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EVALUATION OF QUANTITATIVE ELECTROENCEPHALOGRAPHY FOR ASSESSMENT OF CENTRAL NERVOUS SYSTEM STIMULANT RESPONSE

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EVALUATION OF QUANTITATIVE ELECTROENCEPHALOGRAPHY FOR ASSESSMENT OF CENTRAL NERVOUS SYSTEM STIMULANT RESPONSE

PART I. REPRODUCIBILITY OF CONTROL RESPONSES

PART II. COMPARISON OF QUANTITATIVE ELECTROENCEPHALOGRAPHY TO BEHAVIORAL, PSYCHOLOGICAL AND NEUROENDOCRINE MEASURES OF RESPONSE TO DEXTROAMPHETAMINE

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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AIC	Akaike Information Criteria
AMP	total EEG amplitude - all frequencies
AMPA	total EEG amplitude - alpha band
AMPBI	total EEG amplitude - beta I band
AMPBII	total EEG amplitude - beta II band
AMPD	total EEG amplitude - delta band
AMPT	total EEG amplitude - theta band
ARCI	Addiction Research Center Inventory scales
$AUC_{0\text{-last}}$	area under the drug concentration-time curve through time = t_{last}
AUC_{∞}	area under the drug concentration-time curve extrapolated to time = infinity
$AUMC_{0\text{-last}}$	area under the moment curve through time = t_{last}
$AUMC_{\infty}$	area under the moment curve extrapolated to time = infinity
BLQ	below the limit of quantitation
CBC	complete blood count
C_i	serum concentration at t_i
Cl	apparent total body clearance
C_{last}	the last measured serum concentration
Cl_r	renal clearance
C_{max}	maximum serum concentration observed
CNS	central nervous system
CPT	continuous performance task
CPTAL	average latency on continuous performance task
CPTPC	percent correct on continuous performance task
DA	dextroamphetamine
DBP	diastolic blood pressure
DDA	descriptive data analysis
DFA	difference from actual, expressed as a percent
ECD	electron capture detector
EEG	electroencephalography
E_{max} - (1st 4 hr)	the maximum observed effect during the first four hours
E_{max}	the maximum observed effect (response)
E_{min} - (1st 4 hr)	the minimum observed effect during the first four hours
E_{min}	the minimum observed effect (response)
ET	effect time, calculated as $\Sigma(C_i*t_i)$
F	bioavailability
FID	flame ionization detector

FSH	follicle stimulating hormone
FTLT	finger tapping with left hand
FTRT	finger tapping with right hand
GC	gas chromatography
HR	heart rate
k	terminal (elimination) rate constant
ka	absorption rate constant
LH	luteinizing hormone
MRT	mean residence time
MS	mass spectrometry
NPD	nitrogen phosphorus detector
ϕ	noncentrality parameter
PFB	pentafluorobenzoyl chloride
POMS	Profile of Mood States
PRIH	prolactin release-inhibiting hormone
PRO	prolactin serum concentration
REML	restricted maximum likelihood
RIA	radioimmunoassay
RPA	relative EEG power - alpha band
RPBI	relative EEG power - beta I band
RPBII	relative EEG power - beta II band
RPD	relative EEG power - delta band
RPT	relative EEG power - theta
RSD	relative standard deviation, expressed as a percent
RSDA	RSD of EEG amplitude - alpha Band
RSDD	RSD of EEG amplitude - delta Band
SBP	systolic blood pressure
SIM	selective ion monitoring
SMAC-20	blood chemistries
TCA	trichloroacetyl chloride
TFAA	trifluoroacetic anhydride
t_i	time at the ith hour after dosing
t_{last}	time of the last measured serum concentration
T_{max}	the time from dosing to C_{max}
TP	total EEG power - all frequencies
TPA	total EEG power - alpha band
TPBI	total EEG power - beta I band
TPBII	total EEG power - beta II band
TPD	total EEG power - delta band
TPT	total EEG power - theta band
TSH	thyroid stimulating hormone
W	Shapiro-Wilk statistic

EVALUATION OF QUANTITATIVE ELECTROENCEPHALOGRAPHY FOR ASSESSMENT OF CENTRAL NERVOUS SYSTEM STIMULANT RESPONSE

ABSTRACT

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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The objective of this investigation was to evaluate quantitative electroencephalography (EEG) as a measure of CNS stimulation. The reproducibility and sensitivity of quantitative EEG was compared to neuroendocrine, mood, and psychomotor performance measures.

The study was conducted in two parts. The first part investigated the inter- and intra-individual variability associated with a series of pharmacological response measures under baseline (no drug) conditions. It was an open-label pilot study in which eight healthy male volunteers underwent a series of tests (EEG, visual continuous performance task (CPT), a finger tapping task, and self-rated mood scales) repeated eight times over a 12 hour period on three occasions, one week apart. The second part evaluated the sensitivity of quantitative EEG to dextroamphetamine (DA) compared to other response measures. It was a double-blind, placebo-controlled, four-period crossover study in eight healthy male volunteers. Subjects received 5 mg,

10 mg, or 20 mg DA or placebo orally, and underwent the same series of tests as well as blood collection for serum prolactin and DA determination, eight times over a 12 hour period. A GC method allowing quantitation of 2ng/mL DA in serum was developed.

The greatest between-day, within-day, and intrasubject variability was associated with quantitative EEG. Learning effects were observed for the psychometric tests, and first session effects were apparent for several of the tests including the EEG. EEG response to DA was observed only in the 3 subjects who had baseline alpha activity greater than 35%. There was a statistically significant decrease in serum prolactin levels after DA administration, with the largest decrease observed after the 5 mg dose. Mood scales showed that 3 of 9 subjects experienced dysphoria after DA dosing. The effect on mood was generally greater as the dose increased. One subject was discontinued from the study because he experienced intense dysphoria after the 5 mg dose. Doses could not be distinguished based on the results of the psychometric tests. Effects on mood, serum prolactin levels, and performance as measured by CPT and finger tapping were not correlated with the EEG changes observed. Pharmacokinetic evaluation showed that the rate of DA absorption appears to decrease as the dose increases.

Quantitative EEG conducted under our study conditions and study population was not more sensitive for the assessment of CNS stimulation than the other response measures evaluated. The sensitivity may be improved by screening volunteers to select subjects with higher background alpha activity.

CHAPTER 1

Introduction

Accurate and reproducible measures of drug effect on the central nervous system (CNS) are needed in order to study the pharmacodynamics of centrally-acting drugs.[1] Pharmacodynamics, or the relationship between drug concentration in the systemic circulation and pharmacologic effect, is important because it contributes to the interindividual variability observed in drug response. Determining the association between drug concentration and subsequent response is necessary for optimizing drug therapy. Studies of the pharmacodynamics of centrally-acting drugs have been limited primarily by the difficulty in obtaining quantitative measures of CNS response.[2]

Ideally, a measure of drug effect used in pharmacodynamic studies should be quantitative, objective, and non-invasive. Generally, there should be a gradual, rather than an all-or-none, change in the response measure with changing drug concentration. The measure should be sensitive to small differences in drug concentration. The pharmacodynamic measure should be reproducible both within and between individuals. It is important to be able to measure the response repeatedly in the same individual without changes occurring due to learning or tolerance. Lastly, the response measure should be meaningful; the measured response should relate to the therapeutic or toxic clinical effects of the drug. [2,3]

Various psychometric tests, ranging from self-rating scales of psychologic state to computerized performance tasks, have been used to assess the pharmacodynamics of centrally-acting drugs.[2,4,5] Psychometric tests are noninvasive and the response can be quantitated. However, these tests are not ideal pharmacodynamic measures. Although some tests can measure certain aspects of behavior as a function of drug response, they are more or less subjective and may not be reproducible. Many psychometric tests are not suitable for repeated measures, since learning and motivational factors influence the results of subsequent tests. These limitations may contribute to insensitivity of the measures to small changes in serum drug concentrations. The relationship of performance on psychometric tests to the "real life" behavioral and psychologic effects of drugs are also difficult to define. Therefore, psychometric tests are not entirely acceptable as CNS response measures.

More recently, quantitative EEG has been employed to measure CNS pharmacodynamics.[2,6] Many studies using EEG to profile or classify psychoactive drugs have been conducted, but few studies have attempted to correlate EEG parameters with concurrently measured drug concentrations and response to psychometric tests. Pharmacodynamic modeling of the EEG effects of CNS depressants such as anesthetic agents[7,8,9,10] and benzodiazepines[11,12,13,14] has been performed successfully. Quantitative EEG is objective, noninvasive, and derived parameters change gradually with changes in plasma drug concentration. Repeated or continuous measures of the EEG can be made, although a familiarization session before the study is advisable to avoid a first-session effect due to anxiety.[15] Learning effects on the EEG have not been reported.[2] Recording of the EEG also

requires less subject cooperation than completion of psychometric tests. The reproducibility and sensitivity of quantitative EEG parameters however, requires further evaluation. The behavioral or psychologic meaning of changes in EEG parameters is also unclear. If these issues can be addressed, quantitative EEG may become a preferred measure of CNS pharmacodynamic response.

This study was designed to evaluate quantitative EEG as a pharmacodynamic tool to measure CNS stimulation. Dextroamphetamine was chosen as the model compound for this evaluation. Dextroamphetamine is a sympathomimetic amine known to have potent CNS stimulant effects. The *d* isomer of amphetamine is 3 to 4 times more potent in exciting the CNS than the *l* isomer.[16] Single doses have been administered safely to normal volunteers. Its concentration in the systemic circulation can be measured adequately by gas chromatographic assay methods and it does not have active metabolites that play a clinically significant role after single doses.[17] The renal excretion of dextroamphetamine is dependent on urinary pH and volume, so acidifying the urine will result in constantly enhanced excretion. When urine pH is maintained between 5 and 5.5, the elimination half-life of dextroamphetamine is approximately 7 hours.[18] Dextroamphetamine has been reported to decrease delta activity and increase alpha and beta activity on the EEG.[19,20] Mood changes after dextroamphetamine have been measured using a variety of rating scales.[21,22,23,24,25] It also produces measurable effects on performance tasks.[26,27,28,29,30] Dextroamphetamine affects the neurotransmitters dopamine, norepinephrine, and serotonin.[31] Changes in these neurotransmitter systems result in changes in the release of hormones such as prolactin. The prolactin release after

dextroamphetamine administration could also be used in this study as a pharmacodynamic response measure. Based on these characteristics, dextroamphetamine was chosen as a model compound to test the sensitivity of quantitative EEG as a pharmacodynamic measure of CNS stimulation.

1.1 Objectives

These studies were designed to test the following hypotheses: 1) quantitative EEG is a sensitive and reproducible measure of the CNS's response to sympathomimetic drugs as compared to more widely used methods such as psychometric testing, subjective rating scales, or neuroendocrine tests and 2) changes in the EEG after sympathomimetic drug administration are related to the behavioral, psychological and neuroendocrine effects observed as well as the plasma concentration of the drug.

Two studies were conducted. The purpose of the first study was to investigate the inter- and intra-individual variability associated with a series of potential CNS pharmacodynamic response measures, including quantitative EEG, under baseline (no drug) conditions. Results from this study were used to design a study of quantitative EEG as a pharmacodynamic measure for CNS stimulation. The second study was designed to examine the relationship between EEG changes after administration of dextroamphetamine and 1) performance on automated psychometric tests, 2) serum prolactin levels, 3) subjective response as assessed by self-rated mood scales, and 4) serum concentration of dextroamphetamine. The sensitivity of EEG parameters to dextroamphetamine concentration in serum was compared with that of more subjective measures.

1.2 Rationale

It is necessary to understand the pharmacodynamic and pharmacokinetic factors influencing the response to a drug to optimize drug therapy for the individual patient and to support efficient and rational development of new drugs.[32] To accomplish this task, quantitative, sensitive, accurate, and reproducible measures of drug effect are needed. Identification of a suitable measure of the CNS's response to drugs has proven to be particularly difficult. EEG has been used to qualitatively describe the effects of drugs on the CNS since its development by Hans Berger in 1929.[33] With the advent of the Fast Fourier transform and advanced digital computing, EEG has become a quantitative as well as a qualitative descriptor of brain electrical activity. The sensitivity and reproducibility of the EEG as a pharmacodynamic measure requires further evaluation. In addition, the behavioral and psychological meaning of drug-induced EEG changes remains unclear. These studies were designed to evaluate the sensitivity and reproducibility of EEG as a response measure compared to psychometric tests that are more often used, and to provide a better understanding of the relationship of the EEG to the clinical effects of the drug. A more sensitive, reproducible measure of CNS response is necessary to evaluate the effects of the aging process and various disease states on the pharmacodynamics of centrally-acting drugs. In addition, an improved CNS response measure for sympathomimetic drugs is needed to evaluate the CNS-stimulating properties of drugs such as phenylpropanolamine, where the degree of CNS stimulation and its potential clinical significance in man is controversial.[34]

CHAPTER 2

Literature Review

2.1 Quantitative Electroencephalography

EEG is the recording of changes in the electrical potential of various regions of the brain as measured by electrodes placed on the scalp. These fluctuating electrical potentials or brain waves can be characterized by their voltage or amplitude, and frequency. Brain wave patterns vary depending on the region of the brain being measured, the level of consciousness of the subject, and the age of the subject. EEG is used to record the spontaneous background activity of the brain or the activity evoked by external sensory stimulation.

Attempts to quantitate the patterns observed in the EEG began with the earliest EEG recordings.[35] The EEG tracing shows fairly irregular patterns, and cannot easily be described by explicit mathematical relationships. Rather, it is characterized in statistical terms, by probability distributions and averages.[36] As advances in computing technology have developed, a variety of techniques have become available for analyzing EEG data quantitatively. Some of these include frequency or spectral analysis, bispectral analysis, topographic mapping, compressed spectral arrays, and significance probability mapping.[35,37] EEG signals are processed by Fast Fourier transformation, aperiodic analysis, wavelet analysis, and other techniques.[38] EEG

measurements can be described by various scores (dependent variables) in various domains (independent variables). Domains include the time domain (score versus time), the frequency domain (score versus frequency) and the spatial domain (score versus electrode location). A wide range of scores have been used, including intensity or amplitude, power or the square of the amplitude, coherence, z-scores, relative power, peak frequency, frequency ratios, functions from discriminant analysis, slope descriptors (activity, mobility and complexity), values from period and zero-crossing analysis, and parameters from autoregressive modeling or spectral parameter analysis. Quantitative analysis of the EEG results in a massive amount of information and some sort of data reduction is usually necessary.[36] Various statistical descriptors have been employed for this purpose. A number of computerized systems for quantitative EEG analysis are now commercially available.

Many potential problems in the techniques and the interpretation of quantitative EEG have been identified. The functional significance of changes in quantitative EEG parameters is largely unknown. The role of quantitative EEG in diagnosing conditions affecting the brain and monitoring response to treatment is controversial.[39,35] A considerable amount of research employing quantitative EEG techniques is currently being conducted, but the clinical utility of quantitative EEG remains to be confirmed. Despite the current limitations of quantitative EEG technology and our incomplete understanding of the meaning of quantitative EEG data, it is still one of the only ways to continuously and noninvasively examine the functioning brain with fraction-of-a-second temporal resolution.[40] Significant technological developments, such as computerized tomography and magnetic

resonance imaging provide superior structural resolution, but do not evaluate dynamic brain activity. Positron emission tomography and single-photon emission computerized tomography examine brain function by tracking blood flow and local cerebral metabolism, but lack the temporal resolution of EEG. Interest in the use of quantitative EEG to understand brain physiology and behavior, to diagnose brain dysfunction and to monitor the effects of therapeutic interventions continues.

2.1.1 Use of Quantitative EEG in Drug Research

Evidence of CNS activity for many compounds ranging from aspirin to hormones has been provided by EEG studies.[41] The EEG has been used to examine the effects of drugs on the central nervous system since Hans Berger's descriptions of EEG changes following cocaine, scopolamine, morphine and barbiturate administration in the 1930s.[33] It was not until the 1950s however, that systematic studies of the effects of medications on the EEG were conducted by Bente, Itil, Fink and others.[42] As computer technology and its applications in EEG analysis grew, investigators in the 1960s began using quantitative EEG patterns in an attempt to classify psychoactive compounds and predict responders to therapy.[33,41] Early studies relating EEG changes after drug administration to effects on behavior were not entirely successful, and apparent dissociations between the EEG and behavior resulted in waning interest in the utility of EEG in psychopharmacology from the mid 1960s until the mid 1970s. In the 1970s, renewed interest in EEG and drug research developed.[33] Since that time, technology for quantitating EEG has also grown tremendously. Use of quantitative techniques to examine drug effects has proven

much more useful than visual inspection of EEG records because drugs induce changes in the EEG following acute administration of therapeutic doses that are within normal variability.[41] Controlled experiments and mathematical signal analysis are needed to identify drug-induced EEG changes.

Quantitative EEG has been employed in a wide variety of applications in clinical psychopharmacology including classification of psychoactive drugs[42,43], prediction and investigation of CNS activity early in a drug's development[44,45,46], examination of the CNS toxicity of drugs whose primary action is outside the CNS[47,48,49], comparison of the bioavailability of various dosage forms of psychoactive drugs[42,50,51,52], and investigation of the pharmacodynamics of centrally-acting drugs. Quantitative EEG has not proved to be as useful to study drug abuse or to predict responders to drug treatment.[41] The use of quantitative EEG to evaluate pharmacodynamic relationships will be reviewed in more detail below.

Pharmacodynamic studies examine the relationship between drug concentration in the systemic circulation and pharmacologic effect. Pharmacodynamic studies employing EEG as a measure of pharmacologic effect have investigated the time course, peak effect, and duration of CNS activity for many psychoactive drugs.[41] Because the psychological and behavioral meaning of EEG changes following drug administration is not well defined, the EEG is considered to be a surrogate measure of drug effect.[53,54] The clinical relevancy of EEG changes has not been established. Quantitative EEG has been used most successfully to establish pharmacokinetic-pharmacodynamic relationships for two groups of drugs--anesthetic agents and the benzodiazepines.

Anesthetic drugs cause profound effects on the EEG as subjects progress from consciousness to unconsciousness.[54] By quantitating these EEG effects and applying pharmacodynamic modelling procedures, much has been learned about the clinical pharmacology of anesthetic agents. Quantitative EEG techniques have been used to investigate intravenous anesthetics (thiopental, etomidate, methohexital, and propofol), opiates (fentanyl, alfentanil, sufentanil, and morphine), dissociative anesthetics (ketamine) and benzodiazepines (midazolam).[54] Pharmacodynamic studies of thiopental using the spectral edge of the EEG as a response measure have examined the rate of equilibration of thiopental between the blood and the sites of action, individual CNS sensitivity to thiopental, and whether acute tolerance to the drug develops during repeated infusions.[8,7] Studies of the EEG effects of fentanyl and alfentanil have found that the differences in the time course of the clinical effects of these opioids can be explained by differences in the rate of equilibration between effect site and plasma concentrations.[55] Using the median frequency of the EEG power spectrum as a response measure, investigators have also employed pharmacodynamic modelling to examine differences in the CNS activity of the enantiomers of ketamine.[9] Parameters from aperiodic analysis of the EEG were found to be suitable for measuring the pharmacodynamic effects of midazolam during anesthesia,[13,56] and EEG effects have been used to compare the CNS potencies of benzodiazepines.[14] Pharmacodynamic modelling of EEG effects has also been used to examine the effect of age on the pharmacodynamics of thiopental.[57,58] The use of quantitative EEG in pharmacodynamic investigations has been most successful with the anesthetic agents, perhaps due to the alterations in consciousness and associated

substantial EEG changes produced by these drugs. There is also some evidence that the EEG changes observed with anesthetic agents are related to the depth of anesthesia achieved,[8] so the EEG changes may also be a clinically meaningful response measure.

Pharmacodynamic studies using quantitative EEG have also been productive for the benzodiazepines when the drugs are given in doses and by routes of administration that do not result in loss of consciousness.[59] EEG changes have been used to study tolerance to alprazolam in healthy volunteers,[12] the time course of CNS activity after loprazolam administration in the elderly,[60] and the circadian variation in the CNS effects of midazolam.[61] Measures that have been used to describe benzodiazepine effects on the EEG include percent alpha activity,[61] total amplitude in the 13 - 30 Hz range,[11] and spectral edge.[12] Quantitative EEG is an objective measure of the effects of benzodiazepines, and EEG changes may correspond to changes in sedation, mood, psychomotor performance, and memory.[59]

Much work still remains in realizing the potential of EEG in drug research. Investigations in drug classification with EEG is continuing.[62,63] Quantitative EEG is an objective, noninvasive, continuous response measure which shows promise for understanding the pharmacodynamics of CNS-active drugs. Quantitative EEG has been used most successfully to study the pharmacodynamics of anesthetics and benzodiazepines, but its utility for other drugs requires further work. The relationship between EEG changes and important aspects of performance, mood, and cognition is unclear. The utility of EEG compared to other CNS response measures also requires further investigation.

2.1.2 Methodology in Pharmaco-EEG Studies

A number of methodological issues are important in the studies incorporating quantitative EEG as a response measure. They have been raised by investigators in pharmaco-EEG and quantitative EEG more generally. The quantitative EEG parameters obtained at the end of a study lie at the end of a long chain of physiological, technical, and mathematical steps that are all susceptible to error and artifact. Careful control of experimental conditions in pharmaco-EEG studies is essential. This review will address subject screening, the testing environment, familiarization sessions, control of vigilance, artifact minimization, choice of reference electrode, number of EEG channels recorded, length of data sample, stimulation modalities, definition of classic frequency bands, and choice of EEG-derived parameters for measuring response.

Careful subject screening to ensure a homogenous subject group is essential. Demographic, physical, and psychological attributes can influence the EEG and the response to psychoactive drugs. The EEG depends on factors other than the drug under study, including age, gender, and medical history.[41] Smoking and smoking withdrawal can also alter the EEG.[41] Correlations between quantitative EEG and the menstrual cycle have been described.[41] The EEG response to drugs is influenced by the emotional status and neuropsychological traits of the subject, and the baseline EEG pattern prior to drug administration.[41,64] Some studies require screening EEGs to contain a certain level of alpha activity for example, for the subject to be eligible for entry into the study.[65,66] Drug effects on mood, performance, and the EEG have been shown to depend on psychological selection criteria.[67] Some investigators

recommend that subjects be interviewed before beginning each study period concerning the quality and duration of their sleep the night before dosing.[67] If the sleep is more than one hour under the average length of sleep for the subject, the investigation is delayed by one week. Guidelines for conducting Pharmaco-EEG studies in man suggested by an expert group organized at the Federal Health Office of the Institute for Drugs (Berlin) state that subjects should be as homogeneous as possible with respect to demographic characteristics, medical status, sleep history, use of tobacco, personality characteristics (such as emotional lability, neuroticism, and extroversion/introversion), and reaction to stress.[68] If these variables cannot be controlled, they should as least be documented.

Careful control of the testing environment is critical to the success of pharmaco-EEG studies. The setting in which experiments are conducted and procedures used to collect pharmacodynamic data can affect the EEG.[41] EEG changes can occur with changes in blood pressure, heart rate, sleep habits, and blood sugar.[41] Stressful testing situations can trigger neurohormonal changes that can affect the EEG.[41] Unsystematic changes in the testing environment are particularly problematic and can affect the data enough to alter the results of statistical analysis.[41] Factors important to standardize between treatment periods include time of day for the recording, quality of the previous night's sleep, and type and scheduling of meals.[41] Subjects must be isolated from sensory stimulation and intermittent disturbances during EEG recordings.[41] Some studies have been carried out in sound-attenuated electrically-shielded room with the recording equipment isolated from the subject.[69] Subjects have worn headphones to exclude external noise.[70] In general, experiments should

be designed to avoid spontaneous fluctuations in vigilance. Wong recommended that quantitative EEG measurements be obtained in a quiet, dark environment.[71] The subject should be instructed to remain relaxed and alert. This setting fosters decreased vigilance, and may be inappropriate for studying sedative drugs or potentially useful for studying stimulants.[41] The guidelines suggested by the expert group of the Federal Health Office of the Institute for Drugs state that factors that should be controlled include room temperature, humidity, sound level and light intensity, intermittent disturbing events, organization of the measurement setting, position of the subject, amount and nature of sensory stimulation, degree of interaction with staff, adaptation to the situation, level of vigilance, time of recording, and timing and type of meals.[68]

Familiarization sessions are necessary to acclimate subjects to the testing environment and the study procedures prior to administering study treatments. Irwin and Fink report that less EEG changes and a more stable level of alertness occurred during the first session in pharmaco-EEG studies than on subsequent days.[15] They postulate that this increased alertness is due to unfamiliarity with the testing situation. Herrmann notes that an single blind adaptation day is necessary because subjects are often restless and anxious on the first day in a study.[67]

Vigilance is a term used to describe the behavior of watching for and responding to irregular critical signals.[72] It encompasses the concepts of attention, attentiveness, and arousal. Vigilance is the primary factor affecting the resting EEG.[41] Alpha amplitude is attenuated when subjects reported a subjective feeling of decreased vigilance.[49] Some studies have included both a vigilance-controlled and a

resting EEG recording at each measurement time.[73] During the vigilance-controlled EEG the technician tries to keep the subject alert by arousing the subject when signs of drowsiness appear on the EEG. Pooling data across subjects whose alert EEG patterns are intrinsically different can result in loss of information.[70] Subjects may also be requested to perform a simple auditory continuous performance task to stabilize the level of vigilance.[74] Another method proposed to aid in the maintenance of vigilance is to have subjects continuously press a button.[75] If they release the button, a tone sounds to arouse them. Changes in vigilance are easier to detect in subjects with high background alpha activity.[74] Some investigators have given snacks every few hours rather than heavy meals at usual meal times during pharmaco-EEG studies to reduce the effects of heavy meals on vigilance.[73]

It is important to accurately select artifact-free epochs for quantitative EEG analysis.[76] Artifacts can occur due to eye movement, muscle activity, respiratory motion, glossal and pharyngeal movement, sweating, poor electrode contact, and ambient electrical fields.[77] Some artifacts unique to quantitative EEG also occur, such as leakage and smearing.[35] Leakage results in increased frequency values across the whole spectrum and occurs when data with a nonzero initial and final value is used for a frequency transformation. Smearing is the artifactual broadening of peaks in the frequency domain resulting from the use of certain filters designed to reduce leakage. Detection of artifacts is not an easy task. Automated artifact rejection may be helpful, but the detection rates are not sufficiently high at this point to replace a detailed review of the EEG record for artifacts by the electroencephalographer.[35,78] Artifact monitoring electrodes can be used to measure vertical and horizontal eye

movements to aid in the artifact rejection process. Artifacts are most prominent in the delta and theta frequency ranges.[77] Editing of the EEG record to remove artifacts should be conducted by an individual blinded to the treatment that the subject received to ensure that marking of artifacts is not drug-specific.[67]

Choice of reference electrodes also presents a problem.[35] Many different reference sites have been suggested, including linked clavicles, linked mandibles, chin, linked ears, nose, vertex, an average of an electrodes closest neighboring electrodes, and an average of all other electrodes. Any of these reference sites can become contaminated with EEG or other electrical activity, which obscures the interpretation of the EEG. There is no convincing evidence that one referencing scheme is preferable to another for quantitative EEG.[69] In pharmaco-EEG, where the methodology is highly standardized, the choice of reference may be unimportant as long as the reference is not active.[69] Most investigators use linked ears as the reference point for quantitative EEG studies.[77] Linked ears is an effective reference as long as the potentials are relatively low in amplitude and uncorrelated.[79] If one or both of the ears is very active, the results can be very misleading. Unlike traditional EEG, quantitative EEG techniques often allow for the recording of several reference sites simultaneously. Different references can then be examined during data analysis.

The number of EEG channels recorded during pharmaco-EEG studies is also important. Different numbers of electrodes are applied to the scalp for quantitative EEG measurements by different investigators. Typically, 16 to 32 electrodes are used. Investigators have used as few as 2 and as many as 128. There is no

consensus on the number or placement of electrodes for quantitative EEG beyond the traditional 10-20 system of 21 electrodes.[35] Spatial distribution of EEG effects may be important for assessing the effects of drugs, so a sufficient number of electrodes to permit examination of spatial distributions are necessary.[41] The number of electrodes is limited by the expense of amplification and filtering equipment, computer speed and storage capacity, and difficulty of attaching large numbers of electrodes quickly and accurately.[77]

The epoch length and duration of each recording also requires consideration. Quantitative EEG data is usually acquired and processed in very short segments of time termed epochs or frames. The typical length is several seconds, but can be as long as 30 seconds. Usually, successive epochs are acquired and analyzed, so that the entire recording last for several minutes. The epoch length needs to be short enough to allow for rejection of all artifact contaminated epochs without jeopardizing the entire recording, but long enough so that the frequency analysis can accurately determine the lowest frequency components.[35] The number of epochs recorded is also important. Stability or ergodicity of the EEG refers to changes in the EEG (such as change of state or vigilance) during the recording or between recording sessions. The recording should be short enough to minimize changes in state during the recording, but long enough to gather sufficient artifact-free epochs for further analysis.[35] EEG activity must be sampled over a period that is sufficiently long to insure that it is representative of the state of the subject.[77] Herrmann reports that a 5 minute recording was long enough to ensure that means were not overly dependent on random variation and short enough to minimize the effects of fluctuating vigilance.[67]

A variety of stimulation modalities have been incorporated in pharmaco-EEG studies. Under conditions of no stimulation, spontaneous EEG is measured during some level of alertness such as alert with eyes-open or eyes-closed, drowsy, or in other sleep states. In general, the level of vigilance maintained during spontaneous EEG recordings must be carefully controlled. The improved control of situational variability during sensory stimulation may result in improved sensitivity of evoked phenomena compared to quantitation of spontaneous EEG.[41]

The definition of the classic frequency bands is also an important consideration in quantitative EEG studies. The classic EEG frequency bands are often defined as 0-4 Hz for delta, 5-7 Hz for theta, 8-13 Hz for alpha, and 14 Hz or greater for beta.[35] In quantitative techniques however, different divisions between the bands are sometimes employed. For example, frequency ranges may be defined to allow for even breaks at 4 Hz intervals (4, 8, and 12 Hz). Band definitions are also chosen based on clinical experience or statistical methods such as factor analysis.[77] There is quite a bit of variability between manufacturers and investigators in the definitions of the frequency bands, so it is important to know the delineation used in a particular investigation when evaluating results.[35]

A wide variety of EEG-derived parameters have been used as response variables in pharmaco-EEG studies. Several response variables from spectral analysis have be used for comparing treatments.[77] Power, which is the square of the amplitude, emphasizes very active areas, while amplitudes provide good resolution of mid- and low-range activity. Relative power or amplitude (which is calculated by dividing the total power or amplitude in the given frequency band by the total power or amplitude

across all frequency bands) controls for absolute differences in magnitude between the measures, thus facilitating a direct qualitative comparison between subjects. Spectral edge, or the frequency below which 95% of the total EEG power is located, have been used to quantitate the effects of benzodiazepines and anesthetics. Several parameters from aperiodic analysis have also been used for this purpose.[54] Irwin[65] proposed a measure called the spectral difference index which is a measure of the difference between two relative power spectra. This index was found to be a sensitive EEG discriminator between drug and placebo sessions for a variety of drugs. A similar parameter, the spectral dynamics measure has also been proposed.[80] Response variables may differ in their sensitivities among subjects, and results of studies may differ depending on the response measure under consideration.[65] For general anesthetics for example, different response measures have been useful for each class of drugs.[54] There is no ideal measure of drug response based on the EEG for all drugs. Measures should show within-individual baseline consistency and a minimal response to placebo. Appropriate measures for different classes of drugs and the clinical relevance of changes in these measures requires further investigation.

Obtaining consistent, accurate results in pharmaco-EEG studies depends in large part on carefully controlling the conditions under which the investigation is conducted. Some of the factors that must be considered in designing, conducting and interpreting pharmaco-EEG studies are discussed above. In the next section, statistical issues important for interpreting the results in these studies are reviewed.

2.1.3 Statistical Problems in EEG Analysis

Statistical difficulties arise in the analysis of pharmaco-EEG studies primarily due to the multiplicity of observations: A large number of EEG parameters are derived from at least several electrode sites at several points in time.[81] The studies are usually crossover in design, with placebo and several drugs or dose levels of a drug administered. Other measures such as psychometric tests and mood scales may also be collected. Because of the sizable amount of data and multiple inferential statistical statements, classical methods of confirmatory statistics used in clinical trials cannot be applied directly. The large number of measurements inflates the likelihood of finding chance differences from placebo (alpha error). In addition, data in EEG studies is usually gathered from a small number of subjects. Because of the small number of subjects, statistical generalizations are seldom possible.

Several approaches have been proposed to address the issue of multiplicity of observations. To apply a confirmatory statistical analysis, a few response measures believed to have the must important clinical significance could be chosen for hypothesis testing prior to initiating the study. The p-value must then corrected for the multiple comparisons by methods such as the Bonferroni correction.[82] Another strategy is to reduce the total number of variables for hypothesis testing by pooling variables. This could involve summing across groups of electrodes or frequency bands, or performing techniques such as a principal components analysis.[82] Again, correction of the p-value for the number of comparisons being made is necessary. Yet another strategy, although impractical, would be to use a very large number of subjects in the study.

In many pharmaco-EEG studies, confirmatory statistical analyses are not feasible. It may not be possible to pre-select effect measures to examine before conducting the study, especially for new drugs. It may also be of interest to examine a large number of variables to generate new hypotheses. In these cases, statistical analysis is treated as exploratory in nature. Whenever large numbers of post-hoc analyses are performed in exploratory data analysis, replication of the results in comparable prospective controlled studies is necessary. When this type of validation, which is the most convincing, is not feasible, alternate approaches can be considered, several statistical validation schemes could be used.[78] These strategies involve reserving a portion of the data for testing the results obtained with the rest of the data.

As an alternative to confirmatory and exploratory analysis, Abt has proposed a concept termed "Descriptive Data Analysis" for topographical EEG data.[83,84] In this approach, expected differences between the treatments based on previously reported studies and patterns apparent from examining the data are evaluated statistically without adjustment of the level of significance. The results of these analyses are used to make descriptive inferential statements about the data, but not to reject null hypotheses. This approach takes into account the idea that greater confidence can be placed in statistical results if they have biological relevance (for example, if results are related to dose level, are consistent across subjects, or reflect the behavioral effects or pharmacokinetics of the drug).

Several solutions have been applied to the problem of multiplicity of observations in pharmaco-EEG studies. None of these approaches is entirely suitable

however, and more work is necessary to address this problem.

2.2 Dextroamphetamine

Dextroamphetamine, a sympathomimetic amine with CNS stimulant properties, was chosen as a model compound to study the sensitivity of quantitative EEG as a pharmacodynamic measure. Amphetamines have been used clinically since 1935 to treat conditions such as obesity, narcolepsy, hypotension, and attention deficit disorder. Currently, dextroamphetamine preparations are subject to control under the Federal Controlled Substances Act of 1970 as Schedule II drugs. Characteristics of dextroamphetamine and its effects on the central and peripheral nervous system are described in the following sections.

2.2.1 Chemical Structure

Dextroamphetamine is the *d* isomer of amphetamine (β-phenylisopropylamine). The chemical structure is shown in Figure 2.1. Dextroamphetamine is a basic drug, with a pKa of 9.90. Amphetamine was first synthesized in 1887.[17]

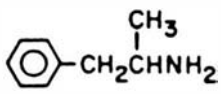

Figure 2.1 The chemical structure of amphetamine

2.2.2 Pharmacology[85]

Amphetamine is an indirectly acting sympathomimetic drug with potent CNS stimulant properties. The *d* isomer, dextroamphetamine, is three to four times more potent as a CNS stimulant than the *l* isomer. The *l* isomer is slightly more potent in its effects on the cardiovascular system. Amphetamines affect the CNS, the cardiovascular system and smooth muscle.

In the CNS, amphetamines stimulate the medullary respiratory center, the cerebral cortex and possibly the reticular activating system. The resulting psychological effects depend on the dose and the state and personality of the person taking it. Amphetamines can cause changes in mood (Section 2.2.6) and in performance on various mental and motor tasks (Section 2.2.7). Usage for long periods of time or at high doses is usually followed by fatigue and depression. Amphetamines have also been reported to decrease appetite perhaps through action on the lateral hypothalamic feeding center.

Amphetamine's action on the CNS appears to result from the release of biogenic amines from their storage sites in the nerve terminals of neurons in the CNS. Release of norepinephrine appears to be related to increased alertness, appetite suppression, and some aspects of motor stimulation. At higher doses, dopamine release appears to be responsible for other aspects of altered motor activity and stereotyped behavior. At even higher doses, release of 5-hydroxytryptamine (serotonin) may result in disturbances of perception. In addition, amphetamine is believed to exercise a direct agonistic effect on serotonin receptors. Release of these various neurotransmitters is associated with changes in the levels of peptide hormones of the anterior pituitary.

These changes are discussed in Section 2.2.5.

The effects of amphetamine on the cardiovascular system result from a combination of release of norepinephrine from peripheral nerve terminals and direct action on peripheral α and β receptors. Administration of amphetamine results in increased systolic and diastolic blood pressure. Heart rate is increased or may be slowed by compensatory reflexive mechanisms. Effects on smooth muscle, as in the gastrointestinal tract, is variable, depending on the state of enteric activity. If activity is high, amphetamine may cause relaxation with slowing of movement of intestinal contents. On the other hand, if the gastrointestinal system is already relaxed, activity may be increased.

Because amphetamines act on multiple receptors and neurotransmitters in the peripheral and central nervous systems, measures of response associated with several body systems can be used to assess amphetamine pharmacodynamics. Although the mechanisms are not well understood, some measures of CNS stimulation, such as mood scales and psychomotor performance tasks are more directly related to pharmacological action on specific neurotransmitters or areas of the CNS than others. For measures based on changes in the EEG after administration of amphetamine, the relationships are even less clear. Changes in the EEG following amphetamine administration are discussed in Section 2.2.4.

2.2.3 Pharmacokinetics

The pharmacokinetics of amphetamine have been studied in normal and drug-dependent volunteers since the 1950s. The pharmacokinetic literature for

amphetamine was recently reviewed by Busto and colleagues.[86] A summary of pharmacokinetic parameters obtained from studies in normal volunteers is shown in Table 2.1. Data on the absorption, distribution and excretion of amphetamine is presented below.

Absorption. Information about the rate and extent of amphetamine absorption from the gastrointestinal tract is limited. Beckett and Rowland[87] analyzed urinary excretion data by the Wagner-Nelson Method and determined that the absorption of amphetamine appears to be complete within 2.5 hours of an oral dose of 10 mg d-amphetamine (1 subject) or 15 mg l-amphetamine (3 subjects). Angrist and colleagues[88] report that maximum concentrations following 0.25 mg/kg and 0.5 mg/kg of amphetamine given orally occurred 2-3 and 3-4 hours after administration respectively. At 1 hr, the average plasma levels were lower for the high dose than for the low dose. The authors hypothesize that differences between the brands of tablets used to prepare the doses in the high and low dose study may account for this observation. Wan and colleagues report that concurrent administration of enteric-coated ammonium chloride or sodium bicarbonate orally did not appear to affect the rate or extent of amphetamine absorption.[18] The presence of food does not appear to alter the absorption of amphetamine.[88]

Distribution. Protein binding of racemic (±), *d*- (+), and *l*- (-) amphetamine was determined using an ultrafiltrate technique at plasma concentrations of 10 to 100 ng/mL. Plasma protein binding was similar for the two isomers, about 16%.[18] Cerebrospinal fluid concentrations are about 80% of that observed in plasma, which reflects the extent of protein binding.[89] The apparent volume of distribution after

Table 2.1 Summary of Amphetamine Pharmacokinetic Studies in Normal Volunteers

Reference	Isomer	Dose/Route	Urine pH	C_{max} (ng/mL)	T_{max} (hr)	Cl (ml/min)	$t_{1/2}$ (hr)
Wan, Matin & Azarnoff[18]	+/-	10 mg PO	alkalinized				17.0 (+) 23.7 (-)
	+/-	10 mg PO	acidified				6.8 (+) 7.7 (-)
	+	10 mg PO	alkalinized				15.6 (+)
	-	10 mg PO	alkalinized				25.0 (-)
Beckett & Rowland[87]	+	15 mg PO	acidified				4.9
	-	15 mg PO	acidified				5.6
	+	10 mg PO	acidified				5.5
	+	13 mg IV	acidified				4.5
Davis, Kopin, Lemberger & Axelrod[90]	+/-	5.8 nM IV	acidified				8-10.5
			alkalinized				16-31
Beckett, Salmon & Mitchard[91]	+	15 mg PO	uncontrolled	44		16-115	
			acidified	49.5		242-539	
Angrist, Corwin, Bartlik & Cooper[88]	+	0.25 mg/kg PO	uncontrolled	39.6[a] (± 2.8)	2-3		
				35.3[b] (± 3.4)	2-3		
	+	0.5 mg/kg PO	uncontrolled	67.25[b] (± 5.45)	3-4		

C_{max} = maximum serum or plasma concentration observed
T_{max} = time at which C_{max} occurs
Cl = apparent total body clearance
$t_{1/2}$ = elimination half-life

a = fasting
b = nonfasting

oral amphetamine is approximately 250 L in normal healthy volunteers[92] and similar between the enantiomers, indicating extensive distribution.[18]

Excretion. Amphetamine is eliminated by excretion unchanged in the urine and by metabolism. In a study of amphetamine pharmacokinetics following 10 mg oral doses, Wan and associates found that plasma amphetamine levels decline monoexponentially following absorption and can be adequately described by a 1-compartment model.[18] Peak concentrations following administration of either isomer were proportional to dose.

The excretion of amphetamine in urine is dependent on the urine pH and urine flow. When the urine is acidified, the excretion of unchanged amphetamine is approximately four times that of the deaminated metabolites (hippuric and benzoic acids). When the urine is alkalinized, excretion of the deaminated metabolites is approximately equal to that of the unchanged drug.[90] The elimination half-life under conditions of acidic urine production (pH $<$ 6.0) is 8 to 10.5 hours, while under alkaline conditions (pH $>$ 7.5), the half-life was prolonged to 16 to 31 hours.[90] The renal clearance of amphetamine also depends on urine flow. When the pH is between 5 and 6, renal clearance is about half as much if flow is less than 30 mL/hr than if flow is 30-125 mL/hr. Diuresis, with flows greater than 125 mL/hr, further increases renal clearance of amphetamine.[93] In a study by Beckett and Rowland[87] however, the influence of urine flow rate on the urinary excretion rate of amphetamine appeared to be minimal. The rate of urinary excretion of amphetamine is directly proportional to amphetamine plasma concentrations when urine is maintained under acidic conditions. This does not hold true when urine pH is allowed to fluctuate.[91] Under

uncontrolled conditions for urine pH, amphetamine renal clearance can be accounted for by glomerular filtration. Under acidic conditions however, the renal clearance is much higher than the glomerular filtration rate, indicating that other processes are contributing to the overall renal clearance. Beckett and associates hypothesize that the drug passes from blood to urine as the urine flows down the kidney tubules due to the high concentration gradient of un-ionized drug across the membrane.[91]

After a 5 mg oral dose of [^{14}C]amphetamine, 90% of the ^{14}C was excreted into the urine (pH not controlled) in 3 to 4 days, with about 60 - 65% excreted on Day 1.[94] On Day 1, approximately 30% of the ^{14}C was excreted unchanged. Metabolites included 4-hydroxyamphetamine (3%), benzoic acid (21%) and hippuric acid (16%). Hydroxyamphetamine is pharmacologically active, but probably does not exert a clinically significant effect after a single dose of amphetamine. d-amphetamine has a shorter elimination half-life than l-amphetamine, because d-amphetamine is metabolized more rapidly.[18] This is more apparent under basic urine conditions, when metabolism is the major route of elimination. Metabolism of amphetamine by deamination is stereospecific[94], and occurs more extensively for the d- isomer. The elimination half-life in chronic amphetamine abusers is significantly longer than in drug-naive control subjects. The difference was not apparent under acidic urine conditions, but was noticeable under alkaline urine conditions.[93] This may be due to increased tissue affinity for amphetamine in drug-dependent individuals.

Amphetamine does not appear to undergo hepatic recycling, as none was found in the bile (free or conjugated) after the administration of 10 mg d-amphetamine sulfate of a cholecystectomy patient with a bile duct fistula.[87]

2.2.4 EEG Changes after Amphetamine Administration

EEG changes following amphetamine administration have been reported in normal volunteers after single doses in several studies. In an early study, Pfeiffer and associates[95] examined EEG changes in 20 male volunteers (21-30 years of age) after receiving racemic amphetamine (0.1 mg/kg) intravenously, dextroamphetamine (15 mg) orally, or placebo intravenously and orally in a crossover fashion. EEG changes were quantitated using a measure termed cortical electrical energy. Activity from the left parietal area was integrated for the intravenous doses, and left occipital activity was integrated for oral doses. After intravenous dosing, the mean cortical electrical energy and its variability were significantly decreased ($p < 0.05$) 20-25 minutes after the infusion compared to control (before dosing). No difference was seen at 0-5, 10-15, 30-35, or 40-55 minutes after the infusion. After oral dosing, mean cortical activity was significantly ($p < 0.05$) decreased at 60-70 minutes after dosing, and its variability was significantly decreased at 30-40, 60-70, and 90-100 minutes after ingestion compared to control. No changes were observed 120-130 minutes after dosing. Changes in cortical electrical energy appear to be short-lived following 15 mg oral doses of dextroamphetamine.

In a later study by Fink and colleagues,[20] single 10 mg doses of dextroamphetamine were administered to female normal volunteers and the EEG was recorded for 30 minutes before and four hours after dosing. To be included in the studies, subjects had to have at least 50% alpha in the occipital region. A bipolar montage with bifrontal and right occipital-vertex leads was used. The state of alertness was maintained using an auditory reaction time task. Data were analyzed

using a period analysis program which produced 19 EEG measures for each 60 sec epoch. Epochs with artifacts in greater than 20% of the sample were deleted. A mean and standard deviation was computed for each measurement time from the artifact-free epochs. These investigators report a significant first session effect. Decreased delta activity compared to baseline was observed soon after dosing and lasted throughout the testing period. Alpha activity increased and remained elevated through 3 hrs of testing.

In another study of the EEG effects of dextroamphetamine in normal volunteers, Hamilton and associates[19] examined twelve healthy male and female subjects aged 20-41 years. Subjects received placebo, dexamphetamine 5 mg and dexamphetamine 10 mg orally. Eyes closed EEG was measured before dosing and at 2.5 and 5.75 hours after dosing. Significant increases compared to placebo were noted only in the alpha (7.5 to 13.5 Hz) and beta (13.5 to 26 Hz) frequency bands following the 10 mg dose at 2.5 hr. The EEG was only examined twice after dosing, so the duration of the effect is difficult to assess. No significant effect at any time was observed however after the 5 mg dose of dextroamphetamine.

Matejcek[72] studied the EEG effects of dextroamphetamine in six healthy male volunteers in a placebo-controlled, randomized, double-blind, crossover study. Subjects were screened for well-defined alpha rhythm in the parietal and occipital regions on a resting EEG with eyes closed to obtain a homogeneous group with respect to EEG characteristics. Subjects underwent a familiarization procedure where they received a placebo and completed all tests as if on a study day prior to randomization to treatments. Subjects received single oral doses of 5 and 10 mg

dextroamphetamine and placebo at intervals of one week during the study. Twenty minutes of resting EEG with eyes closed was recorded from leads O_2-C_z, O_1-C_z, P_4-C_z, and P_3-C_z at 0, 2, 4, 6, and 8 hr after drug administration. These investigators found a dose-dependent decrease in the proportion of delta and theta activity and an increase in that of alpha activity, particularly at the 2 hr measurement. There was a trend toward an increased percentage of beta activity, but it was not statistically significant. They conclude that dextroamphetamine possesses vigilance-promoting properties and that the observed EEG changes correlate with this effect.

In a recent double-blind, placebo-controlled crossover pharmacodynamic study utilizing EEG measures, conducted by Saletu and colleagues,[96] single 20 mg doses of dextroamphetamine and placebo were administered orally to 18 healthy male and female volunteers. EEGs were recorded before dosing at 2, 4, 6 and 8 hours after dosing. Administration of dextroamphetamine resulted in increased total power, decreased delta and theta power, and increased alpha and beta power on the EEG and the changes were observed from 2 to 8 hours after dosing. The peak amphetamine plasma concentrations were approximately 50 ng/mL and occurred about 2 hours after dosing. When the level of vigilance was controlled during the EEG recording, no differences between dextroamphetamine and placebo were observed.

These studies demonstrate that EEG changes occur following doses of at least 15 mg of dextroamphetamine in normal volunteers and they can be followed over time. The duration of effect is unclear however. Increases in fast activity and decreases in slow activity on the EEG are most frequently reported. In his review of drug effects on the EEG, Glaze[97] notes that amphetamines primarily increase beta

and alpha activity, but this is difficult to detect upon visual analysis of the EEG record. Computerized drug profiling studies[43] indicate that the increase in alpha activity after dextroamphetamine administration occurs over the parietal and occipital areas. Correlation of these changes with changes in mood, performance, or serum concentration are not well-described. Studies by Lukas and associates[98] indicate that EEG alpha activity is significantly increased during periods of drug-induced euphoria following intravenous administration of amphetamine. They note that it appears that a threshold level and a relatively rapid rate of increase in plasma drug concentrations is necessary for this euphoria to occur. They also report that it is difficult to study the relationships between mood changes and brain electrical activity without introducing artifacts into the EEG recording. Further work in this area is warranted.

2.2.5 Neuroendocrine Changes after Amphetamine Administration

A wide variety of drugs are known to influence anterior pituitary hormone secretion. Changes in hormone secretion are another potential source of pharmacodynamic response measures. Evidence suggests that drugs with different pharmacological actions have different effects on the secretion of anterior pituitary hormones and that secretion patterns can be used to discern a drug's effects on the CNS.[99] Amphetamines alter secretion of a number of these hormones, including Adrenocorticotrophic hormone (ACTH), prolactin, thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), luteinizing hormone (LH), and growth hormone.[100] Prolactin response was chosen as a representative neuroendocrine measure for dextroamphetamine effects in this study because prolactin levels have

been measured after amphetamine administration in a number of studies and the Clinical Research Center Core Laboratory has considerable experience analyzing serum and plasma prolactin following pharmacologic challenge.

An understanding of the factors influencing the secretion of prolactin are important for interpreting the neuroendocrine response to amphetamine. Many of these factors are reviewed by Kuret and Murad.[101] The synthesis and storage of prolactin takes place primarily in the pituitary lactotrophs. Placental tissue can also synthesize prolactin. Normal plasma concentrations of prolactin range from 5 to 10 ng/mL, with concentrations in males lower than those in females. A number of physiological factors can increase the secretion of prolactin including sleep, stress, hypoglycemia, fluctuations in estrogen concentrations, and exercise. Secretion of prolactin shows a circadian rhythm, with peak concentrations occurring during sleep. In addition, minute to minute fluctuations are also observed due to the pulsatile nature of prolactin secretion. Prolactin's half-life in plasma is approximately 15 to 20 minutes. Prolactin secretion from the pituitary is primarily under negative control by the hypothalamus. Secretion is inhibited by release of prolactin release-inhibiting hormone (PRIH) from the hypothalamus. Some evidence suggests that prolactin secretion is controlled by dopaminergic neurons and that PRIH is actually dopamine. Prolactin secretion is then predominately inhibited by dopamine. Serotonin however stimulates the secretion of prolactin.[99]

Dextroamphetamine has been reported to cause both increases and decreases in the secretion of prolactin. Results from several studies are summarized in Table 2.2. The effect on secretion may depend on the dose and route of administration.

Dextroamphetamine is believed to affect dopamine release at lower doses and serotonin release at higher doses, and thus may cause opposing effects on prolactin secretion. In a study by Nurnberger and associates,[31] normal volunteers were given intravenous doses of amphetamine after pretreatment with haloperidol and amphetamine without pretreatment in a crossover study. No change in prolactin compared to baseline was observed following amphetamine alone, but a large increase was observed when amphetamine was preceded by haloperidol. This increase was much larger than that observed after haloperidol alone in a previous study. Haloperidol blocks dopamine (D_2) receptors, and therefore would be expected to increase prolactin levels. It was hypothesized that the significant rise in prolactin observed after amphetamine plus haloperidol was due to the unopposed effects of serotonin on prolactin secretion. Amphetamine effects through dopaminergic mechanisms, which should decrease prolactin secretion, would be blocked by the haloperidol, and only the stimulatory effects of serotonin would remain. A second possible mechanism was also proposed: amphetamines may release endogenous opiates that may stimulate prolactin secretion. None of these studies have examined the dose-response relationship between dextroamphetamine dose and prolactin secretion over a dosage range including low and high doses. Further work characterizing this relationship is needed.

Table 2.2 Summary of Studies Reporting the Effect of Dextroamphetamine on Prolactin Secretion in Normal Volunteers

Dose	Route	Subjects	Change in Serum Prolactin	Reference
20 mg	Oral	24 (male)	Statistically significant increase compared to placebo	Jacobs, Silverstone & Rees[100]
30 mg	Oral	10 (male and female)	No difference from placebo	Dommisse, Schulz, Narasimhachari, et al[102]
0.3 mg/kg	IV	8 (male and female)	Increase compared to baseline, but not significant	Nurnberger, Simmons-Alling, Kessler, et al[31]
0.10 mg/kg	IV	12 (male)	No difference from baseline	Halbreich, Sachar, Asnis, et al[103]
0.15 mg/kg	IV	12 (male)	Statistically significant increase compared to baseline	Halbreich, Sachar, Asnis, et al[103]
20 mg	Oral	18 (male and female)	No difference from baseline	Saletu, Grunberger, Anderer, et al[96]
10 mg	Oral	9 (male)	Statistically significant decrease compared to baseline	Wells, Silverstone & Rees[104]
20 mg	Oral	9 (male)	Statistically significant decrease compared to baseline	Wells, Silverstone & Rees[104]

2.2.6 Mood Changes after Amphetamine Administration

Amphetamines have been noted to produce alterations in mood in a number of clinical studies.[105] The primary effect on mood is euphoria or feelings of well-being. About 20% of individuals however, experience dysphoria.[102] The subjective effects produced by amphetamine depend on the user, the environment, the dose, and the route of administration.[106] The euphoriant activity of amphetamine is believed to be mediated by dopaminergic mechanisms in the CNS.[105] A summary of selected studies of the effects of oral amphetamines on mood in man are presented in Table 2.3. These studies suggest that the predominate effect of oral amphetamine on mood is euphoria. The effect is greater at higher doses (15 - 30 mg), but can be measured in doses as low as 5 mg. The 1-mg dose did not elicit a measurable response on mood. The maximum effect on mood appears to occur 1 - 3 hrs after dosing and may last longer than 6 hr. The higher the dose, the longer the effect.

Several scales have been used to measure mood changes following amphetamine administration. Both self-rated and observer-rated scales have been utilized. In choosing such a measurement tool for use in a pharmacodynamic study of stimulant response, the validity, reliability and suitability for repeated measures must be considered. Visual analog scales have been used in a number of studies to measure euphoria. Visual analog scales are easy for the subject to complete, easy for the investigator to score, do not require a great deal of motivation from the subject, and the rater is not restricted by demarcations on the scale in how fine a discrimination can be made.[107] Visual analog scales can be valid and reliable for measuring the effects of drugs acting on the CNS, and can be used to measure effects repeatedly

Table 2.3 Summary of Selected Studies Reporting the Effects of Oral Amphetamine on Mood in Healthy Volunteers

Dose	Subjects	Mood Scales	Results	Reference
30 mg	10 (male and female)	Hopkins Mood Scale & The Amphetamine Interview Rating Scale	Statistically significant increase in well-being compared to placebo	Dommisse, Schulz, Narasimha-chari, et al[102]
10 and 20 mg	9 (male)	Visual Analog Scale (Miserable--Happy)	Dose-related increase in subjective rating of mood lasting from 1-3 hr (10mg) and 1->4hr (20mg)	Silverstone Wells & Trenchard[24]
5 mg	31 (male and female)	Profile of Mood States - modified	Increased scores for vigor, friendliness, elation, arousal and positive mood compared to placebo, maximum at 3hr and continuing > 6hr in some	Johanson & Uhlenhuth[108]
1 and 10 mg	9 (female)	Visual Analog Scale (depressed--elated)	No effect with 1 mg; 10 mg produced elevation of mood maximum at 1.5 hr and lasting up to 2.5 hr but not statistically significant	Jain, Kyriakides, Silverstone, et al[109]
5 and 10 mg	12 (males and females)	Visual Analog Scale	Increased alertness, excitation, interest, elation compared to placebo (10>5) at 2 hr, with effects persisting on some measures > 5 hr	Hamilton, Smith & Peck[19]
15 mg	9 (males)	Visual Analog Scale & Symptom Oriented Preference Scale	Increased alertness, extroversion, euphoria and stimulation compared to placebo	Taeuber, Zapf, Rupp, et al[30]

after drug administration. Scores on visual analog scales of euphoria have been used to distinguish doses of amphetamine (See Table 2.3).

Other scales, such as the Profile of Mood States (POMS)[110], the NIMH Self-rating Scale[22] and the Addiction Research Center Inventory (ARCI)[111] scales have been used to measure euphoria. These scales usually take longer to complete and require more subject motivation. A modification of the ARCI has been studied by Martin and colleagues[21] to measure euphoria in studies of amphetamine. They administered questions from the PCAG (sedation), MBG (euphoria), LSD (dysphoria and psychoses) and BG (an empiric amphetamine scale) subscales of the ARCI to male prisoners before and after dosing with amphetamine. Based on the results of this study, they constructed an 11-item amphetamine scale (A) that included those items on the ARCI that showed a significant linear regression of response against dose for 3 doses of amphetamine (7.5, 15 and 30 mg/70 kg). The BG (amphetamine) subscale of the ARCI has also been used by other investigators studying amphetamine response.[23] The ARCI has been shown to reliably distinguish between amphetamine effects.[111] In our studies, we used the MBG subscale of the ARCI and the A scale developed by Martin, modified to measure amphetamine responses over a five-point range.

2.2.7 Changes in Performance after Amphetamine Administration

A large number of studies have investigated the effects of amphetamine on psychomotor and perceptual performance using laboratory based performance tests such as finger tapping, reaction time, critical flicker fusion, digit symbol substitution, tracking tasks, and mental arithmetic.[105] The reported effects of amphetamine on

psychomotor, perceptual, and intellectual function are conflicting. Some studies report no changes while others report changes on some tasks. A summary of selected studies on the effects of oral amphetamine on performance in normal volunteers is presented in Table 2.4.

Several factors impact on the usefulness of psychometric test results in describing pharmacodynamic effects of CNS active drugs.[4] Motivational factors can influence the response to many psychometric tests. Expectations of the subjects, level of payment, intentions of the volunteers and expectations of the investigator can all influence experimental results. The effects of practice, learning and memory on performance must also be considered. Many components, besides the effect of the drug, involving factors such as personality, motivation and expectations determine performance on a psychometric test. These factors should be controlled for as much as possible in the design and conduct of the study to improve the usefulness of psychometric tests. Experiments should be double-blind and placebo-controlled. Subjects must be carefully screened and those administering the tests well-trained. Adequate practice sessions are essential.

For our studies, two tasks were chosen to measure the response to dextroamphetamine. The first was an attentional task (continuous performance task (CPT)) and the second was a motor task (finger tapping). Similar tasks have been used by other investigators to measure the effects of amphetamine on performance. Computerized versions of these tasks were chosen to allow for improved standardization of procedures, accurate measurement and recording of responses, and ease of use.[112] A major disadvantage to these tasks however, is that reliability and

Table 2.4 Summary of Selected Studies on the Effects of Oral Amphetamine on Psychomotor Performance in Healthy Volunteers

Dose	Subjects	Tests	Results	Reference
15 mg	9 (males)	Choice reaction time, simple reaction time, mental arithmetic, critical flicker fusion (CFF)	Significant increase only in CFF and correct solutions on mental arithmetic compared to placebo	Taeuber, Zapf, Rupp, et al[30]
5 and 10 mg	12 (males and females)	Auditory vigilance test, Auditory reaction time, tapping test	For both doses, significant increase in percent correct on auditory vigilance test, decreased reaction time, no difference in tapping	Hamilton, Smith & Peck[19]
0.25 and 0.5 mg/kg	16-low dose, 15-high dose (males)	Motor activity test, skin conductance reaction time, visual continuous performance task, learning task	Increased performance on CPT (high dose), decreased reaction time (low dose), beneficial effects on the learning task (both doses)	Rapoport, Buchsbaum, Weingartneret al[113]
5 mg	8 (males)	Symbol-Digit Substitution Task (DSST), Simple Reaction Time, Pattern recognition, Digit Span, Pattern Memory	No significant differences from placebo	Schmedtje, Oman, Letz, et al[26]
10 mg	11 (males and females)	CFF, Discriminant Reaction Time	No significant differences from placebo	Berchou & Block[114]
10 mg	6 (males)	Tremor, Precision Hole Steadiness, Tracking	No significant differences from placebo except in the compensatory tracking task which requires sustained concentration and motor coordination	Domino, Albers, Potvin, et al[27]
5, 10, and 15 mg	12 (males)	Wobble Board, Pursuit Meter, Delayed Auditory Feedback	Dose-related improvement in stability (eyes closed) on the Wobble Board, and on rapid response on the Pursuit Meter only	Evans, Martz, Lemberger et al[29]

validity for these particular versions has not been established. If some of the subjects are unfamiliar with computers, learning time may be increased. Studies of validity (i.e., is the task truly measuring attention?) are necessary before claims concerning the drugs effects on performance can be made.

2.2.8 Cardiovascular Changes after Amphetamine Administration

Amphetamines affect the cardiovascular system through direct and indirect actions on α and β receptors. Systolic and diastolic blood pressure have been reported to increase following administration of amphetamines. Heart rate may increase or decrease. Gaut, Pocelinko, Abrams and Dalton[115] studied thirteen obese subjects in a double-blind, randomized, crossover design where subjects received 20 mg of dextroamphetamine and placebo. Blood pressure and heart rate were measured before dosing and at 1 and 3 hr after dosing. They found an increase in systolic blood pressure compared to placebo of 21.8% ($\pm$7.4%) and 14.0% ($\pm$4.5%) at 1 and 3 hr following amphetamine dosing respectively. Diastolic blood pressure increased 20.6% ($\pm$6.1%) at 1 hr and 15.2% ($\pm$4.9%) at 3 hr after amphetamine administration compared to placebo. Heart rate increased less dramatically, with a change compared to placebo of 0.8% ($\pm$3.4%) at 1 hr and 12.4% ($\pm$1.9%) at 3 hr after amphetamine dosing. All of these changes were statistically significant ($p < 0.05$) except for heart rate at 1 hr (not significant). Hamilton and associates[19] report a rise in heart rate and diastolic blood pressure, but not of diastolic blood pressure after 10 mg of dextroamphetamine compared to placebo.

Studies of the effects of amphetamine on blood pressure and heart rate in

animals suggest that tonic and reflex (baroreceptor) neural activity obscure the pressor and tachycardic effects of amphetamine on peripheral nerves. Simpson[116] reported that blood pressure and heart rate increases after amphetamine administration to male Wistar rats and that this increase is larger and more sustained in animals pretreated with chlorisondamine. Chlorisondamine is a ganglionic blocker which eliminates tonic and reflex neural activity. This suggests that the conflicting results of the effect of amphetamine on the cardiovascular system, especially heart rate, may be mediated by baroreceptor reflexes or CNS influences on the peripheral responses. In chlorisondamine-pretreated animals, there was a positive relationship between the amount of drug administered and the magnitude of the cardiovascular responses.

CHAPTER 3

Part I - Reproducibility of Control Responses

3.1 Specific Aims

The purpose of this study was to investigate the inter- and intra-individual variability associated with a series of potential CNS pharmacodynamic response measures under baseline (no drug) conditions. These measures included quantitative EEG, automated psychometric tests, and self-rated mood scales. Within day and between day reproducibility was evaluated. Responses for each measure were examined for evidence of circadian changes and learning effects. Results from this study were used to design subsequent studies of quantitative EEG as a pharmacodynamic measure for CNS stimulation.

3.2 Methods

3.2.1 Clinical Study of Control Responses

The clinical portions of this study were conducted at the Clinical Research Center at Virginia Commonwealth University. The Committee on the Conduct of Human Research at Virginia Commonwealth University reviewed and approved the study protocol and the informed consent form before the study began. The study

protocol and consent form are in Appendix A.

This study was an open-labeled pilot study in which healthy volunteers underwent a series of tests (electroencephalography, automated psychometric tests, and self-rated scales of mood) on three occasions one week apart. On each of the three study days, the series of tests were repeated eight times over a 12 hour period. Subjects undertook the study in groups of one or two.

Subjects. Eight healthy volunteers participated in this study. Subjects were recruited from within the hospital and schools at Virginia Commonwealth University. Volunteers were considered for inclusion in the study if they were nonsmokers between the ages of 18 and 35, and determined to be in good health based on the results of a medical history, physical examination, laboratory tests (including a SMAC-20, CBC, and urinalysis), 12-lead electrocardiogram, and vital signs. Volunteers were excluded from the study if they 1) had a history of drug addiction, alcohol abuse, or psychological dependence on drugs, 2) had a first degree relative (mother, father, or siblings) with a history of mental illness or alcohol/drug abuse, 3) took any medications chronically or had taken any prescription or investigational drugs in the four weeks prior to starting the study, or 4) had a normal daily caffeine intake greater than two cups of coffee. Before entering the study, each subject signed an informed consent form attesting that his participation was voluntary and that the study procedures were explained.

Procedure. During each of the three study periods, the following procedure was observed:

Beginning 72 hours before each study day, subjects avoided caffeine, alcohol,

and all medications, including over-the-counter medications. Subjects entered the study facility at 7:00 a.m. on the study day and were released after completion of the 12 hr test battery on the same day. Subjects fasted from midnight on the evening before the study day until after the 4 hr test battery. Lunch was served after the 4 hr test battery and dinner at 10 hr after the baseline test battery. The menu was similar during each study period.

Five-minute segments of 28 channel EEG were recorded for each subject using a NeuroScience Brain Imager (San Diego, CA) with eyes closed at 0, 1, 2, 3, 4, 6, 8, and 12 hrs. Subjects reclined in a hospital bed with the lights off during the recordings. They were asked to count back from 500 by 3s to maintain vigilance. The electrodes were placed using an Electro-cap according to the 10/20 International System with 8 additional electrodes located 50% between the standard 10/20 placement. Omni-Prep ((D.O. Weaver & Co.,Aurora, CO) was used to prepare the scalp and Electro-Gel (Electro-Cap International, Inc., Dallas, TX) was used as the conducting gel. Linked ears were used as a reference. Four additional electrodes were placed to monitor for vertical and lateral eye movements and electromyographic activity. Electrodes in the cap, ear clips, and eye movement monitors were made of tin. Electrode impedances were checked before each recording, and maintained at less than 5.6 kohms and similar between electrodes. Disturbances in the room or subject movement during the EEG was recorded. The Brain Imager filters were set as follows: Low filter - 0.30 Hz, High filter - 40 Hz, Notch filter - off. The raw EEG was stored on optical disks. The system integrity of the Brain Imager was checked weekly throughout the study to ensure stability of channel calibration and

proper filter functioning. EEG recordings on Study Day 1 for Subject 1 (TM) were made simultaneously on the Brain Imager and a standard EEG machine (Grass Instruments Model 8-18D, Quincy, MA). The electro-cap was connected to both input boards by an adapter. The pen recordings from the standard EEG machine were reviewed by a board certified electroencephalographer who determined that recordings from the electro-cap were acceptable.

Each subject completed a computerized visual CPT (NeuroScan, Inc.) at 0, 1, 2, 3, 4, 6, 8 and 12 hr. In this task, the digits 0 through 9 briefly appear on the screen. The subject presses the left button of the mouse when a 0 appears and the right button for all other digits. The interstimulus interval varied from 0.8 to 1.2 seconds. A total of 120 stimuli were presented during each testing session. Two practice sessions were completed before beginning the 0 hr test battery.

Subjects completed a computerized motor task, finger tapping (NeuroScan, Inc.) at 0, 1, 2, 3, 4, 6, 8 and 12 hr. During this task, subjects tap the mouse button as fast as possible for 10 seconds, first with their right hand and then with their left. A total of three trials with each hand were completed during each testing session. Two practice sessions were completed before beginning the 0 hr test battery.

A self-rated scale (Appendix B) based on the MBG (a measure of euphoria) and the A (a measure of amphetamine effects) subscales of the Addiction Research Center Inventory Scales described by Martin et al.[21] was completed by each subject at 0, 1, 2, 3, 4, 6, 8 and 12 hr. The scale consisted of 23 questions which the subject responded to on a scale of 1 to 5. At the same times, a 100 mm visual analog mood scale (Appendix C) was completed.

The test battery was conducted in the following sequence: 1) EEG, 2) visual CPT, 3) rating scales, and 4) finger tapping.

3.2.2 Data Analysis

The measures of response that were examined for the test battery described above are listed in Table 3.1.

Table 3.1 Response Measures Evaluated in Part I - Reproducibility of Control Responses

EEG Variables

TP	Total Power - all frequencies (μV^2)	RPD	Relative Power - Delta band
TPD	Total Power - Delta band (μV^2)	RPT	Relative Power - Theta band
TPT	Total Power - Theta band (μV^2)	RPA	Relative Power - Alpha band
TPA	Total Power - Alpha band (μV^2)	RPBI	Relative Power - Beta I band
TPBI	Total Power - Beta I band (μV^2)	RPBII	Relative Power - Beta II band
TPBII	Total Power - Beta II band (μV^2)		

Psychometric test

FTLT	Finger Tapping with Left Hand (taps/sec)
FTRT	Finger Tapping with Right Hand (taps/sec)
CPTPC	Percent Correct on Continuous Performance Task (%)
CPTAL	Average Latency on Continuous Performance Task (sec)

Mood Scales

RS	Total Score on Self-Rated Mood Scale
VAS	Score on Visual Analog Mood Scale

EEG Analysis. As each EEG was recorded, the signal was processed by a Fast Fourier Transform procedure, to determine the amplitude of the EEG in five frequency bands (Delta: 0.39 - 3.9 Hz, Theta: 4.3 - 7.8 Hz, Alpha: 8.2 - 11.7 Hz, Beta I: 12.1 - 16.0 Hz, and Beta II: 16.4 - 30.0 Hz) at each electrode. Each of the

five-minute recordings was reviewed and edited to remove each 2.5 second epoch (frame) that contained artifacts (eye movements, muscle movement, electrode artifacts, or disturbances noted during the recording).[117,118,35] The remaining frames were averaged using the EEG statistical operations package on the Brain Imager to form an average topographical map representing the five minute recording. Recordings with fewer than 24 artifact-free frames were not processed further and were listed as "missing". To compute the average map, the Brain Imager first forms sub-averages from consecutive groups of eight frames each.[119] The voltage value measured at each of the 28 electrodes for the first eight frames are added together and then divided by 8, the number of frames. This process is then repeated for the next group of 8 frames and so on. The overall average is then formed by averaging the sub-averages. The overall average file contains the average amplitude in each of the 5 frequency bands at each of the 28 electrodes. This file was then transferred from the Brain Imager to an IBM compatible 80386 personal computer. ISTAT (NeuroScience, Inc.) a statistical package for EEG processing was used to prepare ASCII files of the average files. These files were then imported into the Quattro Pro spreadsheet software (Borland International, Scotts Valley, CA) for further processing.

Power was determined for each average recording by squaring the amplitude values at each electrode in each frequency band. Total amplitude and total power in each frequency band was calculated by summing the amplitude or power at each of the electrodes for a given frequency band. Total amplitude and total power across all frequency bands was calculated by adding together the total amplitude or total power

in each of the frequency bands. Relative power in each frequency band was calculated by dividing the total power in the given frequency band by the total power across all frequency bands.

Approximately 6 months after the first EEG editing process, EEGs from one period for three subjects were re-edited by the same investigator to examine the reliability of the EEG editing process over time. These edited EEGs were averaged and transferred as described above, and total power in each frequency band was calculated. These EEGs are referred to as the reliability sample.

Psychometric test analysis. For the computerized visual CPT, latency of response was determined for each trial during the session. The average latency of response and the percent of correct responses for each test session was determined. For the finger tapping task, the average rate (taps/sec) of finger tapping for each hand was calculated for each session by averaging the results of the three trials conducted during each session. The effect of learning on test performance was evaluated by examining plots of the test score versus the cumulative test battery number during the entire study. A total of 30 test batteries were attempted during the study (10 on each of the 3 study days).

Rating scales. A total score on the self-rated mood scale was determined for each test session by summing the scores obtained for each of the 23 items on the scale. A score between 0 and 100 was obtained for the visual analog mood scale for each test session by measuring the number of millimeters between the left end of the scale and the mark placed by the subject.

3.2.3 Statistical Methods

Within-day, between-day and inter-subject variability was examined for each response variable. Within-day variability for each response measure was determined by calculating the mean, standard deviation, and relative standard deviation of the response at each time point for each study day for each subject. Relative standard deviation, also termed coefficient of variation, is defined as the standard deviation divided by the mean and expressed as a percentage. Between-day variability for each response was determined by calculating the mean, standard deviation, and relative standard deviation of the mean response for each study day for each subject. Inter-subject variability was determined by calculating the mean, standard deviation, and relative standard deviation of the response on all study days at all time points for each subject.

To look at the effect of study day and time of day on the response measures, a repeated measures analysis of variance with study day, time of day, and subject as factors and the response as the dependent variable was performed using the PROC GLM procedure[120] in SAS. The residuals were tested for normality using PROC UNIVARIATE[120]. This procedure computes the Shapiro-Wilk statistic, W, for the null hypothesis that the residuals are normally distributed. When the probability of a smaller value of W was less than or equal to 0.1, the null hypothesis of normality was rejected. The hypothesis of normally distributed residuals was rejected for most of the variables tested in this study, so a rank transform was performed on the values for the response measures. The ranks for each response measure for each study day were compared using repeated measures analysis of variance with study day, time of day,

and subject as factors and each rank transformed response as the dependent variable.

To examine the reliability of the EEG editing process over time, Pearson product moment coefficients were calculated between the total power and power in each frequency band obtained during the first and second editing of the reliability sample. The multivariate procedure PROC CANCORR[120] in SAS was used for this computation. The correlation coefficients obtained between the first and second editing are termed stability coefficients.[121]

3.3 Results

Nine male volunteers were entered into the study. Demographic and physical characteristics of the subjects are shown in Table 3.2. All of the subjects were judged to be healthy based on the results of a physical examination, a medical history, and clinical laboratory tests before entering the study. Eight subjects completed the study. Subject 4 (BR) dropped out after the first period for personal reasons. None of the subjects reported adverse events related to the study procedures.

Within-day variability for each of the response measures is presented in Figures 3.1 and 3.2. These figures show the average (M) and range (H - L) of the relative standard deviation of each response at each time point for each study day for each subject. Within-day variability ranged from 0 to 80 percent for the response measures investigated. The highest variability was associated with total EEG power in the delta frequency band and other EEG-derived parameters and the lowest variability was noted for the computerized psychometric tests.

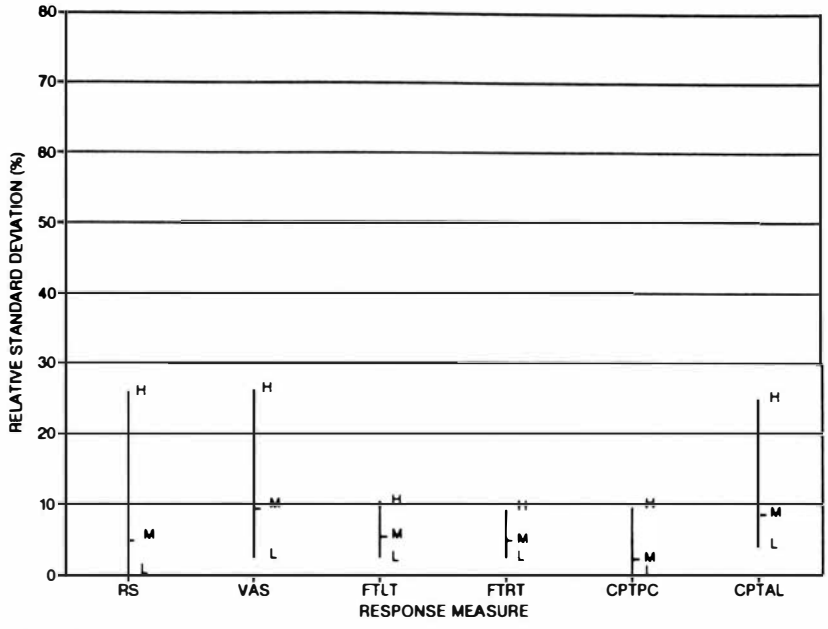

Figure 3.1 Within-day variability for the rating scales and psychometric tests

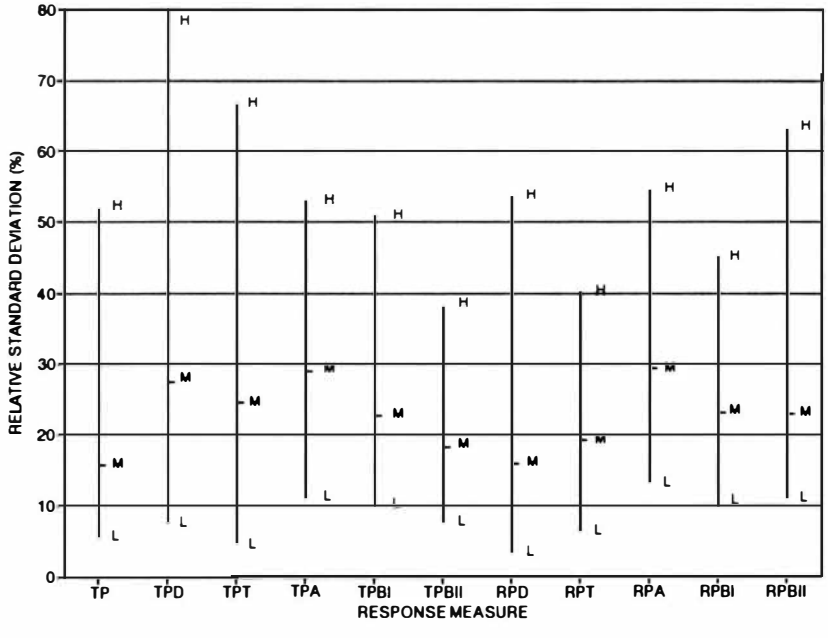

Figure 3.2 Within-day variability for the EEG variables

Table 3.2 Demographic and Physical Characteristics of Participants in Part I - Reproducibility of Control Responses

Subject Number	Initials	Age (years)	Weight (kg)	Race
1	TM	24	91.8	Black
2	MP	39	83.6	White
3	SW	36	78.2	White
4	BR	23	63.0	Black
5	MM	20	69.1	White
6	AT	25	90.9	White
7	JC	34	63.2	Hispanic
8	ML	33	90.9	Black
9	SW	26	70.5	White

Between-day variability for each response measure is presented in Figures 3.3 and 3.4. These figures show the average (M) and range (H-L) of the relative standard deviation of the mean response for each study day for each subject. Between-day variability was in general lower than within-day variability and ranged from 2 to 48 percent. The average within-day variability was less than 20% for all of the measures. Again, the highest variability was associated with parameters derived from the EEG.

Intersubject variability for each response measure is presented in Figures 3.5 and 3.6. These figures show the average (M) and range (H-L) of the relative standard deviation of the mean response for all study days for each subject. Intersubject variability ranged from 2 to 87 percent, with the highest variability again observed for the EEG measures. The lowest intersubject variability was noted for the

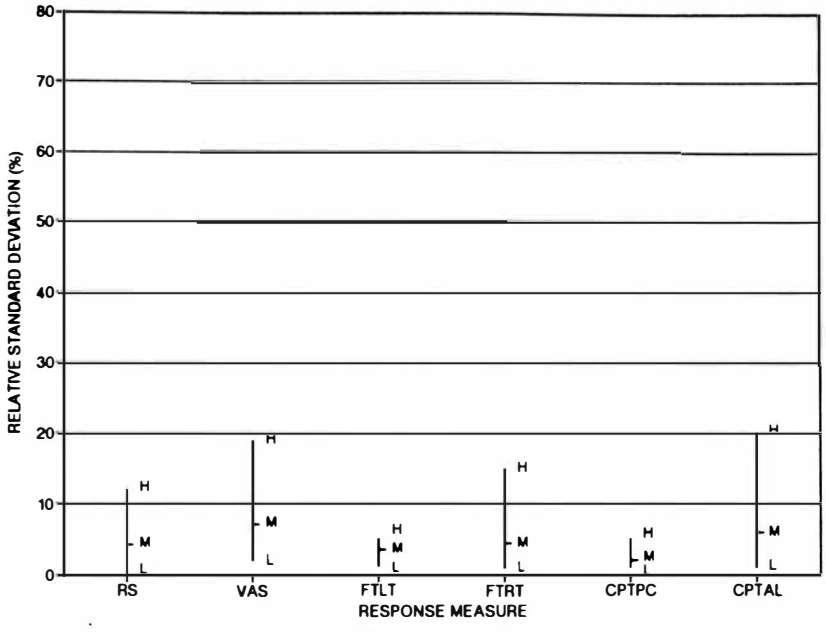

Figure 3.3 Between-day variability for the rating scales and psychometric tests

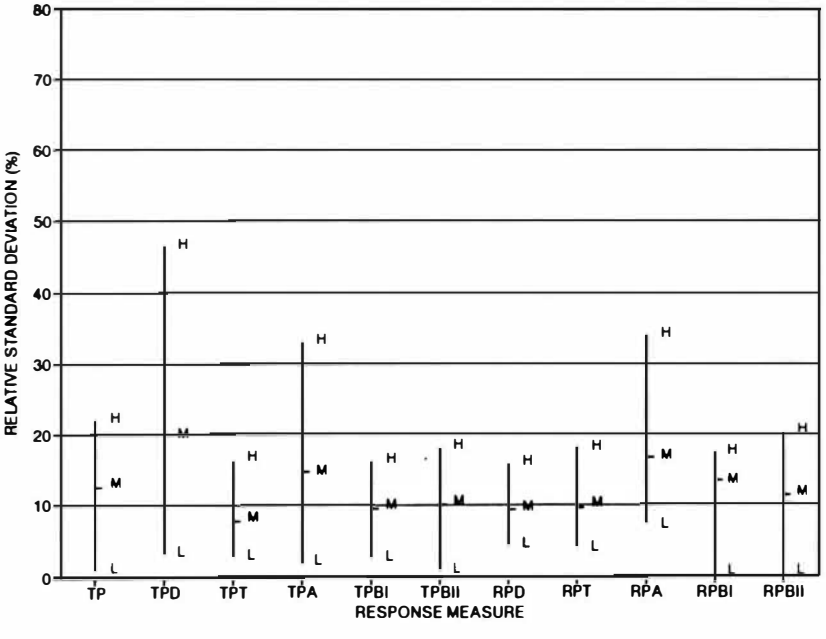

Figure 3.4 Between-day variability for the EEG variables

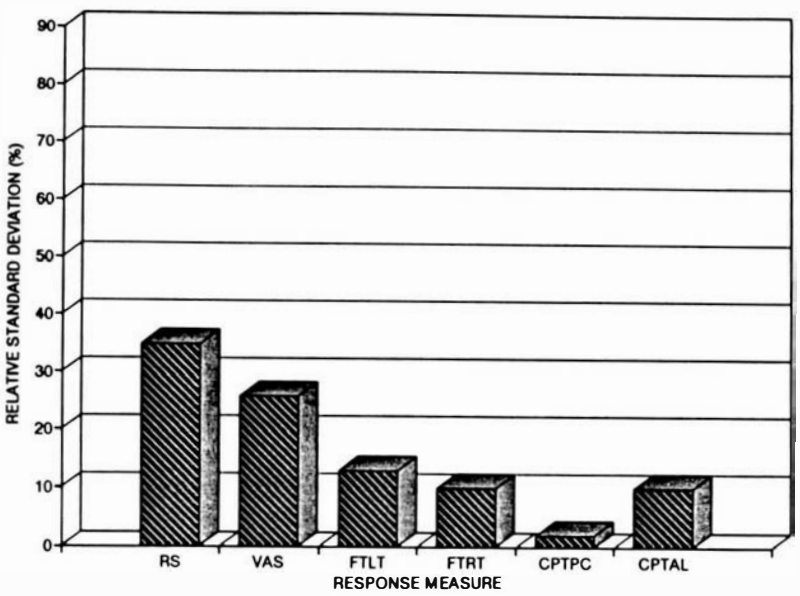

Figure 3.5 Intersubject variability for the rating scales and psychometric tests

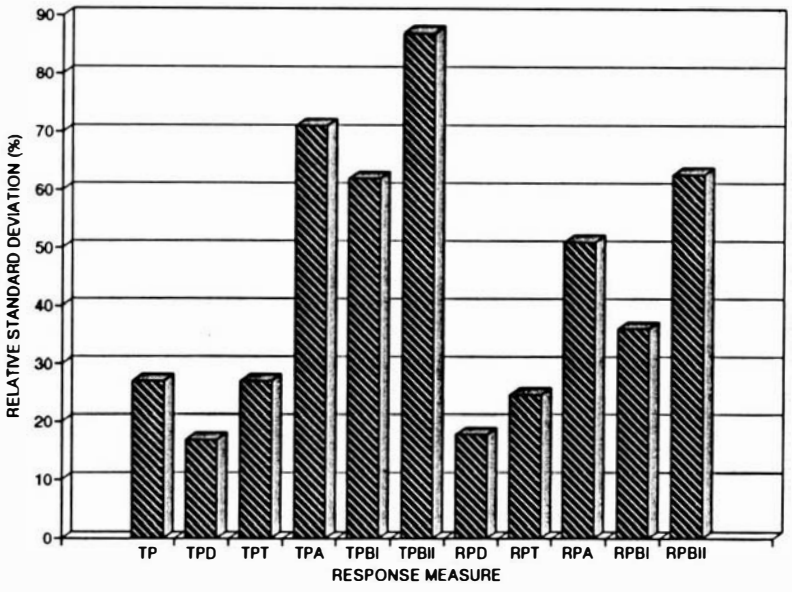

Figure 3.6 Intersubject variability for the EEG variables

computerized performance tests. Intersubject variability was greater than both within-day and between-day variability.

Learning effects were observed for the finger tapping and CPT. Figures 3.7 and 3.8 show representative plots of scores on these tests versus test battery number. Each task was administered a total of 30 times to each subject during the entire study. Similar plots for all subject are presented in Appendix D. These plots show that performance on the visual CPT and finger tapping continues to improve after the first 2 to 6 test sessions for most subjects, when a relatively stable level of performance is achieved. Each study day, the number of test sessions necessary to reach a stable level of performance is decreased.

Results from the analysis of variance showed that the main effect of time of day is statistically significant ($p < 0.05$) for several of the response measures, including the visual analog mood scale, finger tapping, average latency on the CPT, and EEG power in the Theta and Beta II frequency bands. Examples from representative subjects illustrating these effects are shown in Figures 3.9 and 3.10. In a comparison of the results on Study Day 1 with those on Study Days 2 and 3 for the self-rated mood scale, finger tapping with the right hand, percent correct on the CPT and EEG total power in the delta frequency band showed statistically significant ($p < 0.05$) differences. Scores on these measures attained on the first day of testing are different than those achieved on subsequent days. Examples demonstrating this effect are shown in Figures 3.11 and 3.12.

Missing data was problematic for several of the measures studied. Self-rated scales of mood show few if any missing values because they can be completed rapidly

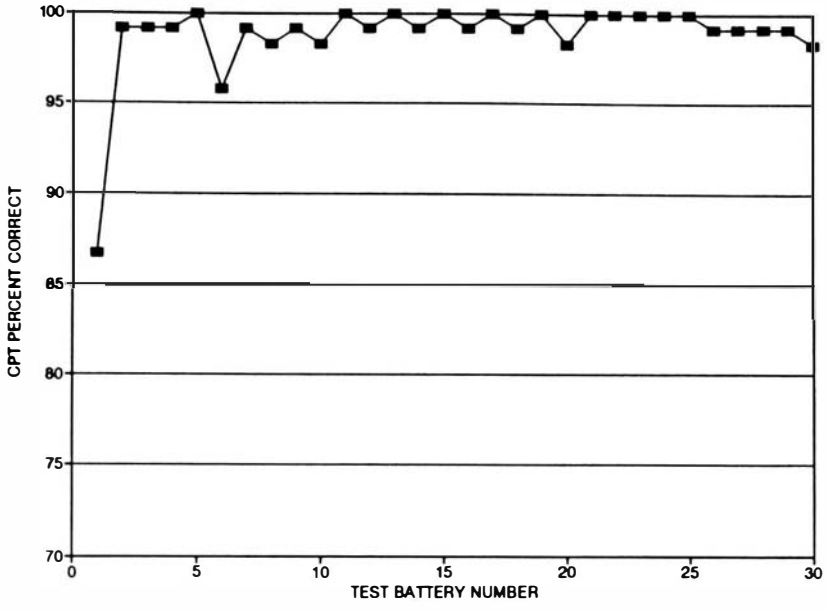

Figure 3.7 Example of the learning effect on the continuous performance task percent correct (Data from Subject 6)

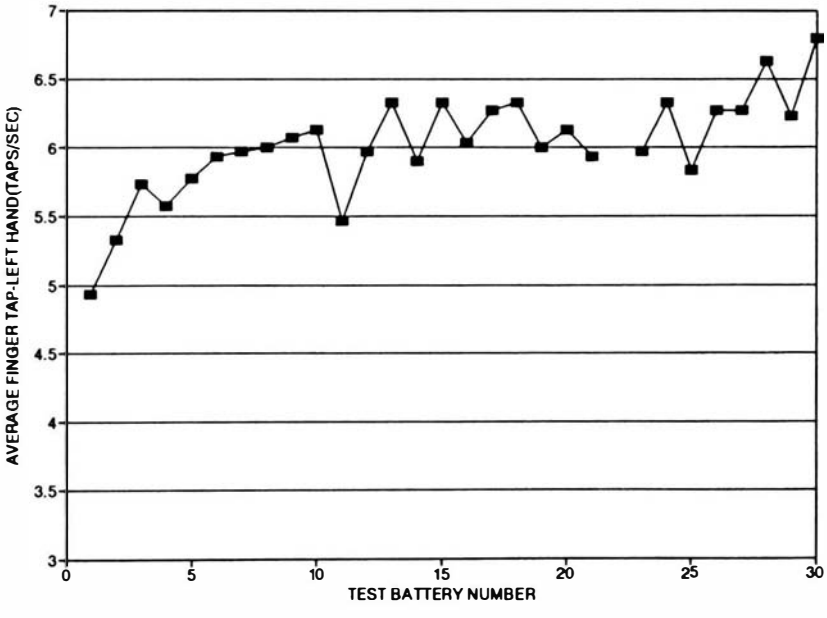

Figure 3.8 Example of the learning effect on the finger tapping task (Data from Subject 3)

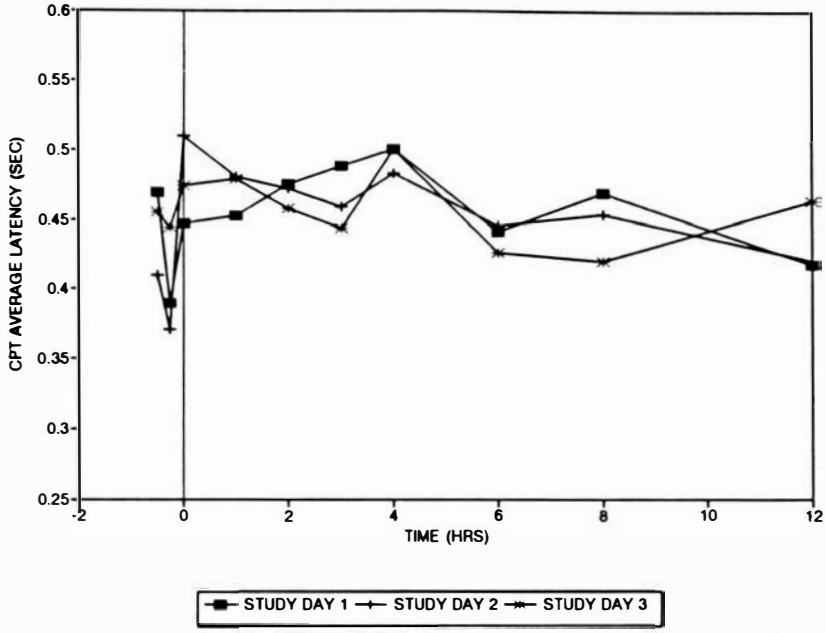

Figure 3.9 Example of the effect of time of day on the continuous performance task average latency (Data from Subject 6)

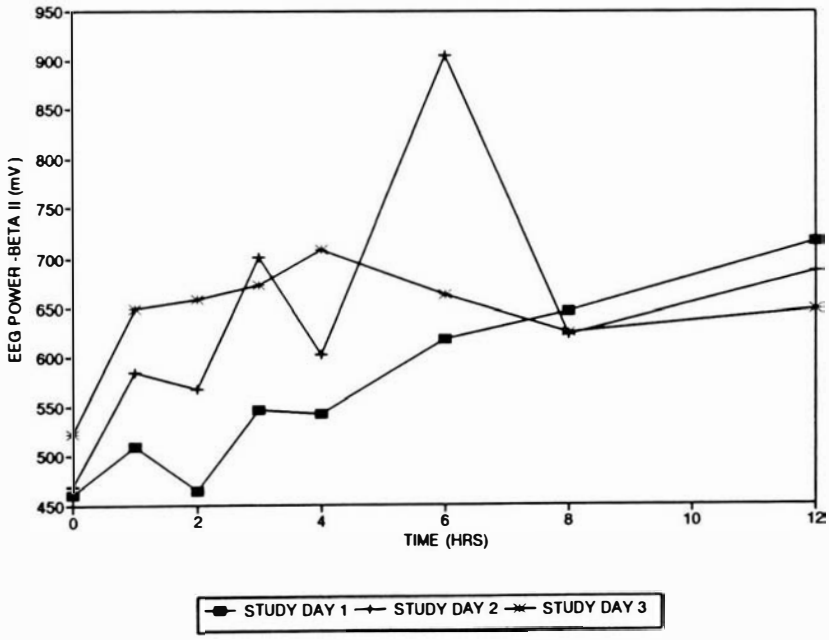

Figure 3.10 Example of the effect of time of day on the EEG power in the beta II frequency band (Data from Subject 1)

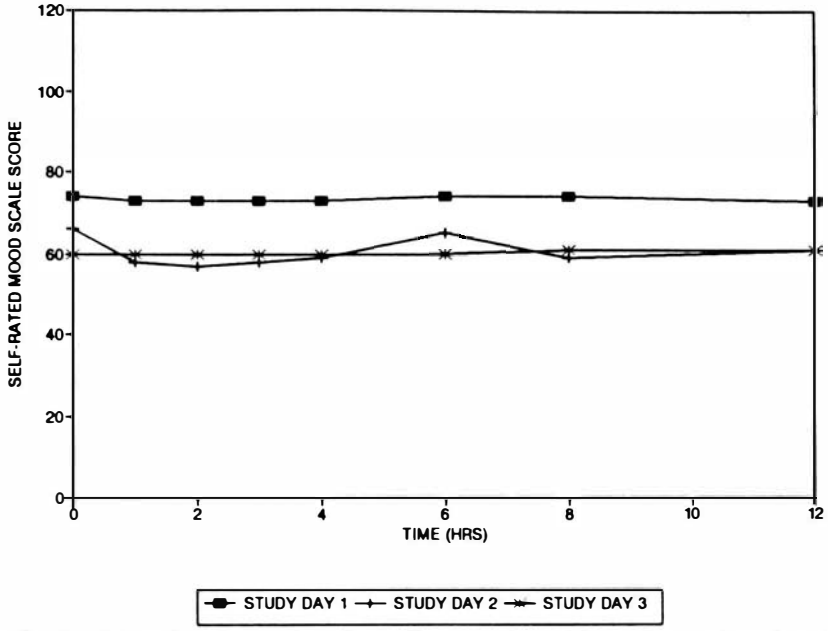

Figure 3.11 Example of the first day effect on the self-rated mood scale (Data from Subject 8)

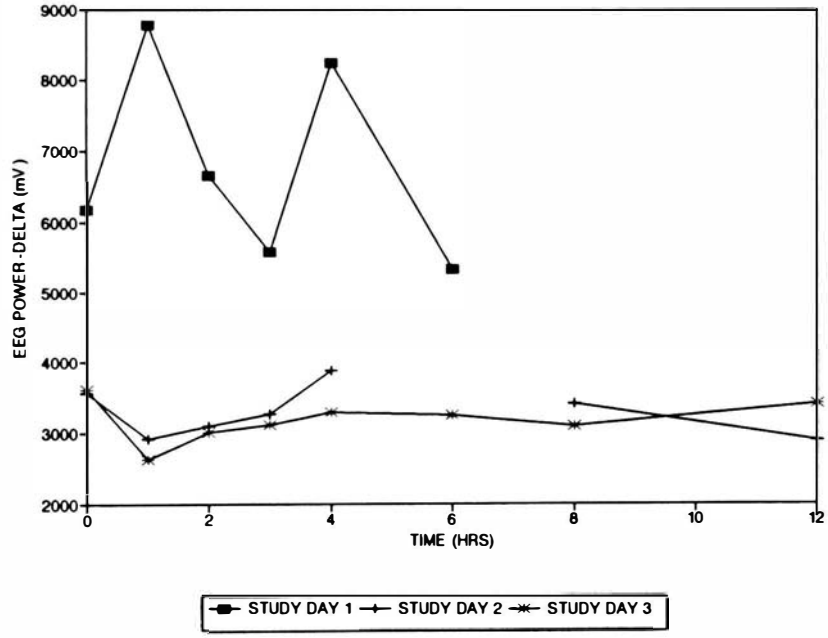

Figure 3.12 Example of the first day effect on the EEG power in the delta frequency band (Data from Subject 2)

and do not require any equipment that is subject to mechanical problems. The computerized psychometric tests showed a higher number of missing data points due primarily to breakdowns of the computerized system, such as failure of the computer hard disk drive for example. Approximately 5% of the CPT and finger tapping tests were lost. Approximately 5% of the measurements were missing for the EEG as well. These measurements were lost primarily due to excessive artifacts present during the recordings.

Results from the reliability of the EEG editing process over time show high correlation (correlation coefficient > 0.90) for all of the EEG-derived measures evaluated. Results from the canonical correlation between the first and second editing are presented in Table 3.3.

Table 3.3 Correlations Between the Results of the First and Second EEG Editing Procedure

EEG Measure	Correlation Coefficient
Total Power - Delta	0.912
Total Power - Theta	0.931
Total Power - Alpha	0.938
Total Power - Beta I	0.992
Total Power - Beta II	0.997
Total Power	0.970

3.4 Discussion

This study was designed to evaluate the variability associated with a series of potential response measures of CNS stimulation and to provide information needed for

planning future studies of quantitative EEG as a pharmacodynamic tool. Response measures evaluated included self-rated assessments of mood, computerized psychometric tests and electroencephalography.

Intersubject variability was greater than intrasubject variability for these response measures, indicating that a crossover design should be considered for future studies. In a crossover design, the comparison of treatments is based on within-subject or intrasubject variability.[122] Because intrasubject variability is less than intersubject variability the crossover design would be more powerful than a parallel group design for examining treatment differences.

Two potential disadvantages to the crossover design must also be considered. The first is the possibility of a differential carryover effect and the second is the impact of missing data. Carryover effect can be minimized by incorporating a sufficiently long washout period between each leg of the study. Determining the duration of the washout period so that it is long enough to ensure that no measurable drug levels remain in the system when the next period begins is relatively simple. Psychological carryover may be more difficult to control. For example, a subject's response on a rating scale may be influenced by previous treatments because the frame of reference changes. Later treatments are compared with the earlier ones. This phenomenon has been observed in studies of psychoactive drugs.[122] Missing data may be a more significant problem. Missing data complicates the statistical analysis and the design loses efficiency. There is an increased opportunity for data to be lost to the analysis in a crossover design, since each subject must provide data on more than one occasion. Loss of computerized psychometric test data could be minimized

by keeping a backup personal computer available to run the STIM program. Loss of EEG data is more difficult to control. Pre-screening subjects and excluding those with excessive eye movement artifacts is one approach that may decrease missing data. Also controlling the testing environment for noise level and temperature may reduce data loss. Excessive noise may increase eye movements and high temperatures may result in poor electrode performance due to sweating of the scalp.[123] Having the subject hold cotton gauze lightly against the closed eyelids may also decrease eye movement artifacts. Despite these limitations, a crossover design was chosen over a parallel design for future studies due to the efficiency of the design when intersubject variability is greater than intrasubject variability and the resulting need for a smaller number of subjects.

Based on the results of this study, incorporation of a placebo period into the crossover design is also desirable. The analysis of variance showed statistically significant effect of time of day for several of the response measures studied, indicating circadian variability in the response under baseline (no drug) conditions. Circadian periodicity in the wakeful EEG has been reported by other investigators.[124,61] For measures with circadian variability, the availability of a placebo period for comparison with drug treatments is preferable to using the baseline (0 hr) as the only control.

EEG and some psychometric test responses may be different on the first day of testing than on subsequent days for some subjects, as indicated by the statistical comparison of responses on Study Day 1 versus those on Days 2 and 3. These first session effects have been previously reported for EEG studies of drug effects.[15]

These authors hypothesize that the during the first session, subjects maintain arousal because of unfamiliarity with the testing environment and study procedures. They suggest the incorporation of a familiarization session in EEG studies prior to study initiation to overcome this limitation. Differences in relative power in the alpha band between EEGS recorded one week apart under no drug conditions has been reported by Sebban and associates.[125] They hypothesize that this variability is linked to an habituation to the testing environment with time. Our results and those of other authors indicate that familiarization sessions are necessary for pharmacodynamic studies using quantitative EEG.

Learning effects were also observed for the psychometric tests. Response measures that show learning effects are more difficult to use in pharmacodynamic studies, because the learning effect can confound the drug effect under study. To ensure that subjects are performing at a relatively stable level before receiving the treatments, practice sessions are necessary. Between 4 and 6 testing sessions were needed on the first study day for the majority of subjects in this study. These practices could be incorporated into the familiarization session. In addition, at least 2 practices are needed each study day based on the performance observed in this study.

This study also demonstrates that it is feasible to conduct a study where subjects undergo testing as often as every hour without unreasonable stress on the study schedule or fatigue for the subjects. A test session could be completed in approximately 15 minutes. Subjects tolerated the procedures well, including wearing the electro-cap continuously for longer than 12 hours. Subjects reported that the informed consent form adequately described the study procedures.

By incorporating a crossover design with a placebo period, practice sessions for the computerized psychometric tests and a familiarization session before the study, these response measures should be suitable for future studies. Average between-day variability for all of the response measures was less than 20 %, which is relatively low compared to expected potential drug effects. The greatest variability was associated with the EEG measures studied when the data is collected in the clinical setting. This may mean that the EEG measures will be the least sensitive of the measures for distinguishing drug effects. The sensitivity of the EEG as a pharmacodynamic measure requires further investigation.

3.5 Conclusions

Based on the results of this study, subsequent studies for the purpose of evaluating quantitative EEG as a pharmacodynamic tool should incorporate a placebo-controlled, crossover design. A familiarization session to acquaint the subjects with the study setting and procedures is necessary to reduce first-session effects. Practice sessions are needed for the psychometric tests to minimize the effects of learning on the comparison of treatments. The testing sessions can feasibly be conducted at least hourly, and subjects can tolerate the procedures for as long as 12 hours. The response measures studied are suitable for future studies to evaluate quantitative EEG as a pharmacodynamic tool for measuring CNS stimulation.

Chapter 4

Part II - Comparison of Quantitative Electroencephalography to Behavioral, Psychological and Neuroendocrine Measures of Response to Dextroamphetamine

4.1 Specific Aims

The purpose of this study was to evaluate the usefulness of quantitative EEG as a measure of CNS response to stimulants. The study was designed to examine the relationship between EEG changes after administration of dextroamphetamine and 1) performance on automated psychometric tests, 2) serum prolactin levels, 3) subjective response as assessed by self-rated mood scales, and 4) serum concentration of dextroamphetamine. The sensitivity of EEG parameters to dextroamphetamine concentration in serum was compared with that of more subjective measures.

4.2 Methods

4.2.1 Clinical Study of Response to Dextroamphetamine

The clinical portions of this study were conducted at the Clinical Research Center at Virginia Commonwealth University. The Committee on the Conduct of Human Research at Virginia Commonwealth University reviewed and approved the study protocol and the informed consent form before the study began. The protocol

and consent form for this study are in Appendix A.

This study was a double-blind, placebo-controlled four-period crossover study with healthy, male volunteers assigned to randomly ordered treatment sequences. Subjects undertook the study in groups of two. The start of each study period was separated by a washout period of at least one week. Subjects received one of four treatments during each study period: dextroamphetamine 20 mg, dextroamphetamine 10 mg, dextroamphetamine 5 mg or placebo as a single oral dose according to the randomization schedule (Appendix E). Eight volunteers were scheduled to be enrolled in the study. The number of subjects included was the minimum that would ensure that at least two subjects were assigned to each treatment sequence.

Subjects. The volunteers were considered for inclusion in the study if they were nonsmokers determined to be in good health based on the results of their medical history, physical examination, and electrocardiography and had no clinically significant deviation from the normal range of values determined in laboratory tests consisting of complete blood count, urinalysis, and clinical chemistry. Volunteers were excluded from the study if they 1) had a history of drug addiction, alcohol abuse, or psychological dependence on drugs, 2) had a first degree relative (mother, father or siblings) with a history of mental illness or alcohol/drug abuse, 3) took any medications chronically or had taken any prescription or investigational drugs in the four weeks prior to starting the study or 4) had a normal daily caffeine intake of greater than two cups of coffee. Before enrolling in the study, all subjects underwent an EEG and psychometric testing familiarization period lasting for at least 4 hours. Subjects with a high number of artifacts on the EEG or who could not tolerate

wearing the electro-cap for extended periods of time were excluded. Before entering the study, each subject signed an informed consent form attesting that his participation was voluntary and the study procedures were explained. The physical examination, electrocardiography, and laboratory tests were repeated within one week of the conclusion of the subject's participation in the study.

Procedure. During each of the four study periods, the following procedure was followed:

Beginning 72 hours before each study day, subjects avoided caffeine, alcohol, and all medications, including over-the-counter medications. Subjects also began a low monoamine diet that was maintained throughout the study period. Tyramine-containing foods such as liver, fermented or dried sausage, canned or dried fish, sauerkraut, fava beans, fermented beverages, and cheese were restricted. The low tyramine diet was instituted as a safety measure, because tyramine can displace norepinephrine from storage sites and indirectly cause a rise in blood pressure. Much of the dietary tyramine is metabolically inactivated presystemically, but the degree to which this occurs may be genetically determined.[126] The degree of influence of tyramine in combination with dextroamphetamine on blood pressure may vary between individuals, so dietary tyramine was maintained at a low level for all subjects. Imposing a very similar diet for all subjects may also serve to make the response to dextroamphetamine more uniform across subjects.

Subjects entered the study facility on the evening of the day preceding each day of dextroamphetamine or placebo dosing and were not released until after the collection of the last blood sample of the study period. Subjects were required to

have a negative urine drug screen and breath alcohol test during each study period before receiving dextroamphetamine or placebo. Subjects fasted from midnight on the evening before dosing until after the 4 hr blood sample was drawn. Water was permitted during the fasting period. Lunch was served after the 4 hr blood sample and dinner at 10 hours after dosing. All meals were low in tyramine content and large quantities of foods potentially promoting alkalinization of the urine (such as milk, nuts, vegetables and fruits) were avoided. The same menu was served on corresponding days of each study period. Subjects began a period of bed rest one hour before dextroamphetamine or placebo administration that continued until after the 6 hr test battery.

Repeated 2 gram oral doses of ammonium chloride (4 x 500 mg enteric-coated tablets, Rugby Laboratories Inc., West Hempstead, NY) were given to acidify the urine and enhance the excretion of dextroamphetamine at the following times: -12, -8, -2, 2, 6, 10, 14, and 18 hr after dextroamphetamine or placebo dosing as described by Wan et al.[18] Subjects received a light snack prior to the -12 and -8 hr ammonium chloride dosing to minimize potential gastrointestinal distress.

Subjects received one of the four treatments: dextroamphetamine 20 mg, dextroamphetamine 10 mg, dextroamphetamine 5 mg, or placebo orally. Both the subjects and the investigator were blinded to treatment. Doses were prepared by the MCV Hospitals Department of Pharmacy and dispensed by the MCV Hospitals Investigational Pharmacy. Dexedrine tablets (SKF Laboratories, Philadelphia, PA) containing 5 mg dextroamphetamine sulfate were used to prepare the doses. The tablets were placed in opaque gelatin capsules to maintain blinding. Sufficient lactose

was added to the capsules to make all doses the same weight. Placebo capsules contained lactose only. Each dose was administered as two capsules.

Prior to dosing, a heparin containing catheter was inserted into a forearm vein for access to blood sampling. Seven-mL samples for determination of dextroamphetamine concentration were collected in red-top tubes with no additives at the following times: pre-dose, 1, 1.33, 2, 2.33, 3, 3.33, 4, 6, 8, 12, 18, and 24 hr after dextroamphetamine or placebo dosing. Blood samples were allowed to clot, centrifuged (within 1 hour of venipuncture) for 10 minutes, serum harvested, and stored at -20 degrees Celsius until analysis. Five-mL samples for the determination of prolactin concentration were collected in red-top tubes with no additives at the following times: pre-dose, 1, 2, 3, 4, 5 and 6 hr after dextroamphetamine or placebo dosing. Blood samples were allowed to clot, centrifuged (within 1 hour of venipuncture) for 10 minutes, serum harvested, and stored at -20 degrees Celsius until analysis.

Subjects completely emptied their bladders just before dextroamphetamine or placebo dosing and the urine pH was determined using a pH meter (Corning) immediately at room temperature after gently shaking the specimen. Two 25 mL aliquots of the urine were retained and frozen until analysis. Urine was then collected over the following intervals after dextroamphetamine or placebo dosing: 0-2 hr, 2-4 hr, 4-8 hr, 8-12 hr, 12-18 hr, 18-24 hr. The pH of the urine voided at the end of each collection interval was determined and the total volume of urine collected during the interval was measured. A 25 mL aliquot of the urine was retained and frozen until analysis for dextroamphetamine concentration. Subjects were required to drink

at least 120 mL of water every hour beginning one hour before dextroamphetamine dosing and continuing through the four hours after dosing.

Five-minute segments of 28 channel EEG were recorded for each subject using a NeuroScience Brain Imager (San Diego, CA) with eyes closed at 0, 1, 2, 3, 4, 6, 8, and 12 hrs. Subjects reclined in a hospital bed with the lights off during the recordings. They were asked to count back from 500 by 3s to maintain vigilance. The electrodes were placed using an Electro-cap according to the 10/20 International System with 8 additional electrodes located 50% between the standard 10/20 placement. Omni-Prep ((D.O. Weaver & Co.,Aurora, CO) was used to prepare the scalp and Electro-Gel (Electro-Cap International, Inc., Dallas, TX) was used as the conducting gel. Linked ears were used as a reference. Four additional electrodes were placed to monitor for vertical and lateral eye movements and electromyographic activity. Electrodes in the cap, ear clips, and eye movement monitors were made of tin. Electrode impedances were checked before each recording, and maintained at less than 5.6 kohms and similar between electrodes. Disturbances in the room or subject movement during the EEG was recorded. The Brain Imager filters were set as follows: Low filter - 0.30 Hz, High filter - 40 Hz, Notch filter - off. The raw EEG was stored on optical disks. System integrity of the Brain Imager was checked weekly throughout the study to ensure stability of channel calibration and proper filter functioning.

Each subject completed a computerized visual CPT (NeuroScan, Inc.) at 0, 1, 2, 3, 4, 6, 8 and 12 hr. In this task, the digits 0 through 9 briefly appear on the screen. The subject presses the left button of the mouse when a 0 appears and the

right button for all other digits. The interstimulus interval varied from 0.8 to 1.2 seconds. A total of 120 stimuli were presented during each testing session. Two practice sessions were completed before beginning the 0 hr test battery.

Subjects completed a computerized motor task, finger tapping (NeuroScan, Inc.) at 0, 1, 2, 3, 4, 6, 8 and 12 hr. During this task, subjects tap the mouse button as fast as possible for 10 seconds, first with their right hand and then with their left. A total of three trials with each hand were completed during each testing session. Two practice sessions were completed before beginning the 0 hr test battery.

A self-rated scale (Appendix B) based on the MBG (a measure of euphoria) and the A (a measure of amphetamine effects) subscales of the Addiction Research Center Inventory Scales described by Martin et al.[21] was completed by each subject at 0, 1, 2, 3, 4, 6, 8 and 12 hr. The scale consisted of 23 questions which the subject responded to on a scale of 1 to 5. At the same times, a 100 mm visual analog mood scale (Appendix C) was completed.

Blood pressure (sitting) and heart rate were measured at the following times: predose and 1, 2, 3, 4, 6, 8, 12, and 24 hr after dextroamphetamine or placebo dosing using a Dynamap (Critikon, Tampa,FL).

When above measurements were scheduled at the same time, they were conducted in the following sequence: 1) urine collection, 2) blood samples, 3) EEG, 4) CPT, 5) rating scales, 6) finger tapping and 7) vital signs with the blood sample being collected at exactly the scheduled time.

All subjects were observed for symptoms and signs of clinical intolerance to the drugs or procedures and asked to report any adverse effects. These were evaluated

by the physician monitor for their clinical significance and potential need for treatment.

At the conclusion of his participation in the study, each subject was asked to identify which treatment he believed he had received during each period.

4.2.2 Sample analysis

Assays for amphetamine in serum were performed by the author at the School of Pharmacy. Method development was conducted with the guidance of Clark March in the Biopharmaceutical Analysis Laboratory and is described in section 4.2.2a. The final method is described in section 4.2.2b. Validation of the method is described in section 4.2.2c. Description of the analysis of the subject samples from the clinical study is in section 4.2.2d. Amphetamine in urine was analyzed by a GC-MS method in the MCVH Toxicology Laboratory under the direction of Dr. Alphonse Poklis. The method is described in section 4.2.2e. Urine samples for subjects 1, 2, 4, 6, 8, 9 and 10 were analyzed for amphetamine concentration. Assays for prolactin in serum were performed using an RIA method by Linda Lawrence, M.S. in the Clinical Research Center Core Laboratory at MCV. Description of the method is found in section 4.2.2f.

4.2.2a Analytical Method Development for Amphetamine in Serum

Analytical method development for amphetamine in serum began in September of 1991, after the completion of the clinical study. Requirements set forth to guide initial method selection included 1) ability to quantitate serum concentrations in the

range of 1 ng/mL (expected concentrations at 24 hours after 5 mg dose) to 60 ng/mL (expected peak concentration after 20 mg dose), 2) 1 mL or smaller serum sample needed for each extraction, and 3) necessary equipment and expertise available within the School of Pharmacy and supplies reasonable in cost. To achieve quantitation at serum levels in the low ng/mL range, gas chromatographic (GC) methods with detection of derivatized drug by mass spectrometry (MS), electron-capture detection (ECD), flame-ionization detection (FID), or nitrogen-phosphorus detection (NPD) are most likely to be useful.[127,17] Of these methods, GC-MS is the most sensitive. Equipment to perform the analysis by GC-MS was not readily available however, so alternatives were considered. A GC equipped with an ECD and an NPD was available, so a method for amphetamine in plasma, urine, and cerebral spinal fluid using GC-NPD[128,129,130] developed by Dr. Narasimhachari at VCU was chosen as the initial method to investigate.

In Dr. Narasimhachari's method, the internal standard β-methylphenethylamine is added to 1-mL aliquots of plasma containing amphetamine. The sample is alkalinized by the addition of 0.5 mL of 2 N NaOH, salinized with 1 gram of NaCl and extracted twice with 5 mL of ethyl acetate. The organic layer is separated, pooled, and then back extracted with 0.5 mL of 0.5 N HCl and the organic layer discarded. The acid extract is alkalinized with 0.5 mL of 2N NaOH and extracted into 5 mL of ethyl acetate. The organic layer is separated and mixed with 0.5 mL CS_2 and set aside for 2 hours. The sample is then washed with 0.5 mL of 0.5 N HCl, the organic layer is evaporated to dryness, and then reconstituted in 100 μL of ethyl acetate and injected onto the GC column. A Hewlett Packard 5840 GC with a

nitrogen-specific detector was used. Three columns were used: 1) a 1.3-m glass column packed with 2% OV-101 on Chromosorb WHP, 2) a 1.3-m glass column packed with 3% SP-2250 on Supelcoport 100-200 mesh and 3) a 0.65-m glass column with 3% OV-225 on Chromosorb WHP. The oven temperature was 140°C or 145°C, the injector temperature was 250°C and the detector temperature was 300°C. This method provided retention times of less than 3 minutes for both the analyte and internal standard.

Dr. Narasimhachari's method required modification for use in our laboratory because of equipment differences. The method was adapted for use with a 5890 Series II GC with nitrogen-phosphorus detector, 7673 Autosampler and controller, 3396 Series II integrator, and capillary column fittings (Hewlett Packard Co., Avondale, PA) as follows:

A 15-m DB5 capillary column with internal diameter of 0.32 mm and film thickness of 0.25 microns (J&W Scientific, Folsom, CA) was used with injections made in the splitless mode. Helium was used as the carrier gas, with a column flow of 1.2 mL/min at an injector temperature of 200°C, detector temperature of 250°C and column temperature of 100°C. To 1-mL samples containing amphetamine and β-methylphenethylamine in water was added 0.5 mL 1N NaOH and 5 mL ethyl acetate. The organic layer was separated and retained, and 0.1 mL CS_2 was added. After 1 hour, the samples were evaporated to dryness and reconstituted with 50 μL ethyl acetate. 1 μL was injected onto the column.

These initial conditions did not provide adequate sensitivity for amphetamine. In addition, an interfering peak at the retention time of the internal standard was

present. The following factors were modified in an attempt to improve sensitivity and chromatography: 1) integrator attenuation (-2, -1, 0, 1 or 2), 2) injection volume (1, 2 or 3 μL), 3) purge valve reset time (0.5, 0.75, 1, or 1.5 μL), 3) injection port temperature (90, 100, or 110°C), 4) oven temperature program (rate = 2, 5, 10 or 15°C/min), 5) injection port liner type (untapered or dual-tapered), 6) reconstitution solvent (ethyl acetate, toluene, toluene:methanol [96:4], dodecane or isooctane), 7) volume of CS_2 added (25, 50, 75, 100, 125 or 150 μL), 8) volume of ethyl acetate for extraction (2, 3, 4, 5 or 6 mL), and 9) choice of internal standard (β-methylphenethylamine or α-phenethylamine. Modifications to these factors improved the sensitivity somewhat, but the interfering peak remained.

Samples of amphetamine and β-methylphenethylamine in serum were prepared and extracted. Many interfering peaks were present, so a three-step extraction procedure similar to that described by Narasimhachari (discussed above) was tried. To each sample, 0.5 mL of 1 N NaOH and 5 mL ethyl acetate were added. The organic layer was separated and 0.5 mL of 0.5 N HCl was added. The organic layer was separated and discarded. To the aqueous layer, 0.5 mL or 1 N NaOH and 5 mL of ethyl acetate was added. The organic layer was separated and 50 μL of CS_2 was added. After 1 hour, the samples were evaporated to dryness, reconstituted with 20 μL of ethyl acetate, and 2 μL were injected. The three-step extraction resulted in fewer interfering peaks, but did not eliminate them. In an attempt to remove or reduce the interfering peaks, the following factors were modified: 1) source of reagents, 2) preparation and storage of reagents, 3) pipet tips and extraction tubes used, and 4) type of extraction solvent (toluene or ethyl acetate). Despite these

modifications, problems with sensitivity and selectivity remained. Selectivity problems may have eventually been solved because the reagents appeared to be responsible, but concentrations in serum of less than 5 - 10 ng/mL were not quantifiable, so sensitivity was the major limiting problem.

Derivatization in gas chromatography can be used to improve chromatography, as with the isothiocyanate derivative of amphetamine[128], or to enhance the detectability of a compound by introducing detector-oriented labels onto it.[131] The latter approach has been particularly successful with derivatives that enhance detection by ECD. A number of halogenated reagents have been used to derivatize primary amines for subsequent analysis by GC-ECD.[132] The decision was made to switch to ECD and investigate alternative derivatizing reagents in an attempt to improve sensitivity and perhaps selectivity.

The next experiments employed the same GC and column with the addition of an ECD to replace the NPD. Helium (grade 5) flow through the column was 1.25 mL/min with an injection port temperature of 200°C, detector temperature of 300°C and column temperature programmed at 110°C for 1.5 min, increase at 10°C/min to 160°C and hold for 10 min. 5% methane in argon was used as the make-up gas flowing at 65 ml/min. Splitless injection was used with a dual tapered liner and purge valve reset time of 0.75 min.

The first derivatizing reagent investigated was trifluoroacetic anhydride (TFAA). Samples of amphetamine and β-methylphenethylamine were extracted using three different extraction solvents: ethyl acetate, toluene, and ethyl ether/hexane (8:2). The derivatizing reagent was added to the organic extract and the mixture was heated

in a water bath at 50°C for 20 min. Samples were evaporated to dryness, reconstituted with ethyl acetate and 2 μL were injected into the GC. No analyte peaks could be detected. Poole & Poole[133] reported the relative response of the ECD to haloalkylacyl derivatives of amphetamine. Responses relative to monochloroacetyl derivatives are as follows: trichloroacetyl 540, trifluoroacetyl < 0.1, pentafluoropropionyl 40, heptafluorobutyryl 90, perfluorooctonyl 230, and pentafluorobenzoyl 770. Based on these responses, trifluoroacetyl derivatives are least detectable, making TFAA a poor choice for derivatizing reagent. Subsequent experiments were conducted with trichloroacetyl chloride (TCA) and pentafluorobenzoyl chloride (PFB) as derivatizing reagents.

When TCA and PFB were compared directly at the same concentration using toluene as the extraction solvent that the derivatization reaction was carried out in, peak height was less than half with TCA than with PFB. The peak shape however, was improved with the TCA derivative. To achieve quantitation as low as 1 ng/mL of amphetamine in serum, it was decided to continue with the PFB derivative which provided greater detector response. The following factors were examined to improve sensitivity and selectivity using the PFB derivative: 1) concentration of PFB chloride (0.001, 0.01, 0.1, 1, 10 or 100%), 2) addition of pyridine as an acid receptor to facilitate the derivatization reaction,[131] 3) concentration of pyridine added (0.01, 0.1, 1 or 10%), 4) volume of serum sample needed (0.5 or 1 mL), 5) storage of diluted derivatizing reagent (store in refrigerator, room temperature or dilute just prior to use), 6) volume and concentration of NaOH and HCl solutions for extraction to determine the minimum acid and base that could be used while still maintaining

adequate pH at each step for effective extraction, 7) source of solvents (Fisher or Burdick & Jackson), 8) source of derivatizing reagent (Aldrich or Regis), 9) removal of trace amounts of water prior to derivatization step, 10) method for washing glassware (with and without a final rinse in toluene), 11) column type (DB-17 or DB-5) and 12) type of extraction and centrifuge tubes (pyrex or borosilicate glass). After adjusting these factors, it appeared that the lowest level of quantitation that could be achieved was 2 ng of amphetamine per mL of serum, which represents a peak height that was eight time the biological noise at the retention time for amphetamine. The retention times of amphetamine and β-methylphenethylamine were 14.3 and 14.6 min respectively.

4.2.2b Description of the Analytical Method for Amphetamine in Serum

The final method that was validated (section 4.2.2c) and used to analyze samples from the clinical study is described below. The reagents and supplies used for the assay are listed in Table 4.1.

Sample preparation. Add 1 mL of serum, 25 μL of internal standard solution (2 μg/mL),and 0.5 mL of 0.5 N NaOH to a culture tube. Vortex briefly. Add 5 mL ethyl acetate to the culture tube and vortex intermittently for 30 sec. Centrifuge for 10 min at 1500 rpm. Transfer the ethyl acetate layer to a clean culture tube. Add 5 mL of 0.001 N HCl. Vortex for 30 sec. Centrifuge for 10 min at 1500 rpm. Aspirate the ethyl acetate layer to waste. Add 0.5 mL of 0.5N NaOH and vortex briefly. Add 1 mL toluene and vortex for 30 sec. Centrifuge for 10 min at 1500 rpm. Transfer toluene layer to centrifuge tube previously rinsed in toluene. Add 5

Table 4.1 Reagents and Supplies Used for Assay of Amphetamine in Serum

1. *d*-Amphetamine, 1 mg/mL in methanol (Alltech Associates, Deerfield, IL)
2. β-Methylphenethylamine, 99% (Aldrich Chemical Co., Milwaukee, WI)
3. Pyridine, silylation grade (Pierce, Rockford, IL)
4. Pentafluorobenzoyl chloride (Regis Chemical Company, Morton Grove, IL)
5. Sodium hydroxide, ASC (Fisher Scientific, Fair Lawn, NJ)
6. Hydrochloric acid, ASC (Fisher Scientific)
7. Methanol, HPLC grade (Fisher Scientific)
8. Toluene, High Purity (Baxter Healthcare Corp., Burdick & Jackson Division, Muskegon, MI)
9. Ethyl Acetate, High Purity (Baxter Healthcare Corp., Burdick & Jackson Division)
10. Distilled, deionized water
11. Helium, grade 5.0 (AIRCO Medical Gases, Ashland, VA)
12. 5% Methane in argon (AIRCO Medical Gases)
13. Nitrogen, Medical grade (AIRCO Medical Gases)
14. Borosilicate glass screw cap culture tubes, 16 X 125mm, with teflon-lined caps (Baxter Diagnostics, Inc., Scientific Products Division, McGaw Park, IL)
15. Borosilicate glass screw cap centrifuge tubes, 10 mL, with teflon-lined caps (Scientific Products)
16. Borosilicate glass transfer pipets, 5 3/4 inches (Scientific Products)
17. Autosampler vial inserts, flat bottom, 200 μL (Sun Brokers, Wilmington, NC)
18. Autosampler vial caps, teflon-lined rubber septum (Sun Brokers)
19. Glass syringes, model 701, (Hamilton Co., Reno, NV)
20. Injection port liner, dual tapered, 4mm ID, 800 μL volume (Hewlett Packard, Avondale, PA)

μL of 1% pyridine and 5 μL of 1% pentafluorobenzoyl chloride. Vortex briefly. Place centrifuge tube in water bath at 50°C for 30 min. Evaporate toluene mixture to dryness under a flow of nitrogen in a Turbo-Vap LV evaporator (Zymark Corp., Hopkington, MA). Reconstitute the residue with 100 μL of ethyl acetate and vortex briefly. Transfer the sample to an autosampler vial with insert and cap. Inject 1 μL into the GC.

Chromatographic Conditions. A 5890 Series II gas chromatograph with nickel 63 electron-capture detector, 7673 autosampler and controller and 3396 Series II integrator (Hewlett Packard Co.,Avondale, PA) was used for the analyses. The column was a 15 m capillary DB-5 column with internal diameter of 0.32 mm and film thickness of 0.25 μm (J & W Scientific, Folsom, CA). The following gas flow rates were used: column - 1.4 mL/min at 80°C (helium), column head pressure - 5 psi (helium), septum purge - 50 mL/min (helium), split - 3 mL/min (helium), make-up gas - 65 mL/min at 300°C and pressure 50 psi (5% methane in argon). Temperatures for the system were: injector - 200°C, detector - 300°C, oven program - 80°C for 1.5 min then 10°C/min to 250°C then 250°C for 1 min. The purge valve reset time was 0.75 min and the splitless injection mode was used.

Data Evaluation. The amphetamine concentration of the prepared standards (50 ng/mL, 20 ng/mL, 10 ng/mL, 5 ng/mL, 3 ng/mL, and 2 ng/mL) was regressed against the peak area ratio of amphetamine to internal standard to obtain calibration curves. The data was better described by a power function than a linear function, because the power function minimized the difference between back-calculated values and actual values for the highest standards. The following regression equation was

used:

$$y = ax^b$$

where x = concentration of amphetamine standard, y = peak area ratio, and a and b are constants. The constants a and b were calculated with the linear regression function of Quattro Pro (Borland International, Inc., Scotts Valley, CA) using a log transform of the data. An example calibration curve is shown in Figure 4.1.

4.2.2c Analytical Method Validation for Amphetamine in Serum

The method was evaluated with respect to specificity, limit of quantitation, linearity, precision within a run, precision and accuracy between runs, extraction recovery, and stability of prepared samples. Analysis of spiked samples whose concentrations were unknown to the analyst were performed to further assess the accuracy of the method. The effects of freezing, thawing, and storage of the samples prior to analysis was also evaluated.

Specificity. Serum collected during the placebo period of the clinical study from nine study volunteers and serum harvested from two additional donors was examined for the presence of potential interferences with amphetamine or the internal standard. 1-mL samples were extracted, derivatized and injected on the GC. These chromatograms were inspected for the presence of interfering peaks at the retention times of amphetamine and the internal standard. Two of the samples had small peaks that interfered with amphetamine that were 12% and 17% of the peak height of the 2 ng/mL standard. These were not considered to be significant interferences for this analysis. Chromatograms from the samples collected from the study volunteers are

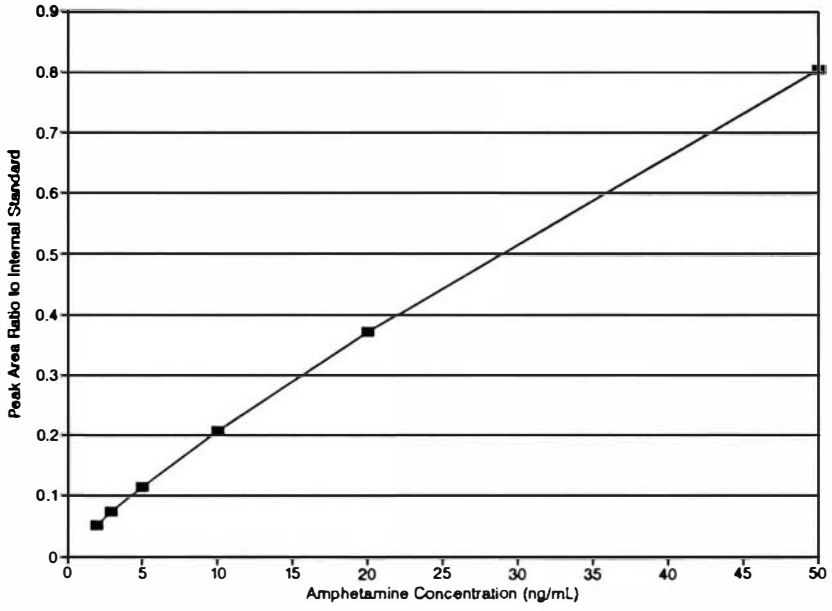

Figure 4.1 An example calibration curve for assay of amphetamine in serum

presented in Appendix F. Serum from these sources was then used to prepare standards and controls for use during the method validation and subject sample analyses.

Limit of quantitation. The lowest standard concentration of amphetamine for this analysis was established at 2 ng/mL of serum. At this concentration, the peak height of amphetamine was eight times the noise of a amphetamine-free biological sample at the retention time of amphetamine. The mean back calculated concentration for the 2 ng/mL standard obtained during the analytical validation runs (average of 12 analyses) was 2.0 ng/mL with a relative standard deviation, expressed as a percent (% RSD), of 7.91 %.

Linearity. Amphetamine serum concentrations over the range from 2 to 50 ng/mL were evaluated for the calibration curve. Peak concentrations for subject samples following the 20 mg dose of amphetamine were expected to be approximately 50 ng/mL. The standards and controls used for this evaluation were made using pooled serum from two of the sources evaluated for possible interferences. Calibration curves from six analytical runs, with duplicates of each standard concentration in each run, were examined. A blank serum sample was also analyzed with each run. Example chromatograms of each standard and control from one run are presented in Appendix G. The constants (a and b) and the correlation coefficients of the calibration curves are presented in Table 4.2. As an additional measure of the appropriateness of the curve modeling, the calculated concentrations of the standards (back-calculated from the regression line) were examined. The back-calculated concentrations are presented in Table 4.3. The RSD (%) indicates the variability in

Table 4.2 Regression Statistics for Serum Calibration Curves

Date	Run Number	a	b	Correlation Coefficient
1/27/92	1	0.0275	0.8414	0.9979
1/28/92	2	0.0281	0.8416	0.9959
1/30/92	3	0.0287	0.8490	0.9992
2/8/92	4	0.0298	0.8422	0.9988
2/15/92	5	0.0323	0.8251	0.9967
2/18/92	6	0.0313	0.8069	0.9975
Mean		0.0296	0.8344	0.9977
SD		0.0019	0.0142	0.0011
RSD (%)		6.41	1.71	0.12

Table 4.3 Concentrations of Standards Back-calculated from the Regression Equations

	Amphetamine Concentration (ng/mL)					
Run No.	50	20	10	5	3	2
1	54.35	19.75	10.41	4.78	2.84	2.13
	53.00	17.35	9.81	4.72	2.88	2.28
2	51.66	19.95	11.57	5.65	3.21	1.89
	43.74	18.87	9.97	5.68	2.85	1.66
3	47.75	19.59	10.17	5.03	2.88	1.94
	51.21	21.26	9.73	5.17	2.82	2.18
4	50.49	20.26	10.81	5.06	3.03	2.04
	50.17	19.94	9.51	4.30	3.11	2.05
5	51.56	20.83	10.80	4.86	3.19	1.86
	41.25	22.22	11.13	4.66	2.68	2.11
6	48.40	18.47	9.54	4.63	3.08	1.96
	47.67	24.23	10.92	4.79	3.24	1.93
Mean	49.27	20.23	10.36	4.94	2.98	2.00
SD	3.62	1.72	0.65	0.39	0.17	0.16
RSD(%)	7.35	8.49	6.26	7.86	5.85	7.91
DFA(%)	-1.46	1.13	3.64	-1.12	-0.53	0.12

the calibration curves and the difference from actual, expressed as a percent (DFA %) shows the accuracy of the calibration curves. DFA (%) is calculated as: DFA(%) = [(mean - actual)/actual] x 100. These results indicate that the range of the calibration curve (2 ng/mL to 50 ng/mL) for amphetamine is appropriate and the variability is acceptable.

Precision within a run. To evaluate precision within a run, six replicates of control samples (prepared by spiking blank serum with amphetamine at 35, 7.54, and 3.5 ng/mL) were analyzed in a single run. The results of these analyses are presented in Table 4.4. The variability, expressed as RSD (%), is acceptable for the purposes of this analytical method.

Precision and accuracy between runs. To evaluate precision and accuracy between runs, control samples (prepared by spiking blank serum with amphetamine at 35, 7.5 and 3.5 ng/mL) were analyzed in six analytical runs. The results are shown in Table 4.5. Precision was assessed by examining the RSD (%) of each control across the 6 runs. Accuracy is reflected in the DFA (%) for each control across the 6 runs. The precision and accuracy between runs for this method was acceptable.

Extraction recovery. Extraction recovery was assessed by comparing results from serum samples spiked with amphetamine and carried through the extraction and derivatization step to results from blank serum samples carried through the extraction and then spiked with amphetamine just prior to derivatization. This process examines extraction recovery, but does not assess the efficiency of the derivatization reaction. To perform the recovery study, blank serum was spiked to amphetamine concentrations of 50, 5 and 2 ng/mL (3 samples at each concentration). These

Table 4.4 Data for Within-Run Precision

	Amphetamine Concentration (ng/mL)		
Replicate No.	35	7.5	3.5
1	34.60	8.03	3.25
2	30.88	7.63	3.26
3	33.02	7.99	3.05
4	34.67	7.66	3.10
5	33.80	7.74	3.41
6	33.16	8.09	3.21
Mean	33.36	7.86	3.21
SD	1.27	0.19	0.12
RSD (%)	3.82	2.36	3.63

Table 4.5 Data for Precision and Accuracy Between Runs

	Amphetamine Concentration (ng/mL)		
Run No.	35	7.5	3.5
1	30.87	7.85	3.49
2	33.22	7.72	3.16
3	32.79	7.41	3.45
4	34.60	8.03	3.25
5	29.56	7.29	3.30
6	32.00	6.90	3.62
Mean	32.17	7.53	3.38
SD	1.78	0.41	0.17
RSD (%)	5.53	5.44	5.03
DFA (%)	-8.09	0.40	-3.43

samples and nine blank serum samples were extracted using the procedure described in section 4.2.2b. Just before the addition of the derivatizing agent, the blank serum extracts were spiked with amphetamine to 50, 5 and 2 ng/mL (3 samples at each concentration). All samples were then derivatized and injected into the GC. Recovery was calculated as the mean peak height of the unextracted samples divided by the mean peak height of the extracted samples, expressed as a percent. The results are presented in Table 4.6. The extraction recovery of amphetamine was 53.6%, 42.5% and 47.4% at 2, 5 and 50 ng/mL respectively. The extraction recovery of the internal standard was 49.5%. Spiking the amphetamine into toluene alone, rather than the toluene-serum extract was attempted initially, but this resulted in greater peak heights for the extracted samples than the unextracted samples. This indicates the presence of a matrix effect in the derivatization or detection of amphetamine.

Stability of prepared samples. To examine the stability of prepared samples, four standard curves were extracted and then exposed to various conditions. The first set of standards were carried through the entire procedure and injected into the GC immediately. The second set of standards were carried through the entire procedure and allowed to sit on the autosampler tray (at room temperature) for 24 hours before they were injected. The third set of standards was carried through the extraction, derivatization and dry-down steps. Prior to reconstitution, the samples were stored in a freezer at -20°C for 24 hours. The samples were then brought to room temperature, reconstituted and injected into the GC. The fourth set of standards was carried through the entire process until they were ready to inject. They were then stored at -20°C for 24 hours. They were then brought to room temperature and injected. The

results of these experiments are given in Table 4.7. These results indicate that the samples can be processed and stored under any of these conditions without loss of the ability to detect amphetamine concentrations in the sample.

Analysis of blinded spiked samples. Ten blank serum samples were spiked with amphetamine at concentrations within the calibration curve range of the assay. The concentrations of amphetamine in these samples were unknown to the analyst at the time of the analyses. These results were used to further evaluate the accuracy of the method. The average DFA (%) for the ten samples was 7.62%. The results are shown in Table 4.8. These results provide further evidence that the accuracy of the method is adequate its intended use.

Effects of freezing and thawing. A set of controls (35, 7.5 and 3.5 ng/mL) was prepared and frozen. The controls were thawed 2 days later and a 1-mL aliquot was analyzed for amphetamine concentration. The remainder of the sample was re-frozen. The controls were thawed again 8 days later, a 1-mL aliquot was analyzed for amphetamine concentration, and the remainder of the sample was re-frozen. The freeze/thaw cycle was repeated 2 days later and then 20 days later (a total of four times) to test the stability of amphetamine in serum to repeated freezing and thawing. The results are presented in Table 4.9. Repeated freezing and thawing did not significantly affect the concentrations of amphetamine in the controls.

Stability of amphetamine in serum samples under storage conditions. This experiment was conducted to study the stability of serum samples containing amphetamine under the same storage conditions as the samples from the clinical study were stored. The first serum samples from the clinical study were collected in May,

Table 4.6 Extraction Recovery of Amphetamine and β-methylphenethylamine from Serum

Compound	Concentration (ng/mL)	Mean Extracted Peak Height	Mean Unextracted Peak Height	Recovery (%)
Amphetamine	2	569	1062	53.6
Amphetamine	5	799	1879	42.5
Amphetamine	50	16154	34080	47.4
β-methyl-phenethylamine	50	59619	120506	49.5

Table 4.7 Results of Stability Study of Prepared Samples

	Amphetamine Peak Area Ratio to Internal Standard					
Standard Set	50	20	10	5	3	2
1	0.601	0.218	0.157	0.109	0.074	0.046
2	0.670	0.344	0.138	0.087	0.061	0.044
3	0.754	0.305	0.139	0.107	0.064	0.053
4	0.687	0.270	*	0.087	0.061	0.034
Mean	0.678	0.284	0.145	0.098	0.065	0.044
SD	0.063	0.054	0.011	0.012	0.006	0.008
RSD (%)	9.29	19.01	7.59	12.24	9.54	17.73

* Sample lost due to autosampler failure

Table 4.8 Results of Analysis of Blinded Spiked Samples

Amphetamine Added (ng/mL)	Amphetamine Found (ng/mL)	Percent Difference From Actual (DFA %)
38.5	39.57	2.78
0	BLQ	0
6.0	6.78	13.00
6.0	6.03	0.50
38.5	37.05	-3.77
3.5	4.24	21.14
0	BLQ	0
3.5	3.84	9.71
24.4	22.12	-9.34
24.4	20.51	-15.94
	Mean	7.62

BLQ = Below limit of quantitation

Table 4.9 Effect of Repeated Freezing and Thawing on the Stability of Amphetamine in Serum

	Amphetamine Concentration (ng/mL)		
Cycle No.	35	7.5	3.5
1	29.17	7.35	3.05
2	32.69	8.22	3.42
3	37.54	7.29	4.17
4	28.22	7.65	3.51
Mean	32.07	7.63	3.54
SD	4.04	0.43	0.47
RSD (%)	12.60	5.64	13.28
DFA (%)	-8.37	1.73	1.14

1991 and were stored at -20°C in polypropylene culture tubes. Stability samples were prepared at this time by spiking blank serum with amphetamine to 10 and 40 ng/mL. The stability samples were stored in polypropylene culture tubes and frozen at -20°C in the same freezer as the study samples until March 1992, when analysis of the study samples was complete. At this time they were thawed for analysis. The concentrations determined for the 10 ng/mL and 40 ng/mL samples were 9.10 and 41.03 ng/mL, respectively. This indicates that amphetamine was stable in serum stored for 10 months at -20°C under the same storage conditions as the study samples.

4.2.2d Analysis of Subject Serum Samples for Amphetamine

Samples were analyzed in batches made up of two standard curves (one at the beginning and one at the end of the run), two of each of three controls (35, 7.5, 3.5 ng/mL), with a control run after each group of 7 study samples, a blank serum sample, and study samples from one subject (39 samples). The following study samples were diluted before analysis by adding 0.5 mL blank serum to 0.5 mL study sample: 20 mg dose - 1, 1.33, 2, 2.33, 3, 3.33, and 4 hr and 10 mg dose - 1, 1.33, and 2 hr. A 35 ng/mL control was similarly diluted and analyzed with each run. Each batch contained a total of 59 samples.

Results from both standard curves were used to determine the regression equation for each batch. In one batch (run on 3/4/92), the first and second halfs of the run were different from one another. The peak height of the internal standard was higher during the second half of the run, so the two sets of standards could not be used to make a single standard curve. The regression of the curve from the first

set of standards was used to calculate concentrations for the first half of the samples and the regression of the curve from the second set of standards was used to calculate concentrations for the second half of the samples. All quality control samples were acceptable using this procedure, and repeat analysis of a portion of the samples from both the first and second half gave similar results to the first analysis. Regression statistics for the standard curves are presented in Table 4.10. Concentrations for the standards back-calculated from the regression equations are shown in Table 4.11. Concentrations for the quality control samples for each batch back-calculated from the regression equations are presented in Table 4.12. When the control samples that fell outside of the precision limits (outside ± 2.58 X SD of the mean obtained for all of the runs) or accuracy limits (more than $\pm 25\%$ of the mean obtained for all of the runs) are excluded, the RSD (%) for the 35 ng/mL (diluted), the 35 ng/mL (undiluted), the 7.5 ng/mL and the 3.5 ng/mL control were 17.0%, 9.4%, 9.8%, and 12.1% respectively.

Samples with values less than 2.0 ng/mL were considered to be below the limit of quantitation and were designated BLQ. Study sample analyses were repeated for the following reasons: 1) the concentration determined was greater than highest standard (50 ng/mL), 2) poor chromatography, 3) the back calculated value for the control run before or after the study sample was either more than $\pm 25\%$ of the mean obtained for all of the runs (outside of accuracy limits) or outside ± 2.58 X SD of the mean obtained for all of the runs (outside of precision limits), or 4) the value for the study sample appeared to be an outlier on a plot of concentration versus time. The original results were considered to be confirmed if the repeat value is within $\pm 20\%$

Table 4.10 Regression Statistics for Standard Curves from Analysis of Subject Samples

Date	a	b	Correlation Coefficient
2/25/92	0.0234	0.8628	0.9933
2/29/92	0.0248	0.8489	0.9942
3/2/92	0.0235	0.8455	0.9942
3/4/92[a]	0.0267	0.8124	0.9913
	0.0190	0.8406	0.9975
3/8/92	0.0241	0.8285	0.9865
3/10/92	0.0233	0.8783	0.9932
3/11/92	0.0183	0.9133	0.9989
3/15/92	0.0219	0.9110	0.9961
3/17/92	0.0190	0.9184	0.9828
Mean	0.0224	0.8660	0.9930
SD	0.0028	0.0360	0.0050
RSD(%)	12.48	4.13	0.46

a = Concentrations for samples from first half of batch calculated from regression of first standard curve and the for the second half from the second standard curve.

Table 4.11 Back-Calculated Concentrations for Standards from Analysis of Subject Samples

Date	50 ng/mL Standard	20 ng/mL Standard	10 ng/mL Standard	5 ng/mL Standard	3 ng/mL Standard	2 ng/mL Standard
2/25/92	56.25	22.28	10.74	5.91	2.76	2.25
	44.87	19.08	7.64	5.51	2.59	1.95
2/29/92	55.24	20.99	10.91	5.62	3.38	2.26
	52.92	16.33	8.23	4.64	2.59	1.95
3/2/92	50.49	22.39	10.66	5.87	3.30	2.17
	54.99	16.77	8.40	4.09	2.90	1.94
3/4/92	58.40	17.30	8.05	5.81	3.35	1.89
	50.41	19.20	11.28	4.39	2.95	2.12
3/8/92	59.08	a	7.79	6.17	3.77	2.43
	55.63	15.08	10.40	4.26	2.80	1.68
3/10/92	43.94	21.14	11.46	4.90	2.88	2.46
	48.71	22.85	8.78	5.76	2.71	1.59
3/11/92	52.39	20.79	9.02	5.12	2.85	2.18
	50.83	19.13	9.99	5.01	2.83	2.09
3/15/92	49.80	19.05	10.90	5.14	3.71	2.09
	52.39	20.41	8.49	5.04	2.56	1.87
3/17/92	31.33	21.27	9.00	4.68	2.76	1.86
	78.71	19.27	10.11	6.01	3.54	1.91
Mean	52.58	19.61	9.55	5.22	3.01	2.04
SD	8.87	2.17	1.26	0.62	0.38	0.23
RSD (%)	16.86	11.08	13.19	11.97	12.59	11.14
DFA	-5.15	1.96	4.53	-4.37	-0.43	-1.92

a = Standard rejected due to poor chromatography

Table 4.12 Back-Calculated Concentrations for Quality Control Samples from Analysis of Subject Samples

Date	35 ng/mL control (diluted)	35 ng/mL control	7.5 ng/mL control	3.5 ng/mL control
2/25/92	30.85	42.62[a]	8.40	3.35
		30.82	6.81	3.55
2/29/92	36.69	33.05	7.81	4.15
		26.87	5.67	2.59
3/2/92	46.16[a]	33.85	8.42	3.60
		32.71	7.00	3.16
3/4/92	40.29	36.42	7.56	2.92
		28.38	7.79	3.30
3/8/92	34.03	35.22	6.52	2.93
		32.19	6.88	2.98
3/10/92	26.24	36.28	7.25	3.72
		27.66	7.31	5.05[a]
3/11/92	26.26	30.04	6.46	3.29
		27.79	6.76	3.27
3/15/92	31.85	33.23	8.17	3.24
		34.09	7.55	3.20
3/17/92	25.53	30.60	8.11	2.65
		30.78	7.33	3.66
Mean	33.10	32.37	7.32	3.37
SD	6.61	3.76	0.72	0.55
RSD (%)	19.98	11.61	9.80	16.43
DFA	5.43	7.52	2.37	3.79

a = Outside accuracy and precision limits

of the original value. If there was a discrepancy between the original and the repeat value, a third assay was performed. If the third assay agreed with the original, then the two values were averaged and reported. If the third assay agreed with the second, then the two were averaged and reported. If the third assay agreed with both the original and the second assay, then the mean of all three values was reported. Seven study samples were repeated due to controls falling outside the accuracy and precision limits, one was repeated because the assayed value was greater than 50 ng/mL, six were repeated due to poor chromatography, and 14 were repeated based on the plot of concentration versus time.

4.2.2e Analytical Method for Amphetamine in Urine

This method was developed in the MCVH Toxicology Laboratory under the direction of Dr. Alphonese Poklis. The extraction and derivatization is based on the method of Meeker and Reynolds.[134] Briefly, urine samples containing amphetamine were spiked with internal standard, made basic, and then extracted with chlorobutane. The analytes in chlorobutane were derivatized with pentafluoropropionic anhydride. The chlorobutane was evaporated, the residue reconstituted in ethyl acetate, and a portion injected into the gas chromatograph with mass selective detector.

Standards and controls. A reference standard at 1.0 mg/mL d-amphetamine in methanol (Radian Corporation, Austin, TX) was used to prepare working standards by spiking drug-free urine with d-amphetamine to 0.2, 0.5, 1.0, 2.0, 4.0, 6.0 and 8.0 μg/mL. The internal standard was d_5-amphetamine (Radian Corporation) with 0.1 mL of a 10 μg/mL solution added to each specimen prior to analysis. Controls were

prepared by spiking drug-free urine to 1 and 4 μg/mL with d-amphetamine.

Apparatus and procedures. Analyses were performed using an HP model 5890 gas chromatograph coupled to an HP model 5971A mass selective detector (Hewlett-Packard, Avondale, CA). Data processing was performed with a HP Chemstation with Version 3.0 software. A 12m x 0.2mm glass capillary HP-1 column was used. The injector temperature was 250°C and the oven temperature was programmed with initial temperature of 180°C held for 2 minutes and then increased 10°C/min to a final temperature of 250°C. The retention time of d-amphetamine under these conditions was 3.95 minutes. The mass selective detector was operated in the SIM mode, with d-amphetamine monitored at ions 91,118 and 190 m/z and the deuterated internal standard at 96,123 and 194 m/z.

Sample preparation. Briefly, 2-mL samples of urine were spiked with internal standard, made alkaline by the addition of ammonium hydroxide, then extracted with 4 mL of chlorobutane. Three mL of the chlorobutane layer was removed and evaporated to 2 mL at room temperature under nitrogen. 100 μL of pentafluoropropionic anhydride (Regis Chemical Co., Morton Grove, IL) was added to the chlorobutane extract, the mixture was capped and heated in a dry heat block at 70°C for 15 min. The mixture was allowed to cool and was evaporated to dryness under nitrogen at room temperature. The residue was reconstituted with 50 μL of ethyl acetate and injected into the GC/MS.

Subject Samples. Samples were analyzed for all three active treatments for Subjects 1, 4, 6, 8, 9 and 10. Only the 5 mg and 20 mg doses were analyzed for Subject 2. Samples were not analyzed for Subjects 3, 5 and 7.

4.2.2f Analytical Method for Prolactin in Serum

The analysis for prolactin concentration in serum was performed in the Clinical Research Center Core Laboratory by Linda Lawrence, M.S. using a radioimmunoassay (RIA) (Amersham Corp., Arlington Heights, IL). The analyses were performed in January and February, 1992. Prolactin is stable in serum over the 9 month period that the samples were stored when frozen at -20°C.[135]

In this method, a known amount of radiolabeled prolactin (prolactin^{125}I) is added to prolactin in the serum sample and competes for a limited number of binding sites on a prolactin specific antibody. The proportion of the prolactin^{125}I that binds to the antibody is inversely related to the concentration of prolactin in the serum sample. The prolactin bound to the antibody is reacted with a second antibody, and the precipitated double antibody complex is separated by centrifugation. The supernatant containing unbound prolactin is discarded, and the proportion of prolactin^{125}I in the precipitate is measured by a gamma scintillation counter. The concentration of prolactin in the sample is determined by interpolation from a serum concentration-response curve established using serum reference standards.

Details of the procedure are described by the manufacturer (Prolactin RIA Kit instructions, Code IM.1061, Amersham Corporation, June 1989). Assays were performed using the long protocol. Standard curves were constructed using 0, 5, 15, 50, 100 and 200 ng/mL prolactin standards. According to the manufacturer's specifications, controls were also run with the study samples (RIA Control Serum, Set 3, Cat. No. 07166120, ICN Biomedicals Inc.). If controls fell outside of the laboratory quality controls limits, then the assays were repeated.

Sensitivity, defined as the smallest amount of prolactin that can be distinguished from zero using this kit, is reported by the manufacturer to be approximately 1.5 ng/mL. This value is the prolactin concentration that causes a decrease in count of two times the standard deviation from the zero prolactin standard using standard counting procedures. Values below 1.5 ng/mL were considered to be below the limit of quantitation (BLQ).

Reproducibility of the Prolactin RIA Kit was evaluated by the manufacturer using freeze-dried control sera. The within assay coefficient of variation was 4.3%, 3.5%, and 2.4% for the low, medium, and high controls respectively. The between assay coefficient of variation was 5.7%, 4.6% and 5.8% for the low, medium and high controls respectively. Cross-reactivity of the antiserum with other pituitary and placental hormones was less than 0.2%.

4.2.3 Pharmacokinetic Data Analysis

Pharmacokinetic analysis based on serum data was performed using both noncompartmental and compartmental approaches. Methods used for the analysis of the serum data are discussed in section 4.2.3a. Pharmacokinetic analysis methods based on urine data is presented in section 4.2.3b. Statistical methods used to compare the pharmacokinetic data obtained for the three doses of amphetamine are described in section 4.2.3c.

4.2.3a Serum Data Analysis

The pharmacokinetic variables estimated from the serum data for this study

were drug concentrations in serum at each sampling time, the maximum drug concentration (C_{max}), the time from dosing to C_{max} (T_{max}), the terminal (elimination) rate constant (k), areas under the drug concentration-time curve extrapolated to infinity (AUC_∞), the apparent total body clearance normalized for bioavailability, F (Cl/F), the mean residence time (MRT) and the absorption rate constant (ka). Determination of these variables is discussed by Gibaldi and Perrier.[136] Plots of amphetamine concentrations in serum versus time, ln amphetamine concentration versus time, and amphetamine concentration divided by dose versus time were constructed. C_{max} was defined as the maximum serum concentration observed in the 24 hr following dosing. T_{max} is the time (relative to dosing) that C_{max} occurred. The elimination rate constant, k, was calculated by log-linear regression as the slope of the final linear portion of the ln serum drug concentration versus time curve. At least three concentration-time points were used to determine k.

AUC_∞, Cl/F and MRT were calculate by noncompartmental methods using the Quattro Pro (Borland International, Scotts Valley, CA) spreadsheet NONCOMP.WK1, Version 2.1 developed by Dr. Jurgen Venitz, Department of Pharmacy and Pharmaceutics, VCU. AUC_∞ was calculated by first determining the AUC from 0 hr to the time (t_{last}) of the last measured concentration (C_{last}) by the linear trapezoidal rule and then extrapolating the area to time ∞ by adding the last observed concentration divided by the terminal rate constant ($AUC_\infty = AUC_{0\text{-}last} + C_{last}/k$). Cl/F was calculated by dividing the dose administered by AUC_∞. MRT was calculated by dividing $AUMC_\infty$ by AUC_∞. $AUMC_\infty$ was calculated by determining area under the moment curve (AUMC) from 0 hr to C_{last} using the linear trapezoidal

rule by summing individual areas calculated by

$$AUMC = (0.5)(t_2 - t_1)(t_1C_1 + t_2C_2)$$

and then extrapolating the area to time ∞ by adding $((t_{last}C_{last}/k) + C_{last}/k^2)$.

The "goodness" of the estimates of AUC_∞ were evaluated by examining the ratio of the extrapolated portion of AUC to AUC_∞ ($[C_{last}/k]/AUC_\infty$). The ratio (expressed as a percent) ranged from 11 to 41% for the 5 mg dose, 7 to 23% for the 10 mg dose, and 9 to 17% for the 20 mg dose. For the 5 mg dose, the ratio was greater than 20% for 3 of the subjects (8, 9, and 10). Less than 20% of the total area was extrapolated for all subjects after the 10 and 20 mg doses. Similarly, estimates of $AUMC_\infty$ were evaluated by examining the ratio of the extrapolated portion of AUMC to $AUMC_\infty$. The ratio, expressed as a percent, ranged from 31 to 73% for the 5 mg dose, 25 to 58% for the 10 mg dose, and 28 to 47% for the 20 mg dose. The estimates of AUC_∞ were better than the estimates of $AUMC_\infty$, because the contribution of the extrapolated portion of the area to the total AUC was smaller.

Because T_{max} appeared to increase as the dose increased for a majority of the subjects, absorption rate of amphetamine at different doses was examined using compartmental pharmacokinetic methods. Pharmacokinetic compartmental models were fit to the serum amphetamine concentration versus time data using the nonlinear regression program PCNONLIN, Version 3.0 (SCI Software, Lexington, KY). Several models, including one-compartment with first order input and first order output with and without a lag time and two-compartment with first order input and

first order output from the central compartment with and without a lag time, and several weighting schemes, including 1, 1/y and $1/y^2$, were evaluated. The Gauss-Newton algorithm with the Levenberg modification was used as the estimation method. Initial values were obtained using the curve stripping program RSTRIP (Micromath, Salt Lake City, UT). The model which best fit the data was chosen based on the following criteria: 1) minimize the estimated standard error of the parameter estimates for k and ka, 2) random distribution of positive and negative deviations of the calculated function values, 3) minimize AIC (Akaike criteria) which is defined as the sum of squares corrected for the number of variables, and 4) random scatter in the plots of weighted residuals versus calculated values and weighted residuals versus the independent variable based on visual inspection of the plots, and 5) minimize the condition number of the matrix of partial derivatives.

Based on these criteria, the one compartment model with first order input and first order output without a lag time was the most appropriate model to describe the data for all subjects and all doses. For most subjects, using a weight of $1/y_2$ resulted in the best fit. Data from subjects 3 (10 and 20 mg dose), 5 (5 mg dose), 6 (20 mg dose), 7 (5 and 10 mg dose), and 8 (10 mg dose) were best modeled using a weight of 1. ka from the most appropriate model and weighting was used to compare treatments.

Values for each of the pharmacokinetic variables were transferred to the VAX computer system (Digital Equipment Corporation) in VCU Health Sciences Computing Services for statistical analysis.

4.2.3b Urine Data Analysis

The pharmacokinetic variables estimated from the urine data for this study were the urinary excretion rate at the midpoint of the each urine collection interval, the elimination rate constant (k), and the renal clearance (Cl_r). The dependency of Cl_r on urine pH and flow was examined. The natural log of the urinary excretion rate (the amount of drug excreted during the collection interval divided by the collection time) was plotted against time at the midpoint of the urine collection interval. The elimination rate constant (k) is equal to the negative of the slope of this line. To calculate Cl_r, the urinary excretion rate was plotted against serum concentration at the midpoint of the urine collection interval. If the serum concentration was not measured at this time, it was calculated by log-linear interpolation from the measurements made before and after the midpoint. Cl_r was determined from the slope of this plot. The first collection interval was excluded when evaluating Cl_r because the serum concentration at the midpoint of this interval was not representative of the entire interval due to the variability in the drug absorption process. The effect of urine flow and urine pH on Cl_r were examined by plotting Cl_r at each collection interval versus urine pH and urine flow at each collection interval in SAS. A smoothing function was used to smooth the response surface.

4.2.3c Statistical Methods

Descriptive statistics (mean, standard deviation and relative standard deviation) were calculated for each pharmacokinetic variable. Dose-dependency of the calculated pharmacokinetic parameters was evaluated by fitting the data for each

pharmacokinetic parameter to a crossover model using a univariate mixed effects analysis of variance (model with both fixed and random effects) of the form

$$Y_{ijk} = \mu + \delta_i + \pi_j + \zeta_{k(i)} + \epsilon_{ijk}$$

$$i = 1,2,3,4$$

$$j = 1,2,3$$

$$k = 1,2,\ldots,8$$

where Y_{ijk} is the response for the kth subject in the ith sequence in the jth period, μ is the overall mean, δ_i is the effect of the ith sequence, π_j is the effect of the jth period, and $\zeta_{k(i)}$ is the effect of the kth subject within the ith sequence, and ϵ_{ijk} is the random error associated with Y_{ijk}. The ϵ_{ijk} are assumed to be normally distributed random variables with mean of 0 and common variance σ_ϵ^2. It is also assumed that the nested effects for subject are random and independently distributed with mean of 0 and common variance σ_ζ^2, and independent of ϵ_{ijk}.

Model fitting was performed using PROC MIXED in SAS.[137] PROC MIXED allows modelling of the mean of y, as in the standard linear model, and also the variance of y. The estimation method used for the covariance parameters was restricted maximum likelihood (REML). The variance of y is modelled by choosing the form of the variance structure matrices. Simple (random effect), unstructured, and time series (autoregressive) structures were evaluated. The autoregressive structure resulted in improved model fitting based on maximization of the Akaike's

Information Criterion for most variables. The autoregressive structure indicates that the correlation between measurements is less if they are made further apart in time. For pharmacokinetic parameters where the analysis with autoregressive variance structure did not converge, the simple structure was used.

For pharmacokinetic parameters where the effect of treatment was significant ($p < 0.05$), multiple comparisons of the treatments were performed using the ESTIMATE procedure.

The residuals were tested for normality using PROC UNIVARIATE[120]. This procedure computes the Shapiro-Wilk statistic, W, for the null hypothesis that the residuals are normally distributed. When the probability of a smaller value of W was less than or equal to 0.1, the null hypothesis of normality was rejected. The residuals were normally distributed for all of the pharmacokinetic variables examined.

4.2.4 Pharmacodynamic Data Analysis

The measures of response that were examined for each of the tests assessing pharmacodynamic effect are listed in Table 4.13.

4.2.4a EEG Analysis

As each EEG was recorded, the signal was processed by a Fast Fourier Transform procedure, to determine the amplitude of the EEG in five frequency bands (Delta: 0.39 - 3.9 Hz, Theta: 4.3 - 7.8 Hz, Alpha: 8.2 - 11.7 Hz, Beta I: 12.1 - 16.0 Hz, and Beta II: 16.4 - 30.0 Hz) at each electrode. Each of the five-minute recordings was reviewed and edited to remove each 2.5 second epoch (frame) that

Table 4.13 Response Measures Evaluated in Part II - Comparison of Quantitative EEG for Assessment of CNS Stimulant Response

EEG Variables

Total Amplitude - all frequencies (μV)
Total Amplitude - Delta band (μV)
Total Amplitude - Theta band (μV)
Total Amplitude - Alpha band (μV)
Total Amplitude - Beta I band (μV)
Total Amplitude - Beta II band (μV)
Total Power - all frequencies (μV^2)
Total Power - Delta band (μV^2)
Total Power - Theta band (μV^2)
Total Power - Alpha band (μV^2)
Total Power - Beta I band (μV^2)
Total Power - Beta II band (μV^2)
Relative Power - Delta band
Relative Power - Theta band
Relative Power - Alpha band
Relative Power - Beta I band
Relative Power - Beta II band

Total Amplitude (Central electrodes) - all frequencies (μV)
Total Amplitude (Central electrodes) - Delta band (μV)
Total Amplitude (Central electrodes) - Theta band (μV)
Total Amplitude (Central electrodes) - Alpha band (μV)
Total Amplitude (Central electrodes) - Beta I band (μV)
Total Amplitude (Central electrodes) - Beta II band (μV)
Total Power (Central electrodes) - all frequencies (μV^2)
Total Power (Central electrodes) - Delta band (μV^2)
Total Power (Central electrodes) - Theta band (μV^2)
Total Power (Central electrodes) - Alpha band (μV^2)
Total Power (Central electrodes) - Beta I band (μV^2)
Total Power (Central electrodes) - Beta II band (μV^2)

Relative Standard Deviation of Amplitude - Delta Band (%)
Relative Standard Deviation of Amplitude - Alpha Band (%)

Total Amplitude (Occipital electrodes) - all frequencies (μV)
Total Amplitude (Occipital electrodes) - Delta band (μV)
Total Amplitude (Occipital electrodes) - Theta band (μV)
Total Amplitude (Occipital electrodes) - Alpha band (μV)
Total Amplitude (Occipital electrodes) - Beta I band (μV)
Total Amplitude (Occipital electrodes) - Beta II band (μV)
Total Power (Occipital electrodes) - all frequencies (μV^2)
Total Power (Occipital electrodes) - Delta band (μV^2)
Total Power (Occipital electrodes) - Theta band (μV^2)
Total Power (Occipital electrodes) - Alpha band (μV^2)
Total Power (Occipital electrodes) - Beta I band (μV^2)
Total Power (Occipital electrodes) - Beta II band (μV^2)

Psychometric tests

Percent Correct on Continuous Performance Task (%)
Average Latency on Continuous Performance Task (sec)
Finger Tapping with Left Hand (taps/sec)
Finger Tapping with Right Hand (taps/sec)

Mood Scales

Total Score on Self-Rated Mood Scale
Score on Visual Analog Mood Scale

Neuroendocrine test

Prolactin serum concentration

Cardiovascular measures

Heart Rate
Diastolic Blood Pressure
Systolic Blood Pressure

contained artifacts (eye movements, muscle movement, electrode artifacts, or disturbances noted during the recording).[117,118] The remaining frames were averaged using the EEG statistical operations package on the Brain Imager to form an average topographical map representing the five minute recording. Recordings with fewer than 24 artifact-free frames were not processed further and were listed as "missing". To compute averages and standard deviations representing the 5-minute recording, the Brain Imager first forms sub-averages and sub-standard deviations from consecutive groups of eight frames each.[119] The amplitude in each frequency band (determined by FFT) measured at each of the 28 electrodes for the first eight artifact-free frames are added together and then divided by 8, to determine the sub-average. The sub-standard deviation is also calculated. This process is then repeated for the next group of 8 frames and so on. The overall average and standard deviation is then formed by averaging the sub-averages and calculating their standard deviation. The overall average file contains the average amplitude in each of the 5 frequency bands at each of the 28 electrodes. The overall standard deviation file contains the standard deviation of the amplitude in the 5 frequency bands at each of the 28 electrodes. These files were then transferred from the Brain Imager to an IBM compatible 80386 personal computer. ISTAT (NeuroScience, Inc.) a statistical package for EEG processing was used to prepare ASCII files of the average and standard deviation files. These files were then imported into the Quattro Pro spreadsheet software (Borland International, Scotts Valley, CA) for further processing.

Power was determined for each average recording by squaring the amplitude values at each electrode in each frequency band. Total amplitude and total power in

each frequency band was calculated by summing the amplitude or power at each of the electrodes for a given frequency band. Total amplitude and total power across all frequency bands was calculated by adding together the total amplitude or total power in each of the frequency bands. Relative power in each frequency band was calculated by dividing the total power in the given frequency band by the total power across all frequency bands.

Relative standard deviation (RSD) for each average recording was determined by dividing the standard deviation in each frequency band for each electrode by the average for the same frequency band and electrode, expressed as a percentage. The mean RSD was then calculated by averaging the RSD for all electrodes for each frequency band.

Total amplitude and power in the central and occipital areas for each frequency band were calculated by adding together the amplitude or power at the central electrodes (C3, CZ, and C4) and the occipital electrodes (O1, OZ and O2) respectively, for a given frequency band. Total amplitude and total power in the central and occipital areas across all frequency bands was calculated by adding together the total amplitude or total power in the central and occipital areas respectively across all frequency bands. Frontal alpha power was not examined because it is more likely to be contaminated by eye movement artifacts.

Values for the response measures calculated were transferred to the relational database Paradox (Borland International, Scotts Valley, CA) for data management and further analysis.

4.2.4b Analysis of Other Response Measures

For the computerized visual CPT, latency of response was determined for trial during the session. The average latency of response and the percent of correct responses for each test session was determined. For the finger tapping task, the average rate (taps/sec) of finger tapping for each hand was calculated for each session by averaging the results of the three trials conducted during each session.

A total score on the self-rated mood scale was determined for each test session by summing the scores obtained for each of the 23 items on the scale. A score between 0 and 100 was obtained for the visual analog mood scale for each test session by measuring the number of millimeters between the left end of the scale and the mark placed by the subject. Values for heart rate, systolic blood pressure and diastolic blood pressure were transcribed from the Dynamap (Critikon, Tampa,FL) output. Serum prolactin concentrations were determined as outlined in section 4.2.2e.

Values for the response measures were transferred to the relational database Paradox (Borland International, Scotts Valley, CA) for data management and further analysis.

4.2.4c Pharmacodynamic methods

Response-time profiles for each response measure were tabulated and plotted for each subject during each treatment. Baseline response for each measure during each treatment was defined as the value obtained at 0 hr, before receiving the study medication. Maximum response for each measure (E_{max}) was determined as the highest response observed during the 12 hours after receiving the study medication.

Minimum response (E_{min}) was the lowest observed effect during the 12 hours after dosing. If more than one value for the response measure was missing, the E_{max} or E_{min} was assigned as "missing". Maximum response during the first 4 hours (E_{max} - (1st 4 hr)) was the highest value observed during the first four hours after dosing for each response measure. Minimum response during the first 4 hours (E_{min} - (1st 4 hr)) was the lowest effect observed during the first 4 hours after dosing. The response during the first four hours was examined separately, because the effect was expected to be greatest during this time period. If one or more values for the response measure were missing, the E_{max} - (1st 4 hr) or E_{min} - (1st 4 hr) was assigned as "missing". Based on previous studies and examination of the data, either the maximum response (for responses that increase with increasing dose) or the minimum response (for responses that decrease with increasing dose) was chosen to represent the drug effect for each response measure.

An effect time (ET) for each response measure after each treatment was calculated by:

$$ET = (|E_1-E_0|*1)+(|E_2-E_0|*2)+(|E_3-E_0|*3)+(|E_4-E_0|*4)+(|E_6-E_0|*6)+(|E_8-E_0|*8)+(|E_{12}-E_0|*12)$$

where E_0 = response at 0 hr; E_1 = response at 1 hr; E_2 = response at 2 hr; E_3 = response at 3 hr; E_4 = response at 4 hr; E_6 = response at 6 hr; E_8 = response at 8 hr; E_{12} = response at 12 hr

This value was used as an indicator of the length of time that the response measure could distinguish a drug effect, weighted toward the later times (and lower concentrations). If a value for the response measure was missing at any time point, the ET was assigned as "missing".

In addition, each subject was given a euphoria score (1 for euphoria and -1 for dysphoria) for each treatment based on the self-rated mood scale scores and the visual analog mood scale scores. If the scores increased after dosing, the subject was given a score of 1 for that treatment. If the scores decreased after dosing, the subject was given a score of -1 for that treatment. During the placebo period a euphoria score of 0 was assigned.

Values for E_{max}, E_{max} - (1st 4 hr), E_0, E_{min}, E_{min} - (1st 4 hr), ET and the euphoria score were transferred to the VAX computer system (Digital Equipment Corporation) in VCU Health Sciences Computing Services for subsequent analysis.

4.2.4d Statistical Methods

Because there are many variables of interest in this statistical analysis, the multiplicity of desired inferential statements about the data becomes problematic. Adjusting the level of significance (α) for the multiple statistical comparisons being made as in traditional confirmatory analysis would result in extremely small α values and virtually no likelihood of detecting any statistically significant differences. Using the concept of Descriptive Data Analysis as described by Abt[138,139], expected differences between the treatments based on previously reported studies and patterns apparent from examining the data were evaluated statistically without adjustment of

the level of significance. The results of these analyses were used to make descriptive inferential statements about the data, but not to reject null hypotheses. Pharmacodynamic parameters that were evaluated using DDA included: decrease in serum prolactin, increase or decrease in mood scores for both rating scales, decreased average latency on the CPT, decreased percent correct on the CPT, increased rate of finger tapping, increase in heart rate and blood pressure, increase in EEG fast activity (total alpha and beta power) and decrease in EEG slow activity (total delta and theta power). Statistical comparisons for other pharmacodynamic parameters were treated as exploratory data analysis. They were used to generate hypotheses rather than to form final conclusions based on the data.

Descriptive statistics (mean, standard deviation and relative standard deviation) were calculated for each pharmacodynamic variable for each response measure. Pharmacodynamic variables obtained during each treatment for each response measure were compared by fitting the data for each pharmacodynamic variable to a crossover model using a univariate mixed effects analysis of covariance of the form

$$Y_{ijk} = \mu + \delta_i + \pi_j + \zeta_{k(i)} + \epsilon_{ijk} + \gamma_{mj}$$

$i = 1,2,3,4$

$j = 1,2,3,4$

$k = 1,2,\ldots,8$

$m = 1,2,(3)$

where Y_{ijk} is the response for the kth subject in the ith sequence in the jth

period, μ is the overall mean, δ_i is the effect of the ith sequence, π_j is the effect of the jth period, $\zeta_{k(i)}$ is the effect of the kth subject within the ith sequence, γ_{mj} is the effect of the mth covariate in the jth period and ϵ_{ijk} is the random error associated with Y_{ijk}. The ϵ_{ijk} are normally distributed random variables with mean of 0 and common variance σ_ϵ^2. It is also assumed that the nested effects for subject are random and independently distributed with mean of 0 and common variance σ_ζ^2, and independent of ϵ_{ijk}.

Model fitting was performed using PROC MIXED in SAS.[137] Because the number of subjects receiving each of the four treatment sequences was not equal, the design was unbalanced. PROC MIXED is particularly useful for analyzing unbalanced designs and data sets with missing values. PROC MIXED allows modelling of the mean of y, as in the standard linear model, and also the variance of y. The estimation method used for the covariance parameters was restricted maximum likelihood (REML). The variance of y is modelled by choosing the form of the variance structure matrices. Simple (random effect), unstructured, and time series (autoregressive) structures were evaluated. The autoregressive structure resulted in improved model fitting based on maximization of the Akaike's Information Criterion for most variables. The autoregressive structure indicates that the correlation between measurements is less if they are made further apart in time. Analysis using the unstructured variance structure would not converge for this data set. For pharmacodynamic parameters where the analysis with autoregressive variance structure did not converge, the simple structure was used.

For pharmacodynamic parameters where the effect of treatment was significant ($p < 0.05$), multiple comparisons of the treatments were performed using the ESTIMATE procedure.

Two covariates, E_0 and the treatment that the subject thought they had received during each period (TRT_{sub}), were used in the analysis of ET scores and E_{max} (or E_{min}). An additional covariate, the euphoria score, was used in the analysis of E_{max} - (1st 4 hr) or E_{min} - (1st 4 hr).

The residuals were tested for normality using PROC UNIVARIATE[120]. This procedure computes the Shapiro-Wilk statistic, W, for the null hypothesis that the residuals are normally distributed. When the probability of a smaller value of W was less than or equal to 0.1, the null hypothesis of normality was rejected. The residuals were normally distributed for the pharmacodynamic variables examined.

To assess the discriminating ability of the statistical tests performed for selected response measures, statistical power was estimated.[140] Because the calculations of power for crossover models is quite complex, the power of the F test for analysis of variance (fixed effect model) was determined. This estimation of power does not take into account the crossover design of the study and therefore is a conservative estimate. The Pearson-Hartley charts of the power of the F test were used to determine power.[140] For this study, the number of degrees of freedom in the numerator of F (ν_1) is the number of treatments minus one, or 3. The level of significance (α) was selected at 0.05. The number of degrees of freedom in the denominator of F (ν_2) which is the number of subjects if not a crossover study (8 subjects x 4 periods) minus the number of treatments (4), or 28. $\nu_2 = 30$ was used to

estimate power from the Pearson-Hartley charts. The noncentrality parameter (ϕ), which is a measure of how unequal the treatment means for the response are, was calculated by:

$$\phi = \frac{1}{\sigma}\sqrt{\frac{n}{r}\sum(\mu_i - \mu)^2}$$

where n = the sample size at each factor level

r = the number of factor levels

σ = the standard deviation (square root of the estimated residual variance)

μ_i = mean for treatment i

$\mu = (\sum \mu_i)/r$

Values for σ were obtained from the model fitting information in the output of PROC MIXED in SAS, and values for μ_i were obtained using PROC MEANS in SAS. With α, ν_1, and ν_2 and estimates of ϕ, power can be obtained from the Pearson-Hartley chart.

Results of the statistical analysis for prolactin, the self-rated mood scales, the computerized psychometric tests and quantitative EEG were used to compare the sensitivity of these response measures.

4.3 Clinical Results

Ten male volunteers were entered into the study. Demographic and physical characteristics of the subjects are shown in Table 4.14. All of the subjects were judged to be healthy based on the results of a physical examination, a medical history, and clinical laboratory tests before entering the study. Eight subjects completed the study. Subject 5 was removed from the study after completing the second study period. During the first period, he received 5 mg of dextroamphetamine, and experienced an intense dysphoric reaction. He became tearful, anxious and withdrawn for approximately two hours after dosing. His mood improved as the morning progressed, and he wished to continue the study. No treatment for the adverse reaction was administered and he fully recovered. He returned for a placebo period (Study Period 2), but did not receive higher doses of dextroamphetamine. Subject 3 was removed from the study after completing the second study period due to an non study-related injury sustained at work between Study Periods 2 and 3. He began taking anti-infective medications prophylactically after the injury, and therefore did not meet the criteria to continue in the study.

Adverse experiences were reported by four of the subjects. Subject 7 reported mild diarrhea and Subject 9 experienced mild pain in the chest during several study periods. The chest pain was not associated with EKG abnormalities and was diagnosed as gastroesophageal reflux by the medical monitor. These adverse experiences were attributed to the administration of ammonium chloride, because they began before the administration of dextroamphetamine. These adverse effects did not require discontinuation of the ammonium chloride and the subjects received no

Table 4.14 Demographic and Physical Characteristics of Participants in Part II - Comparison of Quantitative Electroencephalography to Behavioral, Psychological and Neuroendocrine Measures of Response to Dextroamphetamine

Subject Number	Initials	Age (years)	Weight (kg)	Race
1	TM	25	90.7	Black
2	MM	20	72.0	White
3	JM	32	71.3	White
4	FD	24	77.7	Asian
5	DK	19	67.3	White
6	MC	23	85.5	White
7	BB	21	83.0	White
8	PS	23	105.5	White
9	AW	27	103.0	Asian
10	DA	28	68.0	Black

treatment for these symptoms. Subject 3 complained of feeling restless and "jittery" for approximately 3 hours after receiving the 20 mg dose of dextroamphetamine. Subject 5 experienced an intensely dysphoric mood after 5 mg of dextroamphetamine and was dropped from the study as described above. Laboratory tests, physical exam and electrocardiogram performed at the completion of the study revealed no clinically significant abnormalities compared to tests performed before entering the study.

After each subject completed the study, they were asked which treatment they believed they had received during each period. The results are presented in Table 4.15.

Subjects remained blinded to the treatment throughout their participation in the study. The principal investigator was blinded during the clinical portion of the study and the editing of the EEGs. The principal investigator was unblinded before the analysis of the serum samples and further data analysis. The medical monitor for the study was blinded during the clinical portion of the study, but the blind was broken to evaluate the adverse effects experienced by Subject 5.

4.4 Pharmacokinetic Evaluation

4.4.1 Results

The amphetamine concentration in serum versus time and the log amphetamine concentration in serum versus time plots after dextroamphetamine doses of 5, 10 and 20 mg for each subject are shown in Appendix H. Plots for two representative subjects are shown in Figures 4.2 - 4.5. Amphetamine serum concentration versus time profiles for almost all subjects and doses show multiple peaks during the first 3 -

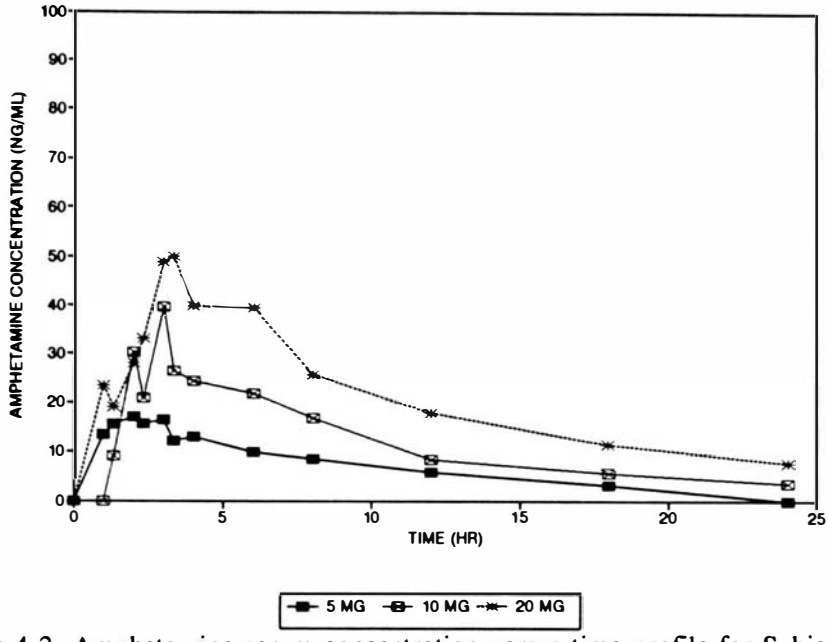

Figure 4.2 Amphetamine serum concentration versus time profile for Subject 2

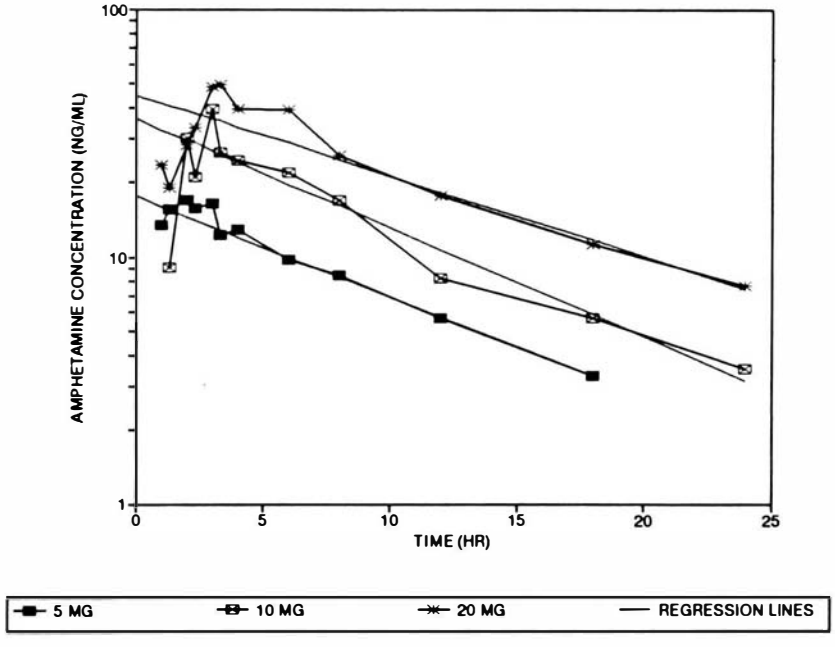

Figure 4.3 Log amphetamine serum concentration versus time profile for Subject 2

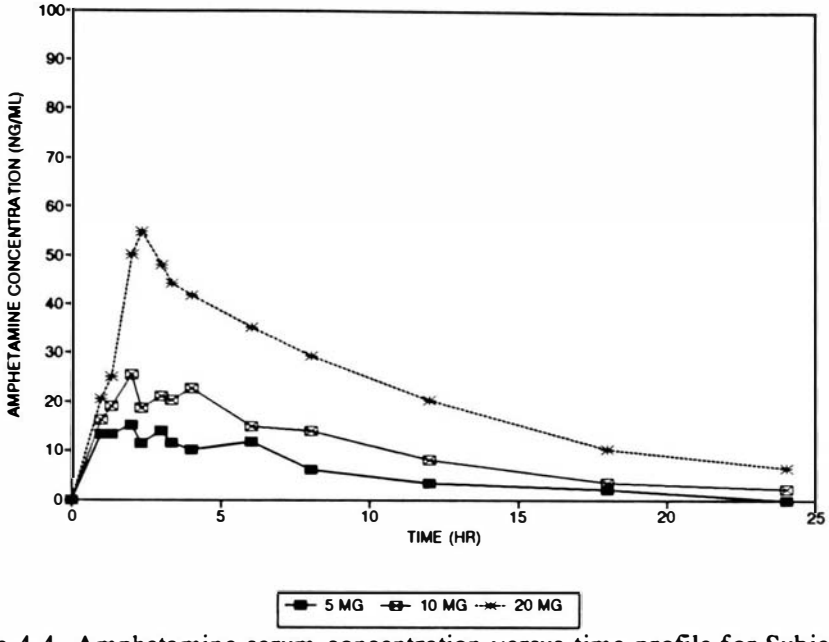

Figure 4.4 Amphetamine serum concentration versus time profile for Subject 4

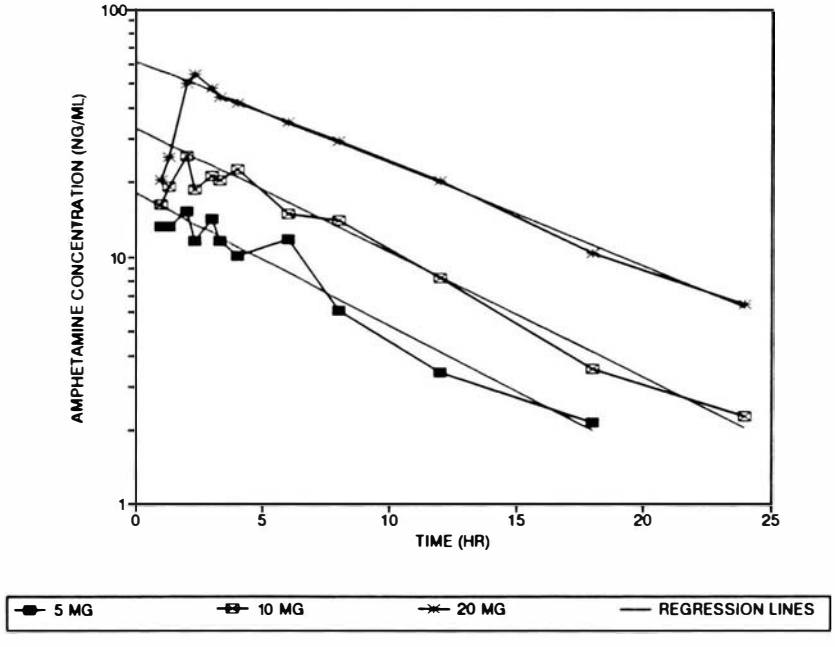

Figure 4.5 Log amphetamine serum concentration versus time profile for Subject 4

4 hours after dosing. After this point, all subjects show a smooth monoexponential decline in serum concentrations. Individual profiles have between one to four peaks in the serum concentration. With the exception of the multiple peaks, the serum data is adequately described by a one compartment pharmacokinetic model with first order input and first order output.

Mean pharmacokinetic parameters determined by noncompartmental analysis of the serum concentration data are presented in Table 4.16. The average C_{max} was 15.4 ($\pm$ 3.7), 30.8 ($\pm$ 5.3) and 54.8 ($\pm$16.3) ng/mL for the 5, 10 and 20 mg doses respectively. The average AUC_{∞} was 148.8 ($\pm$ 30.9), 274.9 ($\pm$ 42.4) and 574.5 ($\pm$ 115.2) ng/mL*hr for the 5, 10 and 20 mg doses respectively. The values for C_{max} and AUC_{∞} are proportional to the dose administered. The elimination rate constants are similar between the doses, and correspond to an elimination half-life of 6.5 to 7.5 hours. T_{max} increased from 2.1 ($\pm$ 0.7) hr at the lowest dose to 2.8 ($\pm$ 0.7) hr at the highest dose, although this was not statistically significant. Values for ka determined by compartmental analysis of serum concentration data are presented in Table 4.17. The absorption rate seems to decrease and T_{max} appears to increase as the dose increases.

Plots of amphetamine concentration in serum divided by dose versus time were used to determine whether amphetamine can be characterized by linear pharmacokinetics in the dose range studied. These plots are located in Appendix I. They demonstrate that amphetamine appears to follow linear pharmacokinetics over the dose range studied. To examine the dose dependency of the calculated pharmacokinetic parameters, the data for each pharmacokinetic parameter were fit to

Table 4.15 Subjects' Ranking of Treatment Order versus Actual Treatment Sequence Received

Subject Number	Subject's Ranking	Actual Treatment Sequence
1	0 - 20 - (10) - (5)	5 - 0 - 20 - 10
2	0 - 10 - 5 - 20	0 - 10 - 5 - 20
3	***	10 - 20 - * - *
4	0 - (10) - (5) - 20	20 - 5 - 10 - 0
5	***	5 - 0 - * - *
6	5 - 20 - 0 - 10	0 - 10 - 5 - 20
7	10 - 20 - 5 - 0	10 - 20 - 0 - 5
8	20 - ( ) - ( ) - ( )	20 - 5 - 10 - 0
9	10 - 0 - 20 - 5	5 - 0 - 20 - 10
10	0 - ( ) - ( ) - 20	0 - 10 - 5 - 20

* = Did not receive treatments
*** = Did not provide a ranking of treatments
( ) = Unsure of ranking

Table 4.16 Mean (± SD) Pharmacokinetic Parameters for Amphetamine Based on Noncompartmental Analysis of the Serum Concentration Data[a]

	Dose		
Parameter	5 mg	10 mg	20 mg
k (1/hr)	0.107 (± 0.022)	0.099 (± 0.021)	0.094 (± 0.012)
C_{max} (ng/mL)	15.4 (± 3.7)	30.8 (± 5.3)	54.8 (± 16.3)
T_{max} (hr)	2.1 (± 0.7)	2.6 (± 0.7)	2.8 (± 0.7)
Cl/F (L/hr/kg)	0.42 (± 0.06)	0.45 (± 0.06)	0.43 (± 0.07)
MRT (hr)	10.6 (± 1.9)	11.3 (± 1.9)	11.8 (± 1.3)
AUC_{∞} (ng/mL*hr)	148.8 (± 30.9)	274.9 (± 42.4)	574.5 (± 115.2)

a = Includes available data from all 10 subjects.

a crossover model using a univariate mixed effects analysis of variance. The effect of treatment was not statistically significant for k, T_{max}, Cl/F, and MRT. C_{max}, AUC_{∞} and ka showed a significant treatment effect ($p < 0.05$). For C_{max} and AUC_{∞}, all treatments were statistically different from one another, with the highest value associated with the highest dose and the lowest value associated with the lowest dose. For ka, the lowest dose was statistically different from the two higher doses. Values for ka plotted against dose are shown in Figure 4.6. Values for ka increase as the dose decreases, indicating that absorption is slowed at higher doses of amphetamine.

Plots of the excretion rate of amphetamine into urine versus time and log of the excretion rate of amphetamine into urine versus time are shown in Appendix J. Plots for two representative subjects are shown in Figures 4.7 - 4.10. Elimination rate constants based on the urine data are presented in Table 4.18. The elimination rate constants obtained from serum data are in general smaller than those obtained from urine data.

Plots of excretion rate of amphetamine into urine versus serum concentration at the midpoint of the urine collection interval for each dose for each subject are presented in Appendix K. In a number of these plots it is evident that the renal clearance is not constant throughout the study period. The influence of urine pH and urine flow on renal clearance of amphetamine were evaluated by examining a three-dimensional plot of renal clearance versus urine pH versus urine flow (Figure 4.11). As urine pH decreases, renal clearance increases. As urine flow decreases, instantaneous renal clearance decreases. Fluctuations in urine pH were relatively small (Range: 4.6 - 6.5). Urine flow ranged from 0.008 to 0.6125 L/hr. Renal

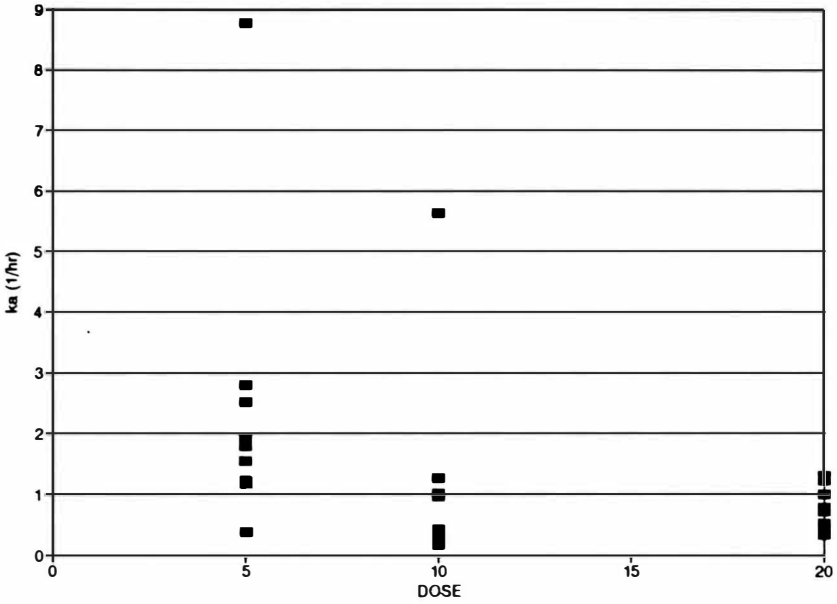

Figure 4.6 Absorption rate constant for amphetamine as a function of dose

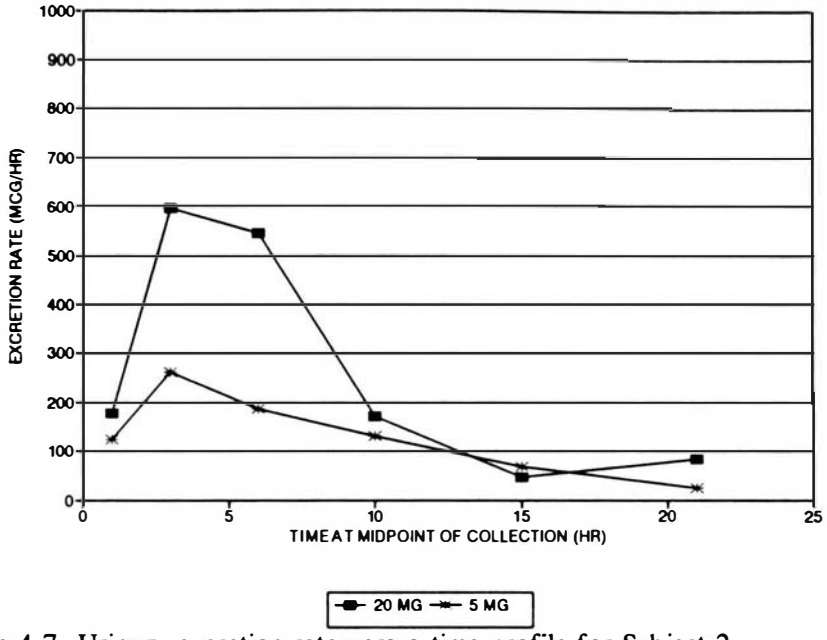

Figure 4.7 Urinary excretion rate versus time profile for Subject 2

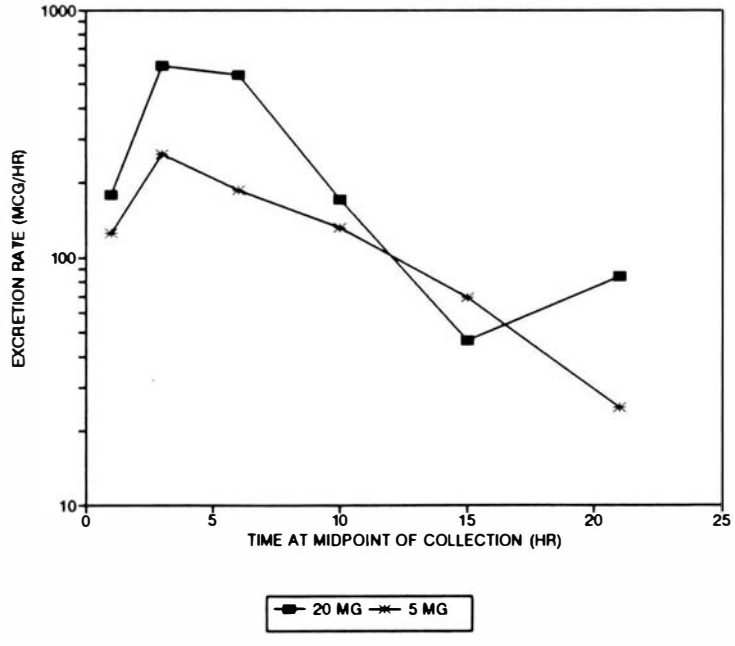

Figure 4.8 Log urinary excretion rate versus time profile for Subject 2

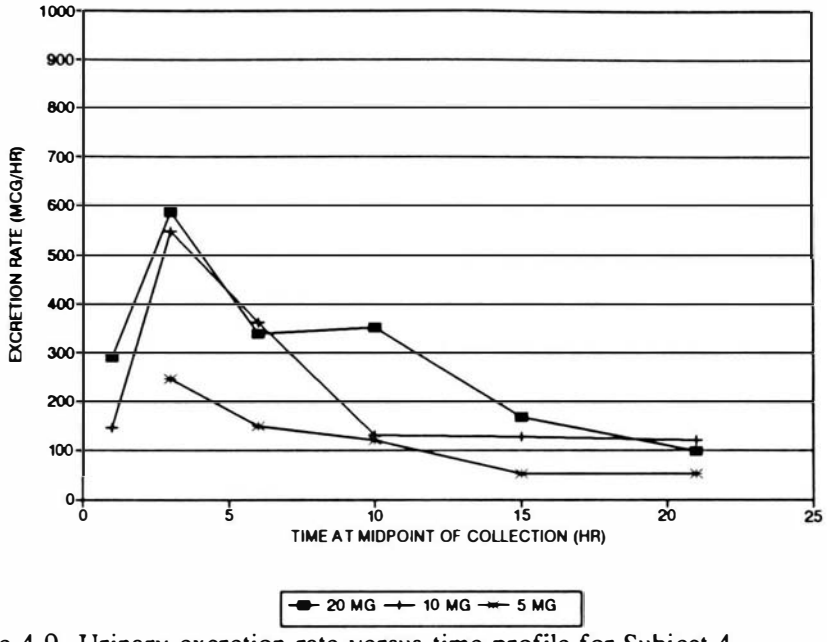

Figure 4.9 Urinary excretion rate versus time profile for Subject 4

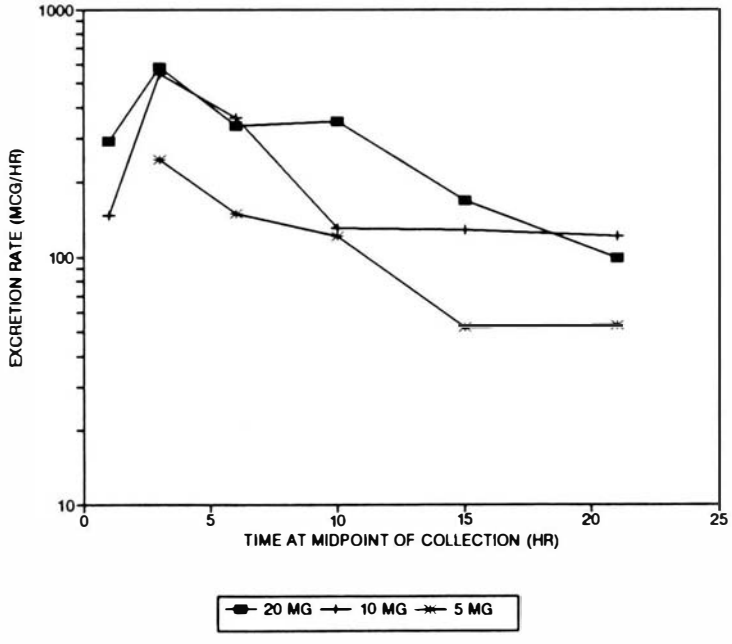

Figure 4.10 Log urinary excretion rate versus time profile for Subject 4

Table 4.17 Values for ka (1/hr) Determined by Compartmental Analysis of Serum Amphetamine Concentration Data

	Dose		
Subject	5 mg	10 mg	20 mg
1	2.52	0.40	1.30
2	1.79	0.43	0.52
3	b	5.63	0.33
4	1.92	0.98	0.53
5	0.38	b	b
6	2.80	1.27	0.41
7	8.77	0.17	0.72
8	1.55	0.32	0.78
9	1.19	0.37	1.23
10	1.22	1.02	1.01
Mean	2.46	1.17	0.76
SD	2.47	1.71	0.35

a = Includes available data for all 10 subjects
b = Subject did not receive this dose

Table 4.18 Elimination Rate Constants (k) for Amphetamine Determined from Serum and Urine Data Analysis

	Urine			Serum		
Dose	5 mg	10 mg	20 mg	5 mg	10 mg	20 mg
Subject						
1	0.080	0.111	0.075	0.073	0.070	0.072
2	0.129	a	0.130	0.093	0.101	0.075
4	0.089	0.084	0.095	0.123	0.116	0.096
6	0.123	0.112	0.095	0.109	0.114	0.100
8	0.157	0.178	0.088	0.099	0.087	0.103
9	0.131	0.140	0.155	0.140	0.131	0.100
10	0.124	0.101	0.125	0.079	0.075	0.097
Mean	0.119	0.121	0.109	0.102	0.099	0.092
SD	0.026	0.033	0.028	0.024	0.023	0.013

a = urine samples not analyzed

clearance values ranged from 0 to 55 L/hr. The urine flow rate appears to have less effect on renal clearance than urine pH does.

4.4.2 Discussion

The pharmacokinetic characteristics of amphetamine observed in this study are consistent with those noted by other investigators (See Section 2.2.3). Amphetamine pharmacokinetics are linear over the dose range studied, with C_{max} and AUC_{∞} proportional to dose. Acidification of the urine by administration of enteric-coated ammonium chloride results in a constantly enhanced excretion of amphetamine. With the exception of the multiple peaks present during the absorption phase, the serum concentration versus time profile is adequately described by a one compartment pharmacokinetic model with first order input and first order output.

Multiple peaks are present in the serum concentration versus time profile for almost all doses for all subjects. Similar profiles have been obtained by other investigators, although their significance has not been addressed. Beckett and colleagues[91] show plasma data from one subject under conditions of acidic and uncontrolled urine pH. Under acidic conditions, two peaks are present in the first 3 hours. Under uncontrolled urine pH, three peaks are present, with the third occurring 9 hours after dosing. Possible explanations for these multiple peaks include analytical variability, enterohepatic recycling,[141,142] resorption of drug from the bladder,[143] delayed gastric emptying of part of the dose,[144,145,146] different rates of absorption at different sites along the GI tract,[147,148] discontinuous absorption,[149,150] variable dissolution of the dosage form,[151] or accumulation in a

post-absorptive storage area (such as hepatic parenchymal tissue) followed by release and reabsorption.[142]

Analytical variability is not the likely cause of the multiple peaks observed in this study, because the analytical method was validated and repeat analysis of samples obtained during the period of multiple peaks for two subjects gave similar results to the first analysis. Analytical variability may contribute to the effect however, since the highest variability in quality control samples was obtained from the high control that had been diluted prior to analysis. Most of the serum samples obtained during the first three hours were higher in concentration and were diluted prior to analysis. Enterohepatic recycling is also not likely to be responsible for the double peaks. Beckett and Rowland[87] found no amphetamine or its conjugates present in the bile of a cholecystectomy patient with a bile duct fistula at 2, 4, 8 and 24 hr after oral administration of 10 mg of dextroamphetamine. Also, peaks due to biliary excretion and reabsorption are often seen after meal times, when the gallbladder contracts, which was not the case in this study. The dosage form administered is probably not responsible for the multiple peaks either, since they have been observed after the administration of an oral solution.[91] It also does not appear that fluctuations can be explained by transfer of drug from plasma to stomach contents with subsequent reabsorption in the intestine because after intravenous administration, less than 1% of the administered dose could be measured in gastric contents during the first 40 minutes after dosing.[91] Based on the data obtained in our study, it is not possible to elucidate the mechanism behind the multiple peaks observed. Any of the remaining hypotheses may be contributing to the phenomenon.

In part because of the multiple peaks observed, relatively large differences in serum amphetamine concentrations are observed between the samples collected at the start of the pharmacodynamic measurements (on the hour) and those collected at the end of the pharmacodynamic measurements (at 20 minutes after the hour). To before pharmacokinetic-pharmacodynamic modeling of the response data, especially for the data collected during the first four hours, the serum concentration at the time the response was actually measured should be estimated. Since the pharmacodynamic endpoints were always collected in the same order and the time of the measurement was recorded, it would be possible to approximate the actual concentration at the time the measurement was made.

The pharmacokinetic parameters calculated in this study are similar to those determined by other investigators. Under conditions of acidified urine, the half-life of amphetamine is approximately 5 to 8 hours. In this study, the half-life of amphetamine was approximately 7 hours. Cl/F was similar to that reported by Beckett & colleagues.[91] Under conditions of acidified urine, they found that Cl/F was 242 - 539 ml/min. In this study, Cl/F was approximately 500 ml/min.

Although not statistically significant, T_{max} appears to increase as the dose increased. This phenomenon was also observed in the study by Angrist and associates,[88] where two groups of subjects received either a high or a low dose of amphetamine. T_{max} was longer for the group who received the higher dose (3-4 hours) than those who received the lower dose (2-3 hours). The dosage forms administered in the Angrist study were tablets, but a different brand was used to prepare each blinded dose level, so differences between the dosage forms was cited as

a possible explanation for the differences in T_{max}. To further examine this observation in our study, ka was estimated for each subject at each dose assuming first order absorption. The blood sampling scheme used in this study was not optimized to characterize the absorption process, so estimates for T_{max} and ka must be interpreted cautiously. Values for ka decreased as the dose increased, indicating the absorption was slowed at the higher doses. Statistical analysis showed that ka was significantly larger at the 5 mg dose, but that there was no difference between the 10 and 20 mg doses.

One possible explanation for the slowing of absorption with increasing amphetamine dose is that amphetamine's effects on the gastrointestinal tract influence its own absorption. Amphetamine can inhibit gastrointestinal smooth muscle activity, thus slowing the intestinal transit time and delaying gastric emptying.[85,152] A slowing of gastric emptying should delay the absorption of a drug like amphetamine.[153] Amphetamine is basic drug with a pKa of 9.9. In the acidic conditions of the stomach, amphetamine is ionized and is unlikely to be absorbed. It must arrive at the small intestine for significant absorption to take place. Amphetamine's influence on its own absorption could be viewed as a pharmacodynamic response measure for effects on the gastrointestinal system.

Another potential explanation for the observed decrease in absorption rate as the dose increases is that the apparent amphetamine absorption rate is a saturable process. Simulation studies performed by Couet and colleagues[154] indicate that T_{max} increases as the dose increases for a drug that undergoes saturable absorption. AUC remains proportional to dose when it assumed that there is no limitation on the time that the

drug is in contact with the absorption site. Similar effects on T_{max} and AUC were observed in our study, so saturable absorption may be possible. Further research is needed to confirm the observations noted in this study and to elucidate the mechanism behind the dose-dependent absorption of amphetamine.

The elimination rate constants calculated based on the serum and urine data were dissimilar for some subjects. Possible explanations for this observation are 1) that estimates of average excretion rate during each collection interval do not adequately represent the instantaneous excretion rate at the midpoint of the collection interval, since the collection interval was approximately equal to the half-life of the drug during later intervals, 2) an insufficient number of data points was available for the urine data to provide a reliable estimate of k, and 3) concentrations of amphetamine were near the limit of quantitation of the urine assay during later collection intervals, and small errors at low concentrations are magnified when multiplied by large urine volumes. Estimates of k from serum data are more reliable in this study.

To obtain a constant renal clearance and minimize the effects of pharmacokinetics on the pharmacodynamic effects observed, control of urine pH and flow rate are essential. In this study, pH was maintained within a fairly narrow range (4.5 to 6.5) and the influence on renal clearance was still apparent. Urine flow also affected renal clearance in this study. Other investigators also observed this effect[93], while others did not[87]. Urine flow was not controlled in this study. Subjects were required to maintain a high level of oral fluid intake (at least 120 mL per hour for the first four hours after dosing), but subjects were allowed additional water and the

actual intake was quite variable between subjects. In future studies, controlled intake of fluids would be more appropriate.

4.4.3 Conclusions

Amphetamine is characterized by linear pharmacokinetics within the dosage range of 5 to 20 mg. Control of urine pH by administration of oral ammonium chloride results in constantly enhanced excretion of amphetamine. Multiple peaks are present in the serum-concentration time profile, indicating that some process of cyclical or discontinuous absorption is occurring. The absorption rate of amphetamine appears to be dependent on the dose. As the dose increases, absorption is slowed. Further studies are needed to determine the mechanisms behind the multiple peaks observed in the serum concentration-time profile and the dose-dependent rate of absorption of amphetamine.

To examine whether amphetamine is affecting its own absorption through its pharmacological effect on gastrointestinal transit, a clinical study similar to that of Robertson and colleagues[155] could be conducted. Each subject should receive placebo, several dose levels of dextroamphetamine given as an oral solution, and an intravenous dose of dextroamphetamine in a crossover design. Along with the amphetamine dose or placebo, subjects would receive a capsule filled with pellets labelled with ^{99M}Tc sodium pertechnetate as described by Coupe and colleagues.[156] A gamma camera could then be used to image the pellets as they move through the gastrointestinal tract, to enable estimation of gastric emptying and small intestinal transit. Frequent blood samples should be obtained during the first three hours after

drug administration to better characterize the absorption process. Values for ka could then be estimated by fitting the serum concentration data to a one compartment pharmacokinetic model and using the method of Wagner and Nelson.[157] Data from both intravenous and oral doses would permit the use of deconvolution techniques to study the absorption of amphetamine.[158] Values for the absorption rate constant could be compared to effects on gastrointestinal transit to provide information about potential mechanisms for amphetamines influence on its own absorption.

4.5 Pharmacodynamic Evaluation

The results of the pharmacodynamic evaluation are presented below. Results, discussion and conclusions for the central nervous system and cardiovascular response measures are in section 4.5.1 and 4.5.2 respectively. The sensitivity and reproducibility of the response measures will be compared in Chapter 5.

4.5.1 Central Nervous System Response Measures

The results for quantitative EEG, serum prolactin, the rating scales, and the psychometric tests are presented and discussed in the following sections. Plots for Subject 3 are not presented because he did not receive a placebo. His data was included in the statistical analysis however.

4.5.1a Quantitative Electroencephalography

4.5.1a(1) Results

Plots of baseline and placebo corrected total EEG power across all electrodes in each of the five frequency bands versus time for each subject who completed the study are presented in Appendix L. The EEG response values plotted at each time point were obtained by subtracting the baseline (0 hr) value for each treatment from the values at later time points for that treatment. The baseline corrected placebo profile was then subtracted from the baseline corrected profiles for the active treatments. Data for Subject 9 are not included because insufficient EEG data was collected during the placebo period. A review of these plots and similar plots of relative EEG power does not reveal EEG patterns consistent with increasing dextroamphetamine dose for most of the subjects. Relative standard deviation in the alpha and delta frequency bands also do not appear to change consistently with dose. Based on the work of other investigators (Section 2.2.4), increases in power in the alpha and beta frequency bands and decreases in power in the delta and theta frequency bands would be expected after amphetamine dosing.

For two of the subjects (Subjects 4 and 6), EEG changes are apparent with increasing dextroamphetamine dose. The effect becomes greater as the dose increases. Subject 4 showed an increase in total power across all frequency bands, an increase in alpha power, and an increase in beta I power. These changes are shown in Figures 4.12 - 4.14. The EEG effects appear to be longer in duration as the dose increases. The increase in alpha power lasted 6 to 8 hours after the 20 mg dose, 6 hours after the 10 mg dose, and 4 to 6 hours after the 5 mg dose. The maximum effect was apparent at 4 hr for all doses. Subject 6 showed an increase in total power, a decrease in theta power, and an increase in alpha power. These changes are

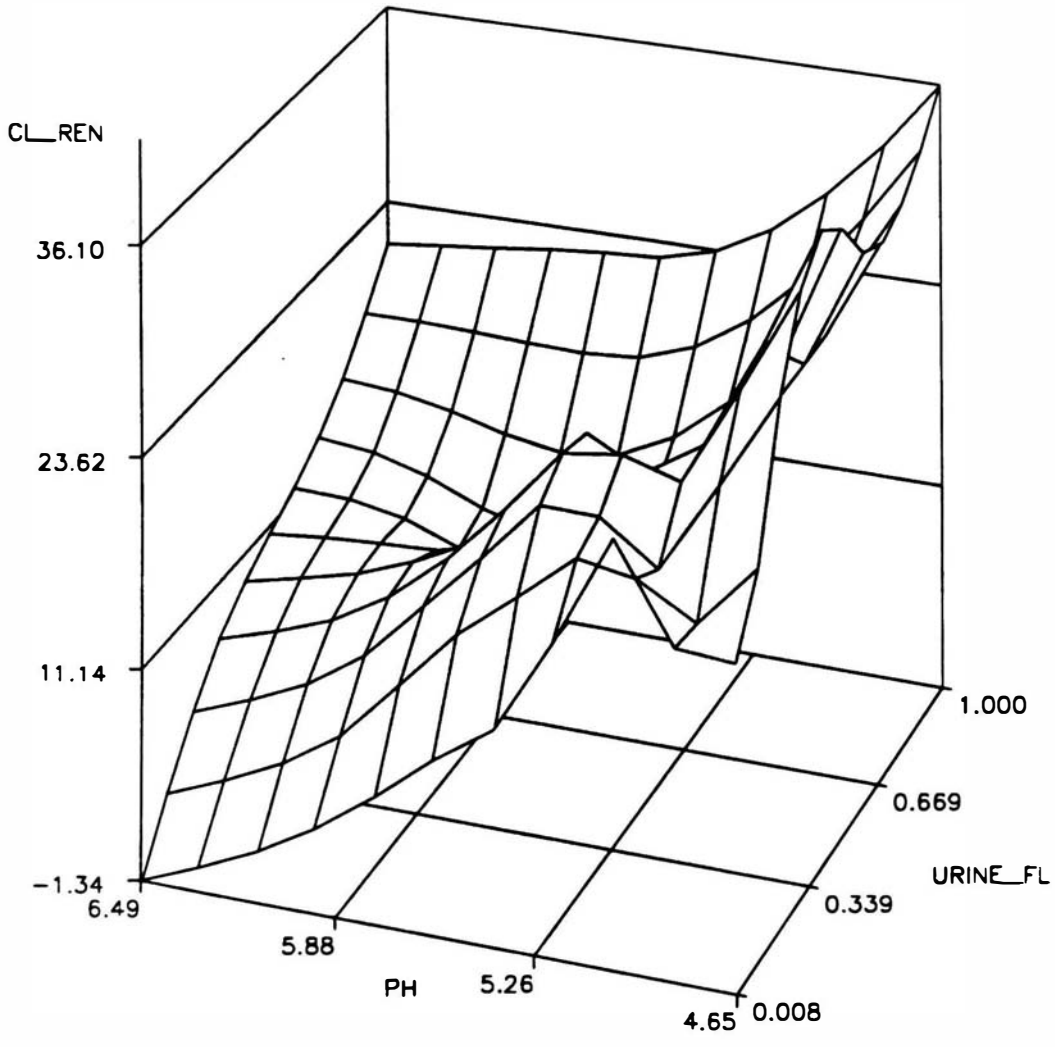

Figure 4.11 Renal clearance as a function of urine pH and urine flow rate

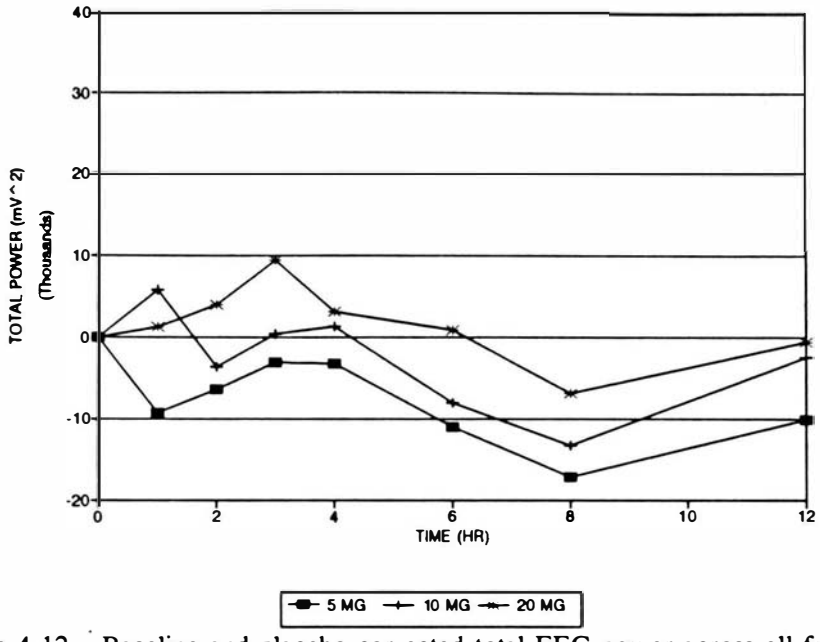

Figure 4.12 Baseline and placebo corrected total EEG power across all frequency bands versus time profile for Subject 4

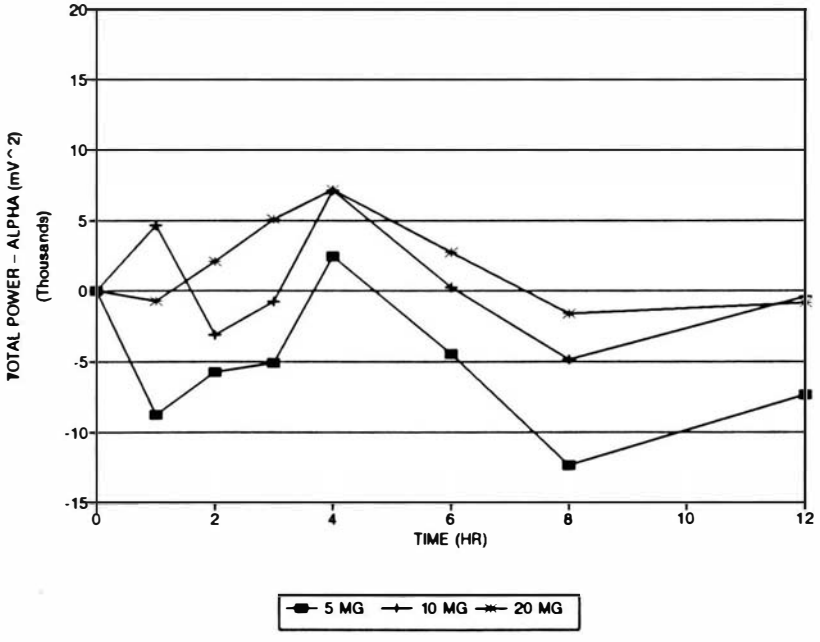

Figure 4.13 Baseline and placebo corrected total EEG power in the alpha frequency band versus time profile for Subject 4

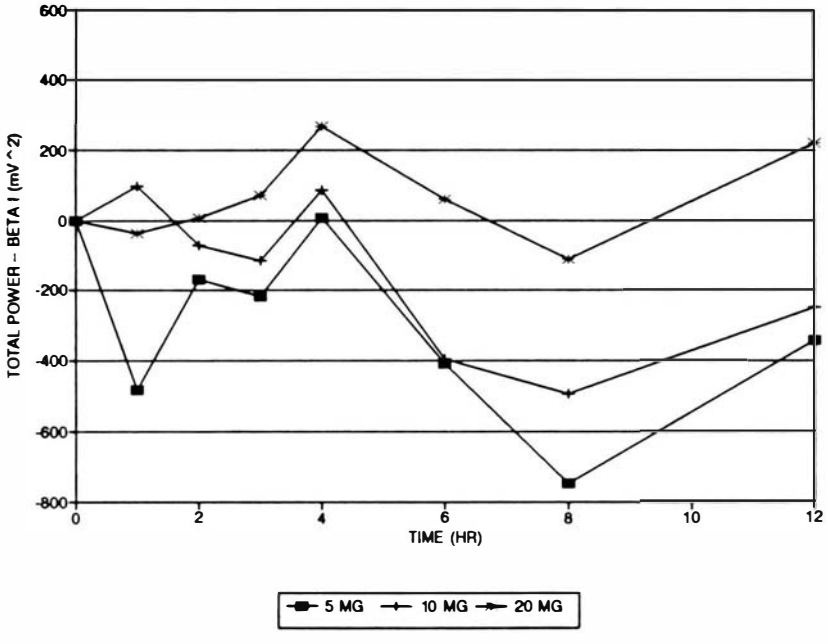

Figure 4.14 Baseline and placebo corrected total EEG power in the beta I frequency band versus time profile for Subject 4

shown in Figures 4.15 - 4.17. The maximum effect occurred at 1 hr for all doses. These changes are more prominent when power from only the occipital electrodes is examined. Changes in alpha power from the occipital electrodes for Subject 6 is presented in Figure 4.18. Examination of plots of power from the occipital and central electrodes for all subjects did not reveal EEG changes other than those seen in plots of power from all electrodes. Changes in alpha power were more pronounced in the occipital region however.

Subjects 4 and 6 are distinguished from the other subjects who completed the study by their baseline EEG characteristics. Average baseline total and relative power across all electrodes for each subject is presented in Table 4.19. Subjects 4, 6 and 8 have much higher total power across all electrodes and in the alpha frequency band. These subjects also show greater than 35% of the total EEG power in the alpha frequency band. Each of these subjects show EEG changes after dextroamphetamine dosing compared to the other subjects, although for Subject 8, the greatest response is observed after the 10 mg dose. The EEG response in total power in the alpha frequency band for all subjects are shown in Figures 4.19 - 4.25 to illustrate this point. The EEG changes do not appear to be related to the mood response of the subjects. Subjects 6 and 8 experienced euphoria, while Subject 4 experienced dysphoria in response to dextroamphetamine.

Statistical comparison of the values for E_{max} (or E_{min}), E_{max} - (1st 4 hr) (or E_{min} - (1st 4 hr)) and ET for each of the EEG response variables for all subjects did not show any significant differences between the treatments that are consistent with increasing dextroamphetamine dose. The residuals were normally distributed for

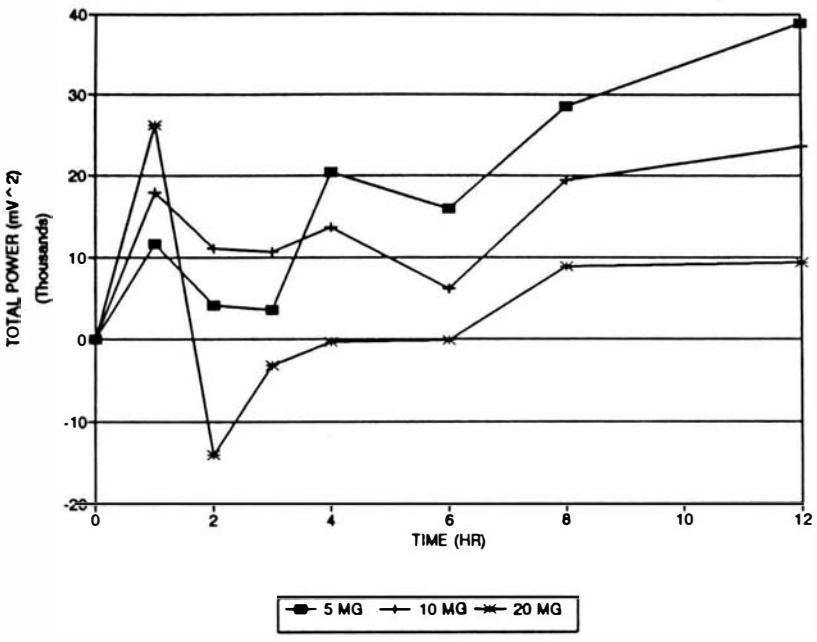

Figure 4.15 Baseline and placebo corrected total EEG power across all frequency bands versus time profile for Subject 6

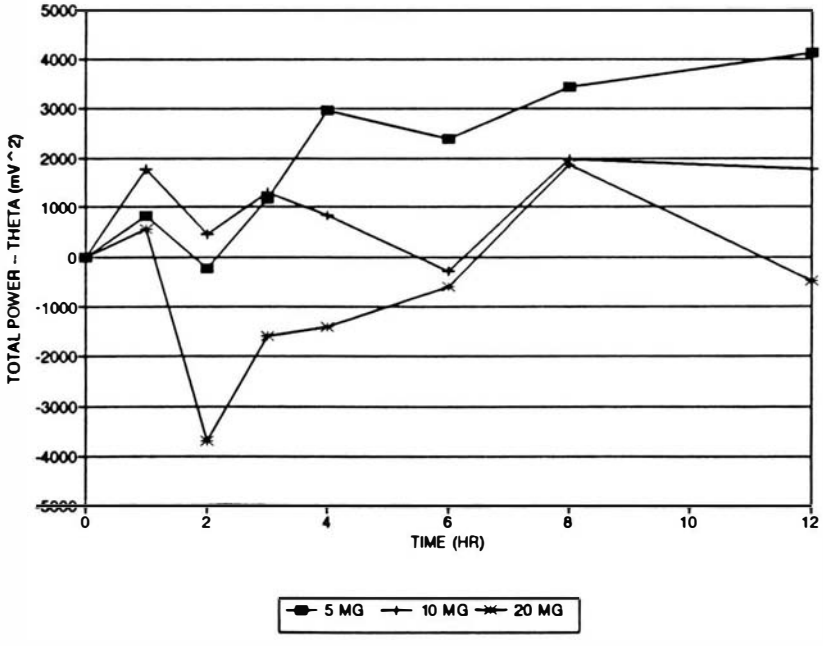

Figure 4.16 Baseline and placebo corrected total EEG power in the theta frequency band versus time profile for Subject 6

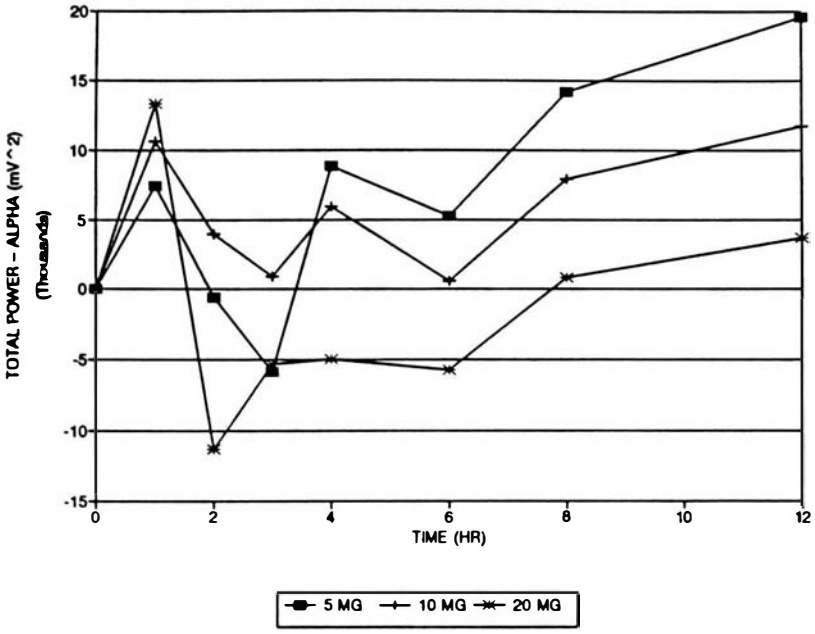

Figure 4.17 Baseline and placebo corrected total EEG power in the alpha frequency band versus time profile for Subject 6

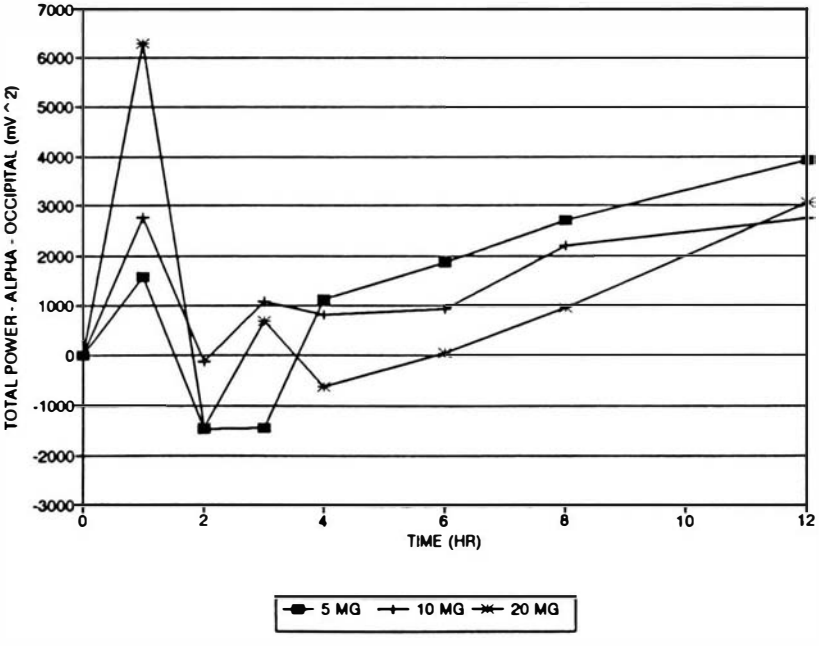

Figure 4.18 Baseline and placebo corrected total EEG power in the alpha frequency band from the occipital electrodes versus time profile for Subject 6.

Table 4.19 Mean (RSD%) Total (TP) and Relative (RP) EEG Power Across All Electrodes at Baseline (0 hr) for Each Subject

Subj	TP (μV^2)	TP Delta (μV^2)	TP Theta (μV^2)	TP Alpha (μV^2)	TP Beta I (μV^2)	TP Beta II (μV^2)	RP Delta	RP Theta	RP Alpha	RP Beta I	RP Beta II
1	7519	7023	878	1340	236	455	0.608	0.118	0.185	0.031	0.059
	(20.7)	(24.5)	(21.1)	(49.8)	(54.7)	(59.0)	(17.2)	(17.9)	(54.2)	(34.7)	(40.1)
2	6547	3913	1107	850	275	403	0.599	0.169	0.130	0.042	0.061
	(8.2)	(8.7)	(17.6)	(20.6)	(23.4)	(14.3)	(7.3)	(12.4)	(17.8)	(15.5)	(7.1)
4	16980	6076	2834	6869	465	736	0.367	0.168	0.396	0.027	0.043
	(11.5)	(19.3)	(12.8)	(39.6)	(31.8)	(24.2)	(29.3)	(13.9)	(30.0)	(25.8)	(17.7)
6	34545	9954	4609	18919	500	563	0.287	0.134	0.549	0.014	0.016
	(50.0)	(60.1)	(53.6)	(48.0)	(51.6)	(64.8)	(21.2)	(14.5)	(8.5)	(36.4)	(75.6)
7	9106	4758	1136	2658	192	362	0.519	0.128	0.295	0.021	0.037
	(22.8)	(28.9)	(12.3)	(20.9)	(37.9)	(72.6)	(10.7)	(23.2)	(16.4)	(29.1)	(49.5)
8	17484	6512	2760	7159	424	630	0.398	0.166	0.374	0.026	0.037
	(38.9)	(38.4)	(32.4)	(59.0)	(26.5)	(38.1)	(38.8)	(27.6)	(41.5)	(19.5)	(22.7)
9	9993	4772	1441	3059	302	419	0.487	0.144	0.298	0.030	0.041
	(15.1)	(14.8)	(15.1)	(45.5)	(13.7)	(35.6)	(24.8)	(1.5)	(37.0)	(8.4)	(25.8)
10	8467	4954	1059	1764	204	486	0.569	0.129	0.218	0.025	0.060
	(24.6)	(42.5)	(15.2)	(25.6)	(18.0)	(17.4)	(18.9)	(20.0)	(37.6)	(30.0)	(28.3)

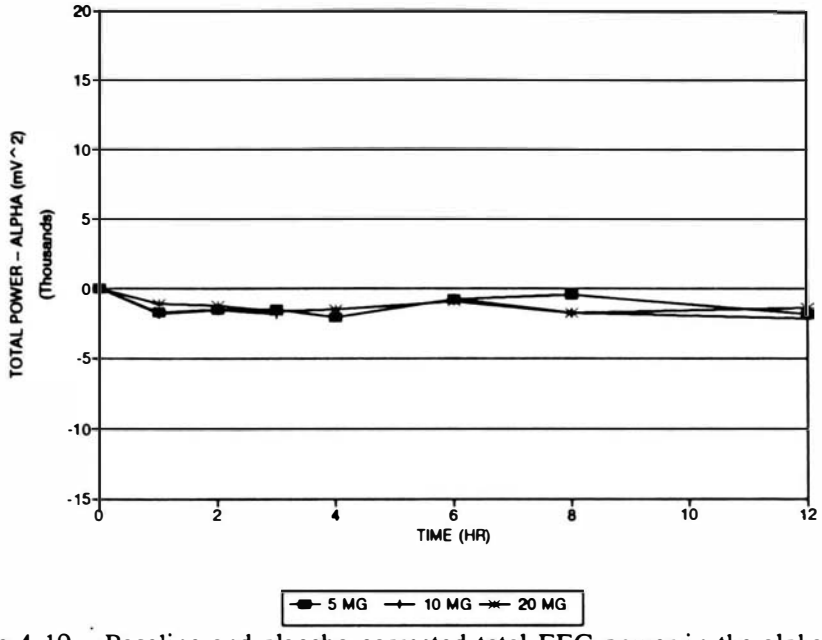

Figure 4.19 Baseline and placebo corrected total EEG power in the alpha frequency band versus time profile for Subject 1

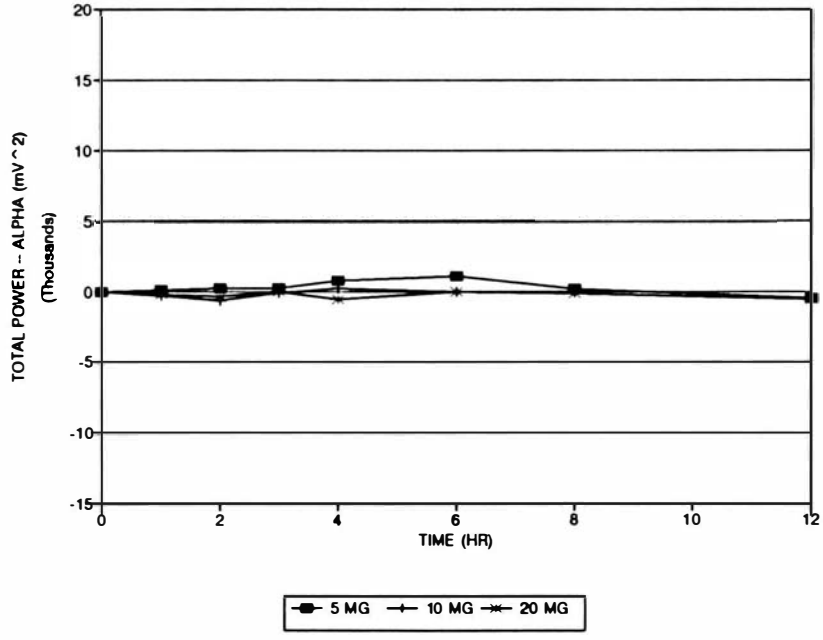

Figure 4.20 Baseline and placebo corrected total EEG power in the alpha frequency band versus time profile for Subject 2

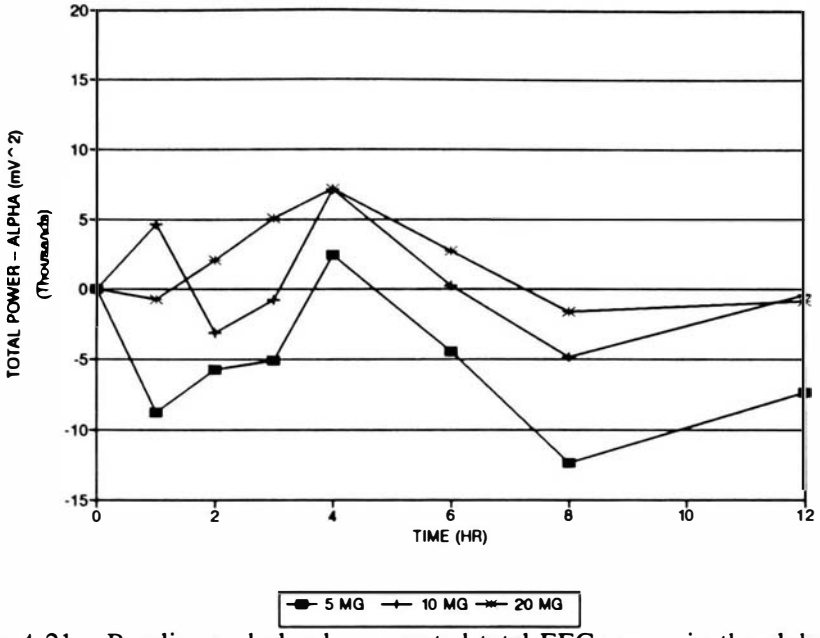

Figure 4.21 Baseline and placebo corrected total EEG power in the alpha frequency band versus time profile for Subject 4

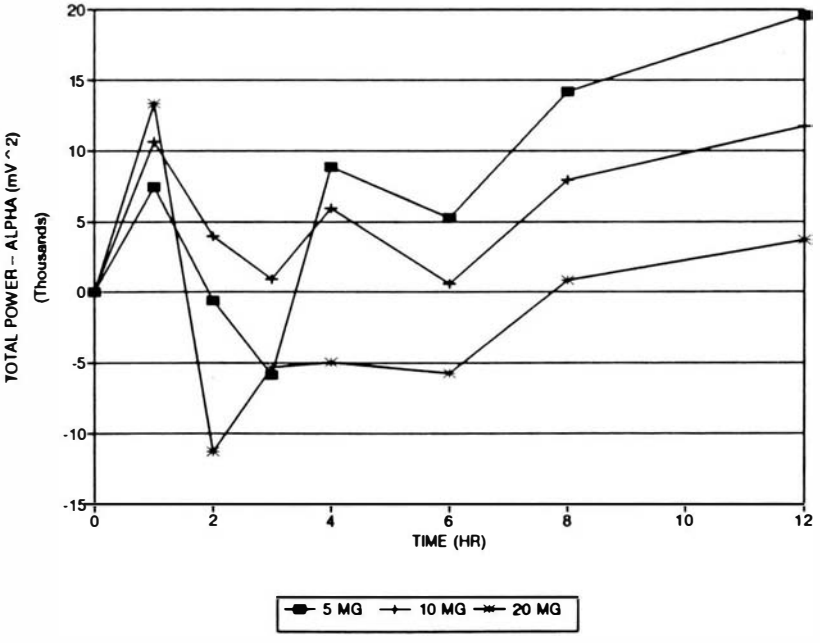

Figure 4.22 Baseline and placebo corrected total EEG power in the alpha frequency band versus time profile for Subject 6

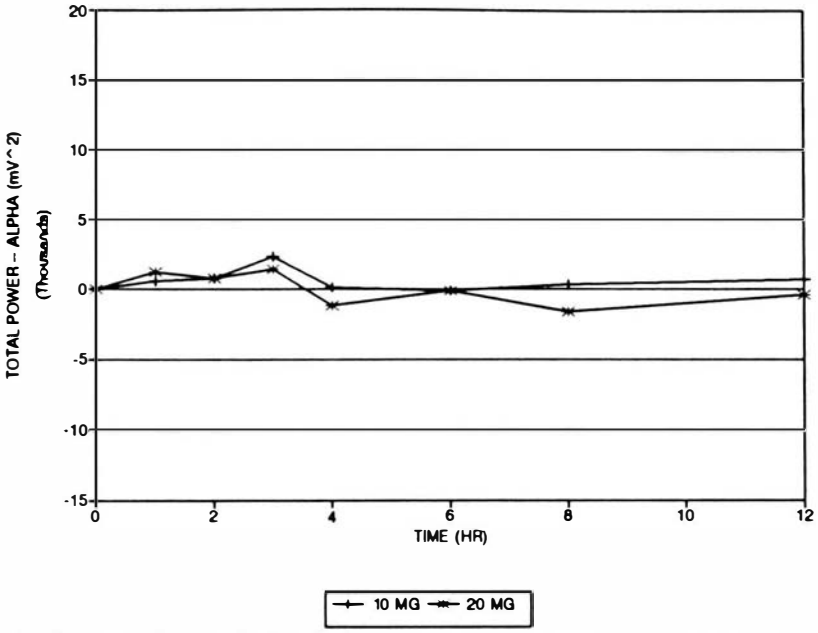

Figure 4.23 Baseline and placebo corrected total EEG power in the alpha frequency band versus time profile for Subject 7

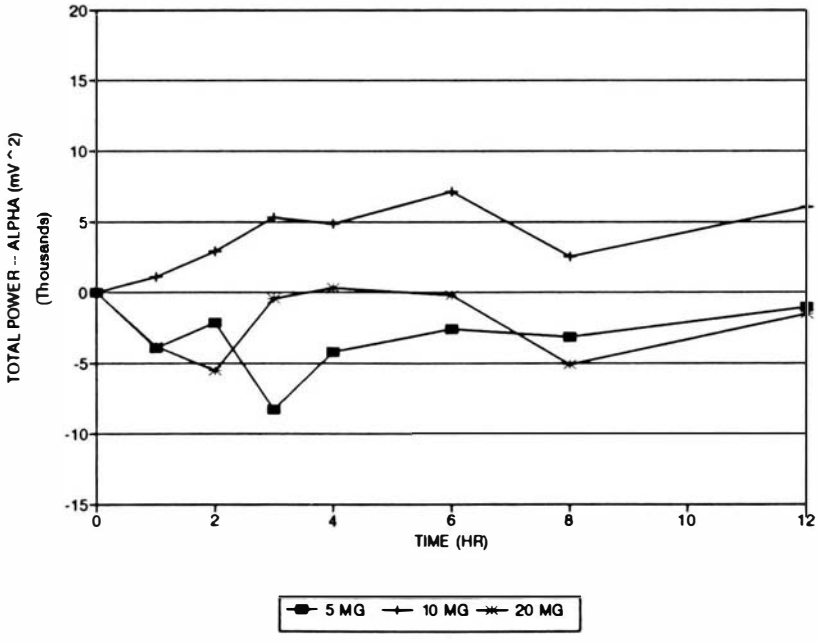

Figure 4.24 Baseline and placebo corrected total EEG power in the alpha frequency band versus time profile for Subject 8

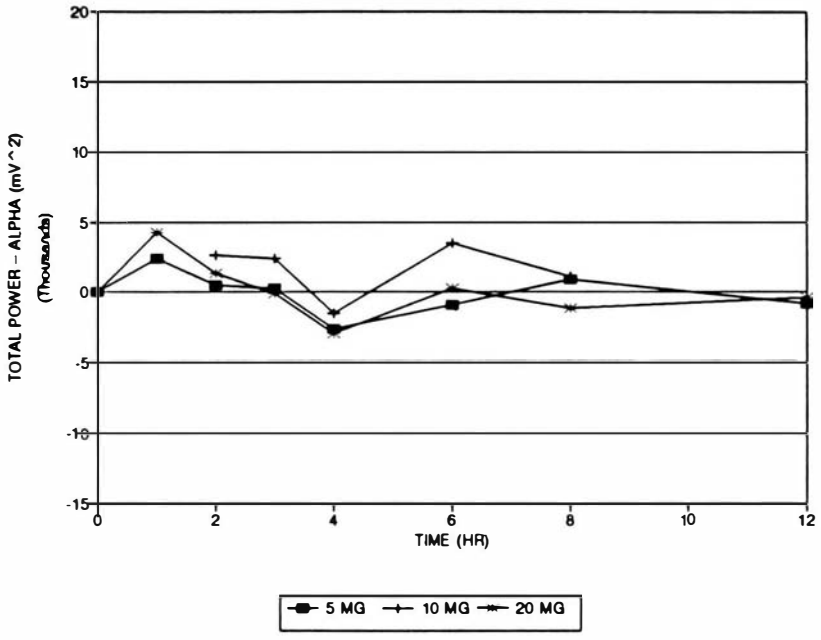

Figure 4.25 Baseline and placebo corrected total EEG power in the alpha frequency band versus time profile for Subject 10

these variables, so transformation of the data was not necessary. Estimates of the power of the statistical tests were low for many of the variables. Statistical power for tests of E_{max} (or E_{min}) for total and relative power ranged from 0.20 to 0.96. Power was lower for relative power than total power and lower in the alpha and beta frequency bands than the delta and theta bands.

Baseline response (E_0) was a significant covariate for the vast majority of the EEG response variables, indicating that baseline response accounted for a significant portion of the variability in E_{max}. The baseline response differed from week to week for all subjects. As can be seen in Table 4.19, the RSD% of baseline values for the EEG response variables for each subject ranged from 2 to 75%. The greatest variability was observed in the alpha and beta frequency bands. There was no apparent relationship between study period and baseline EEG characteristics.

Excessive artifacts were present in 18 of the EEGs collected, resulting in 6% of the EEGs being designated as missing. The baseline EEG was missing for Subject 5 (5 mg dose), Subject 7 (5 mg dose), and Subject 9 (placebo).

4.5.1a(2) Discussion

Changes in the EEG related to dextroamphetamine dose were observed in less than one-half of the subjects who completed the study. Those subjects who demonstrated dose-related changes had similar baseline EEG characteristics. Their percentage of baseline EEG in the alpha frequency band was greater than 35%. They also showed higher total EEG power at baseline than the other subjects. Screening of subjects for a high baseline level of alpha activity may be necessary to study the

pharmacodynamics of CNS stimulation. Since vigilance changes are easier to detect in subjects with high background alpha activity[74], vigilance-promoting effects of CNS stimulants may only be apparent on the EEG for subjects with high baseline alpha activity. In the eyes closed condition, decreased arousal is accompanied by decreased alpha activity, especially in subjects with high background alpha activity.[159] Because alpha power may vary with normal aging, the results obtained in this study may not be generalizable to very young or very old subjects.[160]

Another potential explanation for the variable effect on the EEG between subjects may be the vigilance control method used during the EEG recording. In a study reported after this investigation was completed, Saletu and colleagues reported that EEG changes were evident after 20 mg dextroamphetamine compared to placebo only when EEGs were recorded under resting (not vigilance-controlled) conditions.[96] Vigilance control reduced the magnitude of EEG effects and altered the time course of effects observed. In our study, vigilance was controlled to a degree by instructing subjects to count backward from 500 by 3s during the recording. Compliance with these instructions could not be verified objectively. Other techniques, such as holding a button that alarms if the pressure is relaxed, ensure that vigilance is maintained. In our study, the level of vigilance maintained could have been different between subjects with subsequent differences in the magnitude of the response. Also, vigilance may not have been equally controlled for all of the study periods for an individual subject. The EEG editing process may also have resulted in a potential preferential selection of drowsy records over awake records due to lower artifacts in the drowsy records. If this in fact occurred, the results of the study may be biased

towards the more drowsy, giving different results than if it were a sample of only awake records.

The differences in EEG response between subjects do not appear to be related to the type of mood response to dextroamphetamine experienced by the subject. Mood changes observed in this study are discussed in detail in Section 4.5.1c. Whether subjects felt euphoria or dysphoria did not seem to influence whether EEG changes were observed for an individual. Categorizing subjects as EEG responders or nonresponders does not result in the same grouping as categorizing subjects as euphoric or dysphoric.

Baseline values for EEG variables also play an important role in interpreting the data. Baseline values were different between weeks in the study. Differences in baseline values between the first study period and later periods were not evident, indicating that the familiarization session was sufficient to accustom subjects to the study environment and procedures. It is necessary to take into account the baseline level when comparing treatments for an individual. Baseline values were also a significant covariate in the statistical analysis. Differences in baseline values between weeks may be related to differences in the testing environment, level of stress perceived by the subject, or quality of the subject's previous night's sleep.[41] An adequate determination of the baseline is essential for examining the EEG response to dextroamphetamine. Error in this measurement or loss of this measurement due to artifacts has the potential to greatly influence the outcome of the data analysis. Therefore, making more than one baseline EEG measurement before dosing may be worthwhile.

Examination of smaller groups of electrodes (occipital and central electrodes) did not contribute added discriminating ability over that provided by examining a sum of all of the electrodes. Because alpha activity is most prominent over the occipital region, the effects on alpha power were more evident there. EEG effects were not apparent in the occipital or central regions that were not evident across all of the electrodes.

Statistical comparisons of the treatments revealed no significant differences between the doses for any of the EEG variables. Since only those subjects with high background alpha activity show EEG response to dextroamphetamine, pooling the data across all of the subjects in this study masks any differences in EEG effects between the treatments. The study sample was heterogenous with regard to background alpha activity, and the sample was too small to perform statistical analysis on subgroups of patients. Statistical power was relatively low to detect differences between treatments. An increased sample size or more homogenous subject group with respect to alpha activity is necessary for statistical analysis of treatment differences. Other investigators have noted that differences between the alert EEG patterns of individuals are important factors when attempting to elucidate subtle effects in the EEG indicative of vigilance changes. They suggest that pooling data across subjects results in loss of information.[159]

The number of EEG recordings containing excessive artifacts was similar to that observed in Part I of this study in which no drug was administered (Chapter 3). The pharmacological action of dextroamphetamine and the drawing of blood samples does not appear to significantly increase the number of artifacts obtained on the EEG

recordings. The average variability between baseline EEG responses on each study day was also similar to that observed in Part I of the study.

In conclusion, dose-related EEG changes after dextroamphetamine administration were observed only in a subgroup of patients who had high baseline alpha activity. For these subjects, the EEG response was higher in magnitude and longer in duration as the dose increased. Because the subject group was heterogenous with respect to baseline alpha activity, statistical analysis was not useful for examining differences in EEG response between the treatments. The baseline EEG measurement must also be taken into account when comparing treatments. Future studies attempting to use EEG to measure CNS stimulation should include a screening procedure to obtain a homogenous subject group with respect to background alpha activity and a more dependable determination of baseline values for EEG variables during each period.

4.5.1b Prolactin Response

4.5.1b(1) Results

Plots of baseline and placebo corrected serum prolactin versus time for each subject are presented in Appendix M. The serum prolactin values plotted at each time point were obtained by subtracting the baseline (0 hr) value for each treatment from the values at later time points for that treatment. The baseline corrected placebo profile was then subtracted from the baseline corrected profiles for the active treatments. Representative plots from Subjects 1 and 6 are shown in Figures 4.26 and

4.27. Based on these plots, it appears that there is an inverse relationship between dose and effect on prolactin. Serum prolactin decreases after the administration of dextroamphetamine, with the maximum decrease occurring after the 5 mg dose. The duration of the effect is also longer after the 5 mg dose.

The maximum effect on serum prolactin is observed between 1 and 4 hr. It occurs at the same time after each dose level for an individual. The onset of effect is apparent at 1 hr for the lower doses in most subjects, but is delayed until 2 hr at the higher dose in some subjects. The effects on serum prolactin are usually evident until at least 5 hr. Subject 10 shows little change in serum prolactin in response to dose. Prolactin response does not appear to be related to whether the subject experiences euphoria or dysphoria, since prolactin generally decreased for all subjects regardless of the direction of mood alterations.

Statistical comparison of the maximum decrease in serum prolactin (E_{min}) for each treatment confirms this observation. The mean E_{min} for the baseline and placebo corrected serum prolactin values were 8.8 ng/mL, 8.2 ng/mL, and 4.3 ng/mL for the 5, 10 and 20 mg doses respectively. The mixed effects analysis of covariance showed a statistically significant effect for treatment ($p < 0.05$), with all treatments different from placebo. The power for this statistical test was estimated to be 0.85. The statistical tests could not distinguish between the treatments however.

Baseline serum prolactin values were not statistically significant covariates in the mixed effects analysis of covariance. There was, however, notable variation between the baseline values obtained during each period for many of the subjects. Baseline serum prolactin values for each subject are presented in Table 4.20. There does not

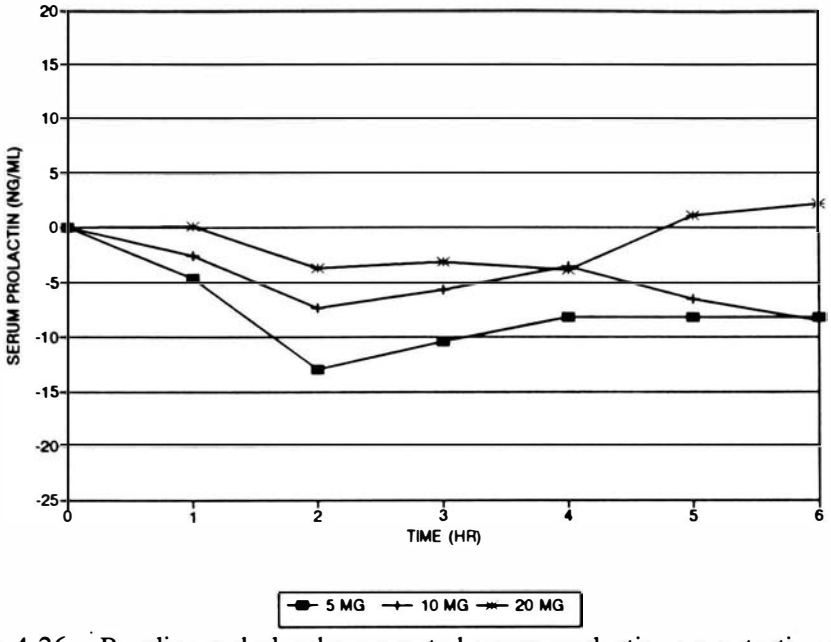

Figure 4.26 Baseline and placebo corrected serum prolactin concentration versus time plot for Subject 1

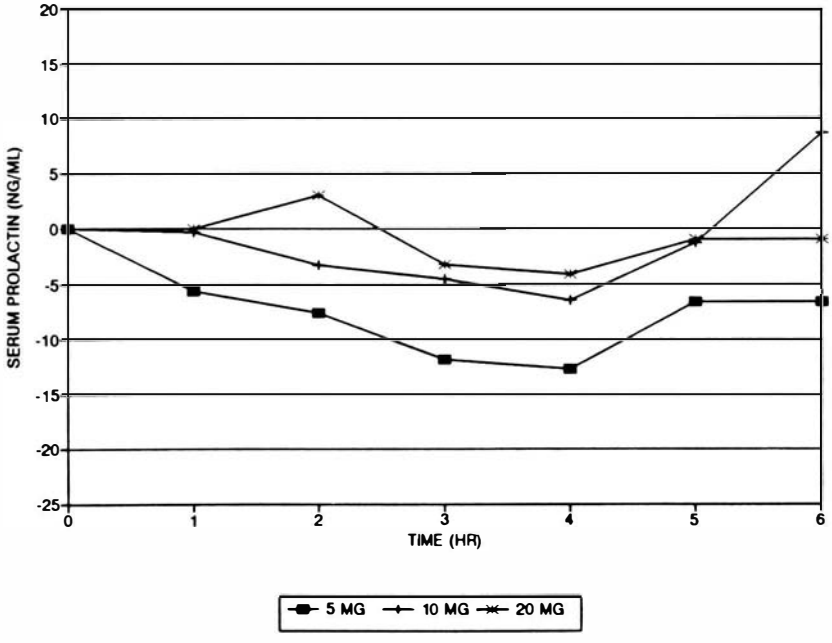

Figure 4.27 Baseline and placebo corrected serum prolactin concentration versus time plot for Subject 6

appear to be a correlation between the serum prolactin level and the study period.

The pattern of prolactin secretion obtained during the placebo period for the subjects who completed the study is presented in Figure 4.28. For most subjects, prolactin levels remain within the normal range of approximately 5 to 10 ng/mL. Subject 2 shows unusually high levels of prolactin for a young healthy male.

When the baseline and placebo corrected serum prolactin concentrations for each subject at each dose level are plotted against the serum amphetamine concentrations obtained at the same time, hysteresis is observed in approximately 70% of the plots. One-half of these hysteresis loops are in the clockwise direction and one-half are in the counterclockwise direction. The direction is consistent within a subject. Representative plots are presented in Figure 4.29 (clockwise hysteresis), Figure 4.30 (counterclockwise hysteresis), and Figure 4.31 (no hysteresis). The direction of the loop does not appear to be related to baseline prolactin levels, mood response, or absorption rate constant of dextroamphetamine observed for each subject.

4.5.1b(2) Discussion

Serum prolactin levels, like the EEG parameters, are a surrogate measure of the effect of dextroamphetamine on the central nervous system. Drugs that increase dopaminergic neurotransmission in the CNS reduce prolactin secretion and drugs which increase serotonergic neurotransmission in the CNS increase prolactin secretion. Dextroamphetamine is believed to affect dopamine release at lower doses and serotonin release at higher doses, and thus may cause opposing effects on prolactin secretion.[85] Amphetamines may also exert direct agonist action at central

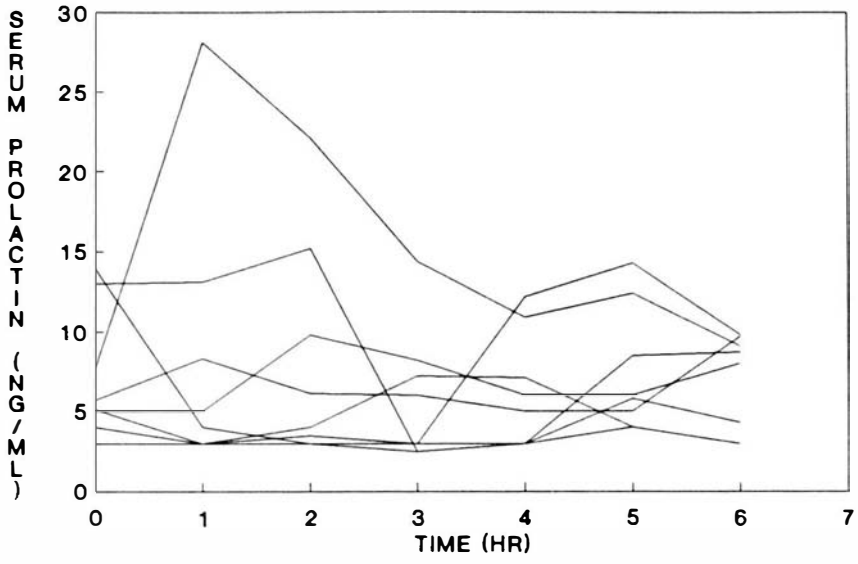

Figure 4.28 Serum prolactin concentration versus time plots for the placebo period for each subject

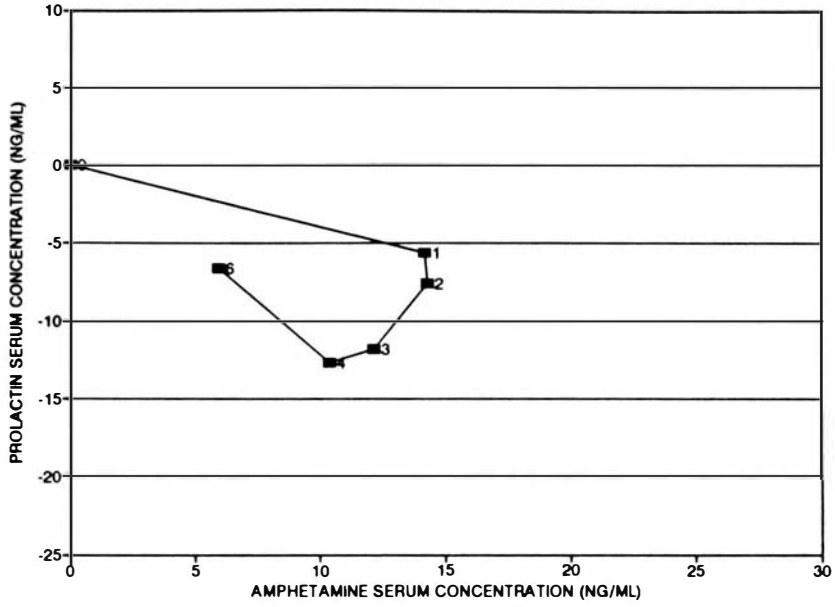

Figure 4.29 Baseline and placebo corrected serum prolactin concentration versus serum amphetamine concentration for Subject 6 (5 mg dose)

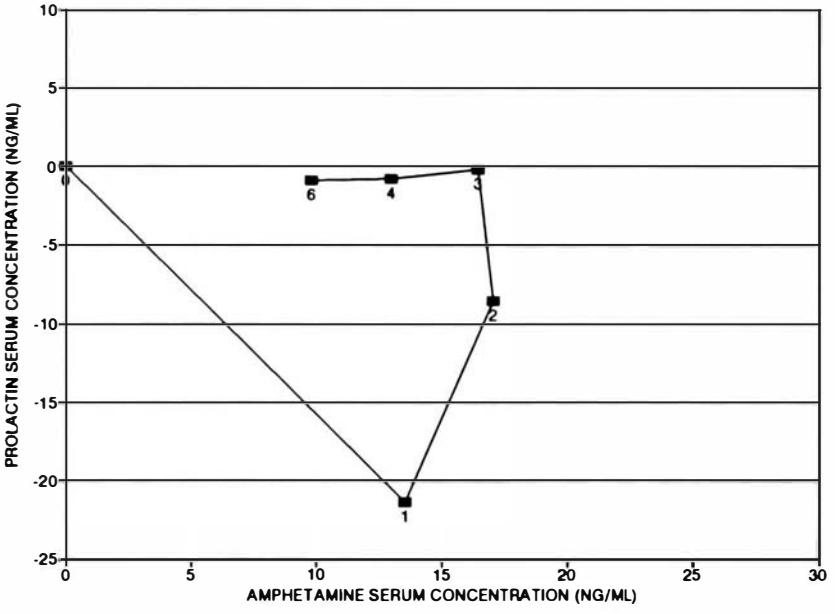

Figure 4.30 Baseline and placebo corrected serum prolactin concentration versus serum amphetamine concentration for Subject 2 (5 mg dose)

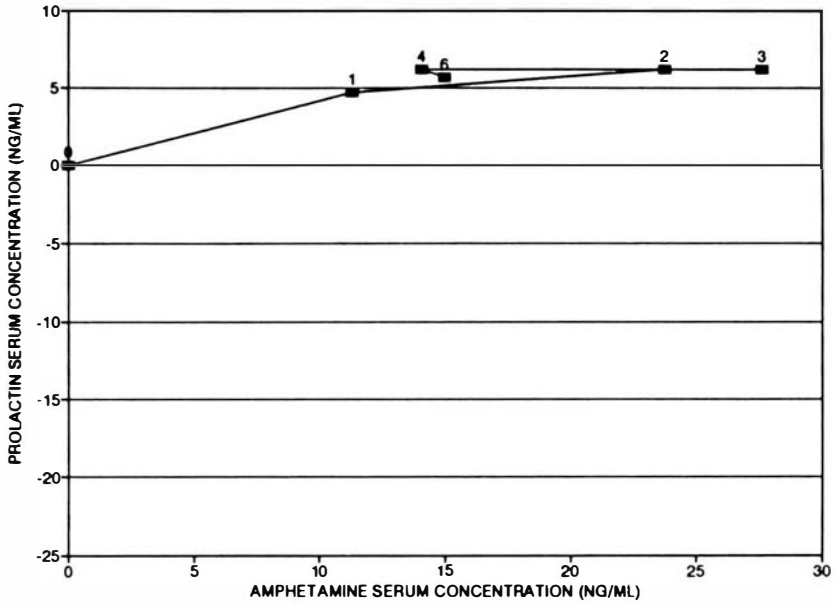

Figure 4.31 Baseline and placebo corrected serum prolactin concentration versus serum amphetamine concentration for Subject 10 (10 mg dose)

Table 4.20 Serum Prolactin Levels at Baseline (0 hr) for Each Subject

Subject	Period 1	Period 2	Period 3	Period 4	Mean (ng/mL)	RSD (%)
1	10.2	5.0	5.9	8.5	7.4	32.2
2	7.6	9.5	5.4	12.9	8.9	35.9
3	3.0	2.0	-	-	2.5	28.3
4	7.8	11.0	6.9	5.7	7.9	28.9
5	8.2	2.0	-	-	5.1	86.0
6	4.0	4.3	10.6	4.0	5.7	56.8
7	9.0	5.7	3.0	7.4	6.3	40.7
8	4.0	4.0	3.0	13.0	6.0	78.0
9	4.9	5.1	3.5	15.0	7.1	74.4
10	14.0	8.3	8.0	11.0	10.3	27.2

serotonin receptors. In this study, prolactin secretion diminished after dextroamphetamine administration, with the greatest decrease occurring after the lowest dose. One potential explanation for this observation is that at the 5 mg dose, dextroamphetamine primarily acts to release dopamine from storage sites in the nerve terminal. As the dose increases, serotonin is also released, which opposes the effect of dopamine on prolactin secretion.

Previous studies have reported that dextroamphetamine causes both increases and decreases in the secretion of prolactin (see Section 2.2.5) The effect on secretion appears to depend on the dose administered. Most studies show no change or an increase in prolactin levels after oral dextroamphetamine doses of 20 - 30 mg. Few studies have reported the effects of lower doses on prolactin secretion. Wells and colleagues observed decreased prolactin levels after 10 and 20 mg oral doses of

dextroamphetamine, with the greatest decrease observed for the 20 mg dose.[104] Our data suggests that a portion of the variability in prolactin response between studies may be due to the doses administered. Our findings have implications for research involving prolactin response to the administration of amphetamines in the study of psychiatric illnesses. Prolactin response in normal controls in these pharmacological challenge studies may depend on the dose administered. Further work is needed to determine whether psychiatric illness alters this dose effect relationship.

Spontaneous fluctuations in prolactin secretion were observed during the placebo period for all subjects. Prolactin secretion shows a circadian rhythm. Superimposed upon this are minute-to-minute fluctuations due to factors such as stress and exercise. To reduce differences in these fluctuations between periods, the subjects were required to remain reclining in bed beginning one hour before dosing until after the 6 hr prolactin levels were drawn. The fluctuating nature of prolactin secretion in the absence of drug treatment emphasizes the necessity of including a placebo period to aid in the interpretation of potential drug effects. Pharmacodynamic models have been developed which include the fluctuation of the hormone secretion.[161] These models have improved predictive capability compared to those which consider the placebo response to be constant.

Baseline serum prolactin levels during each period also showed differences from period to period for all of the subjects. These differences could be due to different levels of stress or other factors which can increase prolactin secretion. Higher baseline levels were not apparent during the first period, when higher levels of stress might be anticipated due to the subjects not being well-acquainted with the study

environment. As with the EEG, it may useful in future studies to make more than one baseline determination of prolactin levels.

Hysteresis loops are observed in plots of serum prolactin concentration versus serum amphetamine concentration for 2/3 of the subjects. Because the direction of the hysteresis differs between subjects, it is difficult to postulate potential mechanisms contributing to the phenomenon. In fact, because it occurs equally in both directions, there may be no true hysteresis present. The fluctuating nature of the serum amphetamine concentrations noted during the first four hours after drug administration may also contribute to the discrepancy observed in the direction of the hysteresis.

As with the EEG measures discussed in the previous section, serum prolactin response does not appear to be directly related to the clinical or therapeutic effects of dextroamphetamine. Alterations in EEG measures and serum prolactin levels were not associated with the direction or magnitude of the mood change experienced by the subjects. The effects of dextroamphetamine on mood in these subjects will be discussed in detail in the next section.

4.5.1c Mood Rating Scales

4.5.1c(1) Results

Plots of baseline and placebo corrected rating scale scores (self-rated mood scale and visual analog mood scale) versus time for each subject are presented in Appendix N. The rating scale scores plotted at each time point were obtained by subtracting the baseline (0 hr) value for each treatment from the values at later time points for that

treatment. The baseline corrected placebo profile was then subtracted from the baseline corrected profiles for the active treatments. Plots of the self-rated mood scale and the visual analog mood scale responses versus time for a subject who experienced euphoria after dextroamphetamine administration (Subject 2) are shown in Figures 4.32 and 4.33. Similar plots for a subject who experienced dysphoria after dextroamphetamine administration (Subject 4) are shown in Figures 4.34 and 4.35.

Dysphoria (based on the self-rated mood scale scores) was reported after 20% of the dextroamphetamine doses given. Subject 4 experienced dysphoria after all 3 doses and Subject 8 became dysphoric only after the 20 mg dose. Subject 5 became intensely dysphoric after the 5 mg dose, and was consequently discharged from the study. At the 5 mg dose level, no change in mood occurred for 4 of the 9 subjects evaluated. At the 20 mg dose, only 1 subject did not experience a change in mood.

When subjects experienced a change in mood (based on the self-rated mood scale scores), it was generally apparent by the 1 hr measurement. The change in mood was typically greatest for the 20 mg dose, although Subjects 4 and 7 showed similar effects at all 3 dose levels. The maximum effect occurred between 1 and 3 hours for most subjects. The duration of the mood changes generally ranged from 3 to 8 hr, with a longer duration noted after the highest dose. The subject who experienced the most intense euphoria following the 20 mg dose (Subject 2) still had elevated mood scale ratings when measurements were discontinued at 12 h.

Statistical analysis of the E_{max} and ET values for the self-rated mood scale showed no significant differences between treatments. When differences between the treatments that the subjects believed they had received during each period were

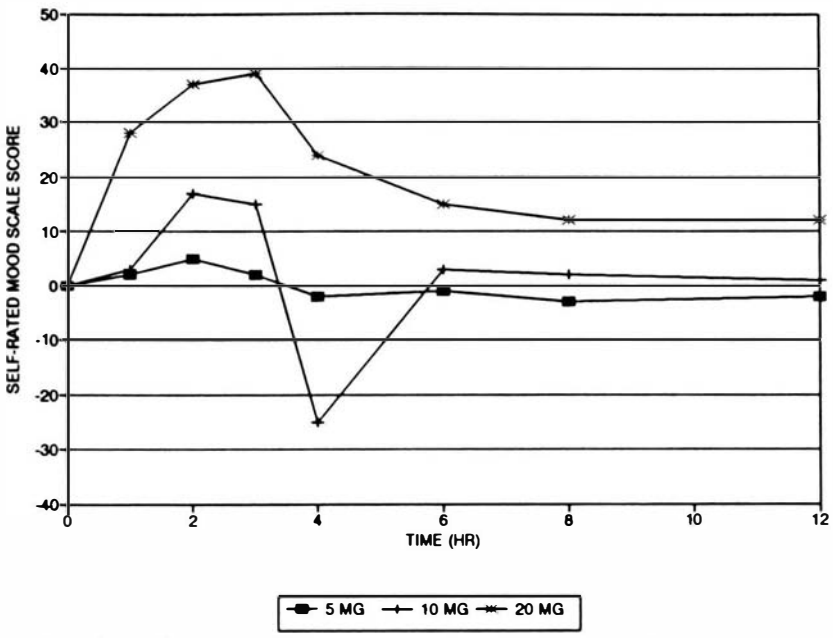

Figure 4.32 Baseline and placebo corrected self-rated mood scale score versus time plot for Subject 2

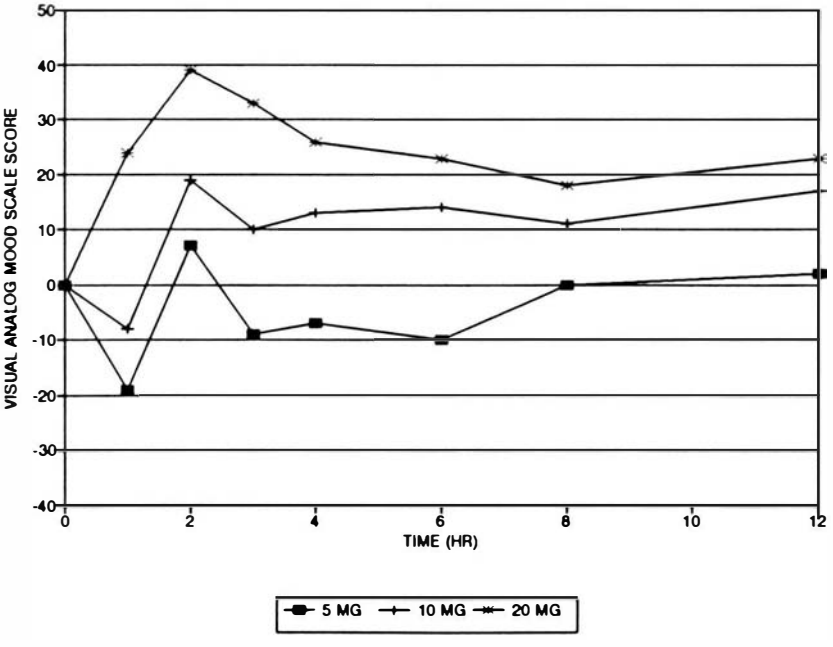

Figure 4.33 Baseline and placebo corrected visual analog mood scale score versus time plot for Subject 2

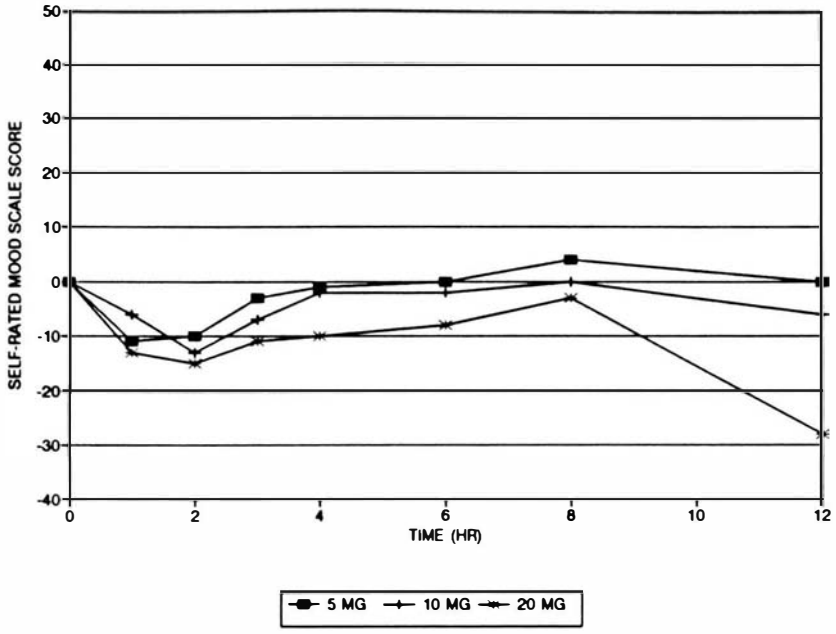

Figure 4.34 Baseline and placebo corrected self-rated mood scale score versus time plot for Subject 4

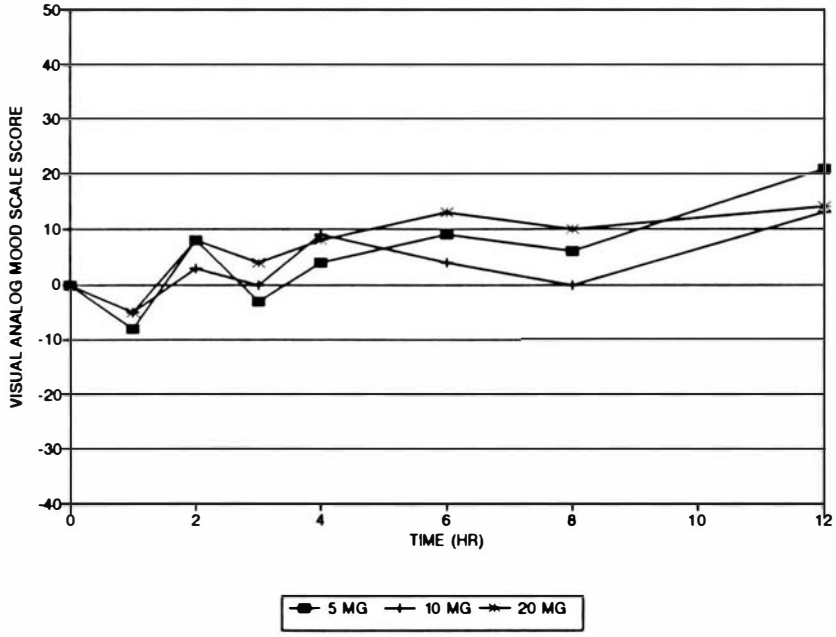

Figure 4.35 Baseline and placebo corrected visual analog mood scale score versus time plot for Subject 4

compared, statistically significant differences were found ($p < 0.05$). During the period when subjects believed that they had received the 20 mg dose, E_{max} was significantly greater than that obtained in the other periods. The other "treatments" were not statistically different. Statistical analysis of the E_{max} values for the visual analog mood scales showed no significant differences between actual treatments or the treatments the subjects believed they had received. Estimated power for these tests was less than 0.3 however. In addition, no significant effect of sequence was noted for the mood ratings.

The visual analog mood scale scores show less consistent change with dose than the self-rated mood scale. After 60% of the administered doses, scores were less than baseline at 1 hr. Subject 2, who showed the highest euphoria based on the self-rated mood scale also has the highest response on the visual analog mood scale. Scores for Subjects 1 and 4 showed little change with dose.

Baseline mood scale scores for both scales were not statistically significant covariates in the mixed effects analysis of covariance. Average baseline mood scale scores for each subject are presented in Table 4.21. There does not appear to be a correlation between the baseline mood scale scores and the study period.

4.5.1c(2) Discussion

A bimodal distribution of mood response was observed in this study. Most subject-dose combinations resulted in euphoria, but some (20%) resulted in dysphoria. The rate of dysphoria observed in this study is similar to that reported by other investigators. Almost 40 years ago, von Flesinger and associates[162] reported that

Table 4.21 Average Mood Rating Scale Scores at Baseline (0 hr) for Each Subject

Subject	Self-Rated Mood Scale		Visual Analog Mood Scale	
Number	Mean	RSD%	Mean	RSD%
1	52.0	6.8	48.3	8.5
2	59.3	8.4	45.5	20.0
4	66.5	3.4	53.3	7.3
5	60.0	25.9	52.0	21.8
6	60.0	4.9	48.5	4.3
7	70.0	1.7	59.0	23.0
8	79.0	1.7	56.5	17.7
9	69.3	0.7	50.8	4.4
10	57.8	2.2	41.3	20.4

20% of normal subjects were dysphoric after receiving amphetamine. Their subjects also underwent psychological testing that showed more immature Rorschach scores for the dysphoric subjects compared to the euphoric subjects. Angrist and colleagues[88] reported that 2 out of seven subjects showed decreased self-ratings of "happy" after 0.25 mg/kg of dextroamphetamine given orally. They also found that under fasting conditions, 4 subjects had increases in self-rating for "happy", while with food, 6 subjects had increases in self-rating for "happy". Dommisse and associates[102] also found a similar rate of dysphoria following 30 mg of oral dextroamphetamine. Psychological or personality differences between subjects may contribute to the heterogeneity in mood response observed in our study, but this cannot be examined because psychological testing was not performed. It may also be related to the study conditions, such as conducting testing while subjects are fasting and drawing blood samples. Subject 5, who experienced intense dysphoria after the 5

mg dose, was the youngest participant in the study, had no experience with the hospital environment, and had never been a research subject before. Dose seems to play a role as well. Subject 8 became dysphoric only after the highest dose. These observations suggest that the mood response to dextroamphetamine results from a complex interaction between individual sensitivity, the study environment, and the dose administered.

Because of the heterogeneity in the mood response to dextroamphetamine, statistical analysis did not show significant differences between the treatments. The study sample was too small to perform subgroup analysis. The maximum self-rated mood scale scores were statistically significantly higher during the period when subjects believed that they had received the 20 mg dose. The subjects expected to feel euphoric or at least better after receiving dextroamphetamine, perhaps because of previous knowledge about the drug or information provided in the informed consent. Consequently, they ranked the period during which they felt the best as the highest dose level. Subject 2, who experienced euphoria, ranked his treatments correctly. Subject 4 on the other hand, experienced dysphoria and ranked his doses in the inverse order.

The visual analog mood scale showed less consistent responses with dose than the self-rated mood scale. The visual analog mood scale asked subjects to rank on a 100 mm line how they felt between "the worst they had ever felt" and "the best they had ever felt". This is a very broad range, and may have been less sensitive than the self-rated mood scale for examining euphoria. In addition, the subject's score on the visual analog scale may have been influenced by feelings other than those measured

by the self-rated mood scale. The self-rated mood scale is designed to measure euphoria, and very similar scales have been shown to discriminate between dose levels of amphetamine in normal volunteers. The self-rated mood scale may be a more specific indicator of euphoria/dysphoria than the visual analog mood scale. There was no significant effect of sequence in the statistical analysis of the results from both mood scales. It might be expected that the subject's frame of reference for rating mood would change based on the experience of prior study periods, obscuring the interpretation of the mood scale results. In our study, the ratings on the scales do not appear to be greatly influenced by the order in which the treatments were received.

Baseline responses were less variable for the mood rating scales than for the EEG parameters and prolactin levels. They also were not significant covariates in the analysis of covariance. The variability was slightly higher for the visual analog scale than the self-rated mood scale. One measurement before dosing appears to be sufficient to characterize the baseline response during each period. No difference in baseline responses between the first period and subsequent periods was evident, indicating that the familiarization session was adequate for most subjects to feel familiar with the testing environment. Subject 5 is an exception to this generalization. His baseline mood response was quite different in Period 1, when he received the 5 mg dose, than in Period 2, when he received the placebo treatment (a score of 49 versus 71 for the self-rated scale, and 44 versus 60 for the visual analog scale). Although he experienced intense dysphoria during Period 1 and was not aware that he was receiving a placebo in Period 2, his baseline mood scores were much higher

during the second period. His anxiety about participating in the study may have influenced his mood response to dextroamphetamine. The familiarization session, which did not include blood sampling and drug administration, was not sufficient to acclimate the subject to the study environment. He was much more at ease prior to dosing during Period 2 than during Period 1. Administering two blinded placebo treatment periods, one prior receiving the drug under study and the second randomized in with the active treatments, would be useful to minimize this effect.

4.5.1d Computerized Psychometric Tests

4.5.1d(1) Results

Plots of baseline and placebo corrected psychometric test scores (CPT average latency, CPT percent correct, and finger tapping rate for the right and left hand) versus time for each subject are presented in Appendix O. The psychometric test scores plotted at each time point were obtained by subtracting the baseline (0 hr) value for each treatment from the values at later time points for that treatment. The baseline corrected placebo profile was then subtracted from the baseline corrected profiles for the active treatments. Representative plots for these response measures from Subject 1 are presented in Figures 4.36 - 4.39.

A review of these plots for each subject reveals no obvious changes consistent with dextroamphetamine dose for either the CPT measures or the finger tapping task. Changes observed after dosing were small and fluctuated around the baseline for most subjects. The baseline and placebo-corrected, percent-correct on the CPT did not

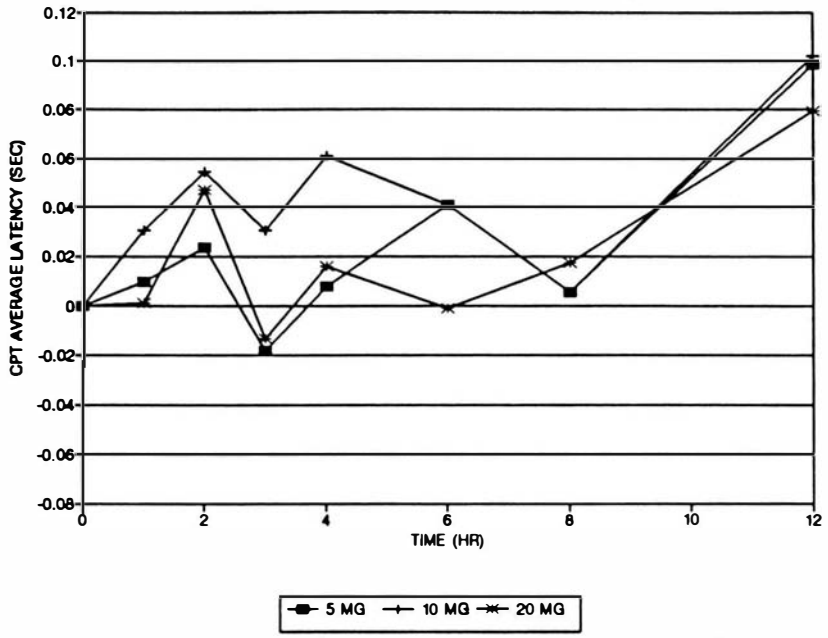

Figure 4.36 Baseline and placebo corrected average latency on the continuous performance task for Subject 1

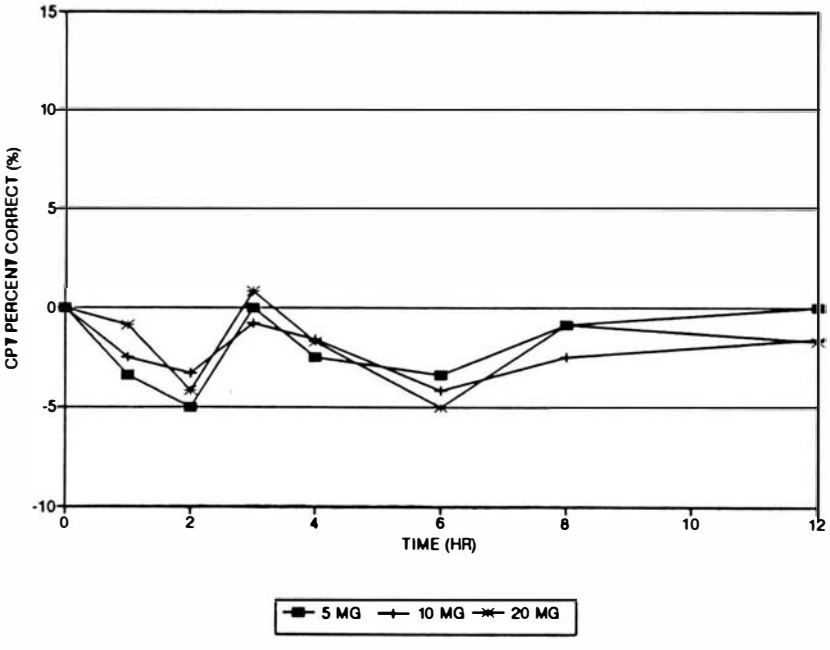

Figure 4.37 Baseline and placebo corrected percent correct on the continuous performance task for Subject 1

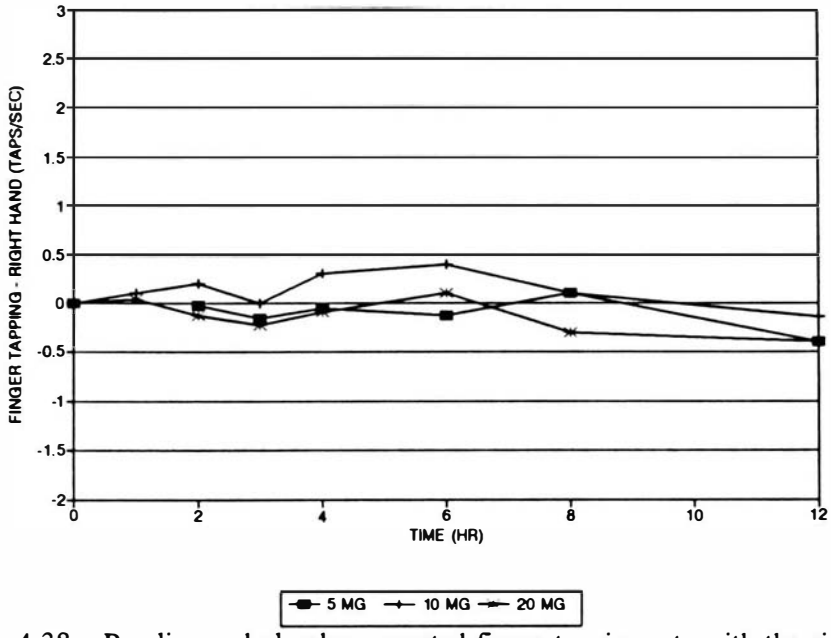

Figure 4.38 Baseline and placebo corrected finger tapping rate with the right hand for Subject 1

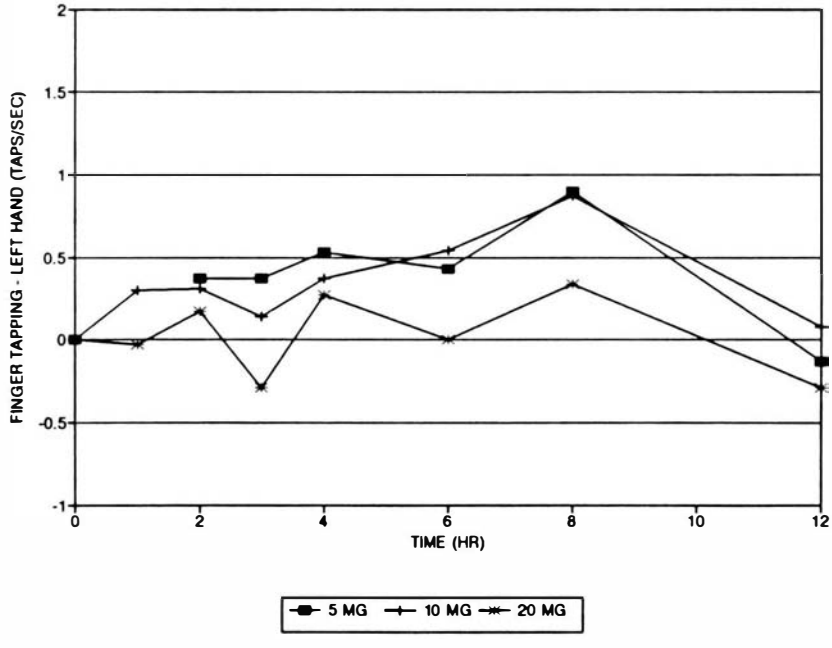

Figure 4.39 Baseline and placebo corrected finger tapping rate with the left hand for Subject 1

increase or decrease by greater than 10% after any of the treatments for any of the subjects. The baseline and placebo corrected average rate of finger tapping did not increase or decrease by more than 3 taps per second for the right hand or 2 taps per second for the left hand for any of the subjects. Statistical analysis of the E_{max} or (E_{min}) and ET values for each response measure showed no significant differences between treatments at the alpha level of 0.05. Estimated power for these statistical tests was 0.45, 0.50, 0.45, and 0.88 for the CPT average latency, the CPT percent correct, the finger tapping right hand, and the finger tapping left hand respectively.

The baseline values were significant covariates in the analysis of covariance for the CPT average latency and the finger tapping (left hand). Average baseline values for the psychometric tests for each subject are presented in Table 4.22. The variability in baseline values between periods was low compared to the other response measures. The CPT average latency was more variable at baseline than the percent correct. Finger tapping with the left hand was more variable than finger tapping with the right hand. Baseline values measured during Period 1 were not noticeably different from those measured during subsequent periods.

4.5.1d(2) Discussion

Based on visual inspection of the baseline and placebo corrected psychometric test response versus time plots and the statistical analysis of the E_{max} (or E_{min}) values for each treatment for each subject, it appears that performance on the psychometric tests was not influenced by the drug treatment administered. This is consistent with the results reported by other investigators (see Section 2.2.7). While some studies

Table 4.22 Average Psychometric Test Scores at Baseline (0 hr) for Each Subject

Subject	Continuous Performance Task				Finger Tapping Task			
	Average Latency (sec)		Percent Correct (%)		Right Hand (taps/sec)		Left Hand (taps/sec)	
	Mean	RSD%	Mean	RSD %	Mean	RSD%	Mean	RSD%
1	0.4071	9.8	99.0	1.1	5.03	1.9	4.38	3.7
2	0.3511	5.3	99.4	0.4	5.71	3.9	4.94	2.7
4	0.3704	6.5	99.3	0.6	6.59	1.2	5.32	2.8
5	0.3603	0.4	99.2	1.2	5.00	5.7	4.40	8.7
6	0.3060	4.1	98.3	1.9	5.08	1.5	4.5	1.6
7	0.2372	7.4	94.2	4.3	6.61	10.6	4.62	5.4
8	0.3315	6.5	99.0	0.8	4.91	18.6	4.23	3.9
9	0.3922	11.3	97.5	3.1	4.95	3.1	4.43	2.2
10	0.3812	5.1	99.6	0.5	4.66	15.2	5.22	10.3

report changes in performance on some tests after subjects receive amphetamine, many others report no differences after drug administration. The computerized tests employed in our testing environment were not able to detect differences between the treatments. The power for the statistical tests for these response measures were relatively low. With a larger sample size, the small differences observed between treatments may have been statistically significant.

Performance on the tasks did not appear to consistently increase or decrease during the dosing intervals. The responses measured as baseline at each period also showed no patterns of increasing or decreasing as the study progressed. This indicates that the practice tests during the familiarization session and prior to baseline measurements during each period were probably sufficient to minimize learning

effects on the test results.

Variability between the baseline measurements for the psychometric tests at each period for each subject were less than those observed for the EEG measures, prolactin levels, and mood scale scores. The average latency was more variable than the percent correct on the CPT. The task was not difficult, and subjects performed it with less than 10% errors after the first few practices leaving little room for improvement. The speed of response as measured by the average latency however is likely to be more variable. Baseline scores for finger tapping with the left hand was more variable than baseline scores for finger tapping with the right hand. All of the subjects in this study were right handed. This may explain why the baseline for the left hand is more variable than the baseline for the right.

These psychometric tests did not prove to be useful for distinguishing the effects of dextroamphetamine on performance. Dextroamphetamine, at the doses studied, may in fact have no effect on elements of performance these tests are intended to measure. Alternatively, the observations may be affected by characteristics of the particular tests administered, the environment in which the tests were given, or characteristics of this subject sample.

4.5.1e Conclusions

A number of response measures were used in this study to assess CNS stimulation. Dose-related EEG changes were observed in only 3 of the subjects. The characteristic that distinguished these subjects from the nonresponders was the level of background alpha activity. Statistical analysis was not useful for comparing

treatments, because there were too few subjects to perform subgroup analysis based on background alpha activity. Future studies of CNS stimulation should exclude volunteers that show less than 35% alpha activity on screening EEG recordings.

Prolactin levels showed dose related changes in the majority of the subjects. In general, prolactin levels decreased after dextroamphetamine administration, with the greatest effect occurring at the lowest dose level. Statistical analysis showed significant differences between placebo and the treatments. The treatments could not be distinguished from each other however. Baseline serum prolactin levels were variable from period to period. Future studies should include more than one measurement of serum prolactin prior to drug administration.

Mood scales also showed a heterogenous response, with some subjects showing euphoria and some showing dysphoria. Dysphoria occurred 20% of the time, which is similar to the rate reported by other investigators. The self-rated mood scale was more useful than the visual analog mood scale for distinguishing between dose levels. The effect on mood may be dependent on dose, since one subject experienced dysphoria only at the highest dose. Another subject experienced intense dysphoria, which may have been related to his anxiety about participating in a study for the first time. Future studies should include two blinded placebo periods, one as the first study period and the second randomized with the active treatments.

The average latency and percent correct on the CPT and the rate of finger tapping with the right and left hand were not useful for distinguishing between the dextroamphetamine dose levels. This result may indicate that amphetamine does not affect attention and motor performance. Alternatively, it may indicate that these

particular tasks administered in our study environment to our study population were not sensitive enough to measure the performance changes that occurred.

The sensitivity, reproducibility, and suitability of each of these tests for pharmacodynamic studies of CNS stimulation will be compared and discussed in Chapter 5.

4.5.2 Cardiovascular Response Measures

The results for blood pressure and heart rate are presented and discussed in the following sections. These responses were measured both for subject safety and as an assessment of stimulant effects outside of the central nervous system. Subject 3 are not presented because he did not receive a placebo. His data was included in the statistical analysis however.

4.5.2a Blood Pressure

4.5.2a(1) Results

Plots of baseline and placebo corrected systolic and diastolic blood pressure versus time for each subject are presented in Appendix P. The blood pressure values plotted at each time point were obtained by subtracting the baseline (0 hr) value for each treatment from the values at later time points for that treatment. The baseline corrected placebo profile was then subtracted from the baseline corrected profiles for the active treatments. Representative plots for these response measures from Subject 2 are presented in Figures 4.40 - 4.41.

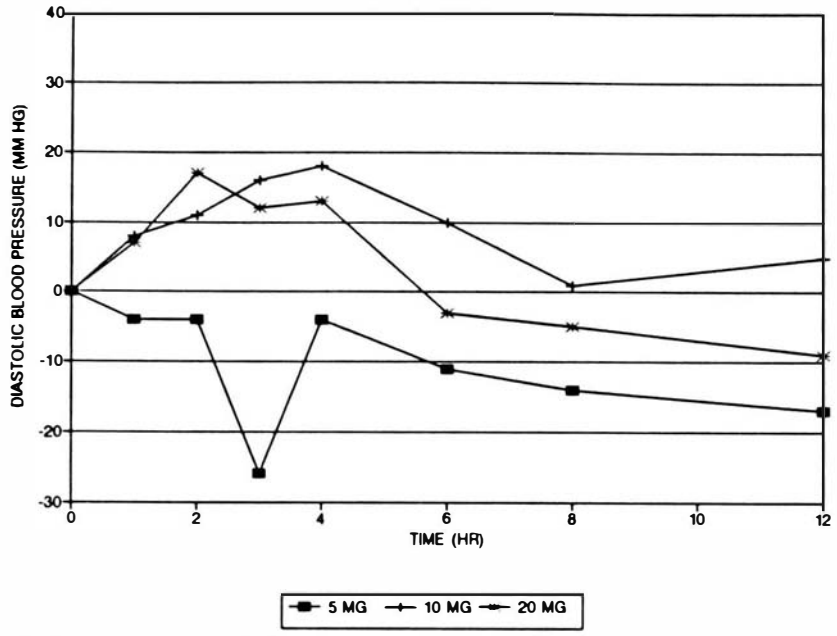

Figure 4.40 Baseline and placebo corrected diastolic blood pressure versus time for Subject 2

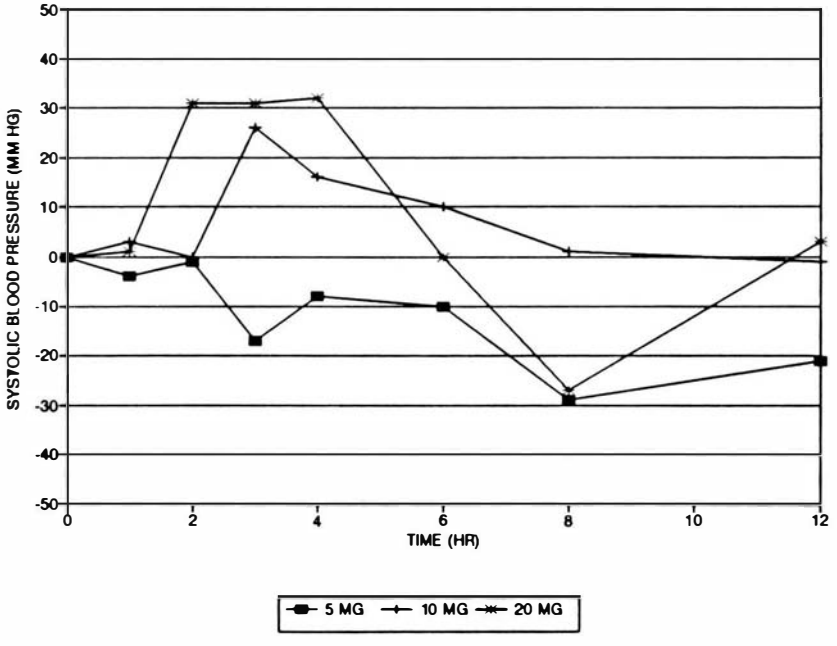

Figure 4.41 Baseline and placebo corrected systolic blood pressure versus time for Subject 2

A review of these plots for each individual indicates that in general, both systolic and diastolic blood pressure rises after the administration of dextroamphetamine. The average increase in the baseline and placebo corrected diastolic blood pressure was 12.2, 13.6, and 16.5 mmHg after the 5, 10, and 20 mg doses respectively. The average increase in the baseline and placebo corrected systolic blood pressure was 15.3 mmHg after the 5 mg dose, 19.1 mmHg after the 10 mg dose, and 29.5 mmHg after the 20 mg dose. The magnitude and duration of the effect varies among individuals.

Statistical analysis of the E_{max} values for diastolic blood pressure showed a significant treatment effect ($p < 0.05$). Power for this statistical analysis was high compared to the analysis of the CNS measures; power was 0.97 for diastolic blood pressure. Statistical comparison of the treatments showed the 10 and 20 mg doses were significantly different than placebo and the 5 mg dose was significantly different from the 20 mg dose. The 5 mg dose was not different from the 10 mg dose, and the 10 mg dose was not different from the 20 mg dose. Statistical analysis of the ET (defined in Section 4.2.4c) values for diastolic blood pressure revealed no significant difference between treatments.

Statistical analysis of the E_{max} values for systolic blood pressure revealed no significant differences between the treatments. The estimated power for this comparison was low (0.6) however. The variability between subjects in systolic blood pressure was greater than for diastolic blood pressure. Baseline systolic blood pressure was also a significant covariate in the analysis of covariance. As with diastolic blood pressure, statistical analysis of the ET values for systolic blood

pressure revealed no significant differences between treatments.

Average baseline values for diastolic and systolic blood pressure for each subject are presented in Table 4.23. The RSD% for baseline blood pressure was less than 20% for all subjects, but for most of the subjects, it was less than 10%. The baseline diastolic blood pressure was a significant covariate in the analysis of covariance. In general, subjects with higher baseline blood pressure showed a lower blood pressure increase.

No consistent pattern of hysteresis is present in plots of baseline and placebo corrected blood pressure versus serum amphetamine concentration measured at the same time. Representative plots from Subject 4 are presented in Figures 4.42 - 4.44.

4.5.2a(2) Discussion

The increase in blood pressure observed in this study is similar to the reported by other investigators (see Section 2.2.8). There was a statistically significant difference in maximum diastolic blood pressure between the treatments, with the largest increase observed for the highest dose level. The rise in systolic blood pressure was more variable between subjects, resulting in lower power for the statistical comparison of treatments. A larger sample size would be necessary to distinguish the treatments based on maximum systolic blood pressure.

The highest diastolic blood pressures observed during the study were 102, 94, and 90 mmHg following the 20 mg dose for Subjects 2, 9, and 4 respectively. These blood pressures were observed 2 to 3 hours after dosing. Blood pressure remained above 90 mmHg for no longer than 1 to 2 hours. Blood pressure increases were not

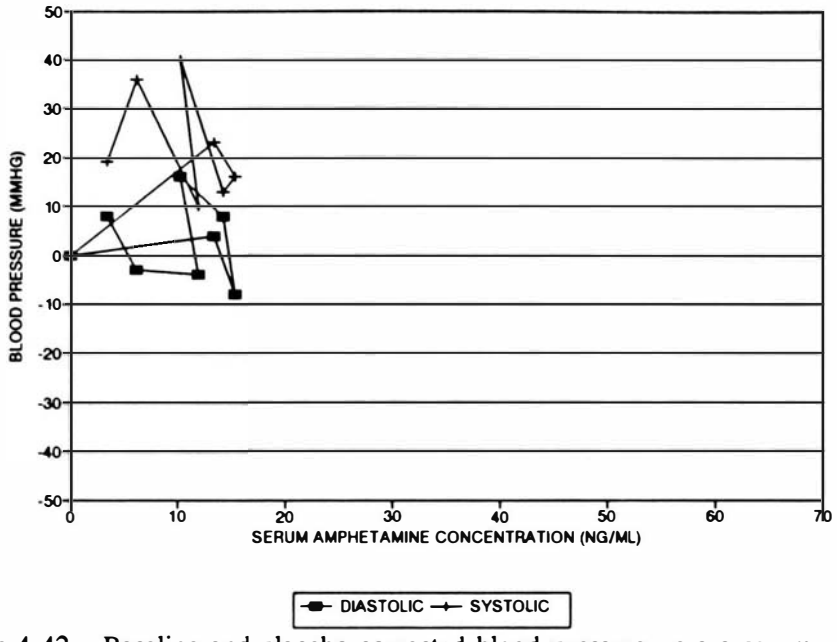

Figure 4.42 Baseline and placebo corrected blood pressure versus serum amphetamine concentration for Subject 4 (5 mg dose)

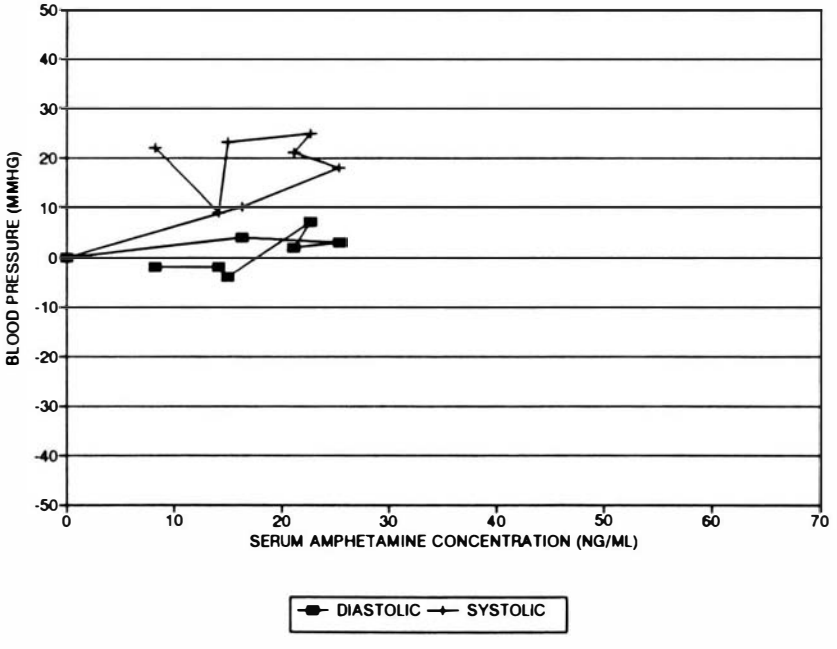

Figure 4.43 Baseline and placebo corrected blood pressure versus serum amphetamine concentration for Subject 4 (10 mg dose)

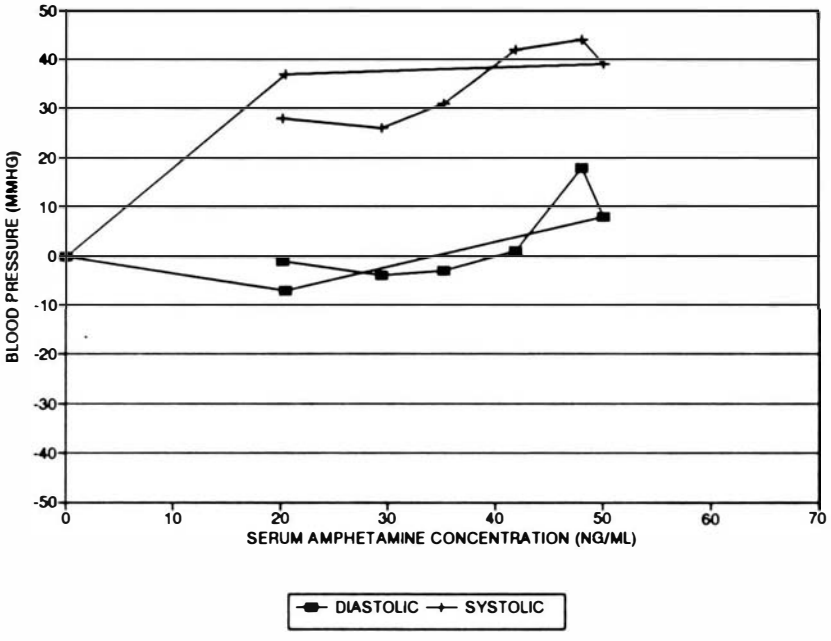

Figure 4.44 Baseline and placebo corrected blood pressure versus serum amphetamine concentration for Subject 4 (20 mg dose)

of sufficient magnitude or duration to require medical intervention for any of the subjects.

Table 4.23 Average Blood Pressure at Baseline (0 hr) for Each Subject

Subject	Diastolic Blood Pressure (mmHg)		Systolic Blood Pressure (mmHg)	
	Mean	RSD%	Mean	RSD%
1	70.5	4.7	125.3	8.7
2	70.0	11.7	116.0	12.9
4	71.3	5.4	127.0	6.4
5	74.0	13.4	122.5	15.6
6	69.0	9.8	131.0	8.9
7	66.5	2.6	128.8	4.1
8	60.3	5.7	125.8	6.3
9	80.3	11.4	136.8	11.0
10	69.3	7.2	112.3	5.5

Baseline blood pressure was a significant covariate in the analysis of covariance, indicating that the baseline blood pressure accounts for a significant amount of the variability in response that was observed. In general, subjects with lower baseline blood pressures showed a higher increase in blood pressure. The variability in baseline values between periods was relatively low, and did not seem to be associated with the period in which it was measured. Baseline blood pressure during Period 1 was not consistently higher or lower than during other periods. One exception to this generalization may be Subject 5, who was withdrawn from the study due to an intense dysphoric reaction. His baseline diastolic blood pressure was higher during Period 1

(81 mmHg) when he experienced dysphoria after the 5 mg dose than during Period 2 (67 mmHg) when he received the placebo. At baseline during Period 1, his blood pressure was elevated and his mood scale scores were lower. His baseline psychological and physical state may have contributed to his dysphoric episode.

Because baseline blood pressure levels contribute to the blood pressure response observed, adequate characterization of the baseline levels is essential. In this study, all measurements were made after subjects had been supine for at least 5 minutes. Taking more than one baseline measurement may improve the determination of the baseline response.

4.5.2b Heart Rate

4.5.2b(1) Results

Plots of baseline and placebo corrected heart rate versus time for each subject are presented in Appendix Q. The heart rate values plotted at each time point were obtained by subtracting the baseline (0 hr) value for each treatment from the values at later time points for that treatment. The baseline corrected placebo profile was then subtracted from the baseline corrected profiles for the active treatments. A representative plot for this response measure from Subject 2 is presented in Figure 4.45.

A review of these plots for each individual indicates that, in general, heart rate increases after the administration of dextroamphetamine. The average increase in the baseline and placebo corrected heart rate was 10.3, 16.4, and 23.1 bpm after the 5, 10, and 20 mg doses respectively. The magnitude and duration of the effect varies

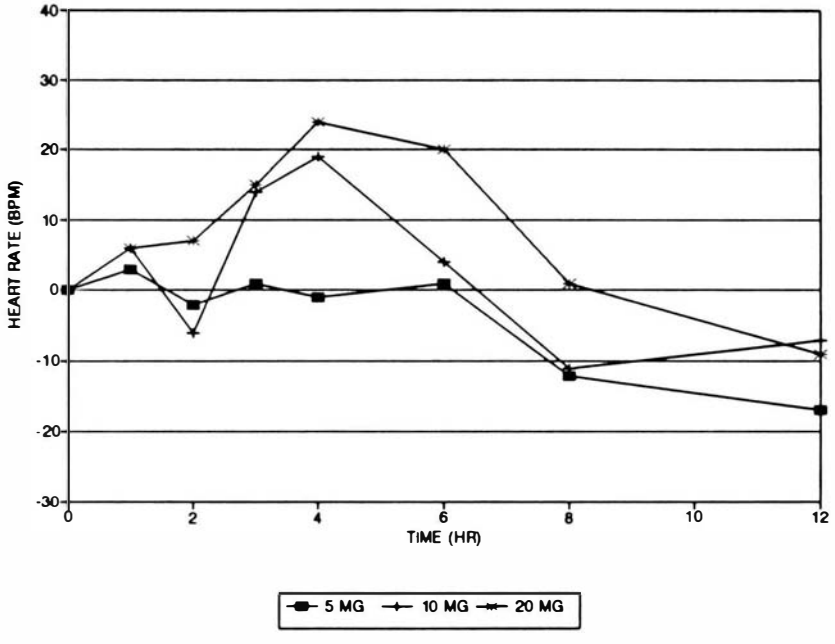

Figure 4.45 Baseline and placebo corrected heart rate versus time for Subject 2

among individuals, but appeared to increase as the dose level increased.

Statistical analysis of the E_{max} values for heart rate showed a significant treatment effect ($p < 0.05$). Power for this statistical analysis was similar to that observed for diastolic blood pressure; power was 0.90 for heart rate. Statistical comparison of the treatments showed the 10 and 20 mg doses were significantly different than placebo and the 5 mg dose was significantly different from the 20 mg dose. The 5 mg dose was not different from the 10 mg dose, and the 10 mg dose was not different from the 20 mg dose. The baseline heart rate was a significant covariate in the analysis of covariance.

Statistical analysis of the ET values for heart rate also revealed significant differences between treatments. The average ET for each treatment was 3.8, 3.9, 5.8, and 9.1 for the placebo, 5 mg, 10 mg, and 20 mg dose levels respectively. Comparison of the treatments showed that response after the 20 mg dose was significantly different than the 5 mg dose and placebo. Other treatments were not statistically different from each other. Power for this statistical analysis was 0.96.

Average baseline values for heart rate for each subject are presented in Table 4.24. The RSD% for baseline blood pressure was less than 12% for all subjects. Average baseline heart rate ranged from 46 to 64 bpm. Plots of baseline and placebo corrected heart rate versus serum amphetamine concentration measured at the same time demonstrated no consistent pattern of hysteresis. Representative plots from Subject 9 are presented in Figures 4.46 - 4.48.

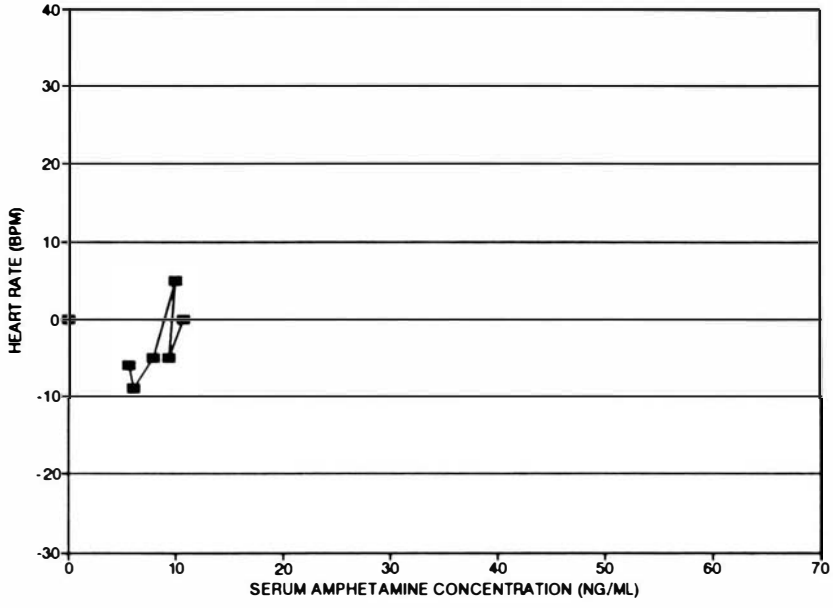

Figure 4.46 Baseline and placebo corrected heart rate versus serum amphetamine concentration for Subject 9 (5 mg dose)

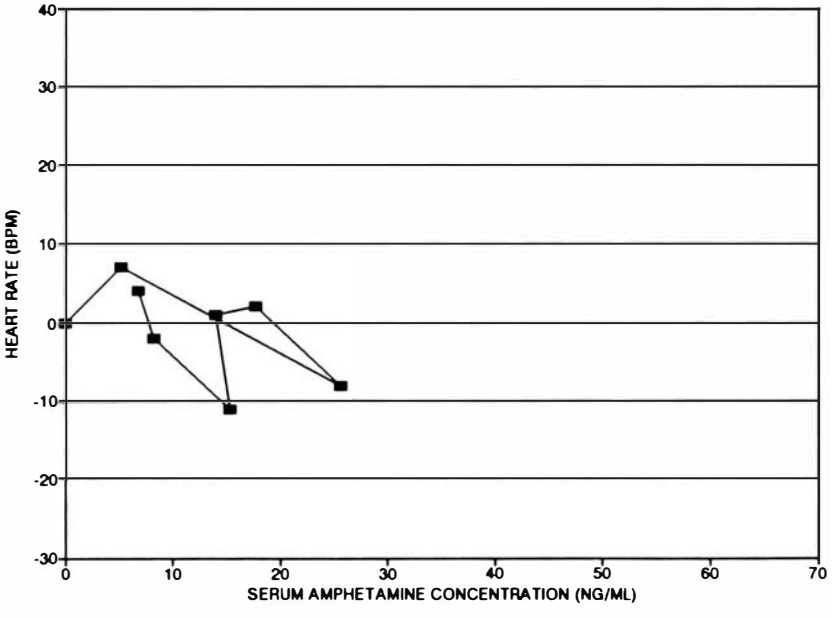

Figure 4.47 Baseline and placebo corrected heart rate versus serum amphetamine concentration for Subject 9 (10 mg dose)

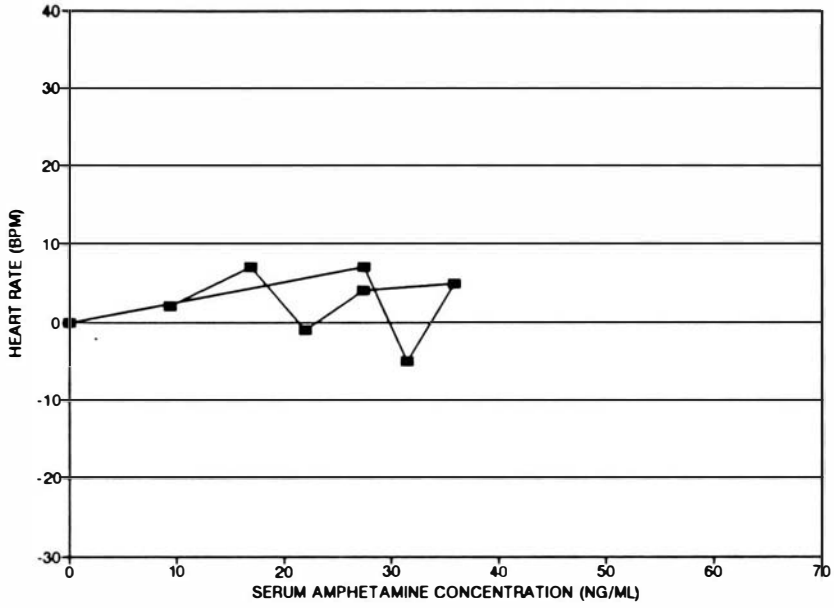

Figure 4.48 Baseline and placebo corrected heart rate versus serum amphetamine concentration for Subject 9 (20 mg dose)

Table 4.24 Average Heart Rate at Baseline (0 hr) for Each Subject

Subject	Heart Rate (bpm)	
	Mean	RSD%
1	55.5	4.3
2	54.5	10.0
4	58.8	10.1
5	53.0	10.7
6	46.5	4.1
7	62.5	11.4
8	46.3	9.9
9	64.0	4.2
10	45.8	5.8

4.5.2b(2) Discussion

In this study, an increase in heart rate was observed for most subjects. Other investigators have reported both increases and decreases in heart rate after dextroamphetamine administration (see Section 2.2.8). There was a statistically significant difference in maximum heart rate between the treatments, with the largest increase observed for the highest dose level. None of the subjects experienced an elevation of heart rate that would be considered tachycardia (> 100 bpm).

The ET was also greater as the dose increased. ET is an indicator of the length of time that the response measure could distinguish a drug effect, weighted toward the later times (and lower concentrations). Heart rate is the only response measure for which there was a statistically significant treatment effect for ET.

Baseline heart rate was a significant covariate in the analysis of covariance, indicating that the baseline heart rate accounts for a significant amount of the

variability in response that was observed. The variability in baseline values between periods for each subject was relatively low, and did not seem to be associated with the period in which it was measured. Baseline heart rate during Period 1 was not consistently higher or lower than during other periods. Baseline heart rate did not appear to be correlated with the magnitude of change in heart rate after dextroamphetamine dosing. As with blood pressure, adequate determination of the baseline heart rate is essential.

4.5.2c Conclusions

Blood pressure and heart rate increased after the administration of dextroamphetamine for most subjects at most dose levels. The increases were not of sufficient magnitude or duration to pose significant risk to the subjects. Only one subject briefly experienced a diastolic blood pressure greater than 100 mmHg.

The maximum blood pressure and heart rate observed was greater as the dose increased. The was a statistically significant treatment effect for diastolic blood pressure and heart rate. The 10 and 20 mg doses were significantly different than placebo and the 5 mg dose was significantly different from the 20 mg dose. The 5 mg dose was not different from the 10 mg dose, and the 10 mg dose was not different from the 20 mg dose.

Adequate characterization of the baseline heart rate and blood pressure is important. Baseline levels contribute to the variability in response observed. In future studies, more than one measurement of heart rate and blood pressure before dosing would be beneficial in establishing the baseline response.

CHAPTER 5

Comparison of Pharmacodynamic Response Measures

In this chapter, the pharmacodynamic response measures used in this study are compared. The relationships between responses on different measures for each subject are examined. The characteristics of each measure are evaluated relative to those an ideal pharmacodynamic measure, including sensitivity, reproducibility, and clinical relevance.

5.1 Results

The direction of change in baseline and placebo corrected response after dextroamphetamine dosing for each response measure at each dose level for each subject is presented in Table 5.1. A review of the data in this table reveals few obvious relationships among the response measures. Increased EEG alpha activity is observed in 3 of the 9 subjects. No other response measures (mood scales, serum prolactin, or psychometric tests), show a similar pattern of response. Serum prolactin decreases in a majority of the subjects, and this change does not directly reflect EEG activity, mood, or psychomotor performance. Mood scales show a dichotomous response. Some subjects experience euphoria and some dysphoria at a given dose level. Subjects with dysphoria do not appear to have unique responses on

Table 5.1 Comparison of Subject Responses on Each Pharmacodynamic Measure

Subj	Dose	EEG Total Alpha Power	Serum Prolactin Level	Self-Rated Mood Scale	Visual Analog Mood Scale	CPT Average Latency	CPT Percent Correct	Finger Tap - Right	Finger Tap - Left	BP	Heart Rate
1	5	↔	↓	↔	↔	↑	↓	↔	↑	↑	↑
	10	↔	↓	↔	↔	↑	↓	↔	↑	↑	↑
	20	↔	↓	↑	↔	↑	↓	↔	↔	↑	↑
2	5	↔	↓	↔	↔	↔	↔	↓	↑	↓	↔
	10	↔	↓	↑	↑	↓	↔	↔	↑	↑	↑
	20	↔	↓	↑	↑	↑	↔	↔	↑	↑	↑
4	5	↔	↓	↓	↔	↓	↔	↔	↑	↑	↑
	10	↑	↓	↓	↔	↓	↔	↔	↑	↑	↑
	20	↑	↓	↓	↔	↓	↔	↔	↑	↑	↑
5	5	–	–	↓	↓	↔	↔	↔	↔	↓	↑
	10	–	–	–	–	–	–	–	–	–	–
	20	–	–	–	–	–	–	–	–	–	–
6	5	↑	↓	↔	↔	↔	↔	↔	↔	↑	↑
	10	↑	↓	↑	↔	↔	↔	↔	↑	↑	↑
	20	↑	↔	↑	↑	↓	↑	↔	↑	↑	↑
7	5	–	↓	↔	↑	↑	↑	↓	↑	↔	↑
	10	↔	↓	↔	↑	↑	↑	↓	↑	↑	↑
	20	↔	↔	↔	↑	↓	↔	↓	↑	↑	↑
8	5	↔	↔	↔	↔	↔	↔	↑	↑	↔	↑
	10	↑	↔	↔	↔	↔	↔	↑	↑	↔	↓
	20	↔	↔	↓	↓	↔	↔	↑	↑	↔	↑
9	5	↔	↔	↔	↔	↑	↓	↔	↑	↓	↔
	10	↔	↓	↔	↑	↑	↓	↔	↑	↑	↔
	20	↔	↔	↑	↑	↑	↓	↔	↑	↑	↔
10	5	↔	↔	↑	↓	↔	↔	↑	↑	↑	↔
	10	↔	↔	↑	↓	↑	↔	↑	↑	↑	↑
	20	↔	↔	↑	↑	↔	↔	↑	↑	↑	↑

other pharmacodynamic measures that could be used to predict the direction of the mood response. In general, the psychometric tests do not show consistent changes across subjects and doses, except that finger tapping rate with the left hand increases in most subjects. Heart rate and blood pressure increase for most subjects, but the cardiovascular changes do not mirror changes in any of the CNS response measures.

To compare the time course of the pharmacologic effects as measured by each pharmacodynamic measure for individual subjects, plots of baseline and placebo corrected response after the 20 mg dextroamphetamine dose versus time were prepared. These are presented in Figures 5.1 - 5.8. The first panel in each plot shows the serum amphetamine concentration for those time points were response measures were made on the left y-axis (-■-) and serum prolactin concentration (-+-) on the right y-axis. The second panel shows EEG total alpha power on the left y-axis (-■-) and self-rated mood scale score on the right y-axis (-+-). The third panel shows CPT average latency on the left y-axis (-■-) and finger tapping rate with the left hand on right y -axis (-+-). The fourth panel shows diastolic blood pressure on the left y-axis (-■-) and heart rate on the right y-axis (-+-). Examination of these plots shows that serum prolactin levels usually peak earlier than or at the same time as the serum amphetamine concentrations. Mood scale scores generally reach their maximum change in either direction before or coincident with the peak serum amphetamine concentrations. When EEG alpha power increases, the time of maximum response does not appear to relate to the time of the maximum serum amphetamine concentration. Maximum diastolic blood pressure occurs before the maximum heart rate, with the peak heart rate usually occurring later than the

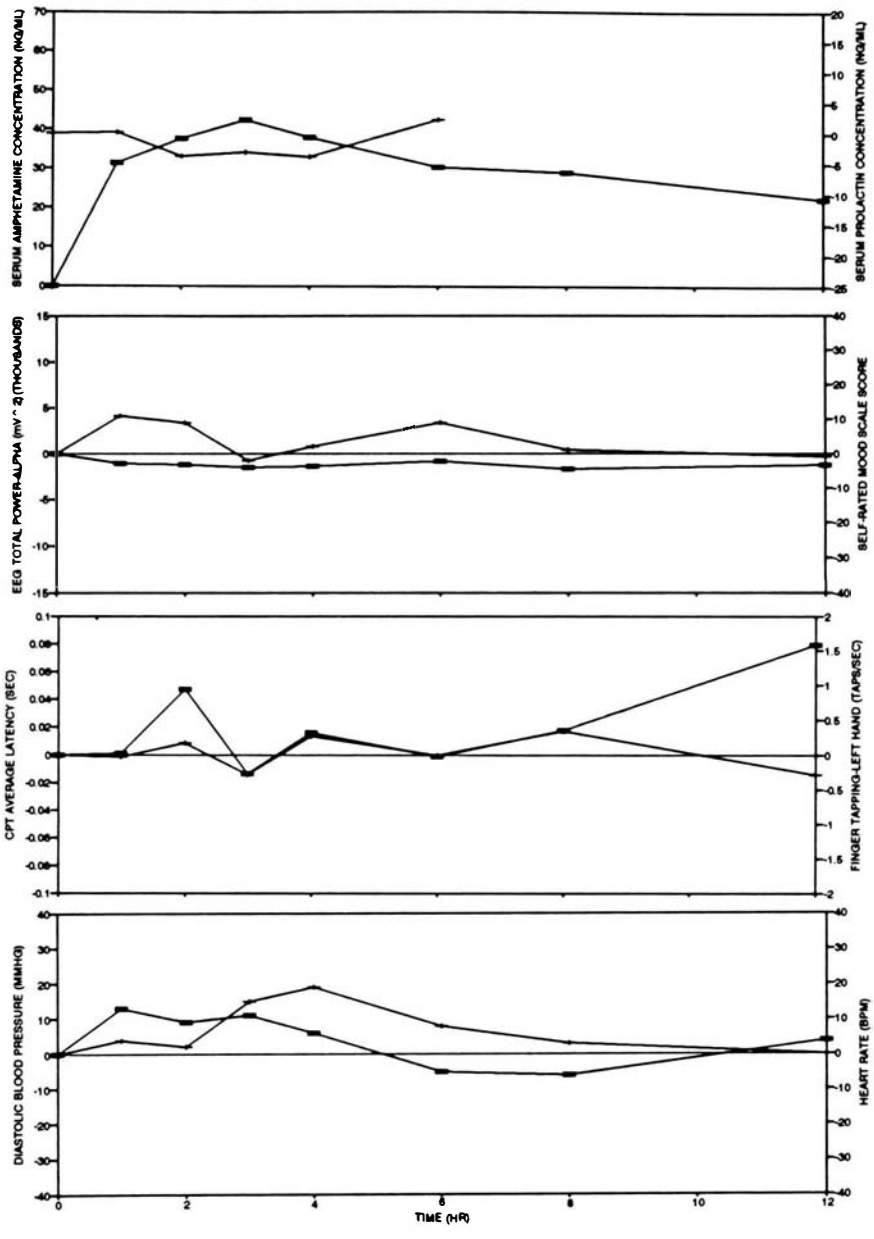

Figure 5.1 Serum amphetamine concentration versus time and baseline and placebo corrected pharmacodynamic responses versus time plots after the 20 mg dose for Subject 1. (-■- refers to left y-axis and -+- refers to right y-axis)

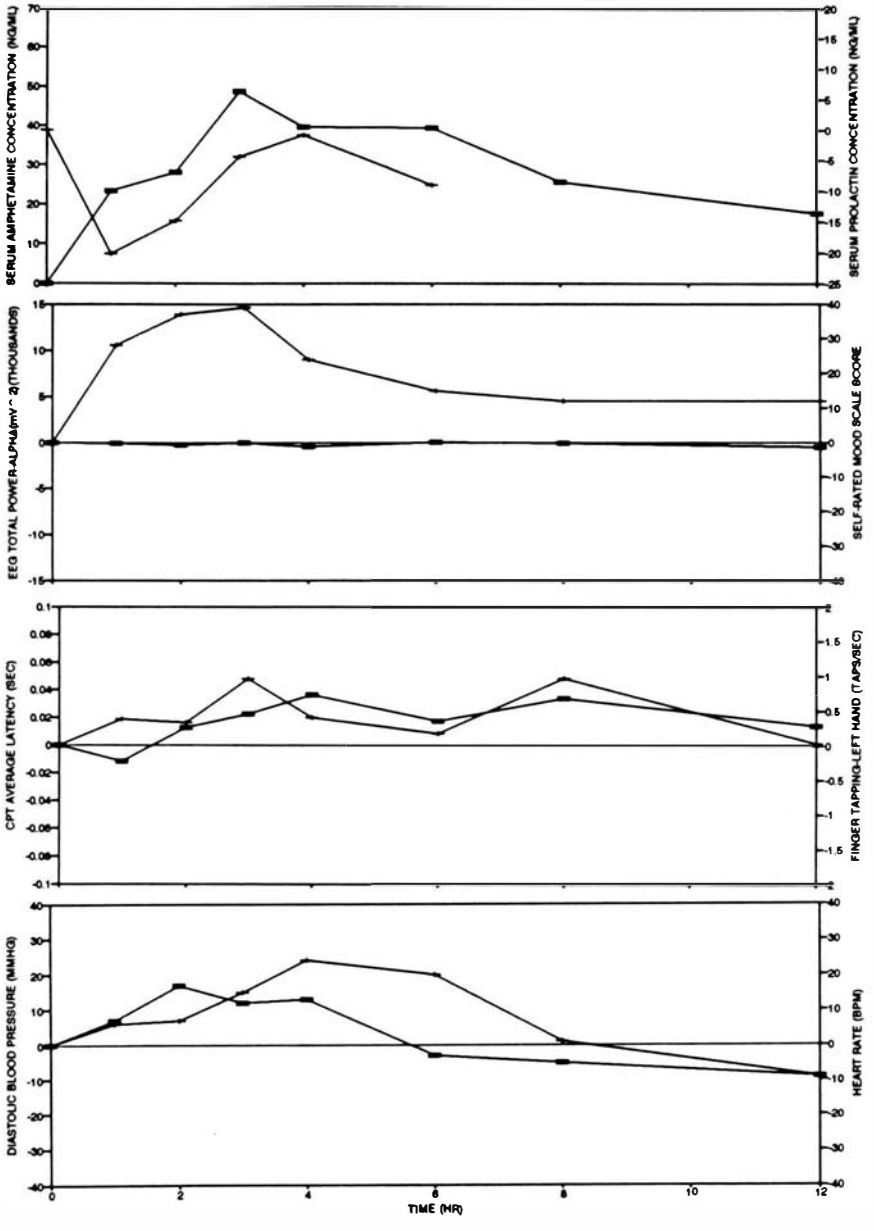

Figure 5.2 Serum amphetamine concentration versus time and baseline and placebo corrected pharmacodynamic responses versus time plots after the 20 mg dose for Subject 2. (-■- refers to left y-axis and -+- refers to right y-axis)

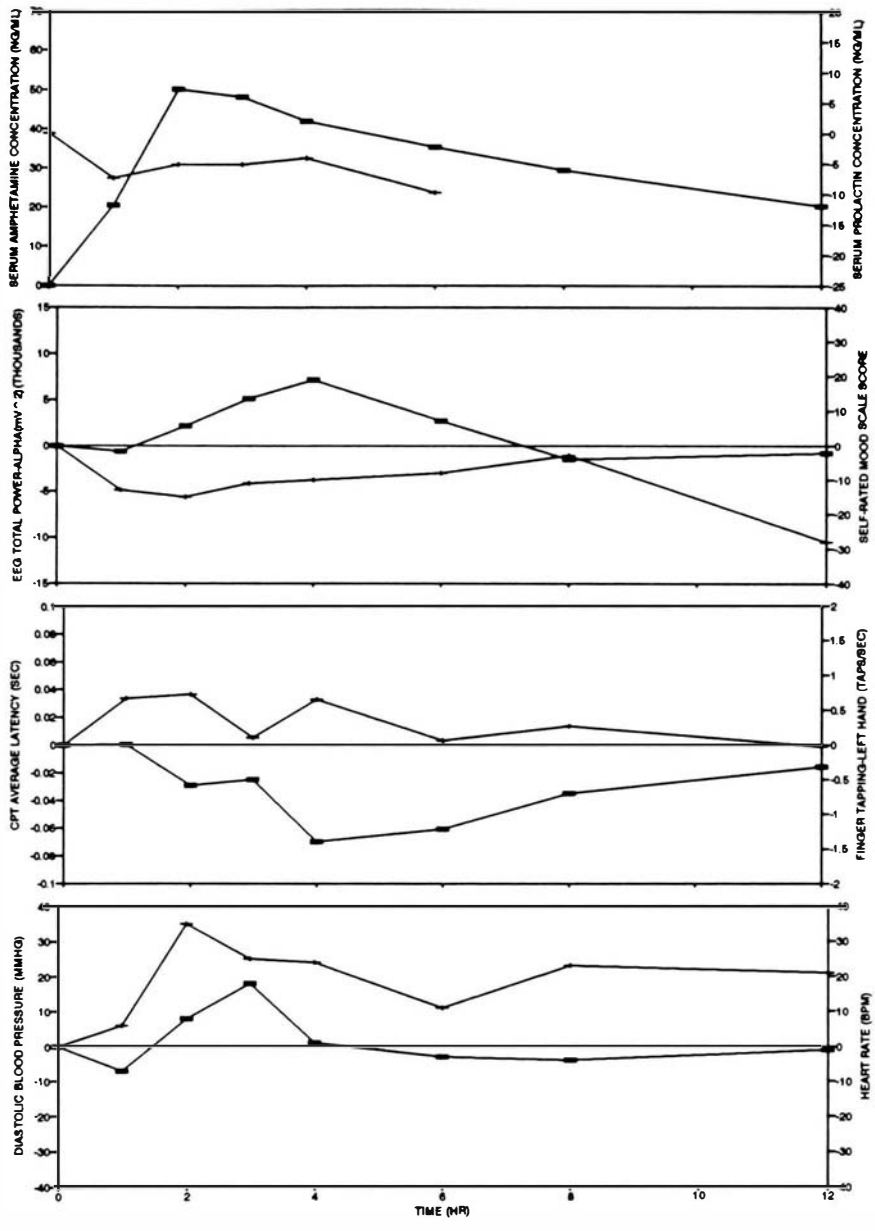

Figure 5.3 Serum amphetamine concentration versus time and baseline and placebo corrected pharmacodynamic responses versus time plots after the 20 mg dose for Subject 4. (-■- refers to left y-axis and -+- refers to right y-axis)

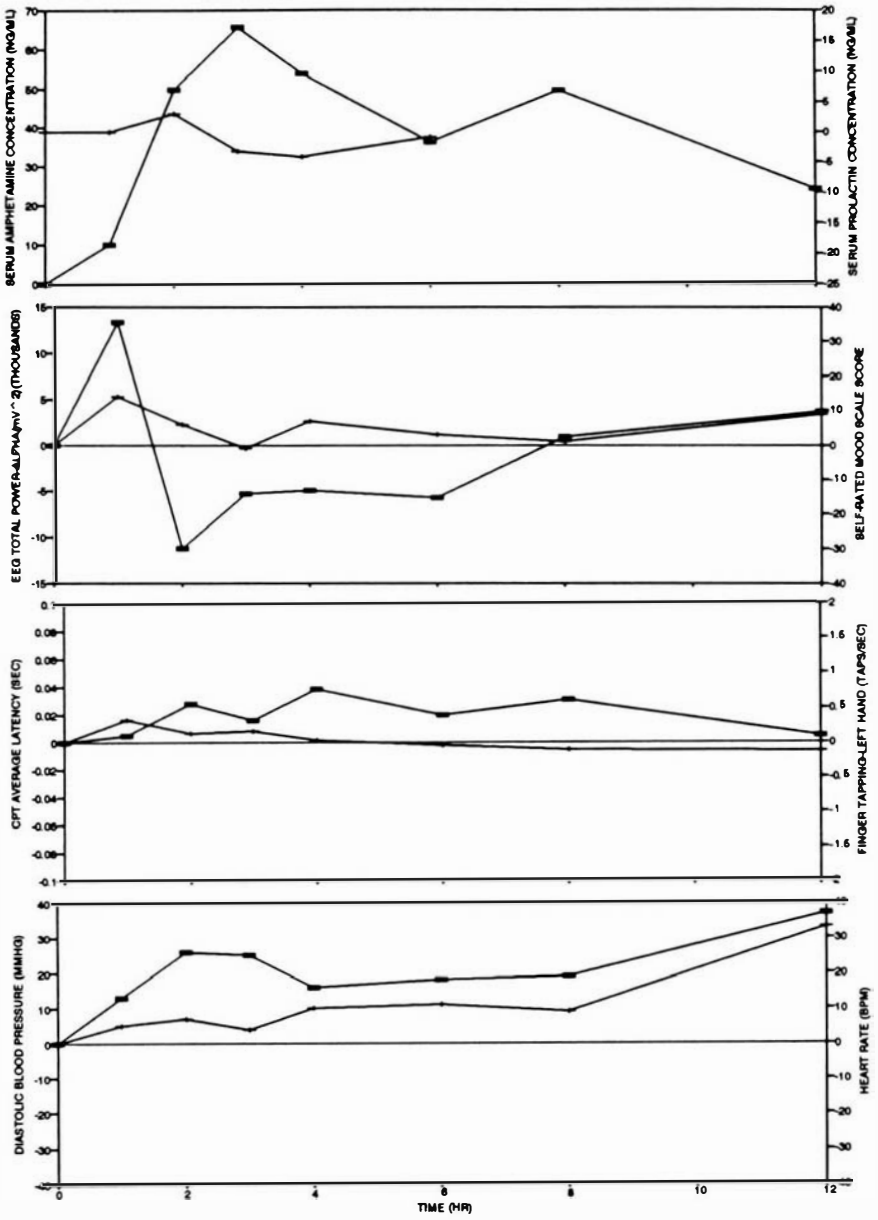

Figure 5.4 Serum amphetamine concentration versus time and baseline and placebo corrected pharmacodynamic responses versus time plots after the 20 mg dose for Subject 6. (-■- refers to left y-axis and -+- refers to right y-axis)

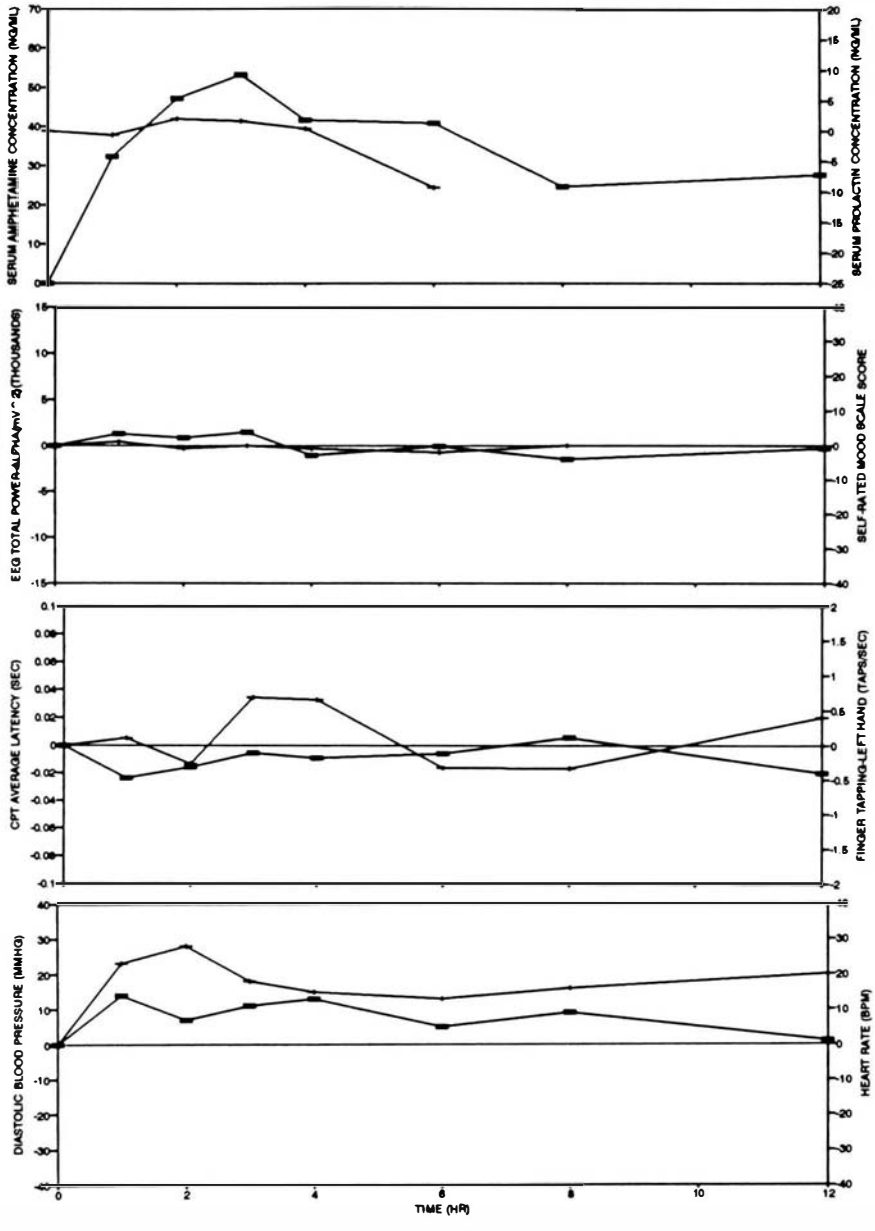

Figure 5.5 Serum amphetamine concentration versus time and baseline and placebo corrected pharmacodynamic responses versus time plots after the 20 mg dose for Subject 7. (-■- refers to left y-axis and -+- refers to right y-axis)

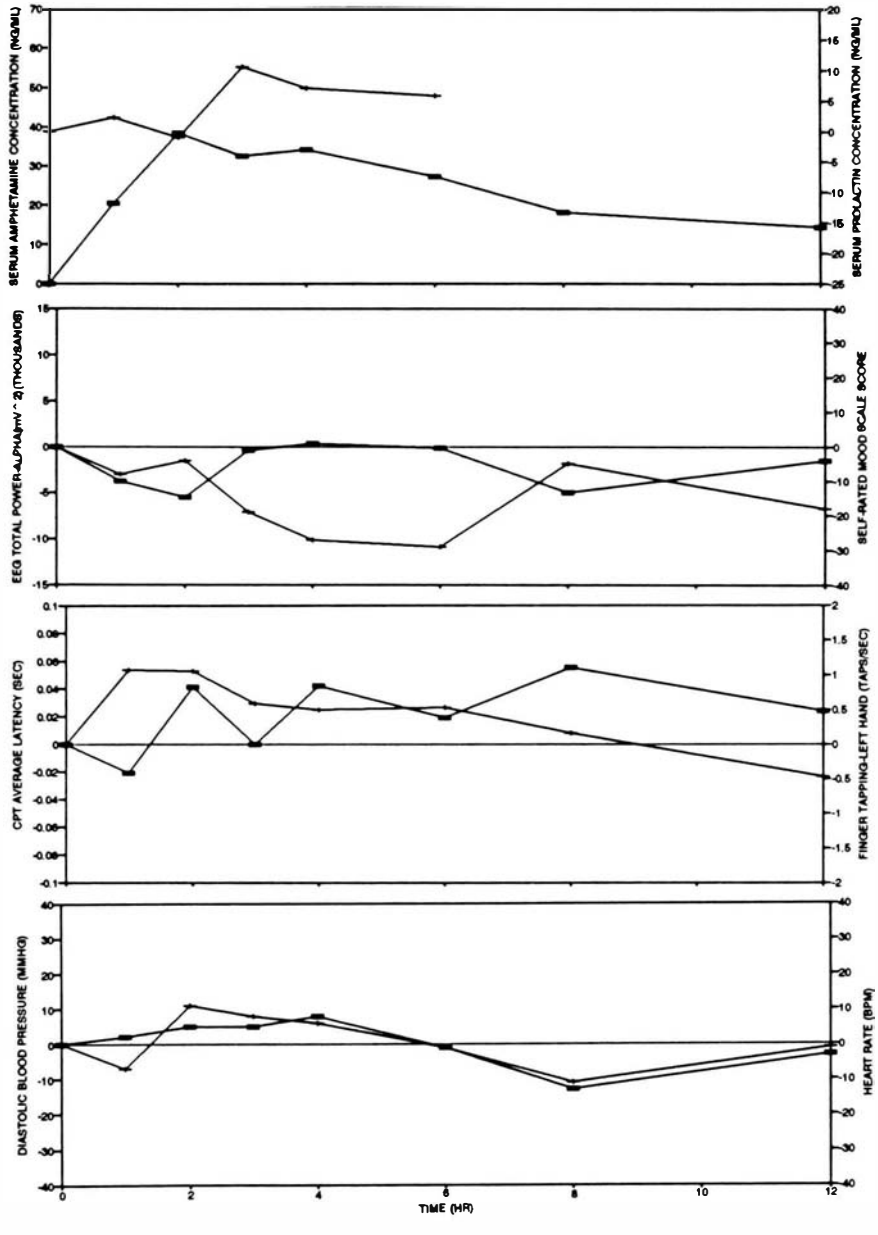

Figure 5.6 Serum amphetamine concentration versus time and baseline and placebo corrected pharmacodynamic responses versus time plots after the 20 mg dose for Subject 8. (-■- refers to left y-axis and -+- refers to right y-axis)

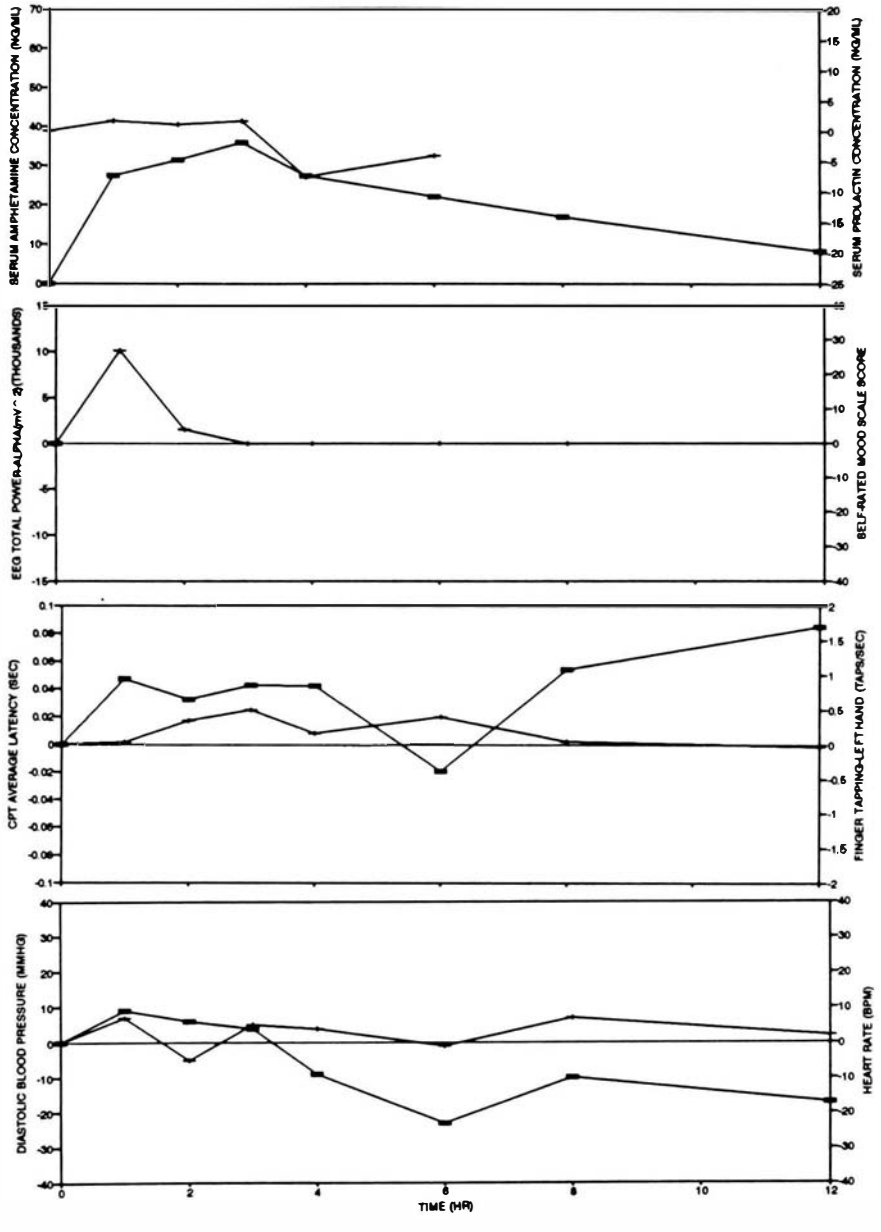

Figure 5.7 Serum amphetamine concentration versus time and baseline and placebo corrected pharmacodynamic responses versus time plots after the 20 mg dose for Subject 9. (-■- refers to left y-axis and -+- refers to right y-axis)

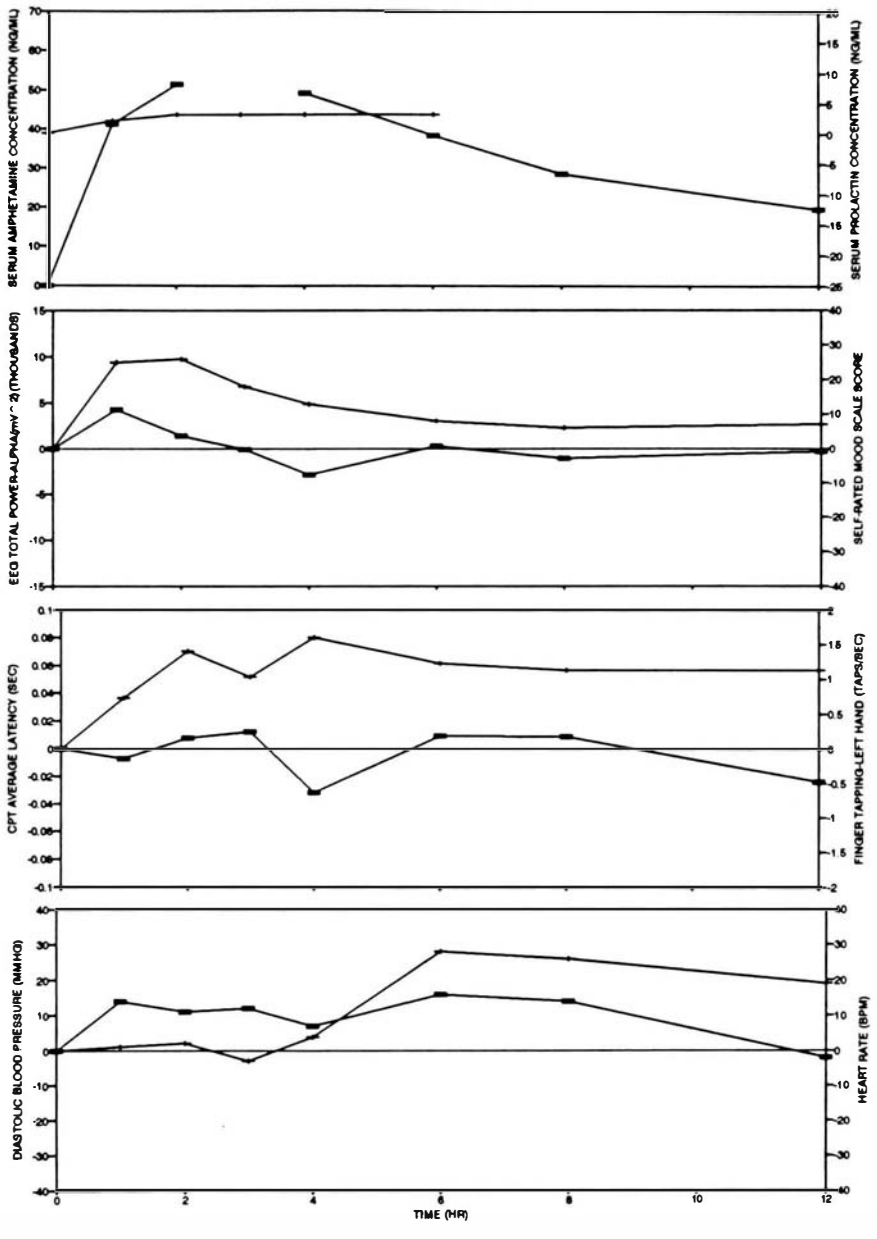

Figure 5.8 Serum amphetamine concentration versus time and baseline and placebo corrected pharmacodynamic responses versus time plots after the 20 mg dose for Subject 10. (-■- refers to left y-axis and -+- refers to right y-axis)

maximum serum amphetamine concentration is achieved.

To examine the relationship between maximum pharmacodynamic response and maximum serum amphetamine concentration, the maximum (or minimum) baseline and placebo corrected response observed after the 20 mg dextroamphetamine dose was plotted against the maximum serum amphetamine concentration. These plots are presented in Figure 5.9. The first row of plots shows the maximum serum prolactin verses the maximum serum amphetamine concentration (left) and maximum EEG alpha power versus maximum serum amphetamine concentration (right). The second row of plots shows the maximum absolute change (increase or decrease) in self-rate mood scale versus maximum serum amphetamine concentration (left) and the minimum CPT average latency versus maximum serum amphetamine concentration (right). The third row shows the maximum finger tapping rate with the left hand (left) and maximum diastolic blood pressure (right) versus maximum serum amphetamine concentration. The fourth row shows the maximum heart rate (left) versus maximum serum amphetamine concentration. The correlations between the maximum CNS response and maximum serum amphetamine concentration are relatively low for most measures. Correlation coefficients of 0.07, 0.34, 0.41, and 0.04 were obtained for serum prolactin levels, mood scale scores, CPT average latency, and finger tapping with the left hand. Correlation with the EEG alpha power was the highest of the CNS measures, with $r = 0.83$. Correlation for the cardiovascular measures was higher than for the CNS measures, with $r = 0.91$ for diastolic blood pressure and $r = 0.87$ for heart rate.

Because this project was designed to evaluate the sensitivity and utility of

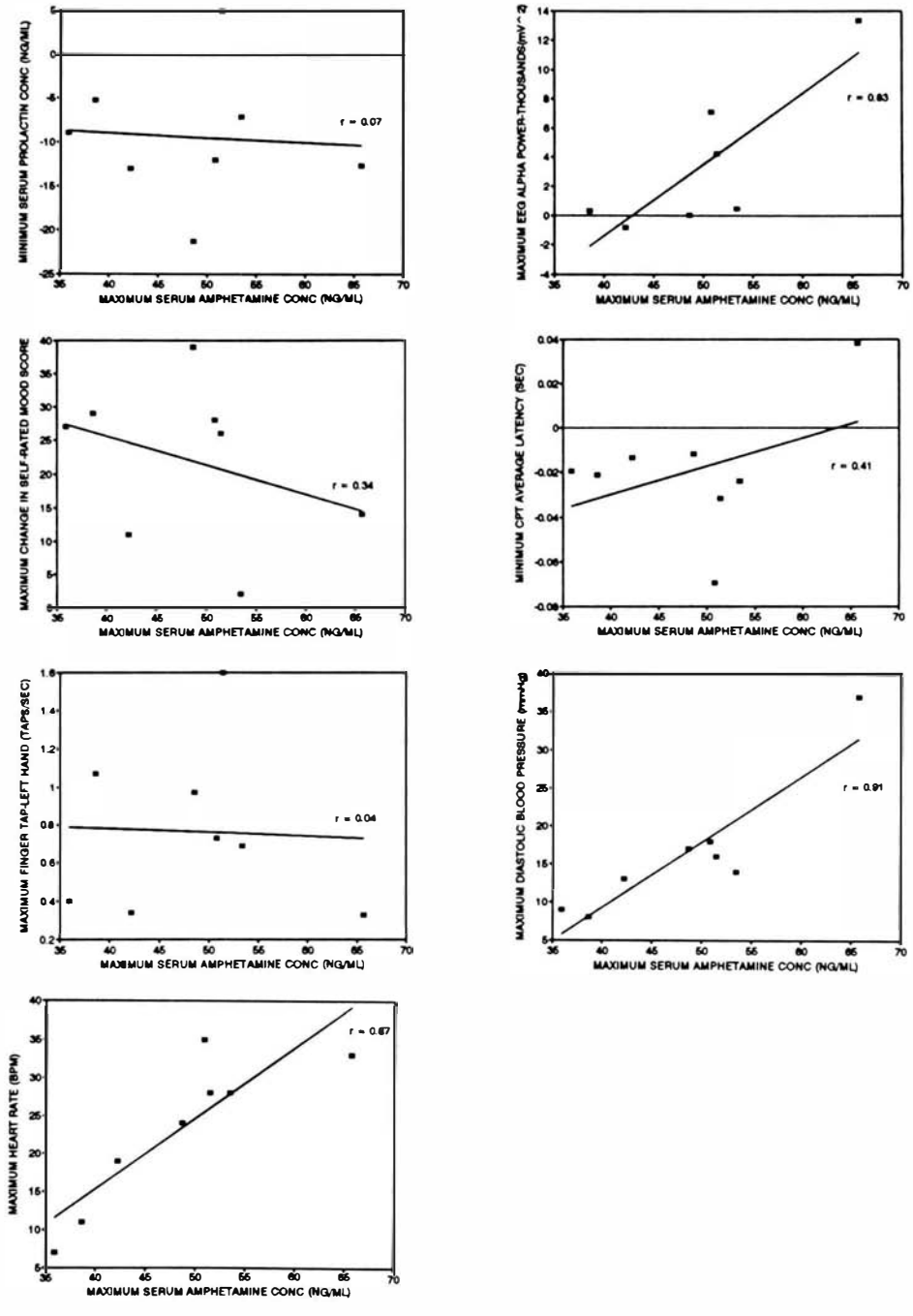

Figure 5.9 Maximum baseline and placebo corrected pharmacodynamic response versus maximum serum amphetamine concentration after the 20 mg dose for each subject

quantitative EEG response to dextroamphetamine compared to other measures, the relationship between maximum EEG alpha power and maximum response for other pharmacodynamic measures after the 20 mg dose of dextroamphetamine was examined. Plots of maximum (or minimum) baseline and placebo corrected prolactin (first row - left), mood (first row - right), CPT average latency (second row - left), finger tapping rate with the left hand (second row - right), diastolic blood pressure (third row - left), and heart rate (third row - right) response versus maximum baseline and placebo corrected EEG alpha power are presented in Figure 5.10. Correlation of the EGG alpha power with the CNS measures is low, while correlation with the cardiovascular parameters is relatively high.

Based on observations made in Part I and Part II of this study, each pharmacodynamic response measure was rated relative to criteria for an ideal pharmacodynamic measure. These ratings are presented in Table 5.2. Justification for these ratings and discussion of the results noted above are addressed in the next section.

5.2 Discussion

The onset, magnitude, and duration of pharmacologic effect observed after dextroamphetamine dosing appears to depend on the response measure and individual subject under consideration. Generalization about the relationships between response on the various pharmacodynamic measures has proven difficult. For example, mood scales show a heterogenous response, while none of the other measures seem to reflect this dichotomy. Physiological or psychological indicators of dysphoria could

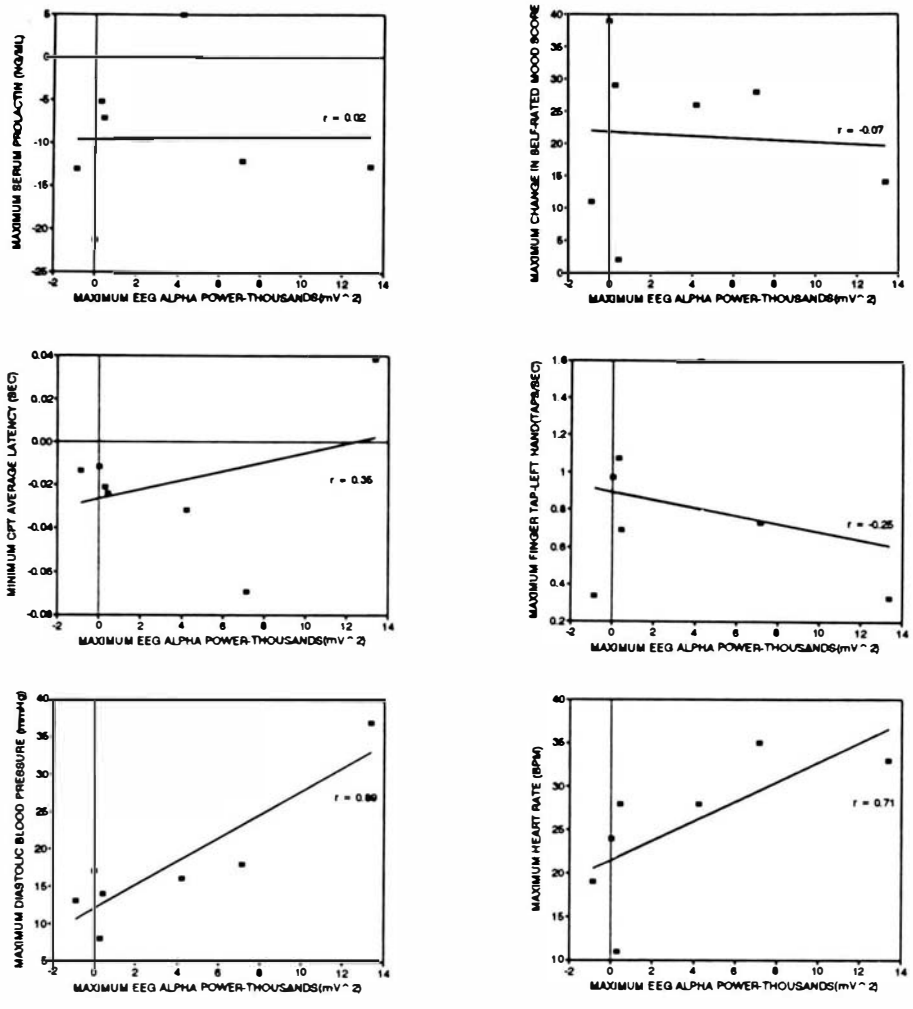

Figure 5.10 Maximum baseline and placebo corrected pharmacodynamic response versus maximum serum amphetamine concentration after the 20 mg dose for each subject

Table 5.2 Characteristics of each CNS Pharmacodynamic Response Measure

	Serum Prolactin Levels	EEG Total Alpha Power	Mood Scales	CPT	Finger Tapping
Non-invasive	−	++	+++	+++	+++
Quantitative	+++	+++	+++	+++	+++
Objective	+++	++	+	+++	+++
Suitable for Repeated Measures	++	+++	++	+	+
Clinically Meaningful	+	+	+++	++	++
Susceptible to First Session Effects	+	++	++	++	++
Low Between-Period Variability of Baseline Values	+	+	++	+++	+++
Low Potential for Missing Data	+	++	+++	++	++
Sensitivity for Distinguishing Dextro-amphetamine Dose Levels	++	+	++	−	−

not be identified in the responses assessed by the other pharmacodynamic measures. EEG response was only observed in 3 subjects, but response on other measures do not reflect this observation. These subjects do not differ from other subjects in response on other measures. Maximum EEG alpha power after the 20 mg dose correlates most closely with the maximum cardiovascular parameters. This may be because EEG alpha power, diastolic blood pressure, and heart rate correlate most highly with serum amphetamine concentration. Patterns in the time course of the effects of the CNS measures were also obscure. Responses from different CNS measures do not seem to follow the same time course or relate directly to the serum amphetamine concentrations. None of the measures reflected the dose-related changes in the absorption rate of dextroamphetamine. This may indicate that the CNS response measures are not measuring the same thing, the measures differ in sensitivity or reproducibility, or the degree of CNS stimulation changes during the testing session.

Based on observations made in Parts I and II of this study, each response measure was evaluated against the criteria for an ideal pharmacodynamic response measure. An ideal measure should: 1) be noninvasive, quantitative and objective, 2) suitable for repeated measures, 3) be insensitive to first session effects, 4) show low variability between periods as baseline, 5) have low potential for missing data, 6) measure a response that relates to some clinically relevant outcome, and 7) be sensitive and reproducible so that changes in dose levels (or serum concentration) of the drug can be discerned. Each of the measures meet some of these criteria, but none fulfills them all. The last criteria listed above, which these studies were designed to address, is the most important.

Each of the measures investigated in this study permits quantitation of the response. The mood scales, CPT, finger tapping, and EEG are noninvasive although the recording the EEG does result in some discomfort to the subject. Collection of serum prolactin levels requires venipuncture, so it is the most invasive of all of the response measures. The serum prolactin levels, CPT, and finger tapping task are the most objective of the measures. The self-rated and visual analog mood scales are the least objective of the measures, since they depend on the subject's individual assessment of the drug effect. The EEG data falls between mood scales and the other measures. The processing of the EEG requires editing, which is subjective in nature. Blinding of the EEG reviewer should reduce the subjectiveness of the process. Another approach, which was not utilized in this study, would be to automate the EEG artifact detection and elimination.

The EEG is the most suitable for repeated measures. Once the electrodes are applied, recordings can be made continuously with little subject cooperation beyond remaining vigilant. Serum prolactin can also be measured repeatedly, but the frequency is limited by the total volume of blood that can be drawn from the subject. Mood scales can also be measured repeatedly, but if administered too frequently, subjects become bored with the questions or their answers may become unduly influenced by the answers on the previous test session. The CPT and finger tapping tasks are the least suitable for repeated measures. During Part I of the study, learning effects were demonstrated for the computerized tests. Although the learning effects can be minimized with adequate practice sessions, these tasks require a higher degree of subject cooperation and motivation than the other tasks. Some subjects complained

that their hand became tired during the finger tapping task, which limits how frequently it can be repeated. The CPT requires that the subject maintain attention and motivation. During Part II of this study, the frequency of the computerized test sessions did not appear to seriously interfere with measurement of response.

In Part I of the study, each measure except serum prolactin was evaluated for first session effects. Scores on the self-rated mood scale, finger tapping with the right hand, percent correct on the CPT and EEG total power in the delta frequency band showed different responses on the first study day than on subsequent study days. This difference may be due to anxiety about participating in the study or other factors associated with being unacquainted with the study procedures. Serum prolactin has the potential to show first session effects as well, since prolactin levels are affected by stress. Prior to Part II of the study, subjects underwent a familiarization session that was similar to a study day except it was shorter and did not involve blood drawing, urine collection or drug administration. When the results of Part II were examined, a first-period effect was not present for most of the subjects, suggesting that the familiarization session was adequate. Study period was not significant in the statistical analysis and the baseline values were not noticeably different during Period 1 than during other periods. One notable exception to this observation is the experience of Subject 5. During Period 1, his baseline CNS and cardiovascular responses were quite different than those measured in Period 2, which may have contributed to the intense dysphoria experienced by the subject. To minimize this type of effect in future studies of CNS active drugs, a familiarization session that is identical to a study period where the subjects receive a placebo and are blinded should

be incorporated into the study design.

An ideal pharmacodynamic measure should also show low variability between periods as baseline. The measure should not vary significantly due to confounding factors, but show a stable response at baseline throughout the study. In our studies, EEG variables and serum prolactin showed the highest baseline variability. These measures are influenced by a number of factors including psychological state, stress, and level of vigilance. The mood scales and psychometric tests showed much lower variability at baseline than the EEG and prolactin. With significant variability at baseline, careful control of the study conditions and accurate determination of the baseline response is necessary.

Pharmacodynamic measures should also have low potential for missing data. The self-rated mood scales are least likely to show missing data. The only time they are missing is if the subject refuses to complete it, the investigator forgets to administer it, or the rating scale forms are misplaced, all of which are highly unlikely and could occur for any of the measures. Psychometric test data and serum prolactin values are also unlikely to show missing data, except when the computer, heparin lock, or RIA assay fails. The EEG is most likely to result in missing data. In our studies, 5 - 10% of the data was classified as missing because less than 24 artifact-free epochs were present after the editing was complete. This occurred despite efforts to minimize artifacts. Missing data complicates statistical analysis and may necessitate studying a larger number of subjects to obtain conclusive results.

A pharmacodynamic measure ideally should have clinical relevance. Change in the measure should have clinically important meaning. This goal has proven elusive

for many CNS pharmacodynamic measures. Intuitively, mood scales seem to be measuring mood alteration, which is clinically relevant for CNS stimulants. The mood scales used in these studies, particularly the self-rated mood scale, have face validity for measuring euphoria or dysphoria. This may be more or less true for a given scale, but in general, changes in these scales have clinical meaning. Each scale must be validated and results with a particular scale may not be consistent with other validated scales. Psychometric tests may also reflect clinically meaningful changes. The tasks used in this study were fairly simple however, and their relevance to complex, "real life" situations is unknown. The behavioral or psychological meaning of changes in serum prolactin levels and EEG variables are much less clear. They may be surrogate measures of more clinically relevant effects, but more work is needed to define these relationships.

Surrogate measures are useful only if response on these measures reflect clinically important changes. One of the objectives of this study was to determine if EEG changes observed after the administration of CNS stimulants correlated with clinical outcomes. The results of this study provided no evidence that the drug-induced EEG changes are correlated with changes in mood or psychometric performance. Similar EEG changes were observed in subjects who experienced euphoria and subjects who experienced dysphoria. The pattern of EEG changes also did not mirror changes in psychometric performance. This study did not suggest psychological or behavioral meaning for changes in serum prolactin levels either. Despite the unclear meaning of surrogate measures, they are still useful in drug development. Changes in these measures can indicate that a drug is influencing CNS

activity and may be useful in studying the influence of disease processes, aging, or other drugs on the CNS. Further work is needed to elucidate clinical correlates for EEG and serum prolactin changes.

Most importantly, pharmacodynamic measures should be reproducible and sensitive to the dose of the drug administered or the concentration of the drug at some collection site (usually serum or plasma). The primary objective of this research was to examine the sensitivity and reproducibility of quantitative EEG compared to other pharmacodynamic measures for the assessment of CNS stimulation. Based on Part I of this study, within-day variability, between-day variability, and intersubject variability was greatest for the EEG measures when no drug was administered. The psychometric tests were the least variable. These results indicate that EEG measures are less reproducible than the other measures under the testing conditions employed in our studies. This was also evident in the variability of the baseline values in Part II of the study. It may be possible to reduce this variability by selecting subjects with very similar background EEG patterns (especially alpha activity) and personality characteristics, more carefully controlling the level of vigilance, and controlling factors in the testing environment such as noise level, temperature, and interaction with staff. Reproducibility also impacts the sensitivity of a measure. If measures are not reproducible, they are less likely to be sensitive to small changes in drug concentration or dose.

The results of Part II of this study provide evidence of the comparative sensitivity of the pharmacodynamic measures investigated. Indicators of sensitivity include: 1) ability to distinguish dose levels based on the maximum or minimum

response observed on the various measures by statistical analysis, 2) the discriminating ability of the measures as indicated by the estimates of power for the statistical analyses, 3) the ability to measure drug effect as serum concentrations of dextroamphetamine are declining as assessed by the ET parameter, and 4) the correlation between maximum serum amphetamine concentrations and the maximum response determined with each response measure.

Statistical analysis of the E_{max} (or E_{min}) values for each response measure showed that not all of the measures could distinguish the dose levels of the drug. Significant treatment effects consistent with dose were observed for serum prolactin, diastolic blood pressure, and heart rate. The mood scales, EEG, psychometric tests, and systolic blood pressure showed no significant differences. Estimated statistical power was also highest for serum prolactin (0.85), diastolic blood pressure (0.97), and heart rate (0.90). Estimated power for the EEG alpha power (0.25), the self-rated mood scale (< 0.20), the visual analog mood scale (0.30), CPT average latency (0.45), CPT percent correct (0.50) finger tapping with the right hand (0.45) and systolic blood pressure (0.60) were lower. Finger tapping with the left hand showed adequate statistical power (0.88), but no treatment effects were observed. Statistical power for the mood scales were low primarily because of the dichotomous response observed in the small study sample. EEG changes were also only present in a portion of the subjects. A larger sample size that permitted subgroup analysis may have shown statistical differences for these measures. Power for the psychometric tests was higher than for the mood and EEG measures, so the performance on psychometric tests, indeed, may not be correlated with dose. Results from the statistical analysis of

E_{max} (or E_{min}) values suggest that the cardiovascular parameters are more sensitive than the CNS measures, and that serum prolactin is the most sensitive of the CNS measures.

The ET was calculated as an indicator of whether the measure was able to detect drug effect as concentrations became lower at later time points. The only measure able to distinguish between treatment based on ET was heart rate. None of the CNS measures showed a significant difference in ET between treatments. These results again indicate that the cardiovascular measures are more sensitive than the CNS measures.

Correlations between maximum serum amphetamine concentration and maximum pharmacodynamic response show a strong correlation for maximum total EEG alpha power ($r = 0.83$), diastolic blood pressure ($r = 0.91$) and heart rate (0.87). Correlation coefficients are less than 0.5 for the other measures. These results imply that the cardiovascular parameters and EEG total power are most sensitive to the maximum serum concentrations of amphetamine.

The indicators of sensitivity evaluated suggest that the cardiovascular measures, especially heart rate and diastolic blood pressure, are sensitive measures of stimulant effects. The cardiovascular measures are more sensitive than the CNS measures. Of the CNS measures, serum prolactin appears to be the most sensitive. The psychometric tests appear to be the least sensitive of the measures. Review of the response versus time plots at each dose level for the self-rated mood scale suggest that the mood scale may also be a sensitive measure. The largest effect is usually observed at the highest dose level. Because the response is dichotomous, statistical

comparisons appear to mask treatment differences. With a larger sample that permitted sub-group analysis, it is likely that significant differences between doses would be apparent. The EEG alpha power may also be a sensitive measure. In this study, there appeared to be a relationship between maximum increase in alpha power and maximum serum amphetamine concentration. Unfortunately, only three subjects showed changes in alpha power after amphetamine dosing, so statistical power was very low. If only subjects with greater than 35% alpha power at baseline were included in the study, significant differences between the treatments may have been apparent.

Our studies did not provide evidence that quantitative EEG is more sensitive than other CNS measures for assessing CNS stimulation. Other investigators have reported that EEG has proved to be sensitive to drug action at drug concentrations lower than where changes are seen in neuropsychological tests.[41] Studies that report this finding have all been performed with benzodiazepines, which cause a decrement in psychomotor performance and cause significant and characteristic EEG changes. This phenomenon has not been reported for CNS stimulants. This has also been viewed as a criticism of quantitative EEG, because EEG changes are present at drug concentrations when no behavioral changes are evident, so the meaning of the changes is unclear. It has been postulated that the sensitivity of EEG may be even greater when specific sensory stimulation modalities are employed, because situational variability (that decreases reproducibility) will be better controlled.[41]

5.3 Conclusions

Each of the CNS measures evaluated in this study fulfills one or more of the criteria for an ideal pharmacodynamic measure to a greater or lesser extent, but none of them can be considered an ideal measurement tool. The study was designed to evaluate quantitative EEG as a pharmacodynamic measure of CNS stimulation, and the following can be concluded from these studies: 1) Quantitative EEG is noninvasive, although it results in more discomfort to the subjects than mood scales and psychometric tests, 2) It is more objective than subject-rated mood scales, but due to the editing process, may not be as objective as the psychometric tests, 3) EEG is the most suitable for repeated measures of all the tools assessed in this study, 4) The behavioral and psychological meaning of quantitative EEG changes is unclear, and the results of this study did not shed much light on this problem, 5) The EEG and other measures evaluated are susceptible to first session effects, so a familiarization period is necessary, 6) The EEG shows the most baseline variability between periods and the lowest within-day, between-day, and intersubject reproducibility of the measures studied, 7) Quantitative EEG has the highest potential for missing data of the measures evaluated, and 8) EEG is not more sensitive than the other measures under the conditions of this study. Further work is needed to identify subject groups or study conditions that can improve the sensitivity of EEG for measuring CNS stimulation.

CHAPTER 6

Overall Conclusions and Significance of Findings

The primary objective of these studies was to test the following hypotheses: 1) quantitative EEG is a sensitive and reproducible measure of the CNS's response to sympathomimetic drugs as compared to more widely used methods such as psychometric testing, subjective rating scales, or neuroendocrine tests and 2) changes in the EEG after sympathomimetic drug administration are related to the behavioral, psychological and neuroendocrine effects observed as well as the plasma concentration of the drug. Based on the results obtained in these studies, quantitative EEG conducted under our study conditions and subject population was not more sensitive for assessment of CNS stimulation than the other CNS response measures evaluated. Quantitative EEG showed higher within-day, between-day, and intersubject variability than the other CNS measures studied, indicating that it is less reproducible under baseline conditions. Dose levels of dextroamphetamine could not be distinguished from placebo in the study population as a whole. A subset of the subjects studied showed notable EEG changes consistent with dose after dextroamphetamine administration. All of these subjects showed higher ($>$ 35%) alpha activity at baseline. Differences in mood, inhibition of prolactin secretion, and psychomotor performance in these subjects compared to the rest of the subjects were not apparent.

Relationships between EEG changes and changes on other CNS measures could not be identified, although maximum change in EEG alpha power was related to the maximum serum amphetamine concentration.

In addition to the findings addressing the primary objectives of the studies, conclusions can be drawn from secondary objectives and observations made during the course of the data collection and analysis:

1) A gas chromatographic method with electron-capture detection of the pentafluorobenzoyl derivative of amphetamine is suitable for the determination of serum amphetamine concentrations as low as 2 ng/mL, which permits characterization of the pharmacokinetics of amphetamine following a 5 mg oral dose.
2) Dextroamphetamine appears to show dose-dependent effects on the rate of absorption, with the fastest rate of absorption observed after the 5 mg dose. This phenomenon has not been described previously for amphetamines.
3) The is an inverse relationship between dose and inhibition of prolactin secretion. The largest decrease in serum prolactin levels occurs after the lowest dose. This dose-response relationship has not been reported previously.
4) In general, the cardiovascular measures (heart rate and blood pressure) were more sensitive than the CNS measures employed in this study. Results from cardiovascular measures can be used to distinguish dose lower doses of dextroamphetamine than results from the CNS measures.
5) The self-rated mood scale (adapted from the scale proposed by Martin, et al.,

based on the ARCI scales) was able to distinguish dose-related euphoria and dysphoria.

6) The psychometric tests employed in this study were not useful for distinguishing dose levels of amphetamine under our study conditions.

The primary significance of the findings from these studies is their importance for the design of future studies in the area. The studies were pilot in nature and intended to generate new hypotheses as well as provide evidence that supports or refutes the hypotheses stated at the start of the investigation. Our results indicate that the placebo-controlled, crossover design is appropriate for pharmacodynamic studies using the CNS measures we investigated. Several of the tests show within day variability and circadian variation in the response, and the intersubject variability is higher than the intrasubject variability. Because many of the responses to drug depend on the baseline response, it is important to accurately characterize the baseline response by carefully controlling the prestudy conditions and measuring the baseline response more than once. In addition, a familiarization period is necessary for all of the tests, and our observations suggest that it should be identical to a study period. This could be accomplished by incorporating two placebo periods into the study, one at the beginning of the study (single-blind) and one randomized with the treatments (double-blind). These design considerations are relevant for any pharmacodynamic study using quantitative EEG. For studies using quantitative EEG specifically to study CNS stimulation, our investigations suggest that prescreening of subjects for background alpha activity greater than 35% is necessary to obtain measurable changes

in the EEG activity after drug administration. Screening subjects for similar personality traits may also improve the reproducibility of quantitative EEG for measuring CNS stimulation. These design modifications should improve the sensitivity and reproducibility of quantitative EEG to measure CNS stimulation. Quantitative EEG shows promise as a measure of CNS effects, but further work is needed before the technique will improve our ability to investigate the pharmacodynamics of weaker CNS stimulants or the effects of age, disease, and other drugs on the pharmacodynamics of CNS stimulants.

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APPENDIX A

PROJECT #:

TITLE: EVALUATION OF QUANTITATIVE ELECTROENCEPHALOGRAPHY (EEG) FOR ASSESSMENT OF CENTRAL NERVOUS SYSTEM (CNS) STIMULANT RESPONSE

PART I. REPRODUCIBILITY OF CONTROL RESPONSES

PART II. COMPARISON OF QUANTITATIVE EEG TO BEHAVIORAL, PSYCHOLOGICAL AND NEUROENDOCRINE MEASURES OF RESPONSE TO DEXTROAMPHETAMINE

INVESTIGATORS:

Principal Investigator:
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HYPOTHESIS AND SPECIFIC AIMS:

Hypothesis

The hypotheses guiding this research project are that 1) quantitative EEG is a more sensitive and reproducible measure of the central nervous system's response to sympathomimetic drugs than more widely used methods such as psychometric testing, subjective rating scales, or neuroendocrine tests and 2) changes in the EEG after sympathomimetic drug administration are related to the behavioral, psychological and neuroendocrine effects observed as well as the plasma concentration of the drug. Dextroamphetamine is a sympathomimetic amine with demonstrated effects on the CNS, and will be used as a model compound for studying quantitative EEG as a response measure.

Specific Aims:

Part 1

The purpose of this study is to investigate the inter-and intra-individual variability associated with a series of potential CNS pharmacodynamic response measures under baseline (no drug) conditions. These measures include quantitative EEG, automated psychometric tests, and subjective self-rating mood scales. Within day and between day reproducibility will be

evaluated. Responses for each measure will be examined for evidence of circadian changes and learning effects.

Part II

The purpose of this study is to evaluate the usefulness of quantitative electroencephalography (EEG) as a measure of CNS response to stimulants. The study will examine the relationship between EEG changes after administration of dextroamphetamine and 1) performance on automated psychometric tests, 2) serum prolactin levels, 3) subjective response as assessed by self-rating mood scales, and 4) serum concentration of dextroamphetamine. The sensitivity of EEG parameters to dextroamphetamine concentration in serum will be compared with that of more subjective measures.

BACKGROUND AND SIGNIFICANCE:

Accurate and reproducible measures of drug effect on the central nervous system (CNS) are needed in order to study the pharmacodynamics of centrally-acting drugs. (1) Understanding pharmacodynamics, or the relationship between drug concentration in the systemic circulation and effect, is important because it contributes to the interindividual variability observed in drug response. Determining the association between drug concentration and subsequent response is necessary for optimizing drug therapy. Studies of the pharmacodynamics of centrally-acting drugs have been limited primarily by the difficulty in obtaining quantitative measures of CNS response. (2)

Ideally, the measures of drug effect used in pharmacodynamic studies should be quantitative, objective, and non-invasive. There should be a gradual, rather than an all-or-none, change in the response measure with changing drug concentration. The measure should be sensitive to small differences in drug concentration. The pharmacodynamic measure should be reproducible both within and between individuals. It is important to be able to measure the response repeatedly in the same individual without changes occurring due to learning or tolerance. Lastly, the response measure should be meaningful; the measured response should relate to the therapeutic or toxic clinical effects of the drug. (2,3)

Various psychometric tests, ranging from self-rating scales of psychologic state to computerized performance tasks, have been used to assess the pharmacodynamics of centrally-acting drugs. (2,4,5) Psychometric tests are noninvasive and the response can be quantitated. However, these tests are not ideal pharmacodynamic measures. Although some tests can measure certain aspects of behavior as a function of drug response, they are more or less subjective and may not be reproducible. Many psychometric tests are not suitable for repeated measures, since learning and motivational factors

influence the results of subsequent tests. These limitations may contribute to insensitivity of the measures to small changes in serum drug concentrations. The relationship of performance on psychometric tests to the "real life" behavioral and psychologic effects of drugs are also difficult to define. Therefore, psychometric tests are not entirely acceptable as CNS response measures.

More recently, quantitative EEG has been employed to measure CNS pharmacodynamics (2,6). Many studies using EEG to profile or classify psychoactive drugs have been conducted, but few studies have attempted to correlate EEG parameters with concurrently measured drug concentrations and/or response to psychometric tests. Pharmacodynamic modelling of the EEG effects of anesthetic agents (7,8,9,10) and benzodiazepines (11,12) has been successfully performed. Quantitative EEG is objective, noninvasive, and derived parameters change gradually with changes in plasma drug concentration. Repeated or continuous measures of the EEG can be made, although a familiarization session before the study is advisable to avoid a first-session effect due to anxiety. (13) Learning effects on the EEG have not been reported. (2) Recording of the EEG also requires less subject cooperation than completion of psychometric tests. The reproducibility and sensitivity of quantitative EEG parameters however, requires further evaluation. The behavioral or psychologic meaning of changes in EEG parameters is also unclear. If these issues can be addressed, quantitative EEG may become a preferred measure of CNS pharmacodynamic response.

This study is designed to evaluate quantitative EEG as a pharmacodynamic tool. Dextroamphetamine was chosen as a model compound for this evaluation. Dextroamphetamine is a sympathomimetic amine known to have potent CNS stimulant effects. Single doses have been administered safely to normal volunteers. Its concentration in the systemic circulation can be measured adequately by gas chromatographic assay methods and it does not have clinically significant active metabolites. It appears to have dose-proportional pharmacokinetics over the dosage range to be used in this study which can be adequately described by a one-compartment body model. (14) The renal excretion of dextroamphetamine is dependent on urinary pH and volume, so acidifying the urine will result in constantly enhanced excretion. With urine pH between 5 and 5.5, the elimination half-life of dextroamphetamine is approximately 7 hours. (15) Dextroamphetamine causes a decrease in delta activity and an increase in alpha and beta activity on the EEG. (16,17) Mood changes after dextroamphetamine have been measured using a variety of rating scales. (18,19,20,21,22) It also produces measurable effects on performance tasks. (23,24,25,26,27) The duration of the central effects of a single dose of amphetamine has been reported to be between 3 and 24 hr. (28) For these reasons, dextroamphetamine will be used to test the sensitivity of quantitative EEG as a pharmacodynamic measure of central stimulation.

Dextroamphetamine affects the neurotransmitters dopamine, norepinephrine and serotonin. (29) The output of pituitary hormones and hypothalamic releasing factors have been used to examine the neurotransmitter pathways involved. (30,31) Following a 20-mg oral dose of dextroamphetamine in normal subjects, statistically significant rises in cortisol, prolactin, growth hormone, TSH, FSH, and LH were observed compared to placebo. (32) The prolactin release after dextroamphetamine administration will be used in this study as an additional pharmacodynamic response measure to aid in the physiologic interpretation of the EEG response.

The significance of this project is two-fold. First, the study will provide information about the usefulness of quantitative EEG as a pharmacodynamic response measure compared with more traditional measures of CNS activity such as psychometric tests. A more sensitive, reproducible measure of CNS response to sympathomimetic drugs is important to evaluate the CNS-stimulating properties of other sympathomimetic drugs such as phenylpropanolamine where the degree of CNS stimulation and its potential clinical significance in man is controversial. (33) Second, an improved measure of CNS response is necessary for evaluating the effects of the aging process and various disease states on the pharmacodynamics of centrally-acting drugs. This study will evaluate quantitative EEG relative to the criteria for an ideal pharmacodynamic measure discussed above, to provide a better understanding of its sensitivity, reproducibility, and behavioral and psychological meaning.

METHODS AND PROCEDURES:

I. Subjects

Eight healthy volunteers will participate in each study. Volunteers will be considered for inclusion if they conform to the following criteria:

1. Demographic: Subjects must be healthy male or nonpregnant female volunteers between the ages of 18 and 30 years and must not deviate more than 15% above or below the range of desirable weights according to the 1979 Build Study, Society of Actuaries and Association of Life Insurance Medical Directors of America (Attachment I.)

   To participate in Part II, female subjects must meet the following criteria:

   As determined by thorough inquiry, women must be found to practice acceptable methods of birth control and have a negative serum beta-hCG pregnancy test. Abstention, oral contraceptives, vaginal

contraceptives or use of contraceptives by the women's partner, do not constitute acceptable birth control. Acceptable methods of birth control will be limited to intrauterine contraceptive devices or surgical sterility. The method of birth control must be recorded in the subject's medical history. A negative pregnancy test is required before enrolling in the study and before each dosing period.

This restriction is mandatory because pregnancy is a contraindication to amphetamine use, especially during the first trimester. (28) The risks to the pregnancy clearly outweigh the benefits of participating in this study.

2. Medical History: Subjects must have no history of renal, hepatic, cardiovascular, gastrointestinal, neurological, pulmonary, or hematologic disease; have no history of drug addiction, alcohol abuse, psychologic dependence on drugs, or psychiatric illness. Subjects must have no first degree relatives (mother, father, or siblings) with a history of mental illness or alcohol/drug abuse. Subjects participating in Part II must also have no history of glaucoma or hypersensitivity to tartrazine (FD&C Yellow dye No.5) or dextroamphetamine. Tartrazine hypersensitivity frequently occurs in those who have hypresensitivity reactions to aspirin.

3. Physical: Subjects must successfully pass a physical examination, demonstrating no evidence of an active disease state or physical or psychologic impairment.

4. Laboratory screen: Subjects must have no clinically significant abnormal laboratory values on a laboratory screen consisting of 1) SMAC-20, 2) CBC and 3) urinalysis. Subjects must have a negative urine drug screen and blood alcohol test. Females must have a negative serum beta hCG test.

5. Electrocardiogram: Subjects must have no clinically significant abnormalities on a 12-lead EKG including a 30 sec rhythm strip.

6. Vital signs: Supine and standing systolic and diastolic blood pressure, heart rate, and oral body temperature must be within normal limits.

7. Other medications: Subjects must not be taking medications chronically and must not have taken any prescription medication or investigational drugs for at least 4 weeks before entering the study. Subjects must have a normal daily caffeine intake equivalent to or less than two cups of coffee. No medication (including OTC medications and vitamins), caffeine or alcohol will be allowed in the 72-hr period before each

study day and on each study day. Subjects must be non-smokers, meaning that they have abstained from smoking for at least 12 months before the start of the study.

8. All subjects participating in Part II will undergo an EEG and psychometric testing familiarization period before enrolling in the study. Subjects with a high number of artifacts on the EEG or who cannot tolerate wearing the electro-cap for extended periods of time will be excluded.

Subjects participating in Part II will be instructed to maintain a low monoamine diet beginning 3 days prior to the start of the study and continuing through the duration of the study.

Within 1 week after study completion, the physical examination, laboratory tests, and electrocardiography will be repeated for subjects participating in Part II. Possible clinically significant abnormalities will be followed up until return to pre-study baseline.

Subjects for this study will be recruited from within the hospital and schools at MCV/VCU.

II. INFORMED CONSENT

Each subject will give written informed consent for study participation before the start of the study. The signed consent forms will be kept in the subjects' confidential medical record as a permanent document.

III. PROCEDURE

A. Part I

During each of the three study periods, the following procedure will be followed:

Subjects will enter the study facility at 7:00 a.m. on the study day and will be released after completion of the 12 hr test battery on the same day (approximately 8:00 p.m.).

Subjects will fast from midnight on the evening before the study day until after the 4 hr test battery. Lunch will be served after the 4 hr test battery and dinner at 10 hrs after the baseline test battery. The same menu will be served during each study period. Water will be permitted during the fasting period. Beverages not containing caffeine may be served with meals.

Electroencephalography

For each subject, five minute segments of 28 channel EEG will be recorded using a NeuroScience Brain Imager with eyes closed at the following times: 0, 1, 2, 3, 4, 6, 8, and 12 hrs. Subjects will be reclined in a reclining chair during the recordings. Subjects will be asked to count back from 500 by 3s to maintain vigilance. The electrodes will be placed using an Electro-cap according to the 10/20 International System with 8 additional electrodes located 50% between the standard 10/20 placement. Linked ears will be used as a reference. Four additional channels will be used to monitor for vertical and lateral eye movements and electromyographic activity. The electrode impedances will be checked before each recording. Impedances should be less than 4.0k ohms and similar between electrodes. Any disturbances in the room or subject movement during the EEG will be recorded by the EEG technician. The raw EEG will be stored on an optical disk.

Psychometric tests

1. A computerized visual continuous performance task (NeuroScan, Inc.) will be completed by each subject at the following times: 0, 1, 2, 3, 4, 6, 8 and 12 hours.

2. A computerized motor task, finger tapping (NeuroScan, Inc.), will be completed by each subject at the following times: 0, 1, 2, 3, 4, 6, 8, and 12 hours.

3. Prior to the 0 hr testing, subjects will practice the computerized tasks two times.

Rating Scales

A self rating scale (Attachment II) based on the MBG (a measure of euphoria) and A (a measure of amphetamine effects) subscales of the Addiction Research Center Inventory Scales described by Martin et al. (18) will be completed by each subject at the following times: 0, 1, 2, 3, 4, 6, 8, and 12 hours.

A 100 mm visual analog mood scale (Attachment III) will be completed by each subject at the following times: 0, 1, 2, 3, 4, 6, 8, and 12 hours.

The tests will be conducted in the following sequence: 1) EEG, 2) CPT, 3) rating scales, 4) finger tapping. A study flow sheet is presented in Attachment IV.

B. Part II

During each of the four study periods, the following procedure will be followed:

Subjects will enter the study facility on the evening of the day preceding each day of dextroamphetamine or placebo dosing and will not be released until after the collection of the last blood sample of the study period. Subjects will fast from midnight on the evening before dextroamphetamine or placebo dosing until after the 4 hr blood sample is drawn. Water will be permitted during the fasting period. Subjects will begin a period of bed rest one hour before dextroamphetamine or placebo administration that will continue until after the 6 hr test battery.

All subjects must have a negative urine drug screen and blood alcohol test each study period before receiving dextroamphetamine or placebo. Female subjects must have a negative pregnancy test (urine beta-hCG) each study period prior to receiving dextroamphetamine or placebo.

All subjects will complete a verbal probe concerning recent medical history and medication use.

Repeated 2 gram oral doses of ammonium chloride will be given to acidify the urine and enhance the excretion of dextroamphetamine at the following times: -12, -8, -2, 2, 6, 10, 14, and 18 hr after dextroamphetamine or placebo dosing as described by Wan et al. (15)

Subjects will receive one of the four treatments: dextroamphetamine 20 mg, dextroamphetamine 10 mg, dextroamphetamine 5 mg, or placebo orally. Both the subjects and the investigator will be blinded to treatment. The time of dosing will be 8:00 a.m. for the first of the two subjects and 8:30 a.m. for the second. Capsules will be taken with 240 mL of water.

Tablets containing 5 mg dextroamphetamine sulfate will be used for dosing, with doses placed in opaque gelatin capsules to maintain blinding. Lactose will be added to prepare capsules with the same weight for all doses of amphetamine. Placebo capsules will contain lactose only.

Blood sampling

Prior to dosing, a heparin containing catheter will be inserted into a forearm vein for access to blood sampling.

1. 7-mL samples for determination of dextroamphetamine concentration will be collected in red-top tubes with no additives at the following times: pre-dose, 1, 1.33, 2, 2.33, 3, 3.33, 4, 6, 8, 12, 18, and 24 hr after dextroamphetamine or placebo dosing. Blood samples will be allowed to clot, centrifuged (within 1 hour of venipuncture) for 10 minutes, serum harvested, and stored at -20 degrees Celsius until analysis by a sensitive, specific, and reproducible gas chromatographic method using isothiocyanate derivatization and a nitrogen detector, modified from the method described by Narasimhachari and Friedel. (34)

2. 5-mL samples for the determination of prolactin concentration will be collected in red-top tubes with no additives at the following times: pre-dose, 1, 2, 3, 4, 5 and 6 hr after dextroamphetamine or placebo dosing. Blood samples will be allowed to clot, centrifuged (within 1 hour of venipuncture) for 10 minutes, serum harvested, and stored at -20 degrees Celsius until analysis by a radioimmunoassay method described by Sinha et al. (35)

The total volume of blood drawn for dextroamphetamine and prolactin determinations during the study will be 504 mL.

Urine collection

Subjects will void just before dextroamphetamine or placebo dosing and the urine pH will be determined immediately at room temperature after shaking using a pH meter. Two 25 mL aliquots of the urine will be retained and frozen until analysis. Urine will then be collected over the following intervals after dextroamphetamine or placebo dosing: 0-2 hr, 2-4 hr, 4-8 hr, 8-12 hr, 12-18 hr, 18-24 hr. The pH of the urine voided at the end of each collection interval will be determined immediately at room temperature after shaking using a pH meter. The total volume of urine collected during the interval will be measured in a graduated cylinder and two 25 mL aliquots of the urine will be retained and frozen until analysis for dextroamphetamine concentration.

Subjects will drink 120 mL of water every hour beginning one hour before dextroamphetamine dosing and continuing through the four hours after dosing. Water will then be available to the subjects as desired.

Electroencephalography

Five minute segments of 28 channel EEG using a NeuroScience Brain Imager will be recorded for each subject with eyes closed at the following times: pre-dose, 1, 2, 3, 4, 6, 8, and 12 hours after dextroamphetamine or placebo dosing. Subjects will be asked to count back from 500 by 3s to maintain vigilance during the recordings. The electrodes will be placed using an Electro-cap according to the 10/20 International System with 8 additional electrodes located 50% between the standard 10/20 placement. Linked ears will be used as a reference. Four additional channels will be used to monitor for vertical and lateral eye movements and electromyographic activity. The electrode impedances will be checked before each recording. Impedances should be less than 4.0k ohms and similar between electrodes. Any disturbances in the room or subject movement during the EEG will be recorded by the EEG technician. The raw EEG will be stored on an optical disk.

Psychometric tests

1. A computerized visual continuous performance task (CPT) will be completed by each subject at the following times: predose, 1, 2, 3, 4, 6, 8, and 12 hours after dextroamphetamine or placebo dosing.

2. A computerized motor performance task, finger tapping, will be completed by each subject at the following times: predose, 1, 2, 3, 4, 6, 8, and 12 hours after dextroamphetamine or placebo dosing.

3. On the evening before dosing for each period, subjects will practice the computerized tasks several times. The exact number of times will be determined based on the results of Part I of this study.

Rating Scales

A self-rating scale (Attachment II) based on the Addiction Research Center Inventory Scales, the MBG scale (a measure of euphoria) and the A scale (a measure of amphetamine effects) described by Martin et al. (18) will be completed by each subject at the following times: predose, 1, 2, 3, 4, 6, 8, and 12 hours after dextroamphetamine or placebo dosing.

A 100 mm visual analog mood scale (Attachment III) will be completed by each subject at the following times: predose, 1, 2, 3, 4, 6, 8, and 12 hours after dextroamphetamine or placebo dosing.

Vital signs

Blood pressure (sitting) and heart rate will be measured at the following times: predose and 1, 2, 3, 4, 6, 8, 12, and 24 hr after dextroamphetamine or placebo dosing.

When above measurements are scheduled at the same time, they will be conducted in the following sequence: 1) urine collection, 2) blood samples, 3) EEG, 4) CPT, 5) rating scales, 6) finger tapping and 7) vital signs with the blood sample being collected at exactly the scheduled time. A study flow sheet is presented in Attachment V.

Diet

On the evening prior to dosing, subjects will receive a light snack prior to the -12 and -8 hr ammonium chloride dosing.

No food or beverages, other than water, will be permitted from 8 hr before dosing until after the 4-hr blood sample has been drawn. Lunch will be served after the 4-hr blood sample and dinner at 10 hours after dosing. A snack will be served in the evening before the 14 hr ammonium chloride dose. All meals and snacks will be low in tyramine content. Large amounts of foods potentially promoting alkalinization of the urine (such as milk and milk products, nuts, vegetables and fruits) will be avoided. Beverages not containing caffeine may be served with meals. The same menu will be served on corresponding days of each study period.

Adverse Effects

All subjects will be observed for symptoms and signs of clinical intolerance to the drugs or procedures and asked to report any adverse effects. These will be evaluated by the physician monitor for their clinical significance and potential need for treatment. For subjects who develop significant nausea, vomiting, or diarrhea, ammonium chloride will be discontinued.

BIOSTATISTICAL DESIGN AND ANALYSIS:

Part I

Design: During this open-labeled pilot study, healthy volunteers will undergo a series of tests (electroencephalography, automated psychometric tests, and

subjective rating scales) on 3 occasions one week apart. On each of the 3 study days, the series of tests will be repeated 8 times over a 12 hr period. Subjects will undertake the study in groups of two.

Data analysis

A. Electroencephalography

Each of the five-minute recordings will be reviewed by a board certified electroencephalographer and edited to remove each 2.5 second epoch that is contaminated with artifacts (eye movement, muscle movement, electrode artifacts, or disturbances noted during the recording). The remaining 2.5 second epochs or artifact-free frames will be averaged to form an average topographical map for each 5 minute recording. The amplitude, power, and relative power of the EEG signal in the 5 classical frequency bands (delta: 0.39 - 3.9 Hz; Theta: 4.3 - 7.8 Hz; Alpha: 8.2 - 11.7 Hz; Beta I: 12.1 - 16.0 Hz; and Beta II: 16.4 - 30.0 Hz) at each electrode will be calculated for each average topographical map. The total amplitude and power for each average map will be calculated. The ratio of total alpha plus beta power to total delta plus theta power will also be calculated.

B. Psychometric tests

1. Visual Continuous Performance Task

Latency of response will be determined for each trial. The average latency of response and the percent of correct responses for each set of testing will be calculated.

2. Finger Tapping Task

The average rate (taps/sec) of finger tapping for each hand will be determined based on three trials at each time point.

C. Rating Scales

A total score on the self-rating scale will be determined at each time point by adding the scores obtained for each item.

A score between 0 and 100 will be obtained for the visual analog scale at each time point by measuring the number of millimeters between the left end of the scale and the mark placed by the subject.

D. Statistical Analysis

The results from each of these tests for each day of testing will be

compared using a multivariate repeated measures analysis of variance with day, time and subject as factors and all responses as dependent variables. Within-day and between-day variance will be determined for each response variable.

Part II

Design: This study will be a randomized, double-blind, placebo-controlled four-period crossover study in healthy volunteers. Subjects will undertake the study in groups of two. The start of each study period will be separated by at least 1 week washout period. Subjects will receive one of four treatments during each study period: dextroamphetamine 20 mg, dextroamphetamine 10 mg, dextroamphetamine 5 mg or placebo as a single oral dose. Each subject will receive each treatment exactly once.

Data analysis

A. Pharmacokinetic analysis

1. Dextroamphetamine serum concentration data

The serum concentrations of dextroamphetamine obtained during the study will be presented in tabular and graphic form for each subject and treatment. Pertinent pharmacokinetic parameters for dextroamphetamine, including elimination rate constant (ke), volume of distribution, apparent total body clearance, mean residence time, maximum concentration (Cmax) and time to maximum concentration (tmax) will be estimated for each treatment for each subject. Descriptive statistics will be calculated for each parameter.

2. Dextroamphetamine urine concentration data

Dextroamphetamine excretion rates during each collection interval will be used to determine the elimination rate constant (ke) and the renal clearance of dextroamphetamine for each treatment. Data will be presented in tabular and graphic form. Descriptive statistics will be calculated for each parameter.

3. Prolactin plasma concentration data

The prolactin plasma concentrations obtained after each treatment will be presented in tabular and graphic form. Secondary parameters such as Cmax, tmax, and area under the effect-time profile (AUE) will be calculated and tabulated. Descriptive statistics will be calculated for each parameter.

B. Electroencephalography

Each of the 5-minute recordings will be reviewed by a board certified electroencephalographer and edited to remove each 2.5 second epoch that is contaminated with artifacts (eye movement, muscle movement, electrode artifacts, or disturbances noted during the recording). The remaining 2.5 second epochs or artifact-free frames will be averaged to form an average topographical map for each 5-minute recording. The amplitude, power, and relative power of the EEG signal in the 5 classical frequency bands (delta: 0.39 - 3.0 Hz; theta: 4.3 - 7.8 Hz; alpha: 8.2 - 11.7 Hz; Beta I: 12.1 - 16.0 Hz; and Beta II: 16.4 - 30.0 Hz) at each electrode will be determined for each average topographical map. The total amplitude and power for each average map will be calculated. The ratio of total alpha plus beta power to total delta plus theta power will also be calculated.Differences from placebo for each of these parameters will be calculated.

C. Psychometric tests

1. Visual Continuous Performance Task

Latency of response will be determined for each trial. The average latency of response and the percent of correct responses for each set of testing will be calculated.

2. Finger Tapping Task

The average rate (taps/sec) of finger tapping for each hand will be determined based on three trials at each time point.

D. Rating Scales

A total score on the self-rating scale will be determined at each time point by adding the scores obtained for each item.

A score between 0 and 100 will be obtained for the visual analog scale at each time point by measuring the number of millimeters between the left end of the scale and the mark placed by the subject.

E. Pharmacodynamic analysis

Response-time profiles for each subject during each period will be tabulated and plotted for each response measure. If appropriate, secondary parameters such as baseline responses, maximum response (Emax), time to reach maximum response, and area under the effect-

time profile (AUE) for each treatment will be listed and descriptive statistics will be calculated. Pharmacokinetic/dynamic modelling will be performed if appropriate.

F. Statistical analysis

Results of the above response measures for each treatment will be compared using statistical techniques appropriate for a 4-way crossover study design with repeated measures. Residuals will be tested for normality. If normally distributed, a repeated measures analysis of variance with subject, dose, period and time as factors will be performed. If not normally distributed, either the data will be transformed or appropriate non-parametric tests will be used. Analysis of variance will be used to examine the dose-dependency of calculated pharmacokinetic and pharmacodynamic parameters.

HUMAN SUBJECT CONCERNS:

Part I

The first part of this study involves undergoing a test battery consisting of EEG, computerized psychometric tests, and self-rating mood scales 8 times on 3 separate occasions for a total of 24 times. Subjects will remain in the study unit for the duration of each day's tests (approximately 13 hours). There risks associated with these tests are minimal. Subjects will be expected to wear the electro-caps throughout the day, which may result in some minor discomfort. No drugs will be administered and no blood samples will be drawn during this part of the study. Subjects may experience smoe discomfort during the pre-study physical exam, EKG and laboratory tests. Subjects will receive no personal benefits to their health from participating in the study, but the procedures will be conducted at no cost to them and they will receive an honorarium for their participation. Any information obtained about subjects from this research will be kept strictly confidential. Subjects will give written informed consent and have the right to withdraw from the study at any time.

Part II

In the second part of this study, subjects will receive oral dextroamphetamine (a controlled substance) and ammonium chloride, have blood samples drawn for dextroamphetamine and prolactin determination, and undergo a series of tests including EEG, automated psychometric tests, and rating scales, repeatedly over a 36 hour period on 4 occasions. Subjects will remain in the study unit for the duration of the testing (approximately 38 hours for each period).

A. Study drugs

Subjects will receive single doses of dextroamphetamine sulfate (5, 10, and 20 mg) and placebo orally in a crossover fashion. Dextroamphetamine is indicated for the treatment of narcolepsy, attention deficit disorder, and obesity. The usual adult dosage of dextroamphetamine sulfate is 5 - 60 mg/day in 2 or 3 divided doses. The dosages administered in this study are within this dosage range. Adverse effects associated with single doses of dextroamphetamine may include: nervousness, insomnia, irritability, talkativeness, increased libido, dizziness, headaches, increased motor activity chilliness, pallor or flushing, blurred vision, mydriasis, hyperexcitability, hypertension or hypotension, tachycardia, palpitations, nausea, vomiting, abdominal cramps, diarrhea, constipation, dryness of the mouth, and metallic taste. (28)

Subjects will also receive a total of 16 grams of oral ammonium chloride over a period of 36 hours during each study period to acidify the urine. The normal adult oral dose of ammonium chloride is 4 - 12 grams daily given in divided doses every 4 - 6 hours. The dosage in this study falls within this range. Adverse effects associated with oral ammonium chloride include: gastric distress, anorexia, nausea, vomiting, thirst, rash, and headache. Symptoms of ammonium toxicity associated with very high doses include pallor, sweating, irregular breathing, vomiting, bradycardia, cardiac arrhythmias, local or generalized twitching, asterixis, tonic seizures, and coma. (36)

Subjects will be monitored for the development of adverse effects to either dextroamphetamine or ammonium chloride by nurses in the CRC. Blood pressure and pulse rate will be determined periodically throughout the study. If subjects develop significant nausea, vomiting or diarrhea, ammonium chloride will be discontinued. Headache may be treated with acetaminophen if necessary. If systolic BP rises above 180 mmHg or greater than 30 mmHg above baseline, the subject will be given 10 mg of nifedipine sublingually. Other adverse effects will be managed as deemed necessary by the medical monitor.

B. Blood sampling

Subjects will have thirteen 7-mL blood samples and 7 5-mL blood samples drawn during each period. A total of 504 mL of blood will be drawn during the study.

C. Test battery

The risks associated with these tests are minimal. Subjects will be

expected to wear the electro-caps throughout the day, which may result in some minor discomfort.

D. Pre- and post-study physical exam and laboratory tests

Some discomfort may be associated with the physical exam, EKG and laboratory tests to be perfromed during screening and at the conclusion of the study.

Subjects will receive no personal benefits to their health from participating in the study, but the procedures will be conducted at no cost to them and they will receive an honorarium for their participation. Any information obtained about subjects from this research will be kept strictly confidential. Subjects will give written informed consent and have the right to withdraw from the study at any time.

Female subjects must be using an acceptable method of birth control (intrauterine contraceptive devices or surgical sterility). They must also have a negative pregnancy test (serum beta-hCG) as a criteria for enrolling in the study and during each period before receiving study drugs. Amphetamine use is contraindicated during pregnancy, especially during the first trimester (28), and teratogenicity studies in animals have not been performed with ammonium chloride, so the risks are unknown. (36) For participation in this study, risks to pregnant females clearly outweigh the benefits.

NEED FOR CRC:

The CRC is needed for the conduct of this study because:

1) A number of specialized tests such as EEG and automated psychometric tests will be performed, requiring a controlled environment.

2) The extensive blood sampling and urine collection schedule in Part II require trained personnel sensitive to the strict timing requirements necessary for pharmacokinetic and pharmacodynamic research.

3) A special diet is necessary during Part II of the study, requiring the services of a dietician for planning and preparing meals.

4) Subjects must be housed overnight due to the blood and urine sampling schedule during Part II.

5) Plasma prolactin determinations are necessary, requiring a laboratory equipped to perform radioimmunoassays.

6) Trained nurses are available throughout the study to handle study-related adverse events.

GRANT SUPPORT:

Sources of funding for this study include:

1. Departmental funds (approximately $1,000) will be used to cover the costs of analysis of samples for dextroamphetamine concentration.

2. A portion of a grant from NeuroScience (approximately $9,000) will be used to cover the costs of subject honoraria, EEG supplies, and screening laboratory tests.

3. The remainder of the study costs are unfunded.

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ATTACHMENT I

Desirable Weights for Men of Ages 25-59 Years[a]

Height in Shoes				
Feet	Inches	Small Frame	Medium Frame	Large Frame
5	2	128-134	131-141	138-150
5	3	130-136	133-143	140-153
5	4	132-138	135-145	142-156
5	5	134-140	137-148	144-160
5	6	136-142	139-151	146-164
5	7	138-145	142-154	149-168
5	8	140-148	145-157	152-172
5	9	142-151	148-160	155-176
5	10	144-154	151-163	158-180
5	11	146-157	154-166	161-184
6	0	149-160	157-170	164-188
6	1	152-164	160-174	168-192
6	2	155-168	164-178	172-197
6	3	158-172	167-182	176-202
6	4	162-176	171-187	181-207

(In indoor clothing weighing 5 pounds and shoes with 1-inch heels.)

Desirable Weights for Women of Ages 25-59 Years[a]

Height in Shoes				
Feet	Inches	Small Frame	Medium Frame	Large Frame
4	10	102-111	109-121	118-131
4	11	103-113	111-123	120-134
5	0	104-115	113-126	122-137
5	1	106-118	115-129	125-140
5	2	108-121	118-132	128-143
5	3	111-124	121-135	131-147
5	4	114-127	124-138	134-151
5	5	117-130	127-141	137-155
5	6	120-133	130-144	140-159
5	7	123-136	133-147	143-163
5	8	126-139	136-150	146-167
5	9	129-142	139-153	149-170
5	10	132-145	142-156	152-173
5	11	135-148	145-159	155-176
6	0	138-151	148-162	158-179

(In indoor clothing weighing 3 pounds and shoes with 1-inch heels.)

[a] 1979 Build Study, Society of Actuaries and Association of Life Insurance Medical Directors of America, 1980.

AMPHETAMINE RATING SCALE

Subject Initials: Date:

Subject Number: Time:

Please respond to the statements below, indicating how you feel at this time.

		disagree strongly	disagree somewhat	neutral	agree somewhat	agree strongly
1.	Today I say things in the easiest possible way.					
2.	Things around me seem more pleasing than usual.					
3.	I have a pleasant feeling in my stomach.					
4.	I feel I will lose the contentment that I have now.					
5.	I feel in complete harmony with the world and those around me.					
6.	I can completely appreciate what others are saying when I am in this mood.					
7.	I would be happy all of the time if I felt as I feel now.					
8.	I feel so good that I know other people can tell it.					
9.	I feel as if something pleasant had just happened to me.					
10.	I would be happy all the time if I felt as I do now.					
11.	I feel more clear-headed than dreamy.					

12.	I feel as if I would be more popular with people today.					
13.	I feel a very pleasant emptiness.					
14.	My thoughts come more easily than usual.					
15.	I feel less discouraged than usual.					
16.	I am in the mood to talk about the feelings I have.					
17.	I feel more excited than dreamy.					
18.	Answering these questions was very easy today.					
19.	My memory seems sharper to me than usual.					
20.	I feel as if I could write for hours.					
21.	I feel very patient.					
22.	Some parts of my body are tingling.					
23.	I have a weird feeling.					

AMPHETAMINE SCALE

Subject initials: Date:

Subject number: Time:

Please mark on the line below how you feel right now.

The worst you have ever felt

The best you have ever felt

ATTACHMENT IV

STUDY DAY FLOW SHEET -- PART I.

		Relative time (hours)								
		0	1	2	3	4	6	8	10	12
EEG		X	X	X	X	X	X	X		X
CPT	XX	X	X	X	X	X	X	X		X
Finger Tap	XX	X	X	X	X	X	X	X		X
Rating Scales		X	X	X	X	X	X	X		X
Meals						X			X	

STUDY PERIOD FLOW SHEET -- PART II.

	Hours relative to d-amphetamine (or placebo) dosing																		
	-12	-8	-2	-1	0	1	1.3	2	2.3	3	3.3	4	5	6	8	10	12	14	18	24
Ammonium Cl dosing	X	X	X					X						X		X		X	X	
Serum sample d-amphetamine					X	X	X	X	X	X	X	X		X	X		X		X	X
Plasma sample prolactin					X	X		X		X		X	X	X						
Urine collection					X	----------		X	----------			X	----------		X	--------	X	--------	X	---X
EEG					X	X		X		X		X		X	X		X			
CPT	XX				X	X		X		X		X		X	X		X			
Finger tap	XX				X	X		X		X		X		X	X		X			
Rating scales					X	X		X		X		X		X	X		X			
Blood pressure					X	X		X		X		X		X	X		X			
Heart rate					X	X		X		X		X		X	X		X			
Meals/snacks	X	X										X				X		X		
Water				X	X	X		X		X		X								
Bed rest				X	----------									X						

CONSENT FORM

Evaluation of quantitative electroencephalography (EEG) for assessment of central nervous system (CNS) stimulant response

Part I. Reproducibility of control responses

Investigators

Patricia W. Slattum, Pharm.D./Ph.D. candidate
William H. Barr, Pharm.D., Ph.D.
Jurgen Venitz, M.D., Ph.D.
Joseph A. Sgro, M.D., Ph.D.
Ananda K. Pandurangi, M.D. (Medical director)

Introduction

You are being asked to participate in this study because you are healthy and not taking stimulant drugs or other medications on a chronic basis. This study is designed to help us learn how people not taking medication perform on various mental tests, and whether performance on the tests changes during the day. We also will study your brain waves (EEG) at various times during the day. The data collected will enable us to better plan future studies looking at the effects of medications on the tests. Eight subjects like yourself will be selected to participate in the present study.

If you agree to participate, you will be expected to provide information about your medical history, have laboratory work done (including blood and urine tests), have a physical examination, and an EKG (electrical tracing of the heart) to determine whether you have any medical condition that would prevent you from participating in the study. Your urine will be tested for drugs of abuse. You will not be permitted to take any over the counter medications (such as antacid, aspirin, vitamins or cold preparations) or any beverages containing caffeine or alcohol for the 72 hours before each study day and on each study day.

You will be expected to report to the study unit one day each week for three consecutive weeks. On each day of the study, you will come to the unit at 7:00 a.m. after an overnight fast (starting at midnight). You may have water, but no other beverage or food. Lunch will be provided 4 hours after the testing has started (approximately 12:00 noon) and dinner at 10 hours (approximately 6:00 p.m.) after the testing has started. After 12 hours of testing, you may go home (approximately 9:00 p.m.).

Inititals _____

The tests that you will be taking repeatedly throughout the day include: two computerized tests, answering 2 questionnaires about you mood, and recording your brain waves (EEG). To have your EEG recorded, you must wear a bathing cap-like apparatus with 28 disks/electrodes. Through a hole in each electrode, your scalp will be cleaned and a small amount of jelly-like substance will be applied to your scalp to make the contact. In addition, six small, round electrodes will be attached to your earlobes and taped on your face above and below your eyes. The cap will remain on your head for most of the day. Each of the individual tests (including EEG) takes less than 5 minutes to complete. These tests will be repeated 8 times during each study day.

Benefits

You are being asked to participate in this study as a volunteer. The study is of no direct medical benefit to you. There will be no charge to you for the screening examination and the results will be made available to you if you want them.

You will be paid $150.00 for the completion of this study. If you elect to withdraw before the end of the study, you will be paid on a prorated basis as described under Withdrawal.

Alternative Therapy

There is no therapeutic benefit to you for participating in this study. Your participation is entirely voluntary; the alternative is not to participate in the study.

Risks, Inconveniences, Discomforts

None of the tests in this study are harmful. There may be some discomfort associated with the physical exam, EKG, and laboratory tests, and with the EEG when applying the cap and wearing it throughout the day. Although the tape and gel used for the EEG are hypoallergenic, they may rarely cause skin irritation. After the cap is removed, you will be able to wash and dry your hair.

Costs of Participation

There will be no charge to you for any laboratory tests or physical examination related to the conduct of this study. This is a time consuming study that may interfere with your employment or other activities. You will be confined to the study unit for the entire day on each study day. You must provide your own transportation to and from the study site.

Initials _____

Pregnancy

Pregnant females are not excluded from this study.

Research Related Injury

Every effort will be made to prevent any injury that could result from your participation in this study. In the event of any physical and or mental injury resulting from your participation in this research project, Virginia Commonwealth University/Medical College of Virginia will not offer compensation. If injury occurs, medical treatment will be available at MCV Hospitals. Fees for such treatment will be billed to you or appropriate third party insurance.

Confidentiality of Records

The investigators will treat your identity with professional standards of confidentiality. Information obtained in this study may be published, but your identity will not be revealed.

Withdrawal

Your participation in this study is voluntary. If you decide to participate, you may withdraw at any time. Neither refusal to participate nor withdrawal will result in any penalty or loss of benefits to which you are otherwise entitled. If you do not complete the study because of premature withdrawal, the honorarium will be prorated based on the amount of usable information which has been collected.

If you have any questions at any time concerning the study procedures, you may contact the study investigators at:

	Office	Home
Patricia W. Slattum William H. Barr Jurgen Venitz Joseph A. Sgro	[redacted]	[redacted]

Dr. Pandurangi is the medical director for this study. He can be reached during office hours at [redacted] and other times at [redacted]. If Dr. Pandurangi is unavailable, you may call Dr. Anthony Pelonero at the same numbers.

You will receive a copy of this consent form.

Initials _____

I have read the above information, and I have had an opportunity to ask questions to help me understand what my participation will involve. I freely give my consent to participate in this study.

Signed _____ Date _____
(volunteer)

Signed _____ Date _____
(witness)

Signed _____ Date _____
(investigator)

CONSENT FORM

Evaluation of quantitative electroencephalography (EEG) for assessment of central nervous system (CNS) stimulant response

Part II. Comparison of quantitative EEG to behavioral, psychological and neuroendocrine measures of response to dextroamphetamine

Investigators

Patricia W. Slattum, Pharm.D./Ph.D. candidate
William H. Barr, Pharm.D., Ph.D.
Jurgen Venitz, M.D., Ph.D.
Joseph A. Sgro, M.D., Ph.D.
Ananda K. Pandurangi, M.D. (Medical director)

Introduction

You are being asked to participate in this study because you are healthy and not taking stimulant drugs or other medications on a chronic basis. This study is designed to study the relationship between changes in your brain waves (EEG) and other mental tests after taking dextroamphetamine. Dextroamphetamine is a central nervous system stimulant drug. Eight subjects like yourself will be selected to participate in the present study.

If you agree to participate, you will be expected to provide information about your medical history, have laboratory work done (including blood and urine tests), have a physical examination, and an EKG (electrical tracing of the heart) to determine whether you have any medical condition that would prevent you from participating in the study. Your urine will be tested for drugs of abuse. You will not be permitted to take any prescription medications for four weeks before the start of the study or during the study. You will not be permitted to take any over the counter medications (such as antacid, aspirin, vitamins or cold preparations) or any beverages containing caffeine or alcohol for the 72 hours before each study day and on each study day. You must maintain a diet low in monoamines for three days before the study according to the instructions the investigators give you. Prior to the start of the study you must undergo a practice session with the EEG (measuring your brain waves) and other tests that will be used during the study.

Initials _____

You will be expected to report to the study unit for a total of 4 study periods on 4 consecutive weeks. During each period, you will come to the unit at 7:00 p.m. on the evening before dextroamphetamine dosing and will not be released until 9:00 a.m. on the day after dextroamphetamine dosing.

You will receive eight, 2 gram oral doses (one every 4 hours) of ammonium chloride tablets to acidify your urine during each study period. On the morning of dosing, you will begin a 7 hour period of bedrest. A catheter will be inserted into your vein and two blood samples (about 12 ml or 2.5 teaspoonsful) will be drawn. You will then receive a single oral dose of dextroamphetamine (5, 10, or 20 mg) or a placebo capsule (a capsule with no active agent) with 8 oz. water during each period. You will receive all four treatments by the end of the study. You will not be told which dose you are receiving during a given period.

After dosing, 18 additional blood samples will be collected through the catheter during each period. A total of 504 ml (about one pint) of blood will be collected during the entire study. If the catheter fails to work, a new catheter will be inserted or it may be necessary to obtain blood samples by sticking a needle directly into the vein. All of your urine will also be collected for 24 hours after taking the dextroamphetamine or placebo.

Beginning just prior to dosing, you will be taking a series of tests repeatedly throughout the day. These tests include: two computerized tests, answering 2 questionnaires about you mood, and recording your brain waves (EEG). To have your EEG recorded, you must wear a bathing cap-like apparatus with 28 disks/electrodes. Through a hole in each electrode, your scalp will be cleaned and a small amount of jelly-like substance will be applied to the scalp to make a good contact. In addition, six small, round electrodes will be attached to your earlobes and taped on your face above and below your eyes. The cap will remain on your head for most of the day. Each of the individual tests (including EEG) takes less than 5 minutes to complete. These tests will be repeated 8 times during each study period. Your heart rate and blood pressure will be monitored periodically throughout the day.

The prestudy physical examination and laboratory tests will be repeated at the end of the study.

<u>Benefits</u>

You are being asked to participate in this study as a volunteer. The study is of no direct medical benefit to you.

Initials _____

There will be no charge to you for the screening examination and the results will be made available to you if you want them.

You will be paid $600.00 for the completion of this study. If you withdraw early or are withdrawn by the medical monitor, the fee will be prorated (See Withdrawal).

Alternative Therapy

There is no therapeutic benefit to you for participating in this study. Your participation is entirely voluntary. The alternative is not to participate in the study.

Risks, Inconveniences, Discomforts

A total of 80 blood samples will be drawn during the study. The total amount of blood will be 504 ml or about one pint over the four weeks of the study, which is about the same as the amount of blood donated at a single donor session. To obtain the blood samples a small catheter will be inserted into a vein in your arm. This procedure may cause some discomfort, pain, or slight bruising around the site of the needle stick. If the catheter fails to work, a new catheter will be inserted or blood samples will be collected directly through a needle inserted into the vein. All of your urine produced during the 24 hours after dosing must be collected. You must remain at bedrest (except for urine collection) for 7 hours during each study period. While on the study unit you will eat only the meals provided by the investigators at times prescribed by the investigators. You will required to remain on the unit for 37 hours during each period. You may receive phone calls during the study, but no visitors will be allowed.

Dextroamphetamine is a stimulant which may cause side effects: nervousness, dizziness, headache, irritability, difficulty sleeping, rapid heart rate, changes in blood pressure, loss of appetite, dry mouth, nausea, changes in sexual desire or false sense of well-being. Ammonium chloride, used to acidify the urine, may cause upset stomach, loss of appetite, nausea, thirst, rash, or headache.

If any undesirable effects occur, you should report them directly to the investigators. Dr. Pandurangi is the medical director for this study and is the person you can contact in the case of a medical emergency. If you can not reach Dr. Pandurangi, you may contact Dr. Anthony Pelonero or proceed to the emergency room for medical treatment.

Initials _____

None of the tests in this study are harmful. There may be some discomfort associated with the EEG when applying the cap and wearing it throughout the day. Although the tape and gel used for the EEG are hypoallergenic, they may rarely cause skin irritation. After the cap is removed, you will be able to wash and dry your hair.

There may be some discomfort associated with the physical exam, EKG, and laboratory work conducted before and after the study.

Costs of Participation

There will be no charge to you for any laboratory tests, physical examination, hospital care, or other tests related to the conduct of this study. This is a time consuming study that may interfere with your employment or other activities. You will be confined to the study unit overnight and for an entire day at each of 4 study periods. You must provide your own transportation to and from the study site.

Pregnancy

For female subjects: You must be using an intrauterine contraceptive device (IUD) or be surgically sterile in order to participate in this study. You will be tested for pregnancy prior to the start of the study and before each dextroamphetamine dose. You will be dropped from the study if you are pregnant.

Research Related Injury

Every effort will be made to prevent any injury that could result from your participation in this study. In the event of any physical and or mental injury resulting from your participation in this research project, Virginia Commonwealth University/Medical College of Virginia will not provide compensation. If injury occurs, medical treatment will be available at MCV Hospitals. Fees for such treatment will be billed to you or appropriate third party insurance.

Confidentiality of Records

The investigators will treat your identity with professional standards of confidentiality. It is important for the United States Food and Drug Administration to be able to inspect the results of this study. By signing this consent form, you authorize release of the portion of your medical records dealing with this study to the FDA. Information obtained in this study may be published, but your identity will not be revealed.

Initials _____

Withdrawal

Your participation in this study is voluntary. If you decide to participate, you may withdraw at any time. Neither refusal to participate nor withdrawal will result in any penalty or loss of benefits to which you are otherwise entitled. If you have any questions at any time concerning the study procedures, you may contact the study investigators:

	Office	Home
Patricia W. Slattum	[redacted]	[redacted]
William H. Barr		
Jurgen Venitz		
Joseph A. Sgro		

Dr. Pandurangi is the medical director for this study. He can be reached during office hours at [redacted] and other times at [redacted]. This is the MCV telepage. They can directly contact either Dr. Pandurangi or Pelonero at home. If Dr. Pandurangi is unavailable, you may call Dr. Anthony Pelonero at the same numbers.

If you do not complete the study because of premature withdrawal, the honorarium will be prorated based on the amount of usable information which has been collected. If the medical monitor terminates your participation in the study you will receive the entire amount.

You will receive a copy of this consent form.

I have read the above information, and I have had an opportunity to ask questions to help me understand what my participation will involve. I freely give my consent to participate in this study.

Signed _____ Date _____
(volunteer)

Signed _____ Date _____
(witness)

Signed _____ Date _____
(investigator)

APPENDIX B

AMPHETAMINE RATING SCALE

Subject Initials: Date:

Subject Number: Time:

Please respond to the statements below, indicating how you feel at this time.

		disagree strongly	disagree somewhat	neutral	agree somewhat	agree strongly
1.	Today I say things in the easiest possible way.					
2.	Things around me seem more pleasing than usual.					
3.	I have a pleasant feeling in my stomach.					
4.	I feel I will lose the contentment that I have now.					
5.	I feel in complete harmony with the world and those around me.					
6.	I can completely appreciate what others are saying when I am in this mood.					
7.	I would be happy all of the time if I felt as I feel now.					
8.	I feel so good that I know other people can tell it.					
9.	I feel as if something pleasant had just happened to me.					
10.	I would be happy all the time if I felt as I do now.					
11.	I feel more clear-headed than dreamy.					

12.	I feel as if I would be more popular with people today.					
13.	I feel a very pleasant emptiness.					
14.	My thoughts come more easily than usual.					
15.	I feel less discouraged than usual.					
16.	I am in the mood to talk about the feelings I have.					
17.	I feel more excited than dreamy.					
18.	Answering these questions was very easy today.					
19.	My memory seems sharper to me than usual.					
20.	I feel as if I could write for hours.					
21.	I feel very patient.					
22.	Some parts of my body are tingling.					
23.	I have a weird feeling.					

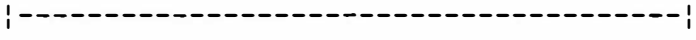
APPENDIX C

AMPHETAMINE SCALE

Subject initials: Date:

Subject number: Time:

Please mark on the line below how you feel right now.



The worst you have ever felt

The best you have ever felt

APPENDIX D

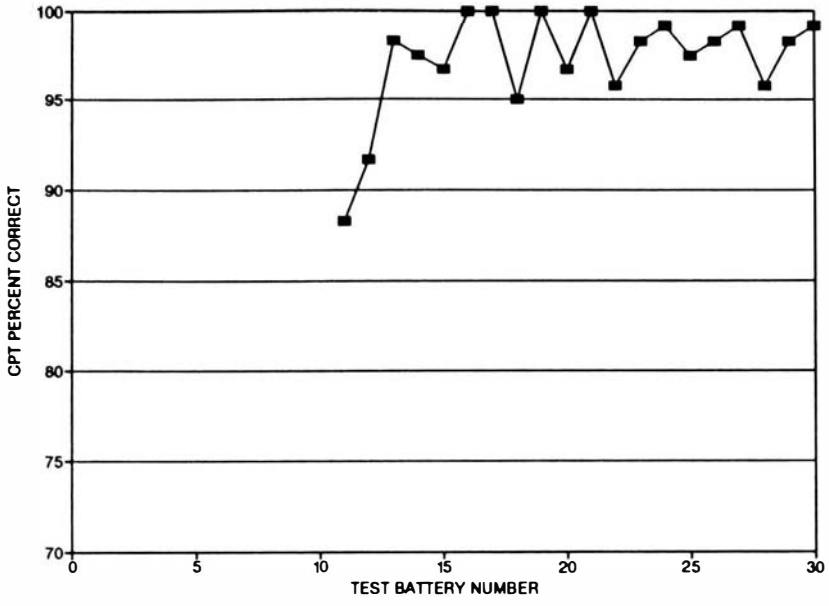


Figure D.1 Learning curve for continuous performance task (percent correct) for Subject 1

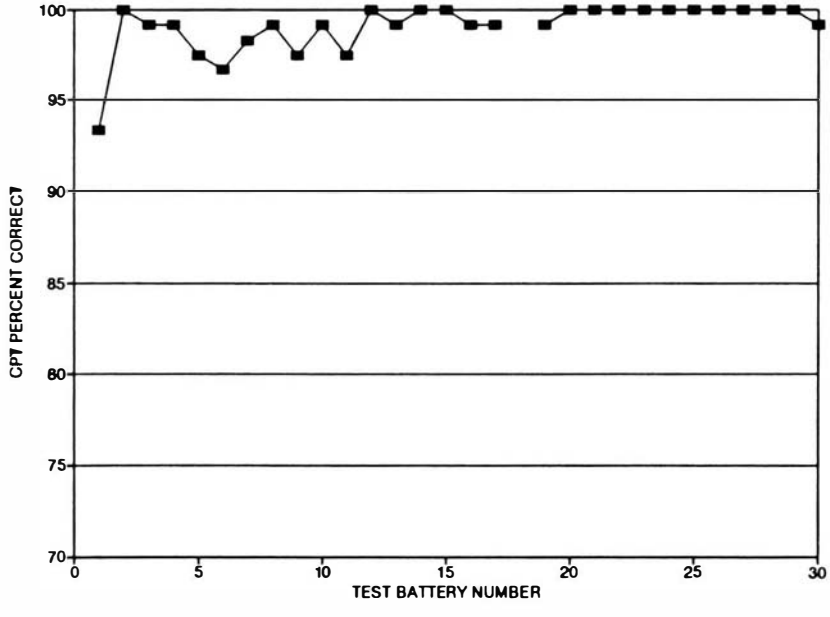


Figure D.2 Learning curve for continuous performance task (percent correct) for Subject 2

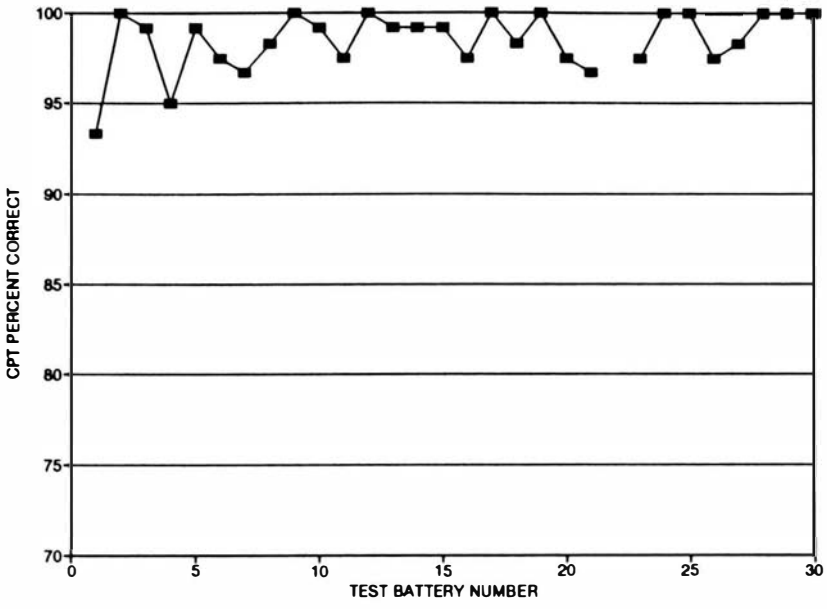


Figure D.3 Learning curve for continuous performance task (percent correct) for Subject 3

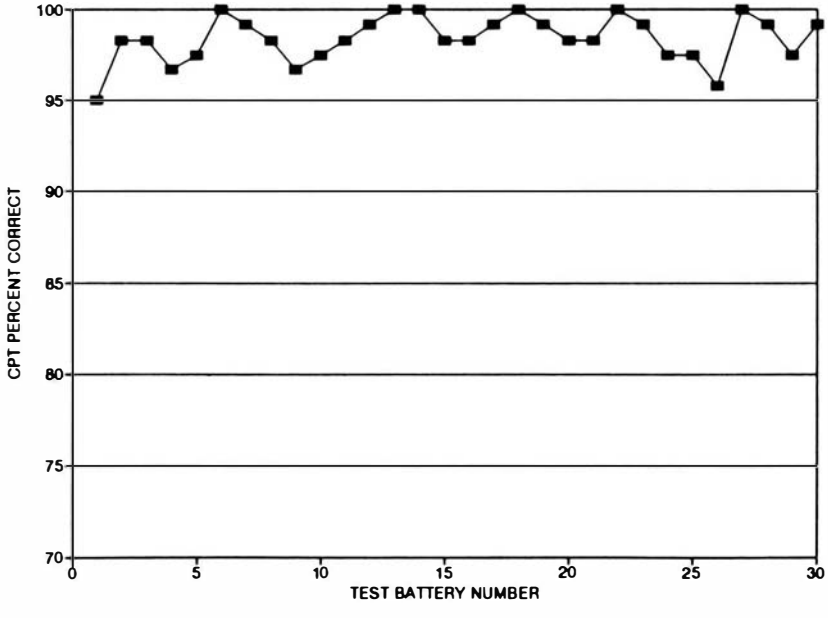


Figure D.4 Learning curve for continuous performance task (percent correct) for Subject 5

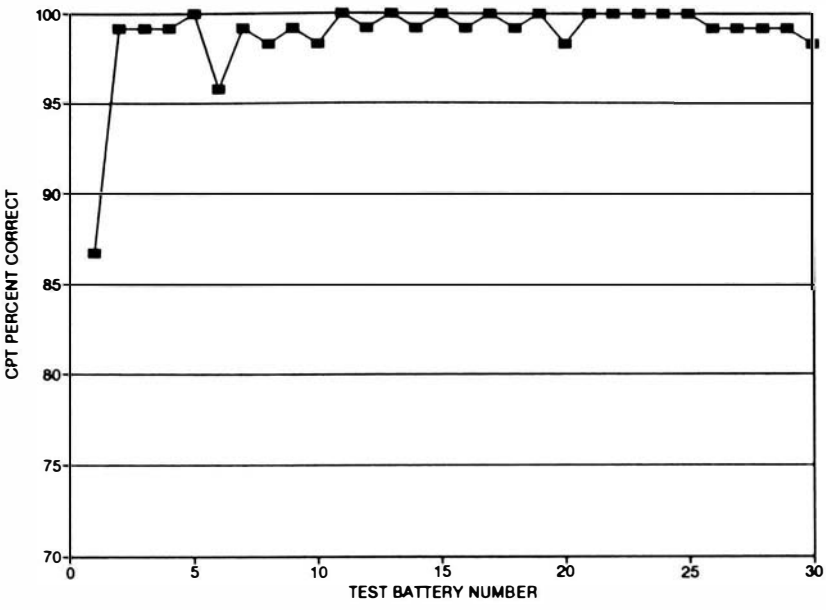


Figure D.5 Learning curve for continuous performance task (percent correct) for Subject 6

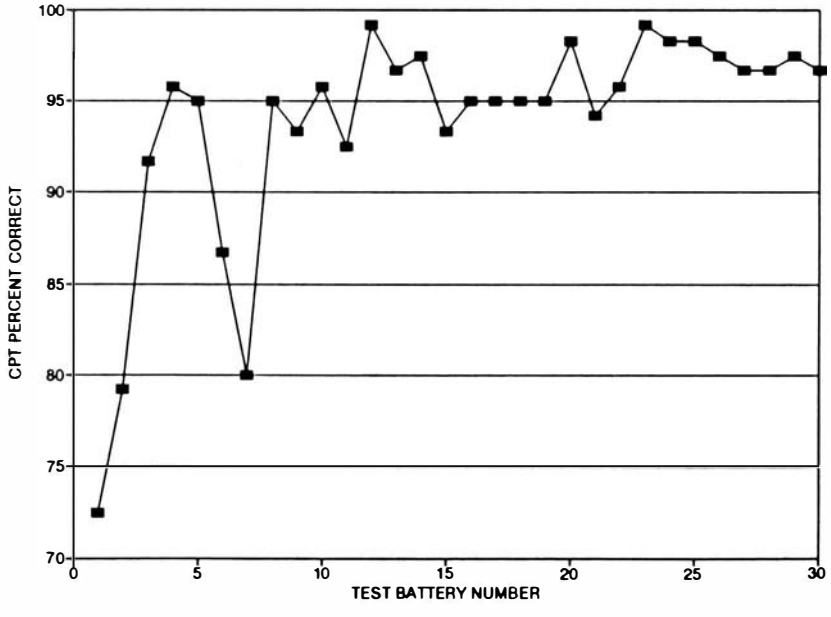


Figure D.6 Learning curve for continuous performance task (percent correct) for Subject 7

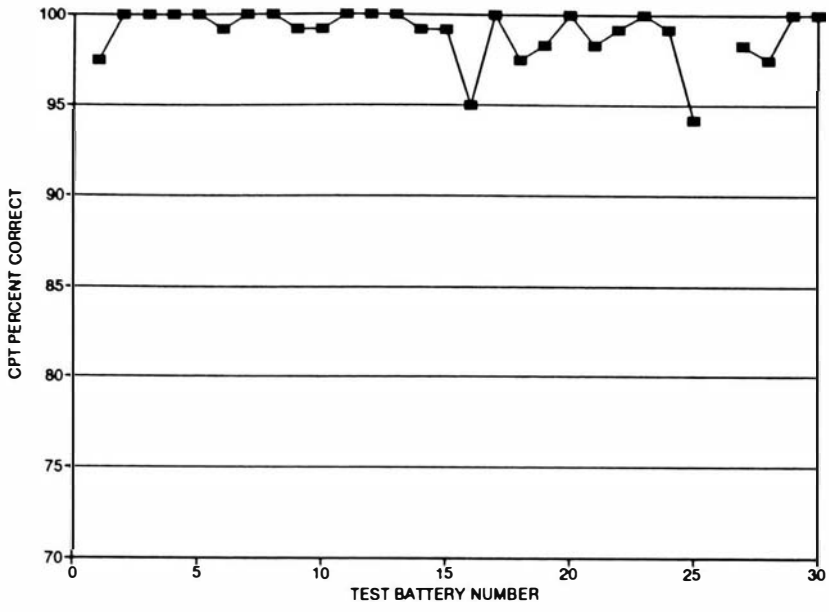


Figure D.7 Learning curve for continuous performance task (percent correct) for Subject 8

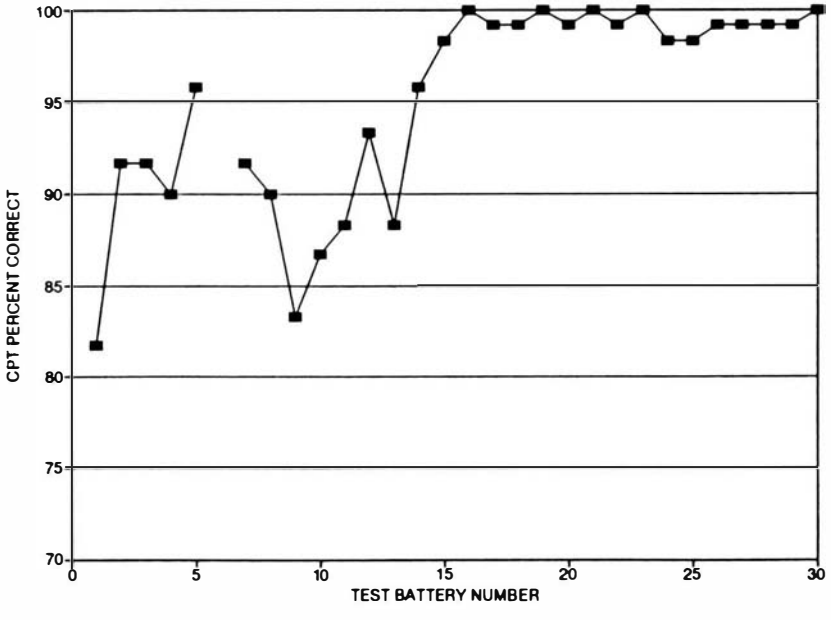


Figure D.8 Learning curve for continuous performance task (percent correct) for Subject 9

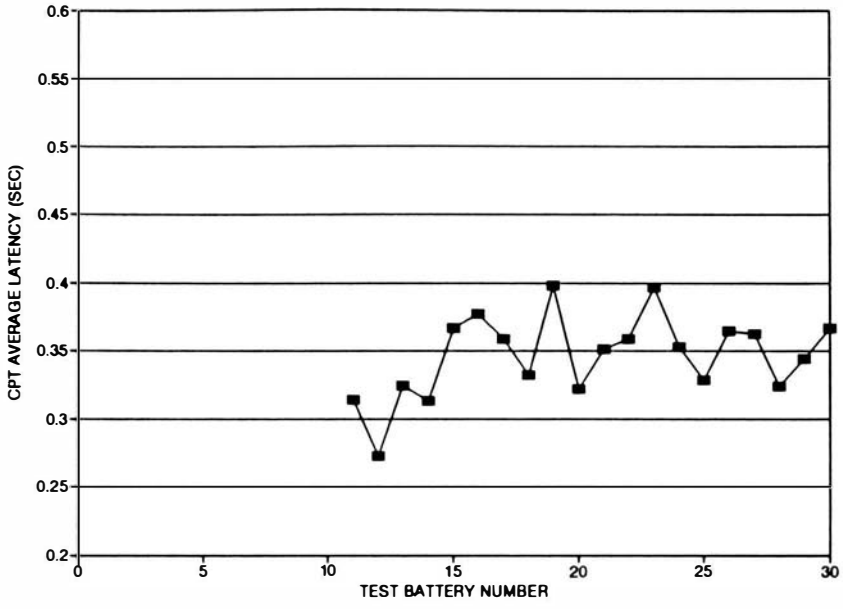


Figure D.9 Learning curve for continuous performance task (average latency) for Subject 1

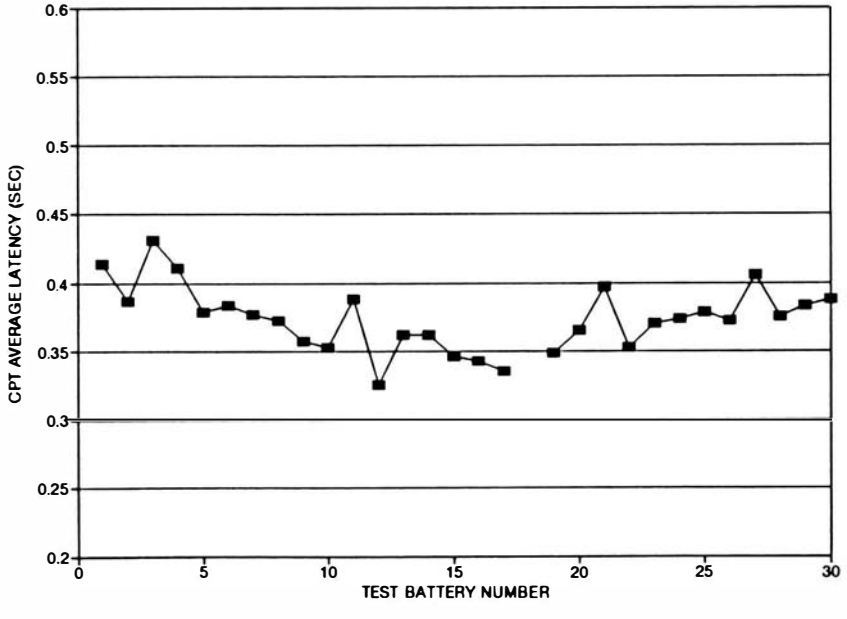


Figure D.10 Learning curve for continuous performance task (average latency) for Subject 2

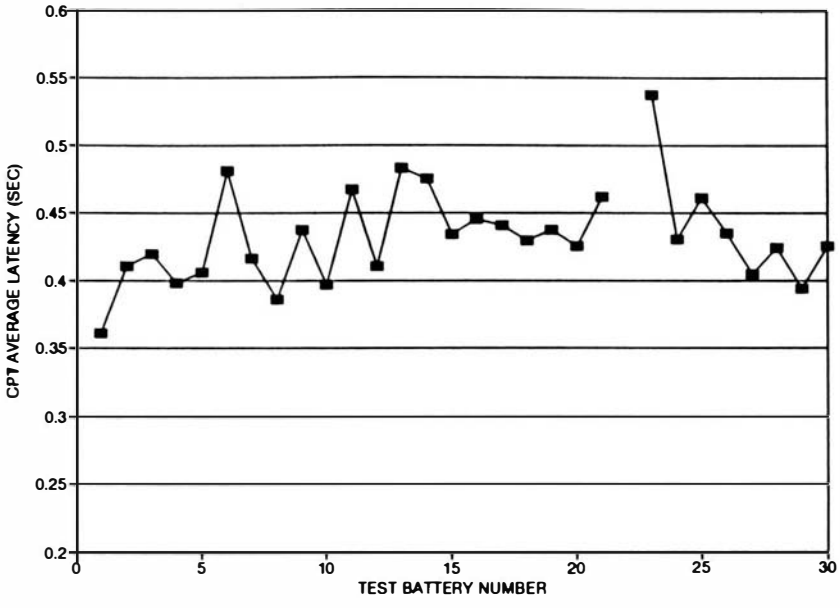


Figure D.11 Learning curve for continuous performance task (average latency) for Subject 3

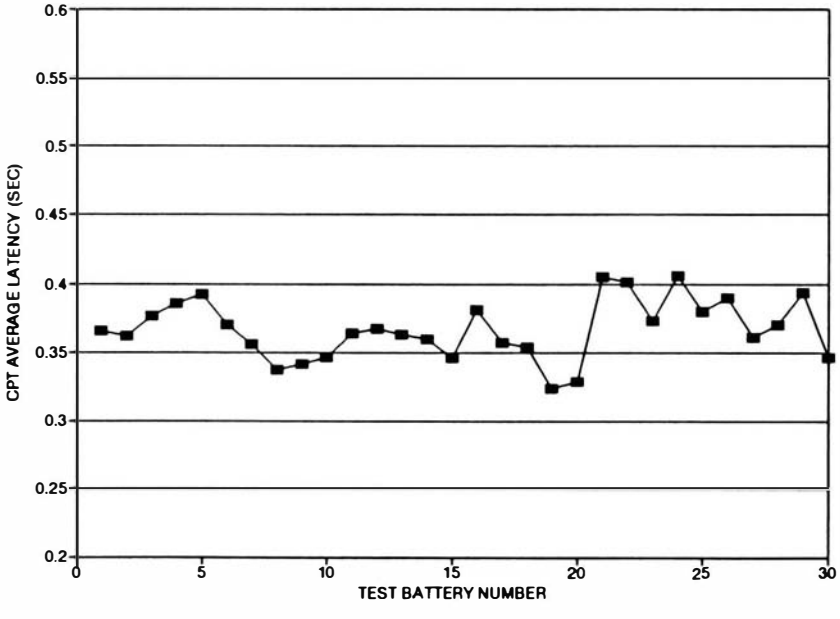


Figure D.12 Learning curve for continuous performance task (average latency) for Subject 5

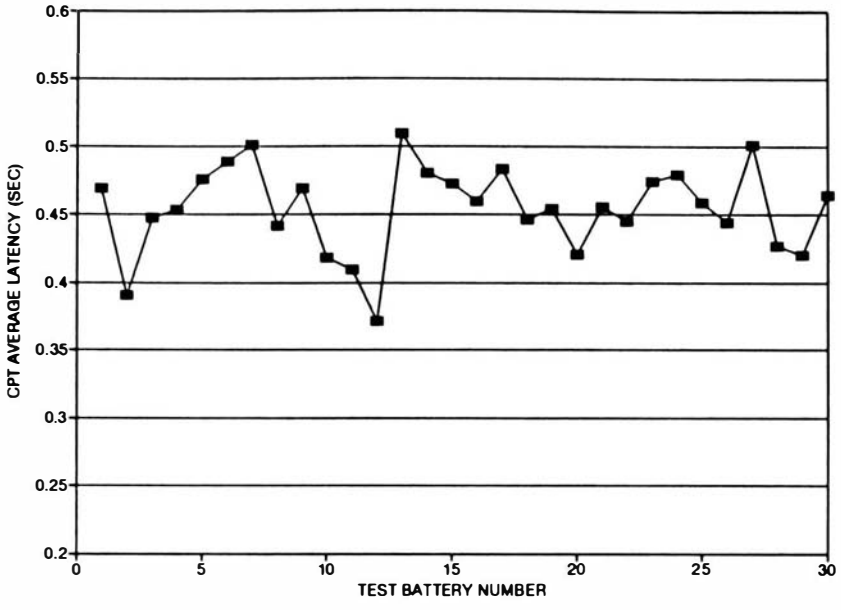


Figure D.13 Learning curve for continuous performance task (average latency) for Subject 6

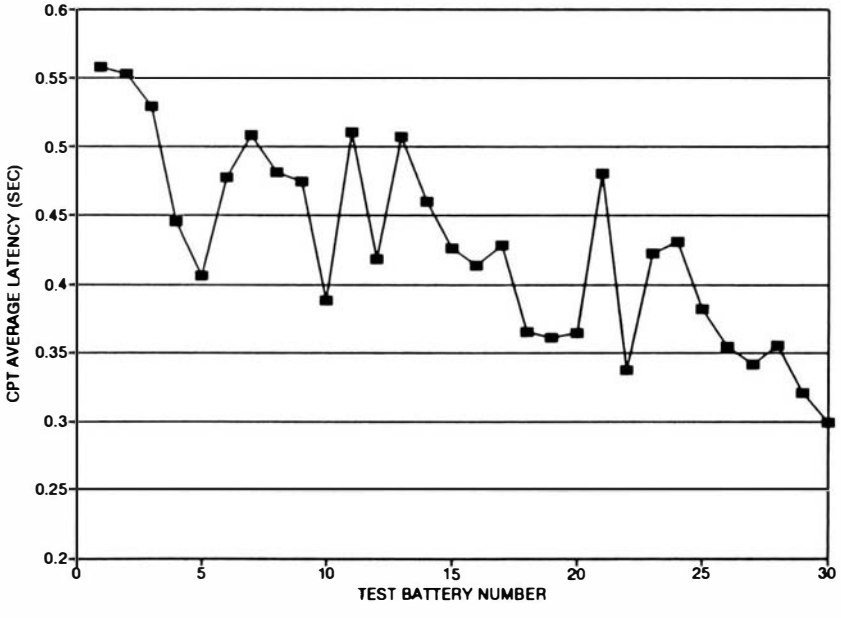


Figure D.14 Learning curve for continuous performance task (average latency) for Subject 7

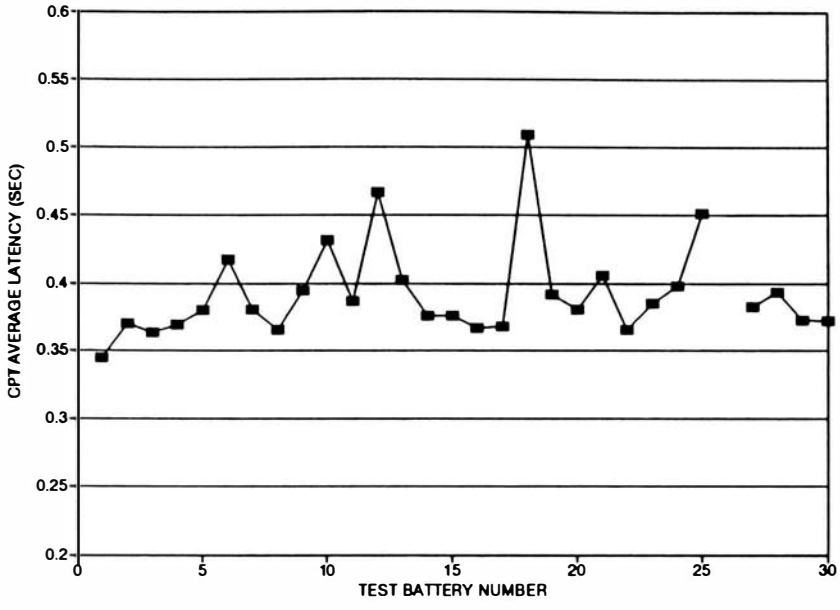


Figure D.15 Learning curve for continuous performance task (average latency) for Subject 8

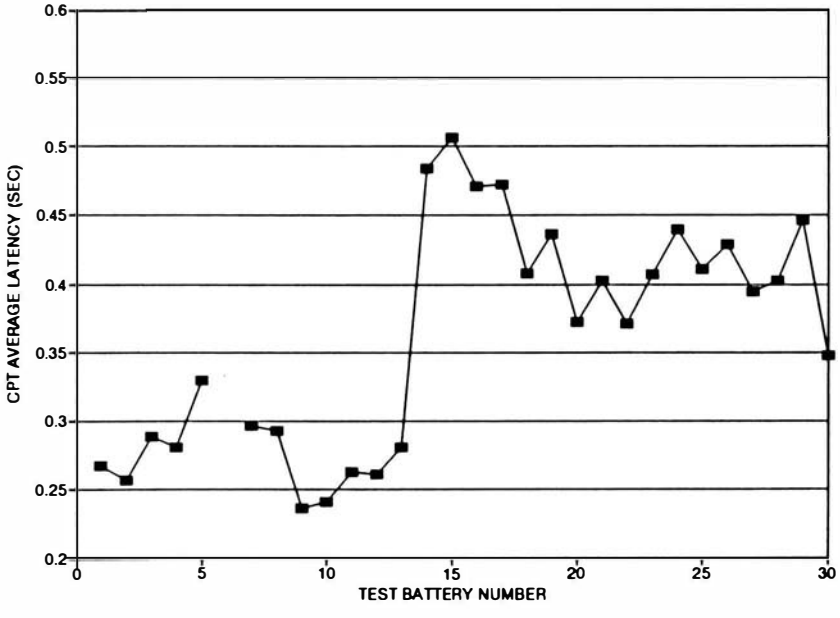


Figure D.16 Learning curve for continuous performance task (average latency) for Subject 9

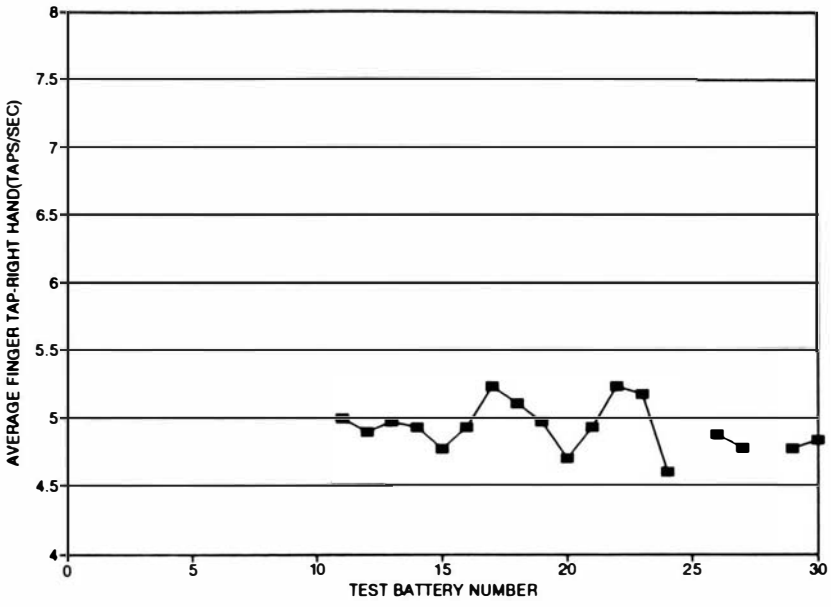


Figure D.17 Learning curve for finger tapping (right hand) for Subject 1

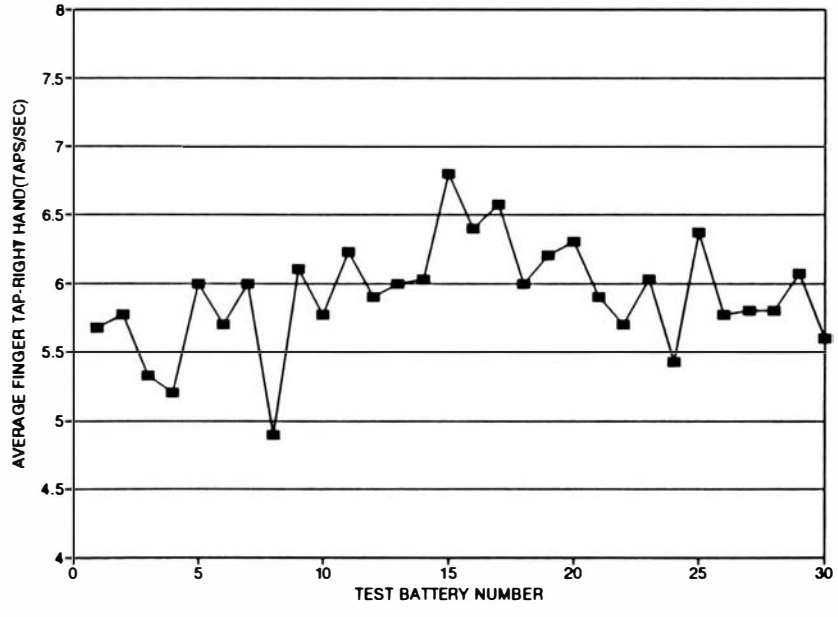


Figure D.18 Learning curve for finger tapping (right hand) for Subject 2

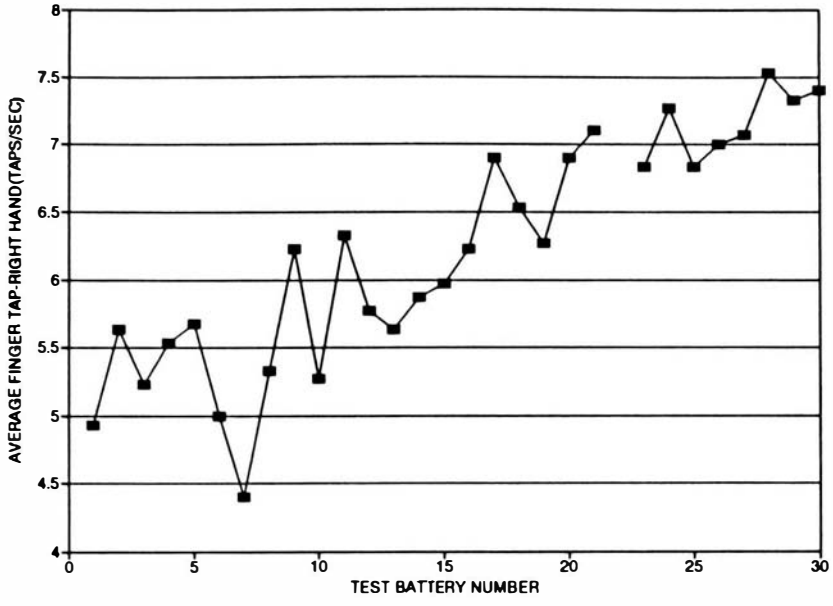


Figure D.19 Learning curve for finger tapping (right hand) for Subject 3

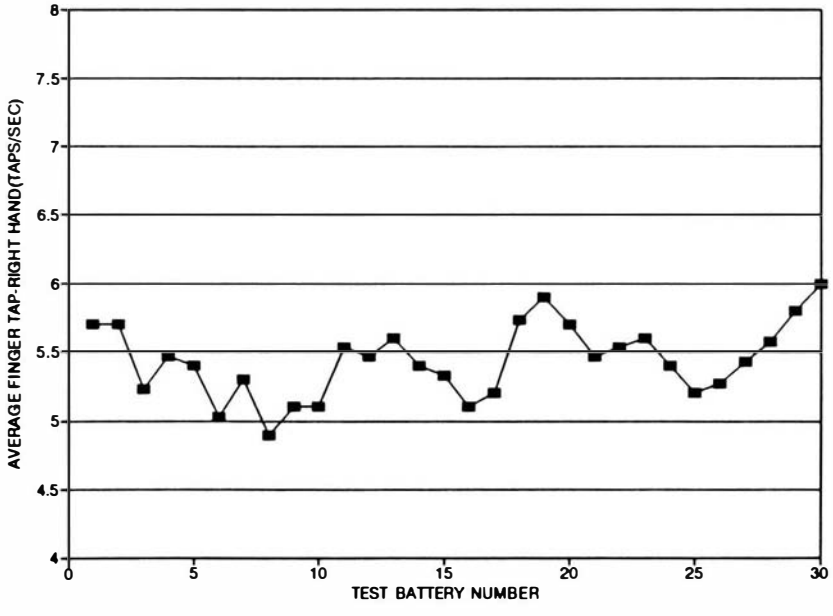


Figure D.20 Learning curve for finger tapping (right hand) for Subject 5

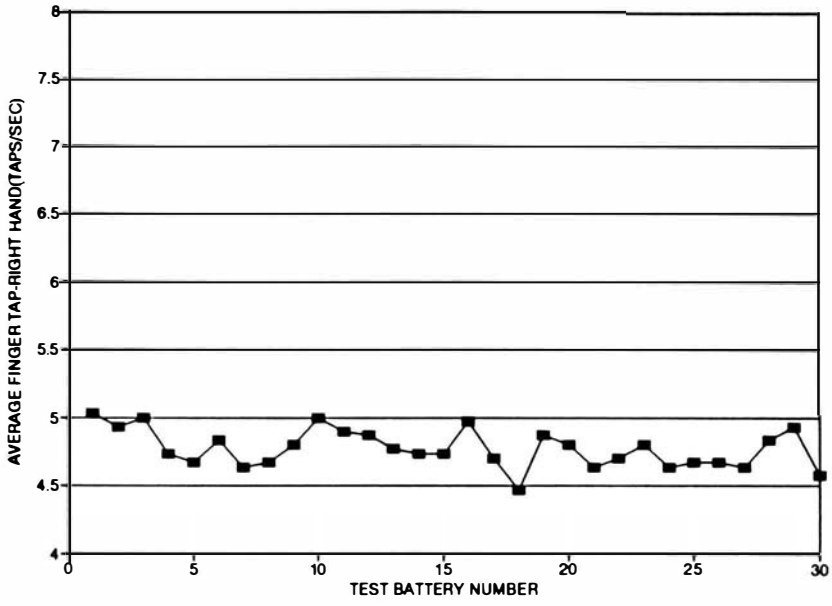


Figure D.21 Learning curve for finger tapping (right hand) for Subject 6

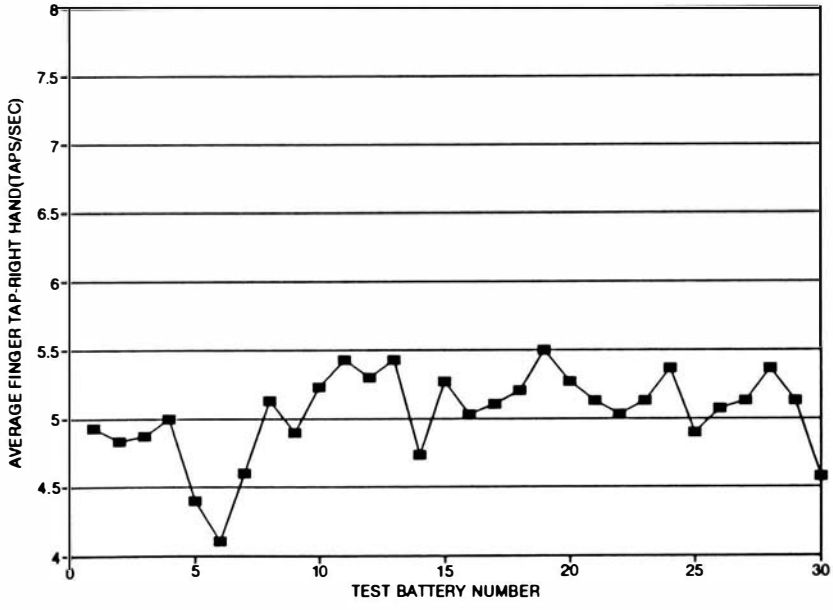


Figure D.22 Learning curve for finger tapping (right hand) for Subject 7

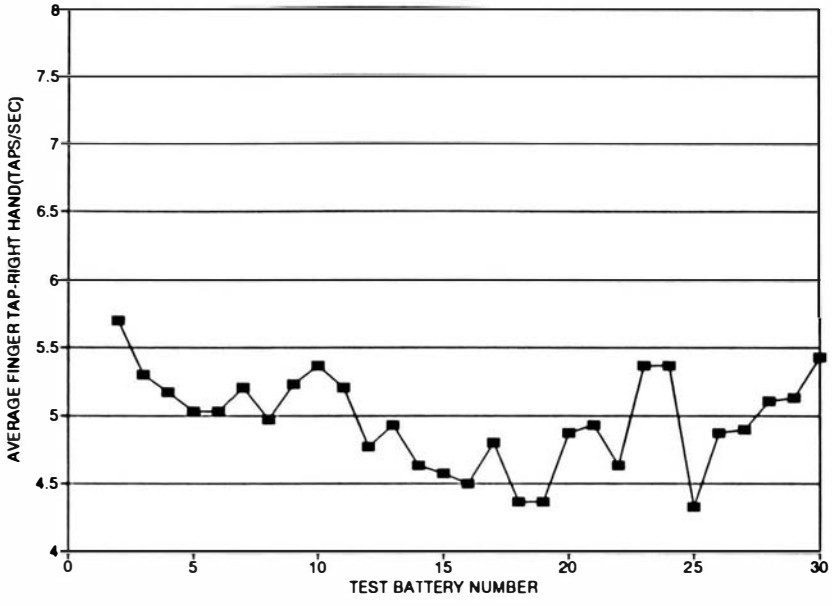


Figure D.23 Learning curve for finger tapping (right hand) for Subject 8

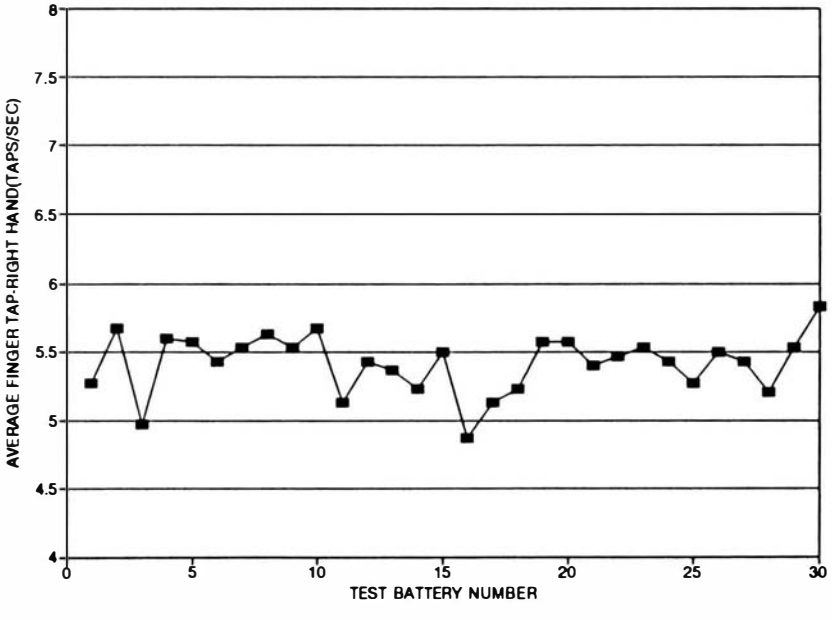


Figure D.24 Learning curve for finger tapping (right hand) for Subject 9

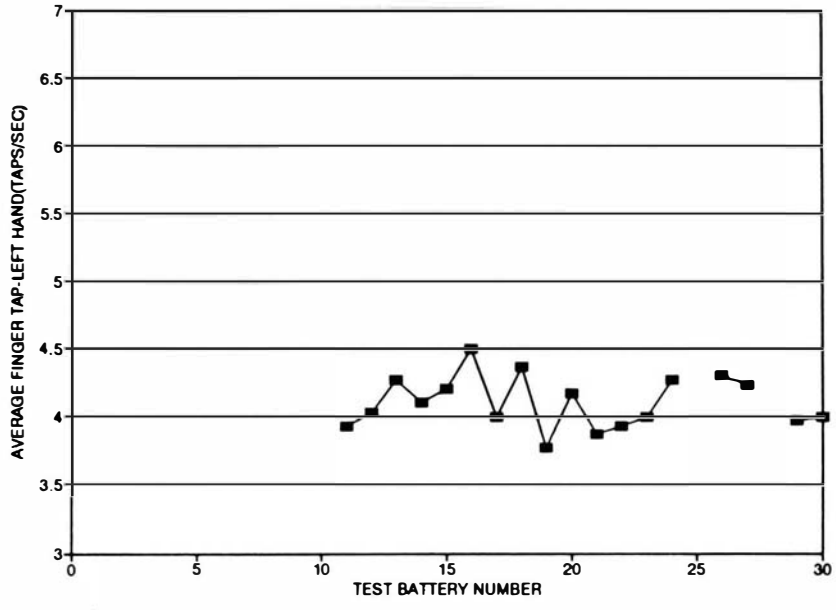


Figure D.25 Learning curve for finger tapping (left hand) for Subject 1

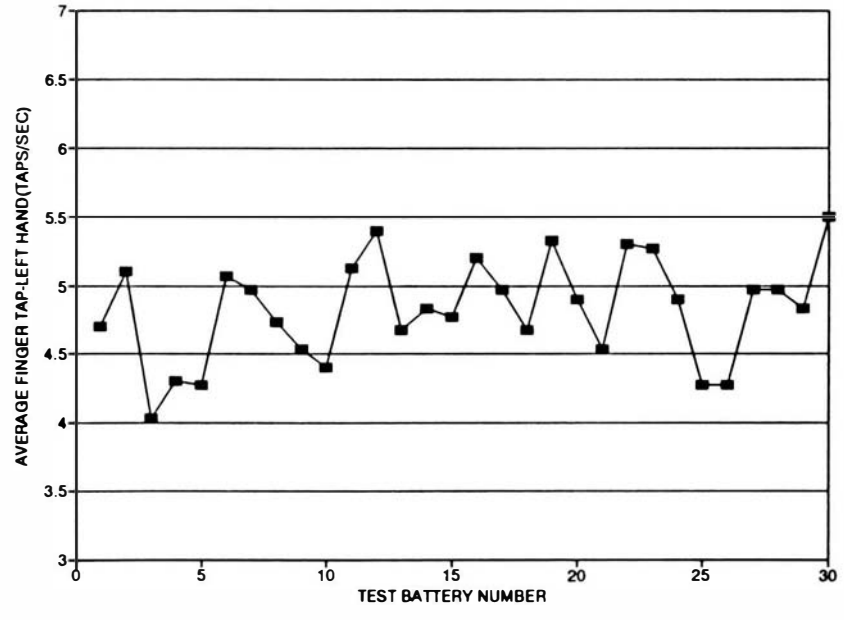


Figure D.26 Learning curve for finger tapping (left hand) for Subject 2

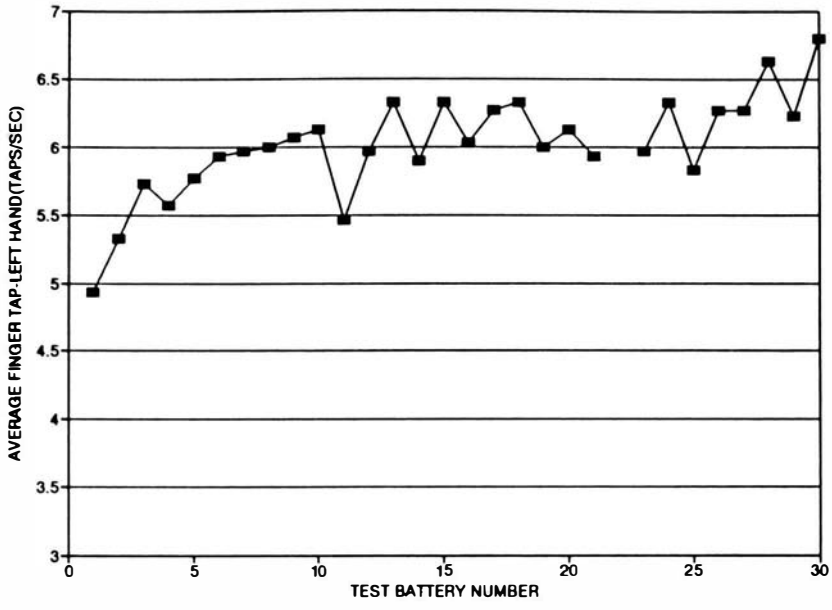


Figure D.27 Learning curve for finger tapping (left hand) for Subject 3

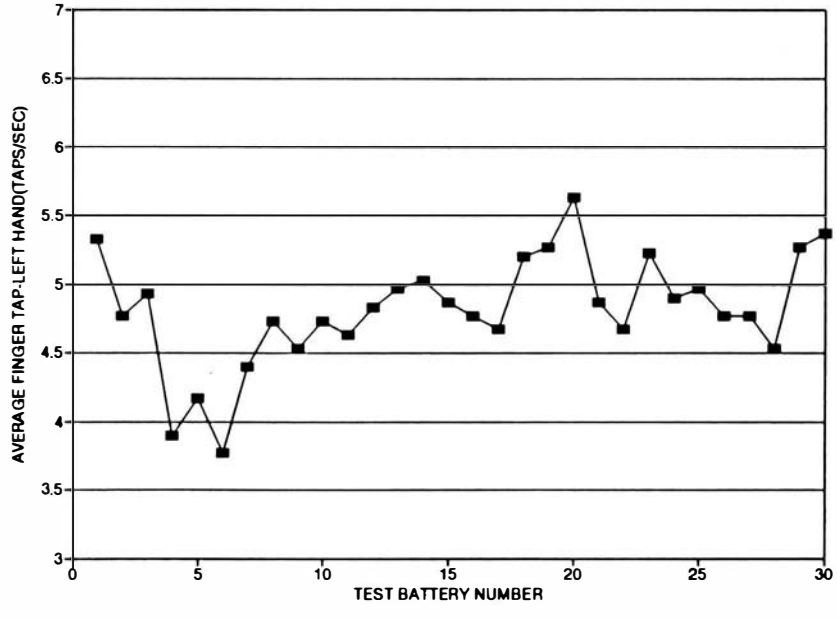


Figure D.28 Learning curve for finger tapping (left hand) for Subject 5

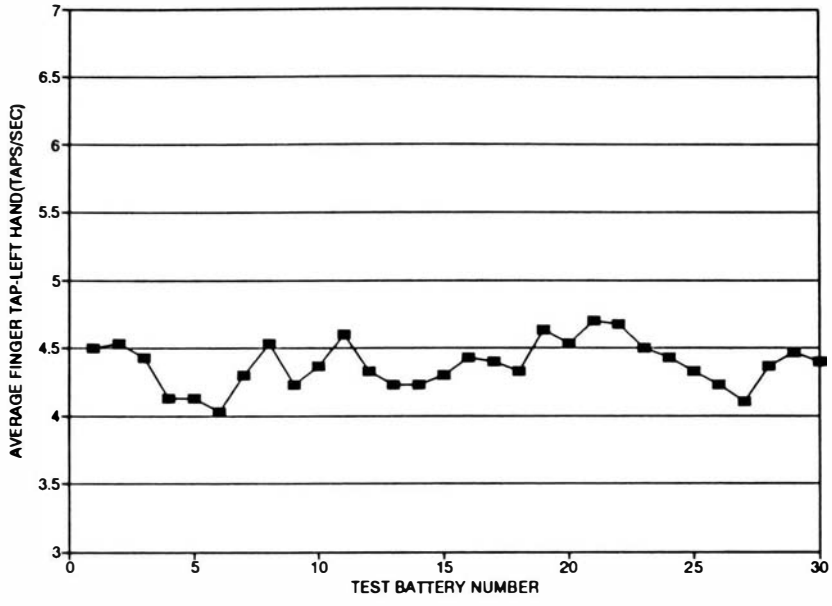


Figure D.29 Learning curve for finger tapping (left hand) for Subject 6

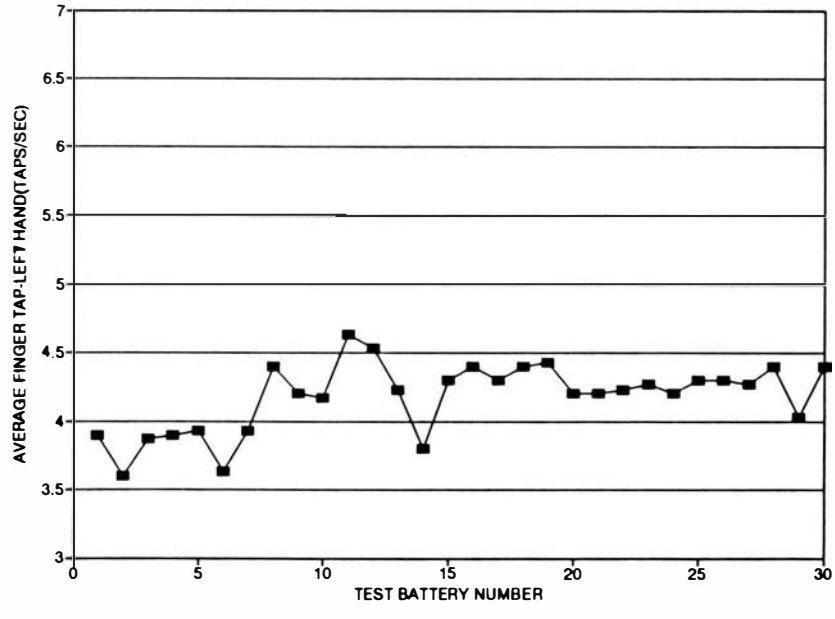


Figure D.30 Learning curve for finger tapping (left hand) for Subject 7

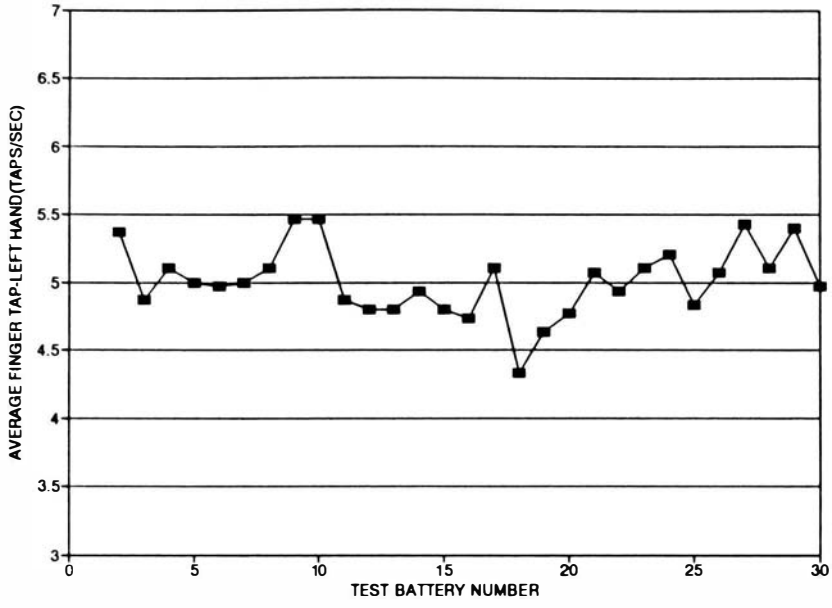


Figure D.31 Learning curve for finger tapping (left hand) for Subject 8

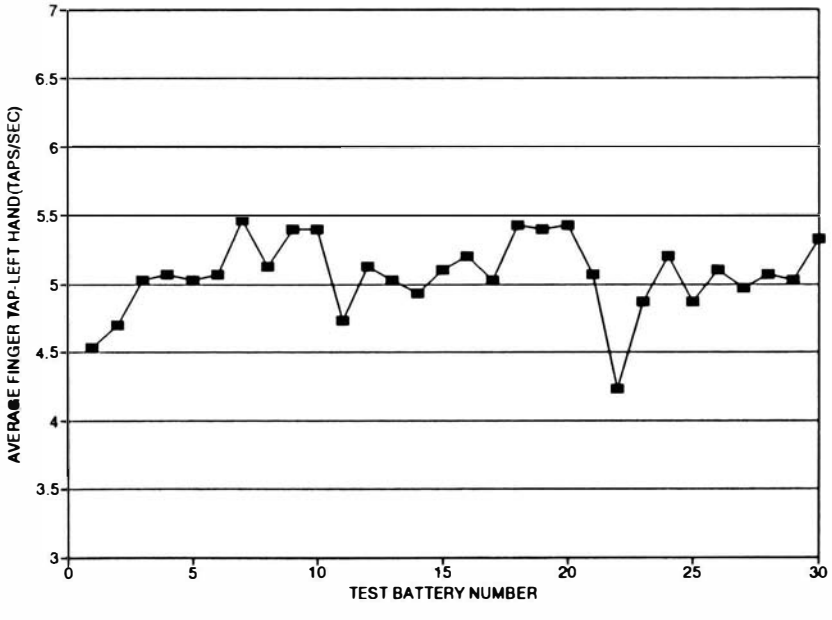


Figure D.32 Learning curve for finger tapping (left hand) for Subject 9

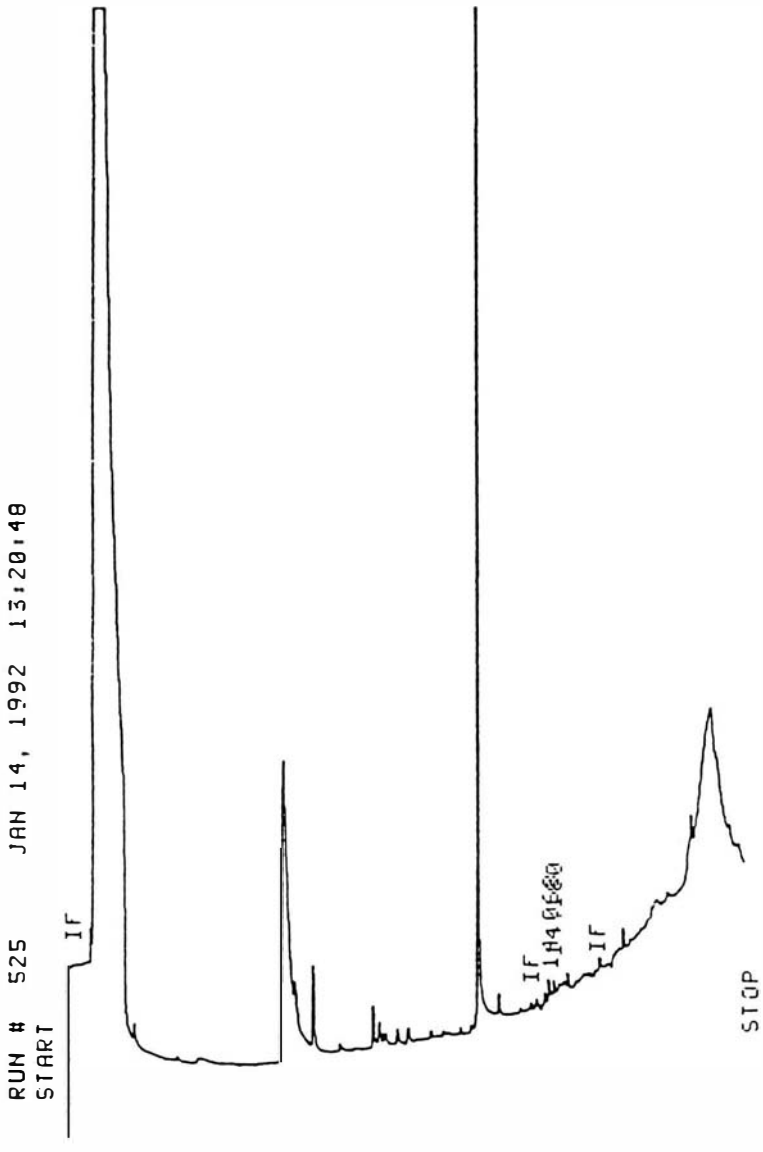
APPENDIX E

Table E.1 Randomization Schedule

SUBJECT NUMBER	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4
1	A	B	C	D
2	B	D	A	C
3	D	C	B	A
4	C	A	D	B
5	A	B	C	D
6	B	D	A	C
7	D	C	B	A
8	C	A	D	B
9	A	B	C	D
10	B	D	A	C

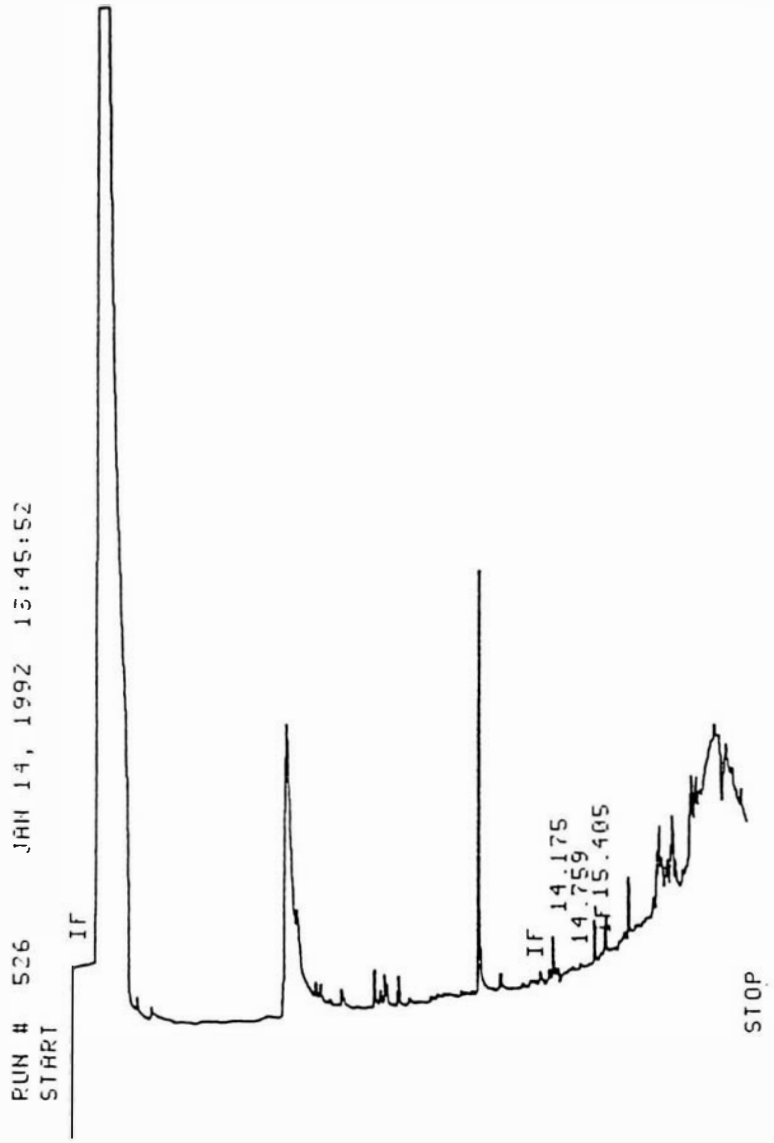
A = 5 MG DOSE (1 X 5 MG CAPSULE + 1 X PLACEBO CAPSULE)
B = PLACEBO (2 X PLACEBO CAPSULES)
C = 20 MG DOSE (2 X 10 MG CAPSULES)
D = 10 MG DOSE (1 X 10 MG CAPSULE + 1 X PLACEBO CAPSULE)

APPENDIX F



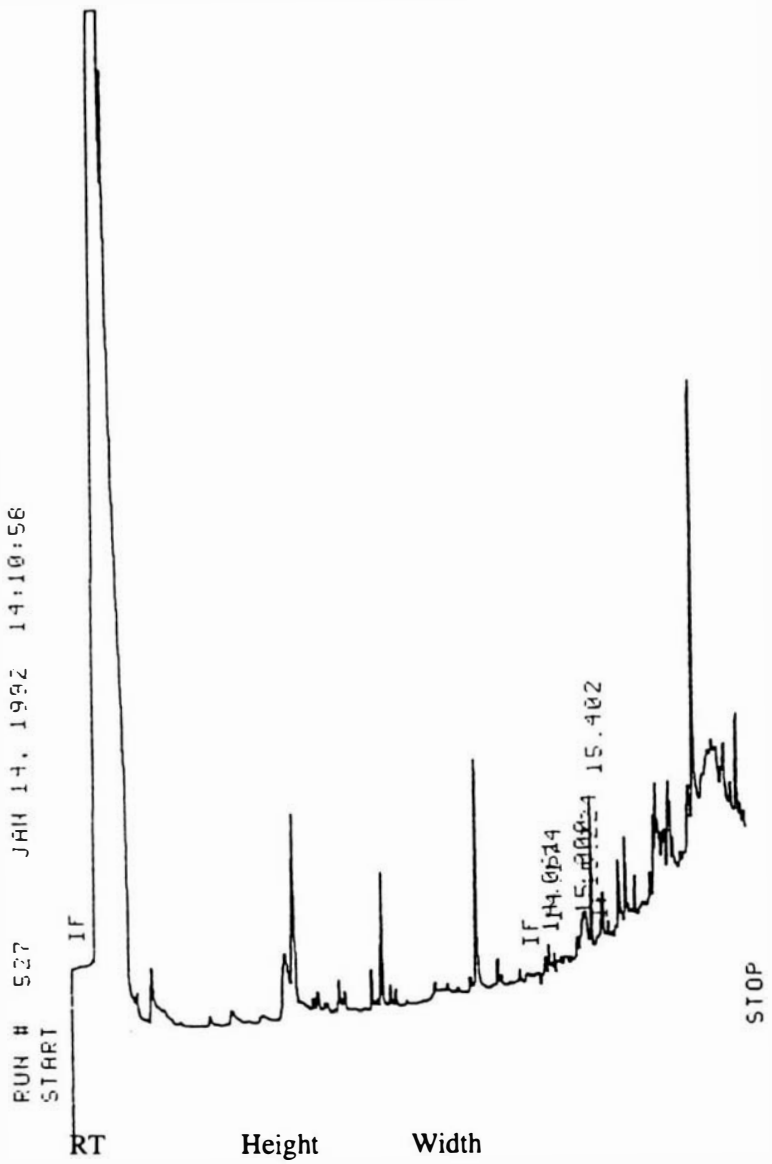
RT	Height	Width
14.068	95	0.068
14.180	142	0.036

Figure F.1 Chromatogram from blank serum of Subject 1



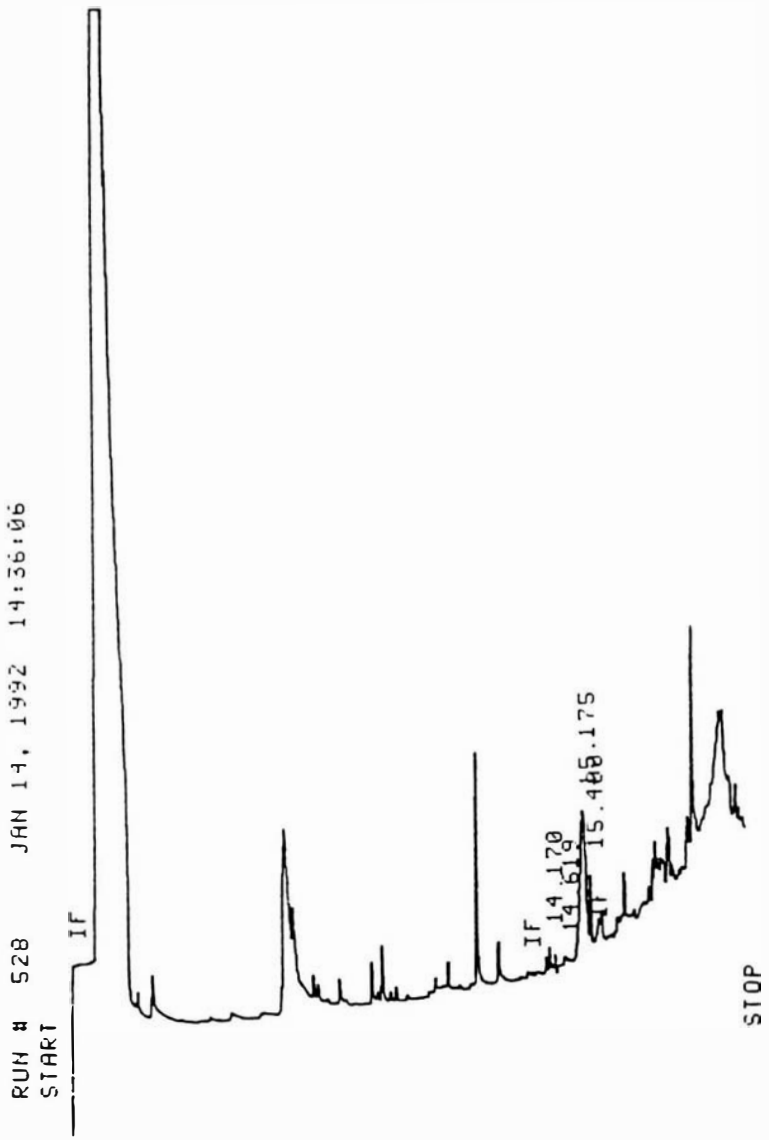
RT	Height	Width
14.175	361	0.048
14.759	61	0.322
15.405	364	0.063

Figure F.2 Chromatogram from blank serum of Subject 2



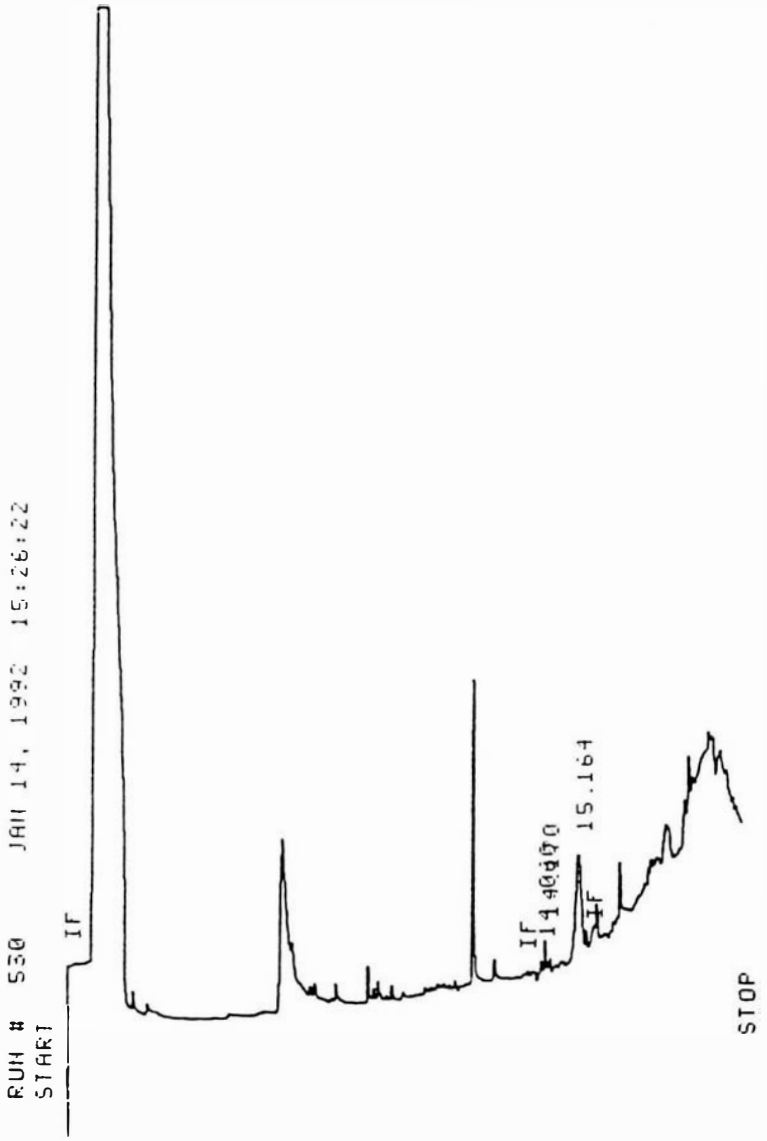
RT	Height	Width
14.061	199	0.091
14.174	270	0.055
15.000	151	0.046
15.224	314	0.186
15.402	1171	0.043

Figure F.3 Chromatogram from blank serum of Subject 4



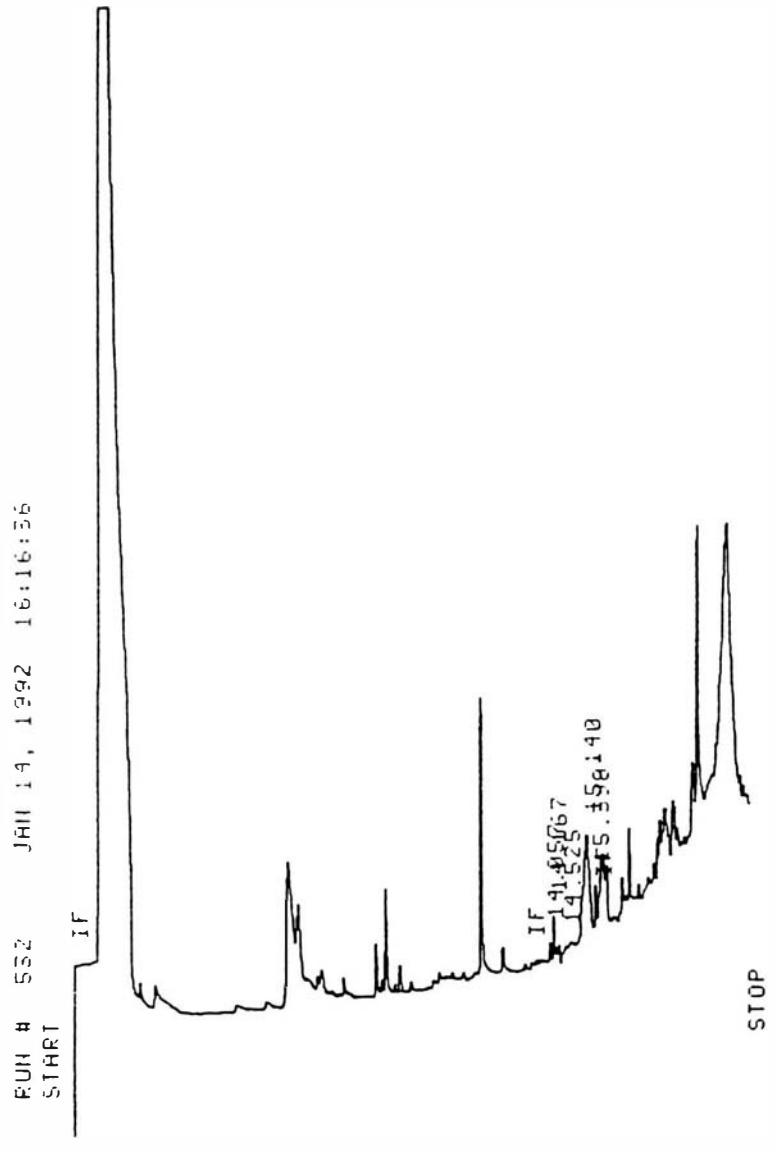
RT	Height	Width
14.170	171	0.037
14.619	45	0.132
15.175	1177	0.205
15.400	666	0.057

Figure F.4 Chromatogram from blank serum of Subject 5



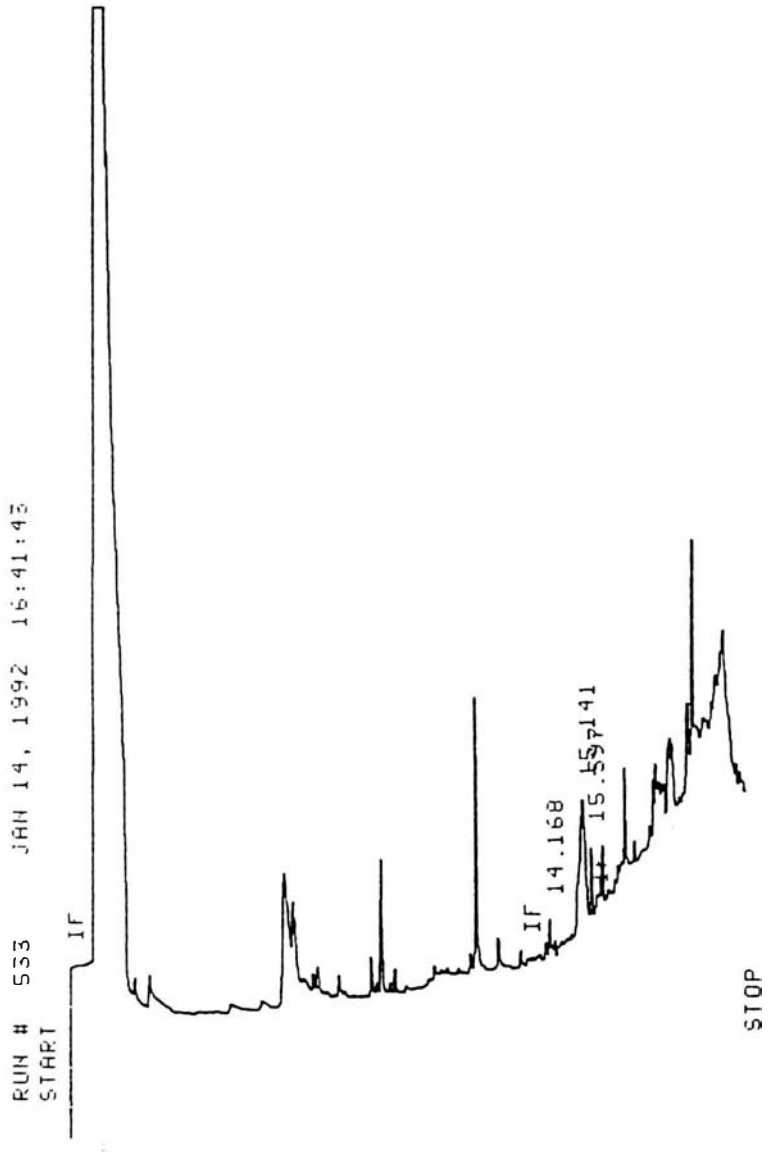
RT	Height	Width
14.060	117	0.081
14.170	231	0.040
15.164	897	0.225

Figure F.5 Chromatogram from blank serum of Subject 6



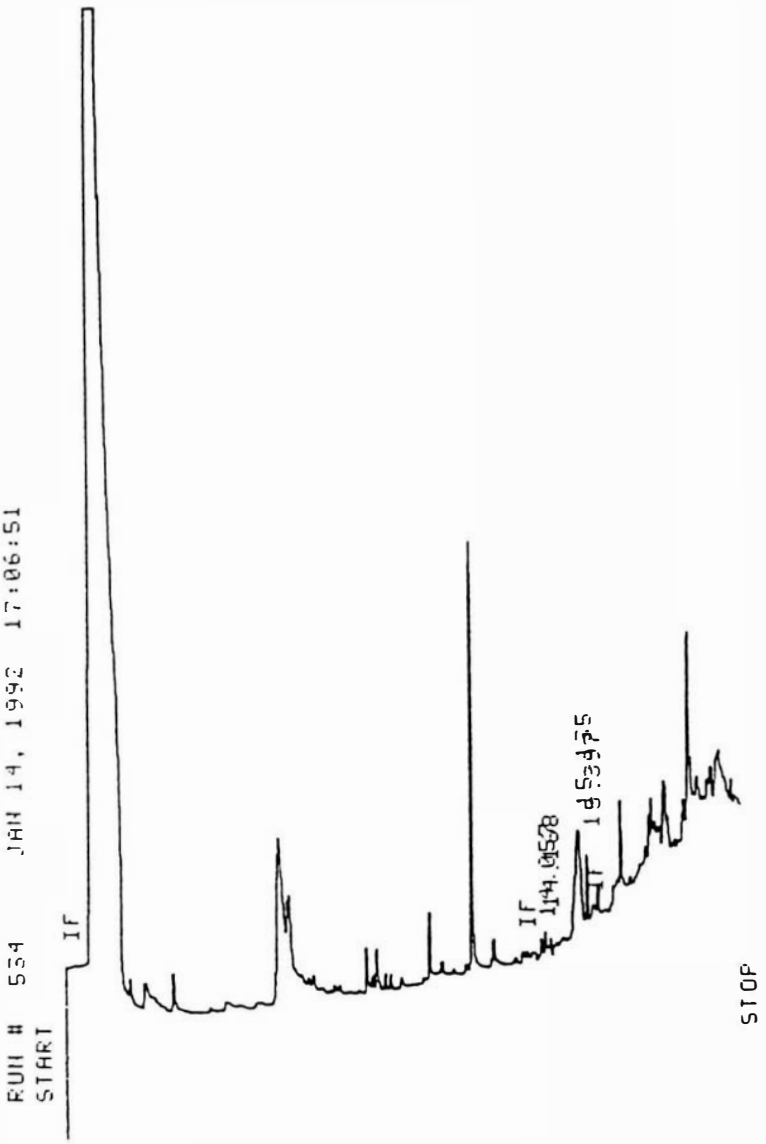
RT	Height	Width
14.057	155	0.052
14.167	367	0.047
14.525	140	0.131
15.140	1012	0.267
15.398	608	0.071

Figure F.6 Chromatogram from blank serum of Subject 7



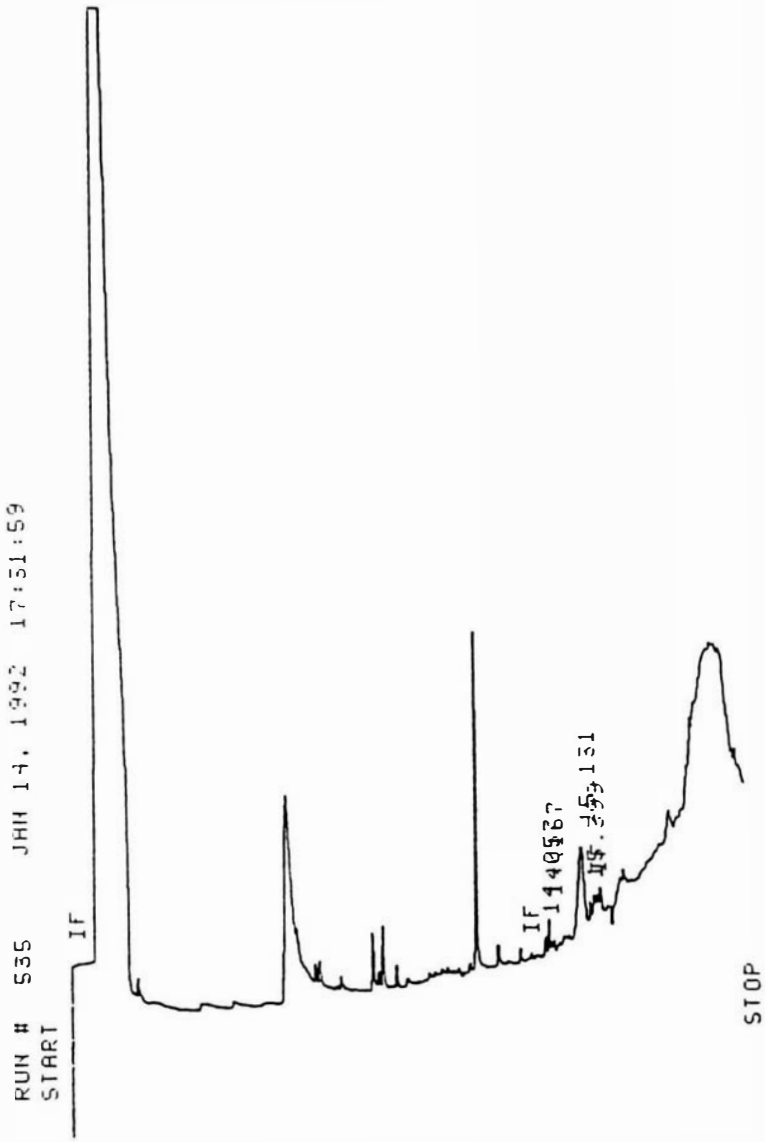
RT	Height	Width
14.168	280	0.045
15.141	1092	0.221
15.397	716	0.061

Figure F.7 Chromatogram from blank serum of Subject 8



RT	Height	Width
14.057	143	0.067
14.168	180	0.047
15.135	869	0.216
15.397	691	0.069

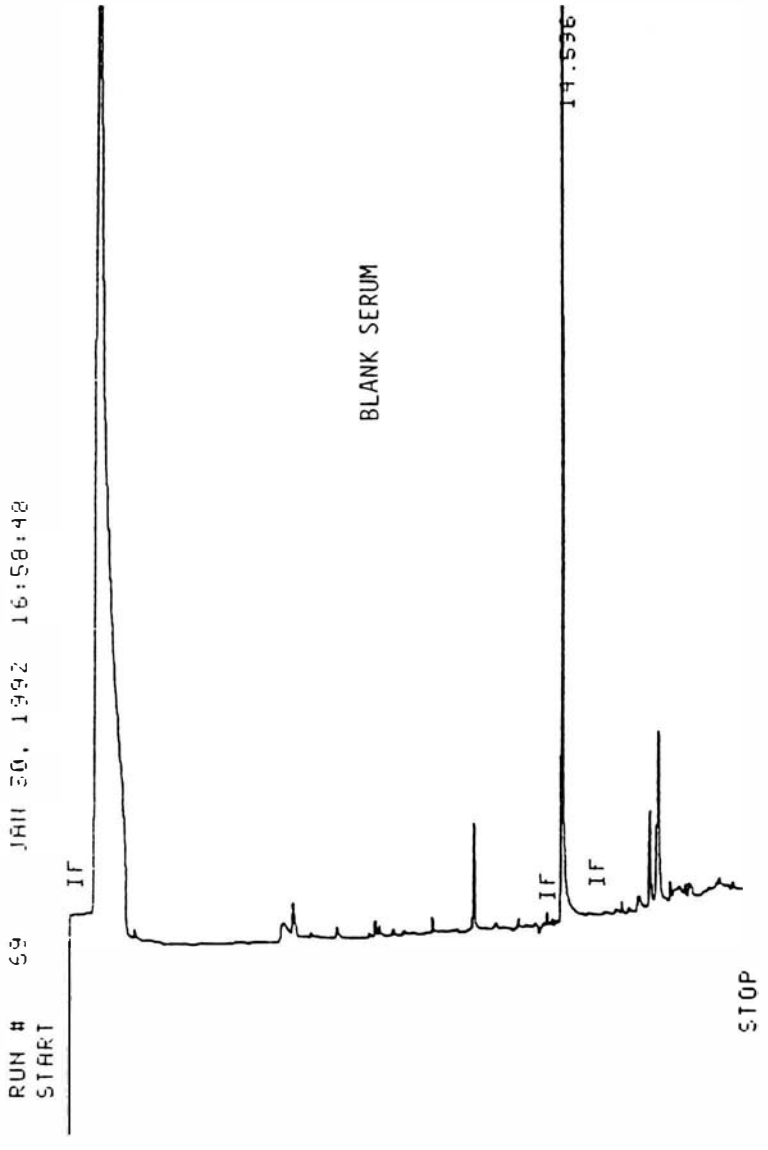
Figure F.8 Chromatogram from blank serum of Subject 9



RT	Height	Width
14.057	133	0.046
14.167	253	0.041
15.131	736	0.222
15.399	293	0.079

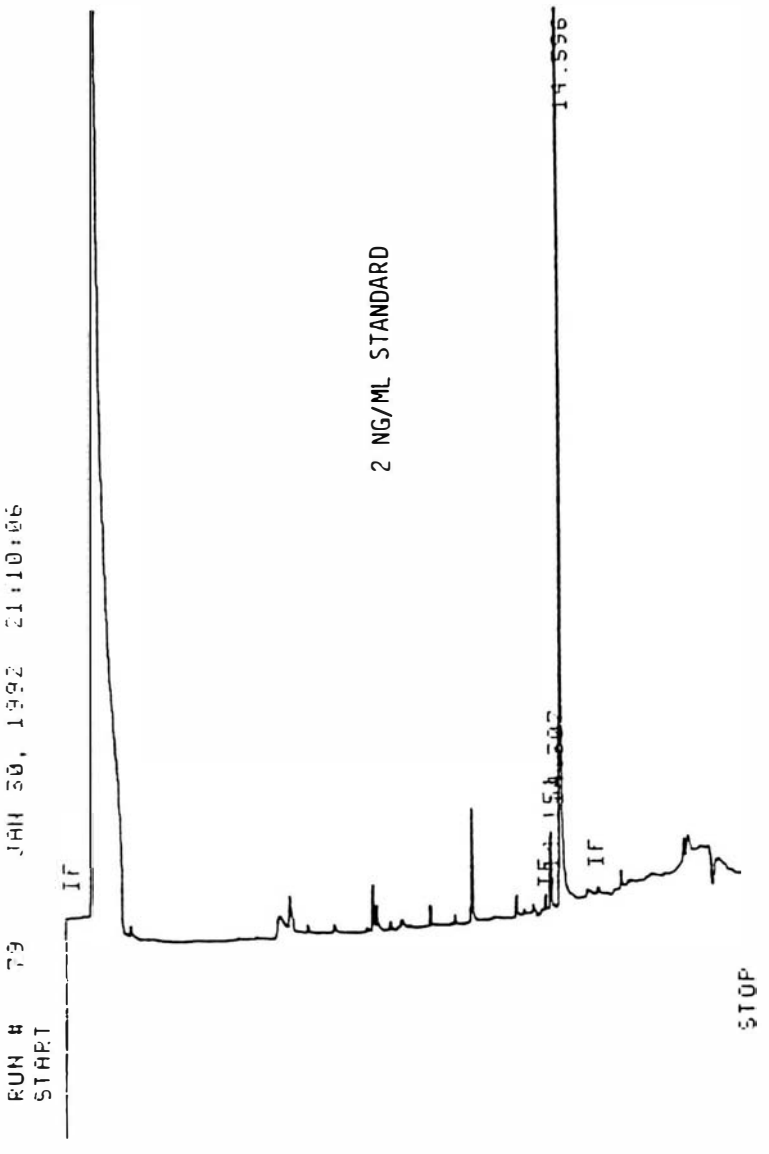
Figure F.9 Chromatogram from blank serum of Subject 10

APPENDIX G



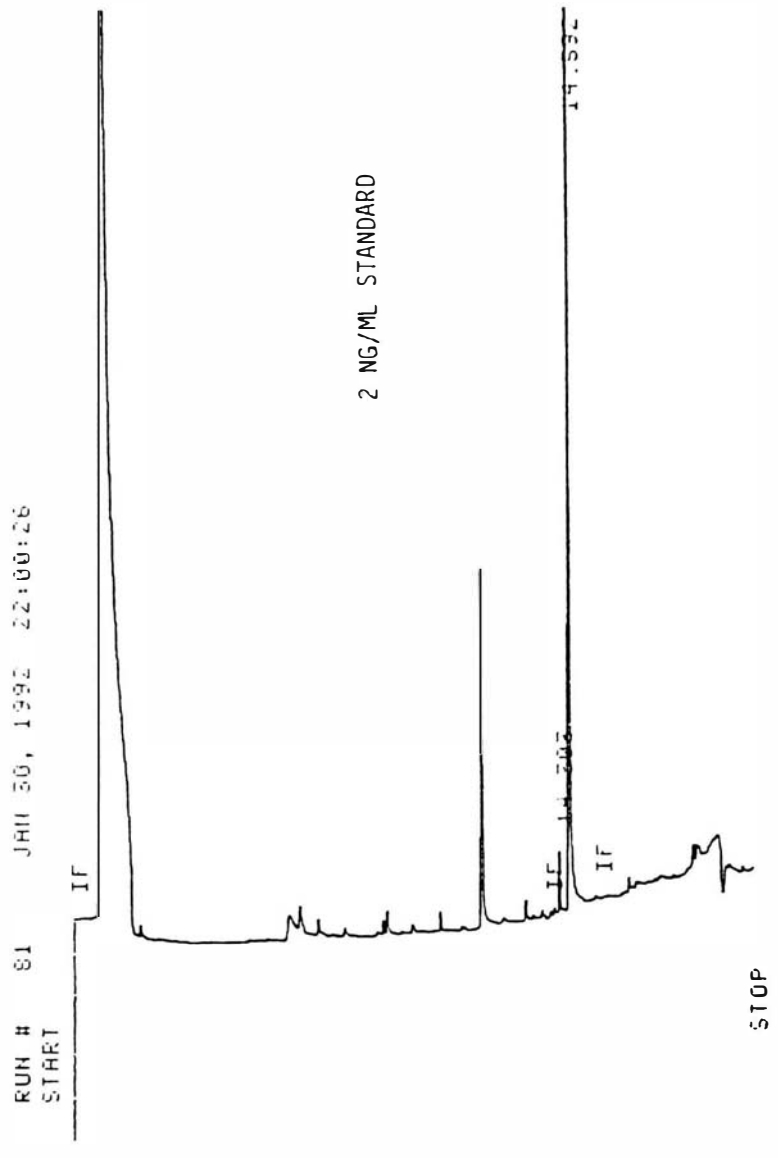
RT	Area	Width
14.596	50010	0.045

Figure G.1 Chromatogram from pooled blank serum



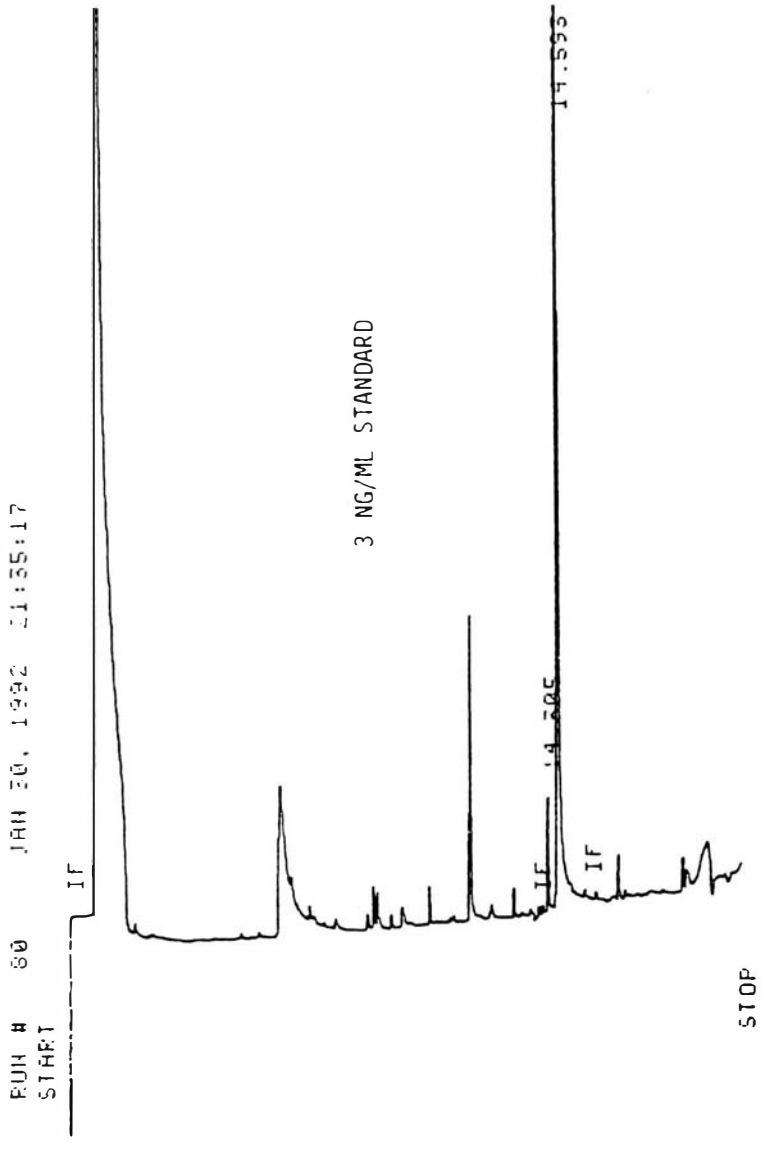
RT	Area	Width
14.151	589	0.038
14.307	3208	0.044
14.596	46282	0.046

Figure G.2 Chromatogram from 2 ng/mL standard - start of run



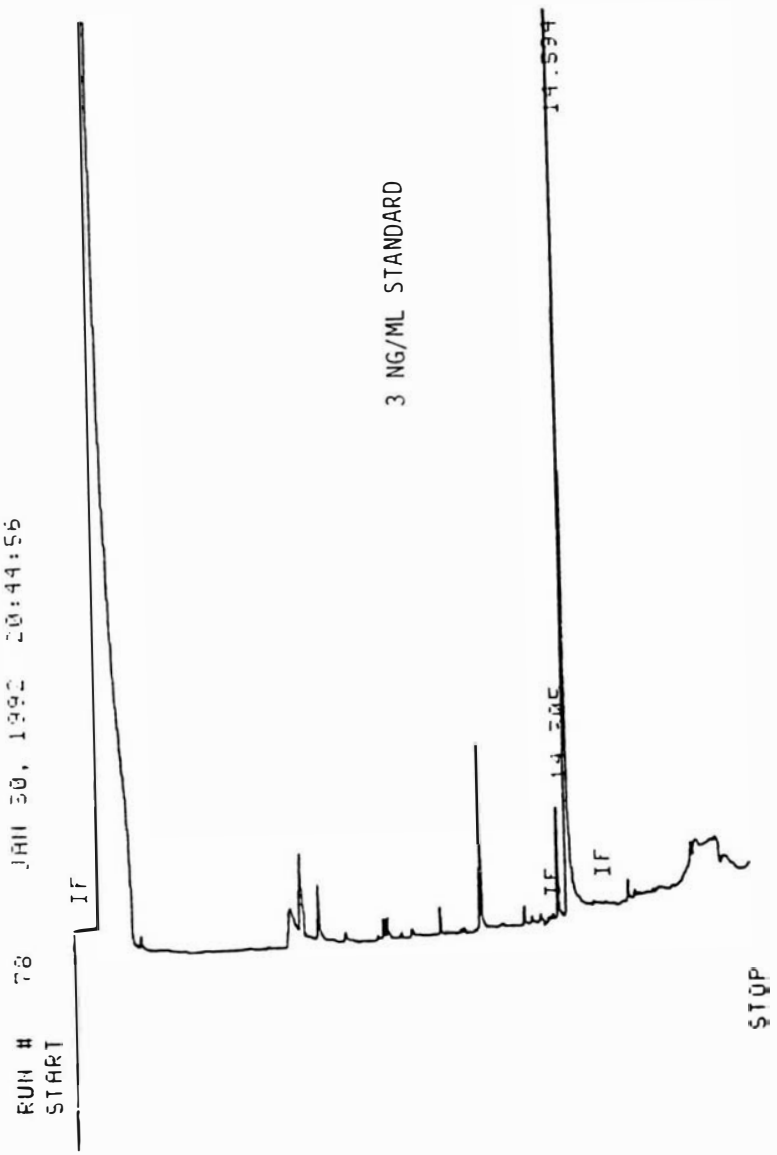
RT	Area	Width
14.303	2608	0.044
14.592	46906	0.047

Figure G.3 Chromatogram from 2 ng/mL standard - end of run



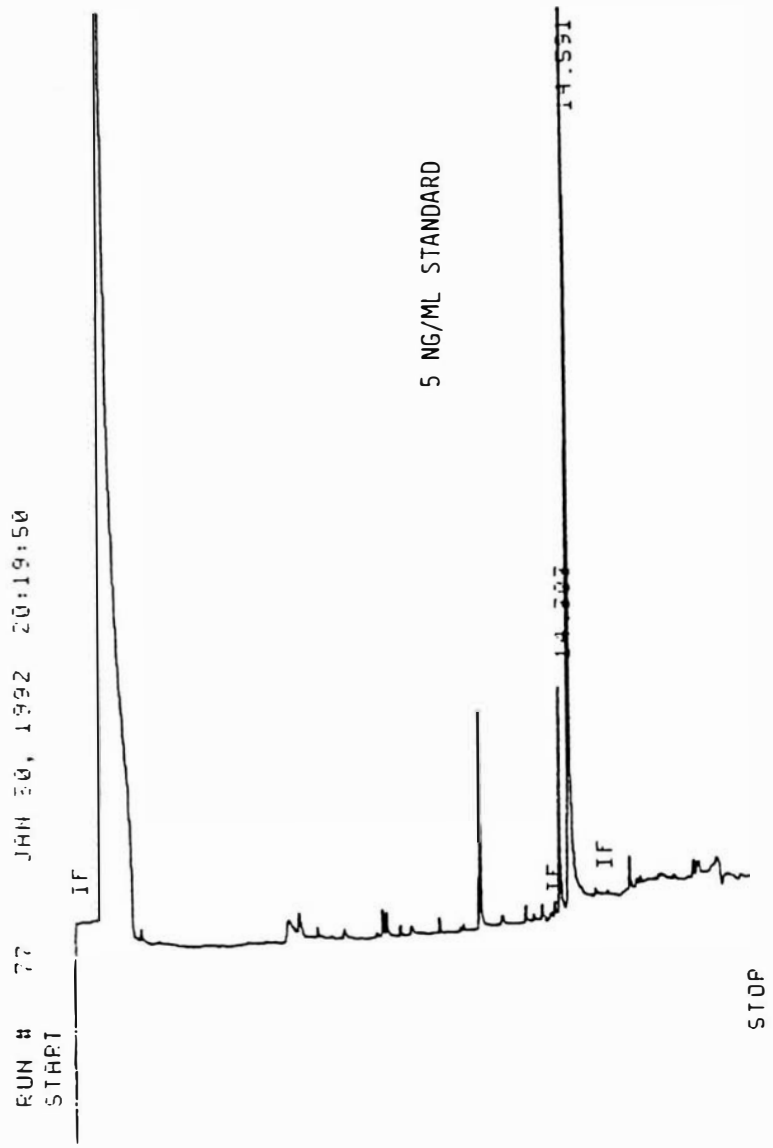
RT	Area	Width
14.305	4784	0.044
14.593	94662	0.043

Figure G.4 Chromatogram from 3 ng/mL standard - start of run



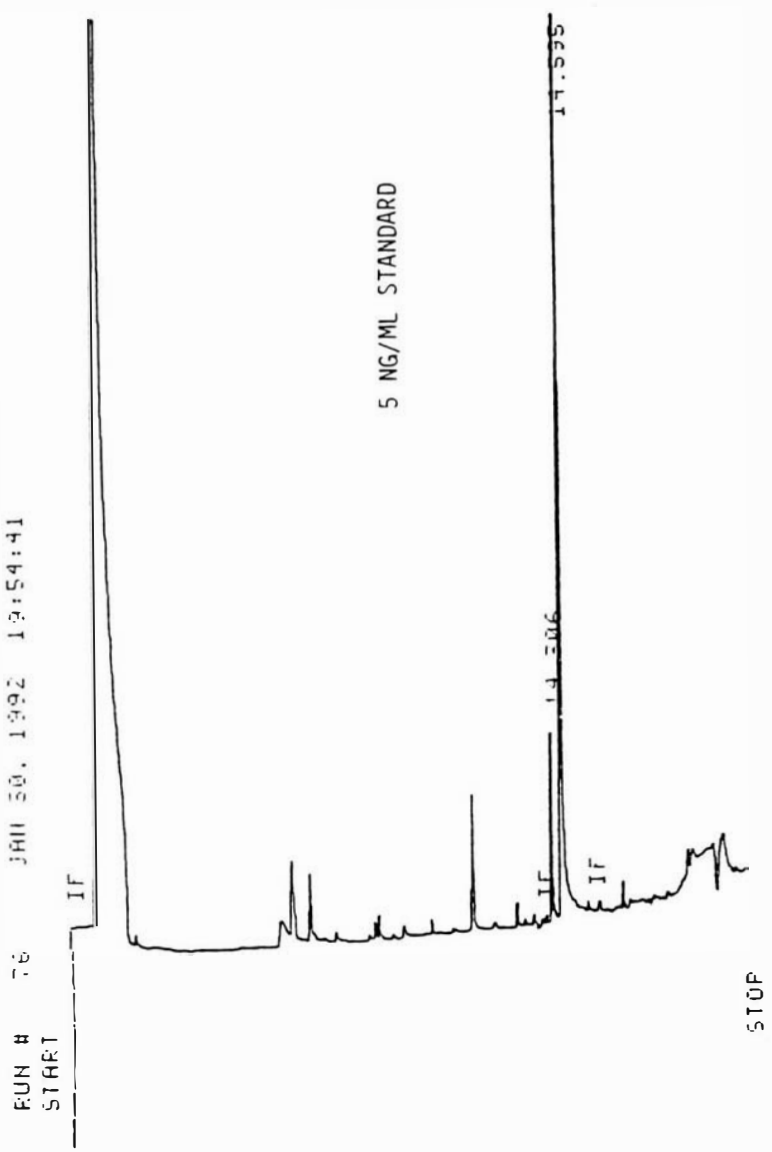
RT	Area	Width
14.305	4675	0.045
14.594	66293	0.045

Figure G.5 Chromatogram from 3 ng/mL standard - end of run



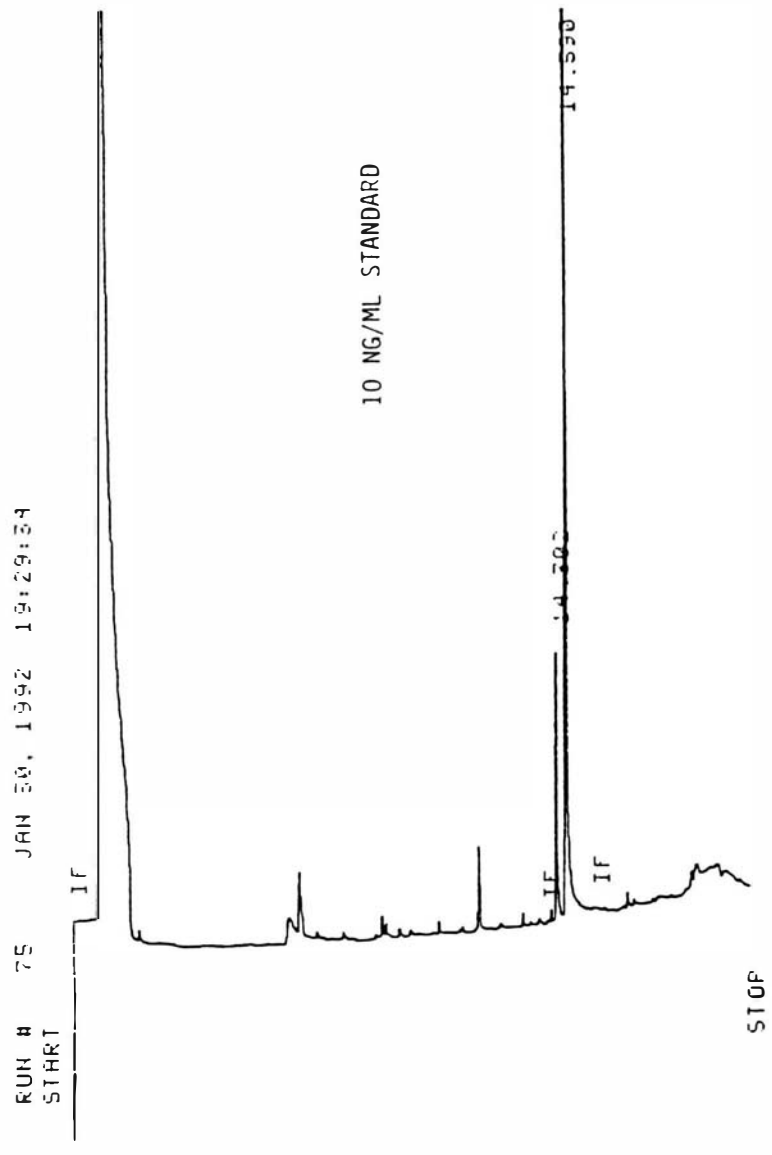
RT	Area	Width
14.303	9501	0.045
14.591	82014	0.043

Figure G.6 Chromatogram from 5 ng/mL standard - start of run



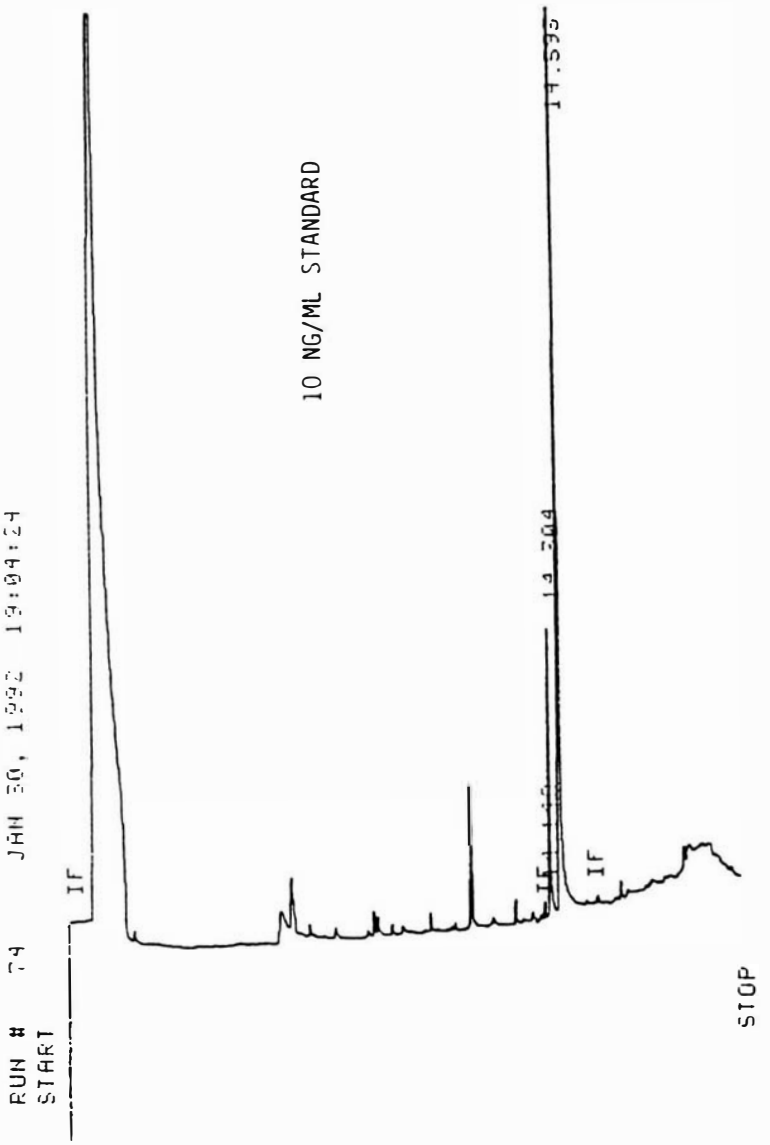
RT	Area	Width
14.306	8220	0.046
14.595	72548	0.046

Figure G.7 Chromatogram from 5 ng/mL standard - end of run



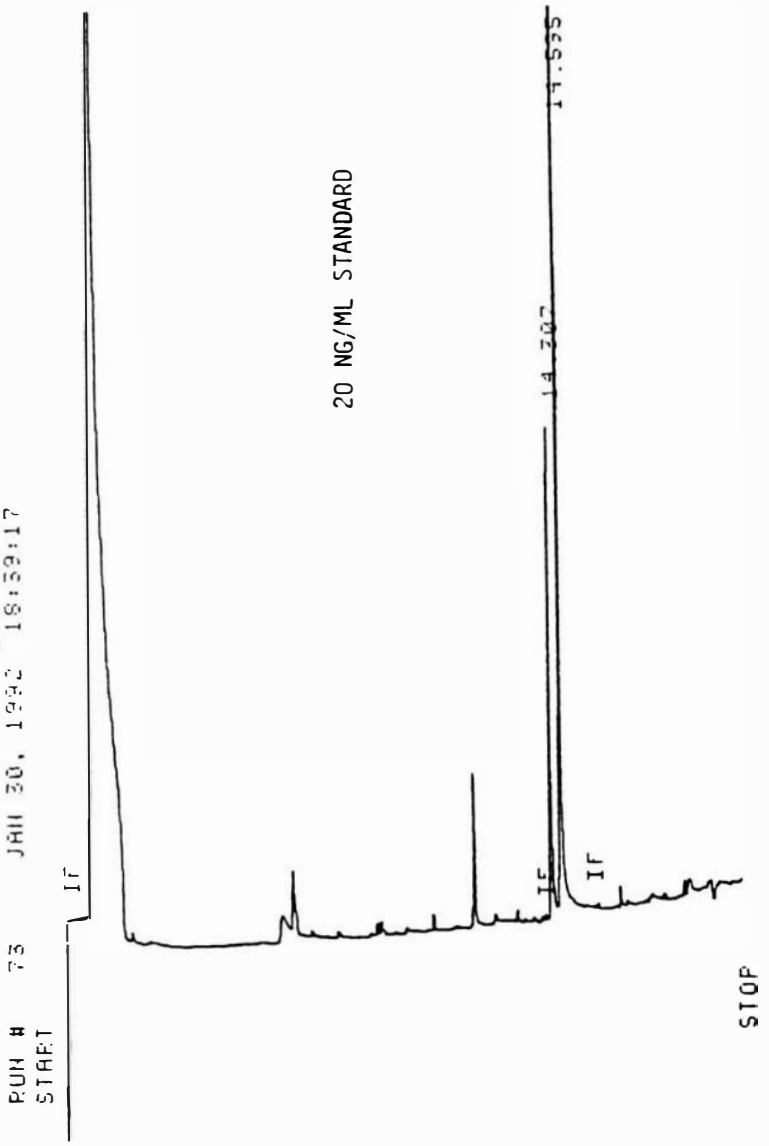
RT	Area	Width
14.302	11903	0.047
14.590	60020	0.046

Figure G.8 Chromatogram from 10 ng/mL standard - start of run



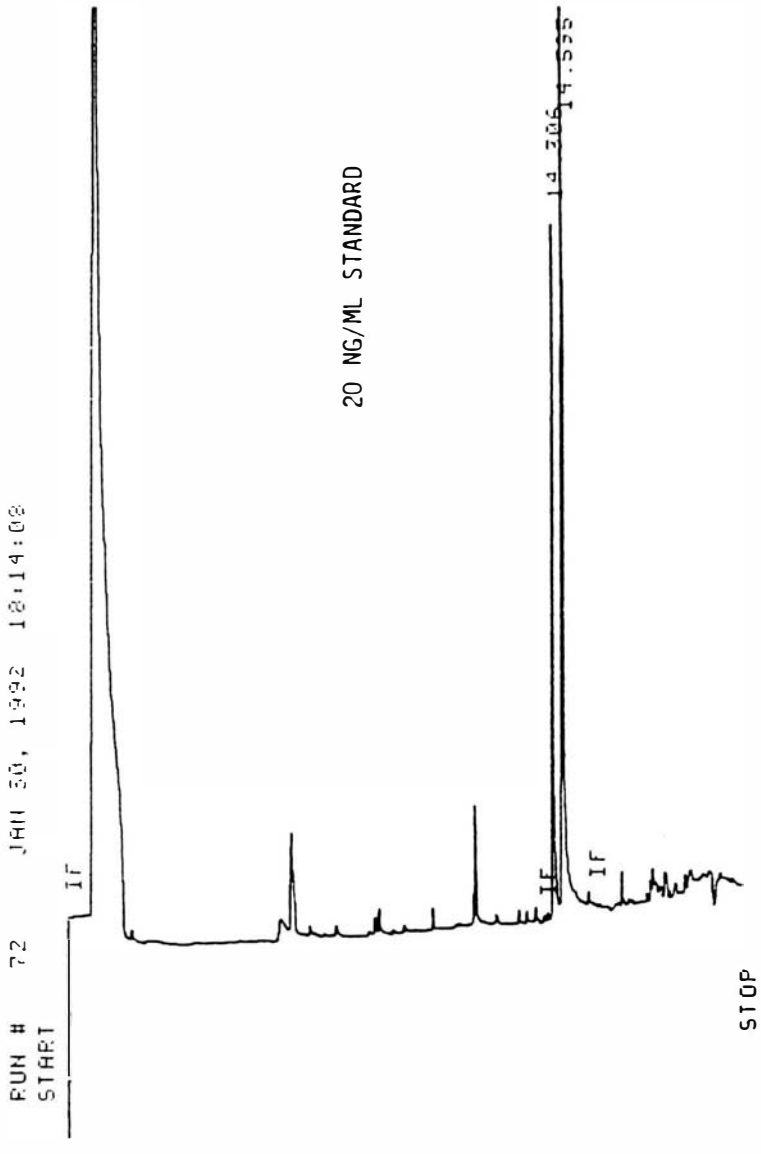
RT	Area	Width
14.148	562	0.037
14.304	12547	0.047
14.593	60916	0.046

Figure G.9 Chromatogram from 10 ng/mL standard - end of run



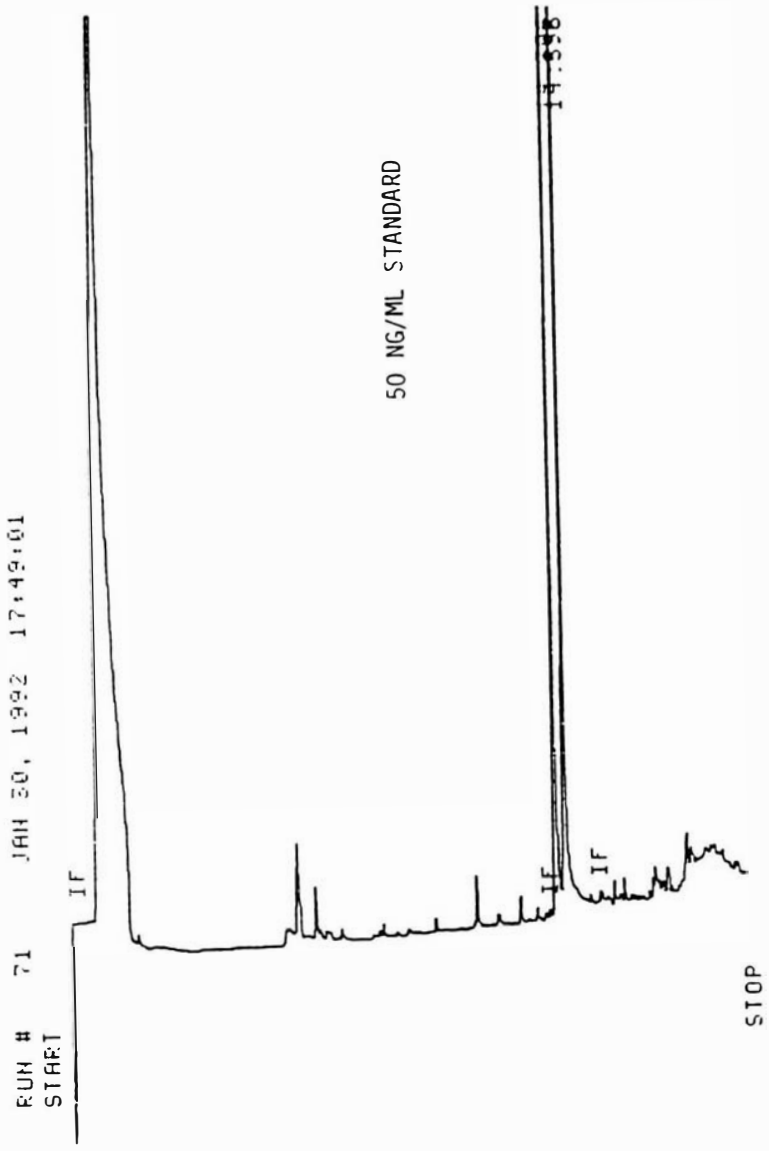
RT	Area	Width
14.307	21552	0.047
14.595	55958	0.045

Figure G.10 Chromatogram from 20 ng/mL standard - start of run



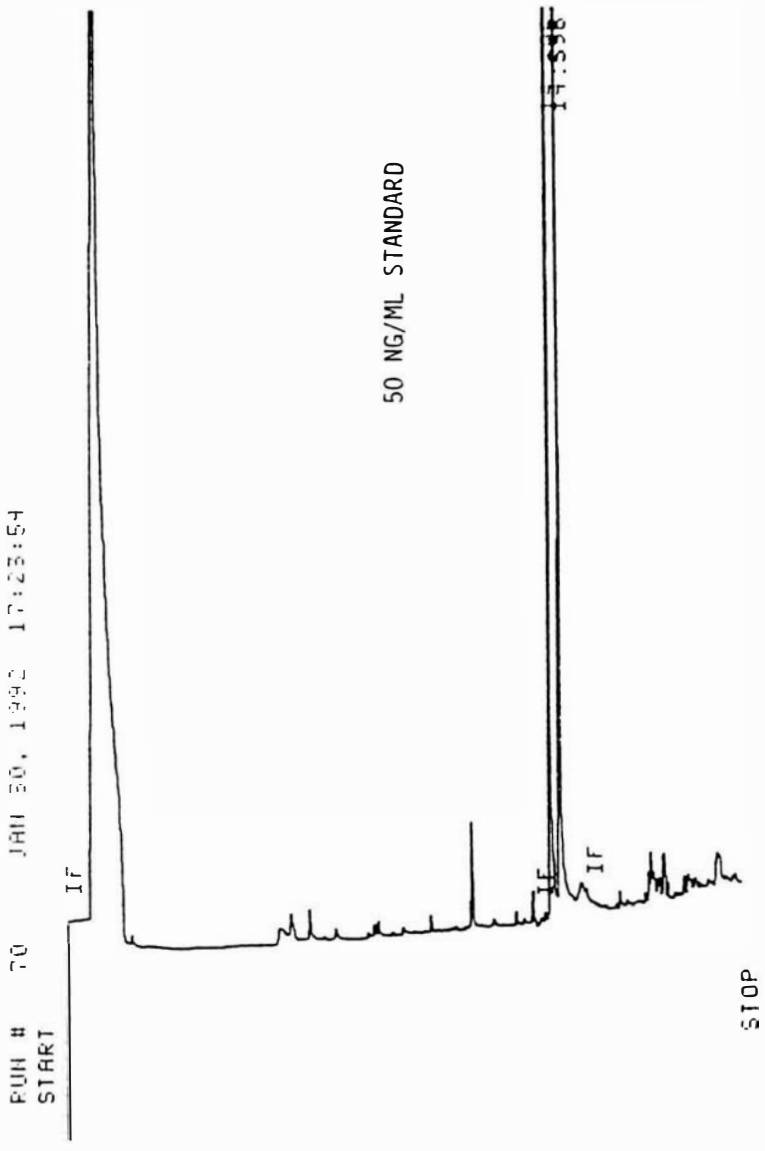
RT	Area	Width
14.306	29358	0.045
14.595	81702	0.044

Figure G.11 Chromatogram from 20 ng/mL standard - end of run



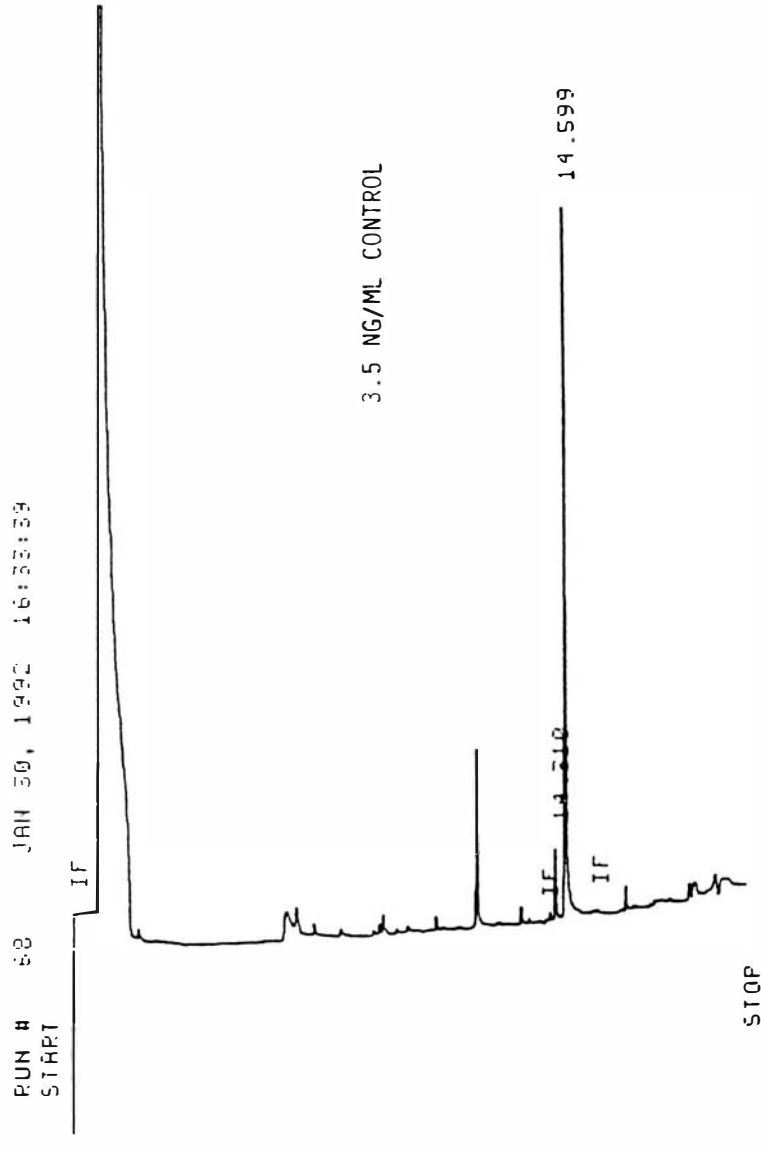
RT	Area	Width
14.306	57243	0.042
14.596	70466	0.045

Figure G.12 Chromatogram from 50 ng/mL standard - start of run



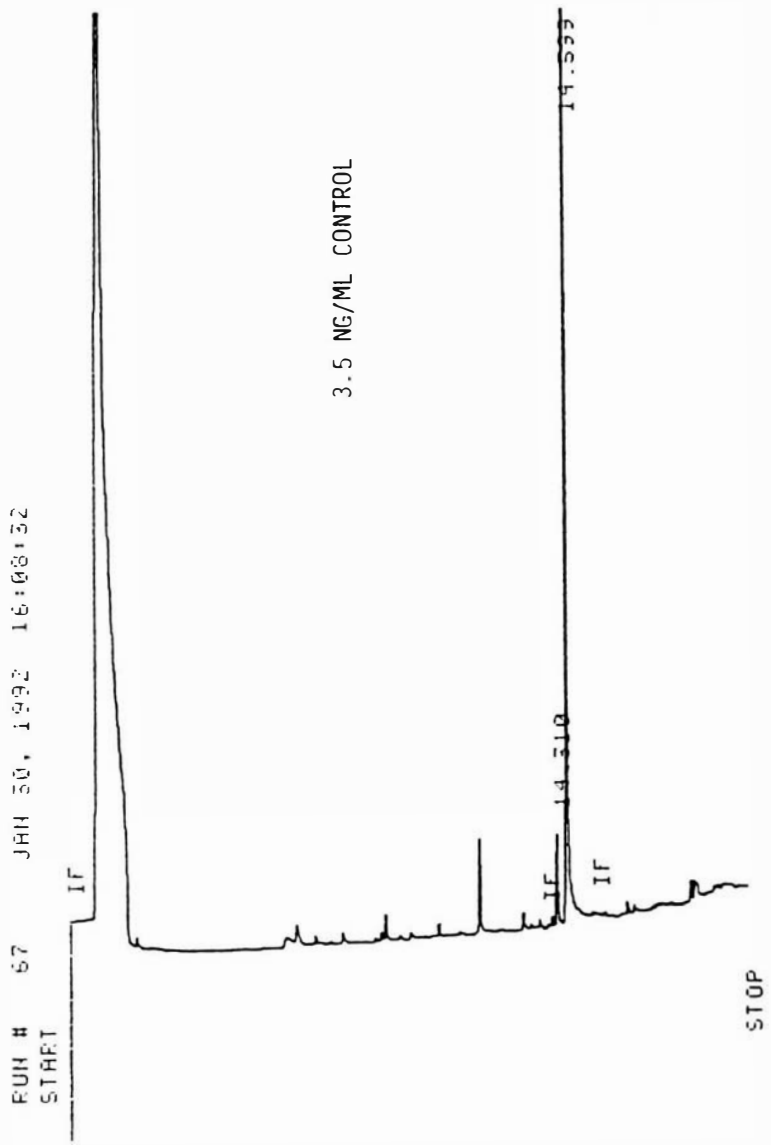
RT	Area	Width
14.306	43136	0.043
14.596	56349	0.044

Figure G.13 Chromatogram from 50 ng/mL standard - end of run



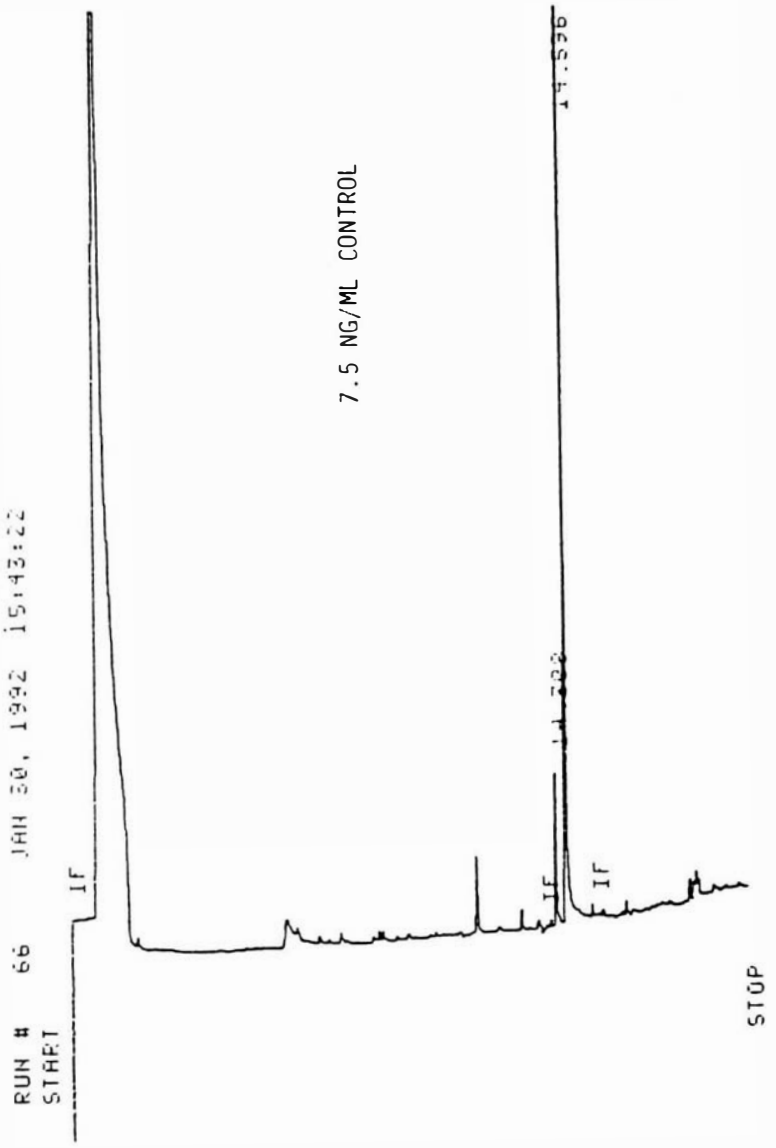
RT	Area	Width
14.310	3043	0.045
14.599	31533	0.047

Figure G.14 Chromatogram from 3.5 ng/mL control - start of run



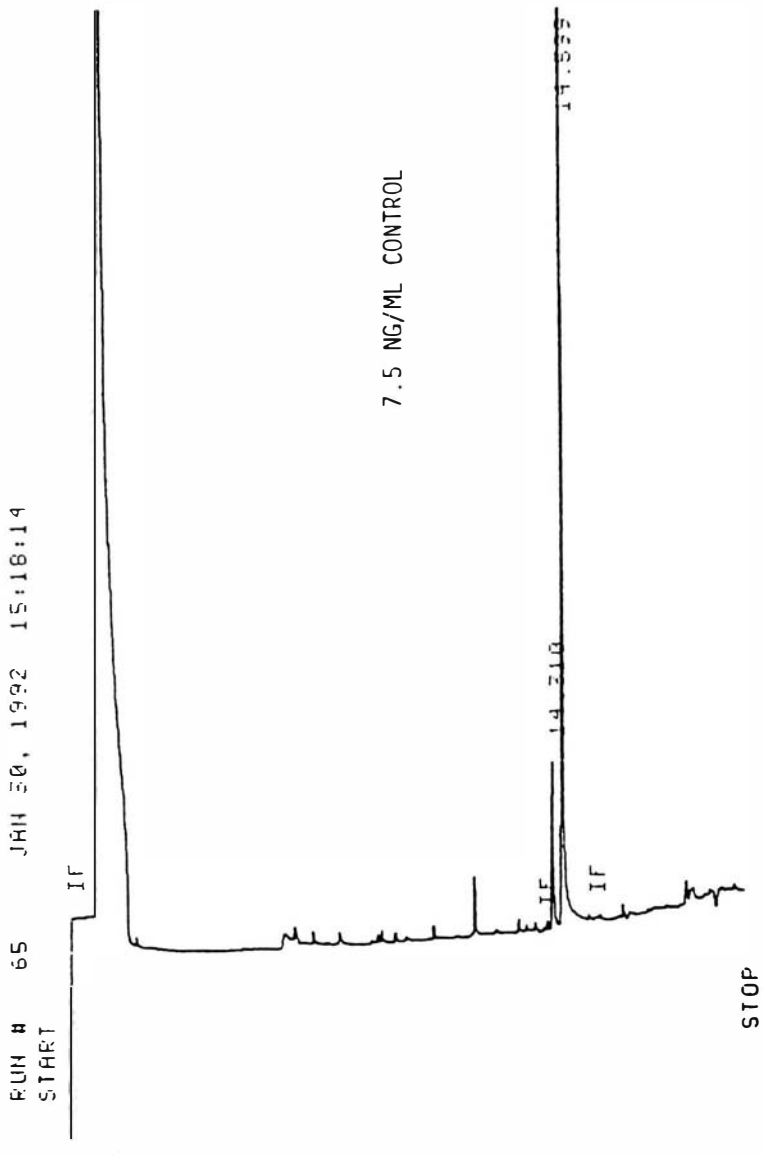
RT	Area	Width
14.310	3833	0.045
14.599	46586	0.047

Figure G.15 Chromatogram from 3.5 ng/mL control - end of run



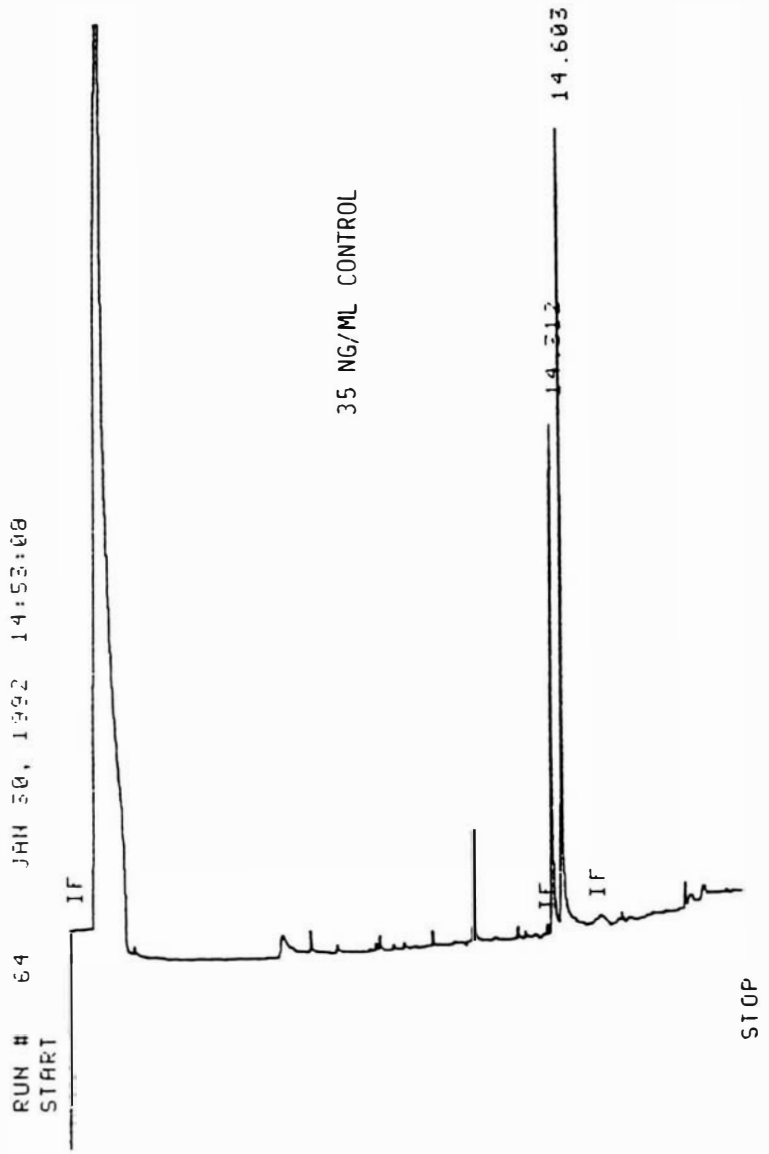
RT	Area	Width
14.308	6405	0.045
14.596	41248	0.047

Figure G.16 Chromatogram from 7.5 ng/mL control - start of run



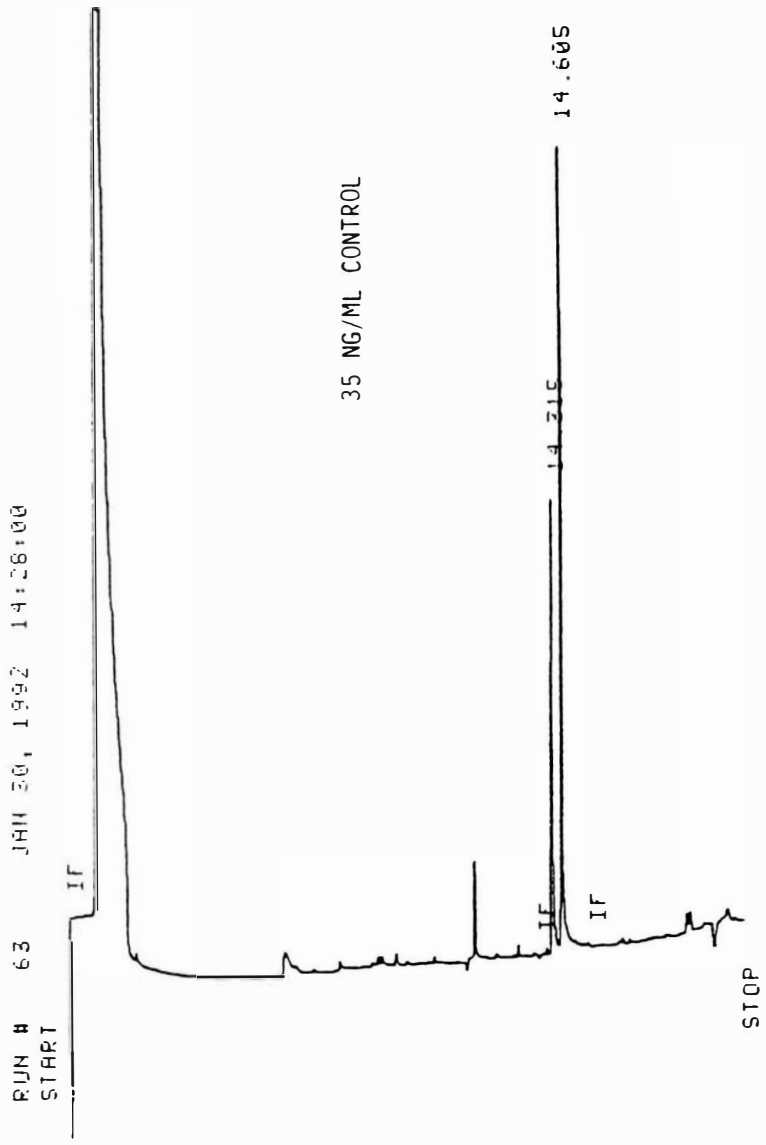
RT	Area	Width
14.310	7020	0.045
14.599	44630	0.047

Figure G.17 Chromatogram from 7.5 ng/mL control - end of run



RT	Area	Width
14.312	21877	0.046
14.603	35055	0.047

Figure G.18 Chromatogram from 35 ng/mL control - start of run



RT	Area	Width
14.315	19123	0.045
14.605	34375	0.045

Figure G.19 Chromatogram from 35 ng/mL control - end of run

APPENDIX H

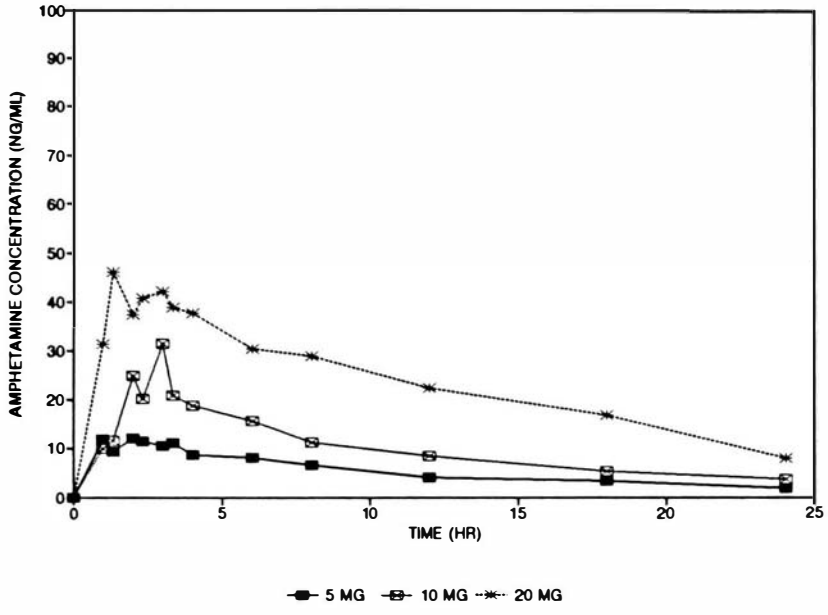


Figure H.1 Serum amphetamine concentration versus time profile for Subject 1

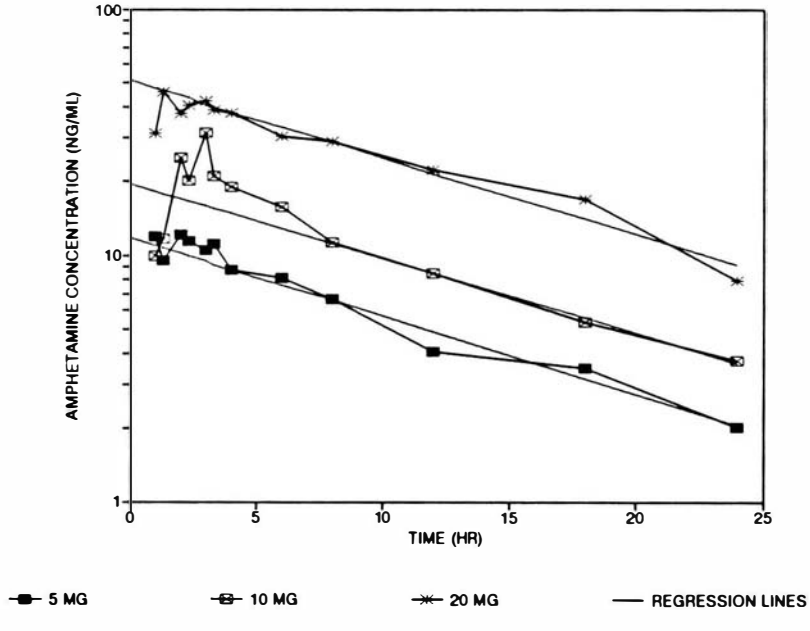


Figure H.2 Log serum amphetamine concentration versus time profile for Subject 1

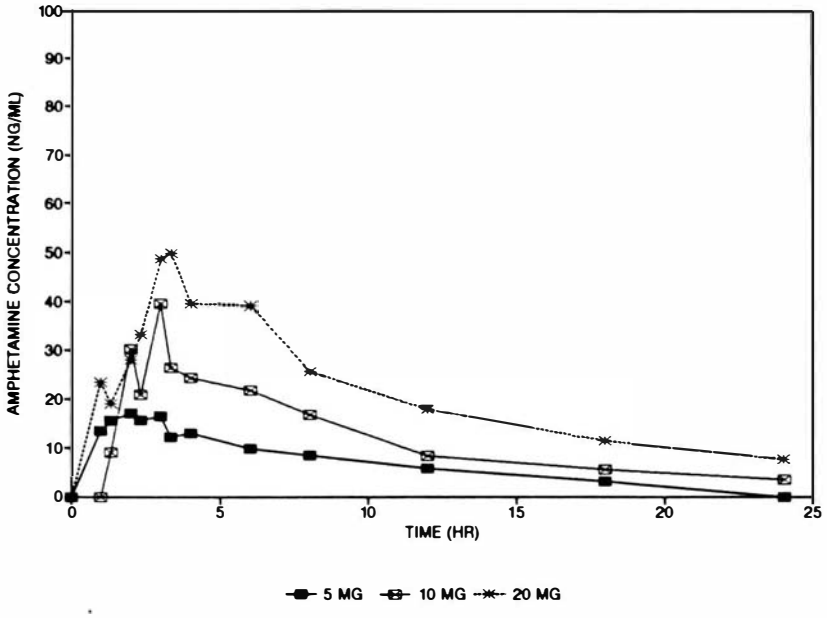


Figure H.3 Serum amphetamine concentration versus time profile for Subject 2

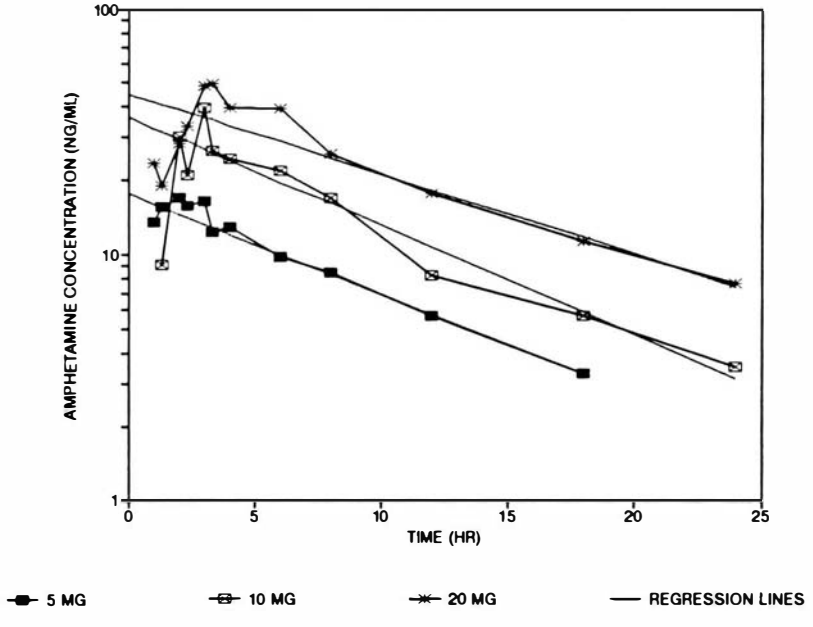


Figure H.4 Log serum amphetamine concentration versus time profile for Subject 2

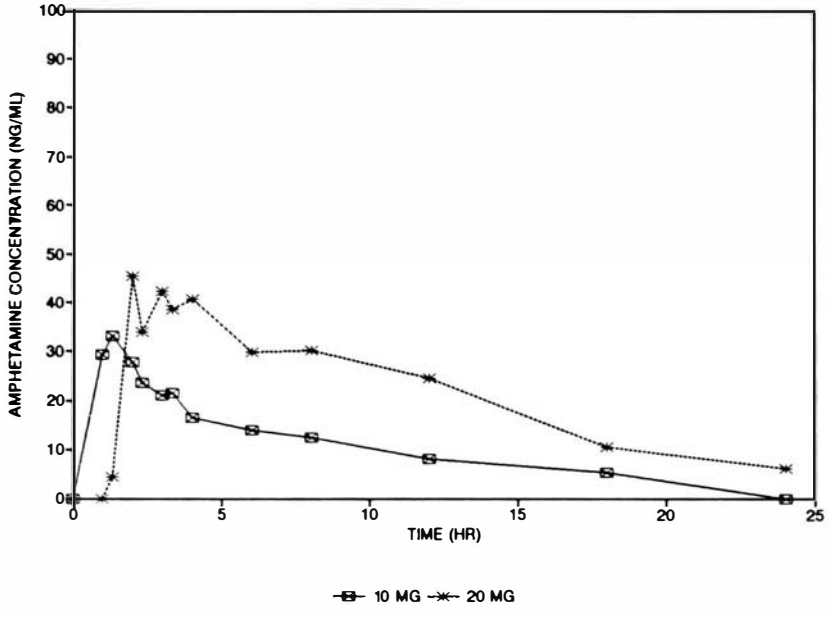


Figure H.5 Serum amphetamine concentration versus time profile for Subject 3

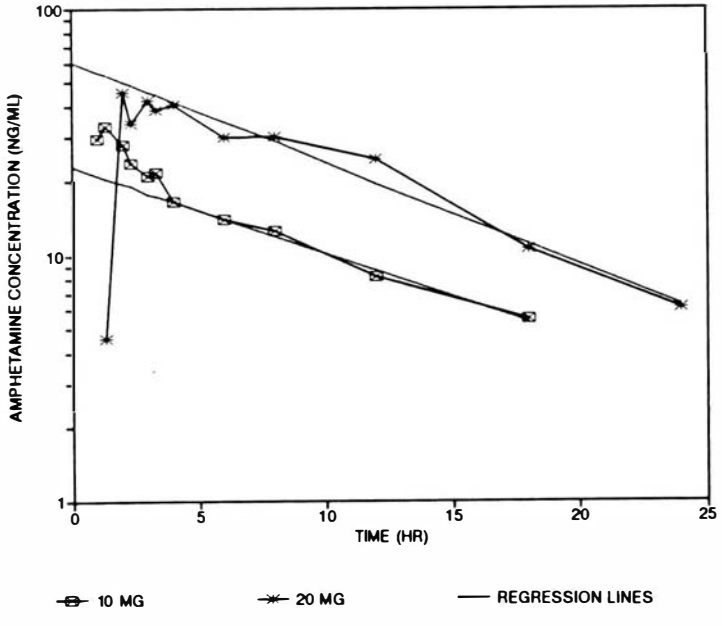


Figure H.6 Log serum amphetamine concentration versus time profile for Subject 3

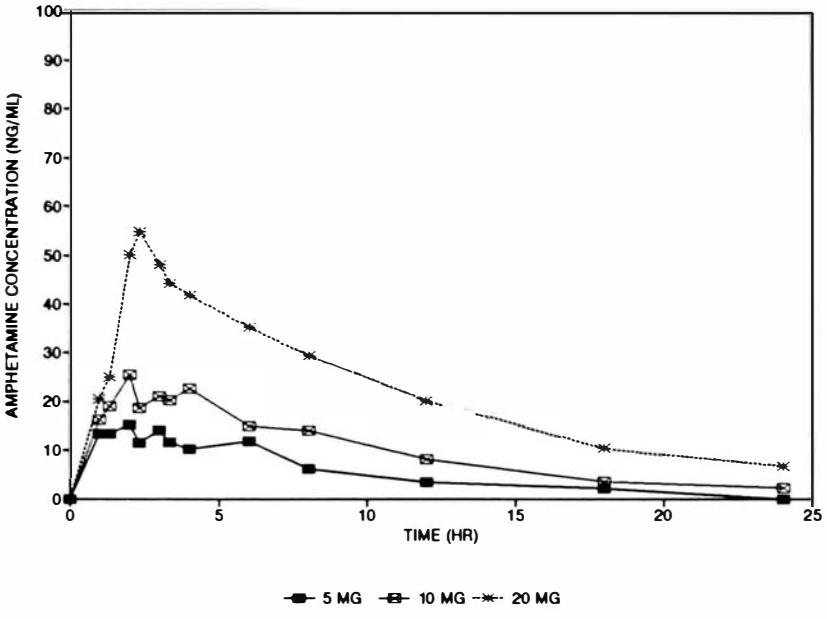


Figure H.7 Serum amphetamine concentration versus time profile for Subject 4

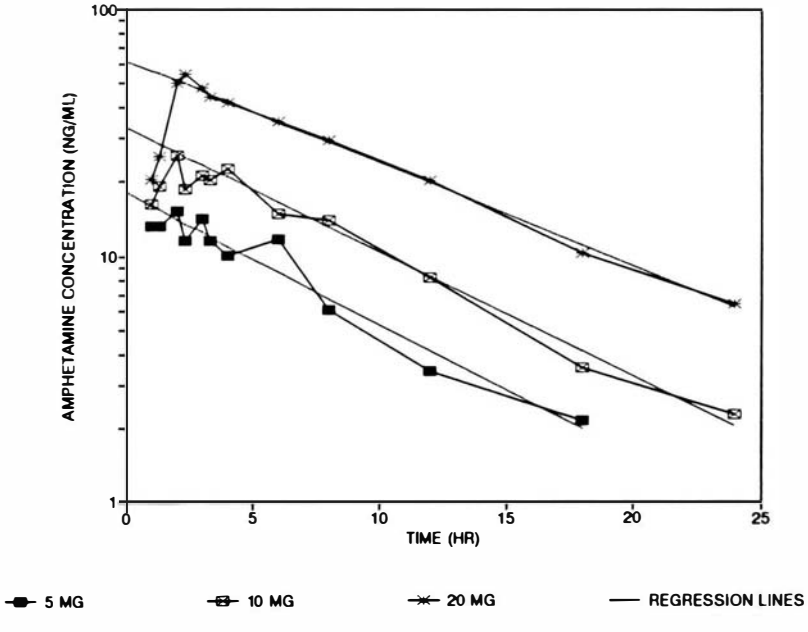


Figure H.8 Log serum amphetamine concentration versus time profile for Subject 4

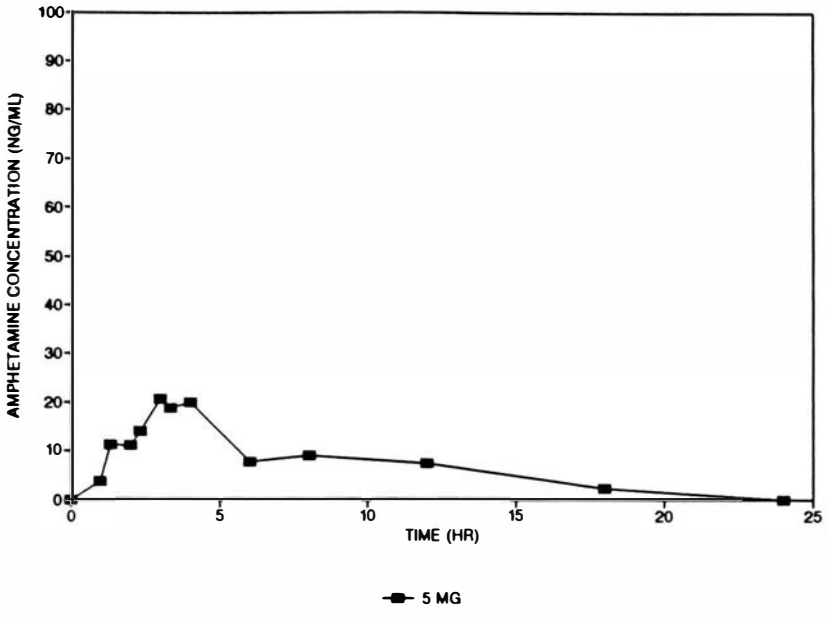


Figure H.9 Serum amphetamine concentration versus time profile for Subject 5

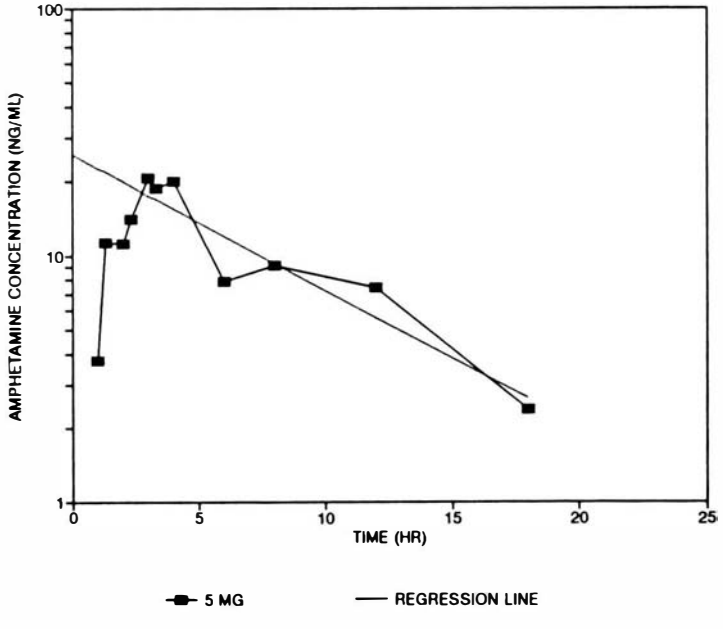


Figure H.10 Log serum amphetamine concentration versus time profile for Subject 5

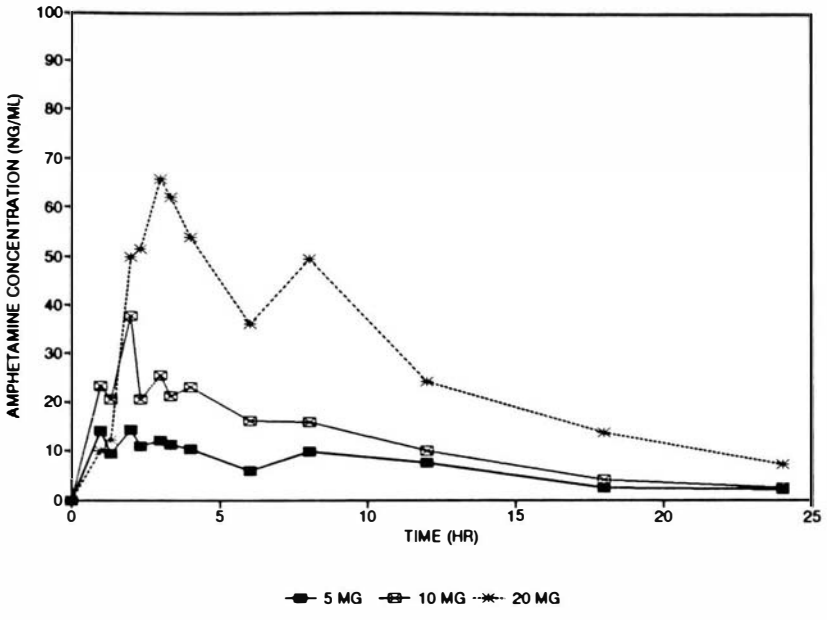


Figure H.11 Serum amphetamine concentration versus time profile for Subject 6

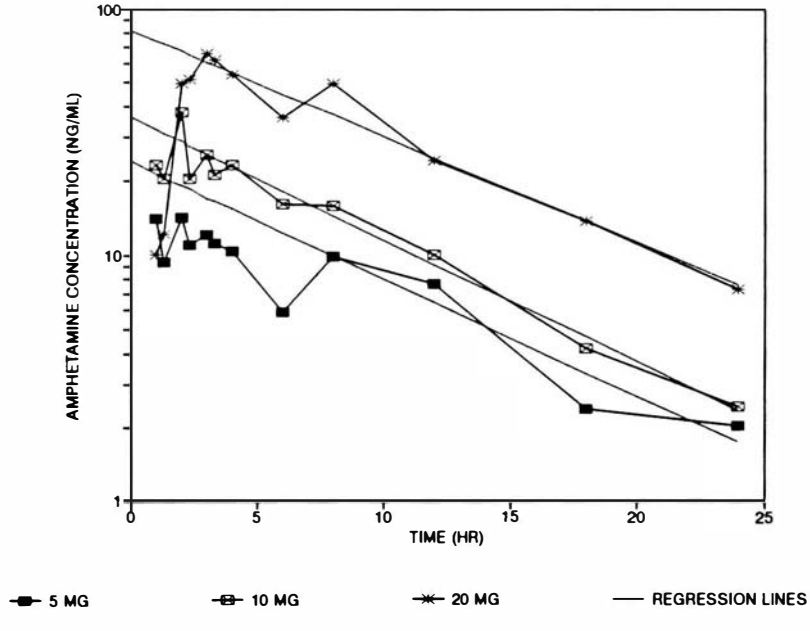


Figure H.12 Log serum amphetamine concentration versus time profile for Subject 6

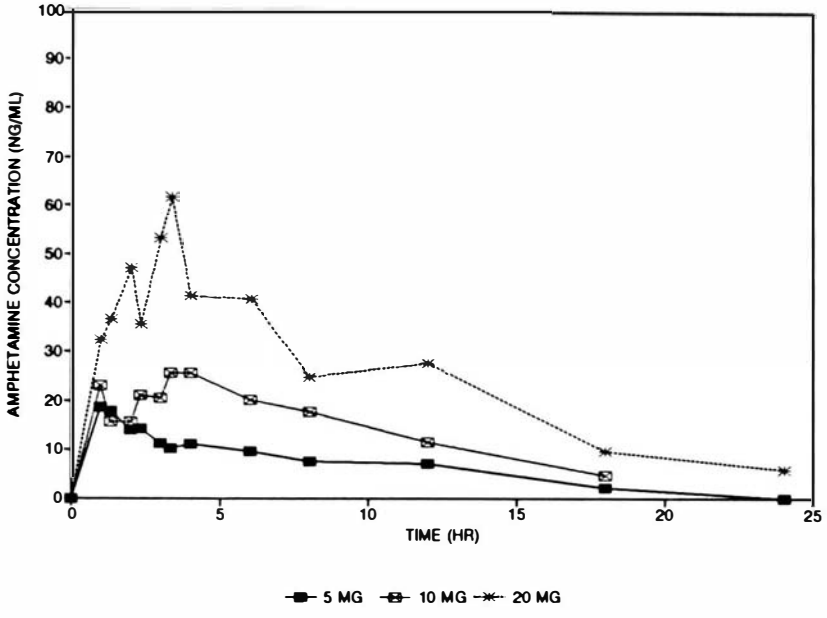


Figure H.13 Serum amphetamine concentration versus time profile for Subject 7

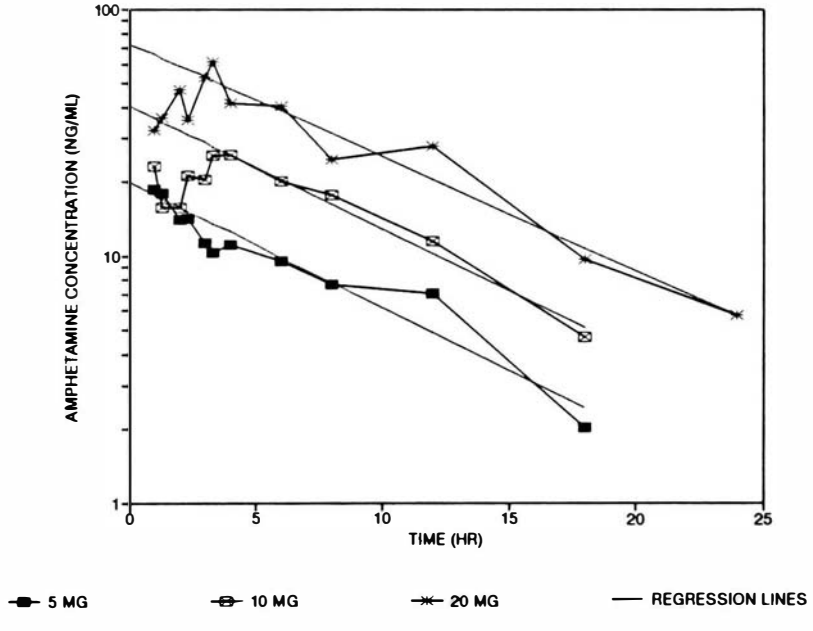


Figure H.14 Log serum amphetamine concentration versus time profile for Subject 7

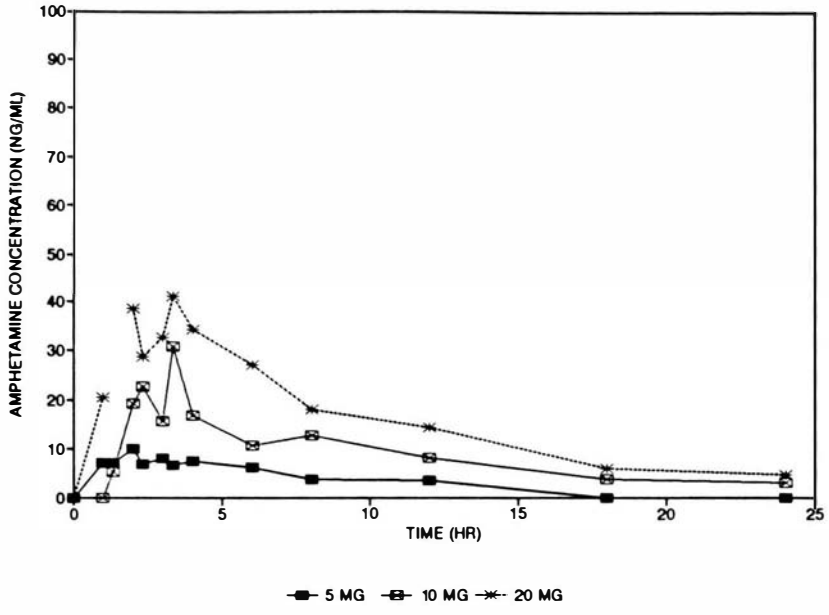


Figure H.15 Serum amphetamine concentration versus time profile for Subject 8

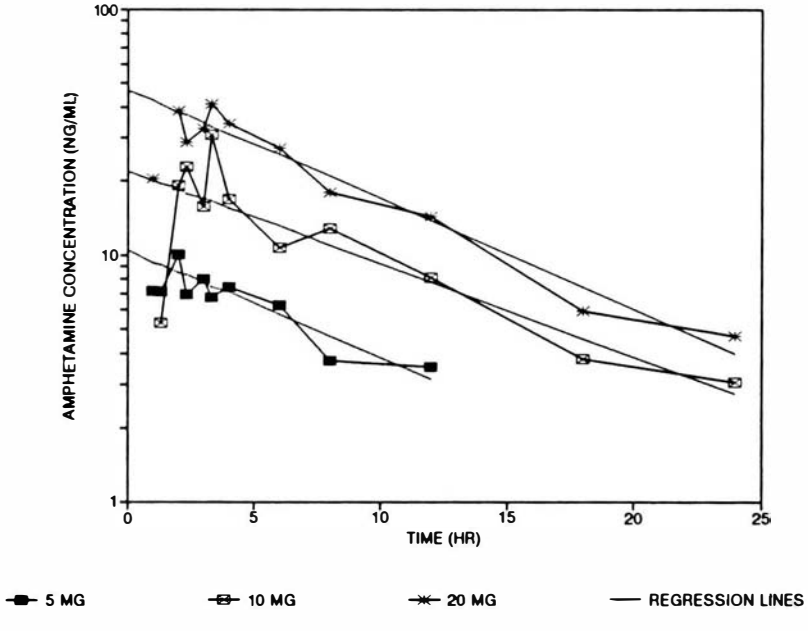


Figure H.16 Log serum amphetamine concentration versus time profile for Subject 8

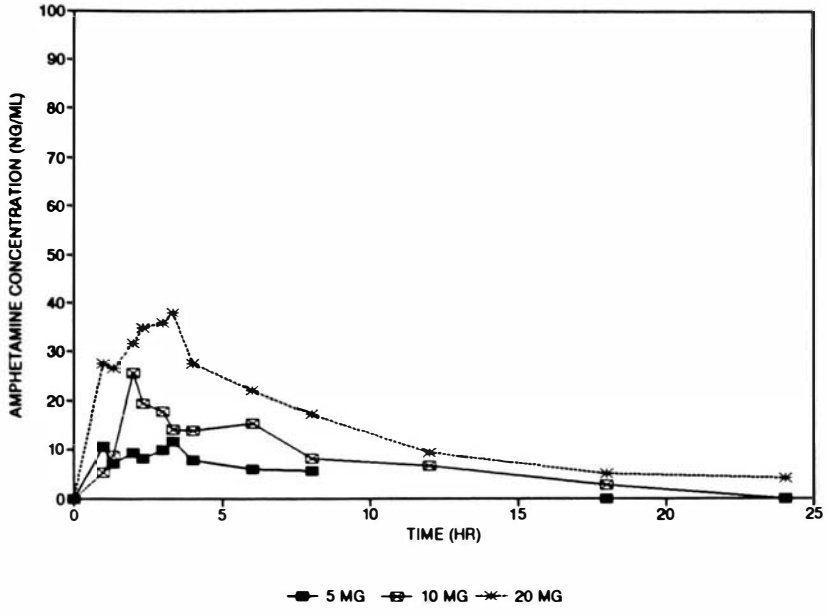


Figure H.17 Serum amphetamine concentration versus time profile for Subject 9

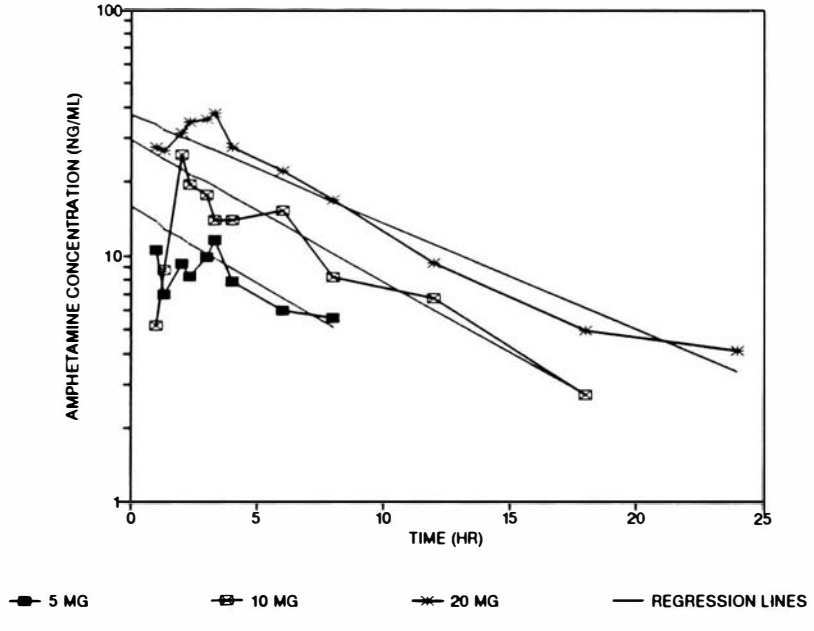


Figure H.18 Log serum amphetamine concentration versus time profile for Subject 9

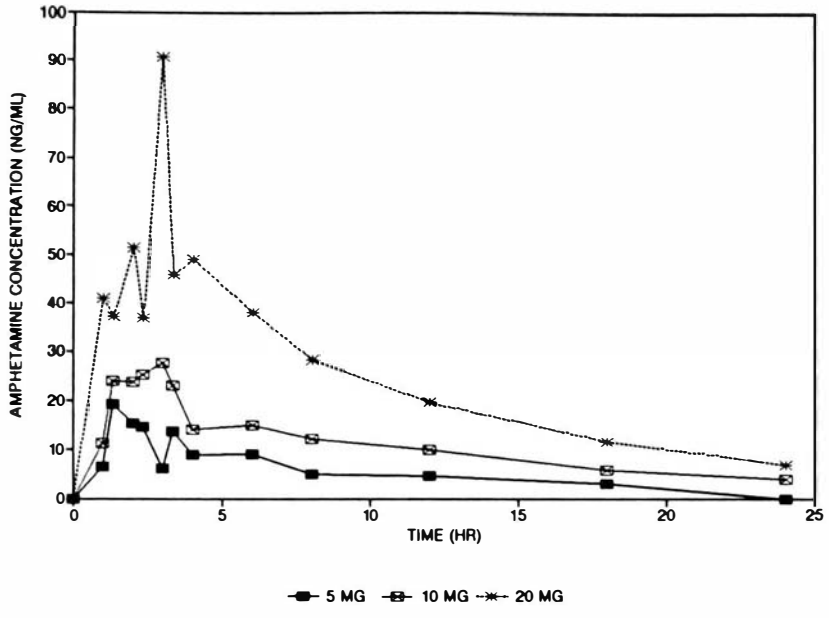


Figure H.19 Serum amphetamine concentration versus time profile for Subject 10

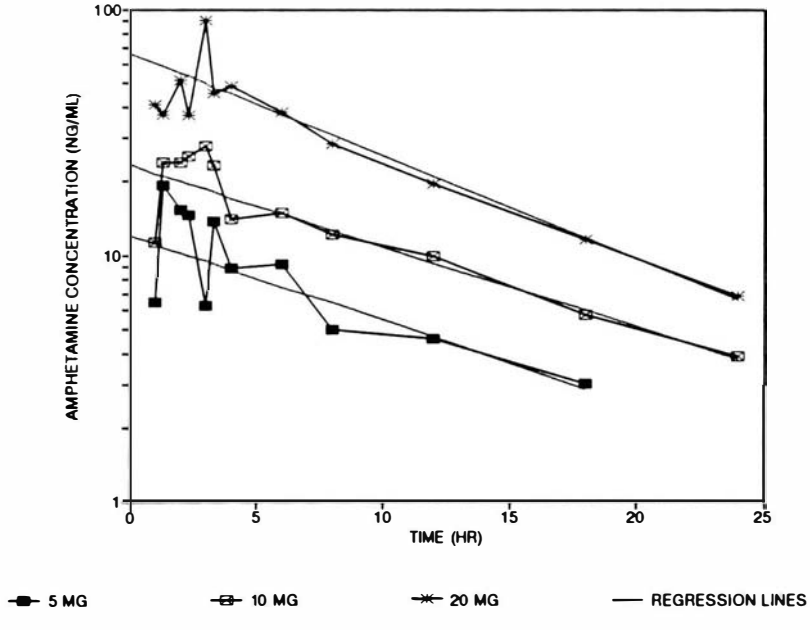


Figure H.20 Log serum amphetamine concentration versus time profile for Subject 10

APPENDIX I

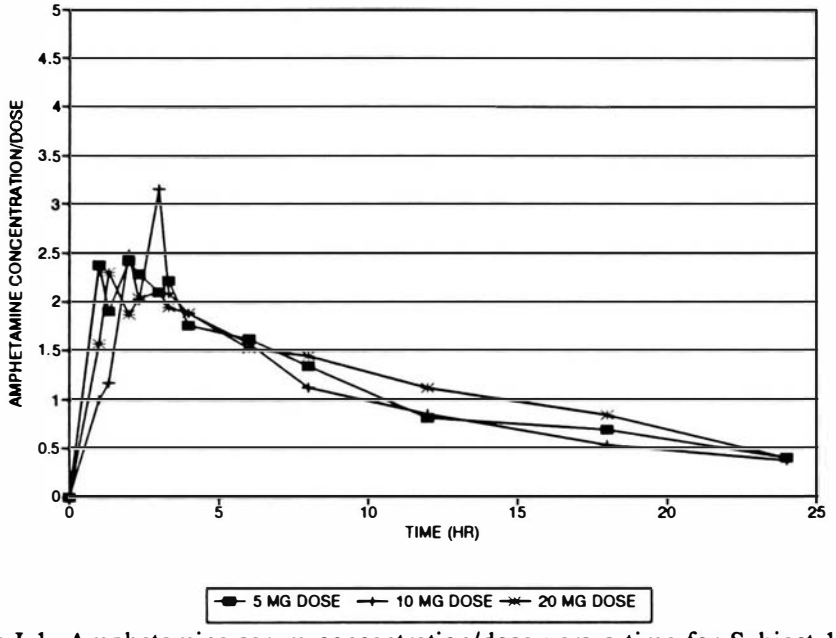


Figure I.1 Amphetamine serum concentration/dose versus time for Subject 1

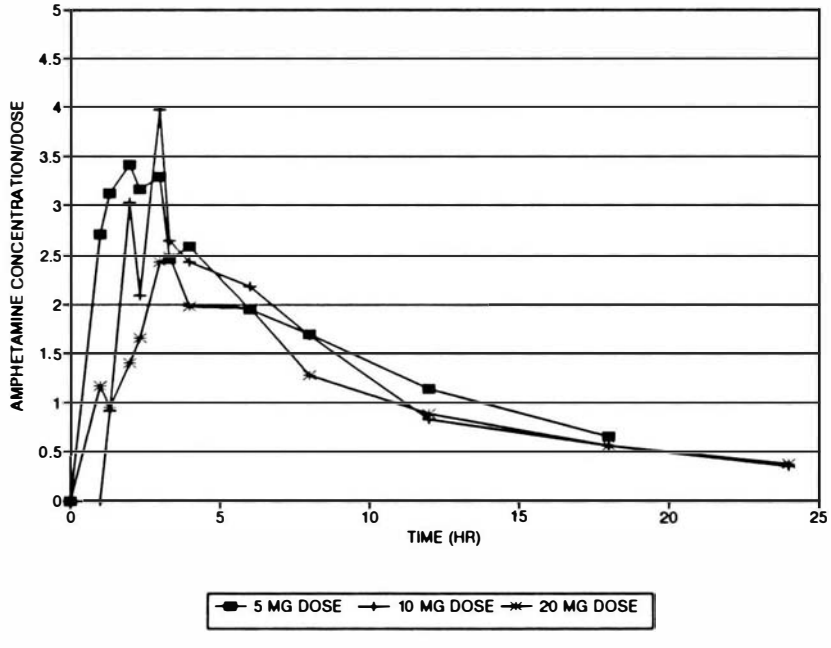


Figure I.2 Amphetamine serum concentration/dose versus time for Subject 2

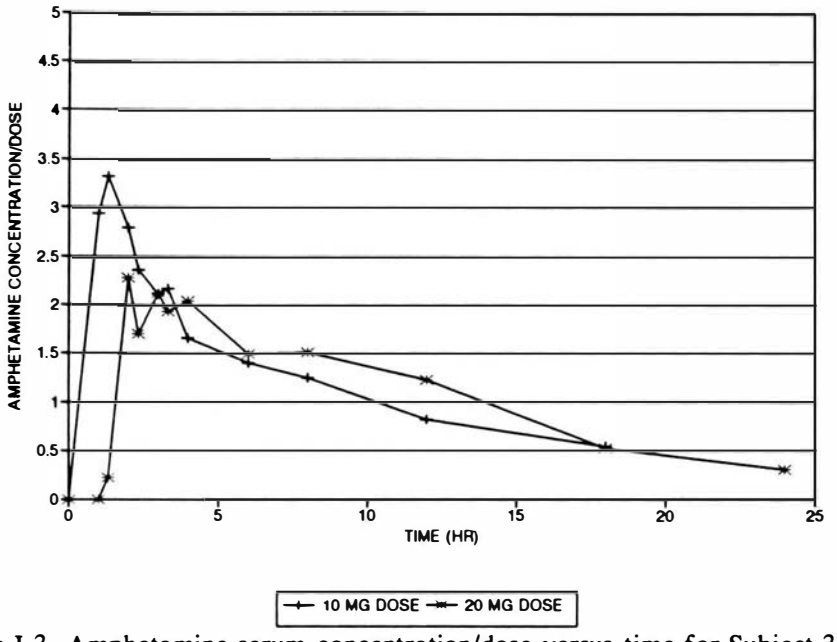


Figure I.3 Amphetamine serum concentration/dose versus time for Subject 3

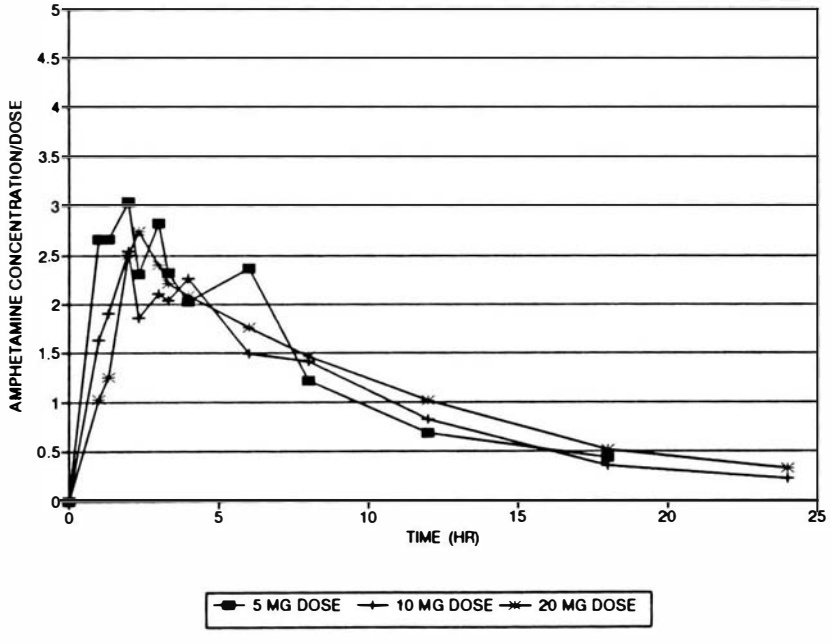


Figure I.4 Amphetamine serum concentration/dose versus time for Subject 4

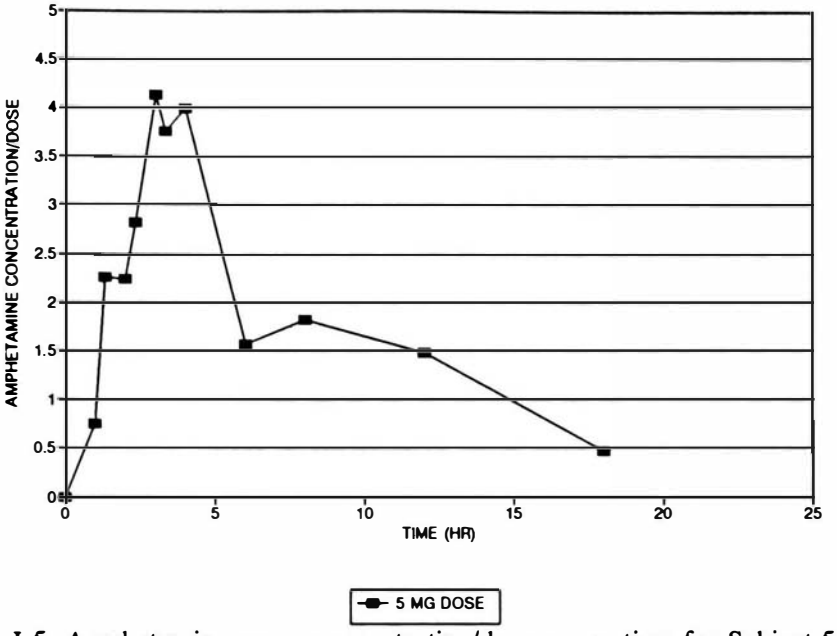


Figure I.5 Amphetamine serum concentration/dose versus time for Subject 5

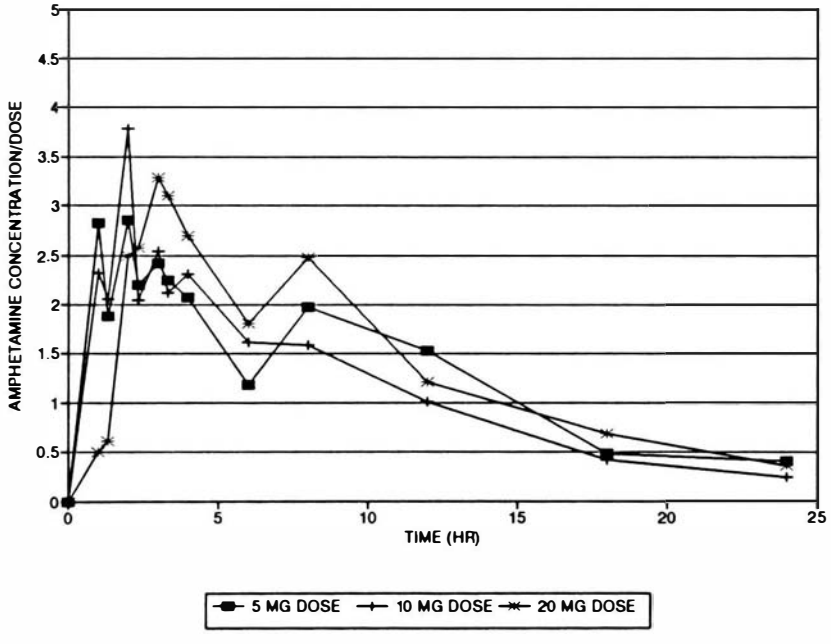


Figure I.6 Amphetamine serum concentration/dose versus time for Subject 6

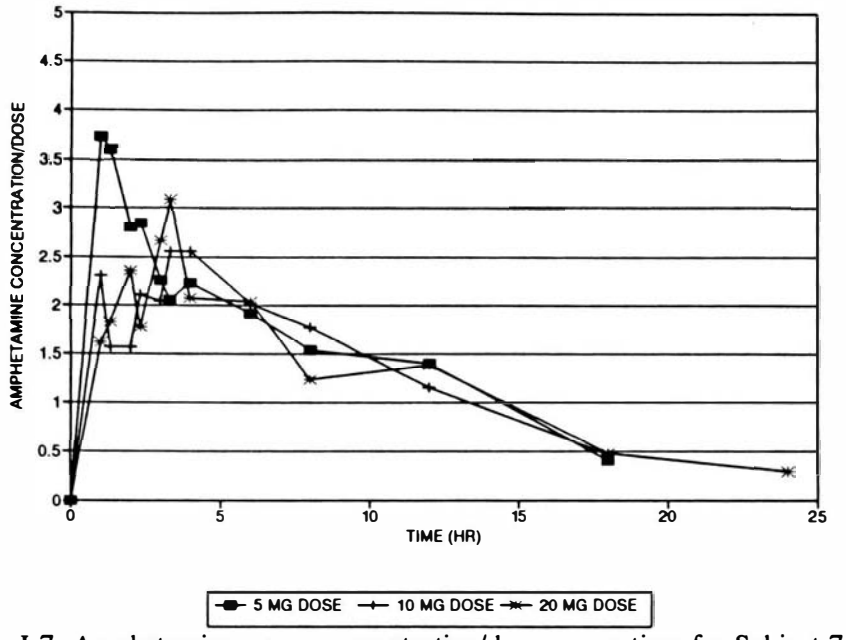


Figure I.7 Amphetamine serum concentration/dose versus time for Subject 7

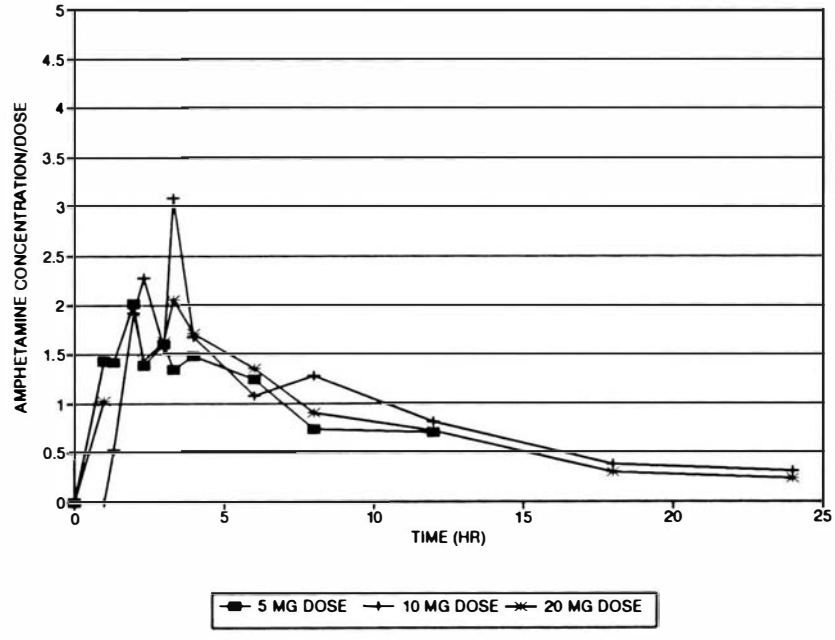


Figure I.8 Amphetamine serum concentration/dose versus time for Subject 8

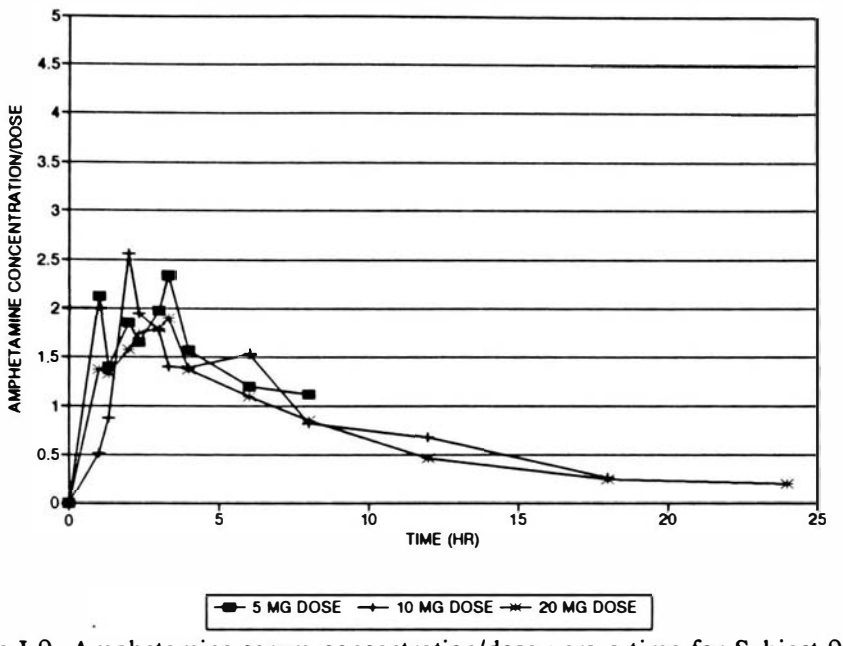


Figure I.9 Amphetamine serum concentration/dose versus time for Subject 9

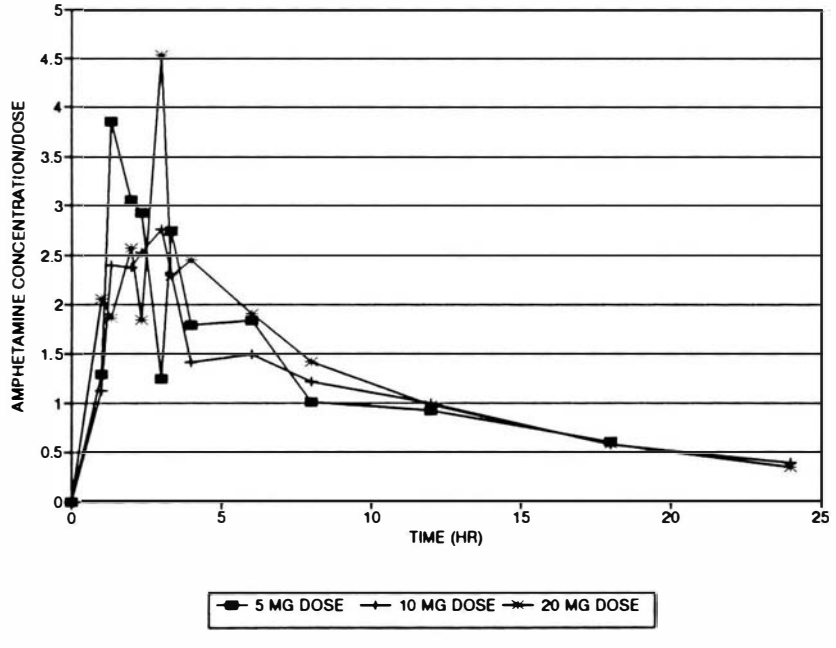


Figure I.10 Amphetamine serum concentration/dose versus time for Subject 10

APPENDIX J

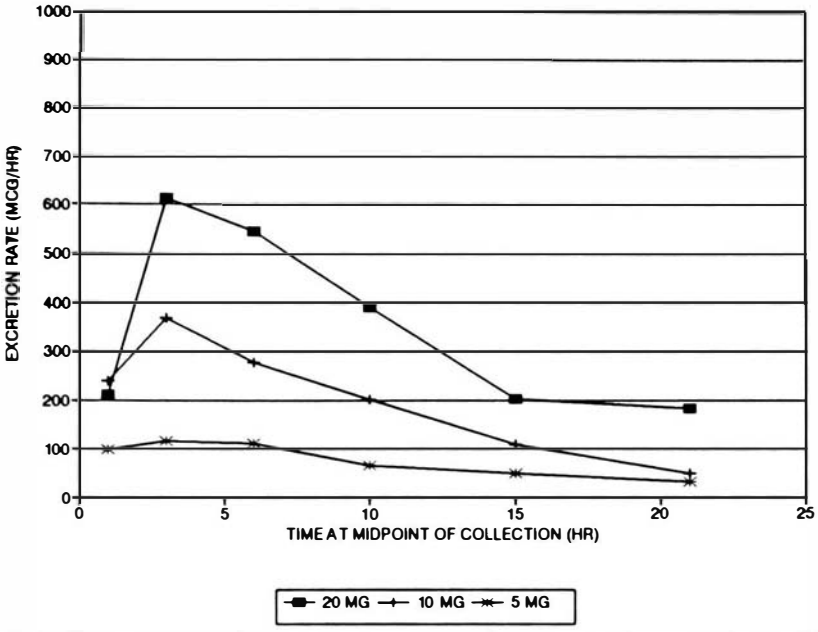


Figure J.1 Urinary excretion rate of amphetamine versus time plot for Subject 1

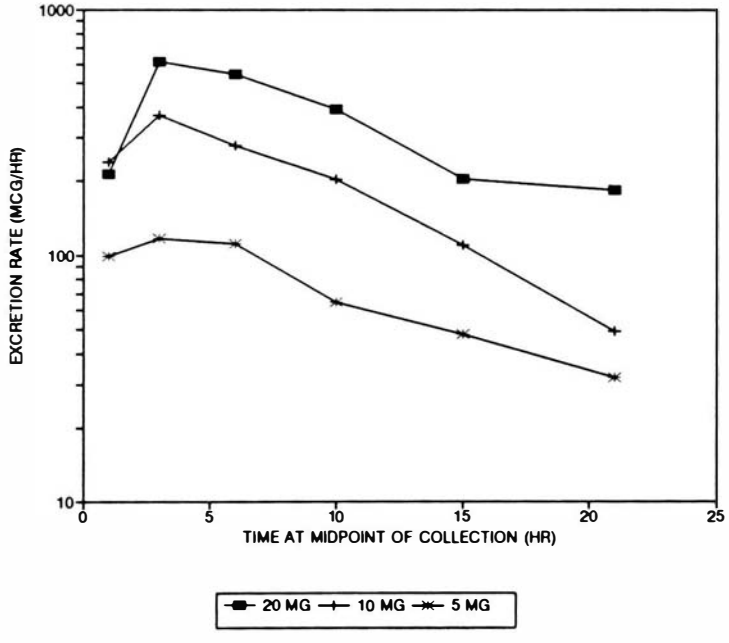


Figure J.2 Log urinary excretion rate of amphetamine versus time plot for Subject 1

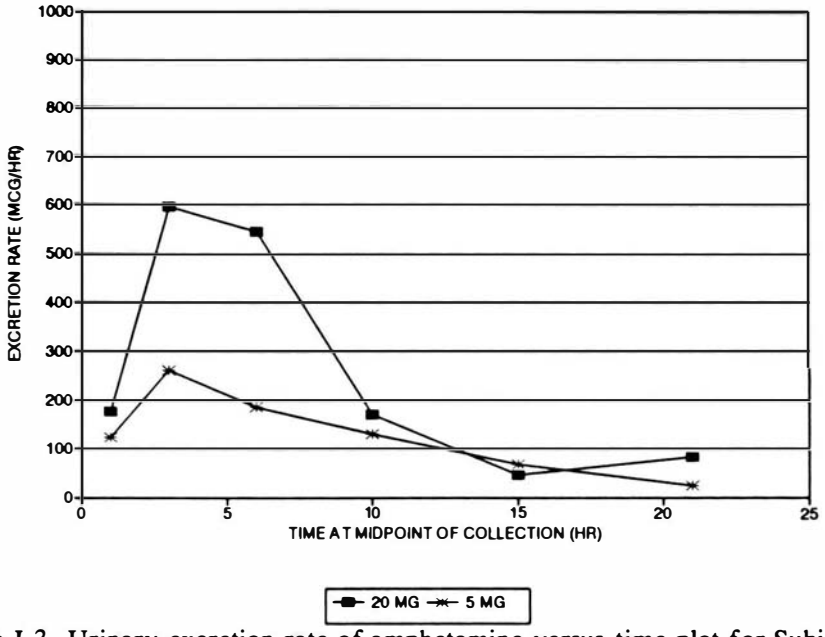


Figure J.3 Urinary excretion rate of amphetamine versus time plot for Subject 2

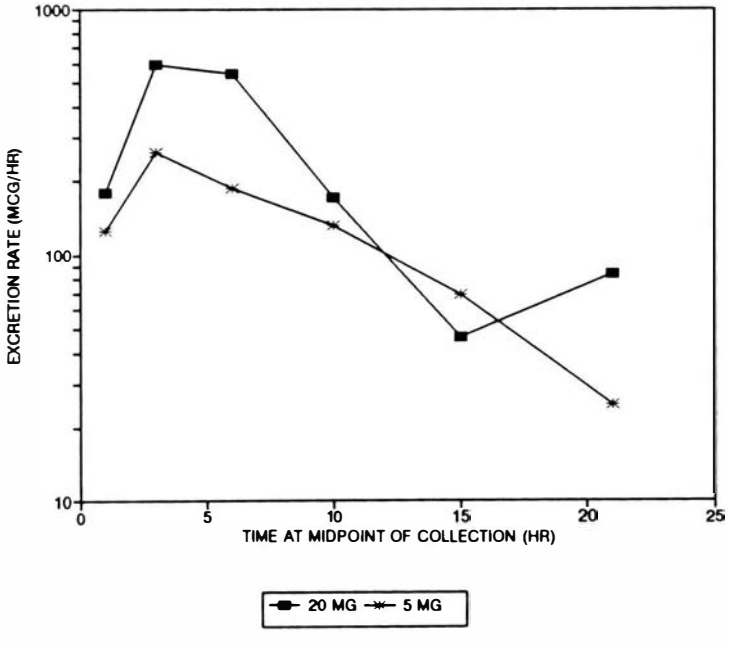


Figure J.4 Log urinary excretion rate of amphetamine versus time plot for Subject 2

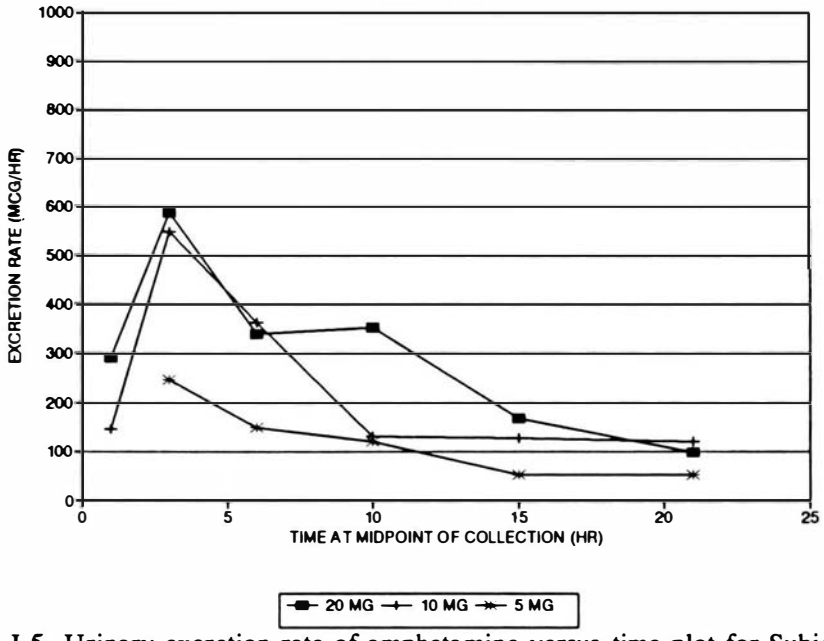


Figure J.5 Urinary excretion rate of amphetamine versus time plot for Subject 4

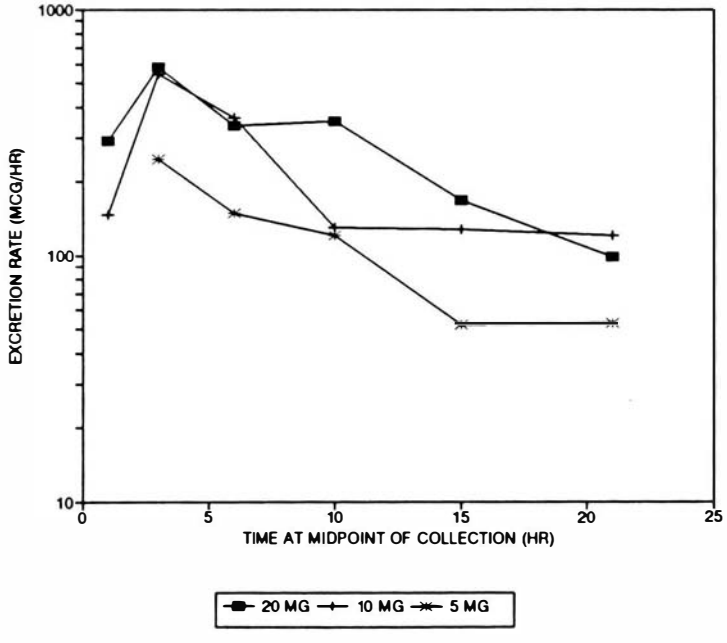


Figure J.6 Log urinary excretion rate of amphetamine versus time plot for Subject 4

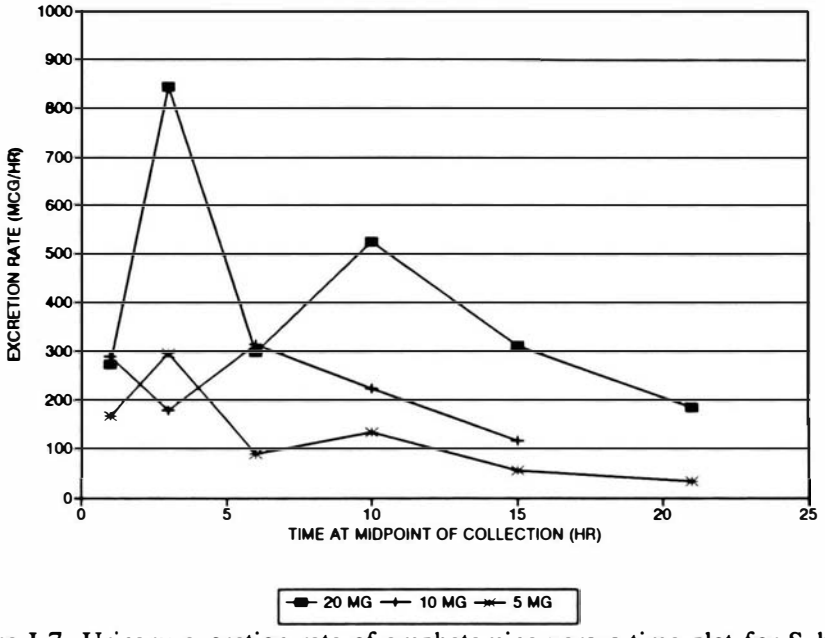


Figure J.7 Urinary excretion rate of amphetamine versus time plot for Subject 6

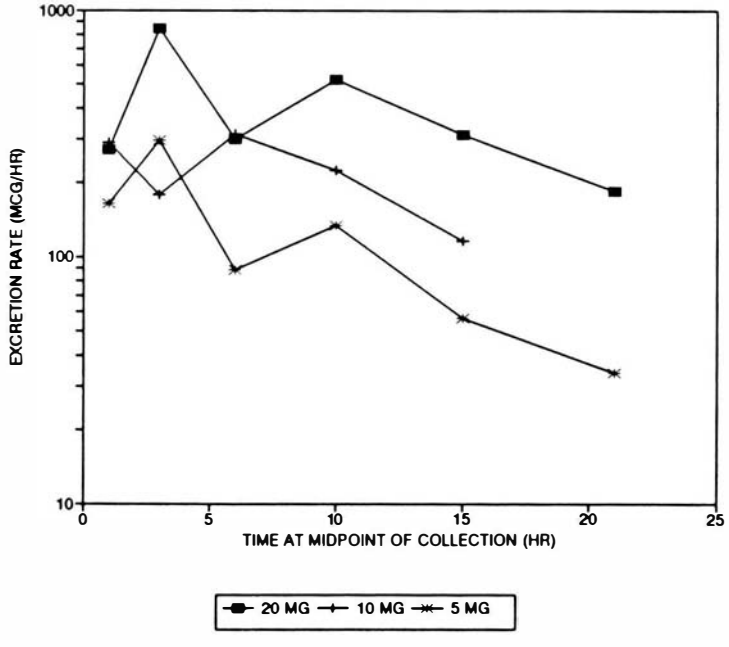


Figure J.8 Log urinary excretion rate of amphetamine versus time plot for Subject 6

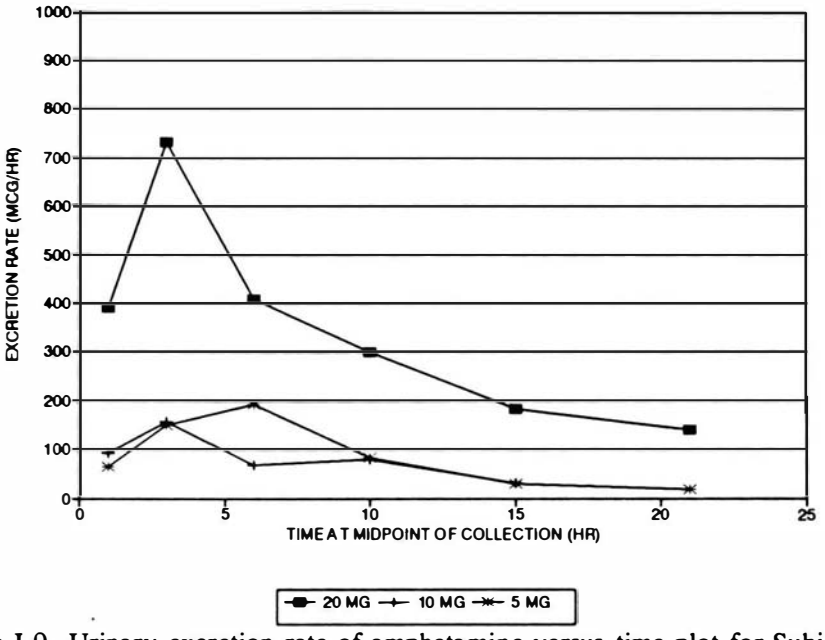


Figure J.9 Urinary excretion rate of amphetamine versus time plot for Subject 8

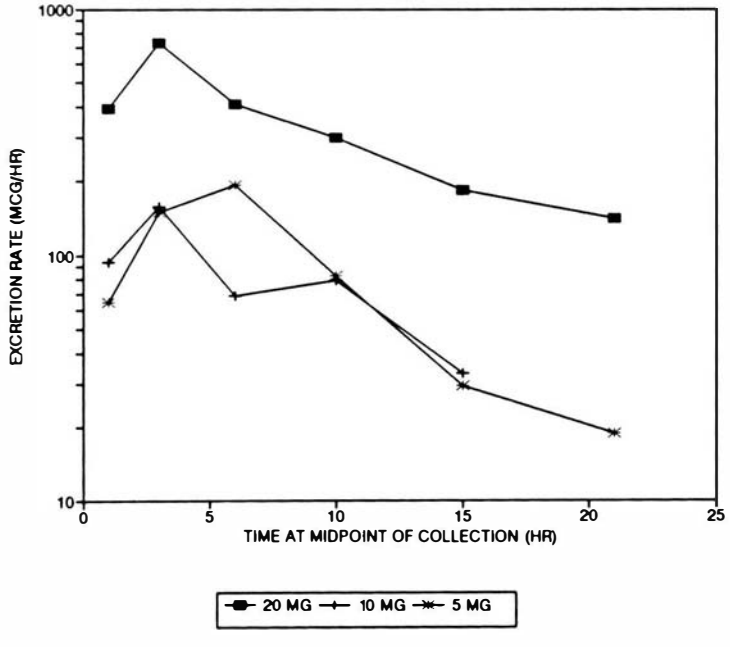


Figure J.10 Log urinary excretion rate of amphetamine versus time plot for Subject 8

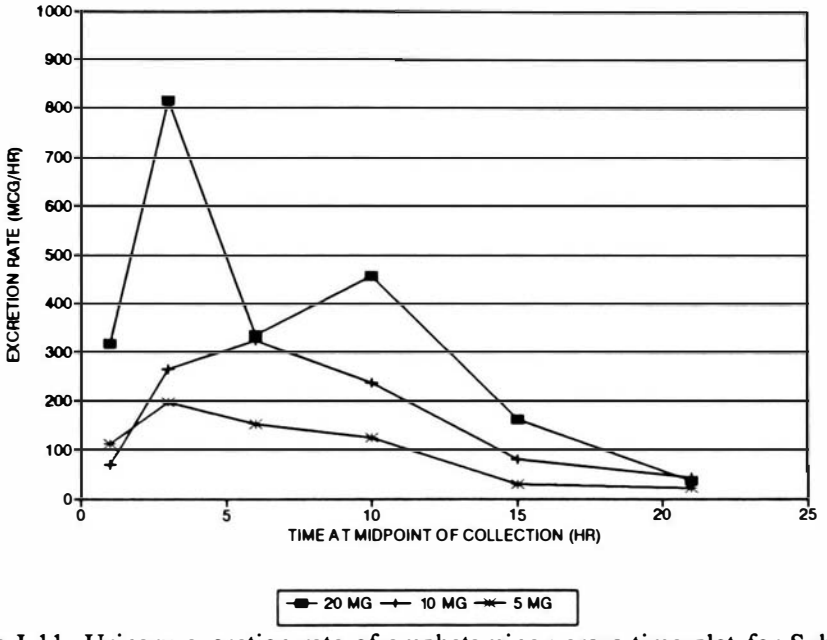


Figure J.11 Urinary excretion rate of amphetamine versus time plot for Subject 9

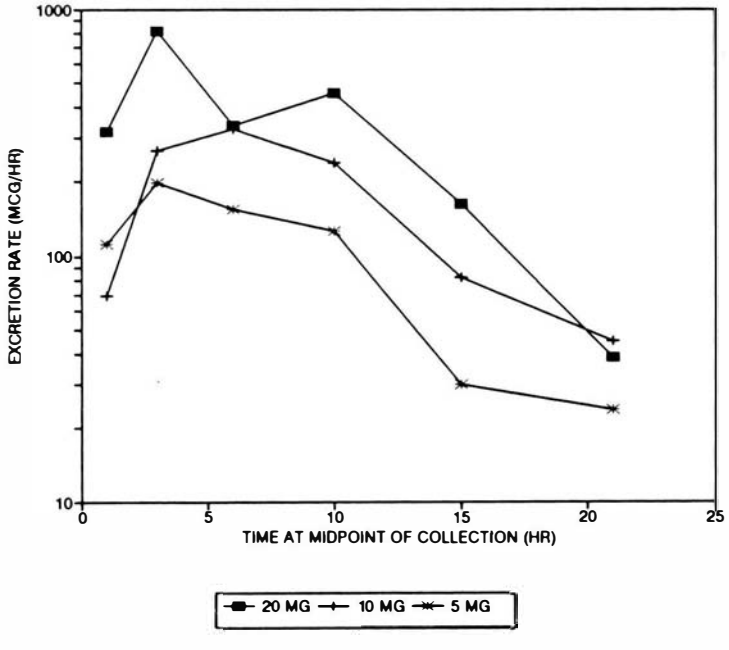


Figure J.12 Log urinary excretion rate of amphetamine versus time plot for Subject 9

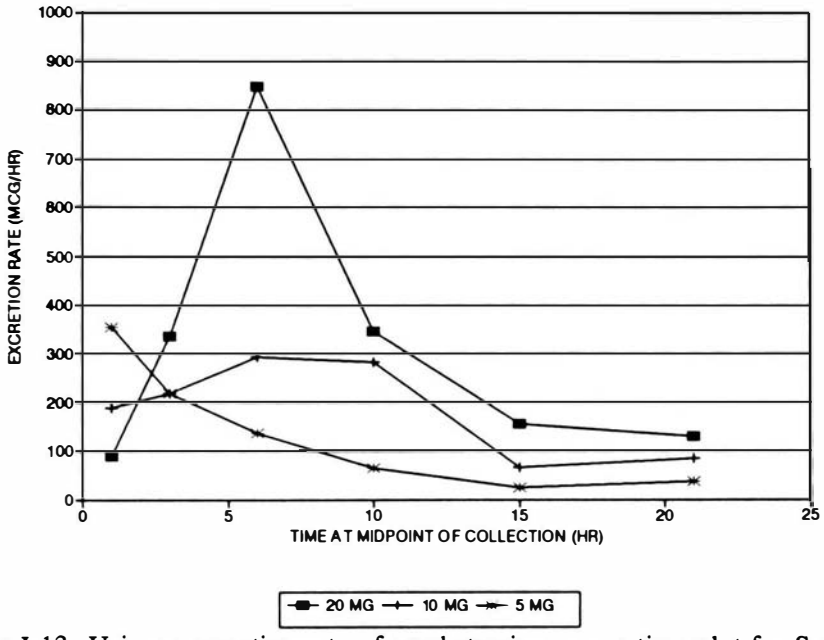


Figure J.13 Urinary excretion rate of amphetamine versus time plot for Subject 10

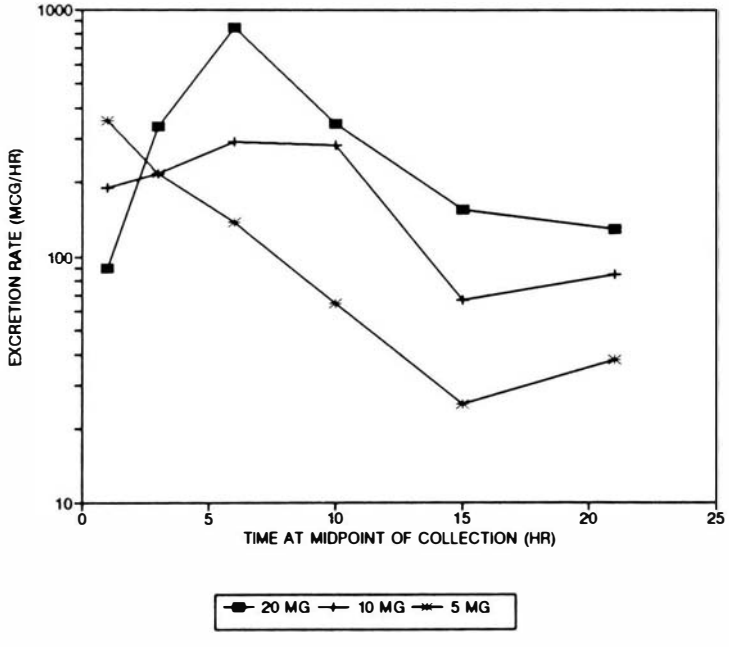


Figure J.14 Log urinary excretion rate of amphetamine versus time plot for Subject 10

APPENDIX K

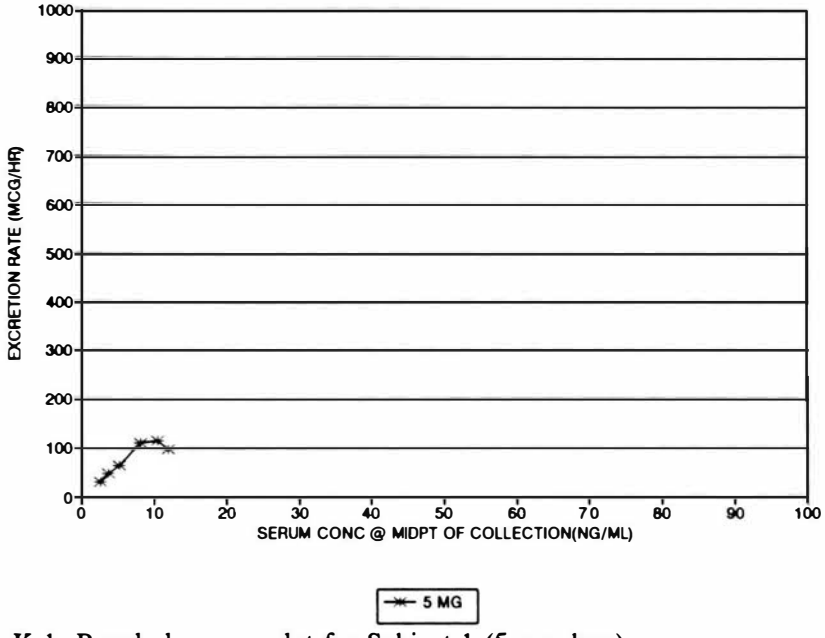


Figure K.1 Renal clearance plot for Subject 1 (5 mg dose)

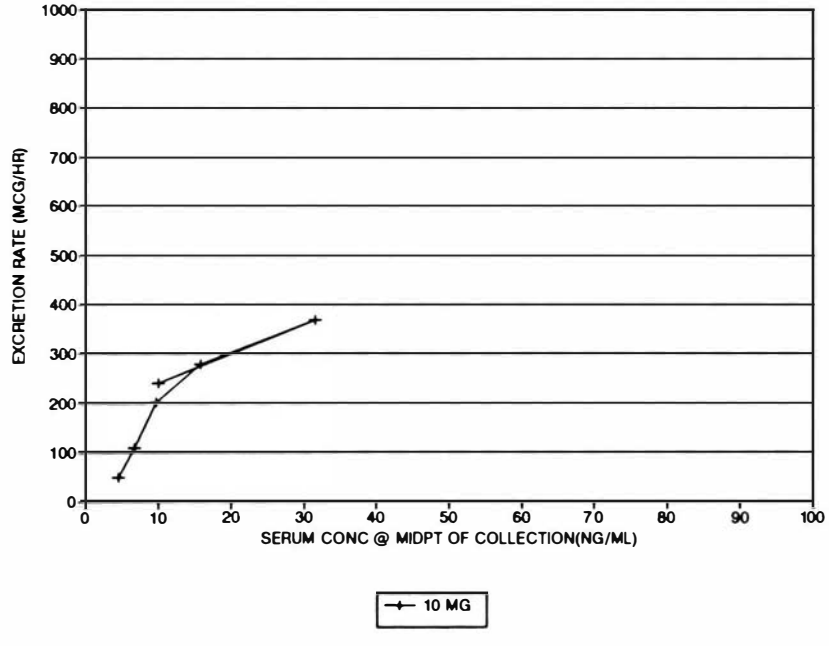


Figure K.2 Renal clearance plot for Subject 1 (10 mg dose)

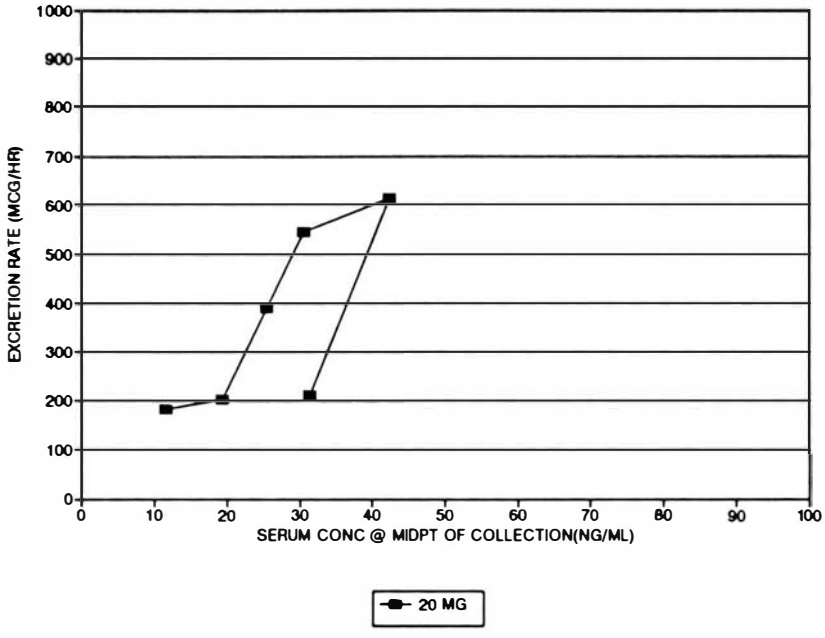


Figure K.3 Renal clearance plot for Subject 1 (20 mg dose)

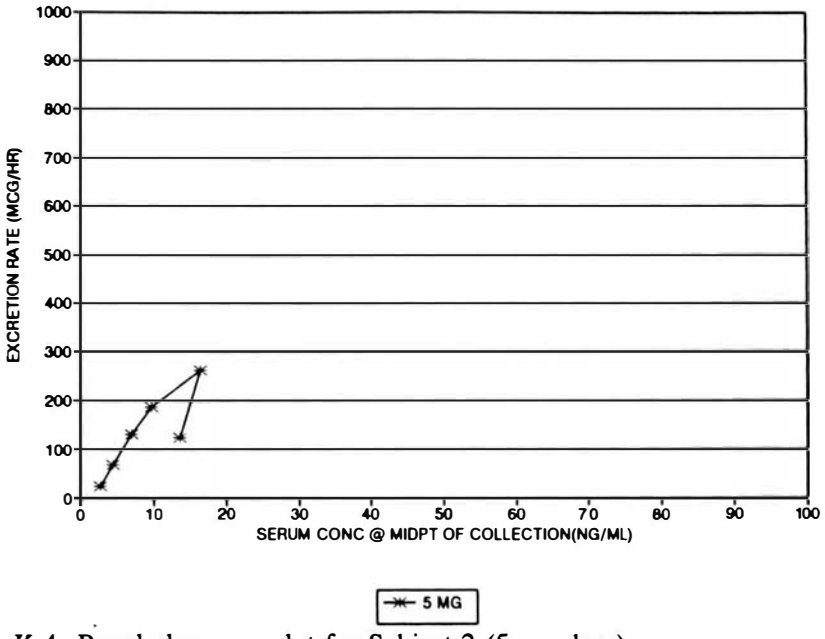


Figure K.4 Renal clearance plot for Subject 2 (5 mg dose)

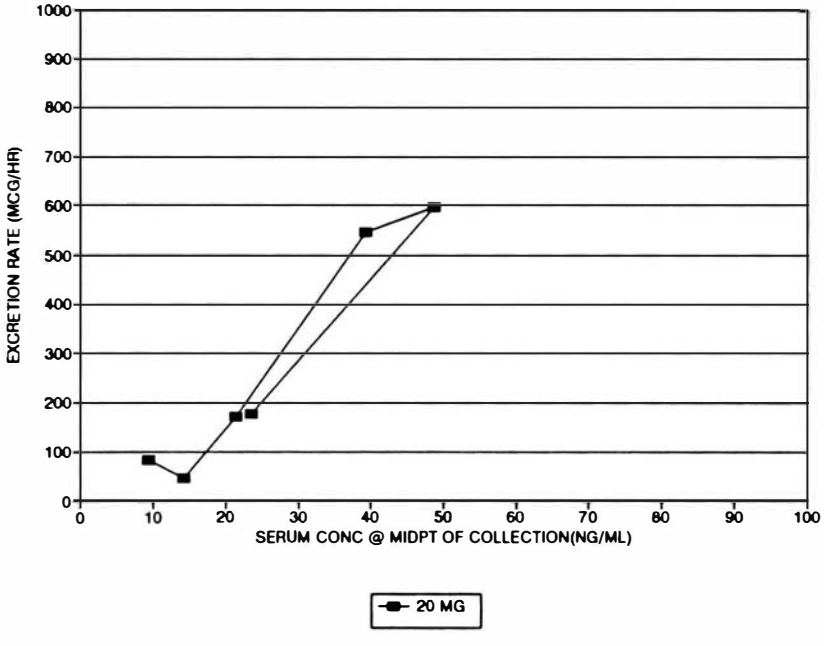


Figure K.5 Renal clearance plot for Subject 2 (20 mg dose)

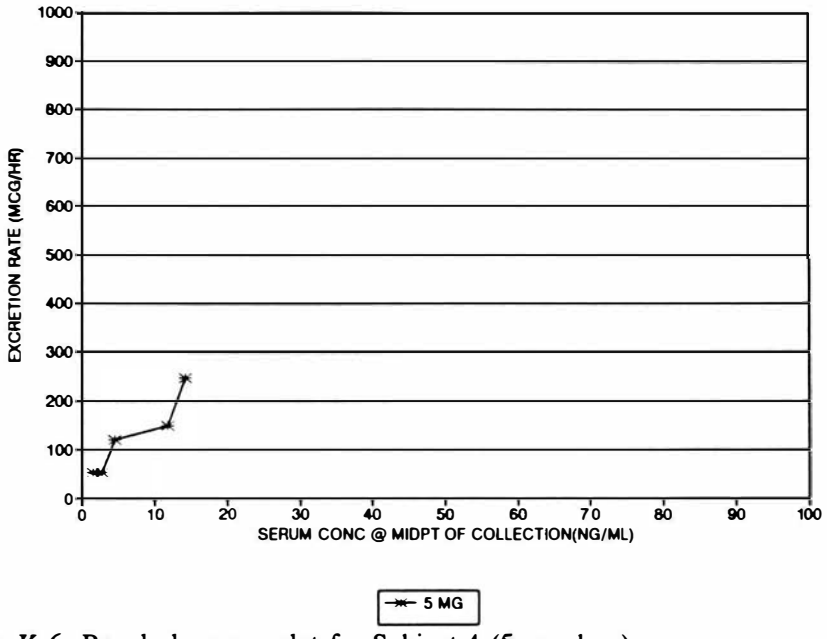


Figure K.6 Renal clearance plot for Subject 4 (5 mg dose)

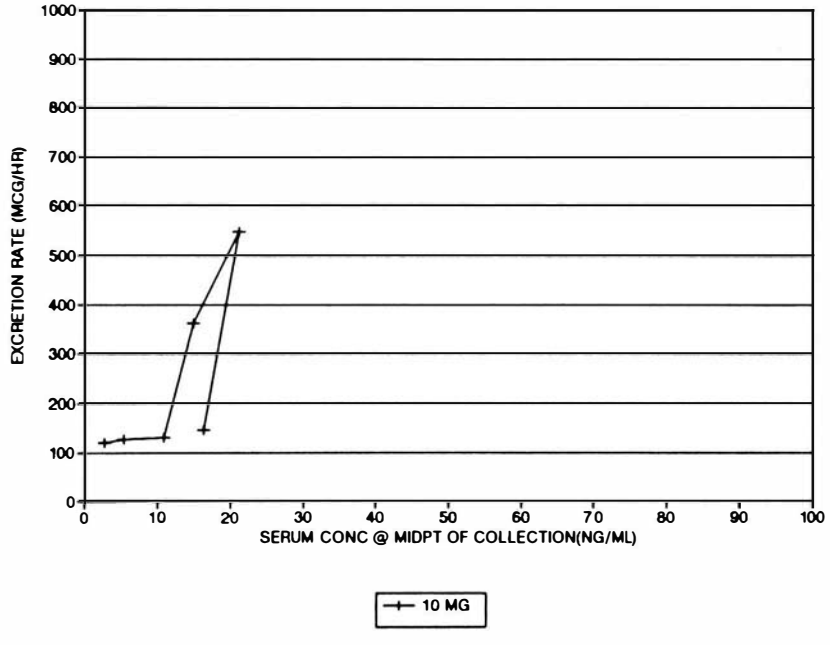


Figure K.7 Renal clearance plot for Subject 4 (10 mg dose)

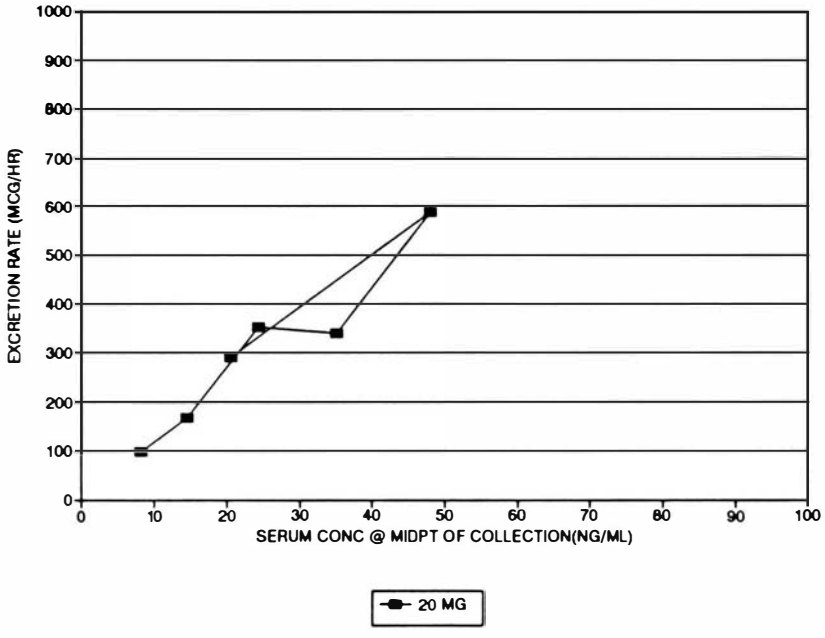


Figure K.8 Renal clearance plot for Subject 4 (20 mg dose)

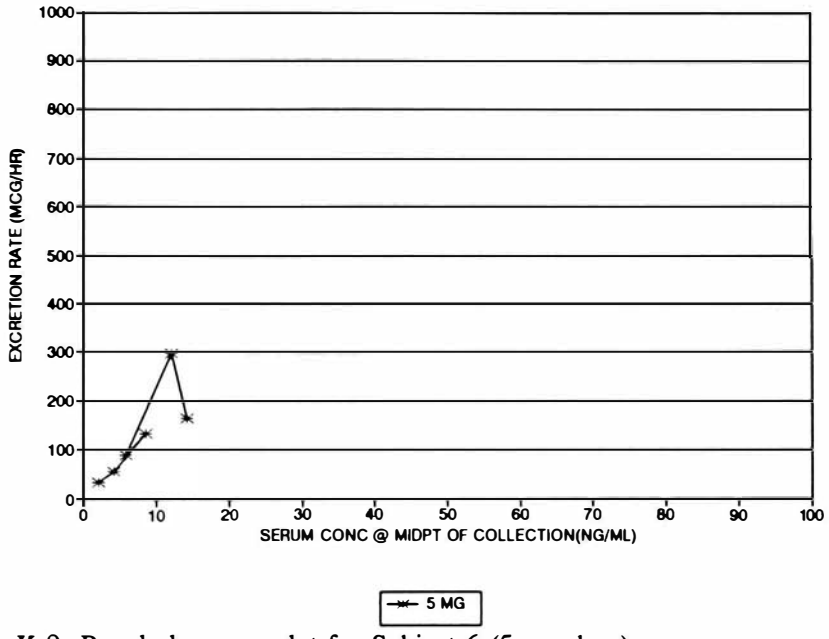


Figure K.9 Renal clearance plot for Subject 6 (5 mg dose)

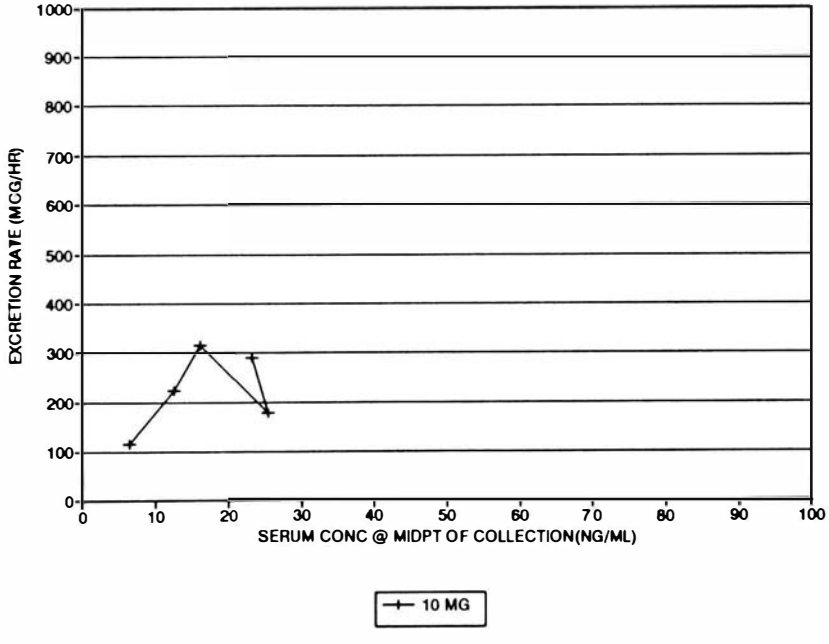


Figure K.10 Renal clearance plot for Subject 6 (10 mg dose)

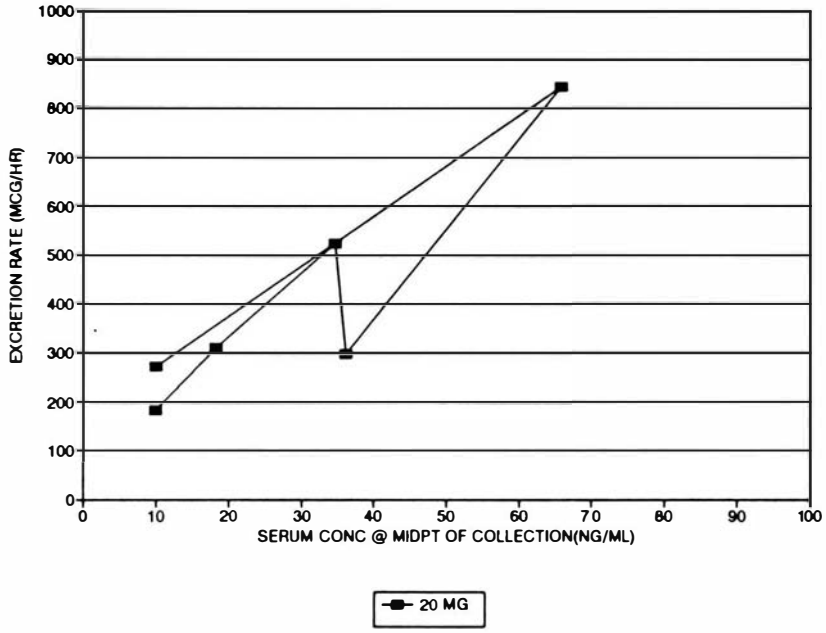


Figure K.11 Renal clearance plot for Subject 6 (20 mg dose)

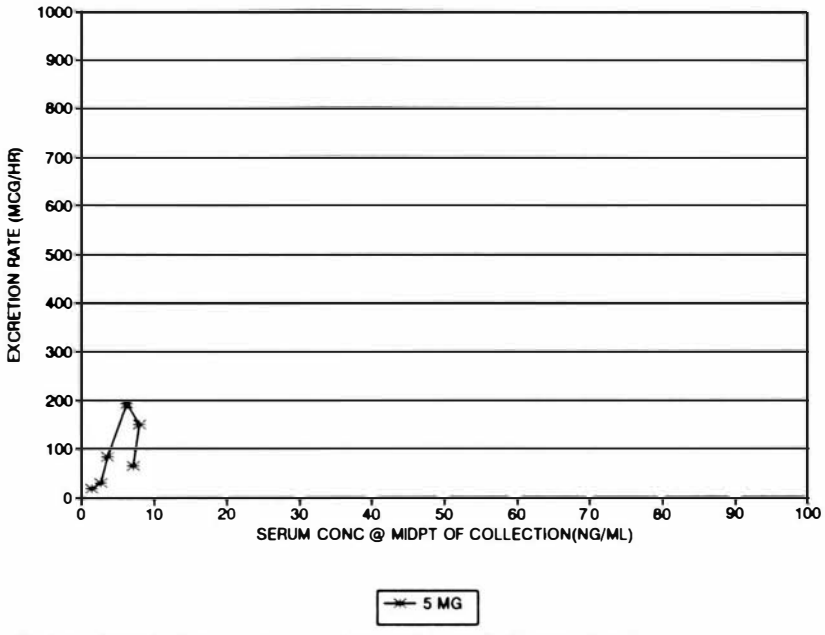


Figure K.12 Renal clearance plot for Subject 8 (5 mg dose)

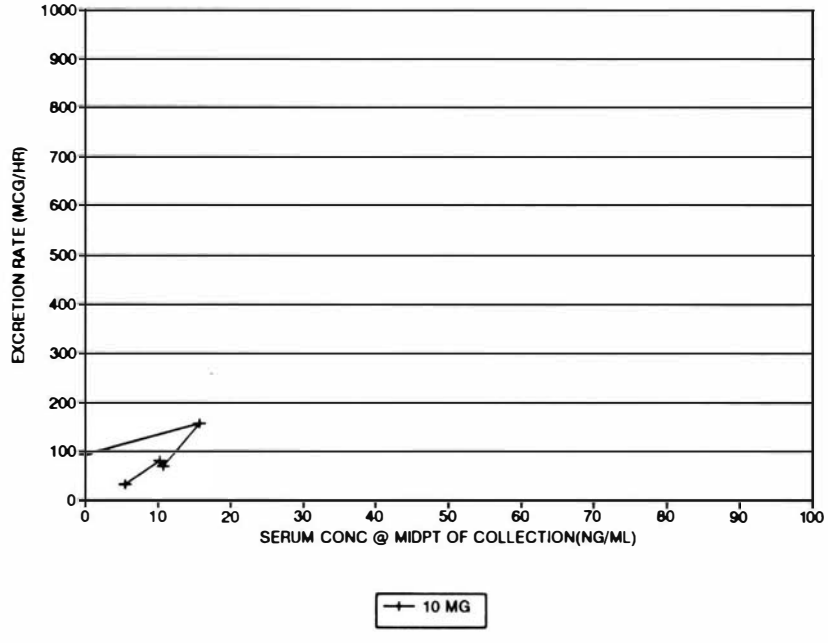


Figure K.13 Renal clearance plot for Subject 8 (10 mg dose)

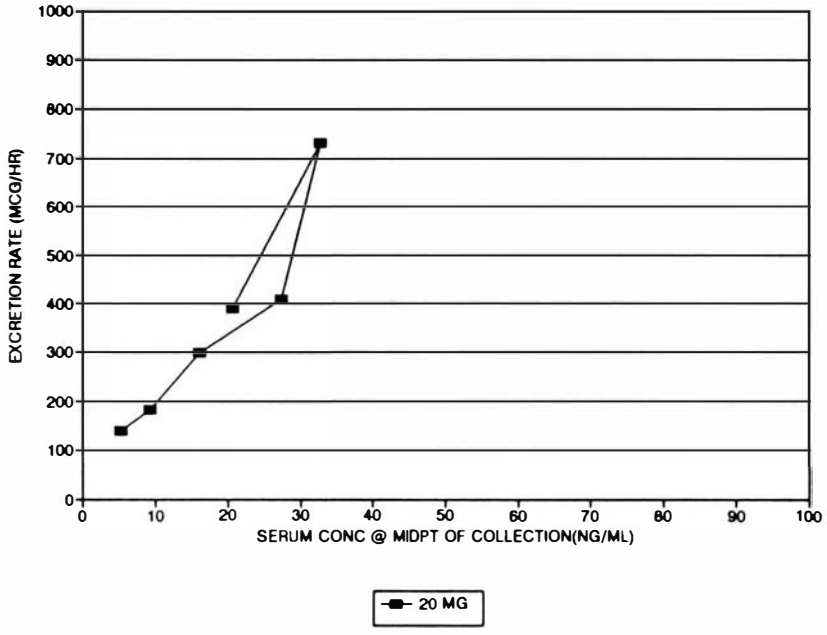


Figure K.14 Renal clearance plot for Subject 8 (20 mg dose)

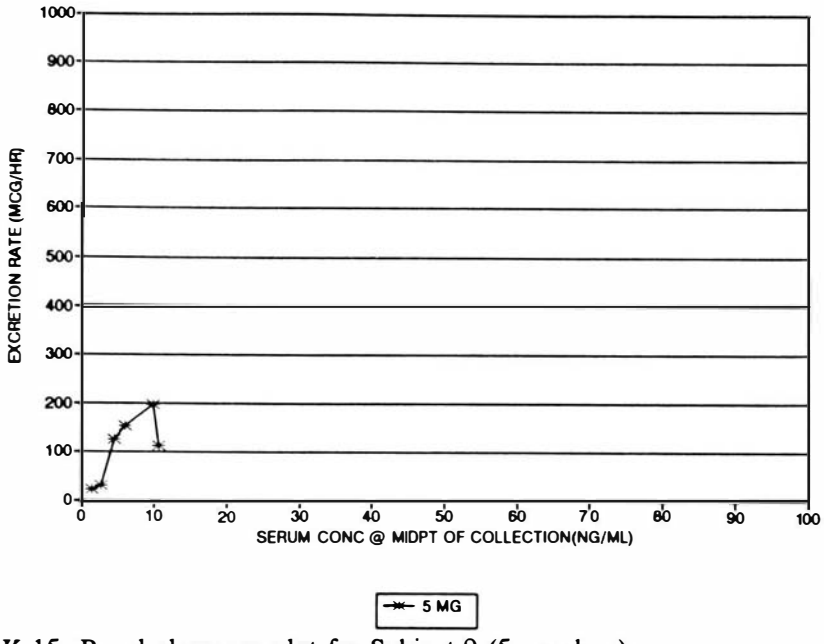


Figure K.15 Renal clearance plot for Subject 9 (5 mg dose)

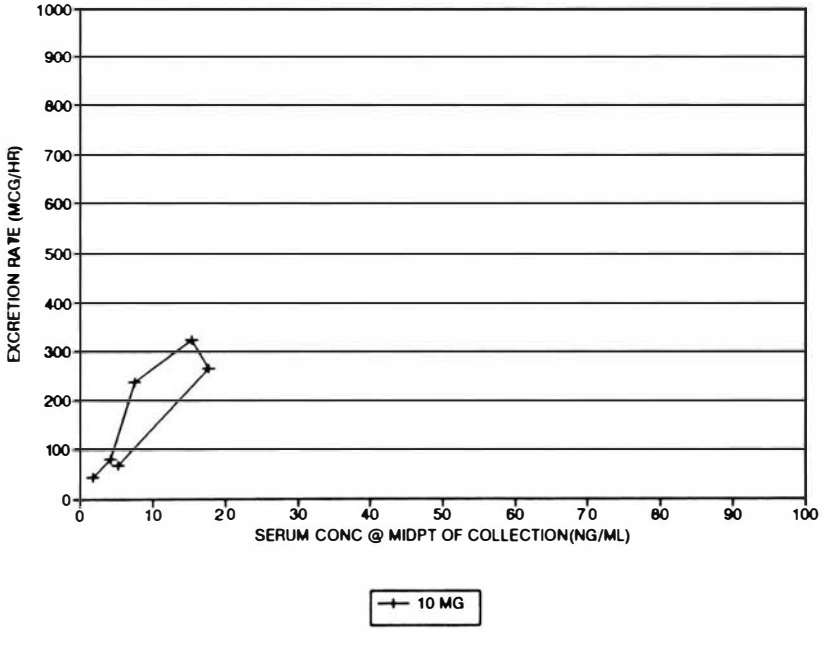


Figure K.16 Renal clearance plot for Subject 9 (10 mg dose)

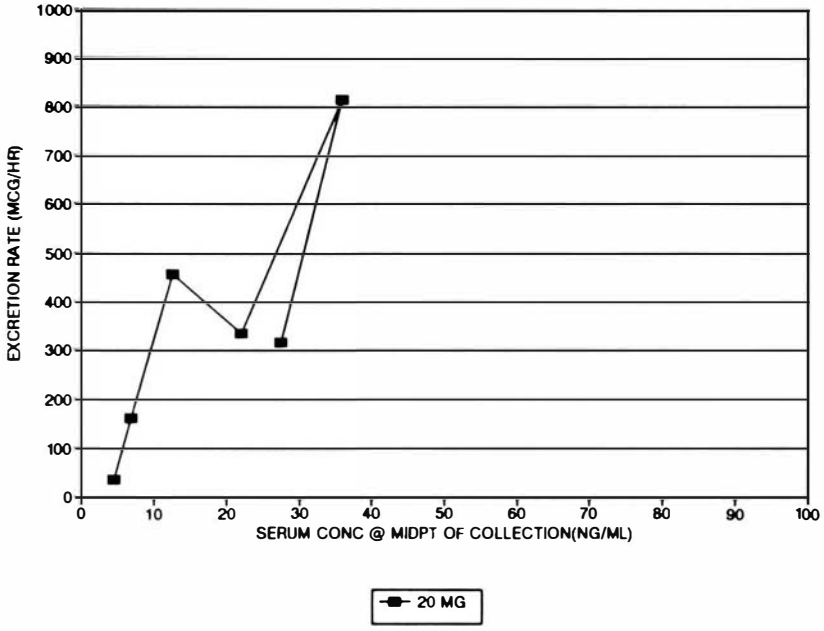


Figure K.17 Renal clearance plot for Subject 9 (20 mg dose)

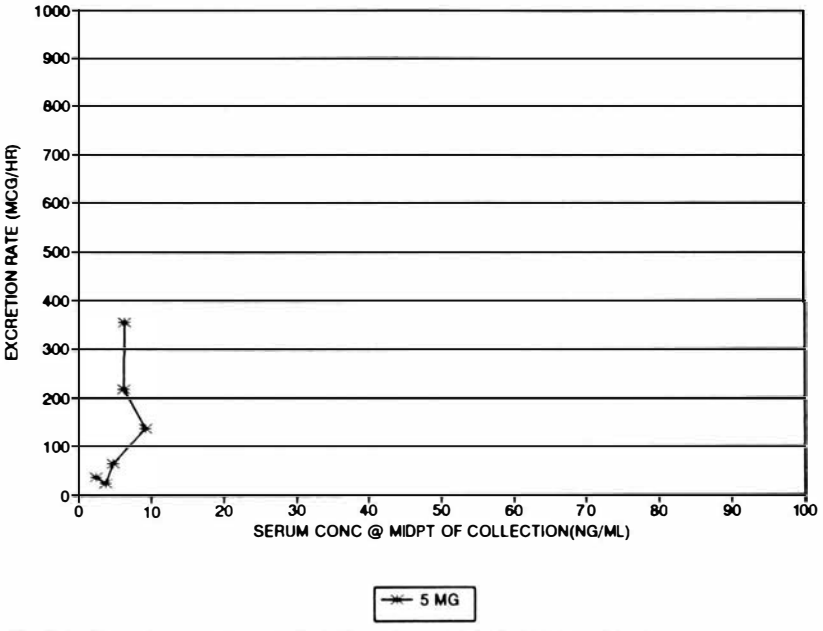


Figure K.18 Renal clearance plot for Subject 10 (5 mg dose)

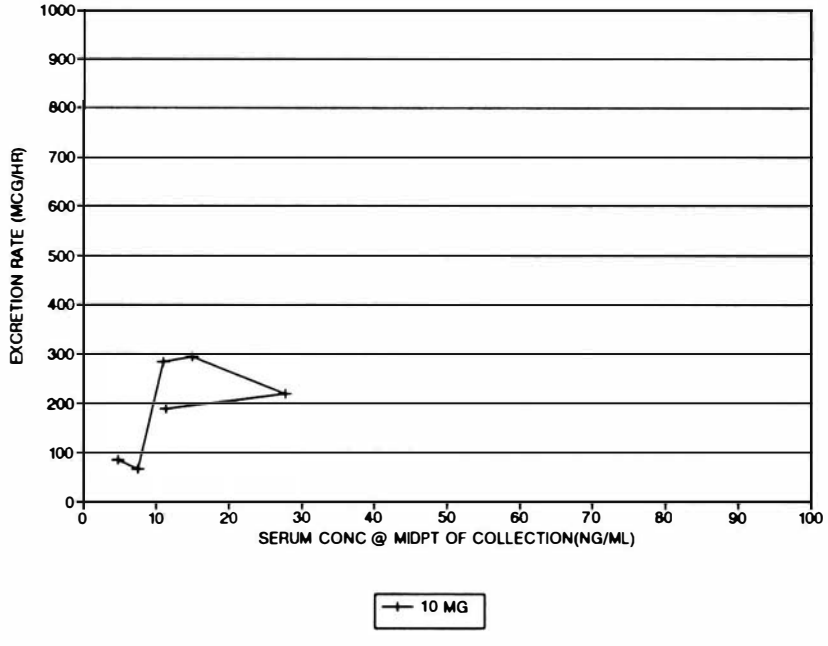


Figure K.19 Renal clearance plot for Subject 10 (10 mg dose)

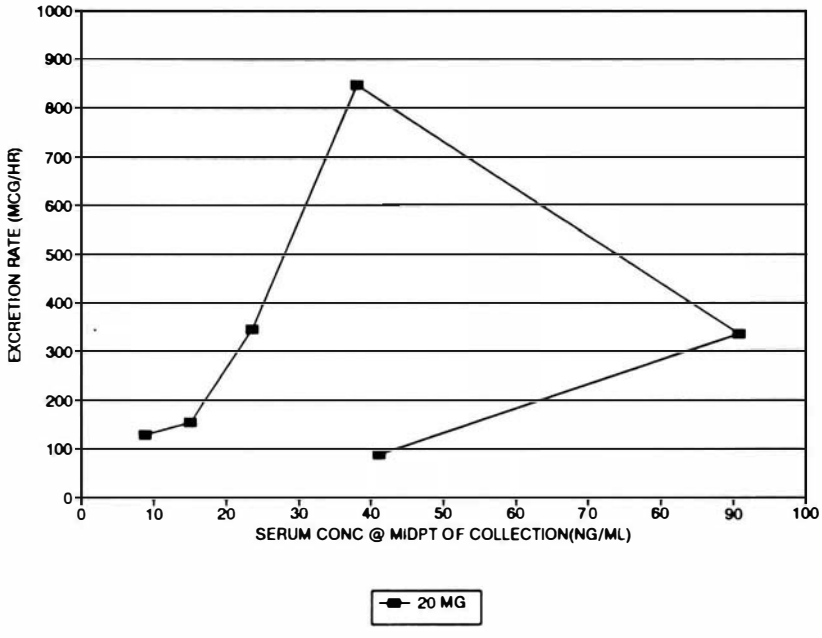


Figure K.20 Renal clearance plot for Subject 10 (20 mg dose)

APPENDIX L

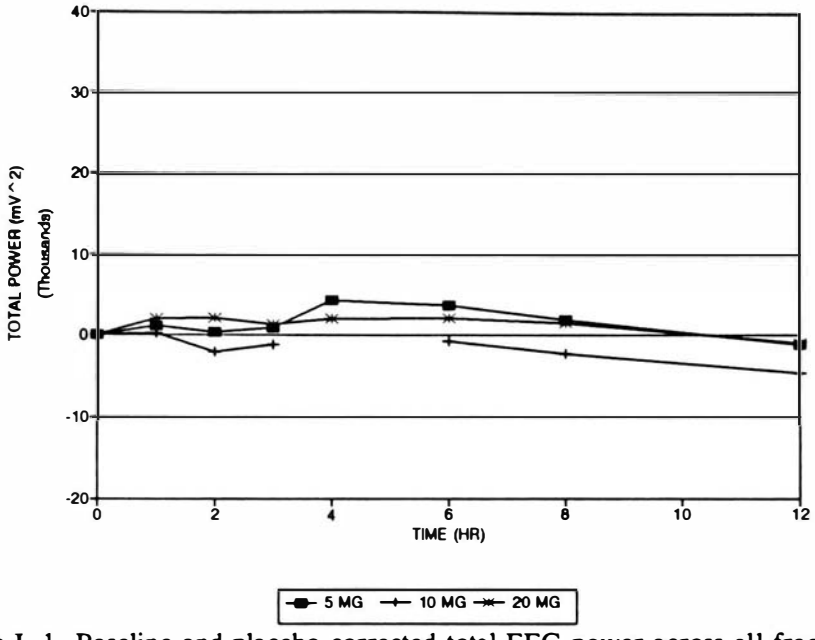


Figure L.1 Baseline and placebo corrected total EEG power across all frequency bands versus time profile for Subject 1

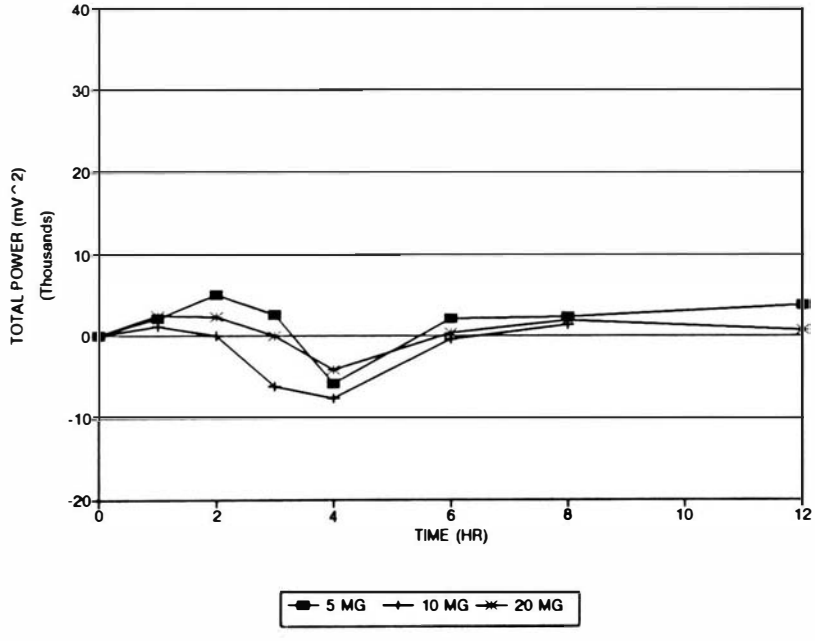


Figure L.2 Baseline and placebo corrected total EEG power across all frequency bands versus time profile for Subject 2

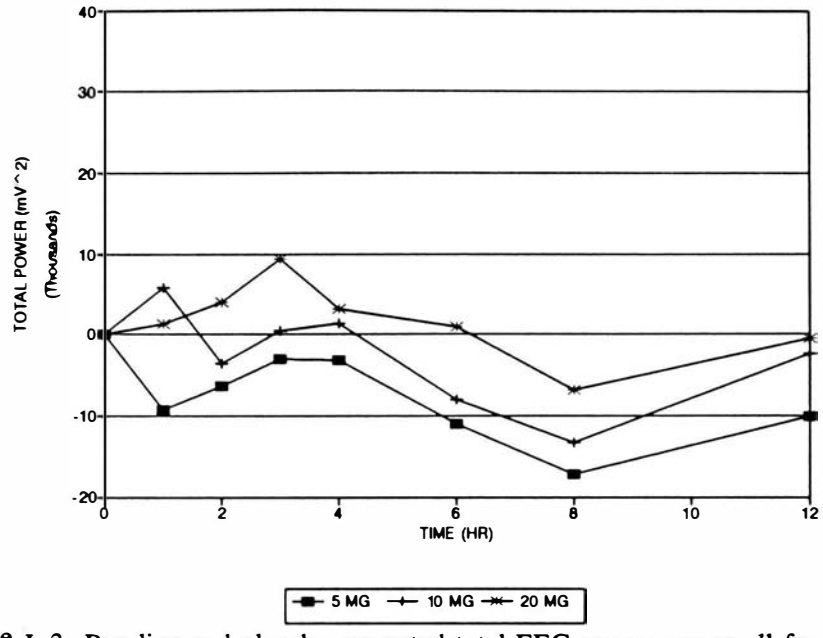


Figure L.3 Baseline and placebo corrected total EEG power across all frequency bands versus time profile for Subject 4

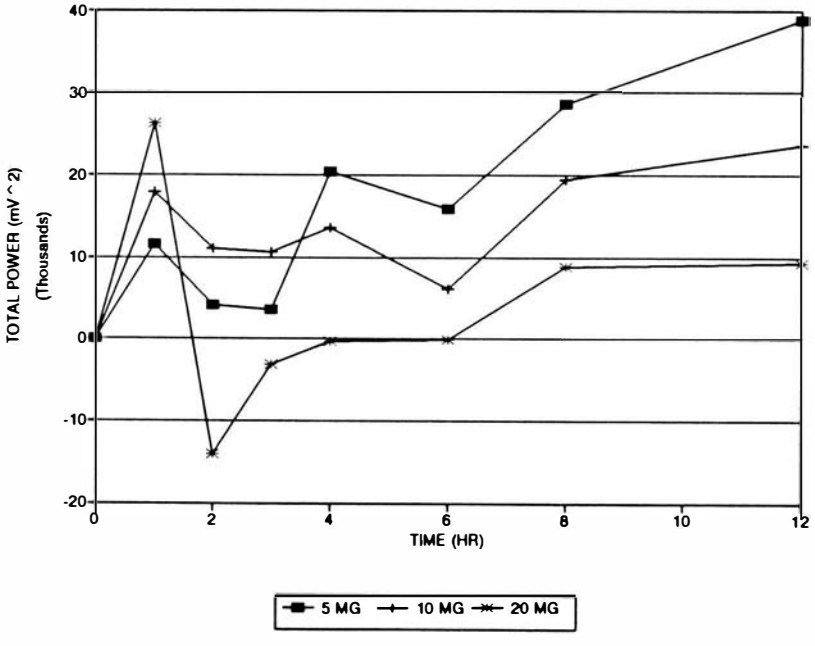


Figure L.4 Baseline and placebo corrected total EEG power across all frequency bands versus time profile for Subject 6

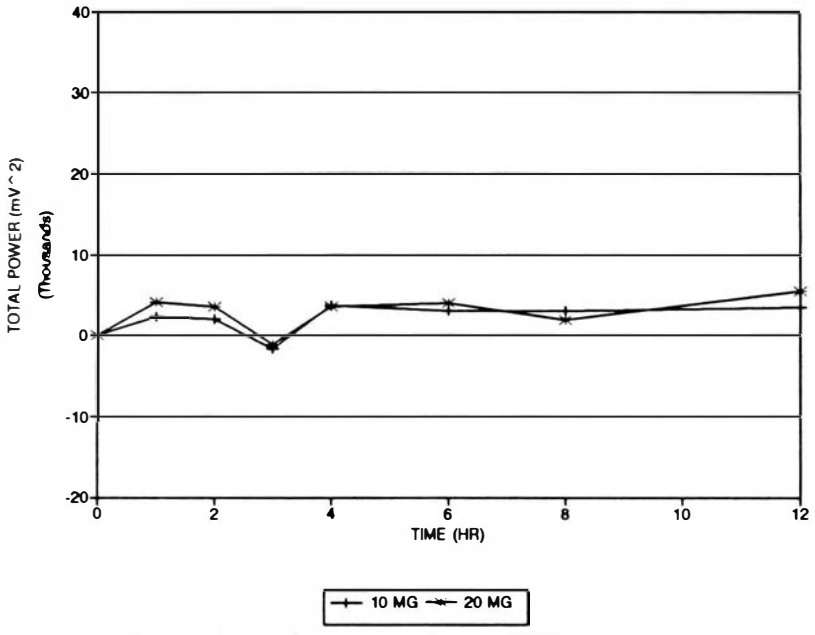


Figure L.5 Baseline and placebo corrected total EEG power across all frequency bands versus time profile for Subject 7

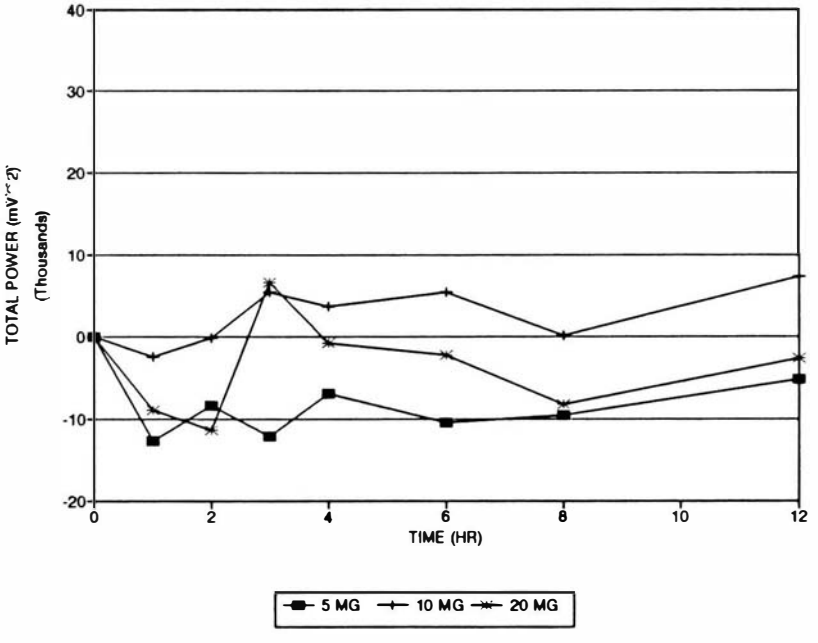


Figure L.6 Baseline and placebo corrected total EEG power across all frequency bands versus time profile for Subject 8

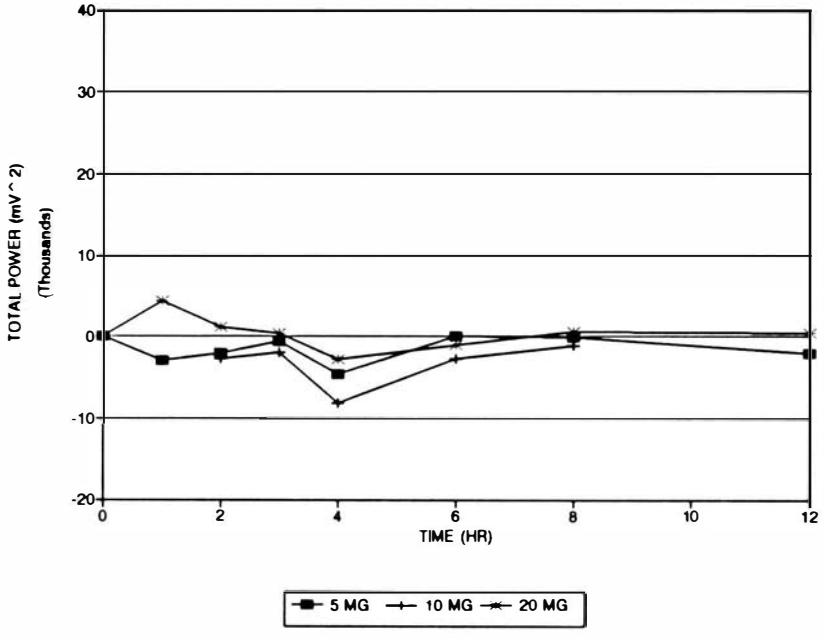


Figure L.7 Baseline and placebo corrected total EEG power across all frequency bands versus time profile for Subject 10

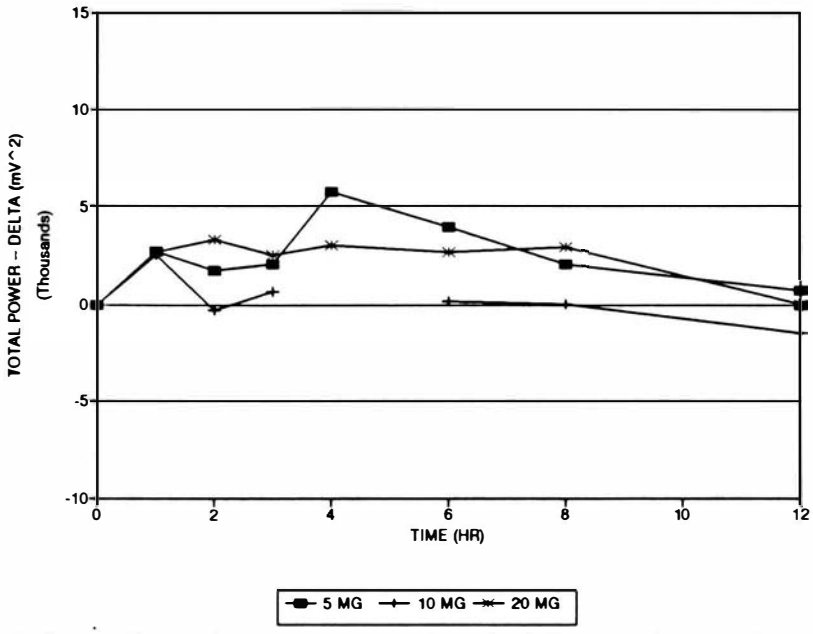


Figure L.8 Baseline and placebo corrected total EEG power in the delta frequency band versus time profile for Subject 1

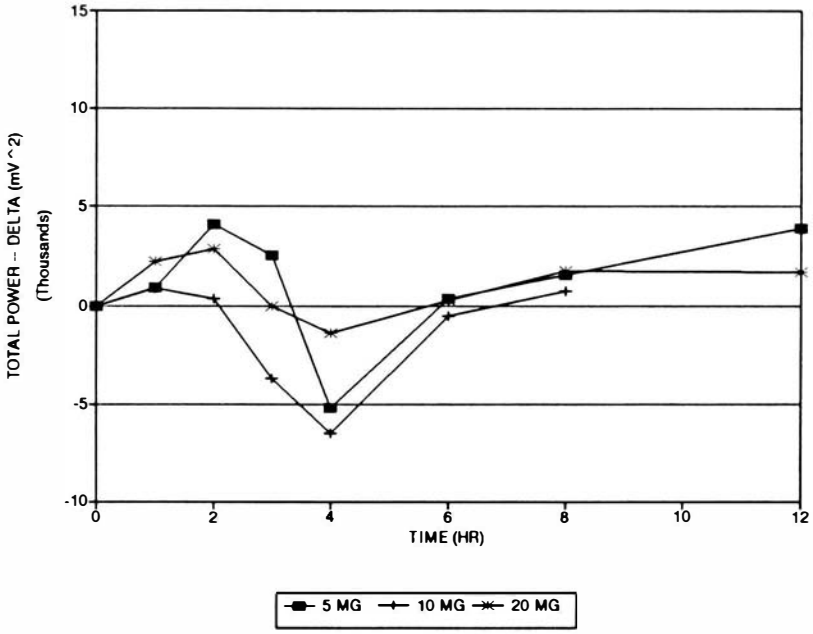


Figure L.9 Baseline and placebo corrected total EEG power in the delta frequency band versus time profile for Subject 2

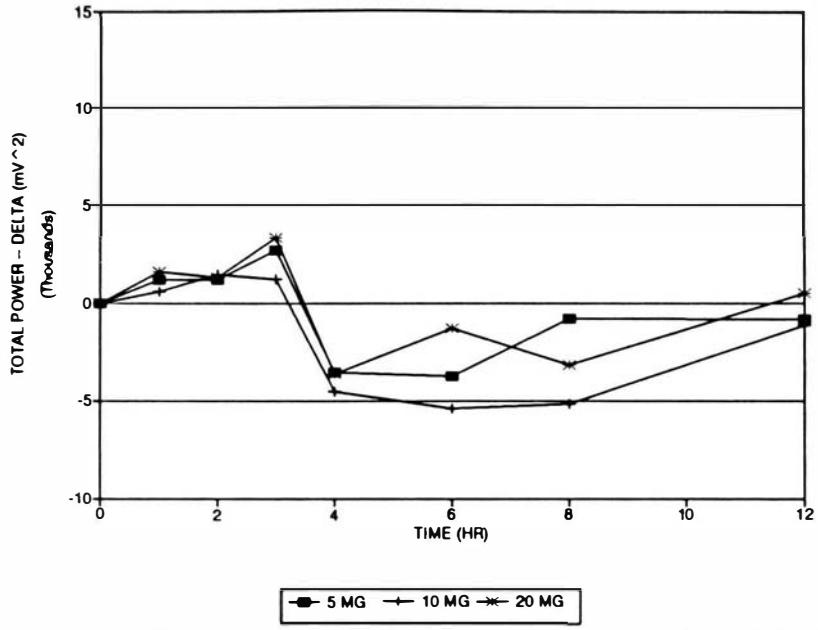


Figure L.10 Baseline and placebo corrected total EEG power in the delta frequency band versus time profile for Subject 4

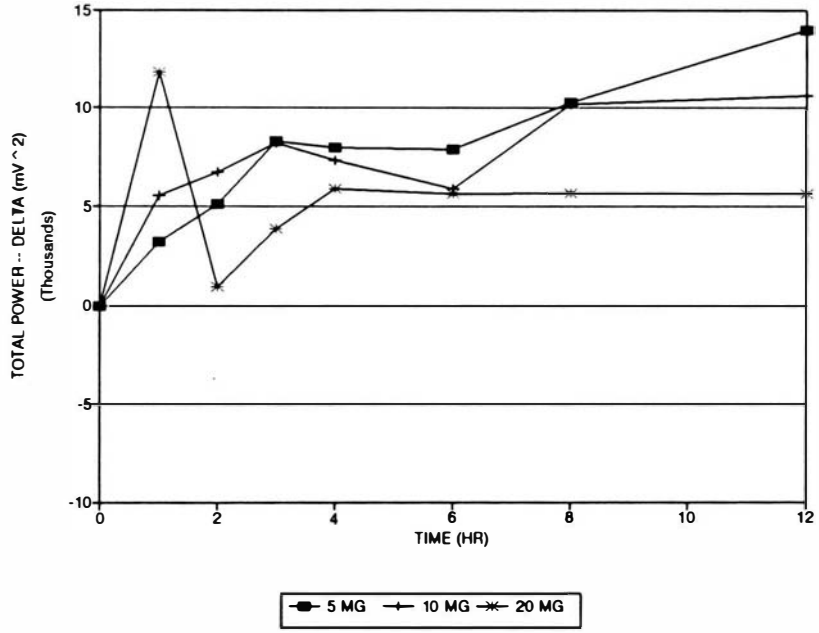


Figure L.11 Baseline and placebo corrected total EEG power in the delta frequency band versus time profile for Subject 6

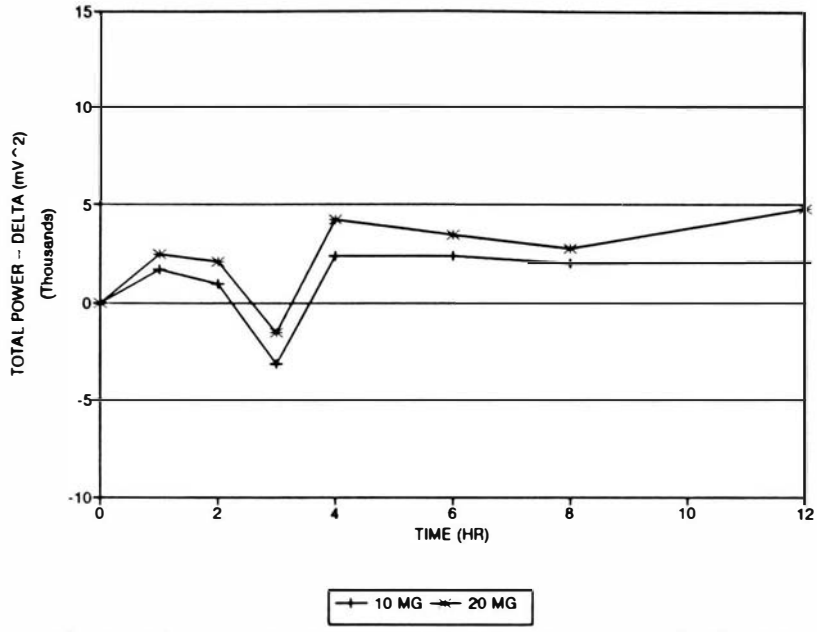


Figure L.12 Baseline and placebo corrected total EEG power in the delta frequency band versus time profile for Subject 7

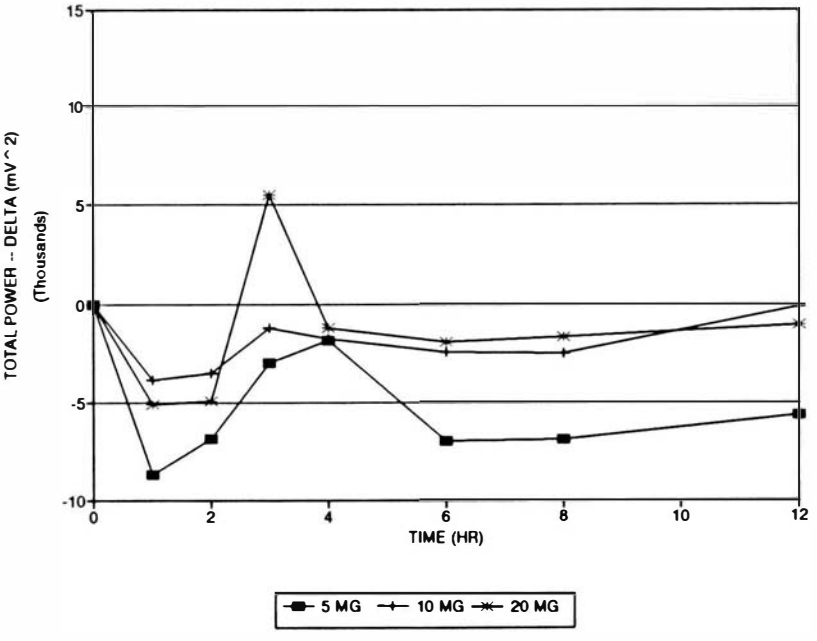


Figure L.13 Baseline and placebo corrected total EEG power in the delta frequency band versus time profile for Subject 8

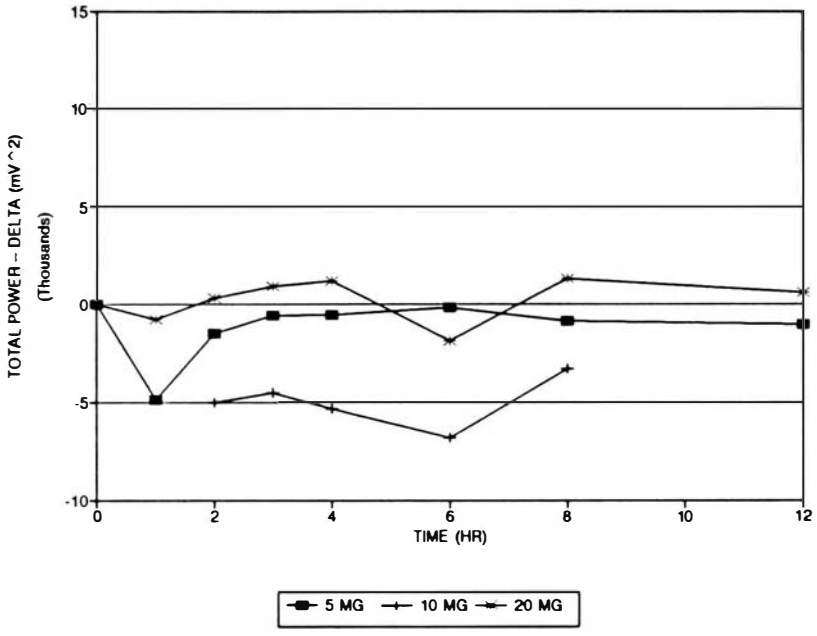


Figure L.14 Baseline and placebo corrected total EEG power in the delta frequency band versus time profile for Subject 10

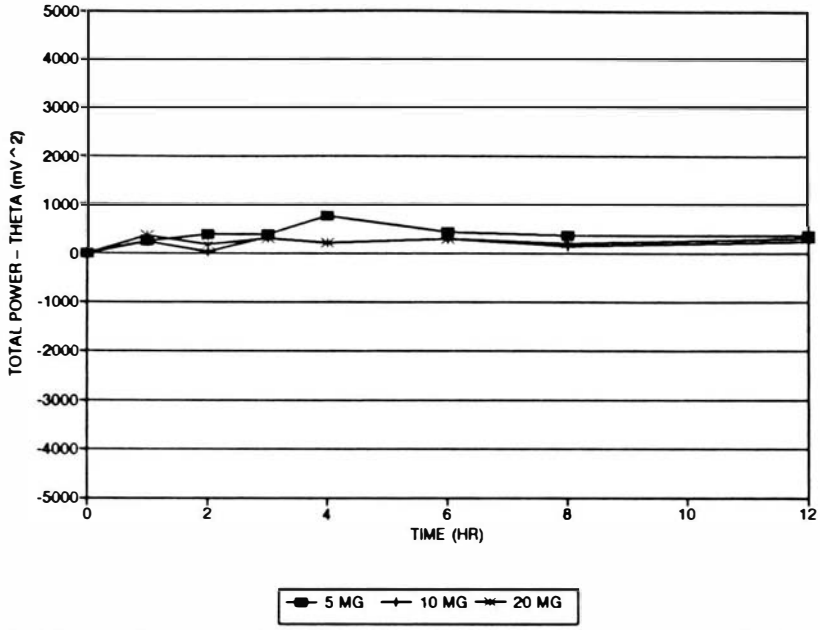


Figure L.15 Baseline and placebo corrected total EEG power in the theta frequency band versus time profile for Subject 1

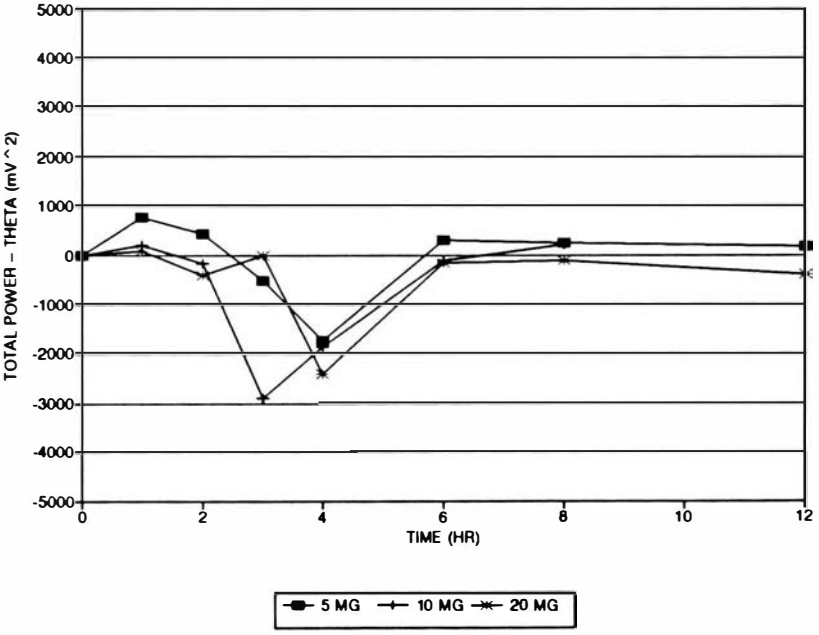


Figure L.16 Baseline and placebo corrected total EEG power in the theta frequency band versus time profile for Subject 2

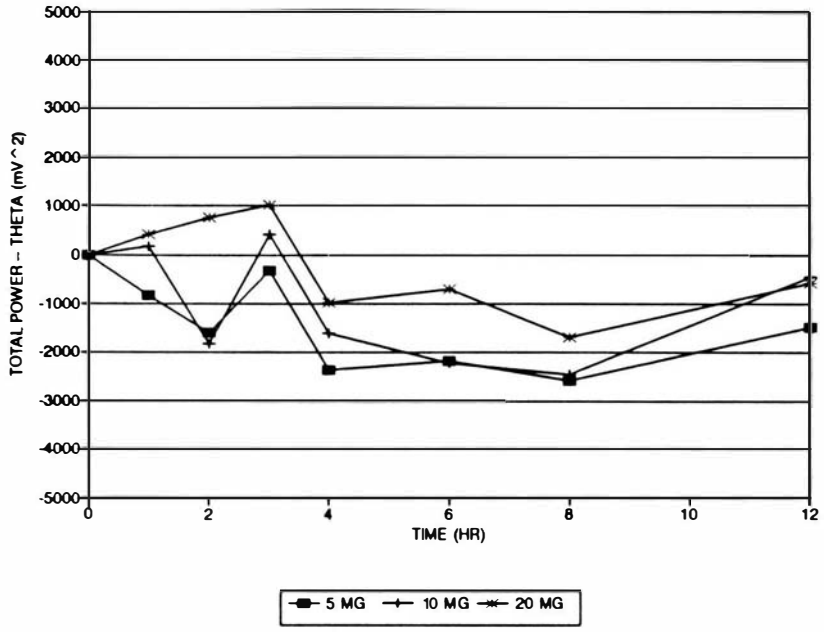


Figure L.17 Baseline and placebo corrected total EEG power in the theta frequency band versus time profile for Subject 4

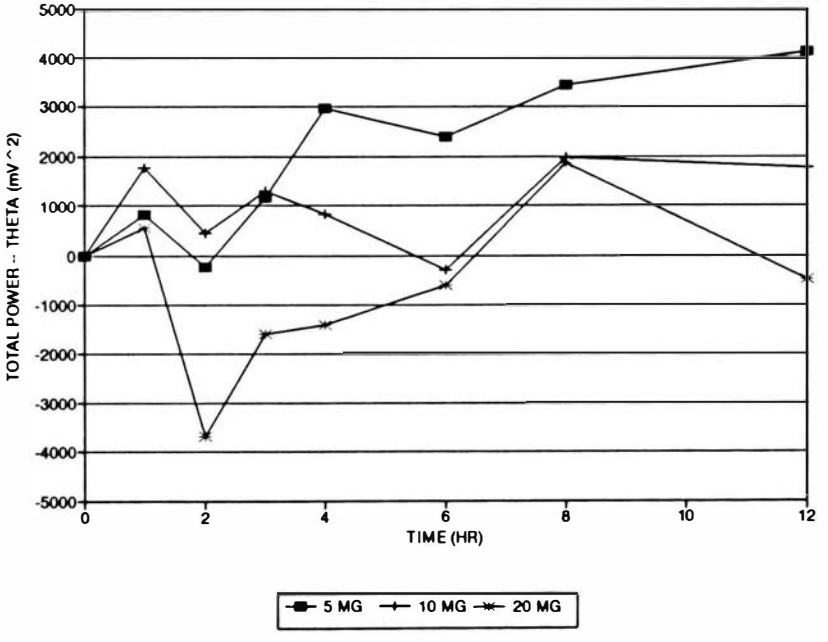


Figure L.18 Baseline and placebo corrected total EEG power in the theta frequency band versus time profile for Subject 6

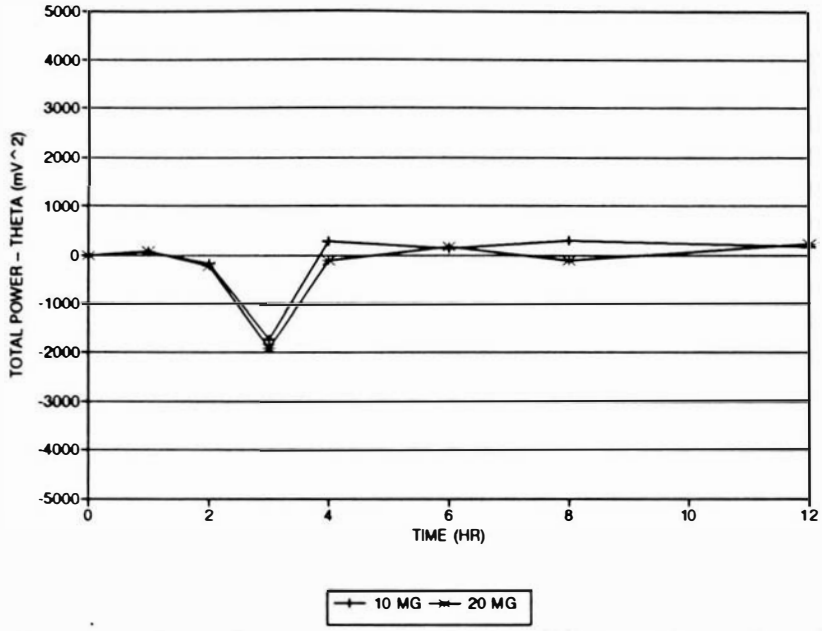


Figure L.19 Baseline and placebo corrected total EEG power in the theta frequency band versus time profile for Subject 7

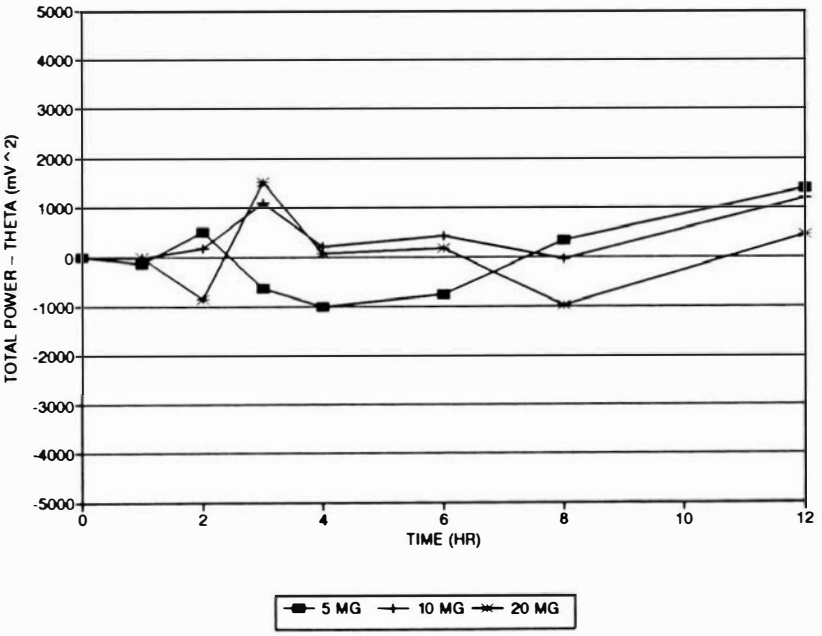


Figure L.20 Baseline and placebo corrected total EEG power in the theta frequency band versus time profile for Subject 8

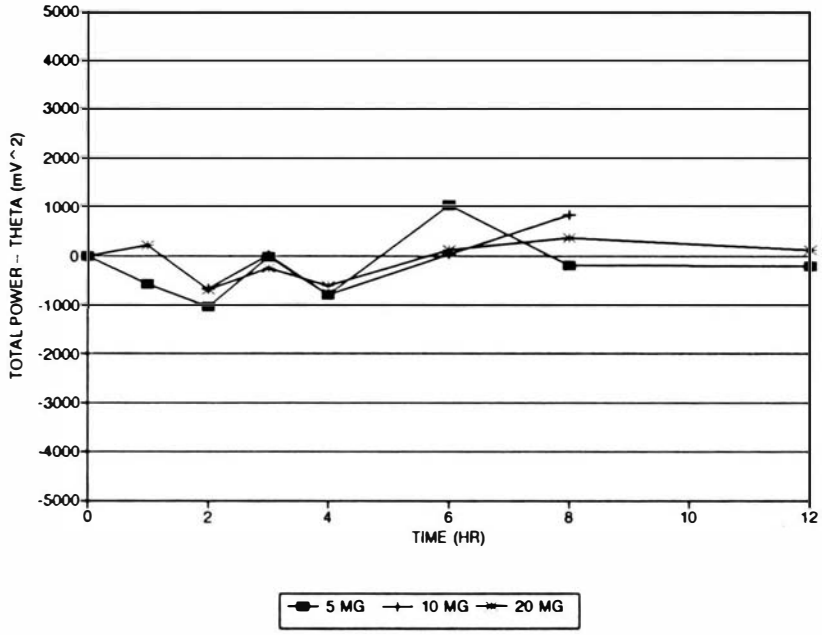


Figure L.21 Baseline and placebo corrected total EEG power in the theta frequency band versus time profile for Subject 10

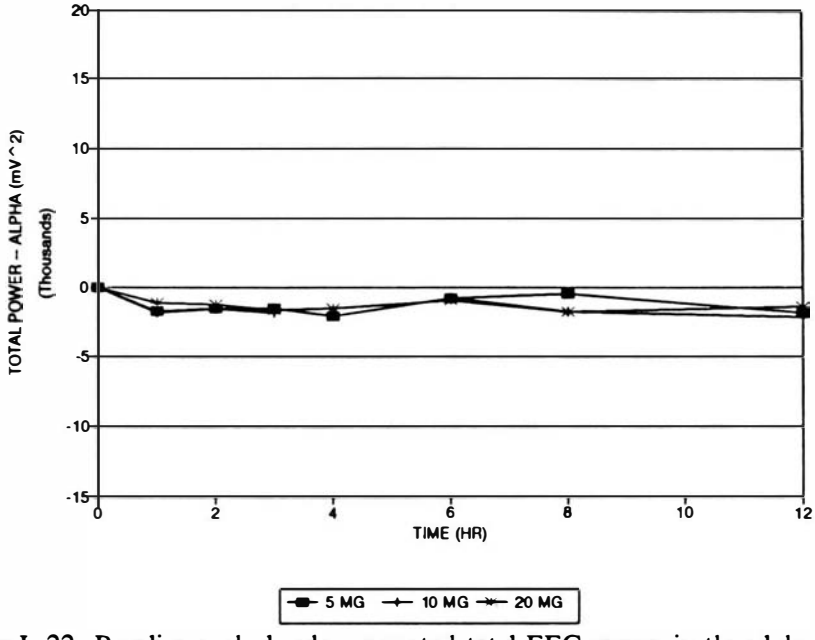


Figure L.22 Baseline and placebo corrected total EEG power in the alpha frequency band versus time profile for Subject 1

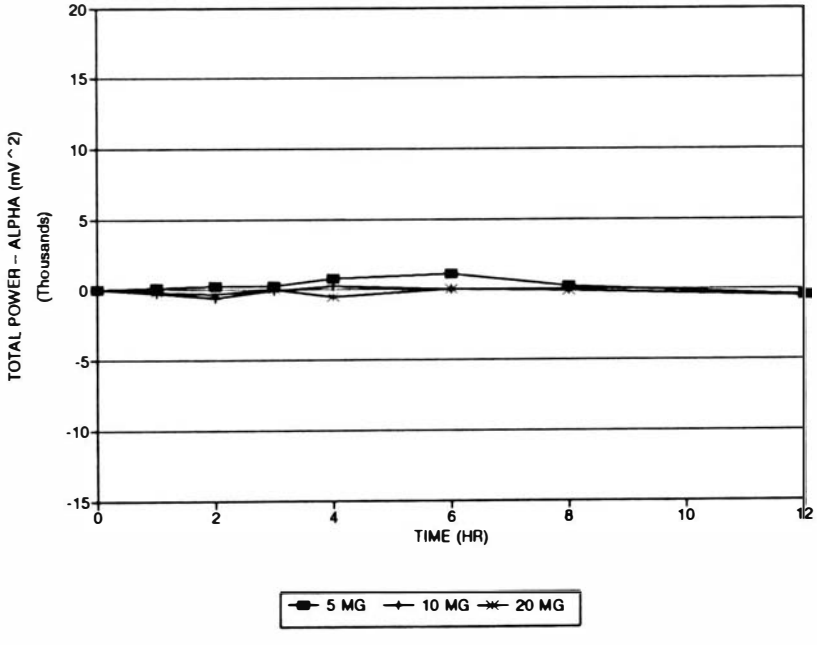


Figure L.23 Baseline and placebo corrected total EEG power in the alpha frequency band versus time profile for Subject 2

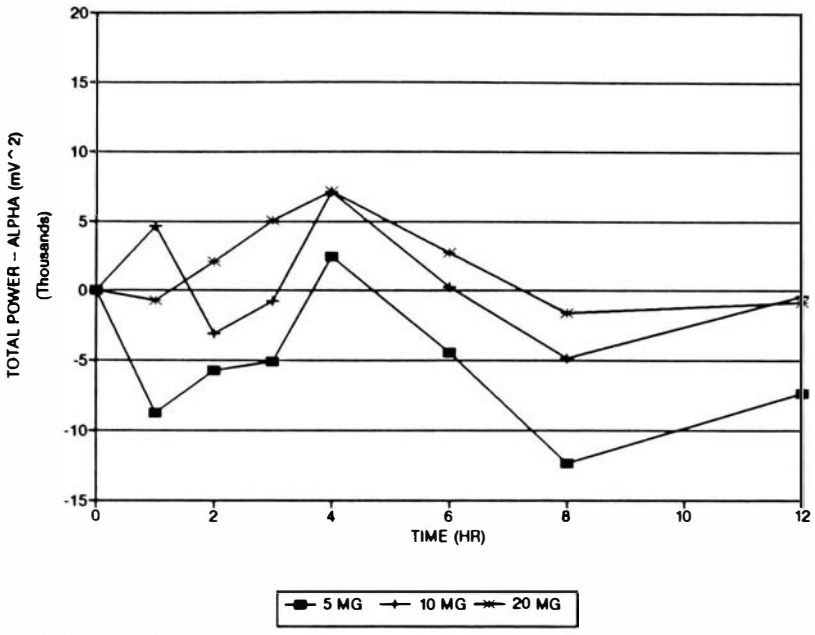


Figure L.24 Baseline and placebo corrected total EEG power in the alpha frequency band versus time profile for Subject 4

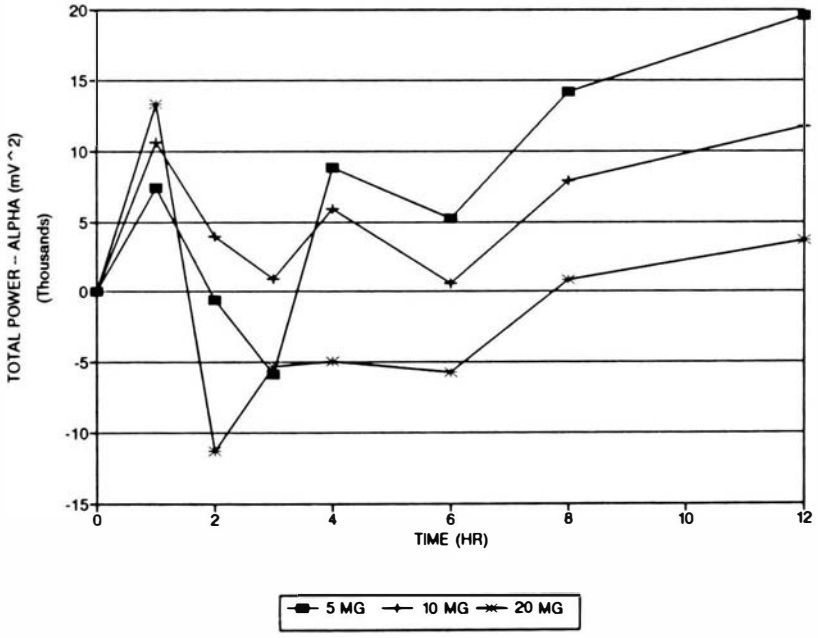


Figure L.25 Baseline and placebo corrected total EEG power in the alpha frequency band versus time profile for Subject 6

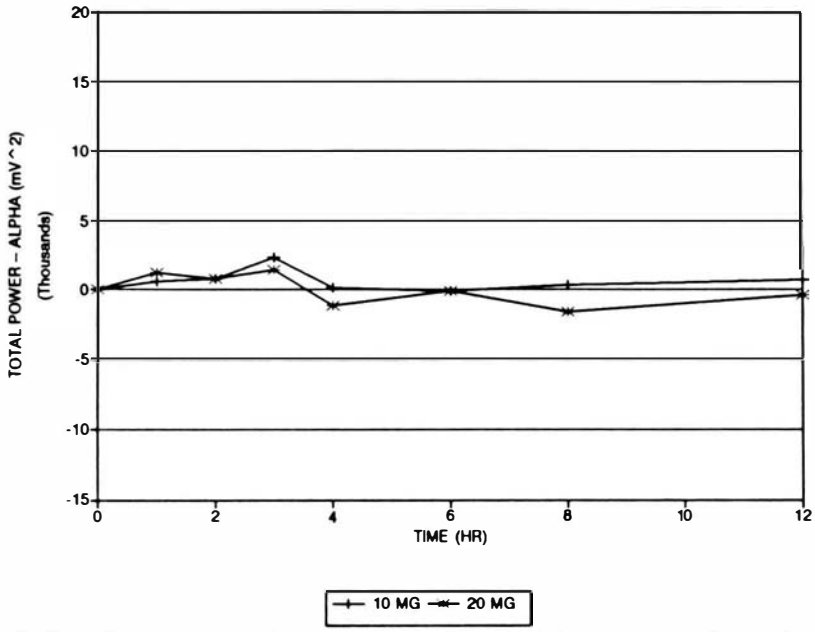


Figure L.26 Baseline and placebo corrected total EEG power in the alpha frequency band versus time profile for Subject 7

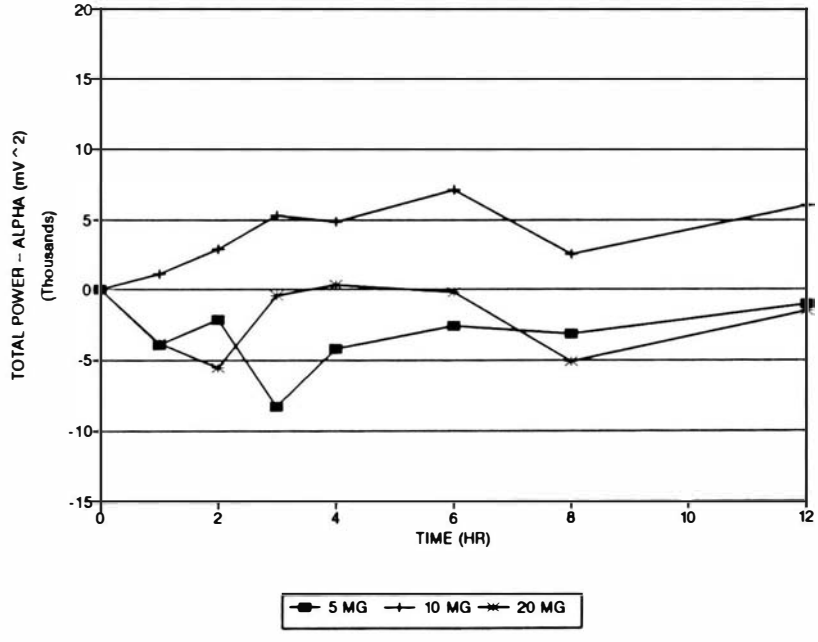


Figure L.27 Baseline and placebo corrected total EEG power in the alpha frequency band versus time profile for Subject 8

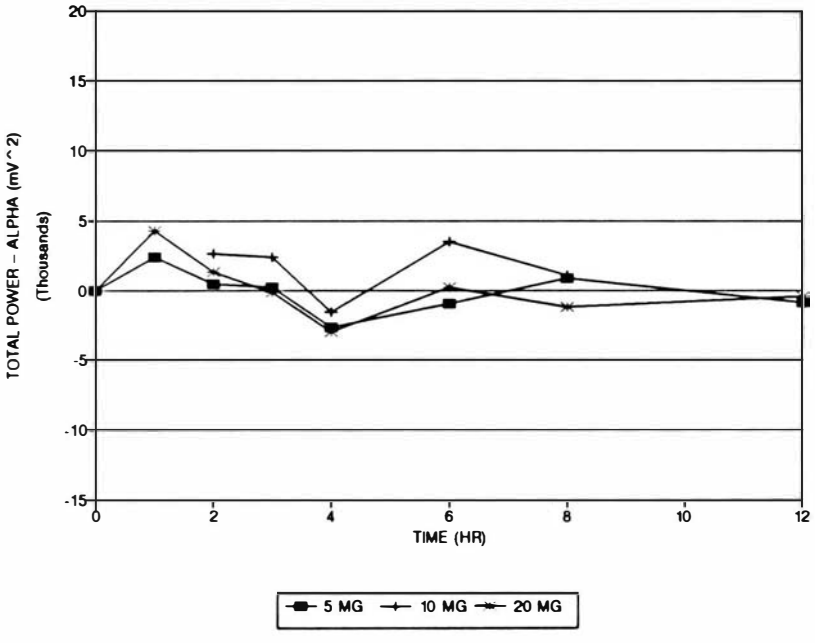


Figure L.28 Baseline and placebo corrected total EEG power in the alpha frequency band versus time profile for Subject 10

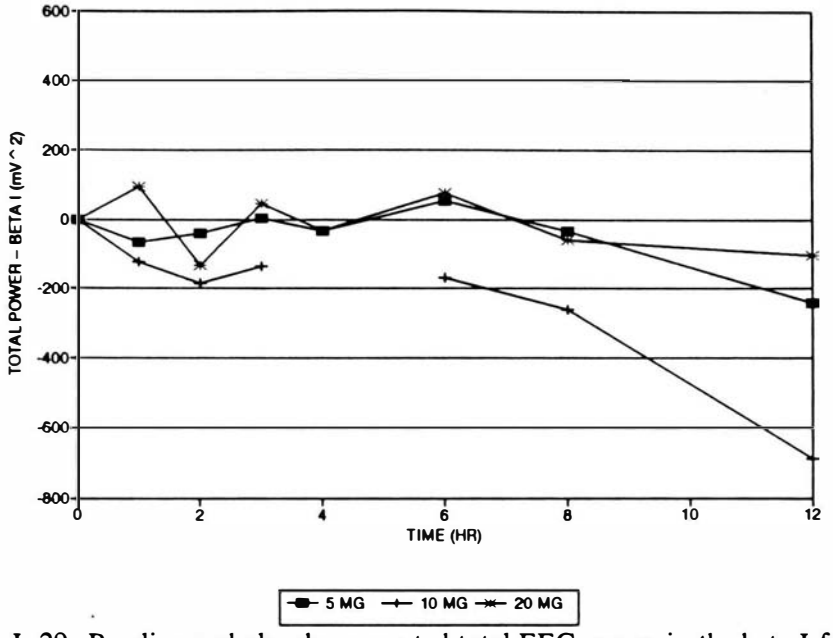


Figure L.29 Baseline and placebo corrected total EEG power in the beta I frequency band versus time profile for Subject 1

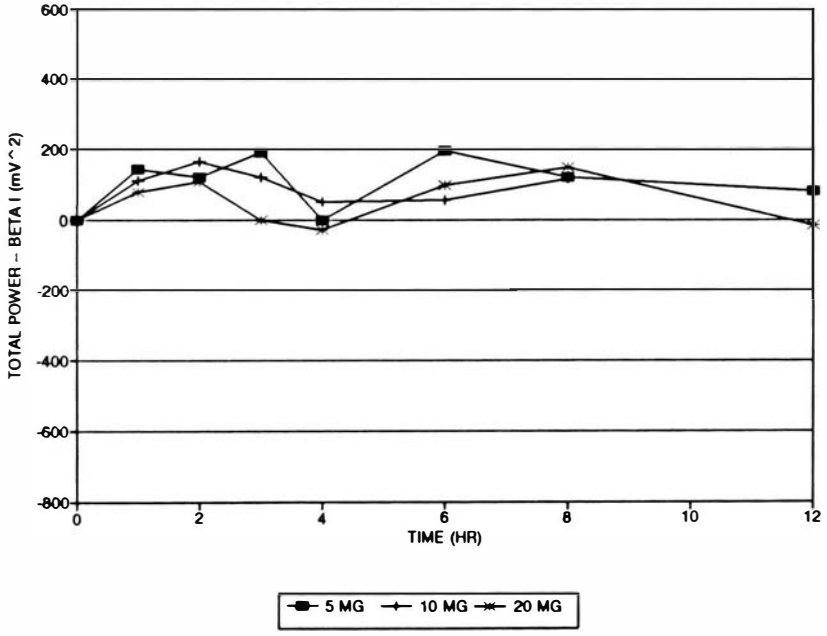


Figure L.30 Baseline and placebo corrected total EEG power in the beta I frequency band versus time profile for Subject 2

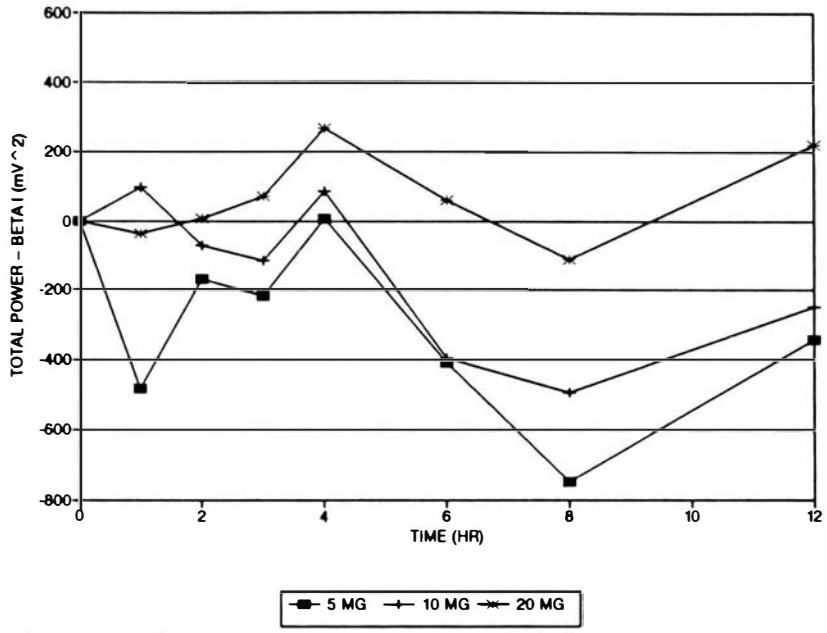


Figure L.31 Baseline and placebo corrected total EEG power in the beta I frequency band versus time profile for Subject 4

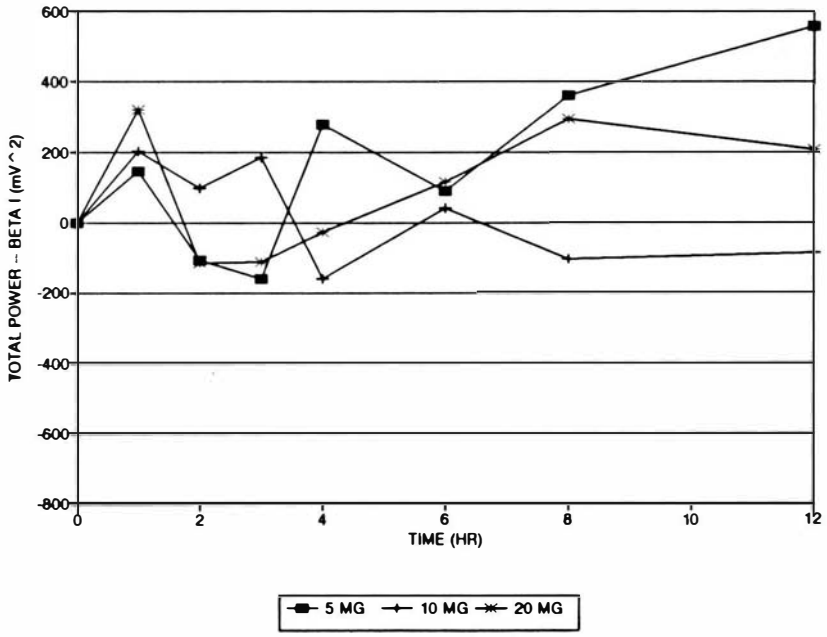


Figure L.32 Baseline and placebo corrected total EEG power in the beta I frequency band versus time profile for Subject 6

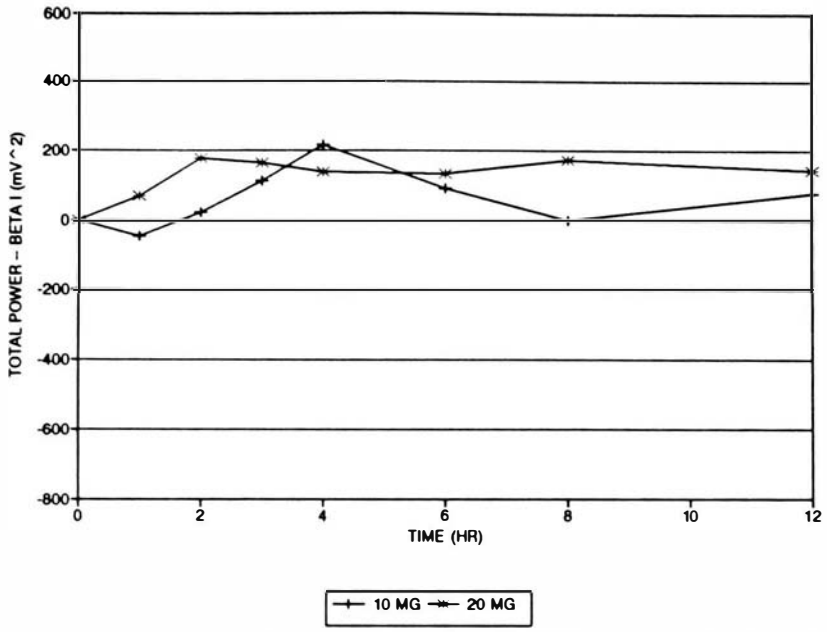


Figure L.33 Baseline and placebo corrected total EEG power in the beta I frequency band versus time profile for Subject 7

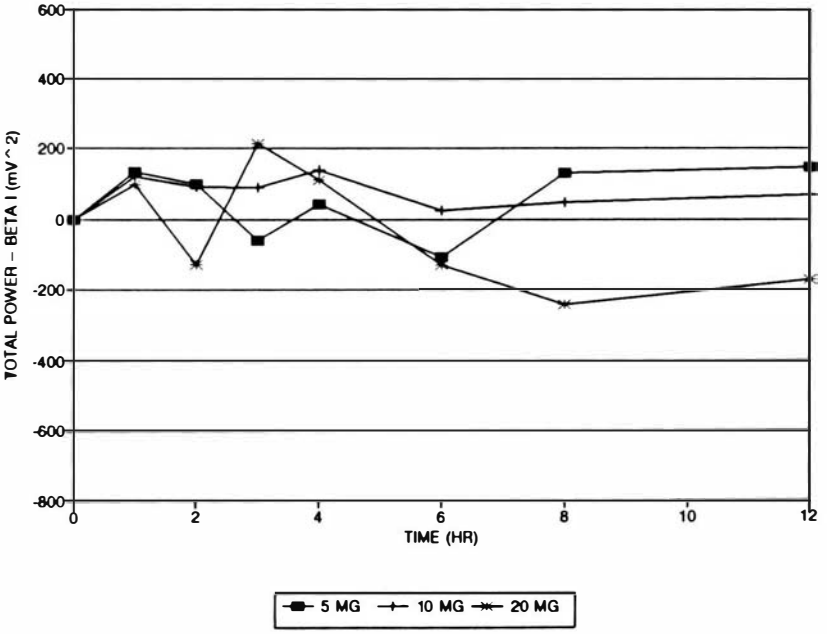


Figure L.34 Baseline and placebo corrected total EEG power in the beta I frequency band versus time profile for Subject 8

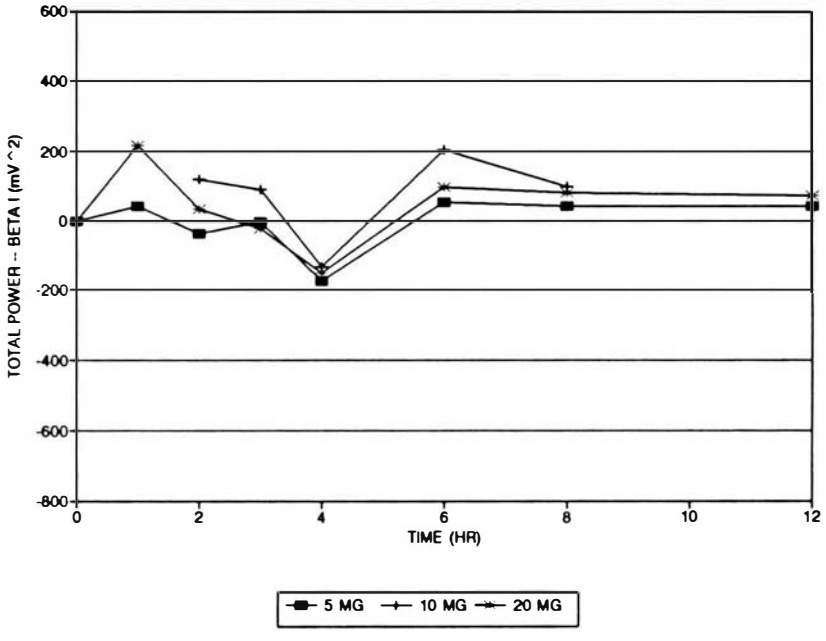


Figure L.35 Baseline and placebo corrected total EEG power in the beta I frequency band versus time profile for Subject 10

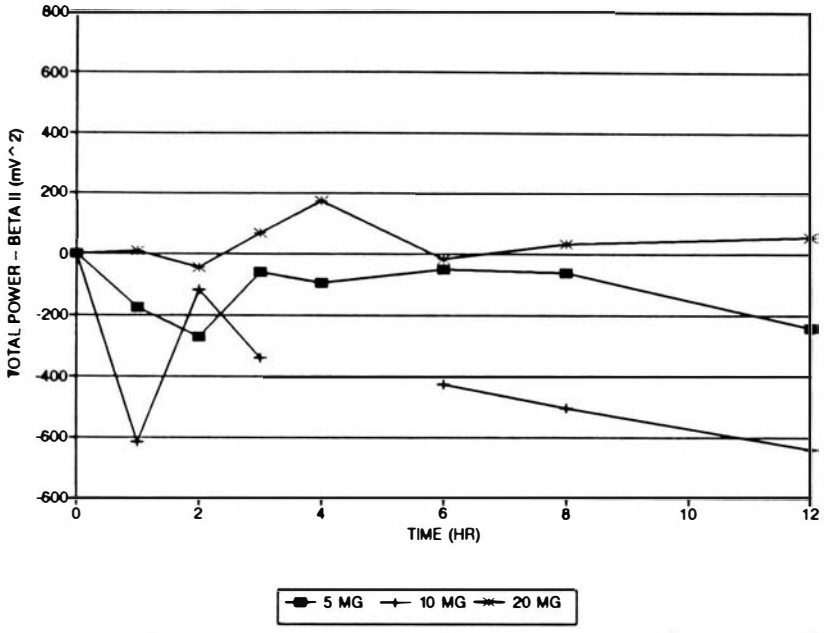


Figure L.36 Baseline and placebo corrected total EEG power in the beta II frequency band versus time profile for Subject 1

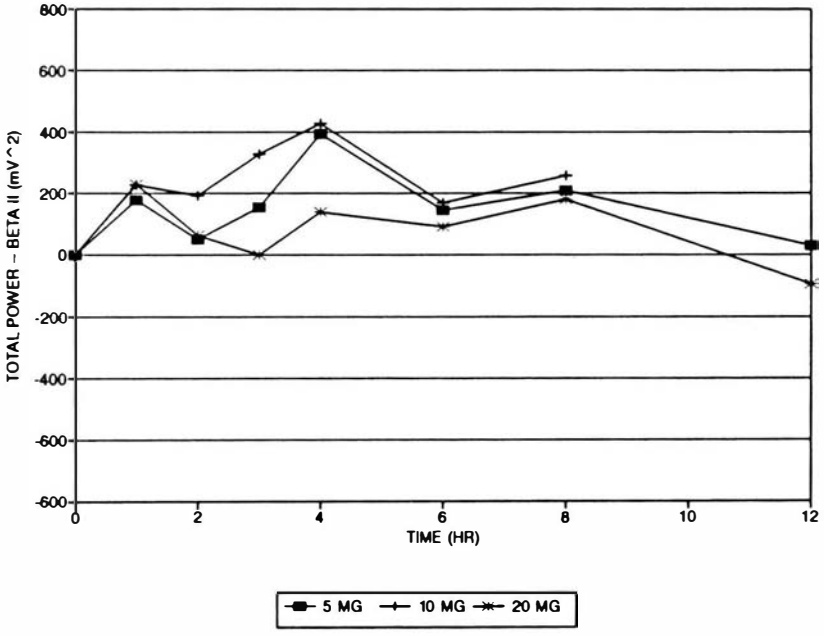


Figure L.37 Baseline and placebo corrected total EEG power in the beta II frequency band versus time profile for Subject 2

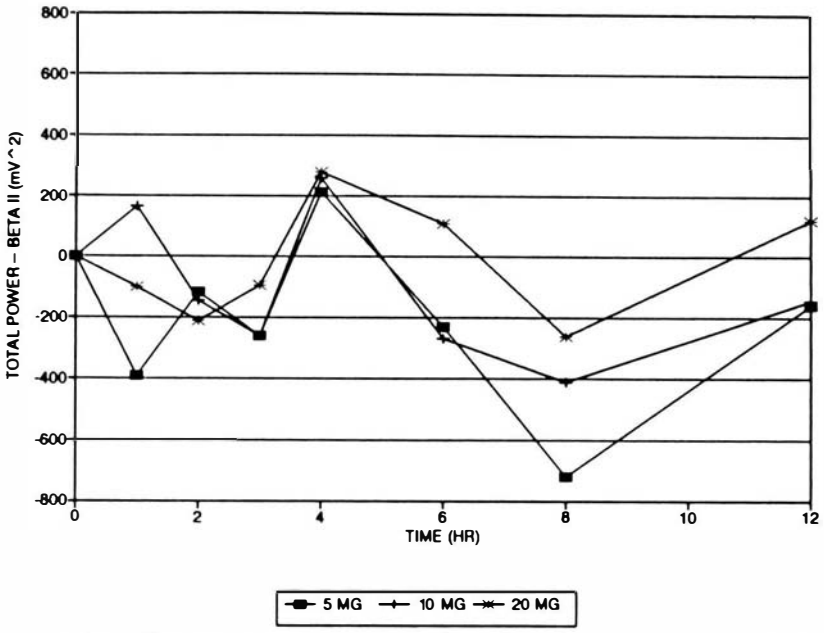


Figure L.38 Baseline and placebo corrected total EEG power in the beta II frequency band versus time profile for Subject 4

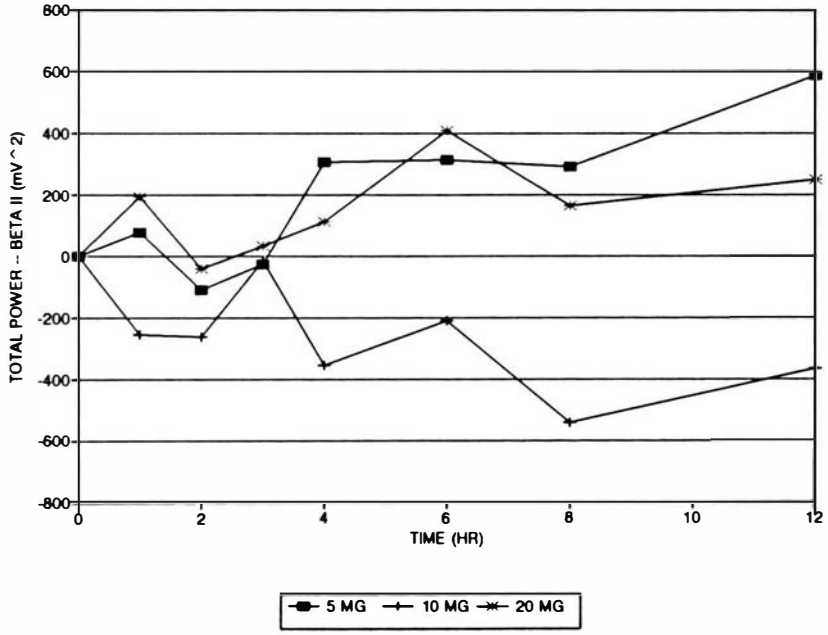


Figure L.39 Baseline and placebo corrected total EEG power in the beta II frequency band versus time profile for Subject 6

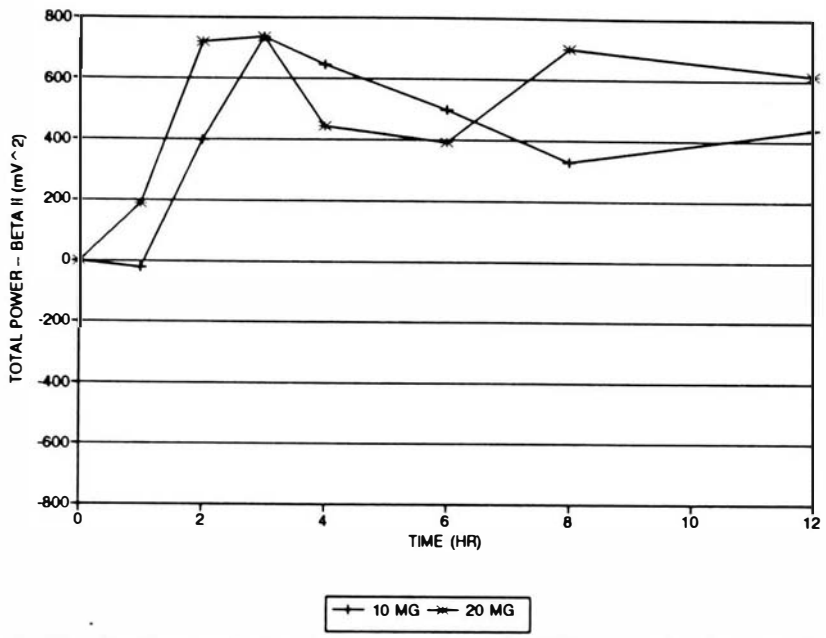


Figure L.40 Baseline and placebo corrected total EEG power in the beta II frequency band versus time profile for Subject 7

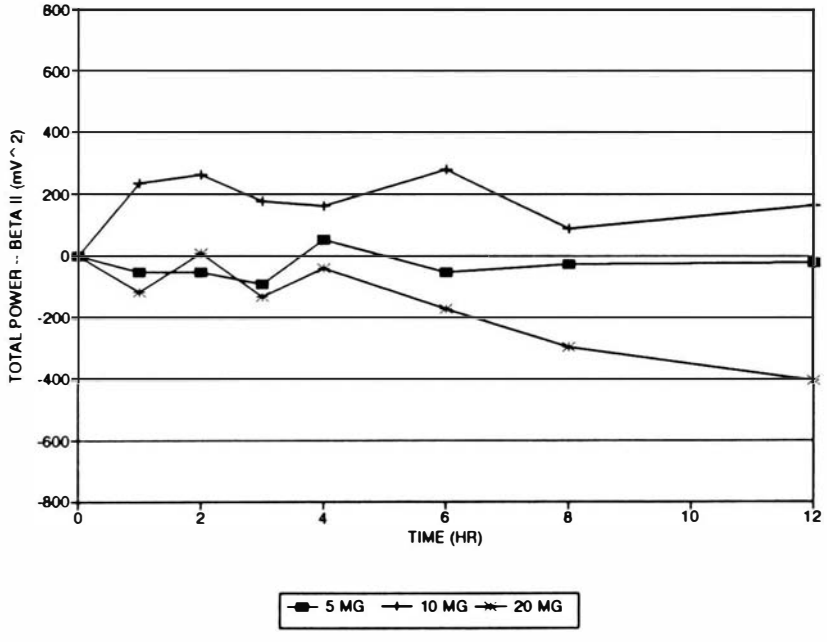


Figure L.41 Baseline and placebo corrected total EEG power in the beta II frequency band versus time profile for Subject 8

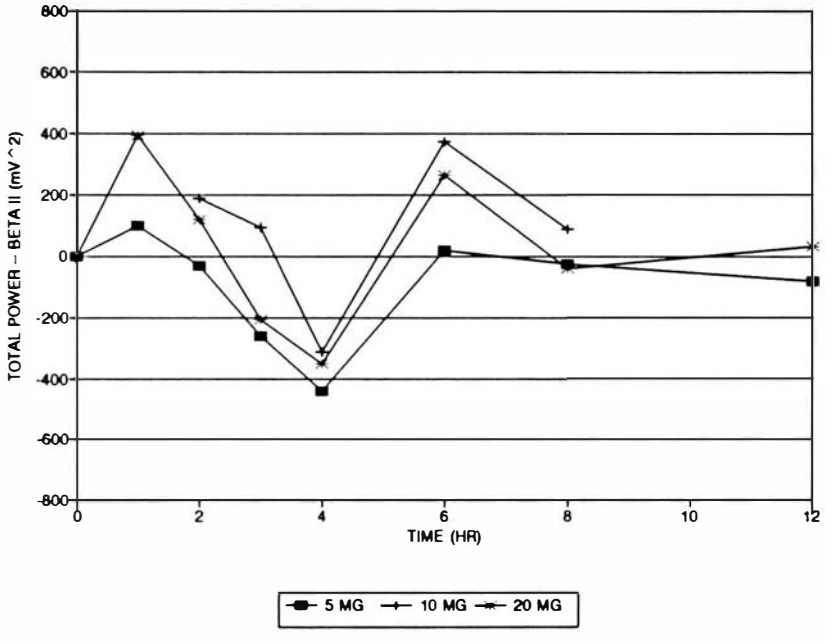


Figure L.42 Baseline and placebo corrected total EEG power in the beta II frequency band versus time profile for Subject 10

APPENDIX M

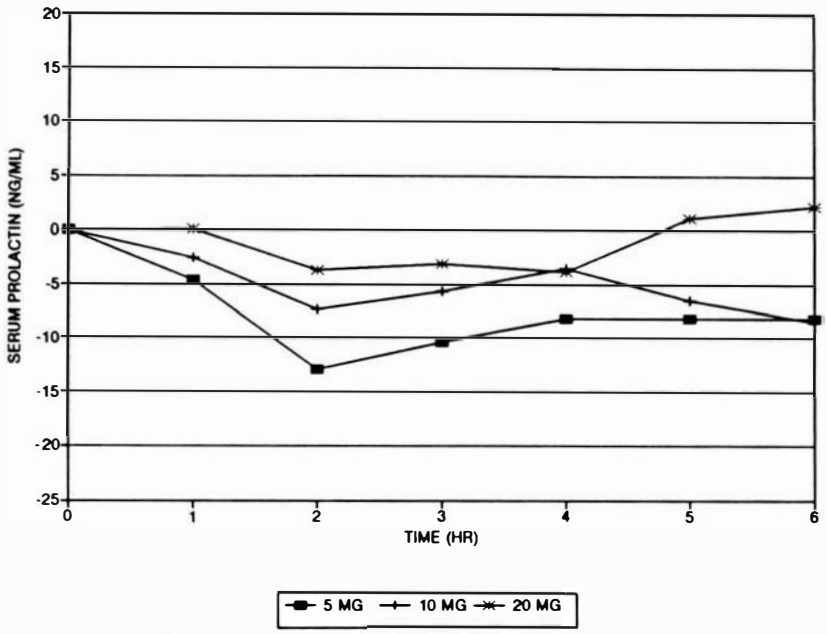


Figure M.1 Baseline and placebo corrected serum prolactin concentration versus time plot for Subject 1

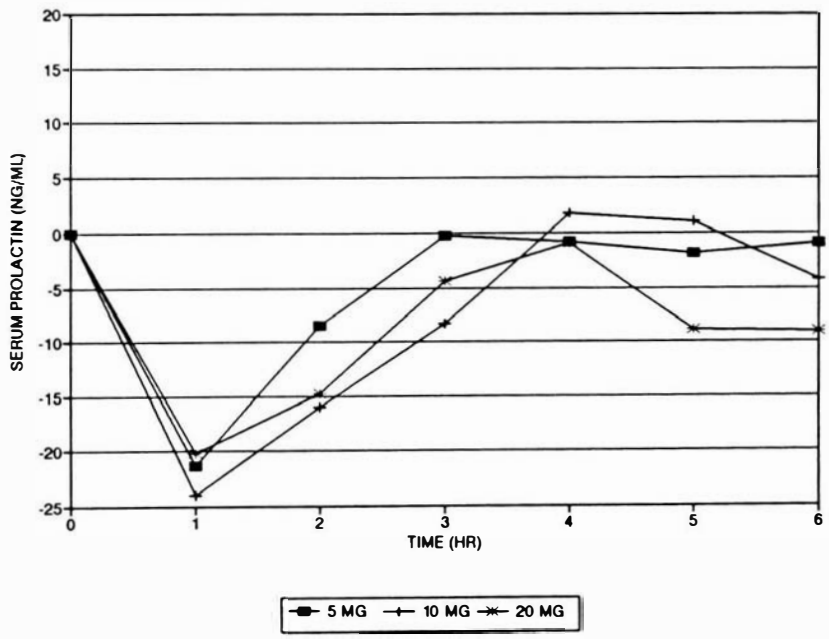


Figure M.2 Baseline and placebo corrected serum prolactin concentration versus time plot for Subject 2

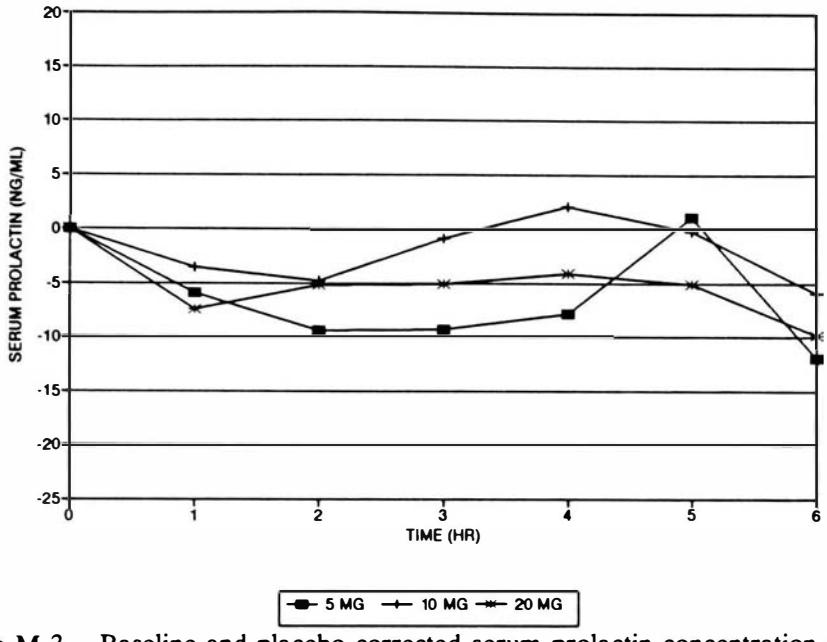


Figure M.3 Baseline and placebo corrected serum prolactin concentration versus time plot for Subject 4

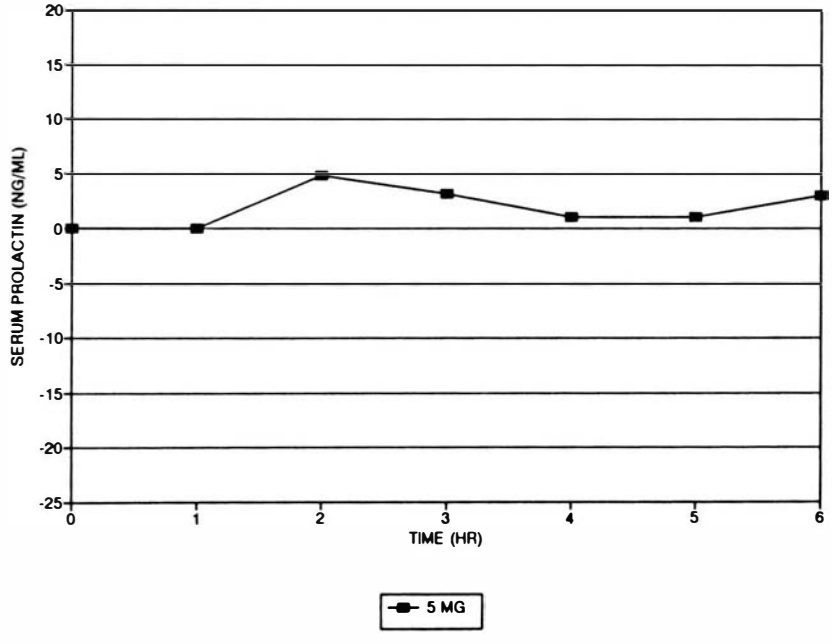


Figure M.4 Baseline and placebo corrected serum prolactin concentration versus time plot for Subject 5

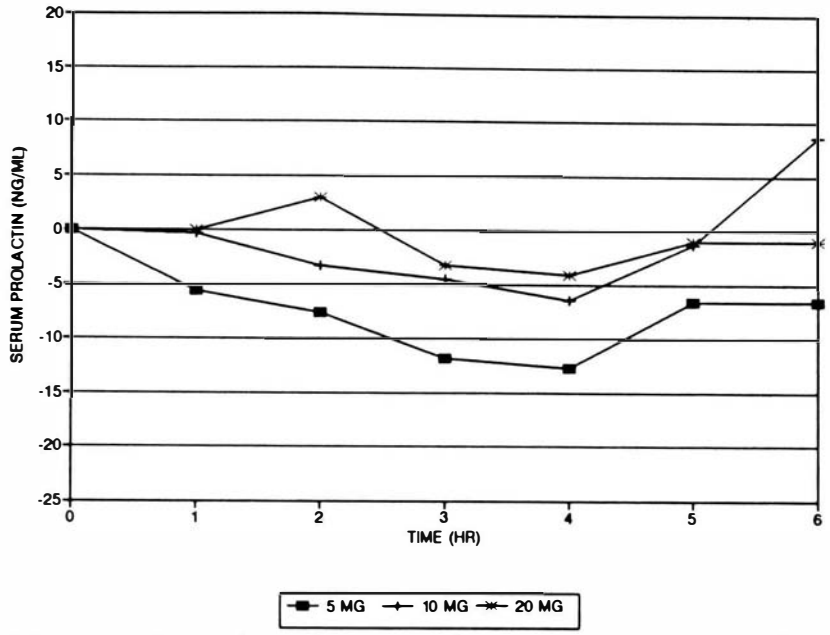


Figure M.5 Baseline and placebo corrected serum prolactin concentration versus time plot for Subject 6

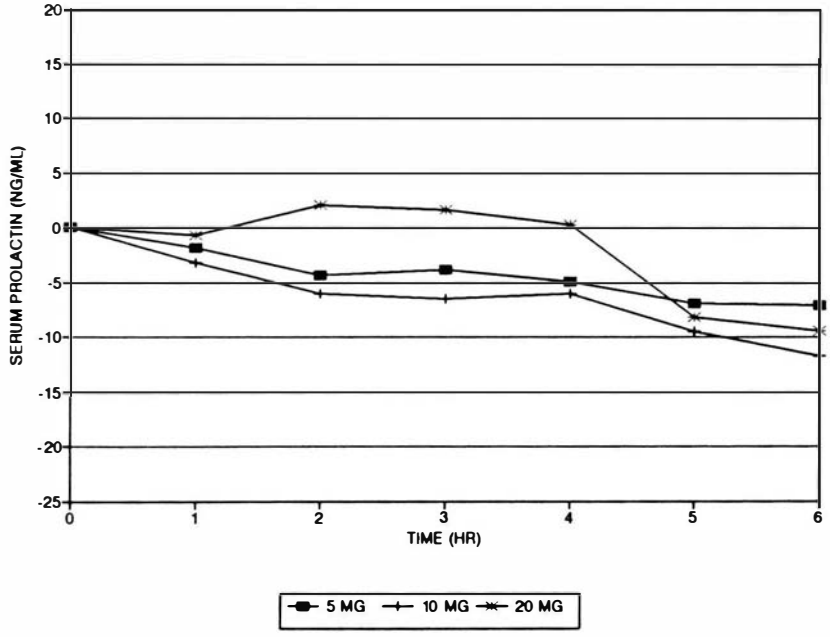


Figure M.6 Baseline and placebo corrected serum prolactin concentration versus time plot for Subject 7

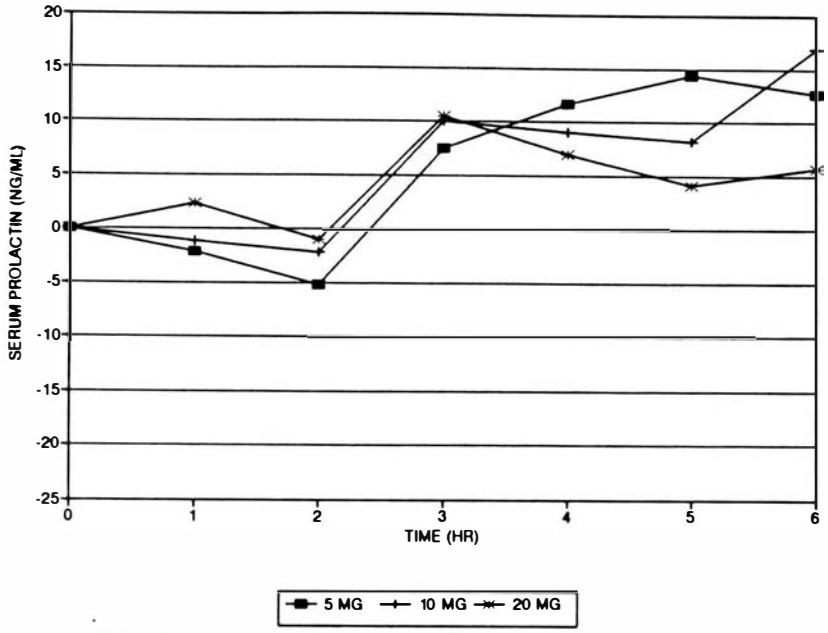


Figure M.7 Baseline and placebo corrected serum prolactin concentration versus time plot for Subject 8

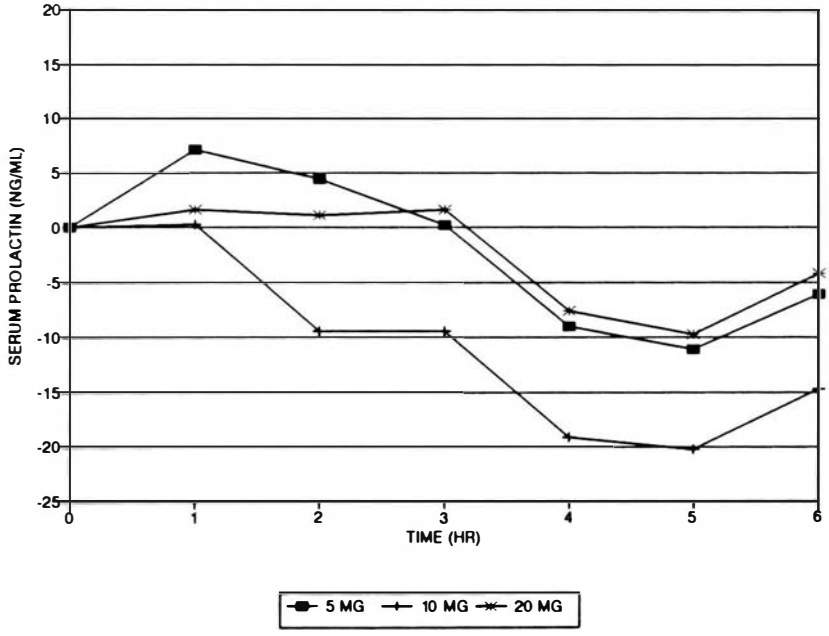


Figure M.8 Baseline and placebo corrected serum prolactin concentration versus time plot for Subject 9

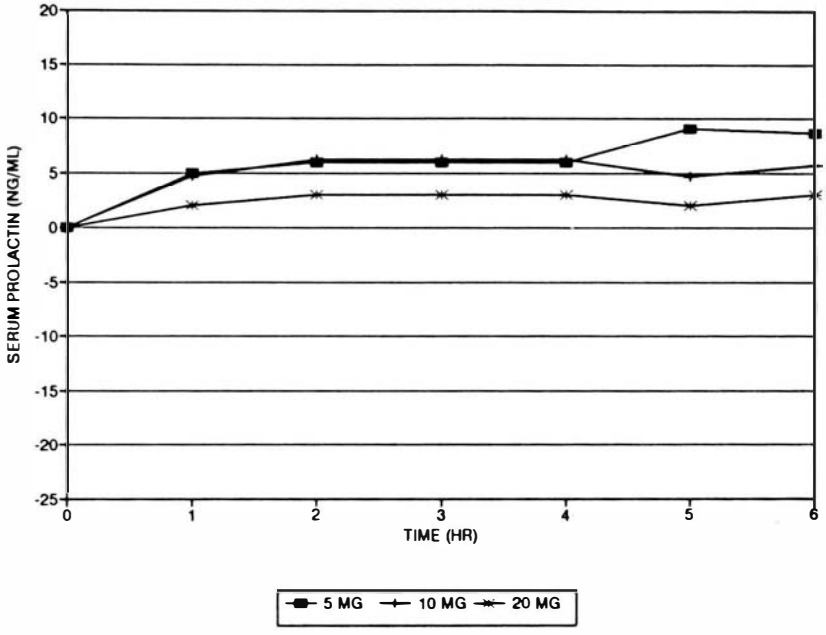


Figure M.9 Baseline and placebo corrected serum prolactin concentration versus time plot for Subject 10

APPENDIX N

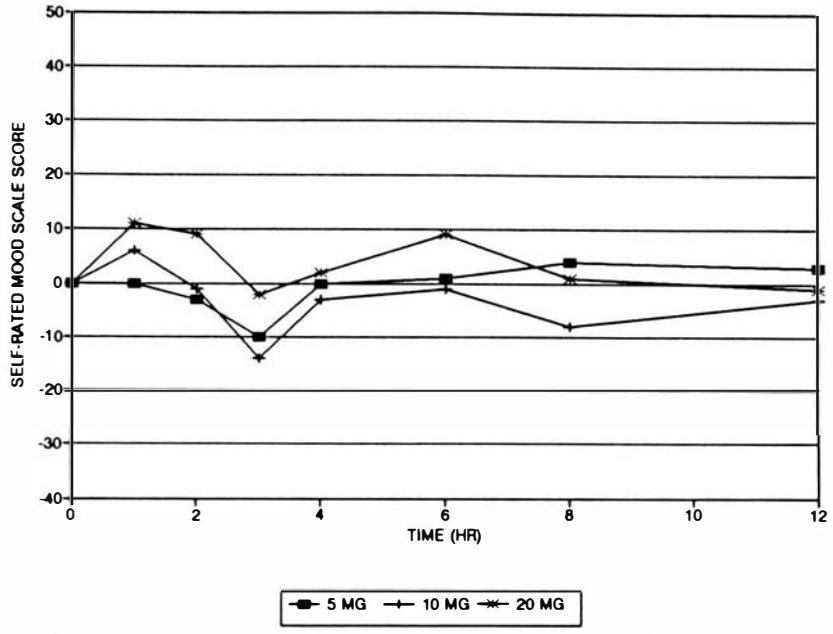


Figure N.1 Baseline and placebo corrected self-rated mood scale score versus time plot for Subject 1

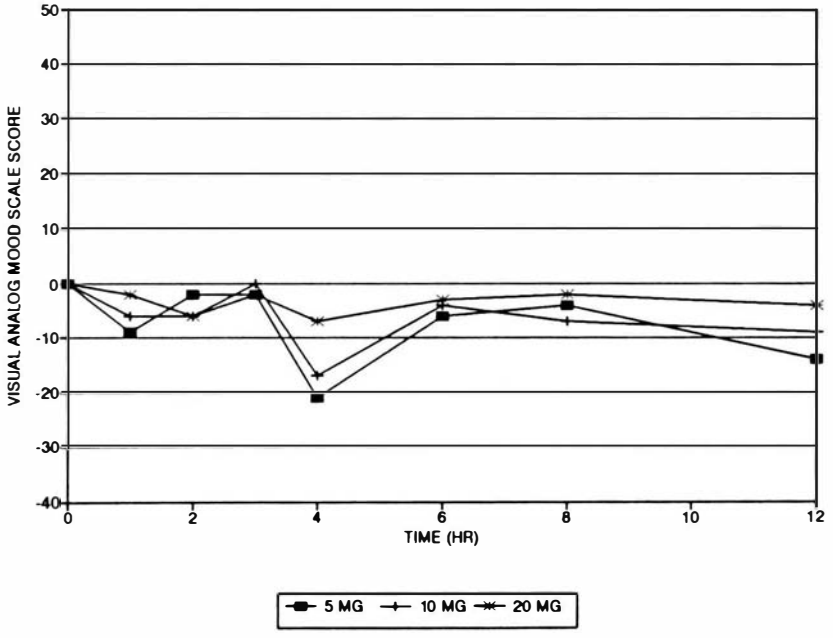


Figure N.2 Baseline and placebo corrected visual analog mood scale score versus time plot for Subject 1

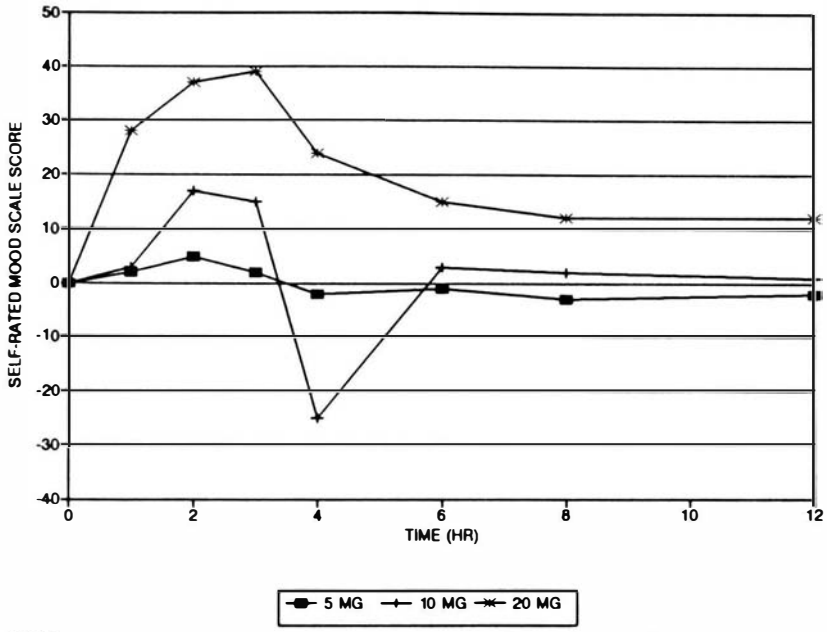


Figure N.3 Baseline and placebo corrected self-rated mood scale score versus time plot for Subject 2

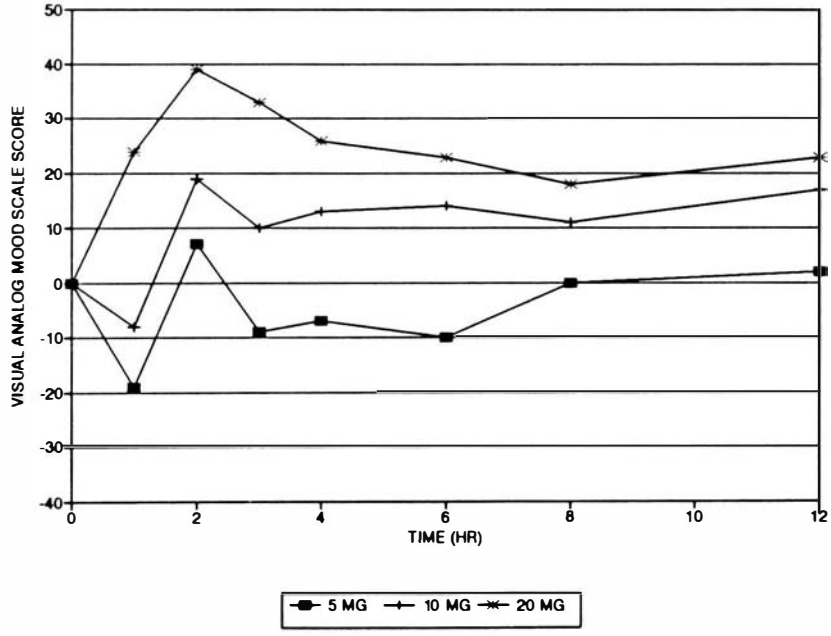


Figure N.4 Baseline and placebo corrected visual analog mood scale score versus time plot for Subject 2

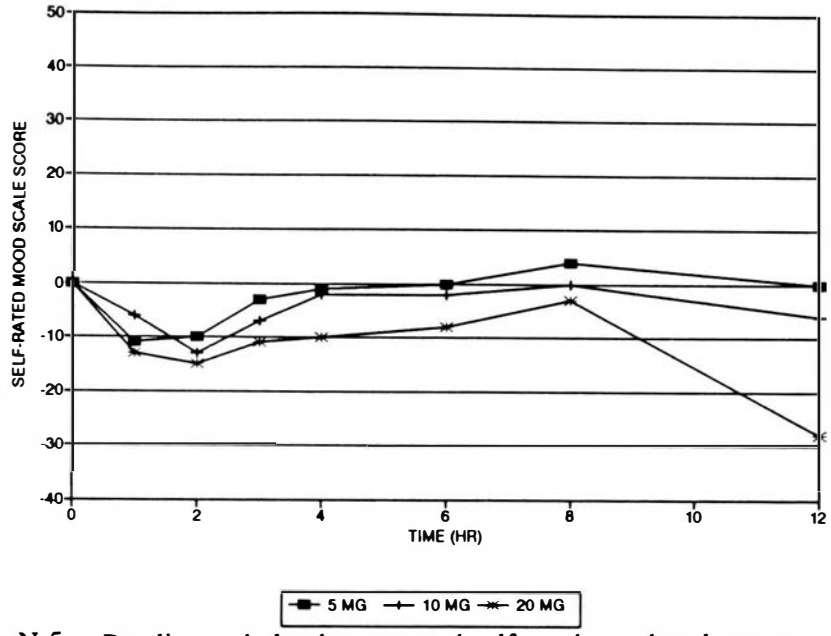


Figure N.5 Baseline and placebo corrected self-rated mood scale score versus time plot for Subject 4

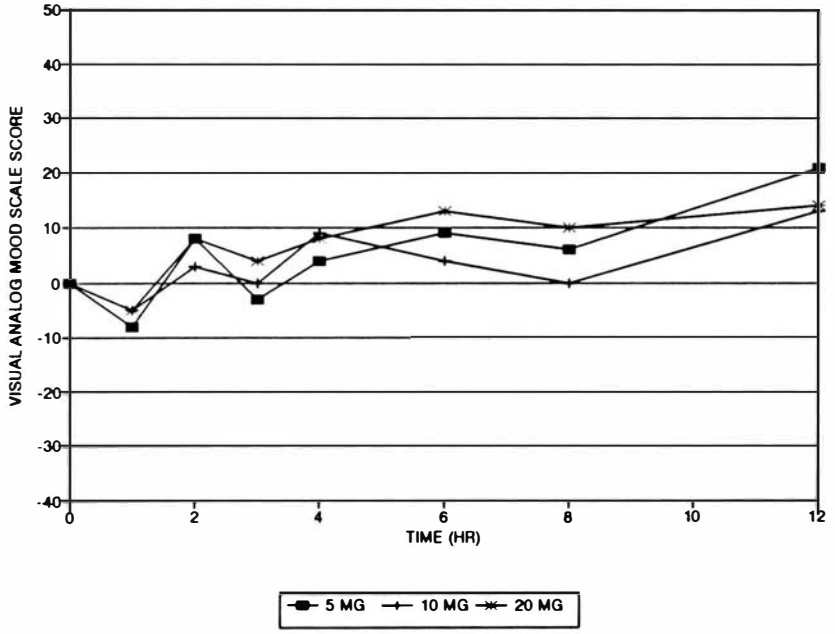


Figure N.6 Baseline and placebo corrected visual analog mood scale score versus time plot for Subject 4

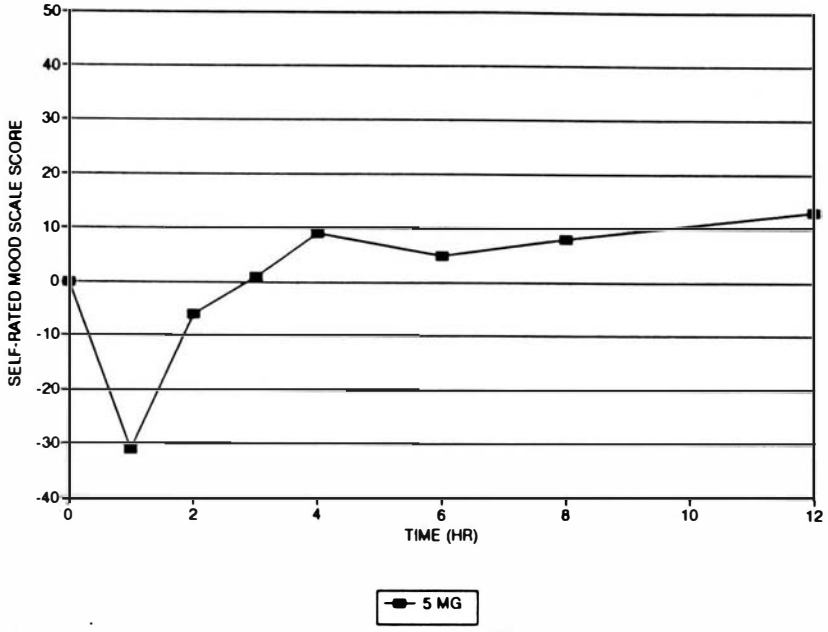


Figure N.7 Baseline and placebo corrected self-rated mood scale score versus time plot for Subject 5

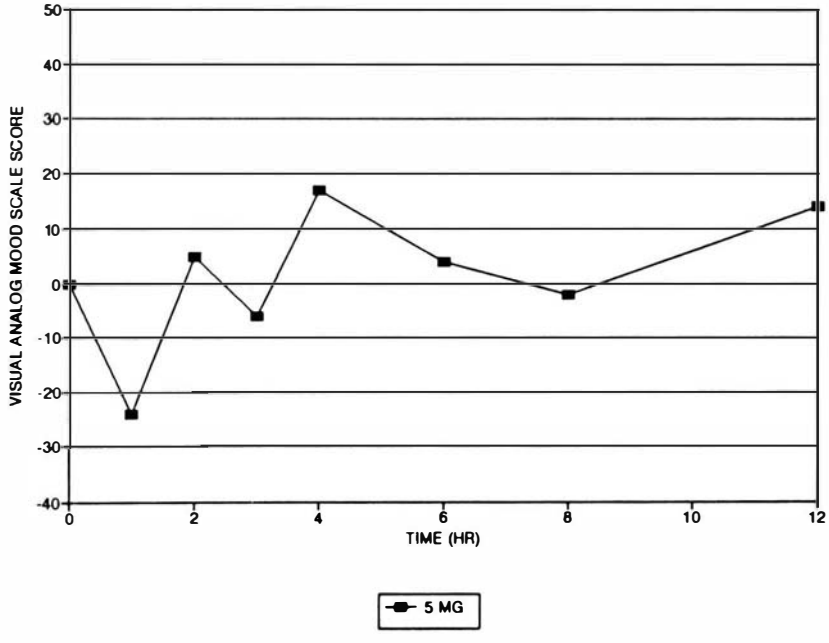


Figure N.8 Baseline and placebo corrected visual analog mood scale score versus time plot for Subject 5

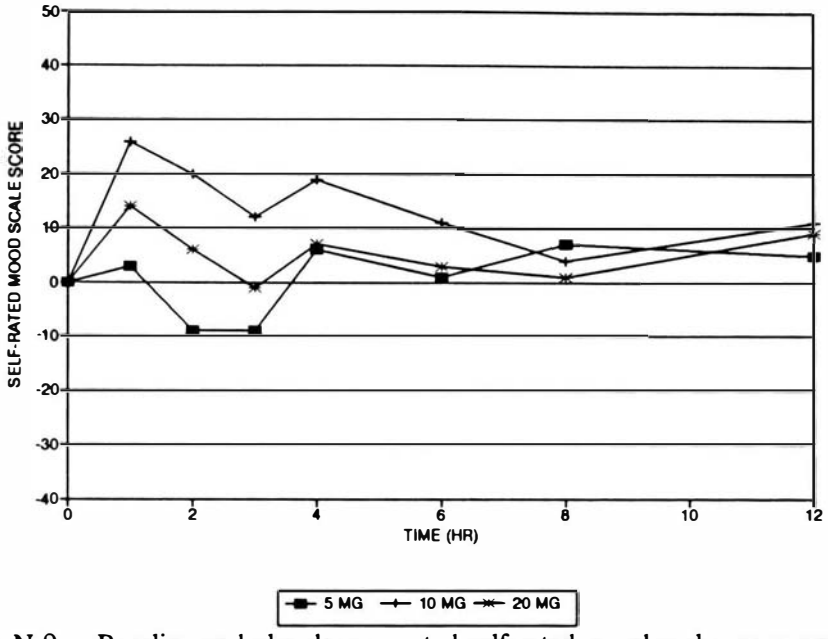


Figure N.9 Baseline and placebo corrected self-rated mood scale score versus time plot for Subject 6

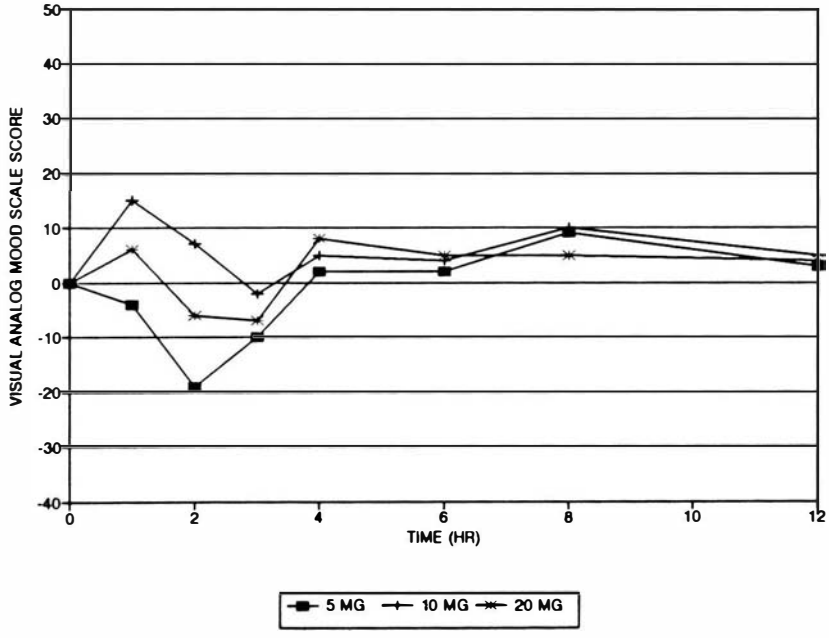


Figure N.10 Baseline and placebo corrected visual analog mood scale score versus time plot for Subject 6

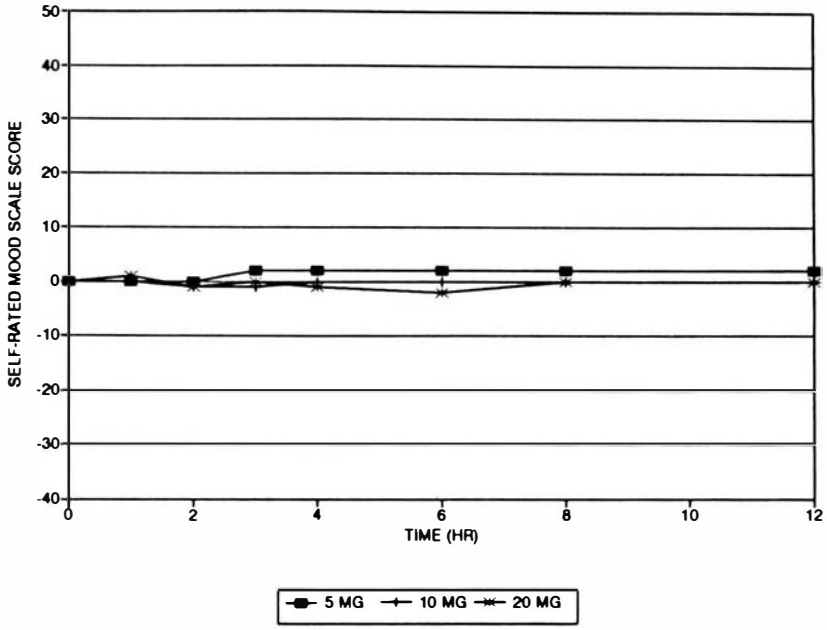


Figure N.11 Baseline and placebo corrected self-rated mood scale score versus time plot for Subject 7

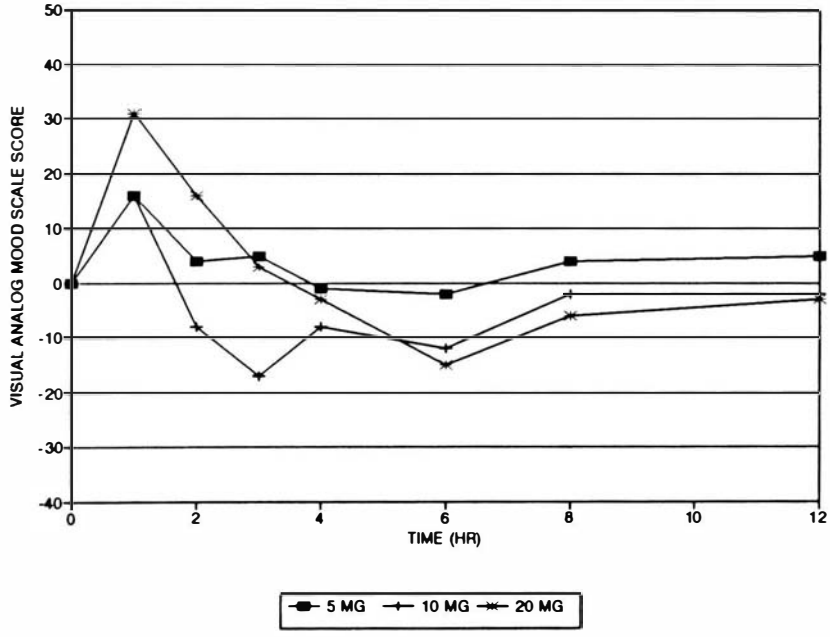


Figure N.12 Baseline and placebo corrected visual analog mood scale score versus time plot for Subject 7

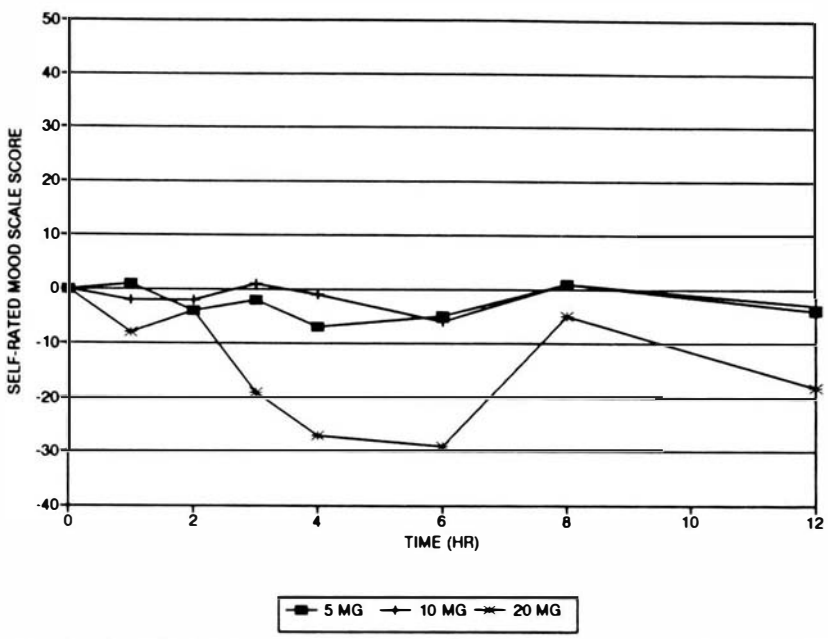


Figure N.13 Baseline and placebo corrected self-rated mood scale score versus time plot for Subject 8

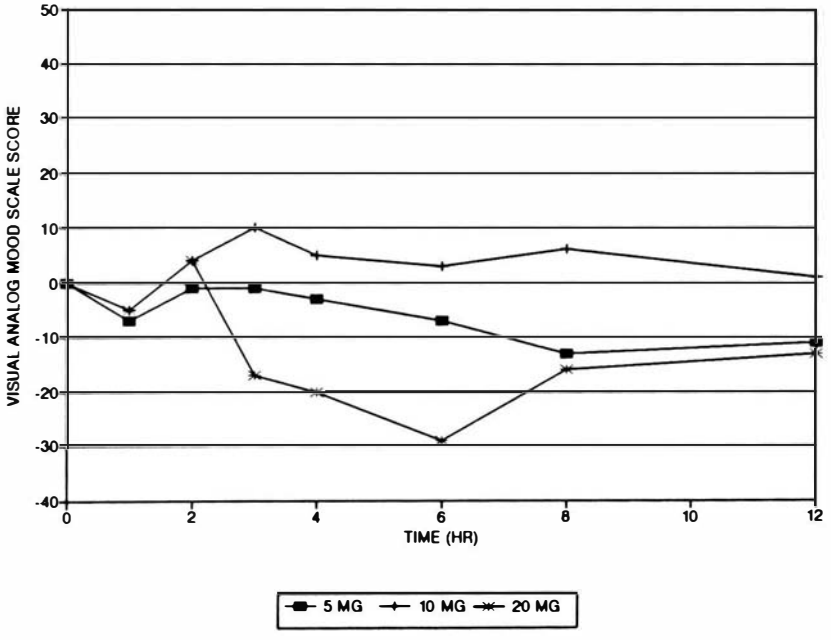


Figure N.14 Baseline and placebo corrected visual analog mood scale score versus time plot for Subject 8

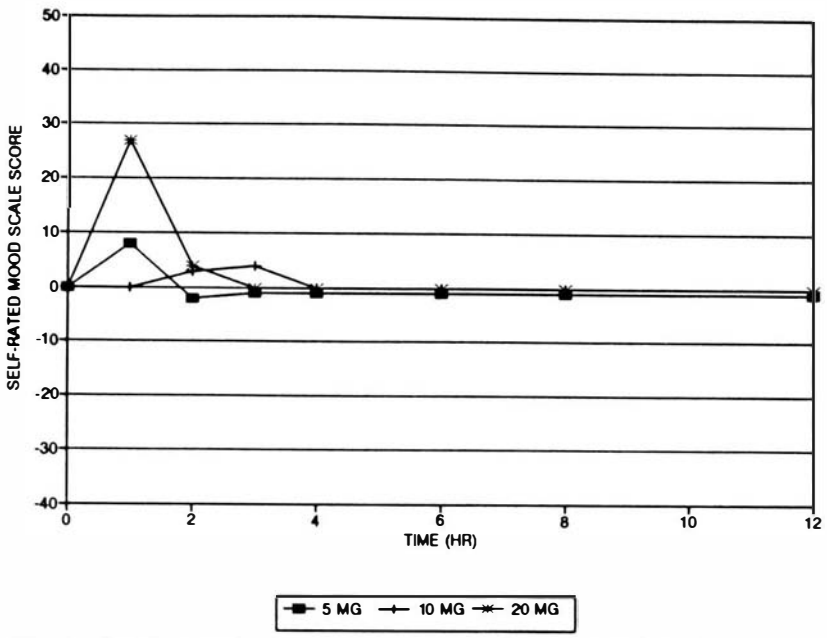


Figure N.15 Baseline and placebo corrected self-rated mood scale score versus time plot for Subject 9

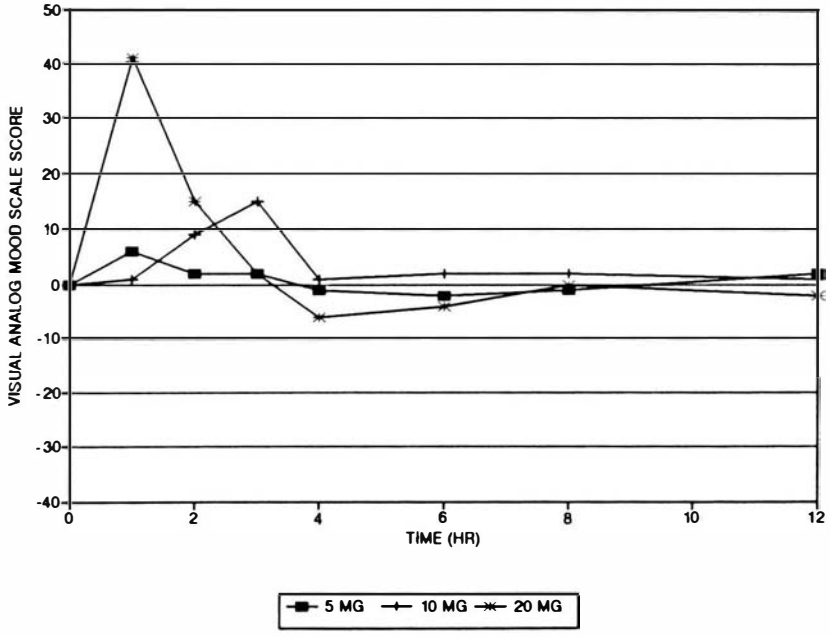


Figure N.16 Baseline and placebo corrected visual analog mood scale score versus time plot for Subject 9

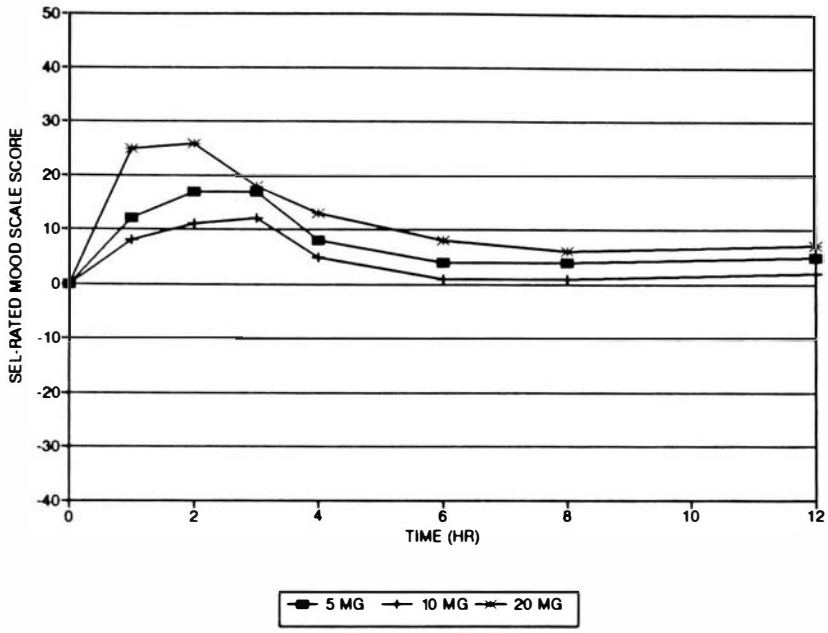


Figure N.17 Baseline and placebo corrected self-rated mood scale score versus time plot for Subject 10

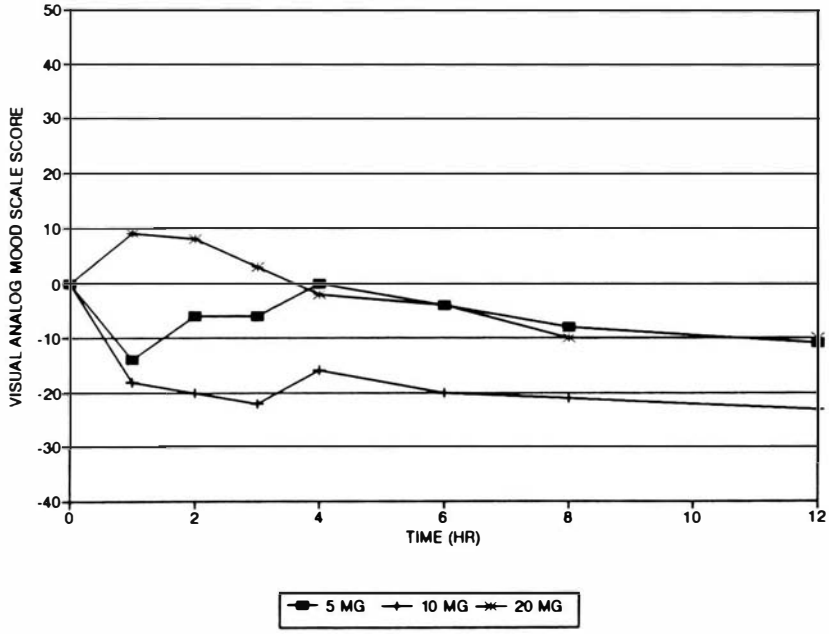


Figure N.18 Baseline and placebo corrected visual analog mood scale score versus time plot for Subject 10

APPENDIX O

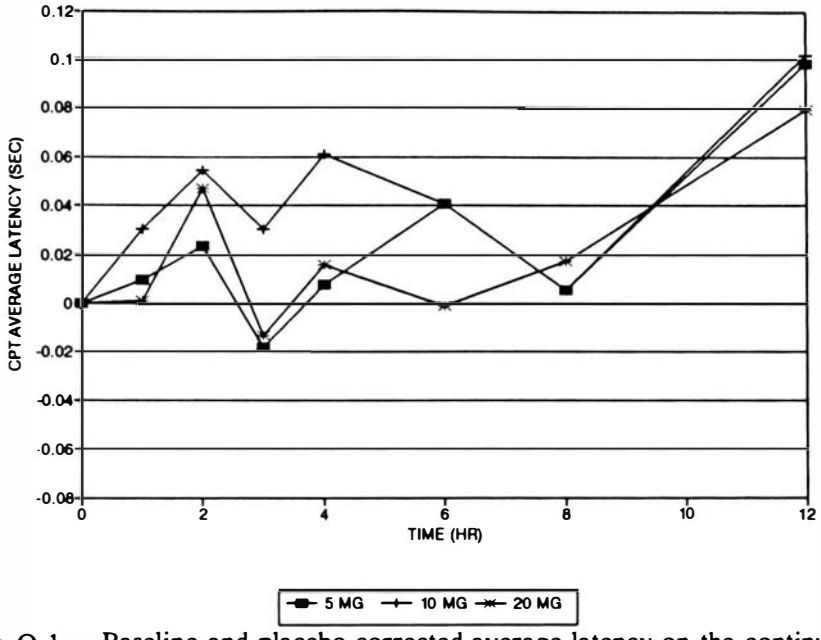


Figure O.1 Baseline and placebo corrected average latency on the continuous performance task for Subject 1

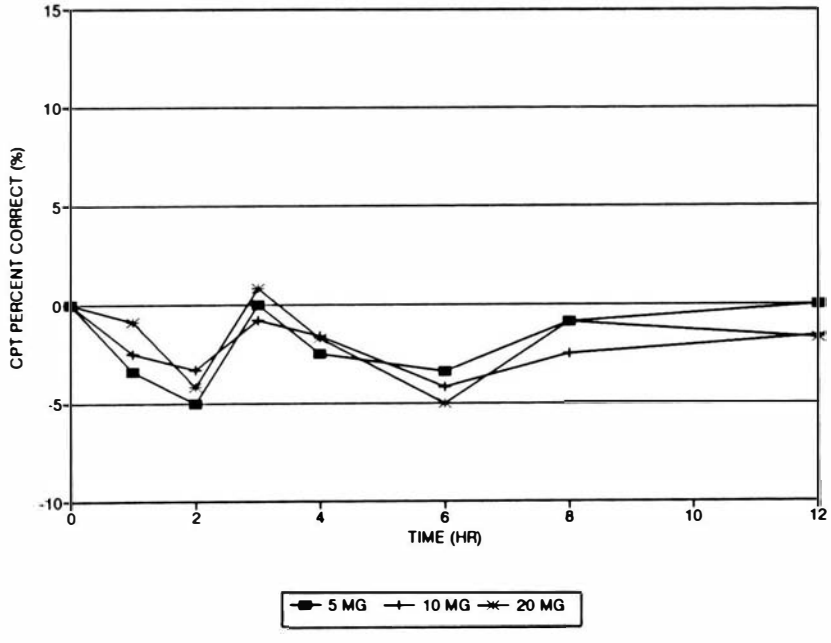


Figure O.2 Baseline and placebo corrected percent correct on the continuous performance task for Subject 1

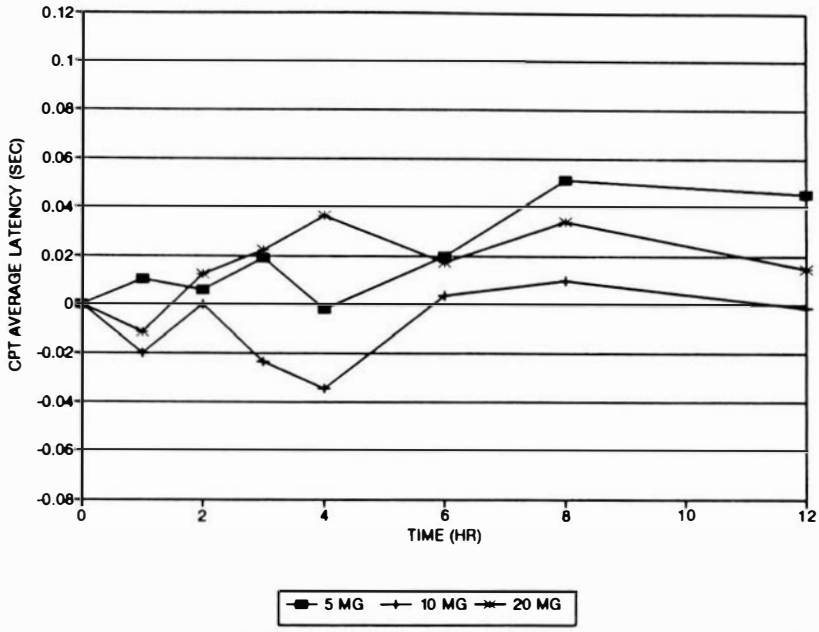


Figure O.3 Baseline and placebo corrected average latency on the continuous performance task for Subject 2

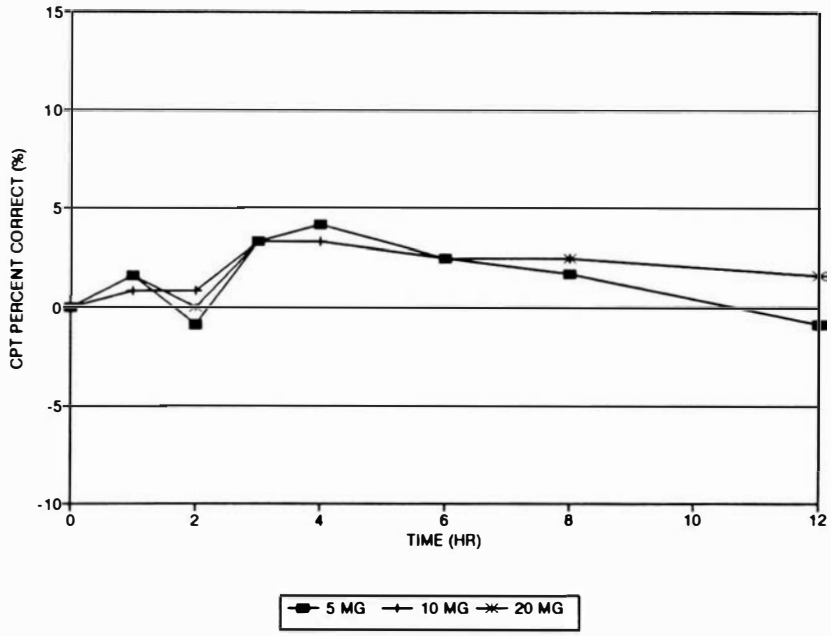


Figure O.4 Baseline and placebo corrected percent correct on the continuous performance task for Subject 2

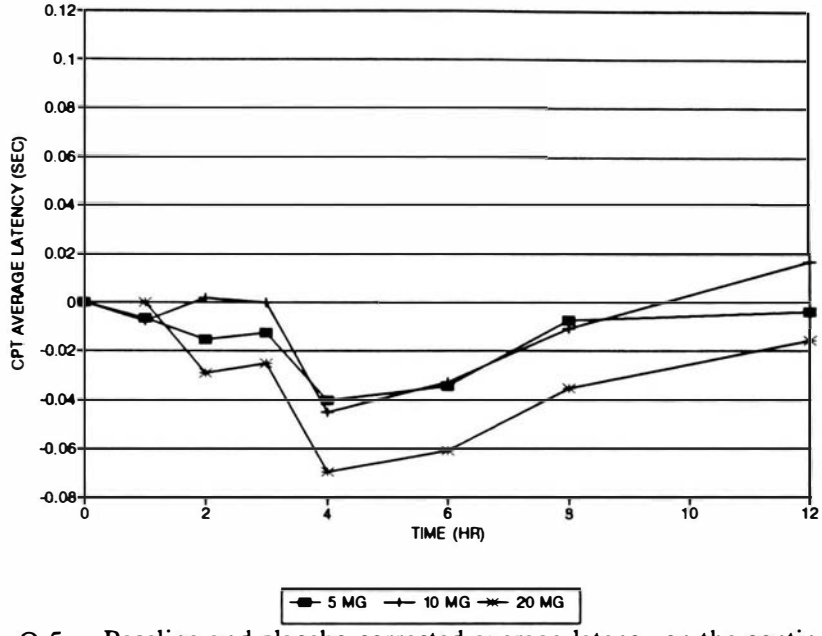


Figure O.5 Baseline and placebo corrected average latency on the continuous performance task for Subject 4

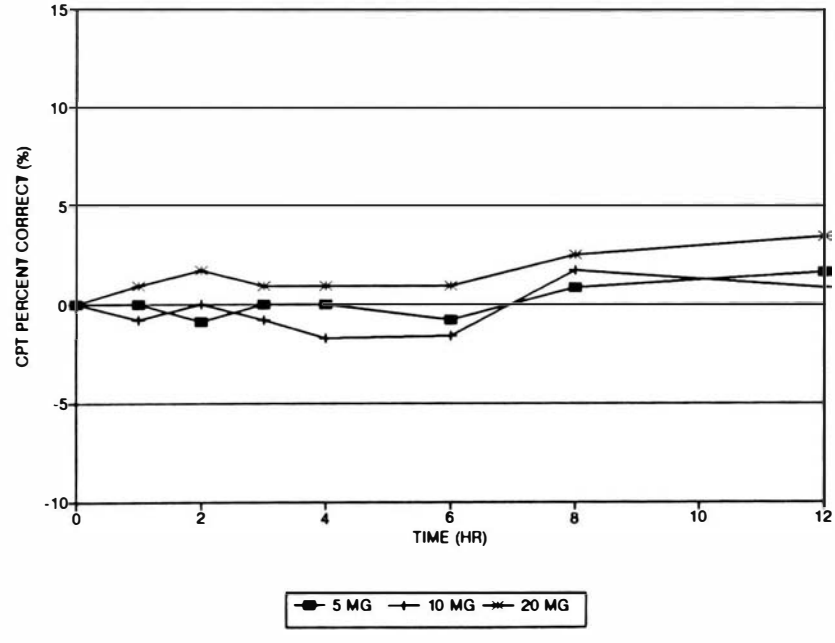


Figure O.6 Baseline and placebo corrected percent correct on the continuous performance task for Subject 4

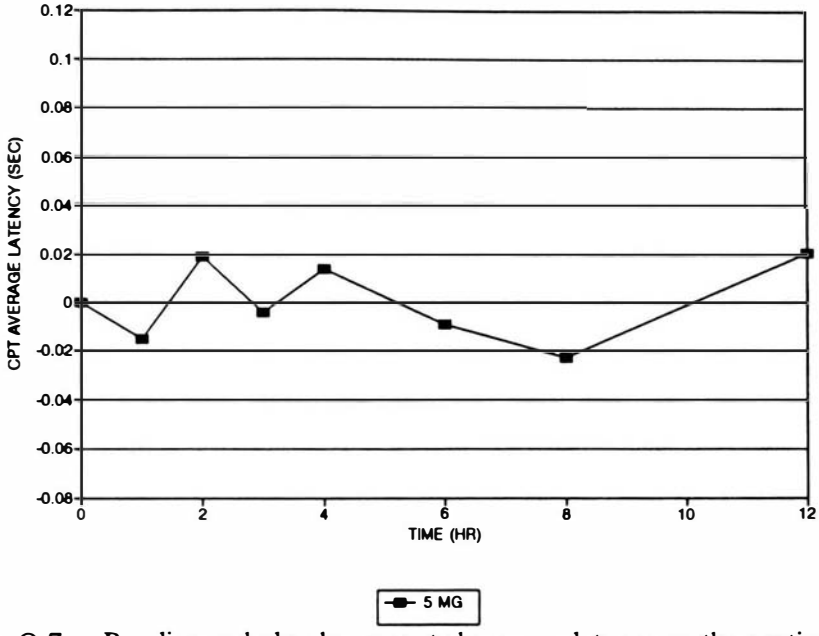


Figure O.7 Baseline and placebo corrected average latency on the continuous performance task for Subject 5

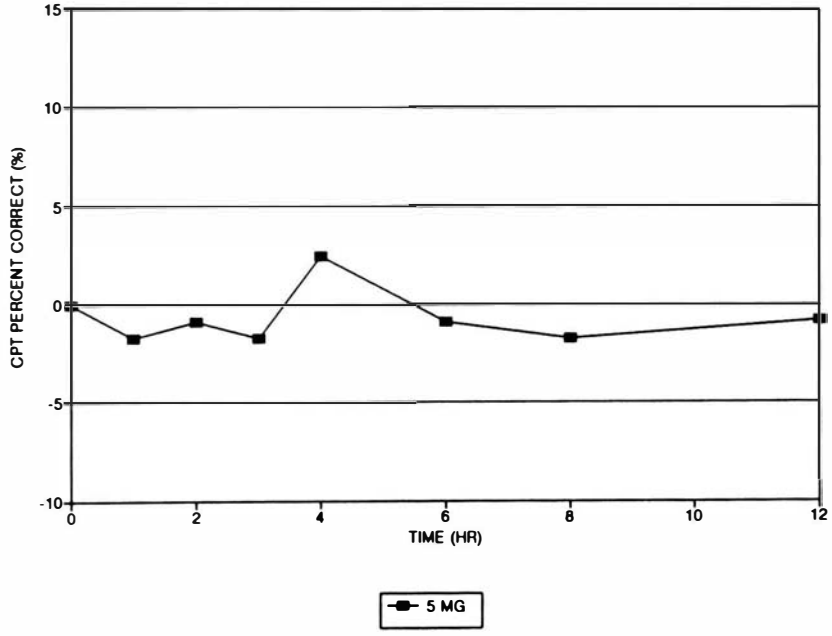


Figure O.8 Baseline and placebo corrected percent correct on the continuous performance task for Subject 5

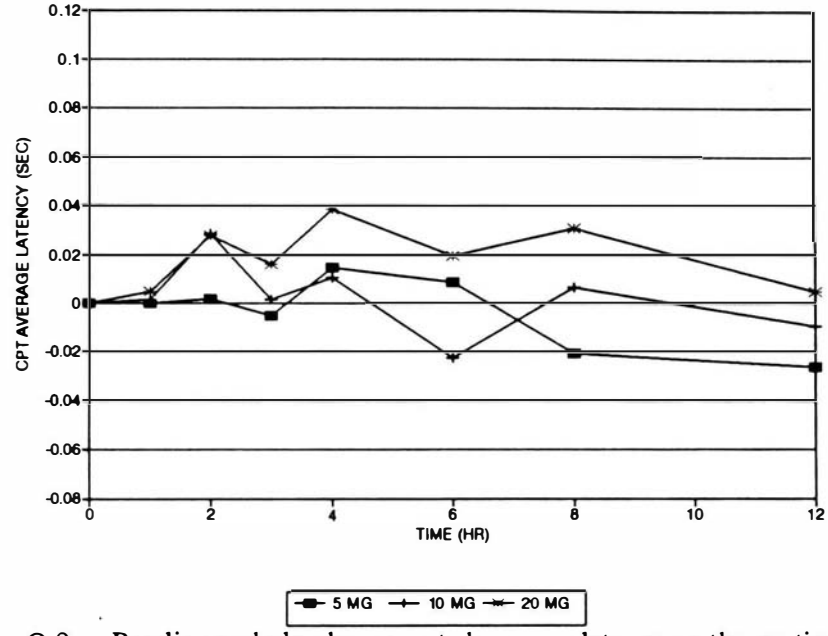


Figure O.9 Baseline and placebo corrected average latency on the continuous performance task for Subject 6

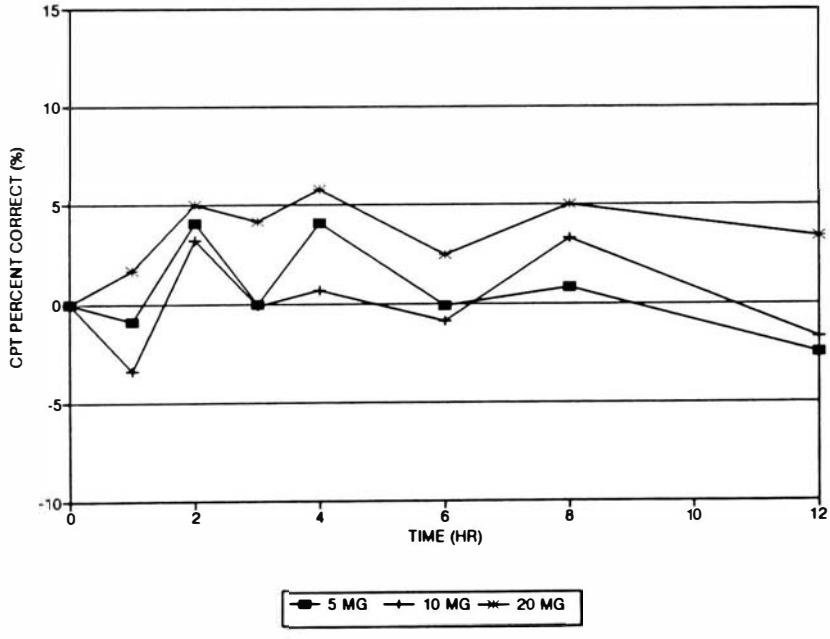


Figure O.10 Baseline and placebo corrected percent correct on the continuous performance task for Subject 6

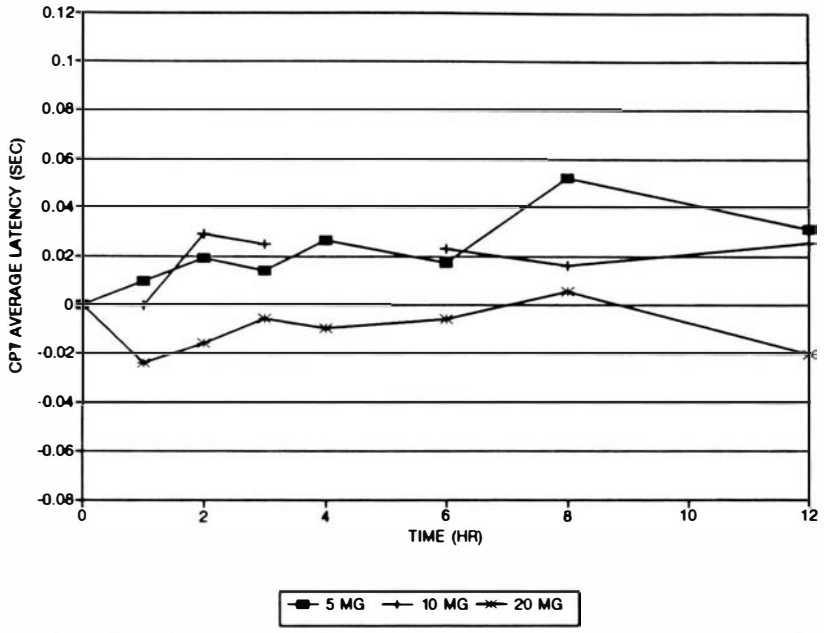


Figure O.11 Baseline and placebo corrected average latency on the continuous performance task for Subject 7

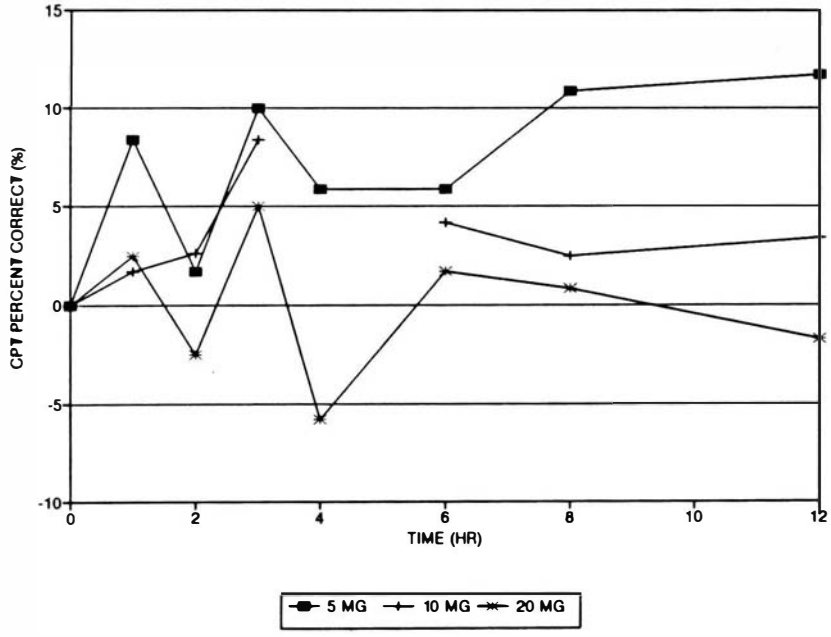


Figure O.12 Baseline and placebo corrected percent correct on the continuous performance task for Subject 7

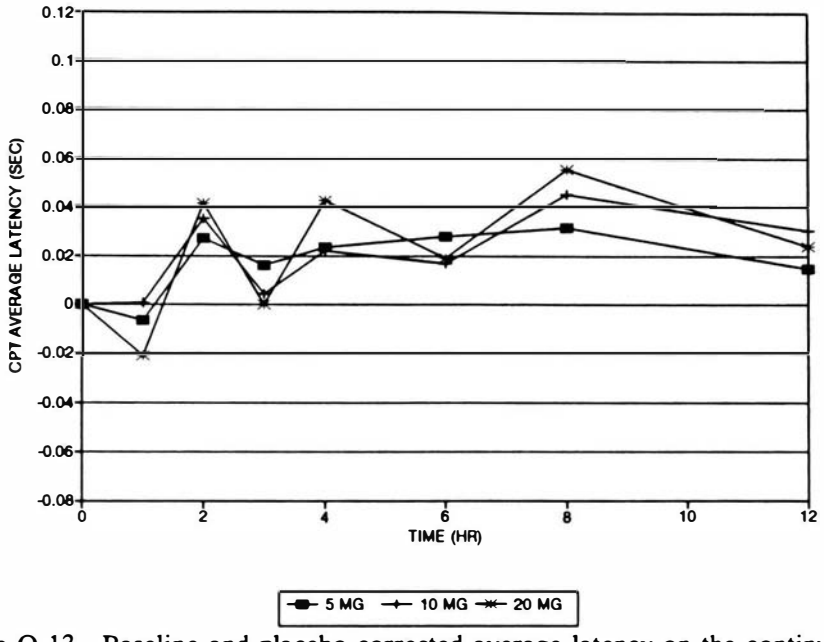


Figure O.13 Baseline and placebo corrected average latency on the continuous performance task for Subject 8

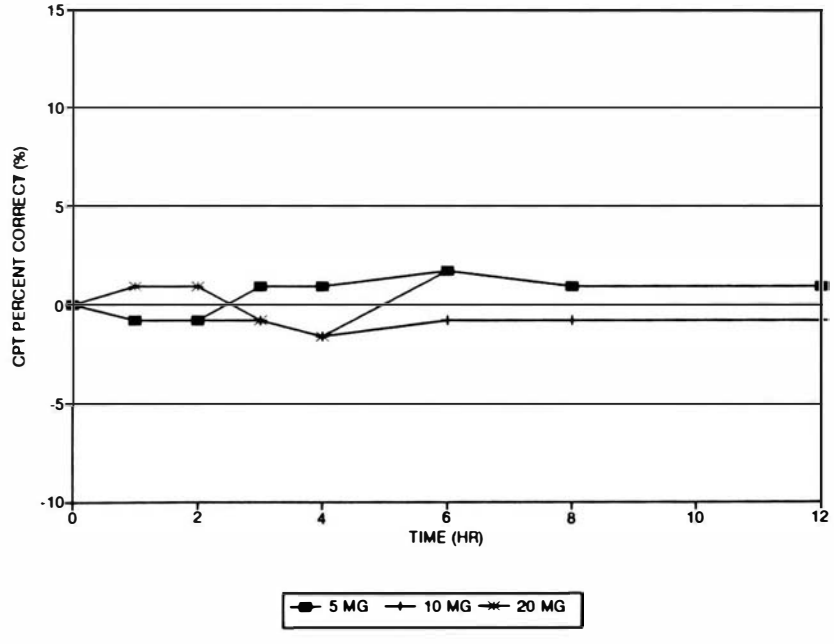


Figure O.14 Baseline and placebo corrected percent correct on the continuous performance task for Subject 8

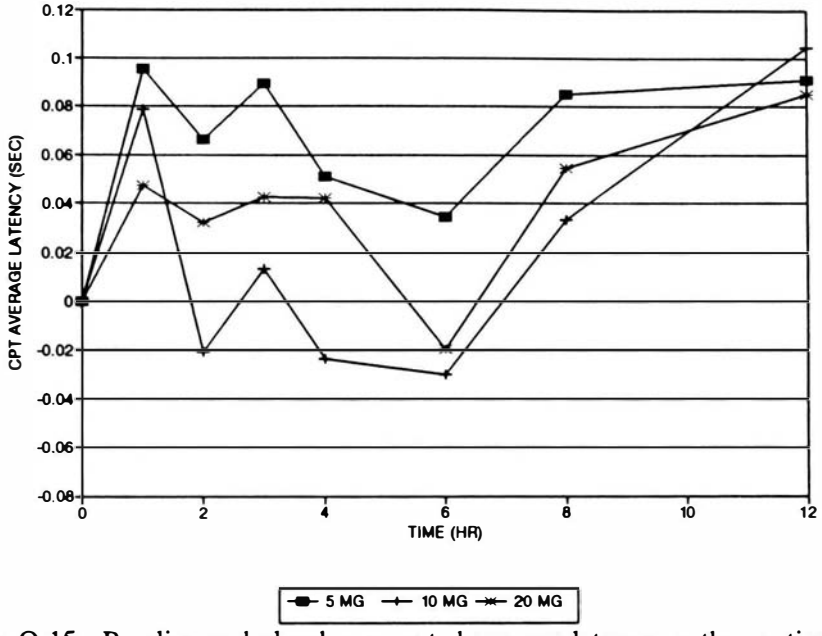


Figure O.15 Baseline and placebo corrected average latency on the continuous performance task for Subject 9

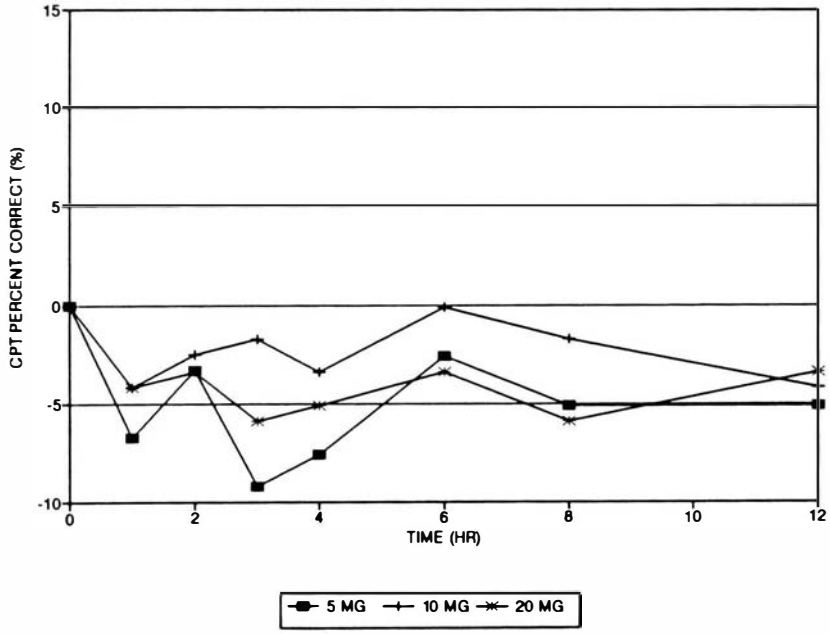


Figure O.16 Baseline and placebo corrected percent correct on the continuous performance task for Subject 9

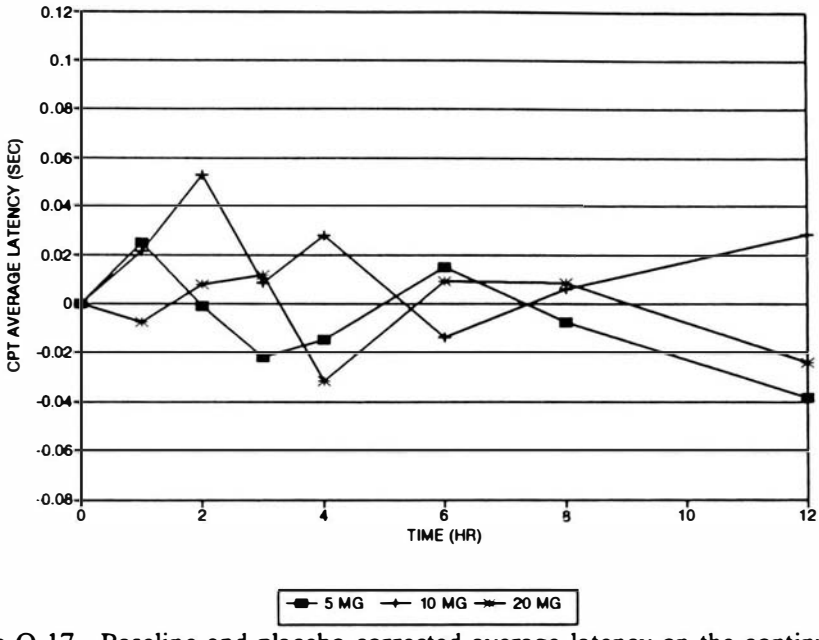


Figure O.17 Baseline and placebo corrected average latency on the continuous performance task for Subject 10

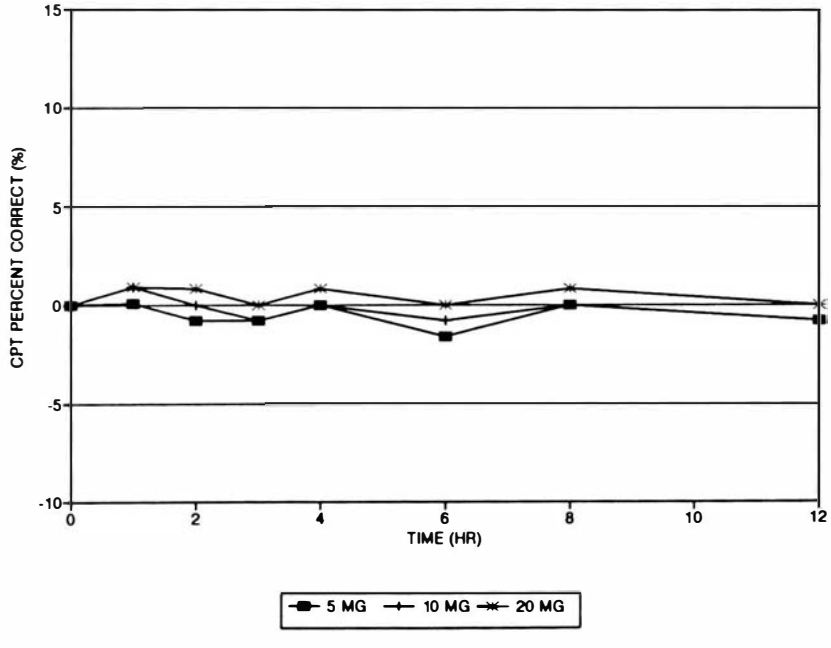


Figure O.18 Baseline and placebo corrected percent correct on the continuous performance task for Subject 10

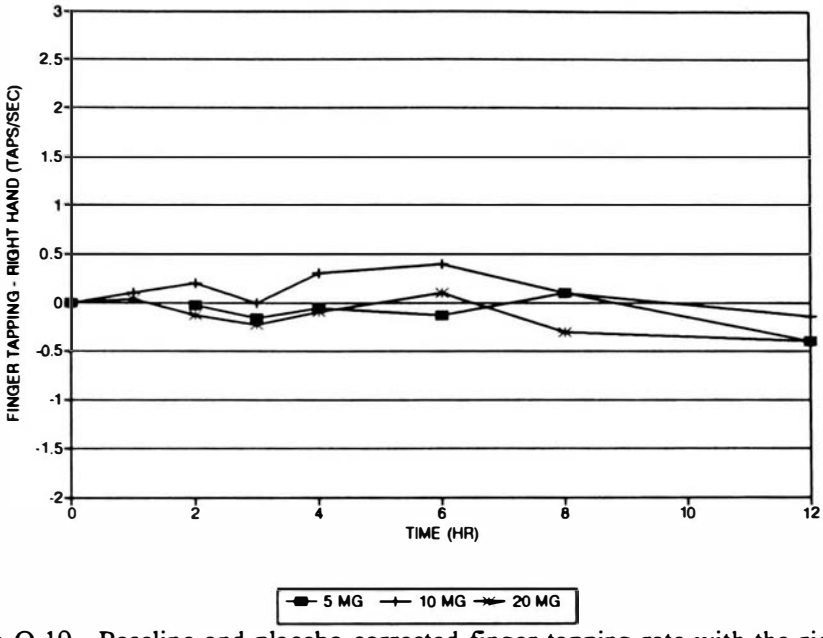


Figure O.19 Baseline and placebo corrected finger tapping rate with the right hand for Subject 1

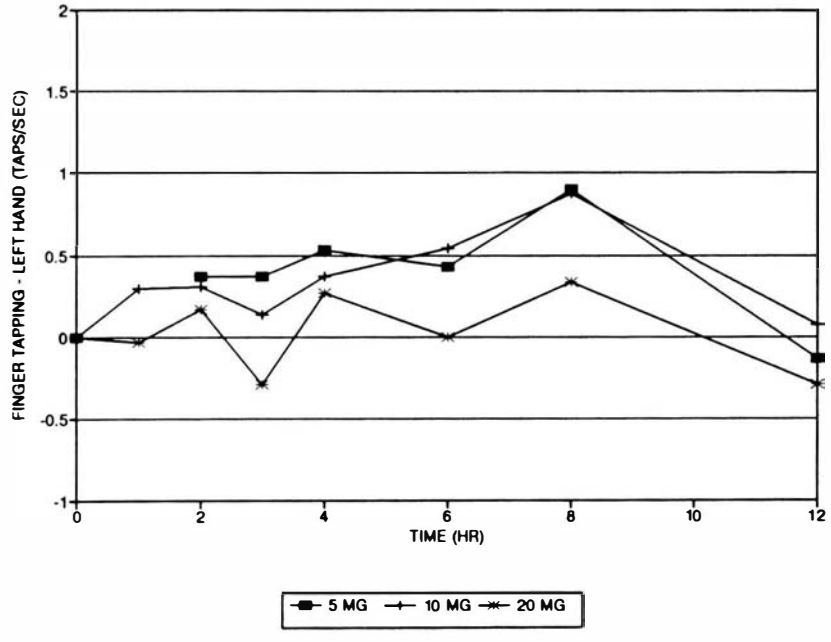


Figure O.20 Baseline and placebo corrected finger tapping rate with the left hand for Subject 1

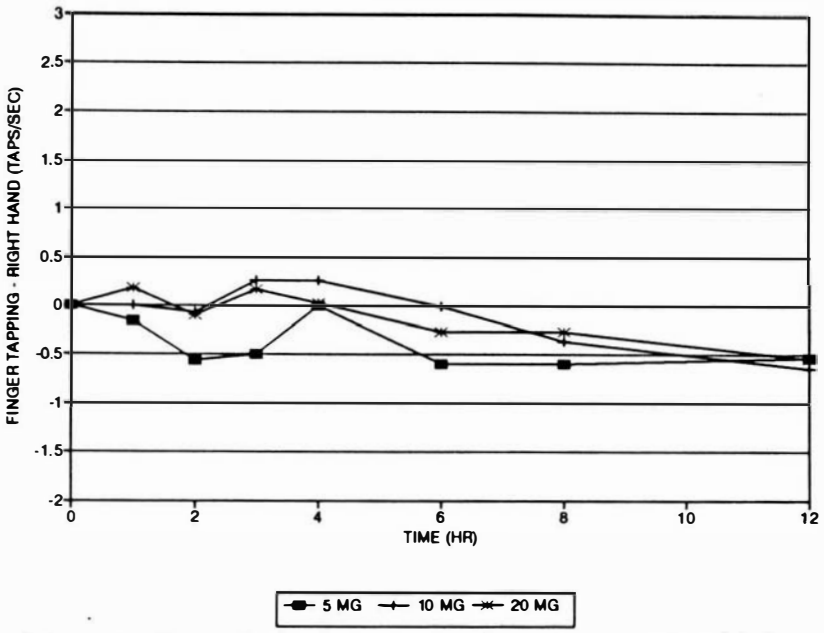


Figure O.21 Baseline and placebo corrected finger tapping rate with the right hand for Subject 2

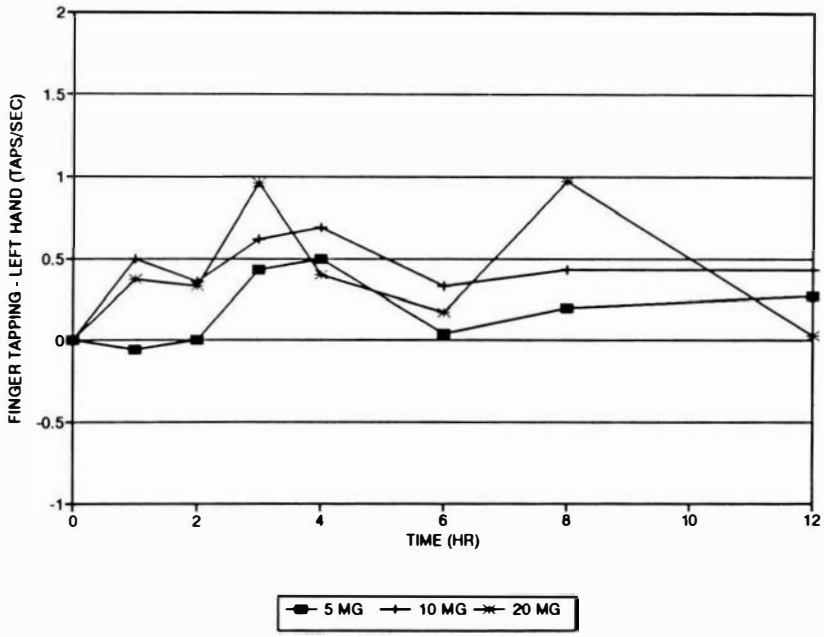


Figure O.22 Baseline and placebo corrected finger tapping rate with the left hand for Subject 2

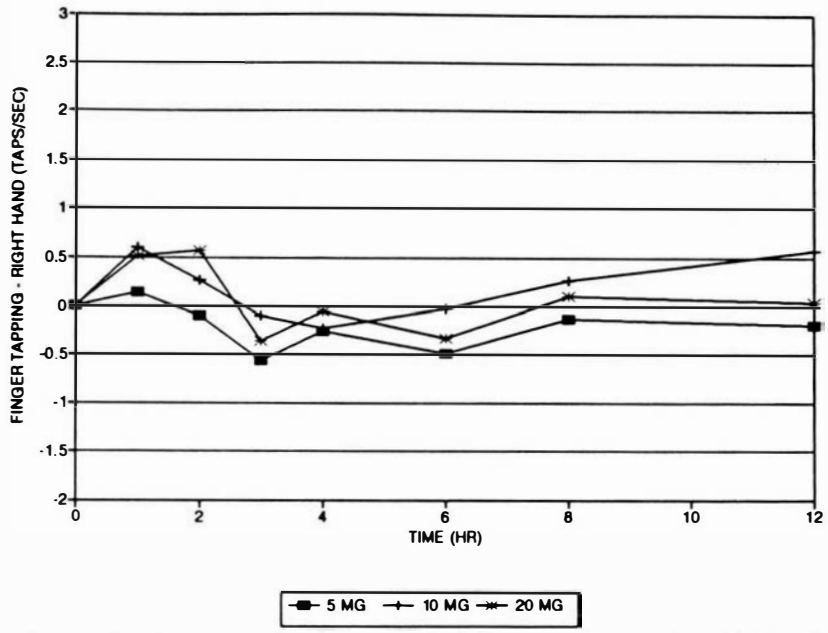


Figure O.23 Baseline and placebo corrected finger tapping rate with the right hand for Subject 4

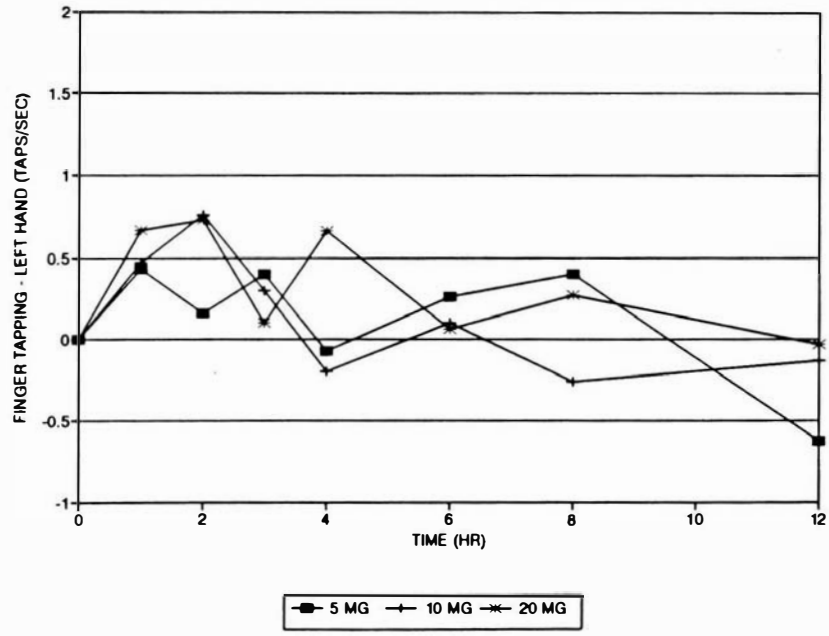


Figure O.24 Baseline and placebo corrected finger tapping rate with the left hand for Subject 4

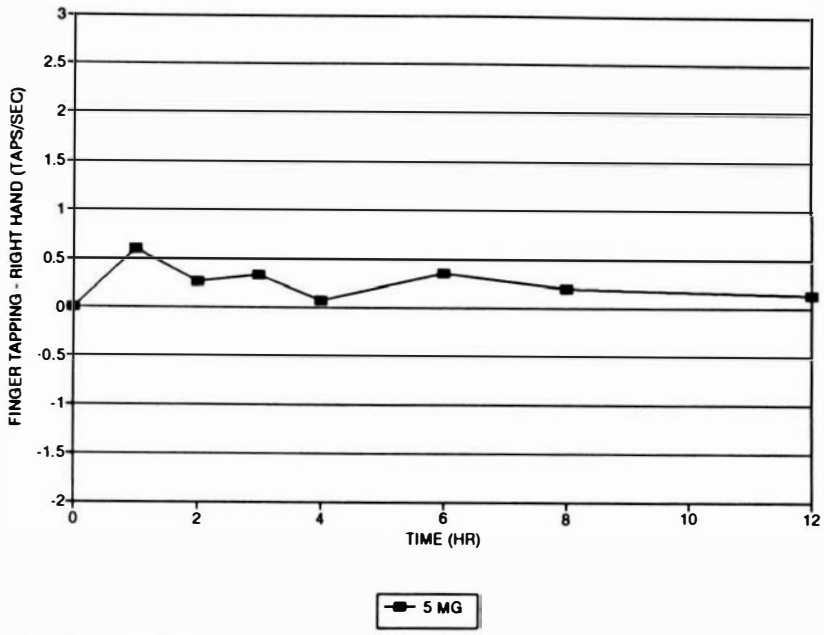


Figure O.25 Baseline and placebo corrected finger tapping rate with the right hand for Subject 5

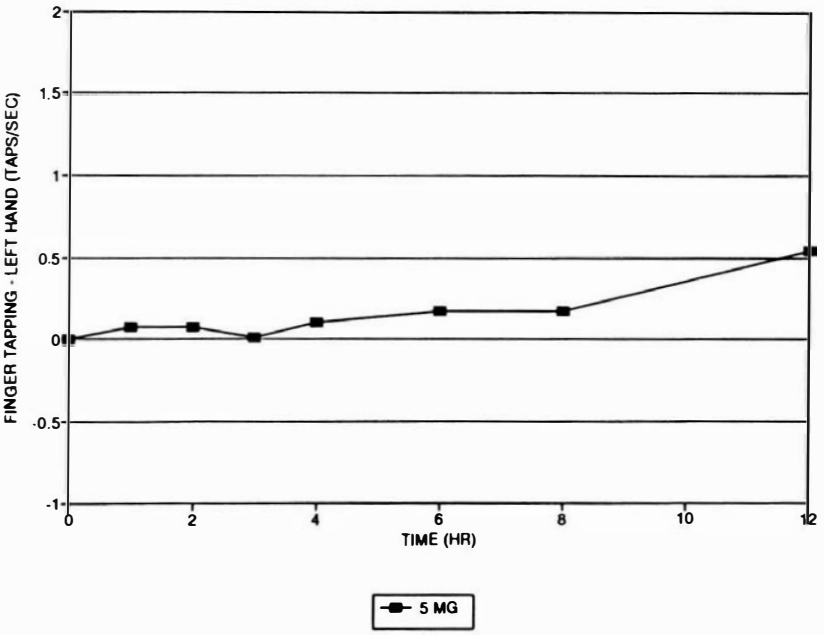


Figure O.26 Baseline and placebo corrected finger tapping rate with the left hand for Subject 5

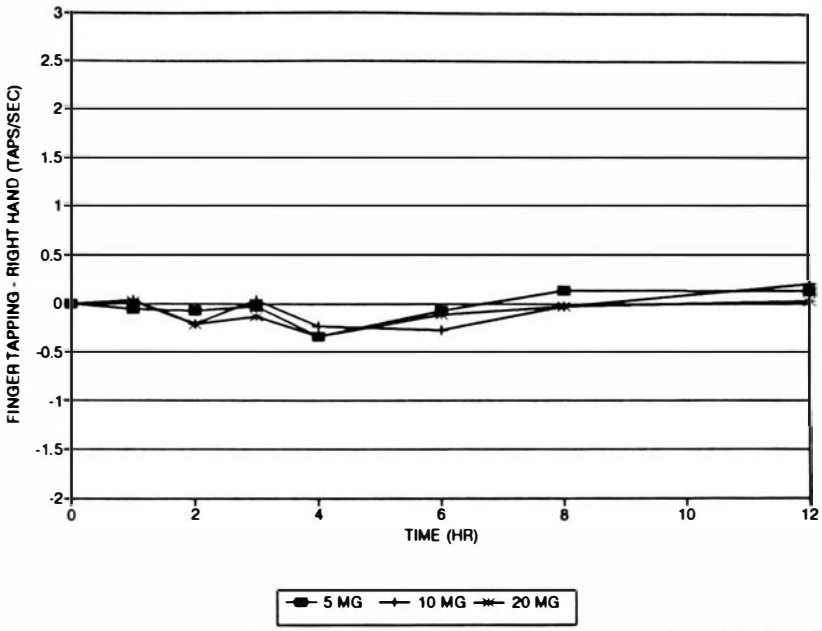


Figure O.27 Baseline and placebo corrected finger tapping rate with the right hand for Subject 6

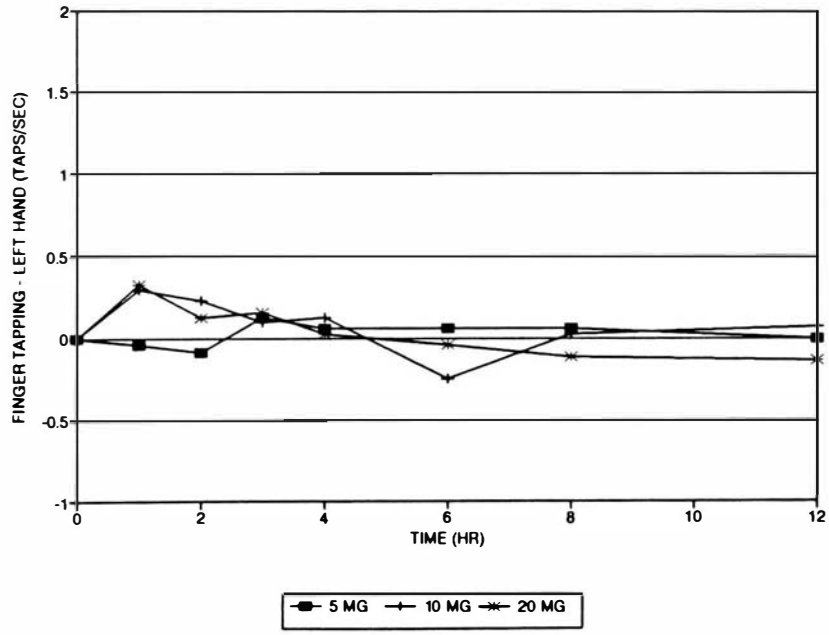


Figure O.28 Baseline and placebo corrected finger tapping rate with the left hand for Subject 6

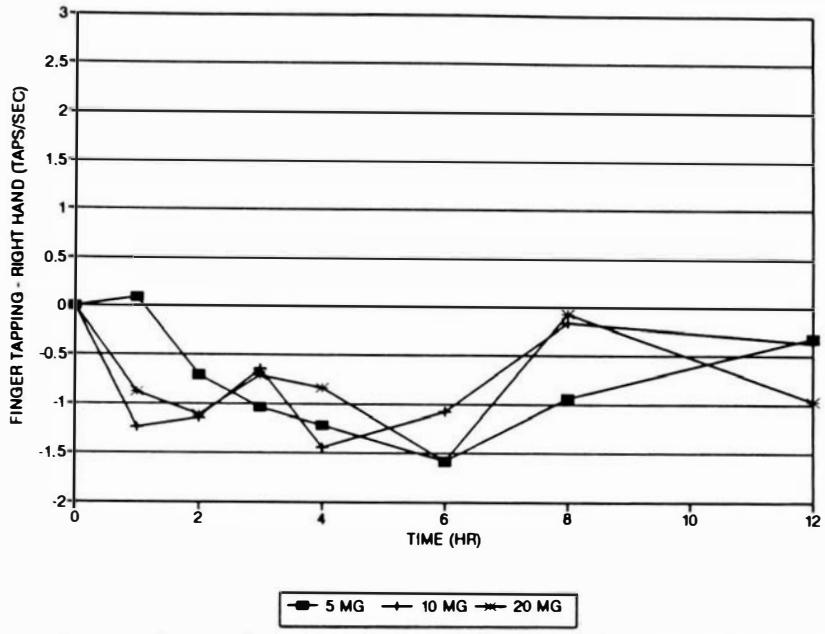


Figure O.29 Baseline and placebo corrected finger tapping rate with the right hand for Subject 7

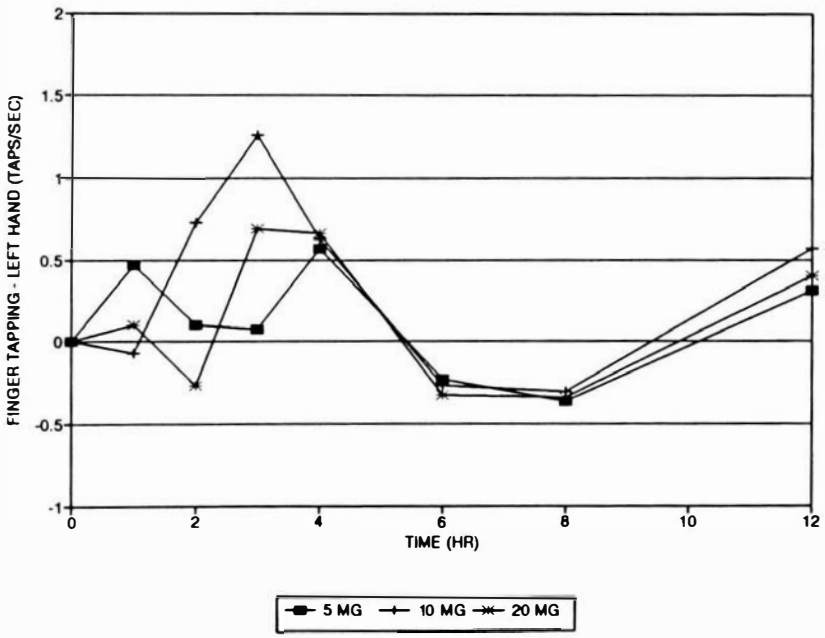


Figure O.30 Baseline and placebo corrected finger tapping rate with the left hand for Subject 7

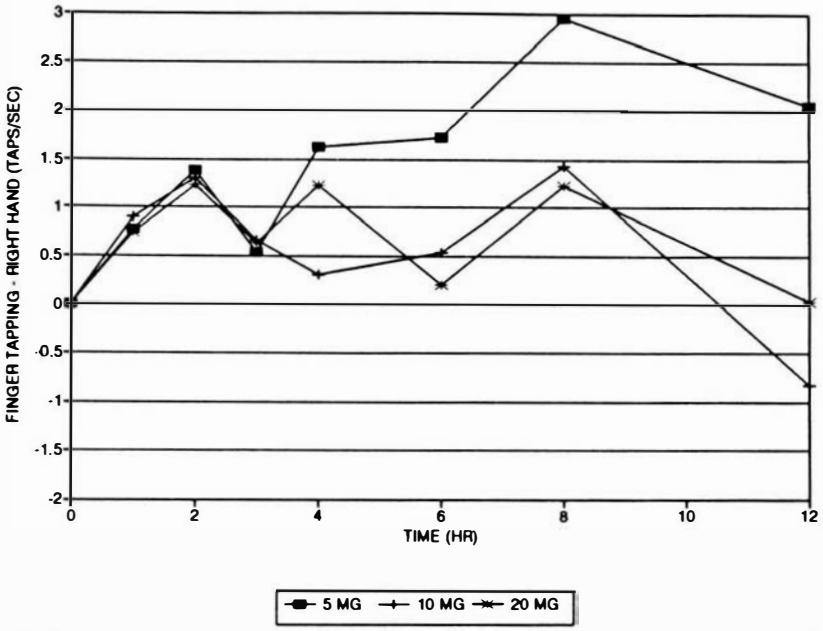


Figure O.31 Baseline and placebo corrected finger tapping rate with the right hand for Subject 8

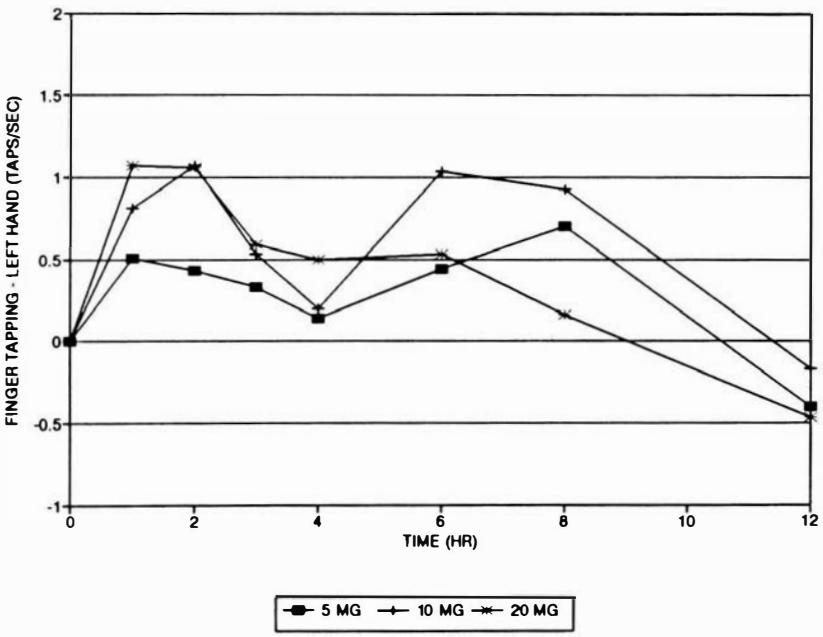


Figure O.32 Baseline and placebo corrected finger tapping rate with the left hand for Subject 8

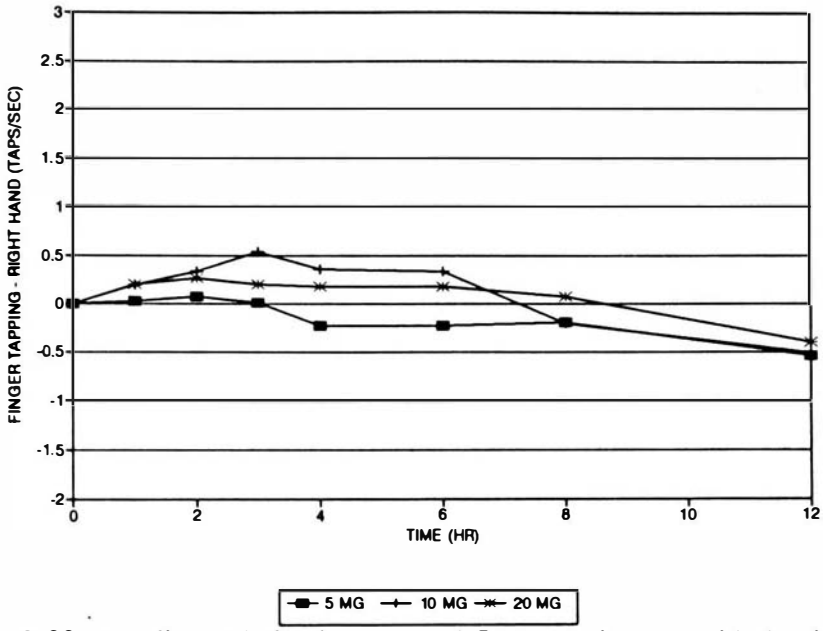


Figure O.33 Baseline and placebo corrected finger tapping rate with the right hand for Subject 9

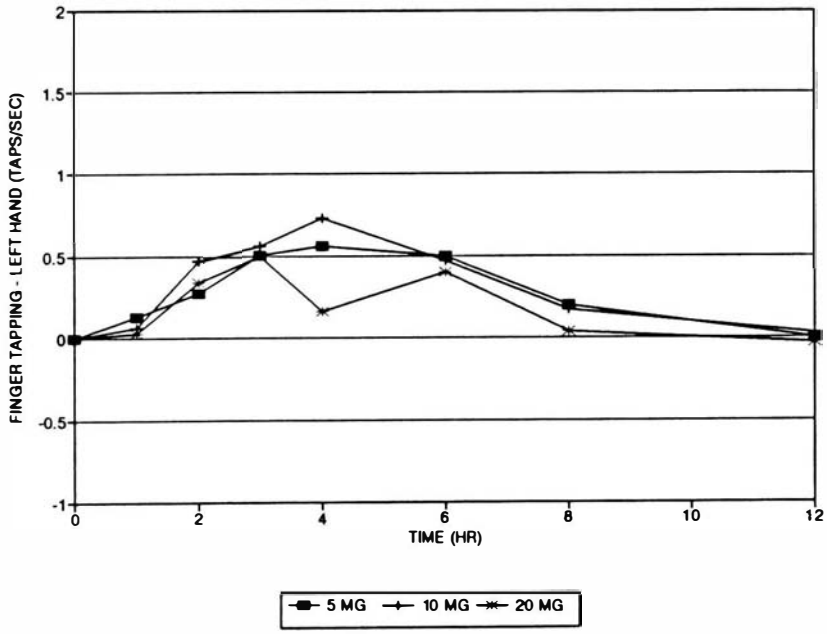


Figure O.34 Baseline and placebo corrected finger tapping rate with the left hand for Subject 9

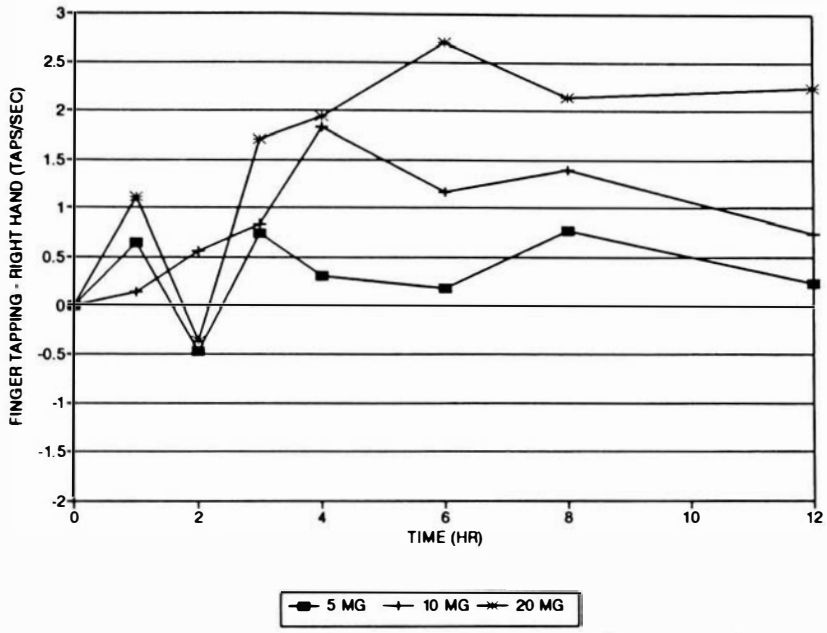


Figure O.35 Baseline and placebo corrected finger tapping rate with the right hand for Subject 10

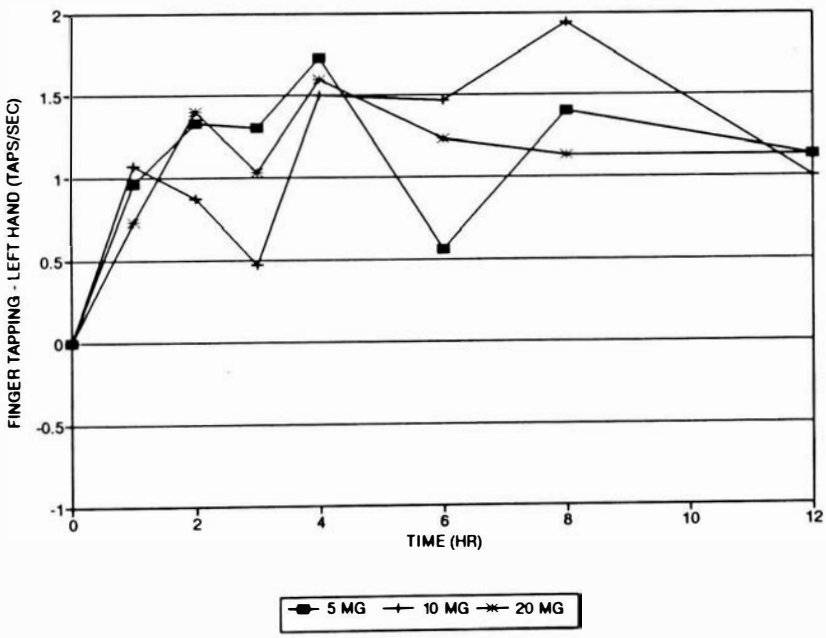


Figure O.36 Baseline and placebo corrected finger tapping rate with the left hand for Subject 10

APPENDIX P

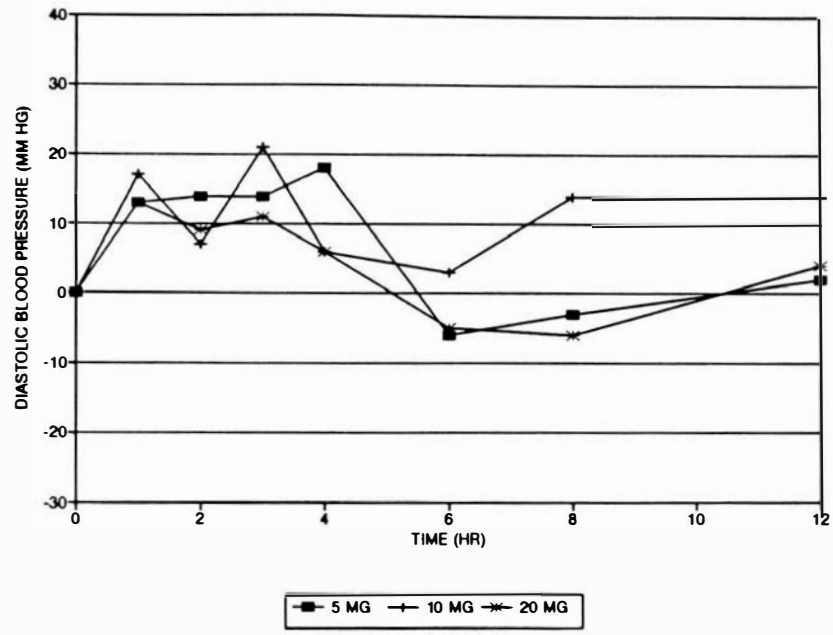


Figure P.1 Baseline and placebo corrected diastolic blood pressure versus time plot for Subject 1

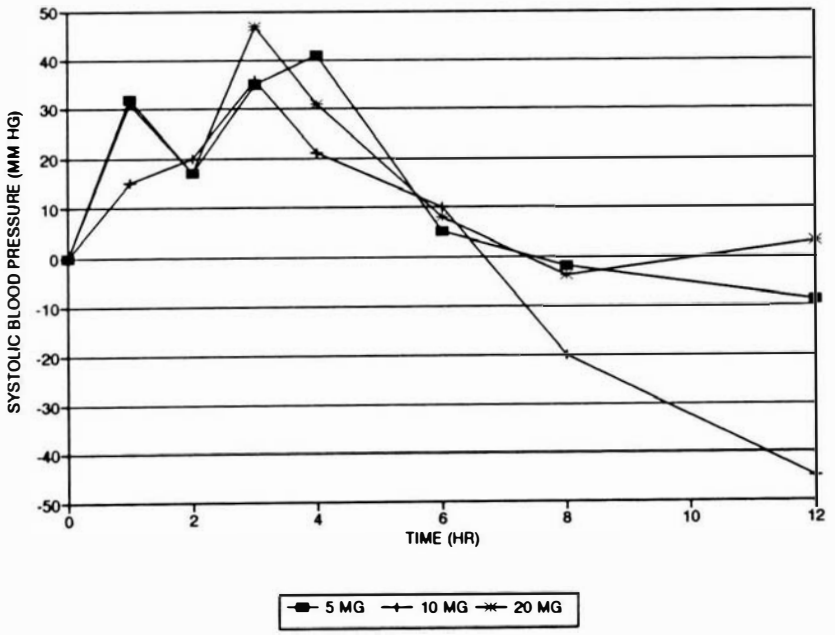


Figure P.2 Baseline and placebo corrected systolic blood pressure versus time plot for Subject 1

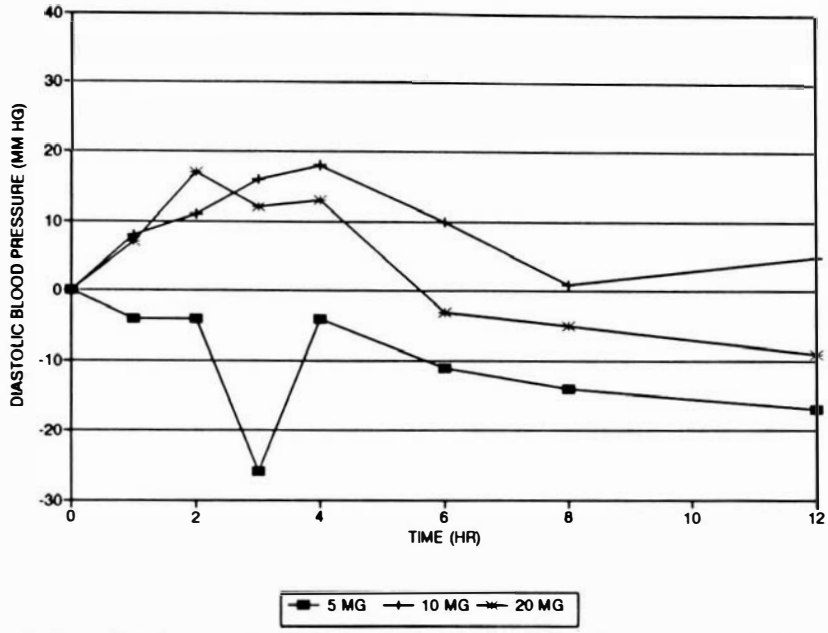


Figure P.3 Baseline and placebo corrected diastolic blood pressure versus time plot for Subject 2

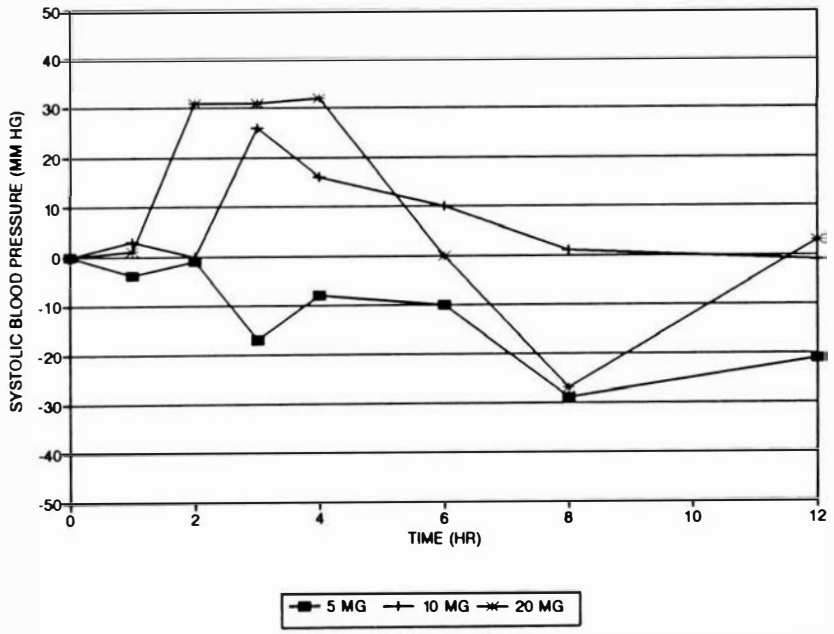


Figure P.4 Baseline and placebo corrected systolic blood pressure versus time plot for Subject 2

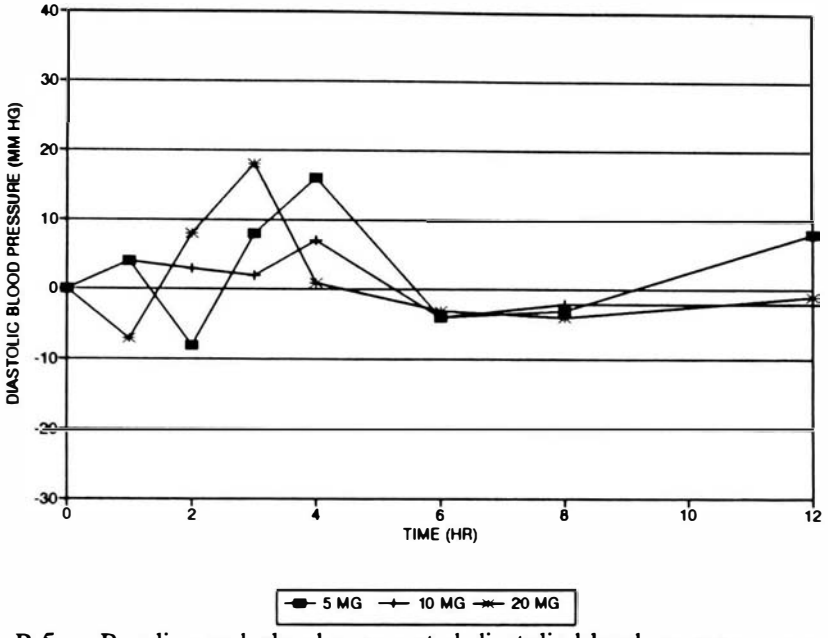


Figure P.5 Baseline and placebo corrected diastolic blood pressure versus time plot for Subject 4

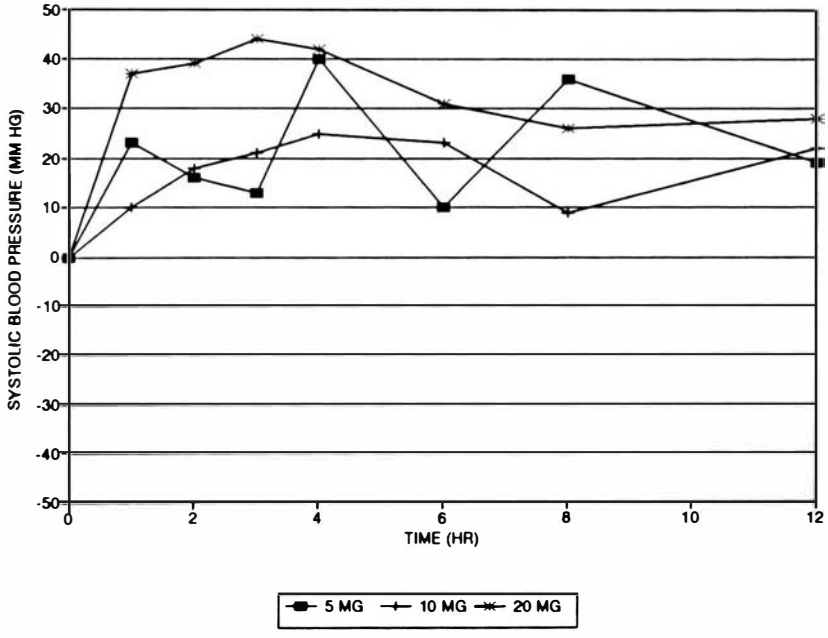


Figure P.6 Baseline and placebo corrected systolic blood pressure versus time plot for Subject 4

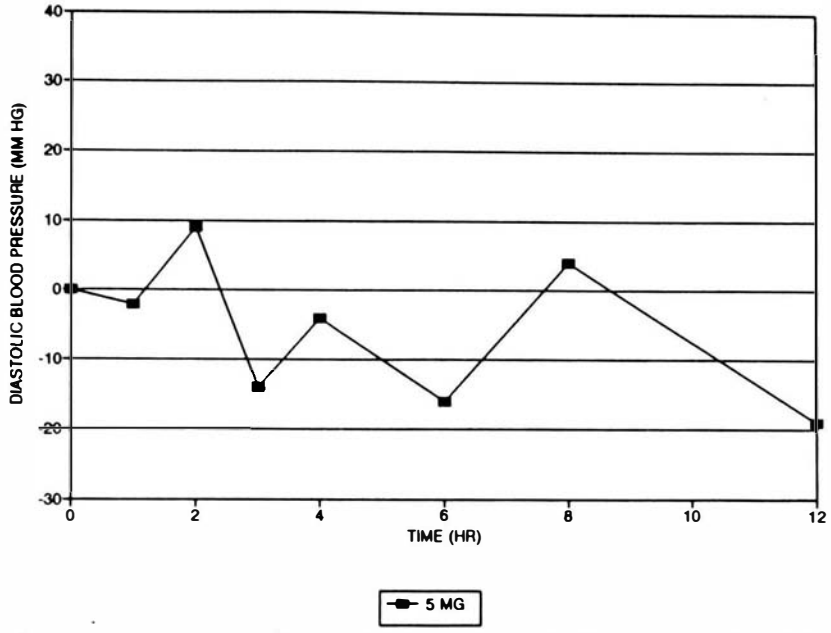


Figure P.7 Baseline and placebo corrected diastolic blood pressure versus time plot for Subject 5

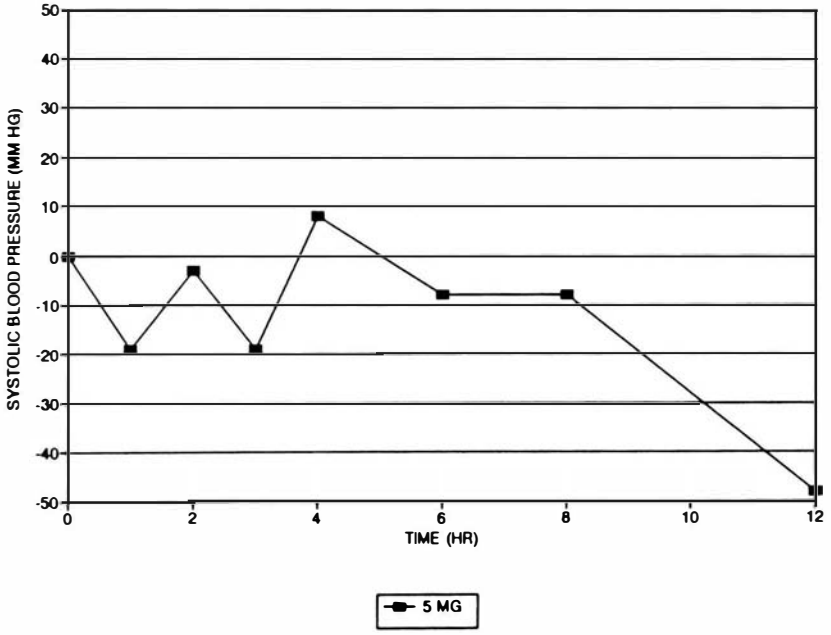


Figure P.8 Baseline and placebo corrected systolic blood pressure versus time plot for Subject 5

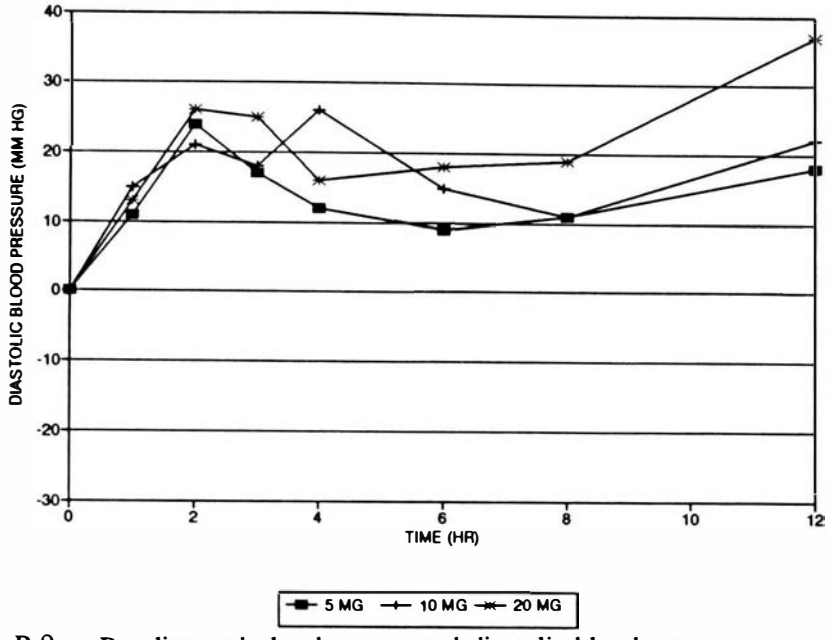


Figure P.9 Baseline and placebo corrected diastolic blood pressure versus time plot for Subject 6

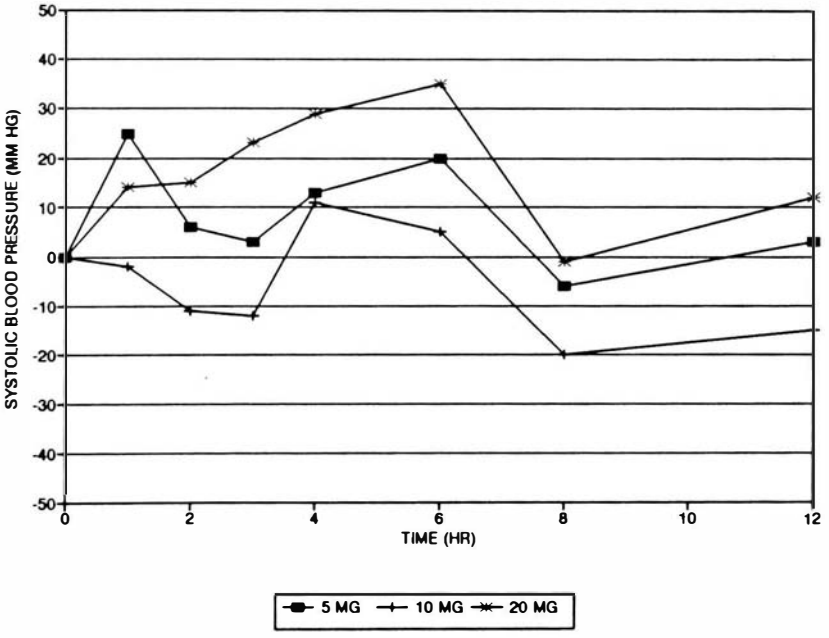


Figure P.10 Baseline and placebo corrected systolic blood pressure versus time plot for Subject 6

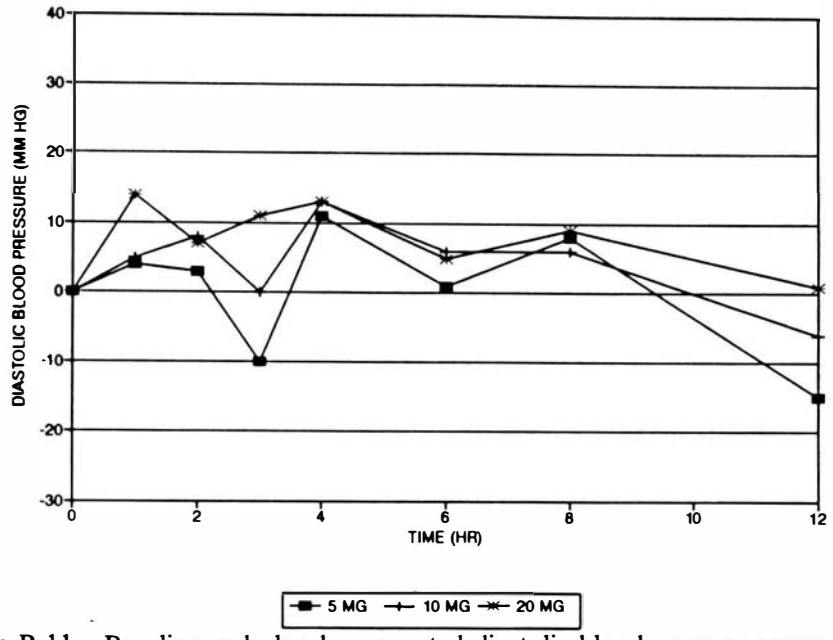


Figure P.11 Baseline and placebo corrected diastolic blood pressure versus time plot for Subject 7

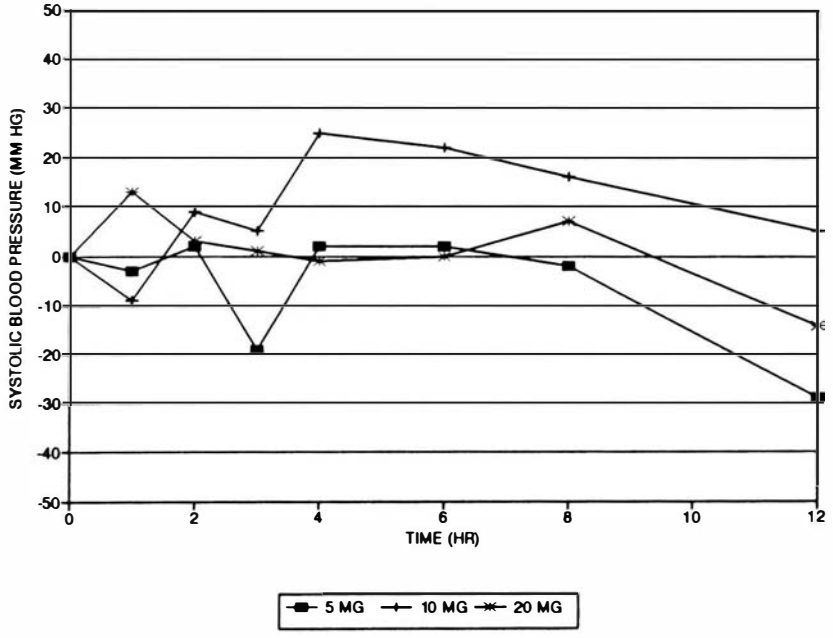


Figure P.12 Baseline and placebo corrected systolic blood pressure versus time plot for Subject 7

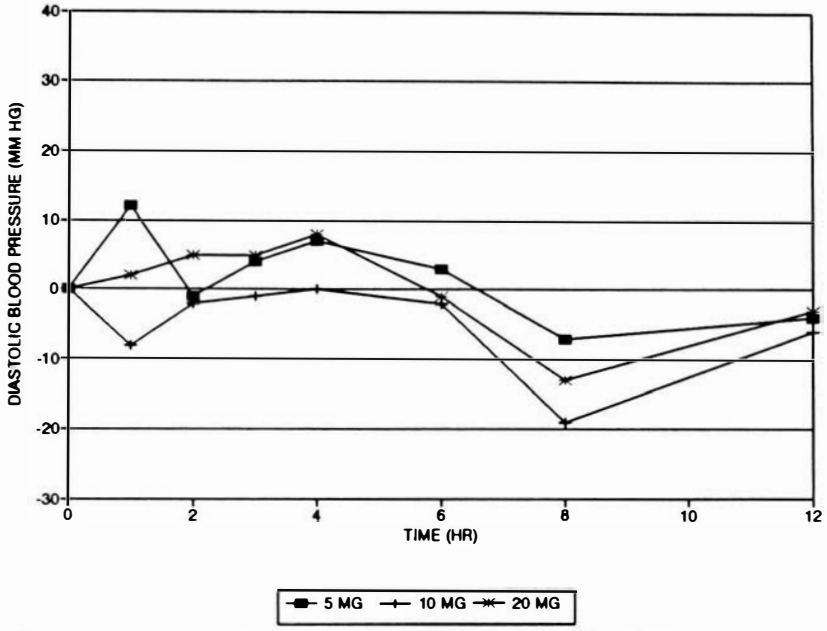


Figure P.13 Baseline and placebo corrected diastolic blood pressure versus time plot for Subject 8

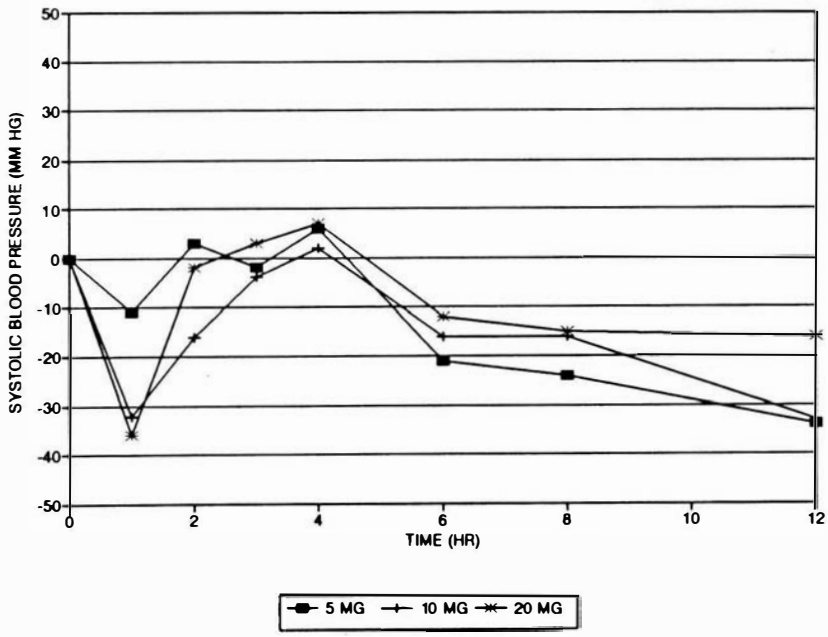


Figure P.14 Baseline and placebo corrected systolic blood pressure versus time plot for Subject 8

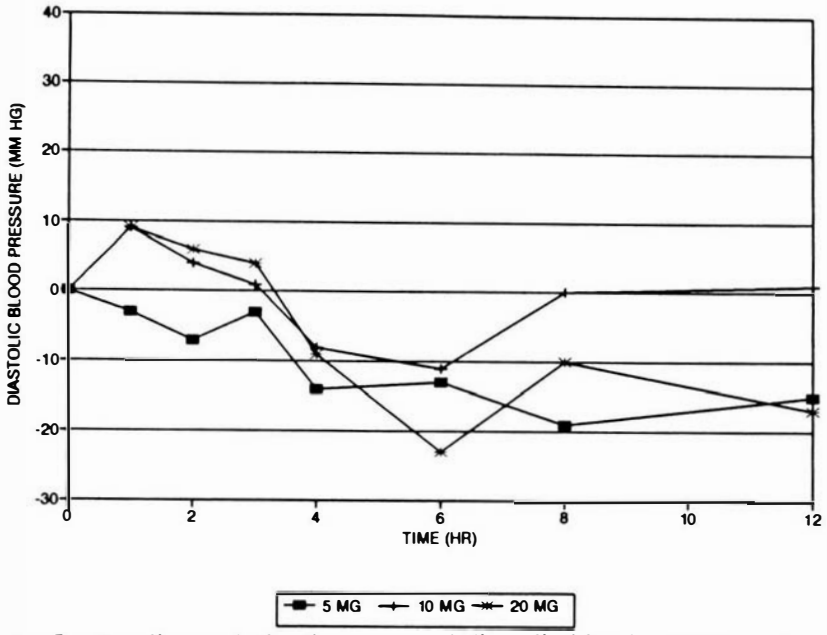


Figure P.15 Baseline and placebo corrected diastolic blood pressure versus time plot for Subject 9

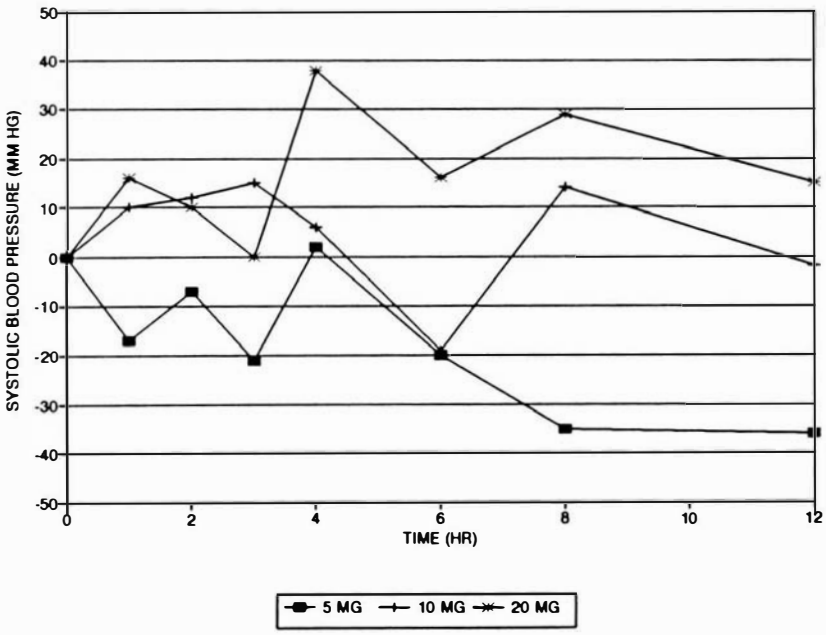


Figure P.16 Baseline and placebo corrected systolic blood pressure versus time plot for Subject 9

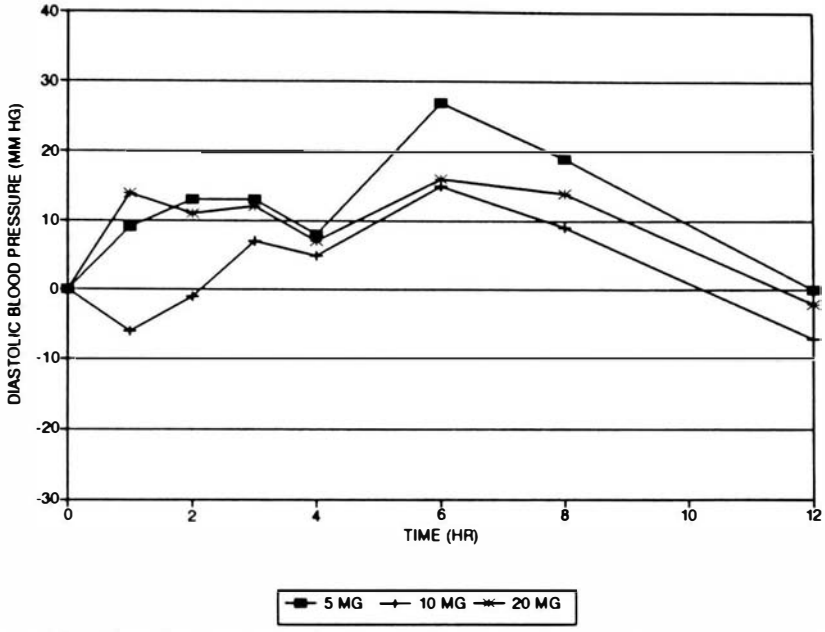


Figure P.17 Baseline and placebo corrected diastolic blood pressure versus time plot for Subject 10

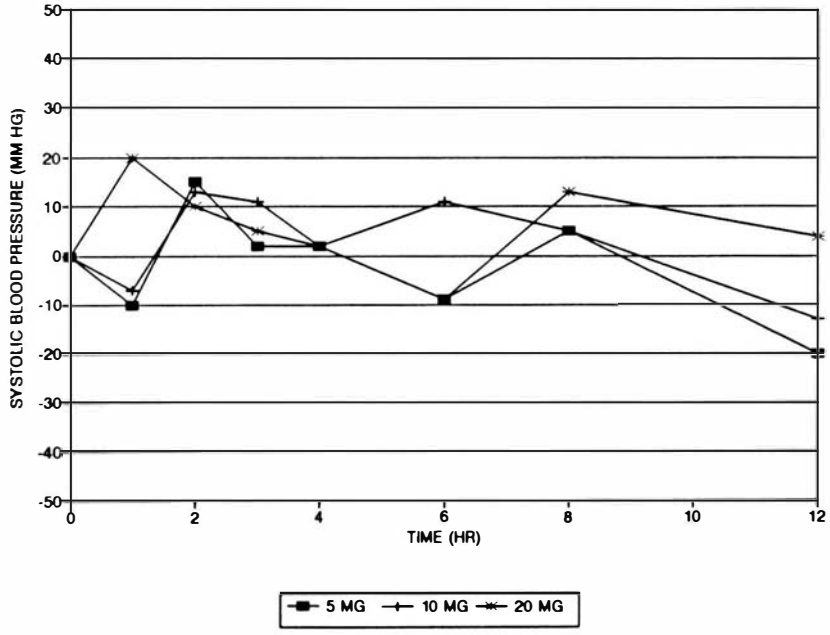


Figure P.18 Baseline and placebo corrected systolic blood pressure versus time plot for Subject 10

APPENDIX Q

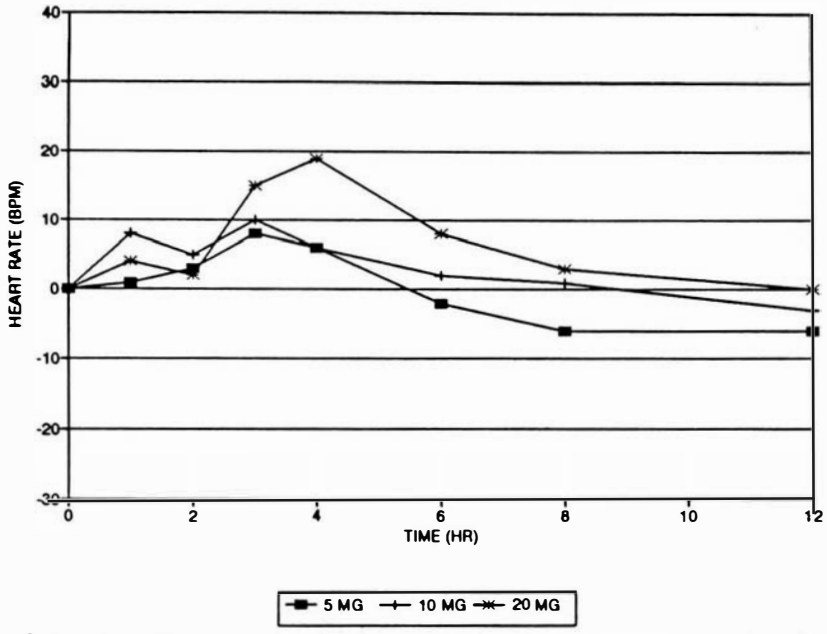


Figure Q.1 Baseline and placebo corrected heart rate versus time plot for Subject 1

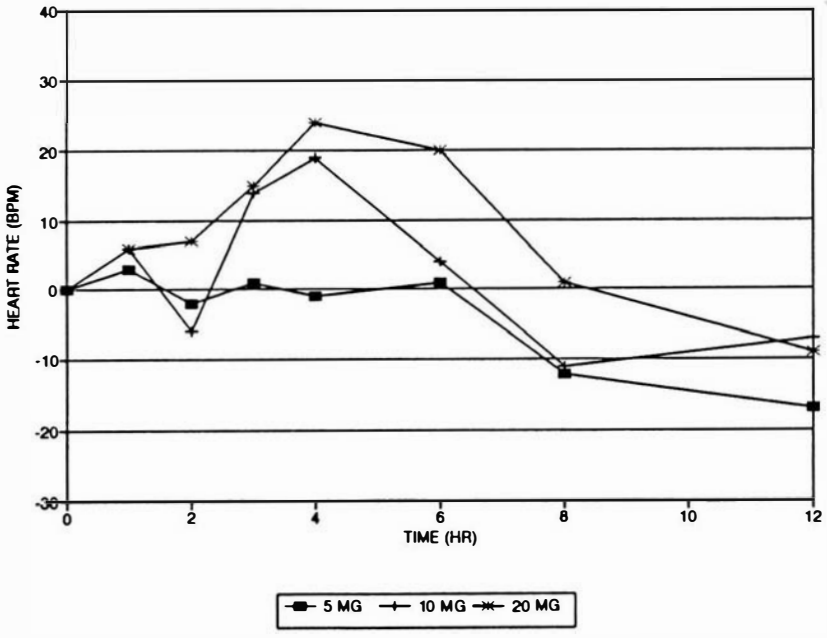


Figure Q.2 Baseline and placebo corrected heart rate versus time plot for Subject 2

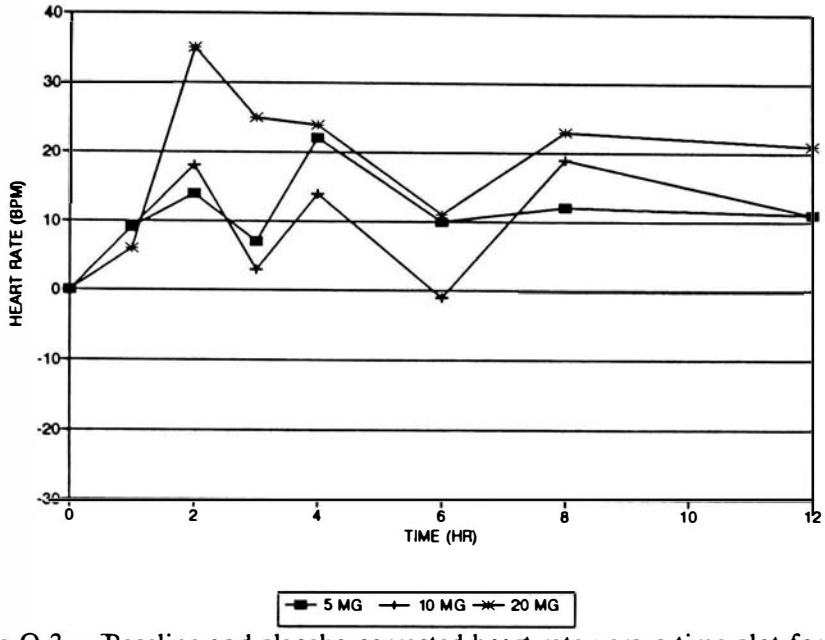


Figure Q.3 Baseline and placebo corrected heart rate versus time plot for Subject 4

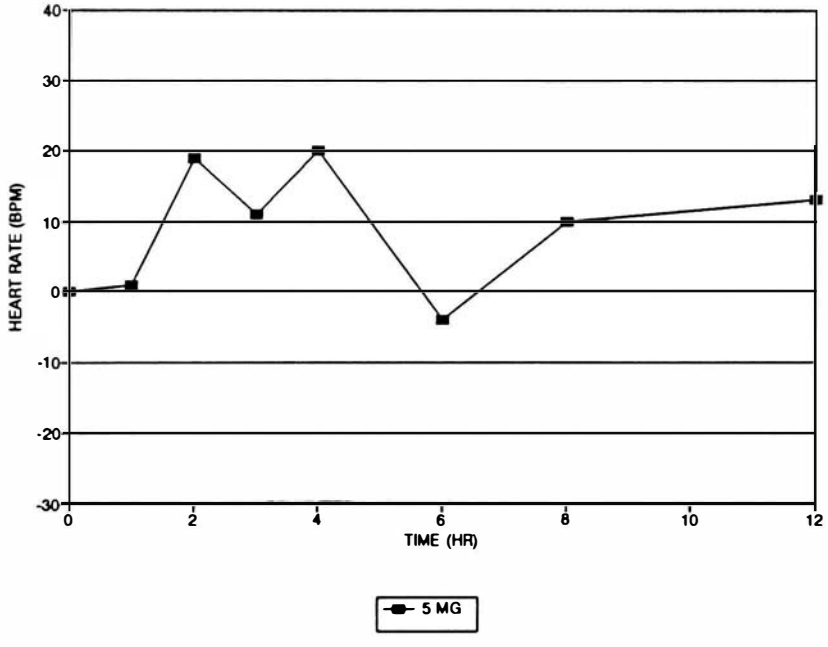


Figure Q.4 Baseline and placebo corrected heart rate versus time plot for Subject 5

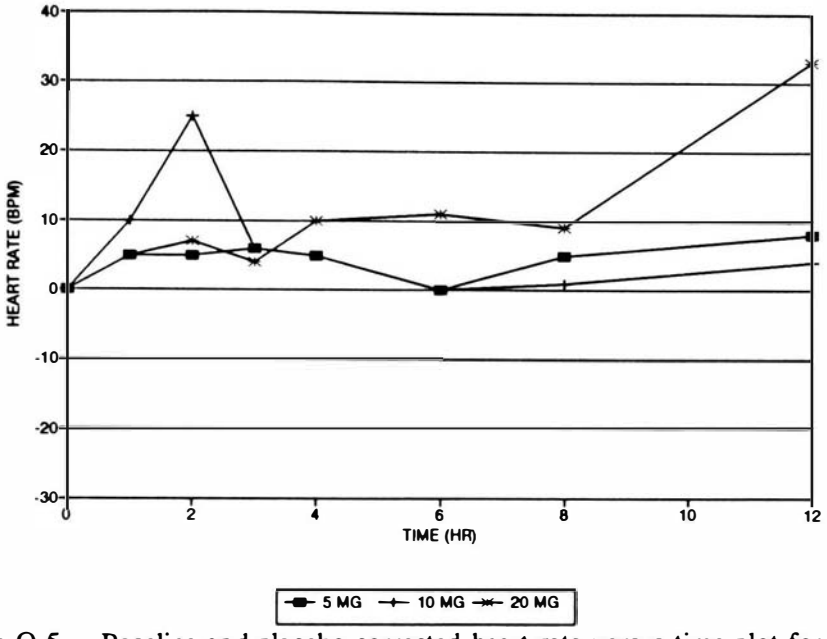


Figure Q.5 Baseline and placebo corrected heart rate versus time plot for Subject 6

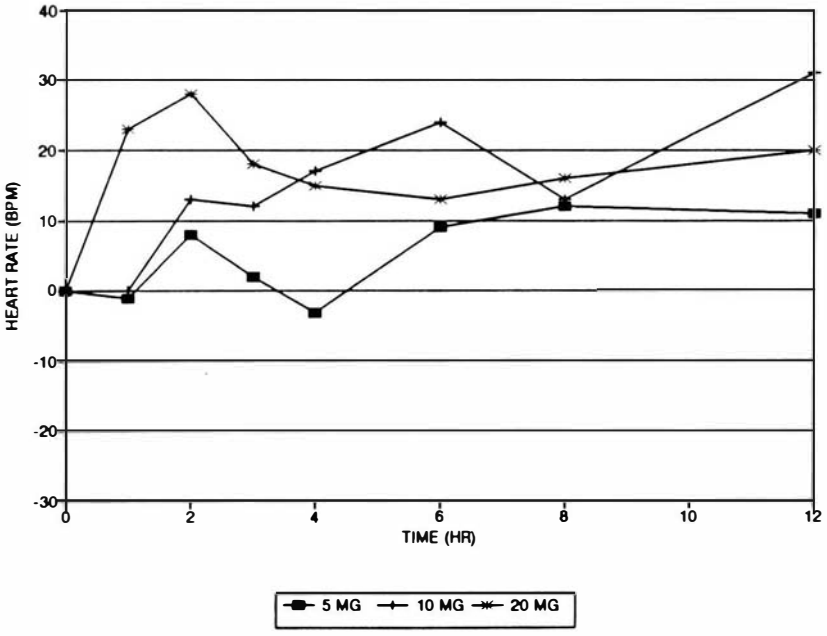


Figure Q.6 Baseline and placebo corrected heart rate versus time plot for Subject 7

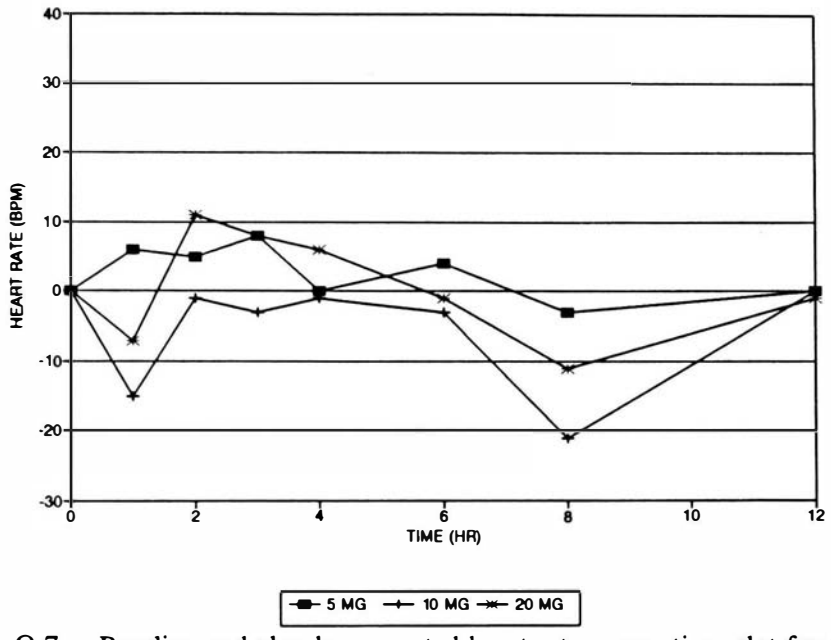


Figure Q.7 Baseline and placebo corrected heart rate versus time plot for Subject 8

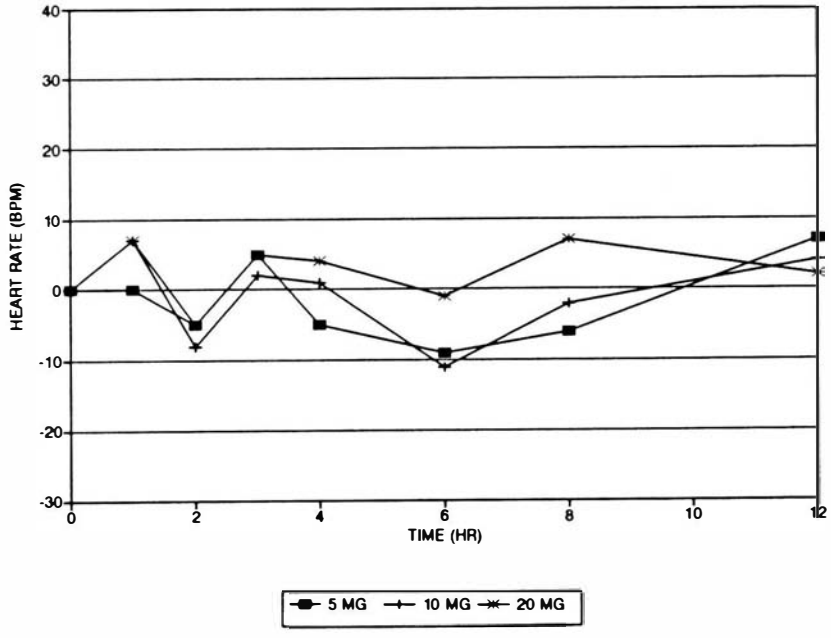


Figure Q.8 Baseline and placebo corrected heart rate versus time plot for Subject 9

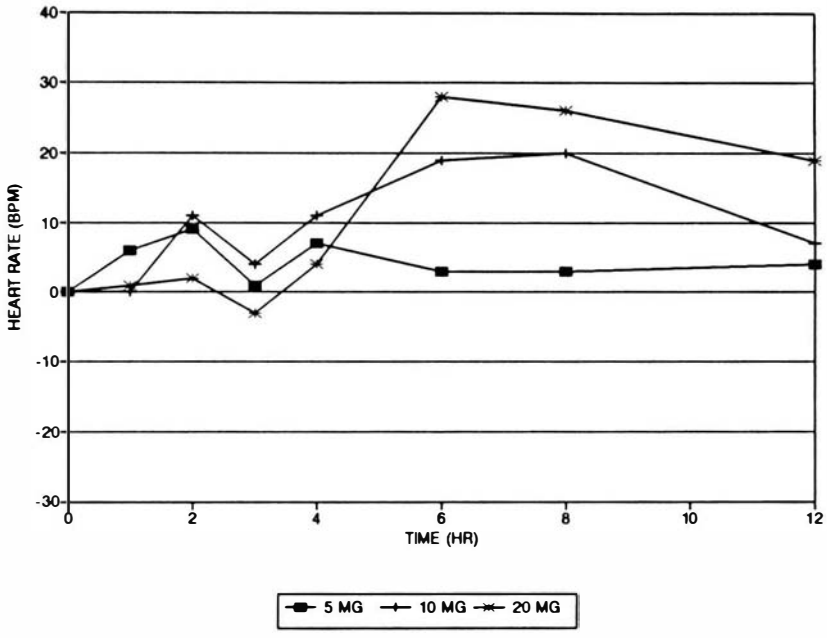


Figure Q.9 Baseline and placebo corrected heart rate versus time plot for Subject 10

Vita