UNIVERSITY OF ALBERTA

The Effects of Amphetamine in Healthy Volunteers

by

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A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF PSYCHIATRY

EDMONTON, ALBERTA

FALL 2002

UNIVERSITY OF ALBERTA

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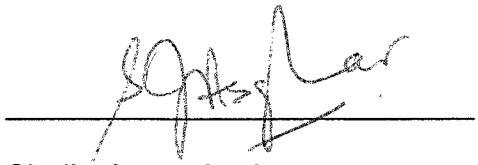
Degree:	Master of Science
Year this Degree Granted:	2002



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The undersigned certify that they have read, and recommend to the faculty of graduate studies and research for acceptance, a thesis entitled EFFECTS OF AMPHETAMINE IN HEALTHY VOLUNTEERS submitted by SHEILA JOYCE ASGHAR in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE !.

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"His power working in us can do infinitely more

than we can ask or imagine"

Ephesians 4:21

Abstract

Acute administration of the stimulant d-amphetamine produces multiple biochemical, physiological, mood, and cognitive changes similar to those seen in mania. The present study investigated the effects of 25 mg of oral d-amphetamine on physiological (blood pressure and heart rate), cognitive (reaction time and errors), subjective (mood) and biochemical (plasma amphetamine, homovanillic acid, 5-hydroxyindole-acetic-3acid, γ-aminobutyric acid, glutamate and tryptophan) changes in 25 healthy male volunteers (18-45 years) using a double-blind, placebo-controlled crossover design over 8 hours. An existing method was modified using electron-capture gas chromatography to measure plasma amphetamine levels. As well, a novel reaction time test was developed to measure time-dependent changes in selective attention and vigilance. Results indicated that peak levels of amphetamine occurred at 3.5 hours and decreased over the subsequent time period studied. These changes were mirrored in subjective, cognitive, and blood pressure changes. The changes seen in biochemicals were inconclusive although a similar pattern was observed.

Acknowledgements

I would like to thank my supervisors Drs. Peter Silverstone and Glen Baker. Dr. Silverstone was instrumental in my undertaking this project and his continual support have made this project possible. Dr. Baker's wisdom provided guidance not only in my work but also in all other aspects. I appreciate the time and interest taken by Drs. Kathryn Todd and Kathleen Hegadoren who have always inspired me to excel in my work.

I cannot find enough words to thank Dr. Veronique Tanay who provided intellectual input and spent endless hours working with me. I would like to acknowledge Drs. Susan Rotzinger, Andrew Greenshaw, Tom Synder, Chris Kelland, Mrs. Gail Rauw, Mrs. Jordyce van Muyden, and Mr. Chris Kuthe for their assistance with various aspects of my work. I would like to thank Marg Arthurs, Maizie Goh, Drs. Alan Wilman, and Peter Allen for providing office space.

My friends Michele Ulrich, Tina O Donnell, Bing Guo, and Kasia Rembarz never failed to provide encouragement at all times. I would like to thank my pastor Bob Peele, my parents Michael and Agnes, sister Rina and brother-in-law Dave who stood behind me all the way. The completion of my work would not have been possible without the unwavering support of my husband, Horacio. Finally, I am grateful to the Lord Almighty for this opportunity, as I believe everything occurred according to His plans and purpose for my life.

Dedication

This thesis is dedicated to the loving memory of my grandparents Sardar Begam and Bharkat Kharku Mall, Mariam and Allah Rakha Asghar, and to my Uncles Sam, Eric and Ezra.

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Abbreviations

ARCI	Addiction Research Center Inventory
AIRS	Amphetamine Interview Rating Scale
ANOVA	analysis of variance
ADHD	attention deficit hyperactivity disorder
BP	blood pressure
BDH	British Drug Houses
X	by
CPT	Continuous Performance Test
CMI	Cornell Medical Index
CO	crossover
°C	degree Celsius
DAF	delayed auditory feedback
d-amphetamine	dextro-amphetamine
(+)-amphetamine	dextro-amphetamine
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
DSST	digit symbol substitution test
DOPAC	3,4-dihydroxyphenylacetic acid
DA	dopamine
DB	double-blind
ECG	electrocardiogram
EEG	electroencephalogram

ECD	electron capture detection
EDTA	ethylenediaminetetraacetic acid
f	female
Fig	figure
FID	flame ionization detector
x	for
FMRI	functional Magnetic Resonance Imaging
G	gauge
GABA	γ-aminobutyric acid
GC	gas chromatography
GLC	gas liquid chromatography
GSC	gas solid chromatography
$t_{1/2}$	half-life
HR	heart rate
HP	Hewlett Packard
HPLC	high perfomance liquid chromatography
HVA	homovanillic acid
hr	hour(s)
5-HIAA	5-hydroxyindole-3-acetic acid
5-HT	5-hydroxytryptamine (serotonin)
In	inche(s)
I.S	internal standard
I.V.	intravenous

K_2CO_3	potassium carbonate
Li	lithium chloride
m	male
MS	mass spectrometry
µl	microlitre(s)
mg	miligram(s)
mg/kg	miligram per kilogram
ml	mililiter
mm	milimetre(s)
mm/Hg	milimetre(s) of mercury
min	minute(s)
MAC	modified Nowlis Mood Adjective Checklist
MAO	monoamine oxidase
MO	Missourie
ng	nanogram
NJ	New Jersey
NH	nitrogen hydride
NPD	nitrogen phosphorus detector
NA	noradrenaline (norepinephrine)
OH	hydroxy
PAL	Paired Associate Learning test
PFBSC	pentafluorobenzenesulfonyl chloride
PI	phosphatidylinositol

POMS	Profile of Mood States
PM	pursuit meter
^{63}Ni	radioactive nickle
R	randomized
RT	reaction time
rpm	rotations per minute
s	second(s)
SC	skin conductance
SEM	standard error of the mean
SH	sulfide
SCOT	support coated open tubular
pH	symbol for the logarithm of the reciprocal of the hydrogen ion concentration
pKa	symbol for the negative logarithm of the ionization constant of an acid
temp	temperature
VAS	Visual Analogue Scale
WCOT	wall coated open tubular
w/v	weight by volume
W	winged
WS	within subjects

CHAPTER 1

GENERAL INTRODUCTION: THE EFFECTS OF D-AMPHETAMINE AS A MODEL FOR MANIA IN HEALTHY VOLUNTEERS

1.1 Introduction

The work presented in this thesis is focussed on d-amphetamine and its suitability as a model of mania in humans. An electron-capture chromatographic method for the measurement of plasma d-amphetamine and a novel reaction time test developed and applied to studies in healthy volunteers receiving d-amphetamine are discussed.

1.2 General overview of amphetamine

1.2.1 HISTORICAL BACKGROUND

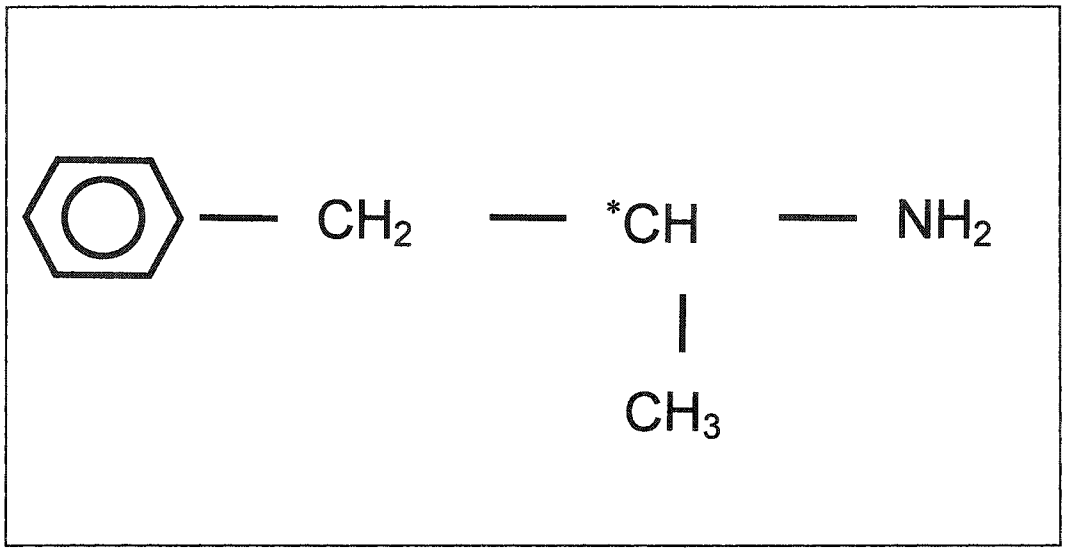
Amphetamine was first synthesized by Edeleanu in 1887. Interest in the molecule emerged steadily once similar compounds were isolated from the herb *Ephedra vulgaris* (Ma Huang) which contained ephedrine and from the evergreen shrub, khat, containing cathinone (Caldwell, 1980; Feldman, 1997). By the 1940s amphetamine was widely used by soldiers during the Second World War to maintain alertness during prolonged periods on duty (Caldwell, 1980; Feldman, 1997). The most famous amphetamine addict was none other than Adolf Hitler (Brust, 1993). By the 1960s, the abuse of amphetamine emerged as a major problem particularly in the United Kingdom, United States, Japan and Sweden (Caldwell & Sever, 1974). The novelty of this drug has been surpassed by cocaine and more recent designer drugs such as Ecstasy (3,4-

methylenedioxymethamphetamine-MDMA) in recent years (Feldman, 1997).

1.2.2 PHARMACOLOGY

Amphetamine, a racemic β-phenylisopropylamine, possesses powerful CNS stimulant actions and peripheral α and β adrenergic actions similar to other indirect acting sympathomimetics (Hoffman, 1996). The basic skeleton of amphetamine (Figure 1-1) is crucial to most of its pharmacological and biochemical properties and consists of an unsubstituted phenyl ring, an α-methyl group, a two-carbon side chain between the phenyl and amine moieties, and a primary amine group (Biel, 1970). Amphetamine exists as two stereoisomeric forms: d-amphetamine (dextro-amphetamine; trade name Dexedrine), and l-amphetamine (levo-amphetamine) (Feldman, 1997). d-Amphetamine is the more potent of the two and has been the focus of this project described in this thesis. The simplicity of its structure has made the molecule a target for molecular modification, accentuating some effects, while abolishing others (Biel, 1970).

Figure 1-1: Structure of d-amphetamine



The unsubstituted phenyl ring, the α-methyl group, two-carbon side chain, and the primary amine group determine the pharmacological and biochemical properties of this compound. The symbol * denotes the chiral carbon.

1.2.3 MECHANISMS OF ACTION

Amphetamine is an indirect agonist of the catecholaminergic systems and has the combined ability to release both dopamine and norepinephrine from presynaptic endings, block their re-uptake and inhibit their catabolism (Groves & Rebec, 1976).

The effects of amphetamine on catecholamine release are concentration-dependent: low doses (1-5 mg/kg) release dopamine from the cytoplasm, whereas high doses of amphetamine release both vesicular and cytoplasmic dopamine (Seiden et al., 1993). Amphetamine also releases serotonin (Sloviter et al., 1978) and may inhibit monoamine oxidase at doses higher than those needed for catecholamine release (Groves & Rebec, 1976).

1.2.4 PHARMACOLOGICAL EFFECTS

The sympathomimetic properties of amphetamine include a rise in blood pressure with reflex slowing of the heart. It displays potent anorexigenic, hyperthermic and, more importantly, CNS-stimulant action (Biel, 1970). In humans, 10 to 30 mg of oral d-amphetamine produces alertness, euphoria, increased motor activity, improved co-ordination, and greater physical endurance as well as pupillary dilatation (Brust, 1993). On chronic administration of higher doses, a paranoid psychosis may be seen (Biel, 1970; Caldwell & Sever, 1974).

The role of specific neurotransmitters in mediating the behavioural effects of d-amphetamine in humans have not been well characterized (Brauer, 1995). However, the behavioural and physiological effects, which are dose-dependent, are believed to be mediated via the neurotransmitters dopamine, norepinephrine (Groves & Rebec, 1976), and serotonin (Sloviter et al., 1978). The arousal seen after amphetamine administration is believed to be mediated via central pathways utilizing dopamine as neurotransmitter (Carlsson, 1970).

1.2.4.1 Pharmacokinetics/Plasma concentration-effect relationship

Amphetamine is a weak base, with a pK_a of approximately 9.9; with a high lipid solubility and a large volume of distribution (Caldwell, 1980). Its oral absorption is slow as most of the drug is positively charged in the acidic stomach and, therefore, unable to pass across the gastric membranes (Feldman, 1997). After a typical dose of 5-15 mg, behavioural and physiological effects are experienced within 30 min (Feldman, 1997). Depending on the urinary pH, the elimination half-life ($t_{1/2}$) can range from 7 to 30 hr (Feldman, 1997). The peak concentration of plasma amphetamine in man following 10 mg d-amphetamine is around 50 ng/ml (Vree, 1970).

Amphetamine is lipophilic and can penetrate the blood-brain barrier with ease. The onset of pharmacological effects occurs when critical concentrations are reached at the site of action i.e. at the peripheral or

central receptors. This is dependent upon the release rate of the pharmaceutical preparation, the route of administration, and the distribution kinetics at the site of action (Farre & Cami, 1991). Therefore, greater lipid solubility is associated with more rapid entry into the brain and is associated with a greater potential for abuse (Farre & Cami, 1991). Other properties such as rapid absorption, high oral bioavailability, short half-life, low protein binding, and small volume of distribution play an important role in the abuse of drugs (Farre & Cami, 1991).

1.2.5 CLINICAL USES

Amphetamine is used in the treatment of narcolepsy, and attention deficit hyperactivity disorder (ADHD) (Barkley, 1997; Caldwell, 1980). The use and withdrawal of amphetamine may invoke features similar to those seen in mania (Mamelak, 1978) however, may not be a good model for bipolar disorder. In higher doses, amphetamine use may mimic paranoid schizophrenia, making it a useful drug model of this illness (Mamelak, 1978).

1.3 Bipolar disorder and mania

Bipolar Disorder is recognized as a serious mood disorder with considerable economic and social burden. It is described as devastating, chronic and often deteriorating (Goldberg & Harrow, 1999). Bipolar

disorder is characterized by cycles between elated (manic) and depressed mood states (APA, 1994).

1.3.1 PREVALENCE

It is a sobering fact that bipolar disorder remains a major public health problem, with a lifetime prevalence of 1 to 1.6% in the general population, most often targeting young adults between 20 and 25 years of age (Hilty et al., 1999). This age range tragically represents the most vocationally productive and child bearing years (Bowden, 1998).

1.3.2 SYMPTOMATOLOGY

Patients with bipolar disorder experience phases of excitement and depression that can occur in an alternate fashion or in various admixtures (Schatzberg, 1998). Bowden and associates (1994) noted that symptoms such as hyperactivity, explosive temper, impaired judgement, insomnia, disorganized behaviour, hypersexuality, grandiosity, and often delusions caused severe functional impairment in patients with mania. This often culminated in divorce, job loss, indebtedness, alienation from family, friends, and co-workers, and other problems of living (Bowden et al., 1994; Manji & Potter, 1997). Eighty percent of bipolar patients, who have had a single episode of mania, will have one or more subsequent episodes (Gelenberg & Hopkins, 1993). These recurring episodes progressively cause a deterioration of inter-episode functioning. Approximately 25% of

patients attempt suicide at some point during their illness, with suicide completion rates of about 15% (Dilsaver, 1989; Gelenberg & Hopkins, 1993).

1.3.3 PATHOPHYSIOLOGY AND TREATMENT

As yet, researchers have been unable to develop a single hypothesis to fully explain the mood changes seen in bipolar disorder. Mania has been successfully treated with mood stabilizers such as lithium, carbamazepine and valproic acid, although neuroleptic agents and potent sedatives are more effective in the agitated phase (Baldessarini, 1995).

1.4 Models of mania

1.4.1 DRUG MODELS IN ANIMALS AND HUMANS

McKinney (1974) proposed four criteria in order to evaluate an animal model. These criteria included similarity of inducing conditions and behavioural states, common underlying neurobiological mechanisms, and reversal by effective treatment.

Animal models for mania are based upon a state of hyperactivity induced by drugs such as amphetamine and its reversal by pretreatment with lithium (Murphy, 1977). Robbins & Sahakian (1980) have further evaluated the other major features of mania, namely elation and irritability in animal models. However, the large gaps in behavioural repertoires

between man and non-human species severely limit the application of these potential models to complex disorders (Murphy, 1977).

In human studies, acute d-amphetamine administration produces a syndrome that mirrors the manic phase of bipolar disorder (Gessa et al., 1995). Studies have also shown that the mood elevating and stimulating effects of d-amphetamine can be blocked by lithium (Angrist & Gershon, 1979; Flemenbaum, 1974; Van Kammen & Murphy, 1974; Van Kammen & Murphy, 1975), pimozide (Silverstone et al., 1980), and α-methyl-*para*-tyrosine (Jonsson et al., 1971). Further, withdrawal of d-amphetamine may cause depression and exhaustion, apathy and fatigue, symptoms often ameliorated by antidepressants (Watson et al., 1972).

1.4.2 EFFECTS OF D-AMPHETAMINE IN HEALTHY VOLUNTEERS

In human research studies, d-amphetamine has been reported to produce elevated blood pressure and heart rate (Angrist et al., 1987; Brauer et al., 1996; Caldwell & Sever, 1974; Caldwell, 1996; de Wit et al., 1997; Hamilton et al., 1983; Hoffman, 1996; Jacobs & Silverstone, 1986; Martin et al., 1971; Morselli et al., 1976; Silverstone et al., 1983; Slattum et al., 1996), elevated blood pressure alone (Dommisse et al., 1984), elevation of mood, in particular arousal as measured using Visual Analogue Scales (de Wit et al., 1997; Miller & Griffith, 1983; Silverstone et al., 1980; Zacny & de Wit, 1989), and elation (Hamilton et al., 1983; Hoffman, 1996; Jacobs & Silverstone, 1986). Other changes induced by

d-amphetamine include a decrease in reaction time (Rapoport et al., 1978; Servan-Schreiber et al., 1998). Some of these studies and their results have been summarized in Table 1-1.

1.4.3 SIMILARITIES BETWEEN MANIA AND EFFECTS OF D-AMPHETAMINE

Studies in manic patients have shown increases in heart rate (Lake et al., 1982) and skin conductance (SC) (Hemsley & Philips, 1975), and increases in plasma norepinephrine (Lake et al., 1982) and cortisol levels (Cookson et al., 1980). As well, response to pharmacological challenges with drugs such as pimozide (Cookson et al., 1981), lithium carbonate (Schou, 1968), and α-methyl-*para*-tyrosine (Brodie et al., 1971) have reduced the manic symptoms. The similarities between symptoms of mania and effects of d-amphetamine have been highlighted in Table 1-2.

1.4.4 RELATIONSHIP BETWEEN PLASMA D-AMPHETAMINE LEVELS AND BIOCHEMICAL, BEHAVIOURAL, PHYSIOLOGICAL, AND COGNITIVE MEASURES

Few studies (Table 1-3) have investigated the relationships between plasma levels of d-amphetamine, behavioural, and biochemical effects, and their time courses in normal adults.

Table 1-1: Effects of oral d-amphetamine in healthy volunteers

AUTHOR	STUDY DESIGN	DEPENDENT MEASURES	RESULTS
de Wit et al. 1997	Within subjects (WS), double-blind (DB), in 42 subjects on 10, 20 mg of d-amphetamine using isolated and social conditions over 60, 120, 180 240 min	• Addiction Research Inventory (ARCI), Visual Analogue Scale (VAS), scales • Digit Symbol Substitution Test (DSST), eye-hand computerized test, observer rating • Blood pressure (BP), heart rate (HR), temperature (temp)	• ⇧ arousal, positive mood, drug liking, and ⇩ratings of hunger • Dose-dependent ⇧ systolic BP, HR, psychomotor performance, stimulated (VAS) and euphoria (ARCI) • ⇩ number of mistakes • ⇧ temp and HR in social conditions
Brauer & de Wit, 1997	DB, 12 [6 male (m), 6 female (f)] on 10, 20 mg d-amphetamine or placebo preceding 8 mg pimozide or placebo randomly over 6 sessions for (x) 5 hr	• Profile of Mood States (POMS), ARCI, VAS, • BP, HR • Digit Symbol Substitution Test (DSST), mistakes (eye-hand coordination test)	• ⇧ in arousal and vigour (POMS) • ⇧ in drug liking and stimulation (VAS) • ⇧ BP, HR • ⇧ number of substitutions on DSST • ⇩ number of mistakes on eye-hand coordination test • No change with pimozide
Brauer & de Wit, 1996	W-S, DB in 10 subjects on 0,10,20 mg d-amphetamine before and after 3 hr preceding pimozide (0,1,2 mg) in 9 weekly sessions x 5 hr	• POMS, ARCI, VAS • HR, BP • DSST, reaction time (RT) test, eye-hand coordination test, • Plasma d-amphetamine levels	• ⇧ in anxiety (VAS) and euphoria (ARCI) • ⇧ in BP, HR • No sig. effect on pimozide pretreatment.except fatigue (POMS) • Plasma levels of d-amphetamine (mean 10 mg=27.5 ng/mL, mean 20 mg=46.1 ng/mL
Slattum et al.1996	DB, placebo-controlled, crossover in 8 m on 5,10 or 20 mg d-amphetamine over 0,1,1.33,2, 2.33,3,3.33,4,6, 8,12,18 ,24 hr	• ARCI • EEG or electroencephalogram , visual continuous performance task (CPT), finger tapping • Serum prolactin, d-amphetamine • BP, HR	• ⇩ in serum prolactin with 5mg • 15.4 ng/ml at 2.1hrs (5 mg), 30.8 ng/ml at 2.6hrs (10 mg) and 54.8 ng/ml at 2.8hrs • ⇧ diastolic BP, HR

AUTHOR	STUDY DESIGN	DEPENDENT MEASURES	RESULTS
Caldwell 1996	6 m and 6 f 30 mg d-amphetamine. (divided doses) at 23 intervals from 12:20am to 10:20pm	• BP, HR	• ⇧ systolic BP in m 1-5 hr, heart rate, • ⇧ systolic BP in f 1-6 hr • ⇧ diastolic BP in m and f 2-6 hr
Brauer & de Wit, 1995	DB, randomized (R), WS in 12 subjects (8m and 4f) on 20 mg d-amphetamine after pretreatment with 4 mg pimozide, 3/6 mg fluphenazine, or 0.5 mg prazosin over 3 studies x 3 hr at hourly intervals	• POMS, ARCI, VAS • DSST, 2 computer tests-RT • BP/ HR	• ⇧ euphoria (ARCI), elation and positive mood (POMS), friendliness and vigor, feel drug, like drug, stimulated and want more (VAS scale) • ⇧ BP • ⇧ performance on DSST & eye-hand coordination • ⇩ sedation (ARCI) • ⇩ hunger (VAS) • Trend for pimozide to ⇩ d-amphetamine induced euphoria ($p<0.06$) • No significant change with fluphenazine or pimozide on euphoria
Zacny & de Wit, 1989	R, DB, WS in 12 adults (6 f, 6m) on 10 mg d-amphetamine in fed versus fasting state, over 1,3 and 6 hr in 8 sessions	• POMS, ARCI and VAS	• ⇧ in elation and vigor in both fast and fed states • ⇧ ability to identify drug in fast state
Jacobs and Silverstone 1986	DB, cross over (CO) in 24 m on 20 mg d-amphetamine x 4 hr half hourly over two sessions; 12 had 2 /4 mg pimozide prior to d-amphetamine DB	• VAS log skin resistance score (SC) • BP, HR • Plasma cortisol, d-amphetamine	• Max drug level occurred 2-4 hr • ⇧ systolic BP, HR • ⇧ cortisol after 2 hr

AUTHOR	STUDY DESIGN	DEPENDENT MEASURES	RESULTS
Kupietz et al.1985	6 adults on 0.25 mg/kg d-amphetamine at hourly intervals x 5 hr	• Paired associate learning task (PAL) • Plasma d-amphetamine levels • Measure of mood (VAS)	• Peak levels at 2-3 hr • ⇩ learning errors 2-3 hr • Correlation between mean errors and mean d-amphetamine over the hourly sessions –0.96 • ⇧ response in happy and energetic state at 2 hr
Rapoport et al. 1980	R, DB, CO in 14 normal, 15 hyperactive boys and 31 men on 0.5 mg/kg (15) and 0.25 mg/kg (16) over 3 sessions weekly at baseline and 2.5 hr	• Motor activity • SC RT test • Sustained–attention using Rosvold's Continuous Performance Test (CPT) • Learning Task • Behaviour observation (Children's Psychiatric Rating Scale) • Self rating scale (van Kammen-Murphy Mood Scale) • Speech–Communication Task	• ⇩ motor activity (low dose) • ⇧ vigilance (CPT) (high dose) • ⇩ in errors of commission or omission errors (high dose) • Euphoria and ⇧ activity in adults,⇧ "feel funny" and "tired" items in boys • ⇧ hypoactivity in boys and ⇩ hyperactivity in hyperactive boys • ⇧ performance on both free and recall • ⇩ reaction time (low dose) • ⇧ memory & ⇧ story telling time in hyperactive boys and high dose adults • ⇩ in speech not task directed in hyperactive and low dose adults
Silverstone et al, 1980	8 f on 10 mg d-amphetamine with/without pimozide for 4 weekly x 6 hr in hourly sessions	• VAS • HR	• ⇧ arousal ratings (max 2 hr after) • ⇩ arousal when pimozide preceded d-amphetamine • ⇩ in hunger with d-amphetamine not affected by pimozide
Evans et al. 1976	R, DB in 12 m on 0,5,10,15 mg/70kg d-amphetamine over weekly sessions at 60 and 90 min	• BP, HR • Wobble Board (standing steadiness) • Pursuit Meter (PM) for attentive motor performance • Delayed Auditory Feedback (DAF) • Cornell Medical Index (CMI)	• ⇧ BP, with dose • no change in HR • ⇧ in stability (eyes closed) • ⇧ performance of PM • no change in DAF • ability to recognize drug at higher doses • ⇧ in anxiety, ⇩appetite

AUTHOR	STUDY DESIGN	DEPENDENT MEASURES	RESULTS
Domino et al. 1972	R, DB, CO in 6 m on 10 mg oral d-amphetamine two sessions one week apart	• Clinical Quantitative Neurological Examination (Tortellotte et al 1965) - resting and sustained tremor, precision hole steadiness, constant force tracking task, random tracking task, critical tracking task	• No sig changes in tremor, resting and sustained or precision hole steadiness • ⇧ performance in compensatory tracking tasks (sustained attention and motor coordination)
Martin et al. 1971	DB,M over 24 hr in 30 subjects on 7.5,15,30 mg per 70 kg d-amphetamine over varying time intervals comparison between d-methamphetamine, methylphenidate, phenmetrazine and l-ephedrine	• BP, HR, respiratory rate, rectal temp, pupillary diameter • ARCI • Urinary catecholamines	• ⇧ BP • ⇧ HR negative correlation between HR and BP except for methylphenidate • ⇧ respiratory rate • ⇧ temp • ⇧ pupillary dilatation except ephedrine • ⇩ appetite, sleep time

DB=double blind; R=randomized; CO=crossover; WS=within subjects; POMS=profile of mood states; ARCI=addiction research center inventory; VAS=visual analogue scale; HR=heart rate; BP=blood pressure; temp=temperature; DSST=digit symbol substitution test; RT=reaction time; EEG=electroencephalogram; CPT=Continuous Performance Task; SC=skin conductance; PAL=Paired Associate Learning task; PM=pursuit meter; DAF=delayed auditory feedback; CMI=Cornell Medical Index; x=for; m=male; f=female

Table 1-2: Comparison between the symptoms of mania and effects produced by the administration of oral d-amphetamine in humans

Dependent measure	Mania	Amphetamine
SUBJECTIVE		
• Elation	⇧	⇧
• Irritability	⇧	⇧
• Alertness	⇧	⇧
• Energy	⇧	⇧
• Restlessness	⇧	⇧
• Mental speed	⇧	⇧
• Sleep	⇩	⇩
PHYSIOLOGICAL		
• Pulse	⇧	⇧
• Blood pressure	⇧	⇧
• Skin conductance	⇧	⇧
BLOOD		
• Cortisol	⇧	⇧
RESPONSE TO PHARMACOLOGICAL AGENTS		
• Pimozide	⇩	⇩
• Lithium carbonate	⇩	⇩
• α-methyl-para-tyrosine	⇩	⇩

Although the table shows many similarities between mania and effects of amphetamine, it is important to consider that the neural mechanisms of mania is uncertain and not well defined.

Brown et al. (1978) correlated serum d-amphetamine levels with elation and vigor as measured by the MACL (modified Nowlis Mood Adjective Checklist). Angrist and colleagues (1987) noted a positive correlation between systolic blood pressure and plasma d-amphetamine levels with 35 mg d-amphetamine administration; this correlation was not seen with 17.5 mg of d-amphetamine. With the lower dose, a positive correlation was observed between energy using visual analogue scales and plasma d-amphetamine levels while negative correlations were observed between diastolic blood pressure and heart rate, and plasma d-amphetamine levels. These researchers also noted a late dissociation between behavioural and physiological effects and plasma levels of d-amphetamine in that while the plasma levels were still rising, the behavioural effects had declined (Angrist et al., 1987). This pattern was also noted in a similar study by Brauer and associates (1996). However, both these studies lacked a placebo group.

Previous research studies have investigated the relationship between the biochemical effects of d-amphetamine and its behavioural responses. Dommisse et al. (1984) reported an increase in serum homovanillic acid (HVA) in six of the ten subjects on 30 mg of d-amphetamine at 120 min, but this increase did not reach statistical significance. No correlation between HVA and behavioural response was found.

Table 1-3: Relationship between plasma d-amphetamine levels and dependent measures

AUTHOR	STUDY DESIGN	PLASMA D-AMPHETAMINE AND OTHER MEASURES	RESULTS
Brauer et al. 1996	6 subjects for 24 hr on 20 mg d-amphetamine	• 40 ng/ml at 4 hr and lasts till 24 hr • peak BP at 3 hr (with 15-20 ng/ml of amphetamine) • peak HR > 6 hr peak euphoria Profile of Mood States (POMS) and Visual Analog Scale (VAS) at 1.5-2 hr with decline by 6 hr	• No correlations calculated
Angrist et al. 1987	Between subjects/double -blind (DB), in 7 subjects on: 0.25 mg/kg (17.5 mg) of d-amphetamine 0.5 mg/kg (35 mg) of d-amphetamine.	• 39.6 ng/ml at 3hr • peak BP at 1 hr • peak subjective effects at 2 hr • 67.25 ng/ml at 4 hr • peak BP at 2 hr • peak HR > 4 hr • peak energy at 2 hr	• Negative correlation with plasma levels and diastolic BP and HR at 3 hr and positive correlations with plasma levels and energy • Positive correlations between plasma levels and systolic BP at 2 hr
Domisse et al. 1984	DB, crossover (CO) in 10 subjects (4 f) for 3 hr on 30 mg of d-amphetamine	• 60.1ng/ml at 2 hr • 2 hr increase in BP • no sig. change in HR • increase in homovanillic acid (HVA) (not sig) • peak plasma growth hormone at 1.5 hr	• Positive correlations between euphoria using Amphetamine Interview Rating Scale (AIRS) and diastolic BP
Morselli et al. 1976	DB, controlled, CO in 6 subjects on 20mg d-amphetamine for 12 hr	• 36 ng/ml at 3 hr 15 min	• none measured

DB=double-blind; CO=crossover; f=female; AIRS=Amphetamine Interview Rating Scale, POMS=Profile of Mood States; BP=blood pressure, HR=heart rate; VAS=Visual Analog Scale; homovanillic acid (HVA)

1.4.5 PROBLEMS WITH THE D-AMPHETAMINE MODEL OF MANIA

Results from different research groups are inconsistent as to the effects of d-amphetamine administration from individual to individual. Other problems include variable doses of d-amphetamine used in these studies, too few measures in a single study, small numbers of volunteers with inclusion of both male and female volunteers, and a short time duration for measurements to be made. To add to this problem, is the dissociation between plasma d-amphetamine levels and its clinical effects, which have not been thoroughly investigated, in healthy volunteers.

1.5 Relevant analytical techniques

1.5.1 GAS CHROMATOGRAPHY

The separation of two or more compounds based on their distribution between a stationary and a mobile phase is termed chromatography (Poklis, 1989). In gas chromatography or GC, the mobile phase or carrier gas containing the mixture of compounds percolates over the stationary phase contained in a narrow tube (the column) which is most often a high-boiling, virtually nonvolatile liquid (Coutts et al., 1985).

The components of a mixture in solution are carried through the column and separate from one another according to their partition coefficients between the carrier gas and stationary phase. Each

component that elutes from the column is detected and displayed as a peak on the chromatogram. The interval between the time of injection and the apex of the recorded peak is called the retention time of the compound and is characteristic of the compound for the GC conditions used (Baker et al., 1982). The retention time may change according to the oven temperature changes, the nature and quantity of stationary phase, the carrier gas–flow rate, as well as the column length and diameter (Coutts et al., 1985).

1.5.1.1 Instrumentation

The gas chromatograph consists of a carrier gas with flow regulators; a heated injection port; a column in a temperature-controlled oven; a detector; and data recorder. The inert carrier gas (usually helium, nitrogen or hydrogen) carries the mixture of compounds through the system.

1.5.1.2 Detectors

Although there are several kinds of detectors that can be used, but only the electron-capture detector (ECD) and mass spectrometer will be discussed here since they were used in the project described in this thesis. With the former, a radioactive isotope (usually ^{63}Ni) releases beta particles that collide with the carrier gas molecules, producing low energy electrons that are collected on electrodes, producing a small, standing

current. These electrons are captured by components of the sample with high electron affinity, causing a loss of standing current which is shown by the recorder as a peak on the graphic output (Coutts et al., 1985; Poklis, 1989). The sensitivity of the ECD is as little as 1 picogram of analyte (Poklis, 1989).

The mass spectrometer can be coupled to a gas chromatograph to serve as a specialized detector. The sample molecules in the gas phase are bombarded with high energy electrons so that they are shattered into ionic fragments, which are separated, and detected according to their atomic masses. The mass spectrum so generated is characteristic of the molecule analysed and is usually displayed as the different masses of the charged fragments and their relative abundance (Poklis, 1989).

1.5.1.3 Columns

Most GC columns currently used are fused silica capillary columns. Wall-coated open tubular (WCOT) and support-coated open tubular column (SCOT) are available. The difference between the two lies in the coating of the inner glass surface of the column, i.e. in the WCOT the liquid phase lies directly on the inner glass without a solid support, whereas in the SCOT the solid support which lies on the inner glass is coated with the liquid phase (Coutts et al., 1985). Fused silica columns are popular due to their inertness, durability, and flexibility and were employed in the analysis of plasma d-amphetamine in the following chapter.

1.5.1.4 Injection systems

The kinds of injection systems that are currently employed include the split and the splitless systems (Coutts et al., 1985). In the former, the gas is split outside the column so that only a small portion of the sample enters the column, thus eliminating the overloading of the column. In the latter, the gas is vaporized in a glass-lined tube extending from the septum cap to the column. This method is used for the analysis of very dilute and wide boiling-range samples (Coutts et al., 1985).

1.5.1.5 Derivatization

In gas chromatography, derivatization is used to chemically modify a molecule that may not be directly amenable to analysis, so that the newly formed product has properties that will permit its analysis (Knapp, 1979). The formation of such derivatives often involve the replacement of the reactive hydrogen atom of polar moieties such as NH, OH, and SH by chemical procedures such as acylation, silyation or condensation (Coutts et al., 1985). Such procedures usually increase the volatility of a compound, making it more amenable for analysis by GC. Such derivatization may also increase sensitivity or selectivity, for a given detection. In addition, derivatives may have less tailing and sharper resolution than the parent compound or provide greater stability in the case of thermally labile compounds (Poklis, 1989).

1.5.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

In high performance liquid chromatography (HPLC), the separation of a mixture of compounds is achieved by flowing a liquid mobile phase under pressure over a stationary phase in a column via their differential equilibration between the mobile and stationary phases. Detectors used for HPLC in this study were fluorescence detection for plasma γ-amino butyric acid (GABA) and glutamate; and electrochemical detection for the biogenic amines.

1.5.3 COGNITION

1.5.3.1 Attention

Attention is a broad term referring to a variety of cognitive phenomena (McDowd, 2000). Strub and Black (1988) have further defined attention as an individual's ability to focus awareness and to attend to a selected environmental stimulus.

1.5.3.2 Vigilance

The ability to sustain attention over an extended period of time is termed vigilance or concentration. Alertness, on the other hand, refers to basic arousal and the ability to respond to any stimulus in the environment. Therefore, an alert individual is not necessary attentive and,

conversely, an attentive person may not necessarily be vigilant (Strub, 1988).

Vigilance is measured by hits, commissions, and reaction time on the traditional Continuous PerformanceTest (CPT).

1.5.3.3 Continuous Performance Test

A CPT is simply a 'vigilance task' used to study sustained attention and to measure 'attention deficits' (Ballard, 2001). The traditional CPT involves a response by an individual by pressing a computer key when a critical signal appears. This critical signal may be specified symbols in a sequence of symbols presented on a computer screen. An omission error occurs when there is failure to press the key on presentation of the critical signal. If the key is pressed at any other time, this constitutes a commission error (Ballard, 2001). The latency between the critical signal onset and the individual's response is termed the individual's reaction time.

1.5.3.4 Factors affecting CPT performance

There are three main categories of factors that may act directly or interactively affect CPT performance. The first includes task parameters such as task duration, infrequent critical signals, and low signal–to-nonsignal ratios, multiple sources of information, low intensity, and brief or degraded stimuli. The speed of stimulus presentation is the most potent

factor, usually confounded by inter-stimulus interval (ISI) and/or stimulus duration.

The second category of factor includes individual characteristics such as age, sleep deprivation, use of CNS stimulants or depressants, socio-economic status, academic achievement and presence of clinical diagnoses of schizophrenia, attention deficit hyperactivity disorder (ADHD), dementia, depression and/or anxiety (Ballard, 2001). The third category includes environmental or situational conditions such as temperature, crowding, noise and other conditions. All these factors may interact, producing differential effects on different performance measures.

1.5.3.5 Selective attention

The great American psychologist and philosopher William James wrote more than a century ago (1890) that selective attention is the taking possession by the mind, in clear and vivid form, of one out of what seems several simultaneously possible objects or trains of thought (cited by Hartley, 1992).

1.5.3.6 Visual spatial paradigm

The act of attending to a particular location in the visual field, or visual spatial attention, has been studied extensively in animals (Posner, 1994). Normal vision involves fixating the eyes on an interesting object; attention may be fixed there, or moved around the visual field until another

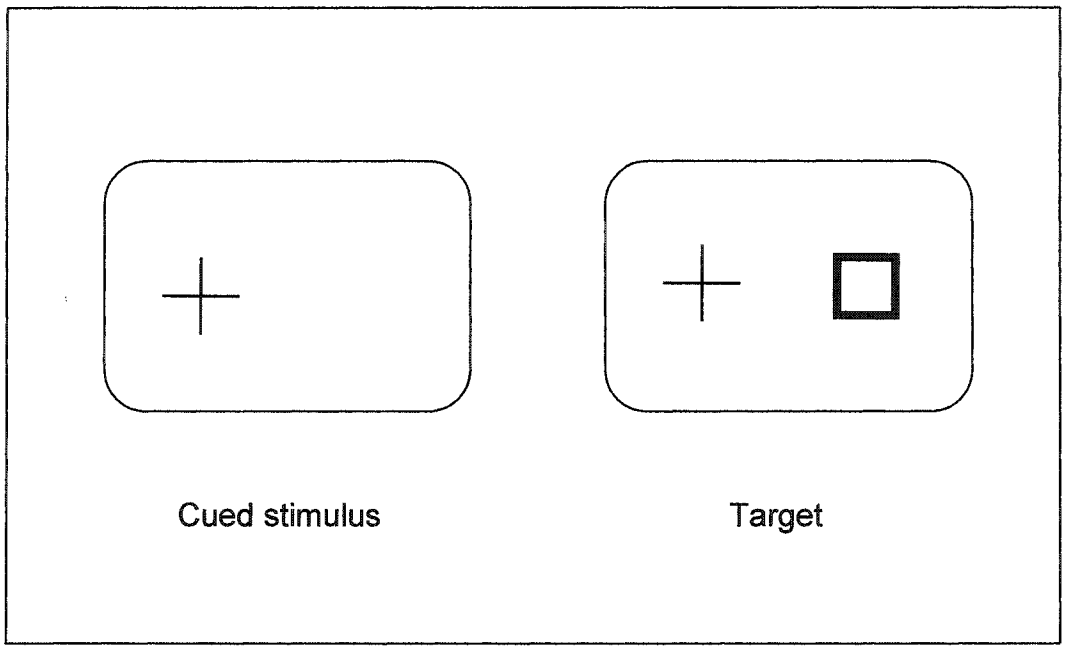
interesting object is seen that may become the target for the next eye movement. Thus, fixating the eyes to a particular spot does not necessarily mean that the attention is focused on that exact spot, but the eyes may move to the location where they are looking at or to any other single area of the visual field (Posner, 1994). These shifts in attention or covert attention may occur independently of eye movement and are fairly rapid (<50 ms) compared with eye movements (200-250 ms).

A useful model using a cued stimulus allows selective attention to be studied in the absence of head or eye movements that may affect reaction times. A cue is used to direct attention and may be presented at the future location of the target or at another place but indicating where the target is most likely to appear (Posner, 1994). The cue may be a central or a peripheral one; the former requires the subject to knowingly choose where to orient, unlike the peripheral cue, which draws the subject's attention more automatically. The paradigm employed in the current study is illustrated in Figure 1-2.

1.5.3.7 Software

Super Lab version 1.04 from Cedrus Corporation, Phoenix, Arizona was used to develop this design.

Figure 1-2: Visual Spatial Paradigm



The cued stimulus is presented as a black cross. The target is presented as a black, thick square with a cross. The volunteer is asked to press the space bar as soon as the target is presented

1.5.3.8 Neuropsychological models of attention

Different networks of brain centers have been hypothesized to be involved in different aspects of attention. Posner and Raichle (1994) proposed a three-circuit model for the different aspects of attention. The 'orienting' circuit consisting of the parietal cortex, superior colliculus, and pulvinar mediates selective attention.

The 'executive control' circuit consisting of anterior cingulate, left frontal cortex, and basal ganglia helps with detection of target stimuli, coordination of multiple subsystems of attention, and start-and-stop mental operations and responses. Sustained attention or vigilance is served by the 'alerting circuit' consisting of right-lateralized noradrenergic connections of the locus coerulus, right prefrontal cortex, and right parietal lobe.

1.5.4 VISUAL ANALOGUE SCALES (VAS)

The VAS have been shown to be sensitive in measuring the subjective effects in healthy volunteers after administration of d-amphetamine (Fischman & Foltin, 1991). Momentary changes in affect are often measured by these scales (Folstein & Luria, 1973), which are shown in Table 1-4. These consist of 100-mm lines, with each end describing the opposite adjective (e.g. like-dislike).

Table 1-4: Visual Analog Scale (VAS)

Dependent measure	Rating of 0	Rating of 10
Anxiety	I don't feel anxious at all	I feel very anxious
Happiness	I feel very miserable or sad	I feel very happy
Alertness	I feel mentally slowed	I feel mentally alert
Physical well-being	I feel physically unwell	Physically I feel fine
Hunger	I don't feel hungry at all	I feel very hungry
Energy	I feel tired and lethargic	I feel very energetic
Concentration	I feel very irritable	I can concentrate well
Irritability	I feel placid and calm	I feel very irritable
Speed of thoughts	My thoughts are slow	My thoughts are speeded up
Light-headedness	I don't feel light-headed	I feel very light-headed
Irritability	I feel physically inactive	I feel physically restless

These are 100-mm lines, each end describing the opposite adjective as illustrated below. The volunteer is required to make a mark along the line rating how they feel along a continuum.

The volunteer is required to make a mark along the line rating how they feel along this continuum.

The advantage of this scale is its rapidity in recording rapid subjective changes in mood (e.g. every 5 min) without much intrusion into an ongoing behaviour (Fischman & Foltin, 1991; Folstein & Luria, 1973).

1.6 Thesis objectives and rationale

A potential problem of the d-amphetamine model of mania has been the paucity of information regarding the relationship between plasma d-amphetamine levels and dependent measures in healthy volunteers. Thus the present study has focussed on this particular area.

Interest in the biochemical pathways (catecholaminergic, and serotonergic) mediating the subjective, physiological, biochemical, and cognitive effects of d-amphetamine has led to the pharmacological manipulation of the effects of d-amphetamine using various drugs such as lithium and pimozide. As well, interest in the mechanisms of action of currently used anti-manic drugs such as valproic acid and lithium has sparked a resurgence of interest in the glutamatergic and gabaergic pathways. The usual method of measuring these neurochemicals in humans has been via CSF or cerebrospinal fluid tap. However, this method is invasive, with a potential risk of infection, and difficult to conduct in time-dependent studies. The neurochemical measures taken in this

study were primarily peripheral measures easily performed in a large group of subjects.

Amphetamine has been measured in plasma samples by several procedures including immunoassay techniques, gas chromatography (GC) and high performance liquid chromatography (HPLC) as well as various derivatizing procedures. Many of the methods employed involved time-consuming extraction, had insufficient selectivity and/or sensitivity, or required expensive equipment (Paetsch et al.,1992). Thus, modification of the previous method of Paetsch and associates (1992) for d-amphetamine analysis in rat tissue samples using GC was essential for the rapid analysis of a large number of human plasma samples obtained in the current study

Since amphetamine has effects on cognition such as decreased reaction time to a target stimulus, a novel reaction time test was developed to measure both selective attention and vigilance using the visual spatial paradigm in a continuous performance test. The test is unique as it can be used over a time-course study where measurements are required within a short span of time. It is also sensitive to measurements of reaction time as small as 50 ms. Further, the software used to develop this test can be readily obtained by most research groups and is cost effective.

Thus, there were four main objectives of the study:

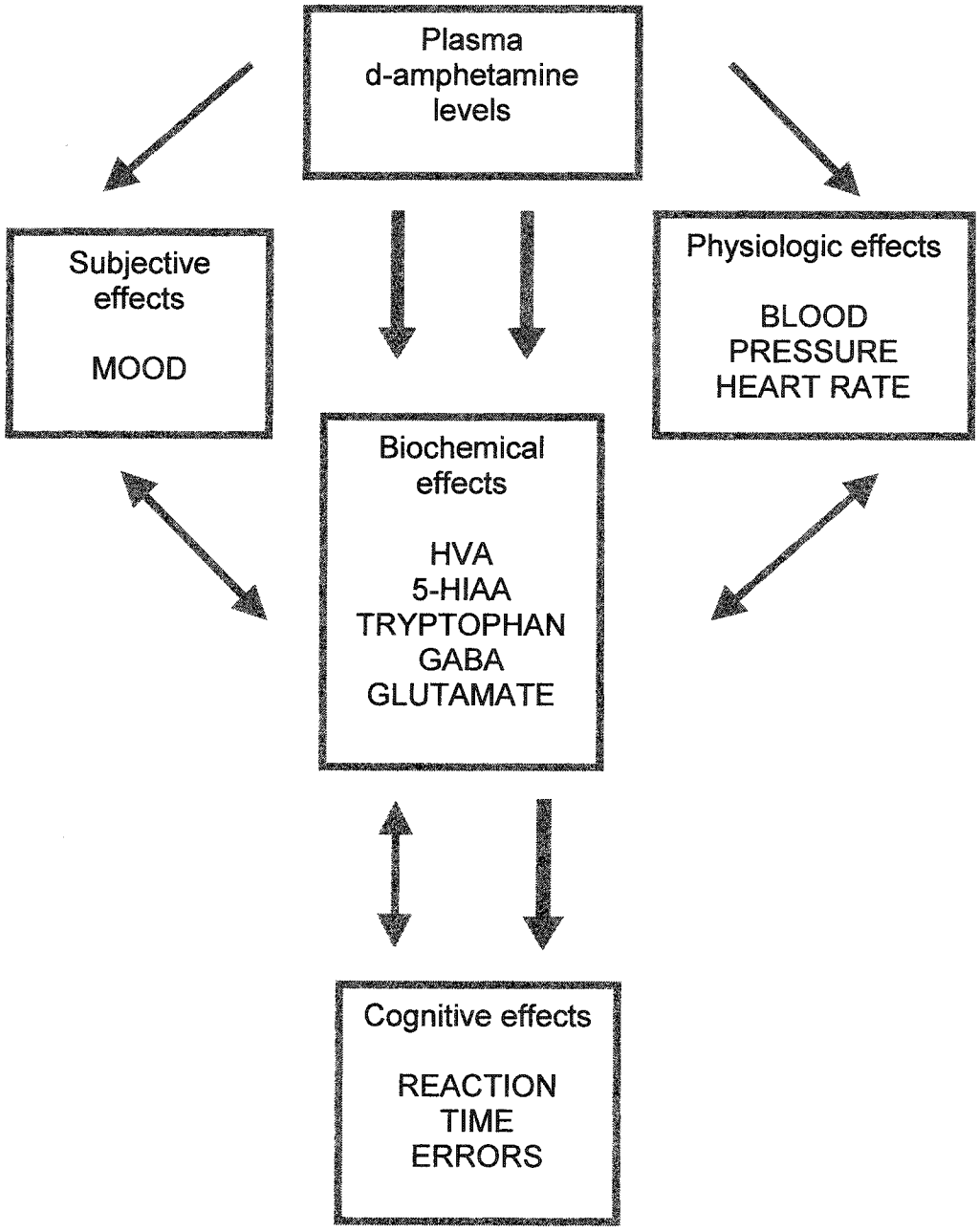
1. To modify an existing gas chromatographic method in order to measure d-amphetamine levels in human plasma samples.
2. To modify existing reaction time tests for measurement of reaction time and errors in an attention task.
3. To further explore the possible relationships between plasma d-amphetamine levels and mood, and psychomotor, physiologic and biochemical measures (Figure 1-3).
4. To measure the effects of d-amphetamine on several biochemicals, including GABA and glutamate not investigated previously in this model.

1.7 Materials and methods

1.7.1 RECRUITMENT AND SCREENING OF VOLUNTEERS

Prospective male volunteers between the ages of 18-45 years were screened before participating in the study. Females were not included since estrogen is known to enhance the effect of d-amphetamine on mood using the Profile of Mood States (POMS), the Addiction Research Center Inventory (ARCI), and two visual analogue scales (Justice & de Wit, 1999). This is dependent on the stage of the menstrual cycle of the woman in that the enhanced effect is greater in the follicular phase as compared to the luteal phase (Justice & de Wit, 2000).

Figure 1-3: Schematic representation of the possible relationships between plasma d-amphetamine and dependent measures evaluated in the study



After a signed consent, a physical examination, full medical interview and electrocardio gram (ECG) were obtained on the first visit. Any history of medical or psychiatric illness, previous drug use, smoking, use of medications or abnormal ECG readings resulted in exclusion from participation in the study.

1.7.1.1 Protocol of the study

A double-blind (DB), placebo-controlled, crossover (CO) design was used in 25 healthy male volunteers. Subjects participated on both days of the study, which were two weeks apart (see Figure 1-4). Either 25 mg of d-amp or an equivalent amount of placebo (lactose powder) was administered to subjects in a randomized fashion so that there was an equal chance of receiving either of the two on day one.

The alternate substance would be administered on day two. The dose of 25 mg was selected based on previous reports by Angrist et al. (1987), Dommisse et al. (1984), and Silverstone at al. (unpublished data 2000) of enhanced psychological effects after 20 mg of d-amp.

1.7.1.2 Venous catheterization

Subjects were required to fast from midnight the previous day and only allowed to drink water on the study day. After a brief debriefing session, volunteers performed a practice reaction time test. Soon after, a nonpyrogenic IV catheter (Insyte-W 22G X 1.0 in 0.9 X 25 mm winged)

was inserted into the basilic vein or cephalic vein, preferably in the nondominant arm after sterilization of the area using an alcohol swab. An extension set with Luer Lock (7 in) was attached to the IV catheter and an injection site interlink was fastened to the extension site. The interlinks were changed after every two to three withdrawals. The area was cleaned, the tubing secured with nonallergenic perforated tape, sterile gauze placed on the wound site and the arm placed in a tubular net nylon dressing to allow mobility.

This system provides easy and rapid blood withdrawal, multiple sampling over time without the unnecessary piercing of the volunteer more than once and, further, allows blood collection using either vacutainer or syringes. To prevent the formation of blood clots and keep the access line patent, 2-3 ml of 0.9% sodium chloride were injected using a 3 ml (W/22G X 1 in) needle after every withdrawal. Further, sodium chloride also served to provide hydration to the fasting volunteer.

1.7.1.3 Plasma sample collection

Blood was withdrawn using a vacutainer holder (blood collection tube holder) with a 21G needle attached to a 10 ml sterile vacutainer with K_4EDTA to prevent the clotting of blood. Two tubes were filled at every withdrawal. The vacutainer system draws blood using vacuum-suction, and thus blood is obtained very rapidly. The blood was promptly placed on ice and centrifuged at 1500 rotations per minute (rpm) for 10 min. Plasma

was removed and stored at –80°C in 1.5 ml microfuge tubes. The remaining portion of the sample was discarded.

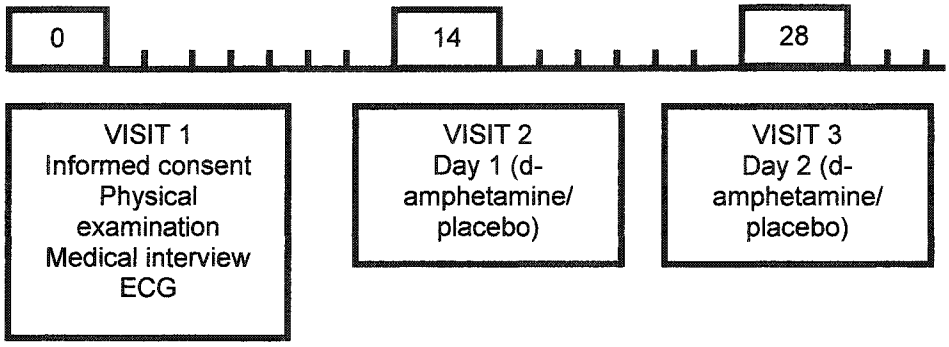
1.7.1.4 Measurements taken

Baseline measurements were taken after the first withdrawal. This consisted of physiological (blood pressure and heart rate), cognitive (reaction time and errors) and mood change measurements using a VAS. Blood pressure was assessed using a sphygmomanometer. The placebo/d-amphetamine capsules were administered after the completion of the baseline measurements. Blood withdrawal and all the measurements were repeated at 30, 60, 90, 150, 210, 240, and 480 min after the administration of d-amphetamine (Fig 1-5).

The blood collected was used for the analysis of plasma d-amphetamine, GABA, glutamate, homovanillic acid (HVA), tryptophan and 5-hydroxyindole-3-acetic acid (5-HIAA).

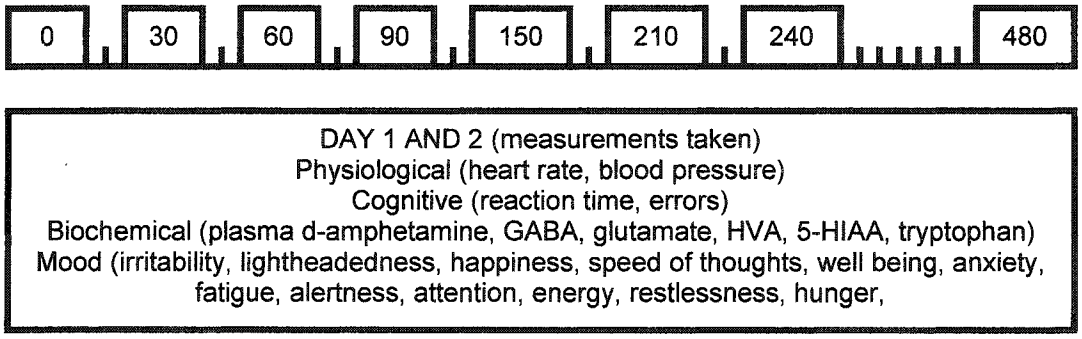
The respective time points were selected based on previous reports which have indicated a steady increase in plasma d-amphetamine from half an hour to three hours, when a plateau is reached (Angrist et al., 1987; Brauer et al., 1996; Morselli et al., 1976).

Figure 1-4: Visit schedule (days)



The above diagram illustrates the study design over a span of 28 days showing when each visit was appointed and what was done at each visit.

Figure 1-5: Protocol (min)



The above diagram illustrates the time course of the study over a span of 480 min and when each of the measurements were carried out. This was repeated over two days with each of the treatments d-amphetamine and placebo administered in a randomized fashion.

After the 240 min withdrawal, the volunteer was given juice and a candy bar and allowed to leave and have a meal without any caffeinated beverages. The volunteer returned after 480 min for the last blood withdrawal and repeated all tests, after which the IV catheter was removed.

At the end of the study, each volunteer was offered 0.5 mg Halcion, a hypnotic, to ensure sleep during the night as d-amp may produce sleeplessness.

1.7.1.5 Disposal and safety techniques

During catheterization and blood withdrawals, sterile gloves were always used. All equipment used was always disposed of in a Sharps disposal container with vacutainer removal device. The same syringes were never used twice for withdrawal purposes.

If any difficulties arose during the catheterization procedure, the volunteer could be taken to the Clinical Investigation Unit at the University of Alberta Hospital. The volunteer was called after 24 hours and then at 48 hours by telephone for follow-up.

1.7.2 CHEMICALS AND REAGENTS

d-Amphetamine was obtained from Health & Welfare Canada (Lot #30603-35403), benzylamine from Sigma and pentafluorobenzenesulfonyl chloride (PFBSC) from Aldrich, as depicted in Table 1-5.

Table 1-5: List of chemicals and reagents with their suppliers used for the d-amphetamine assay.

CHEMICALS	SUPPLIERS
d-Amphetamine	Health & Welfare Canada, Ottawa [Lot # 30603-35403]
Benzylamine	Sigma, St. Louis, MO
PFBSC	Aldrich, St. Louis, MO
Potassium carbonate	Fischer Scientific, Fair Lawn, NJ
Ethyl acetate (glass-distilled) and toluene	Fischer Scientific, British Drug Houses (BDH) Inc. Toronto, Ont
Acetonitrile	BDH Inc. Toronto, Ont
K_2CO_3	Fischer Scientific, Fair Lawn, NJ

The solvents used were commercially pure. The water used for the assay was double-distilled in glass.

1.7.2.1 Saturated Potassium Carbonate (K_2CO_3) (pH=11)

A fresh saturated solution of K_2CO_3 was prepared every month by dissolving the salt in a beaker until the salt could not dissolve any further and using the supernatant.

1.7.2.2 PFBSC preparation

This solution was made by mixing ethyl acetate, acetonitirile and PFBSC in a ratio of 9:1:0.01. The PFBSC solution was stored in the refrigerator at 0-4 °C.

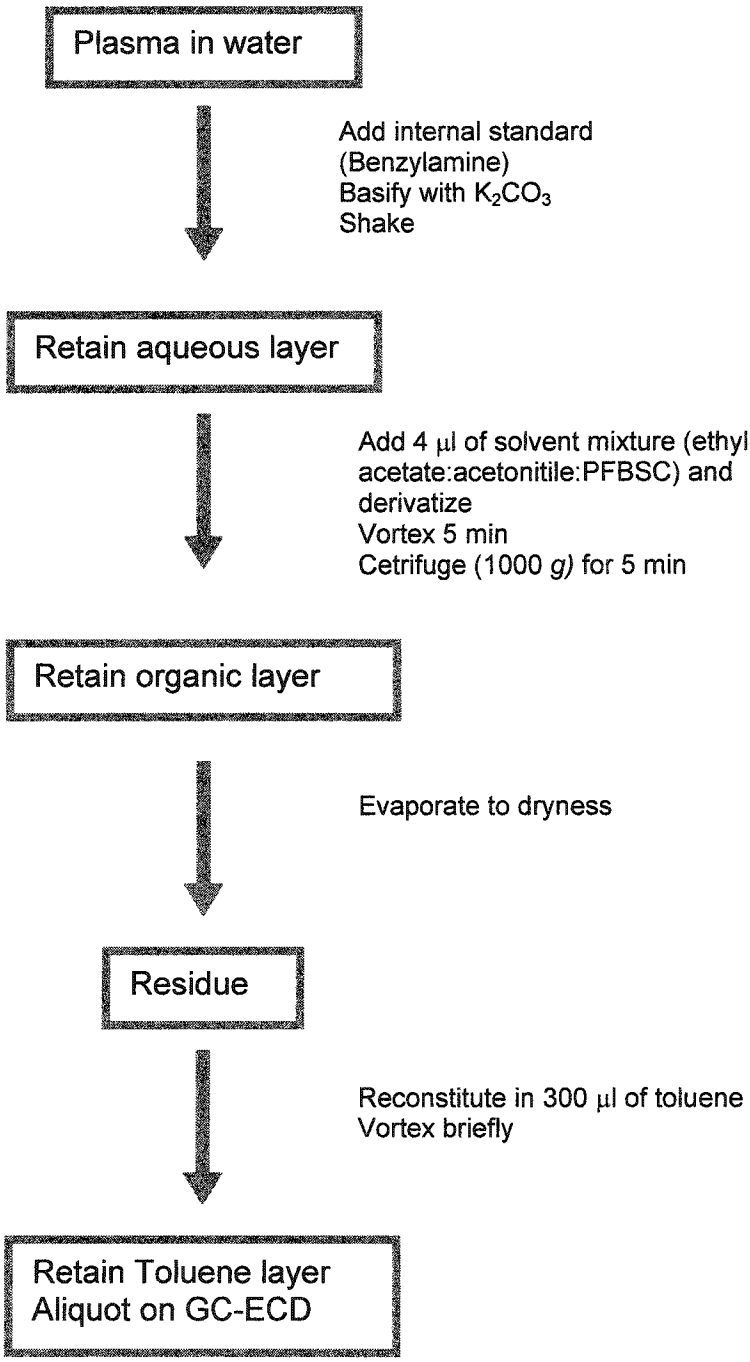
The general procedure developed for the analysis of plasma d-amphetamine is shown in Figure 1-6 and is described in detail in the next chapter.

1.7.3 INSTRUMENTATION AND APPARATUS

1.7.3.1 Gas Chromatography

The analysis was performed using a Hewlett Packard (HP) 5890 gas chromatograph with a HP5 column from Agilent. Helium at a flow rate of 2 ml/min was used as carrier gas with methane-argon (5:95) as the make up gas at a flow rate of 35 ml/min. A temperature of 280°C and 325°C was used for the injection port and detector, respectively.

Figure 1-6: Procedure for the analysis of d-amphetamine in human plasma samples



The oven temperature was set at 105°C for 0.50 min, and programmed to increase at a rate of 5°C/min to a final temperature of 300 °C, which was maintained for 15min.

1.7.3.2 Mass Spectrometry

Combined GC-MS was used to confirm the structures of d-amphetamine and its metabolites.

1.7.3.3 Balance

The compounds used were weighed on a Mettler AE160 electronic balance (Mettler Instrument Corporation, Hightstown, NJ).

1.7.3.4 Centrifuges

A Sorvall GLC-2B General Laboratory Centrifuge (Dupont Instruments, Wilmington, DE) was used.

1.7.3.5 Shaker-mixer

There were two kinds of vortex-shaker used: an Ika Vibrex VXR vortex mixer (Janke and Kunkle Instruments, Staufen, Germany) and a thermolyne Maxi Mix vortex mixer (Sybron/Thermolyne Corp., Dubuque, IO, USA).

1.7.3.6 Vacuum evaporator

A Savant Speed Vac SSI (Savant Instruments, Inc., Farmington, NY) using a vacuum and centrifugal force was used to remove solvent and concentrate samples. The instrument consisted of a concentrator (a rotor chamber with heater), a chemical trap with disposable cartridges, a refrigerated condensation trap, and a vacuum pump.

1.7.3.7 Glassware cleaning

All glassware was scrubbed manually with biodegradable Sparkleen® (Fisher Scientific, Nepean, ON) followed by sonication in an Ultra-Sonic Cleaner (Mettler Electronics, Highstown, NJ) and rinsed in a G7704 Lavador dishwasher (Miele Laboratory Technology, Unionville, ON). Other glassware was rinsed with tap water and then washed with Sparkleen® Dishwater Soap in the dishwasher, which utilized water from a central deionized water source. The glassware was then air-dried in a mechanical oven (Model 28, Precision Scientific Group, Chicago, IL).

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CHAPTER 2

A RAPID METHOD OF DETERMINING D-AMPHETAMINE IN PLASMA SAMPLES USING PFBSC AND ELECTRON-CAPTURE GAS CHROMATOGRAPHY

[The work reported in this chapter forms the basis of a manuscript in press in the Journal of Pharmacological and Toxicological Methods]

2.1 Introduction

The amphetamines and related stimulants display powerful cardiovascular, central stimulant, hyperthermic and anxiogenic properties. They have been a source of attention due to their drug abuse potential as well as therapeutic use in the treatment of narcolepsy and attention deficit hyperactivity disorder (Brust, 1993). As well, amphetamine is a useful research tool in the study of mania as it mimics the physiological, biochemical, and cognitive effects seen in mania (Jacobs & Silverstone, 1986). Symptoms of increased blood pressure and heart rate (Brauer et al., 1996; Caldwell, 1996; de Wit et al., 1997; Jacobs & Silverstone, 1986; Slattum et al., 1996), decreased reaction time (Rapoport et al., 1980; Servan-Schreiber et al., 1998), and elevation of mood (de Wit et al., 1997; Miller & Griffith, 1983; Silverstone et al., 1983; Zacny & de Wit, 1989), have been demonstrated with the acute administration of oral amphetamine

Numerous techniques have previously been used to quantify amphetamine. These include: radioimmunoassay (Schmidt, 1988; Ward et al., 1994); combined gas chromatography-mass spectrometry (GC-MS) (Dasgupta & Spies, 1998; Sato & Mitsui, 1997; Suzuki et al., 1989); high resolution MS (Danielson & Boulton, 1974); GC with flame-ionization detection (Lebish et al., 1970; Kintz et al., 1989), nitrogen-phosphorus

detection (Cheung et al., 1997; Jacob et al., 1995; Terada, 1985) or electron capture detection (GC-ECD) (Coutts et al., 1984; Paetsch et al., 1992); and high-performance liquid chromatography (HPLC) with ultraviolet detection (Farrell & Jefferies, 1983) or chemiluminescence detection (HPLC-CD) (Hayakawa et al., 1989; Nakashima et al., 1992). However, some of these methods are expensive, time-consuming and involve laborious extraction procedures.

Gas chromatography is a relatively inexpensive technique which is accessible to many laboratories. Analysis of amphetamine generally requires derivatization of its amino group to increase sensitivity and selectivity as well as increase volatilility, reduce polarity, and improve chromatographic properties. Derivatives which have been used include acetyl (Lebish et al., 1970), n-propyl (Jacob et al., 1995), trifluoroacetyl (Suzuki et al., 1989), trichloroacetyl (Hornbeck & Czarny, 1989), trichloroethyl chloroformate (Dasgupta & Spies, 1998), heptafluorobutyric (Cheung et al., 1997), pentafluorobenzoyl (Terada, 1985), pentafluorobenzenesulfonyl (Paetsch et al., 1992), pentafluorobenzyl (Sato & Mitsui, 1997), pentafluoropropionyl (Valentine et al., 1995) perfluorooctanoyl (Gjerde et al., 1993; Thompson & Dasgupta, 1994), and 4-carbethoxyhexafluorobutyl (Czarny & Hornbeck, 1989).

A relatively simple, sensitive and reproducible assay for d-amphetamine levels in plasma was desirable for our clinical studies,

where numerous samples were to be analyzed, and for this purpose the procedure of Paetsch et al. (1992), using pentafluorobenzenesulfonyl chloride (PFBSC) for analysis of d-amphetamine in rat brain, was modified as described in the present report.

2.2 Materials and methods

This double-blind, crossover study was part of an investigation of the physiological, cognitive, mood, and biochemical effects of acute d-amphetamine administration in healthy volunteers.

2.2.1 HUMAN VOLUNTEERS

The study was approved by the University of Alberta ethics review committee. Twenty-five healthy male volunteers (18-45 years) were recruited after screening and medical examination. Exclusion criteria included any history of previous drug use, smoking, and use of any medication. On the day of the study, an intravenous (I.V.) catheter was inserted into the antecubital vein on the nondominant arm of the volunteer and a baseline sample taken, followed by the administration of either placebo or 25 mg d-amphetamine in a randomized double-blind manner. Each volunteer served as their own control and returned after two weeks for their second session when they received the other treatment. Blood was withdrawn at intervals of 30, 60, 90, 150, 210, 240, and 480 min after d-amphetamine administration and placed on ice. The samples were

centrifuged at 1000 x *g* for 10 min, and plasma removed and stored in polypropylene 1.5 ml microfuge tubes at –80 °C.

2.2.2 MATERIALS

The chemicals used were d-amphetamine (Health & Welfare Canada [Lot #30603-35403]), benzylamine (Sigma), PFBSC (Aldrich), potassium carbonate (K_2CO_3) (Fisher Scientific, Fair Lawn, NJ), glass-distilled ethyl acetate and toluene (Fisher Scientific and British Drug Houses [BDH] Inc., Toronto, Ont), and acetonitrile (from BDH Inc.). The water used for the assay was double-distilled in glass.

2.3 Methods

2.3.1 EXTRACTION AND DERIVATIZATION

The frozen plasma samples were allowed to thaw on ice for at least an hour. The 1 ml plasma aliquots were vortexed and basified with 300 µl of 25% K_2CO_3. Fresh K_2CO_3 solution (25% w/v) was prepared every month. The samples were then extracted with 4 ml of ethyl acetate:acetonitrile:PFBS (9:1:0.01), vortexed for 5 min and centrifuged (1000 x *g* for 5 min). The organic layers were transferred to another set of tubes and taken to dryness in a SAVANT evaporator. Each residue was reconstituted in 300 µl of toluene and an aliquot (2 µl) was used for GC analysis. A standard curve was constructed for each run with known

varying amounts of d-amphetamine (1-100 ng) and a fixed amount (250 ng) of the internal standard benzylamine added to 1 ml of naive plasma and run in parallel with every set of samples. The peak height ratios of d-amphetamine to internal standard in the plasma samples from the subjects were compared to the ratios from the standard curves to determine the amount of d-amphetamine in each sample.

2.3.2 GAS CHROMATOGRAPHY

The analysis was performed using a Hewlett Packard (HP) 5890 gas chromatograph fitted with a 15-mCi ^{63}Ni linear electron capture detector. The chromatographic column was a narrow-bore fused-silica capillary column (25 m x 0.32 mm I.D., 1.05 μm film of 5% phenylmethylsilicone as a stationary phase; Hewlett-Packard, Palo Alto, CA). Helium at a flow rate of 2 ml/min was used as carrier gas, with methane-argon (5:95) as the makeup gas at a flow rate of 35 ml/min. Injection port and detector temperatures were 280 °C and 325 °C respectively. A splitless injection system was used. The oven temperature was set at 105 °C for 0.50 min, increased at a rate of 5 °C/min to a final temperature of 300 °C and maintained for 15 min.

2.3.3 GAS CHROMATOGRAPHY-MASS SPECTROMETRY

The structures of the derivatives were confirmed using coupled GC-MS. The GC-MS system utilized an Agilent 6890 GC with an Agilent 5973

Mass Selective Detector with a CI source. The system also included an HP X m 600 computer, a HP Laserjet 4050 printer and MSD Chemstation software (Agilent). Operating conditions were as follows: interface temp, 280 °C ; MS Quad, 106 °C ; MS source, 150 °C; column pressure, 10.5 psi; accelerating voltage, 1059 eV; ionization voltage 70 eV and scan speed 3.62 scans/second. The GC column and temperature programs were identical to those used for GC-ECD.

2.4 Results and discussion

The procedure described here is rapid and the derivatives formed are stable with excellent chromatographic properties. The retention times of derivatized benzylamine (the internal standard) and d-amphetamine were 20.1 and 21.8 min respectively (Figure 2-1). The mass spectral analysis was consistent with the structure of N-pentafluorobenzenesulfonyl amphetamine. The standard curves were linear from 1 to 100 ng (r^2>0.99 obtained routinely) (Figure 2-2).

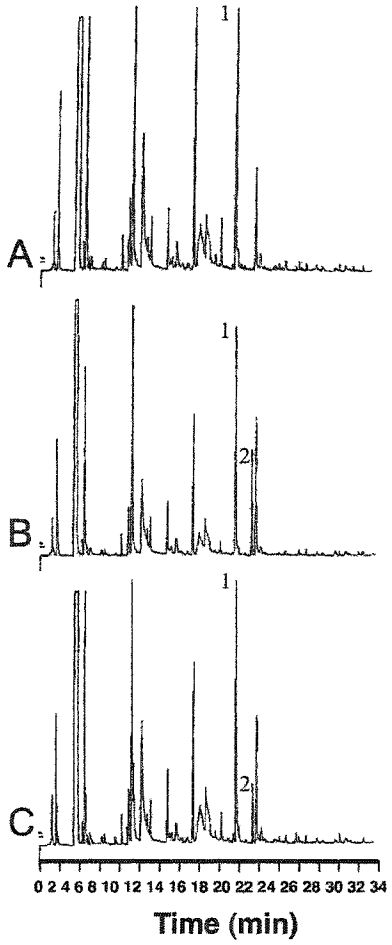


Figure 2-1: Gas chromatographs from human plasma samples after extractive derivatization with PFBSC.

(A) Plasma sample after placebo treatment; peak 1 is the added internal standard. (B) Naïve plasma sample with d-amphetamine (100 ng) (indicated by peak 2) and internal standard (benzylamine) added. (C) Plasma sample from patient treated 3.5 hr previously with d-amphetamine. In A-C above, 250 ng benzylamine was added as internal standard in each case. In samples with no benzylamine added, there were no interfering peaks corresponding to the retention time of derivatized benzylamine.

Figure 2-2: A typical standard curve from the d-amphetamine assay

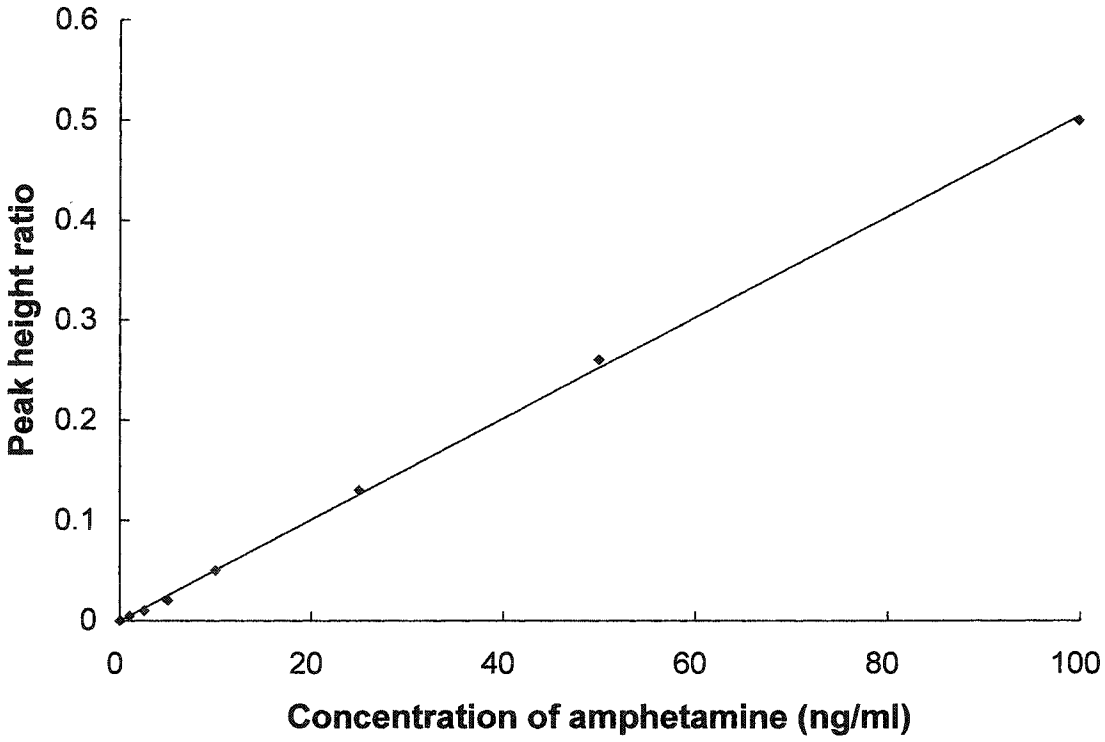
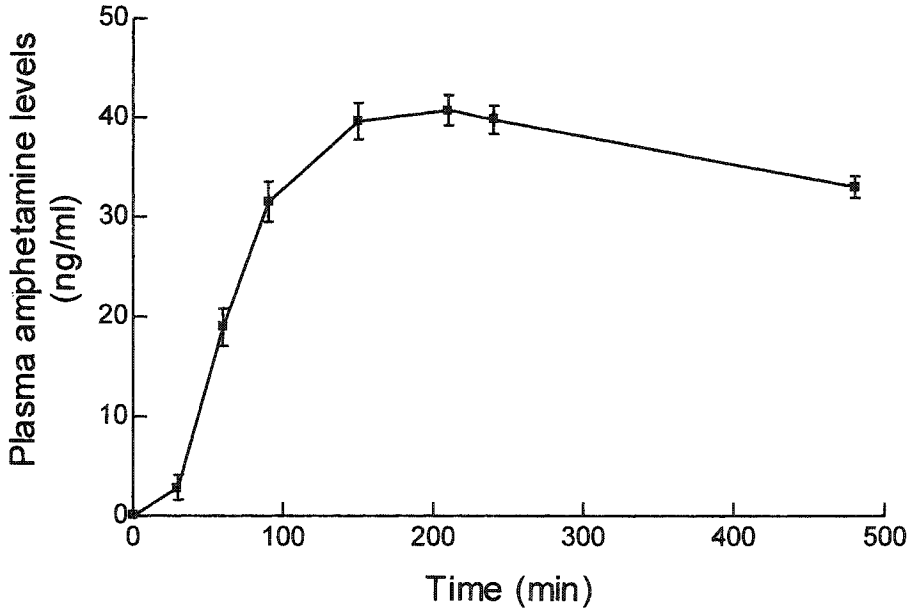


Figure 2-3: Plasma d-amphetamine levels over time in 25 subjects with results expressed as means ± SEM



The procedure was sensitive to <1 ng/ml in plasma, and the mean absolute recovery of 25 ng d-amphetamine was 76.3%. The intra-assay coefficients of variation determined at 50 ng ranged from 2.0 to 2.7% (n=6). The mean inter-assay coefficient of variation for 25 ng samples was 5.46% (n=10). Plasma d-amphetamine levels reached a peak value at 3.5 hr, at which time they were 40.8 ng ± 1.5 ng/ml (n=25)(Figure 2-3). Our findings in this regard are in agreement with those reported by Brauer et al. (1996).

The original method of Paetsch et al. (1992) on which the current procedure is based was developed for analysis of d-amphetamine in rat brain, liver, and urine but had to be modified to provide optimal analysis in human plasma samples, i.e. to provide separation from interfering peaks present in plasma and still retain sensitivity and reproducibility. In the original method, the d-amphetamine was extracted by making the tissue homogenate supernatant slightly basic, shaking with the liquid ion-pairing reagent di(2-ethylhexyl)phosphate (DEHPA) in chloroform and then back-extracting with HCl; the acid phase was then basified and shaken with the PFBSC in ethyl acetate:acetonitrile. The cleanup extraction step with DEHPA was not required with plasma samples, resulting in a more rapid, less tedious procedure, which provided sensitivity and reproducibility as good as the original procedure. Although a fused silica capillary column with 5% phenylmethylsilicone as stationary phase was used in both

methods, a longer column with a larger internal diameter was found to be more appropriate for the present assay procedure on plasma extracts. A slightly different oven program was also used in the procedure reported here to provide separation from possible interfering peaks.

In conclusion, a rapid yet sensitive method of quantifying plasma d-amphetamine using extractive derivatization with PFBSC followed by GC-ECD is described. Pentafluorobenzenesulfonyl chloride has been shown in the past to be useful for the extractive derivatization of amine- and phenol-containing drugs under aqueous conditions (Baker et al., 1986; Urichuk et al., 1997) and for derivatization of tyrosyl peptides (Sentissi et al., 1984), nucleic acid pyrimidine bases (Nazareth et al., 1984), and proteins (Schmid et al., 1985). The method described will be useful to other laboratories as a sensitive, relatively inexpensive method for analyzing large numbers of samples.

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CHAPTER 3

A NOVEL REACTION TIME TEST FOR DETERMINING REACTION TIME AND ERRORS AFTER ADMINISTRATION OF D-AMPHETAMINE TO HEALTHY VOLUNTEERS

3.1 Introduction

There has been an increased interest in understanding how psychoactive drugs affect specific cognitive processes (Halliday et al., 1990). Stimulants, in particular, appear to affect response processing (Callaway, 1984). Callaway and associates (1984) have further proposed that different components of human processing are affected by various neurotransmitters. Dopamine is thought to affect attention (via frontal and mesolimbic circuits) and motor readiness (via nigrostriatal circuits) (Callaway et al., 1994). d-Amphetamine induces the release and blocks the reuptake of dopamine, norepinephrine and serotonin and this may be reflected in the changes seen with reaction time (Halliday et al., 1990).

In addition, the acute administration of d-amphetamine causes multiple cardiovascular, subjective and cognitive effects similar to those seen in mania. Therefore, acute d-amphetamine administration is considered to be a reproducible model for mania (Jacobs & Silverstone, 1986). Most cognitive functions have not been assessed during the manic phase although simple reaction time has been found to be decreased in both depressed and manic patients (Schwartz et al., 1989). Further, selective attention seems to be preserved in euthymic bipolar patients (Martinez-Aran et al., 2000).

Most research studies have used simple reaction time tests to assess response to a target. However, over a longer duration of study, continuous performance tests have been used to assess sustained attention (Ballard, 2001).

In a study of normal prepubertal boys d-amphetamine caused an improvement in vigilance, using Rosvold 's continuous performance task. As well, d-amphetamine was found to have increased reaction time (RT) in the same study (Rapoport et al., 1978).

d-Amphetamine has been reported to decrease reaction time and improve accuracy in a choice-reaction time test (Eriksen Task) used to measure selective attention (Servan-Schreiber et al., 1998b). A dose of 0.25 mg/kg was given to ten subjects (5 men and 5 women, but the results from only 8 subjects were used). The individual was requested to respond to a central letter (H or S) in an array of letters by pressing the corresponding letter on the keyboard (Servan-Schreiber et al., 1998a). The Eriksen task lasted 30-35 min and was measured at two time points, baseline, and 2 hours post d-amphetamine administration (Servan-Schreiber et al., 1998a). There were two types of complexity of the task; requiring the individual to select the letter in a compatible (all letters are identical i.e. HHHHH or SSSSS) and incompatible (the central letter is different from the surrounding letters i.e. HHSHH or SSHSS) task. The drawbacks of this test include the length of the task, not being feasible in a time-dependent study, the small number of subjects, and the design of the study itself, requiring letter recognition and response selection which limits its widespread use.

3.1.1 VISUAL SPATIAL PARADIGM

A useful model using a cued stimulus allows selective attention to be studied in the absence of head or eye movements that may affect reaction times.

A cue is used to direct attention and may be presented at the future location of the target or at another place, but indicating where the target is most likely to appear (Posner, 1994). The cue may be a central cue or a peripheral one; the former requiring the subject to knowingly choose where to orient, and the latter drawing the subject's attention more automatically. The design used in this experiment is based on the above paradigm and has been modified to measure both selective attention as well as vigilance. In particular, the changes in reaction time and errors at different latencies have been explored in this study.

3.2 Materials and methods

3.2.1 SUBJECTS

Twenty-five healthy male volunteers were selected after a preliminary screening conducted via the telephone. Subjects with any history of medical or psychiatric illnesses, previous drug use, current use of medication or smoking were excluded from the study. After explaining details of the study to the participant and obtaining their signed consent, a physical examination, full medical interview and ECG were obtained on the first visit. Any abnormal ECG readings resulted in their exclusion.

3.2.2 PROTOCOL OF THE STUDY

A double-blind, placebo-controlled, crossover design was used. Volunteers participated in two sessions conducted in the Psychopharmacology

Research Unit 2 weeks apart commencing at 7 am and ending 8 hours later. In the first session, either d-amphetamine or placebo (lactose powder) placed in opaque olive coloured gelatin capsules was administered in a randomized fashion so that there were equal chances of receiving either of the two. In the second session, the alternate substance was administered. The study was approved by the University of Alberta Ethics Review Committee. A dose of 25 mg d-amphetamine was selected based on prior reports of enhanced cognitive effects after 20 mg of d-amphetamine (Angrist et al., 1987; Dommisse et al., 1984).

Volunteers were required to fast from midnight the previous day and given only water during the study. They also performed a practice reaction time test for approximately 10 min to acclimatise them to the test. Simultaneously, other measurements such as physiologic, mood and biochemical were also taken. Baseline measurements were taken followed by the administration of placebo/d-amphetamine. The test was repeated at 30, 60, 90, 150, 210, 240, and 480 min based on previous reports that have indicated peak effects of d-amphetamine on physiological, cognitive and mood change measurements within 30-180 min (Jacobs & Silverstone, 1986).

After 4 hours, the volunteer was given juice and candy. They were then allowed to leave for lunch without any caffeinated beverages. At the end of the study, 0.5 mg Halcion, a hypnotic, was given to ensure a restful sleep as

d-amphetamine can cause sleeplessness (Silverstone et al., 1980). Volunteers were called after 24 and at 48 hours by telephone for follow-up.

3.2.3 DESIGN OF TEST

The software used was Super Lab version 1.04, marketed by Cedrus Corporation. The design is shown in Table 3-1.

3.2.3.1 Blocks

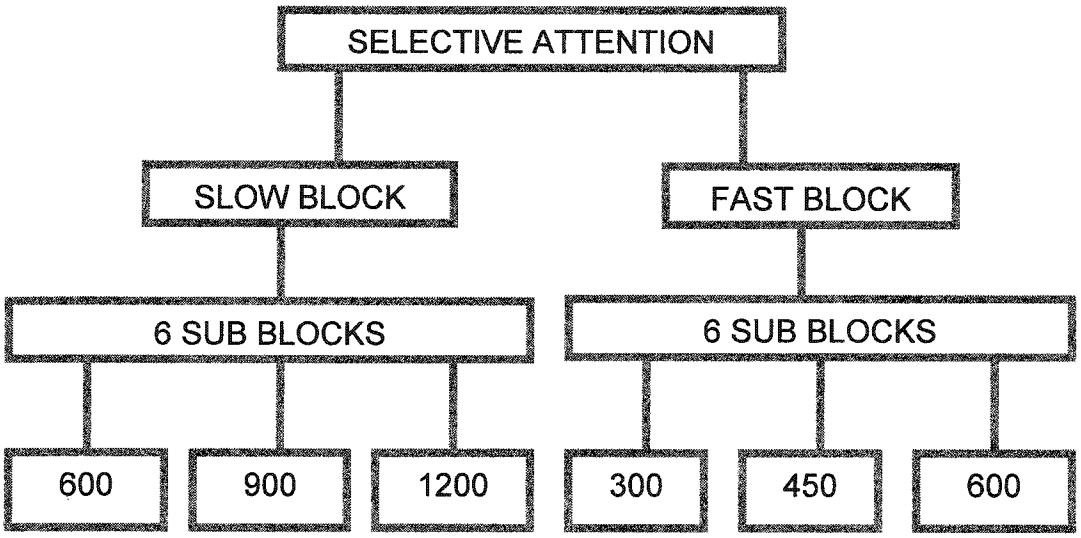
The slow block was subdivided into 6 sub-blocks as shown in Figure 3-1. Each sub-block had 9 target trials and 1 catch trial. The sequence was repeated in a randomized fashion for three different time latencies, namely 600, 900, and 1200 ms. This block was repeated over the 8 time points. Thus, the slow block had 540 trials.

The fast block was subdivided into 6 sub-blocks. Each sub-block had 9 target trials and 1 catch trial. The sequence was repeated in a randomized fashion for three different time latencies, namely 300, 450, and 600 ms. This block was repeated over the 8 time points. Thus, the fast block also had 540 trials.

Table 3-1: Design of novel RT test showing parameters

PARAMETERS	SELECTIVE ATTENTION TEST
• Type of stimuli	Target and catch
• Total # of stimuli per test	120
• Number of blocks	2
• Type of blocks	Slow and fast
• Number of sub-blocks per block	6
• Number of stimuli per block	108 (target) + 12 (catch)
• Number of stimuli per sub-block	9 (target) + 1 (catch)
• Interstimulus interval	6 types depending on block
• ISI variability fast block	300, 450, 600 ms
• ISI variability slow block	600, 900, 1200 ms
• Time intervals	0, 30, 60, 90, 150, 210, 240, 480 min
• Number of times test was repeated	8
• Total time for test	17 min
TARGET STIMULI	
• Definition	Black box with cross preceding a single cross
• Number per block / sub-block	54/9
• Response required	Press spacebar
• Target ISI	According to slow or fast block
• Position variability	10 (randomized)
CATCH STIMULI	
• Definition	Single cross preceding a single cross
• Number per block / sub-block	6/1
• Response required	None
• Target ISI	According to slow or fast block
• Position variability	10 (randomized)
ERRORS MEASURED	
• Omission	Failure to press spacebar when a target stimulus appeared
• Commission	Pressing spacebar when a non-target stimulus appeared

Figure 3-1: Design of the RT test used



The test consisted of 2 blocks, 6 sub blocks, and 3 different time latencies which were repeated 3 times each. The test was repeated over 480 min on two days when either the d-amphetamine or placebo treatment was given.

3.2.3.2 Trials

A trial consisted of the sequence blank screen, screen with black cross (cued stimulus) and followed by either the black cross with a black box (target) or a black cross again. The volunteer was asked to press the spacebar as fast as possible when a target was presented. The reaction time was calculated as the time from the presentation of the cross to the pressing of the spacebar. Both the cross and target were presented at different time intervals starting from the time the blank screen was presented at different latencies. The position of the cross on the screen was varied in 10 different positions and was randomized for each block.

A target trial consisted of the sequence of blank screen for 1000 ms, black cross on screen and followed by target. The time from the presentation of the black cross and target was varied according to the time latencies 600, 900, and 1500 ms in the slow block and 300, 450, and 750 ms in the fast block. The position of the target was varied in 10 different positions in a randomized fashion. The target would remain on the screen until the response was made or 1500 ms has elapsed for the slow block and 750 ms for the fast block.

A catch trial consisted of the sequence of blank screen for 1000 ms, black cross on screen and another black cross. The time at which the second black cross was presented varied according to the slow block 600, 900 and 1500 ms and fast block 300, 450 and 750 ms. The cross remained on the screen for 1500 ms in the slow block and 750 ms in the fast block. The ratio of the target trials to

the catch trials was 9:1 to give an approximate value of 11% of the trials being catch trials.

There were two types of errors assessed during the test namely commission and omission errors. An omission error was defined as failure to press the space bar during the target trial. Pressing the space bar at any other time or during the catch trial resulted in a commission error.

3.3 Data analysis

A change from baseline for reaction time and error measurement was calculated by subtracting the pre-drug score for each post-drug time point. A 6 factor ANOVA was used to identify the variables that may have had an effect on the reaction time. These were treatment (2 levels), time (8 levels), repetitions (3 levels), latencies (3 levels), and blocks (2 levels). A significant drug x time effect $F_{7,18}=2.669$, $p<0.05$ and drug x block x time effect $F_{2,23}=3.902$, $p<0.05$ was seen. The factors repetitions and latencies were collapsed, after which two separate 3 way ANOVAS (RT and time in slow and fast blocks, RT and block in treated and untreated) were run to interpret more clearly which factors were significant. A Student's *t*-test was also run to identify which time points were significant.

3.4 Results

3.4.1 EFFECTS OF D-AMPHETAMINE ON CHANGE IN REACTION TIME

The results indicate a significant decrease in reaction time after d-amphetamine administration. In the slow block, there was a significant time effect $F_{4,326}=4.224$, $p<0.05$. This is shown in Figure 3-2 (drug effect: $F_{1,74}=2.258$, $p=0.14$, and drug x time effect: $F_{5,360}=1.914$, $p=0.09$). In the fast block, there was a significant drug effect ($F_{1,74}=13.225$, $p<0.05$) and time effect ($F_{4,300}=5.618$, $p<0.05$) as seen in Figure 3-3, but the drug x time effect ($F_{5,377}=1.473$, $p=0.20$) did not reach statistical significance. Using Student's *t*-tests in the fast block, the decrease in reaction time was significant at 30, 60, 90, 150, and 210 min. In the slow block, the change in reaction time was significant at 90 and 210 min only.

3.4.2 EFFECTS OF D-AMPHETAMINE ON ERROR CHANGE

There was an increase in mean errors in both the slow and fast blocks, but this did not reach statistical significance as shown in Figure 3-4. There was an increase in commission errors however, the omission errors decreased significantly using Student's *t*- tests $p<0.05$ as shown in Figure 3-5.

Figure 3-2: Time-dependent changes in reaction time for the slow block

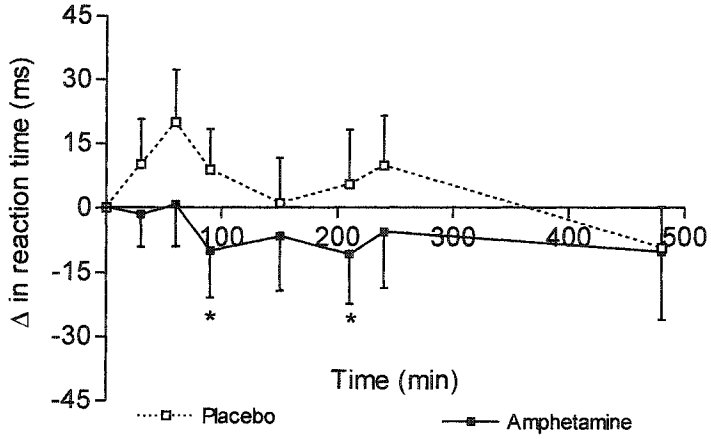
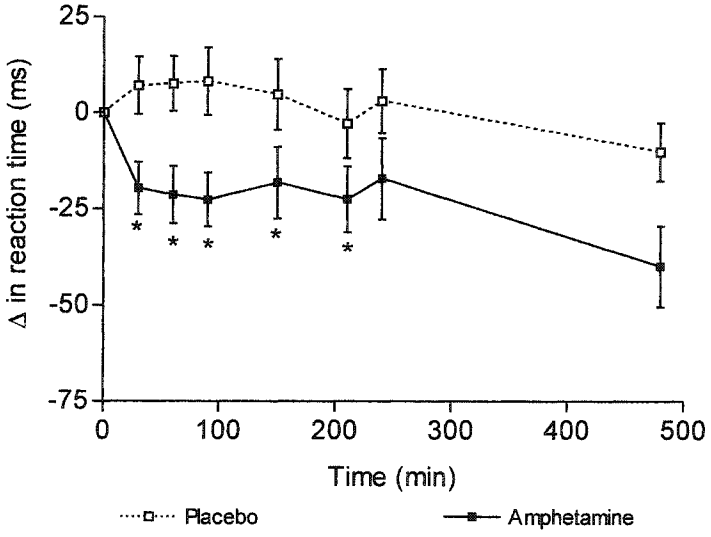


Figure 3-3: Time-dependent changes in reaction time for the fast block



Data in both figures represent the mean ± SEM (n=25) change in reaction time after administration of 25 mg oral d-amphetamine. Statistical analyses were performed by Student's *t* test pairwise comparison between drug and placebo treated group, *p<0.05.

Figure 3-4: Mean errors in slow and fast block

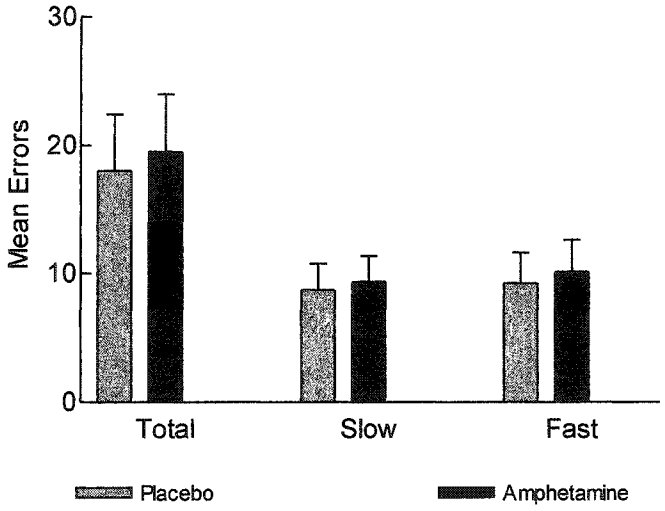
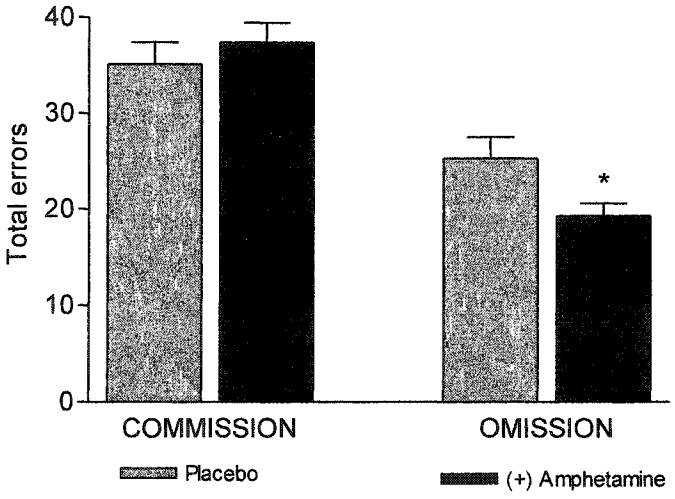


Figure 3-5: Type of error



The data in both the figures above represent the mean ± SEM (n=25) error after administration of 25 mg oral d-amphetamine. Statistical analyses were performed by Student's *t* test pairwise comparison between the drug and placebo treated group, *$p<0.05$.

3.5 Discussion

The results indicate that treatment with 25 mg oral d-amphetamine produces an overall decrease in reaction time, more pronounced in the fast block as compared to the slow block. This means the faster the target was presented the quicker the response time of individuals after the administration of d-amphetamine. Mean number of errors were increased in this study after treatment with d-amphetamine, although there was a statistical significant decrease in omission errors.

These findings are in agreement with the results of a previous study by Rapport and colleagues (1980). After the administration of a high dose (0.5 mg/kg) of d-amphetamine in a group of men performing a CPT, the percentage of omission errors decreased significantly. However, with a lower dose (0.25 mg/kg), both types of errors were increased.

Further, these results are in agreement with the simulation of human processing by a neural network using the Eriksen task (Callaway et al., 1994). This simulation predicted that changing gains (i.e. changing the speed of stimulus and response processing) in the output layers (anticipating and readying the anticipated response) of the neural network changes reaction time without changing speed-accuracy tradeoff functions. This hypothesis was tested in 12 subjects after 10 mg dose of d-amphetamine, performing an attentional task. The

reaction time of individuals was decreased however, the frequency of errors made was not affected (Callaway et al., 1994).

The novel RT test used is sensitive to fast reaction times and can be used to measure RT in an attentional task. The drawback of this design is the duration of the test that may not be long enough to measure a relatively large number of errors to support a time dependent change, unlike the Eriksen task which can be used to measure speed-accuracy trade off.

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CHAPTER 4

RELATIONSHIP OF PLASMA D-AMPHETAMINE LEVELS TO PHYSIOLOGIC, SUBJECTIVE, COGNITIVE, AND BIOCHEMICAL MEASURES IN HEALTHY VOLUNTEERS

[The work reported in this chapter forms the basis of a manuscript to be submitted]

4.1 Introduction

Patients in the manic phase of a bipolar illness exhibit symptoms that include marked euphoria or irritability, distractibility, grandiose ideas, increased arousal and activity, social dysfunction, and a decreased need for sleep (DSM-IV; American Psychiatric Press). Central dopamine overactivity has been proposed to be a factor in the pathogenesis of manic symptomatology (Praag, 1978). Studies have also shown an increase in heart rate and blood pressure (Lake et al., 1982) and a decrease in reaction time (Schwartz et al., 1989). Metabolic changes include a modest increase in plasma levels of catecholamines (Maas et al., 1984), and a decrease in plasma γ-aminobutyric acid (GABA) concentrations (Petty et al., 1993b).

Most studies in manic patients are difficult to conduct; therefore, a variety of animal models have been proposed (Robbins, 1980). Investigations in humans using acute d-amphetamine administration have shown cardiovascular, subjective and cognitive effects, which are similar to those reported in mania. In addition, d-amphetamine has been shown to increase mood, arousal, and activity and to decrease the need for sleep (de Wit et al., 1997; Miller & Griffith, 1983; Silverstone et al., 1980; Zacny & de Wit, 1989). Further, d-amphetamine also reliably increases heart rate and blood pressure (Angrist et al., 1987; Brauer et al., 1996; Caldwell &

Sever, 1974; Caldwell, 1996; de Wit et al., 1997; Hamilton et al., 1983; Hoffman, 1996; Jacobs & Silverstone, 1986; Martin et al., 1971; Morselli et al., 1976; Silverstone et al., 1983; Slattum et al., 1996) and decreases reaction time (Rapoport et al., 1980). Metabolic changes induced by d-amphetamine include an non-significant increase in catecholamine metabolite levels (Diehl & Gershon, 1992; Dommisse et al., 1984). Because of these similarities, d-amphetamine has been found to be a reproducible model for mania (Jacobs & Silverstone, 1986). However, changes in plasma levels of GABA and other amino acids have not been determined in healthy volunteers.

One of the potential confounding variables in the use of d-amphetamine as a model of mania is the relationship between plasma d-amphetamine levels and behavioral and neurochemical effects. To date, few studies have examined this aspect, although Brown and associates, (1978) correlated plasma d-amphetamine concentrations with elation in hyperactive children, while Angrist and colleagues (1987) noted a positive correlation with systolic blood pressure, but not with diastolic blood pressure and heart rate. This, study and that of Brauer et al. (1996), found a dissociation between the behavioral and physiological effects of d-amphetamine and its plasma levels, in that while the plasma levels were still rising, the behavioral effects had declined.

In view of the large number of novel medications proposed for use in patients with bipolar disorder, it is of considerable interest to determine how useful the d-amphetamine model for mania may actually be, especially since recent studies have suggested that this model may not reliably predict which drugs may be effective in the treatment of bipolar disorder (Brauer & de Wit, 1996; Brauer & De Wit, 1997; Brauer, 1995; Silverstone et al., 1998). Therefore, in order to further understand the usefulness of this model the effects of d-amphetamine, specifically the relationship between plasma d-amphetamine concentrations and physiological, subjective, cognitive, and biochemical changes have been examined in a novel and comprehensive manner in healthy volunteers.

4.2 Materials and Methods

Approval for the study was obtained from the Ethics Review Committee of the University of Alberta. A signed informed consent was obtained after written and verbal information was given to all subjects.

4.2.1 SUBJECTS

Twenty-five healthy male volunteers aged between 18-45 (mean=27 years) were selected. All subjects underwent a physical examination, a full medical interview, and an ECG. Any history of medical or psychiatric illness, previous drug use, smoking, use of any medication, or abnormal ECG readings resulted in their exclusion from the study.

The sample consisted of only male volunteers since estrogen enhances the effect of d-amphetamine on mood in women (Justice & de Wit, 1999); this effect is dependent on the stage of the menstrual cycle in that the enhanced effect is greater in the follicular phase as compared to the luteal phase (Justice & de Wit, 2000). The exclusion of female volunteers reduced potential inter-indivudual variation brought about by differences in hormonal stages.

4.2.2 PROCEDURES

A double-blind, placebo-controlled, crossover design was used. Subjects were required to attend two separate study days, each of which was two weeks apart. Each study session started at 7 am and ended eight hours later. Either 25 mg of d-amphetamine or an identical placebo capsule (lactose powder) was administered orally in a randomized fashion so that there was an equal chance of receiving either of the two at session one. The alternate substance was administered at session two. Both substances were packaged in opaque olive-coloured gelatin capsules. The dose of 25 mg was selected based on previous reports (Angrist et al., 1987; Dommisse et al., 1984) and on our own experience of reliable physiological and subjective effects at this dose.

4.2.3 METHODOLOGY

Subjects were required to fast from midnight the previous day and allowed to drink only water on the study day. On arrival, the subjects performed a 10 min practice psychomotor reaction time test to acclimatize them. Soon after, an intravenous (IV) catheter was inserted in the non-dominant arm. Baseline measurements were then taken for plasma levels of d-amphetamine, tryptophan, 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), GABA and glutamate, and for heart rate, diastolic and systolic blood pressure, reaction time, and number of errors on psychomotor testing. In addition, 11 self-rated psychological measurements were taken using 100 mm visual analogue scales (VAS) (Folstein & Luria, 1973), which have been shown to be sensitive to the effects of d-amphetamine (Jacobs & Silverstone, 1986; Silverstone et al., 1983). The variables measured were: alertness, attention, energy, anxiety, light-headedness, racing thoughts, restlessness, mood, appetite, physical well-being, and irritability.

The d-amphetamine (or placebo) capsules were administered after the baseline measurements at time = 0 min. Blood withdrawal was repeated at 30, 60, 90, 150, 210, 240, and 480 min, followed by physiological, cognitive and VAS measurements. The respective time points were selected based on previous reports that indicated maximum d-amphetamine effects within 60-180 min (Jacobs & Silverstone, 1986).

After the 4 hr time point (240 min), subjects were allowed to eat a meal. The last blood withdrawal was taken 8 hr after d-amphetamine or placebo ingestion and all tests were then repeated. The IV catheter was removed soon after. All blood samples were promptly placed on ice and centrifuged at 1500 rpm for 10 min. Plasma was removed and stored at –80 °C in 1.5 ml microfuge tubes until further analysis.

4.2.4 REACTION TIME MEASUREMENTS

A novel reaction time test was developed, using Super Lab Pro (version 1.04), to measure selective attention based on the spatial visual paradigm by Posner (1978). This test involved a cued stimulus consisting of a black cross being presented initially, after which a cross with a black box (target) or another cross appeared on the screen. Subjects were asked to press the space bar as fast as possible when the target was presented on the screen. The time taken to respond to the target was recorded as the reaction time. Errors made were also recorded. In total, 1080 trials were presented over a time period of 17 min.

4.2.5 ANALYSIS OF D-AMPHETAMINE, AMINO ACIDS, AND AMINE METABOLITES

An assay was developed to quantify plasma d-amphetamine (Asghar et al., 2002); the procedure utilized extractive derivatization with pentafluorobenzenesulfonyl chloride (PFBS) followed by detection and

quantitation by gas chromatography with electron capture detection. HPLC with electrochemical detection was used to determine plasma HVA, tryptophan, and 5-HIAA; the procedure was a modification of the method of Baker et al (1987). GABA and glutamate were analyzed by HPLC with fluorescence detection following reaction with o-phthaldialdehyde (Parent et al., 2001; Shiah et al., 2000).

4.2.6 DATA ANALYSIS

Changes from baseline for all measurements were calculated by subtracting the pre-drug score from each post-drug time point. A 6 factor analysis of variance (ANOVA) was used for the psychomotor data (reaction time only), followed by 2 x 3-way ANOVA. A general linear model analysis for repeated measures (RM-ANOVA) was used to interpret the data for each dependent measure for subjective, physiologic, and biochemical measures. Greenhouse-Geisser degrees of freedom corrections for within-subjects designs were used. Post-hoc comparisons were made using Student's *t*-tests for d-amphetamine versus placebo values at each time point. A value of $p \leq 0.05$ was the criterion for statistical significance; Pearson correlations were calculated to examine the relationship between plasma d-amphetamine and changes in biochemical, physiologic, subjective, and psychomotor measures.

4.3 Results

4.3.1 DOSE (MG/KG)

Although a dose of 25 mg was used for all the subjects in the study, a mean dose of 0.32 mg /kg was calculated from the mean weight of the volunteers 78 kg. This ranged from 59.5 to 99.4 kg with a mean dose range from 0.26 mg/kg to 0.43 mg/kg.

4.3.2 PLASMA D-AMPHETAMINE LEVELS

Peak d-amphetamine concentrations reached were 40.77 ng/ml, at 210 min after administration, as shown in figure 4-1 ($F_{1,24}$=629.8, $p<0.05$). The concentration then slowly decreased over time, although it should be noted that during the period 90 min to 480 min post-administration, the concentrations remained at more than 75% of the peak value. This is in agreement with other studies which reported peak concentrations around 3-4 hours after the administration of oral doses of d-amphetamine (Angrist et al., 1987; Brauer et al., 1996; Jacobs & Silverstone, 1986; Morselli et al., 1976; Slattum et al., 1996).

4.3.3 PHYSIOLOGIC MEASUREMENTS

There was a significant increase in systolic blood pressure (Fig 4-2:drug effect: $F_{1,23}$=31.211, $p<0.05$, time effect: $F_{3,60}$=21.567, $p<0.05$, and drug x time effect: $F_{3,70}$=17.079, $p<0.05$), diastolic blood pressure (Fig 4-

3:drug effect: $F_{1,23}$=5.720, p<0.05, time effect: $F_{4,101}$=5.511, p<0.05, drug x time effect: $F_{4,90}$=11.599, p<0.05), as well as heart rate (Fig 4-4:drug effect: $F_{1,23}$=33.775, p<0.05, time effect: $F_{3,65}$=42.843, p<0.05, and drug x time effect: $F_{4,85}$=13.747, p<0.05). The differences between placebo and d-amphetamine treatment were significant from 90 min onward, with peak mean values (changes from baseline) of systolic and diastolic blood pressure occurring at 90 (21.21 ± 1.92 mmHg) and 60 min (5.33 ± 1.56 mmHg) respectively. In contrast, heart rate continued to increase throughout the study period, with the highest change in mean value at 480 min (26.25 ± 2.39 beats/min). Systolic blood pressure correlated significantly with plasma d-amphetamine levels (r =0.476, p<0.05) at 30 and 60 min (r=0.587, p<0.05). No significant correlation existed between d-amphetamine levels and diastolic blood pressure or heart rate.

4.3.4 REACTION TIME

d-Amphetamine significantly decreased the reaction times at 30, 60, 90, 150, and 210 min compared to placebo treatment, as shown in figure 4-5 (drug effect: $F_{1,149}$=11.132, p<0.05, time effect: $F_{5,672}$=9.395, p<0.05 and drug x time effect: $F_{5,798}$=1.635, p=0.143). A mean peak decrease in reaction time was seen at 90 min (-52.85 ± 7.31 ms). Plasma d-amphetamine correlated with decrease in reaction time at 480 min (r=0.443, p<0.05) only.

4.3.5 SELF-RATING SUBJECTIVE MEASUREMENTS

Of the 11 VAS measurements made, significant drug effects were noted for anxiety ($F_{1,23}$=7.058, $p<0.05$), energy ($F_{1,23}$=18.056, $p<0.05$), speed of thoughts ($F_{1,23}$=7.814, $p<0.05$) and light-headedness ($F_{1,23}$=4.771, $p<0.05$) (Figures 4-6,7,8,9).

Significant drug x time effects occurred for alertness ($F_{4,89}$=5.254, $p<0.05$) and attention ($F_{4,97}$=2.840, $p<0.05$) (Figures 4-10 and 4-11). Changes in mean values for anxiety, energy, speed of thoughts, light headedness, alertness and attention were maximum at 60 min (1.56 ± 0.42), 90 min (2.56 ± 0.44), 90 min (2.22 ± 0.45), 60 min (2.24 ±0.63), 60 min (2.02 ± 0.4) and 60 min (0.972 ± 0.51) respectively. Plasma d-amphetamine levels correlated with alertness at 30 min ($r=0.628$, $p<0.05$), with attention at 30 min ($r=0.428$, $p<0.05$), and with speed of thoughts at 30 min ($r=0.545$, $p<0.05$).

Peak mean values for happiness, hunger, restlessness, physical well-being, and irritability occurred at 60 min (1.20 ± 0.29), 90 min (1.23± 0.29), 60 min (1.30 ± 0.48), 60 min (0.27 ± 0.39), and 60 min (1.12 ± 0.48) respectively (Figures 4-12,13,14,15, and 16). These changes were significant for happiness and irritability.

4.3.6 BIOCHEMICAL MEASURES

The plasma levels of five different biochemicals, namely 5-HIAA, tryptophan, GABA, glutamate, and HVA were measured: d-Amphetamine administration caused a significant increase in 5-HIAA levels ($F_{1,23}$=4.432, $p<0.05$), at 480 min (1.59 ± 0.92 ng/ml), as shown in figure 4-17. Apart from this finding, there were no other statistically significant drug, or drug x time effects seen for any of the biochemicals measured. Significant time effects were attained for tryptophan ($F_{1,23}$=40.83, $p<0.05$), glutamate ($F_{1,23}$=17.74, $p<0.05$), 5-HIAA ($F_{1,23}$=8.93, $p<0.05$), and HVA ($F_{1,23}$=27.35, $p<0.05$) with both placebo and d-amphetamine treatment. There was no significant change in plasma GABA levels (Figures 4-18-21).

Figure 4-1: Time-dependent changes in plasma concentration of d-amphetamine

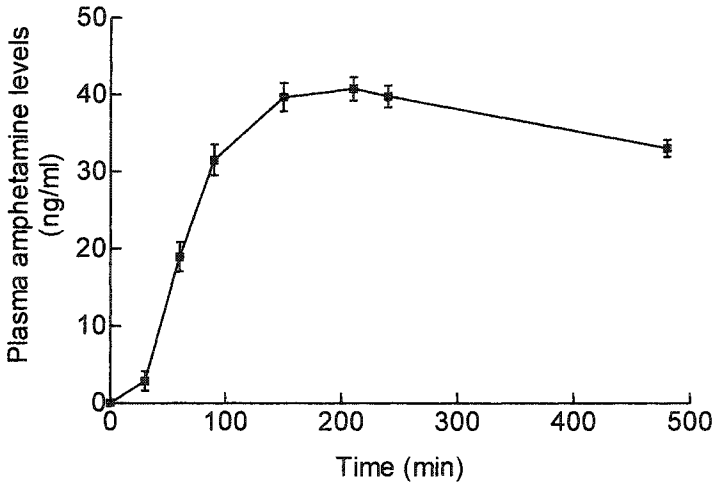
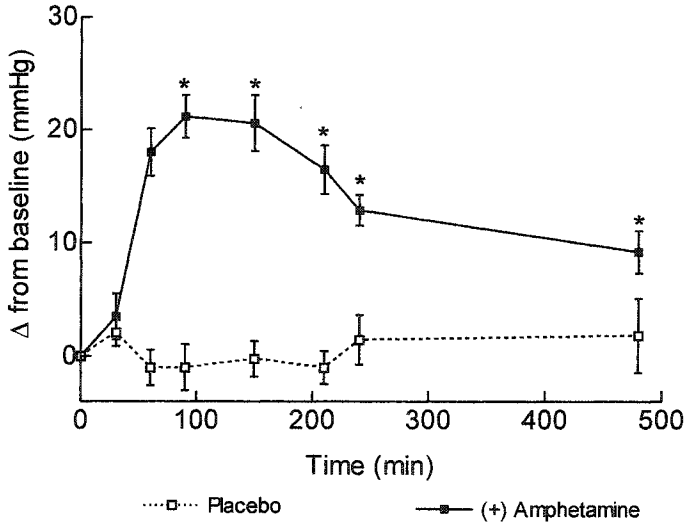


Figure 4-2: Time-dependent changes in systolic blood pressure



Data in both figures represent the mean ± SEM (n=25) change after administration of 25 mg oral d-amphetamine. Statistical analyses were performed by Student's t test pairwise comparison between the amphetamine and placebo treated group, *$p<0.05$.

Figure 4-3: Time-dependent changes in diastolic blood pressure

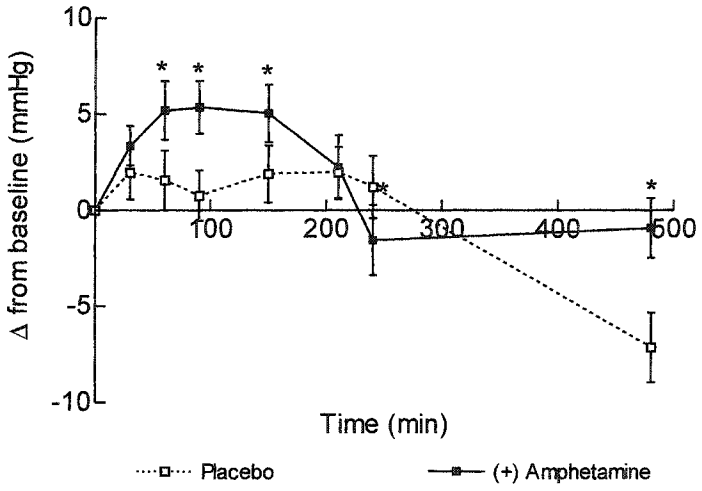
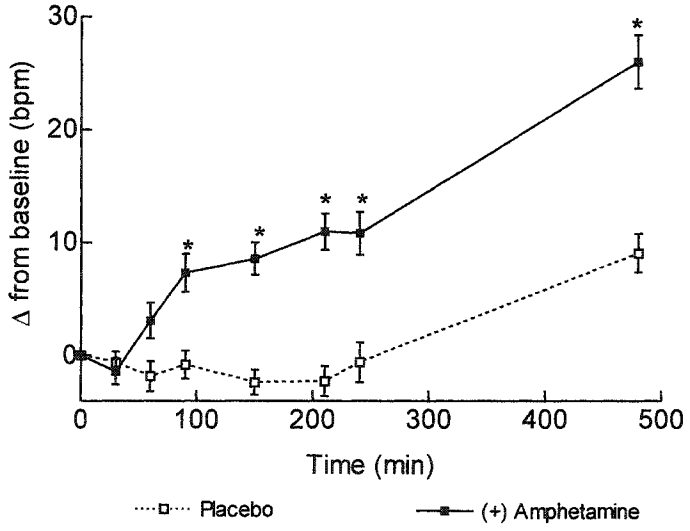


Figure 4-4: Time-dependent changes in heart rate



Data in both figures represent the mean ± SEM (n=25) change after administration of 25 mg oral d-amphetamine. Statistical analyses were performed by Student's t test pairwise comparison between the amphetamine and placebo treated group, *$p<0.05$.

Figure 4-5: Time-dependent changes in reaction time

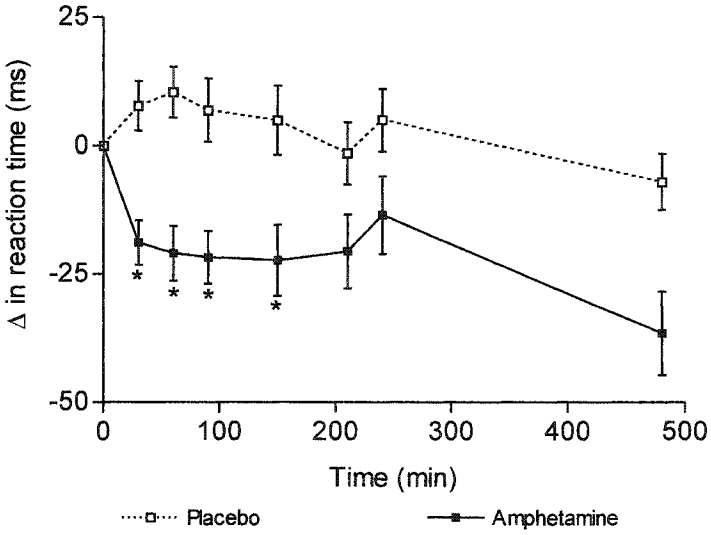
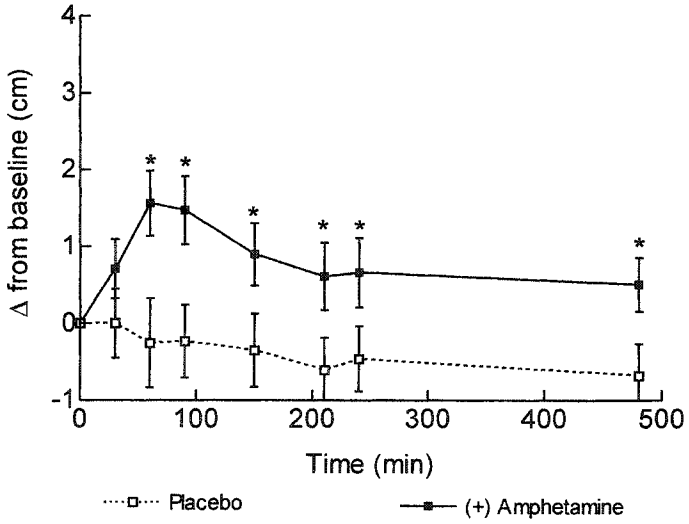


Figure 4-6: Time-dependent changes in anxiety



Data in both figures represent the mean ± SEM (n=25) change after administration of 25 mg oral d-amphetamine. Statistical analyses were performed by Student's t test pairwise comparison between the amphetamine and placebo treated group, *p<0.05.

Figure 4-7: Time-dependent changes in energy

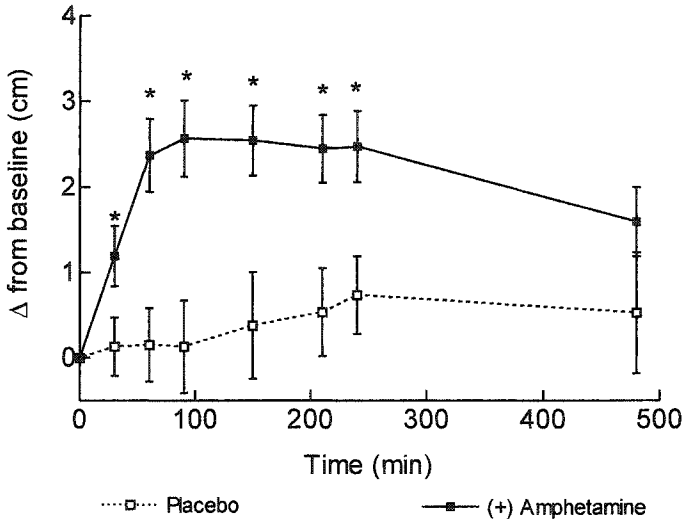
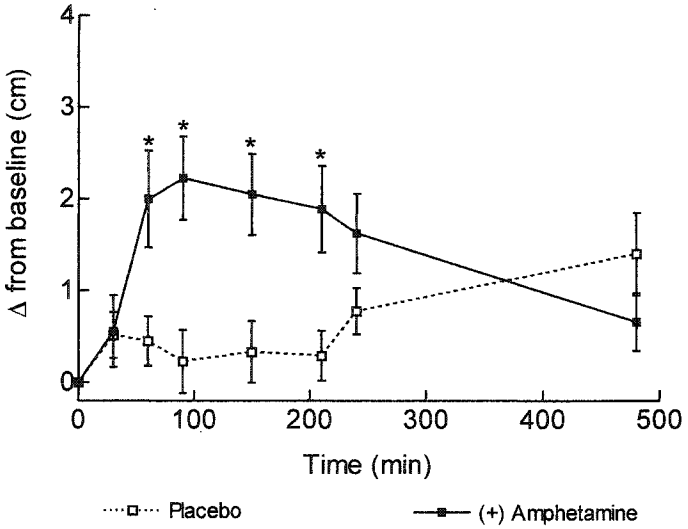


Figure 4-8: Time dependent changes in speed of thoughts



Data in both figures represent the mean ± SEM (n=25) change after administration of 25 mg oral d-amphetamine. Statistical analyses were performed by Student's *t* test pairwise comparison between drug and placebo treated group, *p<0.05.

Figure 4-9: Time-dependent changes in light-headedness

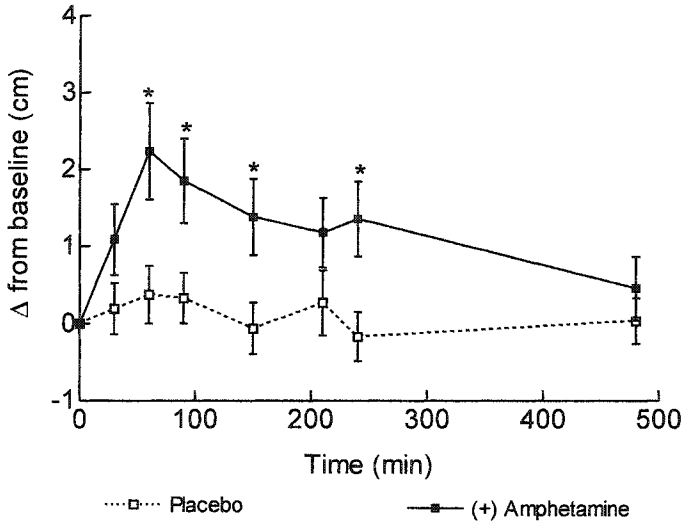
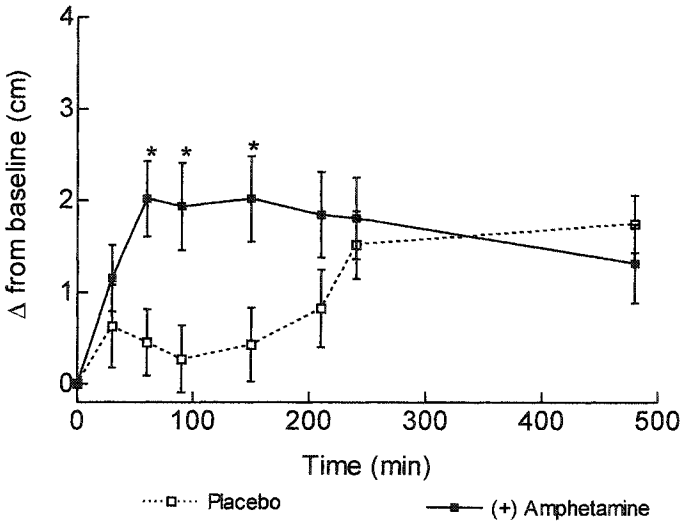


Figure 4-10: Time-dependent changes in alertness



Data in both figures represent the mean ± SEM (n=25) change after administration of 25 mg oral d-amphetamine. Statistical analyses were performed by Student's t test pairwise comparison between the amphetamine and placebo treated group, *p<0.05.

Figure 4-11: Time-dependent changes in attention

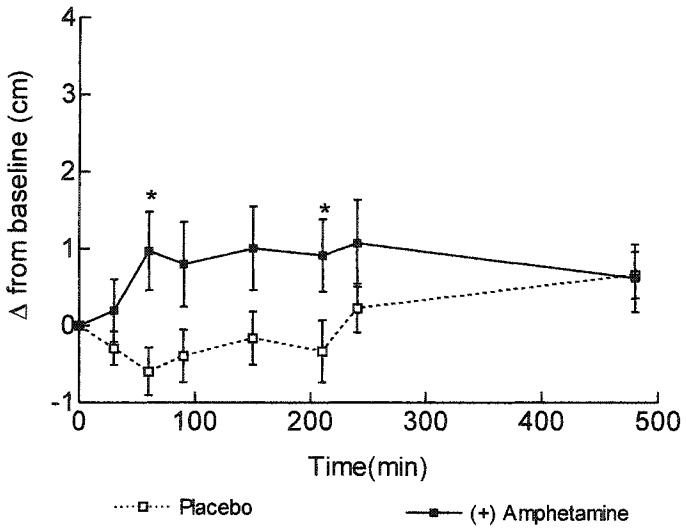
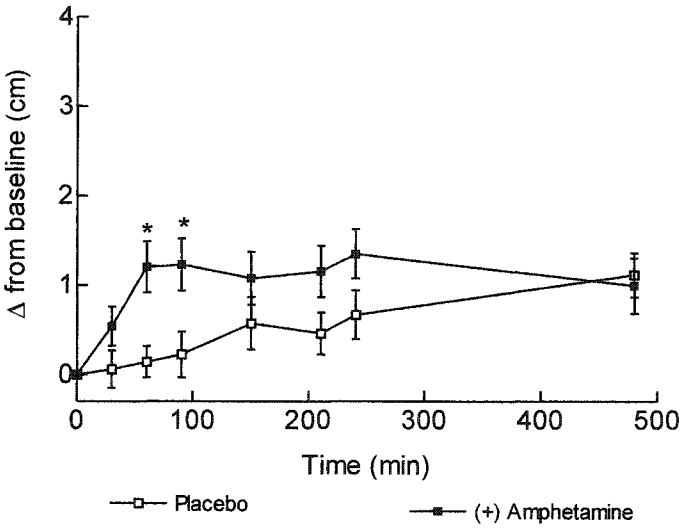


Figure 4-12: Time-dependent changes in happiness



Data in both figures represent the mean ± SEM (n=25) change after administration of 25 mg oral d-amphetamine. Statistical analyses were performed by Student's t test pairwise comparison between the amphetamine and placebo treated group, *$p<0.05$.

Figure 4-13: Time-dependent changes in hunger

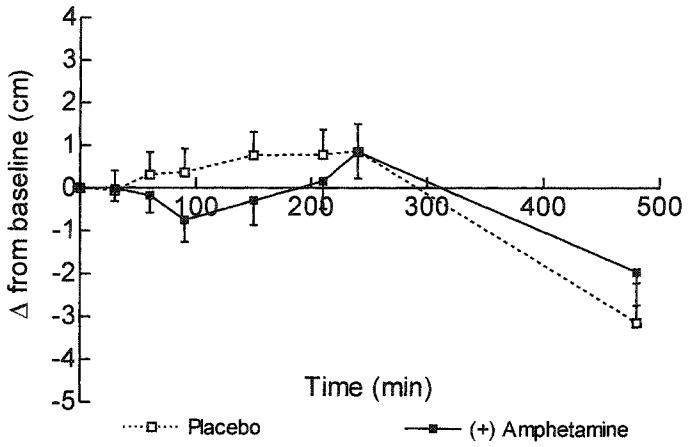
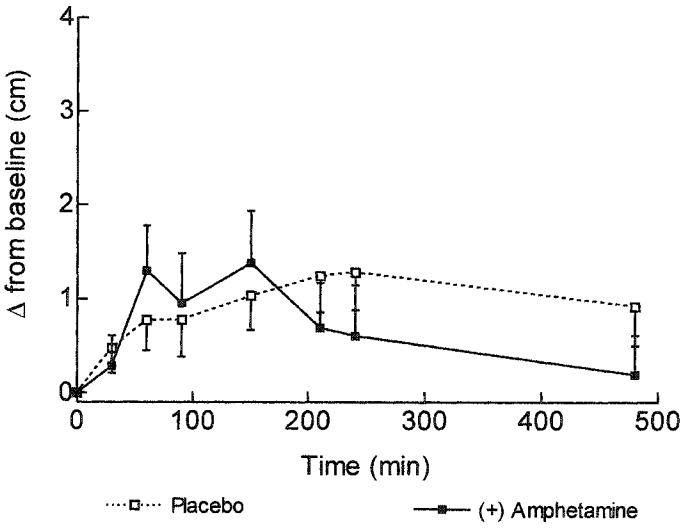


Figure 4-14: Time-dependent changes in restlessness



Data in both figures represent the mean ± SEM (n=25) change after administration of 25 mg oral d-amphetamine. Statistical analyses were performed by Student's t test pairwise comparison between the amphetamine and placebo treated group, *$p<0.05$.

Figure 4-15: Time-dependent changes in physical well-being

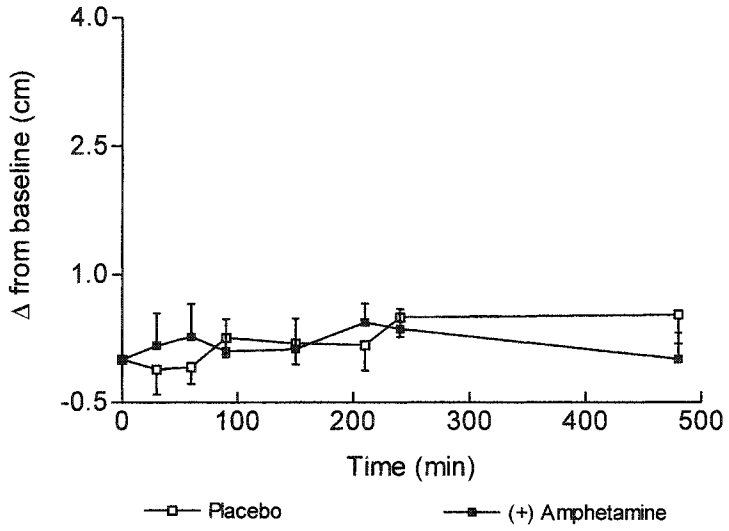
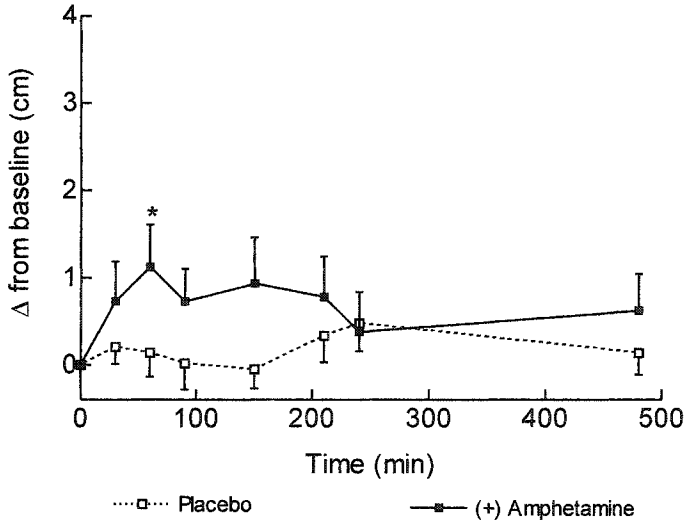


Figure 4-16: Time-dependent changes in irritability



Data in both figures represent the mean ± SEM (n=25) change after administration of 25 mg oral d-amphetamine. Statistical analyses were performed by Student's t test pairwise comparison between the amphetamine and placebo treated group, *$p<0.05$.

Figure 4-17: Time-dependent changes in plasma 5-HIAA concentration

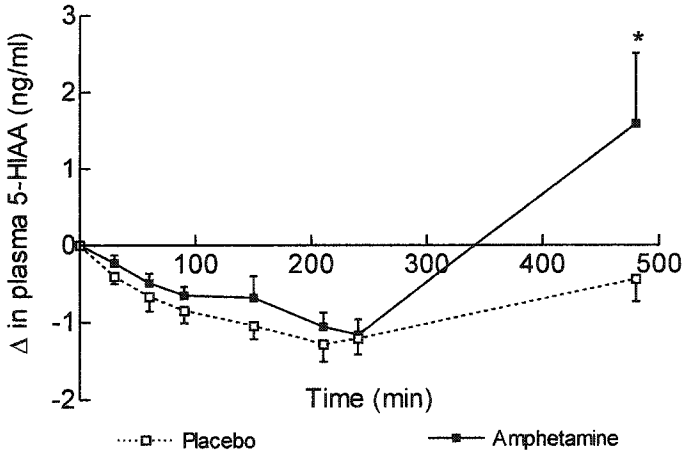
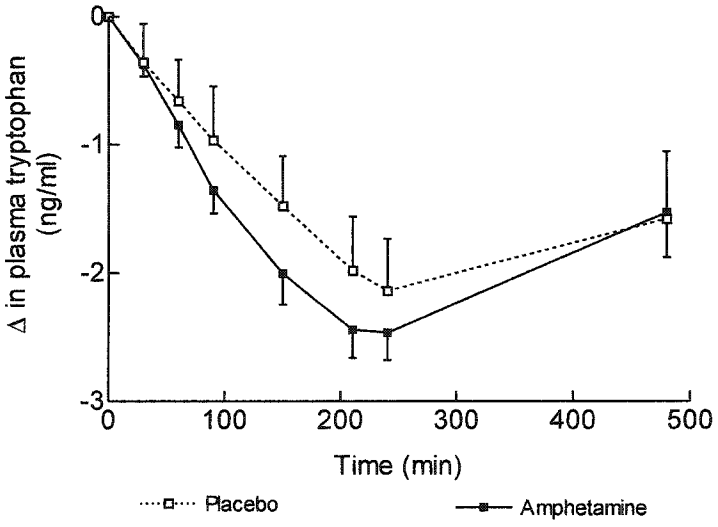


Figure 4-18: Time-dependent changes in plasma tryptophan concentration



Data in both figures represent the mean ± SEM (n=25) change after administration of 25 mg oral d-amphetamine. Statistical analyses were performed by Student's t test pairwise comparison between the amphetamine and placebo treated group, *p<0.05.

Figure 4-19: Time-dependent changes in plasma glutamate concentration

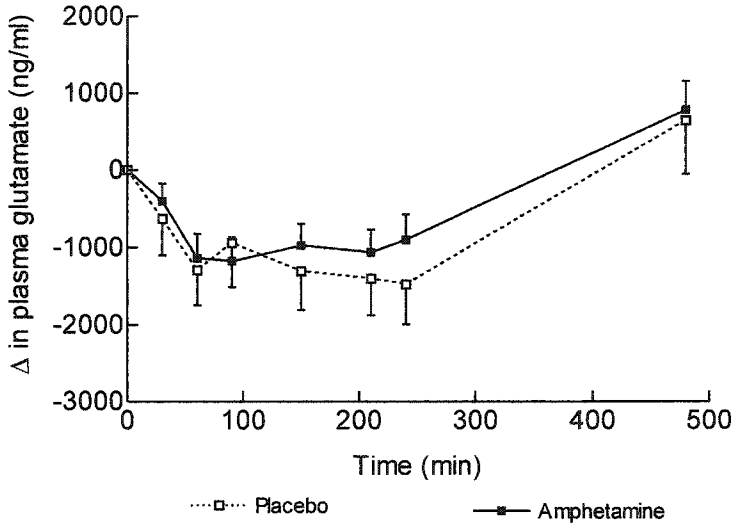
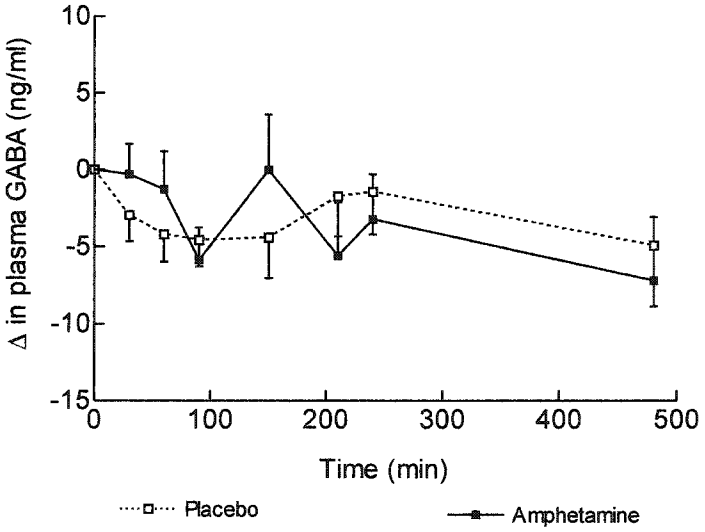
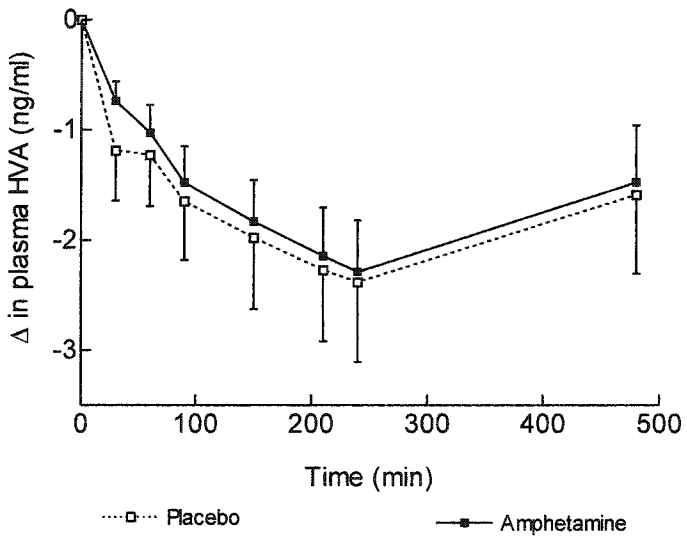


Figure 4-20: Time-dependent changes in plasma GABA concentration



Data in both figures represent the mean ± SEM (n=25) change after administration of 25 mg oral d-amphetamine. Statistical analyses were performed by Student's t test pairwise comparison between the amphetamine and placebo treated group, *p<0.05.

Figure 4-21: Time-dependent changes in plasma HVA concentration



Data in the figure represents the mean ± SEM (n=25) change after administration of 25 mg oral d-amphetamine. Statistical analyses were performed by Student's t test pairwise comparison between the amphetamine and placebo treated group, *$p<0.05$.

4.4 Discussion

In order to appreciate the potential usefulness of the d-amphetamine model for mania, understanding the way in which effects of d-amphetamine are similar to changes that occur during mania is important. Hence, in the current study we were particularly interested in examining the possible relationships between the many different effects of d-amphetamine and its plasma levels.

There have been two similar studies done in the past that have measured plasma d-amphetamine concentrations and some of the dependent measures examined in this study. Angrist et al. (1987) in a research study of 17 subjects reported a peak d-amphetamine level at 3 hr of 39.6 ng/ml on a low-dose 0.25 mg/kg of d-amphetamine. Blood pressure was seen to be maximum at 1 hr with 26 ng/ml of plasma d-amphetamine, while heart rate increased steadily beyond 5 hrs at a plasma d-amphetamine concentration of 39 ng/ml of plasma d-amphetamine. Maximum subjective changes were noted after 2 hr with a plasma d-amphetamine concentration of 39 ng/ml.

In a second study, Brauer and associates (1996) examined 6 men given 20 mg d-amphetamine, and noted peak plasma d-amphetamine levels of 40 ng/ml at 4hrs with maximum blood pressure at 3 hrs with 39 ng/ml plasma d-amphetamine concentration while heart rate was seen

to be increased at 6 hr with 36 ng/ml of plasma d-amphetamine concentration. Maximum subjective effects were observed at 1.5-2 hr with a plasma d-amphetamine concentration of 26-30 ng/ml. Both studies reported a minimum concentration of d-amphetamine of 15-20 ng/ml, producing an increase in blood pressure of >15 mm Hg (Morselli et al., 1976) at the 2-3 hr time point. This early, but unsustained, change in physiologic and subjective effects in spite of elevated plasma d-amphetamine levels is thought to be related to the rapid absorption of d-amphetamine and thus the rate taken by the minimum amount of drug needed to activate receptors at its site of action (Morselli et al., 1976).

Our results compare well with these results, with peak plasma d-amphetamine levels of 40.8 ng/ml at 3.5 hr. Peak blood pressure occurred at 1.5 hr, with a plasma d-amphetamine concentration of 31.5 ng/ml, and subjective effects were maximum at 60-90 min, with a plasma d-amphetamine concentration of 19-31.5 ng/ml. These results indicate that the initial increase in plasma d-amphetamine levels is reflected in a change in subjective, cognitive and blood pressure measurements. However, these do not exactly parallel peak plasma d-amphetamine levels. Interestingly, the subjective, cognitive and blood pressure measures were seen to be elevated over time and did not reach baseline values as previously reported. This may be attributed to the dose used in

our study i.e. 25 mg of d-amphetamine whereas, most studies have used slightly lower doses.

Changes in several subjective measurements, which are consistent with increased mood and arousal, were noted. These changes tended to be maintained, and to be closely correlated to the plasma d-amphetamine concentrations. These results are in agreement with previous studies which have also shown an elevation of mood and arousal (de Wit et al., 1997; Miller & Griffith, 1983; Silverstone et al., 1980; Zacny & de Wit, 1989). It has been suggested that this increase in mood and arousal may be particularly closely correlated to a d-amphetamine-induced increase in central dopamine release (Cho et al., 1999), although recent studies (Brauer & de Wit, 1996; Brauer & De Wit, 1997; Brauer, 1995; Silverstone et al., 1998) indicate that the effects of d-amphetamine, particularly, the euphorigenic effects, may in healthy volunteers be mediated by neurotransmitter pathways other than dopaminergic ones. Our results did not indicate any significant changes in plasma levels of the dopamine metabolite, HVA, which is in agreement with the studies mentioned above (Brauer & de Wit, 1996; Brauer & De Wit, 1997; Brauer, 1995; Silverstone et al., 1998).

It is interesting to note that at 25 mg dose there were no significant changes seen in hunger after d-amphetamine administration. However, at both 20 and 10 mg doses a pronounced decrease in hunger was reported

by Silverstone et al.(1983). Ratings in arousal and mood rating were greater with the 20 mg dose as compared to the 10 mg dose which may imply differences in the underlying neurochemical mechanisms mediating the stimulant and anorectic effects of d-amp.

The present study found that d-amphetamine administration resulted in a decrease in reaction time in a selective attention task. This change was seen at about 90 min after administration of d-amphetamine, at a plasma d-amphetamine concentration of 31.5 ng/ml. This is similar to previous reports that have shown improvements in reaction time produced by d-amphetamine (Rapoport et al., 1980).

Whilst previous studies have shown an increase in total plasma catecholamine and metabolite concentrations during mania (Maas et al., 1984), few studies (Diehl & Gershon, 1992; Dommisse et al., 1984) have demonstrated a significant change in catecholamine levels after acute d-amphetamine administration. The results from the present study did not reveal any significant changes in the biochemicals under investigation with the exception of an increase in 5-HIAA. This may be a reflection of an increased release of serotonin with a higher dose of d-amphetamine (Sloviter et al., 1978). However, a rapid dose-dependent increase in brain dopamine concentration and decreases in the concentrations of the dopamine metabolites DOPAC as well as of the 5-HT metabolite 5-HIAA have been clearly demonstrated in animal studies (Butcher et al., 1988;

Kuczenski & Segal, 1989; Miele et al., 2000; Parada et al., 1988; Schwarting & Huston, 1992; Scorza, 1997). The levels of HVA were not significantly changed (Butcher et al., 1988; Parada et al., 1988). A positron emission tomography (PET) study in seven healthy volunteers revealed a positive correlation between ventral striatal dopamine release and euphoria (Drevets et al., 2001); an IV dose of 0.3mg/kg of d-amphetamine was used in that study. Our results did not indicate such a correlation between HVA levels and euphoria. This may be due to peripheral HVA measurements as compared to CSF HVA, which may be a better reflection of changes in dopamine.

The study reported here was the first to examine the effects of d-amphetamine on plasma GABA and glutamate concentrations. Interest in plasma GABA as a marker for vulnerability to development of mood disorders has emerged over the past decade (Petty, 1994; Petty et al., 1993b). Plasma GABA is thought to reflect GABA levels in the brain extracellular space and in the cerebrospinal fluid (Petty et al., 1993a; Adinoff et al., 1995). However this has been disputed by other schools of thought (Loscher, 1982; Schmidt & Loscher, 1982). Our results showed that d-amphetamine administration had no effect on the plasma concentrations of either of these amino acids. These results contrast with reports that plasma GABA concentrations are decreased in manic patients (Berrettini et al., 1982; Berrettini et al., 1983; Petty, 1995; Petty et al.,

1993a) and increased in brains of rats administered d-amphetamine (Lynch & Leonard, 1978). Studies on brain glutamate levels are contradictory, with reports of a decrease in brain glutamate levels (Miele et al., 2000) while others report an increase in glutamate levels after acute d-amphetamine administration to rats (Del Arco et al., 1999; Wolf et al., 2000; Xue et al., 1996).

In conclusion, the results from this study suggest that several of the effects of d-amphetamine, for example subjective psychological changes, reaction time changes, and heart rate mirror the time-course of plasma levels of the drug. Other changes, such as blood pressure changes, seem to occur rapidly and then return to normal values while d-amphetamine concentrations are still at 75% of peak values. d-Amphetamine, at least in the present study, did not alter amine metabolite or amino acid concentrations. Taking these findings together suggests that, whilst d-amphetamine administration definitely causes several changes which are seen in mania, there are nonetheless several physiological and metabolic differences between these two states which may limit its potential usefulness as a model. As well, it may be important to note that varying oral doses of d-amphetamine may also affect the usefulness of the model itself.

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CHAPTER 5

GENERAL DISCUSSION

5.1 Summary

In Chapter 1 a general overview of amphetamine and bipolar disorder was presented in order to fully appreciate the role of amphetamine as a model of mania used in this study. In Chapter 2, a modification of an existing method for the measurement of plasma d-amphetamine levels was discussed followed by a novel method of measuring reaction time and errors in Chapter 3. The relationship of plasma d-amphetamine levels with dependent measures was used to explore further the usefulness of d-amphetamine as a model for mania in Chapter 4.

5.2 Relationships between measurements

The relationships between plasma d-amphetamine levels and dependent measures have been summarized in Table 5-1. The results indicate a rapid rise in physiologic, subjective and cognitive measures while plasma d-amphetamine levels are still rising. However, these measures rapidly decrease while d-amphetamine levels remained sustained and do not reach baseline levels even at 8 hours of the study. Of note is the fact that heart rate continued to increase throughout the 8 hour study period, similar to the findings of Caldwell (1974) and that this response differs significantly from the changes in blood pressure, which

peaked at 90 min. This discrepancy has been attributed to a peripheral mechanism of blood pressure regulation as compared to a central mechanism for heart rate.

5.3 d-Amphetamine as a model of mania

5.3.1 REPLICATION OF PREVIOUS RESULTS

The results indicate that d-amphetamine at an oral dose of 25 mg may be a more useful model for mania in that its relationship with dependent measures are sustained and do not decrease to baseline values as rapidly as seen with doses of 17.5 mg (Angrist et al., 1987) and 20 mg (Brauer et al., 1996; Brauer & De Wit, 1997; de Wit et al., 1997; Jacobs & Silverstone, 1986) of oral d-amphetamine.

This has been proposed to be due to a depletion of catecholamine stores, to replacement by a 'false neurotransmitter' metabolite of d-amphetamine or to alterations in receptor sensitivity (Kopin, 1965; Kopin, 1968). Others have thought that the initial rapid absorption of d-amphetamine across the blood-brain barrier gave rise to the initial increase in physiologic, subjective and cognitive effects. This was followed by a reduction of the drug absorption due to the vasoconstrictive effect of the drug on the vascular bed of the gastrointestinal tract (Morselli et al., 1976).

Table 5-1: Peak and trough mean values, and F-values of d-amphetamine on physiologic, subjective, cognitive, and biochemical measures (significant p value<0.05 indicated by a *)

Measure	Time (min)	Mean ± SEM (amp)	Mean ± SEM (placebo)	Amp	Hr	Amp x Hr
Physiologic measures						
Systolic bp	90	21.21± 1.92	-0.88 ± 2.06	31.211*	21.567*	17.079*
Diastolic bp	90	5.92 ± 1.27	0.88 ± 1.33	5.720*	5.511*	11.599*
Heart rate	480	26.25 ± 2.39	9.04 ± 1.71	33.775*	42.843*	13.747*
Subjective measures						
Anxiety	60	1.56 ±0.42	-0.26± 0.58	7.059*	3.208*	1.245
Energy	90	2.56± 0.44	0.13± 0.54	18.056*	2.943*	2.444
Speed of thoughts	90	2.22± 0.45	0.23± 0.34	7.814*	1.838	8.806*
Light-headedness	60	2.24± 0.63	0.37 ± 0.37	4.771*	3.845*	1.792
Alertness	60	2.02 ± 0.41	0.448 ± 0.36	3.690	1.971	5.254*
Attention	60	0.972 ± 0.51	-0.60± 0.31	3.555	1.950	2.840*
Happiness	60	1.20 ±0.29	0.14± 0.18	3.239	3.632*	2.144
Hunger	90	1.23± 0.29	0.22± 0.26	0.108	12.342*	2.473
Restless	60	1.30± 0.48	0.77 ± 0.32	0.006	3.801*	1.645
Physical well-being	60	0.27± 0.39	-0.08 ± 0.20	0	1.373	1.745
Irritability	60	1.12± 0.48	0.14 ± 0.28	2.015	0.285	1.904
Cognitive measure						
Reaction time	90	-52.85 ±7.31	7.76 ± 7.80	11.132*	9.395*	1.635
Biochemical measures						
Dextroamp	210	40.36 ± 1.59	0	629.8*	204.03*	204.03*
Tryptophan	240	-2.47 ± 0.21	-2.16 ± 0.41	0.377	40.83*	0.73
GABA	90	-5.86 ± 2.09	-4.58 ± 1.71	0.002	1.737	1.029
Glutamate	90	-1179.01± 304.89	-944.55± 568.77	0.198	17.741*	0.566
5-HIAA	480	1.59 ± 0.92	-0.42 ± 0.30	4.432*	8.935*	3.599
HVA	240	-2.63 ± 0.32	-2.41 ± 0.74	0.001	27.348*	0.294

5.3.2 LIMITATIONS OF THE STUDY

Although this study has attempted to measure the effects of d-amphetamine in a novel way, there are certain limitations. The first of these is the control population group, which is not representative of the patient population, namely individuals with bipolar disorder. As is often the case with clinical studies, a group of 25 volunteers may not be enough to draw statistically significant results as compared to a larger group. In addition, a dose of 25 mg of d-amphetamine was used while most other reported studies have used 20 mg.

Secondly, the reaction time test developed to measure selective attention had not been tested before in a group of volunteers before being used in the study. However, the same test is being used after administration of 25 mg d-amp in healthy volunteers in an ongoing functional Magnetic Resonance Imaging (fMRI) study.

The duration of the study may not have been long enough to assess effects of d-amphetamine after 8 hr since by 8 hr d-amphetamine levels had not declined markedly from peak levels. Ideally, the volunteers should have been followed for at least 24 hours however, this was not feasible at the time.

The biochemical measures taken may not have been representative of central biochemical measures. CSF measurements taken at the same time would have provided a clear indication if the

plasma measures were representative of CSF measures. However, spinal taps are invasive and painful and not ideal for a long study.

5.4 Future research

Similar studies in euthymic bipolar patients may shed further light on the usefulness of the d-amphetamine model.

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