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EFFECT OF CHOLINERGIC PERTURBATIONS ON NEUROMOTOR-COGNITIVE PERFORMANCE

ANNUAL REPORT

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Atropine (d, l-hyoscyamine), an antimuscarinic drug, is widely used as an antidote for anticholinesterase poisoning, as pre-operative medication and for the treatment of conditions, such as peptic ulcers and Parkinsonism. A series of studies was designed to examine the pharmacokinetic-pharmacodynamic relationship of atropine for various cognitive and neuromotor tasks and several physiological measures. In the first study which has been completed, single doses of placebo and 0.5, 1.0 and 2.0 mg of atropine were administered intramuscularly during four test sessions to eight healthy young male volunteers according to a Latin square design balanced for order of dose administration. The psychomotor and cognitive tasks included standing steadiness, reaction time, wheel tracking, visual tracking, divided attention and memory

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19. Abstract (continued)

tasks. Physiological processes were measured by assessing heart rate, blood pressure and body temperature. Plasma levels of stress hormones, ie. arginine vasopressin, adrenocorticotropic hormone, cortisol, catecholamines and growth hormone were also monitored. The pharmacokinetics of atropine were best described by a simple one-compartment model with very rapid first order absorption. The mean elimination half-life, volume of distribution, and clearance were 2.04 (SD=0.23) hr, 132.85 (SD=18.92) L and 45.26 (SD=5.03) L/hr, respectively. The atropine doses used in the present study significantly affected only sitting heart rate and growth hormone levels. Preliminary results indicate a highly similar time course for the changes in plasma atropine levels and plasma growth hormone values during the drug sessions. The implications of the findings from Experiment 1 were discussed and recommendations for future experiments were presented.

Foreword

For the protection of human subjects the investigators have adhered to policies of applicable Federal Law 45CFR46.

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I. Introduction

Atropine sulfate, a well-known anticholinergic agent, has been used for many years in a broad range of clinical applications at moderate doses (1). At higher doses it is used as an antidote for anticholinesterase organophosphorus poisons, including insecticides. Atropine has been distributed since the 1950's to military personnel as self-aid therapy when under chemical attack (2). In light of the highly stressful and complex nature of the tasks required of the modern armed forces, a detailed and comprehensive description of the magnitude and time-course of the effect of higher doses of atropine on different cognitive and neuromotor capacities is important for a complete understanding of potential unwanted side effects. Moreover, Headley (2) lists a number of nontherapeutic or even recreational situations in which anticholinergic drugs may be misused by medically untrained individuals in the absence of organophosphorus nerve gas.

Previous studies have noted differential effects on the peripheral and central nervous systems for antimuscarinic drugs. Peripheral effects include bradycardia and tachycardia, a reduction in systolic blood pressure, a decrease in the activity of sweat glands and in saliva flow, an increase and decrease in skin and oral temperature, pupil dilation and accommodation paresis (2, 3, 4, 5, 6). While dose-dependent effects have been reported for both peripheral and central atropine effects (6), there have been frequent claims in the clinical literature that larger therapeutic doses, ranging from 2 to 3 mg, produce significant peripheral anticholinergic effects but rarely induce central nervous system (CNS) toxicity (7, 8, 9, 10). Many of the early studies, however, were characterized by low doses, small samples of young men, one-time testing and little statistical data analysis (2).

Recent studies have demonstrated that impairment of CNS functioning is detectable at much lower doses of atropine when highly sensitive measures of cognitive and psychomotor performance are used (6, 11, 12). Seppala and Visakorpi (6) have conducted the most comprehensive comparison to date of the effects of 0.85 and 1.7 mg of atropine on a large number of autonomic and behavioral variables. The authors reported not only dose-related effects but also differential impairment time courses for the peripheral and central processes. Similarly, Shader and Greenblatt (10) noted a more prolonged duration of effect for cycloplegia and mydriasis than for sensorium changes. Other studies have demonstrated impairment on mathematical ability, simple reaction time, coordination, divided attention, flicker recognition, standing steadiness and the use of common tools, especially at the higher doses (6, 13, 14, 15).

The influence of anticholinergic drugs on memory has probably been more intensely examined than any other cognitive or psychomotor process. Fairly consistent findings from various studies indicate that the anticholinergic drugs show marked impairment in the acquisition or encoding of material into memory, less effct on memory retrieval, and, at moderate doses, little effect on retrieval from memory of material learned prior to drug administration (11, 12, 16). These effects are shared by other psychotropic drugs affecting memory (for example, diazepam) and thus may be non-specifically related to the anticholinergic properties (11). There is also evidence that nonverbal visual memory is relatively spared (11, 17) and that recent memory recall can be improved with cueing (16) which suggests that subjects can be trained to enhance their performance under the influence of anticholinergic drugs.

Although atropine has been used therapeutically for many years, there is relatively little information on the pharmacokinetics of this drug and on the relationship between its pharmacokinetic and pharmacodynamic properties. A few studies have reported the excretion of 50% of a parenteral dose by the kidney after 4 hours (18) and complete excretion within 24 hours (18, 19). Thus atropine has a rapid elimination half-life of 2 to 4 hours (1, 20). Recently published data also indicate that atropine is extensively distributed and bound to tissue and that the distribution kinetics is dose-dependent (20, 21).

While a few studies have examined the pharmacokinetic-pharmacodynamic relationship for physiological variables (20, 21), there has been no attempt to model this important relationship for cognitive and psychomotor performance. Adams et al. (21) found a high correlation ($r = 0.84$) between heart rate and estimated tissue level of atropine. Using an approach that fitted both pharmacokinetic and pharmacodynamic data to an integrated kinetic-dynamic model, Hinderling et al. (20) determined that the atropine effects on heart rate and saliva flow were related nonlinearly to the drug concentration in the peripheral compartment.

The present study was an initial effort to describe the pharmacokinetic-pharmacodynamic relationship of atropine for various cognitive and neuromotor tasks. Specifically, the objectives of the study included the determination of (1) the sensitivity of different performance tasks and physiological measures to cholinergic perturbation, (2) the time-course of the behavioral and physiological impairment, (3) the concentration-response relationship across time for the different task and physiological response measures, (4) the comparative impairment on the cognitive, neuromotor and physiological measures, and (5) individual variability in atropine responsivity. This report primarily presents the results of an experiment which investigated the effects of 0.5,

1.0 and 2.0 mg doses of atropine on the cognitive, neuromotor and physiological responses of 8 young, healthy men. We are currently completing a second study which uses a similar experimental protocol to administer 1.0, 2.0 and 4.0 mg of atropine to another group of 8 young males and have started to collect data on a third sample of 8 young men using the three higher atropine dosages.

II. Experiment 1: Method

Subjects

Eight normal male volunteers, who were between the ages of 21 and 29 years and within ± 10% of ideal height-weight ratios, participated in the study. Prior to starting the experiment, the subjects were screened for any serious physical and psychological problems and for any history of drug abuse with a physical and psychiatric evaluation, blood tests, urinalysis, glaucoma tests and electrocardiogram. The blood tests included chemistry (with hepatic) profile and complete blood count, and the urinalysis included a urine screen for marijuana. The Vocabulary, Block Design and Digit Symbol Substitution subtests of the Wechsler Adult Intelligence Scale-Revised (22) and the Minnesota Multiphasic Personality Inventory (MMPI) were also administered. Periodic urine screens for marijuana were conducted prior to the scheduled tests session days. After completion of the study, the subjects were given a post-study physical examination. Informed written consent was obtained from each subject after the purpose and procedure of the study as well as the potential physiological and psychological effects of atropine were explained.

None of the participants had any serious physical or mental disorder or were taking anticholinergic or CNS-active drugs during their participation in the study. All of the subjects reported that they were nonsmokers and did not drink alcohol excessively. The mean scaled scores for the Vocabulary, Block Design and Digit Symbol Substitution subtests were 12.9 (± 1.6), 12.4 (± 1.7) and 11.7 (± 3.2), respectively.

Performance Tasks

The effects of atropine over time on fifteen cognitive and neuromotor tasks were measured in the present study. Detailed descriptions of the procedures, response measurements and data analysis programs for the tasks are presented in Appendix A. Different tasks were combined to create three test batteries which required varying periods of time to complete. The ultra-short (briefest) battery consisted of just one task, continuous subcritical tracking (SCT). The medium battery included four tests presented in the following order: standing steadiness in the eyes open condition, digit symbol

substitution (DSS), SCT and abbreviated digit symbol memory tasks. In the long battery, the sequence of the thirteen tasks was standing steadiness with visual feedback and in eyes open and eyes closed conditions, DSS, SCT, continuous performance, divided attention, digit symbol substitution with short term memory test, digit-keypad reaction time, sudden displacement eye saccade, central pendulum eye tracking, lateral pendulum eye tracking and memory encoding. In addition to the three test batteries, a concrete noun memory task was used to assess the atropine effect on verbal memory processes.

Physiological Measurements

Several physiological parameters were measured periodically during the sensitivity and test sessions. Heart rate, oral temperature and blood pressure were taken approximately every 15 minutes (see Tables 1 and 2 for actual times of measurement). Heart rate was monitored continously using a 7830A Hewlett Packard heart monitor equipped with a beat-to-beat audio output for easy identification of the onset of bradycardia or tachycardia. The beats per minute (bpm) heart rate was determined by counting the auditory beats for a duration of 30 seconds and multiplying by 2. Heart rate was taken while the subject was sitting and standing and documented for subsequent time course analysis. This method was cross-checked with the measurement of heart rate obtained by palpitation to ensure proper equipment functioning. In addition, EKG polygraph recordings were made periodically during the test sessions and continously during any periods of tachycardia for detection and identification of arrhythmias.

Diastolic and systolic blood pressure were both determined manually with a blood pressure cuff and stethoscope. Blood pressure was taken by all experimenters during practice sessions to ensure that interexperimenter variations were kept to a minimum. Temperatures, in degrees Fahrenheit, were taken orally using a digital converter and a test probe. The test probes were calibrated with a glass Celsius laboratory thermometer throughout the range of body temperatures and were found to vary within one degree Celsius. Room temperature was also measured and documented during the test session to ensure that fluctuations in body temperature were not caused by changes in the temperature of the room atmosphere. Test room temperature was maintained within a range of 70 to 75 degrees Fahrenheit.

Neuroendocrine Measures

In addition to the blood samples for the atropine assay, plasma samples were drawn during the sensitivity session for the measurement of vasopressin (AVP), adrenocorticotropic hormone (ACTH), cortisol and the catecholamines prior to and at 40, 100, 160 and 400 min following drug administration. During

Table 1

Testing Schedule for the Sensitivity Session

Time (min)	Procedure[a, b]
-90	Breakfast
	Catheter insertion, attachment of electrodes
-30	BP, HR, temperature, medium task battery
	Blood sample
0	Atropine (0.25 mg) injection
2	Medium task battery
20	BP, HR, temperature, medium task battery
40	Blood sample
	Atropine (0.25 mg) injection
42	BP, HR, temperature, medium task battery
60	BP, HR, temperature, medium task battery
80	BP, HR, temperature, medium task battery
100	Blood sample
	Atropine, (1.0 mg) injection
102	BP, HR, temperature, medium task battery
120	BP, HR, temperature, medium task battery
140	BP, HR, temperature, medium task battery
160	Blood sample
	BP, HR, temperature, medium task battery
180	BP, HR, temperature, medium task battery
200	BP, HR, temperature, medium task battery
220	BP, HR, temperature
223	Lunch
280	BP, HR, temperature, medium task battery
340	BP, HR, temperature, medium task battery
400	Blood sample
	BP, HR, temperature, medium task battery

[a] Physiological measures included recording of blood pressure (BP), heart rate (HR) and oral temperture.

[b] The medium task battery is described in the text.

Table 2

Testing Schedule for the Drug Session

Time (min)	Procedure[a]
-105	Breakfast, catheter insertion, attachment of electrodes
- 60	Long task battery, blood sample, BP, HR temperature, Concrete Noun Learning Trial 1
0	Drug administration
10	Blood sample, ultra-short battery
15	Medium task battery
20	Blood sample, BP, HR, temperature after sway task
35	Medium task battery
40	Blood sample, BP, HR, temperature after sway task
55	Medium task battery
60	Blood sample, BP, HR, temperature after sway task
75	Medium task battery
80	Blood sample, BP, HR, temperature after sway task
95	Medium task battery
100	Blood sample, BP, HR, temperature after sway task
114	Concrete Noun Recall Trial 1
115	Medium task battery
120	Blood sample, BP, HR, temperature after sway task
133	Concrete Noun Learning Trial 2
135	Long task battery
160	Blood sample, BP, HR, temperature
165	Long task battery
190	Blood sample, BP, HR, temperature
195	POMS
210	Medium task battery
228	Concrete Noun Recall Trial 2, BP, HR, temperature
230	Long task battery
255	Blood sample, BP, HR, temperature
260	Lunch
290	BP, HR, temperature
330	BP, HR, temperature, medium task battery
350	Long task battery
375	Blood sample, BP, HR, temperature
380	Medium task battery
400	Long task battery
425	Blood sample, BP, HR, temperature
430	Medium task battery
447	BP, HR, temperature
450	Concrete Noun Final Recall and Recognition Tests

[a] Physiological measures and long task battery are explained in Table 1 and the text.

each test session AVP, ACTH, cortisol and catecholamine plasma samples were collected prior to and at 35 and 75 min after drug injection. Additional measurements of AVP and ACTH were taken at 135 min post drug administration. Assessment of the growth hormone levels were conducted at the same testing time points as for the determination of atropine drug concentration. The purpose for monitoring the potential changes in these neuroendocrine variables was to explore the question of (1) whether atropine alters the plasma concentration of stress hormones and (2) whether the impairment effects of atropine interacts with changes in the stress hormone levels. A detailed description of the blood sample collection procedure for atropine and the different hormones is contained in Appendix B.

Procedure

Initially subjects were trained on the behavioral tasks during three or four 2-hour sessions until they showed no evidence of substantial improvement in their performance scores. After the training phase, a sensitivity session was conducted to screen for individuals who might be extremely sensitive to the effects of atropine. Each subject then completed four drug sessions which were scheduled at 2-week intervals.

Except for the differences mentioned below, the same experimental protocol was used for the sensitivity and test sessions. The subjects were instructed to sleep their normal number of hours the night before the test day and to consume no alcohol or drugs during the previous 24 hours. They reported to the laboratory at 07:30 and were given a light breakfast. An obturated intravenous teflon catheter for drawing blood samples was inserted in the forearm. Baseline measurements of the performance and physiological measures and predrug plasma samples were then collected prior to the administration of atropine.

Approximately 1 hr after breakfast, a single injection of the drug was administered. During the sensitivity session, additional injections were given at 40 min and 1 hr and 40 min after the initial injection. The testing schedules for the sensitivity and drug sessions are described in Tables 1 and 2, respectively, which list the times at which blood samples and assessments of the performance and physiological measures were taken following drug administration. All subjects ate a standard lunch, consisting of a meat (usually turkey) sandwich and noncaffeinated soda.

Drug

Individual syringes, containing atropine sulfate or placebo, were prepared by the Duke University Medical Center

Pharmacy for the sensitivity and tests sessions. The concentration of atropine was 1.0 mg/ml. For each sensitivity session, three separate doses of atropine were injected intramuscularly (I.M.) in the ventral aspect of the upper thigh. The dosing schedule was 0.25 mg at 0 min, followed by 0.25 mg at 40 min and 1.0 mg at 1 hr and 40 minutes after the initial injection.

During each of the four drug sessions, a single dose of placebo or one of three doses of atropine, 0.5, 1.0 or 2.0 mg, was given to each subject according to a Latin square design balanced for order of dose administration. Placebo consisted of 2 ml of bacteriostatic water. Similarly, the atropine doses were diluted with sufficient quantities of bacteriostatic water to produce a volume of 2 ml. Using a double-blind procedure, the drug was injected I.M. in the same location as for the sensitivity session.

Assays

Atropine assay. Our objective was to set up a sensitive assay procedure for the detection of atropine in plasma that would allow us to delineate more definitively the pharmacokinetic parameters of atropine in humans. It took about 6 weeks to modify the radioimmunoassay (RIA) method of the Walter Reed Army Institute to a disequilibrium RIA method in order to achieve greater sensitivity. The latter technique is described in greater detail in Appendix C.

Neuroendocrine hormone assays. Different assay procedures were used to determine the levels of AVP, ACTH, cortisol and the cathecolamines. Each assay method is explained in Appendix D.

Data Analysis

The plasma atropine concentration data for individual subjects were analyzed using both compartmental and non-compartmental methods. For the compartmental analysis, initial estimates of the parameters and compartmental configuration were evaluated using ESTRIP (23). Parameter values were further defined using SAS (24). Total plasma clearance (CLp), maximum concentration (Cmax), time to reach concentration maximum (tmax), volume of distribution (V), mean absorption time (MAT), absorption rate constant (ka), absorption half-life (t1/2,ka), elimination rate constant (K) and elimination half-life (t1/2,B or t1/2,K) were calculated in the usual manner (22).

The units of measurement for the performance tasks are explained in Appendix A. Due to the number of comparisons, Bonferroni t-tests were used to compare statistically the performance of atropine vs. placebo conditions at each testing

time point. The t-tests were conducted to determine whether any of the three atropine dosages significantly impaired the behavioral or physiological scores in comparison to the placebo condition.

III. Experiment 2: Method

Subjects

Eight young males with characteristics similar to those of the subjects in Experiment 1 have been recruited for the second study. As in the Experiment 1, all the subjects were given various screening tests and written informed consent was obtained. Eight subjects have completed the sensitivity sessions for Experiment 2. Three of the volunteers have also done all four of the drug sessions, while the remaining five men are currently at varying stages of finishing their participation in the study. Finally, we have started to recruit and collect data on a third group of 8 young men who will also be participating in Experiment 2. Thus, the total sample size for the second experiment will be 16 subjects.

Procedure

Basically the performance tasks and physiological measures as well as the experimental protocol were identical for Experiments 1 and 2. The major change in the second study involved the doses administered during the sensitivity and test sessions. For the sensitivity session of Experiment 2, the initial dose was 0.5 mg at 0 min, followed by 1.0 mg at 40 min and 1.5 mg at 1 hr and 40 min after the first injection. The atropine doses for the drug sessions were 1.0, 2.0 and 4.0 mg. In addition, a computerized method of recording heart rate was introduced in the second experiment (see Appendix A for a description of the procedure). The only results from the second study that will be presented are the pharmacokinetic analyses of the atropine drug levels for the sensitivity session since this is the only part of the experiment that has been completed by the 8 subjects.

IV. Results

Cognitive and Neuromotor Tasks

In general, the dose response curves for performance on the cognitive and neuromotor tasks showed little drug effect at the doses administered in the first study, that is, 0.5, 1.0 and 2.0 mg. The results of the t-tests indicate that there were no significant differences between the scores of the three atropine doses and placebo for any of the behavioral tests. In order to provide a more meaningful perspective for the interpretation of the trends noted, the atropine effects on the

SCT, DSS and sway with eyes open tasks for 2.0 mg were compared to the effects of 1.0 and 2.0 mg of alprazolam (Xanax), a standard anxiolytic drug commonly used with clinical populations (Figures 1 to 3). The 1.0 mg dose of alprazolam is the highest therapeutic dosage usually prescribed, and 2.0 mg is two times this highest recommended prescribed dose. Similarly, the usual clinical pre-anesthetic dose of atropine is 0.8 mg, and 2 mg would be approximately twice that dose. A description of the experimental procedure and results of the study that generated the alprazolam data have been presented previously by Ellinwood et al. (26).

As clearly shown in Figures 1, 2 and 3, the magnitude of the effect of 2 mg of atropine on the SCT, body sway with eyes open and DSS tasks, respectively, is much less than that for either dose of alprazolam. Since the atropine and alprazolam studies were accomplished with different subjects and the sample size of the atropine study was relatively small, direct statistical comparisons between the alprazolam and atropine results were not conducted for Experiment 1. When we have added the data for the remaining 16 subjects tested at the higher 4.0 mg dose to the present atropine subject cohort, we will be able to conduct more systematic statistical analyses and comparisonS of the atropine effects with those of a variety of other psychotropic drugs. This approach will provide a more appropriate perspective for the evaluation of the magnitude of the atropine impairment.

Figures 4 through 9 illustrate the time course of the performance of the 8 subjects in Experiment 1 on a variety of cognitive and neuromotor tasks after I.M. injection of the three atropine doses and placebo but without the comparison drug, alprazolam. Similar impairment profiles over time were observed for the remaining tasks that are not shown. Although none of the neuromotor or cognitive effects was significantly affected by the atropine treatment, there were indications of dose-related trends in some cases. A currently unexplained phenomenon is the occurrence of a second impairment peak at 210 min post drug administration for the SCT task (Figure 4). As soon as the data for the 1.0, 2.0, and 4.0 mg study (Experiment 2) have been collected, the dose-response and concentration-response relationships for the cognitive and neuromotor performance tasks will be re-analyzed and reported. In addition, the question of whether the late impairment peak observed for the 2.0 mg dose on the SCT task is related either to atropine pharmacokinetic or pharmacodynamic processes or to some other random variable will be examined.

Physiological measures

There was the expected dose-dependent increase in sitting heart rate for all the atropine doses, which lasted for approximately 2 hr (Figure 10). There was very little change

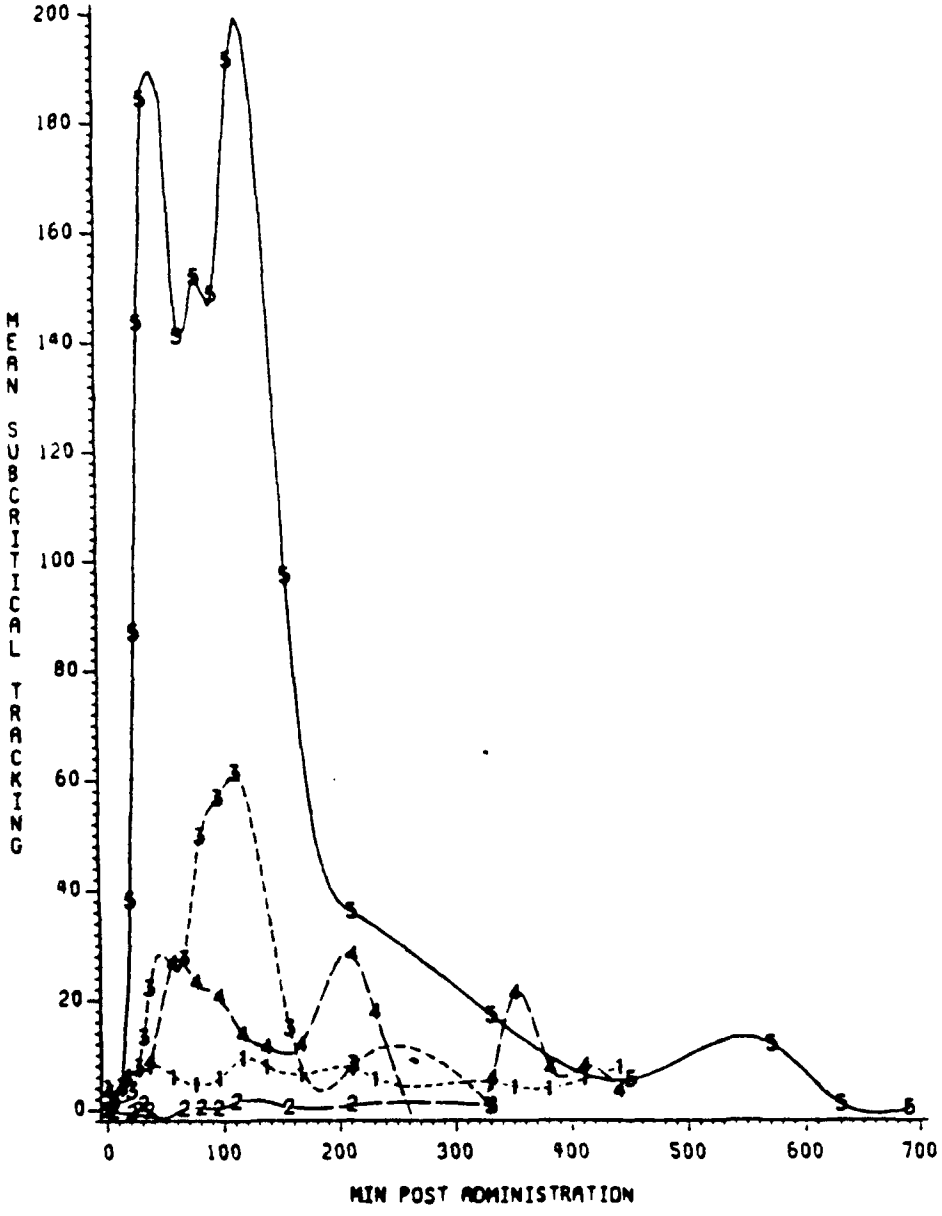

Figure 1. Performance on the SCT task for atropine (Experiment 1) and alprazolam. The atropine dose was 2.0 mg (4) and the alprazolam doses were 1.0 mg (3) and 2.0 mg (5). The placebo sessions for the atropine and alprazolam studies are indicated by 1 and 2, respectively. Performance is represented as unit changes from the predrug score (delta score). The units of measurement for the task are described in Appendix A. A smooth spline fitting procedure was used to generate the curves which connect sequential time points. Zero minutes represent the predrug measurement of the task. (The alprazolam curves are taken from Ellinwood et al. (25, 26)).

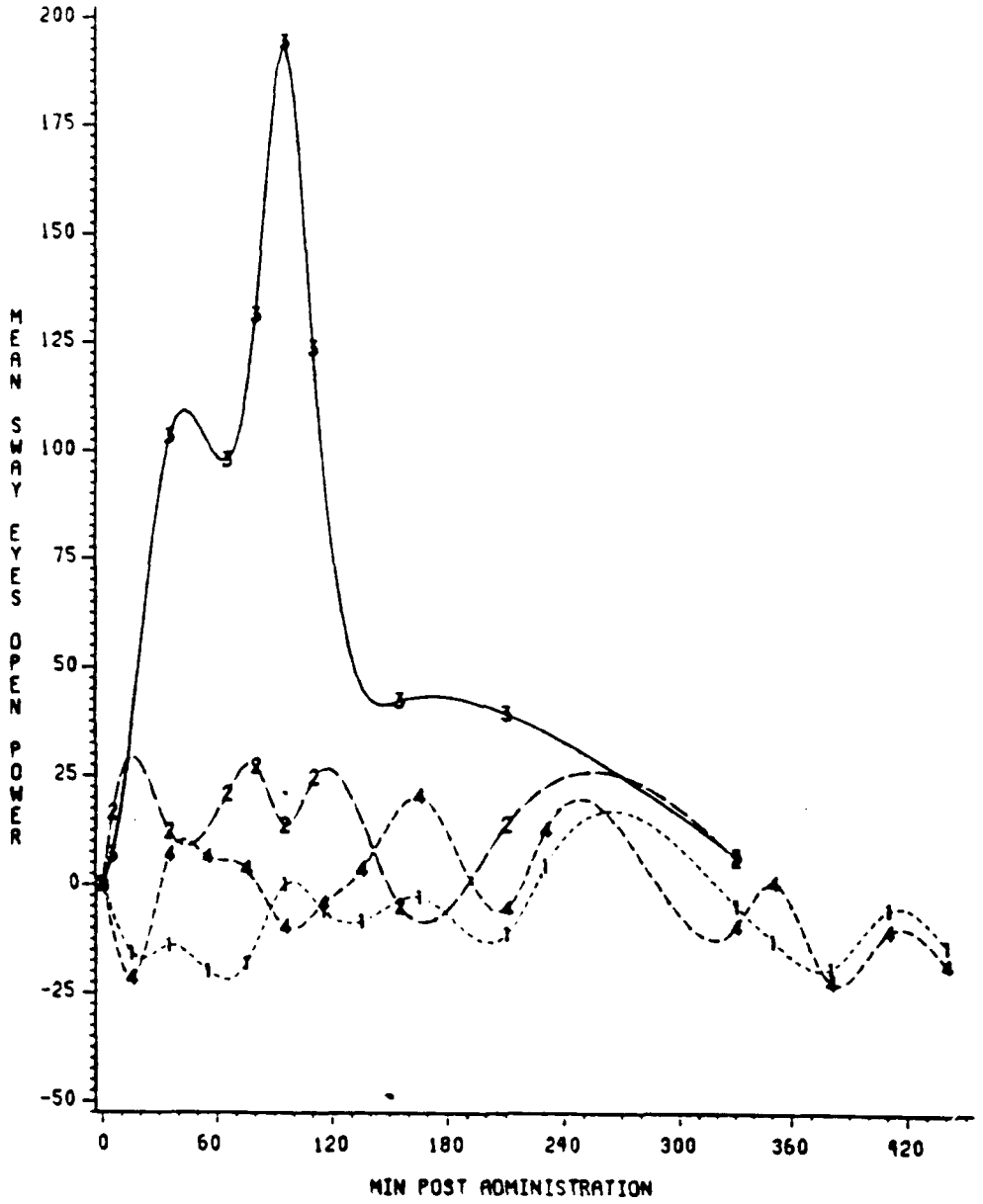

Figure 2. Performance on the sway with eyes open task for atropine (Experiment 1) and alprazolam. The atropine dose was 2.0 mg (4) and the alprazolam dose was 1.0 mg (3). The placebo sessions for the atropine and alprazolam studies are indicated by 1 and 2, respectively. Performance is represented as delta scores. The delta score, units of measurement and curves are described in Figure 1. (The alprazolam curve is taken from Ellinwood et al. (25)).

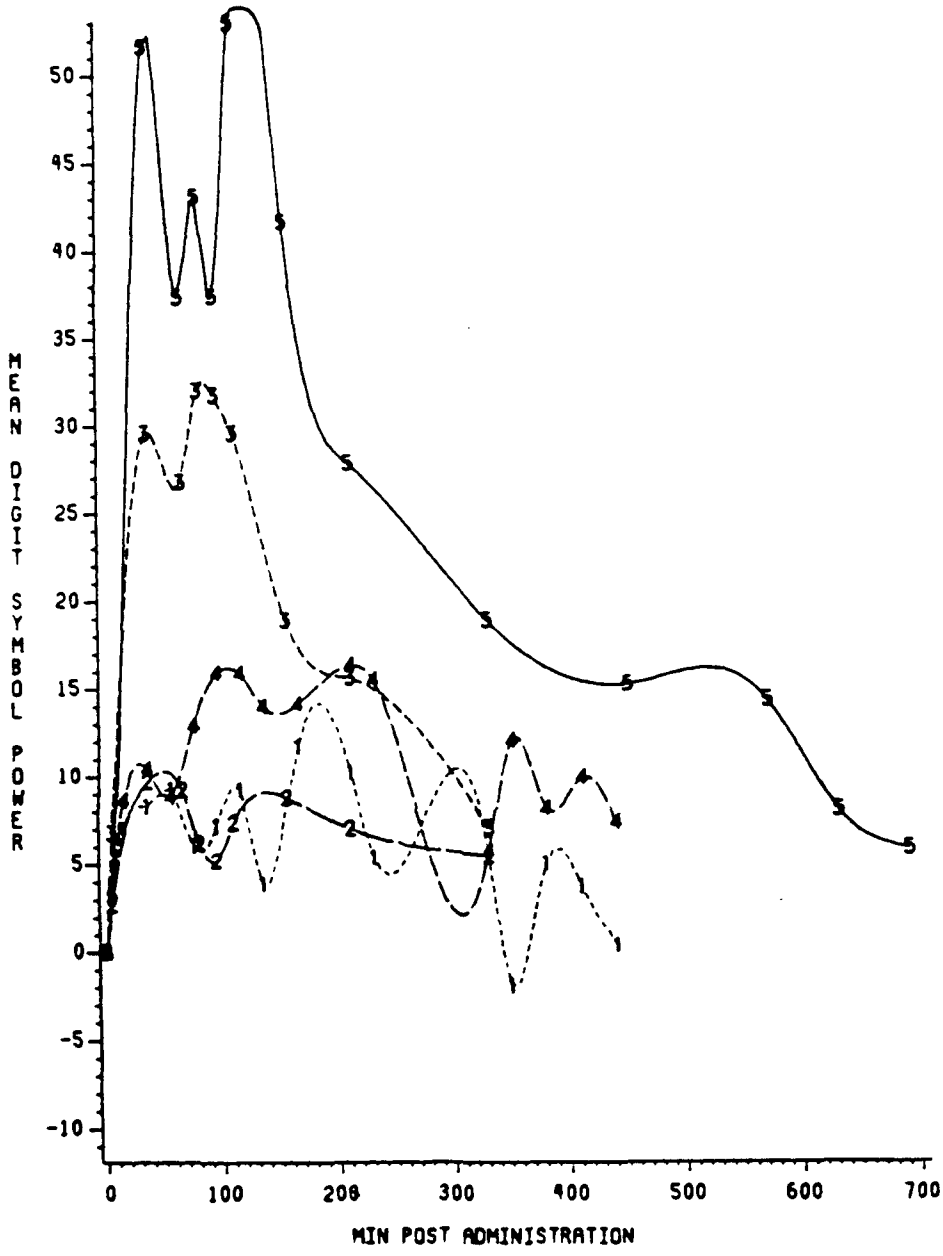

Figure 3. Performance on the DSS task for atropine (Experiment 1) and alprazolam. The atropine dose was 2.0 mg (4) and the alprazolam doses were 1.0 mg (3) and 2.0 mg (5). The placebo sessions for the atropine and alprazolam studies are indicated by 1 and 2, respectively. Performance is represented as delta scores. The delta score, units of measurement and curves are explained in Figure 1. (The alprazolam curve is taken from Ellinwood et al. (25, 26)).

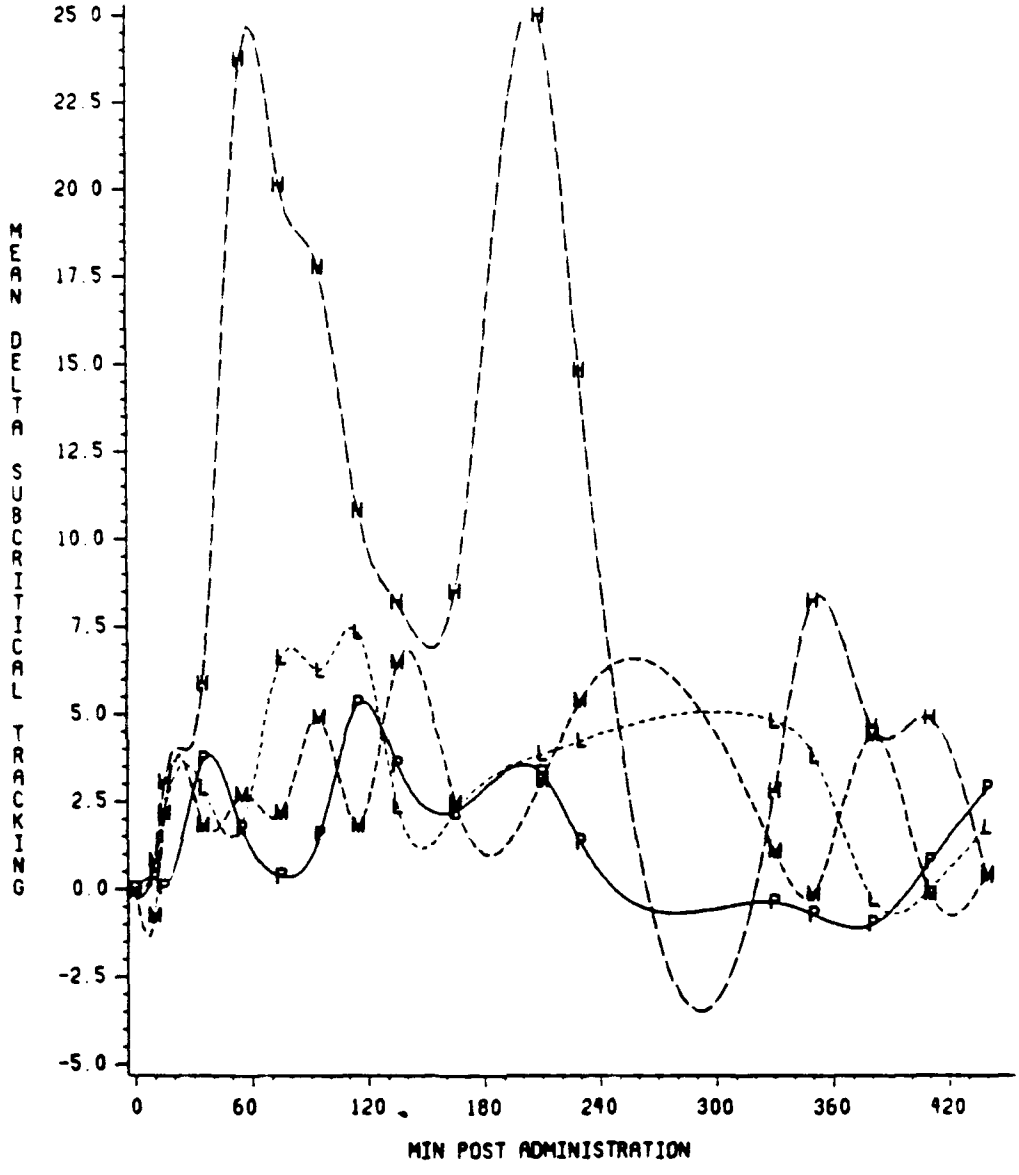

Figure 4. Performance on the SCT task after administration of placebo (P) and 0.5 (L), 1.0 (M) and 2.0 (H) mg of atropine for the eight subjects in Experiment 1. Performance is represented as delta scores. The delta scores, units of measurement for the task and the curves are the same as in Figure 1.

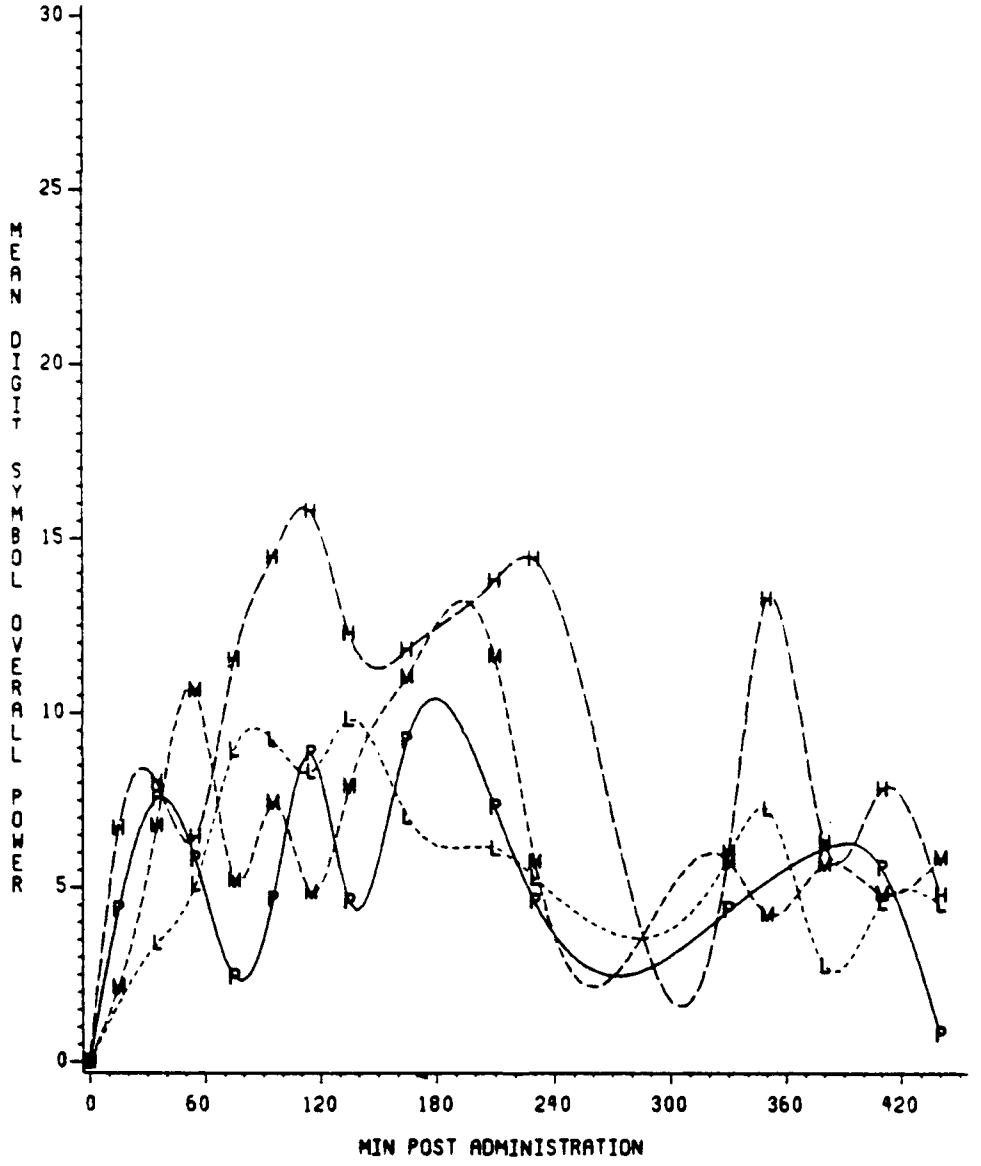

Figure 5. Performance on the DSS task following I.M. injection of placebo (P) and 0.5 (L), 1.0 (M) and 2.0 (H) mg of atropine for the eight subjects in Experiment 1. Performance is represented as delta scores. The delta scores, units of measurement for the task and the curves are described in Figure 1.

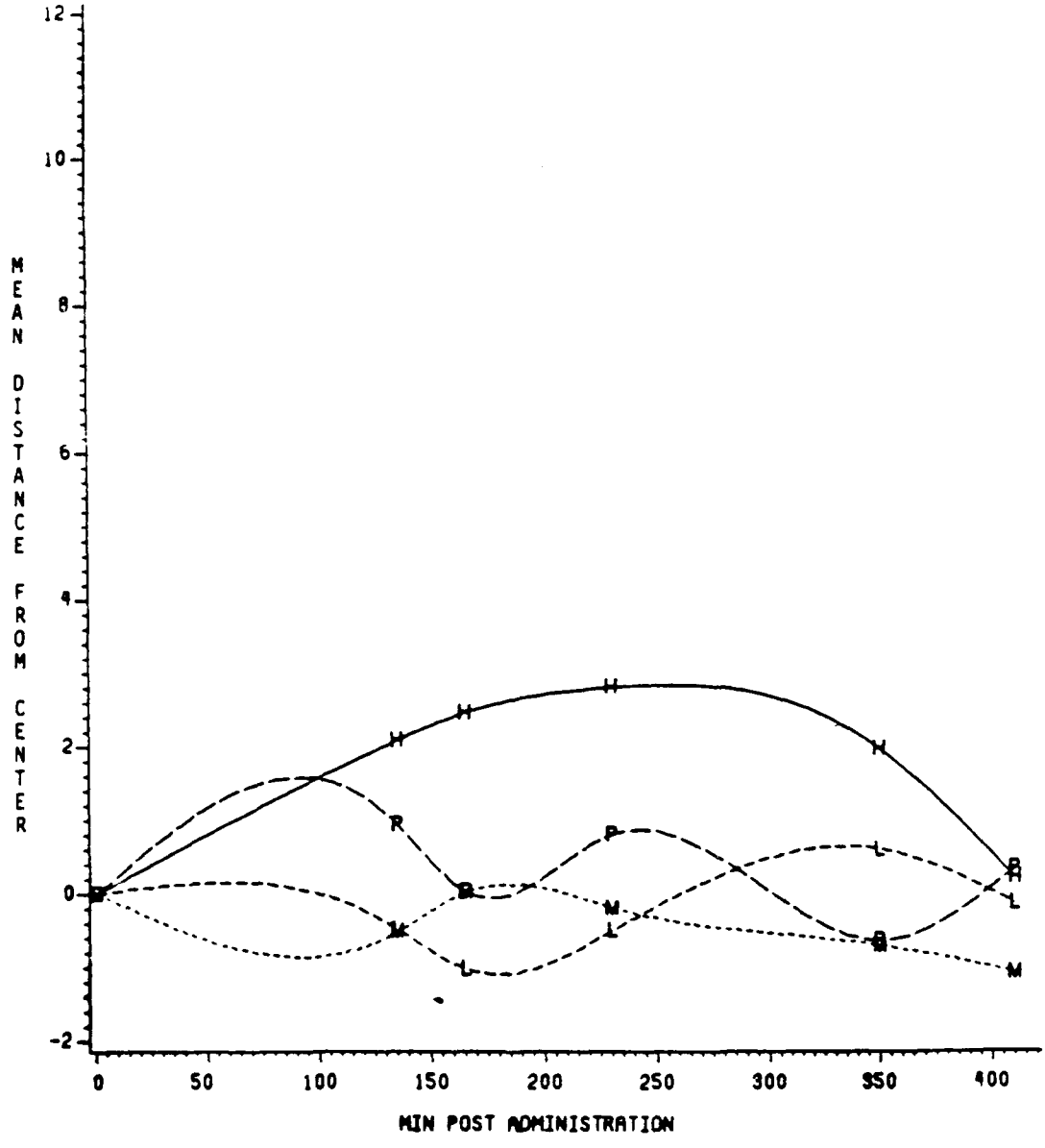

Figure 6. Performance on the sway with visual feedback task for placebo (P) and 0.5 (L), 1.0 (M) and 2.0 (H) mg of atropine for the eight subjects in Experiment 1. Performance is represented as delta scores.An explanation of the delta scores, units of measurement for the task and the curves are presented in Figure 1.

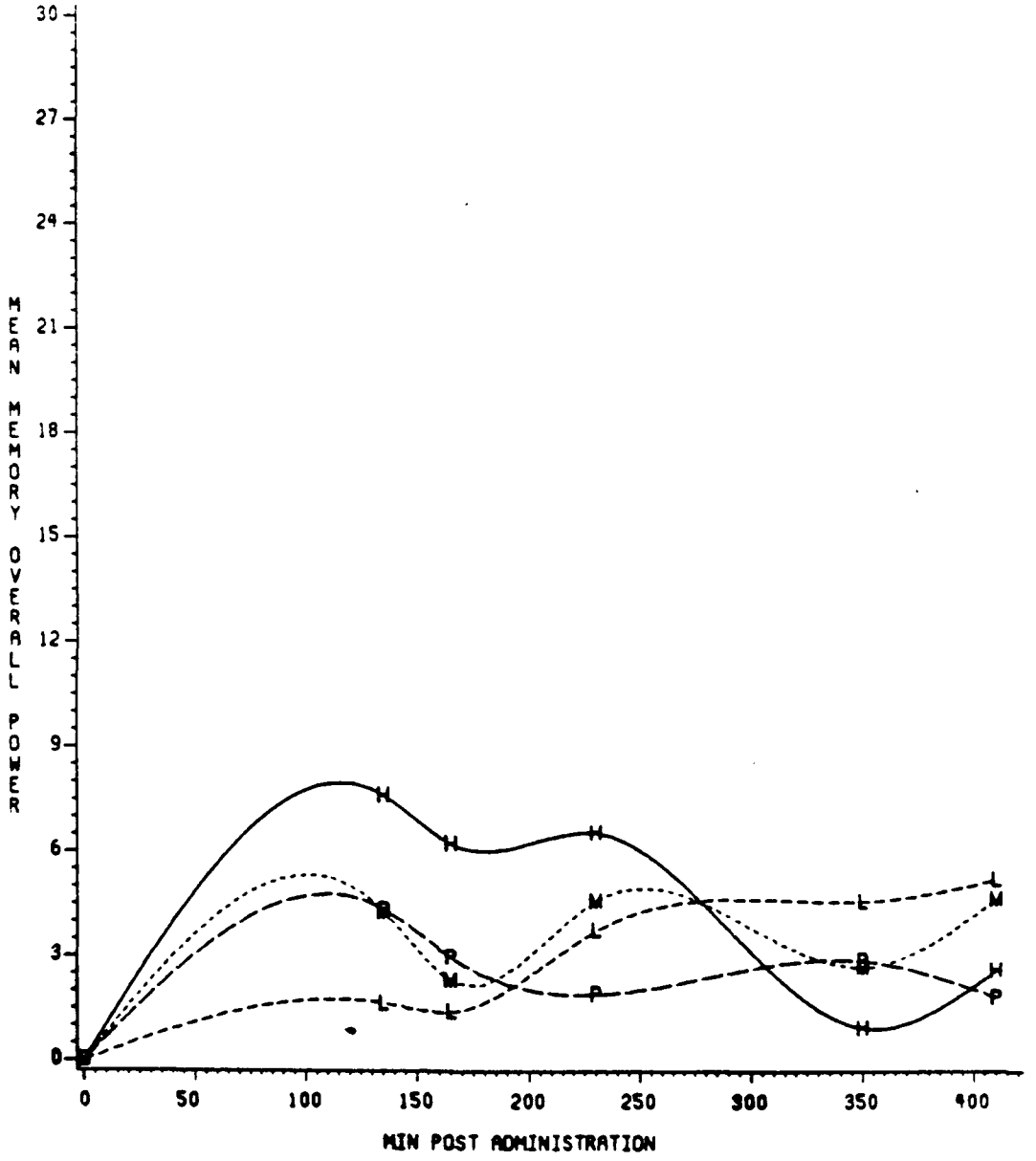

Figure 7. Performance on the recall test of the digit symbol substitution with short term memory task during placebo (P) and 0.5 (L), 1.0 (M) and 2.0 (H) mg atropine drug sessions for the eight subjects in Experiment 1. Performance is represented as delta scores.The delta scores, units of measurement for the task and the curves are described in Figure 1.

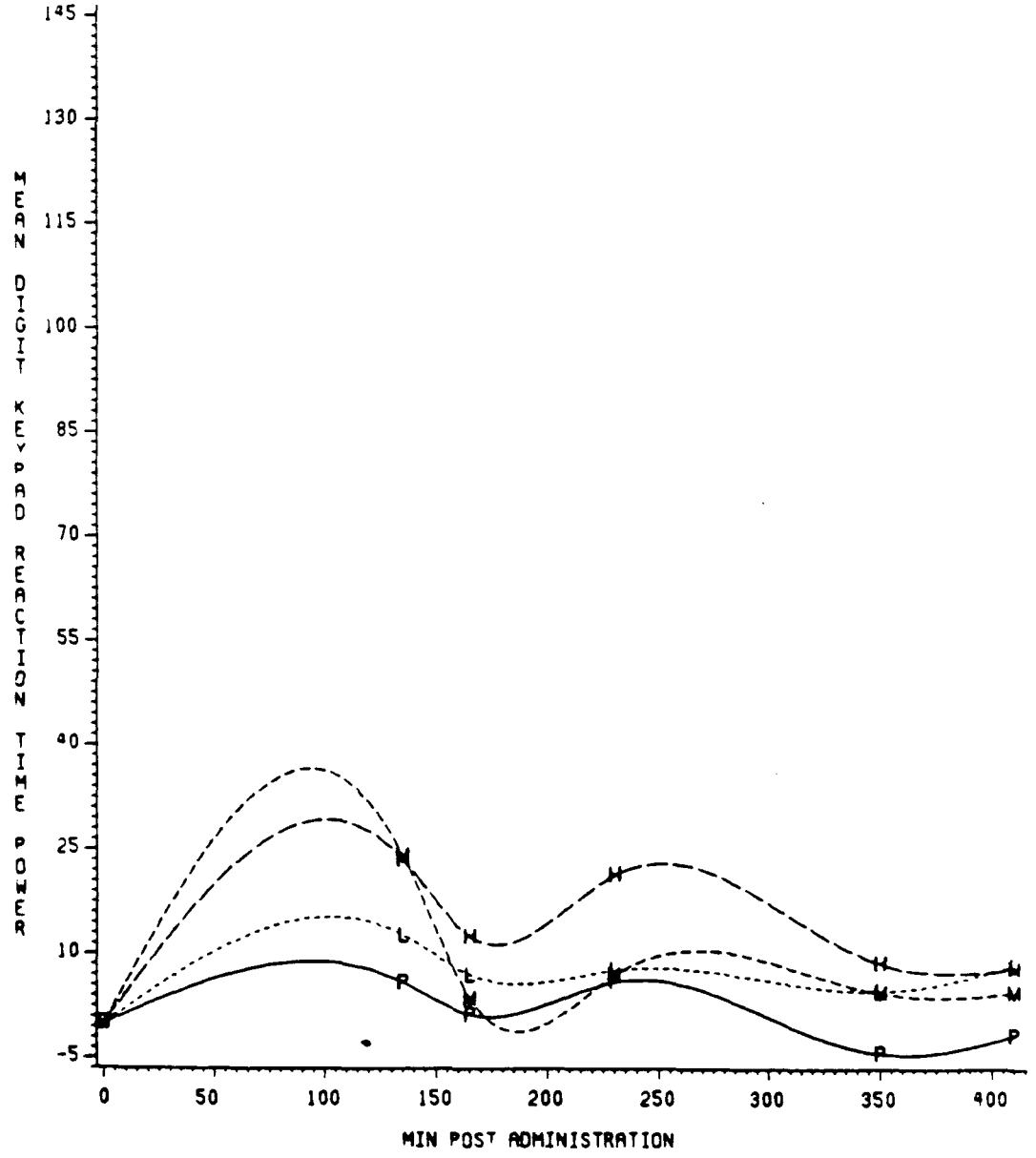

Figure 8. Performance on the digit-keypad reaction time task for the placebo (P) and atropine 0.5 (L), 1.0 (M) and 2.0 (H) mg treatment for the eight subjects in Experiment 1. Performance is represented as delta scores.The delta scores, units of measurement for the task and the curves are explained in Figure 1.

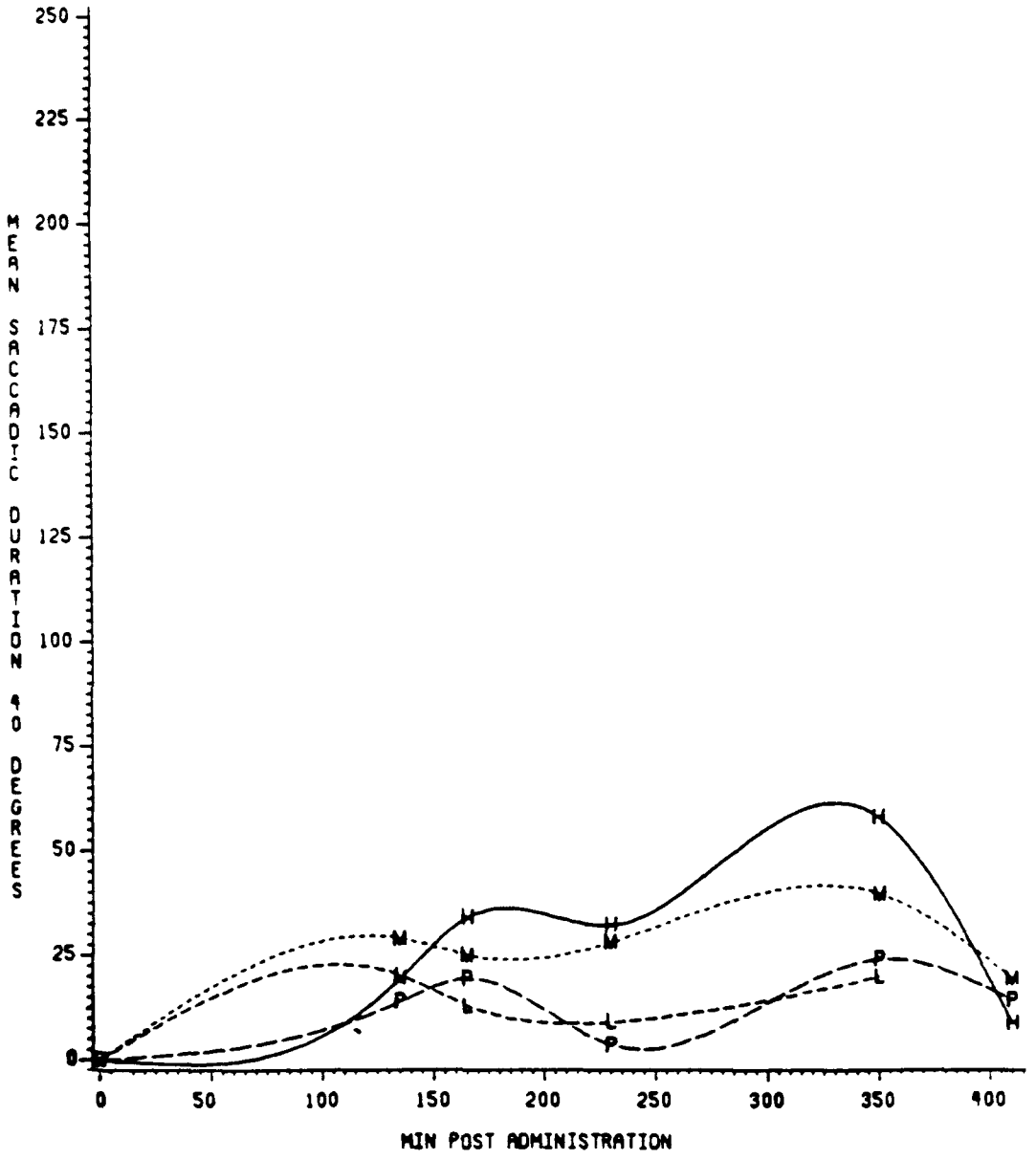

Figure 9. Performance on the sudden displacement eye saccade task after I.M. injection of placebo (P) and 0.5 (L), 1.0 (M) and 2.0 (H) mg of atropine for the eight subjects in Experiment 1. Performance is represented as delta scores.A description of the delta scores, units of measurement for the task and the curves are presented in Figure 1.

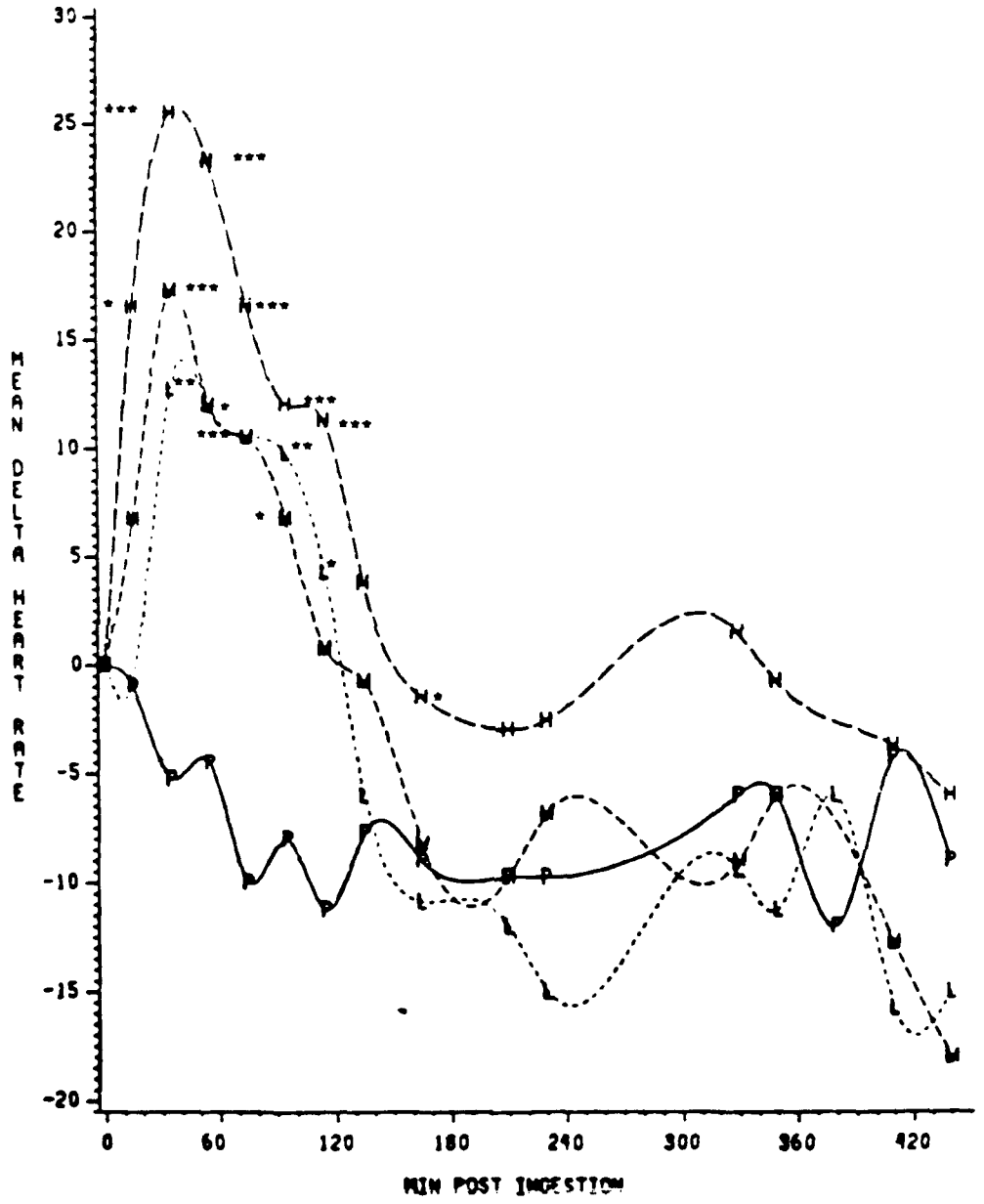

Figure 10. Sitting heart rate measurements (beats per min) during the placebo (P) and 0.5 (L), 1.0 (M) and 2.0 (H) mg atropine test sessions for the eight subjects in Experiment 1. The delta scores and the curves are described in Figure 1. The response measure is explained in the text.

in systolic or diastolic blood pressure, and oral temperature did not change significantly over time. Since there was no significant effect on the cognitive-neuromotor behaviors, sitting heart rate was selected as the pharmacodynamic variable which was used to explore the relationship between the pharmacokinetic and pharmacodynamic properties of atropine. This analysis is discussed further in a later section. Pharmacokinetic-pharmacodynamic modeling of the cognitive and neuromotor behaviors will be conducted as soon as the behavioral and drug concentration data for Experiment 2 become available and are analyzed.

Neuroendocrine Changes

Stress hormone levels were also measured periodically following the various atropine doses during Experiment 1. Plasma AVP, ACTH and cortisol show few, if any, systematic changes across time (Figures 11 to 13). Plasma catecholamine assays have yet to be completed. On the other hand, based on the preliminary results of 4 subjects in Experiment 1, growth hormone appears to demonstrate a time course of responsivity that is very similar to the onset and offset of the atropine plasma levels during the drug treatment sessions (Figure 14). The parallel ascending and descending trends of the changes in atropine drug concentration and growth hormone values across time is further depicted as a hysteresis curve in Figure 15.

Growth hormone secretion is under the control of hypothalamic influences, particularly somatostatin and growth hormone releasing hormone (GHRH). These hormones are under direct neurotransmitter control, and different stimuli activate growth hormone secretion by distinct central mechanisms. For example, stress, physical and psychological, increases growth hormone secretion. Anticholinergic drugs, such as atropine, produce an inhibitory effect on growth hormone secretion when it is stimulated by stress (as in our experimental testing situation) and sleep, but not when stimulted by hypoglycemia. The atropine-growth hormone relationship is under further study. We will be sampling more frequently for this and other hormones and examining more closely in future experiments the relationship of stress hormones to performance and to the effects of atropine on behavioral and physiological variables.

Atropine Pharmacokinetics

Atropine plasma concentration values for Experiment 1 have been determined for six subjects at the 1 mg dose and for four subjects at the 2 mg dose. Mean drug concentration levels are plotted as a function of time following I.M. administration of 0.65 mg and 1.30 mg of atropine as base (equivalent to 1.0 mg and 2.0 mg of atropine sulfate monohydrate) in Figure 16. Application of the F-test indicated that plasma atropine concentration vs. time profiles for individual subjects were

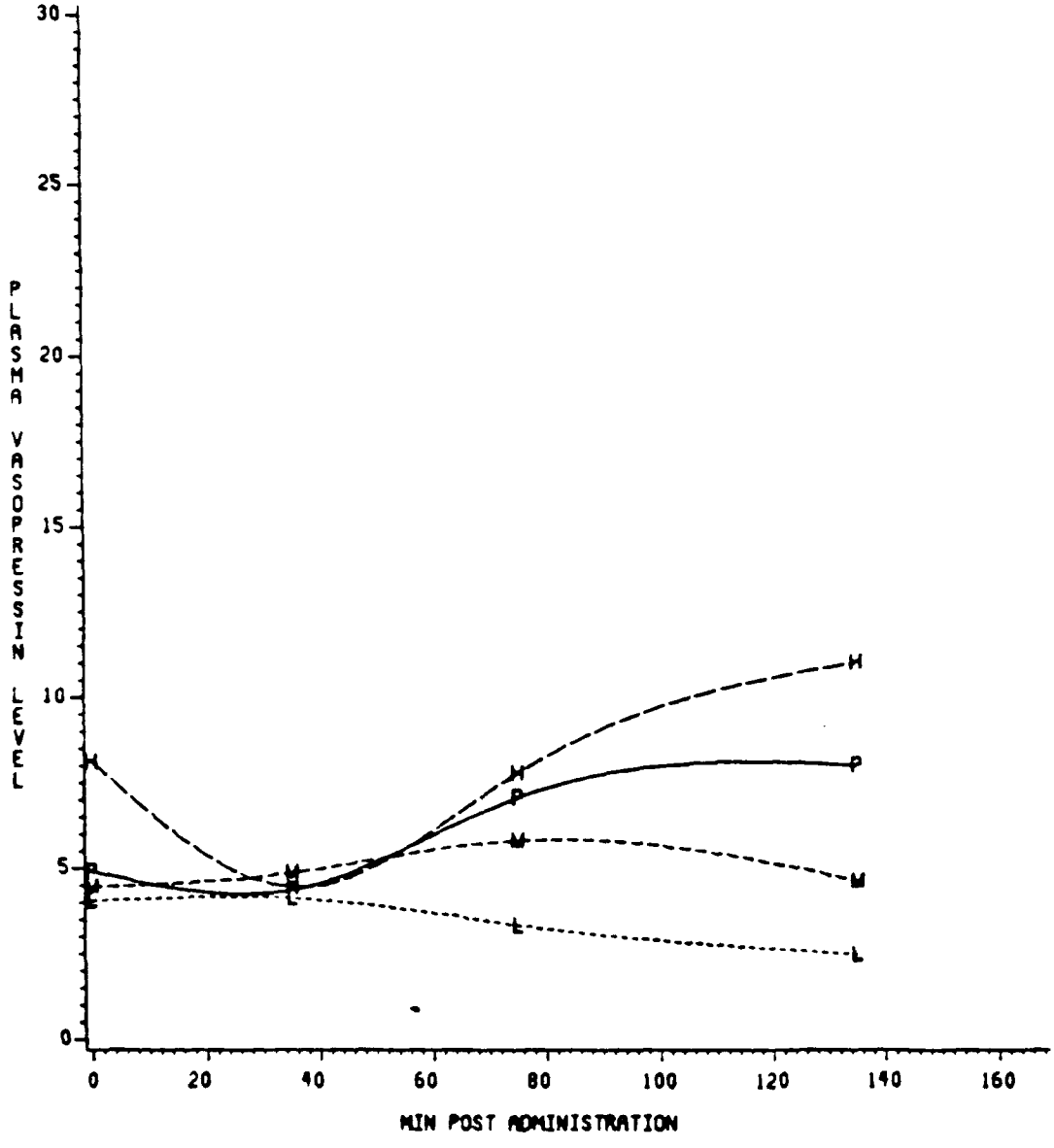

Figure 11. Plasma AVP levels for the placebo (P) and 0.5 (L), 1.0 (M) and 2.0 (H) mg doses of atropine during the drug sessions of the eight subjects in Experiment 1 prior to and at 35, 75 and 135 min after drug administration. The curves were generated by the same method as described in Figure 1.

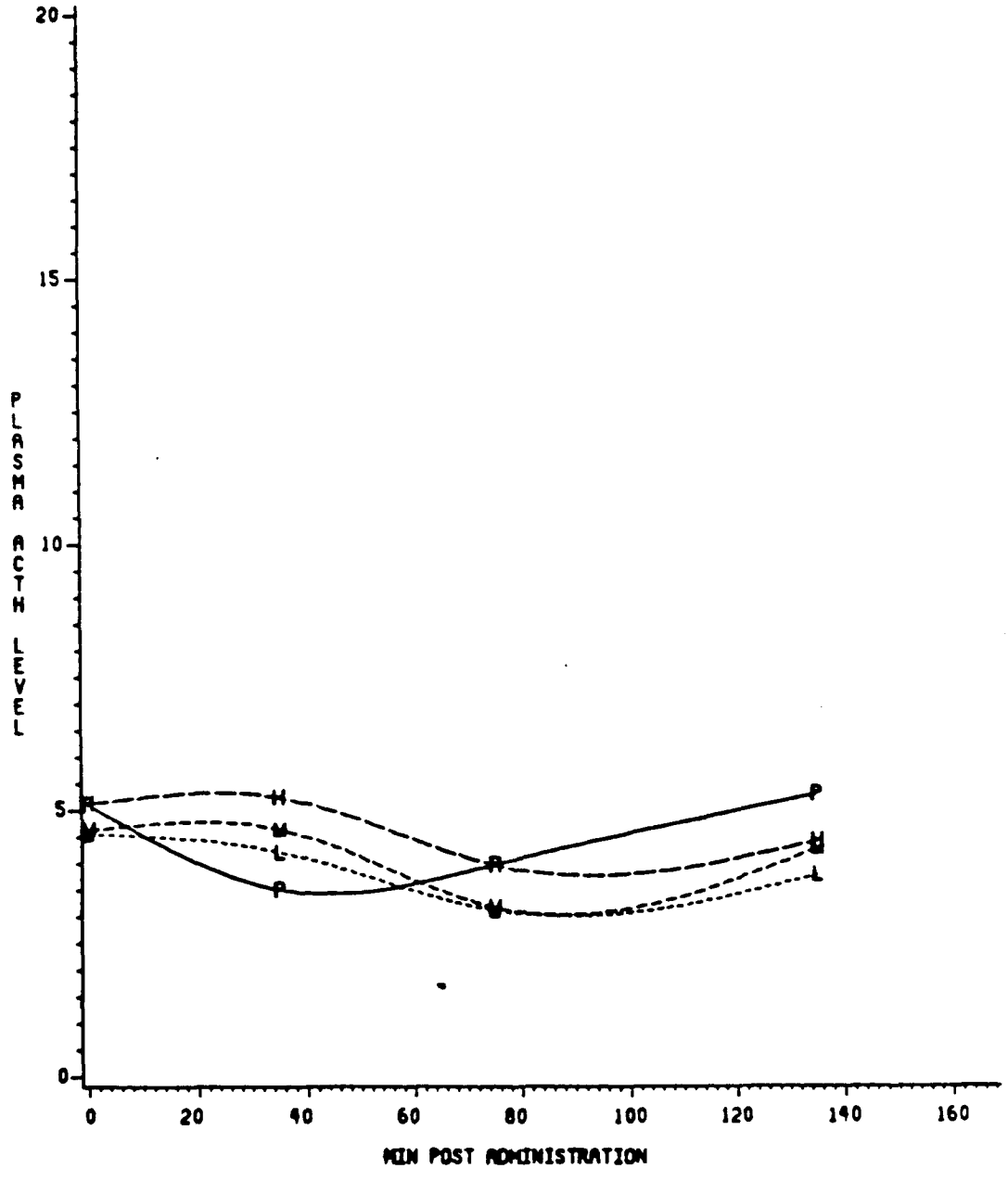

Figure 12. Plasma ACTH levels for the placebo (P) and 0.5 (L), 1.0 (M) and 2.0 (H) mg doses of atropine during the test sessions of the eight subjects in the first study prior to and at 35, 75 and 135 min following drug injection. An explanation of the curves is presented in Figure 1.

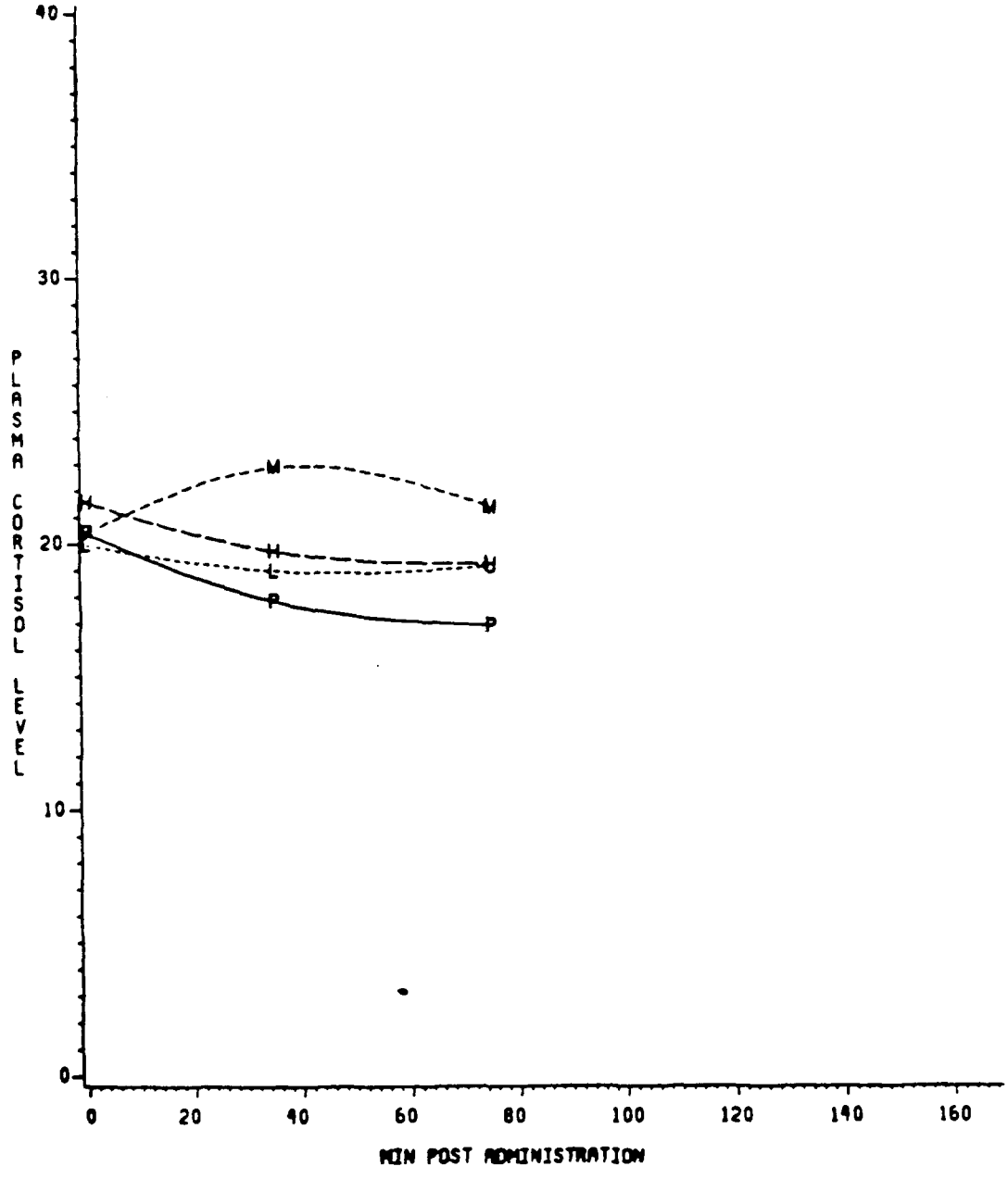

Figure 13. Plasma cortisol levels for the placebo (P) and 0.5 (L), 1.0 (M) and 2.0 (H) mg doses of atropine prior to and at 35 and 75 min after drug administration for the test sessions of the eight subjects in Experiment 1. The curves are described in Figure 1.

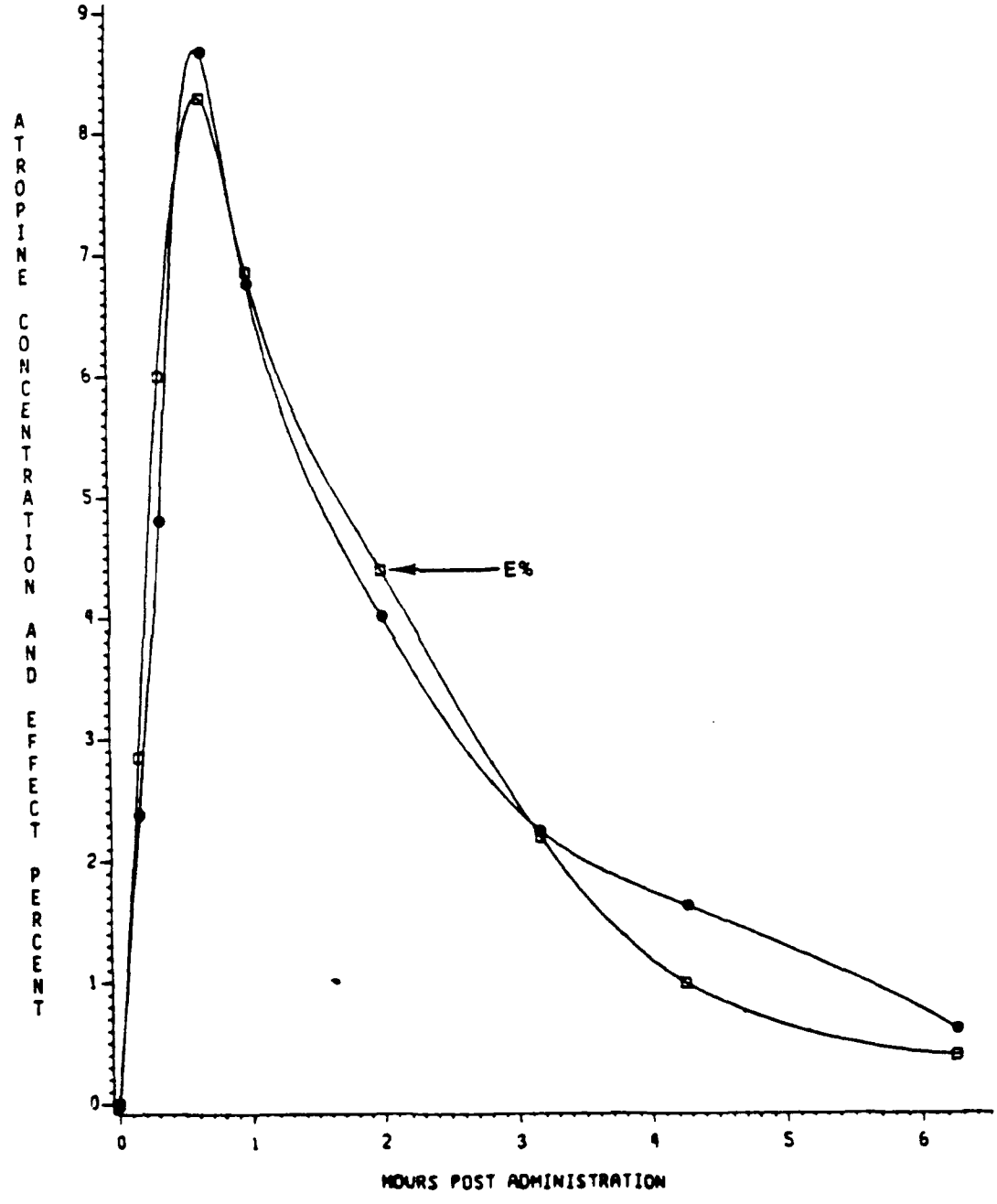

Figure 14. The time course of mean plasma atropine concentration (closed circle) and mean effect percent (open square) following I.M. injection of 2.0 mg of atropine sulfate for four subjects in Experiment 1. Mean effect percent (E%) represents the reduction of the plasma growth hormone levels over time. The times of measurement shown are 0 hr (predrug) and 0.167, 0.333, 0.667, 1.0, 2.0, 3.167, 4.25, and 6.25 hr after drug administration. Construction of the curves are explained in Figure 1.

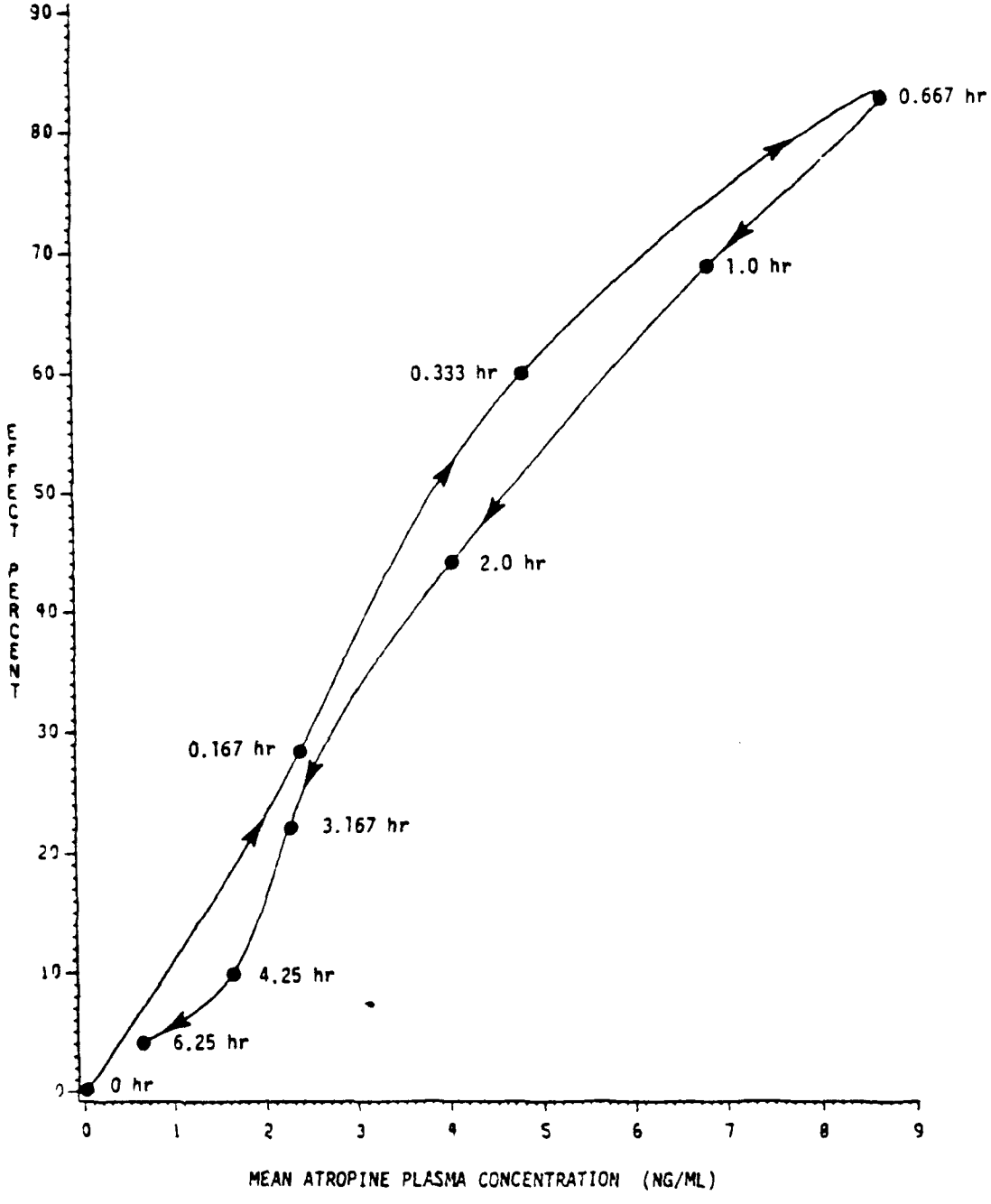

Figure 15. A clockwise hysteresis loop showing the relationship between the mean plasma atropine concentration and effect percent for the data shown in Figure 14. A smooth spline fitting procedure was used to draw the curve and arrows indicate the direction of the consecutive time points at which plasma samples for the atropine and growth hormone assays were drawn. Effect percent is described in Figure 14.

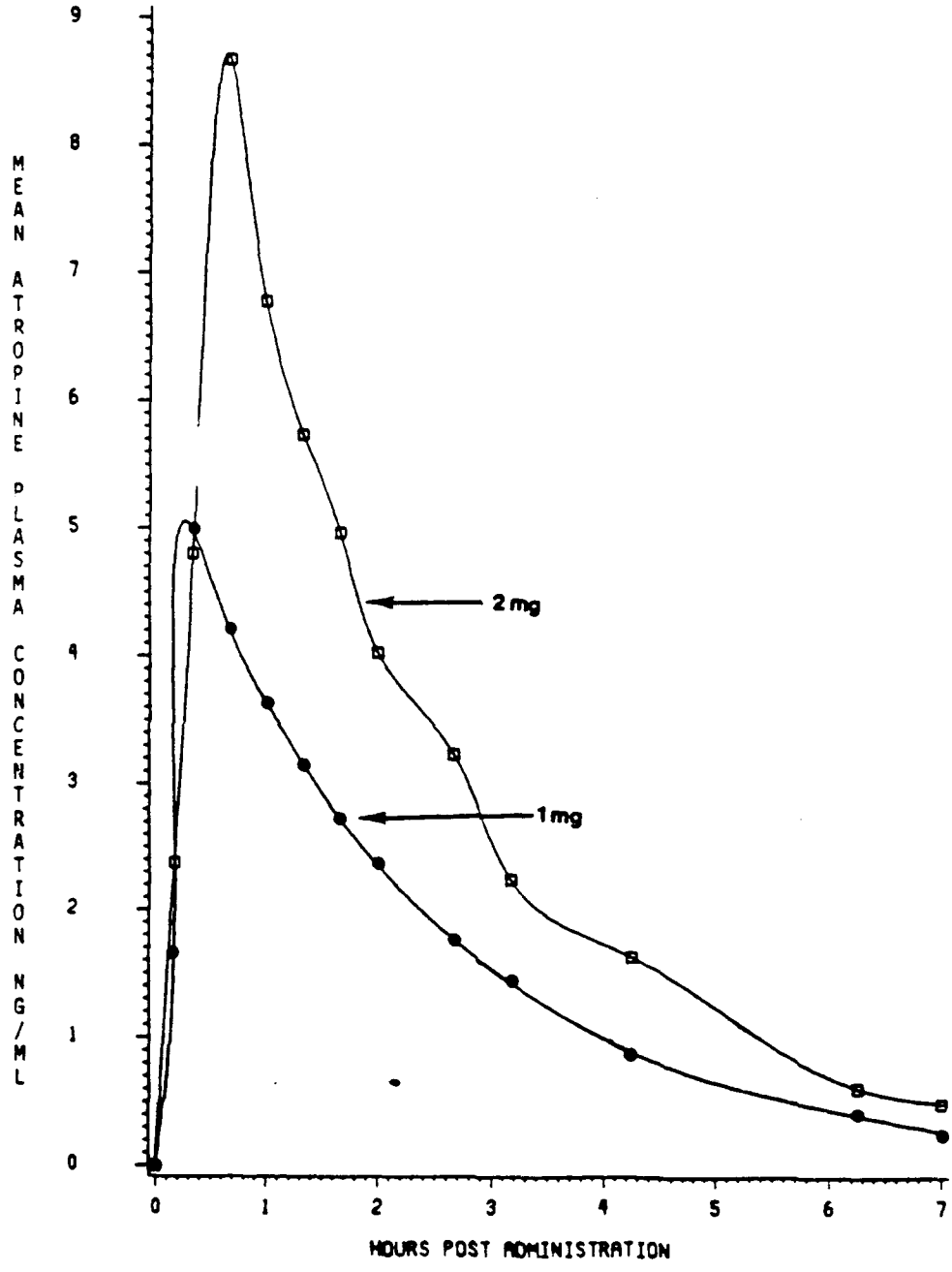

Figure 16. The time courses of the mean plasma atropine concentration following I.M. injection of 1.0 (closed circle) and 2.0 (open square) mg of atropine sulfate. The 1.0 and 2.0 mg curves are based on the analyses of data for six and four subjects, respectively, from Experiment 1. The spline fitted curves are described in Figure 1.

best described by a one-compartment model with very rapid first order absorption. Based on the estimates calculated for the individuals, preliminary average values for the kinetic parameters are summarized in Table 3. The mean t1/2,β of atropine in plasma was 2.04 hr. The mean observed Cmax and tmax values for the 1.0 mg and 2.0 mg dose levels were 4.91 ng/ml at 0.333 hr and 8.67 ng/ml at 0.667 hr, respectively. The Cmax determined from the kinetic parameters in Table 3 for the 1.0 mg and 2.0 mg doses were 5.29 and 8.41 ng/ml, respectively. The average value of the apparent volume of distribution (V/F) was 132.85 L. The mean value of the model-estimated plasma clearance was 45.16/hr. The area under the atropine plasma vs. time curves (AUC), as calculated by the model independent method, increased linearly (17.58± 3.11 ng·hr/ml for the 1 mg dose and 30.96 ± 5.71 ng·hr/ml for the 2.0 mg).

Sensitivity session with multiple dosing procedure. Table 4 lists the mean plasma atropine concentrations for the sensitivity sessions of the eight subjects (ID = A to H) in the first study. Atropine was administered I.M. as follows: 0.25 mg at 0.0 min (09:00), followed by 0.25 mg at 40 min (09:40) and 1.0 mg at 1 hr and 40 min (10:40) after the first dose. In addition, the mean drug levels for the sensitivity sessions of the eight subjects (ID = I to P) in the second study are presented in the same table. The doses used were 0.5 mg at 0.0 min (09:00), followed by 1.0 mg at 40 min (09:40) and 1.5 mg at 1 hr and 40 min (10:40) after the initial injection. The observed mean atropine concentration values in Table 4 and simulated curves based on the estimated pharmacokinetic parameters from Table 3 are presented for the sensitivity session data of Experiments 1 and 2 in Figures 17A and 17B, respectively.

Pharmacokinetic-Pharmacodynamic Relationship

As discussed earlier, consideration of the pharmacokinetic-pharmacodynamic relationship for atropine will be restricted to the sitting heart rate measure in the first study, since this physiological variable was the primary one that showed any significant impairment for the atropine doses used. Average change in heart rate and mean plasma atropine concentration are plotted as a function of time for the 1.0 and 2.0 mg doses in Figures 18A and 18B, respectively. Absorption rate constants based on heart rate data ($t_{1/2,ka}$=2.16 hr^{-1}) and plasma values ($t_{1/2,ka}$=2.93 hr^{-1}) were calculated independently for the 2.0 mg dose and were almost identical (Figure 18B).

A comparison of the relationship between heart rate and atropine plasma concentration during the terminal phase of the curve in Figure 18B is illustrated in Figure 19. Mean changes in heart rate for the final four testing time points were represented as a function of drug levels and the linear

Table 3

Average Pharmacokinetic Parameters[a] for Atropine Following Intramuscular Administration in Experiment 1

Pharmacokinetic Parameters		Mean[a] ± SD
ka	(hr^{-1})	2.93 ± 0.15
$t_{1/2,ka}$	(hr)	0.238 ± 0.03
K	(hr^{-1})	0.34 ± 0.05
$t_{1/2,K}$	(hr)	2.04 ± 0.23
MRT	(hr)	2.96 ± 0.41
MAT	(hr)	0.38 ± 0.02
tmax (1 mg dose)	(hr)	0.333 ± 0.18
tmax (2 mg dose)	(hr)	0.667 ± 0.22
Cmax (1 mg dose)	(ng/ml)	8.67 ± 1.32
Cmax (2 mg dose)	(ng/ml)	4.91 ± 0.76
CLp/F	(L/hr)	45.16 ± 5.03
V/F	(L)	132.85 ± 18.92

[a] The Harmonic mean was calculated for $t_{1/2,ka}$ and $t_{1/2,K}$

ka = absorption rate constant

$t_{1/2,ka}$ = absorption elimination half-life

K = elimination rate constant

$t_{1/2,K}$ = elimination half-life

MRT = Mean residence time
MAT = Mean absorption time
Cmax = maximum plasma concentration
tmax = Time to reach Cmax
CLp = Total plasma clearance
F = systemic availability
V = Volume of distribution

Table 4

Atropine Plasma Levels for the Sensitivity

Sessions of Experiments 1 and 2

Subject ID	Plasma Atropine Concentration (ng/ml)				
	Time (h) / Plasma Sample Number				
	0.0/01	0.667/02	1.667/03	2.667/04	7.0/05
YA01	0.0	1.38	2.32	6.61	1.49
YB01	0.0	1.62	2.57	7.58	1.92
YC01	0.0	1.02	2.01	5.24	0.95
YD01	0.0	1.40	2.20	7.01	1.70
YE01	0.0	2.01	3.12	8.34	2.45
YF01	0.0	1.42	2.15	6.42	1.17
YG01	0.0	0.83	1.48	4.96	0.88
YH01	0.0	1.36	2.25	7.49	1.48
Mean	0.0	1.38	2.26	6.71	1.51
SD	0.0	0.36	0.47	1.16	0.52
YI01	0.0	2.76	6.88	12.40	2.91
YJ01	0.0	3.31	8.02	14.95	4.12
YK01	0.0	2.70	6.56	12.88	2.68
YL01	0.0	1.92	4.95	10.33	1.87
YM01	0.0	2.05	5.83	11.86	2.42
YN01	0.0	1.82	6.12	12.55	2.95
YO01	0.0	4.55	8.84	13.90	4.42
YP01	0.0	3.05	7.86	10.86	1.97
Mean	0.0	2.77	6.88	12.47	2.92
SD	0.0	0.90	1.29	1.51	0.93

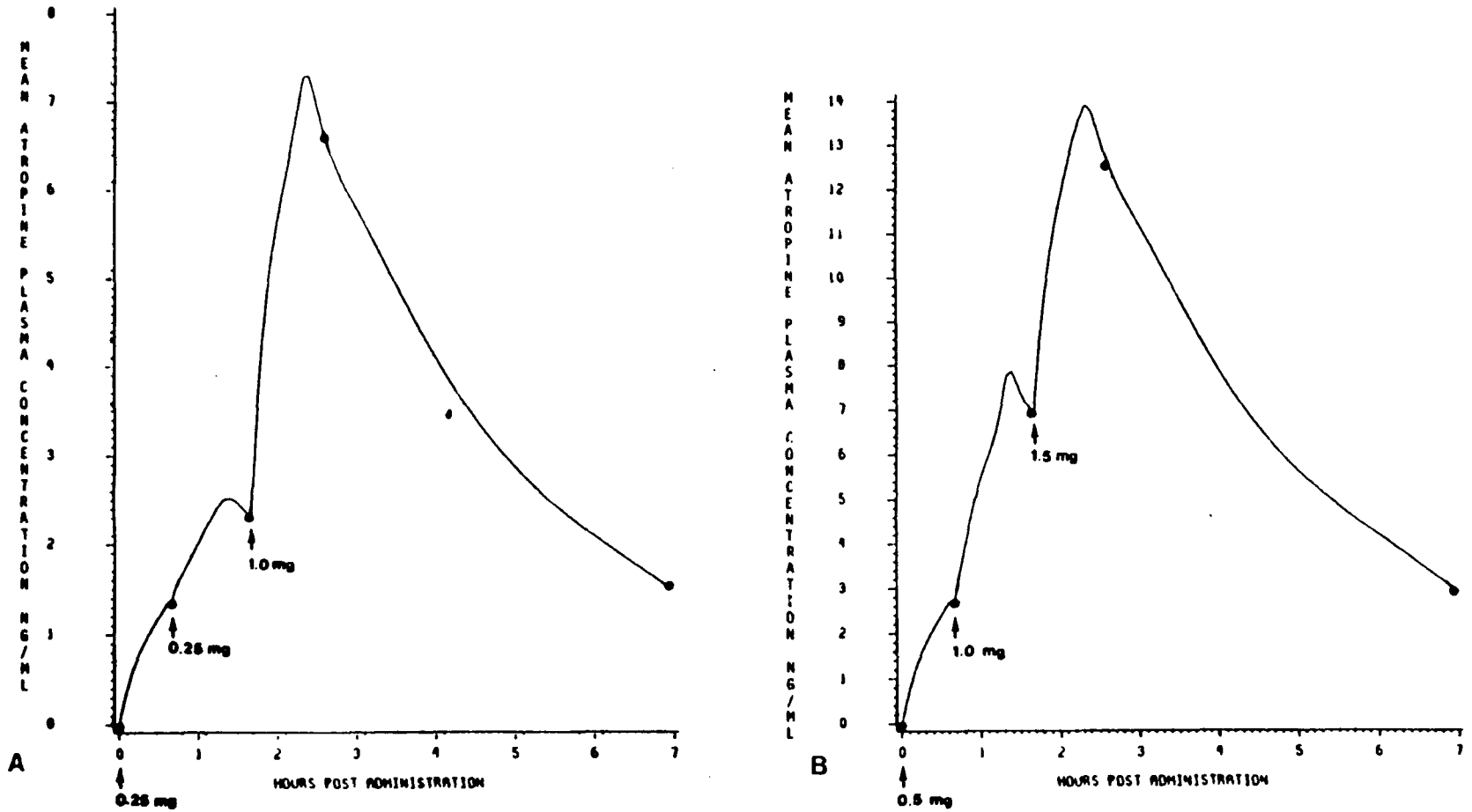

Figure 17. Pharmacokinetic profile of the changes in atropine drug levels over time during the sensitivity sessions for Experiments 1 (A) and 2 (B). The time points at which the three doses were administered for each study are indicated on the curves. Table 1 lists the time intervals at which the plasma samples were taken for the sensitivity sessions. The solid circles represent the mean observed atropine concentrations for the eight subjects in each study (taken from Table 4) and the curves are simulated lines based on the pharmacokinetic parameters of Table 3.

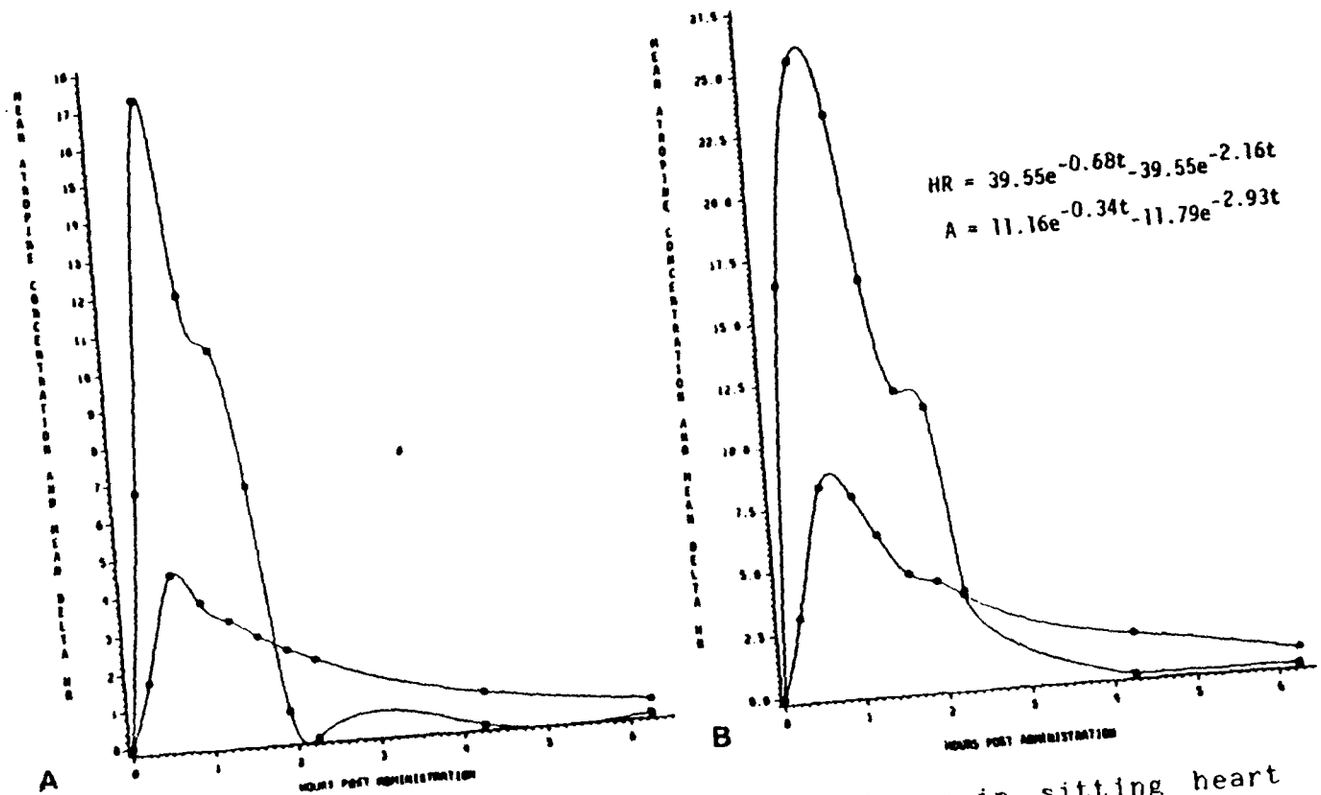

Figure 18. The time courses of the mean change in sitting heart rate (open square) and mean plasma atropine concentration (closed circle) for the 1.0 (A) and 2.0 (B) mg doses. HR=heart rate (bpm). A=Atropine plasma concentration. The 1.0 and 2.0 mg curves represent the results for six and four individuals, respectively, from Experiment 1. The times of measurement shown are 0 hr (predrug) and 0.25, 0.58, 0.92, 1.25, 1.58, 1.92, 2.25, 4.25, and 6.25 hr after drug injection. The delta scores and curves are explained in Figure 1 and the heart rate measure is described in the text.

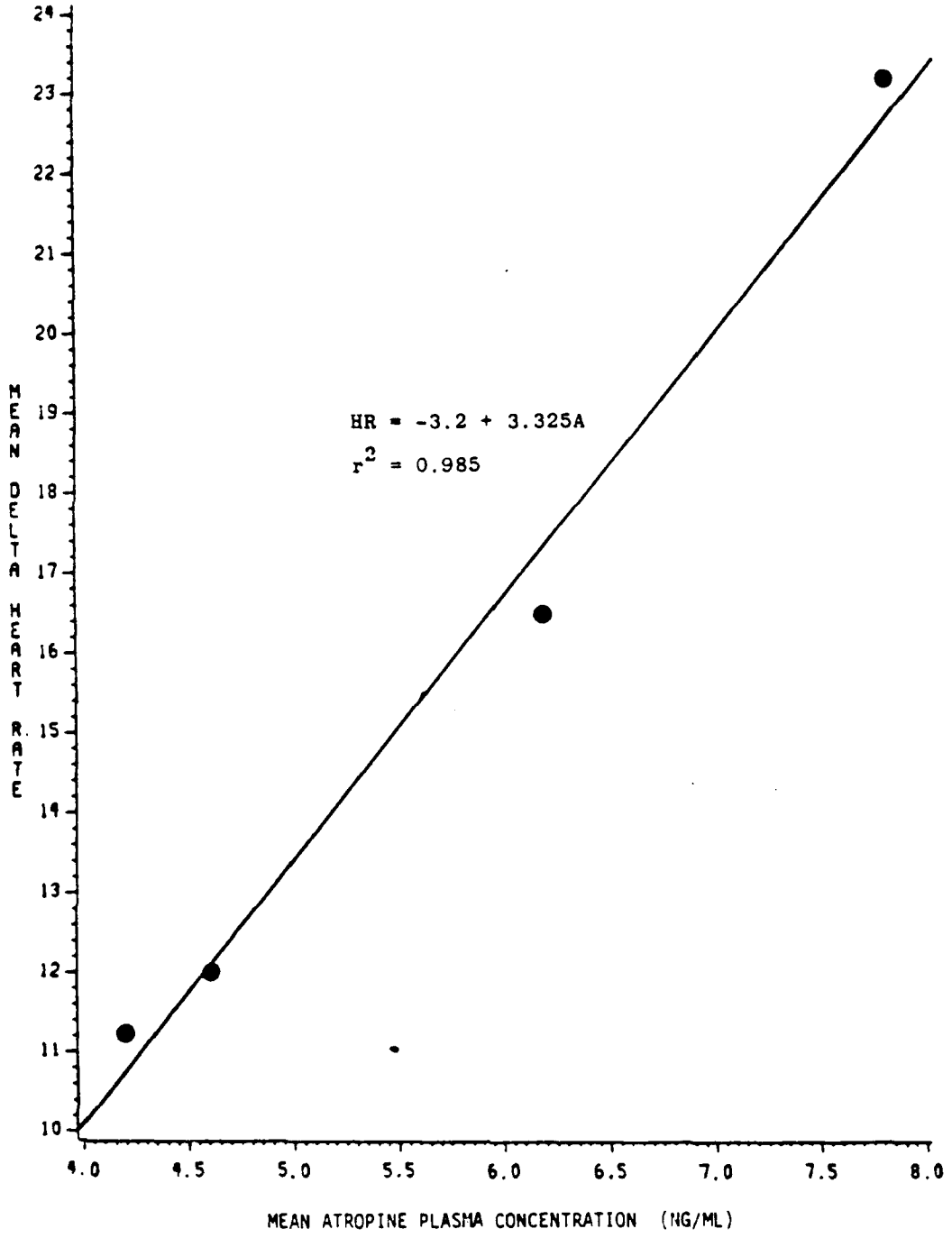

Figure 19. The best fitting linear regression model describing the relationship between mean change in sitting heart rate and atropine plasma concentration for the final four testing time points of Figure 18B after I.M. injection of 2.0 mg. The units of measurement and delta scores are explained in Figure 1 and the text. The equation for this line is shown on the plot. HR=mean heart rate expressed as bpm. A=mean atropine plasma concentration.

regression procedure was used to determine the best fitting line through these points. The equation of the least squares regression line is as follows:

$$HR = -3.20 + 3.325\ C$$

where HR is the average change in heart rate (bpm) and C is the average plasma atropine concentration (ng/ml). The correlation ($r=0.992$, $p<0.01$) between the heart rate and drug level means suggests a very high degree of correspondence between these two measures. Finally, Figures 20A and 20B indicate a clockwise hysteresis relationship between mean heart rate change and average plasma atropine concentration for successive testing time intervals for 1.0 and 2.0 mg, respectively.

V. Discussion

The pharmacokinetic results of Experiment 1 indicate that atropine is absorbed rapidly following I.M. administration with peak drug concentrations appearing at 0.333 hr for the 1.0 mg dose and at 0.667 hr for the 2.0 mg dose. The discrepancy in tmax may be caused by the binding of drug molecules to the tissue at the administration site for the higher dose; consequently, the 2.0 mg dose required a longer time to diffuse. However, the tmax observed in the present study for both doses was comparable to values found in the literature for similar dosages (27, 28). Moreover, the elimination half-life of 2.04 hr obtained in Experiment 1 corresponded to the values of 2 hr and 2.5 to 3 hr reported by others (29-31). Unlike tmax, the terminal phase half-life was not dose-dependent.

The disposition kinetics of atropine appears to be described best by a one-compartment model. The large volume of distribution (132.85 L) in Experiment 1 is consistent with the application of the one-compartment model to estimate the atropine pharmacokinetic parameters. Some authors have even reported a distribution half-life that is as rapid as 1 min (32). The I.M. clearance of atropine was about 45.16 L/hr, which remained fairly constant for the 1.0 mg and 2.0 mg doses. There was no suggestion of a non-linear relationship between the pharmacokinetics of the 1.0 and 2.0 mg doses of the first study.

None of the dose levels in the first study induced significant or substantial impairment on any of the cognitive and neuromotor tasks. Results from previous studies obtained similar results for comparable doses, particularly with complex tasks (2, 5). Since there is evidence that the CNS effects of atropine are dose-dependent (6), higher doses will probably produce significant impairment of cognitive-neuromotor

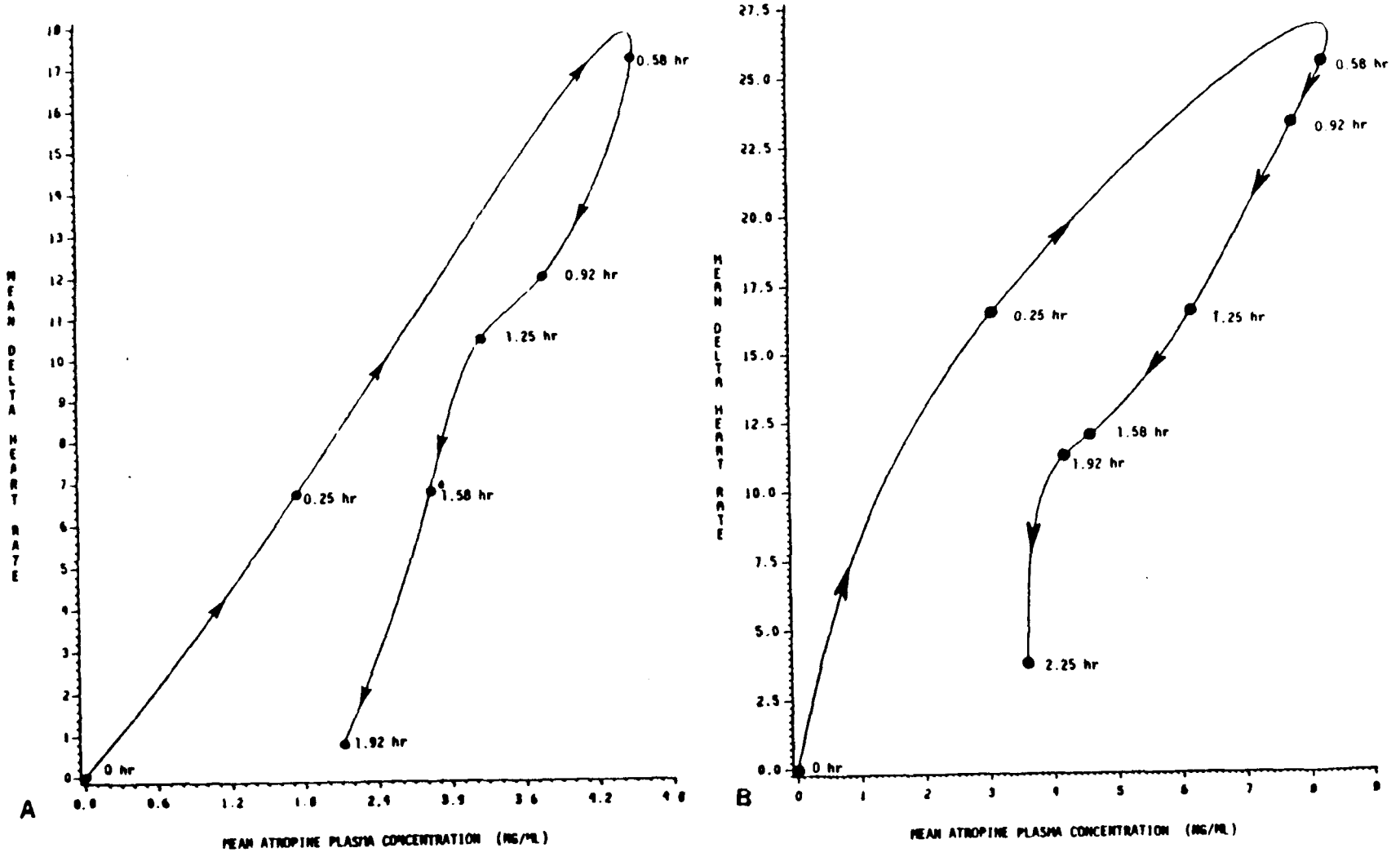

Figure 20. Clockwise hysteresis relationship between mean atropine drug levels and mean change in sitting heart rate following I.M. administration of 1.0 (A) and 2.0 (B) mg of atropine. The data and delta scores are described in Figure 18. The hysteresis curve is described in Figure 15. The time points cover the testing period from 0 min to the time point at which heart rate returned to baseline level for the 1 and 2 mg doses.

performance. In order to test this hypothesis, we are currently conducting further studies in which we are administering 4 mg of atropine to young healthy males.

Heart rate and growth hormone were the only variables that were affected substantially by the atropine doses used in Experiment 1. There was a very high correlation between plasma atropine levels and heart rate change. The observed tmax for this physiological variable corresponded to the tmax of atropine Cmax (Figures 18A and 18B). According to previous studies (33, 34), intermediate dose levels of atropine increase heart rate, whereas low doses often transiently decrease the heart rate. Our results suggest that for 0.5, 1.0 and 2.0 mg of atropine, there is a direct relationship between increases in both atropine level and heart rate. Interestingly, between 140 and 230 min after drug administration, the 0.5 mg dose produced a decrease in heart rate below the corresponding level of the heart rate for the placebo condition. At 410 and 440 min post drug both 0.5 and 1.0 mg of atropine induced lower heart rates than the placebo.

The clockwise hysteresis loop in Figure 20A and 20B for sitting heart rate and drug concentration is pathognomic of acute tolerance at a moderate level. The possible mechanisms for this phenomenon include changes in receptor sensitivity or number, the accumulation of an unknown metabolite (20) which antagonizes the action of atropine at the receptor site, or receptor kinetics and adaptation (26). Ellinwood et al. (26) reported a considerably greater degree of hysteresis for the effect of diazepam on behavioral performance, suggesting that there would be a higher tendency for acute tolerance to develop with cognitive-neuromotor impairment. An important question, therefore, is whether a similar degree of acute tolerance will be observed at higher doses for the atropine effect as for diazepam. This question will be considered in the analysis of the performance data from Experiment 2, which will be using a 4 mg dose (twice the highest dose in Experiment 1).

There was very little hysteresis in the relationship between growth hormone and atropine plasma levels, and the data suggests a close correspondence in the changes over time in the values for these two variables during the drug sessions. As discussed earlier, two competing processes may be influencing the activation of the stress hormones. On the one hand, stressful situations, such as the test session, stimulate the production of these hormones. On the other hand, anticholinergic drugs, including atropine, have an inhibitory effect on the same hormones. A more complete understanding of the interaction between a stressful environment, the use of antimuscarinic drugs and the activation or inhibition of the stress hormones may contribute significantly to elucidating the nature of the effect of atropine on cognitive and neuromotor performance, especially since the activation of different

hormones in response to stress is an integral part of the total behavioral response pattern being measured during the test sessions.

VI. Recommendations

The results of the present study indicate that 0.5, 1.0 and 2.0 mg of atropine did not significantly impair performance on the cognitive and neuromotor tasks used in the present study. The lack of effect may be due to a number of factors: 1) there may be considerable interindividual variability in performance; 2) the atropine doses used in Experiment 1 may be located in the lower asymptotic portion of the dose-response curve; and 3) atropine inhibits activation of the stress hormones and may actually facilitate improvement in performance at the lower doses. In order to address these issues we plan to conduct the following studies:

1. We are currently conducting a study which includes the use of a higher dose of atropine, that is, 1.0, 2.0 and 4.0 mg, to 16 young healthy males. This second study serves two primary purposes. First, the 4 mg dose will allow us to determine the magnitude of impairment induced by higher atropine doses. This is important since the doses of Experiment 1 may represent the lower asymptotic part of the dose-response curve, and the 4 mg dose will allow us to sample from the linear segment of the dose-response curve. Secondly, by increasing the number of subjects we will be able to determine the degreeof interindividual variability in the effect of atropine on cognitive and neuromotor performance. If there is considerable variance in sensitivity to the drug effect, then individuals should be screened for unusual sensitivity to adverse side effects.

2. We plan in the next year to continue development of additional cognitive and neuromotor tasks for possible inclusion in our drug task battery, as described in our original proposal. Thus far, we have completed development of the sudden displacement eye saccade, continuous alternation eye saccade and nystagmometry tasks and are currently working on the hand tremor, hand rapid alternation, finger rapid alternation, pupil diameter, near point of vision, dichotic listening and visual signal detection in a noisy background tasks. Using the basic experimental protocol described for Experiments 1 and 2, we will also determine the sensitivity of the new tasks to the different doses of atropine. We are particularly interested in tasks that may be sensitive to the lower doses. We will continue to compare the drug effects on the behavioral tasks with those on the physiological measures as well as examine the relationship of the pharmacokinetic and pharmacodynamic aspects of atropine for the new tasks.

3. The implications of the influence of atropine on the activation of the stress hormones will be more completely understood when we have completed our investigation of the effect of the higher 4 mg atropine dose on these and other hormones. Therefore, we will continue to measure and analyze the relationship of the stress hormones, such as AVP, ACTH and growth hormones, to atropine induced impairment and/or improvement in the studies that will be conducted within the next year. The study of the influence of atropine on the stress hormones will also provide the needed baseline data for the assessment of the blocking action of atropine on cholinergic agonists when that phase of the project is in operation.

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APPENDIX A

Performance Tasks and Analyses

In order to assess changes in performance at relatively frequent intervals, especially during the period immediately after drug administration, two shorter batteries consisting of selected performance tasks are used to estimate more precisely the rapidity of the onset of impairment. Thirteen tasks used to assess different types of cognitive-neuromotor impairment are included in the long battery. The three testing batteries are as follows: (1) an ultra-short battery consisting of only the subcritical tracking task; (2) a medium battery, including standing steadiness with eyes open, digit symbol substitution, subcritical tracking, and abbreviated digit symbol memory tasks; and (3) a long battery consisting of standing steadiness with visual feedback, standing steadiness with eyes open, and standing steadiness with eyes closed, digit symbol substitution, subcritical tracking, continuous performance, divided attention, digit symbol substitution with short term memory, digit-keypad reaction time, sudden displacement eye saccade, central pendulum eye tracking, and lateral pendulum eye tracking tasks.

During the first year of the present project major changes have been introduced in the procedure programs of the subcritical tracking, continuous performance and divided attention tasks and in the analyses programs of the standing steadiness with eyes open and eyes closed, digit symbol substitution with short term memory, abbreviated digit symbol memory, subscritical tracking, continuous performance, divided attention, central pendulum eye tracking, lateral pendulum eye tracking and concrete noun memory tasks. A new task, sudden displacement eye saccade, was also added to the long task battery. A computerized procedure for recording heart rate has been created and is being used in Experiment 2.

STANDING STEADINESS TASK - EYES OPEN OR CLOSED

Apparatus

The sway table consists of a 45.5 x 45.5 x 1 cm steel platform which is 1 cm above the floor. An 11 x 18.5 x 0.5 cm board is mounted in the center to serve as as indexing mechanism for the placement of the subject's feet. Four strain gauges are used to transduce side-to-side and fore-and-aft movement into a varying voltage. These quantities are digitized and

stored for subsequent analysis.

Instructions

This test will measure your ability to stand steady while staring at a fixed point, and with your eyes closed. When properly positioned on the sway table, with your hands at your sides, you will be instructed to look straight ahead and hold your position until you are told to relax. Following a 10 second rest period, you will be instructed to look straight ahead, close your eyes, and hold your position until you are told to relax. The object in both of these tasks is to stand as steady as you can with as little side-to-side and fore-and-aft movement as possible.

Procedure

The subject is placed in a standing position on the table, placing the long edges of the index board along the inside surface of the feet. Tape marks are used to judge the position fore and aft. When properly positioned, the subject is instructed to place his hands to his sides, look straight ahead with eyes open, and focus on a designated point until instructed to relax. Following a 10 second relaxation period, the subject is instructed to place his hands at his sides, look straight ahead, close his eyes, and hold his position until instructed to relax. Thirty seconds of data are recorded for each task.

Analysis

The standing steadiness data for both eyes open and eyes closed is analyzed using Fast Fourier transforms so that the spectral characteristics may ultimately be examined. In a preliminary analysis it was determined that the low frequency bands (below 2.5 Hz) contained the activity of greatest interest, and most statistical analyses are restricted to them. Fore-aft and port-starboard data are analyzed separately. A single overall measure of sway is fashioned by taking the root of the sum of the low frequency power in both dimensions.

STANDING STEADINESS TASK WITH VISUAL FEEDBACK

Apparatus

The sway table has been described in the previous section, Standing Steadiness Task. The display screen is also used in this task to provide visual feedback to

the subject about his performance. The screen measures 96 cm high and 127 cm wide. The sway table is aligned with the right edge of the screen, and places the subject 1.5 m from the center of the screen.

Instructions

This test will measure your ability to stand steady with visual feedback of how well you are doing. The procedure is the same as the Standing Steadiness task, except the object is to keep the snake-like marker on the screen as close to the center of the circle as possible by making slight shifts in body weight.

Procedure

The subject positions himself on the sway table as in the Standing Steadiness task, except that he faces the display screen instead of looking at a designated point. The subject is instructed to hold as still as possible for 5 sec, during which time the sway table self-calibrates and a mean x-y position of the subject's resting center is determined. Following the calibration period a circle 56 cm in diameter is drawn in the center of the screen and a "+" is displayed to mark the resting center. As the subject sways on the table his position is sampled at a rate of 60 times a second. A dot is drawn on the screen at each sampling, indicating the subject's position relative to his calibrated center. A series of 30 dots, or the data collected for 0.5 sec, is continually displayed. This produces a "snake-like marker" that travels about the screen showing the subject how well he is maintaining a steady position on the sway table. All measurements are recorded as deviations from the subject's mean center and are displayed on the screen as deviations from the center of the circle. In this fashion the screen acts as a mobile window that is centered over the subject's calibrated resting center. The task lasts for 60 sec and all data are recorded and stored.

Analysis

The measures of merit are: mean x (port-starboard), mean y (fore-aft), mean deviation from center and maximum deviation from center.

DIGIT SYMBOL SUBSTITUTION TASK

Apparatus

This task involves the presentation on the display screen of a code consisting of a row of numbers from 1

to 9, and a row of symbols just below the row of numbers. The rows are positioned at 7 and 15 cm below the top of the screen, respectively. Each number is associated by its columnal relationship with a particular symbol (a number positioned over a symbol). A symbol from the code is presented in the center of the display field, 50 cm from the top of the screen. The subject is seated approximately 110 cm from the screen, and employs the response keypad to enter the appropriate digit associated with the symbol. The keypad is attached as an extension to the right armrest of the chair in which the subject is seated. The configuration of the keypad digits, from left to right are, beginning with the top row, 1, 2, 3; middle row, 4, 5, 6; bottom row, 7, 8, 9.

Instructions

You will observe the sequence of numbers from 1 to 9. Below each number is a symbol that corresponds with the number. One at a time, a symbol will be presented in the middle of the screen, and you are to enter the number associated with the symbol via the keypad, which has the same numbers, 1 to 9. Enter the number as fast as you can, as the speed, as well as the accuracy of your response, will be recorded.

Procedure

The numbers of the code are digits 1 through 9. The symbols are the same as in the Wechsler Adult Intelligence Scale- Revised (WAIS). The symbols are always presented in the same sequence on every test. The code, however, is changed in relationship to the digit numbers. This change is made at the beginning of each test. In each test the subject is presented 108 trials, and is required to enter the corresponding number associated with the symbol. The number entered causes the trial symbol to be erased and the number displayed for 0.25 seconds just above the postion of the erased symbol. The duration of the task, although dependent on the subject's response rate, averages 2.0 minutes. If the subject does not respond within 20 sec, a warning message is displayed to the experimenter and the subject is verbally urged to continue.

Analysis

The data are analyzed for the number of correct responses, the average response time, the standard deviation in the response times, and the "power" (the

quotient of the number correct by the mean response time). During the initial trials, this task involves heavy reliance upon visual association while memory is important by its end. Consequently, the performance measures are computed separately for the first, second, third, and fourth quartiles, as well as overall measures at all time periods.

DIGIT SYMBOL SUBSTITUTION TASK WITH SHORT TERM MEMORY TEST

Apparatus

The apparatus used for this task is the same as that used for the Digit Symbol Substitution task.

Instructions

This task is very similar to the Digit Symbol Substitution task. However, the task is divided into four equal parts, or quartiles. Each quartile consists of 27 presentations, which you will respond to just as in the Digit Symbol Substitution task. After the 27 presentations, the code table at the top of the screen will disappear and each symbol will appear one at a time at the center of the screen. You are to respond by entering the appropriate number on the keypad that corresponds to the symbol presented. You will be scored on the number of correct associations you make. Although you will not be scored on your reaction time for this part of the test, it is necessary that you respond as soon as possible so the test will not be delayed. Following the first quartile, the second, third and fourth quartiles will be presented in the same manner, with memory tests given at the end of each group of presentations.

Procedure

This task is very similar to the Digit Symbol Substitution task, except that it is interrupted at the end of each quartile by a memory test. Following 27 presentations, the code table disappears. One at a time, the symbols are displayed in the middle of the screen and the subject keys in the corresponding number by memory. After the memory test, the code table reappears. This sequence is repeated four times. Each symbol is presented three times per quartile, and one time per memory test. The duration of the task depends on two factors; one, the amount of time it takes to perform the task with the code table present which, although dependent on the subject's response rate, averages about 2.0 minutes; and two, the amount of time

it takes to respond to the memory part of the task without the code table present, which varies a great deal (up to several seconds for each of the nine symbols presented). This task, like the Digit Symbol Substitution task, offers the experimenter a warning message if the subject does not respond during a 20 sec interval following symbol presentation.

Analysis

The data are analyzed for the number of correct responses, the average response time, the standard deviation in the response times, and the "power" (the quotient of the number correct by the mean response time). The performance measures are computed separately for the first, second, third, and fourth quartiles, as well as overall measures at all time periods. This task involves many factors (i.e. motor coordination, eye-hand coordination, scanning ability, short-term memory); thus, it will be necessary to do a more in depth analysis of this task after the initial analysis. The in-depth analysis will compare: (1) Keyboard Reaction Task, (2) Digit Symbol Substitution without memory task, (including an explanation of the learning curve), and (3) Digit Symbol Substitution with Memory task. By combining this data, it will be possible to separate out the aforementioned varicus factors. It will then be possible to look at how different pharmacologic agents affect the relative contributions of memory (learning), simple keyboard reaction time, and residual psychomotor performance effect (e.g., eye scan time) on the Digit Symbol Substitution Task.

ABBREVIATED DIGIT SYMBOL SUBSTITUTION TASK WITH MEMORY

Apparatus

The apparatus for this task is the same as that used for the Digit Symbol Substitution with Memory task.

Instructions

This task is equivalent to the first quartile of the Digit Symbol Substitution with Memory task.

Procedure

This task is equivalent to the first quartile of the Digit Symbol Substitution with Memory task.

Analysis

The data from both the Digit Symbol Substitution and the memory portions of the task are analyzed for the number of correct responses, the average response time, the standard deviation in the response times, and the power.

MEMORY ENCODING TASK

Two previously described tasks, Digit Symbol Substitution with Memory and Abbreviated Digit Symbol Substitution with Memory, are designed to assess the subject's ability to recall from "short-term memory" the nine WAIS symbols learned during the course of the test. This memory encoding task is a test of their ability to recall those same symbols from "long-term memory". The use of this test along with the Digit Symbol Substitution with Memory allows for comparative testing of several components of memory and learning which we have found to be more sensitive to drug impairment than other tests.

Apparatus

The apparatus for this task consists of a sheet of paper containing a row of numbers 1-9, and a pencil.

Instructions

Twenty minutes following the completion of the Digit Symbol Substitution with Memory task you will be asked to recall the Digit Symbol Substitution Code Table from that test. We are interested in Long Term Memory under natural conditions, so do not practice or rehearse the material during the other tests. Do the best you can without letting it interfere with your performance on your other tasks.

Procedure

Twenty minutes after the completion of a Digit Symbol Substitution with Memory task the subject is presented with a sheet of paper containing only a row of numbers 1 to 9. The subject then writes under each number the symbol which had been associated with it in the recently-completed Digit Symbol Substitution with Memory task. During the 20 min interval between the Digit Symbol Substitution with Memory task and this memory encoding task the subject is engaged in additional psychomotor testing which should prevent his practicing the number-symbol relationships.

Analysis

The number of correct and incorrect responses is recorded and stored for later analysis.

SUBCRITICAL TRACKING TASK

Apparatus

A 1 cm wide bar is displayed from top-to-bottom in the center of the screen. A portion of the bar, 40 cm from the top of the screen and 24 cm in length, moves right or left across the full width of the screen. The distance from the screen to the top of the wheel is 57 cm. The wheel, 40 cm in diameter, is connected to a 10 turn pot 10 K ohm variable resistor.

Instructions

The aim of this task is to maintain the moving bar in the center of the screen, in line with the fixed bars running from the top and bottom of the screen, with the steering wheel which controls the position of the bar. If the bar moves to the right, for example, turn the wheel to the left or in the opposite direction;if the bar moves to the left of the center, move the wheel to the right. As a hint to controlling the bar, attempt to change the wheel in a smooth motion rather than in a fast jerky motion. You are scored on this task by your ability to keep the tracking bar in the center of the screen.

Procedure

During the first twenty seconds the level of difficulty (lambda) increases to a value of two. Data are collected for 1 min during which the difficulty level remains at lambda 2. During the next 20 sec the level of difficulty increases to lambda 3. Data are again collected for 1 min during the period at which the difficulty remains at lambda 3. The level of difficulty is multiplied by the steering input (position of the pot) and added to the position of the bar, resulting in the bar being repositioned. A simplified equation of this relationship is: Change in bar position = (bar position + pot position) x level of difficulty (lambda). A new bar position is calculated from this equation 60 times a second. For example, if the bar moves to the right of the center line, and the pot is not turned in the opposite direction, then the bar will move further to the right. If the level of difficulty is increased, the bar will be displaced at a

higher rate. Data are recorded for three minutes.

Analysis

A subject's performance on this task is analyzed both in terms of his success in maintaining the bar near the center of the screen, and in terms of the character of the steering inputs he makes. The square root of the mean squared error is quoted as a measure of tracking success both for easier and the more difficult halves of the task. Steering wheel motion is subjected to Fourier transformations and the power spectra condensed into bands one Hz wide. The lower three of these bands, which contain the bulk of the activity are examined usually for drug dependent variations both for lambda 2 and lambda 3 difficulty levels.

CONTINUOUS PERFORMANCE TASK

Apparatus

Numbers (1-9) 13.5 cm tall and 9.5 cm wide are displayed 42 cm below the top of the screen. The subject utilizes the response keypad described for the Digit Symbol Substitution task.

Instructions

Each number displayed will be replaced by another number. You are to respond as fast as you can, as soon as you recognize whether the new number is different, in terms of being odd or even, from the prior number. That is, if the prior number was odd and the new number is even then you would respond. If the new number is odd, then you would not respond even though it may differ in value from the prior number. To review the procedure, you are to respond when either an odd number follows an even number or when an even number follows an odd number. You are not to respond when an even follows an even or an odd follows an odd.

Procedure

One hundred-eight randomly-selected digits (1 - 9) are individually displayed on the screen. Each of the first 54 digits is shown for a duration of 2.0 seconds, then followed immediately by the next digit. Each of the second 54 digits is displayed for a duration of 1.33 seconds, then followed immediately by the next digit. The 1.33 sec trials follow the 2 sec trials without break or interruption. The duration of the

task is three minutes. The sequence of digits is selected from a random number table. In the sequence of 108 numbers, there are 54 appropriate responses and 54 inappropriate (false positive) responses. The subject may enter his response by pressing any one of the keys on the keypad. The subject's reaction time is recorded, as well as the total number of appropriate and inappropriate responses.

Analysis

The number of inappropriate responses is subtracted from the number of appropriate responses before recording the "number correct." The mean response time, standard deviation in response times, and power score are computed using the correct responses.

DIVIDED ATTENTION TASK

This task is a combination of the Subcritical Tracking task and the Continuous Performance task. The Continuous Performance portion of the task is the peripheral, secondary, or distractor task. Consequently, the duration of presentation of the 108 digits remains constant; that is, the digits are displayed at a rate of one every 1.67 sec. In addition, the location of the odd-even digit display is moved closer to the top of the screen so as not to interfere with the tracking bar. The total duration of the task is 3 mins.

DIGIT-KEYPAD REACTION TIME TASK

Apparatus

One hundred randomly-selected digits (1 - 9) are displayed individually in the center of the screen, 53 cm from the top. The size of the digits are 6.5 cm tall by 4.5 cm wide. The subject uses the keypad (previously described in Digit Symbol Substitution) to enter the same number as apppears on the screen.

Instructions

This test is designed to measure simple reaction time. A number will flash onto the screen and you are to respond as quickly as possible by pressing the same number on the keypad. You will be evaluated on both accuracy and speed.

Procedure

One hundred randomly selected digits (1 - 9) are presented individually to the subject. The subject is asked to enter into the keypad the same number as appears on the screen. This causes the trial number to be erased and the subject's response to be displayed for 0.25 sec just above the location of the erased number. Immediately thereafter, another random number is displayed. This task, like the Digit Symbol Substitution task, offers the experimenter a warning message if the subject does not respond within a 15 sec interval following number presentation.

Analysis

The subject's responses are analyzed for the number correct, average response time, standard deviation in response times and power.

CENTRAL PENDULUM TRACKING

Apparatus

A bar 15 cm long is displayed in the center of the screen, 40 cm from the top of the screen. The subject observes the bar movement with his head rested against the chair's headrest, located 110 cm from the screen. EOG electrodes are placed as described for the Sudden Displacement Saccade test. The electrodes are connected to a Grass amplifier with filter settings adjusted to: high frequency setting of 3 KHz, and low frequency of 0.1 Hz. These Grass amplifiers, located in the experimental chamber, are used to amplify the signal to reduce artifact in transmission to the polygraph outside the chamber.

Instructions

You are to rest your head against the headrest and follow the moving bar with only your eyes.

Procedure

The bar moves in a 20 degree arc with a cycle period of two seconds (right-left-right). The top of the bar moves in the same plane as the bottom of the bar, rather than with respect to the imaginary apex of the pendulum. The task is 64 sec in duration.

Analysis

We have implemented an analysis of smooth-pursuit eye movements based upon a statistical method published and evaluated by Lindsey, Holzman, Haberman and Yasillo (1978). This method generates a single figure of merit which reflects the quality of eye tracking movements over any specified time interval. The figure of merit quoted is the natural logarithm of the signal to noise ratio, ln (S/N), where the signal and noise powers are estimated over the time interval of interest.

The analysis proceeds in two steps, corresponding to the computation of signal and noise powers, respectively. First, the amount of signal present in the EOG data is determined by doing a harmonic regression on a sinusoid of 0.375 Hz. This represents the degree to which the eye movements correspond to the actual stimulus motion, and gives a measure of the signal power S. Next this sinusoidal component is subtracted from the EOG data so that only noise remains. A fast Fourier transform is then applied to reduce the effects of various artifacts. Finally, the noise N is computed by summing the power in the range from 0.7 to 8.0 Hz.

LATERAL PENDULUM TRACKING

Apparatus

A 15 cm bar is displayed 40 cm from the top of the screen at a angle of 40 degrees from the center of the subject's visual field. The subject rests his head against the head rest, turns his head in the direction of a light source on the left edge of the screen, and follows the bar movement with his eyes. EOG electrode recording is the same as those described in the Sudden Displacement Saccade test Apparatus section.

Instructions

With your head against the head rest, turn your head so that you are facing the red light on the left edge off the screen. Moving your eyes only, you are to follow the moving bar.

Procedure

The bar moves in a 20 degree arc with a cycle period of two seconds, as described in the preceding task, except the center of the arc is located 40 degrees from the center of the subject's visual field. The task duration is 64 seconds.

Analysis

Data are analyzed using the procedure described in the Pendulum Tracking section.

Apparatus

The apparatus utilized in this task is identical to that in Pendulum Tracking, except for the bar position which has been horizontally displaced.

CONCRETE NOUN MEMORY TASK

This task examines primarily long-term or secondary verbal memory functioning. The learning and recall trials will assess the effects of the drugs on both storage and retrieval.

Stimuli

On each test day the subjects will be shown two lists of concrete nouns, consisting of ten words each. The words were selected from a list of nouns published by Paivio, Yuille, and Madigan (34). All the words had high ratings (above 6.0) on the attributes of concreteness, imagery, meaningfulness, and Thorndike-Forge frequency.

Instructions

For Learning Trials

I am about to show you a list of words which I would like for you to remember. I will ask you to recall the words later. However, when I show you each word, I am going to ask you either to name a category in which the noun belongs, or to tell me how many letters are in the word. Do not say the word, but merely answer my question. Later on, when you are asked to remember the words, you will not be asked about the categories in which you placed them

For Recall Trial 1 (114 min after Learning Trial 1)

Tell me all the words that you can remember from the list that you learned before you got the drug this morning.

For Recall Trial 2 (95 min after Learning Trial 2)

Tell me all the words that you can remember from the list that you learned a short time ago, between the testing batteries.

For Final Recall and Recognition Test (450 min after Learning Trial 1)

Tell me all the words that you can remember from both of the word lists that you learned today. (After the subject has listed all of the words that he can remember, the Recognition Test is done). I am going to show you a set of words. Say "Yes" if you remember being shown the word today, and "No" if you do not.

Procedure

Immediately prior to drug administration in the morning, the subject is shown a series of concrete nouns. The nouns are presented individually on index cards. Upon presentation of each noun, the subject is asked either to categorize the noun, or to tell the experimenter how many letters it contains. The experimenter records the subject's responses without giving any feedback. After 114 min, the subject is asked to recall as many words as possible from the list, without having to remember his earlier responses.

A second list is presented 19 min later, with the same instructions. The subject is asked to recall the second list 95 min after its presentation.

At the end of the testing day, approximately seven hours after the presentation of the first list of words, the subject is asked to recall as many words as possible from the two lists. After that, a list of forty words is shown to the subject, one at a time. The subject is asked whether each word was shown to him at any time during that test session. The recognition list consisted of the 20 words that were presented during the test session and 20 words that were not presented on that day.

Analysis

The data consist of the number of correct and incorrect responses as well as the number of wrong responses from previously learned word lists for each learning plus recall and recall only trial. Overall mean number correct and standard deviation will be calculated for each test day.

HEART RATE MONITORING TEST

A new program has been incorporated into the test schedule after each battery in order to measure and record the changes in heart rate across the course of the day. The heart rate program was designed to minimize human error in measuring tachycardia, and to create a permanent data set which can be analyzed later. The program accomplishes this by integrating the Digital 11/23 computer with the Hewlett Packard 7830A Heart Monitor. The monitor converts the ECG signal into separate components such as the wave form, the audio output, and the heart rate bar graph. The bar graph is the result of a beat to beat cardiotachometer in the heart monitor which measures the time interval between successive R-wave components in the ECG and then integrates the data into a projected heart rate per minute.

The 11/23 computer converts these results from an analog to a digital form, and samples at a rate of 100 times a second for 60 seconds. The 600 data points are then converted by a scaling factor to correspond to data determined by an independent measurement of the heart rate by pulse over a range of 0 to 150 beats per minute with a standard deviation of plus or minus 1 beat per minute. The final form of the data is printed to the screen showing average heart rate over five quartiles and a total average heart rate per minute. Finally, the permanent data sets of raw data with the 600 data points, and the summary data with the 5 quartiles and total average heart rate are stored in separate files like the data from the battery tasks.

APPENDIX B

Procedure for Plasma Collection for Atropine and the Growth Hormones

This outline describes the procedure for the collection of blood samples for atropine and different hormones. Blood samples are collected in two different containers: (1) Green top tubes containing lithium heparin anticoagulant for the atropine, growth hormone, cortisol and catecholamine samples, and (2) purple top tubes containing EDTA anticoagulant for the arginine vasopressin (AVP) and adrenocorticotropic hormone (ACTH) samples. This procedure is necessary because heparin may destroy certain peptides such as AVP and ACTH. In addition, the vacutainers are kept on ice prior to and after blood collection until the plasma sample is frozen. Since atropine is the compound with the highest priority, the phlebotomist draws the atropine sample first (a safeguard in case the catheter becomes clotted and further sampling at that time is prevented). Soon after the blood samples are collected they are centrifuged for 10 minutes at 4 degrees C and 2500 rpm and the following procedures are followed for the different types of samples.

AVP and ACTH: At least 1.0 ml of plasma is necessary for the assay of either AVP or ACTH. The plasma is pipetted and transferred to a polypropylene tube labeled "AVP" or "ACTH," as appropriate. For each assay, 0.2 ml (200 ul) of 1.0 N HCl per 1.0 ml of plasma is added to the tube. Then the tubes are vortexed and then frozen rapidly on dry ice.

Atropine, Growth Hormone, Cortisol and Catecholamines: A minimum of 1.0 ml of plasma is needed for each assay of atropine, growth hormone or catecholamines; 0.5 ml of plasma is needed for the cortisol assay. For the assay of atropine or growth hormone, 1.0 ml of plasma is placed into two separate polypropylene tubes which are appropriately labeled and then frozen rapidly on dry ice. For the catecholamine assay 1.0 ml of plasma is transferred to a polypropylene tube labeled "CAT," and 0.2 ml of a Glutathione solution is added. The Gultathione solution is prepared by adding 500 mg of reduced glutathione to 10 ml of 200 nM HCl. The tube is then vortexed and frozen rapidly on dry ice. For the cortisol assay, 0.5 ml of plasma is transferred to a polypropylene tube labeled "CORT." If osmolality is to be measured, the sample is refrigerated. Otherwise, it is frozen rapidly on dry ice.

APPENDIX C
Radioimmunoassay of Atropine in Plasma

A. Equipment:

The standards and samples are counted on a TRI-CARB 460 CD Liquid Scintillation System (Hewlett-Packard).

B. Reagents:

The atropine antibody dilution (1:1600) is prepared by adding 10 ul of antibody to 15.99 ml of 0.01 M phosphate buffered saline (pH 7.5) in order to have 50-70% binding capacity. Radioligand (3H-atropine sulfate, specific activity = 87 Ci/mol) is diluted in buffered saline to make 4000 dpm counts per 20 ul solution.

C. Standard Solutions:

A stock solution of atropine sulfate monohydrate is prepared with a concentration of 1.16 ug/ml (equivalent to 800 ug/ml atropine) in buffered saline. This solution is then further diluted with buffered saline in order to have atropine concentrations ranging from 62.5 pg to 4 ng in a 10 ul volume. Drug-free plasma (0.99 ml) is added to each tube containing 10 ul of the atropine solutions to produce standard plasma solutions ranging in concentration from 62.5 pg/ml to 8 ng/ml.

D. Quality Control Solutions:

A quality control solution (500 ng/ml) is prepared by adding 6.25 ml stock solution (800 ng/ml) to 3.75 ml buffered saline. This solution is further diluted with buffered saline to prepare atropine concentrations of 0.5 ng and 2.5 ng in a 10 ul volume. Drug-free plasma (0.99 ml) is added to each test tube containing 10 ul of the above solutions to five quality control plasma solutions, 0.5 ng/ml, 2.5 ng/ml and 5 ng/ml, respectively.

E. Assay Procedure:

To a pyrex tube (12 X 75 mm) containing 50 ul of plasma sample, quality control or standard, are added 330 ul buffered saline and 100 ul atropine antibody solution. The tubes are vortexed and then incubated overnight at 4oC. On the following day, a 20 ul volume of radioligand is added to each tube, vortexed and then incubated overnight at 4oC.

The antibody-bound atropine is separated from free atropine by adding 500 ul saturated ammonium sulfate solution. The reaction tubes are vortexed, allowed to incubate 15 minutes at 4oC and a precipate is then obtained by centrifugation at 3000 rpm for 20 minutes. The precipitate is washed once with 1 ml of a 50% saturated ammonium sulfate solution, and then the centrifugation is repeated immediately. The precipitate is dissolved in 1 ml distilled water and transferred to a counting vial containing 8 ml of Aquasol. The test tubes are washed once with 2 ml of Aquasol, the washing being added to the same counting vial. The samples, quality control or standards, are counted on the Hewlett Packard counter for 4 minutes.

APPENDIX D
Assays for Neuroendocrine Hormones

Radioimmunoassay for Vasopressin: Plasma vasopressin is assayed following the Sep-Pak extraction modified from Ysewijn and DeLeenheer (34) by the radioimmunoasaay technique developed by Robertson (35), antiserum supplied by Dr. Tj. B. Van Wimersma Greidanus, synthetic standards from Bachem Chemical Co., and iodinated vasopressing prepared in this laboratory from the same source. Vasopressin is iodinated by a modification of the Chloramine T technique. Plasma samples are adjusted to pH 2.0 by addition of 1N HCl (0.2 ml/ml plasma), and adsorbed onto prewashed C-18 columns (Sep-Pak). Columns are washed with 10 ml of 0.4% trifluoroacetic acid, and the vasopressin is eluted with 5 ml of a 40:60 mixture of methanol and 0.4% fluoroacetic acid. Samples are dried on a Savant evaporator, and reconstituted in assay buffer (0.05 M phosphate buffer, pH 7.5 containing 0.01 M EDTA, 100 KIU/ml aprotinin and 1% normal rabbit serum. Standards are extracted from vasopressin-free plasma in an identical fashion.

Extracted samples are assayed by RIA at 4 degrees Celsius using delayed addition of trace to improve sensitivity and a second antibody precipitation technique. Samples are assayed in a final volume of 0.4 ml containing 4,000 cpm 125 I-vasopressin, 0.1 ml of antiserum (final concentration 1:80,000) and 50 ul sample or standard. The standard curve is run from 0.1 to 100 pg/tube. Samples are incubated with tracer for 24 hrs and then for 24 hrs with antiserum before the addition of second antibody. Goat antirabbit serum is added, samples are centrifuged 24 hrs later, and pellets counted in a Packard gamma counter. The ED90 is 0.15-0.5 ng/tube, and the ED50 ranges from 2-5 pg/tube. The sensitivity of the assay is 0.5 pg/ml plasma.

Catecholamine Assay: Plasma levels of norepinephrine (NE) and epinephrine (E) for human studies will be conducted by high-pressure liquid chromatography (HPLC) with electrochemical detection (36). Catecholamines from 1 ml of plasma are pH adjusted by addition of 1 ml Tris buffer (1 M, pH 8.6), adsorbed onto alumina, washed with 0.01 M Tris, pH 8.6 and eluted with 200 ul of 0.2 N percholoric acid (PCA). The entire aliquot is injected into the HPLC system, which utilizes a "trace-enrichment" method for sample concentration and purification. Samples are first adsorbed onto a cation exchange column and washed with mobile phase of low

ionic strength (0.02 M citrate, 0.02 M potassium acetate, 0.5 mM EDTA, a final pH of 3.5). The samples are separated by reverse-phase chromatography on a C18 bonded 5 um microparticulate silica (Spherisorb ODS) with a mobile phase of higher ionic strength (0.02 M citrate, 0.2 M potassium acetate, 5 mM EDTA, 1.25 mM octanesulfonate as ion pair reagent, pH 5.5). Catecholamines are detected with a TI-5A glassy carbon working electrode maintained at +600 mV vs an Ag/AgCl reference electrode in conjunction with an LC-4A amperometric controller (Bioanalytical System Inc.). An internal standard (3, 4 dihydroxybenzylamine is added at the beginning of the procedure. Standard curves are generated in catecholamine-free plasma.

ACTH and Cortisol Assays: Plasma ACTH (37) and cortisol (38) will be assayed by RIA utilizing methods developed in the Psychiatry Department's Clinical Psychobiology Laboratory by James Ritchie, Jr.