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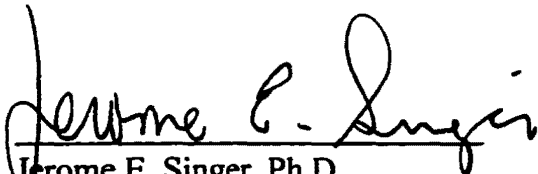


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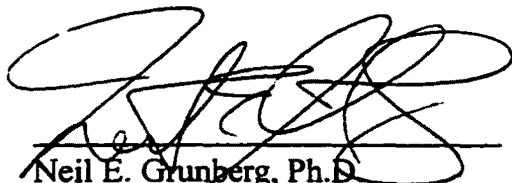
Title of Thesis: "Effects of AZT, ddC, and d4T on Memory in Male and Female Rats"

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A handwritten signature in black ink, reading "Casey Skvorc". The signature is written in a cursive, flowing style.

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ABSTRACT

Title of Thesis: Effects of AZT, ddC, and d4T on Memory in Male and Female Rats

Casey Skvorc, Master of Science, 1998

Thesis directed by: Neil E. Grunberg, Ph.D.

Professor

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Some anti-HIV medications exert behavioral and neurotoxic side effects that deleteriously affect quality of life. The present research examined the effects of three anti-HIV medications -- AZT, ddC, and d4T-- on memory in Sprague-Dawley male and female rats. Memory was chosen as the dependent variable because it is an important psychological construct, its profound effects on quality of life, and its relationship to medication compliance. Three experiments used retention of the active avoidance shuttlebox performance as an index of memory. Experiment 1 (N=60) found that males dosed with medication performed significantly slower (i.e., demonstrating impaired memory function) than females. Females dosed with AZT or ddC performed significantly faster on the second day, compared to the first day, of testing.

Experiment 2 (N=80) examined acute administration of d4T in male and female rats. All subjects showed a decrease in latencies over time. There were no significant drug effects.

Experiment 3 (N=84) examined effects of long-term administration, and subsequent cessation, on latencies of male and female rats chronically dosed with d4T. There were no gender or drug effects in day one or day two of testing. Upon cessation of the drug, animals that had received drug performed more poorly than control animals. Males latencies were longer than

female latencies when comparing the 1.0 mg/kg and 3.0 mg/kg levels to saline. Male latencies were significantly longer than saline at the 0.1 mg/kg drug level.

Overall, male rats were more sensitive to the effects of ddC and AZT on memory. Dosage regimens of d4T, acute vs. chronic, did not have an effect on memory as measured by performance in the active avoidance shuttlebox paradigm. However, effects of d4T on shuttlebox performance were revealed after cessation of the drug.

Effects of AZT, ddC, and d4T on Memory in Male and Female Rats

by

Casey Skvorc

**Master's Thesis submitted to the Faculty of the
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INTRODUCTION

Acquired Immune Deficiency Syndrome (AIDS) is a dreaded and fatal disease affecting increasing numbers of people worldwide and for which there is no cure. The available medications for AIDS and Human Immunodeficiency Virus (HIV) slow the progress of the disease but some of these medications have behavioral and neurotoxic side effects. The purpose of the present research was to examine the effects of three common treatments for HIV and AIDS on memory in an animal model. Most aversive side effects of anti-HIV drugs affect the peripheral nervous system. Memory was used to assess possible central acting effects of these drugs. Memory was examined also because it is an important psychological construct affecting quality of life. An animal model was used because it allows control of relevant variables.

The primary treatment of HIV and AIDS is with nucleoside analogs; these drugs treat the symptoms of HIV disease, but cannot cure it (Carpenter, et al., 1997; Sandberg, et al., 1995). It is important to understand the behavioral and cognitive side-effects of anti-HIV medications so that new drugs can be developed without these side-effects, and to help people deal with expected side effects. Ideally, this research may help to increase medication compliance and to prepare patients to cope with any behavioral or psychological effects of these medications. One important consequence of use of these drugs may be their effects on memory. Nervous system effects can directly affect compliance. For example, peripheral neuropathy can result in non-compliance with the drug regimen for a patient in pain. This basic psychological construct -- memory -- is central to cognitive function and quality of life. Extensive research is required to determine and understand the side effects associated with these medications. This thesis examines the effects of

three nucleoside analog medications azidothymidine (AZT), dideoxycytidine (ddC), and dideoxyhydrothymidine (d4T) on memory in male and female rats.

To place this experiment in context, the Introduction section includes a discussion of HIV and AIDS epidemiology in the United States and the world, followed by a brief description of the disease pathology of HIV and AIDS, a description of nucleoside analog treatments, and a discussion of the cognitive components and potential side effects of three nucleoside analog treatments.

Epidemiology: AIDS and HIV are responsible for approximately 11,700,000 deaths in the world since it was first reported in 1981 (Table 1). There are approximately 30,600,000 people living with AIDS or HIV worldwide (United Nations, 1997). It was recently estimated that at least 10,000,000 young people (ages 10-24) are living with AIDS and HIV (United Nations, 1998). Through December 1997, 641,086 cases of AIDS in the United States were reported to the Centers for Disease Control and Prevention (CDC). Of these AIDS cases, 390,692 have died. It has been reported that there are approximately 331,985 people living with HIV infection and AIDS in the United States (Centers for Disease Control, 1997).

HIV infection continues to increase. CDC reports of HIV infection in states with confidential HIV infection reporting indicate a 14% increase in HIV infection in adults and adolescents from 1996 to 1997 (Centers for Disease Control, 1997). Worldwide, there were about 16,000 new HIV infections per day reported in 1997. The United Nations estimates that one in every 100 adults in the sexually active ages of 15-49 is infected with HIV. The overwhelming majority of HIV-infected people, more than 90%, live in developing countries, and most of them are unaware they are infected (United Nations, 1997).

The disease: HIV and AIDS defeat the body's ability to ward off infections and cancers by compromising the immune system. AIDS was first documented in homosexual men in the United States in 1981 (Gottlieb, et al., 1981; Siegal, et al., 1981; Masur, et al., 1981; United Nations, 1998). HIV, the precursor to AIDS, was identified in 1983 (Centers for Disease Control, 1997). The spread of HIV appears to have begun in the late 1970s and early 1980s among men and women with multiple sexual partners in Africa and among homosexual and bisexual men in urban areas of North and South America, Australia, Asia, and Western Europe. Today, the virus has been transmitted in all countries (United Nations, 1996).

The main route of transmission is blood-borne, which can occur through unprotected sexual intercourse, through blood, blood products, donated organs, semen, or through an infected mother to her fetus or infant during pregnancy or delivery, or when breast feeding (MMWR 1997; United Nations, 1996). Without anti-HIV drug therapy, HIV spreads easily throughout the body. Ongoing HIV replication leads to immune system damage and progression to AIDS. Active replication of HIV infection lowers the number of CD4 white cells over time, and as this occurs, is the cause of progressive immune system damage, with the result that nearly all infected persons will suffer progressive deterioration of immune function resulting in their susceptibility to opportunistic infections, malignancies, neurologic diseases, and wasting, ultimately leading to death (MMWR, 1998). Plasma HIV RNA levels indicate the magnitude of HIV replication and its associated rate of CD4+ T cell destruction, whereas CD4+ T cell counts indicate the extent of HIV-induced immune damage already suffered (MMWR, 1998; O'Brien, et al., 1996). The use of antiretroviral therapy to suppress HIV replication, to achieve maximum suppression of HIV replication is the goal of pharmacotherapy (MMWR, 1998).

Some treatments are available, but not a cure: The HIV infected population of today and tomorrow is clearly no longer predominantly homosexual white men in the western countries. HIV and AIDS have exploded into a worldwide epidemic (United Nations, 1996, 1997; Centers for Disease Control, 1997). Treatment for HIV infection and AIDS in the end of the 20th century must now be focused on large numbers of the global population, and medications must be evaluated for cognitive and behavioral consequences as well as physical side effects.

The first anti-HIV drugs were nucleoside analogs, also called nucleoside analog reverse transcriptase inhibitors (Sandberg, et al., 1995). Among these drugs were AZT (azidothymidine, RETROVIR), ddC (dideoxycytidine, HIVID) , and d4T (dideoxyhydrothymidine, ZERIT). These nucleoside analogs inhibit the action of the enzyme known as reverse transcriptase. Reverse transcriptase allows HIV to transform its genetic material into a form and enter the cell nucleus where it subsumes the cell's genetic material forming long chains of proteins. By this process, HIV makes new copies of itself inside the cells it infects. These new copies of HIV infect other cells in the body. In people infected with HIV, over 10 billion new copies of the virus can be made daily; if the replication process is not arrested, HIV spreads quickly in cells throughout the body. CD4 cells communicate to other infection-fighting cells when to activate. HIV infection lowers the number of CD4 cells over time. As this occurs, the body's ability to resist infections and cancers decreases, and the individual becomes increasingly susceptible to infection and cancers (Markowitz, 1997).

Combination therapy utilizes different anti-HIV medications to attack the virus in different ways (MMWR, April 28, 1998). Drugs that affect the reverse transcriptase process stop HIV just after it enters the cell; and drugs that target protease stop HIV before it leaves the cell. Targeting

two means of the spread of the infection increases the chance of stopping it and protects new cells from infection. This combination therapy process provides the basis of why nucleosides (which target reverse transcriptase) and protease inhibitors work effectively together (Markowitz, 1997).

HIV enters different types of cells in different parts of the body (Markowitz, 1997).

Combination therapies use drugs that differ in how well they attack the virus in these different cells. For example, AZT and d4T enter cells in the spinal cord and the brain better than other drugs (Markowitz, 1997). AZT and d4T work best in infected cells that are actively producing new copies of HIV, while ddC works best in cells that are infected but not yet producing new HIV (Markowitz, 1997).

Furthermore, combinations of anti-HIV drugs may prevent or delay resistance. When one drug is given alone, eventually HIV adapts to resist that drug; if two or three drugs are given together, it is more difficult for HIV to make the changes necessary for resistance (MMWR, 1998). Large studies have shown that AZT and ddC work better than AZT alone, based upon measuring CD4 counts and the amount of virus in the blood (MMWR, 1998).

AZT was an early pharmacological treatment for AIDS patients (PDR, 1997). In addition to its effectiveness in treating adults with AIDS and HIV infection, a short course of AZT has been found effective in reducing perinatal HIV transmission. This regimen has been reported to reduce the rate of HIV transmission to infants of infected mothers by half (United Nations, 1998). The principal side effects of AZT are anemia and granulocytopenia, muscle weakness (Markowitz, 1997), bone marrow suppression, GI intolerance, headache, insomnia, and asthenia (Physicians' Desk Reference, 1997). AZT reaches HIV in the spinal cord and the brain (Markowitz, 1997). ddC has shown effectiveness in combination with AZT, as well as d4T. Side

effects for ddC include pancreatitis and peripheral neuropathy (Markowitz, 1997; Berger, et al., 1993; Anderson, et al., 1993). d4T, like AZT, reaches HIV in the spinal cord and the brain. The principle side effect of d4T is peripheral neuropathy (Davis, et al., under review).

Whereas these drugs, particularly as used in combination therapy (MMWR, 1998; Hammer, et al., 1996; Perrin, et al., 1997), are useful in extending life for people with HIV infection and AIDS, unfortunately they do not cure HIV infection and AIDS. In addition, these drugs have toxic side effects that are likely to lead to reduced compliance, deleteriously affect quality of life, and, perhaps, cognitive and behavioral performance, including decreased memory function.

Side effects associated with HIV and AIDS pharmacotherapy: It is important to understand the behavioral and cognitive side effects of anti-HIV medications so that new medications can be developed with fewer harmful side effects, and to help people deal with expected side effects. Ideally, this understanding will increase compliance and prepare patients to cope with any psychological effects of these medications.

An important goal of the research of nucleoside analogs is to develop and design treatments that minimize cognitive side effects. For example, if one medication is found to result in decreased memory capacity for males with HIV/AIDS but not females with HIV/AIDS, the component of the medication that affects males can, optimally, be isolated and replaced with other, less toxic medications; in the interim this medication could be limited for prescription to females with HIV/AIDS. To achieve this goal of reducing and eliminating cognitive side effects associated with nucleoside analog medications, animal models are used to study neurotoxic effects of drugs in controlled settings and in dosages that would not be possible, or ethical, in

humans. For example, it is possible to obtain large numbers of laboratory rats that are genetically similar to each other and to control environmental experiences. In humans, it is virtually impossible to obtain large samples of individuals so closely related. Laboratory rats are housed and fed in uniform conditions for periods of time that would not be possible with humans. Testing measures, such as the active avoidance paradigm used in this experiment, are conducted on laboratory rats, a process that would not be possible or ethical in humans. Finally, laboratory rats are often euthanized after the experiment so that samples of organs and body fluid samples can be collected. Without the opportunity to conduct animal testing of these medications, it would be necessary to subject humans to experimental procedures without knowing the extent of their side effects.

Physical and cognitive side effects of HIV and AIDS pharmacotherapy: The principal physical side effect of some of the nucleoside analogs is neuropathy (Morse, et al., 1997; Davis, et al., under review). This neurotoxicity raises the question as to what other central nervous system functions are impaired by these drugs. For example, an important cognitive consequence of use of these drugs may be their effects on memory. This basic psychological construct, memory, is central to cognitive functioning and quality of life. Moreover, for patients with HIV, ability to comply with a pharmaceutical regimen is critical to health maintenance. Compliance to medication directions allows patients to have an active role in their treatment, and an opportunity to participate in the management of their disease, a valuable aspect of the patient's retention of personal control over the illness. Non-adherence is a common occurrence in chronic illness and when the individual is asymptomatic but is given the treatment to prevent the onset of symptoms or progression of the disease as with the anti-HIV medications researched in the current work

(Roche-HIV, 1997). Hence, memory was chosen as a dependent variable in this experiment because of its central importance to daily functioning and quality of life.

There are many definitions of memory in the literature. William James (1890) wrote that to remember is to think about an event or concept already experienced, and that was not being thought of immediately before. The concept of associationism defines memory as the ability to form connections between stimuli and responses, and the strength of these associations constitutes the ability to remember (Klatzky, 1975).

This thesis will define memory as the mental faculty of retaining and recalling past experience (Webster, 1988). Memory is the mechanism by which past experiences are utilized to guide current behavior. This construct affects virtually every action of life -- from basic skills of feeding oneself to complex decision-making tasks, such as a driver operating and navigating an automobile or other piece of heavy equipment, an attorney presenting a persuasive argument based upon established case law, a psychologist remembering the elements of cognitive and behavioral therapy, a surgeon performing an appendectomy, or a prison warden determining how to manage a sensitive issue that could ignite a prison riot.

Memory can be studied in many different ways, ranging from simple to complex tasks in the laboratory and in the field. One of the most basic approaches to examine memory is with the active avoidance paradigm used in the shuttlebox. Bovet and colleagues (1969) identified the active avoidance shuttlebox paradigm as advantageous in measuring learning and memory in animals because of the type and the constancy of the motivation adopted. The shuttlebox simulated the need for animals in the wild to stay alert to avoid and escape from predators, one of the basic requirements for survival. The shuttlebox stimuli (light and tone preceding the

escapable adverse event of the electric shock) produce similar conditioning in laboratory animals that animals in the wild acquire and which are reinforced by survival in the wild. The shuttlebox technique, based on an escape reaction, gives reliable and homogeneous results in that it reflects a type of inborn behavior (Bovet, et al., 1969).

Effects of anti-HIV medication on memory in males and females: Current findings reflect sparse clinical research in this area. Damos and colleagues (1997) tested male subjects to determine if treatment with AZT and didanosine (ddI) would have an effect on cognitive functioning. Using a computerized information processing battery that included tests similar to those under consideration for inclusion in military pilot selection batteries and a neuropsychological battery, there were insignificant differences in cognitive functioning between the drug and control HIV+ humans.. A study by Brouwers and colleagues (1997) found that male and female patients with symptomatic HIV disease with evidence of CNS compromise exhibited improvement in memory after treatment with AZT and ddI. Gorman, Mayeux,, and Stern (1993) found that long term AZT use was associated with improved cognitive performance in male subjects with early stage HIV. Baldeweg, Catalan, and Lovett (1995) found no significant benefit from AZT treatment for neurological, neuropsychiatric, and psychiatric measures in HIV infected men. Schmitt, Bigley, and McKinnis (1988), conversely, found that HIV patients receiving AZT showed improved cognition as compared to HIV patients provided with placebo.

Animal studies raise the interesting possibility that effects of nucleoside analogs on memory may differ in males and females. Gender differences have been reported in the effects of many drugs and substances, including nicotine (Grunberg, Winders, & Popp, 1987); fentanyl (Klein, Popke, & Grunberg, 1997); tobacco (Grunberg, Winders, & Wewers, 1991); alcohol (Lex,

1991); and food consumption (Brown & Grunberg, 1996).

With regard to the nucleoside analogs and gender, it has recently been reported by Morse, et al. (1997) and Davis, et al. (under review) that AZT, ddC, and d4T have sex effects in behavioral assay measures that include locomotor activity. These sex differences in behavioral responses to nucleoside analogs are relevant to any measures that include gross body movement, including the active avoidance memory paradigm in this research.

Morse, et al. (1997) reported that AZT had no effect on locomotor activity in female rats, and ddC reduced locomotion, except at the highest dosage levels. Continuing this line of research, Davis, et al. (under review) investigated the effects of AZT and ddC in male rats; for male rats, AZT had no effect on locomotor activity, except in the highest dosages, which had the effect of reducing locomotor activity. ddC increased locomotor activity at high doses. Finally, Davis, et al., (under review) tested the effects of acute administration of d4T in male and female rats. Males and females experienced a decrease in horizontal locomotor activity at the highest dosage of d4T, with vertical activity displaying an inverted U-shaped curve. d4T effects on locomotor activity were more pronounced in females than males. Locomotor activity measures physical rather than cognitive activity. This is an important distinction in that, when both constructs are used to examine effects of a drug, it allows memory to be separated from ability to physically move.

The present research was designed to examine the effects of AZT, ddC, and d4T on a cognitive construct, memory, as operationalized by performance in an active avoidance shuttle box program, with attention given to examining the consistency of non-cognitive effects as found by Morse, et al. (1997) and Davis, et al. (under review). Specifically, where a nucleoside

analogue increased locomotor effect in one gender more than another, would these results be replicated in a shuttlebox model testing the effects of the drugs on memory?

These studies underscore the importance of using multiple behavioral assays to index drug effects in order to ensure findings are applicable to patients across genders. The work included three separate experiments that were designed to examine acute or chronic effects of administration and withdrawal of AZT, ddC, and d4T in rats.

OVERVIEW

The purpose of this research was to examine the effects of three nucleoside analog drugs, AZT, ddC, and d4T, on memory in male and female Sprague-Dawley rats. This project included three separate experiments. Experiments 1 and 2 were conducted to evaluate acute effects of three different nucleoside analogues that are used in the treatment of HIV-infection. Experiment 3 investigated long-term effects of one of these drugs (i.e., d4T because all of these drugs are extremely expensive and this particular drug was available in sufficient quantities for a long-term experiment).

Experiment 1 examined the effects of AZT and ddC, widely used and well-known anti-HIV medications, on memory in male and female rats to determine the feasibility of this model. More specifically, Experiment 1 examined the effects of a bolus intragastric (IG) administration of: 0 (tap water controls), 250 mg/kg AZT, or 500 mg/kg ddC on performance in an active avoidance shuttle box paradigm of 30 male and 30 female Sprague-Dawley rats.

Experiment 2 used a similar paradigm to study another important anti-HIV medication, d4T. Experiment 2 examined the effects of a bolus IG administration of 0 (tap water controls), 32 mg/kg, 250 mg/kg, or 1250 mg/kg d4T on active avoidance shuttle box performance of 40 male and 40 female Sprague-Dawley rats.

In contrast, Experiment 3 evaluated a much longer (i.e., 18 weeks) period of drug exposure than in the previous experiments (2 weeks) in order to collect information regarding exposure that is thought to begin to approximate the human treatment situation. Experiment 3 examined the effects of orally self-administered 0 (tap water controls), 0.1 mg/ml, 0.3 mg/ml, 1.0 mg/ml, or 3.0 mg/ml solutions of d4T on active avoidance shuttle box performance of 42 male

and 42 female Sprague-Dawley rats. The work also investigated consistencies between memory performance and the effects of these drugs on locomotor activity found work by Morse, et al. (1997) and Davis, et al. (under review).

All control and drug substances were administered to the laboratory rats orally, in order to replicate the method by which human patients ingest the medications. The order in which control and drug animals were tested was counterbalanced to avoid an order effect in performance. Rats were used as laboratory animals because of their wide usage in psychopharmacological experiments.

HYPOTHESES

It was hypothesized that:

Hypothesis 1: (Experiment 1). Administration of AZT to male rats would result in longer active avoidance shuttlebox response latencies (i.e., decreased performance) in male rats and no significant effect on latency in female rats.

Rationale: Morse, et al. (1997) reported that AZT had no effect on locomotor activity in females. Davis, et al. (under review) reported that AZT reduced locomotor activity in males at the dosage of 1250 mg/kg. It was hypothesized that there would be similar effects of AZT on active avoidance shuttlebox response latencies as in the locomotor performance measures.

Hypothesis 2: (Experiment 1). Administration of ddC to male and female rats would result in longer active avoidance shuttlebox response latencies for male and female rats in the active avoidance paradigm.

Rationale: Morse, et al. (1997) reported that ddC reduced locomotor activity in female rats. Davis, et al. (under review) reported that ddC reduced locomotor activity in males. It was hypothesized that there would be similar effects of ddC on active avoidance shuttlebox response latencies as in the locomotor performance measures.

Hypothesis 3: (Experiment 2). Acute administration of d4T to male and female rats will result in varying responses depending on dosage. An overall decrease in active avoidance shuttlebox response latencies in male, but not female, rats was predicted.

Rationale: The results of Experiment 1 indicated that acute dosages of two other reverse transcriptase inhibitors, AZT and ddC, resulted in longer latencies in the active avoidance shuttlebox paradigm for male, but not female, rats. These results led to the hypothesis that acute administration of d4T would have similar effects in the active avoidance shuttlebox paradigm.

Hypothesis 4: (Experiment 3). Chronic administration of d4T will lengthen active avoidance shuttlebox response latencies in male and female rats.

Rationale: The effects of acute administration of d4T, as reported by Davis, et al. (in review), should be intensified in a chronic administration paradigm.

Hypothesis 5 (Experiment 3): Cessation of d4T will result in a mild lasting effect that will result in longer active avoidance shuttlebox response latencies in male and female rats.

Rationale: Davis, et al., (under review) reported that d4T has neurotoxic effects. Administration of this drug could result in a change in brain function which would extend past the time of drug clearance, thereby affecting the animal's memory function.

METHODS

EXPERIMENT 1

The purpose of this experiment was to test the hypothesis that two anti-HIV drugs, AZT and ddC, would affect latencies for male and female rats in an active avoidance paradigm.

Specifically, AZT was hypothesized to increase latencies for male rats, and have no effect on female rats. ddC was hypothesized to reduce locomotor activity in male and female rats. These hypotheses were based on the work of Morse, et al. (1997) and Davis, et al. (under review).

Subjects and Housing

Subjects were 30 male and 30 female drug-naive and experimental-naive Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA). All rats were approximately six to seven weeks old and weighed 150-200 grams at the beginning of the experiment. Animals were individually housed in standard polypropylene shoebox cages (35.6 cm x 15.2 cm x 20.3 cm) fitted with a stainless steel grid cage top and equipped with individual sipper-tube water bottles. Cages were lined with absorbent Pine-Dri wood-chip bedding. Water and laboratory chow (Teklad 7001) were available continuously. Animals were maintained under a 12 hour light/dark cycle (lights on at 1900) at approximately 23 degrees C and 50% relative humidity. All phases of the experiment were conducted in the dark cycle. Because rats are nocturnal and the hypothesized effects are reduced in the active period, study during the light cycle (inactive period) would result in a floor effect. This experiment was a between-subjects design.

Drugs and Drug Administration

Drugs: Zidovudine (AZT), National Institute for Allergy and Infectious Diseases, lot AZT (30) 3-92, and zalcitibine (ddC), Hoffmann-LaRoche, lot 008022, were dissolved in deionized

water and the concentrations were adjusted to maintain a constant dose volume of 20 ml/kg. Drug dosages for AZT were 250 mg/kg and 500 mg/kg for ddC. These dosages were selected and prepared based on previous reports (Morse et al., 1997) and reflect the level at which each drug maximally affects locomotor behavior.

Drug Administration: Thirty male and 30 female rats were divided into six experimental groups of 10 rats for each gender. The paradigm used was active avoidance. In this paradigm, there were three treatment groups of ten rats each: control (water), AZT, and ddC. Drug solutions or water were administered by intragastric (IG) infusion using an 18 gauge, 3 inch long curved animal feeding needle with a 2.25 mm ball, attached to a 6 ml plastic syringe. Animals had not been previously exposed to IG administration. Animals were run in the active avoidance paradigm 1 hour subsequent to IG administration.

Apparatus

Behavior was measured using a Gemini Avoidance System shuttlebox, manufactured by San Diego Instruments (San Diego, California). The shuttlebox has two experimental chambers. These chambers consist of plexiglass sidewalls and ceiling, with a stainless steel grid floor. The dimensions are 53 cm wide, 53 cm high, with a diameter of 32 cm. A stainless steel sliding gate separates the two chambers. There are 8 photo beams per chamber that monitor animal movement. Twenty-eight stainless steel floor grids are located perpendicular to the sidewalls and are placed 1.8 cm apart, center to center.

The shuttlebox can be programmed by the user to determine the acclimation period, the type and duration of stimuli (tone, light, electric shock), the time in which the electric gate between chambers opens and closes, and the number of trials for each animal. There are white (50

watt) incandescent houselights mounted on each end wall. The shuttlebox is equipped with a tone device to emit a tone of 85-88 dB, at 2900 Hz. Each chamber of the shuttlebox has an infrared photobeam, which functions to cue the apparatus to terminate shock when an animal crosses from one side to the other. An electric shock apparatus (Coulbourn Instruments, Allentown, PA) is built into the system to allow administration of electric current through the grid floor. The electric shock apparatus has a range of 0.1 - 5.0 mA. The duration of the electrical shock can be programmed for a maximum of 50 seconds. 0.6mA of shock was delivered for no longer than 50 seconds per trial.

Active avoidance paradigm

Active avoidance learning and memory performance measures were conducted in a dedicated room within the Department of Animal and Laboratory Medicine facility of the Uniformed Services University of the Health Sciences. The room was constructed of cinder block walls, acoustic tile ceiling, and steel doors (2) that kept sound to a minimum.

An animal was placed alone into the left side of the shuttlebox chamber for a period of 180 seconds to acclimate to the chamber. At the end of the acclimation period, houselights and tone stimuli occurred simultaneously with the opening of the sliding door to the adjoining chamber. If the animal crossed into the adjoining chamber within 10 seconds, then the door closed and no shock was delivered. If the animal did not proceed to the adjoining chamber within 10 seconds, then a mild electric shock of 0.6 mA was activated through the grid floor. This shock lasted for a period of 50 seconds, or until the animal escaped to the second chamber, whichever occurred first. Methods for all experiments in this research project were based upon the work of Messing and colleagues (1979).

Procedure

Animals were gentled by daily handling for 5 minutes per day for three days prior to testing. This reduces the stress of the animals as they are handled by the investigator during the experiment, thereby minimizing stress as a potential confounding variable. On the first day of testing, the appropriate dosage of drug or water was administered by IG infusion to each animal one hour prior to being tested in the active avoidance paradigm of the shuttlebox. Subjects were placed individually into the shuttlebox, with data collection beginning immediately. All subjects were dosed with drug or water on the first day of testing, and dosed with water on all subsequent days of testing. Male subjects were each run for 15 trials on two consecutive days. Females were run for 15 trials for the first day, and run again 7 days later. An equipment failure occurred after the first day of the female trials; replacement parts arrived in time to run the females seven days later.

Data analyses

Arithmetic mean latencies to leave the lighted chamber were calculated for each subject for Time 1 and 2, separately, based on the 15 individual trials run at each time point. These values were compared among treatment groups by repeated-measures ANOVA, with sex and treatment as between-subjects factors and time as the within-subject factor. Post hoc analyses (Dunnett's t tests) were used to determine whether significant differences existed among treatment groups. All tests were two-tailed with an alpha of 0.05.

Results

Figures 1 and 2 and Table 2 present mean times for active avoidance latencies for male and female rats. In addition, Table 2 provides information from the ANOVAs. Males had longer

latencies than females on both testing times: $F(1,46)=4.44$, $p=0.004$ (time one); $F(1,54)=27.585$, $p<0.001$ (time two). Female rats dosed with AZT or ddC had significantly longer latencies the first testing time compared to the second testing time: $F(28, 378)=25.407$, $p=0.05$.

Brief Discussion

These findings confirmed some consistencies of effects of AZT and ddC on male and female rats in locomotor measures (Morse, et al. 1997, and Davis, et al., under review) and cognitive measures after acute dosage of the drugs. The next experiment was conducted to determine whether the results of Experiment 1 generalized to another reverse transcriptase anti-HIV medication, d4T. As in Experiment 1, memory in male and female rats was examined in an active avoidance shuttlebox measure following acute administration of the drug.

EXPERIMENT 2

The purpose of this experiment was to test the hypothesis that acute doses of d4T, another reverse transcriptase anti-HIV medication, to male and female rats would result in longer latencies in the active avoidance paradigm for males but not female rats. This hypothesis is based upon the findings of Experiment 1, which indicated that two other reverse transcriptase anti-HIV medications, AZT and ddC, resulted in longer latencies in the active avoidance shuttlebox paradigm for males but not female rats.

Subjects and Housing

Subjects were 40 male and 40 female drug and experimental-naïve Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA). All rats were approximately eight weeks old and weighed 160-215 grams. Animals were housed in a manner identical to Experiment 1 except that they were fed Teklad 7001 laboratory chow and were maintained in a 12 h light/dark cycle (lights

on at 0600). All phases of the experiment were conducted in the dark cycle. Temperature and humidity levels were identical to those in Experiment 1. This experiment had a between-subjects design.

Drugs and drug administration

Drug: Dideoxydehydrothymidine (d4T), Bristol Myers Squibb 027857, lot 189 was dissolved in deionized water and the concentrations adjusted to maintain a constant dose volume of 20mg/kg body weight. Dosage levels were as follows: water, 32 mg/kg; 250 mg/kg; and 1250 mg/kg. These dosages were chosen based on Davis, et al., (under review) examining the effect of the drug on locomotor activity.

Drug Administration. Forty male and 40 female were each divided into 4 treatment groups of 10 rats for each gender, as follows: control (water), 32 mg/kg, 250 mg/kg, and 1250 mg/kg of drug. Animals received an acute dosage of either d4T or water. Drug solutions or water were administered by intragastric intubation using an 18 gauge, 3 inch long curved animal feeding needle with a 2.25 mm ball attached to a 6 ml plastic syringe. Animals had not been previously exposed to IG administration. Animals were run in the active avoidance paradigm one hour subsequent to IG administration.

Apparatus.

The apparatus was identical to that used in Experiment 1.

Active avoidance paradigm

The active avoidance paradigm was identical to that used in Experiment 1, except that 35 trials were run for each animal (instead of 15 trials for each animal). This change was made to enhance memory in the animals. Because of the increased number of trials, each treatment group

was run over a period of three days in this experiment, as compared to two days in Experiment 1.

Procedure

Animals were gentled by daily handling for 5 minutes per day for three days prior to testing. On the day of testing, the appropriate dosage of drug or water was administered by IG infusion to each animal, one hour prior to being tested in the active avoidance paradigm. Subjects were placed individually into the shuttlebox, with data collection consistent with the description found in the preceding section. All subjects were dosed with drug or water on the first day of testing, and dosed with water on the subsequent day of testing. Subjects were run 1 hour subsequent to dosing. Forty males and 40 females were run for 35 consecutive active avoidance trials during a period of three consecutive days in the first week, and three consecutive days one week later.

Data Analysis

Arithmetic mean latencies to leave the lighted chamber were calculated for each subject for Time 1 and Time 2, separately, based upon the 35 individual trials run at each time point. These values were compared among treatment groups by repeated-measures ANOVA, with sex and treatment as between-subject factors and time as a within-subject factor. Post hoc (Dunnett's t tests) analyses were used to determine whether significant differences existed among treatment groups. All tests were two-tailed with an alpha of 0.05.

Results

Figures 3 and 4 present active avoidance latencies by mean times for male rats and female rats. Table 3 presents mean active avoidance latencies and information from the ANOVAs. There were no between-subjects effects. Latencies for subjects in all dosage groups decreased

over time, comparing time 1 to time 2, for control ($F[1,18]=5.142$, $p<.05$), 0.32mg/kg ($F[1,18]=7.094$, $p<.05$), 250mg/kg ($F[1,17]=12.971$, $p<.01$), and 1250mg/kg ($F[1,15]=6.297$, $p<.05$) groups.

Brief Discussion

The results of this experiment -- no between-subjects effects -- were unexpected, in light of the results of Experiment 1, in which an acute administration of AZT and ddC resulted in longer latencies in the active avoidance shuttlebox paradigm for male, but not female, rats. These results led to the final experiment, which investigated whether chronic (as opposed to acute), administration of d4T would affect performance of male and female rats in an active avoidance shuttlebox testing paradigm. Chronic dosages were examined because humans with HIV infection who are prescribed d4T on a long-term basis.

EXPERIMENT 3

The purpose of this experiment was to test the hypothesis that chronic dosing of male and female rats with d4T would lengthen active avoidance latencies in male and female rats. An additional purpose of the experiment was to examine effects of d4T subsequent to drug cessation.

Subjects and Housing

Subjects were 42 male and 42 female Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 125 - 150 grams. Animals were individually housed in cages identical to those in Experiments 1 and 2, except that laboratory chow consisted of Teklad 7001, and water was mixed with d4T. Animals were maintained under a 12 h light/dark cycle (lights on at 1900) with all animals dosed and run in the dark cycle. Temperature and humidity levels were identical to those found in Experiments 1 and 2.

Drug and Drug Administration

Drug: d4T(Bristol Myers Squibb, lot R00189) was dissolved and mixed into tap water with concentrations as follows: 0.1mg/kg, 0.3mg/kg, 1.0mg/kg, and 3.0mg/kg. These concentrations were selected and prepared based upon the solubility of d4T in water.

Drug Administration: Forty-two male and 42 female rats were divided into five groups for each gender: control, 0.1, 0.3, 1.0, and 3.0 mg/ml. The control groups had 10 male and female rats; all other groups had 8 male and female rats each. Drug solutions were chronically dosed by infusion into the animal's drinking supply. The animals were exposed to the drug for approximately 7 weeks before this phase of the experiment took place.

Apparatus

The testing apparatus was identical to that used in Experiments 1 and 2.

Active Avoidance Paradigm

The testing apparatus was identical to that used in Experiments 1 and 2.

Procedure

The current experiment was one of a series conducted using these rats. Prior to this experiment, the animals had been exposed to locomotor, tailflick, acoustic startle response, and hotplate experimental procedures during a period spanning 7 weeks.

Subjects were placed individually in the shuttlebox, with data collection beginning immediately. Male and female subjects were counterbalanced and run for 35 consecutive trials on days 1 and 2 (times 1 and 2). Thirty days later, this procedure was repeated. d4T supplements to the drinking water were subsequently discontinued 16 days later, and for 36 days, male and female animals consumed untreated water *ad libitum*. On the 37th day after

discontinuation of drug, the original sample was reduced by 50%, resulting in 20 males and 21 females that were run for 35 consecutive active avoidance trials on one day. This reduction was made as a part of a separate experiment; half of the original sample was conserved to test the effects of drug discontinuation.

Data Analysis

Arithmetic mean latencies to leave the lighted chamber were calculated for each subject for days 1, 2, 32, and 59, separately, based on the 35 individual trials run at each time point. These values were compared among treatment groups by repeated-measures ANOVA, with sex and treatment as between-subjects factors and time as a within-subject factor. Post hoc analyses (Dunnett's *t* tests) were used to determine whether significant differences existed among treatment groups. All tests were two-tailed and an alpha of 0.05 was used.

Results

Figures 5 and 6 and Table 4 present mean active avoidance latencies for male and female rats. In addition, Table 4 provides information from the ANOVAs.

In repeated measures for all subjects, from the first testing day to the second testing day, there was a main effect for time for all subjects, in each drug group. Latencies for all subjects improved from the first testing day to the second testing day; there was a time by sex interaction for this period, such that males performed at greater latencies than females, $F[1,67]=6.94$, $p=0.01$.

There were no between-subjects effects for animals on time one or time two of testing. Cessation group animals provided interesting results. For cessation animals tested on time one, male and female latencies for drug animals were longer than control animals at 0.1 mg/kg ($F[1,7]=45.2$; $p<0.01$), and 1.0 mg/kg ($F[1,7]=8.1$; $p<0.05$). Post hoc analyses indicated that male

rats had significantly longer latencies than female rats at 0.1 mg/kg and 1.0 mg/kg ($p < 0.05$; $p = 0.08$), respectively.

After drug administration had been withdrawn, and animals were subsequently tested on time three, male latencies were longer than female latencies at 1.0 mg/kg, and at 3.0 mg/kg compared to saline ($p = 0.05$; $p < 0.05$), respectively. Male latencies were significantly longer at 0.1 mg/kg compared to saline ($p < 0.05$); there were no other significant differences between drug and saline latencies.

Brief Discussion

An unexpected result of this experiment, taken together with Experiment 2, was that the regimen of dosage — whether acute or chronic — did not affect performance by male or female rats in the active avoidance shuttlebox. Performance impairment appeared, instead, subsequent to cessation of d4T, suggesting that brain function is altered after drug clearance from the rat.

CONFIRMATION AND REJECTION OF HYPOTHESES

Hypothesis 1 (Administration of AZT to male rats would result in longer active avoidance shuttlebox response latencies in male rats and no significant effect on latency in female rats):

Partially confirmed. AZT males had longer latencies than male water controls but these differences did not reach statistical significance. Latencies for AZT females were not significantly longer than control females.

Hypothesis 2: (Administration of ddC to male and female rats would result in longer active avoidance shuttlebox response latencies for male and female rats in the active avoidance paradigm): Rejected. Latencies for ddC male and female animals were not significantly longer than control animals.

Hypothesis 3: (Acute administration of d4T to male and female rats will result in varying responses depending on dosage. An overall decrease in active avoidance shuttlebox response latencies in male, but not female, rats was predicted): Rejected. There were no between-subjects effects.

Hypothesis 4: (Chronic administration of d4T will lengthen active avoidance shuttlebox response latencies in male and female rats): Rejected. There were no between-subjects effects for male and female rats prior to cessation of drugs.

Hypothesis 5: (Cessation of d4T will result in a mild lasting effect that will result in longer active

avoidance shuttlebox response latencies in male and female rats): Confirmed. Subsequent to cessation of drug, male latencies were longer than female latencies at medium and highest levels of drug dosage. d4T male latencies were significantly longer than saline at the 0.1 mg/kg drug level.

GENERAL DISCUSSION

This series of experiments was designed to investigate the effects of AZT, ddC, and d4T on memory in male and female rats. From these experiments it appears there can be direct as well as subtle neurotoxic effects associated with varying dosages of AZT, ddC, and d4T, and that these neurotoxicities can affect memory in the male rat. In an active avoidance shuttlebox paradigm, males performed significantly slower than females. Females dosed with AZT and ddC had significantly shorter latencies on a subsequent day of testing than male rats.

The memory impairments associated with AZT and ddC in the first experiment did not extend to the second experiment, which involved acute dosages of d4T, or to the portion of the third experiment in which chronic dosages of d4T were administered. In both of these experiments, there were no significant between-subject effects to indicate d4T was associated with longer performance on active avoidance shuttlebox measures. Only when the drug was discontinued, and the animals tested approximately five weeks later, after the drug had sufficient time to clear, were significant latencies found. As with the first experiment, where the latencies between subjects significantly differed, it was the males that experienced the greater performance decrement, but not at every level of dosage administration.

These experiments were conducted to investigate the cause of three nucleoside analog drugs, AZT, ddC, and d4T, on learning and memory in male and female rats. The basis of this investigation was to find out whether a behavioral toxicity -- impaired memory-- existed, and if it did, was it consistent between male and female rats. The findings from this research would provide information as to a possible potent side effect of the drugs, diminished memory capacity, and to determine if further research should be conducted to determine if male and female

HIV/AIDS patients should receive different forms of the nucleoside analogs.

This research established that for AZT, ddC, and d4T, when behavioral toxicities to memory existed, it was always males who were affected more than females. Not all dosages of the nucleoside analogs impaired memory performance. Acute dosages of d4T did not produce a significant memory decrement in male or female rats, at any of the 4 dosage levels administered. When chronic dosages of d4T were provided, there were no significant effects of memory decrement. It was not until chronic dosages of d4T ceased, and the passage of five weeks, that some memory decrements were found in males.

There are interesting comparisons to be drawn between the present research and that of Davis, et al. (under review) and Morse, et al. (1997). When Davis, et al. (under review) acutely dosed male and female rats with d4T, there were performance decrements in locomotor activity; in the present research there was no significant presence of performance decrement (decreased memory performance). This difference suggests that memory performance and locomotor activity may not draw upon the same areas of the brain affected by an acute dosage of d4T. In addition, this difference supports the interpretation that the two measures (i.e., shuttlebox and locomotor activity) are separate and not confounded. Indeed, it is this distinction that is important in differentiating the current research with that of Morse, et al. (1997) and Davis, et al. (in review). The prior research tests a construct — locomotor activity — which, while distinct from memory, arguably could be related in a shuttlebox measure that requires some locomotor activity to demonstrate that memory has been acquired. The fact that drug effects on locomotor activity and performance in an active avoidance shuttlebox memory paradigm sometimes diverge manifests the differences in the testing constructs.

Ideally, the first experiment for females would have replicated the two consecutive testing days for males in females. Unfortunately, an equipment failure after the first day of testing the female rats necessitated obtaining replacement parts from the manufacturer. Because there was not sufficient quantities of drug or naive female rats available to start that portion of the experiment over, it was necessary to have a one-week interval between the first and second testing days for females. Intermittent equipment failure, sometimes in mid-trial, resulted in having to discount the results of some of the animals. If this experiment was replicated, this would be rectified.

A wider range of dosages would have been useful for Experiment 1, to establish a dosage response curve to detect levels of behavioral toxicity. Future studies should also address how strain differences in laboratory animals affect the outcome of the effects of anti-HIV medications on memory since it has been demonstrated that medication side effects can be manifested in different ways between strains of laboratory animals (Crawley, et al., 1997). This is relevant because of the pan-global nature of HIV disease.

Manipulating the weight of the rats, specifically reducing the rat weights to mirror the wasting experienced by patients with HIV/AIDS would provide information as it relates to behavioral toxicities that might be body weight-related. Research examining body weight and dosage of AZT, ddC, and d4T would indicate how the drugs affected body mass in male and female animals differently, if at all. This would be of interest since severe weight loss is associated with the progression of HIV disease.

Manipulating hormone levels of the male and female rats, either by gonadectomy or with hormone replacement, would investigate the question to what extent sex hormones, specifically

estrogen and testosterone either protect against or mediate memory decrements when nucleoside analogs are administered. One explanation why, in the current research, female rats seemed to have less of the deleterious effects on memory than male rats is the greater volume of estrogen in the female rat. Resnick, Metter, and Zonderman (1997) collected information on estrogen replacement therapy (ERT) use during visits to the National Institute for Aging for memory testing and substantiated the hypothesis that women who received ERT during the memory testing period performed better than women who had never received treatment. The women in the study were shown the Benton Visual Retention Test, a series of figures were shown for 10 seconds and then were asked to reproduce each figure from memory. Women who were receiving ERT showed a mean difference of about 20% fewer errors than women who were never treated with ERT. Women who had never taken estrogen, but started after they entered the study scored higher on subsequent memory tests than women who did not take ERT. The principal investigator of this study, Dr. Susan Resnick, indicated that estrogen directly influences characteristics of neurons in the brain, particularly in regions important for new learning (National Institute of Aging, December 22, 1997). In this study, it could be hypothesized that estrogen protected against memory deficits associated with AZT, ddC, and d4T. Future studies should manipulate hormone levels in male and female rats in conjunction with administration of the anti-HIV medications to examine how memory is enhanced, reduced, or remains unchanged. This would be of particular importance for male patients who appear to have the greatest liability with memory decrements associated with AZT, ddC, and d4T.

Future studies should test male and female rats with replications of the "HIV cocktails" that utilize combinations of the nucleoside analogs to determine the extent to which combinations

affect memory. Identical tests could be performed with protease inhibitors to determine their effects on memory in male and female rats, and eventually on non-human primates to determine the extent of memory decrements in these species. These tests would indicate if certain combinations of the nucleoside analogs have an effect on memory.

Of primary concern in evaluating the utility of a medication, no matter how effective it is in slowing or curing a disease, are the side effects associated with its administration. Anti-HIV drugs must be taken consistently to reach and maintain sufficient concentrations in infected cells to inhibit HIV replication (Roche-HIV, 1997). A reduction in the concentration of a drug to below a critical level allows the virus to continue replicating and provides ideal conditions for the development of drug resistance leading to a need for increasing drug concentrations to control viral replication. The time spent when the body's drug concentration is reduced allows replication to occur over a longer period of time (Roche-HIV, 1997).

Neurotoxicities resulting from administration of AZT, ddC, and d4T, including impaired memory, could pose significant barriers to complying with a medication regime. People living with HIV/AIDS have been reported as choosing not to adhere to treatment as a means of retaining control or of coping with their illness (Conrad, 1985). Studies of AZT therapy have reported adherence rates of 42-88%; this level of adherence is consistent with studies in other chronic illnesses (Roche-HIV, 1997; Broers, et al. 1994; Samet, et al., 1992; Samuels, et al., 1990; Eldred, et al., 1997; Muma, et al., 1995). In HIV infected people taking AZT, drug toxicity was found to influence adherence, and knowledge of the medication brought about improved compliance (Roche-HIV, 1997; Eldred, et al., 1997).

This research reported some behavioral toxicity associated with AZT, ddC, and d4T

manifested in male rats in an active avoidance shuttlebox paradigm. These results should be confirmed and extended in the paradigms listed in this section, as well as other cognitive assessment measures, to identify the bases for the male rats' vulnerability as well as the relative resistance of the female rats. Additional testing is indicated to explore human gender differences and dosage responses in the field of anti-HIV medication and effects on memory.

By continuing to research how to avoid the toxic side effects of the nucleoside analogs can be effectively avoided. In achieving this objective, the benefit of adhering to a prescribed regimen of anti-HIV drugs can provide a stronger incentive for patient compliance and further extension of the patient's quality of life over the course of the disease.

TABLES AND FIGURES

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Table 1
HIV Epidemiology

Number of deaths from AIDS in 1997.....	2,300,000
Number of deaths from AIDS since 1981.....	11,700,000
Number of people with AIDS or HIV infection in 1997.....	30,600,000
Estimated number of people with AIDS or HIV infection in 2000...	40,000,000
Number of young people (10-24) with AIDS or HIV infection in 1997.....	10,000,000
Number of children < 15 who have lost their mother to AIDS as of 1997.....	8,000,000
Estimated number of children <15 who will lose their mother to AIDS by 2000 (cumulative)	16,000,000

Source: UNAIDS, 1997.

Table 2
Experiment One
Mean +/-SEM Values (Seconds) - Males and Females
and F Values
Testing Times 1 and 2
AZT, ddC, or Water

MALES	Time 1	Time 2
Water	15.06+/-0.97 (n =9)	15.38+/-0.88 (n=10)
AZT	18.3+/-2.6 (n=9)	17.1 +/-0.8 (n=10)
ddC	17.34+/-1.4 (n=10)	17.3+/-2.57 (n=10)

FEMALES	Time 1	Time 2
Water	14.79+/-1.05 (n=9)	11.01+/-0.53 (n=10)
AZT	14.33 +/-0.81 (n=8)	11.69 +/-0.93 (n=10)
ddC	13.4 +/-1.73 (n=7)	10.6 +/-0.75 (n=10)

Males had greater latencies than females:

F(1,46)=4.43; p=0.04; F(1,54)=27.585; p<0.001.

Female rats dosed with AZT or ddC had significantly longer latencies the first time of testing compared to the second testing time.

F(28,378)=25.407; p=0.05.

EXPERIMENT ONE, TIME ONE, BETWEEN-SUBJECTS EFFECTS

SOURCE	SUM OF SQ.	DF	MEAN SQ.	F	SIGNIFICANCE
Sex	95.33	1	95.33	4.438	.041
Drug	17.42	2	8.71	.405	.669
Sex x Drug	39.785	2	19.893	.926	.403
Error	988.107	46	21.481		

EXPERIMENT ONE, TIME TWO, BETWEEN-SUBJECTS EFFECTS

SOURCE	SUM OF SQ.	DF	MEAN SQUARE	F	SIGNIFICANCE
Sex	453.985	1	453.985	27.585	.000
Drug	15.06	2	7.530	.458	.635
Sex x Drug	13.578	2	6.789	.413	.664
Error	888.701	54	16.457		

EXPERIMENT ONE, WITHIN SUBJECT EFFECTS

SEX	SOURCE	SUM OF SQ.	DF	MEAN SQ.	F	SIG.
Female	Time x Drug	711.384	28	25.407	1.491	.054
	Error	6442.924	378	17.045		
Male	Time x Drug	486.945	28	17.391	.476	.990
	Error	13812.876	378	36.542		

Table 3
Experiment Two
Mean Values +/- SEM (Seconds) - Males and Females
and F Values
Testing Times 1 and 2
Acute d4T Administration

MALES	Time 1	Time 2
Control	12.01 +/-1.21 (n =10)	10.08 +/-0.37 (n=10)
32 mg/kg	13.25 +/-1.66 (n=10)	10.14 +/-0.32 (n=10)
250 mg/kg	11.9 +/-0.1 (n=10)	10.84 +/-0.4 (n=10)
1250 mg/kg	13.8 +/-2.0 (n=7)	10.81 +/-0.4 (n=10)

FEMALES	Time 1	Time 2
Control	12.13 +/-1.4 (n=10)	9.77 +/-0.3 (n=10)
32 mg/kg	12.13 +/-1.4 (n=10)	9.41 +/-0.49 (n=10)
250 mg/kg	10.98 +/-0.48 (n=9)	10.00 +/-0.47 (n=9)
1250 mg/kg	11.88 +/-0.3 (n=10)	9.91 +/-0.26 (n=10)

There were no between-subjects effects.

All groups showed decreased latencies over time.

Control:

F(1,18)=5.142, p<0.05

.32 mg/kg:

F(1,18)=7.094, p<0.05

250mg/kg:

F(1,17)=12.97, p<0.01

1250 mg/kg:

F(1,15)=6.297, p<0.05

EXPERIMENT TWO, WITHIN-SUBJECT EFFECTS

DRUG	SOURCE	SUM OF SQ.	DF	MEAN SQ.	F	SIG.
Control	Time	46.010	1	46.010	5.142	.036
	Time x Sex	.462	1	.462	.052	.823
	Error (Time)	161.062	18	8.948		
32 mg/kg	Time	84.972	1	84.972	7.094	.016
	Time x Sex	.380	1	.380	.032	.861
	Error (Time)	215.602	18	11.978		
250 mg/kg	Time	11.05	1	11.05	12.971	.002
	Time x Sex	6.063	1	6.063	.071	.793
	Error (Time)	14.482	17	.852		
1250 mg/kg	Time	52.624	1	56.624	6.279	.024
	Time x Sex	2.563	1	2.563	.306	.588
	Error (Time)	125.715	15	8.381		

Table 4
Experiment Three
Mean Values +/- SEM (Seconds) - Males and Females
and F Values
Testing Times 1, 2, and 3
Chronic Administration of d4T

MALES	Time 1	Time 2	Time 3
Control	11.67 +/-0.73 (n=9)	8.14 +/-0.93 (n=9)	8.05 +/-2.09 (n=4)
0.1 mg/kg	15.14 +/-2.44 (n=8)	10.88 +/-1.81 (n=8)	11.95 +/-0.45 (n=4)
0.3 mg/kg	11.82 +/-0.46 (n=8)	9.0 +/-0.75 (n=8)	9.42 +/-1.58 (n=4)
1.0 mg/kg	16.42 +/-2.54 (n=8)	9.08 +/-1.02 (n=8)	10.47 +/-0.35 (n=4)
3.0 mg/kg	13.12 +/-0.66 (n=7)	9.4 +/-1.17 (n=7)	10.45 +/-0.23 (n=3)

FEMALES	Time 1	Time 2	Time 3
Control	10.04 +/-0.8 (n=9)	7.55 +/-0.83 (n=9)	5.04 +/-1.1 (n=5)
0.1 mg/kg	11.51 +/-0.89 (n=7)	8.72 +/-0.9 (n=7)	4.75 +/-0.96 (n=4)
0.3 mg/kg	11.21 +/-0.22 (n=8)	9.8 +/-0.48 (n=8)	6.02 +/-1.34 (n=4)
1.0 mg/kg	11.32 +/-0.85 (n=7)	8.75 +/-0.95 (n=7)	6.35 +/-1.4 (n=4)
3.0 mg/kg	10.72 +/-4.11 (n=6)	9.22 +/-1.53 (n=6)	7.85 +/-2.13 (n=4)

Male latencies were significantly longer than female latencies for all groups at Times 1 and 2:

$F(1,67)=6.941, p=0.01$; $F(1,30)=26.863, p<0.001$.

For cessation animals tested on time one, male and female latencies for drug animals were longer than control animals at 0.1 mg/kg :
and at 1.0 mg/kg:

$F(1,7)=45.2, p<0.01$,

$F(1,7)=8.1, p<0.05$.

EXPERIMENT THREE - BETWEEN SUBJECTS EFFECTS - TESTING TIMES ONE AND TWO

SOURCE	SUM OF SQ.	DF	MEAN SQUARE	F	SIGNIFICANCE
Sex	101.835	1	101.835	6.941	.01
Drug	101.748	4	25.437	1.734	.153
Sex x Drug	51.938	4	12.984	.885	.478
Error	983.045	67	14.672		

EXPERIMENT THREE - BETWEEN SUBJECTS EFFECTS - CESSATION PHASE

SOURCE	SUM OF SQ.	DF	MEAN SQUARE	F	SIGNIFICANCE
Sex	162.961	1	162.961	26.863	.000
Drug	30.665	4	7.666	1.264	.306
Sex x Drug	26.782	4	6.695	1.104	.373
Error	181.991	30	6.066		

EXPERIMENT THREE BETWEEN-SUBJECTS EFFECTS FOR CESSATION ANIMALS

DRUG	SOURCE	SUM OF SQ.	DF	MEAN SQ.	F	SIG.
Control	Sex	20.134	1	20.134	1.818	.220
	Error	77.542	7	11.077		
.1 mg/kg	Sex	103.680	1	103.680	45.209	.001
	Error	13.76	6	2.293		
.3 mg/kg	Sex	23.120	1	23.120	2.690	.152
	Error	51.575	6	8.596		
1.0 mg/kg	Sex	34.031	1	34.031	8.123	.029
	Error	25.138	6	4.19		
3.0 mg/kg	Sex	11.44	1	11.44	4.093	.099
	Error	13.977	5	2.795		

Figure 1
Experiment One
Males - Testing Times 1 and 2
AZT, ddC, or Water
IG Administration on Time 1

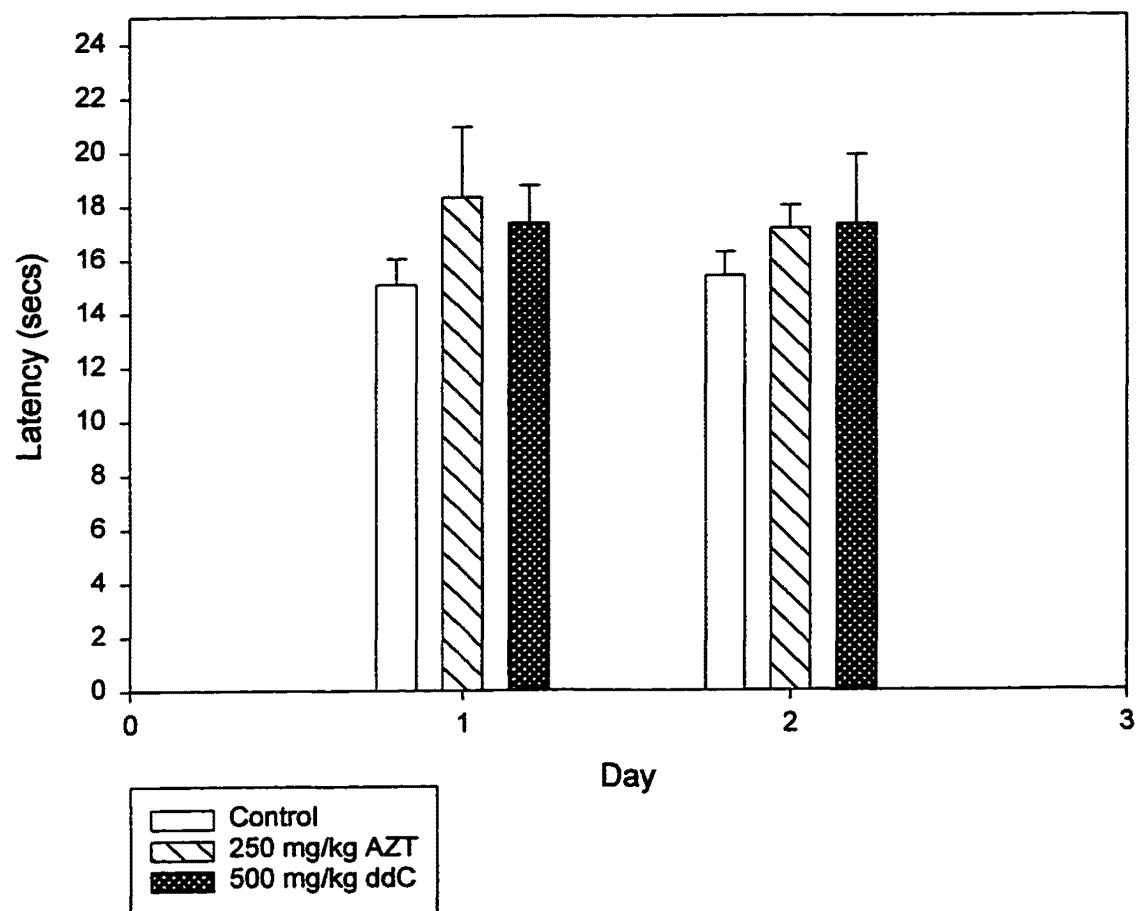


Figure 2
Experiment One
Females - Testing Times 1 and 2
AZT, ddC, or Water
IG Administration on Time 1

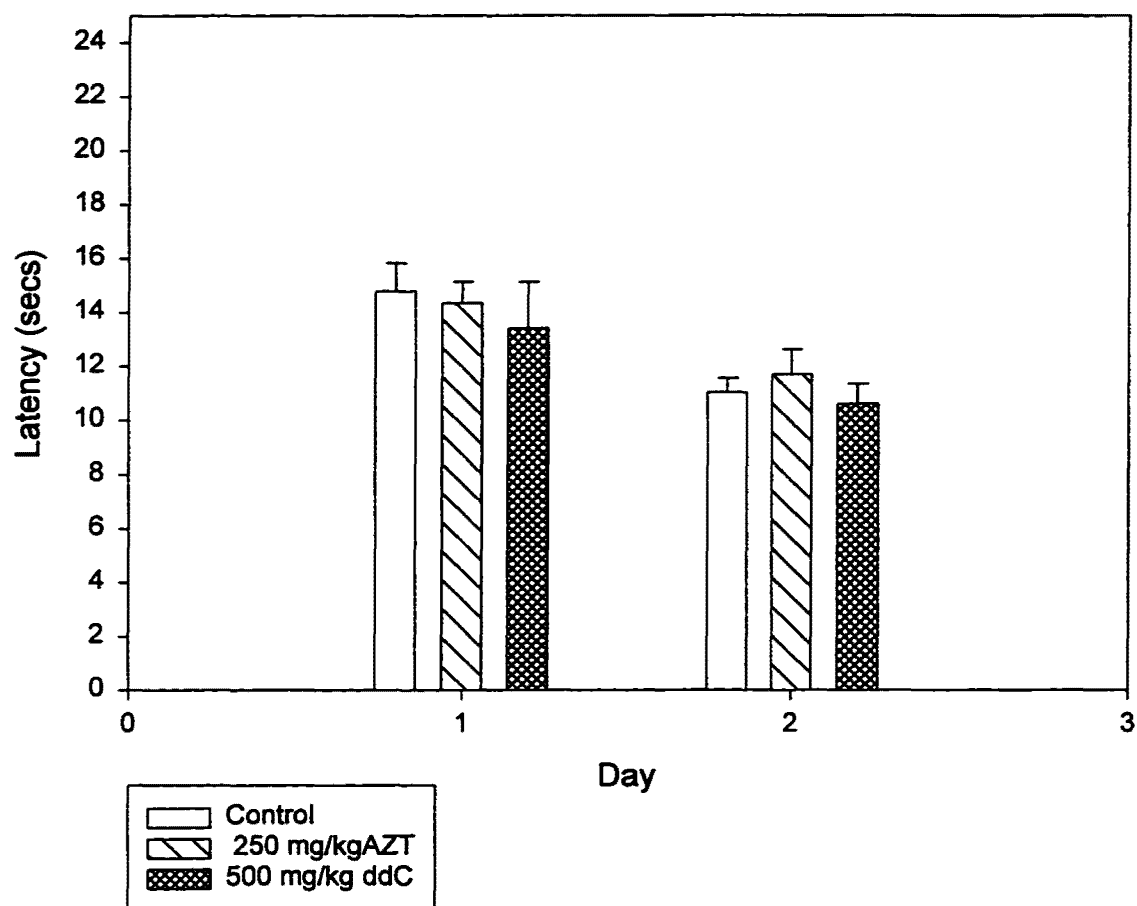


Figure 3
Experiment Two
Males - Testing Times 1 and 2
d4T or Water Administration IG on Time 1

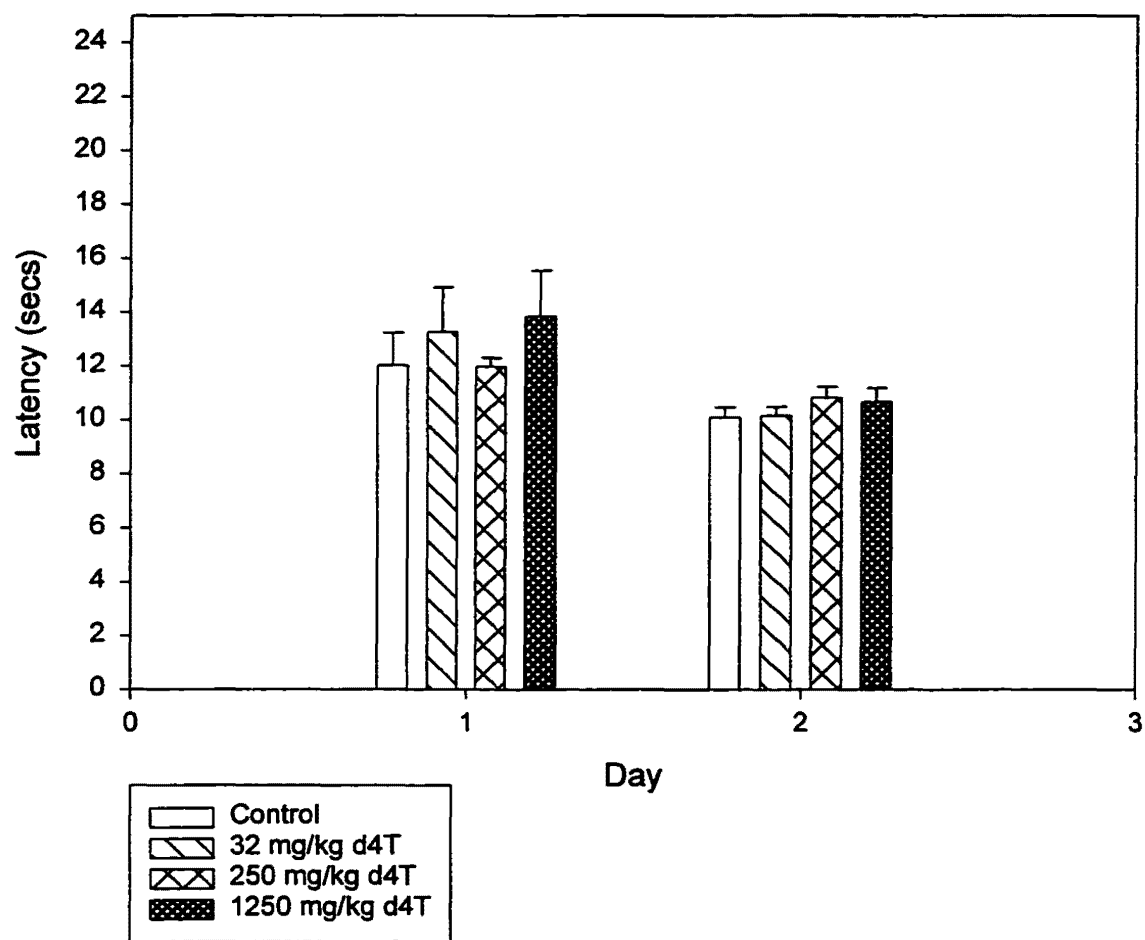


Figure 4
Experiment Two
Females - Testing Times 1 and 2
d4T or Water Administered IG on Time 1

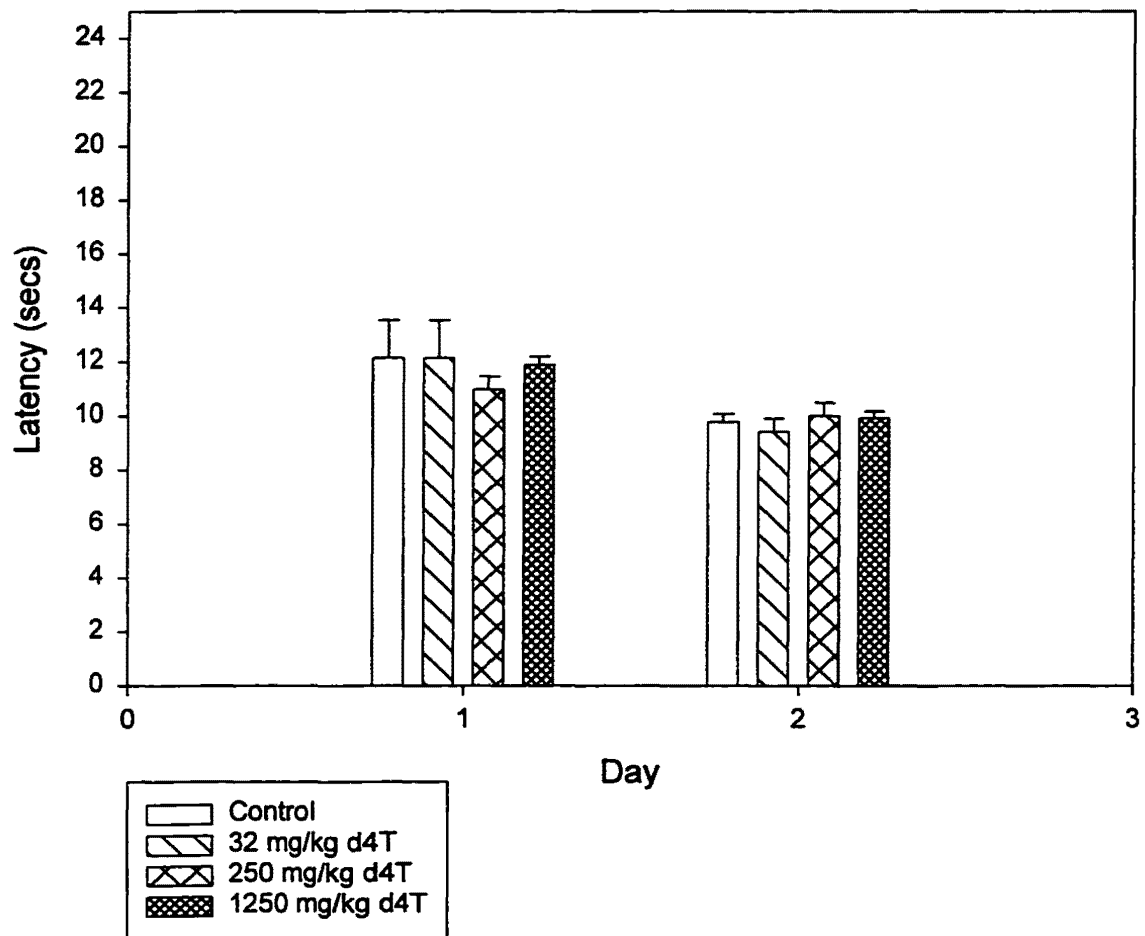


Figure 5
Experiment Three
Males - Testing Times 1, 2, and 3
Chronic Administration of d4T

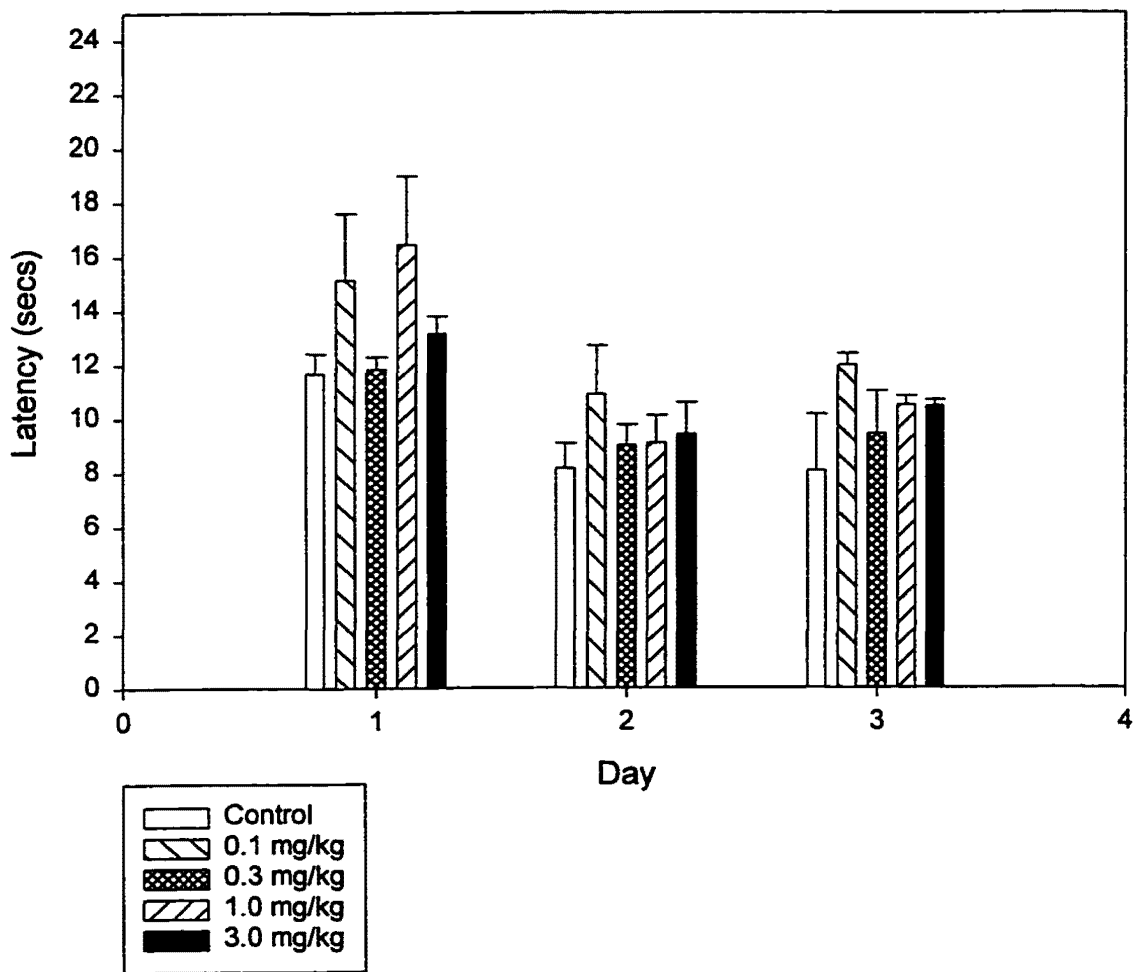
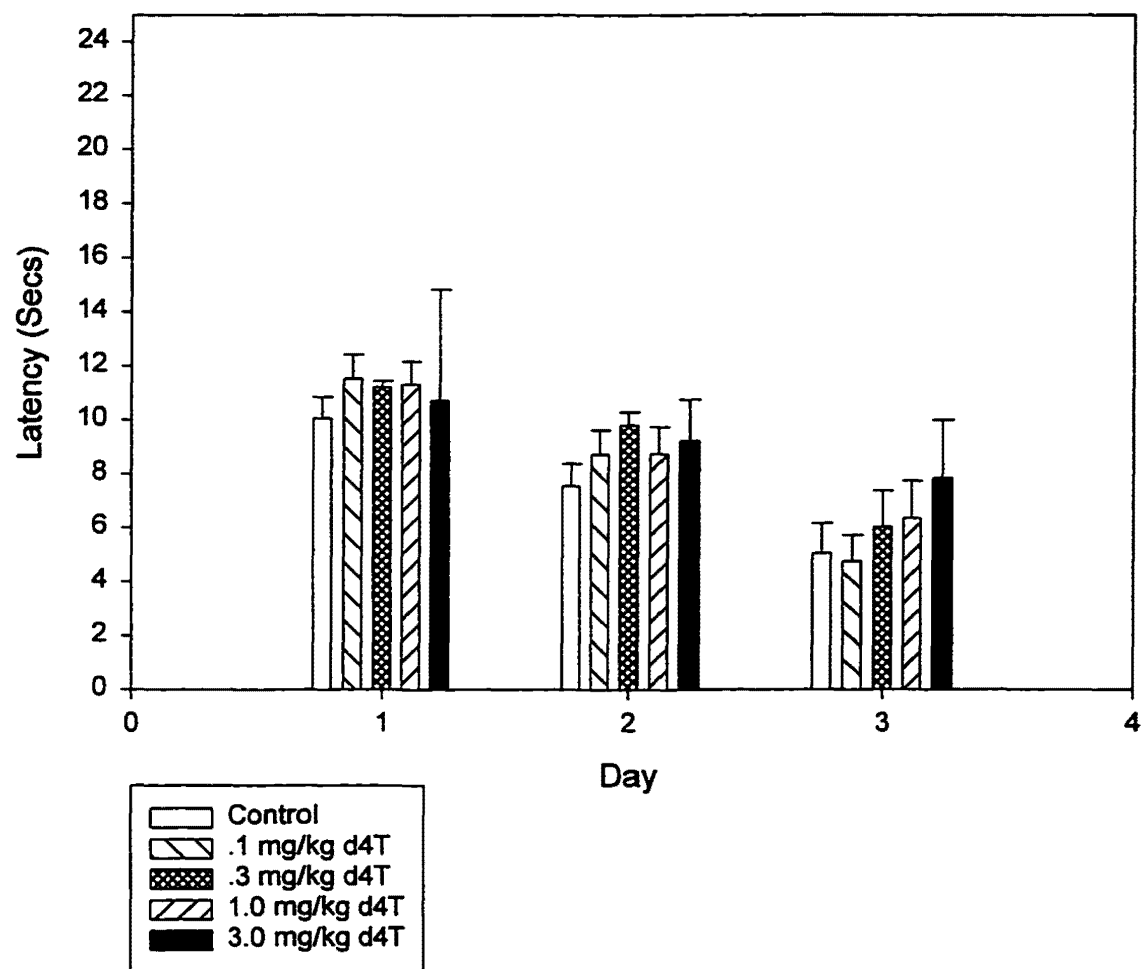


Figure 6
Experiment Three
Females - Testing Times 1, 2, and 3
Chronic Administration of d4T



REFERENCES

- Anderson, T.D., Davidovich, A., & Arceo, R. (1991). Peripheral neuropathy induced by 2',3'-dideoxycytidine: a rabbit model of 2',3'-dideoxycytidine neurotoxicity. Laboratory Investigator, 66, 63-74.
- Baldeweg, T., Catalan, J., Lovett, E. (1995). Long-term zidovudine reduces neurocognitive deficits in HIV-1 infection. AIDS, 9, 589-596.
- Berger, A.R., Arezzo, J.C., & Schaumburg, H.H. (1993). 2',3'-dideoxycytidine (dd) toxic neuropathy: a study of 52 patients. Neurology, 43, 358-362.
- Bovet, D., Bovet-Nitti, F., & Oliverio, A. (1969). Genetic aspects of learning and memory in mice. Science, 163, 139-149.
- Bovet, D., McGaugh, J.L., & Oliverio, A. (1966). Effects of post trial administration of drugs on avoidance learning of mice. Life Sciences, 5, 1309-1315.
- Broers, B., Morabia, A., & Hirschel, B. (1994). A cohort study of drug users adherence with zidovudine treatment. Annals of Internal Medicine, 154, 1121-1127.
- Brouwers, P., Hendricks, M., & Lietzau, J.A. (1997). Effect of combination therapy with zidovudine and didanosine on neuropsychological functioning in patients with symptomatic HIV disease: a comparison of simultaneous and alternating regimens. AIDS, 11, 59-66.
- Brown, K.J., & Grunberg, N.E. (1996). Effects of environmental conditions on food consumption in female and male rats. Physiology and Behavior, 60, 193-297.
- Carpenter, C.C., Fischl, M.A., & Hammer, S.M. (1997). Antiretroviral therapy for HIV infection in 1997: updated recommendations of the International AIDS Society - USA panel. Journal of the American Medical Association, 277, 1962-1969.

Centers for Disease Control and Prevention (1998). Public Health Service Task Force Recommendations for the Use of Antiretroviral Drugs in Pregnant Women Infected with HIV-1 for Maternal Health and for Reducing Prenatal HIV-1 Transmission in the United States. Morbidity and Mortality Weekly Report, 47, 1-30.

Centers for Disease Control and Prevention (1998). Report of the NIH Panel to Define Principles of Therapy of HIV Infection and Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents. Morbidity and Mortality Weekly Report, 47.

Centers for Disease Control and Prevention (1997). USPHS/IDSA Prevention of opportunistic infections working group. 1997 USPHS/IDSA guidelines for the prevention of opportunistic infections in persons infected with human immunodeficiency virus. Morbidity and Mortality Weekly Report, 46.

Centers for Disease Control and Prevention (1997). HIV/AIDS Surveillance Report, 9, 1-43.

Conrad, P. (1985). The meaning of medications: another look at adherence. Social Science Medicine, 20, 29-37.

Crawley, J.N., Belknap, J.K., & Collins, A. (1997). Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. Psychopharmacology, 132, 107-124.

Damos, D.L., John, R.S., Parker, E.S., & Levine, A.M. (1997). Anti-retroviral therapy and cognitive function. Aviation, Space, and Environmental Medicine, 68, 900-906.

Davis, H.D., Grunberg, N.E., O'Donoghue, V.A., Popke, E.J., Brown, K.J., & Morse, D.E. (under review). Effects of ddC and AZT on locomotion and acoustic startle II: acute effects in male rats. Uniformed Services University of the Health Sciences, Bethesda, Maryland.

Davis, H.D., Faraday, M.M., Grunberg, N.E., Morse, D.E., O'Donoghue, V.A., & Ussery, M.A. (under review). Effects of an acute dose of d4T on locomotion and acoustic startle responses in male and female rats. Uniformed Services University of the Health Sciences, Bethesda, Maryland.

Eldred, L., Wu, A., Chaison, R.E., & Moore, R.D. (1997). Adherence to antiretroviral therapy in HIV disease. Washington 1997. Sixth European Conference on Clinical Aspects and Treatment of HIV-Infection, Abstract 251.

Gorman, J.M., Mayeux, R., & Stern, Y. (1993). The effect of zidovudine on neuropsychiatric measures in HIV-infected men. American Journal of Psychiatry, 150, 505-507.

Gottlieb, M.S., Schroff, R., Schanker, H.M., Weisman, J.D., Fra, P.T., Wolf, R.A., & Saxon, A. (1981). Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. New England Journal of Medicine, 305, 1425-1431.

Grunberg, N.E., Winders, S.E., & Wewers, M.E. (1991). Gender differences in tobacco use. Health Psychology, 10, 143-153.

Grunberg, N.E., Winders, S.E., & Popp, K.A. (1987). Sex differences in nicotine's effects on consummatory behavior and body weight in rats. Psychopharmacology, 91, 221-225.

James, W. (1890). The Principles of Psychology (vol. 1). New York: Henry Holt and Co.

Klatzky, R.L. (1975). Human memory, structures and processes. San Francisco: W.H. Freeman and Co.

Klein, L.C., Popke, E.J., & Grunberg, N.E. (1997). Sex differences in effects of predictable and unpredictable footshock on fentanyl self-administration in rats. Experimental and Clinical Psychopharmacology, 5, 99-106.

Lex, B.W. (1991). Some gender differences in alcohol and polysubstance users. Health Psychology, 10, 121-132.

Masur, H., Michelis, M.A., Greene, J.B., Onorato, I., Stouwe, R.A., Holzman, R.S., Wormser, G., Brettman, L., Lange, M., Murray, H.W., & Rundles, S.C. (1981). An outbreak of community-acquired pneumocystis carinii pneumonia. New England Journal of Medicine, 305, 1431-1438.

Markowitz, M. (1997, October). Combination Therapy for HIV Infection: Why to use more than one drug, and which drugs to use. International Association of Physicians in AIDS Care. Available E-Mail: www.thebody.com/iapac/oct97combo.html.

Messing, R.B., Jensen, R.A., Martinez, J.L., Spiehler, V.R., Vasquez, B.J., Soumireu-Mourat, B., Liang, K.C., & McGaugh, J.L. (1979). Naloxone enhancement of memory. Behavioral and Neural Biology, 27, 266-275.

Morse, D.E., Davis, H.D., Popke, J.E., Brown, K.J., O'Donoghue, V.A., & Grunberg, N.E. (1997). Effects of ddC and AZT on locomotion and acoustic startle I: acute effects in female rats. Pharmacology Biochemistry & Behavior, 56, 221-228.

Morse, G.D., Shelton, M.J., & O'Donnell, A.M. (1993). Comparative pharmacokinetics of antiviral nucleoside analogues. Clinical Pharmacokinetics, 24, 101-123.

Muma, R.D., Ross, M.W., Parcel, G.S., & Pollard, R.B. (1995). Zidovudine adherence among individuals with HIV infection. AIDS Care, 7:439-447.

National Institute of Aging. press release. December 22, 1997 -- Estrogen replacement may help slow decline in memory in post-menopausal women. Available E-Mail: www.nih.gov/nia/new/press/estrogen.

O'Brien, W.A., Hartigan, P.M., & Martin, D. (1996). Changes in plasma HIV-1 RNA and CD4+ lymphocyte counts and the risk of progression to AIDS. New England Journal of Medicine, 334, 426-431.

Physicians' Desk Reference, 51st Ed. (1997). Montvale, NJ.

Resnick, S.M., Metter, E.J., & Zonderman, A.B. (1997). Estrogen replacement therapy and longitudinal decline in visual memory: a possible protective effect? Neurology, 49, 1491-1497.

Roche-HIV, 1997 -- International Task Force on Compliance. Available E-Mail: Roche-HIV.com-International taskforce on Compliance.

Samet, J.H., Libman, H. Steger, K.A., & Rajeev, K. (1992). Adherence with zidovudine therapy in patients infected with human immunodeficiency virus, type 1: a cross-sectional study in a municipal hospital clinic. American Journal of Medicine, 92, 495-502.

Schmitt, F.A., Bigley, J.W., & McKinnis, R. (1988). Neuropsychological outcome of zidovudine (AZT) treatment of patients with AIDS and AIDS-related complex. New England Journal of Medicine, 319, 1573-1578.

Siegel, F.P., Lopez, C., Hammer, G.S., Brown, A.E., Kornfeld, S.J., Gold, J., Hassett, J., Hirschman, S.Z., Rundles, C.C., Adelsberg, B.R., Parhman, D.M., Siegal, M., Runcles, S.S., & Armstrong, D. (1981). Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions. New England Journal of Medicine, 305, 1439-1444.

United Nations: 1998 — New Report Finds Five Young People Infected With HIV Every Minute. Available E-Mail: www.unaids.org.

United Nations: 1997 — Report on the Global HIV/AIDS Epidemic . Available E-mail: www.unaids.org.

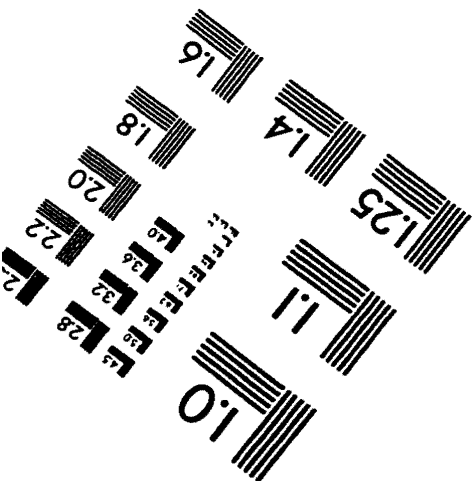
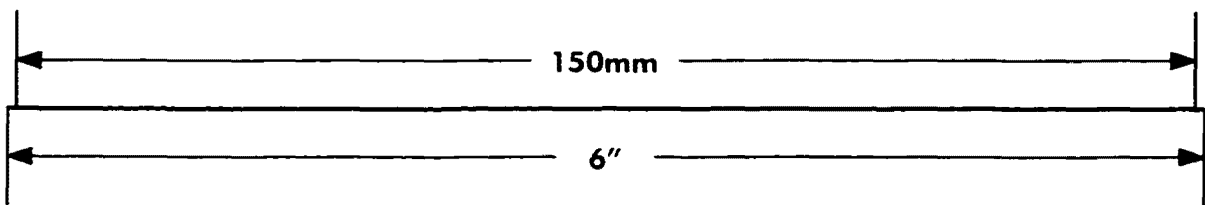
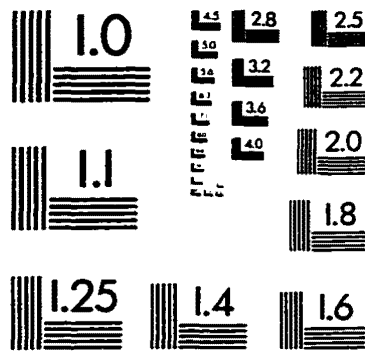
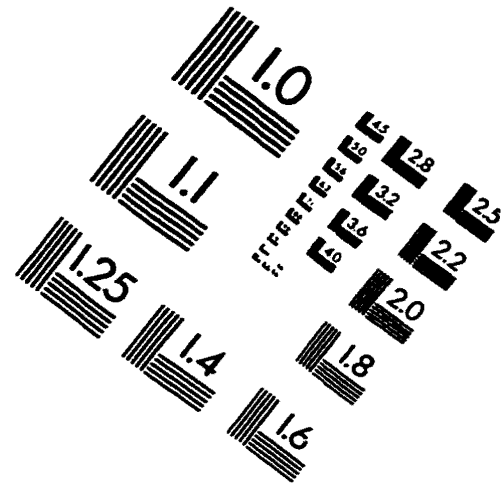
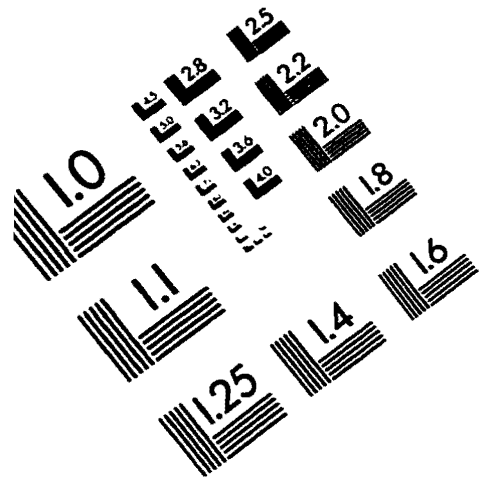
United Nations: 1997a — New UN World AIDS Report Warns that HIV Epidemic is Far Worse Than Previously Thought. Available E-Mail: www.unaids.org.

United Nations: 1996 — The HIV/AIDS Situation in mid 1996. Global and regional highlights, estimates as of 1 July 1996. Available E-Mail: www.unaids.org.

World Health Organization: 1997 — World Health Organization Report on the Epidemic. Available E-Mail: www.hsph.harvard.edu/Organizations/hai/haiwad1997.

Webster's II New Riverside Dictionary (1988). The Riverside Publishing Company: Boston.

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