Serotonin, caffeine and cognition : psychopharmacological studies in human cognitive functioning

Citation for published version (APA):

Schmitt, J. A. J. (2001). *Serotonin, caffeine and cognition : psychopharmacological studies in human cognitive functioning*. [Doctoral Thesis, Maastricht University]. NeuroPsych Publishers.
<https://doi.org/10.26481/dis.20010601js>

Document status and date:

Published: 01/01/2001

DOI:

[10.26481/dis.20010601js](https://doi.org/10.26481/dis.20010601js)

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy

If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Serotonin, caffeine and cognition

Serotonin, caffeine and cognition

*Psychopharmacological studies in
human cognitive functioning*

Neuropsych Publishers, Maastricht

© Jeroen A.J. Schmitt, Maastricht 2001

Production: Datawyse | University Press Maastricht

ISBN 90-75579-12-8

NUGI 744, 746

Neuropsych Publishers is a non-profit organisation which aims at promoting the science of 'Brain and Behaviour' and improving the application of products of this science in health care and education. Neuropsych Publishers accomplishes these aims by publishing books, dissertations and other products of scientific activity, dissemination of educational materials and publication of tests, assessment scales and other psychometric instruments in the field of neuropsychology, neuropsychiatry and other areas within the domain of Brain and Behaviour.

Postal address:

Neuropsych Publishers
Department of Psychiatry and Neuropsychology
Universiteit Maastricht
PO Box 616
NL-6200 MD Maastricht
The Netherlands

Serotonin, caffeine and cognition

Psychopharmacological studies in human cognitive functioning

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Maastricht,
op gezag van de Rector Magnificus, Prof. Dr. Nieuwenhuijzen Kruseman,
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen op
vrijdag 1 juni 2001 om 16.00 uur

door

JEROEN ANTONIUS JOHANNES SCHMITT

Geboren op 6 april 1967 te Roosendaal

Promotor

Prof. Dr. J. Jolles

Co-promotor

Dr. W.J. Riedel

Beoordelingscommissie

Prof. Dr. H.L.G.J. Merckelbach (voorzitter)

Prof. Dr. M.H.J. Maes

Dr. B.J. Sahakian (Cambridge University, United Kingdom)

Prof. Dr. H.W.M. Steinbusch

Prof. Dr. M.N. Verbaten (Universiteit Utrecht)

The studies described in this dissertation were conducted at the Experimental Psychopharmacology Unit (EPU) of the Brain & Behaviour Institute of Universiteit Maastricht (Department of Psychiatry and Neuropsychology). The studies described in chapter 4, 5, 6 and 7 were supported by an unrestricted grant from Pfizer Inc, New York.

Publication of this thesis is financially supported by an unrestricted educational grant from Pfizer Inc, New York.

The author also gratefully acknowledges the financial support for the publication of this thesis from Sigma Tau Ethifarma BV, Douwe Egberts, SmithKline Beecham Farma BV, Novartis Pharma BV, Janssen-Cilag BV, UCB Pharma BV and the 'Internationale Stichting Alzheimer Onderzoek'.

Contents

1	<i>Introduction</i>	9
	Serotonin and human cognition	10
	Caffeine intake and cognitive functioning	13
	Outline and aims of the studies	14
2	<i>Tryptophan depletion impairs memory consolidation but improves focussed attention in healthy young volunteers</i>	19
	Introduction	20
	Methods and materials	22
	Results	28
	Discussion	34
3	<i>SSRIs' pharmacological profile and cognitive effects</i>	41
	Introduction	42
	Methods and materials	44
	Results	48
	Discussion	50
4	<i>Additional dopamine reuptake inhibition prevents vigilance decrement induced by serotonin reuptake inhibition by man</i>	57
	Introduction	58
	Methods and materials	60
	Results	64
	Discussion	68
5	<i>Paroxetine's and sertraline's effects on Critical Flicker Fusion and the influence of concomitant changes in pupil size</i>	77
	Introduction	78
	Methods and materials	79
	Results	81
	Discussion	84

6	<i>Modulation of the Critical Flicker Fusion effects of sertraline and citalopram by pupillary changes</i>	89
	Introduction	90
	Methods and materials	91
	Results	94
	Discussion	97
7	<i>Caffeine improves memory performance during distraction in middle-aged but not in young or old subjects</i>	103
	Introduction	104
	Methods and materials	105
	Results	108
	Discussion	110
8	<i>Low dose of caffeine does not affect memory functions or focussed attention in middle-aged or elderly subjects</i>	117
	Introduction	118
	Methods and materials	120
	Results	124
	Discussion	124
9	<i>General discussion</i>	131
	Serotonin and cognition: general and specific effects	131
	Serotonin and arousal	131
	Serotonin and memory functioning	134
	Summary	135
	Caffeine, age and cognition: a general hypothesis	136
	Concluding remarks: serotonergic drugs and/or caffeine as cognition enhancers ?	138
	<i>Summary</i>	143
	<i>Samenvatting</i>	149
	<i>Dankwoord</i>	155
	<i>Curriculum Vitae</i>	155

General introduction

We are all aware that our ability to concentrate, learn, remember, understand or think may vary from day to day, even from hour to hour. Many factors contribute to fluctuations of brain functions such as memory, attention, comprehension and alertness, generally referred to as cognitive functions. Fatigue, hunger or the use of certain psychoactive substances such as (medicinal) drugs or alcohol are just a few of the factors known to exert detrimental effects on a persons cognitive abilities. Although such instances of cognitive decline may prove to be inconvenient, annoying, or even dangerous, they are generally mild and transient. Other factors, however, are associated with more prolonged or even permanent cognitive deterioration, for example through drug abuse, brain trauma, exposure to neurotoxic agents, and, most commonly, presence of disease states and ageing. Conditions of prolonged cognitive deterioration are likely to pose a substantial long-lasting burden for the affected individual. Even a modest but prolonged cognitive decline can significantly reduce the quality of life, as it interferes with daily activities and social interactions. Therefore, palliative treatment is not only warranted for severe cognitive dysfunction, as seen in dementia and Alzheimer's Disease, but also for milder forms of cognitive deterioration during ageing which exist much more prevalently. For example, in a sample of almost 15.000 persons aged 55 and older, 54% reported that they had (some) difficulty in remembering things in the past year (Cutler and Grams, 1988).

two approaches to the quest for cognition enhancers

The central theme in this thesis is the search for substances and targets to pharmacologically enhance cognitive performance. Given the broadness of this research area, this thesis focuses on two aspects, notably serotonergic mechanisms of cognition and, secondly, the cognitive effects of caffeine. These two focal points of the thesis represent two ways of approaching the issue of pharmacological cognition enhancement. Caffeine is a psychoactive substance that is already a frequently

consumed as ingredient of coffee, tea and several soft drinks. Its widespread use and well-known subjectively experienced activating effects have lead to the study of caffeine as putative cognition enhancer. In this respect, the explanation of mechanisms underlying the effects of caffeine, although interestingly, are not of primary concern. It is known that caffeine's main mode of action is antagonism of adenosine and through this mechanism caffeine may indirectly stimulate multiple neurotransmitters (Nehlig et al. 1992). However, the functional role of these changes in neurotransmission, and the relation with caffeine's behavioural effects, is yet to be established (Fredholm et al. 1999).

Research on the putative role of serotonin (5-hydroxytryptamine, 5-HT) in cognition enhancement is characterised by a different approach. Here, one specific brain mechanism, serotonergic neurotransmission, is singled out and attempts are made to unravel its involvement in normal cognitive functioning and cognitive dysfunction. Such efforts are predominantly triggered by clinical observations of cognitive concomitants to (presumably) serotonergically mediated pathologies, e.g. depression, and by experimental studies exploring the cognitive effects of 5-HT manipulations. Theoretically, once the involvement of 5-HT in cognition is sufficiently charted, specific drugs for specific deficits, aimed at specific subsystems, can be developed. Furthermore, possible adverse cognitive side-effects of existing drugs acting on 5-HT neurotransmission, such as widely prescribed serotonergic antidepressants, may be anticipated and possibly avoided.

Clearly, both approaches to the quest for pharmacological cognition enhancement are overlapping and complementary. For example, clinical studies on the cognitive effects of 5-HT promoting antidepressants suggest that Selective Serotonin Reuptake Inhibitors (SSRIs) may facilitate cognitive performance, but studies with these compounds also provide insight into the role of 5-HT in cognition.

In this thesis, cognitive performance is investigated by means of a number of well-documented and often-used tests and paradigms addressing several aspects of memory, attentional, and executive functioning.

Serotonin and human cognition

Following its discovery, now more than 50 years ago, serotonin has turned out to be a fruitful topic of scientific research into numerous functions and illnesses (for a historical overview see Sjoerdsma and Palfreyman 1990). Despite the fact that only a small

fraction of the serotonin in the body is found in the brain, its functions in the central nervous system (CNS) are the most prominent. In the CNS, serotonin acts as a neurotransmitter, transferring information from one nerve cell to another. Brain serotonergic pathways are extremely widespread, and nerve endings containing serotonin are found even in the remotest areas of the brain (Meltzer et al. 1998). Serotonergic involvement in behavioural functions is equally elaborate. Serotonin is thought to play a role in a diverse range of functions, including sleep and wakefulness, appetite, feeding, thermoregulation, cardiovascular function, respiratory activity, sexual behaviour, mood and cognition (Jacobs and Fornal 1995). Consequently, dysregulation of CNS serotonin has been implicated in the aetiology of many mental disease states, such as depression, anxiety, schizophrenia, eating disorders, obsessive compulsive disorder, and panic disorder.

Several clinical and experimental observations have lead to the presumption that serotonin may play a significant role in human cognitive functioning. First, age-related as well as disease-associated changes in the central serotonergic system may directly underlie concomitant cognitive changes. For example, diminished serotonergic function is found in post mortem and brain imaging studies in Alzheimer's Disease, and to a lesser extent during normal ageing (Altman and Normile 1988; McEntee and Crook 1991; Meltzer et al. 1998). However, the clinical significance of these 5-HT changes remain to be established. While they may contribute to reduced cognitive functioning (Richter-Levin and Segal 1996), it has also been suggested that 5-HT changes are associated with non-cognitive behavioural changes in AD, e.g. aggression and depression (Cross 1990; Meltzer et al. 1998), and, for example, altered sleep in normal ageing (Meltzer et al. 1998). Also, the diminished cognitive capabilities that are often observed in depression (Burt et al. 1995; Siegfried 1989) may arise from reduced 5-HT function. Cognitive deficits in depression may extent to all aspects of cognitive functioning, from memory functions to attention to complex functions like planning and abstract thinking. In fact, cognitive retardation is among the classic features of major depression and may be a core aspect of the disease (Widlocher 1983).

More direct evidence for 5-HT modulation of cognitive functioning is available from numerous experimental animal studies. These largely confirm 5-HT involvement in cognitive behaviour, particularly with regard to memory processes and attentional functions (Buhot et al. 2000; Meneses 1999). However, there is considerable variation in the results, both in the affected cognitive domains and in the direction of the serotonergic effects. Moreover, a major flaw in the current evidence on 5-HT and cognition is that relatively few data are available from experiments with human subjects. Hence, further

identification of the role of 5-HT, particularly in human subjects, would lead to improved insights in the fundamental mechanisms of cognitive dysfunction in normal and pathological conditions, help to prevent prescription drug-induced cognitive impairment, and provide the tools for development of novel drugs in the treatment of cognitive disorders.

serotonergic manipulations in the present thesis

In the present thesis two serotonergic manipulations, namely 5-HT inhibition by acute tryptophan depletion (ATD) and 5-HT stimulation by administration of Selective Serotonin Reuptake Inhibitors (SSRIs) are used. Both methods provide safe, transient, and quite specific alterations in 5-HT neurotransmission and are readily applicable in man.

ATD temporarily reduces the synthesis of 5-HT by reducing the availability of its precursor tryptophan (TRP). This is accomplished by administration of an amino acid drink lacking TRP, and may thus be considered a nutritional manipulation rather than a pharmacological intervention. A detailed overview of the methodological aspects of acute tryptophan depletion method (ATD) is available in several review articles on the topic (Moore et al. 2000; Van der Does 2000; Reilly et al. 1997). There is little doubt that administration of a typical ATD mixture actually reduces 5-HT activity (Moore et al. 2000). In humans, a widespread reduction of brain 5-HT synthesis (Nishizawa et al. 1997) as well as diminished levels of TRP and the 5-HT metabolite 5-HIAA have been demonstrated following ATD (Carpenter et al. 1998; Williams et al. 1999). Given above considerations, ATD is considered a safe, straightforward and well-tolerated research paradigm for investigating 5-HT function in man (Moore et al. 2000; Reilly et al. 1997).

Selective serotonin reuptake inhibitors (SSRIs) are among the most frequently prescribed agents in all of medicine (Stahl 1998b). Although initially introduced as antidepressants (Goodnick and Goldstein 1998), their therapeutic indications have expanded to include anxiety disorders, social phobia, panic disorder, obsessive-compulsive disorder, bulimia nervosa and premenstrual dysphoria (Eriksson and Walinder 1999; Isaac 1999; Montgomery and Den Boer 1998). SSRIs' principle mode of action is blocking of the reuptake of 5-HT into the pre-synaptic neuron, thus enabling 5-HT to exert its effects on post-synaptic receptors for an extended period of time (Stahl 1998b). As a result, serotonergic neurotransmission is stimulated throughout the brain, including the raphe nuclei, hippocampus, striatum, thalamus and cortex (Fuller 1994). This effect is maintained for the duration of uptake inhibition (Fuller 1996). 5-HT neurotransmission may be further enhanced following sub-chronic SSRI administration,

presumably because of downregulation of inhibitory 5-HT_{1A} somatodendritic auto-receptors (Blier et al. 1998; Fuller 1994; Stahl 1998a).

Caffeine intake and cognitive functioning

Caffeine is undoubtedly the most frequently consumed psychoactive compound in the world (Fredholm et al. 1999). It is usually ingested as a constituent of coffee and tea, and as an additive to several soft drinks. Because of its widespread use and its perceived activating effects, the relation between caffeine and cognitive functions has long been a topic of scientific interest. However, following nearly a century of systematic studies on the cognitive effects of caffeine, peaking in the last two decades, the issue is still unresolved. Although the preponderance of the evidence suggests a beneficial effect of caffeine on cognitive functions, it has proven to be extremely difficult to determine which, how, and to what extent cognitive functions are specifically affected by caffeine (Riedel and Jolles 1996; Riedel and Jorissen 1998; Rogers and Démoncourt 1998).

If anything, the vast literature on this topic has identified numerous potential confounders and effect modifiers, which may not only affect the relation between caffeine and cognition, but which may also interact with each other. Possible mediators may constitute of methodological parameters, such as dosage, time of assessment, type of vehicle and type of cognitive test. Other putative modifiers are related to the subjects and include habitual caffeine use, metabolic rate, smoking, menstrual cycle, state of arousal, mood, expectations and age (Fredholm et al. 1999; Nehlig 1999; Nehlig et al. 1992; Riedel and Jorissen 1998; Rogers and Démoncourt 1998; Smith et al. 1999). The influence of many of these factors is still very much a subject of debate.

The notion that caffeine's cognitive effects may vary with age, and particularly the fact that caffeine's beneficial effects may be more pronounced in the elderly (Jarvis 1993; Swift and Tiplady 1988), has raised interest for caffeine as a putative cognition enhancer to combat age-related cognitive decline. Generally, coffee consumption increases with age, peaks during middle-age, and subsequently decreases with old age (Riedel et al. 1995). Therefore, those individuals who may benefit the most from the cognitive effects of caffeine may be the ones who use it the least. Until now, little experimental data is available on the possible interaction between caffeine and age, since the majority of caffeine experiments were done using only young subjects. Therefore, there is a need for experimental studies comparing caffeine's cognitive effects in different age-groups.

Outline and aims of this thesis

In the first part of this thesis, consisting of chapters 2 to 6, the effects of serotonergic manipulations on cognitive functions in healthy human subjects are addressed.

effects of tryptophan depletion on memory and other cognitive functions

In *chapter 2* a study is described in which serotonergic functioning was experimentally reduced by means of the acute tryptophan depletion method. A previous investigation (Riedel et al. 1999) had identified memory functioning, and particularly consolidation of newly learned material, to be sensitive to a lowering of 5-HT activity. In the current study, specific timing of the induction of tryptophan depletion was used to further dissect the observed memory deficit in terms of learning, consolidation and retrieval functions. In addition, the effects of lowered serotonergic function on other cognitive domains, including attention and executive functions, were explored.

In the ensuing chapters the cognitive effects of serotonergic stimulation by SSRI administration are addressed. The results presented in chapters 3, 4 and 5 are derived from a large study on the cognitive effects of sub-chronic administration of the SSRIs sertraline and paroxetine in healthy volunteers. Each chapter, however, deals with a specific component of the study with specific associated hypotheses.

non-serotonergic cognitive effects of serotonin reuptake inhibitors

Chapter 3 is concerned with the cognitive effects of sertraline and paroxetine with special reference to their different non-serotonergic pharmacological properties. Although both SSRIs primarily stimulate 5-HT neurotransmission, additional mild influences on dopaminergic and cholinergic systems, of sertraline and paroxetine respectively, may invoke cognitive changes as well. Healthy volunteers are treated with paroxetine and sertraline for two weeks. Cognitive performance is assessed using tests that are specifically selected based on their sensitivity for manipulations of cholinergic (long-term memory, memory scanning) and dopaminergic neurotransmission (executive functioning, motor functions).

attentional effects associated with serotonin reuptake inhibition

Chapter 4 specifically focuses on changes in attentional functioning, i.e. vigilance (sustained attention), divided attention and focussed attention, as a result of augmentation of serotonergic neurotransmission by serotonergic reuptake inhibitors

(SSRIs). Previous observations suggested vigilance to be specifically affected by serotonergic stimulation (O'Hanlon et al. 1998; Ramaekers et al. 1995). This was further investigated by administering two SSRIs, paroxetine and sertraline, to healthy volunteers for two weeks each. Sertraline's additional dopaminergic properties enabled us to examine the involvement of serotonergic - dopaminergic interactions in drug-induced vigilance impairment.

serotonergic drugs, pupil size and Critical Flicker Fusion

The Critical Flicker Fusion Test (CFF) is frequently used to assess sedating or activating effects of psychoactive drugs. However, CFF changes following drugs affecting serotonergic neurotransmission may be confounded by concomitant changes in pupil size. Serotonergic stimulation increases pupil diameter and pupillary dilation is known to independently raise CFF values. Therefore, claims of activating effects of several serotonin reuptake inhibitors based solely on CFF measurements without control for pupillary changes may be questioned. In two studies, described in *chapters 5 and 6*, the role of pupil size in CFF changes following acute and sub-chronic administration of SSRIs is investigated. Measurements of pupil size and CFF assessments with and without pupillary control, using different CFF devices, were performed following treatment with paroxetine, sertraline and citalopram.

In the second part of the thesis two studies are presented on the cognitive effects of caffeine. In particular, the question whether caffeine exerts different effects, in terms of strength and direction, in different age-groups was addressed.

cognitive effects of caffeine in young, middle-aged and old subjects

First, in *chapter 7*, a study is presented in which caffeine, at a dose comparable to three cups of coffee, is administered to healthy young, middle-aged and elderly subjects. Performance on a memory test with distraction and a memory scanning task is subsequently assessed to identify the possible differences in the cognitive effect of caffeine between the three age-groups.

A subsequent study, described in *chapter 8*, focuses on a possible mechanism of age-related differences to the cognitive effects of caffeine, namely variable levels of caffeine withdrawal symptoms due to differences in habitual caffeine use between age-groups. Furthermore, in this study, the nature of the observed cognitive effects in the previous study is further examined as the effect of caffeine on specific attentional and memory processes is explicitly addressed.

Lastly, in *chapter 9* a general discussion of the combined results presented in this thesis is given.

References

- Altman HJ, Normile HJ (1988) What is the nature of the role of the serotonergic nervous system in learning and memory: prospects for development of an effective treatment strategy for senile dementia. *Neurobiol Aging* 9: 627-38
- Blier P, Pineyro G, el Mansari M, Bergeron R, de Montigny C (1998) Role of somatodendritic 5-HT autoreceptors in modulating 5-HT neurotransmission. *Ann N Y Acad Sci* 861: 204-16
- Buhot MC, Martin S, Segu L (2000) Role of serotonin in memory impairment. *Ann Med* 32: 210-21
- Burt DB, Niederehe G, Zemblar MJ (1995) Depression and Memory Impairment: A Meta-Analysis of the Association, Its Pattern, and Specificity. *Psychological Bulletin* 117: 285-305
- Carpenter LL, Anderson GM, Pelton GH, Gudín JA, Kirwin PD, Price LH, Heninger GR, McDougale CJ (1998) Tryptophan depletion during continuous CSF sampling in healthy human subjects. *Neuropsychopharmacology* 19: 26-35
- Cross AJ (1990) Serotonin in Alzheimer-type Dementia and Other Dementing Illnesses. *Annals of the New York Academy of Sciences* 600: 405-417
- Cutler SJ, Grams AE (1988) Correlates of self-reported everyday memory problems. *Journal of Gerontology: Social sciences* 43:82-90
- Eriksson E, Walinder JE (1999) Growing the therapeutic potential of SSRIs. *International Clinical Psychopharmacology* 14 (Suppl. 2)
- Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE (1999) Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological reviews* 51: 83-133
- Fuller RW (1994) Uptake inhibitors increase extracellular serotonin concentrations measured by brain microdialysis. *Life sciences* 55: 163-167
- Fuller RW (1996) Fluoxetine effects on serotonin function and aggressive behavior. *Annals of the New York Academy of Sciences* 794: 90-97
- Goodnick PJ, Goldstein BJ (1998) Selective serotonin reuptake inhibitors in affective disorders—II. Efficacy and quality of life. *J Psychopharmacol* 12: S21-54
- Isaac M (1999) Where are we going with SSRIs? *European Neuropsychopharmacology* 9: s101-S106
- Jacobs BL, Fornal CA (1995) Serotonin and Behavior. A general Hypothesis. In: Bloom FE, Kupfer DJ (eds) *Psychopharmacology: The Fourth Generation of Progress*. Raven Press Ltd., New York, pp 461-469
- Jarvis M (1993) Does caffeine intake enhance absolute levels of cognitive performance? *Psychopharmacology* 110: 45-52
- McEntee WJ, Crook TH (1991) Serotonin, memory, and the aging brain. *Psychopharmacology* 103: 143-9

- Meltzer CC, Smith G, DeKosky ST, Pollock BG, Mathis CA, Moore RY, Kupfer DJ, Reynolds CF, 3rd (1998) Serotonin in aging, late-life depression and Alzheimer's disease: The emerging role of functional imaging. *Neuropsychopharmacology* 18: 407-430
- Meneses A (1999) 5-HT system and cognition. *Neuroscience and Biobehavioral Reviews* 23: 1111-1125
- Montgomery SA, Den Boer JA (1998) SSRIs in Depression and Anxiety. John Wiley & Sons, John Wiley & Sons
- Moore P, Landolt H, Seifritz E, Clark C, Bhatti T, Kelsoe J, Rapaport M, Gillin JC (2000) Clinical and physiological consequences of rapid tryptophan depletion. *Neuropsychopharmacology* 23: 601-622
- Nehlig A (1999) Are we dependent upon coffee and caffeine? A review on human and animal data. *Neuroscience and Biobehavioral Reviews* 23: 563-576
- Nehlig A, Daval J-L, Debry G (1992) Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Research Reviews* 17: 139-170
- Nishizawa S, Benkelfat C, Young SN, Leyton M, Mzengeza S, de Montigny C, Blier P, Diksic M (1997) Differences between males and females in rates of serotonin synthesis in human brain. *Proc Natl Acad Sci U S A* 94: 5308-13
- O'Hanlon J, Robbe HWJ, Vermeeren A, VanLeeuwen C, Danjou PE (1998) Venlafaxine's effects on healthy volunteers' driving, psychomotor, and vigilance performance during 15-day fixed and incremental dosing regimens. *Journal of Clinical Psychopharmacology* 18: 212-221
- Ramaekers JG, Muntjewerff ND, O'Hanlon JF (1995) A comparative study of acute and subchronic effects of dothiepin, fluoxetine and placebo on psychomotor and actual driving performance. *British Journal of Clinical Pharmacology* 39: 397-404
- Reilly JG, McTavish SFB, Young AH (1997) Rapid depletion of plasma tryptophan: a review of studies and experimental methodology. *Journal Of Psychopharmacology* 11: 381-392
- Richter-Levin G, Segal M (1996) Serotonin, Aging and Cognitive Functions of the Hippocampus. *Reviews in the Neurosciences* 7: 103-113
- Riedel WJ, Hogervorst E, Jolles J (1995) Cognition Enhancers and Aging. In: Jolles J, Houx PJ, Van Bortel MPJ, Ponds RWHM (eds) *Maastricht Aging Study: Determinants of Cognitive Aging*. Neuropsych Publishers, Maastricht, The Netherlands, pp 149-156
- Riedel WJ, Jolles J (1996) Cognition Enhancers in Age-Related Cognitive Decline. *Drugs & Aging* 8: 245-274
- Riedel WJ, Jorissen BL (1998) Nutrients, age and cognitive function. *Curr Opin Clin Nutr Metab Care* 1: 579-585
- Riedel WJ, Klaassen T, Deutz NEP, Van Someren A, Van Praag HM (1999) Tryptophan Depletion in Normal Volunteers Produces Selective Impairment in Memory Consolidation. *Psychopharmacology* 141: 362-369
- Rogers PJ, Dernoncourt C (1998) Regular caffeine consumption: a balance of adverse and beneficial effects for mood and psychomotor performance. *Pharmacology, Biochemistry and Behavior* 59: 1039-1045
- Siegfried K (1989) Depression and dementia of the Alzheimer type: Implications for psychopharmacological research. *Hum Psychopharmacol* 4: 237-245
- Sjoerdsma A, Palfreyman M (1990) History of serotonin and serotonin disorders. *Annals of the New York Academy of Sciences* 600: 1-8

- Smith A, Sturgess W, Gallagher J (1999) Effects of a low dose of caffeine given in different drinks on mood and performance. *Human Psychopharmacology* 14: 473-482
- Stahl SM (1998a) Basic psychopharmacology of antidepressants, part 1: Antidepressants have seven distinct mechanisms of action. *J Clin Psychiatry* 59: 5-14
- Stahl SM (1998b) Mechanism of action of serotonin selective reuptake inhibitors. Serotonin receptors and pathways mediate therapeutic effects and side effects. *J Affect Disord* 51: 215-35
- Swift CG, Tiplady B (1988) The effects of age on the response to caffeine. *Psychopharmacology* 94: 29-31
- Van der Does AJW (2000) The effects of tryptophan depletion on mood and psychiatric symptoms. *Journal of Affective Disorders* in press
- Widlocher DJ (1983) Psychomotor retardation: clinical, theoretical and psychometric aspects. *Psychiatric Clinics of North America* 5: 27-29
- Williams WA, Shoaf SE, Hommer D, Rawlings R, Linnoila M (1999) Effects of acute tryptophan depletion on plasma and cerebrospinal fluid tryptophan and 5-hydroxyindoleacetic acid in normal volunteers. *J Neurochem* 72: 1641-7

Tryptophan Depletion Impairs Memory Consolidation but Improves Focussed Attention in Healthy Young Volunteers

Abstract

Animal and human studies have provided evidence for serotonergic modulation of cognitive processes. However, the exact nature of this relationship is not clear. We used the Acute Tryptophan Depletion (ATD) method to investigate the effects of lowered serotonin synthesis on cognitive functions in 17 healthy young volunteers. The study was conducted according to a placebo-controlled, double-blind, cross-over design. Cognitive performance and mood were assessed at baseline and 5 and 9 hours after administration of ATD. A specific impairment of word recognition, without effects on short-term memory, occurred during ATD. No memory deficits were seen if ATD was induced after acquisition of new words. The Stroop Test and Dichotic Listening Task demonstrated a modality independent improvement of focussed attention after ATD. Fluency was also improved after ATD. ATD did not alter speed of information processing, divided attention or planning functions. These results indicate that serotonin is essential in the process of long-term memory consolidation, primarily in the first 30 minutes after acquisition. Improvement of specific cognitive processes by lowered 5-HT function may be linked to the removal of inhibitory actions of 5-HT in the cortex.

Introduction

Studies into the role of serotonin (5-hydroxytryptamine, 5-HT) in cognitive functioning have provided evidence for a serotonergic involvement in cognitive processes such as memory and attention. However, the exact nature of this relationship is not clear. Most studies in this field are animal studies, which unfortunately have yielded inconsistent results. For example, it has been found that lowering serotonergic function can either impair, facilitate or have no effect on memory functions in rats (for reviews see Sirvio et al., 1994; Altman & Normile, 1988). These discrepancies, which possibly originate from inconsistencies in timing and selectivity of the serotonergic manipulation and type of tests used, make it extremely difficult to reach a uniform conclusion about the role of 5-HT in cognitive functioning. After reviewing the data McEntee & Crook (1991) concluded that, in general, findings indicate an inhibitory effect of 5-HT on memory functions. In contrast, based on animal experiments, Richter-Levin & Segal (1996) concluded that reduced serotonergic modulation in the hippocampus contributes to age-associated memory deficits.

The relationship between 5-HT and cognition is particularly relevant in those illnesses in which cognitive deficits occur along with changes in 5-HT activity. For example, diminished serotonin levels have been found in postmortem studies of patients with Alzheimer's Disease (AD) (Altman & Normile, 1988; Cross, 1990; Bierer et al., 1995). Decreased serotonergic activity may underlie the cognitive deficits in AD (Richter-Levin & Segal, 1993), although it has been suggested that these 5-HT changes relate more closely to the non-cognitive changes associated with the disease, such as aggression (Palmer & DeKosky, 1993). Also, depressed patients, in whom presumably 5-HT function is lowered, often show diminished cognitive capabilities (Burt et al., 1995). Treatment with 5-HT re-uptake inhibitors, which increase serotonergic transmission, often results in improvement of cognitive performance. However, since this improvement coincides with relief of the depression, it is not clear whether these cognitive changes are directly due to altered 5-HT function or are secondary to the alleviation of the depression itself. In healthy human volunteers administration of 5-HT re-uptake inhibitors in general does not affect cognitive performance (Thompson, 1991; Amado-Boccaro et al., 1995).

One method to study serotonergic function in humans is acute tryptophan depletion (ATD) (see Reilly et al., 1997, for a review). This manipulation causes a temporary global reduction of 5-HT synthesis in the brain by decreasing the availability of the 5-HT precursor L-tryptophan (Trp) in the brain (Young, Smith, Pihl & Ervin, 1985).

Oral administration of Trp-free amino acid mixture stimulates protein synthesis, thereby clearing Trp from the blood. Secondly, it reduces the transport of plasma Trp into the brain by increasing the amount of Large Neutral Amino Acids (LNAA) which compete with Trp for the active transport sites across the blood brain barrier. Administration of 100 g of a Trp-free amino acid mixture has been shown to cause a significant lowering of 5-HT synthesis throughout the brain after 5 hours in healthy human subjects (Nishizawa et al., 1997).

So far, few studies have focussed on the effects of ATD on cognitive performance in normal human subjects. Park et al. (1994) reported impairment of learning and retrieval functions after ATD, whereas Coull et al. (1995) found some positive effects of ATD, in that it speeded the response to incompatible stimuli. Recently it was found that ATD impaired long-term memory consolidation in a word learning task. This effect was highly specific since no effect was seen on short-term memory function or performance on perceptual and psychomotor tasks (Riedel et al., 1999). Since memory for words was impaired 30 minutes after their presentation, and although a small non-significant additional decrement was seen 18 hours later, the authors concluded that ATD most probably interfered with memory consolidation primarily during the first 30 minutes after acquisition.

The present study was designed to further investigate the specific effects of ATD on memory functions. If indeed ATD disrupts memory consolidation primarily within 30 minutes after acquisition, the induction of ATD after this 30 minute period should not influence subsequent memory performance. To test this hypothesis we induced ATD one hour after presentation of a wordlist (post-learning) and measured free recall and recognition for these words 5 hours after their presentation. We hypothesised that ATD would have no effect on memory performance at this time. Conversely, when ATD is induced 4 hours before presentation of a new wordlist (pre-learning), we would expect impaired long-term memory retention 30 minutes after that presentation, without an effect on immediate recall. Furthermore, no additional decrement in memory performance would be expected when tested 4 hours after presentation of these words.

The second objective of this study was an exploration of the effects of ATD over a wide range of cognitive functions. To this end we included several neuropsychological tests which assess attentional, memory and psychomotor functions in the cognitive test battery. Since cognitive performance can depend on mood state, and ATD may affect mood (Ellenbogen et al., 1996), this may be a confounding factor. To control for this potential confound we included two visual analogue mood scales in the experiment.

Methods and Materials

Subjects

Twenty healthy subjects (10 male, 10 female) were recruited through advertisements within the university. The subjects were aged 20 to 29 years (mean: 23.3 years, sd: 2.6). Physical and mental health of each subject was assessed by means of a health questionnaire, medical examination, and routine ECG and urine screening. Excluded were those volunteers who suffered from, or had a history of, cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, hematological or psychiatric illness. Other exclusion criteria were excessive drinking (> 20 units of alcoholic a week) pregnancy or lactation, use of medication other than oral contraceptives, smoking, use of drugs, and any sensory or motor deficits which could reasonably be expected to affect test performance. None of the subjects had a family history of depression, as was established by an interview. The study was approved by the Medical Ethics Committee of Maastricht University and the Maastricht University Hospital's Board of Directors. All subjects gave a written informed consent prior to participation.

Design

The study was conducted according to a placebo-controlled, double-blind, cross-over design. The treatment consisted of administration of a placebo or a Trp deletion mixture (ATD) (see below). Treatment order was balanced over the two test days, which were at least 7 days apart.

Amino acid mixtures

The ATD amino acid mixture (100 g) consisted of fifteen amino acids in the following composition: 5.5 g L-alanine, 3.2 g glycine, 3.2 g L-histidine, 8.9 g L-lysine, 12.2 g L-proline, 6.9 g L-serine, 6.5 g L-threonine, 4.9 g L-arginine, 2.7 g L-cysteine, and 3.0 g L-methionine; LNAA's: 8.0 g L-isoleucine, 13.5 g L-leucine, 5.7 g L-phenylalanine, 6.9 L-tyrosine, 8.9 g L-valine.

Reduced Trp/ Σ LNAA ratio's have been reported after a placebo mixture containing 2.3 g (Wolfe et al., 1995) and 3.0 g L-tryptophan (Riedel et al, 1999), and this may underestimate the effect of tryptophan depletion. In order to maintain a constant Trp/ Σ LNAA ratio during the control condition the amount of L-tryptophan in the placebo mixture was increased to 4.6 g. After a comparison of various control mixtures Welzin et al. (1994) found this mixture to be neutral with regard to the Trp/ Σ LNAA ratio.

To provide caloric energy until lunchtime approximately 20 g MD/fat mixture (33% sunflower oil and 67% maltodextrin) was added to the amino acid mixture. Amino acid drinks were prepared prior to administration by adding 300 ml tap water to the amino acid - MD/fat mixture. To compensate for the unpalatable taste the mixture was flavored with either strawberry or banana food flavoring. The amino acid mixtures were administered in two portions. The main portion of 90 grams was given at t0. Subjects were instructed to finish their consumption as fast as possible, but at least within two hours. A second portion of 10 g amino acid mixture, which functioned as a maintenance dose, was administered 6 hours later (t6).

Procedure

All subjects underwent one complete training session to minimize learning effects. During this training session the entire test battery was completed by each subject. On each test day the subjects arrived at the laboratory at 8.30 a.m., after an overnight fast. Subjects were instructed not to use alcohol on the day prior to the experiment, and to arrive well-rested. Figure 1 shows a schematic overview of a test day. During the test day the cognitive test battery (see below) was completed three times: one hour before administration of the amino acid mixture (baseline, t0) and 5 and 9 hours after the AA mixture (t5 and t9). Each cognitive test battery was preceded by a mood evaluation and followed by bloodsampling. Between measurements the subjects remained in a room where they could watch videos, read magazines or books, or play boardgames. Water, decaffeinated coffee and tea, diet softdrinks (but not cola), oranges, apples, grapes and protein-free candy were available ad libitum. Subjects were served a protein-poor lunch and dinner. After completion of the test day bananas and pineapple chunks, which are a rich natural source of L-tryptophan, were served to facilitate a quick recovery from possible incapacitating effects of ATD.

Cognitive Assessment

The cognitive test battery took approximately 50 minutes to complete. For each of the assessments one of six parallel versions of the cognitive tests were used, except for the Stroop Colour Word Test and Motor Choice Reaction Time. The six parallel versions were balanced over two testdays using a 6 x 6 latin square. A separate parallel version of each test was used for the training sessions.

Figure 1: Schematic representation of a test day (Cog = Cognitive test battery; M = Moodscales; B = Bloodsample).

The Visual Verbal Learning Test (VLT) This test is an adapted version of the Auditory Verbal Learning Test (Rey, 1964). Thirty words are presented in the same sequence in three trials on a computer screen. Each trial ends with a free recall of the words (immediate recall). Thirty minutes after the first trial, the subject is requested to recall as many words as possible (delayed recall). This is followed by a recognition test, consisting of 15 previously presented words and 15 other but comparable words, in which the subject has to respond "YES / NO" as quickly as possible to indicate recognition of the word (delayed recognition). The proportion of correctly recognised words (cr) and the proportion of falsely recognised (fr) constitute the nonparametric sensitivity measure: $A' = 1 - 1/4 (fr/cr + (1-cr)/(1-fr))$ (Pollack & Norman, 1964). A' is in fact the proportion of correctly recognised words, corrected for the subject's response tendency. A' is arcsin transformed before it entered statistical analysis because of its skewed distribution.

In this study wordlists were presented at t_0 , t_5 and t_9 . After t_0 , delayed recall and recognition were assessed twice: once after a delay of 30 minutes ($t_0 \rightarrow 0$) and once after a delay of 5 hours ($t_0 \rightarrow 5$). Similarly, for the wordlist presented at t_5 , delayed recall and recognition were assessed after 30 minutes ($t_5 \rightarrow 5$) and again after 4 hours ($t_5 \rightarrow 9$). Delayed recall and recognition for the wordlist presented at t_9 was assessed only once, after a delay of 30 minutes ($t_9 \rightarrow 9$).

Outcome variables were the maximum number of words recalled in either of the three immediate recall trials as a measure of short-term memory, the number of correct words on the delayed recall as a measure of retrieval from long-term memory, sensitivity

measure (A') as a measure of storage in long-term memory, and median reaction time of correctly recognised target words as a measure of speed of retrieval from long-term memory.

Verbal Fluency Test (Luteijn & van der Ploeg, 1983).

The fluency test can be regarded as a measure of strategy-driven retrieval of information from semantic memory. The subjects are asked to produce as many four-letter words as possible starting with a given letter, within one minute. The number of correct responses and errors are recorded. Nonsense words were not accepted, but names, conjugations and plurals were allowed. Starting letters were B, H, R, L, P and M. These letters yielded a high and similar number of correct responses (average: 10.1 to 11.0 words) in a comparative study with 40 subjects (Jolles and Houx, unpublished data).

Stroop Colour Word Test (SCWT) (Stroop, 1935).

The SCWT has often been used to test focussed attention. The test consists of three subtests. First, a card with a hundred colour names must be read as quickly as possible, followed by a subtest in which the same number of coloured patches must be named. In the third subtest colour names are printed in incongruously coloured ink. The colour of the ink must be named, without paying attention to the word itself. The outcome parameters of this test are the time needed to complete each subtest and the interference measure. The latter denotes the percentage of extra time needed to complete card III, relative to the average of card I and II : $(\text{time card III} / ((\text{time card I} + \text{time card II})/2)) * 100\%$.

Dichotic Listening Task.

Dichotic listening can be used to test both focussed attention and divided attention for auditory stimuli. Subjects receive two different auditory stimuli simultaneously though a stereo headphone, one stimulus on each side. The stimuli are numbers ranging from 1 to 200, presented at random and natural-speech spoken by a male voice. This version of the test consists of three subtasks in which subjects are instructed to focus on either the right channel, the left channel or on both channels, and to remember these numbers. The subtasks in which subjects must focus on one side, while ignoring the other, can be regarded as a focussed attention task. The subtask in which stimuli from both sides have to be remembered is a divided attention task. Each of the three subtasks is made up of nine trials, in which 2, 3, or 4 stimuli pairs are presented in random order. After each trial the subjects must identify the appropriate numbers from a list of numbers on a computer

screen. This list is composed of all presented numbers, plus the same amount of other numbers. Outcome variables are the total number of correctly identified stimuli, total number of stimuli identified from the wrong side, and number of false alarms (numbers which were not presented). Performance on each subtask is assessed by calculating sensitivity measures, similar to those used in the VVLT.

Tower of London. (Shallice, 1982).

The Tower of London is a test of planning. The test consists of three coloured balls which must be arranged on three sticks to match a picture with the goal positions. The complexity of the problem is altered by varying the minimum number of moves to reach the goal positions. Each trial consisted of a 2-, 3-, 4-, 5-, 6-, and 7-move problem. Prior to each problem the subjects are informed about the minimum number of moves in which the problem can be solved. The number of moves, time to solve the problem, and time between presentation of the goal positions and the first move (decision time), are recorded. For the Tower of London no baseline measures were taken.

Symbol Digit Modalities Test (SDMT) (Smith, 1968).

This test is an often-used measure of the speed of processing of general information. This version of the SDMT consists of five rows containing, in all, 75 blank squares, each paired with a randomly assigned symbol. Above these rows is a printed key that pairs each of the nine different symbols with a number from 1 to 9. The task is to write down the corresponding number in the blank square below each symbol as quickly as possible. Each test is preceded by a practice trial on the first 10 blank squares. The time required to fill the remaining 65 blank spaces and the number of errors are recorded.

Motor Choice Reaction Time (MCRT) (Houx & Jolles, 1993).

Speed of information processing was assessed by measuring reaction times (RT) as a function of task complexity. The test consists of a simple, choice and incompatible reaction time test. This yields RTs consisting of an initiation phase (time from stimulus onset until release of a hold button) and a movement phase (time from release of the hold button until the response button is pushed). The measures used for analysis are the median initiation RTs of the simple, the choice and the incompatible condition and the overall median movement RTs.

Mood scales

Mood was assessed using 'Bond & Lader Visual Analogue Scales' (Bond & Lader, 1974) and a visual analogue version of the shortened Dutch 'Profile of Mood States' (POMS) (McNair et al., 1971).

Bloodsamples

Blood (10 ml) was collected by venipuncture in sodium heparin tubes. Bloodsamples were placed on ice immediately after collection, and centrifuged at 4°C (5 min. at 5000 rpm) within 30 minutes. Subsequently, 100 µl plasma was mixed with 4 mg sulfasalicyl acid, frozen in liquid nitrogen and stored at -80 °C until quantitative amino acid analysis by high-performance liquid chromatography (Van Eijk et al., 1993).

Statistical analysis

Primary cognitive outcome measure: the VVLT

Dependent variables of the delayed recall and recognition assessments of the wordlists presented at t0 and t5 were analysed separately using a repeated measure analysis of variance (MANOVA using SPSS 6.1 for Macintosh). Within subjects factors were time of the recall and recognition assessments of each wordlist (t0→0 and t0→5 for the wordlist at t0, and t5→5 and t5→9 for the wordlist presented at t5), and treatment (placebo, ATD). Immediate recall at t0, t5 and t9, and delayed recall and recognition assessments at t9 were analysed using treatment as within subjects factor. Sex was entered as a between-subjects factor. Since these comparisons were planned, based on previous findings (Riedel et al., 1999), no correction for multiple comparisons was made.

Secondary cognitive outcome measures, mood scales and bloodparameters

Dependent variables were analysed separately in a repeated measures multivariate analysis of (co)variance, according to a 2 (treatment: placebo, ATD) x 2 (time: t5, t9) factorial model to test the main effect of treatment, time and the treatment by time interaction, using baseline scores (t0) as covariates. Sex was entered as a between-subjects factor. In case of a treatment by time interaction the differences between pairs of treatment conditions at t5 and t9 were examined using paired t-tests (ATD versus placebo, after correction for baseline scores).

Initially, order of treatment was included as between-subjects factor in the analysis. However, since none of the primary outcome parameters showed an effect of treatment

order or an order interaction with treatment, and since treatment order only influenced the effect of ATD in 4 of the 32 parameters (Stroop card III, Dichotic Listening 'right', Tower of London total time and number of steps) this factor was not considered in the remaining analyses.

Results

Seventeen subjects (9 males, 8 females) completed the study. Three subjects withdrew from the study on the first test day due to adverse effects of the amino acid mixture, particularly gastrointestinal discomfort. Two of these subjects (one male and one female) were in the ATD condition, and one female was in the placebo condition.

Primary outcome measures

The results of the delayed recall and recognition trials are depicted in figure 2, immediate recall scores and delayed recognition reactiontimes are listed in table 1.

VVLT: wordlist presented at t0

The ATD group had a significantly lower immediate recall performance before start of the treatment ($F_{1,15}=4.88$, $p<0.05$). Delayed recall showed a main effect of treatment ($F_{1,15}=5.41$, $p<0.05$) and a main effect of time ($F_{1,15}=140.96$, $p<0.001$). Delayed recall in the ATD group was significantly lower than that of the placebo group. Note however, that ATD was not induced until after delayed recall at t_0 ($t_0 \rightarrow 0$). Therefore, effects of ATD can only be observed at t_5 ($t_0 \rightarrow 5$) and not at t_0 ($t_0 \rightarrow 0$). Consequently, an actual effect of ATD should generate an interaction between treatment and time. No treatment by time interaction was found however ($F_{1,15}=0.00$, ns), indicating that the induction of ATD had no effect on delayed recall at t_5 ($t_0 \rightarrow 5$). Delayed recognition performance was only affected by time ($F_{1,13}=15.83$, $p<0.01$), and not by treatment ($F_{1,15}=1.15$, ns), nor was there a treatment by time interaction ($F_{1,15}=0.20$, ns). Reactiontimes of the delayed recognition showed no effects of treatment ($F_{1,15}=0.72$, ns), time ($F_{1,15}=0.00$, ns) or a treatment by time interaction ($F_{1,15}=1.65$, ns).

Figure 2: Means (Standard errors) of the delayed recall and delayed recognition assessments of the VVLT at baseline ($t0$) and at 5 ($t5$) and 9 hours ($t9$) after administration of the amino acid mixture. Delayed recognition performance is depicted in the top box, delayed recall in the lower box. X-axis labels $t \rightarrow y$ denote time of wordlist presentation (x) and time of assessment (y).

Table 1 : Means (standard errors) of the immediate recall and delayed recognition reaction time of the VVLT. Column headings $t \rightarrow y$ denote the time of presentation of the wordlist (x) and time of assessment (y).

Measure	Treatment	$t0 \rightarrow 0$	$t0 \rightarrow 5$	$t5 \rightarrow 5$	$t5 \rightarrow 9$	$t9 \rightarrow 9$
immediate recall (words)	placebo	51.2 (2.0)		47.1 (2.8)	48.7 (2.9)	
	ATD	46.1 (1.9)		43.1 (2.8)	49.1 (2.5)	
recognition RT (msec)	placebo	621 (12)	640 (19)	634 (24)	653 (22)	638 (17)
	ATD	658 (33)	646 (30)	661 (23)	663 (23)	641 (20)

Table 2: Means (standard errors) of the outcome parameters of the secondary cognitive assessments and mood scales at baseline (t_0), and 5 (t_5) and 9 (t_9) hours after start of the treatment, and the results of the repeated measures analyses (* $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$).

Measure	t0		t5		t9		treatment	time	treatment by time
	placebo	ATD	placebo	ATD	placebo	ATD			
<u>FLUENCY</u>									
# of words correct	14.2 (1.0)	12.8 (1.0)	13.4 (0.7)	15.6 (0.8)	15.8 (1.0)	15.0 (1.0)	F1,13=2.06	F1,14=2.18	F1,14=4.82 *
<u>SCWT</u>									
Time card I (sec)	35.2 (1.2)	35.0 (1.1)	35.7 (1.1)	35.1 (1.2)	34.7 (1.1)	33.8(1.0)	F1,14=2.97	F1,15=20.19 ‡	F1,15=0.31
Time card II (sec)	44.6 (1.9)	44.4 (1.6)	43.8 (1.8)	42.0 (1.4)	43.6 (1.7)	42.2 (1.5)	F1,14=7.49 *	F1,15=0.00	F1,15=0.36
Time card III (sec)	64.4 (3.5)	63.5 (3.2)	61.4 (3.1)	56.5 (2.7)	62.5 (2.8)	58.1 (2.8)	F1,14=17.83 ‡	F1,15=3.33	F1,15=0.06
Interference (%)	60.5 (5.1)	59.0 (4.8)	54.4 (5.4)	46.1 (4.5)	60.0 (5.7)	52.4 (4.3)	F1,14=6.09 *	F1,15=6.94 *	F1,15=0.02
<u>DLT</u>									
Left side sensitivity (%)	88.9 (1.9)	90.7 (1.4)	90.3 (1.2)	92.7 (1.3)	91.4 (1.6)	93.5 (1.5)	F1,14=3.28	F1,15=1.23	F1,15=0.12
Right side sensitivity (%)	93.3 (1.2)	92.5 (1.2)	92.2 (1.4)	95.4 (0.8)	93.7 (0.9)	94.8 (1.0)	F1,14=5.17 *	F1,15=0.04	F1,15=0.69
Both sides sensitivity (%)	83.6 (0.9)	83.1 (1.1)	83.8 (1.0)	84.6 (0.8)	84.0 (1.3)	83.9 (0.9)	F1,14=0.86	F1,15=0.10	F1,15=0.04
<u>MCRT</u>									
Simple RT (msec)	277 (6)	283 (7)	270 (5)	270 (6)	269 (5)	272 (5)	F1,14=0.07	F1,15=0.03	F1,15=0.42
Choice RT (msec)	317 (5)	329 (8)	315 (5)	324 (9)	314 (5)	317 (6)	F1,14=0.57	F1,15=0.65	F1,15=1.73
Incompatible RT (msec)	393 (11)	409 (17)	379 (12)	395 (16)	383 (13)	400 (18)	F1,14=0.95	F1,15=0.57	F1,15=0.00
Movement (msec)	102 (4)	103 (4)	104 (4)	100 (4)	100 (4)	98 (4)	F1,14=3.33	F1,15=6.54 *	F1,15=0.04
<u>SDMT</u>									
time (sec)	92.7 (3.2)	95.4 (3.5)	100.9 (3.5)	102.5 (4.3)	103.0 (4.0)	101.6 (4.0)	F1,14=0.39	F1,15=0.13	F1,15=0.75
<u>TOI</u>									
Decision time (sec)	-	-	45.6 (6.3)	37.5 (3.5)	37.7 (4.3)	38.1 (4.6)	F1,10=2.67	F1,10=0.26	F1,10=7.44 *
Total time (sec)	-	-	122.1 (9.1)	109.6 (9.5)	108.1 (7.1)	99.5 (4.8)	F1,12=0.80	F1,12=7.71 *	F1,12=0.20
total # of moves	-	-	36.8 (2.0)	35.5 (1.9)	35.5 (1.5)	34.0 (1.5)	F1,12=0.38	F1,12=1.55	F1,12=0.07
<u>POMS</u>									
Depression	0.95 (0.11)	0.92 (0.11)	0.96 (0.13)	0.93 (0.12)	0.86 (0.14)	0.87 (0.12)	F1,14=0.06	F1,15=2.25	F1,15=0.04
Anger	0.91 (0.10)	0.93 (0.10)	0.86 (0.08)	0.96 (0.12)	0.83 (0.13)	0.85 (0.11)	F1,14=0.34	F1,15=3.40	F1,15=0.16
Fatigue	1.33 (1.67)	1.38 (0.19)	1.48 (0.16)	1.50 (0.15)	1.24 (0.13)	1.57 (0.19)	F1,14=0.59	F1,15=0.56	F1,15=3.38
Tension	0.98 (0.12)	0.97 (0.15)	0.76 (0.11)	0.93 (0.12)	0.79 (0.13)	0.80 (0.13)	F1,14=0.95	F1,15=1.90	F1,15=2.60
Vigor	1.33 (0.18)	1.31 (0.16)	1.53 (0.17)	1.52 (0.17)	1.02 (0.13)	1.50 (0.19)	F1,14=1.05	F1,15=4.67 *	F1,15=13.37 †
<u>BOND & LADER</u>									
Contentedness	7.41 (0.26)	6.84 (0.28)	7.41 (0.30)	7.22 (0.34)	7.49 (0.38)	7.58 (0.35)	F1,14=0.12	F1,15=1.60	F1,15=1.18
Calmness	7.67 (0.30)	7.56 (0.30)	7.52 (0.36)	7.35 (0.43)	8.05 (0.35)	7.69 (0.44)	F1,14=1.45	F1,15=2.18	F1,15=4.42
Alertness	7.09 (0.43)	6.58 (0.39)	6.69 (0.39)	6.56 (0.43)	7.35 (0.37)	6.51 (0.46)	F1,14=1.33	F1,15=2.48	F1,15=0.1

VVLT: wordlist presented at t5.

Treatment did not affect immediate recall performance at t5 ($F_{1,15}=3.10$, ns). Delayed recall of the wordlist presented at t5 showed a main effect of treatment ($F_{1,15}=6.03$, $p<0.05$). Delayed recall was impaired during ATD. A main effect of time ($F_{1,15}=39.05$, $p<0.001$) showed that delayed recall performance at t9 ($t5\rightarrow9$) was lower than at t5 ($t5\rightarrow5$). No interaction between treatment and time was seen ($F_{1,15}=0.03$, ns), indicating that ATD did not cause additional impairment at the $t5\rightarrow9$ recall assessment compared to the $t5\rightarrow5$ assessment. Similar results were seen for delayed recognition sensitivity: a main effect of treatment ($F_{1,14}=8.98$, $p<0.01$) and time ($F_{1,14}=13.71$, $p<0.01$), and no treatment by time interaction ($F_{1,14}=0.69$, ns). Reactiontimes of the delayed recognition were only affected by time ($F_{1,14}=6.14$, $p<0.05$), but not by treatment ($F_{1,14}=0.00$, ns), and no treatment by time interaction was found. ($F_{1,14}=0.05$, ns).

VVLT: wordlist presented at t9.

Immediate recall at t9 was not affected by treatment ($F_{1,15}=0.04$, ns). Also, no treatment effect was seen for delayed recall ($F_{1,15}=0.28$, ns), delayed recognition sensitivity ($F_{1,15}=0.21$, ns) or delayed recognition reactiontimes ($F_{1,15}=0.00$, ns) of the wordlist presented at t9.

Secondary outcome measures

The results of the secondary cognitive assessments and details of the repeated measures analyses are listed in table 2.

Fluency Test

For one subject baseline data were not available. A treatment by time interaction was present for the number of words produced on the fluency test. Subsequent paired t-test analyses revealed that more words were produced in the ATD condition compared to the placebo condition ($t_{15}=-2.49$, $p<0.05$), but this treatment effect was present only at t5 and not at t9.

Stroop Colour Word Test

A main effect of treatment was seen on the interference measure of the Stroop test. Subjects in the ATD condition showed less interference compared to the placebo condition. Also, a main effect of time was present on this measure, with less interference at t5 compared to t9. Improved performance during the ATD condition was also seen for

card II and card III. Performance on card I was not affected by treatment, but showed main effect of time, with an improvement at t9 compared to t5. None of the outcome measures had a treatment by time interaction, and no effects were seen on the number of errors made on each subtask.

Dichotic Listening Task

Performance on subtask 'right' was significantly improved in the ATD condition. The same effect appeared to be present for subtask 'left', but this was not statistically significant. Performance on subtask 'both' did not differ between treatment conditions and none of the subtasks showed main effects of time effects or treatment by time interactions.

Tower of London

Two subjects (ATD / placebo) were excluded from the analyses of all the Tower of London parameters because of missing data. Data were also missing for an additional three subjects (one ATD, one placebo, and one in both conditions) for decision time, and for one subject (ATD) for total time and total number of moves. Total number of moves did not differ between treatment conditions or test times, and no time by treatment interaction was present. Total time to solve the problems was only affected by time but not by treatment, and no treatment by time interaction was found. Decision time was not affected by treatment or time. A treatment by time interaction was found, however, for the decision time. At t5 decision time was shorter for the subjects in the ATD condition compared to the placebo condition ($t_{12} = 2.46, p < 0.05$), but this effect was not present at t9.

Symbol Digit Modalities Test

Performance on the SDMT was not affected by treatment or time, and no treatment by time interaction was found.

Motor Choice Reaction Time.

Overall movement time showed a main effect of time. Otherwise no effects were seen on the outcome variables of the MCRT.

Mood scales

The results of the mood assessments are summarized in table 2. None of the scales of the POMS (depression, anger, tension, fatigue or vigour) or Bond & Lader

(contentedness, calmness, alertness) showed a main effect of treatment. The POMS vigour scale showed main effect of time and a treatment by time interaction. At t9 (but not at t5) subjects in the ATD condition reported to feel more vigorous.

Plasma Amino Acid Levels

Plasma Trp concentrations and the Trp/ Σ LNAA ratios in the placebo and ATD condition at the three assessments are depicted in figure 3. No blood samples could be obtained for one subject at t0 (ATD) and one at t9 (placebo), and invalid plasma leucine content prevented the calculation of the ratio Trp/ Σ LNAA for one subject at t5 (placebo). Analysis of plasma Trp levels showed a main effect of treatment ($F_{1,12}=89.17$, $p<0.001$), a main effect of time ($F_{1,13}=24.87$, $p<0.001$) and a treatment by time interaction ($F_{1,13}=18.81$, $p<0.01$). The ATD mixture caused a mean drop in plasma Trp concentrations of 63% at t5, and 64% at t9, relative to baseline values. In contrast, plasma Trp concentration in the placebo condition increased by 80% at t5, but returned to baseline levels at t9.

Figure 3 : Mean (standard error) plasma ratio Trp/ Σ LNAA and plasma Trp levels at baseline (t0) and 5 (t5) and 9 (t9) hours after start of the treatment.

Plasma ratio Trp/ Σ LNAAs showed a main effect of treatment ($F_{1,11}=313.21$, $p<0.001$), no main effect of time ($F_{1,12}=1.36$, ns), and treatment by time interaction ($F_{1,12}=25.43$, $p<0.001$). In the ATD condition the plasma ratio Trp/ Σ LNAAs fell to 21% of baseline at t5 and to 33% of baseline at t9. The placebo mixture caused a 20% increase in ratio Trp/ Σ LNAAs at t5 relative to baseline, but not at t9.

Sex differences

No sex differences were present for the effects of ATD on cognitive measures, mood scales or blood parameters.

Discussion

The primary aim of this study was to investigate the effects of lowered 5-HT synthesis by ATD on memory functioning. It was hypothesised that ATD would lead to impaired long-term memory performance due to a disruption of memory consolidation during the first 30 minutes after acquisition of new information. Our data show that the induction of ATD one hour after presentation of a wordlist (post-learning) did not affect long-term memory function. Both delayed recall and recognition of those words were not impaired when tested 5 hours later, during a depleted state, indicating that both retrieval and storage were unaffected. This is consistent with the notion that ATD interferes with consolidation primarily within 30 minutes after acquisition. Although unfortunate, the initial values difference for the immediate recall and 30 minute delayed recall does not essentially influence the results. Subsequently, we presented a new wordlist five hours after ATD. ATD impaired both delayed recall and recognition of these words 30 minutes after their presentation. These effects were not due to impaired learning since no effects were seen on immediate recall. Although both recall and recognition of these words were still impaired when tested 4 hours after presentation, no additional impairment was seen after this 4-hour delay. It thus appears that the detrimental effect of ATD is located mainly in the period between immediate recall and 30 minute delayed recall and recognition, ergo within 30 minutes after acquisition. The impairment is then preserved, but not augmented, over time.

Following this premise, memory tests which do not incorporate delayed assessment or do not require long-term storage of new information are likely to fail to detect memory effects of ATD. Park et al. (1994) reported a specific impairment of learning and retrieval processes after ATD. However, except for paired associates learning, where ATD increased the number of trials needed to learn the spatial locations,

none of the memory tests showed an effect of ATD. These memory tests essentially required immediate retrieval or assessed autobiographical memory, and neither of the tests involved consolidation of newly learned material over a longer period of time.

Also, it appears that the timing of the manipulation is important when investigating the role of serotonin in specific memory functions. Our data indicate that reducing central 5-HT activity during or immediately after acquisition would disrupt memory function, whereas a reduction of 5-HT activity pre-retention would not. The timing of the 5-HT manipulation and its cognitive effects have been an issue of debate in the literature on animal studies for several years. Based on their experiments with 5-HT antagonists in mice Altman & Normile (1988) concluded that "pretrain antagonist administration may interfere with some postlearning memory process, only if the drugs were present during or immediately after acquisition". However, as is generally the case with the studies on serotonin and cognition, the results on this matter are far from consistent. The induction of the 5-HT manipulation at different moments in time provides a method to discriminate between the specific effects of serotonin on different memory processes, e.g. learning, retrieval and consolidation. The application of this method in human studies, combined with more rapid serotonergic manipulations, is needed to further clarify the role of serotonin in memory functions.

As for the secondary cognitive assessments, the most striking outcome is the enhancement of focussed attention functions by ATD. Performance on the Stroop Test and the Dichotic Listening Task was improved after ATD, thus showing that the effect occurred for visual as well as auditory stimuli. Findings suggesting a beneficial effect of ATD on focussed attention functions have been reported previously. Stroop performance was improved after ATD in schizophrenic patients (Rosse et al., 1992) and in normal subjects (Rowley et al., 1997). Coull et al. (1995) found that ATD speeded the response to incompatible stimuli by attenuating the interference caused by stimulus - response incongruity in a focussed attention task. However, to our knowledge this study is the first to demonstrate that ATD enhances focussed attention for auditory stimuli as well. This independence of modality emphasizes the robustness of the effect and strengthens the suggestion that central processing rather than perception is augmented by ATD.

Planning functions were not affected by ATD, as reflected by an absence of effects in both accuracy and time needed to solve the problems of the Tower of London. It should be noted, however, that a substantial number of missing data and the lack of baseline values limited the statistical power of this test. Nevertheless, our results are consistent with those of Park et al. (1994), who found no evidence for an effect of Trp depletion on several tests assessing frontal lobe function, including the Tower of

London, working memory and attentional set-shifting. In our study, tryptophan depletion reduced the decision time at t5 without affecting the total time. It appears that subjects started to move the balls sooner but subsequently needed more time to think while moving the balls. This would be indicative of increased impulsivity, rather than an improvement in planning function itself. At t9 these subjects may have applied a more cautious strategy, perhaps being aware of the consequences of an early start. In this study we did however find some evidence for ATD to affect frontal lobe functions. The verbal fluency test is known to be sensitive to frontal lobe dysfunction (Lezak, 1995) and performance on this test was improved during ATD, albeit only at t5. Also, since the frontal cortex is involved in the process of focussed attention (Lezak, 1995), the positive effects of ATD on the Stroop and Dichotic Listening Task may well be due to frontal changes. Removal of the inhibitory actions of 5-HT on other neurotransmitter systems, e.g. dopamine, noradrenaline and acetylcholine, may be involved in this mechanism (Robbins, 1997).

The plasma amino acid analysis showed that a rapid depletion of plasma tryptophan was accomplished in all subjects. The effect of the addition of carbohydrates and fat to the amino acid mixture has been discussed by Riedel et al. (1999) and the MD/fat mix presumably does not alter the effects of ATD on brain Trp availability. The reduction in plasma Trp levels (63%) and Trp/ Σ LNAA ratio (79%) at t5 is somewhat modest compared to the other studies which typically achieve a 75% respectively 90% reduction. In our study subjects were allowed to spread the consumption of the amino acid drink over a two hour period and this is likely to cause a more modest but prolonged state of depletion. Nevertheless, both plasma Trp levels and Trp/ Σ LNAA are convincingly low to assume a reduction of 5-HT activity due to central tryptophan depletion. A neutral Trp/ Σ LNAA ratio during the control condition was not accomplished by increasing the amount of L-Tryptophan to 4.6 g in the placebo mixture. The placebo mixture produced a 20% rise in Trp/ Σ LNAA ratio at t5. Consequently, elevated Trp/ Σ LNAA ratios may have increased 5-HT synthesis during the placebo condition, thereby overestimating the behavioural effects of ATD. One could even pose that this increase may be linked to some of the observed cognitive changes at t5, notably those of the word learning test and fluency test, for which the effects disappear at t9. This would imply that increased 5-HT activity actually improves memory. However, the fact that ATD was found to produce memory deficits compared to a placebo condition in which Trp/ Σ LNAA ratios were reduced as well (Riedel, 1999), strongly argues against this notion. Alternatively, one could speculate that some unknown adaptive response to a reduction in 5-HT activity or even to reduced precursor availability occurred. Once

again, this matter emphasizes the importance of a truly neutral control condition for ATD experiments. As yet we assume that the primary mechanism responsible for the observed effects is lowered 5-HT function during the ATD condition. It has been posed that the use of a Trp-free amino acid mixture may independently increase catecholamine turnover (Danjou et al., 1990). Lowered plasma Trp levels may stimulate the transport of the catecholamine precursor tyrosine into the brain, due to reduced competition for mutual transport sites. However, in our study the ratio between tyrosine and the competing amino acids increased only slightly ($t5: \pm 15\%$, $t9: \pm 14\%$) in both the ATD and placebo condition. It is therefore unlikely that elevated catecholaminergic activity was responsible for the observed differences between treatment conditions. Furthermore, even substantial tyrosine loading will not necessarily increase catecholamine synthesis (Young, 1996). The ratios for phenylalanine, which can be converted to tyrosine, also remained constant (at 10%) between treatment conditions.

No substantial effects of ATD were seen on mood parameters. Although an effect was present for the vigour scale of the POMS, this existed only at $t9$, whereas all observed cognitive changes already occurred at $t5$. Apparently the observed cognitive changes were not mediated by alterations in mood state. The absence of mood effects in this study is consistent with the notion that mood-lowering effects of ATD predominantly occur in genetically vulnerable subgroups (Benkelfat et al, 1994; Klaassen et al., in press). Indeed, in the present study none of the subjects had a family history of depression.

Women have been found to be more susceptible to mood effects of ATD (Ellenbogen et al, 1996), but little is known about sex differences with regard to cognitive effects of ATD. Our data did not show sex differences in the effects of ATD on the cognitive functions or mood assessments. However, the present study was not specifically designed to investigate sex-related differences in the response to ATD, and failure to detect differences may be due to a type II error as a result of small group sizes (9 men vs. 8 women). Since women show a more pronounced reduction in brain 5-HT synthesis after ATD (Nishizawa et al., 1998) it is conceivable that women are more sensitive to the cognitive effects of ATD. Future studies are needed to clarify this. We did not control for phase of the menstrual cycle during testing, consequently some phase dependent variation in mood and perhaps cognitive performance may have occurred. Ideally, female volunteers should be tested in the follicular phase. However, as to memory performance only modest effects of menstrual cycle are expected in healthy women (Sherwin, 1994). Also, lack of sex differences on any of the outcome measures seem to indicate that phase of menstrual cycle did not affect results in a major way.

In conclusion, this study provides corroborating evidence that lowered 5-HT function specifically interferes with long-term memory consolidation in an early phase. Multiple assessments and the induction of ATD after acquisition of new information enabled us to further specify the nature of the memory deficits seen after ATD, showing that retrieval and short-term memory functions remain intact. Exploration of the effects of ATD on other cognitive function particularly identified focussed attention, which showed a modality independent improvement by ATD, as a promising target for future research into the relationship between serotonin and cognition.

References

- Altman, H. J., & Normile, H. J. (1988). What is the nature of the role of the serotonergic nervous system in learning and memory: prospects for development of an effective treatment strategy for senile dementia. *Neurobiology of Aging*, 9(5-6): 627-38.
- Amado-Boccaro, I., Gougoulis, N., Poirier Litre, M. F., Galinowski, A., & Loo, H. (1995). Effects of antidepressants on cognitive functions: a review. *Neuroscience and Biobehavioral Reviews*, 19(3): 479-493.
- Bierer, L. M., Haroutunian, V., Gabriel, S., Knott, P. J., Carlin, L. S., Purohit, D. P., Perl, D. P., Schmeidler, J., Kanof, P., & Davis, K. L. (1995). Neurochemical correlates of dementia severity in alzheimer's disease: relative importance of the cholinergic deficits. *Journal of Neurochemistry*, 64(2): 749-760.
- Benkelfat, C., Ellenbogen, M. A., Dean, P., Palmour, R. M., Young, S. N. (1994). Mood-lowering effect of tryptophan depletion. Enhanced susceptibility in young men at genetic risk for major affective disorders. *Archives of General Psychiatry*, 51(9): 687-97.
- Bond, A., & Lader, M. (1974). The use of analogue scales in rating subjective feelings. *British Journal of Medical Psychology*, 80: 1-46.
- Burt, D. B., Niederehe, G., & Zemblar, M. J. (1995). Depression and memory impairment: a meta-analysis of the association, its pattern, and specificity. *Psychological Bulletin*, 117(2): 285-305.
- Coull, J. T., Sahakian, B. J., Middleton, H. C., Young, A. H., Park, S. B., McShane, R. H., Cowen, P. J., & Robbins, T. W. (1995). Differential effects of clonidine, haloperidol, diazepam and tryptophan depletion on focused attention and attentional search. *Psychopharmacology*, 121(2): 222-230.
- Cross, A. J. (1990). Serotonin in alzheimer-type dementia and other dementing illnesses. *Annals of the New York Academy of Sciences*, 600: 405-417.
- Danjou, P., Hamon, M., Lacomblez, L., Warot, D., & al., e. (1990). Psychomotor, subjective and neuroendocrine effects of acute tryptophan depletion in the healthy volunteer. *Psychiatry and Psychobiology*, 5(1): 31-38.
- Ellenbogen, M. A., Young, S. N., Dean, P., Palmour, R. N., & Benkelfat, C. (1996). Mood response to acute tryptophan depletion in healthy volunteers: Sex differences and temporal stability. *Neuropsychopharmacology*, 15(5): 465-474.
- Houx, P. J., & Jolles, J. (1993). Age-related decline of psychomotor speed: effects of age, brain health, sex and education. *Perceptual and Motor Skills*, 76: 195-211.

- Klaassen, T., Riedel, W.J., van Someren, A., Deutz, N.E.P., Honig, A. & van Praag, H.M. (In press). Mood effects of 24-hour tryptophan depletion in healthy first degree relatives of patients with affective disorders. *Biological Psychiatry*.
- Lezak, M. D. (1995). *Neuropsychological Assessment*. (3rd ed.). New York: Oxford University Press.
- Luteijn, F., & van der Ploeg, F. A. E. (1983). *Handleiding Groninger Intelligentietest (GIT) [Manual Groningen Intelligence Test]*. Lisse, The Netherlands: Swets and Zeitlinger.
- McEntee, W. J., & Crook, T. H. (1991). Serotonin, memory, and the aging brain. *Psychopharmacology Berl*, 103(2): 143-9.
- McNair, D. M., Lorr, M., & Droppleman, L. F. (1971). *Manual for the Profile of Mood States*. San Diego: Educational and Industrial Testing Service.
- Nishizawa, S., Benkelfat, C., Young, S. N., Leyton, M., Mzengeza, S., de Montigny, C., Blier, P., & Diksic, M. (1997). Differences between males and females in rates of serotonin synthesis in human brain . *Proceedings of the National Academy of Sciences U S A*, 94(10): 5308-13.
- Palmer, A. M., & DeKosky, S. T. (1993). Monoamine neurons in aging and Alzheimer's disease. *Journal of Neural Transmission, General Section*, 91(2-3): 135-59.
- Park, S. B., Coull, J. T., McShane, R. H., Young, A. H., Sahakian, B. J., Robbins, T. W., & Cowen, P. J. (1994). Tryptophan depletion in normal volunteers produces selective impairments in learning and memory. *Neuropharmacology*, 33(3/4): 575-588.
- Pollack, I., & Norman, D. A. (1964). A non-parametric analysis of recognition experiments. *Psychonomic Sciences*, 1: 125-126.
- Rey, A. (1964). *L'examen psychologique dans les cas de'encephalopathie traumatique (Psychological assessment in cases of traumatic brain injury)*. Paris: Presses Universitaires de France.
- Reilly, J.G., McTavish, S.F.B. & Young, A.H (1997). Rapid depletion of plasma tryptophan: a review of studies and experimental methodology. *Journal of Psychopharmacology*, 11(4): 381-392.
- Richter-Levin, G., & Segal, M. (1993). Age-related cognitive deficits in rats are associated with a combined loss of cholinergic and serotonergic functions. *Annals of the New York Academy of Science*, 695: 254-257.
- Richter-Levin, G., & Segal, M. (1996). Serotonin, aging and cognitive functions of the hippocampus. *Reviews in the Neurosciences*, 7: 103-113.
- Riedel, W.J., Klaassen, T., Deutz, N.E.P., Van Someren, A., & Van Praag, H.M. (1999). Tryptophan depletion in normal volunteers produces selective impairment in memory consolidation. *Psychopharmacology*, 141: 362-369.
- Robbins, T. W. (1997). Arousal systems and attentional processes. *Biological Psychology*, 45(1-3), 57-71.
- Rosse, R. B., Schwartz, B. L., Zlotolow, S., Banay-Schwartz, M., Trinidad, A. C., Peace, T. D., & Deutsch, S. L. (1992). Effect of a low-tryptophan diet as an adjuvant to conventional neuroleptic therapy in schizophrenia. *Clinical Neuropharmacology*, 15(2): 129-141.
- Rowley, B., Van, F., Mortimore, C., & Connell, J. (1997). Effects of acute tryptophan depletion on tests of frontal and temporal lobe function. *Journal of Psychopharmacology*, 11(3): A60.
- Shallice, T. (1982). Specific impairments of planning. *Philosophical Transactions of the Royal Society of London, Biological Sciences*, 289(1089): 199-209.
- Sherwin, B.B. (1994). Estrogenic effects on memory in women. *Annals of the New York Academy of Science*, 734: 213-230.

- Sirvio, J., Riekkinen, P. J., Jakala, P., & Riekkinen, P. J. (1994). Experimental studies on the role of serotonin in cognition. *Progress in Neurobiology*, 43: 363-379.
- Smith, A (1968). The Symbol Digit Modalities Test: A neuropsychological test for economic screening of learning and other cerebral disorders. *Learning Disorders*, 36: 83-91.
- Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of Experimental Psychology*, 18: 643-662.
- Thompson, P. J. (1991). Antidepressants and memory: a review. *Human Psychopharmacology*, 6(2): 79-90.
- Van Eljk, H.M.,Rooyackers, D.R., Deutz, N.E. (1993).Rapid routine determination of amino acids in plasma by high-performance liquid chromatography with a 2-3 microns Spherisorb ODS II column. *Journal of Chromatography*., 620: 143-8
- Welzin, T.E., Fernstrom, J.D., McConaha, C. & Kaye, W.H. (1994). Acute tryptophan depletion in bulimia: effects on large neutral amino acids. *Biological Psychiatry*, 35: 388-397.
- Wolfe, B. E., Metzger, E. D., & Jimerson, D. C. (1995). Comparison of the effects of amino acid mixture and placebo on plasma tryptophan to large neutral amino acid ratio. *Life Science*, 56(17): 1395-400.
- Young, S. N. (1996). Behavioral effects of dietary neurotransmitter precursors: basic and clinical aspects. *Neuroscience and Biobehavioral Reviews*, 20(2): 313-323.
- Young, S. N., Smith, S. E., Pihl, R. O., & Ervin, F. R. (1985). Tryptophan depletion causes a rapid lowering of mood in normal males. *Psychopharmacology Berl*, 87(2): 173-7.

SSRIs' Pharmacological Profile and Cognitive Effects

Abstract

The current study was carried out to investigate the cognitive effects of two serotonin reuptake inhibitors (SSRIs), sertraline and paroxetine, with special reference to differences in their affinity for other neurotransmitter systems, i.e. anticholinergic activity of paroxetine and putative dopamine reuptake activity of sertraline. The study was conducted according to a double-blind, 3-way cross-over design. During three treatment periods of two weeks, twenty-four healthy middle-aged (30-50 years) subjects of both sexes received sertraline (50 mg on day 1-7, 100 mg on day 8-14), paroxetine (20 mg on day 1-7, 40 mg on day 8-14) and placebo. Paroxetine specifically impaired delayed recall in a word learning test at both the 20- and 40-mg dose. Sertraline did not affect word learning but improved performance on a verbal fluency task at a dose of 50 and 100 mg. Neither drug affected performance on a short-term memory scanning task. These subtle but significant changes in cognitive performance can be explained by subtle differences in pharmacological profiles of these SSRIs. Paroxetine's additional anticholinergic effects could account for its induction of long-term memory impairment. Likewise, sertraline's additional dopaminergic effects could account for its induction of slightly improved verbal fluency. The impairing and facilitating cognitive effects of paroxetine and sertraline respectively, may be more pronounced in the elderly depressed patient.

Introduction

Selective serotonin reuptake inhibitors (SSRIs) have been shown to be effective in the acute treatment of depression. Common to all SSRIs is their ability to powerfully block the reuptake of serotonin into the presynaptic neuron, and this is believed to be the basis of the therapeutic effect. Compared to the older tricyclic antidepressants (TCAs) which affect multiple neurotransmitter systems, SSRIs target the serotonergic system much more selectively. However, SSRIs are not entirely devoid of effects on other neurotransmitter systems, and these additional pharmacological activities may have implications for their clinical profile. The individual potency to affect other neurotransmitter systems may be associated with the efficacy of long-term treatment, treatment of certain subgroups of patients, and the occurrence of unwanted side-effects, including those related to cognitive functioning (Richelson 1996).

Sertraline and paroxetine are the most potent inhibitors of serotonin reuptake currently available (Hyttel 1994). Their pharmacological profile with regard to other neurotransmitter systems, however, is very different. Paroxetine has a relatively high affinity for muscarinic-receptor blockade compared to other SSRIs. Paroxetine's *in vitro* affinity for muscarinic acetylcholine receptors was found to be six times that of sertraline (Hyttel 1994), comparable to that of the TCA imipramine and higher than that of the TCA nortriptyline (Richelson 1996). In contrast, sertraline is a inhibitor of dopamine reuptake compared to other SSRIs, although its dopamine reuptake blocking properties are still 100-fold lower than its serotonin reuptake blockade properties. Sertraline was demonstrated to inhibit dopamine reuptake *in vitro* with one-third the potency of d-amphetamine (Bolden-Watson and Richelson 1993). Sertraline has not been shown to increase prolactin levels in humans (Gordon et al. 1998). Since prolactin release is stimulated by serotonergic input but inhibited by dopaminergic input, this may be indicative of significant dopaminergic stimulation by sertraline *in vivo* in humans.

Improvement of memory functions in depressed patients has often been reported following antidepressant treatment (Allain et al. 1992; Fudge et al. 1990; Siegfried and O'Connolly 1986). It is difficult, however, in clinical studies to rule out the effect of alleviation of depression on memory improvement. In a clinical study we found improvement of long-term memory starting two weeks after treatment with trazodone. Improved memory performance, in contrast to improved mood, reverted to its impaired baseline level, shortly after halting the treatment. This strongly suggested a pharmacologically mediated improvement of memory performance, putatively associated with trazodone's specific serotonergic effects (Riedel et al. 1999b). In healthy volunteers,

the effects of serotonergic manipulations on memory functions are even less well known. Acute tryptophan depletion has been shown to impair learning and memory (Park et al. 1994, Riedel et al 1999a, Schmitt et al. 2000), whereas putative long-term serotonergic depletion in ecstasy users is also associated with impaired memory and other higher cognitive functions (McCann et al. 1999). Augmentation of serotonergic function in healthy volunteers has few effects on cognitive functions (Amado-Boccaro et al., 1995). Acute effects on memory and learning have hardly been reported and studies of (sub)chronic administration of serotonergic antidepressants are entirely lacking in the literature. There is one report however, in which the subchronic administration of the 5-HT_{1A} agonist flesinoxan to healthy elderly volunteers induced improved memory performance (Van Harten et al. 1996).

Based on the pharmacological distinctions between paroxetine and sertraline one may predict specific cognitive functions to be affected by these drugs. Studies with scopolamine have shown that transferal of information to long-term memory and its retrieval during recall is extremely sensitive to muscarinic-receptor blockade (Beatty and Bierley 1986, Riedel et al. 1997). Therefore, paroxetine's anti-muscarinic affinity may cause specific retrieval deficits. Interestingly, none of the studies on the cognitive effects of paroxetine have included memory assessments that required storage of new information into the long-term memory and subsequent retrieval. Impairment of working memory functions, particularly speed of short-term memory search, has also been related to muscarinic receptor blockade by scopolamine. A comparison of several antidepressants revealed a linear relationship between their potency to block muscarinic receptors and the slope of the memory search function in a memory scanning task (Riedel et al. 1997).

There is abundant evidence implicating dopamine in executive functions or cognitive functions which are guided by the prefrontal cortex (Luciana et al. 1998, Volkow et al. 1998, Marié 1999). The fluency task is considered an executive task which has been shown to be sensitive to frontal lobe dysfunction (Lezak 1995). Performance on fluency tasks was found to be sensitive to dopaminergic manipulation. For example, low doses of the D₂ dopamine receptor agonists bromocriptine has been shown to improve fluency performance in subjects with traumatic brain injury, without affecting tasks which do not require executive control (McDowell et al. 1998). In contrast, reduction of dopaminergic activity by administration of the D₁/D₂ receptor antagonist haloperidol in patients with spasmodic torticollis reduced the number of words produced in a verbal fluency test (Berger et al. 1989). Sertraline's dopaminergic activity may thus facilitate performance on a verbal fluency test. Furthermore, given the involvement of dopamine in

motor activation (Luciana et al. 1998; Robbins 1997), sertraline may also speed up reaction times in a memory scanning task by facilitating response selection and execution. Such an effect would be reflected by a decrease of the intercept of the memory search function.

The present study was carried out to assess the specific cognitive effects of subchronic treatment with sertraline and paroxetine, using those tests which are thought to be most sensitive based on drugs' distinct pharmacological profiles. We hypothesized that delayed recall performance in a word learning test would be negatively affected by paroxetine, but not by sertraline. In addition, we expected paroxetine and sertraline to have differential effects on memory scanning performance. Paroxetine was predicted to reduce the speed of short-term memory search, as would be reflected by an increased slope, whereas sertraline would decrease the intercept of the memory search function by facilitating motor activation. Sertraline was further expected to improve performance on a verbal fluency task.

Materials and Methods

Subjects

Twenty-four healthy subjects (13 men, 11 women), aged 30-50 years were recruited through newspaper advertisements. Subjects underwent extensive medical screening, including ECG, blood hematology and chemistry, urine tests, and tests for drug abuse and pregnancy. Subjects were excluded if they suffered from, or had a history of, cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, hematological or psychiatric illness. All subjects scored below the depression threshold (< 50) on the Zung Depression Scale (Zung 1965). Other exclusion criteria were excessive drinking, pregnancy or lactation, use of medication other than oral contraceptives, use of drugs, and any sensory or motor deficits which could reasonably be expected to affect test performance. The study was approved by the Medical Ethics Committee of Maastricht University and the Maastricht University Hospital's Board of Directors. All subjects gave a written informed consent prior to participation.

Design

The study was conducted according to a randomized, double-blind, placebo-controlled, 3-period cross-over design. Treatment periods lasted 14 days and were separated by wash-out periods of 14 days. Treatment order was balanced over the three periods.

Treatment

In the respective conditions, subjects were treated with the following medications: paroxetine 20 mg on day 1 to 7, followed by paroxetine 40 mg on day 8 to 14; sertraline 50 mg on day 1 to 7, followed by sertraline 100 mg on day 8 to 14, and placebo. Medication was taken in the morning as one single oral dose consisting of one (day 1-7) or two (day 8-14) capsules.

Procedure

The subjects underwent three treatment periods of two weeks (figure 1). Approximately one week prior to the first treatment period the subjects practiced the complete cognitive test battery to minimize learning effects. Assessments were done on the day preceding each treatment period (day 0), and on days 7 and 14 of each treatment period. All measurements were done between 12.00 p.m. and 6.00 p.m. Subjects were instructed to arrive at the laboratory well rested. Upon arrival, subjects were seated in a soundproof test room, where they completed the cognitive test battery. Instructions were given through an intercom installation. During the treatment periods subjects were not allowed to drink alcoholic beverages. Consumption of alcohol was limited to 2 glasses a day during the 14-day wash-out period. Female subjects were tested for pregnancy before the start of each treatment period.

Cognitive Assessment

For each of the assessments parallel versions of the cognitive tests were used. The parallel versions were balanced over test times using a latin square.

Figure 1: Schematic representation of the study with an example of a treatment order (sertraline-paroxetine-placebo). For sertraline and paroxetine the dose was doubled in each second week. Diamonds represent the moments of the cognitive assessments, with open diamonds

The Visual Verbal Learning Test (VVLТ).

This test is an adapted version of the Auditory Verbal Learning Test (Rey 1964). Fifteen words are pres

ented in the same sequence in three trials on a computer screen. Each trial ends with a free recall of the words (immediate recall). Thirty minutes after the first trial, the subject is requested to recall as many words as possible (delayed recall). This is followed by a recognition test, consisting of the 15 previously presented words and 15 other but comparable words, in which the subject has to respond "YES / NO" as quickly as possible to indicate recognition of the word (delayed recognition). The proportion of correctly recognized words (cr) and the proportion of falsely recognized (fr) constitute the non-parametric sensitivity measure: $A' = 1 - 1/4 (fr/cr + (1-cr)/(1-fr))$ (Pollack and Norman 1964). A' is in fact the proportion of correctly recognized words, corrected for the subject's response tendency. Outcome parameters were the proportion of the maximum number of words recalled in either of the three immediate recall trials as a measure of short-term memory, the proportion of the number of correct words on the delayed recall as a measure of retrieval from long-term memory, and sensitivity measure (A') as a measure of storage in long-term memory. All outcome parameters are arcsin transformed before they entered statistical analysis because of their skewed distribution.

Verbal Fluency Test (Luteijn and van der Ploeg 1983).

The fluency test can be regarded as a measure of strategy-driven retrieval of information from semantic memory. The subjects are asked to produce as many four-letter words as possible starting with a given letter, within one minute. The number of correct responses and errors are recorded. Nonsense words and names were not accepted, but

conjugations and plurals were allowed. Starting letters were B, H, R, L, P, K, D, G and M. These letters yielded a high and similar number of correct responses (average: 10.1 to 11.0 words) in a comparative study with 40 subjects (Jolles and Houx, unpublished data).

Memory Scanning Task.

The subjects were briefly shown a set of 1 or 3 letters and told to memorize them. This is called the "memory set" (Sternberg 1969). Subjects then saw a series of 75 letters, each displayed to them for 1000 msec. on a computer screen, followed by another 2000 msec. before the next letter appeared. Half of the presented letters were part of the memory set and had to be recognized as targets. The subjects' task was to decide and respond as quickly as possible by pressing either a 'YES' or 'NO' button to indicate whether or not each successive letter was one of those contained in the memory set. The median of the correct responses (targets and non-targets) was taken as a dependent variable. Individual RT * set size function were calculated. This function is defined by the equation $RT = A * Ss + B$, in which A = slope of the function (i.e. the amount of extra time needed per item in the memory set), Ss = size of the memory set, and B = intercept with the Y-axis. The slope is a measure of the speed of scanning short-term memory whereas the intercept measures sensorimotor speed.

Statistical analysis

Outcome variables of the cognitive tests were analyzed using a repeated-measure ANOVA using baseline scores as covariates. *Treatment* (placebo/ sertraline/ paroxetine) and *time* of assessment (7 days/14 days after start of the treatment) were entered as within-subjects factors. Sex was entered as between-subjects factor to investigate the main effect of sex and *treatment* by sex interaction. In addition, separate drug-placebo comparisons were carried out, regardless of the outcome of the overall F tests. This is a legitimate procedure if the comparisons are built into the design or suggested by the theoretical basis of the experiment (Winer, 1971). If no significant *sex* or *sex* by *treatment* effects were found in the initial overall analyses the factor *sex* was omitted in the subsequent drug-placebo comparisons.

Results

Missing data

Twenty-one subjects completed the study. Two men withdrew for reasons unrelated to the study. One woman did not continue after paroxetine treatment in the first treatment period following complaints of nausea and light-headedness. Two of the remaining twenty-one subjects had incomplete data sets. One man could not be tested on day 7 of the placebo period due to personal circumstances, and no memory scanning data were available for one man at the baseline assessment for the sertraline period due to computer failure.

Cognitive tests

The results of the cognitive assessments are presented in table 1.

Visual Verbal Learning Test

Maximal number of words recalled during immediate recall was not affected by sertraline or paroxetine treatment at either dose. Thirty minute delayed recall showed a main treatment effect ($F_{2,35}=4.27$, $p<0.05$), but no main effect of time or a treatment by time interaction. No main effects of sex or sex by treatment interactions were found in either comparison. Separate drug-placebo comparisons revealed that delayed recall performance was decreased during paroxetine treatment ($F_{1,18}=5.26$, $p<0.05$). No treatment by time interaction effect was found, indicating that this effect was similar for the 20 mg and 40 mg dose. Sertraline, on the other hand, did not affect delayed recall performance. Delayed recognition showed no treatment effect, no main effect of time, no treatment by time interaction and no sex differences.

Verbal Fluency Test

No main effects of treatment, time or sex, or interactions between these factors were seen in the overall analysis of the Verbal Fluency Test. However, a separate analysis comparing the effect of sertraline to placebo revealed a main effect of treatment ($F_{1,18}=5.85$, $p<0.05$). The analyses showed no effect of time or a treatment by time interaction. It appeared that sertraline improved the performance on the fluency task at both the 50 mg and 100 mg doses. Although the data showed a similar pattern for

paroxetine, a separate comparison of paroxetine and placebo did not yield any significant main effects of time or treatment or a treatment by time interaction.

Memory Scanning

Neither slope nor intercept showed a main effect of treatment or time, or a treatment by time interaction. No main effects of sex or sex by treatment interactions were found. Separate drug-placebo comparisons did not reveal significant differences.

Table 1 : Means (standard deviations) of outcome parameters of the Visual Verbal Learning Test (VVLТ), Verbal Fluency and Memory Scanning Test at baseline and after 7 and 14 days of treatment with placebo, sertraline and paroxetine. * $p < 0.05$ vs placebo

measure	treatment	baseline	7 days	14 days
VVLТ				
immediate recall (# words)	placebo	13.1 (1.2)	13.3 (1.3)	13.2 (1.8)
	sertraline	13.2 (1.4)	13.2 (1.6)	13.3 (1.8)
	paroxetine	13.2 (1.9)	13.4 (1.1)	13.2 (1.4)
delayed recall (# words)	placebo	12.0 (2.4)	11.8 (2.6)	11.6 (2.7)
	sertraline	11.1 (2.6)	11.8 (2.3)	11.8 (3.3)
	paroxetine	11.1 (2.9)	10.5 (2.3) *	11.0 (2.4) *
delayed recognition (%)	placebo	97.8 (2.1)	98.4 (6.4)	96.4 (3.9)
	sertraline	97.0 (2.6)	97.1 (3.0)	96.2 (3.7)
	paroxetine	96.5 (3.5)	95.5 (4.2)	96.4 (3.2)
Verbal Fluency				
# of correct words	placebo	14.1 (5.3)	12.9 (4.2)	13.7 (4.9)
	sertraline	14.3 (5.3)	14.4 (4.0) *	15.1 (4.9) *
	paroxetine	13.9 (5.8)	13.9 (5.2)	14.6 (4.8)
Memory Scanning				
intercept (msec)	placebo	355 (55)	351 (49)	342 (44)
	sertraline	369 (45)	358 (40)	344 (44)
	paroxetine	364 (53)	359 (57)	354 (66)
slope (msec x memload ⁻¹)	placebo	45.3 (19.7)	47.3 (11.9)	46.6 (13.2)
	sertraline	40.7 (16.8)	43.6 (18.1)	44.9 (15.4)
	paroxetine	44.8 (12.4)	43.2 (16.2)	46.3 (23.8)

Discussion

In the present study we were able to demonstrate differential and specific cognitive effects of sub-chronic sertraline and paroxetine treatment, as was predicted based on their individual pharmacological profiles. Paroxetine treatment impaired long-term memory functioning in that it reduced the number of words recalled thirty minutes after presentation. In contrast, sertraline treatment was associated with improved verbal fluency performance, but had no effects on performance on the word learning test. The hypothesised effect of paroxetine on memory scanning performance, namely an increase in slope of the memory search function, was not found, nor did we find the predicted lowering of the intercept of the memory search function by sertraline. The results will be discussed in terms of each drug's affinity for non-serotonergic neurotransmitter systems, and the clinical implications will be addressed.

Although serotonin has been implicated in memory functions, attention and executive functioning (Altman and Normile 1988; Sirvio et al. 1994; Buhot 1997; Riedel et al. 1999a; Schmitt et al. 2000), the observed differences in direction, as well as the specificity of the cognitive effects of paroxetine and sertraline suggest that these cognitive changes are not due to their shared action on serotonergic transmission. Furthermore, the effects do not appear to be mediated indirectly through global mechanisms, such as sedation or activation, since such a mechanism would most likely affect multiple cognitive functions in a similar direction. Instead, the observed cognitive changes appear to be in line with evidence implicating acetylcholine and dopamine in specific cognitive processes.

Impaired delayed recall performance without effects on delayed recognition is a typical pattern seen following muscarinic blockade by scopolamine (Hasselmo and Wyble 1997; Riedel et al. 1997). In the present study paroxetine induced a similar pattern of impairment on the word learning test, suggesting that the memory effects may indeed attributable to muscarinic receptor blockade. Paroxetine's affinity for the muscarinic cholinergic receptor is often qualified as weak and its clinical relevance has been questioned (Gunasekara et al. 1998; Prakash and Foster 1999). Comparisons of drugs' *in vitro* anticholinergic potency may not necessarily reflect the situation *in vivo*. For example, Pollock et al. (1998) found that serum anticholinergic levels in elderly depressed patients indicated that, at therapeutic plasma concentrations (120 ng/ml), paroxetine had approximately one-fifth the anticholinergic potential of nortriptyline, while *in vitro* data suggested a higher antimuscarinic potency of paroxetine (Richelson 1996).

Nonetheless, it has been shown that even low serum anticholinergic levels are associated with memory impairment, i.e. impaired retrieval of newly learned information from long-term memory, in elderly non-demented subjects (Nebes et al. 1997). In fact, in the present study diminished delayed recall performance could be detected with the lowest dose (20mg) of paroxetine, emphasizing the sensitivity of long-term memory function to anticholinergic actions. Furthermore, it is important to notice that the memory effects of paroxetine persisted throughout the treatment period. This is consistent with the notion that, while the sedating effects of histaminergic or noradrenergic blockade are most pronounced following acute administration and diminish over time due to tolerance, the direct effects of anticholinergic actions on memory performance are resistant to tolerance and may occur as long as the treatment continues (Ramaekers 1998; Thompson 1991).

In the present study detrimental effects of paroxetine on long-term memory performance were detectable in healthy middle-aged subjects. In depressive patients memory functions are already diminished (Burt et al. 1995), and particularly at the initiation of the treatment before onset of the therapeutic response, detrimental effects of antidepressant anticholinergic activity will add to the memory deficits (Lane and O'Hanlon 1999). Furthermore, it has been shown that sensitivity to anticholinergic effects increase with age (Tariot et al. 1996). The combined effects of age-related and depression-induced cognitive decline and enhanced vulnerability to anticholinergic effects of antidepressants may result in more pronounced cognitive deficits than in younger patients (Knegtering et al. 1994, Lane and O'Hanlon 1999). Furthermore, elderly depressive patients are more likely to use co-medication that may have significant anticholinergic properties (Tune et al. 1992). This may lead to significant cumulative anticholinergic effects. Therefore, especially in the treatment of the elderly depressed patient it is important to avoid anticholinergic action when possible.

The observed facilitating effect of sertraline on verbal fluency performance appears to be consistent with dopaminergic modulation of frontal cortical areas involved in 'higher' executive functions. In depressed patients, using SPET (Single Proton Emission Tomography) to assess dopamine D_2/D_3 receptor binding, it was found that reduced striatal dopaminergic activity was associated with impaired verbal fluency performance as well as reduced motor speed (Shah et al. 1997). It is unclear whether the reduction in dopaminergic activity is directly related to depression itself or the result of serotonergic antidepressant medication, which reduces extrastriatal dopamine concentrations (Dewey et al. 1995). However, regardless of the cause, this suggests that a serotonergic antidepressant with some additional dopamine enhancing properties,

such as sertraline, may attenuate a 'hypodopaminergic' state and its associated effects - including those related to cognition - in the depressed patient (Stahl, 1998). The elderly depressed patient may benefit even more from additional dopamine reuptake since age-related decreases in dopaminergic activity have been associated with a decline in motor function and performance on frontal lobe tasks (Volkow et al. 1998). The observed improvement of fluency performance, however, may not be entirely attributable to dopaminergic modulation, as paroxetine also was found to cause a considerable, albeit non-significant, increase in fluency score compared to placebo. Therefore, a direct serotonergic involvement cannot be ruled out, although such an effect needs further investigation.

The memory scanning paradigm has been used in several studies on the cognitive effects of antidepressants. Subchronic sertraline treatment (50 mg increasing to 200 mg daily) did not affect memory scanning performance in healthy men (Williams et al. 1996). Similarly, subchronic treatment with paroxetine 30 mg (Hindmarch 1992) or 20 mg (Kerr et al. 1992) was found to have no effect on memory scanning performance in young and middle-aged respectively elderly healthy subjects. Robbe and O'Hanlon (Robbe and O'Hanlon 1995), however, found subchronic paroxetine treatment to impair memory scanning performance only at the 40 mg dose and not at the 20 mg dose. The effect was small and probably related to reduced sensorimotor function, but it persisted throughout the 8-day treatment period. It seems that the memory scanning paradigm is sensitive to significant muscarinic receptor blockade, for example with scopolamine (Riedel et al. 1995) or amitriptyline (Robbe and O'Hanlon 1995), but may not be able to show effects of weaker muscarinic receptor blockade by paroxetine. In addition, it appears that the paroxetine induced impairment of long-term memory performance can not be explained by concomitant impairment of short-term memory search.

In summary, this is the first study in which SSRIs with different additional pharmacological properties over and above serotonin reuptake inhibition were shown to induce detectable and specific cognitive changes. Paroxetine's anticholinergic activity was most probably related to diminished long-term memory performance, whereas sertraline facilitated executive functioning. The latter effect may be linked to the drug's favorable profile regarding dopaminergic transmission. These effects were modest in healthy volunteers, but there are indications that more pronounced effects may occur in depressed patients, especially the elderly. This is true for the memory impairing effects of paroxetine, as well for the cognition enhancing effects of sertraline.

Acknowledgements

This study was supported by an unrestricted grant from Pfizer Inc. New York. The authors wish to thank Suzanne Teunisse for her work during the data collection

References

- Amado-Boccaro I, Gougoulis N, Poirier Litre MF, Galinowski A, Loo H (1995) Effects of Antidepressants on Cognitive Functions: A Review. *Neuroscience and Biobehavioral Reviews* 19: 479-493
- Allain H, Lieury A, Brunet BF, Mirabaud C, Trebon P, LeCoz F, Gandon JM (1992) Antidepressants and cognition: comparative effects of moclobemide, viloxazine and maprotiline. *Psychopharmacology Suppl* 106: 56-61
- Altman HJ, Normile HJ (1988) What is the nature of the role of the serotonergic nervous system in learning and memory: prospects for development of an effective treatment strategy for senile dementia. *Neurobiol Aging* 9: 627-38
- Beatty WW, Bierley RA (1986) Scopolamine impairs encoding and retrieval of spatial working memory in rats. *Physiological Psychology* 14: 82-86
- Berger HJC, van Hoof JJM, van Spaendonck KPM, Horstink MWI, van den Bercken JHL, Jaspers R, Cools AR (1989) Haloperidol and cognitive shifting. *Neuropsychologia* 27: 629-639
- Bolden-Watson C, Richelson E (1993) Blockade by newly-developed antidepressants of biogenic amine uptake into rat brain synaptosomes. *Life Sci* 52: 1023-9
- Buhot M (1997) Serotonin receptors in cognitive behaviors. *Current Opinion in Neurobiology* 7: 243-254
- Burt DB, Niederehe G, Zemblar MJ (1995) Depression and Memory Impairment: A Meta-Analysis of the Association, Its Pattern, and Specificity. *Psychological Bulletin* 117: 285-305
- Dewey S, Smith G, Logan J, Axelhoff D, Ding Y, King P, Pappas N, Brodie J, Ashby C (1995) Serotonergic modulation of striatal dopamine measured with positron emission tomography (PET) and in vivo microdialysis. *J Neurosci* 15: 821-829
- Fudge JL, Perry PJ, Garvey MJ, Kelly MW (1990) A comparison of the effect of fluoxetine and trazodone on the cognitive functioning of depressed outpatients. *Journal of Affective Disorders* 18: 275-80
- Gordon C, Whale R, Cowen PJ (1998) Sertraline treatment does not increase plasma prolactin levels in healthy subjects. *Psychopharmacology* 137: 201-202
- Gunasekara N, Noble S, Benfield P (1998) Paroxetine: an update of its pharmacology and therapeutic use in depression and a review of its use in other disorders. *Drugs* 55: 85-120
- Hasselmo M, Wyble B (1997) Free recall and recognition in a network model of the hippocampus: simulating effects scopolamine on human memory function. *Behavioural Brain Research* 89: 1-34
- Hindmarch I (1992) A review of the psychomotor effects of paroxetine. *Int Clin Psychopharmacol* 4: 65-7
- Hyttel J (1994) Pharmacological characterization of selective serotonin reuptake inhibitors (SSRIs). *Int. Clin. Psychopharmacol.* 9: 19-26

- Kerr JS, Fairweather DB, Mahendran R, Hindmarch I (1992) The effects of paroxetine, alone and in combination with alcohol on psychomotor performance and cognitive function in the elderly. *Int Clin Psychopharmacol* 7: 101-8
- Knegttinger H, Eijck M, Huijsman A (1994) Effects of antidepressants on cognitive functioning of elderly patients. A review. *Drugs Aging* 5: 192-9
- Lane R, O'Hanlon JF (1999) Cognitive and psychomotor effects of antidepressants with emphasis on selective serotonin reuptake inhibitors and the depressed elderly patient. *German Journal of Psychiatry* 2: 1-42
- Lezak MD (1995) *Neuropsychological Assessment*, 3rd edn. Oxford University Press, Oxford University Press
- Luciana M, Collins PF, Depue RA (1998) Opposing roles for dopamine and serotonin in the modulation of human spatial working memory functions. *Cereb Cortex* 8: 218-26
- Luteijn F, van der Ploeg FAE (1983) *Manual Groningen Intelligencetest (GIT)*. Swets and Zeitlinger, Swets and Zeitlinger
- Marié RM (1999) Relationships between striatal dopamine denervation and frontal executive tests in Parkinson's disease. *Neuroscience Letters* 260: 77-80
- McCann UD, Mertl M, Eligulashvili V, Ricaurte GA (1999) Cognitive performance in (+-) 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") users: a controlled study. *Psychopharmacology* 143: 417-425
- McDowell S, Whyte J, D'Esposito M (1998) Differential effect of a dopaminergic agonist on prefrontal function in traumatic brain injury patients. *Brain* 121: 1155-64
- Nebes R, Pollock B, Mulsant B, Kirshner M, Halligan E, Zmuda M, Reynolds C (1997) Low-level serum anticholinergic activity as a source of baseline cognitive heterogeneity in geriatric depressed patients. *Psychopharmacology Bulletin* 33: 715-720
- Park SB, Coull JT, McShane RH, Young AH, Sahakian BJ, Robbins TW, Cowen PJ (1994) Tryptophan Depletion in Normal Volunteers Produces Selective Impairments in Learning and Memory. *Neuropharmacology* 33: 575-588
- Pollack I, Norman DA (1964) A non-parametric analysis of recognition experiments. *Psychonomic Sciences* 1: 125-126
- Pollock B, Mulsant B, Nebes R, Kirshner M, Begley A, Mazumdar S, Reynolds C (1998) Serum Anticholinergic activity in elderly depressed patients treated with paroxetine or nortriptyline. *Am J Psychiatry* 155: 1110-1112
- Prakash A, Foster R (1999) Paroxetine: a review of its use in social anxiety disorder. *CNS drugs* 12: 151-169
- Ramaekers JG (1998) Behavioural Toxicity of Medicinal Drugs. *Drug Safety* 18: 189-208
- Rey A (1964) *L'examen psychologique dans les cas d'encéphalopathie traumatique*. Presses Universitaires de France, Presses Universitaires de France
- Richelson E (1996) Synaptic effects of antidepressants. *J Clin Psychopharmacol* 16: 7-9
- Riedel W, Hogervorst E, Leboux R, Verhey F, Van Praag H, Jolles J (1995) Caffeine attenuates scopolamine-induced memory impairment in humans. *Psychopharmacology* 122: 158-168
- Riedel WJ, Hogervorst E, Verhey FRJ, Jolles J (1997) Measuring Cholinergic Function and Cognitive Abilities. In: Hindmarch I, Stonier PD (eds) *Human Psychopharmacology: Measures and Methods*, Vol VI. Wiley, Chichester, pp 29-56

- Riedel WJ, Klaassen T, Deutz NEP, Van Someren A, Van Praag HM (1999a) Tryptophan Depletion in Normal Volunteers Produces Selective Impairment in Memory Consolidation. *Psychopharmacology* 141: 362-369
- Riedel WJ, Schoenmakers E, Vermeeren A, O'Hanlon JF (1999b) The Influence of Trazodone Treatment on Cognitive Functions in Outpatients with Major Depressive Disorder. *Human Psychopharmacology* 14: 499-508
- Robbe HWJ, O'Hanlon JF (1995) Acute and subchronic effects of paroxetine 20 and 40 mg on actual driving, psychomotor performance and subjective assessments in healthy volunteers. *European Neuropsychopharmacology* 5: 35-42
- Robbins TW (1997) Arousal Systems and Attentional Processes. *Biological Psychology* 45: 57-71
- Schmitt JAJ, Jorissen B, Sobczak S, Van Boxtel MPJ, Hogervorst E, Deutz NEP, Riedel WJ (2000) Tryptophan depletion impairs memory consolidation, but improves focused attention in healthy young volunteers. *Journal of Psychopharmacology* 14: 21-29
- Shah P, Ogilvie A, Goodwin G, Ebmeier K (1997) Clinical and psychometric correlates of dopamine D2 binding in depression. *Psychological Medicine* 27: 1247-1256
- Siegfried K, O'Connolly M (1986) Cognitive and psychomotor effects of different antidepressants in the treatment of old age depression. *International Clinical Psychopharmacology* 1: 231-243
- Sirvio J, Riekkinen PJ, Jakala P, Riekkinen PJ (1994) Experimental studies on the role of serotonin in cognition. *Progress in Neurobiology* 43: 363-379
- Stahl SM (1998) Basic psychopharmacology of antidepressants, part 1: Antidepressants have seven distinct mechanisms of action. *J Clin Psychiatry* 59: 5-14
- Sternberg S (1969) Memory scanning: mental processes revealed by reaction time experiments. *American Scientist* 57: 421-457
- Tariot PN, Patel SV, Cox C, Henderson RE (1996) Age-related decline in central cholinergic function demonstrated with scopolamine. *Psychopharmacology Berl* 125: 50-6
- Thompson PJ (1991) Antidepressants and memory: A review. *Hum Psychopharmacol* 6: 79-90
- Tune LE, Carr S, Hoag E, Cooper T (1992) Anticholinergic Effects of Drugs Commonly Prescribed for the Elderly: Potential Means for Assessing the Risk of Delirium. *Am J Psychiatr* 149: 1393-1394
- Van Harten J, Olf M, Wesnes K, Strobel W (1996) Flesinoxan Improves Cognition in The Healthy Elderly. *European Neuropsychopharmacology* 6: 186
- Volkow ND, Gur RC, Wang GJ, Fowler JS, Moberg PJ, Ding YS, Hitzemann R, Smith G, Logan J (1998) Association between decline in brain dopamine activity with age and cognitive and motor impairment in healthy individuals. *Am J Psychiatry* 155: 344-9
- Williams SA, Wesnes K, Oliver SD, Rapeport WG (1996) Absence of effect of sertraline on time-based sensitization of cognitive impairment with haloperidol. *J Clin Psychiatry* 1: 7-11
- Winer BJ (1971) Statistical principles in experimental design. New York, McGraw-Hill
- Zung WKK (1965) A self rating depression scale. *Archives of General Psychiatry* 12: 63-70

Additional dopamine reuptake inhibition prevents vigilance decrement induced by serotonergic reuptake inhibition in man

Abstract

The current study was designed to investigate the attentional effects of subchronic treatment with the serotonin reuptake inhibitors (SSRIs) sertraline and paroxetine, according to a placebo-controlled, double-blind, 3-way cross-over design. Twenty-four healthy middle-aged subjects, of whom twenty-one completed the study, underwent three treatment periods of two weeks in which they received sertraline (50 mg on days 1-7, 100 mg on days 8-14), paroxetine (20 mg on days 1-7, 40 mg on days 8-14) and placebo. Treatment periods were separated by 14 days washout periods. Vigilance (Mackworth Clock Test) and selective (Stroop, Dichotic Listening) and divided attention (Dichotic Listening) were assessed at baseline and on days 7 and 14 of each treatment period. Selective and divided attention were unaffected by SSRI treatment. Paroxetine impaired vigilance performance at both the 20 and 40 mg doses. In contrast, no vigilance impairment was found during sertraline treatment. Sertraline is the only SSRI studied so far with no detrimental effects on vigilance. This may be due to sertraline's unique ability to inhibit dopamine reuptake. This study and previous research indicates that serotonergic stimulation is associated with specific reduction of vigilance, which is mediated by serotonergic inhibition of dopaminergic systems.

Introduction

The majority of psychoactive medication poses the potential problem of sedation, a condition generally conceived as decreased or sub-optimal wakefulness. The maintenance of wakefulness is a brain function that is no longer considered a unitary construct. Different neuroanatomical, neurochemical and behavioural functions characterize its multidimensional nature. Stimulus-mediated arousal, tonic readiness to respond, voluntary effort and behavioural inhibition have been outlined as functions controlling wakefulness, distinctly mediated by noradrenergic, dopaminergic, cholinergic and serotonergic neurochemical modulation (Robbins and Everitt 1995; Robbins 1997). Noradrenaline (NA) is mainly involved in rapid but transient changes in cortical arousal in response to outside stimuli, whereas dopamine (DA) and acetylcholine (Ach) are thought to be primarily associated with an internally-driven energetic state, which varies more slowly and is not readily modulated by external stimulation (McGuiness and Pribram 1980; Robbins 1984). Serotonin (5-HT) is thought to dampen the activity of each of these neurotransmitter systems, hereby promoting behavioural inhibition and cortical de-arousal (Robbins 1997).

Indeed, augmentation of 5-HT neurotransmission has been shown to impair human vigilance performance. The specific serotonin reuptake inhibitor (SSRI) fluoxetine, an antidepressant drug which stimulates 5-HT neurotransmission, was found to reduce vigilance performance, as assessed with a classical Mackworth Clock Test, in healthy volunteers at a therapeutic dose of 20 mg throughout the treatment period of three weeks (Ramaekers et al. 1995). Likewise, in healthy volunteers, a reduction of vigilance performance on the Mackworth Clock Test was found following sub-chronic administration of therapeutic doses (75-150 mg) of the antidepressant venlafaxine, which similarly inhibits 5-HT reuptake (O'Hanlon et al. 1998). The authors of the latter study concluded that specific serotonergic anxiolytics and antidepressants consistently impaired vigilance without affecting other attentional functions. The SSRI paroxetine could not be demonstrated to affect vigilance performance in healthy volunteers (Deijen et al. 1989). However, in this study 100 mg maprotiline, an antidepressant drug known to cause subjective drowsiness even at much lower dosages (25 mg) (Amado-Boccaro et al. 1995), did not affect vigilance performance either, suggesting the absence of effects may be caused by insufficient sensitivity of the 'adapted' (otherwise unspecified) vigilance task.

Serotonin is known to inhibit activity of various neurotransmitter systems that play important roles in arousal functions and vigilant behaviour, i.e. noradrenaline,

acetylcholine and DA (Robbins 1997), and such interactions are likely to underlie 5-HT induced vigilance impairments. However, the mechanism responsible for the 5-HT induced vigilance impairment has not been fully elucidated. Particularly, the relative contribution of the various 5-HT interactions with each of these other neurotransmitter systems in inducing the vigilance impairment is largely unknown. A reduction of NA activity does not appear to be a key factor in 5-HT induced vigilance impairment, since concomitant NA stimulation by the dual action 5-HT/NA re-uptake inhibitor venlafaxine proved ineffective in attenuating the impairment of vigilance performance by 5-HT stimulation (O'Hanlon et al. 1998). There now seems to be accumulating evidence from animal studies that NA is not the critical neurotransmitter specifically associated with the maintenance of vigilance (Delagrange et al. 1993; McGaughy et al. 1997). Increased rate of firing of NA locus coeruleus cells in response to novel stimuli, and subsequent habituation after repetitive presentations (Jacobs 1987), seem to support McGuiness and Pribrams' (1980) notion that NA is involved in stimulus-induced phasic arousal. Since habituation for the arousing properties of repetitive external stimulation occurs within minutes, subsequently performance on a 45-minute vigilance tasks would depend heavily on the internal energetic state. This type of arousal has been primarily associated with DA systems (McGuiness and Pribram 1980; Robbins 1984). It is well known that manipulation of DA transmission affects vigilance performance. For instance, DA antagonism by pimozide impaired performance on a sustained attention task in healthy volunteers, whereas DA stimulation by pemoline improved sustained attention (Nicholson and Pascoe 1990). Also, stimulation of DA transmission by amphetamine and methylphenidate was found to facilitate vigilance performance (Koelega 1993). Thus, DA is a likely candidate as mediator of the vigilance effects of 5-HT stimulation.

Sertraline is an SSRI which inhibits 5-HT re-uptake into the presynaptic neuron and thus stimulates 5-HT neurotransmission. However, in addition to its 5-HT activity, it also possesses a relatively high affinity (among SSRIs) for the human DA transporter (Tatsumi et al. 1997) and may therefore facilitate DA neurotransmission as well. It must be noted that its DA reuptake blocking properties are still 100-fold lower than its 5-HT reuptake blockade properties. Nevertheless, sertraline was demonstrated to inhibit DA reuptake *in vitro* with one-third the potency of d-amphetamine (Bolden-Watson and Richelson 1993). Furthermore, in contrast to other SSRIs, sertraline does not generally increase prolactine levels in humans (Gordon et al. 1998). Since prolactine release is stimulated by 5-HT input but inhibited by DA input, sertraline's DA activity may have attenuated 5-HT effects on prolactine release. A similar attenuation of the vigilance impairing effects of 5-HT may occur if the vigilance decrement is mediated by lowered

DA activity. Thus, this dual pharmacodynamic property of sertraline renders it particularly useful in the investigation of the putative role of DA in the 5-HT induced vigilance impairment.

The aim of the present study was to confirm and further dissect the 5-HT mediated reduction of vigilance performance in human volunteers. Based on above considerations, it was hypothesized that vigilance impairment following 5-HT stimulation is primarily mediated by concomitant reduction of DA activity. In order to test this hypothesis, we compared the effects of paroxetine, a SSRI without DA activity, to those of the sertraline, a SSRI with mild stimulating effects on DA neurotransmission. Specifically, it was expected that 5-HT stimulation by paroxetine would impair vigilance performance, whereas sertraline's additional DA activity would attenuate the 5-HT mediated reduction of vigilance performance. Secondly, we sought to confirm the specificity of the effect in terms of attentional functioning. To this end we included two tests of selective and divided attention, which were recently demonstrated to be sensitive to 5-HT manipulation by tryptophan depletion (Schmitt et al. 2000). Lastly, since SSRI administration may alter sleep architecture (Lindsay DeVane 1998), and this in turn may affect daytime vigilance performance, we measured drug-related changes in sleep quality to control for this potential confounder.

Materials and Methods

Subjects

Twenty-four healthy volunteers (13 men, 11 women), aged 30-50 years, were recruited through advertisements in local newspapers. Mental and physical health of each subject was assessed by means of a health questionnaire, medical examination, routine ECG, blood hematology and chemistry, and standard urine screening. A urine test for drug abuse and, for women, a urine pregnancy test was conducted. Excluded were those volunteers who suffered from, or had a history of, cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, hematological or psychiatric illness. All subjects completed the Zung Depression Scale (Zung 1965) and scored well below depression threshold (score < 50). Other exclusion criteria were excessive drinking (> 20 units of alcoholic a week) pregnancy or lactation, use of medication other than oral contraceptives, use of drugs, and any sensory or motor deficits which could reasonably be expected to affect test performance. The study was approved by the Medical Ethics Committee of Maastricht University and the Maastricht University Hospital's Board of Directors. All subjects gave a written informed consent prior to participation.

Design

The study was conducted according to a randomized, double-blind, placebo-controlled, 3-period cross-over design. Treatment periods lasted 14 days and were separated by wash-out periods of at least 14 days. Treatment order was balanced over the three treatment periods.

Treatment

In the respective conditions, subjects were treated orally for 14 days with the following medications: paroxetine 20 mg once daily, increasing to paroxetine 40 mg once daily on the eighth day; sertraline 50 mg once daily, increasing to sertraline 100 mg once daily on the eighth day; and placebo once daily. Medication was taken in the morning in one capsule during the first week and two capsules during the second week.

Procedure

Approximately one week prior to the first treatment period the subjects practiced the test to minimize learning effects. Assessments were done on the day preceding each treatment period (day 0), and on days 7 and 14 of each treatment period. All measurements were done between 12.00 PM and 6.00 PM. Subjects were instructed to arrive at the laboratory well rested. During the treatment period subjects were not allowed to drink alcoholic beverages. Consumption of alcohol was limited to 2 glasses a day during the wash-out period. Female subjects were tested for pregnancy before the start of each treatment period.

Upon arrival, subjects completed the Groningen Sleep Quality Scale (see below) and were then seated in a soundproof test room. They first completed a cognitive test battery, which included the Stroop Color Word Test and Dichotic Listening Task (see below). Subsequently, vigilance performance was assessed using a 45-minute Mackworth Clock Test (see below). Finally, a 10 ml bloodsample was taken.

Attention tests

Mackworth Clock Test.

This test has been extensively used in studies on human vigilance performance (Mackworth 1961). Subjects were seated in front of a computer screen displaying a circular arrangement of 60 dots simulating the second marks on a clock. Dots were briefly illuminated in clockwise rotation at a rate of one per 500 milliseconds. Usually the rotation proceeded with a 6'-jump. Subjects were instructed that at rare, irregular

intervals the target would proceed with a 12'- jump by skipping one of the dots in the normal sequence. This double jump was the signal to which subjects were required to respond by pressing a button as quickly as possible. A response made within 4 seconds after the occurrence of a signal was registered as a correct detection. A total of 30 signals were presented during the 45-min task. Ten signals occurred within each successive 15-min period, with intervals ranging from 8 seconds to 7.2 minutes. Outcome measures were the number of correct detections and the corresponding reaction times, and the number of false detections.

Stroop Color Word Test

The Stroop Test has often been used to test selective attention (Stroop 1935). The test consists of three cards. First, a card with a hundred color names must be read as quickly as possible, followed by a card in which the same number of colored patches must be named. On the third card color names are printed in incongruously colored ink. The color of the ink must be named, without paying attention to the word itself. The outcome parameters of this test are the time needed to complete each card and the interference measure. The latter denotes the percentage of extra time needed to complete card III, relative to the average of card I and II: $(\text{time card III} / ((\text{time card I} + \text{time card II})/2)) * 100\%$.

Dichotic Listening Task

Dichotic listening can be used to test both selective attention and divided attention for auditory stimuli. Subjects receive two different auditory stimuli simultaneously though a stereo headphone, one stimulus on each side. The stimuli are numbers ranging from 1 to 200, presented at random and natural-speech spoken by a male voice. The test consists of three subtasks in which subjects are instructed to focus on either the right channel, the left channel or on both channels, and to remember these numbers. The subtasks in which subjects must focus on one side, while ignoring the other, can be regarded as a selective attention task. The subtask in which stimuli from both sides have to be remembered is a divided attention task. Each of the three subtasks is made up of nine trials, in which 2, 3, or 4 stimuli pairs are presented in random order. After each trial the subjects must identify the appropriate numbers from a list of numbers on a computer screen. This list is composed of all presented numbers, plus the same amount of other numbers. Outcome variables are the total number of correctly identified stimuli, total number of stimuli identified from the wrong side, and number of false alarms (numbers

which were not presented). Performance on each subtask is assessed by calculating sensitivity measures, similar to those used in the VVLT.

Subjective Sleep Quality

Subjective quality of sleep was assessed using the Groningen Sleep Quality Questionnaire (Mulder Hajonides van der Meulen 1981). This questionnaire consists of 14 statements regarding the quality of sleep over the past week. Subjects agreed or disagreed with each of the statements. Maximum score is 14, indicating severely disturbed sleep, and minimum score is 0, indicating no sleeping problems.

Blood sampling

Blood (10ml) was collected by venipuncture on days 0, 7 and 14 at the end of each cognitive test battery. Blood samples obtained in the paroxetine and sertraline periods were assayed for the appropriate drug using a reverse phase high performance liquid chromatography (HPLC) procedure with ultraviolet detection, with a detection limit of 10 ng/ml for the paroxetine assay and 5 ng/ml for the sertraline assay.

Statistical analysis

Results of the attention tests and subjective sleep questionnaire were analyzed using repeated-measures analyses of variance (SPSS 6.1 on an Apple Macintosh). Within subjects factors were *Treatment* (paroxetine, sertraline, placebo) and *Day of assessment* (day 7, day 14). Respective baseline values of each treatment period (day 0) were entered as co-variables to correct for pre-treatment level of performance.

For analyses of the Mackworth Clock Test the additional factor *Time on task* (performance at 15, 30 and 45 minutes) was included and the data were tested for a main effects of *Time on task* and *Treatment by Time on task* interactions. Main effects of *Treatment* ($p < 0.05$) were further investigated using separate comparisons between drug and placebo and between paroxetine and sertraline. In this case, analyses were rerun using two levels of the factor *Treatment* (drug, placebo, or paroxetine, sertraline).

Sex was entered as a between-subject factor in each initial overall analysis, but was omitted in subsequent comparisons if no significant gender differences were apparent. False detections in the Mackworth Clock Tests were not normally distributed and were analyzed using Friedman's non-parametric ANOVA. Outcome variables were not tested for the effects of treatment order. Treatment order was fully counterbalanced for the initial 24 subjects and counterbalancing was not severely compromised by the withdrawal of the three subjects (see below), who each had a different treatment order.

Also, learning effects were minimized by the use of a pre-study training session. Furthermore, the study design, which incorporates multiple baselines and a placebo condition, ensured ample control for possible order effects.

Results

Dropouts and missing data

Twenty-one subjects completed the study (table 1). One woman withdrew in the first period, during paroxetine treatment, with complaints of nausea and light-headedness. Two men dropped out for reasons unrelated to the study; one man was in the sertraline condition, the other withdrew before starting the first treatment period. The subjects who completed the study provided complete data, with the exception of one man who could not be tested on day 7 during placebo because of unforeseen personal circumstances.

Table 1: Demographic characteristics of the subjects who completed the study.

		<u>age (yrs)</u>		<u>height (cm)</u>		<u>weight (kg)</u>	
	<u>n</u>	<u>mean (sd)</u>	<u>range</u>	<u>mean (sd)</u>	<u>range</u>	<u>mean (sd)</u>	<u>range</u>
men	11	36.4 (5.6)	30-49	179.0 (6.7)	171-188	78.7 (9.1)	63-92
women	10	39.4 (6.6)	31-47	166.5 (7.0)	152-177	64.9 (14.6)	50-95

Attention tests

Mackworth Clock Test

The number of correct detections (figure 1) of the Mackworth Clock Test showed main effects of Treatment ($F_{2,35}=3.49$, $p<0.05$) and Time on task ($F_{2,35}=6.04$, $p<0.01$), but no main effects of Day or Sex. There were no significant interactions. Lack of a significant Treatment by Time on task interaction lead to the use of the total number of correct detections over 45 minutes for subsequent analysis of the correct detections. These drug-placebo comparisons revealed that paroxetine reduced the number of correct detections ($F_{1,18}=7.58$, $p<0.05$), whereas sertraline did not significantly affect this task parameter. No main effect of Day or a Treatment by Day interaction was found in either comparison. Also, no main effects or interactions were found in a comparison between paroxetine and sertraline.

Reaction times of the correct detections (figure 1) showed a main effect of Time on task ($F_{2,35}=5.45$, $p<0.01$). In addition, the main effect of Treatment approached a significant level ($F_{2,35}=2.90=0.07$). No main effects of Day or Sex were found, and no significant interactions between Treatment, Time on task, Day and Sex were present. The observed trend for a main effect of treatment lead us to perform additional analyses to explore this effect. No significant main effects or interactions were found in separate drug-placebo comparisons. However, paroxetine showed a trend to prolong reaction times ($F_{1,18}=4.21$, $P=0.06$), whereas sertraline appeared to speed up responses ($F_{1,18}=3.97$, $p=0.06$). A separate comparison between sertraline and paroxetine revealed a main effect of Treatment ($F_{1,19}=7.03$, $p<0.05$), but no main effect of time or a Treatment by Day interaction. No effects were found for the number of false detections.

Stroop Color Word Test

No main effects of Treatment, Day, or Treatment by Day interactions were found for card I, card II, card III or the interference measure of the Stroop Test. None of these analyses showed a main effect of Sex or a Sex by Treatment interaction.

Dichotic Listening Task

No main effects of Treatment or Time, or Treatment by Time interactions were present for the performance on subtasks 'left' and 'right', or on subtask 'both'. No main effects of Sex or Sex by Treatment interactions were found.

Subjective Sleep Quality

Subjective sleep quality showed a main effect of Treatment ($F_{2,37}=7.76$, $p<0.01$) but no main effect of Day or a Treatment by Day interaction. The analysis further revealed a significant Treatment by Sex interaction (figure 2). Consequently, analyses were conducted for men and women separately. It was found that Treatment did not affect sleep quality of the male subjects, but women's sleep quality showed a main effect of Treatment ($F_{2,17}=10.24$, $p<0.001$). No main effects of Day or Day by Treatment interactions emerged from these analyses. Separate drug-placebo comparisons for women revealed that paroxetine significantly reduced women's sleep quality compared to placebo ($F_{1,8}=15.44$, $p<0.01$). Sertraline did not significantly affect women's sleep quality. No main effect of Day or Treatment by Day interaction was found for paroxetine or sertraline. A comparison between paroxetine and sertraline revealed a main effect of treatment ($F_{1,8}=7.89$, $p<0.05$) but no main effect of Day or a Treatment by Day interaction.

Table 2: Mean (standard deviation) number of false alarms of Mackworth Clock Test, and outcome variables of the Stroop Color Word Test and Dichotic Listening task on day 0 (baseline) and after 7 and 14 days of treatment with placebo, paroxetine or sertraline.

MEASURE	Treatment	day 0	day 7	day 14
Mackworth Clock Test				
# false alarms	placebo	2.05 (3.42)	1.00 (1.45)	0.70 (1.13)
	paroxetine	1.40 (1.90)	0.70 (1.03)	0.75 (1.25)
	sertraline	1.00 (2.05)	0.85 (1.39)	0.70 (0.87)
Stroop Color Word Test				
time card I (sec)	placebo	35.6 (4.5)	35.5 (6.0)	36.2 (6.0)
	sertraline	35.4 (4.4)	35.9 (5.2)	36.9 (8.8)
	paroxetine	35.7 (4.9)	35.2 (5.1)	36.0 (7.7)
time card II (sec)	placebo	42.6 (8.7)	41.8 (7.9)	41.1 (7.2)
	sertraline	42.1 (7.5)	41.8 (7.0)	41.7 (9.8)
	paroxetine	42.6 (9.0)	41.3 (7.9)	42.0 (9.7)
time card III (sec)	placebo	61.9 (13.4)	61.2 (13.0)	60.2 (13.8)
	sertraline	64.3 (14.7)	60.4 (12.4)	59.6 (12.6)
	paroxetine	62.2 (15.8)	60.6 (16.2)	60.1 (15.5)
interference (%)	placebo	57.8 (15.7)	58.2 (17.3)	55.2 (20.9)
	sertraline	64.9 (21.7)	55.3 (18.8)	52.2 (14.8)
	paroxetine	57.8 (17.3)	57.4 (20.7)	53.8 (17.6)
Dichotic Listening Task				
left side sensitivity (%)	placebo	89.4 (6.2)	90.4 (7.1)	90.5 (6.1)
	sertraline	86.4 (9.1)	90.9 (6.2)	91.3 (6.1)
	paroxetine	89.5 (10.2)	90.1 (4.5)	91.1 (4.8)
right side sensitivity (%)	placebo	91.2 (6.2)	92.7 (5.8)	92.8 (7.5)
	sertraline	91.7 (8.3)	92.2 (7.6)	91.7 (9.4)
	paroxetine	91.7 (8.1)	93.5 (5.4)	92.7 (5.4)
both sides sensitivity (%)	placebo	84.3 (2.9)	84.6 (2.8)	85.4 (3.0)
	sertraline	84.0 (2.2)	85.0 (3.3)	85.3 (3.3)
	paroxetine	85.5 (2.1)	84.9 (2.9)	85.0 (2.7)

Figure 1 : Mean (standard error) number of correct detections (left) and reaction times for the correct detections (right) in the Mackworth Clock Test, at baseline (day 0) and after 7 and 14 days of treatment with paroxetine, sertraline or placebo. * $p < 0.05$ vs placebo

Figure 2 : Mean (standard error) subjective sleep quality at baseline (day 0) and after 7 and 14 days of treatment with paroxetine, sertraline or placebo, broken down by sex. * $p < 0.01$ vs placebo

Plasma drug levels

Mean (standard deviation) plasma paroxetine concentrations were 36.7 (24.7) ng/ml on day 7 and 70.4 (54.2) ng/ml on day 14. Sertraline concentrations were 17.8 (7.9) ng/ml on day 7 and 41.6 (23.0) ng/ml on day 14. No detectable drug concentrations were present on day 0.

Discussion

The results of the present study show that, in accordance with our hypothesis, vigilance performance is adversely affected by subchronic administration of the SSRI paroxetine. Administration of paroxetine reduced the number of correct detections in the Mackworth Clock Test by approximately 11% compared to pre-treatment level of performance. In addition, the speed of the remaining correct detections appeared to be decreased with paroxetine, although this effect just failed to reach a significant level. A predicted, sertraline had no significant effect on the number of correct detections, although the data suggest that a small, non-significant reduction of the number of correct detections occurred with sertraline as well. However, in contrast to paroxetine, sertraline treatment was associated with a trend to increase response speed. Neither SSRI affected selective or divided attention.

A review of the studies conducted in our laboratory show that impaired vigilance performance following sub-chronic antidepressant administration in healthy volunteers is consistently found (table 3). A distinction must be made, however, between antidepressants that target the 5-HT system specifically (SSRIs) and those with significant affinities for other neurotransmitter systems. Impaired vigilance performance following mianserin (O'Hanlon et al. 1998) and dothiepin (Ramaekers et al. 1995) is most probably due to their antihistaminergic respectively anticholinergic actions, causing general sedation (Riedel et al. 1998). Consequently, these drugs diminish performance in a wide array of cognitive functions (Fairweather et al. 1997; O'Hanlon et al. 1998; Weinstein et al. 1996). The cognitive effects of SSRIs, on the other hand, appear to be limited to a rather specific impairment of vigilance performance. In the current study no effects of paroxetine or sertraline were seen on divided and selective attention. Similarly, venlafaxine reduced vigilance performance without affecting psychomotor functioning (O'Hanlon et al. 1998). However, despite this apparent specificity for impairing vigilance performance, it is possible that 5-HT stimulation may in fact induce general sedation through *indirect* inhibition of cholinergic, histaminergic, DA and/or NA activity. The net effect would be similar to that of *direct* blockade of these neurotransmitter systems by,

Table 3 : Attentional effects of antidepressant drugs and their net effects on neurotransmission related to attentional functions

Substance	Drug Class	Attention			Neurotransmission					Reference
		Sustained	Divided	Selective	5-HT	NA	DA	Ach	HA	
Sertraline	SSRI	0	0	0	+	0	+	0	0	This study
Paroxetine	SSRI	-	0	0	+	0	0	-	0	This study
Fluoxetine	SSRI	-	0	x	+	0	0	0	0	Ramaekers et al, 1995
Venlafaxine	SSRI	-	0	x	+	+	0	0	0	O'Hanlon et al., 1998
Dothiepin	TCA	-	x	x	+	+	0	-	-	Ramaekers et al, 1995
Mianserin 2 nd generation		-	-	x	+	+	0	0	-	O'Hanlon et al, 1998

SSRI: selective serotonergic reuptake inhibitor; TCA: tricyclic antidepressant; +: positive effect; -: negative effect; 0: no effect; x: not measured; 5-HT: serotonin; NA: noradrenaline; DA: dopamine; Ach: acetylcholine; HA: histamine

for example, tricyclic antidepressants, namely general sedation, although these effects may be less pronounced. Frequently, drowsiness is reported subjectively, indicating a potential risk of mild sedation following SSRI administration (Hawley et al. 1997; Hindmarch and Bhatti 1988; Mattila et al. 1988; Robbe and O'Hanlon 1995; Saletu and Grünberger 1988). Mild sedation was also apparent in Critical Flicker Fusion assessments following sertraline and citalopram administration, when control was made for changes in pupil size (Schmitt et al., chapter 6). Certainly, mild de-arousing properties of 5-HT stimulation are most likely to become evident in a 45-minute monotonous, low event vigilance task, in which overall performance is known to show a monotonic relation with an individual's energetic state or arousal. (Warm 1984) (Parasuraman et al. 1998). After reviewing the data on stimulant drugs, Koelega (1993) concluded that 'vigilance tasks appear to be the most sensitive tasks in test batteries'. Performance on other, short-term, high-demanding cognitive tests may be expected remain relatively intact following mild sedation, due to compensatory effort-related mechanisms (Sanders 1998). Subjective self-ratings have been shown to be more sensitive to drug effects than attentional task measures, and this difference may reflect the ability of subjects to allocate greater or lesser amounts of effort to the task depending on drug condition or state (Kahneman 1973; Oken et al. 1995). Thus, the apparent specificity of the 5-HT effect on vigilance performance may merely be the result of modest sedation combined with high sensitivity of the vigilance task.

In addition to the possibility of general sedative actions of 5-HT stimulation, daytime arousal (and consequently vigilance performance) may also be *indirectly*

affected changes in sleep quality (Babkoff et al. 1991), an effect often seen with SSRI treatment (Lindsay DeVane 1998). In the present study paroxetine was found to reduce sleep quality in women, but not in men. Women reported almost twice the number of complaints than men during paroxetine treatment. Sertraline did not significantly modify sleep quality in men or women. However, the absence of significant effects for men and women separately may be due to insufficient statistical power because of small group sizes. A significant reduction in sleep quality was in fact found for sertraline when data for men and women were combined. Diminished sleep quality may therefore have influenced daytime vigilance performance. It must be noted, however, that men and women showed similar vigilance impairments with paroxetine, whereas only women's sleep quality was reduced. Also, despite a similar reduction of sleep quality with sertraline no significant effects could be detected on vigilance performance. We therefore feel that it is unlikely that the observed vigilance effects can be entirely attributed to reduced sleep quality.

The data appear to support the hypothesis of a specific role for DA in mediating the 5-HT related vigilance decrement. More specifically, additional DA stimulation by sertraline appeared to be effective in attenuating the 5-HT induced vigilance impairment, the latter effect being apparent during paroxetine treatment. It could, however, be argued that differences in vigilance effects between sertraline and paroxetine may arise from differences in serotonergic potencies between the two drugs. While sertraline and paroxetine were shown to possess similar *in vitro* potencies for 5-HT reuptake (Hyttel 1994), different pharmacokinetic properties may significantly alter *in vivo* potency. *In vivo* activity can be inferred from other factors that are presumably related to 5-HT activity. The investigated dosages of 50 and 100 mg sertraline versus 20 and 40 mg paroxetine appear to be comparable in terms of efficacy in major depression (Goodnick and Goldstein 1998). Furthermore, sertraline and paroxetine were found to cause nearly identical increases in pupil size (Schmitt et al, chapter 5), and these changes are thought to be the result of enhanced 5-HT stimulation (Barnett and Osborne 1993; Prow et al. 1996). We therefore assume that, at the dosages used, serotonergic activity of both drugs is quite comparable. It is also worth mentioning that paroxetine has a relatively high affinity for muscarinic acetylcholine receptors compared to other SSRIs. Paroxetine's *in vitro* affinity for muscarinic receptors is approximately six times higher than that of sertraline (Hyttel 1994). Theoretically, paroxetine's direct anticholinergic property may have contributed to a reduction of vigilance performance. On the other hand, vigilance impairment was previously found with fluoxetine and venlafaxine, drugs without significant anticholinergic effects. Nevertheless, a more specific serotonergic

drug, for example citalopram (Stahl 1998b), would provide a better reference drug in future studies on 5-HT and vigilance performance.

We propose that SSRI-induced impairment of vigilance performance is primarily mediated by neural circuits modulated by opposing influences of 5-HT and DA. There is now abundant anatomical and experimental evidence indicating an inhibitory influence of the 5-HT system on DA activity. In short, 5-HT projections arising from the dorsal nucleus raphe exert a tonic inhibitory influence over the mesocortical DA system, involved with cognitive functioning, as well as the nigrostriatal DA system, involved in the modulation of motor behaviour (Kapur and Remington 1996). Likewise, the mesolimbic DA system, involved in motivation and reward, is under inhibitory influence of 5-HT neurons from the median nucleus raphe, projecting to the ventral tegmental area (Bonhomme and Esposito 1998). The inhibitory effects are mediated mainly via 5-HT_{2B/2C} inhibitory receptors on DA cellbodies in the ventral tegmental area and substantia nigra, and at terminal endings in striatum and cortex.

Thus, augmentation of 5-HT activity by SSRI administration can be expected to further inhibit DA transmission, thus inducing a 'pseudo-dopamine deficiency' (Stahl 1998a). Such a 5-HT mediated hypo-dopaminergic state has been proposed to underlie several of the side-effects of SSRI treatment, i.e. reduced libido (Stahl 1998a) and extrapyramidal symptoms, such as akathisia, tremor and dystonic reactions (Caley 1997; Kapur and Remington 1996; Stahl 1998a). However, in the majority of the individuals the degree of DA inhibition does not cross the threshold for severe adverse effects such as extrapyramidal symptoms. Nevertheless, a reduction in DA function may become evident from more subtle behavioural changes such as a reduced vigilance performance. Furthermore, it was found that the a single dose of fluoxetine or citalopram caused a dose dependent inhibition of firing rate of ventral tegmental area DA cells, but not of DA cells in the substantia nigra (Bonhomme and Esposito 1998), suggesting that the cognitive and motivational functions of DA are particularly susceptible to the inhibitory effects of 5-HT stimulation.

Diminished DA activity may be linked to vigilance impairment via several pathways. First, as was mentioned in the introduction, DA activity is implicated in tonic, internally-driven arousal, which is particularly important during a long, monotonous vigilance tasks. Second, DA may modulate vigilance performances via its involvement in incentive motivational processes (Levy and Hobbes 1996). During a vigilance test energetic levels inevitably fall below the level required to maintain optimal task performance (O'Hanlon 1981). At this point additional effort is required to compensate for insufficient energetic levels. Motivational state is thought to be the primary modulator

of compensatory effort (Sanders 1998), and motivational mechanism are specifically linked to the mesolimbic DA system, arising from the ventral tegmental area (Kapur and Remington 1996; Robbins and Everitt 1996). Interestingly, in rats, short-term administration of several SSRIs, including fluoxetine, sertraline and paroxetine, was found to cause a slight but significant decrease in the basal firing rate of DA neurons in the ventral tegmental area (Bonhomme and Esposito 1998; Di Mascio et al. 1998). This implies a reduction of mesolimbic DA activity by 5-HT stimulation and provides a neural basis for the role of motivation, and indirectly effort, in SSRI-induced vigilance impairment. Inadequate levels of compensatory effort are proposed to underlie the performance decrements over time in vigilance tasks (Sanders 1998). Thus, diminished motivation (leading to insufficient compensatory effort) would be expected to produce an exacerbation of the normal performance decrement over time. Our data do show treatment related differences on time-on-task effects, but it must be noted that our time-on-task analyses are based on 15-minute blocks. Because performance decrements are most pronounced during the first 15 minutes of a vigilance test (Warm 1984), time-on-task differences between treatments may have occurred mainly within the first 15-minute period. Indeed, our time-on-task data show that major differences were already present after 15 minutes.

DA, particularly the nigrostriatal system, is also involved in motor preparation and execution (Robbins 1997). The tendency for paroxetine to increase reaction times and for sertraline to decrease reaction times certainly is in line with DA involvement via this system. On the other hand, changes in reaction time can originate from increased or decreased efficiency of any of the various stages in the information processing chain (Sanders 1998). Thus, mesocortical DA projections to prefrontal cortex and anterior cingulate (Robbins 1997), the latter being implicated in response selection (Posner and Raichle 1994), may be involved as well. DA manipulation has been suggested to mainly affect response readiness (Iversen and Iversen 1975; Nicholson and Pascoe 1990; Ramaekers et al. 1999), which appears to be primarily associated with response selection and motor activation. Interestingly, according to the cognitive-energetics model by Sanders (1998), target detection and motor preparation are modulated by effort respectively activation (tonic arousal), hereby providing a link between DA mediated global processes and DA's putative influence on specific information processing stages. The relative contribution of each of the proposed mechanisms to the vigilance enhancing effects of DA stimulation is unknown. As is often the case, it is most likely that the behavioural output is the resultant of a combined set of neural mechanisms.

In conclusion, the results of the present study confirm the notion that 5-HT stimulation produces a specific reduction of vigilance performance. This effect has now been demonstrated in three separate studies with three different 5-HT stimulants, namely venlafaxine, fluoxetine and now paroxetine. Moreover, it was shown that 5-HT mediated vigilance reduction can be effectively attenuated by simultaneous stimulation of DA neurotransmission, as was the case with sertraline. We propose that the 5-HT induced vigilance impairment is primarily due to serotonergic inhibition of DA activity. Associated deficits in DA-mediated energetic processes, reduced motivation and possibly reduced target detection and motor activation are speculated to underlie diminished vigilance performance. However, it is clear that the putative role of each of these mechanisms needs to be established in future studies. Also, the relative contribution of 5-HT interactions with other neurotransmitter systems, e.g. acetylcholine, needs to be investigated. Furthermore, assessments of the vigilance effects of non-serotonergic antidepressants, such as reboxetine and bupropion which target NA and DA systems (Lindsay Devane, 1998) are needed to verify the specific role of 5-HT in reducing vigilance.

Acknowledgements

This study was supported by an unrestricted grant from Pfizer Inc. New York. We wish to thank Suzanne Theunisse for her assistance during data collection

References

- Amado-Boccaro I, Gougoulis N, Poirier Litte MF, Galinowski A, Loo H (1995) Effects of Antidepressants on Cognitive Functions: A Review. *Neuroscience and Biobehavioral Reviews* 19: 479-493
- Babkoff H, Caspy T, Mikulincer M (1991) Subjective sleep ratings: the effects of sleep deprivation, circadian rhythmicity and cognitive performance. *Sleep* 14: 534-539
- Barnett NL, Osborne NN (1993) The effect of serotonin on the rabbit isolated iris sphincter muscle. *Curr. Eye Res.* 12: 655-73
- Bolden-Watson C, Richelson E (1993) Blockade by newly-developed antidepressants of biogenic amine uptake into rat brain synaptosomes. *Life Sci* 52: 1023-9
- Bonhomme N, Esposito E (1998) Involvement of serotonin and dopamine in the mechanism of action of novel antidepressant drugs: a review. *J Clin Psychopharmacol* 18: 447-54
- Caley CF (1997) Extrapyramidal reactions and the selective serotonin reuptake inhibitors. *Ann Pharmacother* 31: 1481-1489
- Deijen JB, Loriaux SM, Oriebeke JF, De Vries J (1989) Effects of paroxetine and maprotiline on mood, perceptual-motor skills and eye movements in healthy volunteers. *Journal of Psychopharmacology* 3: 148-155

- Delagrangé P, Canu MH, Rougeul A, Buser P, Bouyer JJ (1993) Effects of locus coeruleus lesions on vigilance and attentive behaviour in cat. *Behav Brain Res* 53: 155-65
- Di Mascio M, Di Giovanni G, Di Matteo V, Prisco S, Esposito E (1998) Selective serotonin reuptake inhibitors reduce spontaneous activity of dopaminergic neurons in the ventral tegmental area. *Brain Research Bulletin* 46: 547-554
- Fairweather DB, DalPozzo C, Kerr JS, Lafferty SV, Hindmarch I (1997) Citalopram compared to dothiepin and placebo: Effects on cognitive function and psychomotor performance. *Human Psychopharmacology Clinical and Experimental* 12: 119-126
- Goodnick PJ, Goldstein BJ (1998) Selective serotonin reuptake inhibitors in affective disorders—II. Efficacy and quality of life. *J Psychopharmacol* 12: S21-54
- Gordon C, Whale R, Cowen PJ (1998) Sertraline treatment does not increase plasma prolactin levels in healthy subjects. *Psychopharmacology* 137: 201-202
- Hawley CJ, McPhee S, Smith VRH (1997) A review of the psychomotor effects of paroxetine. *International Clinical Psychopharmacology* 12: 13-18
- Hindmarch I, Bhatti JZ (1988) Psychopharmacological effects of sertraline in normal, healthy volunteers. *Eur J Clin Pharmacol* 35: 221-3
- Hyttel J (1994) Pharmacological characterization of selective serotonin reuptake inhibitors (SSRIs). *Int. Clin. Psychopharmacol.* 9: 19-26
- Iversen SD, Iversen LL (1975) Behavioural pharmacology. Oxford University Press, Oxford University Press
- Jacobs BL (1987) Brain monoaminergic activity in behaving animals. In: Epstein AN, Morrison AR (eds) *Progress in psychobiology and physiological psychology*. Academic Press, New York, pp 171-206
- Kahneman D (1973) *Attention and effort*. Prentice Hall, Prentice Hall
- Kapur S, Remington G (1996) Serotonin-dopamine interaction and its relevance to schizophrenia. *Am J Psychiatry* 153: 466-76
- Koelega HS (1993) Stimulant drugs and vigilance performance: A review. *Psychopharmacology* 111: 1-16
- Levy F, Hobbes G (1996) Does haloperidol block methylphenidate? Motivation or attention? *Psychopharmacology* 126: 70-74
- Lindsay DeVane C (1998) Differential pharmacology of newer antidepressants. *Journal of Clinical Psychiatry* 59: 85-93
- Mackworth NH (1961) Researches on the measurement of human performance. In: Sinaiko HW (ed) *Selected papers on human factors in the design and use of control systems*. Dover, New York, pp 174-331
- Mattila MJ, Saarialho-Kere U, Mattila M (1988) Acute effects of sertraline, amitriptyline, and placebo on the psychomotor performance of healthy subjects over 50 years of age. *J Clin Psychiatry* 49: 52-58
- McGaughy J, Sandstrom M, Ruland S, Bruno JP, Sarter M (1997) Lack of effects of lesions of the dorsal noradrenergic bundle on behavioral vigilance. *Behav Neurosci* 111: 646-52
- McGuinness D, Pribram K (1980) The neuropsychology of attention. In: Witrock MC (ed) *The brain and psychology*. Academic Press, New York
- Mulder Hajonides van der Meulen WREH (1981) Measurement of subjective sleep quality International European Sleep Conference, Amsterdam The Netherlands

- Nicholson AN, Pascoe PA (1990) Dopaminergic transmission and the sleep-wakefulness continuum in man. *Neuropharmacology* 29: 411-7
- O'Hanlon J, Robbe HWJ, Vermeeren A, VanLeeuwen C, Danjou PE (1998) Venlafaxine's effects on healthy volunteers' driving, psychomotor, and vigilance performance during 15-day fixed and incremental dosing regimens. *Journal of Clinical Psychopharmacology* 18: 212-221
- O'Hanlon JF (1981) Boredom: practical consequences and a theory. *Acta Psychologica* 49: 53-82
- Oken BS, Kishiyama SS, Salinsky MC (1995) Pharmacologically induced changes in arousal: effects on behavioral and electrophysiologic measures of alertness and attention. *Electroencephalogr Clin Neurophysiol* 95: 359-71
- Parasuraman R, Warm JS, See JE (1998) Brain systems of vigilance. In: Parasuraman R (ed) *The attentive brain*. MIT press, Cambridge (MA), pp 221-256
- Posner MI, Raichle ME (1994) *Images of mind*. Scientific American Library, Scientific American Library
- Prow MR, Martin KF, Heal DJ (1996) 8-OH-DPAT-induced mydriasis in mice: a pharmacological characterisation. *Eur. J. Pharmacol.* 317: 21-8
- Ramaekers JG, Louwerens JW, Muntjewerff ND, Milius H, de Bie A, Rosenzweig P, Patat A, O'Hanlon JF (1999) Psychomotor, Cognitive, extrapyramidal, and affective functions of healthy volunteers during treatment with an atypical (amisulpride) and a classic (haloperidol) antipsychotic. *J Clin Psychopharmacol* 19: 209-21
- Ramaekers JG, Muntjewerff ND, O'Hanlon JF (1995) A comparative study of acute and subchronic effects of dothiepin, fluoxetine and placebo on psychomotor and actual driving performance. *British Journal of Clinical Pharmacology* 39: 397-404
- Riedel WJ, Vermeeren A, Van Boxtel MPJ, Vuurman EFPM, Verhey FRJ, Jolles J, Ramaekers JG (1998) Mechanisms of Drug-Induced Driving Impairment: A Dimensional Approach. *Human Psychopharmacology* 13: S49-63
- Robbe HWJ, O'Hanlon JF (1995) Acute and subchronic effects of paroxetine 20 and 40 mg on actual driving, psychomotor performance and subjective assessments in healthy volunteers. *European Neuropsychopharmacology* 5: 35-42
- Robbins T, Everitt B (1995) Arousal Systems and Attention. In: Gazzaniga M (ed) *The Cognitive Neurosciences*. MIT Press, Cambridge MA (USA), pp 703-720
- Robbins T, Everitt B (1996) Neurobehavioural mechanisms of reward and motivation. *Current Opinion in Neurobiology* 6: 228-236
- Robbins TW (1984) Cortical noradrenaline, attention and arousal. *Psychol Med* 14: 13-14
- Robbins TW (1997) Arousal Systems and Attentional Processes. *Biological Psychology* 45: 57-71
- Saletu B, Grünberger J (1988) Drug Profiling by computed electroencephalography and brain maps, with special consideration of sertraline and its psychometric effects. *Journal of Clinical Psychiatry* 49: 59-71
- Sanders AF (1998) Energetics, Stress and Sustained Attention. In: Sanders AF (ed) *Elements of Human Performance*. Lawrence Erlbaum Associates, New Jersey, pp 394-451
- Schmitt JA, Jorissen BL, Sobczak S, van Boxtel MP, Hogervorst E, Deutz NE, Riedel WJ (2000) Tryptophan depletion impairs memory consolidation but improves focussed attention in healthy young volunteers. *Journal of Psychopharmacology* 14: 21-29
- Stahl SM (1998a) Basic psychopharmacology of antidepressants, part 1: Antidepressants have seven distinct mechanisms of action. *J Clin Psychiatry* 59: 5-14

- Stahl SM (1998b) Not so selective serotonin reuptake inhibitors. *J Clin Psychiatry* 59: 343-4
- Stroop JR (1935) Studies of interference in serial verbal reactions. *Journal of Experimental Psychology* 18: 643-662
- Tatsumi M, Groshan K, Blakely RD, Richelson E (1997) Pharmacological profile of antidepressants and related compounds at human monoamine transporters. *Eur J Pharmacol* 340: 249-58
- Warm JS (1984) An introduction to vigilance. In: Warm JS (ed) *Sustained Attention in Human Performance*. John Wiley & Sons, pp 1-13
- Weinstein A, Wilson S, Bailey J, Nutt D (1996) Sedative Antidepressants Impair Visual Detection Mechanisms In Humans. *Journal Of Psychopharmacology* 10: 141-145
- Zung WWK (1965) A self rating depression scale. *Archives of General Psychiatry* 12: 63-70

***Paroxetine's and sertraline's effects on
Critical Flicker Fusion and the influence of
concomitant changes in pupil size***

Abstract

The antidepressants paroxetine and sertraline have been shown to elevate Critical Flicker Fusion (CFF) thresholds. However, this may not necessarily reflect an activating effect of the drugs, as CFF may also rise due to drug-induced increases in pupil diameter (mydriasis). We investigated the effects of paroxetine and sertraline on CFF and pupil size, and their possible interaction, in a double blind, crossover study involving 24 healthy, middle-aged volunteers. Paroxetine and sertraline were respectively administered for 14 days in doses of 20 mg and 50 mg for the first seven days, and in doses of 40mg and 100mg for the remaining days. Pupillary diameter and CFF were assessed with and without the use of an 2-mm artificial pupil (to control for pupillary changes) on baseline and on days 7 and 14 of each treatment period. Sertraline and paroxetine induced significant mydriasis. In case of paroxetine the mydriatic effect was also dose related. Paroxetine and sertraline did not affect CFF threshold, either with or without artificial pupil. Overall, mean CFF decreased by 1.5 Hz lower when an artificial pupil was used. It is concluded that the effects of both paroxetine and sertraline on CFF are neutral. In our CFF device, there was no evidence that SSRI-induced mydriasis affected CFF threshold. However, it is argued that the influence of mydriasis may depend on the CFF apparatus used, and cannot be entirely dismissed as modulator of SSRI-induced CFF changes.

Introduction

Intermittent light is perceived as flicker, but as the frequency increases, the flicker sensation becomes gradually less distinct until successive flicker sensations fuse. This point of fusion, where a flickering light gives the subjective sensation of a steady light, is known as the Critical Flicker Fusion (CFF) threshold. Test devices for measuring CFF have been frequently used in human psychopharmacology for establishing the effects of a wide range of psychotropic drugs on the functioning of the central nervous system (Smith and Misiak 1976; Curran 1990). In general, sedative drugs have been found to decrease CFF in healthy volunteers and patients, whereas stimulants significantly increase CFF. This observation underlies the now commonly held belief that drug induced changes in CFF, reflect a drug's activating or sedating effect on overall CNS arousal and information processing capacity.

Antidepressants are one class of drugs that have been extensively investigated using CFF methodology. Sedative agents such as the tricyclic antidepressants (TCAs) were repeatedly found to decrease CFF in a dose related fashion (Holmberg 1981; Amado Boccarda et al. 1994). TCAs are antagonists of cholinergic, histaminergic and adrenergic receptors, which may cause cognitive impairment and sedation. Selective serotonin reuptake inhibitors (SSRI) are a different class of antidepressants that have no or little affinity for these receptors. Consequently, SSRIs are generally regarded as behaviourally safe drugs that do not adversely affect cognitive function. In fact, the SSRI paroxetine (20-30 mg) was found to increase CFF performance in healthy young volunteers (Kerr et al. 1992; Warrington et al. 1989), healthy elderly (Hindmarch 1992) and also in depressive outpatients (Hindmarch 1992). Similarly, an immediate and dose-related increase in CFF was found following treatment with the SSRI sertraline (50, 75 and 100mg) in healthy young subjects (Hindmarch and Bhatti 1988). Elevation of CFF after sertraline have been reported in healthy elderly in one study (Mattila et al. 1988) but not in another (Hindmarch et al. 1990). Saletu and Grünberger (1988) reported increased CFF scores after 100 to 400 mg sertraline in healthy volunteers, although the effect was stronger for the lower doses. On the basis of these results, it was concluded that paroxetine and sertraline exert an "excitatory" rather than a sedative effect (Hindmarch 1995).

CFF threshold, however, can be influenced by a large number of variables. These variables include light intensity of the stimulus, luminance of the surrounding area, stimulus colour, dark adaptation, circadian rhythm, age, and retinal location, method of assessment and even eye colour (Curran 1990; Curran and Wattis 1998) Many of these

variables can be easily controlled by choosing the appropriate test apparatus and study design. In psychopharmacological research, some factors are of particular importance. CFF has been shown to be sensitive to changes in pupillary diameter since these result in changes in retinal illumination (Davson, 1976). Many psychotropic drugs alter pupillary diameter and cause either pupillary miosis or mydriasis. It is therefore imperative for any investigator in CFF as a measure of CNS functioning to control for variations in pupillary diameter. One of the simplest means of assuring control over this factor is through the use of an artificial pupil. That apparatus is constructed so that subjects view the flickering light through an aperture (2mm) which is close to smallest pupillary diameter possible. In most of the studies above, no control for pupil size was used when measuring the effect of SSRIs on CFF threshold. Yet, it has been repeatedly demonstrated that several SSRIs, e.g. paroxetine, fluoxetine and sertraline, may cause about 2 mm mydriasis in subjects receiving acute or subchronic treatment (Saletu and Grünberger 1988; Raptopoulos and McClelland, 1989; Deijen et al. 1989; McGuirk and Silverstone 1990). The possibility therefore exists that many of the reported elevations in CFF following SSRIs resulted from drug induced changes in pupil size rather than from drug induced changes in arousal. It is noteworthy in this respect that investigators who did control pupillary diameter when measuring the effect of SSRIs on CFF, failed to measure a threshold elevation (Mattila et al. 1988; Ramaekers et al. 1995).

The current study was designed to determine the effect of two SSRIs, i.e. paroxetine and sertraline, and placebo on CFF when measured with and without an artificial pupil. We hypothesised that the CFF elevations following SSRI administration are mediated by increases in pupil size. Thus, it was predicted that CFF threshold would change as a function of pupil size, and that the latter would increase after administration of paroxetine and sertraline.

Material and methods

Subjects

Twenty-four healthy volunteers, 13 males and 11 females, aged 30-50 years, were recruited through newspaper advertisements. Initial screening was done by means of a health questionnaire and a telephone interview. Qualified volunteers subsequently underwent a medical examination, which included routine ECG, blood haematology and chemistry, standard urine screening, a urine test for drug abuse and, for women, a urine pregnancy test. Exclusion criteria were the presence or a history of, cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, haematological or psychiatric illness,

symptoms severity associated with a score < 50 on the Zung Depression Scale (Zung 1965), excessive drinking (> 20 units of alcoholic a week) pregnancy or lactation, use of medication other than oral contraceptives, use of drugs, and any sensory or motor deficits which could reasonably be expected to affect test performance. The study was approved by the standing Medical Ethics Committee of Maastricht University, and carried out in accordance with the Declaration of Helsinki (Hong Kong Modification, 1989). All subjects gave a written informed consent prior to participation.

Design and treatments

The study was conducted according to a randomised, double blind, placebo-controlled, 3-period crossover design. Treatment periods lasted 14 days and were separated by washout periods of 14 days. Treatment order was balanced over the three periods. Subjects received the following treatment regimens during respective treatment periods: paroxetine 20–40 mg, sertraline 50–100 mg and placebo. Subjects received 20 mg of paroxetine and 50 mg of sertraline during the first 7 days of treatment, and respectively 40 and 100 mg during subsequent days. Drugs and placebo were always taken at fixed times in the morning.

Testing procedure

CFF tests were conducted on days 0 (baseline), 7 and 14 of each treatment period. Prior to treatments all subjects were individually trained to conduct the CFF tests. Subjects were not allowed to drink alcoholic beverages during each treatment period and were instructed to arrive at the laboratory well rested. Female subjects were tested for pregnancy before the start of each treatment period. On test days subjects arrived between 12.00 and 18.00 p.m. CFF was assessed in two successive repetitions of the test. During the first session the CFF was measured without a correction for pupil size. During the second session, the subjects' CFF was measured while viewing through an artificial pupil. Pupil size was measured following the second session. Together, CFF and pupil size measurements took approximately 20 minutes.

CFF threshold was measured in a computer-controlled system using a combination of the psychophysical Methods of Limits and Successive Approximations. The subject was seated looking into a visual tunnel that displayed a red light source in Maxwellian perspective. In trials with pupillary control, the visual tunnel covered was an 'artificial pupil' that permitted subjects to view the CFF stimulus through an aperture of 2 mm. To begin, the computer alternatively increased and decreased the source flicker frequency (1:1 light/dark ratio) and the subject responded by pressing separate buttons

whenever his perception changed from one state to the other. After three complete cycles, the approximate location of the subject's CFF was defined according to the Method of Limits. At that point, the program identified two frequencies in 1 Hz steps above, two below and one at the suspected threshold. Each of the five stimuli was shown six times in separate, randomised presentations lasting 3 seconds each. The subject was instructed to withhold responding during the presentation period, and then give one of two responses indicating the perception of flicker or fusion. The proportions of each type of response were used to calculate intersecting linear functions in the frequency domain.

Pupil diameter was recorded using a head-mounted eye tracking system (4000SU eye tracker, Applied Science Laboratories). The beam from an infrared light source illuminates the eye and an optical system focuses an image of the pupil onto a solid state eye camera. Both the illumination beam and the image of the eye are reflected from a helmet visor, which is coated to be reflective in the near infrared region and transitive to visible light. The illuminator, optics and camera are all helmet mounted. Pupil and corneal reflection outlines and centroids are displayed on a pupil monitor over the video image of the eye. Pupillary diameter was recorded for approximately 10 seconds at a rate of 50 measurements per second. Median pupil diameter is taken as the dependent variable.

Statistical analysis

Dependent variables were analysed using a repeated measures, multivariate analysis of variance (MANOVA) adjusted for initial differences at baseline (covariate). Within subjects factors were *Treatments*, *Days* and *Pupillary Control*. In addition, CFF was tested for the overall effect of *Pupillary Control* without covariate adjustment, but with baseline scores added as a third level to the factor *Days*. Univariate, repeated measures analysis for comparing data obtained after sertraline and paroxetine separately with placebo were carried out if the initial analysis revealed an overall effect of *Treatments*.

Results

Missing data

Three subjects withdrew from the study for reasons unrelated to study drugs. Data sets of four subjects were incomplete due to technical malfunctions during time of testing. Only complete data sets (N=17) were used for statistical analysis.

Pupil diameter

Mean (SE), uncorrected pupil size in each treatment condition and day of treatment is shown in Figure 1. MANOVA revealed significant overall effects of Treatments ($F_{2,31}=40.15$, $p<0.001$) and Treatments by Days ($F_{2,15}=3.81$, $p=0.046$). Separate drug-placebo comparisons showed that both paroxetine and sertraline significantly increased pupil size as compared to placebo ($F_{1,15}=55.58$ & 33.05 , $p<0.001$). Paroxetine affected pupil size in a dose related manner, as indicated by a significant Treatments x Days interaction ($F_{1,16}=6.72$, $p=0.02$). Pupil size after sertraline appeared similarly dose related, but in this case the Treatments x Days interaction just failed to reach statistical significance ($F_{1,16}=3.39$, $p=.084$).

Critical Flicker Fusion Threshold

Mean (SE) CFF threshold in each treatment period and day of treatment, with and without pupillary control is shown in Table 1. Overall analysis using baseline values as covariate revealed no significant main effects of Treatments, Days or Pupillary Control, nor were there significant interactions between these factors. With baseline scores included as additional level of the factor Days, the overall effect of Pupillary Control ($F_{1,16}=36.65$, $p<0.001$) on CFF reached statistical significance. The former reflects the general finding that CFF values in trials with an artificial pupil were lower as compared to those in trials without (Figure 2).

Table 1 : Mean (standard error) CFF scores with and without pupillary control, at baseline (day 0) and at days 7 and 14 of treatment with placebo, sertraline or paroxetine.

CFF scores – without artificial pupil

Treatment	Day 0	Day 7	Day 14
Placebo	40.5 (0.9)	40.2 (0.7)	39.3 (0.8)
Sertraline	40.8 (0.7)	39.4 (0.8)	40.5 (0.8)
Paroxetine	39.7 (0.8)	39.7 (0.9)	40.2 (0.6)

CFF scores – with artificial pupil

Treatment	Day 0	Day 7	Day 14
Placebo	38.6 (0.7)	38.6 (0.8)	38.5 (0.9)
Sertraline	38.1 (1.0)	38.1 (0.7)	38.4 (0.9)
Paroxetine	39.8 (0.8)	37.4 (0.9)	38.4 (0.6)

Figure 1 : Mean, uncorrected pupillary diameter (SE) at baseline, and after 7 and 14 days of treatment with paroxetine, sertraline and placebo. * $p < 0.001$

Figure 2 : Mean (SE) CFF threshold as assessed with and without pupillary control and averaged across baselines and days 7 and 14 of treatments. * $p < 0.001$

Discussion

Both paroxetine and sertraline significantly increased pupil size. In the case of paroxetine, the effect on pupil size was dose related. Mydriasis was apparent in the first week of treatment with 20mg, and more so after treatment with the double dose in the second week. A similar dose related increase was observed for sertraline, but that just failed to reach statistical significance. The degrees of mydriasis following paroxetine and sertraline were quite comparable. Relative to baseline, both treatments produced about 1.3 and 1.5 mm increase in pupil size in the first and second week respectively. Together, these observations largely confirm results from earlier studies that demonstrated the mydriatic effect of SSRIs (see Introduction).

The role of a serotonergic mechanism in the control of pupillary diameter has also been suggested by the actions of serotonergic drugs other than SSRIs. It has been reported that 5HT₂ receptor antagonists (Millson et al. 1991; 1992) and 5HT₃ receptor antagonists (Rizzi et al. 1993) cause miosis. In contrast, fenfluramine, m-chlorophenylpiperazine (m-CPP) and 5-HT_{1A} receptor agonists were shown to increase pupil size (Kramer et al 1973; Prow et al. 1996; Benjamin et al. 1997; Broocks et al. 1998). The mydriatic response is probably mediated by enhanced serotonergic stimulation of 5HT_{1A} receptors located on ciliary muscles that regulate pupillary diameter (Barnett and Osborne 1993), and of central 5HT_{1A} receptors evoking the release of noradrenaline on alpha2-adrenoreceptors which produces mydriasis (Prow et al. 1996).

Mean CFF threshold was unaffected by sertraline or paroxetine treatment, regardless of the presence or absence of pupillary control. These results are in sharp contrast with those from studies that demonstrated marked elevations of CFF thresholds following paroxetine (Warrington et al, 1989; Kerr et al., 1992; Hindmarch, 1988) and sertraline (Hindmarch, 1988, Mattilla et al., 1988; Saletu and Grunberger, 1988) in healthy volunteers. Consequently, our data do not support the view that paroxetine and sertraline exert an 'activating' or 'excitatory' effect, as was concluded based on these CFF elevations (Hindmarch, 1995). However, our hypothesis that the previously observed CFF elevations following SSRI administration are secondary to drug-induced mydriasis, can not be confirmed based on the present results either. Particularly the finding that CFF without pupillary control remained unchanged following sertraline and paroxetine, despite a substantial increase ($\pm$ 35%) in pupil size, suggests that CFF thresholds following SSRI treatment are not significantly influenced by mydriatic effects of the drugs. Nevertheless, for reasons which will be discussed below, we feel that such a mechanism can not be entirely dismissed.

The effect of pupillary diameter on CFF threshold is well known. As is stated by the Ferry-Porter law (Davson, 1976), CFF is directly proportional to the logarithm of the light intensity. As a consequence, CFF thresholds will rise following mydriasis and fall following miosis. The effect has been demonstrated using pilocarpine 2% and homatropine 1% eyedrops to produce miosis and mydriasis, respectively (Lawrence et al., 1984). These investigators found that increases in pupil size from 2 to 8 mm produced a steady rise in CFF threshold, which varied between individuals but ranged from 2-9 Hz. In the current study, the CFF measurements gathered with the use of a 2-mm artificial pupil were on average 1.5 Hz lower than those obtained without pupillary control, suggesting that a reduction of pupil size indeed decreases CFF thresholds. Pupil size was held constant (2mm) in trials with artificial pupil, whereas mean values without pupillary control were as large as 5.7 mm. However, in principle, factors other than pupil size may be responsible for the differences in CFF thresholds between the two methods. Because CFF assessments without pupillary control always preceded those with pupillary control, fatigue may have depressed CFF thresholds during the latter measurements. However, the entire procedure lasted 20 minutes at most, making it unlikely that fatigue can fully explain the observed differences.

Given the theoretical and experimental evidence of the relationship between pupil size and CFF, it is rather puzzling that we did not find an elevation of CFF due to drug induced mydriasis. The fact that pupillary diameters and CFF were not measured simultaneously may provide a possible explanation. Pupillary diameter was measured *after* completion of the CFF tests. Pupil size during these CFF tests may not have been influenced by treatments only, but also by the light intensity of the CFF stimuli. The latter by itself might automatically invoke an adaptive miotic response of the pupil depending on its magnitude or brightness. In that case the actual pupil size during CFF tests would be the net result of a drug induced mydriatic effect and miosis caused by the viewing at CFF stimuli. It is therefore possible that present values of pupil size that were obtained after CFF tests are an overestimation of the actual size of the pupil during the tests.

It is important to notice that virtually all studies have used the Leeds Psychomotor Tester to assess CFF changes after SSRIs. The Leeds Psychomotor Tester differs from our apparatus in several aspects. Most importantly, the Leeds Tester yields CFF thresholds which are typically 10-15 Hz below those obtained with the current apparatus. This difference is probably due to a lower light intensity of the CFF stimuli generated by the Leeds Psychomotor Tester. If so, attenuation of SSRI induced mydriasis by an adaptive miotic response would be less pronounced using the Leeds Tester as compared to the current CFF apparatus. The Leeds Psychomotor Tester, was indeed

shown to be highly sensitive to changes in pupil size (Lawrence et al. 1984) and given the dosages, subjects and study design, there is no reason to assume that drug-induced mydriasis did not also occur in previous studies. Consequently, it can not be ruled out that drug-induced mydriasis underlay the previous reports of CFF elevations with sertraline and paroxetine using the Leeds Psychomotor Tester.

In conclusion, we did not find evidence for an activating effect of sertraline or paroxetine, nor did we find that drug-induced mydriasis affected CFF threshold in a significant way. However, we propose that the effects of SSRI induced mydriasis on CFF may be modulated by other factors, i.e. stimulus brightness, and may be dependent upon the type of CFF apparatus used. A future study investigating the CFF effects of SSRI administration in the Euclid device as well as the Leeds Psychomotor Tester, and examining the influence of adaptive miotic responses to light on SSRI-induced mydriasis, will be needed to clarify this matter. At this moment, the possibility that concomitant mydriatic effects of SSRI administration have influenced CFF assessments, cannot be fully excluded. Therefore, claims of activating effects of drugs, particularly SSRIs, based solely on CFF elevations should be made cautiously, especially when putative confounding factors, e.g. changes in pupil size, are not fully controlled for.

Acknowledgements

This study was supported by an unrestricted grant from Pfizer Inc., New York. The authors wish to thank Suzanne Teunisse for her work during data collection.

References

- Amado Boccara I, Gougoulis N, Poirier LM, Galinowski A, Loo H (1994): Effects of antidepressants on cognitive functions. A review. *Encephale* 20: 65-77
- Barnett NL, Osborne NN (1993): The effect of serotonin on the rabbit isolated iris sphincter muscle. *Curr Eye Res* 12: 655-673
- Benjamin J, Nemetz H, Fux M, Bleichman I, Agam G (1997): Acute inositol does not attenuate m-CPP-induced anxiety, mydriasis and endocrine effects in panic disorder. *J Psychiatr Res* 31: 489-495
- Broocks A, Little JT, Martin A, Minichiello MD, Dubbert B, Mack C, Tune L, Murphy DL, Sunderland T (1998): The influence of ondansetron and m-chlorophenylpiperazine on scopolamine-induced cognitive, behavioral, and physiological responses in young healthy controls. *Biol Psychiatry* 43: 408-416
- Curran S (1990): Critical Flicker Fusion techniques in psychopharmacology. In Hindmarch I, Stonier PD (eds), *Human Psychopharmacology*. John Wiley, Chichester
- Davson H (1976): *The eye. Visual functions in man*. Vol2a, NY, London, San Francisco, Academic press.

- Deijen JB, Loriaux SM, Orlebeke JF, De Vries J (1989): Effects of paroxetine and maprotiline on mood, perceptual-motor skills and eye movements in healthy volunteers. *J Psychopharmacol* 3: 148-155
- Fairweather DB, Kerr JS, Harrison DA, Moon CA, Hindmarch I (1993): A double blind comparison of the effects of fluoxetine and amitriptyline on cognitive function in elderly depressed patients. *Human Psychopharmacol* 8: 41-47
- Hale AS, Pinninti NR (1995): Critical flicker fusion threshold and anticholinergic effects of chronic antidepressant treatment in remitted depressives. *J Psychopharmacol* 9: 258-266
- Hindmarch I (1992): A review of the psychomotor effects of paroxetine. *Int Clin Psychopharmacol* 4: 65-67
- Hindmarch I (1995): The behavioural toxicity of the selective serotonin reuptake inhibitors. *Int Clin Psychopharmacol* 4: 13-17
- Hindmarch I, Bhatti JZ (1988): Psychopharmacological effects of sertraline in normal, healthy volunteers. *Eur J Clin Pharmacol* 35: 221-223
- Holmberg G (1981): Critical Flicker Fusion (CFF) test for sedative effects of antidepressants. *Acta Psychiatr Scand Suppl* 290: 289-301
- Kerr JS, Fairweather DB, Mahendran R, Hindmarch I (1992): The effects of paroxetine, alone and in combination with alcohol on psychomotor performance and cognitive function in the elderly. *Int Clin Psychopharmacol* 7: 101-108
- Kramer R, Rubicek M, Turner P (1973): The role of norfenfluramine in fenfluramine induced mydriasis. *J Pharm Pharmacol* 25: 575-575
- Lawrence JR, McEwen J, Stonier PD, Pidgen AW (1982): Pupil size and Critical Flicker Fusion Threshold: a reevaluation. *Drug Development Research Supplement* 1: 67-75
- Mattila MJ, Saarialho-Kere U, Mattila M (1988): Acute effects of sertraline, amitriptyline, and placebo on the psychomotor performance of healthy subjects over 50 years of age. *J Clin Psychiatry* 49: 52-58
- McGuirk J, Silverstone T (1990): The effect of the 5-HT re-uptake inhibitor fluoxetine on food intake and body weight in healthy male subjects. *Int J Obes* 14: 361-372
- Millson DS, Haworth SJ, Rushton A, Wilkinson D, Hobson S, Harry J (1991): The effects of a 5-HT₂ receptor antagonist (ICI 169,369) on changes in waking EEG, pupillary responses and state of arousal in human volunteers. *Br J Clin Pharmacol* 32: 447-454
- Millson DS, Jessup CL, Swaisland A, Haworth S, Rushton A, Harry JD (1992): The effects of a selective 5-HT₂ receptor antagonist (ICI 170,809) on platelet aggregation and pupillary responses in healthy volunteers. *Br J Clin Pharmacol* 33: 281-288
- Prow MR, Martin KF, Heal DJ (1996): 8-OH-DPAT-induced mydriasis in mice: a pharmacological characterisation. *Eur J Pharmacol* 317: 21-28
- Ramaekers JG, Muntjewerff ND, O'Hanlon JF (1995): A comparative study of acute and subchronic effects of dothiepin, fluoxetine and placebo on psychomotor and actual driving performance. *Br J Clin Pharmacol* 39: 397-404
- Raptopoulos P, McClelland GR (1989): The clinical pharmacology of paroxetine in healthy subjects. *Acta psychiatr. scand.*, 80 (suppl 350): 46-48.
- Rizzi CA, Prudentino A, Giraldo E (1993) Effects on general behaviour and neurotransmitter functions of a new 5-hydroxytryptamine₃ receptor antagonist with potential relevance in central nervous system disturbances. *Arzneimittelforschung* 43: 1033-1041

- Saletu B, Grünberger J (1988) Drug Profiling by computed electroencephalography and brain maps, with special consideration of sertraline and its psychometric effects. *J Clin Psychiatry* 49: 59-71
- Smith JM, Misiak H (1976) Critical Flicker Frequency (CFF) and psychotropic drugs in normal human subjects- A review. *Psychopharmacol* 47: 175-182
- Warrington SJ, Dana-Haeri J, Sinclair AJ (1989) Cardiovascular and psychomotor effects of repeated doses of paroxetine: a comparison with amitriptyline and placebo in healthy men. *Acta Psychiatr Scand* 80: 42-44
- Zung WKK (1965) A self rating depression scale. *Arch Gen Psychiatry* 12: 63-70

***Modulation of the Critical Flicker Fusion
effects of sertraline and citalopram
by pupillary changes***

Abstract

The effects of the SSRIs sertraline and citalopram on Critical Flicker Fusion and pupil diameter were investigated in a double blind, cross-over study. During three periods of 15 days, 21 healthy middle-aged (30-50 years) subjects of both sexes received sertraline (50 mg on days 1-8, 100 mg on days 9-15), citalopram (20 mg on days 1-8, 40 mg on days 9-15) and placebo. Assessments were done on days 1, 8 and 15 and consisted of pupillary measurements in a high and low ambient light condition, and CFF with and without pupillary control (a 2-mm artificial pupil) with two different devices; the Leeds Psychomotor tester (LPT) and the Euclid device. Both SSRIs induced an acute and steady increase in pupil diameters, which was similar under high and low ambient light conditions. Sertraline and citalopram did not affect any of CFF measures obtained with the Euclid device. The assessments with the LPT showed reduced CFF thresholds after acute administration of sertraline and citalopram, but only when control was made for pupil size. No effects were seen at day 8, but CFF was again reduced at day 15, with and without control for pupil size. The results indicate that sertraline and citalopram exert a mild detrimental effect on CNS activation and that the effect may be masked by concomitant mydriatic effects of sertraline and citalopram, particularly during acute CFF assessments.

Introduction

The Critical Flicker Fusion threshold (CFF) is the frequency at which a flickering light is perceived as a steady light source. Changes in CFF are indicative of changes in CNS activation (Curran 1990) and have been used extensively to assess the cognitive (side) effects of drugs. Serotonin reuptake inhibitors (SSRIs), a class of antidepressant drugs, were repeatedly shown to increase CFF thresholds (for reviews see Hindmarch 1995; Hindmarch 1999). However, SSRIs also increase pupil size (Deijen et al. 1989; Ramaekers et al. 1995; Saletu and Grünberger 1988; see also chapter 5) and pupil diameter is a well-known independent determinant of CFF threshold (Hart 1992). Hence, the SSRI-induced CFF elevations may have due to enlarged pupils rather than increased CNS activation (Ramaekers et al. 1995; Warrington et al. 1989).

It has been argued that CFF should be included as a primary measure in clinical trials of antidepressants (Hindmarch 1987), and CFF changes have been repeatedly used to rank the behavioural toxicity of antidepressant drugs (Hindmarch 1995; Hindmarch 1998; Hindmarch 1999; Kerr et al. 1991). However, it is clear that the possible confounding effects of pupil size need to be identified and eliminated before CFF can be a valid measure of CNS activation. CFF assessments without control for pupil size may lead to wrongful conclusions regarding the cognitive side effect profile of a drug. Notably, mydriatic effects may lead to a wrongful favourable representation of a drugs cognitive profile, or may even mask a drug's detrimental effect on CNS activation, whereas miotic effects may be misinterpreted as sedative actions. The need for pupillary control in CFF testing, particularly in psychopharmacology research, has been frequently pointed out (Danjou et al. 1992; Lawrence et al. 1982; Smith and Misiak 1976). Nevertheless, pupillary control is rarely used in studies measuring the CFF effects of psychoactive drugs, despite the fact that an effective control for pupillary changes is fairly easy implemented by the use of a 2-mm artificial pupil (Smith and Misiak 1976).

A previous study attempting to identify the role of pupillary changes in the CFF effects of two SSRIs, sertraline and paroxetine, has produced indeterminate results (see chapter 5). While it was found that both SSRIs induced a marked increase ($\pm 1.5\text{mm}$, $\pm 35\%$) in pupil size, CFF thresholds, obtained with and without a 2-mm artificial pupil, were unaffected by either SSRI. The absence of CFF elevation, in contrast to various previous reports (see Hindmarch 1995) and despite a significant increase in pupil size, may be explained by the specific method of CFF assessment in this study. More precisely, CFF in this study was measured using a 'Euclid' device (Euclid Instruments, Maastricht) whereas most other CFF studies, including those reporting SSRI-induced

and pupil related CFF elevations, used a different device; the 'Leeds Psychomotor Tester'. It was speculated that an adaptive miotic response to the relatively bright CFF stimuli of the Euclid device (but not with the Leeds Psychomotor Tester) may have counteracted the SSRI-induced mydriasis and associated CFF elevation. If so, CFF measurements with the Euclid device would reflect the drugs' true effects on CNS arousal. Alternatively, the Euclid device may have lacked sensitivity in general, and may not have been able to detect a presumably modest CFF effect of paroxetine and sertraline.

The present experiment was carried out to further elucidate the role of pupillary changes in CFF changes following SSRI administration. More specifically, the acute and sub-chronic effects of the SSRIs sertraline and citalopram on CFF threshold and pupil size, and a possible interaction, were examined. CFF assessments were done using the Euclid tester as well as the Leeds Psychomotor Tester. This enabled us to compare CFF effects in the two devices and identify differential effects. To establish the role of pupil size, CFF was assessed with and without pupillary control (a 2-mm artificial pupil) in both devices. Pupil size itself was measured with two ambient light intensities (high, low) to investigate a possible interaction between the effects of light and drugs on pupil size. The general hypothesis was that CFF elevations following SSRI administration are directly due to concomitant mydriasis. Thus, sertraline and citalopram are expected to increase pupil size and consequently elevate CFF without pupillary control, but no effects are predicted when CFF is assessed with a control for pupil size. Secondly, the CFF effects of sertraline and citalopram are predicted to be more pronounced with the Leeds Psychomotor Tester than with the Euclid Tester. Finally, it was predicted that pupil size would be inversely related to the ambient light intensity, and that the miotic response to light would counteract drug-induced mydriasis, diminishing pupillary differences between placebo and SSRI conditions.

Materials and Methods

Subjects

Twenty-four healthy subjects, 12 men and 12 women, aged 30-50 years, were recruited through newspaper advertisements. Screening included a telephonic interview, health questionnaire, electrocardiogram, laboratory tests (blood haematology and chemistry, urinalysis, drug and pregnancy screening) and a routine medical examination. Exclusion criteria were the presence or a history of, cardiac, hepatic, renal, pulmonary,

neurological, gastrointestinal, haematological or psychiatric illness, symptoms excessive drinking (> 20 units of alcoholic a week) pregnancy or lactation, use of medication other than oral contraceptives, use of drugs, and any sensory or motor deficits which could reasonably be expected to affect test performance. All subjects scored below depression threshold (< 50) on the Zung Depression Scale (Zung 1965). The study was approved by the standing Medical Ethics Committee of Maastricht University, and carried out in accordance with the Declaration of Helsinki (Hong Kong Modification, 1989). Written informed consent was obtained from each subject prior to participation.

Design

The study was conducted according to a randomised, double blind, placebo-controlled 3-period cross-over design. Nested within this design are two levels of pupil assessments, as well as two (apparatus) by two (pupillary control) levels of CFF assessment.

Treatments

Treatment orders were randomly assigned and double-blind. Treatment periods lasted 15 days and were separated by 14-day washout periods. Treatment regimens consisted of sertraline 50 mg on days 1 to 8, followed by sertraline 100 mg on days 9 to 15; citalopram 20 mg on days 1 to 8, followed by citalopram 40 mg on days 9 to 15, or placebo. Medication was taken at fixed times in the morning.

Testing procedure

Assessments were done on days 1, 8 and 15 of each treatment period on a fixed time between 12.00 and 18.00 p.m. The acute measurements on day 1 were done 5 hours after the first drug administration. Before the start of the study, all subjects were individually trained to conduct the CFF tests. Consumption of alcoholic beverages during the treatment periods was prohibited and subjects were instructed to arrive at the laboratory well rested. Female subjects were tested for pregnancy before the start of each the three treatment periods. CFF assessments were always done in the following sequence: Euclid apparatus without artificial pupil – Euclid apparatus with artificial pupil – LPT without artificial pupil – LPT with artificial pupil. Pupil diameter was subsequently measured with a high ambient light intensity, followed by a low ambient light intensity. The entire procedure lasted approximately 15 minutes.

Critical Flicker Fusion assessment

Critical Fusion was assessed with the Euclid device and the Leeds Psychomotor Tester. Measurements with both devices were performed with and without a 2-mm artificial pupil to control for pupillary changes.

Leeds Psychomotor Tester (Leeds Psychomotor Services, York)

Critical Flicker Fusion threshold was ascertained using four red light-emitting diodes in a binocular foveal fixation, positioned at eye level, with dimmed ambient illumination. Responses were made through a push button, which was attached to the emitting device by a 1-meter long wire. This ensured that the subject viewed the stimuli from standard distance. The psychophysical method of limits was used to determine CFF thresholds, with three alternating ascending and descending trials. In the ascending mode, flicker was increased with 1 Hz/s and subjects had to press the button when they no longer could detect flickering. In the descending mode, the flicker frequency was gradually decreased by 1 Hz/s and responded when they just started to see a flickering. The mean of the six measurements was taken as dependent variable indicating CFF threshold. In the condition with pupillary control, the subjects wore closed eyeglasses with two 2-mm apertures, which could be adjusted to match the position of each subject's eyes.

Euclid apparatus (Euclid Instruments, Maastricht)

Subjects were seated looking into a visual tunnel that displayed a red light source in a Maxwellian perspective. CFF was assessed using the Method of Limits measurement as was described above, providing optimal comparability between the method of CFF assessment in the two test devices. In trials with pupillary control, the visual tunnels were covered with 'artificial pupils' consisting of two plates with a 2-mm aperture.

Pupil diameter

Pupil diameter was measured with two intensities of surrounding light. In the 'low ambient light intensity' condition the test cabin was lit by one indirect light (15 watt), similar to the condition in which CFF measurements took place. In the 'high ambient light intensity' condition the room was directly lit by three TL-lamps, located at the ceiling. Pupil diameter was recorded using a head-mounted eye tracking system (4000SU eye tracker, Applied Science Laboratories). The beam from an infrared light source illuminates the eye and an optical system focuses an image of the pupil onto a solid state eye camera. Both the illumination beam and the image of the eye are reflected from a helmet visor, which is coated to be reflective in the near infrared region and

transitive to visible light. The illuminator, optics and camera are all helmet mounted. Pupil and corneal reflection outlines and centroids are displayed on a pupil monitor over the video image of the eye. Pupillary diameter was recorded for approximately 10 seconds at a rate of 50 measurements per second. Median pupil diameter is taken as the dependent variable.

Statistical Analyses

Pupil diameters were analysed in a multivariate, repeated-measures analysis of variance (MANOVA) according to a $3 \times 3 \times 2$ factorial design. Within-subjects factors were *Treatment* (placebo, sertraline, citalopram), *Time* (days 1, 8, 15) and *Ambient light Intensity* (high, low). CFF thresholds were analysed using a MANOVA according to a $3 \times 3 \times 2 \times 2$ factorial design, with *Treatment* (placebo, sertraline, citalopram), *Time* (days 1, 8, 15), *Apparatus* (Euclid, Leeds Psychomotor Tester) and *Pupillary control* (present, absent) as within-subjects factors. Significant ($p < 0.05$) main effects involving factors with more than two levels (i.e. *Treatment* and *Time*) or interaction effects were further investigated using separate comparisons (MANOVAs or paired t-tests) between the appropriate levels to identify the exact nature of the differences. These analyses were also performed when effects approached a significant level ($p < 0.07$).

Results

Missing data

Three subjects (one man, two women) withdrew from the study after experiencing nausea and diarrhoea following administration of citalopram. Data from the remaining twenty-one subjects were analysed and are reported below.

Pupil diameter

Results of the pupil measurements are depicted in figure 1. Overall analysis of pupillary diameters showed main effects of *Ambient Light Intensity* ($F_{1,20}=150.69$, $p < 0.001$) and *Treatments* ($F_{2,19}=39.96$, $p < 0.001$). As expected, a high ambient light intensity reduced pupil diameters whereas treatment with both SSRIs caused mydriasis. No main effect of *Time* or interaction effects between the factors *Ambient Light Intensity*, *Treatments* and *Time* were found. Separate drug-placebo and drug-drug comparisons revealed that sertraline and citalopram induced a significant increase of pupil diameter compared to placebo at both high (sertraline: $F_{1,20}=38.73$, $p < 0.001$; citalopram: $F_{1,20}=51.47$, $p < 0.001$) and low (sertraline: $F_{1,20}=32.27$, $p < 0.001$; citalopram: $F_{1,20}=88.89$, $p < 0.001$)

ambient light intensity. However, pupil diameters did not differ between sertraline and citalopram. None of the analyses showed a main effect of *Time* or a *Treatment by Time* interaction.

Critical Flicker Fusion

Results of the CFF assessments are presented in table 1 and figure 2. Overall analysis of the CFF data showed a main effect of *Apparatus* ($F_{1,20}=485.86$, $p<0.001$) and *Pupillary Control* ($F_{1,20}=364.62$, $p<0.001$), but no effect of *Treatment* or *Time*. A significant interaction was present between *Apparatus* and *Pupillary Control* ($F_{1,20}=755.40$, $p<0.001$) indicating that the effect of pupillary control on CFF differed between the two test devices. CFF values obtained with the Leeds Psychomotor Tester were generally lower than those gathered with the Euclid device, and although the artificial pupil depressed CFF values in both test devices, the decline was much more robust with the Leeds Psychomotor Tester. No other significant interactions were found, but the *Apparatus by Treatments* interaction approached significance ($F_{2,19}=3.06$, $p<0.07$) suggesting a differential effect of treatments between CFF devices.

Subsequent separate analyses for each apparatus revealed that CFF data obtained with the Euclid device showed a main effect of *Pupillary Control* ($F_{1,20}=140.74$, $p<0.001$) but no main effect of *Treatment* or *Time*, or interactions between the three factors. CFF thresholds with the Leeds Psychomotor Tester showed a main effect of *Pupillary Control* ($F_{1,20}=208.82$, $p<0.001$) and a main effect of *Treatment* ($F_{2,19}=6.68$, $p<0.01$), whereas the main effect of *Time* approached significance ($F_{2,19}=3.36$, $p<0.06$). No interaction effects were found.

In order to specify the effect of the treatments on CFF obtained with the Leeds Psychomotor Tester paired t-tests were used to compare drugs to placebo, with and without artificial pupil, at the three time points. At day 1, sertraline ($t_{20}=2.59$, $p<0.05$) and citalopram ($t_{20}=2.67$, $p<0.05$) significantly reduced CFF when assessed with pupillary control, but no such reduction was found in the CFF assessment without pupillary control. At day 8, no effects of either drug were seen, with or without pupillary control. At day 15, citalopram decreased CFF obtained with ($t_{20}=2.24$, $p<0.05$) and without ($t_{20}=2.72$, $p<0.05$) pupillary control. Similarly, sertraline reduced CFF without pupillary control ($t_{20}=2.41$, $p<0.05$), whereas the reduction of CFF with pupillary control just failed to reach significance ($t_{20}=1.90$, $p=0.07$).

Figure 1: Mean (standard error) pupil diameter after 1, 8 and 15 days of treatment with placebo, sertraline and citalopram, measured with high and low ambient light intensity.

Table 1: Mean (standard deviation) CFF thresholds as measured with the Euclid device, with and without a 2-mm artificial pupil to control for pupil size.

Euclid device - without pupillary control

	Day 1	Day 8	Day 15
Placebo	36.2 (3.2)	36.3 (3.1)	36.1 (3.5)
Sertraline	36.6 (3.4)	36.4 (3.0)	36.0 (2.7)
Citalopram	35.9 (2.9)	36.7 (2.9)	36.3 (2.7)

Euclid device - with pupillary control

	Day 1	Day 8	Day 15
Placebo	34.2 (3.2)	33.6 (3.1)	34.3 (3.6)
Sertraline	34.2 (2.6)	34.1 (2.9)	34.2 (3.3)
Citalopram	34.1 (3.3)	34.2 (3.0)	34.1 (2.6)

Figure 2: Mean (standard error) pupil diameter after 1, 8 and 15 days of treatment with placebo, sertraline and citalopram, measured with high and low ambient light intensity.

Discussion

As was hypothesised, pupil diameter increased significantly during treatment with sertraline and citalopram. Mydriasis occurred quite rapidly following a single acute dose of both SSRIs, already reaching a maximum 5 hours after first administration. Subsequently, mydriasis remained stable at this level throughout the treatment period. Mydriasis following sertraline administration has been previously found (Saletu and Grünberger 1988; chapter 5) and is most likely directly linked to serotonergic stimulation (Barnett and Osborne 1993). Therefore, mydriatic effects of citalopram are expected given the drugs' serotonergic mode of action. However, this is the first study to actually demonstrate the occurrence of mydriasis with citalopram. As expected, pupil diameters were smaller with high ambient light intensity, but, contrary to the hypothesis, the miotic response to light was similar in placebo and SSRI groups. Thus, exposure to bright light did not alter the differences in pupil size between SSRI and placebo groups.

Comparing the two CFF devices, there were marked differences in the outcome of the CFF measurements. First, CFF thresholds obtained with the Leeds Psychomotor Tester were generally 10-15 Hz lower than those obtained with the Euclid device. This is

a common finding, which is most probably attributable to the lower light intensity of the Leeds Psychomotor Tester. Secondly, it was found that the Leeds Psychomotor Tester is more responsive to the use of an artificial pupil. With the Euclid device, the use of an artificial pupil decreased CFF thresholds by $\pm 2\text{--}3\text{ Hz}$ ($\pm 8\%$), whereas a decrease of $\pm 6\text{ Hz}$ ($\pm 22\%$) was seen with the Leeds Psychomotor Tester. More importantly, it appears that the Leeds Psychomotor Tester is considerably more sensitive to changes in CFF threshold following SSRI treatment. SSRI administration caused significant changes in CFF with the Leeds Psychomotor tester (discussed below), whereas no changes were seen with the Euclid device.

A similar absence of CFF effects with the Euclid device was seen in our previous study with sertraline and paroxetine. The pupillary assessments in the present study show that miotic responses to light were similar in SSRI and placebo groups, although it must be noted that ambient light conditions were rather crude as no actual measurements of light intensity were made. Nevertheless, the data strongly suggest differences in pupil sizes between drug and placebo conditions were present while measuring CFF thresholds with the Euclid device. Hence, according to the Ferry-Porter Law (Davson 1976) CFF elevations are expected without the artificial pupil due to increased retinal illumination. Absence of such elevation suggests relative low sensitivity of the Euclid device, although it must be noted that the Euclid device was able to detect CFF elevations following levoprotilline administration (Robbe et al. 1991), as well as CFF decrements following mizolastine (Vuurman et al. 1994), alprazolam and lorazepam (Vermeeren et al. 1995) in healthy volunteers.

The results of CFF assessments with the Leeds Psychomotor Tester are quite diverse and suggest a complex interaction between the relative contribution of pupillary changes, habituation effects and an effect of drug dose. The data show that an acute dose of sertraline and citalopram decreases CFF thresholds when a control is made for pupil size. This indicates that both SSRIs reduce CNS arousal. No such a decrease is seen in the CFF assessment *without* pupillary control, suggesting that the concomitant increase in pupil size has attenuated the performance decrement. Rather unexpectedly, the effects of both drugs disappear following one week of treatment. At this time, CFF thresholds with and without pupillary control show no drug effects. Subsequently, one week of treatment with a double dose (sertraline 100 mg, citalopram 40 mg) results in a decline in CFF, both with and without pupillary control.

The differences in CFF results at different time points cannot be readily explained by variations in pupil diameter since pupils remained steadily enlarged during the full length of SSRI treatment periods. It must be noted that, although the influence of pupil

diameter of CFF is clearly established, there is no experimental data concerning the long-term effects of increased or decreased pupil diameters. A prolonged increase in retinal illumination due to mydriasis may have conceivably induced an adaptive response in subsequent sensory mechanisms. For example, it was found that long-term exposure to bright ambient light produced morphological and biochemical adaptations of the retina in rats to 'regulate the number of photons captured a day and protect against light damage' (reviewed by Penn and Anderson 1991). Adaptation to luminance might be expected to disturb the relation between pupil size and CFF (Ripps and Weale 1976). Indeed, pupil size showed no relation with CFF performance in subjects with naturally wide or small pupils (Miles 1950). Interestingly, all CFF elevations following sertraline were found in acute studies (Hindmarch and Bhatti 1988; Mattila et al. 1988; Saletu and Grünberger 1988) whereas no change was detected following sub-chronic treatment (Hindmarch et al. 1990; Williams et al. 1996). Similarly, the only study to date on the effect of citalopram on CFF showed an elevation of CFF following an acute single (20-mg) dose (Nathan et al. 2000). With the influence of mydriasis diminishing over time, the central mechanism, i.e. CNS activation/arousal, becomes the predominant determinant of CFF threshold during sub-chronic assessments. Thus, the disappearance of CFF effects after one week can be explained by behavioural habituation to the mild CNS deactivating effects of both SSRIs. Increasing the dose may have triggered the reappearance of detrimental effects at week two, but since no acute effect of the doubled dose was measured, this explanation is rather tentative and needs to be confirmed in future studies.

In terms of behavioural toxicity of sertraline and citalopram, the current results of the pupil-controlled CFF assessments indicate that both sertraline and citalopram exert a mild detrimental effect on CNS activation. This is in line with a previous finding of slightly reduced vigilance, as assessed with the Mackworth Clock Test, following sertraline administration (Schmitt and Riedel 1999). Furthermore, vigilance assessments in the present study (results to be published elsewhere) appears to be in perfect accordance with the CFF results when controlled for pupil size. Both sertraline and citalopram reduced vigilance performance following a single acute dose, subsequently, vigilance returned to placebo level after one week of treatment and again decreases after an additional week with the double dose. A similar association between a decline in CFF (controlled for pupil size) and reduction of vigilance was observed with the SSRI fluoxetine (Ramaekers et al. 1995). Also, sertraline was repeatedly found to increase CFF thresholds (obtained without control for pupil size) while the same subjects reported increased feelings of drowsiness (Hindmarch and Bhatti 1988; Mattila et al. 1988; Saletu

and Grünberger 1988). Together, these observations support the notion that CFF is a valid indicator of the effects of SSRIs on CNS arousal *only* when an effective control is made for changes in pupil size.

In conclusion, the present study demonstrated that concomitant mydriatic effects of sertraline and citalopram can indeed significantly modify the outcome of CFF assessments. Our data show that concurrent mydriasis masked the detrimental effects of both SSRIs on CFF, particularly during the acute assessments with the Leeds Psychomotor Tester. Similar confounding is likely to occur with other SSRIs, and indeed with all drugs which intrinsically alter pupil size. Future CFF studies should include an effective control for pupil size or at least take in account the putative effect of pupillary changes following drug administration when interpreting the results. Our results raises questions regarding the validity of the assessment of the behavioural toxicity of SSRIs based on CFF measurements without ample control for pupil size, especially when these concern acute measurements. A critical reappraisal of the data, for example by comparing CFF results with other measures of CNS arousal and sedation, may be warranted.

Acknowledgements

This study was supported by an unrestricted grant from Pfizer Inc., New York. The authors wish to thank Anita van Oers, Karin Eikmans and Annette Heldens for their logistic support and work during data collection.

References

- Barnett NL, Osborne NN (1993) The effect of serotonin on the rabbit isolated iris sphincter muscle. *Curr. Eye Res.* 12: 655-73
- Curran S (1990) Critical Flicker Fusion techniques in psychopharmacology. In: Hindmarch I, Stonier PD (eds) *Human Psychopharmacology*. John Wiley, Chichester
- Danjou P, Warot D, Hergueta T, Lacomblez L, Bouhours P, Puech AJ (1992) Comparative study of the psychomotor and antistress effects of ritanserin, alprazolam and diazepam in healthy subjects: some trait anxiety-independent responses. *Int Clin Psychopharmacol* 7: 73-9
- Davson H (1976) *The Eye*. Academic Press, Academic Press
- Deijen JB, Loriaux SM, Orlebeke JF, De Vries J (1989) Effects of paroxetine and maprotiline on mood, perceptual-motor skills and eye movements in healthy volunteers. *Journal of Psychopharmacology* 3: 148-155
- Hart WMJ (1992) The temporal responsiveness of vision. In: Adler FH, Hart WMJ (eds) *Adler's physiology of the eye*. Mosby, St. Louis
- Hindmarch I (1987) Antidepressant drugs and cognitive function. In: Biziere K, Garattini S, Simon P (eds) *Diagnosis and treatment of depression*. Medsi/McGraw, Paris, pp 356-365

- Hindmarch I (1995) The behavioural toxicity of the selective serotonin reuptake inhibitors. *Int Clin Psychopharmacol* 4: 13-7
- Hindmarch I (1998) The behavioural toxicity of antidepressants: effects on cognition and sexual function. *Int Clin Psychopharmacol* 13 Suppl 6: S5-8
- Hindmarch I (1999) Behavioural toxicity of antianxiety and antidepressant agents. *Human Psychopharmacology: Clinical and Experimental* 14: 137-141
- Hindmarch I, Bhatti JZ (1988) Psychopharmacological effects of sertraline in normal, healthy volunteers. *Eur J Clin Pharmacol* 35: 221-3
- Hindmarch I, Shillingford J, Shillingford C (1990) The effects of sertraline on psychomotor performance in elderly volunteers. *J Clin Psychiatry* 51: 34-36
- Kerr JS, Sherwood N, Hindmarch I (1991) The comparative psychopharmacology of 5HT reuptake inhibitors. *Human Psychopharmacology Clinical and Experimental* 6: 313-317
- Lawrence JR, McEwen J, Stonier PD, Pidgen AW (1982) Pupil size and Critical Flicker Fusion Threshold: a reevaluation. *Drug Development Research Supplement* 1: 67-75
- Mattila MJ, Saarialho-Kere U, Mattila M (1988) Acute effects of sertraline, amitriptyline, and placebo on the psychomotor performance of healthy subjects over 50 years of age. *J Clin Psychiatry* 49: 52-58
- Miles PW (1950) Flicker fusion fields, effects of age and pupil size. *Am. J. Ophthalmol.* 33: 769-772
- Nathan PJ, Stough C, Siteram G (2000) Serotonin and information processing: a pharmacological study on the effects of citalopram on cognitive and psychomotor function. *Human Psychopharmacology* 15: 306-307
- Penn JS, Anderson RE (1991) Effects of light history on the rat retina. In: Osborne NN, Chader GJ (eds) *Progress in Retinal Research*. Pergamon Press, Oxford
- Ramaekers JG, Muntjewerff ND, O'Hanlon JF (1995) A comparative study of acute and subchronic effects of dothiepin, fluoxetine and placebo on psychomotor and actual driving performance. *British Journal of Clinical Pharmacology* 39: 397-404
- Ripps H, Weale RA (1976) Temporal analysis and resolution. In: Davson H (ed) *The eye*. Academic Press, New York, London, San Francisco
- Robbe HWJ, Schoenmakers EAJM, O'Hanlon JF (1991) Comparison of laboratory and driving performance measures under the influence of levoprotiline and doxepin. In: Mulder LJM, Maarse FJ, Sjouw WPB, Akkerman AE (eds) *Computers in psychology: Applications in education, research and psychodiagnostics*. Swets & Zeitlinger, Amsterdam, Netherlands, pp 171-177
- Saletu B, Grünberger J (1988) Drug Profiling by computed electroencephalography and brain maps, with special consideration of sertraline and its psychometric effects. *Journal of Clinical Psychiatry* 49: 59-71
- Schmitt JAJ, Riedel WJ (1999) Serotonergic antidepressants effects on vigilance and cognition. *European Neuropsychopharmacology* 9: S241
- Smith JM, Misiak H (1976) Critical Flicker Frequency (CFF) and psychotropic drugs in normal human subjects- A review. *Psychopharmacol* 47: 175-182
- Vermeeren A, Jackson JL, Muntjewerff ND, Quint PJ, Harrision EM, O'Hanlon JF (1995) Comparison of acute alprazolam (0.25, 0.50 and 1.0 mg) effects versus those of lorazepam 2 mg and placebo on memory in healthy volunteers using laboratory and telephone tests. *Psychopharmacology* 118: 1-9

- Vuurman EFPM, Uiterwijk MMC, Rosenzweig P, O'Hanlon JF (1994) Effects of mizolastine and clemastine on actual driving and psychomotor performance in healthy volunteers. *Eur J Clin Pharmacol* 47: 253-259
- Warrington SJ, Dana-Haeri J, Sinclair AJ (1989) Cardiovascular and psychomotor effects of repeated doses of paroxetine: a comparison with amitriptyline and placebo in healthy men. *Acta Psychiatr Scand* 80: 42-44
- Williams SA, Wesnes K, Oliver SD, Rapeport WG (1996) Absence of effect of sertraline on time-based sensitization of cognitive impairment with haloperidol. *J Clin Psychiatry* 1: 7-11
- Zung WWK (1965) A self rating depression scale. *Archives of General Psychiatry* 12: 63-70

***Caffeine improves memory performance
during distraction in middle-aged,
but not in young or old subjects***

Abstract

The present study evaluated the effect of caffeine (225 mg) on cognitive performance in young, middle-aged, and old subjects in a placebo-controlled parallel groups design (n=60). Groups were matched for level of education and sex. Positive effects of caffeine, as compared to placebo, were found in middle-aged subjects in the first trial of the word learning test. In contrast, caffeine had negative effects on the speed of searching short-term memory in young subjects. Caffeine had no effect on the intercept, which is an indicator for sensorimotor speed, of a memory scanning task. The middle-aged subjects appeared to regularly consume twice as much caffeine as the young and old subjects. These results were similar to earlier findings in a large population study. Although statistical analyses with habitual caffeine consumption as a covariate did not yield different results, a caffeine withdrawal effect was hypothesized to be responsible for the reduced cognitive performance of middle-aged subjects receiving placebo. The habitual use of large amounts of caffeine by middle-aged subjects may be a means to overcome the age-related decrease in cognitive functioning that is caused by changes in information processing.

Introduction

Caffeine is thought to act as a central stimulant and to have effects on cognitive functioning by enhancing alertness and vigilance (Goldstein & Kaizer, 1969; Lieberman, Wurtman, Emde, Roberts, & Coviella, 1987). Although it has been reported that caffeine has no direct effects on memory functions (Loke, 1988), it was recently found that 250 mg of caffeine could attenuate scopolamine-induced memory dysfunction without increasing psychomotor speed (Riedel, Hogervorst, Leboux, Verhey, van Praag, & Jolles, 1995). This is of interest for studies of cognitive aging, in view of the decrease in memory functioning with age and the development of cognition enhancers.

The present study evaluated the effects of caffeine on memory and memory-related processes in a cross-sectional study involving three age groups (young, middle-aged, and old). Effects of caffeine in middle-aged subjects are especially interesting because of known differences in habitual caffeine use between cohorts (Riedel, Hogervorst, & Jolles, 1995) and because cognitive performance has been found to be diminished in the fourth and fifth decades of life (Houx, Vreeling, & Jolles, 1991; Jolles, Houx, vanBoxtel, & Ponds, 1995). Moreover, most studies generally compare young and old subjects. There have been relatively few studies of the effect of caffeine in ageing subjects. In a large population study of 7414 subjects, improved performance was associated with higher levels of caffeine consumption. Elderly subjects appeared more susceptible to the performance improving effects of caffeine on memory, visuospatial reasoning, and reaction time tasks (Jarvis, 1993). Yu, Maskray, Jackson, Swift and Tiplady (1991) found that the performance of elderly subjects on a continuous attention test improved with caffeine relative to placebo. The elderly subjects also felt more alert, energetic, and interested with caffeine. Another study (Swift & Tiplady, 1988) reported that young and old subjects responded differently to caffeine. The authors suggested that the beneficial effects of caffeine in elderly subjects were possibly due to central mechanisms, as reflected by a decrease in the number of errors on cognitive tasks. The beneficial effects were not mediated by an increase in psychomotor speed, as they were in the younger subjects. Lorist, Snel, Mulder, and Kok (1995) however, did not find substantial differences between the effects of caffeine on cognitive functions in elderly and young subjects. They found that caffeine could only partly counteract the decreased energetical resources of elderly subjects during task performance, in that caffeine counteracted the age-related performance decrement related to perceptual processes. However, caffeine had a similar beneficial effect in young, well rested subjects on

stimulus evaluation. Caffeine did not affect central processes, i.e. the speed of searching short term memory.

It is thus currently unclear whether caffeine has a different effect in young or old subjects and at which levels of information processing caffeine acts. In the present study, the effect of 225 mg of caffeine on sensorimotor and memory functions was investigated in young, middle-aged, and old subjects. Extreme care was taken to include only healthy subjects in view of the studies of Houx and Jolles that health-related factors influence neurocognitive performance in otherwise healthy subjects (Houx, Vreeling, & Jolles, 1991; Houx & Jolles, 1993; Jolles, Verhey, Riedel, & Houx, 1995). Because we were mainly interested in the performance of middle-aged subjects, we used a newly developed word learning test with distraction in which subjects had to ignore distracting auditory information. This test was used because it is potentially more sensitive for detecting subtle effects in middle aged subjects as compared to the young subjects. We expected caffeine to have different effects in the three age groups.

Method

Subjects

Sixty healthy subjects were recruited by means of an advertisement in a local newspaper. The mean age of the young group ($n=20$) was 29.4 (range: 26-34 years), the mean age of the middle aged group ($n=20$) was 49.6 (range: 46-54 years), and the mean age of the eldest subjects ($n=20$) was 69.1 (range: 66-74 years). Subjects were pre-selected over the telephone. Only subjects without any previous adverse health-related factors were included (Houx et al, 1991; 1993). Factors screened for were treatment by a neurologist / neurological disorders (e.g. cerebrovascular attacks, epilepsy, Parkinson's Disease, Multiple Sclerosis, or any other muscle or nerve disease, severe head trauma, encephalitis, or meningitis); cardiovascular disease (e.g. hypertension, arrhythmia); internal disease e.g: cancer, diabetes, thyroid disease, and renal dialysis); treatment by a psychiatrist and/or the presence of severe psychiatric disturbances (e.g. depression and electroshock treatment); the use of medication liable to induce cognitive deficits; and the excessive use of alcohol (> 40 consumptions/ week) or the use of addictive drugs. Lastly, subjects who had sensory and/or motor impairments which could be expected to affect psychomotor performance were excluded. Groups were matched for sex and level of occupational ability (LOA). Each group contained 10 males and 10 females. The LOA may be a better estimator of intelligence than education, especially in older subjects. For this purpose, occupation was given a four-digit code, based on a detailed functional

description (CBS, 1985) that was transformed to a 7-point score, which ranged from 1 (simple labour requiring no education or a low level of skills) to 7 (highly specialized labour at an academic level) (van den Brandt, Goldbohm, van't Veer, Volovics, Hermus, & Sturmans, 1985). The LOA was derived from the Directorate General for Employment Policies in the Netherlands (1989). Each group contained subjects with 10 low and 10 high LOAs, the average LOA was 4.5. Subjects were asked how much coffee or tea they consumed a day. Caffeine consumption for each age group is shown in fig 1.

Figure 1: Caffeine intake in cups per day in young, middle-aged and old subject

Procedure

The study was a double blind, placebo controlled design, matched for sex and LOA. Groups were order balanced for time of testing to avoid the influence of circadian factors. All testing was done in the morning. The entire procedure took 1.5 hours. Subjects were asked not to drink any alcohol containing beverages 24 hours preceding testing procedures and to arrive at the laboratory well rested. Subjects were asked not to drink any beverages containing caffeine or related substances, such as theophylline (coffee, chocolate, tea, or coca cola) on the morning of testing. On arrival in the laboratory, a saliva sample was collected and subjects were instructed to drink 225 mg of caffeine in a total of 3 cups of coffee in 15 min. For the placebo condition, 225 mg of decaffeinated

coffee was used. After half an hour there was a 15-min training session. Thereafter, subjects started the test procedure, which took approximately 30 min. Subjects were seated in front of an IBM compatible computer in a sound proof room. Standardized instructions were given through an intercom. After the experiment, subjects rinsed their mouth with water and a saliva sample was collected.

Saliva caffeine analysis

Saliva samples were taken before and after (about 75 min after caffeine consumption) experimental sessions. Samples were stored at -220 C. Saliva caffeine concentration was analysed using a high-performance liquid chromatography method.

Psychological testing

Two cognitive tests that are sensitive to the effects of ageing were used.

Visual Verbal Learning Test with Auditory Distraction (VVLTD).

One of twelve parallel lists of 15 monosyllabic meaningful nouns, matched across word frequency, was presented on a computer screen connected to an IBM compatible computer at a rate of 2 seconds per word (Brand & Jolles, 1985). Simultaneously, 15 similar but different nouns were presented auditorily. Subjects were asked to verbally recall as many of the visually presented words as possible after the presentation had stopped, ignoring the (distracting) auditory words. The first trial was followed by four more trials in which the same words were repeated in the same order. The number of correctly recalled words was noted after each trial. The first trial was taken as an index of short term memory. The trial with the maximum number of recalled words is also taken as an dependent variable (immediate recall). Twenty min after completion of the fifth trial, subjects were asked to recall as many of the previously learned words that were presented visually as possible (delayed recall or long term memory).

Memory Scanning.

Subjects were briefly shown a set of unrelated consonants and told to memorise them. This is called the "memory set" (Sternberg, 1969; Brand & Jolles, 1988). Subjects then looked at a series of 48 letters displayed for 1000 ms on a computer screen, connected to an IBM compatible computer; after 2000 ms the next letter appeared. Half of the presented letters were part of the memory set and had to be recognised as targets. The subjects' task was to respond as rapidly as possible by pressing either 'YES' or 'NO' to indicate whether or not each successive letter was one of those from the memory set.

The median of the response time (RT) for correct responses (on targets and non targets) was taken as a dependent parameter. This task was performed with memory sets consisting of 1, 2, and 3 letters, respectively. Individual RT x set size functions were calculated. This function is defined by the equation $RT = A \cdot Ss + B$, in which: A = slope of the function (i.e. the amount of extra time needed per item in the memory set), Ss = size of the memory set, and B = intercept with the Y-axis. The slope is a measure of the speed of scanning short term memory, and the intercept is a measure of sensorimotor speed.

Statistics

The results were analysed using a two-way analysis of variance (ANOVA) with the factor Drug (caffeine, placebo) and the factor Age (young, middle-aged, old). For habitual caffeine use non-parametric analyses (resp. Kruskal-Wallis and Mann-Whitney runs test) were carried out. All analyses were done with the SPSS-PC statistics package for Apple Macintosh. The cognitive test performance of the various groups is given in table 1.

Table 1: Outcome (mean and SD) of test performance in young (n=20), middle-aged (n=17), and elderly subjects (n=20) with caffeine (n=10) or placebo (n=10).

Test-subtest	Measure	Young		Middle-aged		old	
		Caffeine	Placebo	Caffeine	Placebo	Caffeine	Placebo
VVLT-trial1	# words	5.0(1.7)	6.0(1.5)	5.3(1.1)*	4.0(1.1)†	4.3(1.8)	4.1(1.2)
Immediate recall	# words	12.6(1.3)	12.9(1.1)	12.2(1.7)*	11.5(1.2)†	11.4(1.7)	11.3(1.7)
Delayed recall	# words	10.6(1.5)	11.3(1.8)	9.8(1.8)*	9.0(3.0)†	8.7(2.8)	9.8(2.4)
Memory Scanning	Intercept	425(47)	433(38)*	466(40)†	455(46)†	510(72)	502(71)
	Slope	56(15)	38(17)†	41(15)†	53(17)†	47(15)	57(18)

* n=9, † n=8

Results

Saliva caffeine concentrations

Three (one caffeine, two placebo) subjects in the middle-aged group had pre-treatment saliva caffeine concentrations slightly over 2.0 mg/L, which suggested that they had not adhered to the abstinence instructions. Data for these subjects, who had an average habitual caffeine use, were not used in the analyses. The overall average pre-treatment saliva caffeine concentration in the remaining subjects was .34 mg/L (SD= .44), which

was not different between conditions (CAF:M=.37 (SD.45), PLAC:M=.31 (SD.43), $F_{1,54}=0.32$, $p=n.s.$). Saliva caffeine concentrations were higher in the subjects that had received caffeine containing coffee ($M= 3.12$ mg/L, $SD= 1.47$) than the subjects who had received decaffeinated coffee ($M= .33$ mg/L, $SD= .46$) ($F_{1,54}=93.2$, $p<.0001$).

Performance

A 2 (drug) $\times$ 3 (age) analysis of variance of performance on the VVLT-D revealed a main effect of age on the number of words recalled during the first trial ($F_{2,51}=4.10$, $p<0.05$). There was no significant main effect of caffeine ($F_{1,51}=0.09$, $p=n.s.$). There was a trend significant interaction effect of age and caffeine on trial 1 ($F_{2,51}=2.99$, $p=0.06$). Figure 2 shows that the middle-aged subjects receiving placebo stored less words in short-term memory than did the subjects receiving caffeine.

There was a significant main effect of age on the maximum number of words recalled over the 5 trials ($F_{2,51}=4.57$, $p<0.05$) (table 1). Caffeine had no main effect on the maximum number of words recalled ($F_{1,51}=0.14$, $p=n.s.$). Also, no interaction effects were seen between age and caffeine on the maximum number of words recalled ($F_{2,51}=0.55$, $p=n.s.$). There was a significant main effect of age on the delayed recall ($F_{2,51}= 3.32$, $p<0.05$) (table 1). Caffeine did not have a main effect on delayed recall ($F_{1,51}=0.44$, $p=n.s.$) and also no interaction effect between age $\times$ caffeine could be detected ($F_{2,51}=0.85$, $p=n.s.$).

No main effect of age ($F_{2,47}=0.53$, $p=n.s.$) was seen on the slope of the Memory Scanning Task. Hence, age did not affect the speed of searching short-term memory. In addition, no main effect of caffeine was detected ($F_{1,47}=0.12$, $p=n.s.$). However, there was a significant interaction effect between age and caffeine on the slope ($F_{2,47}=4.58$, $p<0.05$). Figure 3 shows that in the young group, the slope was significantly lower for the subjects who received placebo than for the subjects who received caffeine. Age clearly affected sensorimotor speed, as a 3 (age) $\times$ 2 (caf/placebo) ANOVA on the 1-intercept showed ($F_{2,49}=9.67$, $p<0.001$). However, caffeine did not affect sensorimotor speed ($F_{1,49}=0.05$, $p=n.s.$) and no interaction effect of age and caffeine was detected ($F_{2,49}=0.15$, $p=n.s.$).

Habitual use

The three age groups differed in the average amount of caffeine consumed per day ($\chi^2_{(57)}=10.89$, $p<0.005$). Young subjects consumed 3.2 (median: 2.8, $SD=2.6$) cups of coffee/ day, middle-aged subjects consumed 5.4 (median: 5.3, $SD= 2.8$) cups of coffee/day, and old subjects consumed 2.6 (median: 2.1, $SD=2.0$) cups of coffee/day.

The mean amount of caffeine was similar for the young and old subjects (2-3 cups of coffee/day, $Z=-.69$, $p=ns$) and significantly lower than that of the middle-aged subjects (vs young: $Z=-2.60$, $p<.01$; old: $Z=3.06$, $p<.005$).

Figure 2 (left): Number of words recalled on trial 1 of the VVLT with caffeine and placebo over age groups. $t(15)=2.51$, $p<0.05$

Figure 3 (right): Speed of search in short-term memory of the memory scanning task with caffeine or placebo over age groups. $t(15)= 2.51$, $p<0.05$

Discussion

The objective of the present study was to evaluate the effects of caffeine on cognitive functions in three age groups: young, middle-aged, and old healthy subjects. The results show that caffeine had selective effects on cognitive performance in the three age groups. First, as expected, age affected performance in the verbal learning test with distraction and also clearly affected sensorimotor speed, as measured from the intercept

of the memory scanning task. Age did not affect the speed of searching short-term memory, as measured from the slope of the memory scanning task. Furthermore, young subjects who drank caffeine-containing coffee needed significantly more time to search short-term memory than did young subjects who drank decaffeinated coffee (placebo). By contrast, in the middle-aged subjects, caffeine had a positive effect on short-term memory functions, as measured with the first trial of the word list task. More words were retained in memory after consumption of caffeine than after consumption of decaffeinated coffee. Caffeine did not affect maximum and delayed recall of the word list task, or the sensorimotor speed parameters of a memory scanning task.

There are various factors which may explain the different effects of caffeine on short term memory functions in the three age groups. In the first place, since the pharmacokinetics of caffeine do not change with age (Blanchard & Sawers, 1982), the difference in the response to caffeine in the different age groups is thought to have a pharmacodynamic basis. However, there was a difference in habitual use of caffeine in the three age groups. The middle-aged subjects drank almost twice as much coffee a day as the young and old subjects. The groups were not *a priori* matched for habitual caffeine consumption in order to conform to standard procedures in cross-sectional studies in cognitive ageing research, where caffeine use is not a matching criterion. Similar cohort differences in the regular use of caffeine have been reported earlier (Riedel, Hogervorst, & Jolles, 1995), where young subjects were seen to drink on average 3 cups of coffee / day, middle aged 5 cups, and old subjects 3.5 cups. Cohort differences in the use of nicotine have also been found (Hogervorst, Riedel, & Jolles, 1995). These differences are usually controlled by statistical procedures. We also performed statistical analyses with the number of cups of coffee consumed per day as a covariate. The results remained essentially the same and F-values remained in the same order. This can be taken to indicate that inter-subject differences in habitual caffeine consumption were not the major determinant of differences in the effects of caffeine found in the present study. Yet, habitual caffeine use has been shown to affect the outcome of tasks which measure memory or memory-related processes. Loke (1988), for example, found that regular caffeine users (388-928 mg/week) recalled more words after a delay than very low caffeine (<178 mg) users. Likewise, Smith, Rafferty, Lindgren, Smith, and Nespor (1991) reported that moderate to high habitual users of caffeine (> 200 mg/day) had increased sensitivity on a vigilance task. This may explain the absence of effects in our low caffeine consuming elderly subjects.

Further, a high habitual use may also render subjects more sensitive to caffeine withdrawal effects. This could be a second explanation for the findings of the present

study. However, the effect of caffeine withdrawal on cognitive functions is disputed (James, 1994). The performance of habitual caffeine consumers on simple tasks is reported to decrease during caffeine deprivation (Silverman, Evans, Strain, & Griffiths, 1992). Furthermore, in coffee-drinking managers (Streufert, Pogash, Miller, Gingrich, Landis, Lonardi, Severs, & Roache, 1995) cognitive effectiveness during complex task performance was diminished during caffeine deprivation. Lieberman, Wurtman, Emde, Roberts, & Coviella (1987) showed that even very low doses of caffeine (32 mg) enhanced cognitive function (vigilance and RT) after caffeine abstinence for 12 h. Some dispute whether caffeine deprivation in habitual caffeine consumers is the only explanation for the enhancing effect of caffeine on cognitive functions (Rogers, Richardson, & Dernoncourt, 1995; Warburton, 1995). In the study performed by Warburton (1995) the effect of low doses of caffeine (75 and 150 mg) was investigated after a minimal caffeine abstinence of 1 h. Attention, problem solving, and delayed recall were improved but immediate recall and short-term memory were not. However, performance in the placebo condition was close to the maximum, giving little margin for improvement. Hence, even without caffeine deprivation, subjects in the caffeine condition still improved their cognitive performance. Yet, in view of the fact that our middle-aged subjects used nearly twice as much caffeine as the young and the old subjects, the effect of caffeine withdrawal was probably much more pronounced in the middle-aged subjects than in the other subjects. This is interesting because it may provide clues as to the mechanisms of action of changes in cognition in relation to age. Young subjects, for example, have an optimal cognitive performance and it is possible that middle-aged subjects use coffee to compensate for the deterioration of cognitive functions with age. Also, many studies have shown that a decline in cognitive performance is already evident in the fourth decade (Jolles et al, 1995; Houx et al, 1991; 1993). This hypothesis is currently being tested in the Maastricht Aging Study, which is a large scale longitudinal study involving 1980 subjects aged 25 to 80 (Jolles et al, 1995).

The effect of caffeine taken together with the deterioration of information processing resources with age may explain the differences in caffeine/age effects in the present study. Salthouse (1988) suggested that the age-related decrease in short-term memory capacity reflects the decrease in resources available with advancing age. According to the model of Hasher & Zacks (1979), sensitivity to difficult tasks which require effortful information processing, increases with age because these tasks require attention and energetic capacity, or resources. Moreover, elderly subjects are more sensitive to distracting information and find it difficult to actively suppress irrelevant information (Carlson, Hasher, Zacks, & Connelly, 1995; Kane, Hasher, Stoltzfus, Zacks,

& Connelly, 1994). Caffeine positively affects the ability to focus attention (Lorist, Snel, Kok, and Mulder, 1994; Lorist, Snel, Mulder, & Kok, 1995). The model of Hasher & Zacks combined with the positive effect that caffeine has on selective attention may explain why caffeine had an effect on performance in the first trial of the word list in middle-aged subjects, but not in young subjects. Caffeine increased the number of words recalled by reducing the susceptibility to the distracting, auditorily presented words, but only in the middle-aged subjects because these subjects are more sensitive to distraction and will profit from the effect of caffeine on selective or focused attention. The more structural deficits (loss of neural connections) of elderly subjects may explain the absence of positive effects of caffeine in this age-group. A third explanation of our findings thus involves the known effects of caffeine on attentional functions (Lorist, Snel, Kok, & Mulder, 1994; Nehlig, Daval, & Debry, 1992). Similarly to our finding, others have reported negative effects of caffeine on the slope of a memory scanning task performed by young subjects (Anderson, Revelle, & Lynch, 1989). However, caffeine has also been reported to have significant positive effects on sensorimotor speed (intercept) (Anderson, Revelle, & Lynch, 1989; Lorist, Snel, Kok, and Mulder, 1994; Lorist, Snel, Mulder, & Kok, 1995). The absence of intercept effects in the present study may be because our task was of short duration and caffeine is known to increase the ability to sustain attention or to maintain vigilance (Nehlig, Daval, & Debry, 1992; Pons, Trenque, Bielecki, Moulin, & Potier, 1987; Yu, Maskray, Jackson, Swift & Tiplady, 1991). Furthermore, as for the effects of caffeine on other aspects of information processing, in the present study caffeine did not affect maximum and delayed recall. Hence, these data suggest that caffeine does not affect storage and consolidation functions.

In sum, age-related changes in information processing may thus underlie the effects of caffeine on cognitive performance. It remains to be established whether there is a dose-dependent relationship in this respect. There is convincing evidence that in young subjects caffeine does not affect performance in a linear dose-dependent manner. Rather, the dose-response relationship follows an inverted U-shaped curve analogous to the relation of performance and arousal which is described by the Yerkes-Dodson law (1908). For instance, Loke (1988) reported in her literature review that memory span increases with low (e.g. 162 mg) but not with high (325 mg) doses of caffeine. In the present study, 225 mg of caffeine had a negative effect on the short-term memory performance of young subjects, but did not affect delayed recall. Anderson et al (1989), who showed a negative effect of caffeine on short-term memory functions, used a comparable dose of 4 mg/kg caffeine. Interestingly, delayed recall has been shown to increase with 200 mg but to decrease with 400 mg of caffeine, while 325 mg had no

effect (see also: Smith, Kendrick, & Maben, 1992; Terry & Phifer, 1986). It may be the case that, because arousal decreases with age, the Yerkes-Dodson curve moves to a position more to the right. This would explain why caffeine had negative effects in young subjects, positive effects in our middle-aged subjects and no effect in the elderly subjects.

Concluding, results show that age has subtle effects on memory functions under conditions of distraction. In addition, caffeine has different effects on cognitive performance in different age-groups. The decreased performance in the middle-aged subjects who drank decaffeinated coffee may be due to a caffeine withdrawal effect, which is stronger than in subjects of other ages because the habitual caffeine consumption in middle-aged subjects was twice as high. The present findings are important for cognitive ageing research in view of the fact that cross-sectional studies usually do not control for caffeine use. In addition, the neurocognitive paradigm used (measurement of memory performance under distraction) may prove relevant to cognitive ageing research. The habitual use of large amounts of caffeine by middle-aged subjects may be a means to overcome the age-related decrease in cognitive functioning that is caused by changes in information processing.

Acknowledgement

The caffeine saliva sample analyses were made possible by a grant from the Institute for Scientific Information on Coffee, Paris.

References

- Anderson, K.J., Revelle, W., & Lynch, M.J. (1989). Caffeine, impulsivity, and memory scanning: A comparison of two explanations for the Yerkes-Dodson effect. *Motivation and Emotion*, 8, 614-624.
- Brand, N., & Jolles, J. (1985). Learning and retrieval rate of words presented auditorily and visually. *Journal of General Psychology*, 112, 201-210.
- Brand, N., & Jolles, J. (1988). Neuropsych: Computer-assisted neuropsychological assessment. In: *Computers in Psychology: Methods, Instrumentation, & Psychodiagnostics*. Maarsse, P.J., Mulder, L.J.H., Sjouw, W.P.B., & Akkermans, A.E. (Eds), Swets & Zeitlinger, Amsterdam, pp. 149-157.
- Brandt, P.A. van den, Goldbohm, R.A., van't Veer, P., Volovics, A., Hermus, R.J.J., & Sturmans, F. (1985). A large-scale prospective cohort study on diet and cancer in the Netherlands. *Journal of Clinical Epidemiology*, 43, 285-295.
- Blanchard, J., & Sawers, S.J.A. (1983). Comparative pharmacokinetics of caffeine in young and elderly men. *Journal of Pharmacokinetics and Biopharmacology*, 11, 109-126.
- Centraal Bureau voor Statistiek-CBS (1985). *Beroepenclassificatie 1984* (2nd edition) CBS: Voorburg.

- Carlson, M.C., Hasher, L., Zacks, R.T., & Connelly, S.L. (1995). Aging, distraction, and the benefits of predictable location. *Psychology and Aging*, 10(3), 427-436.
- Directoraat-Generaal voor arbeidsvoorzieningen (1989). Handleiding voor de functie-analyse (Manual for job analysis). SDU Uitgeverij: Den Haag.
- Goldstein, A., & Kaizer, S. (1969). Psychotropic effects of caffeine in man, III. A questionnaire survey of coffee drinking and its effects on a group of housewives. *Clinical Pharmacology Therapy*, 10, 477-488.
- Hasher, L., & Zacks, R.T. (1979). Automatic and effortful processes in memory. *Journal of Experimental Psychology: General*, 108(3), 356-388.
- Hogervorst, E., Riedel, W.J., van Boxtel, M.P.J., & Jolles, J. (1995). Smoking and cognitive complaints. In: *The Maastricht Aging study*. Jolles, J., Ponds, R., van Boxtel, M.P.J., & Houx, P. (Eds). Neuropsych Publishers: Maastricht, pp. 105-111.
- Houx, P.J., Vreeling, F.W., & Jolles, J. (1991). Age-associated cognitive decline is related to biological life events. In: *Alzheimer's Disease: basic mechanisms, diagnosis, and therapeutic strategies*. Iqbal, K., McLachlan, D.R.C., Winblad, B., & Wisniewski, H.M. (Eds). John Wiley & Sons Ltd: Chichester, pp. 353-359.
- Houx, P.J., & Jolles, J. (1993). Age-related decline of psychomotor speed: effects of age, brain health, sex, and education. *Perceptual and Motor Skills*, 76, 195-211.
- James, J.E. (1994). Does caffeine enhance or merely restore degraded psychomotor performance? *Biological Psychology/ Pharmacopsychology*, 30, 124-125.
- Jarvis, M.J. (1993). Does caffeine intake enhance absolute levels of cognitive performance. *Psychopharmacology*, 110, 45-52.
- Jolles, J., Ponds, R., van Boxtel, M.P.J., & Houx, P. (1995). *The Maastricht Aging Study*. Neuropsych Publishers: Maastricht.
- Jolles, J., Verhey, F.R.J., Riedel, W.J., & Houx, P.J. (1995). Cognitive impairment in elderly people: predisposing factors and implications for experimental drug studies. *Drugs and Aging*, 7(6), 459-479.
- Kane, M.J., Hasher, L., Stoltzfus, E.R., Zacks, R.T., & Connelly, S.L. (1994). Inhibitory attentional mechanisms and aging. *Psychology and Aging*, 9(1), 103-112.
- Lieberman, H. R., Wurtman, R. J., Emde, G. G., Roberts, C., Coviella, I.L.G. (1987). The effects of low doses of caffeine on human performance and mood. *Psychopharmacology*, 92(3), 308-312.
- Loke, W.H. (1988). Effects of caffeine on mood and memory. *Physiology and Behavior*, 44, 367-372.
- Lorist, M.M., Snel, J., Kok, A. & Mulder, G. (1994). Influence of caffeine on selective attention in well-rested and fatigued subjects. *Psychophysiology*, 31, 525-534.
- Lorist, M.M., Snel, J., Mulder, G., & Kok, A. (1995). Aging, caffeine, and information processing: An event-related potential analysis. *Electroencephalography and Clinical Neurophysiology*, 96(5), 453-467.
- Nehlig, A., Daval, & Debry, G. (1992). Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic, and psychostimulant effects. *Brain Research Reviews*, 17, 139-170.
- Pons, L., Trenque, T., Bielecki, M., Moulin, M., & Potier, J-C. (1987). Attentional effects of caffeine in man: comparisons with drugs acting upon performance. *Psychiatry Research*, 23, 329-333.

- Riedel, W. J., Hogervorst, E., Lebox, R. L. A. M., Verhey, F. R. J., Praag, H. M. van, & Jolles, J. (1995). Caffeine enhances Memory after Cholinergic Blockade. *Psychopharmacology*, 122(2), 158-168.
- Riedel, W.J., Hogervorst, E., & Jolles, J. (1995). Cognition enhancers and aging. In: *The Maastricht Aging Study*. Jolles, J., Ponds, R., van Boxtel, M.P.J., & Houx, P. (Eds). Neuropsych Publishers: Maastricht, pp. 149-157.
- Rogers, P.J., Richardson, N.J., & Démoncourt, C. (1995). Caffeine use: is there a net benefit for psychomotor performance. *Neuropsychobiology*, 31, 195-199.
- Salthouse, T.A. (1988). Resource reduction interpretations of cognitive aging. *Brain and Cognition*, 7, 238-272.
- Silverman, K., Evans, S.M., Strain, E.C., & Griffiths, R.R. (1992). Withdrawal syndrome after the double blind cessation of caffeine consumption. *The New England Journal of Medicine*, 327 (16), 1109-1113.
- Smith, A.P., Kendrick, A., Maben, A. (1992). Use and effects of food and drinks in relation to diurnal rhythms of mood and cognitive performance. *Proc Nutr.Soc.Dec*, 51(3), 325-333.
- Smith, B.D., Rafferty, J., Lindgren, K., Smith, D.A., & Nespor, A. (1991). Effects of habitual caffeine use and acute ingestion: Testing a biobehavioral model. *Physiology and Behavior*, 51, 131-137.
- Sternberg, S. (1969). Memory scanning: mental processes revealed by reaction time experiments. *American Scientist*, 57(4), 421-457.
- Streufert, S., Pogash, R., Miller, J., Gingrich, D., Landis, R., Lonardi, L., Severs, W., & Roache, J.D. (1995). Effects of caffeine deprivation on complex human functioning. *Psychopharmacology*, 118, 377-384.
- Swift, C.G., & Tiplady, B. (1988). The effect of age on the response to caffeine. *Psychopharmacology*, 94, 29-31.
- Terry, W.S., & Phifer, B. (1986). Caffeine and memory performance on the AVL T. *Journal of Clinical Psychology*, 42, 860-863.
- Warburton, D.M. (1995). Effects of caffeine on cognition and mood without caffeine abstinence. *Psychopharmacology*, 119, 66-70.
- Yerkes, R.M., & Dodson, J.D. (1908). The relation of strength of stimuli to rapidity of habituation. *Journal of Comparative Neurology and Psychology*, 18, 459-482.
- Yu, G., Maskray, V., Jackson, S.H.D., Swift, C.G., & Tiplady, B. (1991). A comparison of the central nervous system effects of caffeine and theophyllin in elderly subjects. *British Journal of Clinical Pharmacology*, 32, 341-345.

***Low dose of caffeine does not affect
memory functions or focussed attention in
middle-aged or elderly subjects***

Abstract

The cognitive effects of a low dose (100 mg) of caffeine in middle-aged and elderly subjects were investigated according to a double blind cross-over design. Sixteen middle-aged (45-60 years) and fourteen elderly (60-75 years) healthy men and women, who were regular caffeine consumers, received 100 mg caffeine or placebo on two separate days. A cognitive test battery was administered 30 minutes later. Caffeine did not affect short-term memory span or speed, or long-term memory retrieval functions, as was assessed with a word learning test, digit span test and memory scanning test. Although caffeine improved performance on card II of the Stroop Colour Word Test, performance on other sub-tests or the Stroop interference measure were unchanged, showing caffeine did not affect focussed attention. Cognitive performance and effects of caffeine did not differ between middle-aged and old subjects. Also, habitual caffeine use did not influence cognitive performance as such, nor did it alter the effects of caffeine in either age-group. The results indicate that caffeine withdrawal does not substantially affect (short-duration) cognitive performance, even in subjects who normally consume relatively large amounts of caffeine. Absence of cognitive effects of caffeine administration may be due to the relatively low dose (100mg), which may not be effective in middle-aged and elderly subjects.

Introduction

Despite several decades of extensive experimental research, the precise and distinct effects of caffeine on cognitive functioning remain quite elusive. Attempts to relate caffeine actions to specific cognitive domains, such as learning and memory, are hampered by inconsistent and contradicting results (for reviews see Fredholm et al. 1999; Riedel and Jorissen 1998; Rogers and Dernoncourt 1998). While there are some indications that caffeine, under certain conditions, can influence specific functions, for example long-term memory (Riedel et al. 1995), it appears that caffeine's most consistent effect is an increase in arousal (Herz 1999; Kenemans and Lorist 1995; Watters et al. 1997). Thus, caffeine can be considered a non-specific stimulant (Kenemans and Verbaten 1998) that can indirectly improve human cognitive performance by increasing alertness.

Traditionally, the effect of a non-specific stimulant is seen as reducing the amount of cues that are being utilised while performing a certain task (Easterbrook 1959). Thus, caffeine and other stimulant drugs may be expected to increase selectivity of information processing, in other words, induce a 'narrowing' of attention and reduce distractibility to irrelevant stimuli (Anderson et al. 1989; Kenemans and Lorist 1995; Kenemans and Verbaten 1998). Experimental studies with caffeine have provided evidence supporting such a view (Kenemans et al. 1999; Kenemans and Lorist 1995; Lorist et al. 1994; Lorist et al. 1995). This effect of caffeine is particularly interesting with regard to the results of our previous study, in which caffeine improved performance on a word learning task with distraction (Hogervorst et al. 1998). The effect was interpreted in terms of improved selective attentional processing, i.e. caffeine improved the subjects' ability to focus on the (visually presented) target words, and reduced distraction by the auditory distractor words. However, in a conventional word learning task (without distraction) the affected task parameter, i.e. number of words recalled after one presentation trial, can be seen as an indicator of short-term memory span (Lezak 1995). Thus, an increase in short-term memory span may also underlie the effect. Indeed, caffeine has recently been shown to improve performance on a digit span test (Rees et al. 1999).

Another issue in caffeine research, one that was specifically addressed in our previous study, is a possible interaction between age and the effects of caffeine. The cognitive effects of caffeine may be more pronounced in elderly subjects (Jarvis 1993; Rees et al. 1999; Swift and Tiplady 1988; Yu et al. 1991), although age-related differences in the cognitive effects of caffeine were not always apparent (Hameleers et al. 2000; Lorist et al. 1995; Rogers and Dernoncourt 1998; Smith et al. 1999). In our

previous study (Hogervorst et al. 1998) it was found that 250 mg caffeine diminished speed of short-term memory search in young subjects only, whereas an improvement was seen of short-term memory span in middle-aged subjects. We proposed that differences in habitual caffeine consumption between age-groups may have caused the differential caffeine effects. Specifically, we hypothesised that the high caffeine consumption in the middle-aged group may have rendered them particularly vulnerable to caffeine withdrawal effects in the placebo condition, and this may have lead to poor cognitive performance.

The present study was designed to further investigate the above issues. The aim of the present study was threefold. First, we sought to establish whether the previously observed performance increment in the word learning task was due to an increase in short term memory span or was alternatively caused by narrowing of attention. To this end, we measured the effects of caffeine on a similar word learning task *without* distraction, as well as on a specific short-term memory span test (digit span). We also included the Stroop Colour Word Test to specifically assess selective attention. It was hypothesised that caffeine would not affect performance on either memory test, but would improve performance on the Stroop Test. Second, we wanted to investigate whether the observed differences in performance between middle-aged and old subjects were due to differences in habitual caffeine consumption. Therefore, we matched our age-groups to a level of habitual caffeine use that was similar to that of the middle-aged group in the previous study. It was explicitly decided *not* to try to avoid putative withdrawal effects by using only non-caffeine users. Instead, we aimed to induce similar caffeine withdrawal effects in both age-groups, as it was hypothesised that withdrawal effects were (at least partly) responsible for our previous findings. Also, it would be expected that the facilitating effect of caffeine would be similar in magnitude with a lower dose of caffeine (100 mg) since even small doses are effective in relieving withdrawal effects (Mitchell et al. 1995). Finally, in our previous study there was some indication that caffeine may reduce the slope of the memory scanning function in middle-aged and old subjects, but no significant effect could be detected. In the present study the effects of caffeine on this task were re-investigated according to a cross-over design, which may be more sensitive to detect effects. In addition, stimulus quality in the memory scanning task was modulated to specifically investigate the effect of caffeine on the perceptual processes.

Methods and Materials

Subjects

Thirty healthy subjects were recruited from a pool of subjects that had responded to newspaper advertisements requesting volunteers for scientific research. The group consisted of 16 middle-aged subjects, aged 46 to 60 years, and 14 old subjects, aged 60 to 74 years (see table 1 for subject characteristics.). Subjects were pre-selected over the telephone. They reported to feel healthy (on average 4 on a 5-point scale ranging from 1: 'not healthy at all' to 5: 'very healthy') and not to use any medication or drugs liable to influence cognitive functioning. Subjects with excessive alcohol consumption (≥ 5 drinks a day) were excluded. Groups were matched for sex, intelligence, and habitual caffeine consumption (in mg/day). Intelligence was inferred from LOA (Level of Occupational Ability).

The LOA is based on a detailed functional description (Centraal Bureau voor de Statistiek 1985), each subjects' occupation was transformed to a score that ranged from 1 (simple labour requiring no education or low level of skills) to 7 (highly specialised labour at an academic level) (Directoraat-Generaal voor de arbeidsvoorziening 1989). The LOA may be a better estimator of intelligence in older subjects than years of education.

Habitual caffeine intake was calculated on reported weekly consumption of caffeinated coffee, tea and soft drinks, in cups (150ml), mugs (175 ml), glasses (150 ml) or cans (330 ml). Habitual use in milligrams of caffeine a day was calculated using the following average caffeine contents in the Netherlands: coffee: 85 mg/ 150 ml, tea: 30 mg/ 150 ml, cola: 20 mg/ 150 ml (Willems 1986-Voeding in de praktijk-VII-B6-I)

Table 1 : Subject characteristics (mean and standard deviation in brackets) of Age, Level of Occupational Ability (LOA), Habitual Caffeine consumption in mg / day (incl. tea and cola).

	MIDDLE-AGED	OLD
Age	54.6 (3.7)	67.7 (5.1)
LOA (1- 7)	4.1 (1.8)	4.5 (1.3)
Caffeine (mg /day)	427 (180)	349 (175)
N	16	14

Design and procedure

The study was conducted according to a double-blind, cross-over design. Treatment orders (caffeine-placebo, placebo-caffeine) were balanced for each age-group over the two test days. One week before the experiment started, subjects were trained to perform the cognitive tests. During this training session each subject completed the full test battery. Subjects were instructed not to drink any alcohol containing beverages 24 hours preceding testing procedures, and to arrive at the lab well rested. Also, subjects were asked not to drink any beverages containing caffeine or related structures (coffee, chocolate milk or tea) on evening before and the morning of testing. All testing was done in the morning to reduce abstaining effects of caffeine.

Upon the subject's arrival, two capsules containing either placebo or a total of 100 mg caffeine were dissolved in one cup of decaffeinated coffee. Subjects were instructed to finish the drink within a period of 5 min. Cognitive tests were performed thirty minutes after finishing the drink. The test battery took approximately 25 minutes to complete. Subjects were seated in front of a computer screen and were given standardised instructions by the same experimenter. After one week, subjects returned for another session that was similar except for the content of the drink. Subjects were paid f 25, - for their participation.

Cognitive Assessments

Two parallel versions of the Word learning Test and Digit Span test were used for the experiment. A third version of the tests was used for each training session. The order of the two experimental versions was balanced over test days. A single version of the Stroop Colour Word Test was used in all assessments.

Digit Span

The Digit Span test is a sub-test of the Wechsler Adult Intelligence Scale (Wechsler 1981) and is used to test working memory storage capacity or memory span (Lezak 1995). A sequence of 3 randomly chosen and unique digits (from 1 to 9) is slowly read aloud to the subject. This is usually well produced, and a second set of 3 digits is presented. If this is also correctly reproduced, the examiner augments the set with one digit. If the subject failed at two consecutive items, the memory span was assumed to be the last sequence that was correctly produced.

Word Learning Test (WLT)

A list of 15 monosyllabic meaningful nouns matched across word frequency is presented on a computer screen connected to an IBM compatible computer with a rate of one word per two seconds. Subjects are asked to verbally recall as many of the presented words as possible as soon as the presentation had stopped (Brand and Jolles 1985). This first immediate recall trial was followed by two more trials in which the same words were repeated in the same order. The number of correctly recalled words was noted after each trial. The first trial was taken as the dependent variable for storage capacity in working memory. When the third trial was completed, subjects performed subsequent activities. Twenty minutes after the last presentation, subjects were asked to recall the previously learned words; the delayed recall, which was taken as a measure of long term memory.

The Stroop Colour Word test (Stroop 1935)

The Stroop is a well-known test for the ease of shifting perceptual sets to conform to changing task requirements (Lezak 1995). The test consists of three subtests (Bohnen et al. 1992). In subtest I, 10 rows by 10 columns of color names (red, blue, green, and yellow) are printed in black on white cardboard. In subtest II, the same number of correspondingly colored patches are printed, whereas subtest III contains a number of color names, printed in incongruously colored ink. For instance, the word "red" can be printed in green. The protocol for administering the test in this study was as follows. For subtest I ('Color Word Reading'), the subject was requested to "read the color names row by row, as fast as you can, without making any mistakes". The time needed to complete the whole card was recorded with a stopwatch in sec. For the second subtest ('Color Naming') the instruction was to "name the colored patches". The third subtest ('Color-Word Interference') involved naming the color of the ink the color words were printed in, without paying attention to the word itself. Time to complete each card is recorded, as well as number of the errors. The susceptibility to interference is computed with the formula: $(\text{Time to read card III} - (\text{Time card I} + \text{card II}) / 2)$ and can be regarded as a measure of focused attention.

Memory Scanning

Subjects were briefly shown a set of unrelated consonants and told to memorise them. This is called the "memory set" (Sternberg 1969). Subjects then looked at a series of 120 letters displayed on a computer screen, connected to an IBM compatible computer. Half of the presented letters were part of the memory set and had to be recognised as

Figure 1: An example of a normal (left) and degraded (right) stimulus in the memory scanning test.

targets. Half of the targets and non-targets were degraded by the addition of speckled noise to the letters in a graphics programme (see figure 1). The subjects' task was to respond as rapidly as possible by pressing either a 'YES' or 'NO' button to indicate whether or not each successive letter was one of those from the memory set. The mean of the response time (RT) for correct responses (on targets and non targets) for degraded and non-degraded stimuli separate was taken as a dependent parameter. This task was performed with memory sets consisting of 2 and 4 letters, respectively.

Statistical analysis

The outcome variables of the word learning test, Digit Span and Stroop test were analysed with a 2 x 2 repeated measures Analysis of Variance (MANOVA) according to a 2 (treatment) x 2 (age-group) factorial model to test the effect of treatment (caffeine 100mg / placebo), the effect of age (middle-aged / old) and the interaction effects between caffeine and age-group. Two additional within-factors were added to the analysis of the Memory Scanning task. These were memory load (2 letter, 4 letters) and stimulus quality (degraded, non-degraded). Since habitual caffeine intake was not entirely identical in both age-groups this variable was included as covariate in the above manova's. In order to test the effect of habitual use on the effect of treatment and the interaction between age and treatment, individual differences in performance in the caffeine and placebo conditions were calculated. Differences were then analysed using a one-way analysis of co-variance (ANCOVA) with age-group as main factor and habitual caffeine intake as co-variate. All analyses were done with the SPSS 6.0 statistics package for Apple Macintosh.

Results

The results of the cognitive assessments are summarised in table 2. The outcome measures of the word learning test, i.e. number of words in trial 1, immediate recall and delayed recall, showed no main effects of age-group or treatment, or an interaction between age-group and treatment. Similarly, digit span performance showed no main effects of age-group or treatment, and there was no treatment by age-group interaction.

Performance on Card I of the Stroop test showed no main effects of age-group or treatment, or an age-group by treatment interaction. A main effect of treatment was found for card II ($F_{1,28}=4.50$, $p<0.05$), but no effect of age-group or an interaction was found. Caffeine reduced the time needed to complete card II in both age-groups. Time to complete card III and the interference measure showed no main effects of age or treatment or an interaction between treatment and age. The error rate was very low and was not statistically tested. The mean number of errors (card I + II + III) was less than one during caffeine and placebo conditions in both age-groups.

Analysis of the memory scanning revealed a main effect of memory load ($F_{1,28}=79.53$, $p<0.001$), as well as a main effect of stimulus quality ($F_{1,28}=6.88$, $p<0.05$). As is to be expected, responses slowed as memory load increased, and responses were also slower for degraded stimuli as compared to non-degraded stimuli. There was no interaction between memory load and stimulus type. No main effects of treatment or age-group were seen for the memory scanning test, and no significant interactions between factors were found.

Covariance analysis to establish the effect of habitual caffeine intake revealed that habitual caffeine explained a significant amount of variance of the main effect of age-group on Digit span performance. However, correction for habitual caffeine intake did not alter the results, i.e. the effect of age-group remained non-significant. Results of other cognitive assessments were unaffected by the inclusion of habitual caffeine intake as covariate in the analyses.

Discussion

In the present study, we examined the effects of 100 mg of caffeine and placebo on memory functions and selective attention in middle-aged and old subjects. Caffeine was found not to affect short-term memory span or speed, or long-term memory retrieval functions, as was assessed with a word learning test, digit span test and memory

Table 2: Means (standard deviations) of the outcome variables of the cognitive assessments, broken down by age-group and treatment. Results printed in *italic* show a main effect of caffeine.

	MIDDLE-AGED				OLD			
Measure	caffeine		placebo		caffeine		placebo	
<i>Word Learning Test</i>								
WLT 1 (# words)	6.9	(2.1)	6.6	(2.1)	6.4	(1.7)	6.4	(1.5)
Immediate recall (# words)	28.8	(6.0)	28.1	(5.9)	26.4	(5.7)	26.3	(5.3)
Delayed recall (# words)	9.6	(2.8)	9.2	(2.8)	8.3	(2.8)	8.5	(2.9)
<i>Digit span (# digits)</i>	5.6	(1.2)	5.5	(0.8)	5.7	(1.4)	5.6	(1.1)
<i>Stroop Colour Word Test</i>								
card I (sec)	43.4	(5.5)	44.8	(6.0)	44.4	(6.7)	44.8	(6.3)
card II (sec)	54.4	(8.8)	57.4	(9.1)	54.9	(10.8)	55.9	(10.4)
card III (sec)	84.5	(15.3)	87.9	(15.0)	90.4	(23.2)	91.0	(22.0)
Interference (%)	72.4	(18.8)	71.7	(17.5)	81.5	(33.9)	80.4	(31.0)
<i>Memory Scanning</i>								
Memory load 2 letters								
Degraded stimuli RT	567	(64)	585	(64)	620	(150)	587	(62)
Non-degraded stimuli RT	562	(56)	567	(73)	594	(61)	578	(78)
Memory load 4 letters								
Degraded stimuli RT	726	(150)	681	(116)	720	(91)	717	(92)
Non-degraded stimuli RT	721	(191)	655	(100)	669	(95)	663	(90)

scanning test. Caffeine improved performance on card II of the Stroop Colour Word Test, but no caffeine effects were seen on other sub-test or on the Stroop interference measure. Cognitive performance and effects of caffeine did not differ between middle-aged and old subjects. Also, habitual caffeine use was found not to influence the cognitive performance as such, nor did it alter the effects of caffeine in either age-group.

In general, cognitive performance was not different in the caffeine and placebo groups. This suggest that no withdrawal effects, as to cognitive functioning, occurred under placebo in our study groups, despite relatively high habitual caffeine intake. Adverse effects following short-term caffeine deprivation are well known, and these consist mostly of headache, drowsiness and reduced mood (Griffiths and Mumford 1995; Rogers and DERNONCOURT 1998). However, caffeine withdrawal effects are less consistently found on objective measures of cognitive performance, especially on short

duration tasks (Lane 1997; Phillips-Bute and Lane 1998; Rogers and Démoncourt 1998). Cognitive withdrawal effects appear to be most pronounced in long duration vigilance-like tests (Lane and Phillips-Bute 1998) and perhaps after longer (48 hours) caffeine deprivation periods (Fredholm et al. 1999). The present results seem to fit this general idea.

Based on the present results, we cannot confirm that improvement of memory test performance in the middle-aged group in our previous study was caused by withdrawal effects due to relatively high habitual caffeine intake in this group. Habitual caffeine intake in the present middle-aged group (± 425 mg/day) was comparable to that of the middle-age group in the previous study (± 450 mg/day), while the intake in our current old group was somewhat lower than that (± 350 mg/day). Despite the fact that caffeine consumption in our previous study may be an underestimation because caffeine-containing soft drinks were not included in the calculation, similar withdrawal effects may still be expected to occur in the present study, at least in the middle aged group. It must be noted that, because no baseline measurements are taken, withdrawal effects could only be inferred by comparison of performance under placebo versus caffeine. Hence, it can be argued that withdrawal effects indeed occurred in the present study, but the lower caffeine dose of 100 mg was less effective in removing this effect than the 225 mg dose that was used in our previous study. However, Mitchell et al. (1995) have shown that doses of caffeine that are low compared to habitual caffeine intake are sufficient to prevent withdrawal symptoms. Therefore, we assume that if withdrawal effects were present, administration of 100 mg would have effectively removed these effects, resulting in a better performance under caffeine. We conclude that we found no evidence for caffeine withdrawal effects occurring in the placebo condition, even in subjects with relatively high habitual caffeine use.

Consequently, if differences in habitual caffeine intake cannot explain the differential effect of caffeine in the middle-aged and old group in our previous study, this may have reflected a true age-related difference in cognitive response to caffeine. However, in our present study, we found no evidence for such a age by caffeine interaction either. The existence of an age-related difference in the effect of caffeine on cognitive function is indeed disputed (Amendola et al. 1998; Smith et al. 1999). However, in the present study methodological factors may be considered to underlie the lack of an age by caffeine interaction. We used contiguous age-groups, whereas in our former study age-groups were separated by ten years. Average difference between the middle-aged and old group in the present study was approximately 13 years, whereas age-groups differed nearly 20 years in the previous study. The fact that that age-groups

in the present study were less distinct may have made it more difficult to detect age-related differences. This is supported by the fact that test performance showed no effects of age itself, despite the fact that performance on each of the current tests has been shown to decline with age (Houx et al. 1993; Kausler 1991; La Rue 1992). It must be noted that the absence of age effects is not likely to be the result of insufficient sensitivity due to procedural factors, since the well known task-related effects on the memory scanning task, i.e. effects of memory load and stimulus quality (Sternberg, 1969) were shown to occur. The relatively low dose of caffeine (100 mg) may also be held responsible for the absence of cognitive effects of caffeine, particularly since caffeine withdrawal effects were not apparent. However, doses of caffeine below 100 mg have been reported to facilitate vigilance performance (Lieberman et al. 1987) as well as attention and memory (Durlach 1998; Smith et al. 1999), and even in the absence of withdrawal effects (Warburton 1995). However, these effects were observed in relatively young subjects (<40 years), whereas older subjects may benefit more from higher doses (Hogervorst et al. 1998).

The specific results of the current cognitive assessments need to be interpreted with caution, given the general issues addressed above. Caffeine was found to affect neither short-term memory (first immediate recall trial, digit span) nor focussed attention (Stroop interference) measures. It is clear that this implies that no statement can be made regarding the nature of the previously observed improvement of the first immediate recall trial in the previous study. As was discussed, absence of the proposed withdrawal effects, in combination with differences in subject characteristics and dosage, compromises a valid comparison between the present study and our former study. The literature shows that, in young subjects, stroop interference may be increased (Foreman et al. 1989), decreased (Kenemans et al. 1999) or unchanged (Edwards et al. 1996) by an acute dose of 250 mg caffeine. No studies on the effects of caffeine on Stroop performance were done using older subjects. It has been suggested that, since elderly may be more sensitive to distraction and have more difficulty suppressing irrelevant information (Carlson et al. 1995), they may be particularly sensitive to possible facilitating effects of caffeine on focussed attention (Hogervorst et al. 1998). In the present study, the dose may have been too low to influence focussed attention, although performance on card II (colour naming) was improved under caffeine. Interestingly, in a study by Rees et al. (1999) 250 mg caffeine was seen to act differently in young (20-25) and old (50-65) subjects on a number of tests. In young subjects tapping and response time on a rapid processing task was improved under caffeine, whereas caffeine elevated digit span and focussed attention (as measured with a digit cancellation test) in the

elderly subjects. Important in this respect was that subjects were allowed to consume their normal caffeine intake until 1.5 to 2 hours before the experiment, hereby minimising any possible withdrawal effects. These data are in line with our current and previous findings, suggesting that elderly may benefit from a higher dose of caffeine (250 mg) particularly on span and/or focussed attention tasks, independent of withdrawal effects.

In conclusion, results of this study indicate that caffeine withdrawal does not substantially affect (short-duration) cognitive performance, even in subjects who normally consume relatively large amounts of caffeine. Certainly, lack of cognitive withdrawal effects needs to be confirmed by incorporation of a baseline measure during which performance is tested under normal caffeine intake. Also, it would be interesting to investigate putative withdrawal effects during prolonged cognitive testing, as this would perhaps reflect a more realistic situation. Secondly, the present results suggest that lower doses of caffeine may be less potent in terms of cognitive effects in elderly subjects. It would be interesting to investigate the effects of higher caffeine dosages in elderly subjects, particularly with regard to focussed attention and short-term memory span.

Acknowledgements

We thank Suzanne Teunisse and Natalie Bougie for their efforts during data collection. We also wish to thank Edwin Klinkenberg for his insights during the analysis of the data.

References

- Amendola CA, Gabrieli JDE, Leberman HR (1998) Caffeine's effects on performance and mood are independent of age and gender. *Nutritional neuroscience* 1: 269-280
- Anderson KJ, Revelle W, Lynch MJ (1989) Caffeine, impulsivity, and memory scanning: A comparison of two explanations for the Yerkes-Dodson effect. *Motivation and Emotion* 8: 614-624
- Bohnen N, Jolles J, Twijnstra A (1992) Modification of the Stroop Color Word Test improves differentiation between patients with mild head injury and matched controls. *Clinical Neuropsychologist* 6: 178-184
- Brand N, Jolles J (1985) Learning and retrieval rate of words presented auditorily and visually. *Journal of General Psychology* 112: 201-210
- Carlson MC, Hasher L, Connely SL, Zacks RT (1995) Aging, distraction, and the benefits of predictable location. *Psychology and Aging* 10: 427-436
- Centraal Bureau voor de Statistiek (1985) Beroepenclassificatie 1984. CBS, CBS
- Directoraat-Generaal voor de arbeidsvoorziening (1989) Handleiding voor de functie-analyse. SDU Uitgeverij, SDU Uitgeverij

- Durlach PJ (1998) The effects of a low dose of caffeine on cognitive performance. *Psychopharmacology* 140: 116-119
- Easterbrook JA (1959) The effect of emotion on cue utilization and the organization of behavior. *Psychological Review* 66: 183-201
- Edwards S, Brice C, Craig C, Penri-Jones R (1996) Effects of caffeine, practice, and mode of presentation on Stroop task performance. *Pharmacol Biochem Behav* 54: 309-15
- Foreman NP, Barraclough S, Moore C, Mehta A, et al (1989) High doses of caffeine impair performance of a numerical version of the Stroop task in men. *Pharmacology, Biochemistry and Behavior* 32: 399-403
- Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE (1999) Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological reviews* 51: 83-133
- Griffiths RR, Mumford GK (1995) Caffeine: a drug of abuse ? In: Bloom FE, Kupfer DJ (eds) *Psychopharmacology: the fourth generation of progress*. Raven Press, New York, pp 1699-1713
- Hameleers PAHM, Van Boxtel MPJ, Hogervorst E, Houx PJ, Buntinx F, Riedel WJ, Jolles J (2000) Habitual Caffeine Consumption is Associated with Improved Long-Term Memory Performance and Simple Response Speed Across Multiple Age Groups. *Human Psychopharmacology* 15: in press
- Herz RS (1999) Caffeine effects on mood and memory. *Behaviour research and therapy* 37: 869-879
- Hogervorst E, Riedel WJ, Schmitt JAJ, Jolles J (1998) Caffeine improves memory performance during distraction in middle-aged, but not in young or old subjects. *Human Psychopharmacology Clinical And Experimental* 13: 277-284
- Houx PJ, Vreeling FW, Jolles J (1993) Stroop interference: aging effects assessed with the Stroop Color-Word Test. *Experimental Aging Research* 19: 209-224
- Jarvis M (1993) Does caffeine intake enhance absolute levels of cognitive performance ? *Psychopharmacology* 110: 45-52
- Kausler DH (1991) *Experimental psychology, Cognition and Human aging*, 2nd edn. Springer-Verlag, Springer-Verlag
- Kenemans JL, Lorist MM (1995) Caffeine and selective visual processing. *Pharmacol Biochem Behav* 52: 461-471
- Kenemans JL, Verbaten MN (1998) Caffeine and visuo-spatial attention. *Psychopharmacology* 135: 353-360
- Kenemans JL, Wieleman JS, Zeegers M, Verbaten MN (1999) Caffeine and stroop interference. *Pharmacol Biochem Behav* 63: 589-98
- La Rue A (1992) *Aging and Neuropsychological Assessment*. Plenum Press, Plenum Press
- Lane JD (1997) effects of brief caffeinated-beverage deprivation on mood, symptoms and psychomotor performance. *Pharmacology Biochemistry and Behavior* 58: 203-208
- Lane JD, Phillips-Bute BG (1998) Caffeine deprivation affects vigilance performance and mood. *Physiology & Behavior* 65: 171-175
- Lezak MD (1995) *Neuropsychological Assessment*, 3rd edn. Oxford University Press, Oxford University Press

- Lieberman HR, Wurtman RJ, Emde GG, Roberts C, Coviella ILG (1987) The effects of low doses of caffeine on human performance and mood. *Psychopharmacology* 92: 308-312
- Lorist MM, Snel J, Kok A, Mulder G (1994) Influence of caffeine on selective attention in well rested and fatigued subjects. *Psychophysiology* 31: 525-534
- Lorist MM, Snel J, Mulder G, Kok A (1995) Aging, caffeine, and information processing: An event-related potential analysis. *Electroencephalogr Clin Neurophysiol Evoked Potentials* 96: 453-467
- Mitchell SH, de Wit H, Zacny JP (1995) Caffeine withdrawal symptoms and self-administration following caffeine deprivation. *Pharmacol Biochem Behav* 51: 941-5
- Phillips-Bute BG, Lane JD (1998) Caffeine withdrawal symptoms following brief caffeine deprivation. *Physiology and Behavior* 63: 35-39
- Rees K, Allen D, Lader M (1999) The influences of age and caffeine on psychomotor and cognitive function. *Psychopharmacology (Berl)* 145: 181-8
- Riedel W, Hogervorst E, Lebox R, Verhey F, Van Praag H, Jolles J (1995) Caffeine attenuates scopolamine-induced memory impairment in humans. *Psychopharmacology* 122: 158-168
- Riedel WJ, Jorissen BL (1998) Nutrients, age and cognitive function. *Curr Opin Clin Nutr Metab Care* 1: 579-585
- Rogers PJ, Dernoncourt C (1998) Regular caffeine consumption: a balance of adverse and beneficial effects for mood and psychomotor performance. *Pharmacology Biochemistry and Behavior* 59: 1039-1045
- Smith A, Sturges W, Gallagher J (1999) Effects of a low dose of caffeine given in different drinks on mood and performance. *Human Psychopharmacology* 14: 473-482
- Sternberg S (1969) Memory scanning: mental processes revealed by reaction time experiments. *American Scientist* 57: 421-457
- Stroop JR (1935) Studies of interference in serial verbal reactions. *Journal of Experimental Psychology* 18: 643-662
- Swift CG, Tiplady B (1988) The effects of age on the response to caffeine. *Psychopharmacology* 94: 29-31
- Warburton DM (1995) Effects of caffeine on cognition and mood without caffeine abstinence. *Psychopharmacology Berl* 119: 66-70
- Watters PA, Martin F, Schreier Z (1997) Caffeine and cognitive performance: the nonlinear Yerkes-Dodson Law. *Human Psychopharmacology* 12: 249-257
- Wechsler D (1981) Wechsler Adult Intelligence Scale-Revised. Manual. Psychological Corporation, Psychological Corporation
- Yu G, Maskray V, Jackson SH, Swift CG, Tiplady B (1991) A comparison of the central nervous system effects of caffeine and theophylline in elderly subjects. *Br J Clin Pharmacol* 32: 341-5

General discussion

In this thesis, the cognitive effects of caffeine and role of serotonin (5-HT) in human cognition were investigated by means of pharmacological manipulations in healthy subjects. Rather than presenting a chapter to chapter account of the outcomes and a discussion of their implications, in this final chapter an attempt is made to present a viewpoint spanning multiple chapters. In other words: how do the current results converge into an overall picture regarding the three main elements in this thesis: serotonin, caffeine and cognition. However, in addressing these issues an initial distinction is made between the association between serotonin and cognition, and that of caffeine and cognition. In the final section of this chapter, for both associations some shared aspects will be discussed, notably their potential as cognition enhancing properties.

Serotonin and cognition: general and specific effects

Serotonin and arousal

The combined results of the studies presented in the current thesis indicate that stimulation of 5-HT neurotransmission induces mild sedation. It is proposed that these mild sedating effects are, in general, easily counteracted by compensatory mechanisms, presumably by the investment of additional effort. Consequently, 5-HT induced sedation is not readily observable in short duration tests, but surfaces only in situations in which the role of additional effort as compensatory mechanism is somehow diminished or even excluded. The experimental evidence supporting this notion, consisting of three main observations, will now be discussed.

The terms 'sedation' and 'activation' will be used to indicate decreases and increases in arousal respectively. Arousal is defined as a non-specific energetic state of a subject which may vary along a continuum, ranging from deep sleep to high alertness and excitement (Anderson et al. 1989). However, arousal should not be considered as

an all-encompassing unitary construct. Several theories and models have been put forward in which a passive low-level energetic construct can be modulated by a higher-level cognitive arousal system. This second system will actively compensate for fluctuations in the low-level arousal system in order to maintain an optimum energetic state for the performance on a specific task (Broadbent 1971; Pribram and McGuinness 1975; Sanders 1998).

serotonergic stimulation and arousal

Stimulation of 5-HT appears to be associated with a selective and consistent reduction of performance on a vigilance test. The studies presented in chapters 3 and 4, but also previously reported studies (O'Hanlon et al. 1998; Ramaekers et al. 1995), show that impaired vigilance emerges following 5-HT stimulation by several drugs, and in the absence of 5-HT induced deficits in other attentional measures, memory or psychomotor functions. As was discussed in chapter 3, cognitive changes that did occur after sertraline and paroxetine administration, i.e. improved verbal fluency and reduced long-term memory respectively, can be ascribed to non-serotonergic actions of both drugs. As for vigilance, it is well known that overall performance on a vigilance test is strongly related to arousal levels (Parasuraman et al. 1998; Warm 1984). Although additional effort may be initially allocated to attenuate arousal related performance decrements, as time proceeds such compensatory actions cannot be maintained (Sanders 1998). With the diminishing contribution of effort the de-activating and thus performance impairing actions of 5-HT become apparent. Hence, as was discussed in more detail in chapter 4, vigilance is particularly sensitive to the mild sedative effects of 5-HT stimulation.

Secondly, it appears that if performance is assessed in a way in which compensatory effort cannot affect the outcome, the detrimental effects of 5-HT on arousal can be objectified in short tests as well. This is demonstrated by the assessments of the critical flicker fusion threshold (CFF), as described in chapters 5 and 6. It was shown that 5-HT stimulation reduced CFF thresholds, which are indicative of reduced CNS arousal and sedation. Importantly, this effect only became apparent following the elimination of the modifying influence of concomitant changes in pupil size on CFF results.

Thirdly, the mild sedative effect of 5-HT stimulation is also apparent in subjectively reported changes in arousal / activation. While such measurements were not taken in the current studies on 5-HT stimulation, studies reporting increased subjective drowsiness following 5-HT stimulation through SSRI administration are abundant (Hawley et al. 1997; Hindmarch and Bhatti 1988; Mattila et al. 1988; Robbe

and O'Hanlon 1995; Saletu and Grünberger 1988). The specific role of 5-HT in this effect is underlined by preliminary data showing drowsiness is also evident in subjective measures following 5-HT stimulation by L-tryptophan loading (Cleare and Bond 1995; Sobczak et al. in preparation-a). Thus subjective measures appear to be sensitive to such mild de-arousing effects (Oken et al. 1995), presumably because they include feelings of increased difficulty (effort) to maintain a certain level of performance in the laboratory, but also, and perhaps foremost, in daily activities.

serotonergic inhibition and arousal

If 5-HT stimulation has mild sedative effects, it may be expected that inhibition of 5-HT may exert the opposite effect, notably activation. However, mild activation is likely to be even more difficult to detect than mild sedation. Nevertheless, the results of the tryptophan depletion study in chapter 2 presents some evidence to support such a notion. It must be noted that since the presumed effects of 5-HT on arousal are mild, the concomitant cognitive effects may not always present themselves in a clear and concise manner, such as an overall improvement of performance. Again, subjective measures appear to be most sensitive to changes in arousal. Tryptophan depletion was associated with increased vigour ratings of a subjective mood scale. The effect emerged at the end of the lengthy test day (following a stay of almost 10 hours in the laboratory), suggesting that diminished 5-HT activity had attenuated fatigue.

It may also be argued that the activating effects of a 5-HT reduction may have underlain the observed improvement of performance on selective attention tasks in that same study. According to Easterbrook's theory of cue utilisation (Easterbrook 1959), the amount of cues (units of information) that is used during performance on a certain task shows a direct negative relation with the level of arousal. Thus, as arousal increases the number of cues that are used is reduced. In what way this affects performance, in terms of improvement or impairment, strongly depends on the characteristics of the particular task. Under circumstances requiring attention to be directed to several information sources at once, a narrowing of attentional focus is likely to adversely affect performance. By contrast, in specific focussed attention tasks, as were used in the TRP depletion study, limited utilisation of the distracting cues (by elevated arousal) facilitates task performance due to reduced distractibility.

serotonin and sleep

One of the many processes in which serotonin is implicated is sleep. On first sight, the notion of sedative actions of 5-HT stimulation appears to be in contradiction with a

reduction of sleep quality following SSRI administration (Chapter 4, (Lindsay DeVane 1998; Oberndorfer et al. 2000). However, the role of serotonin in the sleep / wake cycle is still quite equivocal. Inhibition of 5-HT activity (by lesions, neurotoxins or inhibition of synthesis by PCPA) results in loss of sleep in animals, whereas sleep is restored by small doses of the 5-HT precursors 5-hydroxytryptophan and L-tryptophan (see Portas et al. 2000 for a review). However, the view of serotonin as sleep inducing substance is contradicted by the fact that the activity of 5-HT neurones in the nucleus raphe is most pronounced during waking, considerably lower during slow-wave sleep, and completely abolished during rapid eye movement (REM) sleep (Portas et al. 2000). However, it has been hypothesised that these changes in 5-HT firing are associated with the suppression of tonic motor behaviour during REM sleep (Jacobs and Fornal 1995), rather than reflecting arousal changes. Decrements in REM sleep are indeed most consistently found following SSRI administration (Lindsay DeVane 1998; Oberndorfer et al. 2000). It may be tentatively hypothesised that 5-HT related disturbance of REM sleep may be secondary to insufficient suppression of motor activity, as involuntary movements may produce sleep interruptions.

Serotonin and memory functioning

One obvious counterpoint to the foregoing discussion is the rather robust impairment of long-term memory consolidation seen following tryptophan depletion. The impairing effects of 5-HT inactivation on storage of information in long-term memory have now been repeatedly found (Riedel et al. 1999a; Rubinsztein et al. in press; Schmitt et al. 2000; Sobczak et al. 2000). While such an effect is hard to reconcile with mild activation, it is quite well possible that serotonin's arousal effects and those relating to memory reflect two distinct and separate modes of 5-HT involvement in cognition. It is well known that a specific neurotransmitter may exert different functions depending on the target localisation in the brain and differential interactions with other neurotransmitters. The hippocampus has long been associated with memory functioning (Hasselmo and Wyble 1997) as is the neurotransmitter acetylcholine (Decker and McGaugh 1991; Riedel et al. 1997; Hasselmo 1999). Thus, the hippocampus and the functional interaction between serotonin and cholinergic pathways is of particular interest when addressing 5-HT involvement in memory functions (Richter-Levin and Segal 1996; Steckler and Sahgal 1995).

There is little doubt that the serotonergic and cholinergic systems display important functional interactions in memory functioning (Buhot et al. 2000; Cassel and Jeltsch 1995; Steckler and Sahgal 1995). Serotonin is able to modulate cholinergic

septo-hippocampal neurones both directly as well as indirectly by interacting with GABAergic interneurones (Cassel and Jeltsch 1995; Richter-Levin and Segal 1996). This dual mechanism has important implications since *direct* 5-HT actions would *inhibit* cholinergic activity, whereas 5-HT may *indirectly stimulate* cholinergic activity by reducing inhibitory actions of GABA interneurones. The latter mechanism has been suggested to be predominant at the level of the hippocampus (Richter-Levin and Segal 1996). Hence, it has been proposed that a reduction of serotonergic modulation of hippocampal interneuron activity may contribute to age-related cognitive deficits (Richter-Levin and Segal 1996).

Experimental data, mostly derived from animal studies, support the notion that serotonin is particularly implicated in memory functions (Altman and Normile 1988) (Buhot 1997; Buhot et al. 2000; Meneses 1999; Meneses and Hong 1997). However, the present data do not permit a clear-cut conclusion as no clear pattern of effects has as yet emerged. This is illustrated by the fact that some authors have concluded that 5-HT generally inhibits memory functioning (Buhot et al. 2000) (McEntee and Crook 1991), whereas others provide evidence supporting the opposite conclusion (Meneses 1999; Richter-Levin and Segal 1996). Inconsistencies may partially be explained by methodological issues, e.g. differences in type of cognitive tests, the selectivity of the intervention, the timing of the 5-HT manipulation, the targeted brain area, various dosages, subjects characteristics (Buhot 1997; Meneses and Hong 1997). Furthermore, the nature of the 5-HT system, with its wide-spread innervations, interactions with multiple neurotransmitters systems (Cassel and Jeltsch 1995; Mongeau et al. 1997; Robbins 1997; Steckler and Sahgal 1995), and a wide range of heterogeneously distributed auto- and heteroreceptor subtypes (Buhot 1997; Roth 1994) adds to the complexity of the issue. Nevertheless, recent human experiments employing TRP depletion have quite consistently demonstrated that long-term memory functioning, particularly storage and consolidation of new information, is compromised following global 5-HT inhibition by TRP depletion. The robustness of the effect is underlined by the observed impairments in the storage of new words (Riedel et al. 1999a; Schmitt et al. 2000; Sobczak et al. 2000), pictures (Sobczak et al. in preparation-b) as well as abstract visual patterns (Rubinsztein et al. in press).

Summary

In summary, the results presented in the current thesis provide evidence for two distinct mechanisms of 5-HT involvement in cognitive functioning. One may assume that both mechanisms are based on relatively specific neural pathways and interactions with

distinct neurotransmitter systems. First, serotonin exerts a general inhibitory influence on behavioural activation and arousal levels. Consequently, stimulation of 5-HT results in de-activation, or mild sedation, whereas inhibition of 5-HT activity induces mild activating effects. Evidence for such a premise is found in impaired performance on vigilance tasks, reduction of CFF thresholds and subjective ratings of increased drowsiness following 5-HT stimulation, and attenuation of fatigue and possible narrowing of attention following 5-HT inhibition. Diffuse inhibition of the neurotransmitter systems maintaining arousal, i.e. cholinergic, noradrenergic and dopaminergic systems, by 5-HT (Robbins 1997) may explain the de-arousing properties of 5-HT. As was discussed in chapter 4, particularly inhibition of mesocorticolimbic dopaminergic activity is hypothesised to play a major role in the observed vigilance impairment following 5-HT stimulation.

Secondly, 5-HT modulates long-term memory functioning, particularly consolidation of new information, which is impaired after 5-HT inhibition. This is apparent from the study described in chapter 2, as well as other studies (see above) showing decreased performance on various long-term memory tasks. These specific memory effects of 5-HT may be related to interactions with cholinergic neurones in the hippocampus.

Caffeine, age and cognition: a general hypothesis

The two closing experimental chapters in this thesis focussed on the cognitive effects of caffeine and how these may vary across the human life span. In chapter 7 the cognitive effects of caffeine were explored and compared across three groups with subjects of 26 to 74 years of age. A subsequent chapter focused on the elderly portion of this range consisting of middle-aged and elderly subjects. It is this part of the population that exhibits the preponderance of cognitive complaints and actual impairments and would therefore benefit most from facilitation of cognitive functioning by for example caffeine. Although hope was raised by initial observations of a more pronounced effect of caffeine in the elderly (Jarvis 1993; Swift and Tiplady 1988; Yu et al. 1991) - an effect which moreover appeared to be mediated by pharmacodynamic rather than pharmacokinetic mechanisms (Massey 1998) - an unequivocal confirmation of such an effect is yet to be demonstrated. In fact, recent evidence appears to argue against a differential effect of caffeine with age (Amendola et al. 1998; Hameleers et al. 2000; Smith et al. 1999). However, as is quite characteristic for the quest for mapping caffeine's cognitive effects, results on the matter are quite inconsistent.

attenuation of fatigue

One intriguing explanation for previous findings of more pronounced effects of caffeine in elderly is the possibility of a complex interaction between age, fatigue, duration of cognitive testing, task complexity and caffeine. Caffeine's stimulating effects are thought to be most pronounced when arousal is low (Herz 1999; Mitchell and Redman 1992). While completing a series of cognitive tests, elderly subjects may be fatigued at an earlier point in time than younger subjects (Rees et al. 1999). Therefore, at this point in time, elderly may benefit more from caffeine than younger subjects. Consequently, the temporal position of a particular cognitive test in a test battery may not only determine the effect of caffeine on performance on that test, but may also produce an interaction between age and the effect of caffeine on task performance. Since the temporal position of a test in a test battery is not generally incorporated in the analyses or considered in the interpretation of the effects, this is likely to be a source of inconsistent and contradicting effects.

Some recent experimental evidence supports the above hypothesis. It was found that the beneficial effects of a low dose of caffeine was greatest towards the end of the test session (Smith et al. 1999). This may be taken to indicate that caffeine's cognitive effects are most prominent in fatigued subjects, although pharmacokinetic differences (time to absorb and metabolise caffeine) or sensitivity of particular tests cannot be ruled out. Comparing different age-groups, Rees et al. (1999) showed that caffeine particularly facilitated cognitive performance in the elderly subjects, but that this primarily reflected an attenuation of performance decrements over time.

possible influence of task difficulty

The matter may be further complicated by the theorised differences in optimal arousal levels for different cognitive tasks. According to the Yerkes-Dodson law (Yerkes and Dodson 1908) arousal and task performance follows a negative quadratic relationship (inverted-U hypothesis) and the optimal arousal level for simpler tasks is higher than that of a more complex task (task-difficulty hypothesis). Experiments using caffeine to manipulate arousal have provided support for the inverted U-curve hypothesis as small doses improve performance and higher doses may reduce performance below a subject's baseline level (see (Watters et al. 1997). The task difficulty hypothesis has been proven to be more difficult to confirm, not in the least because it is quite difficult to justify the labelling of a task as 'difficult' or 'easy'. (Neiss, 1988; Anderson, 1994). Nevertheless, conform the task difficulty hypothesis, performance on simpler tasks, such as reaction time tests, is more likely to benefit from the activating effects of caffeine,

whereas caffeine may diminish performance in more complex tasks due to over-arousal. Hence, the stimulating actions of caffeine may reduce performance on a complex task in both young and old subjects when the test is administered at the *beginning* of a test battery (when arousal is high), but improve performance only in fatigued elderly (in whom arousal is low) when the same test is administered at the *end* of a lengthy test battery. Conversely, simple task performance is expected to improve with caffeine in both old and young subjects, and at the beginning as well as the end of a test battery. This would account for the fact that caffeine's facilitating effects are mostly found on reaction time tests and vigilance tasks (Amendola et al. 1998; Bryant et al. 1998; Mitchell and Redman 1992), whereas more complex functioning is much more variably influenced by caffeine (Riedel and Jorissen 1998).

Concluding remarks: serotonergic drugs and/or caffeine as cognition enhancers ?

Given the results of the 5-HT studies presented in this thesis, one may be inclined to uphold a rather pessimistic view regarding the putative role of serotonergic drugs as cognition enhancers. After all, both 5-HT reduction and stimulation were associated with diminished performance at specific cognitive tasks. Moreover, there was little evidence of improvement of these same tasks with the opposite manipulation. For example, inhibition of 5-HT induced memory deficits (chapter 2) but memory function was not improved when 5-HT activity was stimulated (chapter 3). A narrowing of attention was found following a 5-HT reduction by TRP depletion (chapter 2). However, such an effect can be beneficial or detrimental, depending on the specific requirements of the situation.

It should be kept in mind, however, that all experiments were done using healthy subjects, who may have performed at or close to their maximum. Therefore, in such subjects, there may have been little room for improvement. Indeed, it is in this context that the notion of cognition enhancement must be viewed. Drugs or manipulations that turn us all into geniuses, are unlikely to be ever discovered. However, in situations in which an individual's cognitive capabilities are somehow compromised, interventions may be able to restore diminished cognitive abilities, whereas other interventions may help prevent cognitive deterioration, for example during ageing. Therefore, rather than 'cognition enhancers', such drugs may be better referred to as 'cognition restorers' or 'cognition maintainers'.

There are indications that pro-serotonergic drugs may indeed be useful as cognition restorers depending on the specifics of the situation. As it was shown that

decreased 5-HT neurotransmission is associated with memory deficits, serotonergic stimulation is likely to be effective in elevating memory dysfunction in conditions of lowered 5-HT function. Recently, it was shown that in patients with depression long-term memory functioning was improved following treatment with trazodone, which potently stimulates serotonergic neurotransmission (Riedel et al. 1999b). The effect appeared to be independent of mood, since cessation of the treatment resulted a decline of memory function to baseline levels, whereas mood continued to be improved. Less encouraging cognitive results are obtained following SSRI administration in patients with Alzheimers Disease ((Meltzer et al. 1998). However, the development of more specific 5-HT drugs, for example targeting post-synaptic 5-HT_{1A} receptors in the hippocampus (Meneses 1999), may prove a useful strategy in the facilitation of diminished memory functioning.

As for caffeine, its potential as cognition restorer is probably mainly associated to a temporary relief of a state of lowered arousal, due to caffeine's actions as a non-specific stimulant. However, caffeine may have more specific cognitive effects, as it was found to attenuate diminished long-term memory function following cholinergic blockade with scopolamine in humans (Riedel et al. 1995). Furthermore, since it was found that high daily caffeine consumption was positively related to cognitive performance and this was most pronounced in the elderly (Jarvis 1993), habitual caffeine intake may exert a protective influence on age-related cognitive decline.

References

- Altman HJ, Normile HJ (1988) What is the nature of the role of the serotonergic nervous system in learning and memory: prospects for development of an effective treatment strategy for senile dementia. *Neurobiol Aging* 9: 627-38
- Amendola CA, Gabrieli JDE, Leberman HR (1998) Caffeine's effects on performance and mood are independent of age and gender. *Nutritional neuroscience* 1: 269-280
- Anderson KJ, Revelle W, Lynch MJ (1989) Caffeine, impulsivity, and memory scanning: A comparison of two explanations for the Yerkes-Dodson effect. *Motivation and Emotion* 8: 614-624
- Broadbent DE (1971) *Decision and Stress*. Academic Press, Academic Press
- Bryant CA, Farmer A, Tiplady B, Keating J, Sherwood R, Swift CG, Jackson SH (1998) Psychomotor performance: investigating the dose-response relationship for caffeine and theophylline in elderly volunteers. *Eur J Clin Pharmacol* 54: 309-13
- Buhot M (1997) Serotonin receptors in cognitive behaviors. *Current Opinion in Neurobiology* 7: 243-254
- Buhot MC, Martin S, Segu L (2000) Role of serotonin in memory impairment. *Ann Med* 32: 210-21

- Cassel JC, Jeltsch H (1995) Serotonergic Modulation of Cholinergic Function in the Central Nervous System: Cognitive Implications. *Neuroscience* 69: 1-41
- Cleare AJ, Bond AJ (1995) The effect of tryptophan depletion and enhancement on subjective and behavioural aggression in normal male subjects. *Psychopharmacology* 118: 72-81
- Coull JT (1998) Neural correlates of attention and arousal: Insights from electrophysiology, functional neuroimaging and psychopharmacology. *Progress in Neurobiology* 55: 343-361
- Decker MW, McGaugh JL (1991) The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory. *Synapse* 7: 151-68
- Easterbrook JA (1959) The effect of emotion on cue utilization and the organization of behavior. *Psychological Review* 66: 183-201
- Hameleers PAHM, Van Boxtel MPJ, Hogervorst E, Riedel WJ, Houx PJ, Buntinx F, Jolles J (2000) Habitual Caffeine Consumption and its Relation to Memory, Attention, Planning Capacity and Psychomotor Performance across Multiple Age Groups. *Human Psychopharmacology* 15: in press
- Hasselmo M, Wyble B (1997) Free recall and recognition in a network model of the hippocampus: simulating effects scopolamine on human memory function. *Behavioural Brain Research* 89: 1-34
- Hawley CJ, McPhee S, Smith VRH (1997) A review of the psychomotor effects of paroxetine. *International Clinical Psychopharmacology* 12: 13-18
- Herz RS (1999) Caffeine effects on mood and memory. *Behaviour research and therapy* 37: 869-879
- Hindmarch I, Bhatti JZ (1988) Psychopharmacological effects of sertraline in normal, healthy volunteers. *Eur J Clin Pharmacol* 35: 221-3
- Jacobs BL, Fornal CA (1995) Serotonin and Behavior. A general Hypothesis. In: Bloom FE, Kupfer DJ (eds) *Psychopharmacology: The Fourth Generation of Progress*. Raven Press Ltd., New York, pp 461-469
- Jarvis M (1993) Does caffeine intake enhance absolute levels of cognitive performance ? *Psychopharmacology* 110: 45-52
- Lindsay DeVane C (1998) Differential pharmacology of newer antidepressants. *Journal of Clinical Psychiatry* 59: 85-93
- Massey LK (1998) Caffeine and the elderly. *Clinical Pharmacology* 13: 43-50
- Mattila MJ, Saarialho-Kere U, Mattila M (1988) Acute effects of sertraline, amitriptyline, and placebo on the psychomotor performance fo healthy subjects over 50 years of age. *J Clin Psychiatry* 49: 52-58
- McEntee WJ, Crook TH (1991) Serotonin, memory, and the aging brain. *Psychopharmacology* 103: 143-9
- Meltzer CC, Smith G, DeKosky ST, Pollock BG, Mathis CA, Moore RY, Kupfer DJ, Reynolds CF, 3rd (1998) Serotonin in aging, late-life depression and Alzheimer's disease: The emerging role of functional imaging. *Neuropsychopharmacology* 18: 407-430
- Meneses A (1999) 5-HT system and cognition. *Neuroscience and Biobehavioral Reviews* 23: 1111-1125
- Meneses A, Hong E (1997) A Pharmacological Analysis of Serotonergic Receptors: Effects of their Activation of Blockade in Learning. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 21: 273-296

- Mitchell PJ, Redman JR (1992) Effects of caffeine, time of day and user history on study-related performance. *Psychopharmacology* 109: 121-126
- Mongeau R, Blier P, de Montigny C (1997) The serotonergic and noradrenergic systems of the hippocampus: their interactions and the effects of antidepressant treatments. *Brain Research Reviews* 23: 145-195
- O'Hanlon J, Robbe HWJ, Vermeeren A, VanLeeuwen C, Danjou PE (1998) Venlafaxine's effects on healthy volunteers' driving, psychomotor, and vigilance performance during 15-day fixed and incremental dosing regimens. *Journal of Clinical Psychopharmacology* 18: 212-221
- Oberndorfer S, Saletu-Zyhlarz G, Saletu B (2000) Effects of selective serotonin reuptake inhibitors on objective and subjective sleep quality. *Neuropsychobiology* 42: 69-81
- Oken BS, Kishiyama SS, Salinsky MC (1995) Pharmacologically induced changes in arousal: effects on behavioral and electrophysiologic measures of alertness and attention. *Electroencephalogr Clin Neurophysiol* 95: 359-71
- Parasuraman R, Warm JS, See JE (1998) Brain systems of vigilance. In: Parasuraman R (ed) *The attentive brain*. MIT press, Cambridge (MA), pp 221-256
- Portas CM, B. B, Ursin R (2000) Serotonin and the sleep/wake cycle: special emphasis on microdialysis studies. *Progress in Neurobiology* 60
- Pribram KH, McGuinness D (1975) Arousal, activation, and effort in the control of attention. *Psychological Review* 82: 116-149
- Ramaekers JG, Muntjewerff ND, O'Hanlon JF (1995) A comparative study of acute and subchronic effects of dothiepin, fluoxetine and placebo on psychomotor and actual driving performance. *British Journal of Clinical Pharmacology* 39: 397-404
- Rees K, Allen D, Lader M (1999) The influences of age and caffeine on psychomotor and cognitive function. *Psychopharmacology* 145: 181-8
- Richter-Levin G, Segal M (1996) Serotonin, Aging and Cognitive Functions of the Hippocampus. *Reviews in the Neurosciences* 7: 103-113
- Riedel W, Hogervorst E, Leboux R, Verhey F, Van Praag H, Jolles J (1995) Caffeine attenuates scopolamine-induced memory impairment in humans. *Psychopharmacology* 122: 158-168
- Riedel WJ, Hogervorst E, Verhey FRJ, Jolles J (1997) Measuring Cholinergic Function and Cognitive Abilities. In: Hindmarch I, Stonier PD (eds) *Human Psychopharmacology: Measures and Methods*; Vol VI. Wiley, Chichester, pp 29-56
- Riedel WJ, Jorissen BL (1998) Nutrients, age and cognitive function. *Curr Opin Clin Nutr Metab Care* 1: 579-585
- Riedel WJ, Klaassen T, Deutz NEP, Van Someren A, Van Praag HM (1999a) Tryptophan Depletion in Normal Volunteers Produces Selective Impairment in Memory Consolidation. *Psychopharmacology* 141: 362-369
- Riedel WJ, Schoenmakers E, Vermeeren A, O'Hanlon JF (1999b) The Influence of Trazodone Treatment on Cognitive Functions in Outpatients with Major Depressive Disorder. *Human Psychopharmacology* 14: 499-508
- Robbe HWJ, O'Hanlon JF (1995) Acute and subchronic effects of paroxetine 20 and 40 mg on actual driving, psychomotor performance and subjective assessments in healthy volunteers. *European Neuropsychopharmacology* 5: 35-42
- Robbins TW (1997) Arousal Systems and Attentional Processes. *Biological Psychology* 45: 57-71
- Roth B (1994) Multiple Serotonin Receptors: Clinical and Experimental Aspects. *Annals of Clinical Psychiatry* 6: 67-78

- Rubinsztein J, Rogers RD, Riedel WJ, Mehta MA, Robbins TW, Sahakian BJ (in press) Acute dietary tryptophan depletion impairs affective shifting and delayed visual recognition in healthy volunteers. *Psychopharmacology* : in review
- Saletu B, Grünberger J (1988) Drug Profiling by computed electroencephalography and brain maps, with special consideration of sertraline and its psychometric effects. *Journal of Clinical Psychiatry* 49: 59-71
- Sanders AF (1998) Energetics, Stress and Sustained Attention. In: Sanders AF (ed) *Elements of Human Performance*. Lawrence Erlbaum Associates, New Jersey, pp 394-451
- Schmitt JA, Jorissen BL, Sobczak S, van Boxtel MP, Hogervorst E, Deutz NE, Riedel WJ (2000) Tryptophan depletion impairs memory consolidation but improves focussed attention in healthy young volunteers. *Journal of Psychopharmacology* 14: 21-29
- Smith A, Sturgess W, Gallagher J (1999) Effects of a low dose of caffeine given in different drinks on mood and performance. *Human Psychopharmacology* 14: 473-482
- Sobczak S, Honig A, Riedel WJ (in preparation-a) Serotonergic challenge effects on mood and cognition in 1st degree family members of patients with bipolar depression.
- Sobczak S, Riedel WJ, Booij L, aan het Rot M, Deutz N, Honig A (in preparation-b) Cognitive performance following ATD, differences between first-degree relatives of bipolar patients and healthy matched controls.
- Sobczak S, Riedel WJ, Honig AH (2000) Memory Performance and Cortisol Response Following Acute Tryptophan Depletion 4th Dutch Endo-Neuro Meeting, Doorwerth, pp 83
- Steckler T, Sahgal A (1995) The role of serotonergic-cholinergic interactions in the mediation of cognitive behaviour. *Behavioural Brain Research* 67: 165-199
- Swift CG, Tiplady B (1988) The effects of age on the response to caffeine. *Psychopharmacology* 94: 29-31
- Warm JS (1984) An introduction to vigilance. In: Warm JS (ed) *Sustained Attention in Human Performance*. John Wiley & Sons, pp 1-13
- Watters PA, Martin F, Schreter Z (1997) Caffeine and cognitive performance: the nonlinear Yerkes-Dodson Law. *Human Psychopharmacology* 12: 249-257
- Yerkes RM, Dodson JD (1908) The relation of strength of stimuli to rapidity of habituation. *Journal of Comparative Neurology and Psychology* 18: 459-482
- Yu G, Maskray V, Jackson SH, Swift CG, Tiplady B (1991) A comparison of the central nervous system effects of caffeine and theophylline in elderly subjects. *Br J Clin Pharmacol* 32: 341-5

Summary

The central theme in this thesis is the search for substances and targets to pharmacologically enhance cognitive performance. The thesis focuses on two aspects of this vast research area. First, the role of serotonin in human cognitive performance is investigated. Manipulation of serotonergic neurotransmission may prove to be a successful approach in the quest for cognitive enhancing strategies. The cognitive effects of caffeine, the most widely used psychoactive substance, and possible differences in these effects between different age groups, comprise the second part of this thesis.

In *chapter 1* the rationale and general structure of the thesis is given. Several clinical and experimental observations have lead to the presumption that serotonin may play an important role in human cognitive functioning. Diminished serotonergic function in for example depression and Alzheimer's Disease may be directly associated with the concurrent cognitive deficits. Animal studies generally confirm a relationship between serotonin and cognition, especially with regard to memory and attentional functions. However, human studies on this topic are scarce and therefore necessary. By contrast, the effects of caffeine on human cognitive performance have been investigated abundantly. Nevertheless, it has proven to be extremely difficult to determine which, how and to what extent cognitive functions are specifically affected by caffeine. Numerous potential confounders and effect modifiers to the effects of caffeine have been identified, including age. The cognitive effects of caffeine may be more pronounced in the elderly, making caffeine a putative cognition enhancer to combat age-related cognitive decline. As of yet, however, few studies have focussed on the possible interaction between age and the cognitive effects of caffeine. Chapters 2 to 6 describe several studies on the role of serotonin in human cognitive functioning, whereas chapters 7 and 8 address the interaction between age and the cognitive effects of caffeine.

In *chapter 2*, serotonergic activity in healthy human subjects was temporarily reduced by acute tryptophan depletion (ATD). A previous study had shown that consolidation of

newly learned material was specifically impaired by ATD. In the current study, this effect was further investigated by selective timing of the serotonergic manipulation and subsequent memory assessments. Also, the effects of ATD on other cognitive domains and mood were tested. According to a double-blind cross-over design, 17 healthy, young subjects received 100 g of an amino acid mixture with tryptophan (placebo mixture) or without tryptophan (active mixture). Memory, attention, executive functioning, psychomotor functions, information processing and mood were assessed before intake of the amino acid mixture, and 5 and 9 hour after intake. ATD impaired 30-minute delayed recognition of previously learned words without affecting learning capability itself. No effects of ATD on word recall or recognition were found when the mixture was administered 1 hour after acquisition of a wordlist. Focussed attention for visual (Stroop Colour Word test) as well as auditory stimuli (Dichotic Listening), and performance on a verbal fluency task was improved during ATD. No effects were found on speed of information processing, divided attention, planning or mood. The results suggest an essential role for serotonin in the consolidation of newly learned words in the long-term memory, particularly in the first 30 minutes after acquisition. Serotonergic modulation of focussed attention is a promising target for future research in this area.

In *chapters 3, 4 and 5* the cognitive effects of serotonergic stimulation by SSRI administration are addressed. SSRIs are antidepressant drugs that selectively inhibit the reuptake of serotonin into the pre-synaptic neuron and thus stimulate serotonergic neurotransmission. The chapters describe the results of a large study on the cognitive effects of sub-chronic administration of the SSRIs sertraline and paroxetine in healthy volunteers. Each chapter deals with a specific component of the study with specific associated hypotheses. During three treatment periods of two weeks, separated by wash-out periods of two weeks, twenty-four healthy middle-aged (30-50 years) subjects of both sexes received sertraline (50 mg on day 1-7, 100 mg on day 8-14), paroxetine (20 mg on day 1-7, 40 mg on day 8-14) and placebo, according to a double-blind 3-way cross-over design.

In *chapter 3*, the cognitive effects of sertraline and paroxetine with special reference to their different non-serotonergic pharmacological properties are addressed. Although both SSRIs primarily stimulate 5-HT neurotransmission, additional mild influences on dopaminergic and cholinergic systems of sertraline and paroxetine respectively, may invoke cognitive changes as well. Cognitive performance was assessed using tests that are specifically selected based on their sensitivity for manipulations of cholinergic (long-

term memory, memory scanning) and dopaminergic neurotransmission (executive functioning, motor functions). The SSRIs induced specific detectable (albeit mild) cognitive changes. Paroxetine specifically impaired delayed recall of a previously learned wordlist. Sertraline did not affect memory functions but improved performance on a verbal fluency task. Neither SSRI affected speed of searching the short term-memory or sensorimotor speed, as was measured using a memory scanning task. The results are discussed in terms of anticholinergic and pro-dopaminergic effects of paroxetine and sertraline respectively. It is further argued that, while the observed cognitive effects are relatively small in the investigated group of healthy middle-aged subjects, the effects may be more pronounced in the elderly depressed patient.

Chapter 4 specifically focuses on SSRI-induced changes in attentional functioning, i.e. vigilance (sustained attention), divided attention and focussed attention. Previous observations suggested vigilance to be specifically diminished by serotonergic stimulation. In the present study the selectivity of the effect was examined by measuring several different attentional functions. Moreover, sertraline's additional dopaminergic properties enabled us to examine the involvement of serotonergic - dopaminergic interactions in SSRI-induced vigilance impairment by comparison of sertraline's (mild dopaminergic stimulation) effect with that of paroxetine (no effect on dopamine). Changes in sleep quality, a possible confounder for changes in day-time alertness, were also recorded. Paroxetine and sertraline did not affect focussed attention (Stroop Colour Word Test) or divided attention (Dichotic Listening). However, paroxetine was found to reduce vigilance, which was assessed using a 45-minute Mackworth Clock Test. Sertraline had no effect of vigilance, and even appeared to speed up responses in the Mackworth Clock Test. Both SSRIs reduced sleep quality, but this did not appear to be related to diminished vigilance performance. This study and previous research indicates that serotonergic stimulation is associated with mild sedation, which is mediated by serotonergic inhibition of dopaminergic systems, resulting in a specific impairment of tasks requiring prolonged attention.

The Critical Flicker Fusion Test (CFF) is frequently used to assess sedating or activating effects of psychoactive drugs. SSRIs, including paroxetine and sertraline, have been attributed 'activating' effects based on their ability to raise CFF values. However, CFF changes following drugs affecting serotonergic neurotransmission may be confounded by concomitant changes in pupil size. Serotonergic stimulation increases pupil diameter and pupillary dilation is known to independently raise CFF values. Therefore, claims of

activating effects of several serotonin reuptake inhibitors based solely on CFF measurements without control for pupillary changes may be questioned. The role of pupillary changes in the CFF effects of SSRIs is examined in chapters 5 and 6.

In *chapter 5*, the effects of sertraline and paroxetine on pupil diameter and CFF are described. CFF was measured with and without a 2-mm artificial pupil to control for possible changes in pupil diameter. The results show that both SSRIs indeed invoked a significant increase in pupil diameter. Nevertheless, the accompanying CFF assessments – both with and without pupillary control – showed no effects of sertraline or paroxetine treatment. It was concluded that the influence of sertraline and paroxetine is essentially neutral and that the results provide no evidence for CFF to be influenced by pupillary changes. However, it was also argued that the influence of pupillary changes on CFF may depend on the type of CFF apparatus.

In order to further clarify this matter a subsequent study was undertaken, described in *chapter 6*. During three periods of 15 days, twenty-four healthy middle-aged men and women received the SSRIs sertraline (50 mg on days 1-8, 100 mg on days 9-15) and citalopram (20 mg on days 1-8, 40 mg on days 9-15), and placebo. In this study an acute measurement was done 5 hours after the first drug administration in each treatment period. Subsequent measurements were performed on days 8 and 15 of each treatment period. Pupil diameter was assessed under a high and low ambient light condition, and CFF was measured with and without a 2-mm artificial pupil using the same device as in the previous study (Euclid device). In addition, identical CFF measurements were obtained using a another often-used device; the Leeds Psychomotor tester (LPT). The latter device was used in all studies reporting elevated CFF thresholds following SSRI administration. The results from the current study showed that sertraline and citalopram induced an acute and steady increase in pupil diameters, which was similar under high and low ambient light conditions. In accordance with the results of the previous study, CFF thresholds obtained with the Euclid device remained unchanged by treatment. By contrast, the assessments with the LPT showed reduced CFF thresholds after acute administration of sertraline and citalopram, but only when control was made for pupil size. No effects were seen at day 8, but CFF was again reduced at day 15, with and without control for pupil size. The results indicate that sertraline and citalopram exert a mild detrimental effect on CNS activation. During the acute CFF assessments this effect appeared to be masked by concomitant mydriatic effects of both SSRIs. The data also suggest habituation to the detrimental effects after one week, but subsequent re-

appearance of the effects following the dose increments. Although mydriasis was still present at this point, this did not appear to affect the CFF values since similar effects were found with and without pupillary control. The latter may be due to an unknown adaptive response to prolonged light exposure due to enlarged pupils. The results raise questions regarding the validity of the assessment of the behavioural toxicity of SSRIs based on CFF measurements without ample control for pupil size, especially when these concern acute measurements.

Chapters 7 and 8 relate to the cognitive effects of caffeine and their interaction with age. In *chapter 7*, a study is presented in which caffeine, at a dose comparable to three cups of coffee, is administered to healthy young, middle-aged and elderly subjects ($n=60$), according to a double-blind placebo-controlled parallel groups design. The age groups were matched for level of education and sex. Positive effects of caffeine, as compared to placebo, were found in middle-aged subjects in the first trial of the word learning test. In contrast, caffeine had negative effects on the speed of searching short-term memory in young subjects. Caffeine had no effect on the intercept, which is an indicator for sensorimotor speed, of a memory scanning task. Habitual caffeine intake in the middle-aged group was twice as high as in the young and old group. Although statistical analyses with habitual caffeine consumption as a covariate did not yield different results, a caffeine withdrawal effect was hypothesized to be responsible for the reduced cognitive performance of middle-aged subjects receiving placebo. The habitual use of large amounts of caffeine by middle-aged subjects may be a means to overcome the age-related decrease in cognitive functioning that is caused by changes in information processing.

A subsequent study, described in *chapter 8*, focuses on a possible mechanism of age-related differences to the cognitive effects of caffeine, namely variable levels of caffeine withdrawal symptoms due to differences in habitual caffeine use between age-groups. Furthermore, the nature of the observed cognitive effects in the previous study, in terms of attention and memory functions, is further examined. A low dose (100 mg) of caffeine and placebo were administered to 16 healthy middle-aged (45-60 years) and 14 elderly (60-75 years) men and women, according to a double blind cross-over design. Habitual caffeine intake in both age groups was similar and comparable to that of the middle-aged group in the previous study. Caffeine did not affect short-term memory span or speed, or long-term memory retrieval functions, as was assessed with a word learning test, digit span test and memory scanning test. Although caffeine improved performance

on card II of the Stroop Colour Word Test, performance on other sub-tests or the Stroop interference measure were unchanged, showing caffeine did not affect focussed attention. Cognitive performance and effects of caffeine did not differ between middle-aged and old subjects. Also, habitual caffeine use did not influence cognitive performance as such, nor did it alter the effects of caffeine in either age-group. The results indicate that caffeine withdrawal does not substantially affect (short-duration) cognitive performance, even in subjects who normally consume relatively large amounts of caffeine. Absence of cognitive effects of caffeine administration may be due to the relatively low dose (100mg), which may not be effective in middle-aged and elderly subjects.

In *Chapter 9* a general discussion of the combined results of this thesis is given. The current studies provide evidence for two distinct mechanisms of serotonergic involvement in cognition. First, serotonin appears to exert a mild sedating effect. It is proposed that this mild reduction of arousal can be temporarily overcome by the investment of additional compensatory effort. Consequently, 5-HT induced sedation is not readily observable in short duration tests, but surfaces only in situations in which the role of additional effort as compensatory mechanism is somehow diminished or even excluded. Evidence for such a premise is found in impaired performance on vigilance tasks, reduction of CFF thresholds and subjective ratings of increased drowsiness following 5-HT stimulation, and attenuation of fatigue and possible narrowing of attention following 5-HT inhibition. Diffuse inhibition of the neurotransmitter systems maintaining arousal, i.e. cholinergic, noradrenergic and presumably particularly dopaminergic systems, by serotonin may explain the de-arousing properties of serotonin. Secondly, serotonin appears to play a essential role in the consolidation of newly learned information in the long-term memory, as is apparent from deficits in this particular memory function after tryptophan depletion. It is proposed that this may be linked to serotonergic – cholinergic interactions in the hippocampus. The results from the caffeine studies indicate that caffeine improves short-term memory functions in middle-aged subjects. It further appears that the effect is not due to withdrawal effects during placebo. A beneficial cognitive effect of caffeine in middle-aged and elderly subjects may be detectable only at higher (>100 mg) caffeine doses. The putative role of serotonergic substances and caffeine as cognition enhancers is probably limited to restoring sub-optimal task performance in specific situations.

Samenvatting

Het thema van dit proefschrift is de identificatie van stoffen en mogelijke aanknopingspunten waarmee via farmacologisch interventie het cognitief functioneren van de mens kan worden verbeterd. Het proefschrift richt zich op twee aspecten van dit brede onderzoeksterrein. Ten eerste wordt de rol van de neurotransmitter serotonine in het menselijk cognitief functioneren onderzocht. Manipulatie van de serotonerge neurotransmissie is mogelijk een aangrijpingspunt voor de ontwikkeling van nieuwe cognitieverbeterende stoffen. De cognitieve effecten van de meest gebruikte psychoactieve stof cafeïne, en mogelijke verschillen hierin tussen verschillende leeftijdsgroepen, vormen het tweede aspect van dit proefschrift.

In *Hoofdstuk 1* wordt de rationale en opbouw van het proefschrift weergegeven. Klinische en experimentele gegevens suggereren dat serotonine een belangrijke rol speelt in processen zoals geheugen en aandacht. Een verminderd functioneren van het serotonerge systeem bij bepaalde aandoeningen, zoals depressie en de ziekte van Alzheimer, is mogelijk direct gerelateerd aan de geassocieerde cognitieve stoornissen. Dierexperimenteel onderzoek bevestigt het bestaan van een relatie tussen serotonine en cognitie, maar humaan onderzoek op dit gebied is vooralsnog schaars en dus noodzakelijk. Wat betreft de mogelijke invloed van cafeïne op cognitie is reeds veel humaan onderzoek verricht, maar het in kaart brengen van de specifieke effecten is moeilijk gebleken, met name door het grote aantal variabelen dat verstoring of modifierend zou kunnen werken. Eén van deze variabelen is leeftijd. Bij ouderen is de positieve invloed van cafeïne mogelijk het sterkst en cafeïne is daarom interessant als mogelijke cognitieve-verbeterende stof bij leeftijdsgerelateerde cognitieve achteruitgang. De invloed van leeftijd op het effect van cafeïne is tot op heden echter nog weinig onderzocht. Hoofdstuk 2 tot en met 6 richt zich op de relatie tussen serotonine en cognitie, terwijl hoofdstuk 7 en 8 de invloed van cafeïne bij verschillende leeftijdsgroepen behandelt.

In de studie uit *hoofdstuk 2* werd door middel van de tryptofaan depletie methode de serotonerge activiteit bij gezonde mensen tijdelijk geremd. Eerder onderzoek had

aangetoond dat met name het consolideren van nieuwe informatie in het lange-termijn geheugen gestoord was na tryptofaan depletie. Dit effect werd in het huidige onderzoek uitgediept door selectieve timing van de tryptofaan depletie en bovendien werd het effect van tryptofaan op een groot aantal andere cognitieve functies en op stemming onderzocht.

In een dubbel-blind cross-over experiment werd aan 17 gezonde, jonge proefpersonen op twee verschillende dagen 100 gram aminozuur mengsel zonder tryptofaan (actief mengsel) of met tryptofaan (placebo mengsel) toegediend. Geheugen, aandacht, executieve functies, psychomotoriek, informatieverwerking en stemming werden gemeten vóór inname van het mengsel, en na 5 en 9 uur na inname. Tryptofaan depletie verstoorde de herkenning van 30 minuten daarvoor geleerde woorden, zonder het leren op zich te beïnvloeden. Er waren echter geen effecten op het terughalen of herkennen van woorden wanneer tryptofaan depletie na het leren van die woorden werd geïnduceerd. Selectieve aandacht, zowel voor visuele (Stroop test) als auditieve stimuli (dichotische luistertaak), en prestatie op een verbale fluency taak bleken verbeterd tijdens tryptofaan depletie. Geen effect werd gevonden op snelheid van informatie verwerking, verdeelde aandacht, planning of stemming. Deze resultaten tonen aan dat serotonine essentieel is voor de consolidatie van nieuw geleerde woorden in het lange-termijn geheugen, met name in de eerste 30 minuten na de acquisitie. Bovendien is met name de rol van serotonine in selectieve aandachtsfuncties een interessant onderwerp voor toekomstig onderzoek.

In de hoofdstukken 3, 4, 5 staan de cognitieve effecten van de twee SSRIs (Selective Serotonin Reuptake Inhibitors) sertraline en paroxetine centraal. SSRIs remmen de heropname van serotonine in het neuron en stimuleren daardoor de serotonerge neurotransmissie. De drie betreffende hoofdstukken beschrijven de resultaten van een uitgebreid onderzoek naar de subchronische effecten van sertraline en paroxetine bij gezonde vrijwilligers. Ieder hoofdstuk behandelt een specifiek aspect van de studie, elk met een specifieke onderzoeksvraagstelling en geassocieerde hypothesen. Tijdens het betreffende onderzoek werd sertraline, paroxetine en placebo elk twee weken lang toegediend aan 24 gezonde mannen en vrouwen (waarvan 21 het complete onderzoek doorliepen) in de leeftijd van 30 tot 50 jaar, volgens een dubbel-blind, cross-over design. De proefpersonen ontvingen dagelijks 50 mg sertraline, 20 mg paroxetine in de eerste week van elke medicatie periode en vervolgens dagelijks respectievelijk 100 mg sertraline en 40 mg paroxetine in de tweede week, of placebo. De metingen vonden

plaats vóór aanvang van elke medicatieperiode, en op dag 7 en 14 dagen. Tussen de medicatie perioden was steeds een wash-out periode van twee weken ingelast.

In *Hoofdstuk 3* worden eerst de cognitieve effecten van sertraline en paroxetine die gerelateerd kunnen worden aan hun niet-serotonerge farmacologische eigenschappen behandeld. Hoewel beide SSRIs voornamelijk serotonerge neurotransmissie stimuleren, hebben ze additionele, zij het relatief milde, effecten op andere neurotransmitter systemen. Sertraline heeft een licht stimulerende invloed op dopaminerge neurotransmissie, terwijl paroxetine een licht anti-cholinerge werking heeft. Cognitieve functies die verondersteld zijn gevoelig te zijn voor dopaminerge (executieve functies, motor functies) en cholinerge (lange-termijn geheugen, memory scanning) manipulaties werden getest. Beide SSRIs veroorzaakten verschillende, subtiele maar detecteerbare, cognitive veranderingen. Paroxetine verminderde het terughalen van eerder geleerde woorden uit het lange-termijn geheugen. Sertraline verbeterde de prestatie op een verbale fluency test. De zoeksnelheid in het korte termijn geheugen en de basale sensorimotorische snelheid, zoals gemeten met een memory scanning taak, was onveranderd onder invloed van sertraline of paroxetine. De resultaten worden besproken in termen van de anticholinerge en pro-dopaminerge effecten van respectievelijk paroxetine en sertraline. Verder wordt beargumenteerd dat, hoewel de gevonden effecten in gezonde vrijwilligers subtiel zijn, deze bij depressieve patiënten, en met name bij de ouderen onder hen, in versterkte mate kunnen optreden.

Hoofdstuk 4 richt zich op de effecten van sertraline en paroxetine op verschillende aandachtsfuncties, met name vigilantie (volgehouden aandacht), selectieve aandacht en verdeelde aandacht. Voorgaande onderzoeken hadden een specifieke daling in vigilantie geconstateerd na behandeling met SSRIs. In het huidige onderzoek werd de selectiviteit van dit effect verder onderzocht door de effecten van sertraline en paroxetine op verschillende aandachtsfuncties te vergelijken. Bovendien, kon de rol van serotonerge- dopaminerge interacties in dit effect onderzocht worden door de effecten van paroxetine (geen effect op dopamine) en sertraline (milde stimulatie van dopamine) vergelijken. Veranderingen in slaapkwaliteit, als mogelijke confounder voor de veranderingen in waakzaamheid gedurende de dag, werden eveneens bepaald. Paroxetine en sertraline hadden geen effect op selectieve of verdeelde aandachtsfuncties, gemeten door middel van de Stroop test en Dichotische Luistertaak. Paroxetine verminderde de prestatie op de vigilantie taak, een 45-minuten durende Mackworth Clock Test. Sertraline had geen negatief effect op vigilantie, en leek zelfs de

reactiesnelheid te verbeteren. Hoewel beide SSRIs de slaapkwiteit verminderden, leek dit niet samen te hangen met de prestatie op de vigilantie taak. Deze resultaten suggereren dat serotonerge stimulatie, mogelijk via inhibitie van het dopamine systeem, milde sedatie kan veroorzaken, hetgeen vooral tot uiting komt bij langdurige taken.

De 'Critical Flicker Fusion' (CFF) test is een veelgebruikte methode om de sederende of activerende effecten van psychoactieve middelen te meten. Een verandering van CFF is een indicator voor verandering in activatie van het centrale zenuwstelsel (CZS). Van SSRIs wordt beweerd dat zij een activerende werking hebben, een claim voornamelijk gebaseerd op SSRI geïnduceerde verhogingen van CFF waarden. Echter, serotonerge stimulatie verwijdt de pupil en pupilverwijding kan de CFF onafhankelijk verhogen. Verhoogde CFF waarde na SSRI toediening is dus mogelijk een vals positief resultaat. De mogelijke invloed van pupilverwijding op CFF veranderingen na SSRI toediening is het onderwerp van hoofdstuk 5 en 6.

In *hoofdstuk 5* worden de effecten van sertraline en paroxetine op de pupildiameter en CFF beschreven. CFF werd gemeten mét en zonder een 2-mm artificiële pupil als controle voor mogelijke veranderingen in pupil diameter. Er werd gevonden dat sertraline en paroxetine een significante pupilverwijding veroorzaakten. Desalniettemin, de CFF metingen, zowel met als zonder artificiële pupil, vertoonden geen verandering onder invloed van sertraline of paroxetine. Geconcludeerd werd dat het effect van sertraline en paroxetine op CFF neutraal is en er geen bewijs gevonden kon worden dat pupilveranderingen de CFF resultaten na SSRI toediening beïnvloeden. Echter, eveneens werd geopperd dat de invloed van pupilverwijding mogelijk afhankelijk is van het specifieke apparaat dat gebruikt wordt om CFF te meten.

In een vervolgstudie, beschreven in *hoofdstuk 6*, werd deze materie verder onderzocht. Gedurende drie perioden van 15 dagen ontvingen 24 gezonde mannen en vrouwen, in de leeftijd van 30 tot 50 jaar, sertraline (50 mg op dag 1-8, 100 mg op dag 9 – 15), citalopram (20 mg op dag 1-8, 40 mg op dag 9-15) en placebo. Deze keer werd eveneens een acute meting verricht, 5 uur na inname van de eerste capsule. Vervolgens werd gemeten op dag 8 en 15 van elke medicatieperiode. De metingen bestonden uit een bepaling van de pupildiameter in omgevingslicht met een hoge en lage lichtintensiteit, en CFF bepalingen mét en zonder 2-mm kunstmatige pupil met het apparaat zoals dat in de vorige studie was gebruikt (Euclid device). Dezelfde CFF metingen werden eveneens uitgevoerd met een ander veelgebruikt apparaat; de Leeds

Psychomotor Tester (LPT). Alle studies waarbij een verhoging van CFF na SSRI toediening werd gevonden maakten gebruik van de LPT. De huidige studie toonde aan dat sertraline en citalopram de pupildiameter significant vergrootten. De grootte van het effect was gelijk onder beide lichtcondities. Evenals in de vorige studie werden geen veranderingen gevonden in CFF waarden gemeten met het Euclid device. Echter, de CFF resultaten met de LPT lieten een daling zien na acute toediening van sertraline en citalopram, maar *alleen* wanneer gecontroleerd werd voor pupilgrootte. Op dag 8 werd geen effect gevonden, maar CFF was wederom verlaagd op dag 15, dit keer zowel met als zonder kunstmatige pupil. Deze resultaten tonen aan dat sertraline en citalopram een milde daling van CZS activatie veroorzaken en dat dit effect gemaskeerd kan worden door gelijktijdige pupilverwijding onder invloed van deze SSRIs, met name tijdens acute metingen. De invloed van pupilverwijding op CFF lijkt te verminderen bij subchronische toediening, mogelijk als gevolg van een vooralsnog onbekend adaptief mechanisme.

De hoofdstukken 7 en 8 richten zich op het effect van cafeïne op cognitieve functies, en een mogelijke interactie met leeftijd. In *hoofdstuk 7* worden de resultaten beschreven van een studie waarin het effect van cafeïne (225 mg) op cognitief functioneren in jonge, middelbare en oudere personen, wordt onderzocht. De studie werd uitgevoerd volgens een placebo-gecontroleerd dubbel-blind parallele groepen design (n=60). De drie leeftijdsgroepen (jong; 26-34 jaar, middelbaar; 46-54 jaar, oud; 66-74 jaar) waren gematched voor opleiding en geslacht. Cafeïne verbeterde de prestatie op de eerste immediate recall trial van een woordenleertaak met distractie bij middelbare proefpersonen. Bij jonge proefpersonen had cafeïne een negatief effect op de zoeksnelheid in het korte termijn geheugen. Cafeïne had geen effect op basale sensorimotorische snelheid, zoals bleek uit de Memory Scanning taak. In deze studie was de habituele cafeïne opname in de middelbare leeftijdsgroep ongeveer twee keer zo hoog als dat in de jonge of oude groep. Het optreden van onttrekkingsverschijnselen tijdens de placebo conditie zou mogelijk een daling van de prestatie in de middelbare groep kunnen verklaren.

In *hoofdstuk 8* wordt een studie gepresenteerd waarin, via een cross-over design, het effect van 100 mg cafeïne op geheugen en selectieve aandacht bij 16 middelbare (45-60 jaar) en 14 oude (60-75 jaar) gezonde mannen en vrouwen werd onderzocht. In deze studie was het habitueel cafeïnegebruik zoveel mogelijk gelijk gehouden tussen de twee leeftijdsgroepen, en dit lag op een niveau dat overeenkwam met dat van de middelbare leeftijdsgroep in hoofdstuk 7. Cafeïne had geen effect op korte of lange termijn

geheugenfuncties, gemeten met een woordenleertaak, digit span taak, en een memory scanning taak, of op selectieve aandacht gemeten met een Stroop test. Er was geen verschil in de cognitieve prestatie of het cafeïne effect tussen de leeftijdsgroepen, noch was er een invloed van habitueel cafeïnegebruik. De resultaten tonen aan dat onttrekkings-verschijnselen geen substantieel negatief effect uitoefenen op (kortdurende) cognitieve prestaties, zelfs niet bij een relatief hoge dagelijkse cafeïne inname. Het uitblijven van cognitieve effecten in de huidige studie suggereert dat een lage dosis cafeïne (100 mg) minder effectief is bij middelbare en oudere proefpersonen.

Hoofdstuk 9 eindigt dit proefschrift met een algemene discussie van de in dit proefschrift beschreven resultaten. De resultaten leveren aanwijzingen dat serotonine het cognitief functioneren in ieder geval via twee mechanismen kan moduleren. Ten eerste heeft serotonine een algemene remmende invloed op arousal niveaus. Op kortdurende taken kan deze milde vermindering van arousal tijdelijk gecompenseerd worden door de inzet van extra inspanning (effort). Vermindering van arousal kan verklaard worden door een diffuse inhibitie van andere neurotransmitter systemen, met name cholinerge, noradrenerge en mogelijk vooral dopaminerge systemen, door serotonine. Ten tweede lijkt serotonine vooral betrokken te zijn bij de opslag en consolidatie van nieuw geleerd materiaal in het lange-termijn geheugen. Dit effect is mogelijk gekoppeld aan interacties met cholinerge neuronen in de hippocampus. Wat betreft cafeïne is gebleken dat deze stof met name korte-termijn geheugenfuncties lijkt te verbeteren in mensen van middelbare leeftijd, dat dit effect niet gerelateerd is aan habitueel cafeïnegebruik en geassocieerde onttrekkingsverschijnselen, en dat dit effect mogelijk pas hogere een hogere dosis cafeïne (>100 mg) bereikt wordt. Het potentieel van cafeïne en serotonerge stoffen als cognitieverbeteraars is waarschijnlijk beperkt tot het opheffen van een suboptimaal prestatieniveau in specifieke situaties.

Dankwoord

De oplettende lezer zal, getikt in kriebellettertjes aan het begin van de meeste hoofdstukken of weggestopt tussen discussie en de referenties, een aantal namen hebben opgemerkt. Deze mensen hebben op een of andere manier bijgedragen aan de inhoud van dat hoofdstuk. Veel andere mensen waren voor dit proefschrift minstens net zo belangrijk, maar hun bijdrage is moeilijk aan een hoofdstuk of specifiek gedeelte te verbinden. Zij zijn de mensen die de condities hebben geschapen waardoor ik kon werken, schrijven en onderzoeken voor dit proefschrift. Ik blij dat ik deze mensen op het eind van dit proefschrift nog eens specifiek en persoonlijk kan bedanken.

Jelle Jolles, promotor, ik wil je danken voor de gelegenheid, het vertrouwen en de vrijheid die je me steeds gegeven hebt om onderzoek te doen. Als we elkaar spraken gaf je vaak waardevol advies en inspiratie.

Wim Riedel, co-promotor en begeleider, ik ben je vanzelfsprekend zeer dankbaar. Je opmerkingen, sturing, raad en de discussies hebben me voor een groot deel gevormd als wetenschapper. Ook buiten de universiteit was je aangenaam gezelschap, onder andere door je scherpe humor en waardevolle tips voor succesvol 'congressen'.

Anja de Bruin en Roy van de Ven, goede vrienden, dank ik voor het paranimf zijn. Anja, je kritische blik op de wetenschap, de gespreken en gezelligheid worden hier nog steeds gemist. Roy, het feit dat je als proefpersoon het tryptofaan depletie drankje zonder probleem naar binnen werkte illustreert de interesse die je altijd hebt gehad voor mijn onderzoek. Het toont bovendien aan dat als je eenmaal in ons studentenhuus geleefd hebt, je alles binnen kunt houden. Ik ben blij dat jullie 1 juni naast me staan.

Een belangrijk deel van plezierig werken is de aanwezigheid van plezierige mensen om je heen, en dat geldt natuurlijk met name voor je kamergenoten. Eef Hogervorst, nu beroemd Oxford-geleerde, maar tijdens mijn eerste jaren als promovendus mijn onovertroffen kamergenote. Dank voor alle goede raad, steun en vooral de lol die toen - en nu ook nog - het werk verlicht. Sjacko Sobczak was in de

tweede helft van mijn promotietijd mijn kamergenote, en ik haar zeer leren waarderen als iemand die op vele manieren slim is en bovendien grappig en gezellig. En als we het over lachen hebben komen we vanzelf op Brenda Jorissen, iemand bij wie het gewoon leuk vertoeven is en wiens lach aanstekelijk werkt op hele treincoupees en volle restaurants.

Een aantal andere collega's wil ik nog speciaal noemen en danken: Jan Ramaekers en Annemiek Vermeeren, beiden bescheiden maar stiekem toch briljante wetenschappers en bovendien bijzonder vrolijk gezelschap; Caroline Lamers en Sven Stapert, lotgenoten bij psychologie, bij wie als je niet oppast een lunch wel drie uur kan duren; Eric Vuurman met zijn ongeëvenaarde talent voor techniek en droge humor. Bedankt voor jullie wetenschappelijke en andersoortige bijdragen.

Edwin Klinkenberg, Petra Hurks en Mariska den Hartog plus velen hierboven genoemden, dank ik voor de vele vrijdagmiddagen en -avonden, waarop ik in hun gezelschap na menig zware wetenschappelijke werkweek kon 'unwind'-en.

Martin van Boxtel, Peter Houx, Nico Rozendaal en Elsa Misdom wil ik danken voor de vele uiteenlopende vormen van hulp, ondersteuning en raad die ze me gaven tijdens de afgelopen 4 jaar.

Ik dank ook de stagiaires en assistenten die samen met mij, en menig maal ook zonder mij, de vaak lange onderzoeksdagen draaiden en zeer veel werk hebben verzet: Brenda Jorissen en Sjacko Sobczak (daar heb je ze weer), Monique Kruizinga, Suzanne Teunisse, Natalie Bougie, Anita van Oers, Karin Eikmans en Annette Heldens.

Alle proefpersonen bedank ik voor hun grote inzet en motivatie tijdens de experimenten.

Heel belangrijk is het thuisfront geweest, mijn familie en vrienden in meer westelijke regionen. Op hun steun kon ik altijd rekenen. En ja, iedereen, mijn 'scriptie' is nu eindelijk af!

Marjolein, sinds een jaar mijn vrouw maar al veel langer de allerbelangrijkste persoon in mijn leven, zonder jou had ik dit zeker niet kunnen doen. En, hoezo zou je tijdens het afronden van je proefschrift geen tijd meer hebben voor andere dingen?

Curriculum Vitae

Jeroen Schmitt werd op 6 april 1967 geboren te Roosendaal. In 1985 behaalde hij het Havo diploma aan het Gertrudis Lyceum in Roosendaal, twee jaar later gevolgd door het Atheneum-B diploma. Vervolgens vertoefde hij als dienstplichtig militair bij de Genie een jaar in Duitsland. In 1990 startte Jeroen met de studie Gezondheidswetenschappen, afstudeerrichting Biologische Gezondheidkunde, aan de Universiteit Maastricht, waar hij in 1995 afstudeerde. Na zijn afstuderen was hij werkzaam als onderzoeksassistent bij de vakgroep Humane Biologie in Maastricht. In 1997 kreeg hij de kans om als onderzoeksmedewerker bij de vakgroep Psychiatrie en Neuropsychologie promotie onderzoek te verrichten op het gebied van de cognitieve psychofarmacologie. Vanaf 2001 is hij daar als onderzoeker aangesteld. Jeroen Schmitt is getrouwd met Marjolein Jonkman en verwacht deze zomer zijn eerste koter.

Serotonin, caffeine and cognition

Psychopharmacological studies in human cognitive functioning

A pill that enhances our ability to concentrate, think, remember and understand, a cure for cognitive incapacitating illnesses such as dementia or even mild age-related cognitive decline, the availability of 'pure' psychoactive medication without adverse cognitive effects. Although presently none of the above notions are a reality, they may and probably will be in a not too distant future. Research efforts on this area are extensive and still expanding.

This thesis' main theme is the quest for substances and targets to pharmacologically enhance cognitive performance. Given the broadness of this research area, two main focal points are singled out. These topics are the function of the neurotransmitter serotonin in human cognitive functioning and, secondly, the influence of caffeine intake on human cognitive performance. In this book, in a total of seven chapters, experimental findings in human subjects on these issues are described. Manipulations of serotonergic activity and the consequent cognitive effects provide insight in the role of serotonin in cognitive processes. Such insights aid the development of new and safer drugs. Caffeine is the most widely consumed psychoactive substance. The putative role of caffeine as cognitive enhancer, particularly in the elderly population, is investigated in two experimental studies. The main findings and implications are discussed in the concluding chapter.