. 90% of the magnocellular elements were AChE rich and the majority of these were ChAT positive, indicating that there was a 10% non-cholinergic element.

The Ch4 group, found adjacent to the globus pallidus, was further divided into four segments. Ch4a, the anterior section, is posterior to the olfactory tubercule and overlaps with the crossing of the anterior commissure. The Ch4al (antero-lateral) section continues to the periamygdaloid area and the Ch4am (antero-medial) is bounded by the Ch2 group, the preoptic area, and the Ch3 group. The Ch4i (intermediate) group is located from the periamygdaloid area and the globus pallidus to the preoptic region, the hypothalamic grey and the Ch3. This subregion is further divided into Ch4id (intermediodorsal) and Ch4iv (intermedioventral) by the ansa peduncularis. The Ch4p (posterior) is delimited by the putamen, globus pallidus and optic tract, anteriorly and consists of a band of neurons between the putamen and globus pallidus, posteriorly.

The Ch4 has a large component of compact neurons and an interstitial component which penetrates fiber bundles

coursing through this area. Jones et al. (1976) estimates that 100-150 cells are included within these "island" clusters. The interstitial component consists of hyperchromic, AChE rich, ChAT positive cells, which are embedded within the internal capsule, ansa lenticularis, inferior thalamic peduncle, stria terminalis, interior and exterior medullary lamina of the globus pallidus and anterior commissure. Relative to other definitions of the NBM, such as Substantia Innominata, nucleus of the ansa lenticularis (Gorry, 1963), the Ch4 definition is more inclusive, especially of these interstitial elements. In a separate study using similar techniques, Mesulam et al. (1984b) observed that the most significant difference between the Ch1-4 system in the rodent as compared to the primate or human was that the Ch4 was not as well differentiated and was more diffusely organized. In a study using concurrent HRP retrograde transport and ChAT immunoreactivity, it was concluded that the topography of Ch4 projections is not as specific as in the primate (Rye et al., 1984) In addition, the rodent Ch4 was not as relatively larger than other Ch1-4 structures as is observed in the primate and human. In addition, although there is dispute about the localization of the NBM/Ch4 in primate and human, there is more argument concerning the corresponding structure in the rodent.

Mesulam et al. (1983) also identify a group of hypothalamic neurons which project to cerebral cortex. One

group (Hce1) consisted of the supraoptic and paraventricular neurosecretory cells, the other (Hce 2) is located in the lateral hypothalamus and extends into the lateral perimammillary region. 50% of these neurons are AChE rich, but are negative for ChAT immunoreactivity. Whereas in the Ch1-4 areas, perikarya, dendrites and axons were positive for AChE, in the hypothalamic region, the axons were not positive. Pearson et al. (1983) also reports labelling in hypothalamus after HRP injection into various areas of cortex. Therefore, it is unclear whether these are indeed cholinergic neurons, although these neurons may be cholinoceptive.

In order to describe the ultrastructure of NBM neurons, Walker et al. (1983) utilized light and electron microscopy to examine cells identified by HRP injected into areas 17,18, and 19 of occipital cortex. These studies were performed in the squirrel monkey (Saimiri sciureus), ranging in age from 1-10 years. Labelled neurons located within the dense cluster of NBM were compared with cells embedded in the internal and external medullary lamina of globus pallidus, which are included in the interstitial component of the structure. Sections were stained with either toluidine blue for light microscopy, or lead citrate or uranyl acetate and lead citrate for electron microscopy. The mean area of cell somata was 437μm^2, with the cells of the dense component being larger (505 versus 437 and 383μm^2 for internal and external laminae, respectively) and rounder

than the interstitial component. Otherwise, the cells in these areas were similar. Electrophysiological findings, based upon single unit recordings in the behaving animal, show similar firing patterns among the two cell groups (DeLong et al.,1972). The somata were frequently accompanied by satellite neuroglial cells and were surrounded by large myelinated and small unmyelinated axons, dendritic profiles and glial processes. The nuclei of these cells are heterochromatic, moderately involuted and eccentric in position. The surrounding cytoplasmic rim is wide and contains many organelles. Lipofuscin pigment was observed in these cells, in greater amounts in the older primate. Contact specializations are not seen and myelinated and unmyelinated nonterminal axons come into contact with the plasmalemma without specialized contacts. There are few synaptic contacts between somata and proximal dendrites, the mean number of synapses per section being 2.8 or 4% of the membrane perimeter per section occupied by synapses. Variety in axosomatic synapses is observed. A small amount are Grey Type 2 synapses, symmetrical synapses with flattened presynaptic vesicles. Most of the axosomatic synapses are symmetrical with pleomorphic vesicles and dense core vesicles are observed in axon terminals. The synapses surrounding the NBM cells are asymmetrical, with round vesicles. The unlabelled cells in NBM, presumably those which do not project to cortex, are very similar to the labelled cells.

Studies in the Human

The findings in primate brain reported by Mesulam, et al. (1983) were similar to that in the human brain examined. Exceptions in the human were noted, in that the cell density, primarily in Ch4, is greater and there is a lesser extention of the interstitial elements into such adjacent structures as the globus pallidus. Subsequent studies report variability in localizing Ch4 segments.

Hedreen et al. (1984) studied human material with cresyl violet staining and have demonstrated that NBM neurons range from 8.71-41μm in size and stain intensely for Nissl, which is found in one or more foci in the perikaron. Interstitial neurons stain less intensely and have Nissl clusters that are not as peripherally located. The large, AChE positive neurons are also found in lateral hypothalamus, putamen and nucleus accumbens. These investigators report that the Ch1-4 segments are identifiable in human, but Ch2 is less clearly seen.

Saper and Chelimsky (1984) performed Nissl and AChE histochemical staining, alone and combined, on eight human brains (post mortem interval ranging from 2-19 hours). AChE positive neurons were seen in hypothalamus, with the population in supraoptic and paraventricular nuclei showing 20-30μm round and multipolar perikarya and a second group scattered through lateral and posterior hypothalamus, which were 25-35μm multipolar neurons. The NBM was characterized

as being organized in a series of clusters, with variation seen among the eight brains examined. The intensely stained AChE positive neurons were pyramidal or multipolar in shape and measured 35-50μm. The AChE stained proximal dendrites could be followed for 50μm or more from the cell body. The perikarya contained dense granular Nissl substance, a large vesicular nucleus containing a large nucleolus. Smaller, fusiform neurons, 20-25μm, were scattered within the medial septum and diagonal band nuclei. As in the primate, the horizontal limb of the diagonal band contained few AChE positive neurons, the cells in this region being fusiform or multipolar neurons (10-15μm in diameter). Magnocellular perikarya stain positively for AChE in diagonal band and medial septal areas. This was not observed to the same extent in the primate. The intermediate segments of NBM (Ch4iv,id) were found in some of the the human brains, but not the anterior segments. The posterior segment appears to be more extensive in the human, with cells found lateral to the amygdala and medial to the tail of the caudate.

In contrast, Arendt et al. (1985) were able to localize four segments of NBM (Ch4am,al,i,p) using cresyl violet and silver staining of five human brains. Cell densities were estimated by counting nucleolated, large cells (greater than 30μm) across every tenth tissue section. In all cases studied, a greater number of neurons were seen in the right NBM. The fewest neurons were seen in the Ch4p segment, intermediate levels in Ch4al and Ch4i, and the most neurons

in Ch4am. Samples from Alzheimer's disease patients show variable degeneration within Ch4 segments.

In summary, the basal forebrain cholinergic system has been characterized in primate and human brain, as well as in the rodent. Most of the findings for human and primate are consistent, but there is dispute over the segmentation of NBM and the ability to localize all segments in the human brain.

Connectivity

Efferent Projections to Cerebral Cortex

Mesulam et al. (1983) demonstrated that HRP injections into hippocampal formation, adjacent parahippocampal cortex, and posterior hypothalamus produced the greatest amount of labelling in Ch2 and to a lesser extent in Ch1 groups. After olfactory bulb injection, most labelling was seen in Ch3. After cortical and amygdala injection, Ch4 had the greatest labelling density. However, combined HRP-AChE showed that 1-12% of HRP projections to olfactory bulb were AChE rich, 70-77% to hippocampus and 86-100% to cerebral cortex. Given that AChE may not be the most specific marker for cholinergic neurons, concurrent study of ChAT immunoreactivity and HRP would be more conclusive.

There was no specificity of labelling based upon neuronal morphology. HRP label in Ch4 was found in large fusiform, multipolar and pyramidal neurons and small, fusiform neurons. In regard to the hypothalamic cortical projections, primarily larger neurons were labeled. A small

number (1.8%) of labeled neurons were found in the side contralateral to injection in the Ch1-4 and hypothalamic projections.

There was a topography demonstrated by the projections of Ch4 to cortex. The components of Ch1-4 which contained the highest percent of labeled neurons and the elements which project to cerebral cortex will be described. Ch4am projected to the medial parts of the hemispheres, such as mid principalis, medial frontal pole, subcallosal, cingulate, dorsomedial motor and medial parietal cortices. Lesser densities were seen after injection into ventrolateral orbital, inferior parietal lobule and peristriate visual cortices. The highest concentration of label was found in Ch4al after injection in fronto-parietal opercular regions and ventral opercular aspects of somatosensory cortex, posterior parietal cortex and amygdala and lesser concentrations after injection of ventrolateral orbital and middle insula cortices. Dense labelling in Ch4id and Ch4iv was seen after injection to ventrolateral orbital cortex, anterior insula, posterior and midperiarcuate, posterior principalis and peristriate visual cortices. Label in Ch4id alone was seen after injection into frontal operculum, middle and posterior insula, anterior periarcuate, inferotemporal and parahippocampal cortices. In Ch4iv, label was selective for inferior parietal lobule. Injections into anterior and posterior auditory association cortex or temporal pole produced labelling in Ch4p.

Labelling was also seen in the interstitial components after injections to all areas to cortex. In terms of the hypothalamic groups, some degree of labelling was seen after injection to cortical areas, primarily to Hce2 with injections into midprincipalis, ventrolateral orbital, ventral posterior, insula, posterior and midperiarcuate and peristriate visual cortices. Similar results have been reported by other groups (Pearson et al.,1983, Jones et al., 1976). For example, Jones et al. (1976) demonstrated that injections of HRP into precentral, postcentral gyrus, parietal operculum and parietal cortex produced labelling in NBM, especially in the anterior "islands". Pearson et al. (1983) concludes from HRP injection into cortex, that the anteromedial half of NBM projects to frontal lobe and the postero-lateral part to temporo-parietal cortex. The posterior interstitial extention in between putamen and globus pallidus is related to the visual areas.

A subsequent study by Mesulam et al. (1986a) was performed to address the methodological concerns raised by the previous studies. A stabilized tetramethylbenzedine chromagen was used to increase the sensitivity of HRP detection. Retrograde transport of HRP and ChAT immunohistochemistry were performed concurrently to test the validity of the projections previously outlined. Significantly larger injections of HRP (50 times that of previous studies) were used to attempt to define cortical topography more clearly. Two juvenile macaques were

examined, given injections into either the frontoparietal operculum (lateral area 12, ventral area 6, ventral S1 and M1 and ventral inferior parietal lobule) or the dorsal aspect of the anterior portion of superior temporal gyrus. The results obtained were consistent with previously reported topographic findings, although the results did not yield a more precise topography. In this more specific study of cholinergic projections, it was estimated that 96% of Ch4 projections were cholinergic, relative to the 90% estimate based on previous studies with concurrent AChE/HRP histochemistry.

In order to further characterize the cholinergic pathways to cortex, Kitt et al. (1987) utilized light microscopic autoradiographic tracing studies of anterograde transport of tritiated amino acids. In a series of adult (5-26 years) macaques, ChAT immunocytochemistry, AChE histochemistry and combined autoradiography and immunocytochemistry were performed. Tritiated amino acids, ([^{3}H] amino acid, a combination of leucine and proline, were injected into NBM. In this study, neurons of the NBM were identified by electrophysiological recordings based on specific behavioral criteria and the tritiated amino acid was injected directly into NBM. Sections processed for autoradiography were also stained with cresyl violet or thionin. AChE histochemistry and ChAT immunocytochemistry were performed with techniques similar to that of Mesulam et al. (1983;1984a).

Injections were placed within the portion of NBM which has the highest density of neurons, but the amino acid tracer spread to the horizontal limb of the nucleus of the diagonal band (corresponding to Mesulam's Ch3 region, an area which has few cholinergic cells which do not project to cortex) and in small amounts to ventral globus pallidus and putamen. Based upon the analysis of the anterograde tracing patterns and considerable overlap with enzyme activity, five major pathways of cholinergic axons were delineated. One group of axons passed through the nucleus of the diagonal band and medial septum, traversed the cingulum bundle to innervate cingulate, medial frontal and parietal cortices. Within the main component of the NBM, fibers were seen laterally behind striatum and pallidum, which passed through the external and extreme capsules to the corona radiata, to innervate insular and dorsolateral aspects of temporal, parietal and occipital cortices. Another group of axons projected to temporal lobe, hippocampus and basolateral amygdala. A fourth group of axons passed through the fibrea orbitofrontales to orbitofrontal, rostral frontal and olfactory cortices. The last group projected ventrally, mostly from the horizontal band nuclei, through the fimbria/fornix to the entorhinal cortex and subiculum, terminating in hippocampal formation, primarily prosubiculum. The latter two pathways represent primarily non-NBM input. The small amounts of fibers were found within the corpus callosum and anterior commissure, which project

to the contralateral hemisphere. Due to the method of identification of the NBM in this study and the extent of the tracer injection into the nucleus of the diagonal band, it is difficult to integrate these findings with the results from retrograde transport studies. These results do correspond to these previous findings and identify the major NBM to cortex pathway. An important potential study would involve specific injections of tracer into the five subdivisions of Ch4 to describe the corresponding pathways of the topographical projections. The medial and lateral pathways defined in this study, correspond to components of the topography identified by Mesulam et al. (1983).

Walker et al. (1985) sought to determine the degree of collateralization of NBM axons and to define the cortical fields innervated by individual neurons. Fluorescent dyes (fast blue and nuclear yellow) were injected into the left principle sulcus (in portions of superior and mid frontal gyrus) and left superior parietal gyrus in two macaques and into inferior and superior gyri surrounding the interparietal sulcus and superior frontal gyrus in a third macaque. These areas were chosen because the Ch4am segment projects to both frontal and parietal cortices and independent studies have shown cortico-cortico connections between these two areas. Therefore, it is most probable that the Ch4am neurons have overlapping projections. None of the NBM neurons were labelled with both dyes, although double labeled cells (about 5% of total labelling) were found in

locus coeruleus. Approximately 4% of the NBM neurons were labelled. Single labelled cells were found in Ch4am and Ch4i, located adjacent to each other. ChAT immunoreactive cells were also found in this area. These results are consistent with those of Mesulam et al. (1983), in demonstrating topographic NBM projections. However, these results indicate that NBM axons do not collateralize in cortex, but the neurons projecting to these areas are intermingled. The functional significance of proximity of these neurons is unknown. These cholinergic projections are very different from noradrenergic projections from locus coeruleus, in which there is a high degree of collateralization. This may have functional implications in that the NBM projections may have a more limited behavioral role. Many questions remain as to the actual pattern of NBM ennervation to cortex.

Studies in the Human

Saper and Cheminsky (1984) delineated two pathways in the human, a medial pathway from medial NBM, medial septum and diagonal band nuclei, running through septum with some fibers entering the fornix. The ventral pathway traversed the Substantia Innominata, between the putamen and amygdala and entered the external capsule before terminating in the lateral wall of the cerebral hemisphere. Other projections were observed passing through the extreme capsule to the

insula. These are similar to the pathways described by Kitt et al. (1987).

In summary, these results demonstrate a substantial degree of topography within the Ch4 cortical projections and delineate certain cortical areas such as ventrolateral orbital, insular, posterior parietal and peristriate cortices which receive multiple Ch4 projections. Relative to thalamic projections to cortex, the cholinergic Ch4 projections are more diffuse. There is no organization according to functional division of sensory, motor, association and limbic cortices nor are the cell groups projecting to a given cortical area as specific as in the thalamic efferent system. Preliminary data in the human suggest overlap with primate findings.

Afferents to NBM/Ch4, Primarily from Cerebral Cortex Studies in the Primate

The approach of tracing anterograde transport of amino acids using light microscopic autoradiography to characterize afferent input to Ch4 was applied by Mesulam et al.(1984a). The tracer was injected into various areas and labelling within divisions of Ch4 was observed. After injection into medial and lateral septum, nucleus accumbens-ventral pallidum and medial hypothalamus, significant label was seen in Ch4am, al, id and iv areas and in the Ch4p segment only after nucleus accumbens-ventral pallidum and medial hypothalamus injection. Many cortical injections were

performed. For the Ch4am section, slight labelling was seen in prefrontal-orbitofrontal and prepyriform olfactory cortex. In contrast, for Ch4al, highest label was seen after orbitofrontal injection and to lesser degrees with prefrontal, medial visual association, anterior insula, entorhinal, prepyriform and temporopolar cortices. In terms of Ch4id and Ch4iv, significant labelling was seen after injection to orbitofrontal, prefrontal, medial visual association, anterior insula, prepyriform and temporopolar cortices. Finally for Ch4p, significant labelling was seen after temporopolar and superior temporal cortical injection. In this study, the orbitofrontal and anterior insula labelled fibers passed through external and extreme capsules before entering Ch4. The medial and polar temporal fibers passed through the uncinate fasciculus en route to Ch4. The projections from septal nuclei traversed through the diagonal band of Broca. The axons from hypothalamus, prepyriform cortex and accumbens-pallidum were widely dispersed. Since the NBM is embedded within fiber bundles and the light micrographic technique cannot determine whether the label is within the terminal field of synaptic contact or axons of passage, these results require ultrastructural corroboration.

In earlier studies of [^{3}H] amino acid anterograde transport, Jones et al.,(1976) describes labelling in NBM after injection into lateral preoptic areas of hypothalamus.

However, injections into cortex produced labelling in thalamus, striatum and claustrum, but not NBM.

Russchen et al.,(1985) performed studies of retrograde HRP transport, anterograde [^{3}H] amino acid transport and acetylcholinesterase histochemistry, immunocytochemistry for ChAT, enkephalin and Substance P-like immunoreactivity in a series of macaques. It is important to note that these authors do not use the terminology by Mesulam et al.,(1983) and refer instead to the Substantia Innominata which include the NBM, Nucleus of the horizontal limb of the Diagonal Band and an area equivalent to the ventral pallidum in cat and rat, located ventromedial to the Nucleus Accumbens. This distinction was based upon morphological, rather than neurochemical properties of labelled cells, since the presence of non-cholinergic elements was under investigation. Afferent projections from cortex demonstrated by Mesulam et al.,(1984a) were confirmed in this study. In addition, afferents were identified from basal nuclei of the amygdala, from striatum to intermediate and posterior NBM (which were Substance P and enkephalin immunoreactive), from lateral preoptic, mammillary, paraventricular and supraoptic nuclei to anterior NBM. Afferents from Substantia Nigra, dorsal raphe, subthalamic nucleus and pendunculopontine nucleus were shown, but may be attributed to adjacent ventral pallidum and striatal structures and may not specifically project to NBM. Since these authors use such a general definition of basal forebrain cholinergic

structures, it is difficult to determine which afferents are specific to NBM.

The results from these studies indicate that the vast projections from Ch4 to cortex are not reciprocal. Afferents were not demonstrated from such areas as dorsolateral frontal, posterior parietal, peristriate, lateral temporal, posterior insula and cingulate cortices. However, the topography displayed by the cortical afferents is consistent with the topography of efferents demonstrated by previous HRP retrograde transport studies and the pathways consistent with those delineated by Kitt et al.,(1987). In terms of non-cortical afferents, areas such as hypothalamus, nucleus accumbens, septum, amygdala have been demonstrated to show such projections. It is important to note that there is little or no published data demonstrating afferents to NBM conducted with human material.

Distribution of Cholinergic Enzyme Markers and Receptors

Studies in the Primate

Using AChE histochemistry and ChAT immunocytochemistry as described previously, Mesulam (1984a) studied the distribution of these enzymes in four macaques. These results paralleled the previous findings with concurrent HRP transport and enzyme determination (Mesulam et al.,1983) supporting the Ch1-4 classification and confirming the presence of hypothalamic cortical projections. In addition

it was shown that AChE positive neurons were located in layer six of cerebral cortex, in the absence of staining for ChAT. Using the identical technique with the same antibody, cortical ChAT positive cells were visualized in the rodent (Wainer et al.,1984). The lack of ChAT staining in cortex was also reported by Hedreen et al.,(1983) using a monoclonal antibody against ChAT obtained from rat brain. It appears that there may be species differences in the existence or distribution of cortical cholinergic neurons.

Two studies have examined regional cortical differences in the distribution of AChE and ChAT in the macaque (Mesulam et al.,1986b; Lehmann et al.,1984). In both studies, quantitative ChAT determinations were performed by a radiochemical assay (values reported in nmol/min/mg protein). Lehmann et al.,(1984) found significant regional and individual variation in ChAT, but not in glutamic acid decarboxlase (GAD). Such variation is not observed in the rodent (Lehmann and Fibiger, 1979). Highest levels of ChAT were found in superior and inferior temporal, precentral and anterior cingulate cortices; intermediate levels in superior, middle and inferior frontal and superior and inferior parietal cortices; low levels in superior and inferior occipital cortices. Mesulam et al.,(1986) also demonstrated high variability across regions and subjects. The regional variation in ChAT was significantly correlated with variation in AChE (r=0.97,0.89 respectively per animal $p<0.001$), although the magnitude of correlation varied

across subjects. The amount of ChAT detected in cortex ranges from 5-34% of that which is found in such ChAT rich areas as NBM or amygdala. Based on sampling of thirty-three cortical areas, highest levels were seen in nonisocortical paralimbic areas (paraolfactory, caudal and midorbitofrontal, insula, temporopolar areas). Moderate levels were seen in primary sensorimotor regions, with primary motor and somatosensory versus primary visual being the highest and lowest areas, respectively within these regions. ChAT was found to be lowest in association areas (peristriate and temporal visual association, somatosensory,auditory, premotor, dorsolateral prefrontal, frontopolar, inferior parietal lobule, superior temporal and caudal inferotemporal cortices) and cingulate gyrus. While exhibiting the highest enzyme activity, the paralimbic areas also have extensive projections back to NBM (Mesulam et al., 1984a). Changes in innervation correspond to changes in cytoarchitecture, in that nonisocortical areas have higher concentrations than isocortical areas.

The distribution of muscarinic receptors has been described. Yamamura et al.,(1974) studied muscarinic receptor binding in the macaque, using [^{3}H] quinuclidinyl benzilate binding assays. Determinations of ChAT and AChE and [^{3}H] choline uptake were also performed. In the areas of the cortex examined, binding was 50% that of caudate and putamen and approximately equal to that of amygdala and hippocampus. Specific binding did not vary significantly (2-

25%) among these areas of precentral, postcentral, cingulate, pyriform cortices and frontal and occipital poles. However, pyriform cortex showed highest ChAT activity and [^{3}H] choline uptake. Recent studies distinguishing between high and low affinity muscarinic receptor subtypes (M1/M2) in the macaque brain have shown that these receptors show regional variation (Mash and Mesulam, 1986). The M1 receptor is thought to be located postsynaptically, whereas the M2 receptor may be a presynaptic autoreceptor (Cortes et al., 1987). M1 receptor binding was widely distributed, to a lesser extent in primary sensory and motor cortices, and seen primarily in superficial cortical layers. M2 receptors were shown to be most dense in primary koniocortical visual somatosensory and auditory areas, the hippocampal formation (CA3 and subiculum) and parahippocampal area (ventral entorhinal and prorhinal). Premotor and motor areas showed intermediate levels of receptor density.

Studies in the Human

Few studies have been conducted in the human and there do not appear to be any studies demonstrating ChAT immunoreactivity in cortex. Using a radiochemical assay, MacKay et al. (1978) determined the localization of ChAT and AChE in cerebral cortex and summarized the findings of previous studies for comparison with their results. In terms of percent activity relative to the caudate nucleus, a lesser degree of ChAT/ AChE was found in orbitofrontal

(18/29%), convexity frontal cortex (16/25%), cingulate (16/26%), sensory (26/25%), motor (26/26%), parietal (11/30%), calcarine(22/27%) and temporal (14/31%) cortices. These results indicate that there is a fairly uniform distribution of these enzymes in human brain, relative to the primate brain.

Some degree of regional variation (4-64%) was shown by Fishman et al. (1986) for the molecular forms of AChE (G4 membrane bound and G1 monomeric,soluble) as determined by sucrose density gradients of cortical tissue. The G4/G1 ratio was highest in Brodmann's frontal and temporal areas 10,11,21,22 and lower in areas frontal, occipital and temporal and parietal areas 9,17,20,40. Relative to these cortical areas, hippocampus and amygdala had higher ratios. It is important to note that in this study, the correlation between the AChE ratio and ChAT determinations from a separate study for the same cortical areas was significant (r=0.96, $p<.01$), indicating that the ratio is a fairly specific marker of cholinergic activity.

Significant ChAT concentration in human NBM was demonstrated by Davies et al. (1982). Five brains were studied, with a post mortem delay that did not exceed 12 hours. A radiochemical procedure was utilized, the author acknowledging that " at present there would seem to be no acceptable histochemical or immunohistochemical reagent for localization of ChAT activity." In a recent study, ChAT immunohistochemistry was performed, with an antibody

purified from human neostriatum (Mizukawa et al.,1986). Only results for hindbrain structures were described in this report. Therefore, the localization of ChAT is human cortex is still controversial.

Several receptor binding studies have been performed by Cortes et al.,(1984; 1986; 1987), examining human material using quantitative light microscopic autoradiographic techniques. Muscarinic receptors were characterized by n-methylscopolamine binding with carbachol or pirenzepine displacement to distinguish between M1/M2 muscarinic binding sites. Visual cortex (area 17) was examined and found to contain high densities of receptors, mostly in lamina II and III. Some receptors were seen in lamina IV. As in the primate, M1 sites were densely observed (70% of total) and concentrated in relatively superficial layers, whereas M2 sites were found in deeper layers (40% of those in lamina IV). The Substantia Innominata was found to be rich in M2 receptors.

Two subsequent studies established the topography of muscarinic receptors in brainstem and forebrain, using the same technique. Receptor densities in brainstem were found to be lower than that of forebrain. At the level of the midbrain, intermediate binding densities were noted in the superficial layers of the superior colliculus, mesencephalic nucleus of the trigeminal nerve, cuneiform and subcuneiform nuclei, area surrounding the brachium of the inferior colliculus, nucleus raphe centralis and higher densities in

periaqueductal grey area, mesencephalic grey area, the ventral tegmental area of Tsai, dorsomedial half of the inferior colliculus and nucleus of the troclear nerve. Highest levels of binding were noted in the interpeduncular nucleus. At the pontine level, highest levels of binding were noted in the nuclei of the facial and trigeminal motor nuclei and the griseum pontis. Intermediate levels were noted in the parabrachial nuclei, lateral lemniscus, pontine reticular nucleus, mesencephalic and principle nuclei of the trigeminal nerve and nucleus raphe magnus. At the medullary level, highest levels of binding were observed in the hypoglossal nucleus, arcuate nucleus, nucleus solitarius and pars ovalis. Intermediate levels were noted in the accessory olivary nucleus, nucleus ambiguus, spinal nucleus and Substantia Gelatinosa of the trigeminal nerve and along the arcuate-cerebellar tracts. The addition of carbachol to block binding to the high affinity site, resulted in a 70% inhibition of binding in the areas previously described. Areas containing mainly low affinity receptors were the Substantia Nigra, Olivary nucleus, pars principalis and the Substantia Gelatinosa of the trigeminal nerve, in which only 30-40% of binding was inhibited by carbachol.

The same technique was applied to determine receptor localization in the forebrain. The forebrain contains predominantly low affinity receptors, whereas the brainstem contains mainly high affinity receptors. Receptor density was negatively correlated with age, which reached

statistical significance in the striatum and Substantia Innominata. High binding concentrations were observed in the caudate and putamen, intermediate levels in the claustrum and low levels in the globus pallidus and subthalamic nucleus. The M1 receptor type accounted for 80% of the sites in caudate, putamen, claustrum and globus pallidus, while M2 site were found in the subthalamic nucleus. High binding densities were found in the olfactory tubercule and nucleus accumbens, primarily of the M1 subtype. Intermediate to low levels of binding were observed in the basal forebrain nuclei (septal nuclei, diagonal band and Substantia Innominata), mainly of the M2 type. Within the hypothalamus, an equal proportion of M1 and M2 sites were observed, the weakest binding within the supraoptic and paraventricular nuclei and the highest in the lateral tuberal nuclei of the ventral hypothalamus. Highest densities of binding within the thalamus were found in the anterior, medial (medialis dorsalis) and lateral geniculate nuclei, primarily of the M2 type. The amygdaloid complex contained a homogeneous distribution of intermediate to high densities, mostly of the M1 type. Within the hippocampal formation, highest binding was observed in the strata oriens and pyramidalis of the CA1 subfield and the molecular layer of the dentate gyrus, primarily of the M1 subtype. In the cerebral cortex, intermediate levels of binding were detected, without differential binding densities across cortical areas. Slightly greater binding densities were noted in primary

visual cortex (Area 17). Cortical muscarinic receptors were shown to be primarily of the M1 subtype, but M2 receptors were also shown to account for 25-40% of total binding sites. The ratio of M1/M2 receptors was similar across all cortical areas. As shown for visual cortex in earlier studies, superficial layers (I-III) contained greater receptor densities than deeper layers (IV-VI). The superficial layers contained more M1 sites, whereas the deeper layers contained more M2 sites.

Recent reports of *in vivo* muscarinic receptor imaging (using [^{3}H] quinuclidinyl benzilate as a ligand with single photon emission tomography) represent promising technical developments, which could potentially further the knowledge about the human, cholinergic system and its functional correlates (Holman et al.,1985; Eckelman et al.,1984). Studies in neurologically normal humans show uniform distribution across cortex and in a patient with Alzheimer's Disease, decreased receptor density in temporo-parietal cortex consistent with the site of greatest cortical degeneration.

Summary

The characterization of the cortical cholinergic system in terms of efferent and afferent projections and enzyme and receptor distributions demonstrates a diffuse cholinergic representation across cortical areas with some degree of topography in these projections. These results must be

considered relative to the constraints inherent in the available techniques for acetylcholine analysis. Questions remain as to the presence of cholinergic interneurons, the significance of hypothalamic inputs to cortex and the afferent projections to NBM. Many investigators have attempted to correlate functional parameters with features of the cholinergic system. Cholinergic deficits have been found in neurodegenerative disease, most notably Alzheimer's disease, but the specificity and relation of these findings to other pathological changes in such disease states is unclear. The cholinergic system is vulnerable to pathology, especially the NBM projections to cortex. The significance and etiology of this vulnerability cannot be evaluated based on current knowledge of cholinergic neuroanatomy. The further elucidation of the cholinergic system is essential, before such correlations can be performed.

The purpose of the next section is to review anatomical and functional aspects of the cerebral circulation, with an emphasis upon the cerebral cortex, the area monitored by the rCBF technique. In addition, the issues of CBF/Metabolism coupling, intrinsic regulation of CBF and metabolism and neurotransmitter innervation of the vasculature will be discussed.

CEREBROVASCULAR ANATOMY

The common carotid artery originates at the bifurcation of the innominate artery (left branch) and the aorta (right branch). Each branch bifurcates into two external and internal carotid arteries, at the level of the C4 spinal cord. The external carotid artery divides into the superficial temporal and internal maxillary arteries at the mandibular level. The distribution of the external carotid is mainly extracranial, but several of the branches supply the dura of the basal and lateral brain surfaces. The internal carotid artery supplies the cerebral hemispheres and ipsilateral eye. The internal carotid artery ascends from the carotid bifurcation, to enter the carotid canal in the petrous portion of the temporal bone. The internal carotid artery ascends to the middle cranial fossa and continues upward toward the posterior clinoid process and lateral to the wall of the sphenoid sinus. After ascending medially to the anterior clinoid process, the internal carotid artery perforates the dura to enter the subarachnoid space, below the optic nerve (Carpenter and Sutin, 1983; Day, 1987).

The internal carotid artery reaches the lateral aspect of the optic chiasm and runs to the anterior perforated space at the medial end of the Sylvian fissure. There, it bifurcates into the anterior and middle cerebral arteries. Other branches which arise from this segment, include the

opthalmic artery, the posterior communicating artery, the anterior choroidal artery and many small perforating arteries.

The anterior cerebral artery passes above the optic chiasm to join the contralateral artery, through the anterior communicating artery, to form the anterior portion of the Circle of Willis. On the baso-medial and medial, the anterior cerebral artery supplies the medial portion of the orbital gyri, gyrus rectus, olfactory bulb and tract, corpus callosum, cingulate gyrus, superior frontal gyrus, paracentral lobule and precuneus. The anterior cerebral artery also supplies the superior frontal gyrus and the superior portions of the precentral, central and postcentral gyri, on the lateral surface.

The middle cerebral artery diverges from the internal carotid artery at the medial end of the Sylvian fissure. One segment runs through the lateral fissure and branches into the lenticulostriate perforating arteries. The inferior and superior branch of the middle cerebral artery runs within the Sylvian fissure over the insula to supply the cerebral cortex. The middle cerebral artery supplies the majority of the lateral surface, the insular and opercular surfaces, the temporal pole and its lateral inferior surface and the lateral part of the orbital surface of the frontal lobe. The middle cerebral artery joins with the anterior cerebral artery as part of the Circle of Willis. The vertebral artery, the largest branch of the subclavian artery, enters

the foramen magnum and passes toward the medulla to join with the contralateral vertebral artery, forming the basilar artery. The basilar artery has several branches: the anterior inferior cerebellar artery, superior cerebellar artery and the posterior cerebral artery. The posterior cerebral artery is the terminal branch of the basilar artery, which encircles the upper mesencephalon, runs medial to the hippocampus and inferior to the optic tract, enters the ambient cistern, then, the quadrageminal cistern and runs to the anterior limit of the calcarine fissure, projecting medially to the occipital lobes. The three branches of the posterior cerebral artery are: the central branch to the brainstem, the ventricular branch to the choroid plexus and the cortical branches. The cortical branches are the inferior temporal arteries, which supply the hippocampus and temporal lobe, the terminal branches (parieto-occipital and calcarine arteries), that supply the medial occipital and parietal lobes and the splenial vessels. The posterior cerebral artery is joined to the middle cerebral artery by the posterior communicating artery to complete the Circle of Willis (Carpenter and Sutin, 1983; Day, 1987). Figure 1 depicts the arterial supply of the lateral surface of the cerebrum from the internal carotid artery and its branches.

Figure 1

The Arterial Distribution of the Cerebral Cortex- Schematic of the arterial distribution of the cerebral hemispheres (Day 1987). ACA=Anterior Cerebral Artery, MCA=Middle Cerebral Artery, PCA=Posterior Cerebral Artery

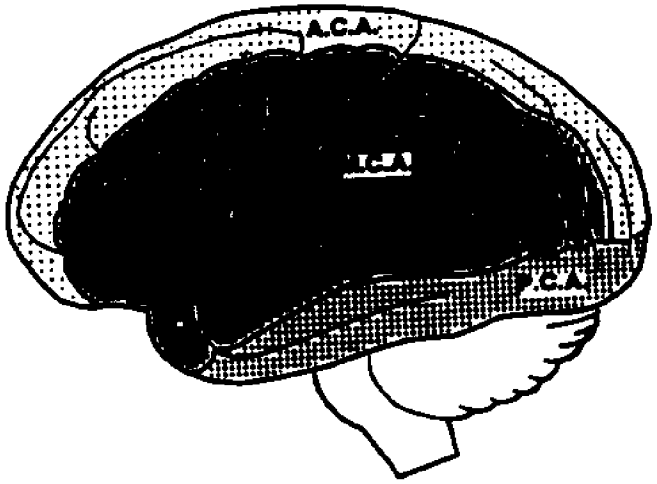


Figure 1

THE COUPLING OF CEREBRAL BLOOD FLOW AND METABOLISM

The precise mechanism responsible for the coupling of neuronal activity, CBF and metabolism has been debated since Roy and Sherrington (1890) hypothesized that the brain possesses an intrinsic regulatory mechanism by which blood flow is varied in accordance with variations in functional activity. This hypothesis has been supported by such studies as Greenberg et al.,(1979), who used the quantitative autoradiographic technique to demonstrate a significant correlation between local CBF, measured with the tracer ^{14}C-iodoantipyrine, and local cerebral metabolic rate of glucose (CMRGl), measured with the tracer ^{14}C-2-deoxyglucose. The topography of activation, elicited by discrete vibrissal stimulation in the rodent, was identical for flow and metabolism. However, in pathophysiological states such as hyperthermia and status epilepticus, CBF increases more than the metabolic requirements (Nilsson et al.,1978). Fox and Raichle (1986) demonstrated greater increases in blood flow than cerebral metabolic rate for oxygen ($CMRO_2$) in the human after somatosensory stimulation. These findings were focal and were not observed in the rest of the brain, but were probably due to a decrease in the oxygen extraction fraction. In contrast, Roland et al.,(1987) recently

demonstrated concommitant increases in rCBF and $CMRO_2$, in response to a visual imagery activation task. The oxygen extraction fraction was unchanged in this study, as was regional cerebral blood volume. The coupling between flow and metabolism, then, is consistent under conditions of cognitive activation, but as described in a subsequent section, the stimulation of certain central nuclear groups can produce an uncoupling of CBF and metabolism.

Several theories have been proposed to account for the flow-metabolism coupling mechanism. The factors previously studied as candidates include PCO_2, extracellular pH and adenosine (Lassen, 1959, Skinhoj, 1966, Rubio et al.,1975). Most recently, Lou et al.,(1987) have proposed that flow and metabolism are regulated by a third factor, neurotransmitter innervation. Increased metabolic demands are met by neurally governed vasodilation. The putative neurotransmitter for flow and metabolic coupling is VIP (Vasoactive Intestinal Peptide). The following sections will describe the evidence for neurotransmitter innervation and putative intrinsic regulators of CBF and metabolism.

NEUROTRANSMITTER AND NEUROPEPTIDE INNERVATION OF THE CEREBROVASCULATURE

Noradrenergic Sympathetic Innervation

Norepinephrine containing nerve fibers form plexi around blood vessels at the base of the brain and on the

cerebral surface and innervate intraparenchymal blood vessels. The terminals of these fibers are located in close proximity to the vessel wall. The arteries of the carotid system are more densely innervated than those of the vertebral system. These fibers originate from the ipsilateral superior cervical ganglion and also contain Neuropeptide Y (Edvinsson et al.,1982). Administration of norepinephrine to pial or cerebral vessels results in a dose dependent vasoconstriction (Edvinsson and Owman, 1974). In the human, vasoconstriction is mediated by $alpha_1$-adrenoreceptors (Edvinsson et al.,1982). Dilation induced by norepinephrine administration to serotonin pre-contracted vessels is inhibited by $beta_1$-adrenoreceptor antagonists (Edvinsson and Owman, 1974). The role of intracerebral noradrenergic systems remains controversial. Fibers from the locus coeruleus are found proximal to the vasculature (Edvinsson et al.,1973). The sympathetic system serves its primary role in the prevention and attenuation of excessive cerebral vasodilation, as in extreme hypertension (Edvinsson et al.,1987a).

Dopamine and dopamine receptor agonists produce contraction of cerebral arteries via activation of alpha-adrenoreceptors and serotonin receptors (Edvinsson et al.,1987a) and increase global CBF and oxygen consumption and increase regional blood flow and glucose metabolism in a pattern that does not entirely overlap with dopaminergic projections (McCulloch, 1979).

Cholinergic Parasympathetic Innervation

The origin of cholinergic innervation is not well established, but recent studies implicate the sphenopalantine ganglion (Hara et al.,1985). Concentrations of ChAT, AChE and acetylcholine and high affinity choline uptake processes have been demonstrated in capillaries, cerebral arteriolar and arterial tissues. Using the ligand [^{3}H] quinuclidinyl benzilate, muscarinic receptors are found in cerebral arteries, but at much lower densities than in cerebral cortex (Edvinsson et al.,1977).

The intracranial distribution of cholinergic fibers is similar to that of adrenergic fibers. Dense plexi are found in the vessels originating from the carotid artery, as compared with the vertebral system, which is less well innervated (Edvinsson et al.,1977). There is anatomical evidence for interaction between cholinergic and noradrenergic innervation. Electron microscopic studies reveal adrenergic and cholinergic membranes in close proximity (20nm), which form axo-axonic contacts. In addition, adrenergic fibers contain cholinergic receptors which inhibit norepinephrine release (Edvinsson et al.,1977). The smooth muscle cells of the vessel walls contain muscarinic receptors that mediate vasodilation with low acetylcholine concentrations and vasoconstriction (Edvinsson et.al, 1977).

Cholinomimetics mediate relaxation in isolated cerebral arteries, which is antagonized by atropine (Edvinsson et

al., 1977).This effect is less prominent in cerebral arteries, relative to peripheral vessels. Duckles (1982) has hypothesized that cholinergic nerve innervation affects vascular function through either modulation of norepinephrine release or blood-brain barrier transport.

In summary, anatomical and biochemical studies demonstrate structural evidence for a role of cholinergic innervation in the regulation of CBF. These observations await corroboration from physiological studies.

Neuropeptide Innervation

The trigemino-cerebrovascular system, originating from the trigeminal ganglion is a source of nerve fibers that innervate cerebral vessels, containing Substance P, Neurokinin A and Calcitonin gene related peptide (CGRP). Relaxation of pial arteries produced by these peptides is not affected by propranalol, cimetidine or atropine (Edvinsson et al.,1987b). This system is thought to be involved in the transmission of pain of intracerebral vascular origin, as in migrane, and protects the brain from vasoconstriction of the large arteries as observed during subarachnoid hemorrhage, by the release of vasodilators (McCulloch, 1982).

VIP and Cholecystokinin (CCK) provide local intracerebral cerebrovascular innervation. The perikarya of both substances are located in close proximity to the innervated vessels. The functional significance of CCK is

unknown, but it may be involved in either microvascular regulation or influence blood-brain barrier permeability (McCulloch, 1984). VIP produces dilation in cerebral arteries and is hypothesized to maintain the coupling of neuronal activity, CBF and metabolism (Edvinsson et al.,1987a).

INTRINSIC CONTROL OF CEREBRAL BLOOD FLOW AND METABOLISM

The mechanism responsible for the control and regulation of CBF are poorly understood. The studies reviewed in this section have examined the role of structures within the central nervous system as intrinsic regulators of CBF.

Two vasodilatory systems have been identified in the brainstem. Miura and Reis (1969) initially characterized the fastigial pressor response. Stimulation of the cerebellar fastigial nucleus produces an elevation of arterial blood pressure, tachycardia and abolition of autoregulation and alterations in motivated behavioral states such as feeding, drinking and attack (Reis et al.,1973). Increases in CBF are observed after stimulation of the rostral ventromedial pole of the fastigial nucleus, without change in arterial pressure and blood gasses. The magnitude of change in cerebral cortex parallels that of hypercarbia induced CBF increase. This response persists after removal of the superior cervical ganglion and transection of either the

spinal cord, the sympathetic trunk or seventh cranial nerve outflow. Corresponding changes in CMRGl were not observed in all areas, for example in cerebral cortex and basal telencephalon areas which do not receive a direct input from the fastigial nucleus. As yet, the pathway mediating the fastigial nucleus response is unknown. Lesions of the Substantia Innominata abolish fastigial nucleus-induced vasodilation in the ipsilateral cerebral cortex, but not elsewhere in the brain. Atropine administration produces a similar inhibition of vasodilation (59% reduction) and acetylcholine release is reduced during fastigial nucleus stimulation (Arneric et al.,1987). Therefore, this response is thought to be mediated by intrinsic neuronal projections.

Stimulation of sites within the dorsal medullary reticular formation produce changes in CBF. Specifically, the area involved is located between the nucleus parvocellularis and nucleus gigantocellularis dorsalis. Increases in CBF, arterial pressure and bradycardia are elicited through electrical stimulation. The increase in rCBF is generalized, persists after lesion of either the cervical sympathetic trunk or the spinal cord and is accompanied by proportional changes in CMRGl. The stimulation effect is not blocked by atropine (Reis, 1984).

A third nuclear complex, the parabrachial nucleus, reduced CBF with and without accompanying change in CMRGl, depending on area of stimulation within the nucleus. CMRGl reduction occurred with stimulation in medial portions of

the nucleus, but no change was observed in lateral portions. Stimulation of the locus coeruleus, adjacent to the parabrachial nucleus, did not affect CBF.

Sites within the central nervous system have been implicated in the control of CBF. It is important to emphasize that these findings have been derived from experimental stimulation studies and generalization to normal and pathophysiological states is not yet possible. In addition, the exact pathways and their neurochemical nature have not yet been characterized.

Consideration of the issues raised in this section is important for the evaluation of scopolamine's effects on neural function. There are several possible mechanisms for the alterations induced by scopolamine. First, scopolamine may affect the cholinergic innervation of the vasculature directly and may affect the arterial territories supplying the cortex differentially. Second, scopolamine may interact with neuropeptide function to produce effects on perfusion and metabolism. Third, scopolamine may act at subcortical sites, such as the fastigial nucleus, to modify rCBF and metabolism. These possibilities will be evaluated in the discussion.

RATIONALE

Study 1: Scopolamine Dose-Response

1. The specificity of scopolamine's effects on cognitive function and rCBF were established by the administration of two doses of scopolamine (6.1 and 7.3µg/kg,IV) in a between subject design.
2. The topography of scopolamine effects in the cerebral cortex were examined and the extent to which this overlaps with neurotransmitter receptor topography assessed.
3. The cognitive deficits produced by scopolamine were evaluated and the relation of these deficits to regional cortical change examined.
4. The significance of the neuropsychological and perfusion deficits were assessed relative to other putative neurochemical interactions and to neurodegenerative diseases.

Study 2: Scopolamine Reversal by Physostigmine/Neostigmine

1. In order to distinguish between central versus peripheral-vascular effects of scopolamine, physostigmine and neostigmine, central and peripheral cholinesterase inhibitors, respectively, are administered to antagonize the effects of scopolamine.
2. The relative contribution of cholinergic mechanisms to the rCBF and cognitive effects of scopolamine was

determined by evaluating the efficacy of cholinesterase inhibitors in reversing these deficits.

3. The neuropsychological deficits produced by scopolamine were further characterized and evaluated relative to the topography of cortical deficits.

METHODS

Subjects

Subjects were recruited through advertisements placed at various locations within the Columbia Presbyterian Medical Center and in the campus newspaper. In a brief telephone interview, the study was described and the potential subject questioned as to significant medical history and previous radiation exposure. If subjects expressed interest in participating in the study and did not report medical contraindications, the subjects were invited to the laboratory for a formal screening. At this time, subjects underwent an extensive interview of past medical history, medical and neurological examination, electrocardiogram, urinalysis and laboratory blood testing. Specific exclusion criteria are shown in Appendix 1. In Study 1, one subject was rejected from participation due to a history of asthma. In Study 2, three subjects chose not to participate after the screening session, one subject was rejected for questionable psychiatric history, another for low body weight and questionable nutritional status and a third for history of a depressive episode and suspected cardiac arhythmia.

The procedures for both studies were approved by the Institutional Review Board at the New York State Psychiatric Institute and all subjects provided informed consent before beginning the study (for the consent form see Appendix 2).

Subjects were reimbursed at the rate of ten dollars per hour for participation in the study. None of the subjects were currently taking medication. Several subjects were excluded from the analysis: one subject was given too low a dose of neostigmine, the time course of procedures was disrupted in the case of another subject, who experienced gastroenterological discomfort and a third subject showed monexponential flows in one study, questioning the integrity of the rCBF data.

Study 1 included fifteen subjects, ten received high dose scopolamine (6 males/4 females, mean age 28.9, range 20-48 years) and five low dose scopolamine (4 males/1 female, mean age 27.4, range 20-35 years). Nine subjects completed both conditions of the second study (7 males, 2 females mean age 25.3, range 20-34).

Side Effects

The majority of subjects in both studies reported dry mouth after scopolamine administration and were given water. One subject in the scopolamine/neostigmine condition reported blurred vision. Otherwise, all subjects could be easily engaged in the neuropsychological testing and none of the subjects appeared drowsy to the extent that it would interfere with testing.

All subjects who participated in Study 1 completed the procedures without complication. For the second study, two subjects showed significant reductions in blood pressure

after neostigmine administration and were given atropine (0.5mg,IV) to reverse this effect. These two subjects were excluded from the analysis and the dose of neostigmine was lowered for the remaining subjects in the study (as described in the section concerning drug and dosage selection).

Procedures

Neuropsychological Assessment

The cognitive assessment procedures in Study 1 consisted of the Digit Span subtest of the Wechsler Adult Intelligence Scale-Revised (Wechsler, 1981) and the Selective Reminding Test, 12 item version (SRT, Buschke, 1973). Several parameters of the SRT were analyzed: total recall, long term storage, long term retrieval, consistent long term retrieval (an example of the SRT is given in Appendix 3 and procedures for administration in Table 3). Study 2 included these procedures with the addition of other tests of attention and vigilance, non-verbal memory and response inhibition (procedures, duration of each test and rationale are shown in Table 3). The SRT and Visual Reproduction subtest were administered at the beginning of each testing session, so that delayed recall could be assessed subsequently. In both studies, the order of remaining tests was randomized and counterbalanced, to account for potential time dependent drug effects. In the case of the SRT and Visual Reproduction subtest of the

TABLE 3: NEUROPSYCHOLOGICAL ASSESSMENT PROCEDURES FOR SCOPOLAMINE STUDY 2

Testing at Baseline and Post-Scopolamine and Physostigmine/ Neostigmine Administration

Selective Reminding Test (12-item version,Buschke, 1973)

Time: 10 -15 minutes

Rationale: This is a test of the immediate and delayed recall of verbal material. It is sensitive to effects of normal aging, Alzheimer's disease and scopolamine's effects (Larabee et al., 1986; Buschke and Fuld, 1977; Stern et al., 1988).

Description: The examiner reads a list of 12 words and asks the subject to repeat each word immediately after. The subject is then given 60 seconds to recall the list. If the subject omits words from the list, the examiner reminds the subject of the words not mentioned and than asks the subject to recall the entire list. This procedure continues until the subject can recall all 12 words on two consecutive trials. Subjects are not provided with verbal cues to assist recall, but are encouraged to continue with the test if they begin to fatigue or become distracted. At the end of the entire testing session, subjects are asked to recall the words.

Parameters Derived:

Total Recall- Total number of words recalled per trial, regardless of whether a reminder was provided.

Table 3-Continued

Long Term Storage-After a word is retrieved on two consecutive trials without a reminder, the word is considered to be entered into storage. The word does not have to be mentioned after the two trials in order to receive credit.

Long Term Retrieval-After a word is retrieved on two consecutive trials without a reminder, the word meets criteria for long term retrieval. The word does must be mentioned on the trial in order to receive credit.

Consistent Long Term Retrieval- Once a word is consistently retrieved on every trial until the termination of testing, it meets criteria for consistent long term retrieval.

Wechsler Memory Scale-Visual Reproductions Subtest (Wechsler, 1955)

Time: 5 minutes

Rationale: This test involves the immediate and delayed recall and recognition of geometric figures A non-verbal, visuo-spatial memory test was included to evaluate whether non-verbal memory is impaired, relative to verbal memory.

Description: Subjects are presented with a geometric figure for 30 seconds. The figure is removed and subjects are asked to draw it from memory. After the three figures are presented, the subjects are asked to choose the correct figure from four choices presented visually. Both recall and

Table 3-Continued

recognition of the figures is performed at the end of the testing session.

Wechsler Adult Intelligence Scale-Revised, Digit Span Subtest

(Wechsler, 1981)

Time: 5 minutes

Rationale: This test measures attention and sequencing processes, which would interfere with memory performance if disrupted (Lezak 1983).

Description: Subjects are asked to repeat a sequence of digits after the examiner, in the same order as they are read. The sequences consist range from three to eight numbers, two trials of each. Testing is terminated when the subject incorrectly repeats two consecutive trials, within a pair. Then, the subject is asked to repeat the digit sequence backwards, ranging from 2 to 7 digits. Testing is again terminated when the subject incorrectly repeats two consecutive trials, within a pair.

Stroop Test (Stroop, 1935)

Time: 6 minutes

Rationale: This test is sensitive to frontal lobe dysfunction in assessing perceptual interference and response inhibition

Description: The subject is presented with three visual arrays in sequence: an array of colors (red,green, blue), names of colors in black print (red,blue and green) and the

Table 3-Continued

name of a color written is a different color. In each case, the subject is asked to read through the array as quickly and as accurately as possible. The examiner records total time to completion and number of errors.

Cancellation Tests- Numbers, Shapes, Trigrams

Time: 3 minutes

Rationale: Measures visual scanning, vigilance and attention.

Description: Subjects are asked to scan the visual array and cross out all of the number sixes, diamonds or TMX trigrams for the number, shape, trigram arrays, respectively. The examiner records total time to completion and number of omissions.

Controlled Oral Word Association Test (CFL,FAS; Benton et al., 1983)

Time: 4 minutes

Rationale: Sensitive to frontal lobe dysfunction and showed impairment in Alzheimer's disease (Benton 1968, Rosen 1980)

Description: The subject is given a letter of the alphabet and is asked to name as many words as possible beginning with that letter in 60 seconds. Subjects are instructed not to use proper nouns and the examiner records the words retrieved.

Table 3-Continued

Category Naming

Time: 1.5 minutes

Rationale: Sensitive to memory impairments in Korsakoff's syndrome and Alzheimer's disease (Talland, 1965; Rosen, 1980)

Description: The subject is given a category (animals or foods) and asked to name as many items as possible, belonging to the category. Testing is terminated after 60 seconds.

Testing After the Clinical Effects of Physostigmine/ Neostigmine - Residual Scopolamine Effect (89 minutes after scopolamine infusion)

Selective Reminding Test

Wechsler Memory Scale, multiple forms of the tests were available, previously shown to be equivalent (Hannay, et al., 1985; Wechsler, 1955).

rCBF Procedures

Instrumentation

rCBF was measured using the ^{133} Xenon inhalation technique, with a commercial system (Novo Cerebrograph 32c). This technique is based upon the Kety-Schmidt (1945) principle that the rate of uptake/clearance of an innert diffusible gas is proportional to blood flow in tissue. The clearance of a radioactive tracer is monitored by extracranial detectors, thereby providing a measure of tissue clearance as an index of rCBF.

A face mask is positioned over the subjects nose and mouth to administer the tracer, ^{133} Xenon (Mediphysics). This inert, diffusible tracer is inhaled in a mixture of room air for one minute. After inhalation, the gas passes from the lungs via the pulmonary circulation to the heart and then to the brain. In the brain, ^{133} Xenon diffuses through the tissue and is not metabolized.

A leak detector signals the escape of ^{133} Xenon from the mask, should the mask seal loosen. The mask contains a thin catheter attached to a scintillation detector to monitor ^{133} Xenon concentrations in expired air. A respired air curve is reconstructed, based upon sampling at 5/16 seconds. The air curve is illustrated in Figure 2. This

Figure 2

Example of an Air Curve, representing sampling (every 5/16 second) of ^{133}Xenon in respired air. This provides end tidal values which accurately estimate arterial Xenon concentrations in the absence of pulmonary disease.

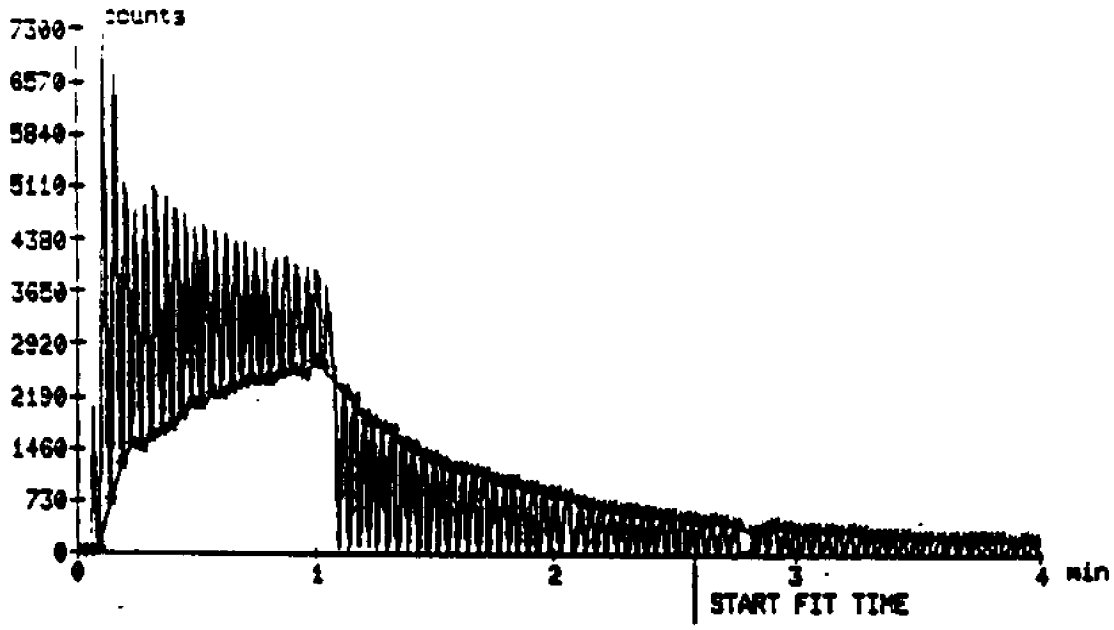

counts
7300
6570
5840
5110
4380
3650
2920
2190
1460
730
0
0
1
2
3
4
min
START FIT TIME

Figure 2

provides end tidal values which accurately estimate arterial ^{133} Xenon concentrations, in the absence of pulmonary disease. In addition, the air curve is used to correct the regional detector curves for tracer recirculation. The cerebrograph contains both CO2 and O2 monitors for expired air.

Regional clearance curves are derived from the monitoring of tracer diffusion by thirty-two collimated scintillation detectors, each of which contain a Sodium Iodide crystal (3/4" D X 3/4" H) and a photomultiplier tube. The detectors are set to scintillate when collision occurs with gamma particles emitted by ^{133} Xenon. The detectors are mounted perpendicular to the skull, in a helmet specifically designed to minimize 'cross-talk' or intra-hemispheric contamination of homologous brain regions (Prohovnik et al., 1983). Relative detector placement is shown in Figure 3. The helmet is positioned by alignment with light markers that indicate the cantho-meatal line to insure reproducibilty of placement. The resolution of this technique is 20-30mm at the surface of the cerebral cortex. Other structures are imaged indirectly, as they affect cortical function.

Calculation of rCBF

Count rates are integrated at five second intervals, from which a curve is reconstructed for each regional detector location. Before calculating flow, the curves are corrected for background and remaining activity. Background

Figure 3

Detector Localization-Detector positions on the dorsolateral surface of the cerebral cortex relative to sulcal landmarks, for the Novo Cerebrograph (32c). F=Frontal Cortex, C=Central Cortex, T=Temporal Cortex, P=Parietal Cortex, O=Occipital Cortex, R=Right Hemisphere, L=Left Hemisphere.

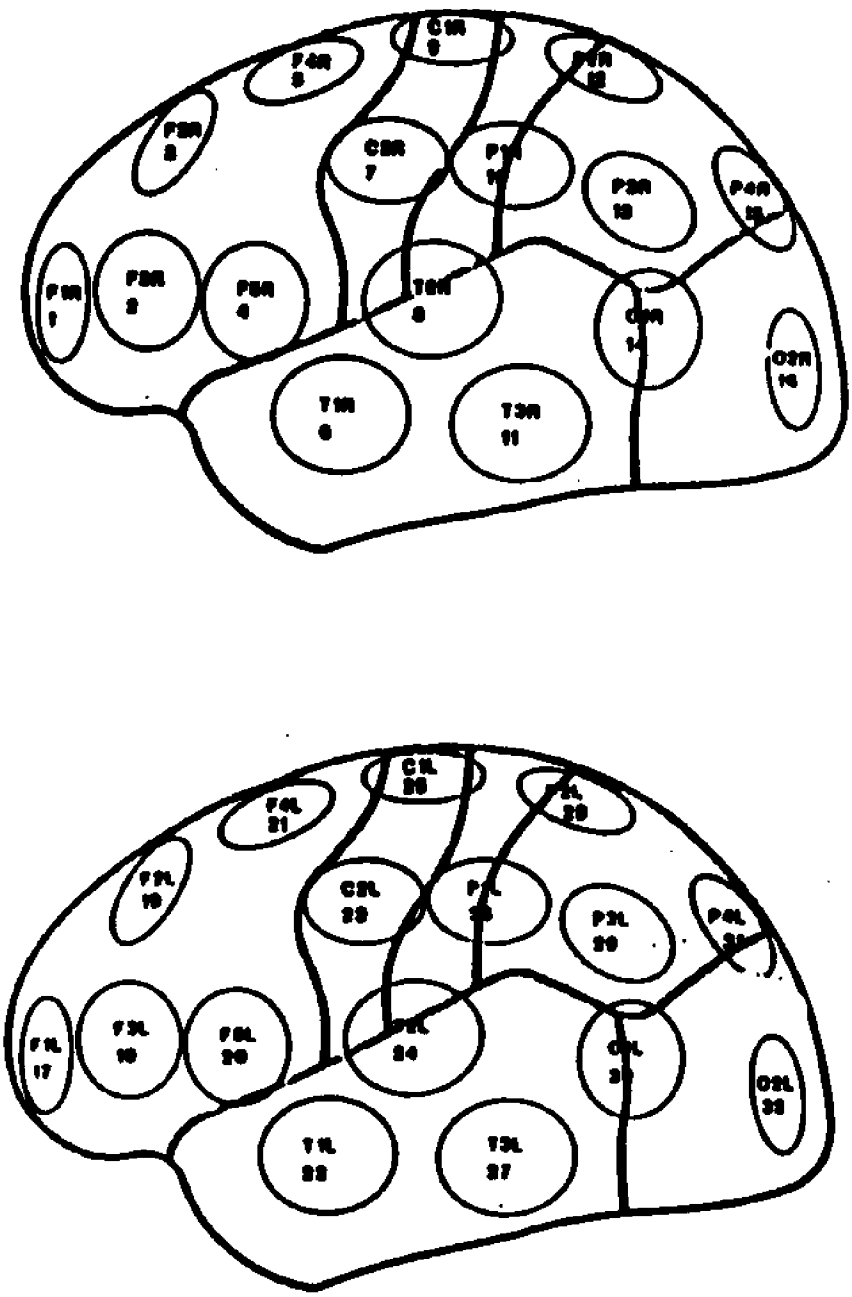


Figure 3

activity is calculated for thirty seconds prior to the initial measurement, the mean of which is subtracted for correction of the detector curves. For repeated determinations, a remaining activity correction is applied. A monexponential decay curve is derived and the remaining activity count rate values are subtracted from the detector curve data.

A six unknown, dual artifact model is applied to the corrected data (Prohovnik et al., 1983). This analysis yields the size and clearance rate for the fast (grey matter, p1,k1) and slow (white matter, p2,k2) compartments and the estimation of two artifacts, which will be discussed in the next section. Flow is calculated from these coefficients. Grey matter flow is more accurately estimated, white matter flow being contaminated by extracerebral tissues. Figure 4 depicts an example of an detector curve, with the compartments and artifacts indicated.

For the purposes of these studies, the f1 parameter, a measure of grey matter flow will be reported. This is a fully quantitative index of gray matter flow (in ml/100g/minute, Prohovnik et al., 1983). Within normal to high flow ranges, this index is most sensitive (Risberg and Prohovnik, 1981). The f1 is calculated as shown:

$$f1 = k1 * lg * 100$$

In this equation, lg represents the partition coefficient for ^{133} Xenon, a constant calculated relative to hemoglobin

Figure 4

Example of a Detector Curve. The fast and slow compartments, grey and white matter respectively, and the arterial and air-passage artifacts are depicted.

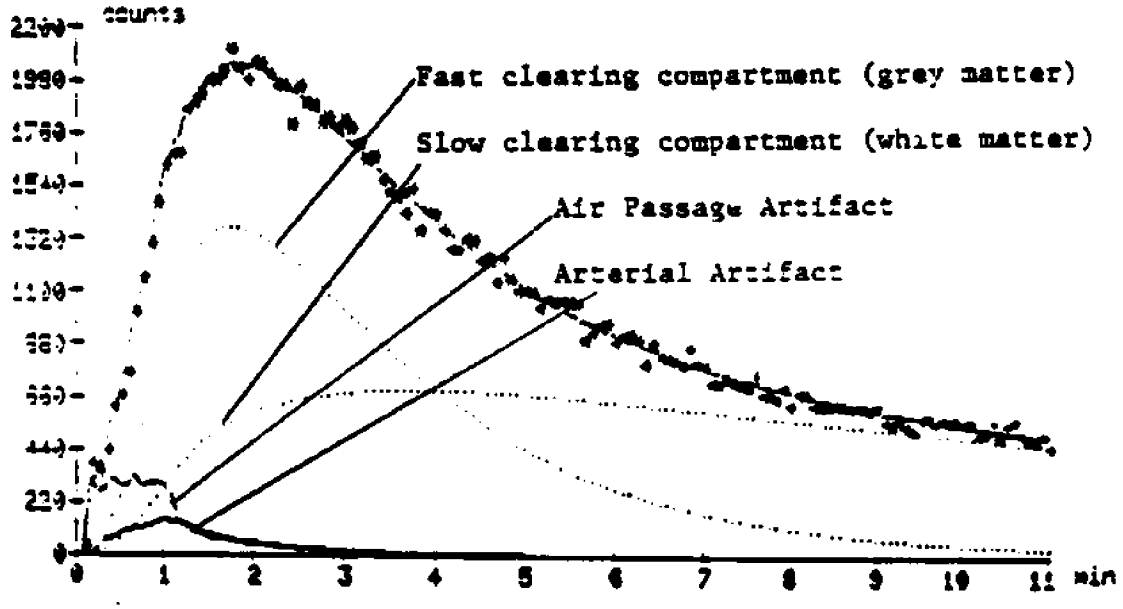

counts
Fast clearing compartment (grey matter)
Slow clearing compartment (white matter)
Air Passage Artifact
Arterial Artifact
0
1
2
3
4
5
6
7
8
9
10
11
min

Figure 4

levels (g/100ml). The partition coefficient is the ratio of the concentration of ^{133} Xenon in blood to that in the tissue, at steady state conditions. k1 is the clearance rate of the fast compartment.

Artifact Correction

Two artifacts are most prominent with the ^{133} Xenon inhalation technique. The air-passage artifact refers to counts from ^{133} Xenon in respiratory air due to presence of ^{133} Xenon in the nasopharyngeal passages. The first attempt to eliminate this factor was to begin curve fitting (delay the start fit time) when ^{133} Xenon concentrations in respiratory air were reduced to 20% its maximum value, 1.5 minutes after the beginning of inhalation (Obrist et al., 1975). Limitations to this approach include the elimination of the initial portion of the curve from analysis, thereby decreasing the number of samples. In addition, changes in respiration patterns can significantly alter start fit time. A second artifact involves the presence of ^{133}Xenon in the vasculature, the distribution of which parallels the location of extracranial arteries (Prohovnik et al., 1983). Both the air- passage and vascular artifacts contaminate flow estimates in inferior frontal and anterior temporal detector locations. The equation for bicompartmental analysis with dual artifact correction is :

$$H(t) = [P1 * EXP(-K1*t) + P2 * EXP(-K2*t)] \ \$A(t) + P3* Av(t) + P4* A(t)$$

In which: H(t) represents the observed detector curve, a(t) the end tidal curve, Av(t), the average air curve, P3, size of the air passage artifact, P4, size of the vascular compartment.

Subsequent validation for the vascular artifact correction has been provided by data from both animal models and human clinical material (Prohovnik et al., 1987).

PCO2 Correction

Hypercapnia (elevation in PCO2 after CO2 inhalation) has been demonstrated to produce vasodilation and increase CBF, while hypocapnia causes vasoconstriction and decreased blood flow (Lassen, 1959). Therefore, correction of CBF for change in PCO2 has been employed. In a study of hypercapnic reactivity in normals (Maximillian et al., 1980), a change in PCO2 of 10.55mmHg was shown to produce a 34% increase in flow, yielding a correction factor of 2.3 ml/mmHg. This is consistent with other reported correction factors (eg. Obrist et al., 1975) and is used routinely in this laboratory. To eliminate variance due to inter- and intra-subject variability in PCO2, for Study 1 and Study 2 the subjects' flow values were corrected to the mean PCO2 at baseline for all subjects. For Study 1, this value is 40.3 mmHg and for Study 2, 40.6 mmHg.

Conditions of Study

The studies in this report were conducted in a resting, unstimulated state, in a dark room. Subjects are instructed

to relax, with eyes closed, ears unoccluded. Once placed into the helmet, the mask is placed on the subjects' nose and mouth and they are given several minutes to adjust their respiratory pattern. Ambient room noise consists of cooling fans. Pulse and blood pressure were continuously monitored throughout the study. Statistical analyses utilize the pulse and blood pressure values that are obtained immediately following the rCBF study.

Drug Selection and Pharmacokinetic Considerations

The available cholinergic antagonists and cholinesterase inhibitors must be used with caution in humans. Many such agents affect cholinergic transmission irreversibly (such as hemicholinium, which blocks choline uptake and the irreversible cholinesterase inhibitors such as diisopropyl fluorophosphate and soman). The remaining substances have narrow therapeutic windows for central and peripheral effects and are highly toxic. Peripheral cholinergic metabolites and muscarinic receptors are difficult to assay and available measures only weakly correlate with central cholinergic activity (Pomara and Stanley, 1986). To insure adequate absorption of the drug, intravenous administration is preferable, given that oral administration of such agents results in poor absorption into the blood stream (Weiner, 1980).

The design of these studies was influenced by such considerations. Scopolamine hydrobromide (Elkins-Sinn, Inc.)

was chosen as a muscarinic cholinergic receptor antagonist. Two doses of scopolamine, 6.1 µg/Kg (low dose) and 7.3 µg/Kg (high dose) were administered intravenously to insure absorption. The doses were selected based upon the existing literature (as reviewed in Table 2), suggesting that higher doses of the drug produce more disruptive peripheral side effects (dry mouth, mydriasis,urinary retention, emesis, increased intraocular pressure), sedation and attentional deficits, which would confound interpretation of results.

Scopolamine functions as a competitive antagonist of muscarinic cholinergic receptors and competes with acetylcholine to bind to both the high affinity presynaptic (M1) muscarinic receptor and the low affinity postsynaptic (M2) receptor.As described previously, postsynaptic receptors are distributed diffusely throughout the cerebral cortex and presynaptic receptors are located in such areas as Nucleus Basalis of Meynert, the area in which the cell bodies of cortical cholinergic projections are found (Cortes et al., 1987). Therefore, scopolamine can potentially exert its effects in both areas.

The scopolamine induced antagonism of acetylcholine can be reversed by administering an agent which increases the concentration of acetylcholine at the receptor site. This can be accomplished by the use of a cholinesterase inhibitor which inhibits the breakdown of released acetylcholine. Such agents are used clinically to treat anticholinergic overdose (Feldman and Quenzer, 1984). The available acetylcholine can

act at the unoccupied muscarinic receptor sites to activate the remaining receptors and reverse the effects of cholinergic antagonism.

N-methylscopolamine, an anticholinergic which does not cross the blood brain barrier, is unavailable for parenteral use in the United States and could not be used as a control for the peripheral effects of scopolamine. Therefore, Study 2 was designed to control for central versus peripheral effects by reversing scopolamine induced deficits with physostigmine salicylate (antilirium, Forrest Pharmaceuticals) and neostigmine methylsulfate (prostigmin, Hoffman LaRoche, Inc.), central and peripheral cholinesterase inhibitors, respectively. The dosages chosen were relatively modest (physostigmine 0.022mg/kg,IV; neostigmine 0.011mg/kg,IV). Previous studies have shown that these two doses are equivalent because neostigmine produces more potent side effects than physostigmine in equal doses (Risch et al., 1986). To avoid serious side effects such as seizures, these agents must be slowly infused, preferably over a ten minute period. After two subjects showed declines in blood pressure after neostigmine, the dose was reduced to 0.007mg/kg,IV. Five subjects received "high dose" neostigmine and four "low dose" neostigmine. Such inter-subject variability in response to scopolamine and physostigmine has been reported by Serby et al. (1984) who observed significant cognitive and cardiovascular impairment in several subjects within a larger sample. Such response

could not be attributed to age, sex or body weight differences. Due to the potent side effects of cholinesterase inhibitors, these agents could not be administered independently of an anticholinergic to assess the effects of cholinesterase inhibition separately. The design of Study 2 allows dissociation of central from peripheral effects of scopolamine given the limitations inherent in cholinergic manipulation in humans.

A final consideration is the timing of procedures. The purpose of the first study was to determine the time course of scopolamine's effects, since there was no available data establishing the time course of scopolamine's effect on brain metabolism in the human. A recent study, using a radioreceptor assay and autonomic indices showed that the peak activity of scopolamine occurred at 45-60 minutes after administration of 0.02mg/kg,PO (Markkanen et al., 1987). In the case of physostigmine and neostigmine, the duration of action is better established and peak activity occurs from 45-60 minutes after administration (Forest Pharmaceuticals, Hoffman-La Roche, Inc.). The neuropsychological battery was limited to 30 minutes, so that the rCBF and testing would fall within the range of clinical effects of these compounds.

Design of Study

The timetable of procedures for both studies is shown in Tables 4 and 5. The purpose of Study 1 was to establish a dose response and time course of the effects of scopolamine.

TABLE 4: TIMETABLE OF PROCEDURES FOR SCOPOLAMINE STUDY 1

-35 minutes	Neuropsychological Testing (10 Minutes)
-23 minutes	Baseline rCBF begins (11 minutes)
-2 minutes	Baseline rCBF ends
0 minutes	Scopolamine administered
+ 2 minutes	rCBF 2 begins (16 minutes)
+18 minutes	rCBF 2 ends
+20 minutes	rCBF 3 begins(16 minutes)
+36 minutes	rCBF 3 ends
+38 minutes	Neuropsychological Testing begins (10 minutes)
+50 minutes	rCBF 4 begins(16 minutes)
+66 minutes	rCBF 4 ends

TABLE 5: TIMETABLE OF PROCEDURES FOR SCOPOLAMINE STUDY 2

-45 minutes	Neuropsychological Testing (30 minutes)
-13 minutes	Baseline rCBF begins (11 minutes)
-2 minutes	Baseline rCBF ends
0 minutes	Scopolamine administered
+2 minutes	Run 2 begins (16 minutes)
+16 minutes	Physostigmine/Neostigmine infusion begins (10 minutes)
+18 minutes	Run 2 Ends
+21 minutes	Run 3 Starts(16 minutes)
+26 minutes	Physostigmine/Neostigmine Infusion Ends (note: this time coincides with the end of the background activity measure, prior to the rCBF recording)
+37 minutes	Run 3 Ends
+39 minutes	Neuropsychological Testing begins (30 minutes)

Table 5-Continued

+71 minutes	Run 4 Begins(16 minutes)
+87 minutes	Run 4 ends
+89 minutes	Neuropsychological Testing begins (10 minutes)

For both studies, all subjects were told to fast for three hours prior to the study. Tobacco and coffee were not permitted in the hour preceding the study. Baseline rCBF and neuropsychological testing was conducted, followed by administration of either low or high dose scopolamine. Neuropsychological testing was repeated at approximately forty minutes after scopolamine administration. rCBF measurements were conducted at approximately 5 minutes, 25 minutes, and 50 minutes after scopolamine administration. These times reflect the intervals at which rCBF is monitored, with the inclusion of 5 minute monitoring for estimation and correction of remaining background activity and time required for subject preparation.

In the case of Study 2, all participants were blind to the drug condition of the subject, except the study nurse, who prepared the drugs for injection. Subjects were studied on two separate occasions, separated by approximately one week. Neuropsychological testing and rCBF measurements were performed at baseline, followed by administration of the high dose of scopolamine.

Two minutes prior to the end of the second rCBF measurement, the infusion of physostigmine or neostigmine was begun, lasting for ten minutes. The third rCBF study was conducted during the peak rCBF effect of scopolamine (as determined by the results from study 1) and the peak clinical effect of physostigmine/ neostigmine. Neuropsychological testing was repeated, immediately

followed by a fourth rCBF study. A final SRT was administered after the fourth rCBF to assess the residual effects of scopolamine, after termination of the clinical effects of the cholinesterase inhibitors.

Statistical Analysis

For Study 1, repeated measures analysis of variance (Statistical Package for the Social Sciences, SPSS-X, 1987) was performed with dose (low/high-two levels) as a between subjects factor and time relative to scopolamine infusion (baseline, acute scopolamine, 20 minute post-scopolamine-3 levels) as a within subjects repeated measures factor. It is important to note that for the variables with greater than two levels, multivariate F tests are computed and for the remaining variables, univariate F tests are computed. The data from the fourth rCBF study were incomplete due to technical problems and were not used in statistical analyses, but mean values will be reported. Global grey matter flow was analyzed and separately, regional flow at each detector location. The regional analyses showed that the maximal flow change occurred in the anterior five detectors. In order to increase statistical power and reduce the 32 detectors for each condition, the five anterior detectors were grouped into a frontal region and the effects compared to the remaining eleven detectors for each rCBF study. The main effect of hemisphere (right/left) was not significant in initial analyses and was eliminated

subsequently. The digit span and SRT were also subject to repeated measures analysis of variance with dose (low/high - two levels) as a between subjects factor and time (pre/post -two levels) as a within subjects factor. Pearson product moment correlations were computed between change in rCBF and change in cognitive function.

The data for Study 2 were also analyzed by repeated measures analysis of variance. For the rCBF data, analyses included the main effects of drug condition (physostigmine/ neostigmine-2 levels), time (baseline, scopolamine, scopolamine + physostigmine/neostigmine, washout-4 levels). To assess the potential effects of neostigmine dose, an additional between subject factor was added to evaluate the effects of high/low dose neostigmine. As in the previous study, the detectors were divided into anterior/posterior (region factor-2 levels), again based on the observation of maximal flow change in the anterior five detectors. Cognitive tests were analyzed similarly, with drug condition (physostigmine/ neostigmine-2 levels) and time (pre/post-2 levels) as within subject factors. Post-hoc comparisons were performed by Student's T-test, to evaluate the relevant interactions.

RESULTS OF STUDY 1: SCOPOLAMINE DOSE-RESPONSE

Autonomic Indices

The means, standard deviations and repeated measures analysis of variance results for autonomic variables are shown in Appendix 4. A marginally significant increase in PCO2 was observed (F [2,12]= 3.16, p=.08), maximal at 25 minutes post-infusion. As described in the Methods section, this necessitated a correction for PCO2.

Diastolic blood pressure was unaffected, while systolic blood pressure shows a significant dose by time interaction (F [2,12]= 4.37, p=.04). Systolic blood pressure decreased in the low dose group and increased in the high dose group. Post-hoc t-tests indicate that these between group differences are significant at 5 and 25 minutes post scopolamine (t [14]=-3.83, p=.002; t [14]=-2.29, p<.001, respectively). The results for pulse reveal significant main effects for time (F [2,12]= 3.88, p=.05) and dose (F [2,12]= 8.09, p=.01), but a non-significant interaction for these variables. Thus, the groups differed in pulse throughout the studies, the pulse rate of the high dose group was higher. Pulse increased at 5 minutes and decreased at 25 minutes post-infusion, for both groups combined.

Therefore, scopolamine increased PCO2 at 25 minutes post-scopolamine. Systolic blood pressure increased at 5 minutes and decreased at 25 minutes post-infusion while diastolic blood pressure was unaffected. Similarly, pulse

increased at 5 minutes and decreased at 25 minutes post-infusion.

Neuropsychological Measures

For the cognitive variables, the means and standard deviations are shown in Table 6 and the repeated measures analysis of variance results are shown in Appendix 5. For both groups, performance on the SRT declined significantly after scopolamine administration as revealed by the significant main effect of time ($F\ [1,13]=117.76$, $p<.001$). These findings are shown in Figure 5. Multivariate analysis of the SRT parameters revealed a significant effect for scale ($F\ [3,11]=32.35$, $p<.001$) and time by scale interaction ($F[3,11]=15.00$, $p<.001$). This indicates a differential magnitude of decline across scales, as depicted in Figure 6. Post hoc t-tests to evaluate the interaction effect showed that the magnitude of decline in total recall was less than that of decline in long term retrieval ($t[14]=6.45$, $p<.001$), long term storage ($t\ [14]=5.80$, $p<.001$) and consistent long term retrieval ($t\ [14]=4.17$, $p=.001$). Although comparisons among long term storage, consistent long term retrieval and long term retrieval were non-significant, the percent change values indicated that the consistent long term retrieval parameter was most affected. Therefore, SRT performance was significantly impaired by both doses of scopolamine. However, there was a trend toward greater impairment with

Table 6 SCOPOLAMINE STUDY 1 : EFFECTS ON NEUROPSYCHOLOGICAL FUNCTION

Means and Standard Deviations for the Selective Reminding Test

Low Dose			High Dose		
Baseline	30 minutes Post-Drug	%Δ	Baseline	30 minutes Post-Drug	%Δ
Total Recall					
128.4±7.9	104.4±9.4	19%	121.5±12.0	88.1±20.4	27%
Long Term Retrieval					
122.8±10.9	87.2±17.6	29%	114.3±17.1	66.5±29.1	42%
Consistent Long Term Retrieval					
112.4±18.4	57.2±11.5	49%	92.2±32.3	39.4±33.5	57%
Long Term Storage					
128.4±8.4	93.8±16.4	27%	120.8±13.5	73.5±25.7	39%

Means and Standard Deviations for Digit Span

Low Dose			High Dose		
Baseline	30 minutes Post-Drug	%Δ	Baseline	30 minutes Post-Drug	%Δ
Digit Span Forwards					
7.8±2.6	8.8±0.8	1.13%	8.6±1.8	7.7±1.4	10%
Digit Span Backwards					
6.4±0.9	6.6±2.3	1.03%	7.0±2.1	7.4±1.4	1.06%

Figure 5

Scopolamine Study 1: Effects on the Selective Reminding Test -Total Recall Score. Results for low and high dose groups at baseline and 25 minutes post-scopolamine infusion. For both groups, performance on the SRT declined significantly after scopolamine administration as revealed by the significant main effect of time (F [1,13]=117.76, $p<.001$).Note that the magnitude decline is greater for the high dose relative to the low dose group.

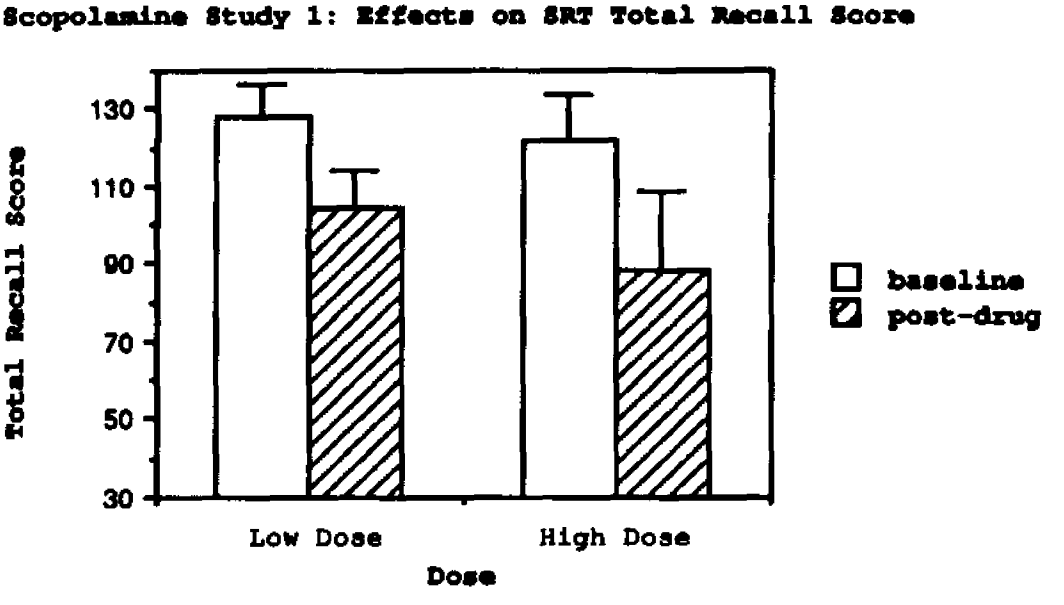
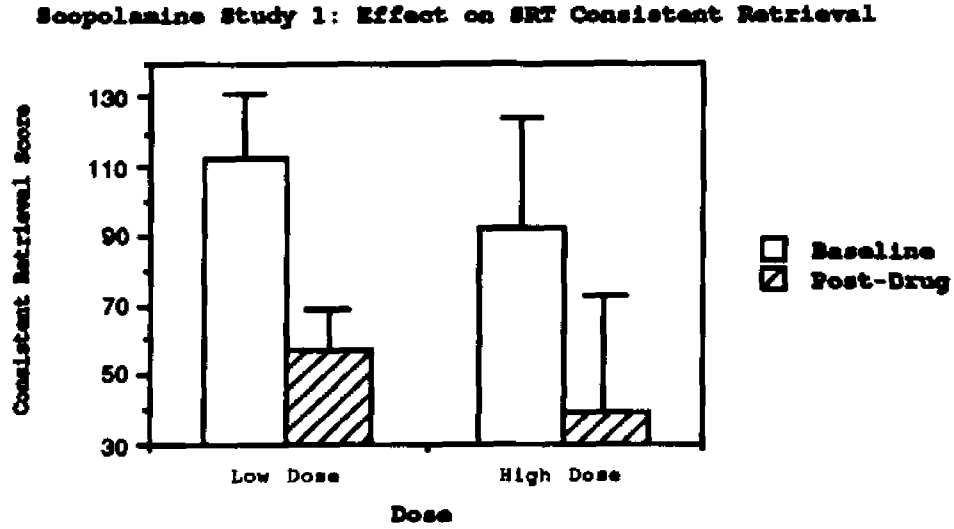
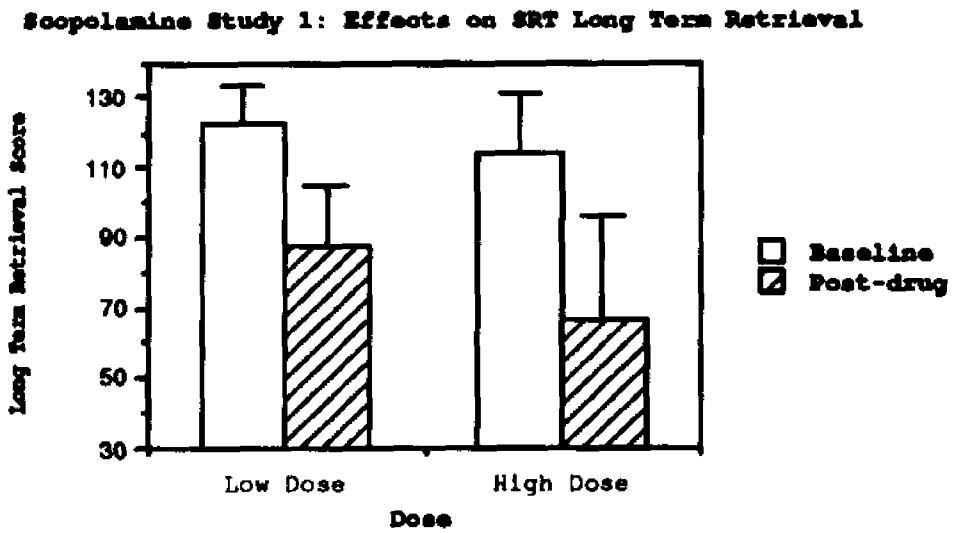

Scopolamine Study 1: Effects on SRT Total Recall Score
Total Recall Score
130
110
90
70
50
30
Low Dose
High Dose
Dose
baseline
post-drug

Figure 5

Figure 6

Scopolamine Study 1: Effects on the Selective Reminding Test Parameters. Results for low and high dose groups at baseline and 25 minutes post-scopolamine infusion. Multivariate analysis of the SRT parameters revealed a significant effect for scale ($F [3,11]=32.35$, $p<.001$) and time by scale interaction ($F[3,11]=15.00$, $p<.001$).


Scopolamine Study 1: Effect on SRT Consistent Retrieval
Consistent Retrieval Score
130
110
90
70
50
30
Low Dose
High Dose
Dose
Baseline
Post-Drug


Scopolamine Study 1: Effects on SRT Long Term Retrieval
Long Term Retrieval Score
130
110
90
70
50
30
Low Dose
High Dose
Dose
Baseline
Post-drug

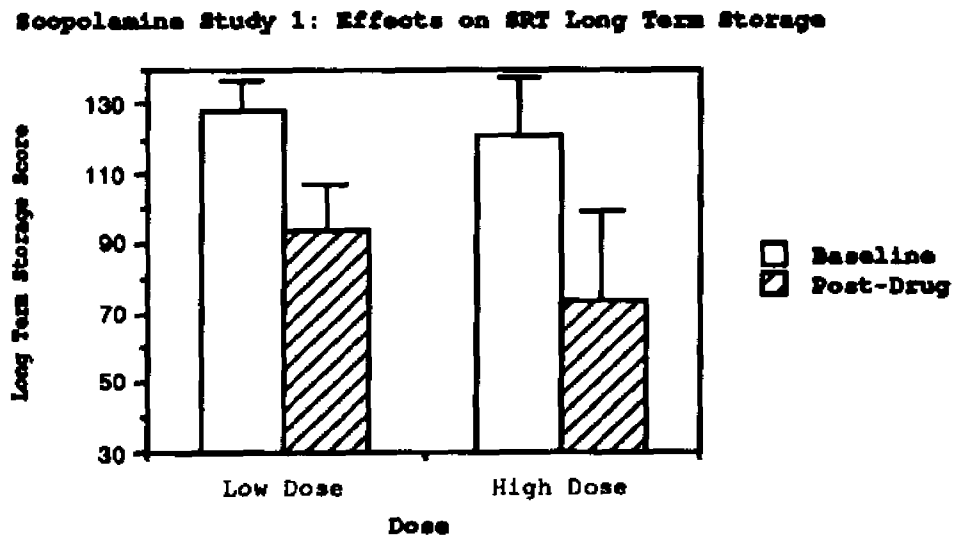

Scopolamine Study 1: Effects on SRT Long Term Storage
Long Term Storage Score
130
110
90
70
50
30
Low Dose
High Dose
Dose
Baseline
Post-Drug

Figure 6

high dose scopolamine. The aspect of SRT performance most affected was the consistent long term retrieval parameter.

There was no significant effect on digit span backward, while digit span forward reveals a significant dose by time interaction. Post-hoc t-tests for this interaction show that the low dose group increased in number of digits recalled, while the high dose groups showed a slight decrement (t [14]=2.33, p=.04).

rCBF Results

The means and standard deviations for anterior and posterior rCBF values, the anterior/posterior ratio and global flow values are shown in Table 7. Repeated measures analysis of variance results (as shown in Appendix 6) indicated significant main effects for time (F [2,12]= 20.05, p<.001) and region (F [1,13]= 88.35, p<.001) and significant dose by time (F [2,12]= 19.48, p<.001) and region by time (F [2,12]= 8.46, p=.01) interactions. The main effect of dose and the dose by region and dose by region by time interactions were non-significant, perhaps reflecting the small sample size in the low dose group. However, the magnitude of change in rCBF was much smaller for the low dose group. The significant main effect of region indicates the maintenance of relative hyperfrontality throughout the rCBF studies. The effect of time shows that the maximal global change in rCBF occurred at 25 minutes post-infusion. The dose by time interaction reflects the

TABLE 7 SCOPOLAMINE STUDY 1: EFFECTS ON RCBF

Means and Standard Deviations [in ml/100g/minutes]

(corrected to the mean baseline PCO2)

Time from infusion (minutes)		
	Low Dose	
Baseline	5min post	25min post
	Anterior Perfusion	
75.8±15.7	73.2±12.1	74.1±11.5
	Posterior Perfusion	
68.0±12.8	68.2±13.3	69.6±10.3
	Anterior/Posterior Ratio	
1.11±0.03	1.09±0.07	1.07±0.05
	Global Perfusion	
70.5±13.7	69.9±12.9	71.0±10.6
	High Dose	
	Anterior Perfusion	
81.5±11.8	77.7±14.8	65.3±12.5
	Posterior Perfusion	
71.4±12.0	70.6±14.7	60.4±12.6
	Anterior/Posterior Ratio	
1.15±0.05	1.10±0.04	1.09±0.08
	Global Perfusion	
74.5±11.9	72.8±14.7	61.9±12.4

differential effect on global flow. Post-hoc t-tests of the dose by time interaction showed that between group differences were non-significant at 5 minutes post-scopolamine, but were highly significant at 25 minutes post-scopolamine (t [14]=6.10, p<.001). At this time, the low dose group increased and the high dose group decreased in global flow. This interaction is illustrated in Figure 7. For the region by time interaction, the anterior detectors showed greater decrements relative to the posterior detectors at both 5 and 25 minutes post-scopolamine (t [14]=-3.70, p=.002; t[14]=-4.82, p<.001, respectively, see Figure 8).

To establish the time course of scopolamine's effects, additional studies were conducted at 60 minutes post-administration. Due to technical problems, there were missing data for one subject. For nine subjects in the high dose condition, results indicated that flows began to return to baseline levels at 60 minutes post-infusion (mean rCBF: baseline = 75.0±12.2, 5 minutes = 73.7±14.9, 25 minutes = 62.4±12.8, 60 minutes = 68.9±11.9).

Correlations between rCBF and Cognitive Function

Pearson product moment correlations were computed for the absolute values and for the change scores between autonomic and cognitive variables and rCBF. A more stringent probability value was used due to the number of

Figure 7

Scopolamine Study 1: Effects on Global Grey Matter Perfusion. Results for low and high dose groups at baseline, 5 and 25 minutes post-scopolamine infusion. Repeated measures analysis of variance results indicated a significant main effect for time ($F [2,12] = 20.05$, $p < .001$) and a significant dose by time ($F [2,12] = 19.48$, $p < .001$) interaction.

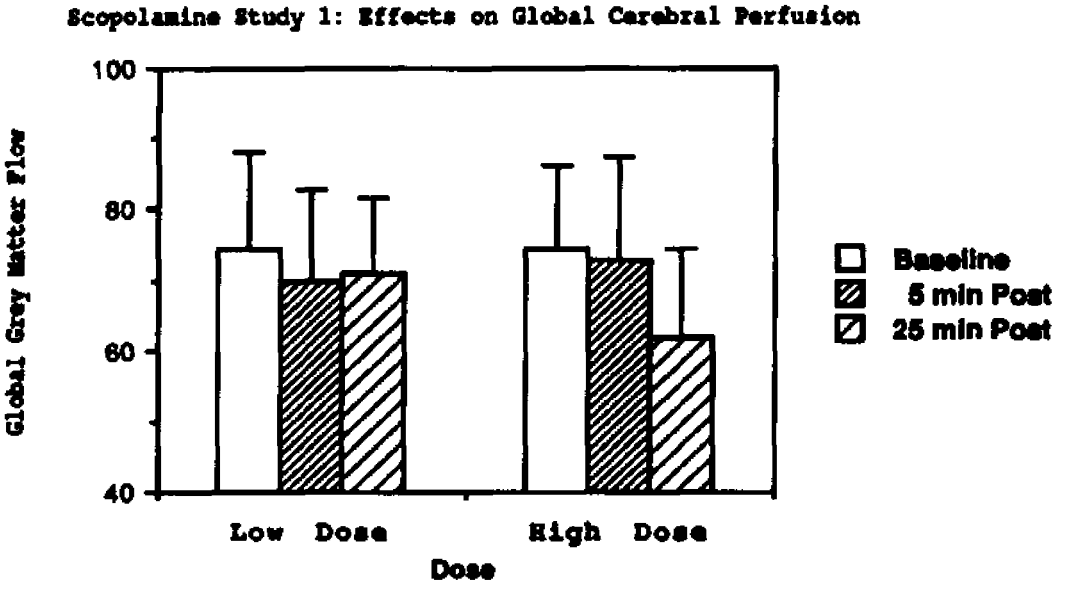

Scopolamine Study 1: Effects on Global Cerebral Perfusion
Global Grey Matter Flow
100
80
60
40
Low Dose
High Dose
Dose
Baseline
5 min Post
25 min Post

Figure 7

Figure 8

Scopolamine Study 1: Effects on Regional Grey Matter Perfusion. Results for low and high dose groups at baseline, 5 and 25 minutes post-scopolamine infusion. Repeated measures analysis of variance results indicated a significant main effect for region ($F [1,13] = 88.35$, $p < .001$) and a region by time ($F [2,12] = 8.46$, $p = .01$) interaction.

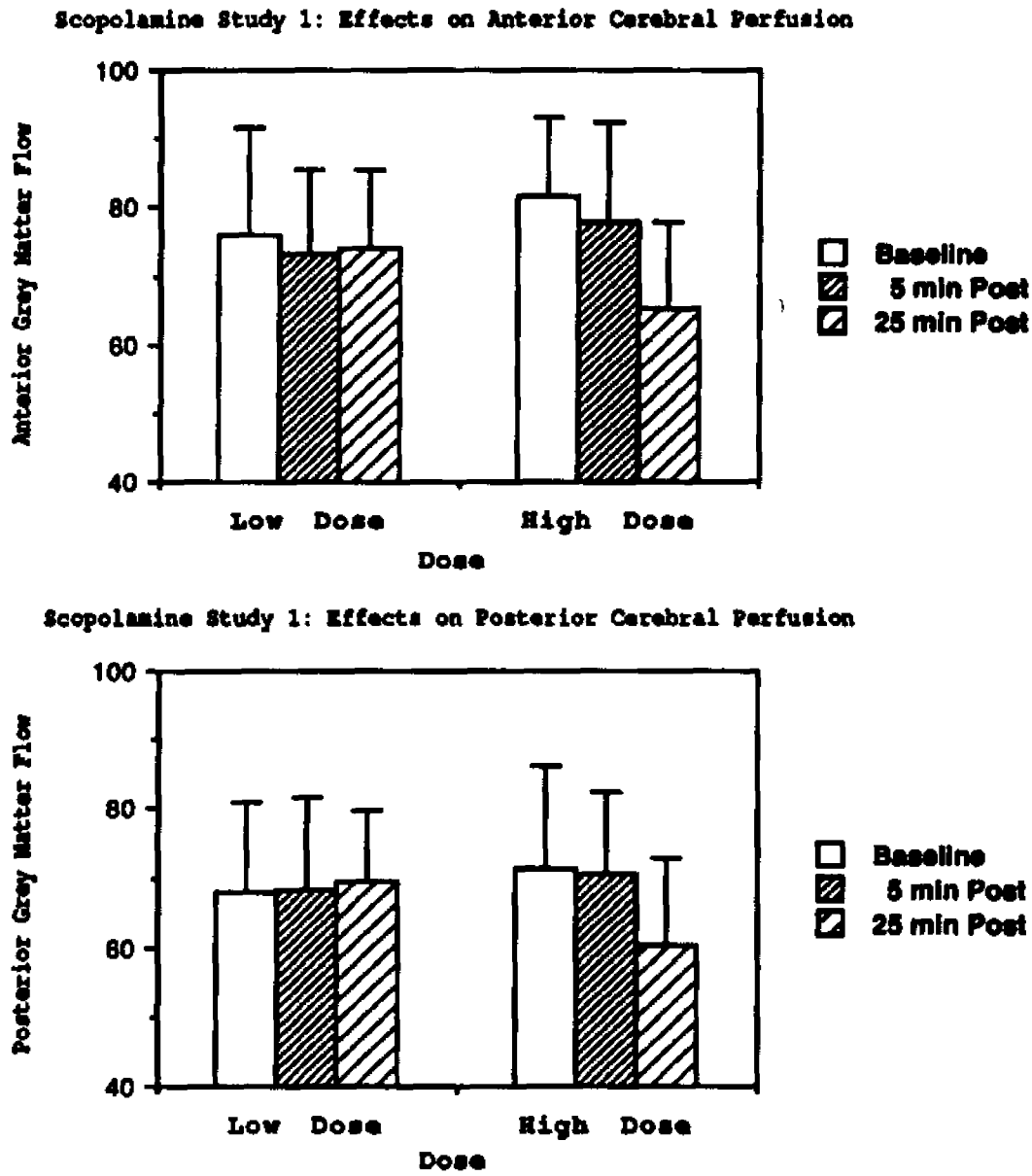

Scopolamine Study 1: Effects on Anterior Cerebral Perfusion
Anterior Grey Matter Flow
100
80
60
40
Low Dose
High Dose
Dose
Baseline
5 min Post
25 min Post
Scopolamine Study 1: Effects on Posterior Cerebral Perfusion
Posterior Grey Matter Flow
100
80
60
40
Low Dose
High Dose
Dose
Baseline
5 min Post
25 min Post

Figure 8

correlations computed. None of the coefficients were significant at the $p<.01$ level for the low and high dose groups, separately. For both groups combined, the change in digit span forward was significantly correlated with the change in anterior, posterior and global flow (r=0.61, 0.65, 0.65, $p<.01$).

DISCUSSION OF STUDY 1

Interpretation of Results

Scopolamine produced maximal cardiovascular effects at 25 minutes post-infusion. Except for systolic blood pressure, these results are not observed in a dose dependent manner. The rCBF results should not be influenced by these cardiovascular changes because the range of blood pressure values are within the limits governed by autoregulation (McCulloch, 1988) and the flow values were corrected for the elevation in PCO2.

Significant declines in number of words recalled on the SRT were evident at both scopolamine doses, although the effect was smaller in the low dose group. While significant impairments were observed in long term storage and retrieval, the consistent long term retrieval parameter was most affected. The dose by time interaction for digit span forward was statistically significant, revealing an improvement in performance after the low dose and a decrement in performance after the high dose. These changes are small, would not be considered to be clinically

significant and are within the range of variability for this measure.

A correlation between change in rCBF and digit span forward was observed for the total subject sample. Since differential response was noted on this measure (increase for the low dose group, decrease for the high dose group) the variability was sufficient to obtain a significant correlation. Whereas on the SRT, all subjects showed decline and the range of values was more restricted. If the processes responsible for SRT performance are localized to such subcortical structures as hippocampal formation, the rCBF technique would not directly visualize these regions. Finally, normal topography was maintained (i.e. relative hyperfrontality) after scopolamine administration and is lost in conditions such as Alzheimer's disease in which cognitive function is correlated with metabolism (Smith and Prohovnik, 1987). The correlation between Digit Span Forward and rCBF may reflect change in arousal, correlated with global CBF. The neuropsychological findings demonstrate significant impairment in memory after both doses of scopolamine and equivocal effects on attention with high dose scopolamine. Memory impairments are observed at both scopolamine doses, although as was the case for rCBF, there appears to be a trend toward smaller reductions in the low dose group. rCBF shows a dose response relationship, in that global deficits are observed in the high dose group only.

Therefore, the cognitive and rCBF measures show differential sensitivity to the effects of scopolamine.

Clinical Significance of Findings

The magnitude of decline in rCBF was significant, relative to other clinical conditions. Although, the range of flow values remain within the range observed in young normal adults (eg. Smith and Prohovnik, 1986), a twenty percent reduction in anterior rCBF and an eighteen percent reduction in global CBF are greater than change observed after other manipulations such as amphetamine administration, acutely post-bilateral electroconvulsive therapy in patients with Major Depressive Disorder and cerebrovascular challenge via CO2 inhalation (Kahn et al., 1987; Prohovnik et al., 1986; McCulloch, 1988). A global decrement in flow would be expected, if scopolamine produced a purely vasoconstrictive effect.

Several clinical conditions studied with neuroimaging techniques show a similar pattern of deficits as that observed after scopolamine administration. Declines in global and frontal perfusion have been observed in normal human aging (Smith and Prohovnik, 1986). In addition, loss of muscarinic receptors in frontal cortex have also been reported (White et al., 1977). Other neurodegenerative disorders associated with frontal flow/ metabolism deficits are Parkinson's Disease, Progressive Supranuclear Palsy, Down's syndrome, Pick's disease and late-stage Alzheimer's

disease (Bes et al. 1983; Litvan et al. 1988; Schwartz et al.1983; Risberg and Gustafson, 1988). Alzheimer's disease is characterized by a presynaptic cholinergic deficit, presumably attributable to a loss of cholinergic neurons of the Nucleus Basalis of Meynert (Davies and Maloney 1979; Whitehouse et al., 1983). Neuroimaging studies have demonstrated most severe deficits in temporo-parietal areas (Smith and Prohovnik et al., 1987). Although it may be surprising that scopolamine does not reproduce the rCBF pattern observed in Alzheimer's disease, it must be emphasized that the cholinergic deficit is only one component of the complex pathology in Alzheimer's disease.

The relatively greater deficit in consistent long term retrieval has also been reported after chronic anticholinergic treatment in dystonia patients and in the normal aging process (Stern et al. 1988; Larabee et al. 1986). The neuropsychological battery for the second study was designed to evaluate this deficit in the context of the assessment of other neuropsychological functions such as attention. A further discussion of this finding with be presented in subsequent sections.

The physiological significance of the cognitive and rCBF effects remains to be addressed, such as the relation of rCBF change to neurotransmitter topography and cortical connectivity and the relation of the rCBF deficits to the cognitive dysfunction produced by scopolamine. The assumption of the following sections is that receptor

antagonism results in reduced metabolic demands and therefore, declines in rCBF and metabolism. Other processes may account for the flow and metabolic deficits, such as secondary effects on neuroendocrine or cerebrovascular function (eg. Risch et al., 1986).

Neurotransmitter Topography and rCBF Effects of Scopolamine

McCulloch (1982) concluded that "although neuronal perikarya are likely responsible for a considerable portion of the glucose use in a region, the contribution of terminals is likely to be considerably greater." In order to evaluate the relation of metabolic change to neurotransmitter distributions, markers of neuronal perikarya (eg. synthetic enzymes) and terminals (eg. degradative enzymes and receptors) must be evaluated. The distribution of cholinergic markers in the cerebral cortex in non-human primates and humans was discussed in the Introduction. In summary, the cholinergic ennervation of cerebral cortex is diffusely organized, with muscarinic receptors (Cortes et al., 1987), projections from the Nucleus Basalis of Meynert (Mesulam et al., 1983) and choline acetyltransferase and acetylcholinesterase positive fibers (Mesulam et al., 1986) found in all areas of the cerebral cortex to approximately the same extent. The global decrement in rCBF after scopolamine administration is consistent with this diffuse distribution (particularly on the dorsolateral surface). As far as the anterior cortical

deficit is concerned, this cannot be explained by a specificity of cholinergic projections and there is no evidence for the vascular supply to this area, the middle and anterior cerebral arteries, to contain a differential representation of cholinergic innervation. Two possibilities can be considered. First, the anterior deficit may represent the involvement of non-cholinergic projections, preferentially distributed in anterior cortex. The depression of cholinergic function produced by scopolamine would produce secondary effects on another neurotransmitter system. Secondly, the effects of scopolamine may reflect interconnections between areas of the cerebral cortex, beyond the known cholinergic projections. Such a dissociation of effects, that of a pharmacological agent exerting an influence beyond its neurotransmitter topography, to reflect anatomical pathways has been described previously for other pharmacological agents (such as agents effecting dopamine, acetylcholine and gamma-amino butyric acid systems). Evidence for and the integration of these hypotheses will be presented.

Non-Cholinergic Projections to Frontal Cortex: Interactions Between Acetylcholine and Dopamine

Of the major neurotransmitter systems represented in the cerebral cortex, dopamine is preferentially localized to frontal cortex (Nieuwenhuys, 1985). The projections to frontal cortex originate from cells of the ventral tegmental

area and medial third of the Substantia Nigra, pars compacta, ascend in the medial forebrain bundle and project dorsally into both mesial frontal and anterior cingulate cortices (Moore and Bloom, 1978). Noradrenergic projections from locus coeruleus also show a selective prefrontal cortex distribution (Moore and Bloom 1979). Goldman-Rakic (1987) has demonstrated that prefrontal cortex (the cortex surrounding the Principal sulcus of the primate) contains the greatest levels of the catecholamines dopamine and norepinephrine and has described a gradient across the cerebral hemispheres, in which prefrontal cortex has the highest and primary visual cortex the lowest concentrations.

Dopaminergic projections may be relevant especially given the documented interactions between dopaminergic and cholinergic systems. de Belleroche et al. (1982) have shown that administration of dopamine inhibits release of acetylcholine from cerebral cortex (resting release 54%, K^+ evoked release 29%) and striatum. In a subsequent study, the effects of cholinergic manipulation on dopamine release from nucleus accumbens was examined (de Belleroche et al., 1985). Oxotremorine was shown to facilitate K^+ evoked dopamine release (25% increase), an effect antagonized by both pirenzepine and scopolamine. These results may be extended to cerebral cortex because the cells providing dopaminergic innervation to nucleus accumbens are also derived from the ventral tegmental area and evidence supports the preponderance of high affinity acetylcholine receptors in

cerebral cortex (Cortes et al., 1987, de Belleroche et al., 1985), the same receptor population which antagonized the oxotremorine induced dopaminergic release. Generalization from these findings is problematic because these interactions have not yet been demonstrated in vivo. Dopaminergic agonists such as apomorphine increase glucose metabolism in cerebral cortex, whereas antagonists such as haloperidol, decrease metabolism (McCulloch et al., 1979; 1982). Cholinergic antagonism, then, may inhibit dopaminergic activity to further depress rCBF and these effects may be observed specifically in anterior cortex.

As described in the Introduction, the neuropeptide CCK is located in close proximity to cerebral vessels and is though to be involved is cerebrovascular regulation. CCK is co-localized with dopamine in the ventral tegmental area and may become activated if these neurons are effected by scopolamine (Hokfelt et al., 1980). VIP, another putative modulator of cerebrovascular function, is co-localized with acetylcholine in the cerebral cortex (Eckenstein and Baughman 1984) and may also exert a hypothetical influence on the rCBF deficits produced by scopolamine.

Effects of Pharmacological Agents on Cerebral Metabolism

The second hypothesis to be evaluated concerns the relation of the topography of scopolamine's effects to cortico-cortico connections. Investigations of other neurotransmitter systems examining the effects of

pharmacological manipulation on glucose metabolism (with the ^{14}C deoxyglucose autoradiographic technique) have shown effects on metabolism that are distinct from known neurotransmitter projections. Examples will be drawn from the study of dopaminergic, gamma-amino butyric acid and cholinergic systems

Dopamine

McCulloch et al. (1979,1982) have examined the effects of dopaminergic agonists and antagonists (apomorphine and haloperidol, respectively, both non-selective for the D1 and D2 dopamine receptors) on glucose utilization. The results from these studies demonstrated that the effects of these agents are not confined to regions with dopaminergic innervation and that the magnitude of change in glucose utilization is not proportional to the receptor density. Metabolic change was observed in some areas which contain no dopaminergic receptors, such as the dentate gyrus, cerebellar hemispheres, inferior olivary nucleus and red nucleus. Significant alterations were observed in the striatum and Substantia Nigra, which send fibers of passage through the inferior olivary nucleus and red nucleus, to terminate in the cerebellum. Change observed in dentate gyrus may represent effects on dopaminergic afferents from the septo-hippocampal pathway. The pattern of alteration in cortical metabolism was such that change in specific thalamic nuclei was accompanied by changes in the

corresponding areas of cortical innervation. After haloperidol administration, for example, reductions in anteromedial and anteroventral nuclei were observed, with concomitant alterations in the principal cortical projections, anterior and posterior cingulate gyrus. Finally, myelinated fiber tracts, such as corpus callosum, which can be visualized with the autoradiographic technique, were shown to be affected by drug manipulation. This further suggests that neurochemical manipulation has an impact on efferent pathways.

Selective frontal lobe effects of dopaminergic agents have been demonstrated in pathological conditions. Studies in primates treated with n-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have shown that administration of L-dopa increases frontal lobe metabolism (Porrino et al., 1987). Such an effect was not observed in control primates. Administration of piribidil, a dopamine agonist, reversed the hypofrontal rCBF deficit observed in unmedicated schizophrenic patients (Geraud et al., 1987). Normal controls showed no change in rCBF after administration of the same dose and diffuse increase after a higher dose (Guell et al., 1982). These studies suggest that dopamine agonist have differential effects in pathological versus normal function and that the primary locus of these effects in pathology is the frontal lobes.

Gamma-Amino Butyric Acid

Similar dissociation are noted in manipulations of the gamma-amino butyric acid system (Palacios et al. ,1982, Helen and London, 1984). Muscimol (a gamma-amino butyric acid agonist, nonspecific with respect to the gamma-amino butyric acid A and B receptors) produced a pattern of glucose metabolic deficit after administration, which did not correlate with [^{3}H] muscimol binding sites. Muscimol reduced metabolism in the majority of regions studied, including diffuse reductions in cerebral cortex, anterior nuclei of thalamus and medial geniculate body, which overlap with receptor densities. No change was observed in Substantia Nigra, pars reticulata, which is an area of high receptor density. Other areas such as superior olivary nucleus, lateral lemniscus and inferior colliculus do not contain gamma-amino butyric acid receptors, but do represent structures associated with the auditory pathway. While prominent metabolic reductions occur in auditory cortex and medial geniculate nucleus, secondary effects are observed in other structures related to the auditory pathway.

Acetylcholine

Studies concerning the effects of scopolamine on cerebral metabolism are summarized in Table 8. Weinberger et al. (1979) have demonstrated decreases in glucose utilization using a dosage of the drug (0.4mg/kg,IV) which produces cognitive impairment, but not sedation. The

TABLE 8: SUMMARY OF STUDIES CONCERNING THE EFFECTS OF SCOPOLAMINE ON CEREBRAL METABOLISM IN THE RODENT

Author	Species, Weight	Drug Dosage, Route of Administration	Time of Drug Administration
Weinberger, 1979	Wistar Rats 200-250g	Scopolamine 0.4mg/kg, IV	25 minutes pre-study
Piercy, 1986	Sprague-Dawley rats 300-400g	Scopolamine 3mg/kg, IP	30 minutes pre-study
Dow-Edwards 1981	Fisher-344 rats	Scopolamine 2.5mg/kg, IP	12 minutes pre-study
Dam 1982, 1984		Oxotremorine 0.1-1.0mg/kg, IP	2 minutes pre-study

IV=intravenous, IP=intraperitoneal

greatest reductions were seen in globus pallidus (57% of treated relative to control animals), which includes the NBM in the rat, in addition to auditory, frontal and parietal cortex (67%,71%,70%), Ammon's horn (61%),dentate gyrus (72%) and lateral thalamus (73%). The brain stem and cerebellum were unaffected. No reductions in caudate nucleus were observed, an area which contains the greatest concentration of cholinergic receptors, but the depletions seen in globus pallidus may represent the contribution of afferents from the caudate nucleus. Since, in the rodent, the Nucleus Basalis of Meynert is contained within the ventromedial globus pallidus, the deficit in this region may reflect inhibition of the Nucleus Basalis of Meynert. The pattern of reductions are consistent with the septo-hippocampal and thalamo-cortical systems, both cholinergic pathways. At a relatively high dose of scopolamine (3mg/kg,IV), Vogelsang and Piercy et al. (1986) observed diffuse reductions, maximized in the dense band of the hippocampus, lateral and ventral thalamus and in cortical areas (motor cortex, sensory cortex, parietal cortex, auditory cortex and anterior cingulate cortex). Piracetam (2-pyrrolidone acetamide, a cerebral metabolic enhancer) pre-treatment attenuated the reductions in hippocampus and anterior cingulate cortex.

Several studies (eg. Dam et al.,1982,1984, Dow-Edwards et al. 1981) demonstrated reductions in glucose metabolism after scopolamine administration, which were reversed by

administration of oxotremorine, a muscarinic agonist. The areas of greatest deficit and reversal were presubiculum and postsubiculum of hippocampus, anterior thalamic nuclei, retrosplenial cortex, medial septum, postcommissural fornix and mammilothalamic tract, In addition, oxotremorine increased metabolism in entorhinal, auditory, visual sensorimotor and frontal cortices. The remainder of the cerebral cortex, pyramidal and extrapyramidal motor nuclei and cerebellum were unchanged by scopolamine. Contrary to the previous study, areas with high densities of muscarinic binding sites, such as dentate gyrus, pyriform and entorhinal cortices were unaffected, however, decline was observed in mammillary bodies, which contain low densities of muscarinic receptors. These studies used a smaller dose of scopolamine relative to Weinberger et al. (1979) and Vogelsang and Piercy (1986), which may account for the lack of effects in hippocampal structures.

These studies indicate that cholinergic manipulation affects glucose metabolism in many areas of cerebral cortex and hippocampus and thalamic nuclei which receive afferents from limbic structures and project to cerebral cortex. Acetylcholine is one of the neurotransmitters implicated in this pathway (Mesulam et al., 1983). The effect of scopolamine on paralimbic, thalamic and hippocampal structures, with established connectivity (Amaral, 1987), has important implications for the cortical effects of scopolamine, which will be discussed in the next section.

Cortical Connectivity in Relation to the rCBF Deficits Produced by Scopolamine

The previous section established that the effects of pharmacological agents on cerebral metabolism do not entirely mimic the distribution of the specific neurotransmitter receptors or projections, rather the effects correspond to connectivity across structures. As described in the Methods section, the limitation of the rCBF technique is that cortical activity is monitored and therefore, the contribution of subcortical structures is indirectly measured. This section will describe cortico-cortico connections, with an emphasis upon connectivity of the frontal lobes. Secondly, the involvement of paralimbic cortices, limbic and thalamic structures will be addressed. These areas are not visualized directly by the rCBF technique, but are affected by scopolamine as demonstrated by the studies of glucose metabolism reviewed in the previous section.

Recent investigations have elucidated cortico-cortico pathways. Goldman-Rakic (1987) has characterized reciprocal parietal-prefrontal connections, which are organized in a columnar manner. These interconnections consist of an intermingling of callosal and associational pathways. The areas involved are the inferior parietal lobule (areas 7a, 7m, 7ip, 7b in the primate) and prefrontal cortex (cortex surrounding the Principal sulcus). The inferior parietal

lobule receives afferents from visual association cortex (eg. the medial portion of Brodman's area 19 , above the calcarine fissure), primary and secondary somatosensory areas (SI and SII) and multi-modal association cortex (eg. the upper bank of the superior temporal sulcus). Reciprocal connections are found from the hippocampal formation to both the prefrontal cortex and the inferior parietal lobule via the posterior cingulate gyrus. Double labelling studies show that both frontal and parietal areas project to opercular and superior temporal cortices and limbic cortices including entorhinal, parahippocampal, cingulate, subicular and retrosplenial cortices. These areas share a common thalamic input from the medial pulvinar nucleus. As will be discussed in a subsequent section, the prefrontal cortex has been shown to be involved in aspects of attentional and mnestic processing. This complex network represents connections and integration throughout the cerebral cortex and limbic structures. It is conceivable that a decline in cortical metabolism, mediated by cholinergic antagonism would produce a secondary depression in this pathway, perhaps manifested by an accentuation of decline in frontal lobe metabolism. Prefrontal cortex may be most vulnerable to the anti-dopaminergic effects of scopolamine due to the increased concentration of catecholamines in this area.

Another possibility is that scopolamine may exert its primary effect on the hippocampal formation, as suggested by the reductions in glucose metabolism observed after

scopolamine administration in the rat (eg. Weinberger et al., 1979). Due to the extensive connectivity of this area, this may influence efferent pathways, particularly the entorhinal cortex, which has extensive connections with paralimbic areas (eg. cingulate gyrus) and most areas of the cerebral cortex, especially the homotypical association areas (Amaral, 1987). Interruption of this pathway, secondary to a reduction of metabolism in hippocampal formation, could create a generalized reduction in cortical function, perhaps accentuated in anterior cerebral cortex. Mesulam (1983, 1986) has shown that the hippocampal formation, entorhinal cortex and paralimbic cortices have the greatest density of cholinergic innervation, so that inhibition of this pathway by scopolamine probably contributes to the cortical deficits. Neuroanatomical investigations of post-mortem brain samples from Alzheimer's disease patients have shown severe degeneration in these areas, resulting in an "isolation" of the hippocampal formation from cerebral cortex (Van Hoesen and Damasio, 1987). In vivo neuroimaging studies of these patients demonstrated global decline in rCBF and metabolism and frontal deficits in the latter stages of the illness (Smith and Prohovnik, 1987).

In summary, several compatible possibilities may account for the generalized and focal anterior decline in rCBF after scopolamine administration. Primary effects on cerebral cortex may be observed by the antagonism of

diffusely localized cholinergic receptors, producing a global reduction in flow. Secondly, a primary locus of effect may occur in the hippocampal formation-entorhinal cortex-paralimbic cortical areas, producing secondary effects on cerebral cortex. Finally, primary cholinergic antagonism produces a secondary inhibition of catecholaminergic function, resulting in a reduction of anterior flow. The analysis of the cognitive deficits produced by scopolamine will be discussed in the next section. The functional role of prefrontal cortex is reviewed, and its contribution to these deficits assessed.

Relation of rCBF Deficits to Cognitive Deficits of Scopolamine

As discussed previously, the cognitive deficits produced by scopolamine consist of a global effect on memory performance as assessed by the SRT. Although retrieval and storage seen to be equally impaired, the consistent long term retrieval measure shows the greatest impairment, almost twice that of the other parameters. The consistent long term retrieval parameter represents the subject's ability to sequence and keep track of information. The subject must maintain simultaneously a representation of words recalled on the current trial, words recalled on previous trials and words immediately read by the examiner. This information must then be organized into a verbal output. The functions represented by this parameter are similar to the abilities

required to successfully perform the delayed response task, which had been described as a measure of "a synthetic capacity that has at least three subdivisions: access to appropriate information, ability to hold that information 'on line' for the temporal interval over which a decision or operation is to be performed and the initiation of a motor command" (Goldman-Rakic, 1987, pp.378). This ability is referred to as short term representational memory. Deficits on the delayed response task are specific to lesions of the frontal lobes; lesions of posterior parietal and temporal cortices do not produce this deficit (Jacobsen 1936). Task performance is specifically disrupted by lesions of the catecholamine pathways (Goldman-Rakic 1987). Prefrontal lesions were produced by the catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA), with co-administration of desmethylimipramine to preserve noradrenergic terminals. This disrupted performance of the delayed response task and administration of either clonidine (an a_2 receptor agonist), L-dopa or apomorphine restored performance (Arnstein and Goldman-Rakic, 1986).

Clinico-pathological and behavioral observations support the role of catecholamines in cognitive deficits. Relevant to the consideration of cortical dopamine projections are recent case reports of patients presenting with intellectual decline, extra-pyramidal movement disorders and depressive symptomatology, who show significant cell loss in the ventral tegmental area and

hippocampal formation (Toback and Morris, 1988). Age-related declines in both acetylcholine and catecholamine markers have been reported (Selkoe and Kosik, 1983) in addition to deficits on the SRT, most prominent in the consistent long term retrieval parameter (Larabee et al. 1986).

The contribution of representational memory abilities and the prefrontal-catecholaminergic circuitry responsible for this aspect of mnestic function may account for one aspect of the mnestic deficit produced by scopolamine, the impairment in consistent long term retrieval. The storage and retrieval deficits observed may be linked to deficits within the hippocampal formation-entorhinal cortex-paralimbic cortex-cerebral cortex pathway. Experimental lesions studies in primates and human clinical material has implicated the hippocampal formation and related structures in memory deficits (eg. Mishkin, 1982; Squire and Zola-Morgan; 1984, Butters and Cermak; 1980, Squire and Cohen; 1982). To summarize this vast literature, lesions within these pathways produce deficits in the acquisition of new information (anterograde amnesia) and the recall of information acquired before the insult (retrograde amnesia). As described in the Introduction, cholinergic antagonism has been shown to produce storage and retrieval deficits. Performance on verbal learning tests such as the SRT are typically impaired in patients with Korsakoff's Syndrome, Post-encephalitic amnesia, lesions of the nucleus medialis dorsalis of the thalamus and hippocampus and normal subjects

after administration of cholinergic antagonists (Squire and Cohen, 1982). Therefore, impairments in limbic structures by cholinergic antagonism must be considered an important contribution to the mnestic deficits observed.

Conclusions

1. Scopolamine administration produces significant global declines in rCBF and focal diminutions of anterior perfusion.

2. Significant effects on memory are observed, in storage, retrieval and consistent retrieval of verbal material and equivocal effects on short term recall of digits.

3. The rCBF effects of scopolamine may occur by several possible mechanisms: first, a primary antagonism of muscarinic receptors diffusely distributed in cerebral cortex; a second, primary inhibition of limbic (eg. hippocampal formation, entorhinal cortex) and paralimbic structures, resulting in a secondary reduction in cortical metabolism; and an inhibition of cortico-cortico connections involving frontal, parietal and temporal areas, third, regional differences due to an inhibition of catecholaminergic pathways secondary to cholinergic antagonism, with a locus in frontal cortex.

4. The mnestic effects of scopolamine may be attributable to inhibition of the hippocampal formation, producing

long term storage and retrieval deficits and antagonism of the frontal cortex catecholaminergic system, producing deficits in consistent long term retrieval.

RESULTS OF STUDY 2: SCOPOLAMINE REVERSAL BY PHYSOSTIGMINE / NEOSTIGMINE

Preliminary analyses (not shown) were conducted to assess potential order effects. Data were analyzed in order of sequential study days to evaluate sensitization or carry-over effects. None of the main effects or interactions were significant for the rCBF, cognitive or autonomic variables. These analyses demonstrate the lack of a practice effect for the cognitive tests. Analyses of the effect of neostigmine dose were conducted. These analyses failed to show significant differential effects on the antagonism of rCBF deficits or the reversal of cognitive deficits. Two effects reached statistical significance. A significant main effect for neostigmine dose on systolic blood pressure (F [1,7]= 8.10, p=.03), indicating that the high dose group had higher systolic blood pressure throughout all studies. A neogroup x time interaction (F [2,6]= 5.51, p=.05), indicating that across both physostigmine/neostigmine conditions, the groups responded differentially. The most revealing test for the rCBF results was the the comparison of difference scores for anterior and posterior flows, between the groups. The differences between the scopolamine +neostigmine run and the baseline run (anterior:t [8]=-0.17, p=.87, posterior:t [8]=-0.05, p=.96) and differences between the scopolamine +neostigmine run and the acute scopolamine run (anterior:t [8]=-0.42, p=.69, posterior:t [8]=-0.40, p=.70) were non-significant. These data are shown in Appendix 7.

Given the timing of procedures for this this study, neuropsychological testing could not be conducted after scopolamine administration to coincide with the 5 minute acute scopolamine study. Therefore, the neuropsychological findings must be discussed as a reversal to baseline performance. For the SRT and digit span test, the magnitude of change in scores can be compared with the results of the first study to assess whether the administration of physostigmine/neostigmine prevented the decline in SRT performance. Such comparisons must be made with caution given the limitations inherent in generalizing between subjects. For the rCBF studies, two issues can be addressed: that of reversal of deficits to baseline levels (by comparing the scopolamine +physostigmine/neostigmine condition to the baseline condition) or antagonism of scopolamine's effects; can administration of physostigmine/ neostigmine block the rCBF deficit produced by scopolamine (by comparing the scopolamine +physostigmine/neostigmine condition to the acute scopolamine condition)?

Autonomic Indices

The means, standard deviations and repeated measures analysis of variance results for the autonomic measures are shown in Appendix 8. For PCO2, significant main effects for time ($F\ [1,7]= 31.7$, $p<.001$) and drug ($F\ [2,6]= 5.04$, $p=.06$) were observed PCO2 increased in the acute scopolamine condition and returned to baseline levels in the

scopolamine+physostigmine/neostigmine condition and the washout condition. PCO2 was higher on the physostigmine drug condition, relative to the neostigmine condition. No significant effect on diastolic blood pressure was observed. For pulse, a significant main effect of time was noted (F [1,7]= 77.43, $p<.001$), indicating slight elevations at 5 minutes and reductions at 25 minutes post-infusion for both neostigmine and physostigmine conditions.

Neuropsychological Measures

Means and standard deviations for the neuropsychological variables are shown in Table 9 and repeated measures analysis of variance results in Appendix 9. The results for SRT total recall and the individual parameters are shown in Figures 9 and 10, respectively. The SRT showed significant main effects for scale (F [3,5]=12.6, $p=.007$) and time (F [1,7]=41.5, $p=.001$) and significant interactions for drug by time (F [1,7]=7.7, $p=.03$)$p=.03$) and scale by time (F [3,5]=5.3, $p=.05$). Scores began to return to baseline levels after physostigmine infusion, but then worsened when the clinical effects of physostigmine dissipated, representing the residual scopolamine effects. Post-hoc t-tests were performed to evaluate the scale by time interaction, in order to determine differential effects on the SRT subscales, as a function of drug manipulation.

TABLE 9 SCOPOLAMINE STUDY 2: EFFECTS ON NEUROPSYCHOLOGICAL MEASURES

Physostigmine			Neostigmine		
Baseline	Post-Drug	%Δ	Baseline	Post-Drug	%Δ
Selective Reminding Test					
Total Recall					
133.4±8.5	126.8±7.8	5%	136.3±4.3	115.8±10.2	14%
Long Term Retrieval					
129.9±11.3	120.2±12.7	7%	134.6±5.6	104.9±16.5	22%
Consistent Long Term Retrieval					
124.4±18.4	103.0±29.6	17%	131.4±9.7	79.9±33.1	39%
Long Term Storage					
131.6±8.7	122.4±10.2	7%	135.3±4.8	111.6±15.2	18%
Delayed Recall					
11.1±0.9	10.0±1.5±		11.4±0.9	9.6±2.1	
Digit Span					
Digit Span Forwards					
10.0±2.1	10.0±1.9		10.3±2.3	9.6±1.8	
Digit Span Backwards					
8.4±3.1	8.8±3.2		9.0±2.9	9.2±2.5	

Table 9-Continued

Visual Reproduction

	Physostigmine		Neostigmine	
	Baseline	Post-Drug	Baseline	Post-Drug
Recall				
Immediate	12.2±1.7	12.7±2.2	12.2±1.4	11.4±3.0
Delayed	10.8±2.3	9.6±3.6	12.7±1.8	9.4±4.6
Recognition				
Immediate	3.2±1.1	3.4 ±0.8	3.1±0.9	3.0±1.0
Delayed	2.7±1.2	3.2 ±1.2	2.3±1.4	2.7±0.8

Stroop Test

Time				
Word	46.9±11.9	46.0±11.2	47.3±7.3	46.3±7.4
Color	66.0±21.5	66.6±15.7	71.3±27.7	77.0±20.0
Color/word	112.1±21.9	117.4±34.4	109.4±12.4	113.4±13.3
Errors				
Word	0.3±0.7	0.4±0.7	0.1±0.4	0.1±0.4
Color	3.0±2.3	1.6±1.8	1.3±1.0	2.8±2.9
Color/word	1.6±1.3	3.3±2.6	2.1±1.2	5.3±6.5

Table 9-Continued

	Physostigmine		Neostigmine	
	Baseline	Post-Drug	Baseline	Post-Drug
Word Fluency				
	53.1±11.3	60.0±15.1	49.3±11.8	49.4±16.3
Category Naming				
	22.9±6.0	23.4±4.6	24.3±7.7	21.7±4.0
Cancellation				
Number(6)				
TIME	32.1±6.5	30.0±5.7	28.6±3.9	34.7±11.3
OMISSION ERRORS	0.3±0.8	0.6±0.5	0.7 ±1.3	0.7 ±1.1
Shapes(Diamond)				
TIME	35.6±5.2	37.0±8.8	41.7±24.9	41.7±10.6
OMISSION ERRORS	2.7 ±3.9	1.6 ±2.6	2.7 ±1.9	2.2±2.3
Trigram(TMX)				
TIME	46.5±7.3	45.3±5.4	46.2±10.9	49.6±13.3
OMISSION ERRORS	0.6±0.7	0.5±0.8	0.3±0.7	1.1±1.8

Figure 9

Scopolamine Study 2: Effects on the Selective Reminding Test- Total Recall Score. Results for physostigmine and neostigmine conditions at baseline and 35 minutes post-scopolamine infusion.

The Selective Reminding Test showed significant main effects for scale ($F [3,5]=12.6$, $p=.007$) and time ($F [1,7]=41.5$, $p=.001$) and significant interactions for drug by time ($F [1,7]=7.7$, $p=.03$) and scale by time ($F [3,5]=5.3$, $p=.05$).

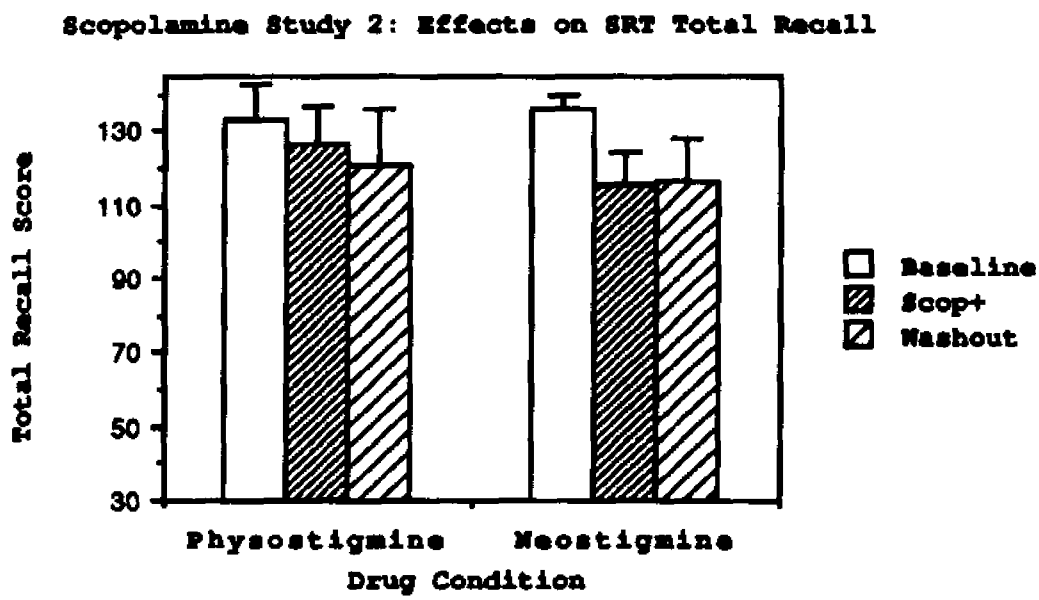

Scopolamine Study 2: Effects on SRT Total Recall
Total Recall Score
130
110
90
70
50
30
Physostigmine
Neostigmine
Drug Condition
Baseline
Scop+
Washout

Figure 9

Figure 10

Scopolamine Study 2: Effects on the Selective Reminding Test Parameters. Results for physostigmine and neostigmine conditions at baseline and 35 minutes post-scopolamine infusion.

The Selective Reminding Test showed a significant main effect for scale ($F [3,5]=12.6$, $p=.007$) and a significant interaction for scale by time ($F [3,5]=5.3$, $p=.05$).

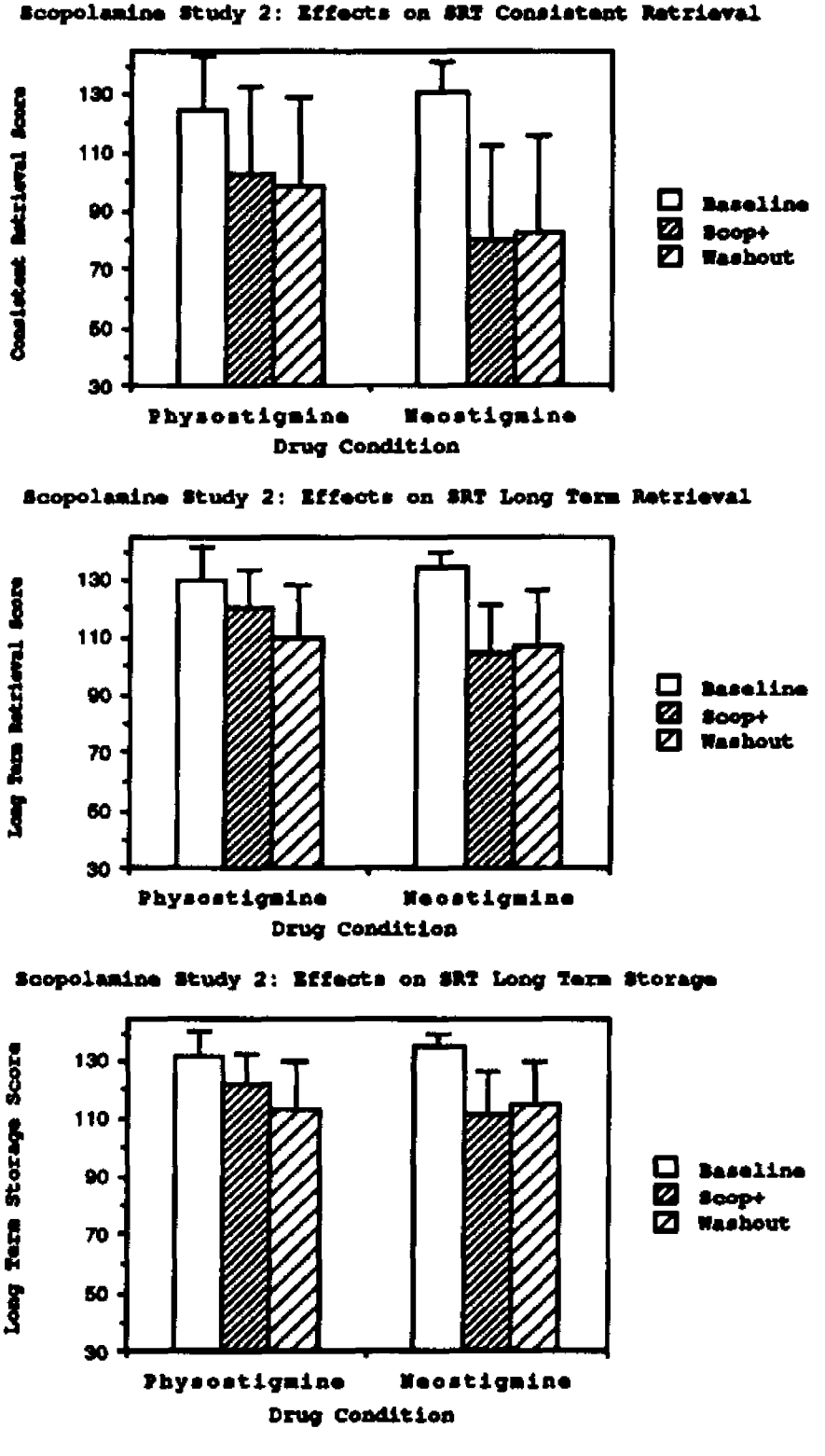

Scopolamine Study 2: Effects on SRT Consistent Retrieval
Consistent Retrieval Score
130
110
90
70
50
30
Physostigmine
Neostigmine
Drug Condition
Baseline
Scop+
Washout
Scopolamine Study 2: Effects on SRT Long Term Retrieval
Long Term Retrieval Score
130
110
90
70
50
30
Physostigmine
Neostigmine
Drug Condition
Baseline
Scop+
Washout
Scopolamine Study 2: Effects on SRT Long Term Storage
Long Term Storage Score
130
110
90
70
50
30
Physostigmine
Neostigmine
Drug Condition
Baseline
Scop+
Washout

Figure 10

Significant declines in consistent long term retrieval (t [8]=4.4, p=.002), long term retrieval (t [8]=6.6, p<.001) and long term storage (t [8]=9.2, p<.001) were shown relative to baseline. T-tests were computed to evaluate the differential effects on each scale after physostigmine versus neostigmine administration. The magnitude of deficit was significantly greater after neostigmine than after physostigmine for consistent long term retrieval (t [8]=2.50; p=.04), long term retrieval (t [8]=2.84, p=.02) and marginally significant for long term storage (t [8]=2.06, p=.07). In a separate examination of the physostigmine condition, all parameters significantly differed from baseline, indicating that physostigmine failed to reverse the SRT deficit (total recall: t [8]=2.65, p=.03; consistent long term retrieval: t [8]=2.81, p=.02; long term retrieval: t [8]=2.71, p=.03; and long term storage (t [8]=2.73, p=.03). Although greater deficit was observed after neostigmine verses physostigmine administration, scores did not return to baseline levels after physostigmine.

In regard to differential decline across parameters, decline in consistent long term retrieval was significantly greater than decline in total recall (t [8]=2.33, p=.05) or long term retrieval (t [8]=2.25, p=.05). For the neostigmine condition, decline in consistent long term retrieval was significantly greater than decline in total recall (t [8]=3.51, p=.008), long term retrieval (t [8]=2.68, p=.03)

or long term storage (t [8]=2.73, p=.03). Decline in long term retrieval was significantly greater than change in total recall (t [8]=3.94, p=.004) and long term storage (t [8]=2.71, p=.03).

Physostigmine did not reverse the memory deficit produced by scopolamine, especially in the consistent long term retrieval parameter. However, physostigmine administration prevents the decline in SRT to the extent observed in either the high or low dose groups in the first study (for example, total recall deficit post-treatment score relative to baseline: low dose-19%, high dose-27%, scopolamine +physostigmine-5%). The magnitude of deficit produced by the scopolamine +neostigmine combination is comparable to the effects of low dose scopolamine (for example, total recall deficit -post-treatment score relative to baseline: scopolamine +neostigmine-14%). A significant main effect of time on delayed recall was observed (F [1,7]=16.66, p=.01), indicating that after both physostigmine and neostigmine, subjects had difficulty in retrieving the words after a time interval.

For the digit span test, there was no effect on digit span forward, but a marginally significant drug by time interaction (F [1,7]=4.7, p=.06) was observed for digit span backward. Post-hoc t-tests reveal a marginally significant difference between conditions, in that performance on scopolamine +neostigmine decreased slightly and on scopolamine/ physostigmine, increased (t [8]=2.2, p=.06). As

in the first study, this deficit would not be considered to be clinically significant. There was a marginally significant effect of time on the delayed Visual Reproductions test ($F_{[1,7]}=4.10$, $p=.08$), indicating that scores were lower in comparing the post-infusion to baseline scores. Given the lack of effect on delayed recognition, this may reflect a retrieval deficit. The main effect of drug for the word fluency test was significant ($F=[1,7]=10.30$, $p=.01$), indicating that scores prior to and after infusion were higher in the physostigmine, than the neostigmine condition.

Other neuropsychological measures were not affected significantly. The more difficult condition for the Stroop Test, shows greater time to completion and more errors after drug-infusion for both conditions. Performance on the cancellation tests (trigram and number) and category naming tests was slightly, but non-significantly impaired after scopolamine +neostigmine administration. The lack of significant results may be attributable to effect size. Also of interest is that physostigmine does not dramatically enhance performance over baseline levels. This may be due to dosage considerations, because the physostigmine dose used in this study is lower relative to previous studies (see Table 2). Since the magnitude of deficit after scopolamine +neostigmine on the SRT was similar to that of low dose neostigmine, it is difficult to evaluate whether greater

deficit on the other measures would have been observed with scopolamine alone.

rCBF Results

Means and standard deviations for the rCBF data are shown in Table 10. Results of repeated measures analysis of variance are provided in Appendix 10. In examining change at individual detector locations, the maximal flow change was consistent with the anterior detector grouping used in the first study. A significant main effect was observed for drug (F [1,7]=74.06, p=.001) and a marginally significant effect for time (F [2,6]=4.83, p=.06). Significant interactions were obtained for drug by time (F [2,6]=5.31, p=.05), drug by region (F [1,7]=13.58, p=.01), time by region (F [2,6]= 9.34, p=.02) and drug by time by region (F [2,6]=7.13, p=.03).

The drug by time interaction is illustrated in Figure 11. Post-hoc tests were performed to examine this interaction. Comparisons of global flow were computed between drug and time conditions. The only significant difference which emerged was the difference in global flow between the 5 minute acute scopolamine study and the 25 minute scopolamine+neostigmine study (t [8]=2.22, p=.05). This indicates that neostigmine failed to antagonize scopolamine's effect on global flow, whereas physostigmine prevented the further reduction in flow.

TABLE 10 SCOPOLAMINE STUDY 2: EFFECTS ON RCBF

Means and Standard Deviations [in ml/100g/minutes]

(corrected to the mean baseline PCO2)

Physostigmine			
Baseline	Scopolamine	Scopolamine +Physostigmine	Washout
Anterior Perfusion			
71.5±9.7	67.4±10.0	73.9±8.1	68.8±5.9
Posterior Perfusion			
62.6±8.2	62.7±9.3	66.9±6.9	63.9±5.8
Anterior/Posterior Ratio			
1.14±0.04	1.08±0.06	1.10±0.04	1.08±0.05
Global Perfusion			
67.2±8.2	64.2±9.4	70.2±7.5	66.5±5.8

Neostigmine			
Baseline	Scopolamine	Scopolamine +Neostigmine	Washout
Anterior Perfusion			
73.9±12.6	77.8±13.0	71.2±7.8	76.3±10.2
Posrerior Perfusion			
65.5±11.4	71.1±12.5	66.2±7.8	71.1±9.5
Global Perfusion			
70.1±11.5	73.2±12.6	68.3±7.7	73.2±10.3
Anterior/Posterior Ratio			
1.13±0.04	1.10±0.05	1.08±0.04	1.07±0.04

Figure 11

Scopolamine Study 2: Effects on Global Grey Matter Perfusion. Results for physostigmine and neostigmine conditions at baseline, 5,25 and 70 minutes post-scopolamine infusion. A significant main effect was observed for drug ($F[1,7]=74.06$, $p=.001$) and a marginally significant effect for time ($F[2,6]=4.83$, $p=.06$). A significant interaction was obtained for drug by time ($F[2,6]=5.31$, $p=.05$).

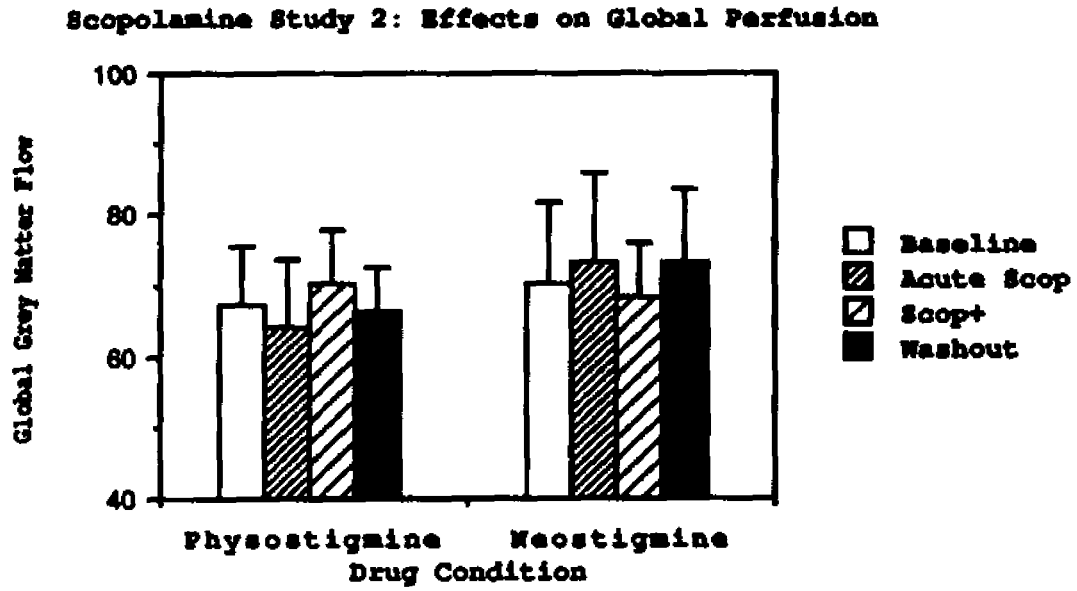

Scopolamine Study 2: Effects on Global Perfusion
Global Grey Matter Flow
100
80
60
40
Physostigmine
Neostigmine
Drug Condition
Baseline
Acute Scop
Scop+
Washout

Figure 11

Post-hoc tests for the drug by region interaction were computed to determine regional flow differences averaged across the four rCBF studies for each individual study day. These tests showed that the comparison of anterior to posterior flows within each drug condition were significant (physostigmine condition : t [8]=6.98,p<.001, neostigmine condition: t [8]=9.10, p<.001). This indicated that hyperfrontality was maintained throughout the four measurements on each of the two study visits. However, comparisons between anterior and posterior regions across drug conditions were non-significant, due to the fact that flows varied across time and that this variation was masked by averaging across time.

To evaluate the significant time by region interaction, post hoc t-tests were computed for the regional flow differences across drug conditions for each time condition (baseline, acute scopolamine, scopolamine +physostigmine/neostigmine, residual scopolamine). Anterior/posterior comparisons within each time condition were significant (baseline: t [8]=9.90, p<.001, 5 minute: t [8]=6.90, p<.001, 25 minute: t [8]=8.60, p<.001, 60 minute: t [8]=5.20, p=.001), indicating the maintenance of relative hyperfrontality for the time conditions. Comparisons of difference scores within regions, across time conditions were non-significant. This indicates that differential anterior versus posterior flow change was non-significant when averaging across drug conditions.

To examine the drug by time by region interaction, post-hoc tests were computed between the difference scores for each condition and region. These comparisons allow for the analysis of differential regional effects between drug and time conditions. This interaction is illustrated in Figure 12. For anterior rCBF, significant comparisons were observed in the difference between the 5 minute acute scopolamine study and 25 minute scopolamine +neostigmine study (t [8]= -3.27, p =.01), the difference between 5 minute acute scopolamine study and 25 minute scopolamine+ physostigmine study (t [8]=2.00, p =.08) and the difference between the 5 minute acute scopolamine study and 25 minute scopolamine +neostigmine study versus the difference between the 5 minute acute scopolamine study and 25 minute scopolamine +physostigmine study (t [8]=4.26, p=.003). Anterior flows significantly increased after physostigmine administration and decreased further after neostigmine administration. The differential response between neostigmine and physostigmine conditions was statistically significant. Therefore, physostigmine antagonized the further decline in flow from 5 to 25 minutes after scopolamine administration and neostigmine did not block the effects of scopolamine. For the neostigmine condition, anterior flows continued to decline at 25 minutes after drug administration, as observed in the high dose group in the first study.

Figure 12

Scopolamine Study 2: Effects on Regional Grey Matter Perfusion. Results for anterior and posterior regions in physostigmine and neostigmine conditions at baseline, 5,25 and 70 minutes post-scopolamine infusion.Significant interactions were obtained for drug by region (F [1,7]=13.58, p=.01), time by region (F [2,6]=9.34, p=.02) and drug by time by region (F [2,6]=7.13, p=.03).For the neostigmine condition, anterior flows continued to decline at 25 minutes after drug administration, as observed in the high dose group in the first study. Physostigmine antagonized the decline in anterior rCBF at 25 minutes.

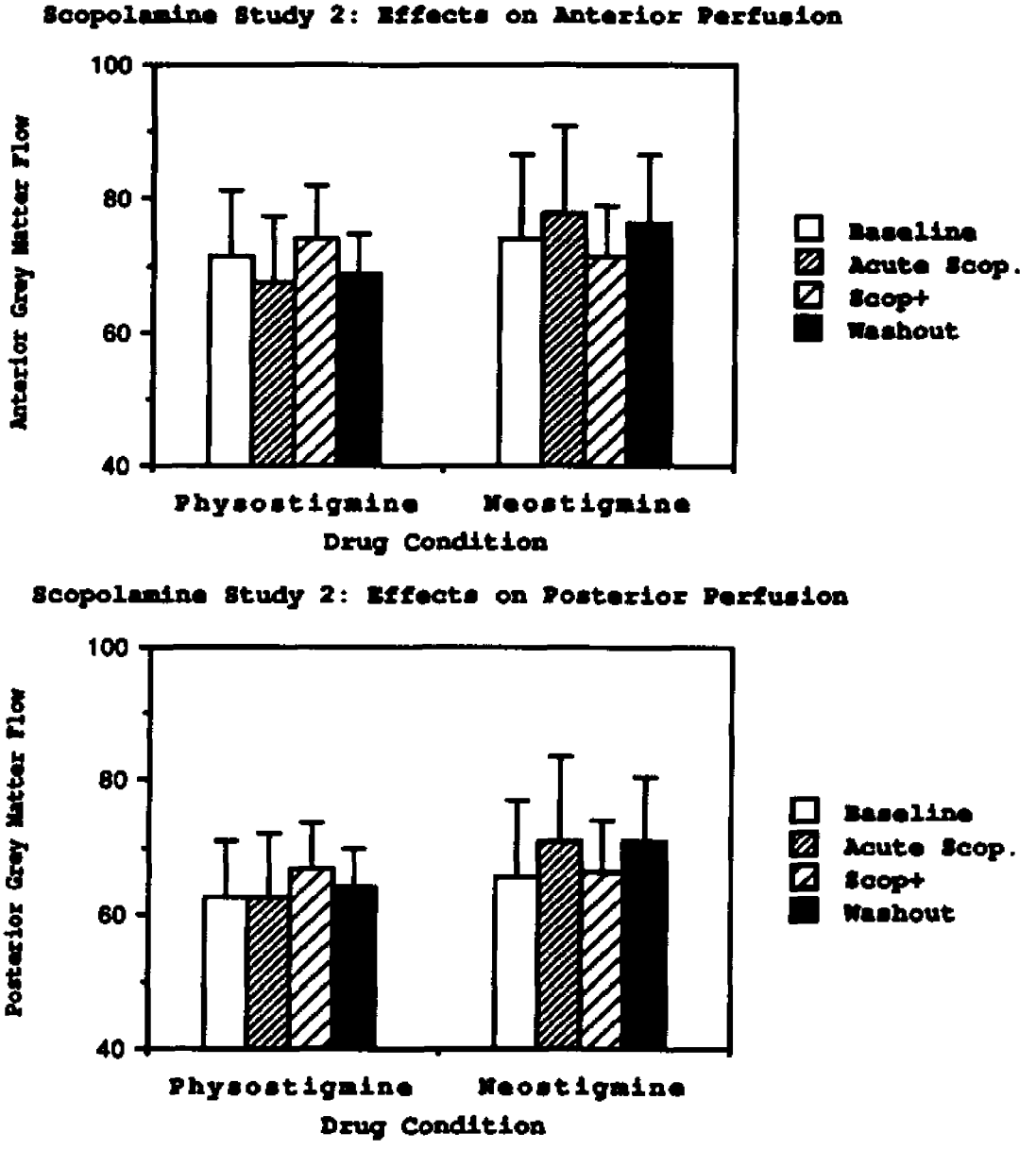

Scopolamine Study 2: Effects on Anterior Perfusion
Anterior Grey Matter Flow
100
80
60
40
Physostigmine
Neostigmine
Drug Condition
Baseline
Acute Scop.
Scop+
Washout
Scopolamine Study 2: Effects on Posterior Perfusion
Posterior Grey Matter Flow
100
80
60
40
Physostigmine
Neostigmine
Drug Condition
Baseline
Acute Scop.
Scop+
Washout

Figure 12

For posterior rCBF, the difference between the 5 minute acute scopolamine study and 25 minute scopolamine +neostigmine study was marginally significant (t [8]=-2.18, p=.06). The difference between the 5 minute acute scopolamine study and 25 minute scopolamine +neostigmine study versus the difference between physostigmine conditions was significant (t [8]=4.26, p=.003). A slight decline in flow was observed in posterior areas in the neostigmine condition, but no change occurred in the physostigmine condition. Again, the differential response was statistically significant. Neostigmine failed to antagonize the effects of scopolamine in posterior cortex, whereas physostigmine prevented the further reduction in flow.

Post-hoc t-tests were computed to assess differential change in anterior versus posterior regions. The comparison of change for anterior and posterior areas demonstrated that change in anterior areas was greater than change in posterior areas for the scopolamine and scopolamine +physostigmine effects relative to baseline (5 minute acute scopolamine study: t [8]=6.30, p<.001; 25 minute scopolamine +physostigmine study: t [8]=2.80, p=.02 and 60 minute residual scopolamine study: t [8]=5.39, p=.001) and for the scopolamine +neostigmine condition relative to baseline (25 minute scopolamine +neostigmine study: t [8]= 2.80, p =.02 and 60 minute residual scopolamine study: t [8]= 2.60, p =.03). Anterior flows showed significantly greater decline after acute scopolamine, greater increase after

physostigmine and decrease after neostigmine, relative to baseline. Significant differences were obtained for comparisons between the 5 minute acute scopolamine study and 25 minute scopolamine +physostigmine study (t [8]= 4.55, p =.002) and a marginally significant difference between the 5 minute acute scopolamine study and 25 minute scopolamine +neostigmine study (t [8]=-2.08, p =.07). These results indicate that anterior areas were subject to greater antagonism by scopolamine, further reduction after neostigmine and elevation by physostigmine.

Correlations between Neuropsychological Performance and rCBF

For the physostigmine condition, the change from the third rCBF study to baseline was significantly correlated with change in visual reproductions, delayed (r=0.75, 0.77, 0.77 , p=0.02,0.01,0.01 for anterior, posterior and global change, respectively) and change in the Stroop test, time to read color names (r=0.89, 0.88, 0.88; p=0.001,0.002,0.002).

Significant correlations were also observed for the change in scopolamine +neostigmine relative to baseline and change in Stroop Test, color-word interference, total time (r=0.79,0.82,0.82, p=0.02, 0.01, 0.01, respectively), the most difficult Stroop subtest. All other correlations were non-significant.

DISCUSSION OF STUDY 2

The administration of physostigmine or neostigmine after scopolamine produced similar, non-significant changes in autonomic function. Deficits on the SRT persisted after administration of both agents, the magnitude of decline greater with neostigmine. The magnitude of impairment is smaller after physostigmine administration than after either dose of scopolamine alone, as shown in Study 1. The consistent long term retrieval parameter was most affected and most resistant to reversal. Therefore, physostigmine partially antagonized the decline in memory function to the degree observed after administration of scopolamine alone. The finding of a SRT deficit after neostigmine administration, consistent with low dose, rather than high dose scopolamine may reflect either inter-subject variation or the relative contribution of peripheral anticholinergic effects to the memory deficit observed.

As in the first study, the SRT shows striking impairments in verbal memory, particularly in consistent long term retrieval. However, the subjects were not impaired in the ability to retrieve verbal material when a phonemic or semantic category was provided (verbal fluency and category naming) Chronic anticholinergic administration has been shown to produce greater deficit in consistent long term retrieval without impairments in delayed recall or recognition (Stern et al., 1988). The preservation of

retrieval on tasks which provide a strategy, indicates that the ability to organize information and spontaneously impose a mnemonic strategy is impaired. Consistent with this hypothesis is the observation that when questioned after testing, the majority of subjects did not report having adopted a particular mnemonic strategy to perform the SRT. Other investigators have reported similar patterns of impairment after scopolamine administration (eg. Beatty et al., 1986).

In the absence of a deficit on tasks measuring attentional processes, the consistent long term retrieval impairment appears to represent the phenomena of "forgetting to remember" as described by Hecaen and Albert (1978) in characterizing memory deficits in patients with frontal lobe injury. Squires (1987) interprets this as a "disconnection of knowing and doing". This implies a disorganization of memory and a lack of insight into mnestic processes or "metamemory". Scopolamine may impair the subjects' ability to evaluate their performance subjectively.

Greater impairment on the consistent long term retrieval parameter has been observed in normal aging (Larabee et al., 1986). Recently, Flicker et al. (submitted) demonstrated that scopolamine produces greater deficits in tasks of psychomotor speed, visuo-perceptual function (recall, recognition memory and praxis) and spares immediate memory and language function. These results are

consistent with the observation that the cognitive profile produced by scopolamine better mimics normal aging than Alzheimer's disease (Drachman and Leavitt, 1974). As described in the Discussion of the first study, the pattern of anterior rCBF deficits is similar to that observed in normal aging, rather than the temporo-parietal deficit observed in Alzheimer's disease (Smith and Prohovnik, 1986). Therefore, based on dissociations between the cognitive and rCBF effects of scopolamine and findings in Alzheimer's disease, scopolamine administration may provide a better model of the normal aging process.

The remaining neuropsychological measures were not significantly affected. Several possibilities must be considered to account for these findings. First, these measures may not be difficult for young, unimpaired subjects to perform. However, measures, such as the Stroop test and cancellation tests are timed and not likely to show "ceiling effects". Level of task difficulty must be considered in light of the findings by Sitaram et al. (1979), demonstrating that subjects who showed greatest proficiency at baseline memory testing were the most resistant to the effects of scopolamine. Another factor is effect size, in that a larger sample may be needed to demonstrate significant differences.

The lack of a significant effect on the remaining neuropsychological tests is contrary to other investigations. For example, deficits have been reported in

cancellation tasks, the Stroop test and category naming in young and elderly controls (Parrott, 1986; Wesnes and Revell, 1984; Caine et al., 1981; Sunderland et al., 1987). These studies all conducted neuropsychological testing at least one hour after drug administration, but due to the different route of administration of scopolamine it is difficult to equate the dosages. In these studies, scopolamine was not administered with neostigmine or another agent which would reverse the peripheral anticholinergic side effects. The lack of effect on the remaining neuropsychological tests indicates relative preservation of such functions as non-verbal memory, attention, vigilance and response inhibition. Although the rCBF profile indicates a frontal lobe locus of effect, the subjects do not exhibit classical frontal lobe deficits. This may be due to the fact that throughout all conditions, the hyperfrontal distribution was maintained, even though anterior rCBF showed the greatest amount of change. Therefore, the maintenance of hyperfrontality may be significant in preserving performance on these measures.

The rCBF results indicated that the physostigmine antagonized the effects of scopolamine on both global and anterior rCBF. Neostigmine failed to antagonize the decline in global and anterior flow. The decline in flow is attenuated after neostigmine, relative to that observed in the first study. After scopolamine administration, physostigmine elevated flow levels, primarily in anterior

cortex, and did not significantly increase posterior flow. Clinical studies have revealed that physostigmine produced relative increases in rCBF in temporo-parietal cortex of Alzheimer patients and rCBF in frontal cortex of patients with Progressive Supranuclear Palsy (Gustafson et al., 1987; Litvan et al. 1988).

Several possibilities may account for the the reversal of the anterior flow deficit with physostigmine. The most direct explanation involves the increased levels of available acetylcholine preferentially acting upon unoccupied muscarinic receptors in the frontal lobe. Secondly, acetylcholinesterase has been characterized in non-cholinergic neurons, for example non-cholinergic neurons of the Substantia Nigra, pars compacta and ventral tegmental area (Butcher et al., 1978). Administration of an acetylcholinesterase inhibitor may activate these cholinoceptive neurons, which may be among the neuronal population that projects to frontal cortex. Physostigmine failed to reverse the consistent long term retrieval deficit to baseline levels. This may be due to the relatively low dose of physostigmine used. If this deficit is mediated by dopaminergic mechanisms, a dopaminergic agonist may be more efficacious. To more directly evaluate the role of dopaminergic systems in the scopolamine effect, the ability of a dopaminergic agonist to reverse the resulting deficits should be evaluated.

The issue of vascular or peripheral components of scopolamine's effects must be evaluated. Although, neostigmine is considered a peripheral cholinesterase inhibitor, several studies have shown minimal effects on cognitive and autonomic function, probably due to indirect, peripheral activation. Ketchum et al. (1973) administered either saline, physostigmine (60µg/kg,IM) or neostigmine (60µg/kg,IV) to reverse the effects of atropine (175µg/kg,IM). Neostigmine produced an immediate reduction in heart rate and improved performance on a digit span test several hours later The decline in heart rate induced by neostigmine differed significantly from the saline condition. Physostigmine both reduced heart rate and immediately improved performance on a digit span test. The doses of physostigmine and neostigmine used were much greater than the doses used in this study. Therefore, neostigmine produces potent cardiovascular effects and may affect cognitive performance at a longer time interval. Risch et al. (1986) studied the effects of physostigmine and neostigmine on neurohormone markers in psychiatric patients. Significant elevations in ACTH, cortisol, dopamine, norepinephrine and epinephrine were noted after physostigmine and slight elevations after neostigmine administration. Neostigmine exerts minimal effects, through peripheral mediation, although physostigmine exerts a more potent effect on cognitive and neurochemical function.

A series of investigations conducted by Davis and colleagues have examined peripheral and central mechanisms contributing to the the cholinergic modulation of local cortical blood flow (LCBF). The effects of centrally and peripherally-acting cholinesterase inhibitors and muscarinic agonists were evaluated, using the hydrogen clearance technique in the rat (Davis, personal communication). Physostigmine administration (0.1 or 0.32mg/kg, SC) increased LCBF, but the same dose of neostigmine (the higher of which is lethal) did not affect LCBF. Scopolamine, but not n-methylscopolamine, antagonized the physostigmine elevation in LCBF. Similarly, the effects of physostigmine (0.1mg/kg) or arecholine (3.2mg/kg, both administered with n-methylscopolamine, 0.32mg/kg) in enhancing LCBF are attenuated when administered after neurotoxic lesion of the Nucleus Basalis of Meynert. Therefore, centrally, not peripherally-acting cholinesterase inhibitors increase LCBF and the effects of centrally acting muscarinic agonists and cholinesterase inhibitors can be antagonized by manipulations that affect central (scopolamine administration or lesion of the Nucleus basalis of Meynert), but not peripheral (n-methylscopolamine administration) cholinergic systems. Similar findings have been reported by Triguero et al. (1988), who demonstrated that neostigmine failed to effect CBF, (30µg/kg, IV), but physostigmine increased CBF (30µg/kg, IV), as measured with the hydrogen clearance technique in the goat. In light of these findings,

the differential response to scopolamine across studies (scopolamine alone versus scopolamine +neostigmine) is not attributable to activation of peripheral cholinergic mechanisms,which affect rCBF, but favors an interpretation of inter-subject variability.

There are two instances of dissociation between preserved rCBF but mnestic deficit: the low dose group in the first study and the physostigmine group in the second study. This indicates that there may be differential thresholds and time courses for rCBF and cognitive function. Cognitive function may be more sensitive to the effects of cholinergic manipulation and memory performance in young normals can be impaired in the absence of a flow deficit. This may indicate a compensatory ability of the nervous system, which is present in intact individuals and can be elicited through challenge of the system. In pathological states, such as the degenerative dementias, this compensatory ability may be lost, in which case rCBF and cognitive function are correlated (Prohovnik et al., 1988). The manifestation of such compensatory mechanisms has been observed in animal models of cholinergic impairment (London et al., 1984; Smith, 1988). One month after neurotoxic lesion of the nucleus Basalis of Meynert, persistent deficits in cortical cholinergic activity and memory function are observed. However, deficits in glucose metabolism manifest acutely after lesion are reversed at this time. Two examples of such structural/ functional

dissociations can be cited from human studies. Recent work has shown that normal aging is accompanied by structural change, (i.e. cortical atrophy), but not by cerebral glucose metabolic alterations (De Leon et al., 1987). rCBF studies in presenile onset Alzheimer's disease reveal a significant reduction in the weight of the grey matter compartment , but no reduction in perfusion, relative to senile onset Alzheimer patients (Prohovnik et al., in press). The elucidation of these compensatory mechanisms may provide useful insight into the etiology and treatment of neurodegenerative disease

Conclusions

1. The global decline in rCBF is antagonized by physostigmine.

2. The anterior rCBF deficit is antagonized by physostigmine, not neostigmine.

3. Impairments in neuropsychological function are seen in the SRT. The parameter most affected is consistent long term retrieval as demonstrated in the first study. The mnestic deficit is more severe in the neostigmine condition, although the deficit persists after physostigmine administration. Other areas of neuropsychological function are unimpaired, such as phonetic and semantic word retrieval, attention, vigilance and response inhibition.

GENERAL DISCUSSION

Several implications have emerged from the studies reported herein. Analysis of the effects of scopolamine on rCBF permit evaluation of the relation of neurochemical manipulation to neurotransmitter topography and neural connectivity, the dissociations between rCBF effects relative to the underlying neural pathways; the effects of such manipulation on neuropsychological function, and the implications of reversal of impairments for the specificity of the deficits produced.

The effects of scopolamine on rCBF represent an example, in the human, of the dissociation between neurotransmitter distribution and drug effects previously observed in the rodent in several neurotransmitter systems (eg. McCulloch, 1982; Palacios et al., 1982). The findings from such studies suggest that the administration of neuropharmacological agents produce a topography of change in neurometabolism that overlaps with functional anatomical pathways, rather than producing changes limited to the those areas with the highest receptor or enzymatic concentrations. Another aspect of the metabolic effect is the interaction of a particular agent with other neurotransmitter systems, which could also contribute to the dissociation between metabolic change and known neurochemical pathways.

Several alternative explanations for scopolamine's effects on rCBF have been offered. The diffuse reduction in

rCBF parallels the diffuse distribution of muscarinic receptors, choline acetyltransferase and acetylcholinesterase levels in the cerebral cortex, the projections of which originate from the Nucleus basalis of Meynert in the ventral forebrain (Mesulam et al., 1983). The ability of a pharmacological agent to produce such widespread effects on rCBF and mnestic function emphasizes the vast influence of the cholinergic system on higher cortical activity. A further anatomical substrate for this effect is provided by the identification of cortico-cortico connections (Goldman-Rakic, 1987; Amaral, 1987), reciprocal circuits between secondary sensory areas and multimodal association cortices. These areas of cerebral cortex project via entorhinal cortex to the hippocampal formation, which sends reciprocal efferents to these structures via the entorhinal cortex. Depression of activity in this pathway would cause diffuse diminutions of rCBF. The selective anterior rCBF deficit cannot be explained by a selective cholinergic projection, but could be accounted for by another neurotransmitter system, preferentially distributed in frontal cortex, which interacts with acetylcholine. The logical choice in this regard is dopamine, particularly the neurons of the ventral tegmental area and the Substantia Nigra, par compacta-the origin of the mesocortical dopamine projection (Moore and Bloom 1978; Goldman-Rakic, 1987). Subpopulations of these cells exhibit acetylcholinesterase immunoreactivity and the ability of cholinergic manipulation

to affect dopaminergic release from these cells has been reported (Butcher et al, 1978; de Belleroche et al., 1982; 1985). Therefore, the inhibition of diffuse cholinergic projections throughout cortex after scopolamine administration produces global depression of rCBF and the secondary antagonism of dopaminergic function results in further inhibition of the mesocortical dopamine pathway to reduce anterior CBF. In addition, the widespread inhibition of cortical activity, across highly interconnected regions, eliminates the major input to the hippocampal formation.

This scenario can also account for the behavioral deficits produced by scopolamine. The impairment in mnestic processes, as shown by deficit on the SRT, represents deficits in the storage, retrieval and especially, the consistent retrieval of verbal material. Such anterograde deficits, are typical sequelae of lesions to the hippocampal formation and amygdala (eg. Squire and Cohen, 1984) and the severe disruption of consistent recall is similar to the cognitive impairment noted after damage to prefrontal cortex, specifically lesions of the catecholaminergic innervation (Goldman-Rakic, 1987). Involvement of the multiple pathways described is also consistent with the pattern of neuropsychological deficits.

Within the studies reported, there were two instances in which cognitive deficit were observed without concomitant flow reductions: in the low dose scopolamine condition and the scopolamine +physostigmine condition in the second study

. This is underscored by the lack of correlation between change in rCBF and change in cognitive function. These findings may indicate a compensatory ability in the intact nervous system, revealed when the system is challenged. In disease states such as the degenerative dementias, cognitive function is correlated with rCBF and other indices of brain function (eg. Prohovnik et al., 1988). The simultaneous assessment of rCBF and cognitive function allows for observation of neural connectivity and provides new insights into localization of function, previously limited to the assessment of brain-damaged subjects. A more direct method of examining the cognitive effects of scopolamine would involve the performance of a mnestic activation during an rCBF study, at which point task performance could be more directly correlated with regional flow change.

FUTURE DIRECTIONS FOR STUDY

These studies highlight the complexity of the organization of the cerebral cortex and support the simultaneous assessment of cognitive function and neural metabolism to elucidate the role of neurotransmitter systems in brain function. Several questions remain to be addressed. The exact interaction between cholinergic and other neurotransmitter systems must be clarified and their role in the regulation of cerebral blood flow and metabolism addressed. First, the efficacy of a receptor agonist versus

a cholinesterase inhibitor in reversing scopolamine's effects should be examined. The use of a muscarinic agonist to reverse scopolamine's effects might reveal a less diffuse pattern of reversal. The administration of a dopaminergic agonist would allow the opportunity to examine cholinergic/dopaminergic interactions. Other relevant studies would involve the evaluation of the cognitive and metabolic effects of other agents implicated in higher cortical function and neurodegenerative disease, such as the excitatory neurotransmitter glutamate or the effects of the glucocorticoids, which have been implicated in the degenerative changes of normal aging (Van Hoesen and Damasio, 1987; Sapolsky and McEwen, 1986). These agents may prove to better mimic the changes observed in Alzheimer's disease.and other neurodegenerative diseases. Finally, the application of these paradigms to normal aging and degenerative dementias may reveal other aspects of the pathophysiology of these entities.

The studies described have elucidated some of the interactions between cholinergic function, cerebral perfusion and cognitive function. Several methodological limitations should be addressed. Due to practical limitations as discussed in the Methods section, the studies did not include a rigorous dose finding phase for the compounds utilized. The question that remains is whether a greater dose of physostigmine would have reversed the SRT deficit. Several issues remain regarding the

neuropsychological effects of scopolamine. First, whether the lesser degree of SRT deficit in the scopolamine+ neostigmine condition is due to reversal of peripheral anticholinergic side effects or inter-subject variability. Secondly, whether more difficult neuropsychological tests would have revealed deficits in attention, response inhibition and other frontal lobe functions. Finally, the use of tests to measure subjective perception of memory performance and the comparison of perfsrmance on tests with structured versus unstructured task demands would further elucidate the nature of the consistent long term retrieval impairment.

The central/peripheral contributions to the effects of scopolamine should be evaluated. The best approach would be to use Positron Emission Tomography, with [^{15}] O as a tracer to evaluate the vascular effects of cholinergic manipulation, then a deoxyglucose tracer to examine the effect on cerebral metabolism.

With the advent of improved neuroimaging techniques, more detailed studies of the relation between the neurotransmitter function and cerebral metabolism will be possible. Further improvements in the resolution of Positron Emission Tomography studies will permit imaging of the subcortical structures, such as hippocampal formation which are involved in memory processes and neurodegenerative disease. In evaluating the role of the cholinergic system, for example, many new ligands are available such as

quinuclidinyl benzilate for Single Photon Emission Tomography and [^{11}C] scopolamine for Positron Emission Tomography (Eckelman et al., 1984; Frey et al., 1987), which can be applied to studies of cognitive activation and to elucidate the mechanisms of neurodegenerative disease.

APPENDIX 1: SUBJECT EXCLUSION CRITERIA

EXCLUSIONARY CRITERIA FOR SCOPOLAMINE STUDIES

1. History of sensitivity to anticholinergics and cholinesterase inhibitors.
2. History of cardiac, gastrointestinal or respiratory disorders: including, diabetes, hypertension, heart disease, glaucoma, asthma, ulcers, collitis.
3. History of neurological, neuromuscular or opthalamic disorders.
4. History of psychiatric illness.
5. History of drug or alcohol abuse.
6. Radiation exposure-more than three radiation procedures within the last year.
7. Significant clinical findings on urinalysis, laboratory blood testing or electrocardiogram.
8. Subjects must not be taking any medication which may influence rCBF, cognitive function or interact with anticholinergics.

APPENDIX 2: SUBJECT CONSENT FORM

CONSENT FORM

IRB Protocol # 1418

Effects of Cholinergic Agents on Regional Cerebral Blood Flow in Normal Subjects

Purpose of Study

I voluntarily agree to participate in a study of the effects of cholinergic drugs on regional Cerebral Blood Flow (rCBF, a noninvasive measure of blood flow in the brain).

Study Procedures

I will be invited first to a screening session, during which the purpose and schedule of the experiment will be explained again in detail. I will then undergo medical examinations, to ensure that I have no medical problems that would interfere with the tests. The screening tests will include EKG, interviews, physical exam, and the drawing of a small amount of blood for laboratory tests. I will also be asked about allergic reactions to any of the drugs used, and to Atropine. I am not allergic to any of those, to the best of my knowledge.

After the initial screening session, I will have to come for two visits. On each of these I will undergo one rCBF measurement,as well as EEG and a measurement of finger sweat. Then I will receive medication. The three drugs I will receive (in a random and unknown order) are scopolamine, neostigmine and physostigmine. All three are well-known drugs, and approved for use by the FDA as,

respectively, a medicine to prevent nausea and vomiting, a drug to reverse problems after surgery and one to counter the effects of anticholinergic drugs. After the medicine, I will have a repeat set of measurements. I will then have some psychological tests administered to assess my memory and mood. Then I will receive another drug, and the the measurements will be repeated. Before each laboratory visit, I must not have any food and drink for three hours. If I am a smoker, I must not smoke for at least 30 minutes before coming to the laboratory. During the study, I must not take other medications or drugs without asking the investigators, except in an emergency.

The rCBF procedure requires that I lie on a stretcher, with my head in a helmet-like device. I should simply relax and keep my eyes closed. A small amount of a radioactive gas (133-Xenon) will be added to the air I breathe. Xenon is a noble gas, and in the small amount used here does not have any effect on my body. It simply passes through the lungs and into the blood, much like oxygen. The blood carries the Xenon to the brain, where it is measured by sensitive radiation detectors placed around the head. The blood then washes the Xenon out of the brain and carries it back to the lungs, where it is exhaled. The blood flow in each region of the brain is calculated by the rate at which the Xenon enters and then leaves the areas under the detectors.

For each measurement I will be asked to lie on the bed, with my head in the helmet, for about 15 minutes. It is

important that I do not move my head during this time, and therefore my head will be lightly restrained. During the measurement, I will breathe through a face mask (such as pilots wear), covering my nose and mouth. Someone will be with me at all times, and the mask is equipped with a microphone so that I can easily communicate if necessary. It is important that the mask fit snugly; a mask will be selected that best fits my face, and will be fastened with rubber bands. The procedure is completely painless, and there is no discomfort besides breathing through the mask and lying still for about 15 minutes.

Risks and Benefits

I understand that there will be a maximum of eight rCBF measurements in total. The total amount of radiation I will receive from all eight procedures is less than that of other common X-ray procedures. It is also less than the levels considered safe by the FDA for either occupational exposure or for testing of new radioactive procedures. Such rCBF studies have been performed in many hospitals and with thousands of subjects, and no risks have been found. The consensus of medical opinion is that health risks from this low level of radiation, if they exist, are too small to be measurable. The possible existence of such hazards cannot, however, be excluded. Other than this minimal risk, there is no known danger associated with these measurements. The only individuals who may be at a somewhat greater risk are those who have already been exposed to large radiation doses in

the past year; therefore, I will be asked to complete a form regarding my previous radiation exposure.

Scopolamine may cause a dry mouth and blurred vision as transient side effects. Scopolamine may also produce temporary memory impairment and disorientation. At doses higher than the one I will be given, some people experience visual hallucinations and get upset. Physostigmine and neostigmine are used to reverse the effects of drugs like scopolamine and may cause salivation and nausea. If these side-effects do occur, they are likely to be brief and end before I leave the laboratory. The doses used in this experiment are not likely to result in any other side effects, and many subjects have been given these drugs without any report of ill effects. The only known risks consist of irregular heart rhythm and low blood pressure. Should any of these occur, the experiment will be stopped immediately and appropriate medications given.

The EEG and stimulus tests involve electrodes being applied to my scalp and finger tips with conductive paste resembling toothpaste. This takes about 45 minutes. During the response tests either a light will be flashed in my eyes or a click will be heard.These tests will be conducted 4 times and will take about 4 minutes each time. I understand that there is no risk associated with these tests in subjects with no history of convulsive disorder.

Research Standards and Rights of Participants

I understand that all data collected will be used for research purposes only. Information that can be identified as associated with me will not be released outside of the research team without my consent. All information will be coded by number. I understand that there are no benefits of this study to me, except for money paid to me for my time ($10 per hour; or $120 for finishing the study, which takes about 10 hours). All my questions about the study have been answered; if I have further questions, I can call the investigators: Dr. Prohovnik (960-5853) or Dr.Decina (960-5558). I understand that I may refuse to participate or withdraw from the study at any time without any penalty (other than the loss of remuneration), and without any loss of benefits to which I am otherwise entitled. The amount paid to me will be prorated if I withdraw, that is, $10 per hour for the time I do spend in the study.

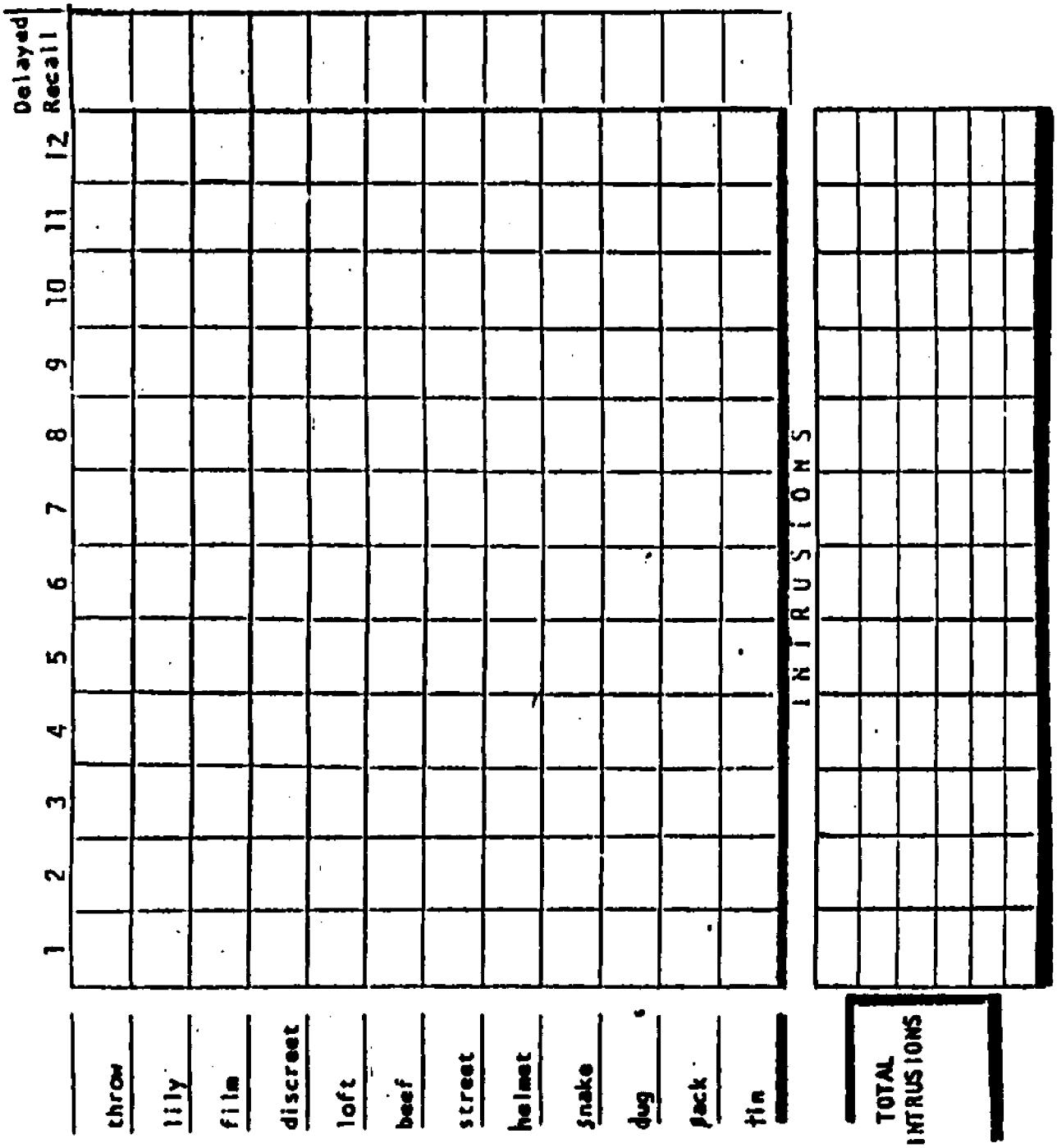
I understand that if, as a result of my participation in this research, injuries occur from the known or unknown risks of the study, immediate medical care and treatment (including hospitalization, if necessary) will be available. Emergency medical treatment, within the capability of the New York State Psychiatric Institute, will be provided free of charge. I understand, however, that funds are not available to cover the costs of additional medical treatment or other compensation.

I understand that this study has been approved by the Institutional Review Board (IRB) of the New York State Psychiatric Institute and the Department of Psychiatry, College of Physicians and Surgeons of Columbia University, as well as by the Columbia-Presbyterian Medical Center Joint Radioisotope Committee. If I have any questions about my rights as a research subject, I may call the IRB at 960-5757.

I have received a copy of this consent form.

_____	_____
Subject's name (print)	Subject's signature
_____	_____
Witness' name (print)	Witness' signature

APPENDIX 3: EXAMPLE OF THE SELECTIVE REMINDING TEST



APPENDIX 4 SCOPOLAMINE STUDY 1: EFFECTS ON AUTONOMIC FUNCTION

Means and Standard Deviations for Autonomic Variables

Low Dose			High Dose		
Time from scopolamine infusion (minutes)					
Baseline	5min post	25min post	Baseline	5min post	25min post
PCO2 [mmHg]					
38±3	39±2	39±3	42±5	43±5	44±5
Systolic Blood Pressure [mmHg]					
113±7	106±5	107±10	110±9	115±7	114±8
Diastolic Blood Pressure[mmHg]					
71±9	71±6	72±4	75±6	75±6	73±7
Pulse Rate[beats/minutes]					
60±9	65±10	58±4	73±6	76±11	70±11

Appendix 4-Continued

Results of Repeated Measures Analysis of Variance for Autonomic Variables

PCO2

	df	F	p
Dose	1,13	3.02	0.11
Time	2,12	3.16	0.08
Dose x Time	2,12	0.67	0.53

Systolic Blood Pressure

	df	F	p
Dose	1,13	1.45	0.25
Time	2,12	0.31	0.74
Dose x Time	2,12	4.37	0.04

Diastolic Blood Pressure

	df	F	p
Dose	1,13	0.84	0.38
Time	2,12	0.12	0.99
Dose x Time	2,12	0.47	0.64

Pulse

	df	F	p
Dose	1,13	8.09	0.01
Time	2,12	3.88	0.05
Dose x Time	2,12	0.06	0.94

APPENDIX 5 SCOPOLAMINE STUDY 1: RESULTS OF REPEATED MEASURES ANALYSIS OF VARIANCE FOR NEUROPSYCHOLOGICAL MEASURES

Selective Reminding Test

	df	F	p
Dose	1,13	2.32	0.15
Time	1,13	117.76	<0.001
Scale	3,11	32.25	<0.001
Dose x Time	1,13	1.59	0.23
Dose x Scale	3,11	0.83	0.50
Time x Scale	3,11	15.00	<0.001
Dose x Time x Scale	3,11	0.44	0.73

Digit Span

Digit Span: Forward

	df	F	p
Dose	1,13	0.03	0.86
Time	1,13	0.01	0.90
Dose x Time	1,13	5.41	0.04

Digit Span: Backward

	df	F	p
Dose	1,13	1.03	0.33
Time	1,13	0.82	0.38
Dose x Time	1,13	0.01	0.94

APPENDIX 6 SCOPOLAMINE STUDY 1: RESULTS OF REPEATED MEASURES ANALYSIS OF VARIANCE FOR rCBF

	df	F	p
Dose	1,13	0.00	0.95
Time	2,12	20.05	<0.001
Region	1,13	88.35	<0.001
Dose x Region	1,13	1.01	0.33
Dose x Time	2,12	19.48	<0.001
Region x Time	2,12	8.46	0.01
Dose x Region x Time	2,12	0.39	0.69

APPENDIX 7 SCOPOLAMINE STUDY 2: NEOSTIGMINE DOSE EFFECTS

Means and Standard Deviations for Autonomic Variables

Baseline	Acute Scopolamine	Scopolamine+	Washout
	PCO2 [mmHg]		
	Physostigmine		
low dose neostigmine			
38±3	40±3	40±2	39±2
high dose neostigmine			
42±3	43±2	41±2	42±4
	Neostigmine		
low dose neostigmine			
40±5	40±4	39±4	38±3
high dose neostigmine			
38±6	39±6	38±5	38±6
	Systolic Blood Pressure [mmHg]		
	Physostigmine		
low dose neostigmine			
103±8	107±4	107±3	104±9
high dose neostigmine			
115±5	116±5	116±4	107±5
	Neostigmine		
low dose neostigmine			
107±4	108±5	107±4	105±5
high dose neostigmine			
112±8	113±5	108±9	110±8

Appendix 7-Continued

Baseline	Acute Scopolamine	Scopolamine+	Washout
	Diastolic Blood Pressure [mmHg]		
	Physostigmine		
low dose neostigmine			
69±8	69±3	66±6	66±6
high dose neostigmine			
70±5	74±4	74±6	65±5
	Neostigmine		
low dose neostigmine			
68±8	69±4	66±6	68±5
high dose neostigmine			
67±8	73±5	68±2	69±3
	Pulse Rate [beats/min]		
	Physostigmine		
low dose neostigmine			
64±11	66±8	51±6	51±8
high dose neostigmine			
70±9	74±6	59±3	63±3
	Neostigmine		
low dose neostigmine			
65±8	67±8	55±9	58±9
high dose neostigmine			
68±11	71±12	53±8	58±4

Appendix 7-Continued

Repeated Measures Analysis of Variance for Autonomic Measures

PCO2

	df	F	p
Neogroup	1,7	0.07	0.79
drug	1,7	5.04	0.06
time	3,5	31.70	0.001
Neogroup x drug	1,7	2.60	0.15
Neogroup x time	3,5	4.67	0.07
drug x time	3,5	0.85	0.52
Neogroup x drug x time	3,5	0.15	0.93

Systolic Blood Pressure

Neogroup	1,7	8.10	0.03
drug	1,7	0.00	0.99
time	3,5	2.08	0.22
Neogroup x drug	1,7	1.93	0.21
Neogroup x time	3,5	0.26	0.86
drug x time	3,5	0.48	0.71
Neogroup x drug x time	3,5	1.09	0.43

Appendix 7-Continued

Diastolic Blood Pressure

	df	F	p
Neogroup	1,7	1.21	0.31
drug	1,7	0.03	0.86
time	3,5	2.03	0.23
Neogroup x drug	1,7	0.50	0.50
Neogroup x time	3,5	1.35	0.36
drug x time	3,5	2.55	0.17
Neogroup x drug x time	3,5	0.83	0.53

Pulse

Neogroup	1,7	1.38	0.29
drug	1,7	0.08	0.79
time	3,5	77.43	<0.001
Neogroup x drug	1,7	7.24	0.03
Neogroup x time	3,5	2.80	0.15
drug x time	3,5	0.38	0.78
Neogroup x drug x time	3.5	0.82	0.54

Appendix 7-Continued

Means and Standard Deviations for Selective Reminding Test

Baseline	Post Scop+ Physostigmine	Baseline	Post Scop+ Neostigmine
Total Recall			
low dose neostigmine			
133.4±9.1	127.2±10.0	135.8±3.3	109.2±9.0
high dose neostigmine			
133.5±9.1	126.3±5.2	137.0±5.8	124.0±2.9
total			
133.4±8.5	126.8± 7.8	136.3±4.3	115.8±10.2

Results of Repeated Measures Analysis of Variance for Selective Reminding Test

	df	F	p
Neogroup	1,7	0.96	0.36
drug	1,7	1.50	0.26
time	2,6	19.29	0.002
Neogroup x drug	1,7	1.70	0.23
Neogroup x time	2,6	1.66	0.27
drug x time	2,6	4.73	0.06
Neogroup x drug x time	2,6	1.60	0.28

Appendix 7-Continued

Effects on rCBF

Physostigmine

Baseline	Acute Scopolamine	Scopolamine+	Washout
Anterior Perfusion			
low dose neostigmine			
72.7±5.6	66.9±13.0	74.6±9.2	66.7±5.7
high dose neostigmine			
70.0±14.3	68.1±6.1	73.1±7.8	71.4±5.6
Posterior Perfusion			
low dose neostigmine			
63.7±5.5	62.4±11.2	67.9±8.2	62.4±7.0
high dose neostigmine			
61.2±11.7	63.1±7.8	65.8±6.0	65.9±3.4
Anterior/Posterior Ratio			
low dose neostigmine			
1.14±0.03	1.07±0.05	1.10±0.04	1.07±0.04
high dose neostigmine			
1.14±0.06	1.08±0.07	1.11±0.04	1.08±0.07
Global Perfusion			
low dose neostigmine			
68.3±5.3	63.7±11.7	70.2±7.5	66.4±5.8
high dose neostigmine			
65.7±11.7	64.7±7.0	70.9±9.0	64.8±6.9

Appendix 7-Continued

Neostigmine

Baseline	Acute Scopolamine	Scopolamine+	Washout
Anterior Perfusion			
low dose neostigmine			
66.1±4.0	76.17±15.0	70.3±8.8	70.4±6.3
high dose neostigmine			
83.5±13.4	79.8±11.9	72.4±7.5	83.7±9.6
Posterior Perfusion			
low dose neostigmine			
57.8±4.3	69.6±12.8	65.3±6.9	66.4±6.5
high dose neostigmine			
75.1±10.1	73.1±13.8	67.4±9.8	77.0±10.0
Anterior/Posterior Ratio			
low dose neostigmine			
1.15±0.02	1.10±0.05	1.08±0.04	1.06±0.02
high dose neostigmine			
1.11±0.06	1.10±0.06	1.08±0.05	1.09±0.06
Global Perfusion			
low dose neostigmine			
70.1±11.5	73.2±12.6	68.3±7.7	73.2±10.3
high dose neostigmine			
62.6±4.2	71.6±13.4	66.8±7.1	68.1±6.9

Appendix 7-Continued

Repeated Measures Analysis of Variance Results for rCBF

	df	F	p
Neogroup	1,7	1.17	0.32
drug	1,7	74.06	<0.001
time	2,6	4.83	0.06
region	1,7	0.23	0.65
Neogroup x drug	1,7	0.18	0.68
Neogroup x time	2,6	5.51	0.05
Neogroup x region	1,7	0.11	0.75
drug x time	2,6	5.31	0.05
drug x region	1,7	13.58	0.01
time x region	2,6	9.34	0.02
Neogroup x drug x time	2,6	2.11	0.22
Neogroup x drug x region	1,7	1.03	0.34
Neogroup x time x region	2,6	1.17	0.41
drug x time x region	2,6	7.13	0.03
Neogroup x drug x time x region	2,6	0.21	0.88

APPENDIX 8 SCOPOLAMINE STUDY 2: EFFECTS ON AUTONOMIC FUNCTION

Means and Standard Deviations for Autonomic Variables

Baseline	Acute Scopolamine	Scopolamine+	Washout
		PCO2 [mmHg]	
		Physostigmine	
41±3	41±3	41±2	40±3
		Neostigmine	
39±5	39±5	38±4	38±4
		Systolic Blood Pressure [mmHg]	
		Physostigmine	
108±9	111±6	109±7	105±7
		Neostigmine	
109±6	110±6	107±6	107±7
		Diastolic Blood Pressure [mmHg]	
		Physostigmine	
68±7	71±4	69±7	65±5
		Neostigmine	
67±7	71±5	67±4	68±4
		Pulse Rate [beats/min]	
		Physostigmine	
67±10	69±8	55±6	56±9
		Neostigmine	
66±9	69±9	54±8	58±7

Appendix 8-Continued

Repeated Measures Analysis of Variance for Autonomic Measures

PCO2

	df	F	p
drug	1,7	5.04	0.06
time	3,5	31.7	0.001
drug x time	3,5	0.85	0.52
Systolic Blood Pressure			
drug	1,7	0.00	0.99
time	3,5	2.08	0.22
drug x time	3,5	0.48	0.71
Diastolic Blood Pressure			
drug	1,7	0.03	0.86
time	3,5	2.03	0.23
drug x time	3,5	2.55	0.17
Pulse			
drug	1,7	0.08	0.79
time	3,5	77.43	<0.001
drug x time	3,5	0.38	0.78

APPENDIX 9 SCOPOLAMINE STUDY 2: RESULTS OF REPEATED MEASURES ANALYSIS OF VARIANCE FOR NEUROPSYCHOLOGICAL MEASURES

Selective Reminding Test

	df	F	p
scale	3,5	12.6	0.007
time	1,7	41.51	0.001
drug	1,7	1.41	0.28
drug x time	1,7	7.7	0.03
scale x drug	3,5	0.7	0.56
scale x time	3,5	5.3	0.05
scale x drug x time	3,5	2.4	0.18

Degrees of Freedom for the following tests= Drug 1,7,: Time 1,7: Drug x Time 1,7

Selective Reminding Test, Delayed Recall

drug	0.01	0.91
time	16.66	0.01
drug x time	1.18	0.31

Digit Span Forward

drug	0.04	0.85
time	1.29	0.29
drug x time	2.03	0.20

Appendix 9-Continued

Digit Span: Backward

	F	p
drug	0.10	0.76
time	0.73	0.42
drug x time	4.01	0.09

Visual Reproductions

Recall

Immediate	drug	1.1	0.3
	time	0.6	0.8
	drug X time	2.9	0.1
Delayed	drug	1.9	0.2
	time	4.1	0.08
	drug X time	2.4	0.2

Recognition

Immediate	drug	0.9	0.4
	time	0.00	1.0
	drug X time	0.6	0.5
Delayed	drug	1.9	0.2
	time	1.4	0.3
	drug X time	0.0	0.5

Appendix 9-Continued

Stroop Test

Time

		F	p
Color	drug	0.1	0.9
	time	0.1	0.7
	drug X time	0.0	1.0
Word	drug	3.7	0.1
	time	0.2	0.9
	drug X time	0.0	1.0
Color/Word	drug	.22	0.7
	time	0.8	0.4
	drug X time	.01	0.9

Errors

Color	drug	0.8	0.4
	time	0.8	0.8
	drug X time	0.8	0.8
Word	drug	0.2	0.7
	time	0.0	0.9
	drug X time	3.8	0.09
Color/Word	drug	1.6	0.3
	time	2.5	0.2
	drug X time	0.4	0.6

Appendix 9-Continued

Word Fluency

drug	10.3	0.01
time	1.4	0.3
drug X time	2.6	0.2

Category Naming

	F	p
drug	0.1	0.8
time	0.2	0.7
drug X time	1.4	0.3

Cancellation Tests

Number(6)

Time

drug	0.1	0.8
time	1.0	0.4
drug X time	3.4	0.1

Omission Errors

drug	0.5	0.5
time	0.2	0.7
drug X time	0.1	0.7

Shapes

Time

drug	1.6	0.3
time	0.0	0.9
drug X time	0.0	0.9

Appendix 9-Continued

Omission Errors

drug	0.9	0.8
time	1.6	0.2
drug X time	0.6	0.5

TRIGRAM (TMX)

Time

	F	p
drug	1.7	0.2
time	0.1	0.8
drug X time	0.5	0.5

Omission Errors

drug	0.1	0.8
time	1.5	0.3
drug X time	2.6	0.2

APPENDIX 10 SCOPOLAMINE STUDY 2: RESULTS OF REPEATED MEASURES ANALYSIS OF VARIANCE FOR rCBF

	df	F	p
drug	1,7	74.06	<0.001
time	2,6	4.83	0.06
region	1,7	0.23	0.65
drug x time	2,6	5.31	0.05
drug x region	1,7	13.58	0.01
time x region	2,6	9.34	0.02
drug x time x region	2,6	7.13	0.03

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