20030128292 AD_____ The Effects of CW-Related Chemicals on MC FILE COP!"

Annual Report

Bradford N. Bunnell W. Ben Iturrian

October 1, 1985

September 30, 1984 - September 29, 1985

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-83-C-3260

University of Georgia Athens, Georgia 30602



21

88 1

013

DOD DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

	DOCUMENTATIC	N PAGE	TION PAGE OMB No. 0704-0188				
1. REPORT SECURITY CLASSIFICATION		1b. RESTRICTIVE	MARKINGS				
Unclassified		1					
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION	AVAILABILITY OF	REPORT			
26. DECLASSIFICATION / DOWNGRADING SCHEDU	JLE	Approved for public release; distribution unlimited					
4. PERFORMING ORGANIZATION REPORT NUMB	ER(S)	5. MONITORING	ORCANIZATION R.	PORT NUMBER(5)		
6. NAME OF PERFORMING ORGANIZATION	66. OFFICE SYMBOL	7a. NAME OF M	ON TORING ORGAN	IZATION			
University of Georgia	(if applicable)						
5c. ADDRESS (City, State, and ZIP Code)		7b. ADDRESS (Cit	ty, State, and ZIP C	ode)			
Athens, Georgia 30602							
Ca. NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMEN	TINSTRUMENT IDE	NTIFICATION NU	IMBER		
Research & Development Command	L		. DATUIT -0.	J-U-J200			
Ic. ADDRESS (City, State, and ZIP Code)		10. SOURCE OF F	UNDING NUMBERS	TASK	WORK LINIT		
Fort Detrick Frederick, Maryland 21701-5012		ELEMENT NO. 62734A	NO. 3M1- 62734A875	NO. AE	ACCESSION NO		
11. TITLE (Include Security Classification)		1			1		
The Effects of CW-Related Chemi	cals on Social 1	Behavior and	Performance				
12. PERSONAL AUTHOR(S)							
Bradford N. Bunnell; W. Ben Itu	rrian						
I3a. TYPE OF REPORT	OVERED	14. DATE OF REPO	RT (Year, Month, D	ay) 15. PAGE	COUNT		
AIIIIUAL REPORT PROM <u>41</u>	30/84 10 9/29/85	UCTOD	er UI	5	8		
	Cont& Fre	Pq.					
	18. SUBJECT TERMS (Continue on reverse	if necessary and	identify by bloci	k number)		
7. COSAT! CODES				Charles D	iazepam;		
7. COSAT! CODES FIELD GROUP SUB-GROUP	Social Behavio	or; Operant;	Performance;	Screas; D	• •		
7. COSAT! CODES FIELD GROUP SUB-GROUP 06 16 15	Social Behavio Pyridostigmine	or; Operant; e; Caffeine	Performance;	Scress; D			
7. COSAT! CODES FIELD GROUP SUB-GROUP 06 16 06 06 15 06	Social Behavio Pyridostigmine	or; Operant; e; Caffeine	Performance;	Stress; D	• ´		
7. COSAT! CODES FIELD GROUP SUB-GROUP 06 16 06 15 9. ABSTRACT (Continue on reverse if necessary	Social Behavic Pyridostigmine and identify by block m	or; Operant; e; Caffeine umber)	Performance;	Stress; D			
7. COSAT! CODES FIELD GROUP SUB-GROUP 06 16 06 15 9. ABSTRACT (Continue on reverse if necessary)	Social Behavic Pyridostigmine and identify by block m	or; Operant; e; Caffeine umber)	Performance;	Scress; D	. <i>,</i>		
7. COSAT! CODES FIELD GROUP SUB-GROUP 06 16 06 15 9. ABSTRACT (Continue on reverse if necessary)	Social Behavic Pyridostigmine and identify by block m	or; Operant; e; Caffeine, umber)	Performance;	Scress; D			
7. COSAT! CODES FIELD GROUP SUB-GROUP 06 16 06 15 9. ABSTRACT (Continue on reverse if necessary)	Social Behavic Pyridostigmine and identify by block n	or; Operant; e; Caffeine umber)	Performance;	Stress; D	. ,		
7. COSAT' CODES FIELD GROUP SUB-GROUP 06 16 06 15 9. ABSTRACT (Continue on reverse if necessary)	Social Behavic Pyridostigmine and identify by block m	or; Operant; e; Caffeine umber)	Performance;	Stress; D			
7. COSAT' CODES FIELD GROUP SUB-GROUP 06 16 06 15 9. ABSTRACT (Continue on reverse if necessary)	Social Behavic Pyridostigmine and identify by block m	or; Operant; e; Caffeine, umber)	Performance;	Scress; D	. ,		
7. COSAT' CODES FIELD GROUP SUB-GROUP 06 16 06 15 9. ABSTRACT (Continue on reverse if necessary)	Social Behavid Pyridostigmine and identify by block n	or; Operant; e; Caffeine umber)	Performance;	Scress; D			
7. COSAT' CODES FIELD GROUP SUB-GROUP 06 16 06 15 9. ABSTRACT (Continue on reverse if necessary)	Social Behavic Pyridostigmine and identify by block n	or; Operant; e; Caffeine umber)	Performance;	Stress; D			
7. COSAT' CODES FIELD GROUP SUB-GROUP 06 16 06 15 9. ABSTRACT (Continue on reverse if necessary)	Social Behavic Pyridostigmine and identify by block n	or; Operant; e; Caffeine, umber)	Performance;	Scress; D			
7. COSAT' CODES FIELD GROUP SUB-GROUP 06 16 06 15 9. ABSTRACT (Continue on reverse if necessary)	Social Behavic Pyridostigmine and identify by block n	or; Operant; e; Caffeine, umber)	Performance;	Scress; D	. ,		
7. COSAT' CODES FIELD GROUP SUB-GROUP 06 16 06 06 15 9 ABSTRACT (Continue on reverse if necessary	Social Behavic Pyridostigmine and identify by block n	or; Operant; e; Caffeine, umber)	Performance;	Stress; D			
7. COSAT' CODES FIELD GROUP SUB-GROUP 06 16 06 15 9. ABSTRACT (Continue on reverse if necessary	Social Behavic Pyridostigmine and identify by block n	or; Operant; e; Caffeine, umber)		Scress; D			
7. COSAT' CODES FIELD GROUP SUB-GROUP 06 16 06 06 15 9 ABSTRACT (Continue on reverse if necessary 0 D. DISTRIBUTION / AVAILABILITY OF ABSTRACT Image: Comparison of the comparison	Social Behavic Pyridostigmine and identify by block m	21. ABSTRACT SEC Unclass	URITY CLASSIFICAT	ION	. ,		
7. COSAT' CODES FIELD GROUP SUB-GROUP 06 16 06 06 15 9 9. ABSTRACT (Continue on reverse if necessary 0. DISTRIBUTION / AVAILABILITY OF ABSTRACT Image: Comparison of the struct of t	Social Behavid Pyridostigmine and identify by block m PT. DTIC USERS	21. ABSTRACT SEC Unclass 22b. TELEPHONE (W	URITY CLASSIFICAT ified xlude Area Code)	ION	MBQL		
7. COSAT' CODES FIELD GROUP SUB-GROUP 06 16 06 06 15 9 9. ABSTRACT (Continue on reverse if necessary 0 D. DISTRIBUTION / AVAILABILITY OF ABSTRACT Image: Comparison of the second s	Social Behavic Pyridostigmine and identify by block m PT DTIC USERS	21. ABSTRACT SEC Unclass 22b. TELEPHONE(11 301/663-7	URITY CLASSIFICAT if ied rclude Area Code) 325	TION 22c. OFFICE SY SGRD-RM	MBOL I-S		

1. N.Y.

۰.

Table of Contents

Section	Page
Title Page	1
Table of Contents	2
Summary, Abstract, or Digest	3
Foreword	3
Body of the Report	3
A. Dverview B. Monkey Colony	3
C. Activity Tests and Drug Dose Selection	7
D. Social Behavior and Organization	8
E. Open Field Testing	25
F. Complex Problem Solving	30
G. Operant performance	36
H. Plasma Hormones - Baseline and Stress	45
I. Equipment and Facilities	48
J. Personnel	49
Future Work	50
References	52
Appendix: A - Social Behavior Matrices	53
B - Post Injection Delays	- .
and DRL Performance	56
Distribution List	57
LACCE IN THE	



-2-

Summary, Abstract, or Digest

This report summarizes work accomplished in the second year of a three year project aimed at developing a battery of tests of social behavior and performance that will be sensitive to the effects of CW-related chemicals considered for use as antidotes or prophylactics against 💭 agents. Procedures for assessing social behavior in nonhuman primates are described and compared. Performance scores on three operant schedules, a test of complex problem solving, and behavior in a novel environment are presented and correlations between the social effects and performance variables are examined. The of atropines on several of the social and performance measures are reported as are data from plasma hormone assays for cortisol and prolactin. Their Wordsing

Chemical Worfor.

Foreword

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Facilities and Care and Use of Laboratory animals" (DHEW Publicaion No. (NIH) 80-23 prepared by the Institute of Laboratory Animal Resources, revised 1978, reprinted 1980, National Research Council, and in the United States Department of Agriculture's regulations and standards.

Body of the Report

A. Overview:

This report describes the work conducted during the second year of a three year contract which involves the development of a battery of individual tests for use in studying the effects of chemical warfare (CW) related chemicals on social behavior and performance. The specific objectives of the project are: (1) To evaluate and develop a set of behavioral tests for studying social behavior, individual performance, and the relationships between individual performance and social behavior in nonhuman primates. (2) To evaluate the utility of this battery of tests by examining the effects of CW-related chemicals that might be used as antidotes or prophylactics for CW agents on social behavior and performance. (3) To develop procedures and provide facilities for testing the long term behavioral sequalae of non-lethal exposure of nonhuman primates to CW agents.

During the year considerable effort was devoted to evaluating the various procedures used in obtaining social data with an eye toward selecting the most efficient methods of collecting data. A modified group scan/focal animal procedure was chosen as the best compromise fur daily data collection, with a two-observer scheme to be used for drug studies. Extensive work with indoor social tests on various combinations of animals has led to the selection of two procedures for inclusion in the battery. In one, stranger males and females are introduced to selected pairs during the social observations. In the second, a pellet feeder is activated to increase the amount of agonistic social behavior seen during an observation period. Two studies of atropine effects on social behavior were conducted during the year.

Studies of the effects of atropine sulphate and atropine methyl nitrate on behavior in an open field were conducted with both an empty open field and with novel objects present in the field. As a result, the open field test with novel objects has been chosen as the test of choice for inclusion in the test battery. Correlations were found between the the social ranks of the monkeys and their willingness to enter the open field in both types of open field tests.

Tests of complex problem solving were conducted in the WGTA with an object quality reversal learning set paradigm. Dose dependent disruptions of performance were obtained with both atropines. A very interesting effect was an interaction between the degree of disruption of performance and the social status of the animals. High status animals suffered less disruption of performance than low status monkeys. A number of significant correlations between social status, agonistic behavior frequencies, and WGTA performance were obtained.

Operant tasks included the study of atropine effects of DRL, FI, and RI schedules of reinforcement. The last schedule included both 100% and partial reinforcement contingencies. The atropines produced a number of changes in performance on these schedules but there were considerable individual differences in the dose response curves. Omission of reinforcement on the RI schedule did not consistently produce the response bursting MO had expected and we plan to substitute a multiple RI -RI extinction schedule for present schedule in future work. There were no consistent correlations between performance on the RI tasks and social variables. However, there were consistent, marginally significant, correlations between frequency of aggressive responses and response bursting and between high social rank and efficient performance on the DRL schedule. At the end of the year we began examining performance under 3 response suppression paradigm in which footshock was delivered on a random schedule while the animals were working on the RI schedule for food reward. This task is now being evaluated.

A series of experiments to study plasma stress hormone responses to social manipulations and to footshock were initiated and the procedures seem to be working well. A related study of diurnal variations was completed which allowed us to determine the baseline levels of cortisol and prolactin throughout the day.

A new computer system was received and installation of the SKED software system was accomplished during the last month of the reporting year. The new machine is now being interfaced to our existing operant chambers.

The results of the year's work are described in detail in the sections which follow.

B. Monkey Colony:

Animals. As of 30 September 1985, the colony consisted of 103 Macaca fascicularis monkeys (variously known as cynomolgous, crab-sating, or Java macaques). During the year there were 17 births and 5 deaths in the colony. The monkeys are housed i four groups for the purpose of studying social behavior and organization. Two groups, named T-Troop and NT-Troop are breeding groups that contain all age/sex classes of animals. The third and fourth groups, I-Troop and C-Troop, are both all-male units. T-, NT-, and I-Troop are housed in outdoor compounds and the members of these groups are together at all times except when they are undergoing testing in the laboratory or when experimental manipulations of the social organization are being performed. The two breeding troops were formed in December, 1974; I-Troop was formed in the spring and summer of 1978 from surplus males taken from T- and NT-Troop. C-Troop was formed in the spring of 1984. It consists of young adult males that were removed from NT-Troop. C-Troop animals are housed in individual cages in the laboratory and are brought together only during social behavior testing. A fifth group consists of young adult and females belonging to T-Troop. These animals are being trained on a visual social preference task. They are still considered part of T-Troop, although they are removed from the parent troop from time to time to facilitate the training process. They have been designated TF-Troop for purposes of identification. The composition of the various groups is given in Table 1:

Table 1

Group Composition as of 29 September 1985 (Number of monkeys in each age/sex category.)*

	Ad	ult	Suba	adult	Juve	mile	Infa	int
TRAAP	M	F	M	F	M	F	M	F
11001								
"T" N= :	6	19	2	2	8	7	2	4
("TF")		(5)						
"NT" N≔31:	6	12 `	2	ο	5	5	2	6
"I" N= 8:	8	ο						
"C" N= 6:	6	ο						

* Males (M) over 6 years old and females (F) over 4 years old are classified as adults. Males 4-6 and females 4 years old are subadults. Juveniles are over 1 year old (both sexes).

-5-

Housing. T-, NT-, and I-Troops are each housed in outdoor compounds 14.1 m long, 3.1 m wide, and 2.0 m high. Each compound is equipped with perches, swings, and a water fountain and contains an observer station, 1.6 m square, in the center from which observations of social behavior are taken. The compounds are connected to heated and airconditioned indoor quarters by runways that are 1.2 m in cross section. The runways are partially covered to provide shelter from rain and sun when the animals are outside. The indoor guarters are cages 6.1 m long x 1.2 m wide x 2.0 m high which are equipped with water fountains and perches. Small guillotine doors on the sides of these cages are used to collect the animals in transport boxes for testing in the laboratory. Guillotine doors between the indoor cages and the runways, and between the runways and the compounds, allow the animals to be moved to different sections of the living quarters during social testing and daily cleaning.

The 6 males of C-Troop are housed in a battery of individual cages in a separate colony room in the laboratory. An adjacent suite contains a cage, measuring 1.8 m x 1.8 m x 1.8 m, in one room and an observer station, equipped with one way windows, in the other. The C-Troop monkeys are brought from their colony cages and placed in this cage for studies of activity and social behavior.

Yet another room contains 18 individual cages that are used as a holding facility during laboratory testing and to house the TF females when they are being kept in the laboratory.

Capture and handling. The behavioral testing performed in the laboratory requires that the monkeys serving as subjects be removed from their social groups, weighed, and brought to the test apparatus. They also must be kept adapted to the restraint device used to hold the animals while drugs are injected or blood drawn for assay for stress hormones. The animals have been trained to enter transport cages from their social cages; they are then weighed, placed in the restraint device where they are handled for a real or simulated drug injection or blood draw, and then taken to the rooms where the behavioral tests are administered. This capture and handling procedure is a part of the daily routine for all animals undergoing experimental testing.

Health; deaths and births. The entire colony was tested for TB three times during the contract year. All animals were negative. A recurrence of diarrhea in several animals in August, 1984, had prompted us to have cultures for shigella and salmonella done on the entire colony. Twenty six shiqella carriers, primarily females and juveniles, were identified, treated, and recultured during the fall of 1984. Twelve of these animals that had a past history of recurrent diarrhea were rechecked for shigella twice in February and once in March, 1985 and all were negative. In late spring, stool samples from 10 animals (8 males and 2 females) that had shown one or more bouts of diarrhea during the winter and spring were analyzed for parasites. A variety of organisms were detected, but no consistent pattern which would establish any one parasite as the primary organism was found. In the opinion nf the consulting microbiologist, the occasional appearance of diarrhea in these animals is probably related to social or environmental stressors.

There were 17 live births and 1 stillbirth during the year. Two liveborn infants died within a few days of birth, apparently from maternal mistreatment and neglect. Two juveniles, one male and one female, died of injuries received from other monkeys. A 15 year old male from NT-Troop died in August. Necropsy revealed that he had a variety of ailments, including esophogeal candidiasis (the apparent proximal cause of death), chronic enterocolitis, myocarditis, interstitial nephritis, and a cortical adenoma of the adrenal.

The floor of the indoor monkey quarters was scraped and repainted in March.It will have to be repainted annually unless and until a satisfactory permanent floor covering can be found. The old floor had begun to peel badly and made proper sanitation increasingly difficult. The monkey facility was inspected by USDA veterinarians in March and again in August. At the first inspection, aside from the condition of the floor in the indoor animal quarters which was being corrected at the time, no major problems were identified. Minor items involving moving some cleaning solutions away from the food storage area and replacing a deteriorating sink cabinet were corrected. No discrepancies were noted at the second inspection.

C. Activity Tests and Drug Dose Selection:

Procedures for observing general activity and for selecting the initial doses of the drugs to be used in the project were described in Annual Report #1 on the project. The C-Troop animals are released individually into the C-Troop social observation cage and observed through the one-way glass windows. Locomotor movement within the cage, which is divided into 8 imaginary 2 m cubes, is recorded by the observer who also records the behavior of the animal using a rating scale similar to that used in scoring social behavior that is described in the next section - the animals sometimes interact with their images in the one-way glass. The rating scale also contains additional codes for various behaviors that are directed toward the environment. After 10 min, the observer

-7-

dons a rubber fright mask and enters the observation room. Activity and behavior in response to the masked observer are recorded for 90 sec. The test is concluded by having the observer wave a length of garden hose in front of the monkey a live rat snake was used in place of the hose on some of the tests during the current year. In addition to recording activity and behavior, the observer notes all physical changes as they appear, such as changes in respiration, pupillary dilation, speed and coordination of movements, etc. The monkeys are then returned to their home cages and monitored by an observer until all overt signs of drug effects have returned to normal. The animals are given food and water at this time and the latencies to eat and drink are recorded, as well as the kind of food that is eaten first (monkey biscuit, vegetable, fruit, etc.). In these tests, the onset of overt behavioral and physiological changes is used in the initial determination of the time that will be used between administering a drug and the beginning of any behavioral test.

A series of these tests, conducted during the summer of 1985, resulted in the selection of diazepam doses of 1.6, 0.8, and .16 mg/kg with a 15 min delay between injecton of the drug and the beginning of testing for use in the first studies to be done with this drug. There is to be a minimum of 72 hours between doese of diazepam for all but the lowest dose and special diazepam vehicle is to be used as the placebo. The highest dose results in some lack of locomotor coordination, while the intermediate dose is close to that (1.0 mg/kg) which others (e.g. Delgado, et al 1976) have found to alter social behavior in macaque monkeys. It is likely that we will also want to try a dose of 0.4 mg/kg, at least in tests of social behavior and of behavior in the open field situation.

D. Social Behavior and Organization:

During the year, considerable social data were gathered and analyzed from the four groups of monkeys. Over 400 hours of observations were recorded from the three troops in the outdoor compounds and another 122 tests of social behavior were done on C-Troop in the indoor social cage. Work with the troops in the outdoor compounds generally followed the procedures which have been described previously in the RFQ for the contract and in Annual Report Number 1. An important modification to the procedure was the decision to increase the amount of time devoted to group scan observations during the daily observation periods. This is done at the expense of time spent in focal animal observations. Since we have also decided that the use of focal animal observation techniques is essential for studies of drug effects on social behavior, our future studies of the social effects of drugs will employ two simultaneous observers, one using scan technique and the other focal animal technique. A review of the procedures employed with T-, NT-, and I-Troops is given below (more details are available in Annual Report Number 1) followed by a summary of some of the results obtained. Results from studies in which we sought correlations between social variables and performance on various laboratory

-8-

tasks are presented in later sections of the report under the headings of the various laboratory tests which were used. We also did extensive work with C-Troop in an effort to develop and standardize a satisfactory procedure for testing dyadic interactions between monkeys which would allow the repeated tests with the same pairs that are necessary for obtaining usable dose response data in an efficient manner. These efforts also will be described in this section.

Group social behavior. Observations of social behavior are done using the behavior categories given in Table 2. The observers record the code for the animal exhibiting the behavior, a code for the behavior itself, and then a code for the animal that is the recipient of the behavior. The two procedures utilized in gathering data are the "group scan" and the "focal animal" techniques. In a group scan, the observer watches the entire group and records every behavior that occurs as it happens; a modified version of a group scan involves looking at each monkey in sequence and recording what it is doing at the instant it is scanned. The focal animal procedure involves attending to only one animal for a period of time and recording the direction and nature of all behavior it either does or receives during that time.

The behaviors which each animal directs toward every other member of the troop and the behaviors which it receives from every other member in its troop are used to construct matrices which summarize the dyadic interactions in each group. These are then used to define and analyze the social organization. A very important element of the social organization of the primate groups is the presence of dominance hierarchies. The adult males have such a hierarchy among themselves and each animal's social rank within this hierarchy is determined by defeats. The occurrence of a submissive behavior in a monkey indicates that the monkey is inferior in rank to the animal toward which the submissive signal is directed. Knowledge of each male's status with regard to all of the other males defines the hierarchy.

Another critical element in this species' social organizaton is the hierarchy of matriarchies, such that each female and her daughters are a social unit and each such unit has a social rank within the troop.

The means by which one animal establishes and maintains dominance over another (e.g., by attack, threat, teaming up with another animal) varies from animal to animal, from group to group, and from situation to situation. By recording and analyzing the entire range of social behavior in our animals we define both the behavioral constancies and the range of variation of each of our subjects. This provides a more detailed picture of social status and social organization than a simple assignment of rank.

Table 2

M. fascicularis Behavior Categories

Agonistic Behaviors:

Aggressive

Chase Threat (open-mouth) Charge Slap Bite

Submissive

Avoid Grimace Squeal Flee

Other Agonistic

Lid Lip Smack Enlist Demonstrate

Sexual Behaviors:

Sexual Present Mount (no thrusting) Mount (with thrusting) Masturbate Genital Manipulation (other animal) Genital Sniff (other animal)

Other Social Behaviors:

Present to Groom Groom Ventral-Ventral Hug Ventral-Dorsal Hug Sit-Next-To (Physical contact) Play (not included in analysis)

Non-Social Behaviors:

Self Groom Move Sit - No Social Interaction

In analyzing social behavior, the group scan data are summarized by a laboratory computer which provides a listing of the frequencies of each behavior performed by each monkey and the frequencies with which it directs these behaviors to each of the other monkeys in the troop. These data are then used to produce a series of matrices which describe the basic social organization and dynamics of the group. Usually, several days' data are combined in these analyses. In this procedure, the computer goes through all of the data and determines the social rank of each animal on the basis of who is defeated by whom, using the submissive behavior categories listed in Table 2. This defines the social dominance hierarchy for the troop. The computer then prints a series of six matrices in which the animals are listed in the order of their social rank. In each matrix, the frequency of occurrence of each behavior, or class of behaviors selected for inclusion in that matrix, is given for each animal with respect to every other animal in its troop. (Presently, we are limited to 24 x 24 matrices; in scoring the behavior in T- and NT-Troops this year, the behavior of the 23 oldest animals in each group was scored and the 24th slot was used to represent all the remaining infants and juveniles in the troop). Four of the six matrices are used to summarize the combinations of behaviors listed under the functional categories Aggressive, Submissive, Sexual, and Other Social as given in Table 2. For

the other two matrices, any individual behavior of interest may be selected. Thus, we might look at threat - a measure of noncontact aggression - in order to compare it with the matrix for overall aggression, or obtain separate matrices for grooming, which is included in the Other Social matrix and play, which is not. Examples of these matrices may be found in Appendix A.

The data from each focal animal observation can be analyzed individually or summarized across observations to provide baseline information on response frequencies and directions to which the data from observations during experimental manipulations can be compared. It is also cossible to use the matrix programs with these data by combining focal data for several animals for one or more days. Under certain circumstances, useful information is obtained by combining both scan and focal data in a single matrix analysis.

The use of focal animal observations is essential to the study of drug effects on social behavior since it ensures that each subject is observed in the same way, and for the same length of time, during each session. The procedure does has disadvantages, however, in that social interactions between other members of the troop are not recorded as with the scan procedure. Information about such interactions is often critical for achieving many of the objectives of the contract, so we have to utilize both scan and focal procedures in gathering our social data. Thus, it is important that the overall social structure of each group be closely monitored and updated while at the same time sufficient data on the social interactions of individuals must be available for monitoring

-11-

the effects of drugs and other experimental manipulations and correlating social behaviors with the various performance measures being obtained.

As noted in the first annual report we had made only limited use of the focal animal procedure in our previous work with these groups, so it has been necessary to compare and contrast data obtained by the scan and focal animal techniques to determine the best combinations of the procedures for use in each aspect of the project. During the past year we have sought answers to the following issues:

> 1. The extent to which the social behavior matrices are equivalent when they are generated from data using focal animal as opposed to group scan techniques. Included in this question are subsidiary questions such as the number of focal observation periods in which only the adult males are observed that are required to define (a) the male dominance hierarchy in the troop and **(b)** the social ranks of the other animals in the troop that interact with the focal males. A related question is the extent to which a change in the frequency of specific behaviors throughout the troop is accurately reflected by the frequencies of this behavior obtained from the focal data; yet another is the identification of those behaviors that may not be picked up at all using the focal procedure.

> 2. The relative sensitivity of both procedures for detecting short term changes in the social structure that may be induced by either removing or replacing animals in the troops or by administering a drug.

> 3. The frequency with which observations of either kind must be made in order to maintain an accurate picture of the social organization of the troop and provide a baseline against which the experimental manipulations can be imposed. Gathering these data is a very labor intensive operation and we are interested in determining the most efficient schedules for each experimental objective of the project.

Several observers were trained to collect social data during the late winter and spring of the first contract year. For the rest of that year and during the first quarter of the second year, most of the data collected on each troop employed a single observer who used both scan and focal techniques during each observation period. The procedure used most frequently with the two large troops, T- and NT, began with a systematic scan in which the behavior of each of the 23 monkeys being scored was sampled in turn for 30 sec. This was followed by a 5-min focal observation of each adult male in the troop and then the observation period was concluded with another systematic 30-sec scan. The order in which the animals were observed was different each day for both types of observations. Thus, about 22 min of scan data and 30-40 min of focal data were obtained each day from T- and NT-Troops. In I-Troop, observations began with a general scan which lasted 10 min (20

min in a few instances) during which all social interactions between the animals were recorded as they occurred; this was followed by 5-min focals on each of the 8 monkeys and the session concluded with another 10-min general scan. As with the larger troops, the order in which the focal observations were made was changed each day.

Analyses of scan and focal data. For the 23 oldest monkeys whose social behavior is scored in each of the two large breeding troops, T- and NT-, there are 253 possible different combinations of pairs excluding the "other" category animals which are all scored under one code. In order to have a complete picture of the social rank structure of these animals, the dominance/submission relationship between the members of each pair must be known. Several months of daily observations may be needed before all possible dominance/submission relationships can be observed and noted. There are several reasons for this. Dominance/submission relationshionships, once established, tend to be relatively stable and require minimal overt agonistic behavior to maintain. There is a gradient in the expression of agonistic behavior such that the adult males and the highest ranking females and the members of their matriarchies show the highest frequncies of these behaviors. Agonistic behavior within matriarchies and between lower ranking matriarchies is less frequent. Subadult and the older juvenile males tend to interact largely with each other and with young juveniles rather than with other troop members. Exceptions to this gradient can also occur, however, which sometimes makes the determination of the dominance/submission relationships between of higher ranking animals difficult. In the adult male hierarchy, some animals may be virtual social isolates while others may have alliances that reduce the frequency of agonistic interactions between members of а particular pair so that weeks may go by before the observer can verify the relationship. Fortunately, changes in relationships are readily apparent because agonistic behavior increases during a change and may last for several days.

To help us evaluate the scan procedures used in gathering the social data during the summer and fall of 1984, we were able to use data obtained in some of our earlier work in which we had employed 40-min general scans in scoring the 24 oldest members of T-Troop. These data were analyzed to determine the number of dominance/submission relationships that were actually observed across different numbers of observation periods and the kinds of relationships that were easily identified vs those which were rarely or never observed. Three months of data containing 15 general scan observations for June, 16 for July, 1979 (Data and 14 for November, were examined. for August-October of that year were not comparable and were not data were taken in August and used since focal group composition was manipulated in September and October.) The results are summarized in Table 3. A total of 538 submissive behaviors was recorded in June; this enabled us to resolve "he dominance/submission relationships between 129 of the 276 pairs possible in the 24 x 24 matrix (47%). Adding the July data

-13-

which contained 343 submissive responses increased the total number of resolved relationships to 170 (62%). Data from the third month, in which there were 480 submissive behaviors, increased the total number of identified relationships observed in three months to 204 (74%) for the 45 days of observations.

Identification of the dominance relationships between animals was most rapid among the higher ranking animals. In the first month, 90 submissive responses identified 93% (14/15) of the relationships between the six adult males present in the troop. In the top 8 monkeys, which included to the two hinhest ranking females, 86% (24/28) of the dominance/submission relationships were actually observed. In the top half of the group -12 monkeys- 70% (46/66) of the relationships were observed. These figures confirm the gradient described above, since the number of relationships actually observed in the entire troop was just 47% during the first month. Subsequent observations during July and November primarily served to clarify the relationships among the lower ranking monkeys.

When we began the anaylsis of the social data gathered in the summer and fall of 1984 it became evident that cutting the scan observations by about 50% in order to include a series of focal observations in each observation period drastically dominance/submission reduced our ability to identify relationships from the scan data. The next to last column of Table 3 contains the data for 31 days of observations of T-Troop obtained from late August through early November, 1984. (The total amount of observation time is approximately equal to that for one month - about 15 observations - during 1979). Only 204 submissive responses were recorded, enabling the identification of just 19% (48/253) of the dominance/submission relationships. Only 8/15 relationships among the 6 adult males could actually be confirmed from these and the data relationships among the higher ranking females and between these females and the adult males were not observed in many cases. In fact, it was not possible to specify the ordinal ranking of the top 8, let alone the top 12, animals in the group from these data alone.

Adding the focal observation data to the scan data for this period improved the picture somewhat. The percentage of dominance/submission relationships increased to 28% (71/253) and 11/15 relationships among the adult males were observed. The improvement involved only the higher ranking animals - only 2 relationships were identified out a possible 66 among the bottom 12 monkeys in the group.

Since the daily 20-min scan observations obtained from the two large troops did not provide an efficient way of obtaining sufficient data to keep up with the social rank structure of the troops, the procedures were modified to provide more scan data. First, several weeks of data were gathered on T- and NT-Troops during April and May, 1985, using a systematic scan with each of the 23 monkeys being observed twice and with the observers instructed to record all agonistic activity whenever and wherever it occurred. Observation periods generally lasted between 45 and 55 min. Data from T-Troop for the month of May, 1985 are given in the last column of Table 3.

A total of 720 submissive behaviors by the 23 monkeys being scored enabled us to actually identify 53% (134/253) of the possible dominance submission relatonships from 21 days of observations. Although 3 of the 6 adult males in the group ranked in the bottm half of the heirarchy, 13 of the 15 intermale relationships were identified. (Actually, 11 of 15 were seen during the first 9 days of observations.) Once again, relationships were clearest among the higer ranking animals, with 23/28 relationships being verified among the top eight animals, 3 males and 5 females. Overall, the top 12 monkeys accounted for 116 of the 134 relationships identified. These data compare very favorably with that obtained from the 40 min general scans used with T-Troop in 1979.

However, the May, 1985 scan data from NT-Troop produced only 100 submissive behaviors over 19 days of observations and these allowed the identificaton of only 11% (27/253) of the relationships, including 11 of the 21 dominance/submission relationships among the 7 adult males. Adding 16 more days for June and July increased the totals to 22% (55/253) overall and to 17/21 of the intermale relationships. These figures are low, but are somewhat better than those obtained with the 20 min scans on NT-Troop in 1984. An 18 day sample from September, 1985 in which each observation period contained 40 min of systematic scans (in addition to 5-min focals) yielded only 93 submissive behaviors from the scan data but allowed us to verify 22 additional relationships which we had not seen before. Agonistic activity in this group has been low since the spring of 1985 and the social rank structure has been quite stable for many months.

Table 3

Comparisons of Scan Data Obtained From T-Troop Using Different Scan Procedures

(1979 Data are Cumulative Across Three Months)

	40	Min Scans 1979		20 Min Scans 1984	45 Min Scans 1985	
	June	+July	+Nov.	AugNov.	May	
Number of						
Observations	15	31	45	31	20	
# Submissive						
Behaviors	538	881	1361	204	720	
		(+342)	(+480)			
Dominance						
Submission	129/	170/	204/	48/	134/	
Relationships	276	276	276	253	253	
Identified		(+41)	(+34)		•	
Intermale	14/	15/	15/	8/	13/	
Relationships	15	15	15	15	15	
Identified		(+1)				
Top Eight	24/	27/	27/	Ranks	23/	
Relationships	28	28	28	Unknown	28	
Identified		(+3)	×			
Top Twelve	46/	56/	62/	Ranks	48/	
Relationships	66	66	66	Unknown	66	
Identified		(+10)	(+6)			

-10

Although 40-50 min of scan data produced much more than twice as much information about the social rank structure than our 20 min scans, at least in T-Troop, extending the length of the daily observation periods much beyond 40 min did not produce proportionately more data about the rank structure. The agonistic interactions observed on any one day are likely to involve the same animals, so while extending the observation periods to 60 min or more might be expected to increase the total frequency of aggressive and submissive behaviors recorded, we find that it does little to increase the identification of dominance/submission relationships.

The focal observation procedure is obviously not geared to producing complete dominance/submission matrices since the interactions between animals that are not themselves focal animals are excluded from consideration. Even among the animals that are being scored, the observer will miss interactions that that may occur between monkeys that do not happen to be under observaton at that moment in time. For example, when we examined 19 days of 10-min focal observations of 8 adult monkeys (6 males and 2 females) in T-Troop that were obtained in August, 1979, we found that, at the end of 19 days, submission had been recorded in only 12 of the possible 28 relationships among these 8 animals and in 23 more interactions between these 8 and the remainder of the troop. The 1979 scan data from any one of the other three months represented in Table 3 obviously does a better job of identifying dominance/submission relationships at varous levels of the group structure than the focal procedure. Similar findings were obtained from the 1984 daily 5-min focal data that were gathered on the same days that scan data were obtained. As noted earlier, combining scan and focal data from these days improveds the percentage of dominance submission relationships that could be identified, at least among the higher ranking animals, but the combination does not provide a substitute for the information provided by additional scan data.

Since I-Troop contained only the 8 adult males, we thought that the focal procedure might do a better job of efficient identification of the 28 possible dominance/submission relationships than was the case in the two large troops. Twenty days of 5-min focal observation of each I-Troop male during June, 1984 produced 84 submissive behaviors and allowed 20/28 relationships to be identified. By way of contrast, 18 days of 40-min general scan data obtained in June, 1985, yielded 166 submissive behaviors and identified 26/28 dominance/submission showed relationships. An analysis of the July, 1985 scan data that all 28 relationships appeared in the 21 days of data that fact, 27/28 contained 181 submissive responses. In relationships were identified from the first 11 days of scan data. Use of the scan procedure is clearly preferable for dominance/submission relationships identifying and reconstructing the social rank hierarchy. The July, 1985 data are reproduced in Appendix (A) which also serves to illustrate the social data matrices produced by the analyses we are using. The comparisons of the T-Troop 1984 data with the 1979

data, the analyses and comparisons of the 1984 focal and scan data from all three troops, and the results of the extended scans obtained in the spring of 1985 led to a major change in the procedures used during the summer and fall of 1985. Beginning in late May, daily observations of T- and NT-Troops were changed such that each observation period began and ended with a 20-min systematic scan. Four 5-min focal observations of the adult males in each troop were inserted in the middle of the observation period. Since there are 8 adult males in NT-Troop and 6 in T-Troop and only four focals are done each day, the order in which the animals are observed is rotated 50 that each male is a focal animal two or three times a week. То make up for the loss of focal animal data resulting from the increased scan time, future experimental studies of drug effects on social behavicr will utilize two observers so that simultaneous scan and focal data are obtained throughout the observation period. The additional observer training required to implement this procedure was completed during the summer of 1985 and we now have at least two observers capable of identifying and scoring each of our troops. We think that the new procedure will substantially improve the quality of the data obtained and compensate for the increased observer time required. The usefulness of the 1985 focal data obtained during the daily observations will be reevaluated this coming winter. It is possible that we can save some observation time by dropping the daily focals from the procedure. This would ease the demand on observer time and make the two-observer scheme easier to schedule.

<u>Manipulations of social group structure.</u> During the year, several adult males were removed from the troops for varying periods of time and then replaced to study the effects of these manipulatons of the social behavior and organization of the groups. During the year, the top ranked male (the "alpha" male) in NT-Troop and the second ranked, or "beta" male from T-Troop were removed and then returned to their troops. Four similar manipulatons were conducted with I-Troop using the alpha male (twice) and the beta and fifth ranked monkeys.

The NT-Troop manipulation produced several changes in the the seven-member male dominance hierarchy. First, following removal of the alpha male, Barker, there was a marked increase in agonistic behavior and the beta male, Eju, lost out to the third ranked male, Weed, who became the tempoporary alpha. Hobbit, the seventh ranked animal moved up two ranks. Eju, who had held the beta position because he had an alliance with Barker, fell into a tie with Allen in the rank below Weed. After several days, Weed was injured in an unobserved fight, treatment of and had to be taken out of the troop for his wounds. Upon Barker's return, three weeks following his removal, he was attacked by Allen. Allen and the fifth ranked Barker animal, Tag, were injured and removed for treatment as reestablished himself as the alpha male. The return of Barker also reestablished the original ranks among the bottom animals in the hierarchy. Weed was returned two days after Barker and became the beta male, once again displacing Eju. Over the next

three weeks, agonistic behavior remained at a high level and there were several more injuries which caused animals to be removed from the troop for one or more days before the situation stabilized.

In T-Troop, the beta male, Easy, received a bite wound and had to be removed for treatment. Instead of being returned to the troop immediately after his wound had healed, he was held out for 12 days and then reintroduced. His removal produced little change in agonistic activity in the troop and no change in the social rank structure. When he was returned, he immediately assumed his former rank without being challenged by any of the other males. However, there was a significant increase in the frequency of agonistic interactions throughout the troop on the day of his return. There were 40 such interactions on the day of his return, only 5 of which involved Easy. The mean number of such interactions over the three days prior to his return was 10.3 and on the three days after his return, 10.7. The agonistic interactions were largely confined to threats and submissive gestures and there were no injuries to any animals during this period.

In I-Troop, the removal and replacement (after 18 days) of Cracker, the 5th ranked male, had no effect-on the frequency of agonistic interactions or social rank in the troop during the time he was out. On the day of his return we recorded 28 agonistic behaviors (aggressive and submissive behaviors combined) whereas the mean over the previous 17 days had been 12.3 per observation period. There was no change in the rank structure of the group and aggression.dropped back to a low level the next day. In another manipulation, Alabama, the beta male, was returned to the troop after an absence of three months. (During this time he was used as a social stimulus animal in the work with C-Troop which is described in the next section of this report.) There were 105 agonistic behaviors recorded on the day of his reintroduction compared with a mean of 10.1 for 6 days prior and 7.7 for 14 days after his return.

Gus, the alpha male, was removed from I-Troop for 18 days. Unlike the upheavai in NT-Troop which followed Barker's removal and replacement, the rank structure remained stable and aggression in I-Troop was at normal levels during Gus' absence. Upon his reintroduction, he was immediately recognized as the alpha male without his having to attack or threaten the other animals in order to regain his status - he made only one threat toward another monkey on the day he reentered the group. There was, however, an overall increase in agonistic behavior in the group during and immediately after his return. During the 7 days before Gus' return, there was a mean of 11.3 agonistic behaviors per day; The 53 submissive behaviors (7.3/day) allowed us to verify 17/21 dominance/submission relationships. On the day of his return, there were 55 agonistic behaviors observed, including 24 submissive responses). The latter identified 8/28 dominance/submission relationships, although none of these involved Gus(!). The mean frequency of agonistic behaviors over the 14 days after Gus' return was 18.0 and the 197 submissive responses recorded allowed us to identify 26/28

፟ዿ፝ዀፚዀፚዀፚዀፚዀፚዀፚዀፚዀፚዀፚዀፚዀዾዀዾዀዾዀዾዀፚኯፚዀ

-20-

dominance/submission relationships. In the next calendar month. with the groups still intact, the level of agonistic behavior began to decline over 15 days of observations to a mean of 14.6: 159 submissive behaviors identified 21/28dominance/submission relationships during this time. Another manipulation in which Gus was removed for 9 days and then replaced produced a smaller and shorter-lived increase in agonistic behavior in the troop, also without any changes in the rank structure.

From these results we conclude that removal and replacement of selected males is an effective way of generating increases in agonistic behavior throughout the monkey groups. This effects are related to the status of the animal removed and replaced, the relative stability of the group at the time the manipulation is conducted, and the length of time the monkey is out of the troop. With a stable social structure, the high ranking animals may be removed for two or more weeks and returned without seriously disturbing the rank hierarchy and with a low potential for physical injuries. In less stable groups, low ranking animals may also be used in this fashion, but the amount of agonistic behavior induced is low and the use of low ranking animals is not an effective way of generating agonistic behavior for use in studying drug effects on this class of social behaviors. However, the removal and replacement of high ranking males, particularly the alpha male, from an unstable group may produce profound changes in the structure of the group that can confound the interpretation of drug effects. In addition, the potential for injuries is increased, which is undesirable for both experimental and animal welfare considerations. The amount and intensity of agonistic behavior generated by Barker's removal and replacement in NT-Troop interfered with both operant testing and drug testing in this group for several weeks, since stable baselines were unobtainable and several animals had to be removed from the testing program for varying periods of time.

During the coming year, drug testing will be conducted both with the groups intact and following removal and replacement manipulations and the results will be compared. It may be that the removal and replacement procedure will be the most efficient way to study drug effects and that it can be used as the primary method to obtain these data in the outdoor groups.

Nonagonistic social behavior in a group might be expected to decrease as agonistic behavior increases following a removal and reintroduction manipulation. Although there is a small decline in these behaviors, the change is small and most behaviors are present with sufficient frequency to allow the detection of both increases and decreases following drug or other experimental manipulations. Table 4 gives the mean I-Troop frequencies of "other social", allogrooming, and sexual behaviors per monkey per day for the 7 days before Gus' reintroduction, the day of his reintroduction, and for the following 7 days during the manipulation described earlier. There was a decrease in "other social" behaviors, including grooming, on the day of reintroduction and sexual behavior disappeared. During the following week, the frequencies recovered to near the preintroduction levels. Mean sexual behaviors actually increased slightly. Intermale mounting in these animals is both a sign of affiliative behavior and a way in which interanimal relationships are confirmed.

Table 4

Nonagonistic Social Behavior in I-Troop for 7 Days Before, During and 7 Days After Gus' Reintroduction. Data are Given as Mean Frequencies/Animal/Day

Behavior Category	7 Days Pre- Reintroduction	Reintroduction Day	7 Days Post- Reintroduction
Other Social	8.4	6.9	7.8
Grooms	3.4	2.5	2.9
Sexual	2.0	0.0	2.3

** Grooms is included in the "Other Social" category (See Table 2)

Social behavior in C-Troop. One of the purposes for setting up the group of C-Troop males was to allow tests of dyadic interactions between selected pairs of monkeys. Because we are interested in cooperative behavior, we also investigated the utility of using enlisting behavior, in which one monkey solicits assistance from another during an agonistic encounter with a third animal. This procedure involved placing both familiar and unfamiliar animals in with pairs of C-Troop males and recording the ensuing behaviors. In addition to tests with both dyads and tryads, observations were also made of the troop as a 6-animal social unit both by themselves and in the presence of unfamiliar males and females from other troops. pellet Finally, an operant panel containing feeder, a manipulanda, and cue lights and sounds was placed on one wall of the social test cage and the social behavior of the animals, both in pairs and as a group, was observed during the delivery of food pellets on a 30-sec variable time schedule.

Tests of the effects of caffeine, atropine sulphate, and conducted atropine methyl nitrate were during group observations of C-Troop in the fall of the year. A failure to find any effects was attributed to the low level of agonistic behaviors present during this time. (See the next section for more details.) During the winter and spring of 1985, the social behavior of the C-Troop males was observed in a variety of situations in an effort to standardize a procedure for observing social behavior in group, triadic, and dyadic interactions which would produce sufficient agonistic behavior to make drug induced reductions in aggression detectable. These efforts also involved an attempt to induce enlisting behavior as a measure of cooperation among the monkeys.

The first procedure involved the brief introduction of new animal to the five member group. First, the animals were placed together in the observation cage for nine days. A five-min scan was followed by a five-min focal observation of each animal, in random order, and the session was concluded with another five min scan. Blood for plasma hormone assays was drawn before the 8th day of observations and after the observatins were concluded on the 9th day. On day 10, a "strange" male was introduced to the group following the completion of the regular observation period and observations continued for 10 min with the new animal serving as the focal animal. Blood was draawn at the conclusion of this test. Two more days of observations followed, with the stranger absent. Blood was drawn before the observations were made on the 12th day. (The male used in the introduction was a young adult, named Defeat, that had been removed from NT-Troop and kept in the laboratory for use as a stimulus animal in the tests of social behavior with the C-Troop males. Since the C-Troop animals were originally taken from NT-Troop, Defeat was not a complete stranger, but the C-Troop males had had no contact with him for eight months prior to this test.)

During the next five days of observations, the five C-Troop animals were put together in the social cage each morning and kept together for the rest of the day. Observations (5 min -tan - 5 min individual focals - 5 min scan) were made during the middle of the day each day. On the 4th day the cage was baited with fruit during the observation period and on the 5th day the monkeys.were given one of their daily feedings during the observation period in an attempt to increase social interactions.

For the next nine days, 10 min tests of pairs of animals were done. The animals were paired randomly, and four pairs were observed per day with different pairs each day - all possible combinations were tested from 2 to 5 times. Following this, three days of group observations were taken using the scan-focal-scan procedure to see if the paired exposures had generated any increase in enlisting behaviors. We then examined diadic/triadic interactions for four days. In this procedure, a pair of animals was placed in the cage and observed for 10 min. A third animal was then introducd and the observations continued for another 10 min. One member of the original pair was then removed and the two remaining animals were observed for 10 min after which a new animal was introduced to form Four triadic combinations another triad, and so on. were observed each day. This was followed by five days on which the "strange" male, Defeat, was introduced to pairs of C-Troop males. Defeat was introduced for 10 min following 10 min observation of each C-Troop pair. Three diad/triad sets were observed each day.

Two days of scan-focal-scan observations of the entire group were followed by tests involving exposure to a different strange male. This male, Alabama, is the second ranked male in I-Troop and it was felt that he might be a better stimulus than

-22-

Defeat because he is an aggressive, fully adult male who had been removed from NT-Troop before most of the C-Troop animals had been born. During the next eighteen days of observations, C-Troop was first tested with Alabama placed in a small cage outside the social test cage; observations of the reactions of both the entire group and of selected pairs were made. Then the small cage containing Alabama was placed inside the social test cage and group reactions recorded. Next, individual C-Troop males were observed with Alabama still in the small cage: this was followed by releasing Alabama in the social test cage with each of the C-Troop males separately. The series concluded by observing triads composed of selected pairs of C-Troop animals plus Alabama for two days and both Alabama and Defeat (introduced successively to each pair) for three days.

Next, the C-Troop males were tested with strangers placed directly into the cage with them. Tests were conducted using individuals and pairs of C-Troop males. The strangers were males of intermediate rank and nonpregnant, nonnursing females that were taken from T-Troop just for the time it took to conduct these tests and then returned to their own group. Twenty days of tests were employed, during which there were four days on which no strangers were introduced in order to alow us to look at baseline interactions. There was intense interest in the female strangers that was accompanied by some agonistic activity. The three highest ranking C-Troop animals attacked the stranger males on a number of occasions; several fights had to be broken up by the observer, and both stranger and C-Troop males sustained some minor injuries during these tests.

The results of all these observations were:

a. There was very little overt agonistic social behavior among the C-Troop males in the group situation, in pairs, or when triads were observed. The most frequent agonistic behaviors were "lid", a low intensity and "lipsmack", somewhat ambiguous aggressive behavior and а low level submissive or appeasement behavior. There was virtually no "enlisting" behavior. Most social behavior involved sitting next to each other, grooming, and occasional hugging and mounting. Thus, simply keeping the animals in individual cages and bringing them together for just an hour or two a day did not induce an increase in frequency of agonistic interactions or in the intensity of such interactions. The five males formed a dominance hierarchy, but the social structure was apparent only when data from a good many days of observations were combined. Such tests of social behavior are adequate for detecting overall increases in social behavior and increases in in agonistic behavior, but of little_use studying CW-related agents that might produce decreases in social interactions and agonistic behaviors. The absence of enlistina situations behaviors in these Wass disappointing, since it was hoped that this behavior would provide a useful index of cooperation between animals.

b. Keeping the animals together as a group during

several days did little to enhance the frequency or intensity of social interactions when the procedure of using brief daily exposures to each other was reinstated. Baiting the cage with fruit or feeding the animals during the observation period produced brief flurries of social activity, but these habituated rather quickly during an observation period. Such procedures do not appear to be very useful for experiments which will require repeated daily tests.

c. Exposure to the young adult male, Defeat, a monkey that had very low social rank in its former troop, produced little agonistic behavior. Exposure to Alabama, a high ranking male from I-Troop, did elicit agonistic behavior during early exposures when Alabama was caged inside the social cage and when he was free to interact directly with the C-Troop males.. Habituation of agonistic behavior across days was rapid, however, and little enlisting behavior occurred. These r esults indicate that it will be necessary to keep changing stimulus animals in order to generate appreciable amounts of agonistic behavior in the laboratory we have been using. Caution must be exercised in selecting the stimulus animals to be tested with different individuals and pairs from C-Troop to minimize the potential for injuries. However, the procedure does work and it has a definite place in the behavioral test battery, although it may not be practical to use it on an everyday basis because of the habituation problem.

In September, 1985, a banana pellet dispenser was installed in the social testing cage and the monkeys have been trained to take pellets that are delivered automatically on a VT-30 sec schedule. The early results of pair and group social testing during dispenser operaton have been very encouraging in that the monkeys increased their agonistic activities significantly in competing for access to the feeder. Additional data are being gathered on the efficacy of using the feeder to increase agonistic behavior and tests of diazepam and atropine effects in this situation are scheduled for the coming fall and winter. It now appears that some combination of tests utilizing both the introduction of strangers and the social behavior generated during dispenser operation will provide good test situations for assessing drug effects on social behavior in the laboratory setting.

Drugs and social behavior. Group social behavior in the five C-Troop males was examined following injections of caffeine sodium benzoate and atropine sulphate in a study which was begun at the end of September, 1984 and continued through the fall of last year. Testing consisted of a five min group scan followed by five min of focal observations of each animal and was concluded by another 5 min scan. For the first 20 days of the experiment, the alpha male always received a saline injection each day while the other four animals alternated drug and placebo days with two animals getting the drug each day. During the last four days of the study, the alpha male was also given the drug on alternate days. Caffeine sodium benzoate doses were 4, 12, and 36 mg/kg. Atropine sulphate doses were .032, .08 (3 times to each animal), and .20 (3 times to each animal). The delay between the last injection and the beginning of testing was 5 min for all caffeine doses. Delays of 5, 30 and 60 min were used with the three administrations of the .08 and .20 mg/kg of atropine. At the end of this study, a pilot study was run with doses of .08 and .20 atropine methyl nitrate and a 30 min delay using the same behavioral testing procedures.

The results can be summarized by the statement that there were no effects of caffeine, atropine sulphate, or atropine methyl nitrate on social behavior at any dose or post injection delay. The C-Troop males exhibited very little agonistic behavior at any time - most of their interactions involved grooming and playing. The outcome of this study pointed up the problems with the social testing procedures being employed with C-Troop and led to the series of behavioral studies described in the preceding section. The studies with the atropines will be repeated in the coming year using the newer procedures outlined above.

The effects of atropine sulphate on social behavior in the eight I-Troop males was examined with doses of .032, .08 and ,20 (twice) mg/kg and an injection/testing interval of 30 min. The Mondays of each of the two weeks of the study were placebo days for all animals; the monkeys alternated drug and placebo days the rest of the time, with half of the animals receiving the drug each day. In this experiment there was a drug effect at the .08 and .20 doses. The frequency of agonistic behavior both aggression and submission - was sharply reduced in the group on the days when some of the animals were given atropine. The mean frequency of agonistic encounters on the two days when all monkeys got the placebo was 19; for the days half the animals got atropine the mean was 5 for the four days some animals got .20 mg/kg, 6 for the two days some got .08 mg/kg, appears to be and 12.5 for the .032 mg/kg days. The effect specific to agonistic behavior and does not reflect a general depression of social activity since no consistent changes in frequencies of grooming behavior were observed. It appears likely that the failure to see any drug effect in C-Troop Was due to the low baseline levels of agonistic interactions in that group. We have not yet repeated this experiment with atropine methyl nitrate but will do so during the coming year. A removal/replacement manipulaton will also be performed using both forms of atropine.

E. Open Field Testing:

Oper field testing is conducted to study the monkeys' willingness to enter a strange environment, the amount of exploration that they do in that environment, and their responses to stimuli, either inanimate objects or other animals, placed in the field during testing. Earlier work with this test situation (see Bunnell, 1982) showed a relationship

-25

between scores in the open field and social behavior during initial, but not subsequent, behavior in the situation.

Testing is conducted in a square open field, 3.7 m on a side and 1.8 m high that is located in a large room in the laboratory building. Walls and floor are painted white, and the floor is divided into 16 squares by a painted grid. Five threaded studs, one in the center and the other four arranged in a square pattern equidistant from the center and the walls. are imbedded in the floor. These are used to attach the novel objects that are used as stimuli in some of the tests. The open field is covered by chain link fencing and is illuminated by four 150 watt floodlights placed above the ceiling. There are two guillotine doors located at diagonally opposite corners of the arena by which animals may be introduced into the field. An elevated platform located along one wall outside the arena is used for observing and scoring behavior. Opaque curtains and a one way window prevent the monkeys from seeing the observers during testing.

Monkeys being tested are brought to the open field in transport cages; these cages are placed outside a guillotine door to the arena for 5 min before the door is opened and the animal allowed access to the field. In a typical test, the animal is allowed 5 min to emerge into the field. (On some tests, if this time is exceeded, the animal is gently forced into the field and the test is continued). "Emergence" requires that the animal enter the arena and move beyond the first square in the field (a distance of @ 1 m). When the animal has emerged, the guillotine door is closed behind it and its behavior during the ensuing 5 min is recorded by the observers. At the end of 5 min, the guillotine door is reopened and the monkey is allowed to return to its transport cage. When the animals are tested in the bare field, without novel objects being present, the following measures are taken:

(1) Head Out Latency: Time from opening the guillotine door until the animal pokes its head through the door into the arena.

(2) Body Out Latency: Time from opening the guillootine door until the animal enters the square of the arena that is directly in front of the guillotine door.

(3) Number of Returns: Number of times monkey returns to transport cage after entering the first square ("body out").

(4) Emergence Latency: Time from opening the guillotine door until the animal "emerges" as defined above.

(5) Exploratory Moves: Number of squares traversed by the animal during the 5 min following its emergence into the field.*

(6) Return Latency: Time from reopening of the door following the 5 min exploratory period until the animal reenters its transport cage.

(7) Return Moves: Number of squares traversed during the return latency period.*

* Time spent on the floor is differentiated from that spent moving about on the ceiling during these periods.) When novel objects are present in the arena, the frequencies of occurrence of the following additional behaviors are also recorded:

- (8) Lip Smacking
- (9) Orientation toward object(s)
- (10) Manipulation of object(s)
- (11) Threats toward object(s)
- (12) Bites (object)
- (13) Other contacts with object(s)
- (14) Vocalizations
- (15) Self directed behaviors (groom, masturbate, etc.)

If two or more animals are observed simultaneously in the open field, the social behaviors listed in Table 2 are also scored for both animals.

Effects of atropine on open field behavior. A study of the effects of atropine sulphate and atropine methyl nitrate on emergence and activity in the empty open field was completed using the seven adult males from NT-Troop. Doses of .032, .08. .20, and .40 mg/kg of both drugs were alternated with placebo days (physiological saline) until all animals had received all doses. There was a delay of 30 min between injection of the drug and the beginning of testing. Emergence time and locomotor activity data for each drug day were compared with the means of these measures for the 10 saline days. Monkeys failing to enter the field within 5 min of the opening of the guillotine door were gently forced into the field. This happened on two occasions during testing with the placebo but not during tests with the drugs. The data for each monkey are given in Table 5.

There were no consistent effects of either drug on emergence or locomotor activity. There were several very long emergence latencies, particlularly with atropine sulphate, but these were not well correlated with the doses given. Instances of both increases and decreases in locomotor activity were observed at all doses of both drugs when compared with the means for saline days, but in most cases the scores were within the range exhibited during saline days. Order of administration of the various doses was not related to the responses. Thus. doses of these drugs which disrupted operant and complex problem solving behavior (see later sections of this report) had minimal effects on this task. This was surprising in that the pilot work on activity with C-Troop had demonstrated a reduction in general activity at the .20 mg/kg dose.

In a second study, a novel object was placed in the center of the field and the animals were tested with doses of .08. .20, and .40 mg/kg of both atropines. To minimize habituation different objects were used each day. Two objects were used each day with different animals, such that some animals were exposed to a specific object on a drug day while others were exposed to the same object on a placebo day. Objects were either large toys, such as a hobby horse, or household items such as a ladder, a vacuum, a bucket, etc. Drug and placebo days were alternated until all monkeys had received all three doses of both drugs. The results are given in Table 6.

Table 5

Effects of Atropine Sulphate (AS) and Atropine Methyl Nitrate (AMN) on Emergence and Locomotor Exploration in the Empty Open Field

Emergence Latency (Sec)

Drug Dose

	P	Placebo *		.032		.08		.20		.40	
Animal	(+	/- SEM)	AS	AMN	AS	AMN	AS	AMN	AS	AMN	
Barker	2.5	- (0.34)	1	2	2	2	4	3	• 4	1	
Eju *	8.1	(3.30)	5	4	5	14	9	12	100	3	
Weed	2.8	(0.36)	4	3	2	3	6	3	1	1	
Tag *	21.0	(10.80)	106	25	19	3	143	3	1	4	
Hobbit	3.2	(0.65)	3	5	5	2	2	2	6	2	
Kukla	24.2	(8.50)	259	3	9	14	132	10	104	11	
Allen	13.4	(4.16)	260	6	7	13	29	6	105	2	

Number of Moves

Barker	61 5	(8 92)	80	53	40	77	73	108	34	53
Eju	23.3	(5.03)	24	28	33	18	26	5	6	29
Weed	205.4	(8.01)	228	216	191	195	178	205	232	294
Tag	102.2	(6.62)	102	96	126	81	147	59	111	101
Hobbit	69.8	(6.68)	69	67	58	97	96	73	65	35
Kukla	46.8	(4.84)	32	39	20	22	50	32	34	37
Allen	46.1	(6.02)	79	25	29	29	7	39	48	52

*Placebo n = 10 days except 9 days emergence latency for Eju and Tag who each had a forced entry on one day.

Table 6

Effects of Atropine Sulphate (AS) and Atropine Methyl Nitrate (AMN) or Behavior in the Open Field Containing a Novel Object

Emergence Latency (Sec)

Drug Dose

	P1	Placebo *		.08		20	. 40	
Animal	(+/	- SEM)	AS	AMN	AS	AMN	AS	AMN
Barker	1.3	(0.25	1	2	2	4	5	3
Eju +	2.7	(0.29	2	3	18	6	88	3
Weed	2.3	(0.63)	1	2	2	4	3	2
Tag	2.3	(0.48)	2	2	3	2	2	2
Hobbit	3.5	(0.87)	3	6	5	3	9	1
Kukla	17.0	(2.68)	4	5	13	9	3	1
Allen	4.8	(1.38)	2	2	12	4	10	3

Number of Moves

Barker	48.8	(3.83)	41	38	67	52	6	36
Eju	31.8	(6.82)	32	20	45	51	17	25
Weed	198.8	(12.43)	189	240	192	218	137	279
Tag	81.8	(11.99)	104	63	73	102	68	44
Hobbit	42.5	(5.01)	54	18	82	80	31	47
Kukla	56.5	(4.30)	69	50	33	44	33	39
Allen	83.0	(8.53)	78	79	57	108	6	- 34

* Placebo n = 4 days except 3 days for Eju who had an emergence latency of 25 sec on one day.

In tests with a novel object present in the field, the .40 mg/kg dose of atropine sulphate produced a decrease in locomotor exploratory behavior in all seven monkeys. A few animals also had a decrease at .20 mg/kg, while no differences appeared with.08 mg/kg. Four animals had reduced activity scores with the .40 mg/kg dose of atropine methyl nitrate, 2 were unchanged, and 1 exhibited an increase. In the 4 animals with the lower scores, the reductions were smaller than those obtained with atropine sulphate. The results with the.40 dose are the first consistent differences between atropine sulphate and atropine methyl nitrate effects which we have seen on any of our tests with these drugs. The differences appear only with a relatively large dose. On the emergence latency measure, 4 of 7 had longer latencies and 1 was much shorter with the .40 mg/kg dose of atropine sulphate whereas latencies tended to be shorter or unchanged in all but 1 monkey with the same dose of atropine methyl nitrate. Effects at the two lower doses were inconsistent and added little to an understanding of the drug

-29-

effects. The overall picture suggests that there is an interaction between the central and peripheral effects of the two drugs on open field behavior. It might be worthwhile to pretreat the animals with atropine methyl nitrate and then give varying doses of atropine sulphate. This could provide an indirect assessment of central effects on this task.

<u>Correlations between open field and social variables</u>. In an earlier experiment using the NT-Troop males, we had found a high positive correlation between the monkeys' social rank in the group and the percentage of their responses to the novel objects that were contact responses. However, this relationship was not present on placebo days in the atropine study described above. There had been changes in social rank prior to the more recent study and these changes were not accompanied by the corresponding changes in percent contact that would have been predicted from the earlier correlation. In addition, low ranking monkeys showed an overall increase in contact responses relative to their performance the first time around. It appears that there has been some habituation to the general test situation such that lower ranking animals are now more willing to approach and contact the objects.

There were high correlations between short emergence latencies and high social rank in the NT-Troop males on placebo days during both the empty field (+.79) and the novel object present (+.78) phases of the atropine experiment. A similar relationship (+.86) had been obtained with the I-Troop males during testing in the empty open field a year earlier. We had not seen this before in NT-Troop, but the earlier data contained many instances of nonemergence in these males. Because of the correlations between social variables and performance in the open field and because of the sensitivity of this test to drugs, it will be retained in the battery. However, it does not seem necessary to utilize both the empty field and the field with novel objects present. Because the latter procedure seems to tap more dimensions of the process of entering and exploring a potentially threatening environment and because the monkeys show habituation to the empty field with repeated exposures, use of a novel object will be the procedure of choice in the future work with drugs.

F. Complex Problem Solving:

The six oldest adult males in T-Troop were tested on an object quality - reversal learning set task. A study of the effects of atropine sulphate and stropine methyl nitrate was completed. Correlations between social variables and performance on this task and on a modification of the task which involved introducing "false" reversal cues were examined.

In this task, which is conducted in a modified Wisconsin General Test Apparatus (WGTA), the monkeys are given four new object quality - reversal learning set problems each day with lengths of 10, 11, 12 and 13 trials. (The order of presentation of problems of different length is counterbalanced across

Each problem requires that the animal l'earn days). presented discrimination between two objects that are simultaneously. Reversals occur on the fifth trial of the 10-trial problems, the sixth trial of the 11-trial problems. O place, the object that has been correct up to that trial of the problem is no longer rewarded and the other object of the pair now becomes the correct stimulus for the remaining five trials on that problem. Criterion performance is 17 out of 20 correct critical trial responses in 20 consecutive problems. The critical trial on a problem is the first trial after the reversal trial. The intertrial interval is 30 sec and the monkey is allowed a maximum of 10 sec to respond to each stimulus presentation. There are a total of 46 trials per daily

session and each session is 25-30 min long. Raisins are used as

reinforcers. Measures of learning and performance obtained on this task are: Habit Formation - the intraproblem performance on each new problem up until the reversal trial is given, measured as the number of correct responses on initial learning of each day's four problems. Concept Formation - assessed on both the object quality learning set and the reversal learning set portions of the problems. Correct responses on the second trial of each new problem across successive problems constitute the measure of object quality learning set performance and correct responses on the critical trials (above) across problems are the measure of reversal learning set. In addition, total errors, anticipatory errors, and response patterns, e.a. perseveration of responding to particular positions or objects, the development of response strategies, and the like, can also be examined. To provide flexibility in the testing program, three assistants have been trained to conduct the tests so that the monkeys are used to performing for different experimenters. Details of the training and testing procedures may be found in Bunnell and Perkins (1980).

Effects of atropine on WGTA performance. Doses of .032, .08, and .20 of atropine sulphate and atropine methyl nitrate were given to the six monkeys who were performing at criterion levels at the beginning of the experiment. The initial study in this series, reported last year, had found severe disruptions in performance with doses of .40 mg/kg and .20 mg/kg when a delay bewteen drug administration and testing was used. Because performance tended to be worse at the end of each session than it was at the beginning, this interval was increased to 30 min in the present experiment. Three monkeys received the .20 mg/kg dose of atropine sulphate and 3 got the same dose of atropine methyl nitrate. All 6 animals were given the .08 and .032 mg/kg doses of both drugs. Drug daya alternated with placebo (physiological saline) days, except that there was a 72 hr delay between the largest dose and the next test with saline. The results are presented in Table 7. Since there were a number of response failures during the tests, errors were calculated at percentages of the total responses actually performed.

-31-

Table 7

Effects	of	Atropine	Sulphate	(AS)	and	Atropine	Methyl	Nitrate
		(4	AMN) on WO	STA P	erfor	rmance		
		•	Means	(+/-	SEM)		

Response	. 20	C	- 0	Э	. 03	2	
Measure	AS	AMN	AS	AMN	AS	AMN	SALINE
n =	2*	3	6	6	6	5*	6
% Habit	32	23	21	18	14	15	18
Errors	(02)	(05)	(05)	(06)	(04)	(03)	(04)
% Total	28	27	21	20	16	16	17
Errors	(05)	(02)	(05)	(03)	(03)	(02)	(03)
# No Res-	29.0	2.3	9.5	20.8	0.7	0.0	1.0
ponses	(5.0)	(1.2)	(4.7)	(7.6)	(0.5)		(0.6)
Trial 2	2.0	2.3	2.2	2.2	3.3	2.6	3.6
Correct	(0.0)	(0.3)	(0.5)	(0.8)	(0.2)	(0.5)	(0.2)
Reversals	0.5	3.0	2.0	1.5	2.8	3.0	3.8
Correct	(0.5)	(0.0)	(0.7)	(0.6)	(0.3)	(0.6)	(0.2)

A dose dependent impairment of performance was produced by both atropines. The 3 monkeys that received the .20 mg/kg dose of atropine sulphate performed somewhat worse than the 3 that got the same dose of atropine methyl nitrate. These differences were due primarily to the large number of response failures in the atropine sulphate group. One animal made only one response and his data are not included in the table. Examination of the data from the experiment that used a 15 min injection-test delay and included a .20 mg/kg dose of atropine sulphate suggests that the apparent differences_may_be a function of the particular individuals that got the atropine sulphate in the present experiment. It will be necessary to repeat this dose of both drugs with all 6 monkeys to resolve the issue. Certainly, the response failures were no greater with atropine sulphate than with atropine methyl nitrate at the .08 mg/kg dose where all 6 animals received both drugs. With the .08 mg/kg dose, the slight increase in errors over saline days is nonsignificant. but the impairment on both learning set and reversal

Dose (mg/kg)

performance is real as is the increase in response failures. Here there is no difference between the two forms of atropine. Some, but not all of the deficits in learning set and reversal performance are attributable to response failures since animals that continued to respond made fewer correct choices on the criterion trials for these measures. At the .032 mg/kg dose, the effects have largely disappeared although reversal performance is down slightly in the atropine sulphate group.

There is a potentially interesting relationship between the social rank of the monkeys and their performance under atropine. Using a combination of Trial 2 and Reversal Trial scores across as an index of overall performance, the rank order correlation between high social rank and performance on placebo days is a nonsignificant +.61; with the .032 mg/kg dose of the atropine (combined) it is only +.20; but at .08 mg/kg, it is +.89 which yields a p < .05, two-tailed, despite the small n involved. At .20 mg/kg, the correlation is only +.76, but this compares closely with a +.74 obtained from data from the earlier study where all 6 monkeys received .20 of atropine sulphate. This indicates that, at least at the moderate .08 mg/kg doses, the drug effects interact with social status such that high status monkeys show less impairment of performance than lower ranking animals. Further examination of the data for the .08 mg/kg dose yielded a correlation of -.99 between social rank and number of response failures - the higher the animals' rank, the fewer the trials on which he failed to respond. To test the robustnesss of this finding, it will be necessary to manipulate the social status of the individual monkeys and see if the apparent drug-induced interaction between status and deterioration of performance still obtains. We plan to conduct this experiment during the coming year.

performance and social variables. Although our WGTA earlier work had demonstrated a relationship between high social rank and poor performance during training on the various stages of this task (Bunnell and Perkins, 1980), once criterion performance has stabilized these relationships disappear or change (see the next paragraph and Table 8). In the 1980 paper, we showed that extinction of the reversal learning set was also related to social rank, in that high ranking animals took longer to reach criterion when they were required to learn to ignore the reversal cue and continue to respond to the stimulus that was correct on initial learning. Extinguishing the reversal learning set took many weeks and the procedure would too time consuming for inclusion in the test battery. Ł especially since relearning the reversal set following extinction takes about as long as original learning. However, we have tried a procedure in which a single reversal extinction problem ("false reversal cue") is given on one of the four daily problems. We found that performance following a reversal extinction trial was disrupted to varying degrees in the different monkeys and was least affected in the animals that were the best performers on the regular trials. There is 00 obvious correlation between changes in performance produced bv the false reversal cues and any of the social variables.

Nevertheless, the effect of the procedure on performance is such that we plan to evaluate it further by including it in some of the drug testing during the coming year.

rank, The correlations among social frequency of submissive, aggressive, and "other social" behaviors, mean daily Trial 2 Correct responses, mean daily Reversal Trial Correct responses, and mean total reinforced responses on all 46 daily trials (an indicant of overall daily performance in the WGTA) is given in Table 8. The data cover 20 days of social observations during May, 1985 for which 18 days of WGTA data were also obtained. Although the small number of animals requires that the correlations be interpreted with caution, some interesting relationships are apparent. Trial 2 Correct responses on initial learning and total reinforcements received are negatively correlated with frequency of submissive behaviors and positively correlated with high social rank. Thus, monkeys that make few submissive responses do well on the object quality learning set part of the task and make more correct responses overall each day when they are performing at criterion levels over an extended period of time. As noted above, this is quite different from the relationships obtained in the earlier study involving acquisition and extinction where high ranking animals were slower reaching criterion performance and took longer to extinguish the reversal set.

Although both Trial 2 Correct and Reversal Correct scores are positively correlated with overall performance as measured by total reinforcements received, their intercorrelation is a nonsignificant +.41. This indicates that the two parts of the task are tapping different dimensions of the monkeys' performance in the complex problem solving situation.

Our overall assessment of this task is that it should be included in the test battery, even though it is fairly labor intensive to administer. Initial learning takes a long time, but retraining following a layoff is fairly rapid - a matter of 2 or 3 weeks after breaks in testing of up to 3 or 4 months. The task is sensitive to drug effects which interfere with performance and various aspects of the test are related to agonistic social variables. Most important, the atropine data show that it can be useful in detecting sociopharmacological effects, i.e., interactions between social variables, such as status, and the degree of interference in performance that is produced by a drug.

-35-

Ta	Ь	1	e	8
----	---	---	---	---

Correlations Between Social Variables and Performance on the WGTA

	NUMBER SUBMIS- SIVE	NUMBER AGGRES- SIVE	NUMBER OTHER SOCIAL	TRIAL 2 CORRECT	REVERSAL TRIAL CURRECT	TOTAL REIN- FORCERS
SOCIAL RANK	94 *	.83 *	.20	.81	. 54	.87 *
NUMBER SUBMIS- SIVE		60	37	99 ¥	37	- . 83 *
NUMBER AGGRES- SIVE	·		.14	.56	.07	.54
NUMBER OTHER SOCIAL				.50	.14	.31
TRIAL 2 CORRECT					.41	.84 ×
REVERSAL TRIAL CORRECT						.83 *

* p<.05

G. Operant Performance:

DRL schedules. The seven oldest males from NT-Troop had been trained on a differential reinforcement of low rate of response with a limited hold during 1984. The schedule, а DRL-18 sec, LH-10 sec, required the animals to delay 18 seconds between responses before receiving a reinforcement; responding within the 18 seconds reset the timers and instituted another 18 sec delay. The limited hold required that the animal make a response within 10 seconds once the 18 sec delay requirement had been met, otherwise no reinforcer was given. During June and August of 1984, the effects of caffeine on performance on these schedules was assessed. In September, 1984, the first experiment on the effects of atropine on this schedule was conducted with atropine sulphate. The results of these earlier experiments were reported in Annual Report Number 1. Two followup studies were conducted in the fall and winter of 1984-85. In the first, the delay between injecting the drug and the beginning of testing was increased from 15 to 30 min. In the second, the effects of both atropine sulphate and atropine methyl nitrate were examined.

Animals were allowed to earn 40 reinforcements (banana pellets) during each session; sessions were terminated after 60 min if the animals had not finished. Three measures of performance were obtained: Efficiency Index (EI) - the reciprocal of total responses divided by number of reinforcements obtained. An EI of .50 or larger indicates that the monkey is averaging two or less resonses per reinforcement. (This is generally indicative of highly efficient performance on the DRL schedule. However. when responding drops to a very low rate, such that the limited hold requirement is exceeded repeatedly, EI's may remain relatively high although it takes the monkey considerably longer to obtain its 40 reinforcements.) Response Bursting - the 18 second schedule requirement was divided into six 3 second response bins and bursting was defined as the number of responses in the first bin (interresponse time <IRT> distributions were also obtained, these allow a study of response patterning), and Limited Holds - the number of times the animal exceeded the limited hold requirement during a session. Total number of reinforcements received and total responses during a session were also recorded.

Atropine and DRL performance. The results of the initial experiment on the effects of atropine DRL On performance were given in the previous annual report, cited above (pp. 32-33 and Table 8). Atropine sulphate produced a severe disruption of performance at the two highest doses (-40)and .20 mg/kg) and a more variable impairment at the lowest dose (.08 mg/kg). Efficiency indexes were generally lower, bursting was reduced (overall responding was also .ower), and the frequency with which limited holds were exceeded increased. In many cases the monkeys did not complete the task within the allotted 60 min and so failed to receive all 40 reinforcers.

This experiment utilized a delay of 15 min between injection and the beginning of testing.

The next experiment repeated the .20 and .08 mg/kg doses of the first experiment, but utilized a 30 min delay between injection of the drug and the beginning of testing. There had been considerable individual differences in the degree of disruption of performance in the first experiment and we felt that the animals which worked most quickly might be completing more of the task before the full effects of the drug had time to occur when tests were begun 15 min after injection. The experiment, conducted 5 weeks after the initial study, utilized one presentation of each of the two doses of atropine sulphate and three placebo days.

At the .20 mg/kg dose, efficiency indices (EI's) were substantially lower than they had been with the 15 min delay in 3 of the 7 monkeys and bursting was increased in 2 animals. There were no differences in the effects of atropine on the limited holds exceeded or total number of number of reinforcements received with the two delay intervals. At the 30 min delay, the .08 dose had very little effect on performance whereas, in the first experiment, 6 of the 7 monkeys exhibited substantial changes on one or more performance indices at the 15 min delay. This appears to indicate that some behavioral tolerance had developed. Overall, the effect of increasing the drug/test interval was rather small. However, the reduction in EI's that occurred with the .20 mg/kg dose was seen in the three monkeys that were the most efficient performers under baseline conditions. Consequently, a delay of 30 min between injection and testing was used in the last experiment in this series. Data comparing the effects of the two delay intervals are given in Appendix B. This appendix also includes some data from the third experiment, described below, so that the effects of repeated doses can be examined. The trend toward behavioral tolerance described above had largely disappeared in the third experiment which was begun three months after the second study was finished.

The third experiment compared the effects of atropine sulphate and atropine methyl nitrate on DRL performance. Atropine methyl nitrate, the quarternary nitrogen derivate of atropine, is presumed to have largely peripheral effects as a muscarinic receptor blocker since it does not readily pass the blood brain barrier (Weiner, 1980). It has been reported that it does not have much effect on most behavioral and EEG measures (Russell, 1982) when compared with atropine sulphate.

The experiment used the 7 NT-Troop males and doses of .20, .08, and .032 mg/kg of both drugs. There was a 30 min delay between the im administration of the drugs and the beginning of testing. Placbo (physiological saline) days alternated with drug days with Mondays always being a placebo day. The order of administration was .08, .20, and .032 mg/kg of one compound followed by the same order for the other compound. Four monkeys received the atropine sulphate (AS) series first followed by the atropine methyl nitrate (AMN) series. The other three animals received atropine methyl nitrate first. A partial replication using the .032 and .08 mg/kg doses with selected animals was added to the main study to check some of the

a da piñerar

ኯዀኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯኯ

-37-

initial findings. There were a total of 8 drug days and 12 placebo days in the experiment. See Table 9.

TABLE 9

Effects of Atropine Sulphate (AS) and Atropine Methyl Nitrate (AMN) on DRL Performance

					DOSE				
		.2	0	. (08	.03	52	SALINE *	
	Animal	AS	AMN	AS	AMN	AS	AMN	(+/- SEM)	
a.	Efficiend	zy Index	:						
	Barker	.08	.24	.16	.13	.54	.34	.57 +/04	
	Eju	NR**	NR**	. 39	NR**	.58	.49	.65 .03	
	Hobbit	.33	.32	.27	.36	.62	.55	.46 .03	
	Tag	.12	.19	.15	.12	.14	.16	.17 .01	
	Allen	.33	.35	.52	.33	.40	.31	.46 .03	
	Kuk la	.13	.22	.32	.38	.37	.27	.44 .03	
	Weed	.31	.24	.30	.22	.56	.38	.50 .03	
ь.	Number of	f Reinfo	rcements	out of	40:				
	Barker	3	6	36	11	40	26	40 +/	
	Eju	1	2	27	2	40	34	40 -	
	Hobbit	40	40	40	40	40	40	40 -	
	Tag	40	40	40	40	40	40	40 -	
	Allen	3	14	40	18	40	28	34.8 2.8	
	Kukla	2	2	23	10	27	26	39 0.7	
	Weed	14	40	30	37	40	40	40 -	
c.	Limited H	Hold Exc	eeded:						
	Barker	139	112	124	125	21	101	5.7+/-0.9	
	Eju	152	145	113	154	21	105	19.5 3.6	
	Hobbit	39	38	31	37	27	24	24.5 2.4	
	Tag	19	57	33	99	18	47	42.2 4.9	
	Allen	149	125	67	147	53	103	62.8 3.5	
	Kukla	141	149	118	133	121	99	53.4 9.1	
	Weed	109	56	78	69	8	27)	9.9 4.1	
d.	Bursting	(# 1st	bin resp	onses):					
	Barker	18	12	• 7	40	24	28	13.2+/-2.9	
	Eju	0	0	9	1	5	6	6.5 1.5	
	Hobbit	17	36	62	19	10	15	23.6 3.7	
	Tag	246	142	200	239	229	181	163.7 16.8	
	Allen	2	3	11	1	33	10	21.5 4.8	
	Kukla	5	3	21	2	15	25	24.0 4.3	
	Weed	13	70	38	67	13	23	20.9 2.5	

* Scores for Saline days are the means of 12 sessions. ** NR - not enough responses made to calculate a score.

The data for the .20 and .08 mg/kj doses of AS were quite similar to those from the previous study which used the 30 min delay between injection and testing, although the .08 mg/kg dose produced deficits that were more severe. The primary results were:

a. At the .20 mg/kg dose, performance was disrupted by both AS and AMN. All monkeys showed changes in one or more mealines of performance, i.e. efficiency index, total reinforcements received, number of limited hold periods exceeded, and changes in response bursting (first bin responses). In 6 of the 7 subjects, AS produced somewhat greater decrements in performance than AMN; this occurred whether atropine sulphate or atropine methyl mitrate was the first drug given at this dose.

b. At the .08 mg/kg dose, AMN produced severe deficits in performance in 5 animals, a moderate deficit in 1, and a slight deficit in 1. AS produced deficits in 5 animals that were less severe than those produced by ANN, a clear improvement in 1 monkey, and had no effect on performance in 1. The deficits persisted in those monkeys (n=3) which received a second administration of AMN at this dose but were smaller with the second administration of this dose of AS (n=4).

c. Th .032 mg/kg dose of AMN resulted in impaired performance in 5 monkeys, no change in 1, and slight improvement in 1. This dose of AS produced slight to moderate deficits in 3 monkeys, no change in 2, and slight improvement in 2. All 4 of the monkeys that got a second adminstration of this dose of AS performed slightly to moderately above their baseline (placebo) performances, but the 3 animals that got a second dose of .032 mg/kg AMN exhibited little change in performance between the two administrations.

The poorer performance under atropine sulphate as compared with atropine methyl nitrate at the dose of .20 mg/kg suggests the operation of a central effect of atropine at this relatively large dose. At .08 and .032 mg/kg, deterioration in performance may be due primarily to peripheral effects and the central effects actually may be exerting a racilitatory effect on performance, particularly with the smallest dose. Deficits tended to be smaller with atropine sulphate and there were some instances where performance was actually better than mean placebo performance. However, we do not know whether or not the time between administration of the two drugs and their production of peripheral effects could be sufficiently different to account for our data.

Social behavior and DRL performance. Comparisons between social behavior and performance on the DRL schedule were made for data obtained in the fall of 1984 during the time of the first atropine experiment. There was a marginally significant correlation of +.71 between frequency of aggressive responses and bursting and a nonsignificant correlation +.65 between high efficiency and high social rank. The correlation between rank and efficiency ratios on the 12 placebo days during the last atropine experiment rose to +.73; there had been a reorganization of the rank structure between the first and second atropine experiments. No detailed social data were available for this time because of bad weather, so it is not known if the aggression/bursting ratio relationship changed. We have seen these same two correlations between social behavior and performance in the past and it is interesting that they keep appearing despite changes in rank, aggression, and performance. High ranking animals are the moost efficient performers on this schedule and aggressive monkeys tend to show more response bursting than nonaggressive monkeys, whatever their rank.

There was no discernible interaction between the performance with any dose of either atropine and social variables during the first experiment. In other words, no score on any social variable, including rank, was related to the nature or magnitude of performance changes induced by the drug.

Fixed Interval schedules and atropine. In Annual Report Number 1, we reported on the initial findings from a study of the effects of atropine sulphate on performance on a fixed interval (FI) schedule. This experiment was completed during the fall of 1984 using 7 of the 8 adult males in I-Troop.

A FI-30 sec schedule was used in which the monkey received a reinforcer (banana pellet) for a response made after 30 seconds had passed since its last rewarded response. In well trained animals, the cumulative response curves on this schedule are scalloped, that is, there is a pause after а rewarded response followed by a gradual increase in responding as the end of the next 30 sec interval approaches. Scalloping may be identified by comparing response frequencies early in the interreinforcement interval with those late in the interval and it can be quantified by calculating an Index of Curvature (Fry, Kelleher, & Cook, 1960). For a second measure of performance, the 30 sec interval was divided into six 5-sec bins and response frequency in the first bin used as a measure following of response bursting reinforcement. Other ā performance measures obtained were the ratios of responses to reinforcements and the total number of reinforcements received. (The maximum was 40 and, if the monkey had not earned 40 pellets at the end of 60 min, the session was terminated).

The first part of the study had used doses of .08 and .20 mg/kg AS and delays between injection of the drug and the start of testing of 15 and 60 min for each dose. Completion of the experiment involved testing the animals with doses of .20 and .032 mg/kg with a delay of 30 min between injection of the drug and the start of testing.

Performance under the .20 mg/kg dose was severely disrupted with the 30 min delay. The findings were similar to those seen at the other delay intervals in that the monkeys performed slowly and did not finish the sessions, temporal discrimination was lost, and first bin responding WAS decreased. Some individual differences animal's in the responses at the 30 min delay were apparent in that 50-ae monkeys performance was similar to their behavior following the 15 min delay while the response patterns of others were more

similar to those they exhibited with the 60 min delay. Thus, it appears ⁺ at there are individual differences in the rabidity with which atropine affects performance. The .032 mg/kg dose of AS had little or no effect on performance.

This completed testing on the FI schedule - the schedule was used primarily to study caffeine effects on FI performance and the I-Troop monkeys were then trained and tested on a random interval schedule as described in the next section.

Random (Variable) Interval schedules. Upon completion of testing of the effects of AS on FI performance, the I-Troop males were retrained on a random interval (RI)schedule. On this schedule, a random (or variable) interval-1 min schedule (RI-1 min), responses are reinforced on the average of once per minute, but the actual intervals between the availability of reinforcers are produced by a random interval generator. Such a schedule tends to produce а moderately high rate of responding and a fairly constant rate of responding during the intervals which, in this case, were divided into 12 sec bins. Performance measures include total responses, responses per reinforcement, proportion of total responses in the first bin (a measure of response bursting), and number of reinforcements received out of a possible total of 40 within the 60 min test session. As a variant of this schedule, reinforcement may be randomly omitted 10 percent of the times the monkey completes the schedule requirements - this is done by dropping the banana pellet through the bottom of the food hopper before the monkey can reach it. The omission of reinforcement procedure is designed to frustrate the animal and produce response bursting in the first bin of the post omission interval.

Effects of atropine on RI performance. Seven of the 8 males in I-Troop had reached stable performance on the RI-1 min schedule by June, 1985 (The eighth performed poorly and was not included in these experiments) and testing of the effects of AS and AMN on this schedule was begun. Doses were .032, .08, and .20 mg/kg and the interval betweem drug administration and the start of testing was 30 min. The first part of the experiment was run with the animals on 100% reinforcement; the second was done with random omission of reinforcement on 10% of the completed intervals. Sessions were terminated after an animal earned 40 banana pellets or at the end of 60 minutes under both reinforcement conditions. Drug days were alternated with placebo (physiological saline) days with some monkeys receiving AS and the rest AMN on a given day. The order in which the doses were administered was .08, .20, and .032 mg/kg; this order was then repeated so that all animals received all doses of both drugs under the 100% reinforcement condition. During testing with omission of reinforcement, the order of the doses was the same, but the monkeys received a given dose of both drugs before being tested on the next dose; once again, drug days were alternated with placebo days. One monkey became ill during testing with omission of reinforcement and he was removed from the experiment while undergoing treatment.

In the 100% reinforcement condition there were dose

ለምድ ውጤም ይሉ ውጥ ውጥ ውጥ አንበሩ የፍም አንበሩ የሌላ

dependent changes in responding under both drugs. At .20 mg/kg there was a depression in responding. Four of 7 monkeys had very low response rates and did not complete either the session. One monkey finished the AS session, 1 finished the AMN session, and 1 finished both sessions. At .08 mg/kg, responding increased and 5 of the 7 animals finished one or both sessions. All monkeys earned 40 pellets under both drugs at the .032 mg/kg dose; total responding was still slightly depressed in two animals buc was elevated in two others at this dose. The data for each animal are summarized in Table 10 which gives total responses, number of reinforcments, and the proportion of total responses that occurred in the first 12 sec bin of the interreinforcement intervals (bursting). Two additional things to be noted from the table are: (1) There are changes in response distribution in 5 of the monkeys such that they make a greater proportion of responses during the first bin. particularly at the higher doses of one or both drugs, indicating a disruption of normal response patterning in addition to the general reduction in responding. (2) It appears that the two drugs may have differential effects on the performance of different animals, i.e., Cracker's performance is more seriously disrupted by the .20 mg/kg dose of AS than AMN while Equal's is poorer under this dose of AMN than . it is with .20 mg/kg of AS. These differences are _accounted _for___by the order of administration of the two drugs to the monkeys. With one exceeption, the drug administered first at a given dose had the greatest effect on performance. (The exception is Yuk who received the .08 mg/kg dose of AS before he got the same dose of AMN). We conclude that there is little difference in the effects of AS vs AMN on performance on this schedule. Instead, it appears that experience with a given dose may lead to behavioral adaptation such that drug effects on performance are reduced with repetition of that dose.

The data from the 90% reinforcement condition are given in Table 11. Omission of reinforcement failed to produce an increase in responding after nonreinforced intervals. The responses/nonreinforced interval on placebo days were no greater than responses/reinforced interval. (This can also be seen by comparing the bottom sets of data in Tables 10 and 11.) The atropine data add little or nothing to what was found with 100% reinforcement.

<u>RI schedules and social behavior</u>. No significant relationships were found between any performance measure on either of the RI schedules and any of the social behavior variables. Since there was no bursting with omission of reinforcement, the expected relationships (Bunnell, 1782, Bunnell, et al, 1979) between high rank and high bursting could not be obtained.

Table 10

Effects of Atropine Sulphate (AS) and Atropine Methyl Nitrate (AMN) on RI-1 min Responding with 100% Reinforcement

Drug Dose

Number of Reinforcements

. - -

	P16	acebo 🕈	• (032	• •	28		20
Animal	(+/·	- SEM)	AS	AMN	AS	AMN	AS	AMN
Cracker	40.0	(0.0)	40	40	40	40	19	40
Equal	40.8	(0.0)	40	40	40	26	31	10
Gus	40.0	(0.0)	40	-	12	14	3	4
Quotation	39.4	(0.6)	40	40	19	10	3	4
Spiro	40.0	(0.0)	40	40	40	40	10	34
Yamamoto	40.0	(0.0)	40	40	40	24	40	24
Yuk	40.0	(0.0)		40	40	40	40	40

Total Responses

Cracker	178.0	(12.5)	161	193	140	140	32	101
Equal	440.4	(40.4)	422	408	287	237	324	76
Gus	689.0	(118.5)	940	-	61	54	6	26
Quotation	79.6	(3.5)	105	105	35	11	4	5
Spiro	603.8	(48.5)	264	411	237	1173	38	151
Yamamoto	916.8	(178.8)	637	773	1149	176	875	108
Yuk	2132.6	(138.7)		2113	1948	528	465	1461

Responses First Bin/Total Responses (Bursting)

Cracker	.50	(.01)	.48	.54	. 46	.22	.34	. 17
Equal	.31	(.02)	.31	.30	.38	.27	.26	.24
Gus	.35	(.02)	.35		.54	.46	.50	.38
Quotation	.01	(.004)	.02	.04	.05	.09	.50	.00
Spiro	.14	(.01)	.28	.25	.24	.27	.24	.18
Yamamoto	.29	(.03)	.34	.42	.31	.44	.27	. 48
Yuk	.21	(.02)	-	.19	.20	.28	.63	.27

Responses/Reinforcement

Cracker	4.5	(0.3)	4.0	4.8	3.5	3.5	1.7	2.5
Equal	11.0	(1.0)	10.5	10.2	7.2	9.2	10.5	7.6
Gus	19.5	(1.9)	23.6	-	5.1	3.9	2.0	6.5
Quotation	2.0	(0.1)	2.6	2.8	1.8	1.1	1.3	1.2
Spiro	15.1	(1.2)	6.6	10.2	5.9	29.3	3.8	4.4
Yamamoto	24.4	(4.0)	15.9	19.3	28.7	7.3	21.9	4.5
Yuk	53.3	(3.5)	~	52.8	48.7	13.2	11.6	36.5

* Placebo n = 5 days. Blanks are for apparatus failures on these days.

Table 11

Effects of Atropine Sulphate (AS) and Atropine Methyl Nitrate (AMN) on RI-1 min Responding with 90% Reinforcement

Drug Dose

Number of Reinforcements

	Placebo *		.032		.08		.20	
Animal	(+/-	- SEM)	AS	AMN	AS	AMN	AS	AMN
Cracker	39.7	(0.3)	40	-	40	40	39	20
Equal	40.0	(0.0)	40	40	40	14	40	10
Quotation	40.0	(0.0)	40	40	19	8	2	3
Spiro	39.9	(0.1)	40	-	40	27	5	20
Yamamoto	40.0	(0.0)	40	40	40	40	40	40
Yuk	40.0	(0.0)	40	40	40	40	5	40

Total Responses

Cracker	270.2	(29.5)	233	~	263	135	154	85
Equal 👘	439.1	(45.5)	469	247	637	28	314	57
Quotation	117.4	(7.9)	115	75	39	11	5	11
Spiro	676.9	(128.0)	279	-	309	128	19	77
Yamamoto	678.4	(74.1)	312	619	959	1361	448	875
Yuk	1742.1	(163.7)	1549	1447	1998	2052	20	1023

Responses First Bin/Total Responses (Bursting)

Cracker	.61	(.06)	. 57	-	.14	.41	.29	.24
Equal	.32	(.03)	.41	.44	.25	. 39	.35	.16
Quotation	.04	(.02)	.02	.04	.00	.00	.00	.18
Spiro	.20	(.03)	- 38	-	.24	.33	.11	.23
Yamamoto	.30	(.02)	.54	.36	.40	.30	.35	.30
Yuk	.24	(.02)	.26	.28	.21	.22	.35	. 32

Responses/Reinforcement Following Nonreinforcement

Cracker	4.7	(0.8)	3.6	-	4.0	5.2	2.0	3.0
Equal	13.0	(2.8)	7.0	6.8	27.8	×	11.5	7.0
Quotation	2.9	(0.6)	4.3	1.5	1.0	2.0	1.0	×
Spiro	7.8	(1.4)	11.5	-	21.5	6.0	x	1.8
Yamamoto	17.5	(3.5)	×	9.0	9.0	52.0	24.0	44.5
Yuk	32.3	(4.7)	70.3	32.5	70.0	34.3	×	40.7

* Placebo n = 8 days. Blanks (-) are for apparatus failures on these days.

x = random programmer did not omit reinforcement on this day.

H. Plasma Hormones - Baseline and Stress:

Measures of the hormones cortisol and prolactin are used as indicants of stress. Levels of plasma cortisol and plasma prolactin were determined by radioimmunoassay using kits obtained from Cambridge Medical Diagnostics, Inc. The assays were done in the nutrition laboratories of the College of Home Economics at the University of Georgia. Graduate assistants on the project assisted the laboratory personnel with the assays. In the assay for cortisol, recovery runs between 92-96% with intra-assay and inter-assay coefficients of variation of 4% and 7.5% respectively. Frolactin recovery is 98-110% with coefficients of variation of 8.5% and 11.1%.

In obtaining blood samples, the monkeys were restrained using the device mentioned earlier in this report and blood was collected in heparinized tubes from the saphenous veins of the animals' legs. Samples were centrifuged and stored at -18 C until assay. During the first 18 months of the project, samples were taken between 0900-1100 hours. In obtaining baseline data, 2 ml samples were drawn no more often than every second or third day for a period of 10-15 days. During the summer of 1985, a study of diurnal variations in hormone levels was performed with the C-Troop males. Some of the results are described below.

Samples collected from C-, NT-, and I-Troops during 1984 and the winter of 1985 were analyzed in February, 1985. The 230 samples included two social manipulations. In the first of these, Gus, the alpha male in I-Troop was removed for 2 weeks and then returned. The second manipulation involved the introducton of Defeat, a young adult male, into C-Troop.

Baseline prolactin levels were elevated during the first blood draws, but habituated fairly quickly to levels consistent with those we had obtained in some of our previous work in which the samples had been assayed at the Walter Reed Army Institute of Research. Individual monkey's prolactin responses to the social manipulations varied tremendously. Some exhibited large increases, some did not change, and some had slight decreases with respect to baseline values. In rats, prolactin levels have been shown to vary as a function of the intensity and duration of the stressor (Kant, et al, 1983). In our monkeys, we did not find a correlation between either baseline levels of prolactin or changes in prolactin and any of the social variables being measured. Cortisol levels did not habituate very well under baseline conditions and social manipulations often produced readings that were at or near the upper limits of the assay (75ug/100ml). However, our initial concern that there might be a problem with the assay itself, with our handling of the samples, or with the time of day the samples were collected, proved to be unfounded. Reassay of some of the old samples produced interassay coefficients of variation of around 7% and more frequent sampling produced better habitutation.

Because of the high baseline values for cortisol, we were concerned that taking blood during the 0900-1100 hours window was too close to the peak of the diurnal cortisol rhythm and that we would not be able to detect the effects of social manipulations or punishment on hormone levels. We also did not know how frequently we could take blood samples without producing problems in the animals or altering plasma hormone readings. To examine these issues, we did a study with the C-Troop males in which samples were obtained at different times of the day and in which we examined the effects of taking 2 ml samples at intervals of 1, 2, and 3 days on hematocrit values. Table 12 gives the mean values obtained for both cortisol and prolactin for a series of samples taken at different times of the day.

Table 12

Mean (+/- S.E.M.) Plasma Cortisol and Plasma Prolactin Levels for Six Monkeys Sampled at Four Different Times of Day

Time Window:	0800-0900	1000-1230	1400-1530	2030-2130
Number of Days	4	6	3	2
Cortisol	25.99	26.99	19.42	12.29
(ug/100 ml)	+/- 0.81	+/- 0.95	+/- 1.74	+/- 1.21
Prolactin	7.83	5.14	6.71	6.88
(ng/ml)	+/- 1.72	+/- 1.05	+/- 2.19	+/- 1.66

The plasma cortisol levels were within the normal range seen in other species of macaques, such as rhesus monkeys (Holaday, Meyerhoff and Natelson, 1977) and are 30-50% lower than those we obtained during the first year of the project. As expected, there is a marked diurnal effect such that cortisol values are highest in the morning at the times when we do most of our experimental work. However, the range of response available above these baseline values is sufficient to detect the effects of stressors and other stimuli and we probably no longer have to worry about a ceiling effect. No circadian rhythm in prolactin was present and none was expected. As noted above, the absolute values for prolactin are comfortably within the range we had obtained from assays done elsewhere on blood samples taken from I-Troop males in 1971.

Hematocrits obtained from the samples in the heparinized tubes averaged about 35% and did not vary appreciably in repeated daily sampling with sample sizes of 2 ml. (Hematocrits taken directly from the vein ran about 40-42%; doing hematocrits on the heparinized blood provided a small but significant saving in time and was justified because our primary interest was in making certain that red cell counts were not dropping due to the daily sampling procedure.)

Table 13 presents the data obtained from C-Troop following a 20 min exposure to 1.5 ma constant current inescapable footshock delivered on a 1 min variable time schedule and following a 50 test of social behavior during which time all 6 monkeys were together. There was no overt agonistic behavior during the social test of the group.

Table 13

Mean (+/- S.E.M.) Flama Cortisol and Plasma Frolactin Levels Followin Exposure to Shock and Social Group for Six monkeys in C-Troop

	CONTROL	FOOTSHOCK	SOCIAL
Cortisol	21.48	31.83	29.11
(ug/1 00 ml)	+/- 2.45	+/- 0.73	+/- 2.32
Prolactin	3.71	6.49	3.99
(ng/ml)	+/- 0.85	+/- 1.84	+/- 1.10

Footshock produced small increases in both cortisol and prolactin. Observation of the animals during the footshock showing sessions suggested that they were considerable behavioral adaptation to the shock. After the first two or three shocks, the monkeys sat immobile and tense for the rest of the session. The marginal increase in prolactin over the control day indiates that the stress produced by footshock was not very great. In the social situation which produced no overt agonistic behavior, there was no rise in prolactin although cortisol levels were up a bit. Since adrenal glucocorticoids tend to respond in all or none fashion (Kant, et al, 1983) to stressors of a wide range of intensities, we do not judge this particular test to have been very stressful.

The cortisol and prolactin values obtained before, during, and after the reintroductions of Cracker and Alabama into I-Troop in the spring of 1985 are presented in Table 14. The reintroduction of Cracker, a low ranking male, produced little overt agonistic behavior in the troop. The reintroduction of Alabama, the second ranked animal, which is described in the social behavilor section of this report, resulted in considerable agonistic interaction. For a baseline, 4 samples were taken over eight days prior to Cracker's introduction, 1 on the day of introduction, 1 two days later, 1 on the day of Alabama's introduction four days later, and 3 over the seven days following Alabama's introducton. The values for Cracker and Alabama are not included in the Table until two days after their introductions. Cracker's mean preintroducton cortisol was 14.8 ug/100 ml and on the day of introducton it was 30.9; his prolactin values were 6.1 ng/ml and 21.2. Alabama's baseline cortisol was 34.0 um/100 ml and it rose to 70.6 on the day of introduction. Prolactins were 13.1 ng/ml and 14.8.

Table 14

Mean (+/- S.E.M.) Plasma Cortisol and Plasma Prolactin Levels Followin Social Manipulations in I-Troop

	PRE	CRACKER IN	POST	ALABAMA IN	POST
Number of D	ays 4	1	1	1	3
Cortisol	33.00	36.06	33.09	45.73	38.51
(ug/100ml)	+/- 2.05	+/- 2.96	+/- 5.83	+/- 6.28	+/- 2.92
Prolactin	12.95	16.75	24.93	22.37	18.65
(ng/ml)	+/- 2.83	+/- 4.67	+/- 5.39	+/- 6.66	+/- 8.23

Cortisol levels increased following Alabama's introduction and were still slightly elevated eight days later. Cracker's introduction had no effect on mean cortisol on that day. The most interesting effect is the large increase in the standard error that is seen two days after Cracker's introduction and on the day of Alabama's introduction. This means that some animals were responding to the social manipulations with moderate to large increases while others were largely unaffected. This is certainly the case for the prolactin scores where the large increases in standard errors obscure the mean increases following the introductions. There were significant na correlations between individual response frequencies in any social behavioral category and individual values for either hormone. Thus, if social stress is operationally defined by hormone measures, it cannot be assessed by looking at the individual monkey's agonistic response frequencies.

Overall, the current sampling procedure seems to be working well and providing reasonable baselines against which to assess the effects of experimental manipulations so long as animals are kept habituated to the procedure. The assays are reliable and valid. Because blood samples are generally drawn in the mornings when the performance testing is done, cortisol baselines tend to be higher. We plan to evaluate the effect of shifting the light-dark cycle of the C-Troop animals on baseline and stress induced hormonal responses. Turning on the lights 4 hours earlier may reduce baselines by 25% during the morning and provide more room between baseline and ceiling responses.

I. Equipment and Facilities:

The PDP-8 laboratory computer which is used to run the operant programs and to analyze the social data continued to give problems. There were several breakdowns in the handware and these, coupled with poor performance by the field maintenacte personnel assigned to repairs under the maintenance contract with Digital Equipment Corporation, interfered with daily operant testing on a number of occasions and hampered the analyses of the social data. A new PDP 11/73 computer system, together with associated interfaces and software to run the

-48

SKED-11 operating system was ordered, received, and installed. (The University of Georgia provided \$2400 toward the purchase of additional software for this system.) Because of hardware problems with the new machine, the SKED operating system was not installed until the end of September, 1985. Rewiring of the operant chambers to increase the inputs and outputs of the chambers is being done on a non-interference basis with the ongoing research and it is expected that the conversion to the new system will be completed sometime this coming winter. As soon as time permits, the social analysis programs will be rewritten for the new machine. In the meantime, the PDP-3 will continue in use for social data analysis and this machine 15 being continued under a field maintenance contract.

New shock grids were built and installed in the operant chambers during the summer. These are now being used in the studies on response suppression and are available for free operant avoidance testing when the new computer system comes on line.

An operant panel together with a pellet feeder was installed in the indoor cage used for testing social behavior and activity with the C-Troop animals. The use of this panel is described in the section on social behavior. A centrifuge for doing hematocrits was purchased from University funds and is being used in conjunction with the hormone studies described in the preceding section of the report.

The floor of the indoor quarters for I-, T-, and NT-Troops was scraped and painted in the early spring using funds granted by the University's Research Foundation for the purpose. This is going to have to be done on an annual or semiannual basis until some arrangements can be made to install a satisfactory, permanent new floor covering.

There were no other major changes in facilities or equipment during the reporting period.

J. Personnel:

Dr. Bunnell, of the Department of Psychology, serves as principal investigator for the project. Dr. Iturrian, from the Department of Pharmacology and Toxicology, is coprincipal investigator. The consulting veterinarian is Dr. Willy L. Chapman, Jr. from the Department of Pathology of the College of Veterinary Medicine at the University of Georgia. Additional veterinary care and support are provided as needed by the staff of the veterinary college and by the university's Animal Care Coordinator from the Office of the Vice President for Research. The full time animal caretaker left 'the project in July and was replaced by a person with B.S. in animal science and considerable experience in caring for farm animals. She did not work out and was replaced in September by a former graduate student on the project who was given a temporary appointment while we search for a suitable permanent replacement. A full time research technician, with electronic and computer interfacing skills, manages the day-to-day schedule for the project and as well as overseeing the computer operations. He has a batchelors degree in psychology and has served as an electronics technician in the U.S. Army. He is enrolled as а

part time graduate student in psychology at the University of Georgia and has been assigned the development of the operant cooperative behavior task as a Masters thesis project. Additional backup support for laboratory and electronic maintenance are provided by the University's Instrument and Electronic Design and Maintenenace shops. Three part-time graduate research assistants are employed on the project. Two of these are replacements for assistants who left the project at the end of the summer. The replacements began work prior ta the termination of those they were replacing in order to provide continuity in the testing program. One assistant is a doctoral student in physiological, the other two are working on their PhDs in the field of primatology. All three have been trained to administer all of the tests used in the project and each one is able to collect social data from at least two of the social groups. The new animal caretaker will also he trained to collect social data from two groups of monkeys and to serve as an observer on the tests in the open field. The projected use of two observers on tests of drug effects on social behavior makes it essential that we have a well trained cadre of social observers continually available.

Future Work

We still have to evaluate the use of a multiple RI -RI extinction schedule using the I-Troop males. This schedule should produce increased responding in the second component of the schedule, i.e., a behavioral contrast effect. If we fail to see behavioral contrast with the multiple schedule, then WP will probably recommend returning to a fixed interval schedule with omission of reinforcement for inclusion in the test battery. Fixed ratio (FR) schedules will be tried with C-Troop first, and depending on how this works in the social situation. we will put manipulanda in the NT-Troop compound and allow the animals access to food on the FR schedules while in the social group. A similar test has been used with rhesus monkeys (Bartlett & Meier, 1971) and we expect the results to be very interesting. We will continue testing the adult males in NT-Troop on the DRL schedule to maintain their proficiency and to complete drug testing with this schedule. The DRL schedule will be included in the final set of tests in the battery as will the WGTA learning set problems and the open field with novel objects present. Suppression of responding on the RI schedule is being examined in I-Troop, with footshock as the punishing stimulus. This is working fairly well, but the monkeys show considerable behavioral adaptation to the shock. We still must evaluate a free operant avoidance task and will use the T-Troop males for this. Because of the behavioral adaptation to footshock we have seen with the C- and I-Troop males, we are not very confident that the avoidance paradigm is going to be useful. (We cannot chair the monkeys for shock delivery without interfering with the study of the social variables.) If the free operant avoidance task does not work reasonably well, we will evaluate a conditioned suppression paradigm as a possible alternative. A test of preferences for social stimuli is being developed. It utilizes a simultaneous

presentation of pairs of visual stimuli, including pictures of troop members, unfamilar animals, and familiar humans. Following a forced exposure phase which insures that the subject attends to both stimuli of a given pair, the preference for one stimulus is measured in terms of the length of time the monkey will hold down a button which causes that picture to be projected on the screen. We are using 5 young adult females from T-Troop in this project. Our attempts to study naturally occurring cooperative behavior by looking at enlisting behaviors in the C-Troop males have not been very successful, although they may improve with the use of the pellet feeder procedure. We will continue to monitor enlisting behaviors, as well as affiliative behaviors in the groups that are living together. A successful operant task of cooperative behavior will probably require that the partners in the task live together continuously (Mason & Hollis, 1963). We are trying to work out caging arrangements that will allow us to do this using pairs of C-Troop males. The task will require that both monkeys pull simultaneously on a rope in order to bring a tray containing a food reward within reach.

Studies of social behavior will continue using the procedures described in the body of the report. Use of the pellet feeder in the C-Troop test cage promises to substantially increase the quantity and quality of the data obtained from the dyadic interaction tests and should allow the assessment of social behavior changes produced by drugs which decrease social interactions.

The assays for plasma prolactin and cortisol are working well and we will continue monitoring these hormones in conjunction with the performance measures and the social observations.

A protocol for using diazepam has been submitted and we expect to complete most of these tests during the coming winter. We plan to do more work with the atropines and social behavior in the outdoor troops and we still must examine the effects of pyridostigmine 2-PAM on both social behavior and our performance tasks.

-51-

References

- Bartlett, D. P. & Meier, G. W. Dominance status and certain operants in a communal colony of rhesus macaques. <u>Primates</u>, 1971, 12, 209-219.
- Bunnell, B. N. Ferformance correlates of social behavior and organization in non-human primates. Final Technical Report, Contract DADA17-73-C-3007, USAMRDC, 1982.
- Bunnell, B. N. & Perkins, M. N. Performance correlates of social behavior and organization: Social rank and complex problem solving in crab-eating macaques (M. fascicularis). Primates, 1980, 21, 515-523.
- Bunnell, B. N., Kenshalo, D. R., Jr., Allen, J. D., Manning, F. J., & Sodetz, F. J. Performance correlates of social behavior and organization: Social rank and omission of reinforcement in rhesus monkeys (<u>M. mulatta</u>). Frimates, 1979a, 20, 77-86.
- Delgado, J. M. R., Grau, C., Delgado-Garcia, J. M. & Rodero, J. M. Effects of diazepam related to social hierarchy in rhesus monkeys. Neuropharmacology, 1976, 15, 409-414.
- Fry, W., Kelleher, R. T. & Cook, L. A mathematical index of performance on fixed interval schedules of reinforcement. <u>Journal of the Experimental Analysis of Behavior</u>, 1960, 3, 193-199.
- Holaday, J. W., Meyerhoff, J. L. & Natelson, B. H. Cortisol secretion and clearance in the rhesus monkey. Endocrinology, 1977, 100, 1178-1185.
- Kant, G. J., Bunnell, B. N., Mougey, E. H., Pennington, L. L. & Meyerhoff, J. L.Effects of repeated stress on pituitary cyclic AMP, and plasma prolactin, corticosterone, and growth hormone in male rats. <u>Pharmacology Riochemistry</u> and Behavior, 1983, 18, 967-972.
- Mason, W. A. & Hollis, J. H. Communication between young rhesus monkeys. <u>Animal Behaviour</u>, 1962, 10, 211-221
- Waal, F. B. M. de, Van Hooff, J. A. R. A. M., & Netto, W. J. An ethological analysis of types of agonistic interaction in a captive group of Java-monkeys (Macaca fascicularis). Primates, 1976, 17, 257-290.

APPENDIX A

Social Behavior in I-Troop: The following tables are the matrices derived from the analyses of 11 days of 40 min group scan social data recorded from I-Troop during the period 1 July - 16 July 1985. I-Troop was intact during this time - all 8 adult male monkeys were present during all observation periods. The matrices labeled SUBMISSIVE, AGGRESSIVE, NONAGONISTIC SOCIAL, and SEXUAL contain the behaviors listed in these categories in Table 2 of the main text. The GROOMS matrix is for social grooming (allogrooming) and contains this behavior only. GROOMS is contained within the NONAGONISTIC matrix as well. In each matrix, the frequency with which each monkey directs a given class of behavior toward every other animal in the troop is read across the horizontal rows. The frequency with which each monkey receives each class of behavior is read down the vertical columns. Row, column, and matrix totals are at the right margin and the bottom of each matrix. The SUBMISSIVE matrix establishes the social rank hierarchy in terms of who submits to whom. The other matrices are constructed using this same order. Notice that the SUBMISSIVE matrix shows that 27 of the 28 possible dominance/submission relationships have been identified during the 11 observation periods. Only the relationship between Yuk and Quotation was not directly observed.

SUBMISSIVE:

	G	Α	S	Y	С	Y	Q	E	Т
	U	L	P	A	R	U	U	Q	0
	S	Α	I	M	Α	κ	O	U	т
		B	R	Α	С		т	Α	A
		Α	O	M	к		A	L	L
GUS		, O	0	0	0	0	0	0	0
ALABAMA	2		0	0	0	0	0	0	2
SPIRO	1	1		0	0	0	0	0	2
YAMAMOTO	Ę	4	5		0	0	0	0	14
CRACKER	2	4	7	1		0	0	0	14
YUK	1	2	2	6	1		Э	0	12
QUOTATION	2	4	7	5	3	0		0	21
EQUAL	5	. 6	9	6	7	7	2		42
TOTAL	18	21	30	18	11	7	2	0	107

-53-

APPENDIX A

AGGRESSIVE:

	G U S	A L A	S P I	Y A M	C R A	Y U K		E Q U	Т 0 Т
		В	R	A	C		T	A	A
		A	U	m	к		A	L	Ļ
ຣນຣ		0	0	5	4	2	2	ο	13
ALABAMA	0		0	2	0	0	4	0	6
SPIRO	0	0		5	4	0	3	0	12
YAMAMOTO	0	0	0		0	5	0	0	5
CRACKER	0	0	0	0		4	4	6	14
YUK	0	· 0	0	0	0		1	13	14
QUOTATION	0	0	0	0	0	0		1	1
EQUAL	, O ,	0	0	0	0	0	0		0
TOTAL	0	0	0	12	8	11	14	20	65

NONAGONISTIC SOCIAL:

	G U S	A L A B A	S P I R O	Y A M A M	C R A C K	Y U K	Q U D T A	E Q U A L	T 0 T 0 L
GUS		37	1	3	2	1	0	0	44
ALABAMA	11		6	4	2	4	6	5	38
SPIRO	0	1		55	6	39	37	35	173
YAMAMOTO	0	0	24		7	61	7	29	128
CRACKER	6	5	3	0		4	35	78	131
YUK	0	0	2	23	0		6	6	37
QUOTATION	O	0	1	1	8	0		1	11
EQUAL	0	1	18	4	37	25	4		89
TOTAL	17	44	55	90	62	134	95	154	651

-54-

APPENDIX A

UROOMS:

	6 U S	A L A B A	S P I R O	Y A M A M	C R A C K	Y U K	Q U D T A	E Q U A L	T T A L
GUS ALABAMA SPIRO YAMAMOTO CRACKER YUK QUOTATION EQUAL	3002000	16 0 1 0 0 0	0 3 16 2 0 0 11	1 1 21 0 15 1 1	0 0 1 0 2 29	0 1 19 23 1 0 13	0 2 16 0 15 2	0 3 14 12 21 10 0	17 13 70 52 42 17 3 55
SEXUAL:					-	57	56	70	267
	G U S	A L A B A	S P I R O	Y A M A M	C R A C K	Y U K	Q U T A	E Q U A L	T 0 T 0 L
GUS ALABAMA SPIRO YAMAMOTO CRACKER YUK QUOTATION EQUAL	000000000000000000000000000000000000000	0 0 0 0 0 1	000000	0 0 0 0 0 0	0 0 0 0 1 0	0 0 1 0 0	0 2 0 0 0 0	0 5 1 0 0 0	0 7 2 0 0 0 0 3
TOTAL	0	1	2	0	1	1	2	6	13

-55-

APPENDIX B

Effects of 15 min vs 30 min Delays Between Atropine Sulphate Injection and Start of Testing on DRL Performance

			DOSE mg/kg							
			.08			•	20		SALINE *	
	Min Delay	: 15	30(1)	30(2)	15(1)	15(2)	30(1)	30(2)	(+/- SEM)	
	Animal									
a.	Efficiency	' Inde	<:							
	Barker	.26	.38	.16	.16	.30	.11	.08	.58 +/01	
	Eju	.80	NR	.39	.30	.67	.06	NR	.59 .03	
	Hobbit	.28	.48	.27	.17	.23	.28	.33	.45 .03	
	Tag	.06	.16	1.15	.13	.13	.12	.12	.17 .01	
	Allen	.33	.39	.56	.41	.38	. 37	.33	.41 .03	
	Kukla	.07	. 25	.32	.15	.07	.19	.13	.32 .06	
	Weed	.22	40		• • 29 •	• • • • • • • • • • • • • • • • • • • •	. 10	.31	.43	
ь.	Number of	Reinfo	prcement	s out o	f 40:					
	Barker	13	40	17	3	5	13	3	40 +/	
	Eju	4	NR	27	3	2	2	1	40 -	
	Hobbit	40	40	40	36	40	40	40	40 -	
	Tao	10	40	40	19	17	21	40	40 -	
	Allen	34	40	40	32	29	38	3	37 2.4	
	Kukla	9	40	23	8	9	16	2	39.5 0.3	
	Weed	39	28	30	23	25	11	14	40 -	
c.	Limited Ho	ld Exc	:eeded:							
	Barker	152	64	124	142	121	136	139	8.9+/-3.2	
	Eju	166	NR	113	147	127	157	152	22.3 2.8	
	Hobbit	69	27	31	91	63	55	39	18.8 4.7	
	Tag	152	7	33	117	9 9	131	19	29.1 13.1	
	Allen	116	25	67	107	79	108	149	42.4 16.6	
	Kukla	147	60	118	136	9 9	137	141	47.7 4.7	
	Weed	82	98	78	100	92	107	109	18.5 8.6	
d.	Bursting (# 1st	bin res	(ponses	•					
	Barker	12	27	7	15	5	70	18	10.7+/-1.3	
	Eju	0	NR	9	2	1	21	0	9.4 3.8	
	Hobbit	64	28	62	9	89	62	17	26.7 2.8	
	Tag	124	157	200	143	87	115	246	186.1 13.2	
	Allen	9	14	11	6	7	15	2	21.2 2.2	
	Kukla	81	58	21	26	83	41	5	44.7 10.5	
	Weed	71	17	38	23	34	40	13	23.7 6.3	

-56