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Grooming in the rat: Novelty or stressful stimulation?

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GROOMING IN THE RAT:

NOVELTY OR STRESSFUL STIMULATION?

by

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ABSTRACT

Groups of subjects were observed in an Open Field apparatus under various levels of illumination and white noise stimulation. The measure of relative heart rate decrement was used as an objective measure of fear in an attempt to determine whether the grooming produce by rats in novel and stressful situations is related to fear.

The results showed that although there were differences in the amount of fear experienced, as measured by relative heart rate decrement, no differences in grooming existed either in terms of the amount of grooming or the manner in which this behaviour was distributed in time.

It was concluded that the experience of fear is not related to the occurrence of grooming in novel situations. The present data offer support for the view that such grooming merely represents the spontaneous elicitation of a routine everyday response.

PREFACE

The present study was undertaken in the tradition of the pure researcher, i.e., one who wishes simply to increase his knowledge and understanding. I am grateful to all those who participated in this endeavour, both men and rats, for all that they taught me.

I should like to express my indebtedness to Dr. A. A. Smith, whom circumstance obliged to assume the chairmanship of the Thesis Committee at a late stage, for his guidance and encouragement. Dr. H. W. Kirby, under whose direction the research was begun and who later served as external examiner, deserves special thanks. I am grateful also to Dr. A. H. Warner, Dr. G. Namikas, and Dr. W. G. Bringmann, who served as readers and made many constructive suggestions. I should like finally to thank my wife, Barbara, for her support and encouragement throughout.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The laboratory rat has served as a subject in psychological research for more than sixty years. During this time the rat has been used mainly in learning experiments and relatively little attention has been given to the etiology of the routine, everyday activities performed by these animals (Beach, 1950). It has been suggested (Bindra, 1959; Bindra and Claus, 1960; and Bindra and Seely, 1959) that an understanding of the factors governing the elicitation of these spontaneous responses would assist our understanding of the acquisition and performance of instrumental responses. Included in the rat's repertoire of everyday activities is the grooming response, generally considered to be innately patterned and highly resistant to modification by experiential factors (Barnett, 1963; Horvath, 1966). Detailed descriptions of rat grooming behaviour have been presented by Barnett (1963), Horvath (1966), and Rosenblatt and Lehrman (1963).

The nature of the grooming response, i.e., cleaning and self-licking, suggests that the behaviour is typically elicited by the presence of dirt, parasites, or other skin

irritants. However, one is struck, even in casual observation, by the frequency with which grooming responses occur. Bolles (1960) has reported that adult male rats may spend fifteen to twenty per cent of their waking time engaged in grooming activities. This seems to be more than the demands of cleanliness should require. Barnett (1963) comments,

> "The behaviour at least seems to be performed regardless of need: the animal does not wait until there are parasites or dirt in its hair....no experiments have been performed on the factors influencing the build-up of the readiness to groom....There is scope here for a detailed study."

An additional peculiarity of the grooming response in rats is that this behaviour is often initiated in seemingly inappropriate circumstances. For example, one may commonly observe grooming during the intertrial intervals of a series of conditioning trials. When rats are placed in a novel environment they sniff vigorously, move about cautiously, and almost invariably produce a bout of grooming. The term "displacement" is used by Ethologists to denote the occurrence of a behaviour in a situation not directly appropriate. Displacement behaviours are hypothesized by some Ethologists to occur either when the motivation to perform some instinctive response is high but the appropriate object is not present, or when two mutually exclusive behaviours are simultaneously elicited. In both cases there is blockage of the appropriate behaviour(s)

and an "irrelevant" response is performed (Tinbergen, 1951; Thorpe, 1963). A more recent view is that displacement behaviours may be explained in one of the following ways, i.e., instances in which the displacement behaviour is a byproduct of stimulation, such as being a consequence of autonomic nervous system arousal, and instances in which inhibition on the behaviour is removed and it is expressed even though the immediate motivation to perform the response is not high (Barnett, 1963).

The occurrence of grooming behaviour in novel situations may be interpreted in at least two ways. On the one hand grooming may be a reaction to fear produced by the strange stimuli. The grooming response would be familiar self-stimulation which the rat could provide for itself when the environmental stimuli were frightening. Thus, grooming may be a response to the autonomic components of a state of fright, possibly having anxiety-reducing properties (Hinde, 1966; Hinde, personal communication). This interpretation predicts that relatively more fearful situations will give rise to relatively more grooming.

An alternative possibility is that grooming occurs in response to the novelty aspect of the situation, rather than to any fear that may be induced. Since the animal is in a situation that is novel, it will not have a directly appropriate response. The behaviours most likely to occur

are those that are well-practised in everyday life, such responses as sniffing, walking, and grooming. The likelihood that a particular response will occur would be a function of its place in the response heirarchy that the animal brings into the novel situation (Bindra, 1961). There is a difficulty associated with this hypothesis in predicting the amount of grooming as a function of environment novelty since, if novelty is anxiety-inducing as suggested by Montgomery (1955), there will always be a confounding between the degree of novelty and the amount of fear experienced. However, if the degree of novelty was held constant and level of fear manipulated, this view would predict that the amount of fear experienced by the rat will be unrelated to the amount of grooming produced.

Several investigators have attempted to identify the variables that evoke grooming in novel and fearful situations. A review of these studies is presented below.

Bindra and Spinner (1958) observed 100 day old male Hooded rats in novel situations. Groups of animals previously maintained under identical conditions were tested in chambers representing different degrees of novelty, relative to the home cage. The results showed a direct relationship between the degree of novelty and the amount of grooming, i.e., the more novel the test situation the more grooming occurred. In addition, all groups showed an increasing frequency of grooming responses during the first

12 minutes of the test, and a decline during the remaining 3 minutes. Bindra and Spinner concluded that grooming could not be a manifestation of fear induced by the novel stimuli since the frequency of grooming responses increased throughout most of the test period. These authors assumed that novelty-induced fear would be greatest at the outset, and would decrease as a function of time in the chamber.

Woods (1962) maintained groups of 90 day old Sprague-Dawley rats, for 24 hours prior to a critical test, in environments that varied in stimulus complexity. These environments were described as: FREE, a 24 in. by 44 in. by 12 in. cage containing a maze, tunnel, and see-saw; NORMAL, a standard laboratory cage; and RESTRICTED, a cage with four solid walls. All subjects were tested for 24 minutes in a grey, well-lighted, 30 inch square Open Field. Woods found that the FREE environment rats groomed much more than either the NORMAL or RESTRICTED groups. Woods makes the point that novelty lies in the amount of difference between the stimuli of the test situation and the manipulated home environment, regardless of which has greater stimulus complexity.

Gray, Levine, and Broadhurst (1965) observed the grooming behaviour of 100 day old Maudsley Reactive and Nonreactive rats. The test chamber was a 10 in. by 12 in. by 7 1/2 in. wire mesh cage, except that the sides were solid metal. The subjects were exposed to 85 decibels (db)

intensity white noise (simultaneous presentation of all sound frequencies) throughout a 10 minute test. The Reactive rats groomed much more under these conditions than the Nonreactives. However, in another experiment using similar apparatus and subjects, except that the white noise was set at 60 db intensity, no differences in the amount of grooming were observed (Gray, 1965). Evidently a certain level of stress must be imposed on these animals before the strain differences manifest themselves.

Bolles (1960) attempted to determine whether novel situations give rise to grooming because they are fear producing or simply "stimulating". Bolles tested 100 day old male rats in a 12 in. by 12 in. by 12 in. white chamber. The experimental treatment consisted of placing the animal in the chamber and observing it there for 30 minutes. The control "stimulation" treatment consisted of placing the subject in and then immediately returning it to its home cage and observing it there for 30 minutes. Each animal was observed three times under each condition so that directly comparable data were obtained. Defecation scores indicated that the experimental treatment was the more fearful but more grooming occurred under the control "stimulation" condition.

It will be necessary to digress for a moment to explain the above reference to the 'defecation score'. Broadhurst (1957) has shown that certain types of intense

stimulation, such as intense illumination and intense white noise, evoke urination and defecation from the rat. This autonomic reactivity is interpreted by him, and others, to be a fear response. It has become common practice to use the number the fecal boluses deposited (the defecation score) as an index of the animal's level of fear or "emotionality".

Singer (1963) observed groups of male Sprague-Dawley rats aged 60 to 180 days in fear and non-fear test conditions. The test chamber was a 17 in. by 16 in. by 5 1/2 in. box constructed entirely of wood except for two wire mesh sides. An adjacent compartment contained a door buzzer, a door bell, and a 150 watt lamp. The fear condition consisted of continuous presentation of the buzzer, bell, and light stimuli throughout a 90 second test period. The defecation scores were higher in the fear than in the non-fear condition, in which the animal was observed in the same chamber but without the buzzer, bell, and light stimulation. Animals in the fear condition groomed reliably less than those in the non-fear situation.

Doyle and Pratt-Yule (1959) tested 60 day old male rats in an Open Field apparatus. Each subject was given three minutes exposure for twelve consecutive days. The purpose of the experiment was to establish whether the incidence of grooming and freezing behaviours bore any relationship to measures of ambulation and defecation, and

might therefore be useful as additional indices of emotionality. It was found that grooming appeared only when other measures indicated that emotionality had reached a low level, and the authors concluded that grooming is not a useful measure of emotionality.

It is clear that the experiments reviewed above report contradictory results. In the one comparison in which the stimuli of the test conditions differed only quantitatively, the Gray (1965) and Gray, Levine, and Broadhurst (1965) experiments, it was shown that more grooming occurred under more intense, and presumably more frightening, stimulation. As was pointed out earlier, whenever the attempt is made to manipulate the stimuli of the test (or home) chamber in a qualitative way, so as to create different degrees of novelty, an automatic confounding of this factor with fear may be introduced. The Bindra and Spinner (1958), Singer (1963), and Woods (1962) experiments are therefore impossible to interpret clearly. The unknown effects of handling and repeated exposure to the test situation make the Bolles (1960) and Doyle and Pratt-Yule (1959) experiments difficult, if not impossible, to compare with other studies. Taken together, the experiments reviewed above do not establish whether more fearful situations give rise to more grooming behaviour. Throughout these studies there is a lack of procedural consistency in terms of the age of testing (from 60 to 180 days of age), the type of test ap-

paratus used (varying size test chambers, home cages, and Open Fields), and the length of the test session (from 90 seconds to 30 minutes). These uncontrolled factors further impede, if not preclude, meaningful comparisons.

The literature contains several studies which report the effects of various drugs on grooming and other behaviours (Bindra and Baran, 1959; Bindra and Blond, 1958; Ryall, 1958; and Singer, 1963). Experiments involving the use of drugs must always be interpreted cautiously since the potential for unknown effects is everpresent. Meaningful interpretations must await several replications reporting consistent results. This difficulty may be illustrated by the drug Chlorpromazine, used by Bindra and Baran (1959), Ryall (1958), and Singer (1963). Chlorpromazine is generally considered to be fear reducing, or fear preventing, by way of blocking sympathetic nervous system activation, although its exact site and mode of action are not fully known (Gordon, 1967). Using a dosage of 2.0 mg/kgm body weight and testing 60 minutes after injection Singer (1963) reports increased grooming, Ryall (1958) reports decreased grooming, and Bindra and Baran (1959) report no effect on amount of grooming. It would seem that, at this stage, the drug experiments can offer no assistance to the understanding of the relationship, if any, between fear and grooming.

No satisfactory measure of fear was present in any of the experiments reviewed above. The defecation score, used by Bolles (1960), Singer (1963), and Doyle and Pratt-Yule (1959), is of doubtful utility under conditions of prolonged observation (McCleary, 1954) and has been questioned as a valid indicator of fear (Bindra and Thompson, 1953; O'Kelly, 1940). It would seem appropriate to measure directly some autonomically controlled function, for example, the heart rate (HR). The heart rate is regarded as a valid indicator of sympathetic and parasympathetic activity (Malmo, 1961; Schore, 1959), and the likeliest measure through which to assess anxiety in the rat (McCleary, 1954).

McCleary's (1954) attempt to use heart rate as a measure on anxiety in rats was not altogether successful; he concluded that either the measure was not useful, or that "rats are always anxious". More recent studies, however, report fairly consistent data regarding the HR response to various stressful situations and stimuli. Word, Stern, Sines, and McDonald (1959) reported that 74 per cent of normal rats respond with heart rate decrement to their first exposure to electric shock. Boyles, Black, and Furchtyott (1965) reported that Wistar rats responded with HR decrement to initial presentations of white noise stimulation. Similar decrements in heart rate were noted by Stern and Word (1961) upon initial presentations of tone,

and electric shock stimuli. Holdstock and Schwartzbaum (1965) reported that, in rats, a classically conditioned heart rate response to electric shock took the form of a rate decrement. Wenzell (1961) noted that cats' heart rates accelerated on presentation of a stimulus previously associated with food, but decelerated on presentation of a stimulus previously associated with electric shock. Snowdon, Bell, and Henderson (1964) measured rats' ambulation and defecation in a brightly illuminated (100 foot candles) Open Field, and compared these scores to measures of heart rate taken in a similar, but dimly illuminated, compartment. The results showed that a consistent association existed between low heart rates and indicies of high emotionality (i.e., low ambulation and high defecation scores). In a similar experiment, Harrington and Hanlon (1966) reported that Maudsley Reactive rats responded to a stressful Open Field (78 db white noise and 165 candle power illumination) with a marked heart rate deceleration. Finally, Candland, Pack, and Matthews (1967) reported that rats placed in a novel, but relatively non-stressful situation (a 24 in. by 24 in. by 24 in. light-proof and sound-proof box) responded with heart rate acceleration.

The studies reviewed above consistently show that when rats are first exposed, or conditioned, to stressful stimulation their cardiac response is one of HR decrement. Data presented by Candland, Pack, and Matthews (1967) shows

that rats respond to novel but relatively non-stressful situations with heart rate acceleration.

The present experiment used the measure of relative heart rate decrement as an objective measure of fear in an attempt to test the hypothesis that rats respond to relatively more intense, or fearful, stimulation by producing relatively greater amounts of grooming.

CHAPTER II

METHODOLOGY

The procedures employed in this study, and in particular certain controls employed with respect to handling and to temperature variation in the Open Field, were developed as a result of a fairly extensive pilot study. The results of this study are of some interest in their own right; they are reported in Appendix A.

Subjects

The subjects were 30 naive male Long-Evans Hooded rats obtained from Canadian Research Animal Farms, Bradford, Ontario. The animals were received three weeks prior to the beginning of the experiment and during this time they were housed in pairs in standard 10 in. by 7 in. by 7 in. stainless steel cages. They were maintained on ad lib. food and water, and on a 24 hour light-dark cycle such that the period from 10 a.m to 10 p.m was the dark phase. Evidence presented by Marler and Hamilton (1966) indicates that three weeks was ample time for the animals to become accustomed to this light-dark cycle. All Ss were between 100 and 120 days of age on the day of testing, and were randomly assigned to the treatment groups.

The design of this study called for the testing of 30 subjects. The Experimenter found it necessary to prepare a total of 40 animals with the telemetry transmitter packet (described below). Four animals were discarded because they had destroyed their packets during the interval between preparation and testing. Two subjects were discarded because of apparatus failures during the test, and data from four other animals could not be used because the heart rate records obtained were not scoreable.

Apparatus

The test apparatus was a standard Open Field (Broadhurst, 1960). The Open Field consists of a white circular arena, 32 3/4 inches in diameter and 12 1/2 inches in height. Starched muslin, mounted on a steel superstructure, provided a fairly efficient one-way vision screen into the Open Field arena. Two 15 watt lamps, mounted symetrically over the arena, provided a uniform 3 ft.c. illumination in the arena when the experimental room was otherwise darkened. Five 5-inch speakers and five 375 watt photoflood lamps (Sylvania, R-30, Type EBR) were mounted in a circular array 60 inches above the arena floor. The lamps and speakers were mounted on a sheet of plywood such that the position of lamps and speakers alternated on the circumference of a 21 inch circle. The plywood sheet was then centered over the Open Field arena. This positioning

of the lamps and speakers provided fairly uniform (variation < 10%) sound and illumination levels in the arena at all intensities. Rheostat devices enabled the Experimenter to vary the intensity levels of the sound and illumination stimulation continuously, and independently. A diagram of the Open Field test apparatus is presented in Appendix B.

The lamps were found to produce a considerable amount of heat, as well as illumination. Measures indicated that the temperature in the Open Field arena would rise until the heat output from the lamps reached an equilibrium with the cooling capacity of the air conditioning system. The amount of temperature change, and the equilibrium temperature itself, depended on the starting temperature of the room and the intensity setting of the lamps. The equilibrium temperature for the highest illumination setting used (1000 ft.c.) was 77°- 79°F. Since the design of the study called for the collection of data at several intensities, and since attempts to reduce the heat output of the lamps were unsuccessful, it was decided to collect all data at the equilibrium temperature of the most intense illumination setting to be used. A 1,300 watt electric range element, mounted in an aluminium bowl eight inches above the starched muslin, provided an alternative source of radiant heat energy. This device enabled the Experimenter to compensate for the reduction of heat output when the photoflood lamps were off, or operating at reduced intensity.

Appropriate settings were found on the switch controlling the range element to enable the Experimenter to maintain relative temperature stability in the Open Field. When the experimental design called for a change in the stimulus conditions the heat output of the range element was adjusted appropriately.

Temperature was measured with a mercury bulb thermometer mounted on a wooden block such that the mercury bulb was suspended one-half inch above the floor's surface. White noise stimulation was provided by a Lehigh Valley model 1524 white noise generator. The intensity of the white noise was measured by a Scott model 412 sound level meter. Illumination intensity was measured at floor level by a Macbeth model 6800 illuminometer.

The Open Field arena rests directly on the terazzo floor of the experimental room. A grid pattern drawn on the floor, within the arena, permits ambulation to be measured by counting the number of grid lines crossed by the animal. The grid pattern is shown in Appendix C. Multiplication by a suitable constant converts these scores into distance ambulated in conventional units of measure (Broadhurst, 1960). In the present experiment the raw ambulation scores were multiplied by 8.6 and thereby converted to inches of ambulation.

An Easterline-Angus event recorder was used to measure ambulation and grooming behaviour. The heart rate

measure was obtained through the use of biometric telemetry apparatus. A tiny transmitter, and HR electrodes, mounted on the subject's body relays the HR signal to a polygraph recorder. The rat is not bound by any tether and may move about with complete freedom. The apparatus used was an E&M model FM-1100-E3 telemetry transmitter, an E&M model FM-1100-6 receiver, and an E&M model DMB Four-A physiograph.

The collection of the HR data involved mounting a telemetry transmitter on the animal. This procedure, described below, required the use of a plastic cylinder to house the transmitter. The barrel of a 12 guage plastic shotshell was used for this purpose. Electrode leads were fashioned of copper wire, of the type used in the construction of electric motors. An adhesive known as Skin Bond cement (United Surgical Co., Port Chester, N.Y.) was used in the attachment procedure. Also used in the attachment procedure were ordinary 16 mm wound clips.

A compartment constructed entirely of black opaque plexiglas and measuring 12 in. by 12. by 12 in. was used as a pre-test holding chamber. The compartment had a hinged lid and a two-part hinged floor. This arrangement permitted the Experimenter to remove a subject from the compartment by simply lifting the compartment up and away, thereby depositing the animal on the floor gently and without the necessity of handling. A diagram of the

holding compartment is presented in Appendix D.

Procedure

The following technique was developed to mount the telemetry transmitter on the rat's body. The method consists of attaching a packet to the body which serves as the transmitter housing and HR electrode placement. The transmitter packet consists of a plastic cylinder secured with adhesive tape to a 1/2 inch wide strip of upholstery plastic material. Electrode lead wires are inserted through the base of the cylinder and placed beneath the plastic strip, one to each side. An electrode lead wire is brought up through the tape-plastic strip about 1/2 inch from each end. Finally, a strip of adhesive tape is applied to the undersurface so as to secure the position of the electrode lead wires (See Figure 1, page 19). The electrode lead wires in the cylinder terminate as transmitter input jacks. When the animal is not being tested these may be folded back into the cylinder and the open end stopped with a suitable plug. Each transmitter packet was equipped with a removable weight equal to the weight of the telemetry transmitter. This weight was removed when the transmitter was inserted into the packet for testing.

The transmitter packet was attached to the rat in the following manner. Once anaesthetized (sub-cutaneous injection of Sodium Pentobarbital, 50 mg/kgm body weight) the animal's back was shaved in a two inch wide band for

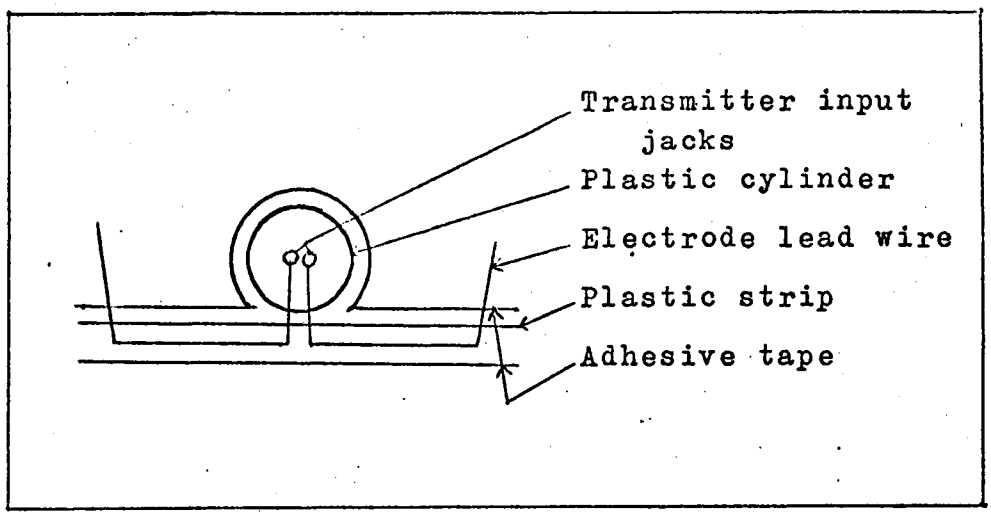


Figure 1. End view of transmitter packet.

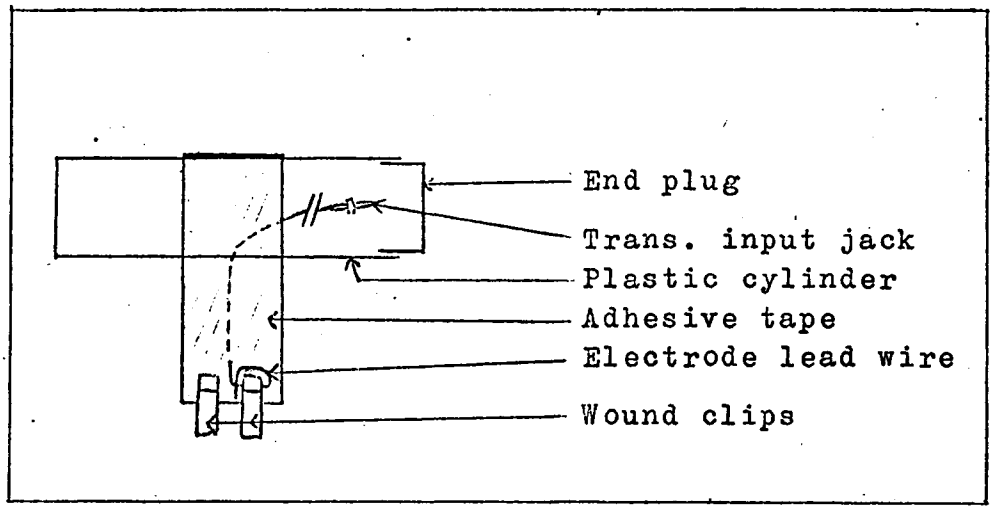


Figure 2. Side view of transmitter packet showing electrode hookup.

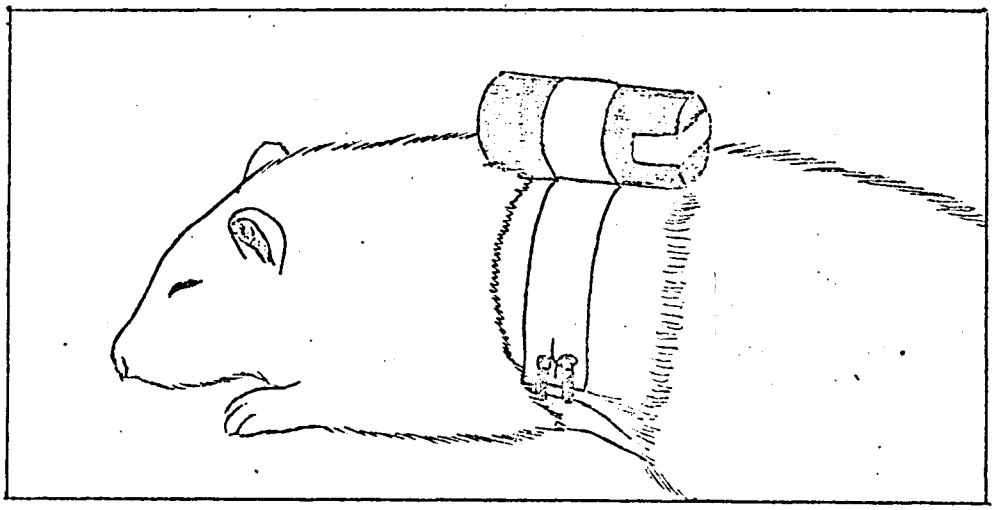


Figure 3. Typical S with transmitter packet mounted.

approximately three inches to either side of the spine in the upper lumbar region. The underside of the tape strip of the transmitter packet was coated with Skin Bond cement, and placed on the body with the cylinder directly over the spine. Each end of the tape strip was clipped to a fold of skin with a pair of 16 mm wound clips. On each side of the body the free end of the electrode lead wire was lead into and around the eyelet of a wound clip (See Figures 2 and 3, page 19). The wound clips served the dual purpose of securing the transmitter packet to the animal and of being the heart rate electrodes. Finally, a coat of Skin Bond cement was applied over all points of animal-packet contact. The cement seemed to inhibit the tendency to chew at the packet, and may also have provided the exposed HR electrodes with some insulation.

Immediately upon being fitted with a transmitter packet, the animals were returned to the colony room and placed in clean individual cages. Each subject was tested from 50 to 58 hours after preparation, by which time the effects of the anaesthetic had worn off (Gay, 1965; Kirby, 1963).

Each animal was observed in the Open Field for 30 consecutive minutes, divided into two 15 minute periods. There were two treatment conditions. The SFN groups (Stimulus OFF-ON) were exposed to a quiet, dimly illuminated (3 ft.c.) Open Field for the first 15 minutes, and to

white noise and bright illumination stimulation during the second 15 minutes. The SNF groups (Stimulus ON-OFF) were bombarded with white noise and bright illumination during the first 15 minutes, followed by quiet and dim illumination during the remaining 15 minutes. This design was chosen because it incorporated the four possible combinations of novelty and fearfulness, i.e., novel and fearful, novel and non-fearful, non-novel and fearful, and non-novel and non-fearful. During the first 15 minutes the SFN subjects were exposed to a novel, but relatively non-stressful situation. During the second 15 minutes the stressful, and presumably fear inducing, stimuli were present but by this time the Open Field environment was no longer a novel situation. The SNF subjects receive both novel and stressful stimulation during the first 15 minutes but are in a relatively non-novel and non-stressful situation for the second 15 minutes.

Data were collected under both treatment conditions (SFN and SNF) at three levels of stimulation intensity: LOW (60 db white noise and 100 ft.c. illumination); MEDIUM (75 db white noise and 550 ft.c. illumination); and HIGH (90 db white noise and 1000 ft.c. illumination). The entire compliment of treatment groups, each having five members, is presented below in Table 1.

The test procedure was as follows. The Open Field

laboratory was placed in the Stimulus-OFF condition (quiet and dark, except for 3 ft.c. illumination in the arena), and the electric range element above the arena was turned on to an intensity which produced a heat output equivalent to that given off by the lamps at 1000 ft.c. intensity. The plexiglas holding compartment was placed on the floor in the center of the Open Field arena. Each subject was then wheeled, home cage and all, into the recording laboratory adjacent to the Open Field room. The Experimenter inserted the telemetry transmitter into the S's packet, then carried the rat into the Open Field room and placed it into the pre-test compartment.

Table 1

Treatment Groups

Group Designation	First 15 Minutes		Second 15 Minutes	
	ft.c.	db	ft.c.	db
L-SFN	3	none	100	60
L-SNF	100	60	3	none
M-SFN	3	none	550	75
M-SNF	550	75	3	none
H-SFN	3	none	1000	90
H-SNF	1000	90	3	none

The recording of the S's heart rate began 90 minutes after placement in the pre-test chamber. Pilot study data, and data reported by Black, Fowler, and Kimbrell (1964)

indicated that the heart rate response of a rat to a handling experience, which takes the form of acceleration, has almost completely recovered by 90 minutes. The recording of heart rate, once initiated, continued uninterrupted throughout the test session.

Five minutes after the initiation of heart rate recording, the Experimenter entered the Open Field room and lifted the holding chamber out of the arena, thereby depositing the subject in the center of the Open Field arena floor. If any fecal boluses, deposited during the pre-test interval, dropped out of the holding compartment they were immediately removed from the arena with the aid of a plastic fork. This was necessary on only three occasions for one animal in each of three separate treatment groups. The starched muslin curtain, which had been folded back to allow access to the Open Field so that the Experimenter could remove the pre-test compartment, was lowered and the recording of grooming and ambulation began immediately.

Grooming and ambulation were both recorded by means of a hand switch connected to an Easterline-Angus event recorder located in the recording laboratory adjacent to the experimental room. Grooming was defined as the duration of activities wherein licking responses were directed at any part of the body. A pen of the recorder was deflected while this behaviour was in progress, and a measure of grooming in seconds was obtained. For the ambulation

measure, a pen of the recorder was briefly deflected each time the animal crossed a line of the grid pattern on the floor of the Open Field.

If the animal was a member of the SNF groups, the white noise and illumination stimuli were turned on simultaneously with the release of the S into the arena, at which time the heat output of the electric range element was reduced or turned off, depending on the intensity of the illumination for that subject. The intense stimuli were turned off (and range element turned on) at the end of 15 minutes, or vice-versa, as called for by the experimental design. At the end of 30 minutes the Experimenter turned off all stimuli (and range element if on), removed the animal from the Open Field and placed it into a cage in the recording laboratory. The Experimenter then recorded the temperature in the Open Field (this was also done just prior to each S's release into the arena), noted the number of fecal boluses that had been deposited during the test, cleaned the arena floor with damp paper towels and replaced the pre-test compartment in the center of the arena. The experimenter then recovered the telemetry transmitter and returned the S to the colony room. The Open Field room was allowed to return to its original starting temperature, which required from 45 to 60 minutes, and then the procedure was begun again with another animal.

All data were collected in a twelve day period between the hours of 12:00 noon and 8:00 p.m., during which time up to three subjects could be processed. On any given day, data were collected at one level of white noise and illumination intensity, i.e., LOW, MEDIUM, or HIGH. The Macbeth illuminometer and Scott sound level meter were used to reset the level of the white noise and illumination stimuli before each day's testing. Data were collected for one day at each stimulus level in turn, until the experiment was complete. Within each day's testing the order of treatment (SFN and SNF) was alternated. The testing order, and times at which data were collected, are presented in Appendix E.

CHAPTER III

RESULTS

In addition to the main measures taken in this experiment, those of grooming, heart rate, and ambulation, a number of subsidiary measures were taken upon which data analyses were performed. These subsidiary measures, subjects' weights, Open Field temperature, and time of testing, represent factors which may potentially confound the main data if not controlled. Thus, in order to aid interpretation of the main findings, these subsidiary data will be presented first in this section.

Subsidiary Measures

Subjects' Weights

Each animal was weighed on the day that it was prepared for the experiment, i.e., had a telemetry transmitter packet attached to its back. Since the weight of the packet and transmitter (about 11 gm) would be more easily borne by larger animals, it is necessary to establish whether the treatment groups differed in this respect. A summary of the analysis of variance of the subjects' weights

is presented below in Table 2.

Table 2

Analysis of Variance of Subjects' Weights by Treatment Group (N=30)

Source of Variation	SS	df	MS	F
Treatment groups	5,215.8	5	1,043.2	1.01*
Error	24,714.4	24	1,029.8	

*p >.05

Table 2 shows that there were no reliable differences in subjects' weights between the various treatment groups ($F=1.01$, $p > .05$).

Open Field Temperature

The Experimenter attempted to maintain a constant temperature in the Open Field arena throughout the observations. The temperature in the Open Field was recorded immediately before each animal was released into the arena and immediately upon termination of the 30 minute test session. The direction of temperature change from start to finish of each test was always that of an increase, but in no instance did the amount of temperature increase exceed three Fahrenheit degrees. The overall mean start temperature was 76.0°F, and the mean finish temperature was 77.6°F. A summary of the analysis of variance of the starting temperatures in the Open Field arena is presented in

Table 3, below.

Table 3

Analysis of Variance of Start Temperature in the Open Field by Treatment Group (N=30)

Source of Variation	SS	df	MS	F
Treatment groups	8.2	5	1.64	0.56*
Error	70.8	24	2.95	

*p >.05

Table 3 shows that there were no reliable differences in the starting temperature of the Open Field between the treatment groups (F=0.56, p >.05).

Time of Testing

Since the range of times at which animals were tested spanned several hours (from 12:00 noon until 8:00 p.m.), it was decided to examine these data for possible biases in time of testing. The time at which each S was tested was recorded in military time; a summary of the analysis of variance of the time of testing is presented below in Table 4. The data in Table 4 show that there were no differences in time of testing between the treatment groups (F=1.66, p >.05).

Table 4

Analysis of Varinace of Time of Testing by Treatment Group (N=30)

Source of Variation	SS	df	MS	F
Treatment groups	471,414.17	5	94,282.83	1.66*
Error	1,365,090.00	24	56,878.75	

*p >.05

Main Measures

The purpose of this study was to examine the relationship between the amount of fear experienced by the rats, as measured by relative heart rate decrement, and the amount of grooming behaviour produced, in stressful situations. Although the main dependent variable was grooming, it is of prime importance in interpreting these data to establish whether the animals did indeed experience different amounts of fear in the various test conditions. Therefore, the heart rate data will be presented first.

Heart Rate

The heart rate of each animal was recorded during the five minutes immediately preceeding the test, these being the last five minutes of the pre-test period. This measure was taken so that it could be determined whether any differences in HR existed between the treatment groups

at the beginning of the test session. The mean heart rates of the treatment groups, in beats per minute, during the pre-test period and during the first and second 15 minutes of the test are presented in Table 5, below.

Table 5

Mean Heart Rate, by Treatment Group, by Measurement Period (N=30)

Treatment Group	Pre-test Period	1st 15 Min.	2nd 15 Min.
L-SFN	366	438	398
L-SNF	388	422	388
M-SFN	369	429	388
M-SNF	365	409	405
H-SFN	376	430	381
H-SNF	377	386	380

A summary of the analysis of variance of the pre-test heart rate means is presented below in Table 6.

Table 6

Analysis of Variance of Pre-test Heart Rate Means by Treatment Group (N=30)

Source of Variation	SS	df	MS	F
Treatment groups	1,801.8	5	360.4	0.16*
Error	53,872.6	24	2,244.7	

*p >.05

Table 6 shows that there were no reliable differences in

pre-test heart rates between the treatment groups ($F=0.16$, $p > .05$).

The data in Table 5 indicate that all subject groups showed some increment in mean heart rate during the first 15 minutes of the test, relative to pre-test rates. A repeated measures analysis of variance of the mean HR scores during the pre-test period, and 1st 15 minutes of the test, is summarized below in Table 7. In this kind of analysis comparisons are made between the results yielded by different groups of subjects (between Ss variation), and between scores yielded by the same subjects at different times (within Ss variation).

Table 7

Analysis of Variance of Heart Rate Mean Scores during Pre-test Period and First Fifteen Minutes of Test by Treatment Group (N=30)

Source of Variation	SS	df	MS	F
Between Subjects				
Treatment groups(T)	4,645.68	5	929.14	0.41
Error between Ss	54,733.80	24	2,280.58	
Within Subjects				
Measurement period(M)	31,236.01	1	31,236.01	14.73*
TxM	6,039.29	5	1,207.86	0.57
Error within Ss	50,888.20	24	2,120.34	

*$p < .05$

The data in Table 7 show that there were no overall differences in heart rate between the treatment groups (Fac-

tor T, F=0.41, p >.05). It can also be seen from Table 7 that a highly reliable overall difference existed between heart rates during the pre-test period, and during the first 15 minutes of the test (Factor M, F=14.73, p <.05). Table 7 also shows that the treatment group by measurement period interaction was not significant (Factor TxM, F=0.57, p >.05). Although this small interaction might at first seem to imply that there were no group differences in HR increment, inspection of the mean scores (See Table 5, page 30) reveals that there were in fact large differences. It was therefore decided to perform an analysis of variance for simple effects on the measurement period factor (Winer, 1962, p. 237); this analysis is summarized in Table 8, below.

Table 8

Analysis of Variance for Simple Effects on HR Scores during Pre-test Period and First Fifteen Minutes of Test by Treatment Group (N=30)

Source of Variation	SS	df	MS	F
L-SFN	12,888.10	1	12,888.10	6.08*
L-SNF	2,992.90	1	2,992.90	1.41
M-SFN	9,120.40	1	9,120.40	4.30*
M-SNF	4,708.90	1	4,708.90	2.22
H-SFN	7,344.10	1	7,344.10	3.46**
H-SNF	220.90	1	220.90	0.10
Error within Ss	50,888.20	24	2,120.34	

*p <.05, **p <.10

The data in Tables 5 and 8 indicate that all of the groups for which the illumination and white noise stimuli were not present during the first half of the test session tended to increase their heart rates during this period, as compared with pre-test rates. None of the groups which were exposed to the light and sound stimulation during the first 15 minutes showed a significant HR increment.

The design of this study calls for the data of the first and second 15 minutes of the test to be analysed by a latin square analysis of variance. The computational procedures appropriate for the present data are given by Winer, 1962, pages 554-562. A summary of the latin square analysis of variance of the heart rate mean scores during the first and second 15 minutes of the test session is presented below in Table 9. It can be seen from Table 9 that no reliable differences in heart rate were attributable to the levels of intensity of the white noise and illumination stimuli (Factor I, $F=0.67$, $p>.05$). There was a highly reliable difference in mean heart rates between the first and second 15 minute periods (Factor M, $F=18.97$, $p<.05$). Examination of the data in Table 5 shows that heart rates were lower during the second 15 min. The measurement period by stimulus intensity interaction was found to be non-significant (Factor MxI, $F=0.41$, $p>.05$). It can be seen from Table 9 that a reliable overall difference in heart rate occurred between periods when the

light and sound stimuli were present and when they were absent (Factor P, F=4.68, p <.05). Examination of the data in Table 5 indicates that heart rates were lower when the white noise and illumination stimuli were present. Table 9 also shows that there was no interaction between the presence of the stressful stimuli and the level of magnitude of the stimuli (Factor PxI, F=0.74, p >.05), indicating that the LOW, MEDIUM, and HIGH levels of the intense stimulation were equally effective in producing heart rate decrement.

Table 9

Latin Square Analysis of Variance of Mean Heart Rate Scores by Measurement Period by Treatment Group (N=30)

Source of Variation	SS	df	MS	F
Between Subjects				
Stimulus intensity(I)	3,403.4	2	1,701.7	0.67
MxP between Ss	2,232.6	1	2,232.6	0.88
MxPxI between Ss	1,062.1	2	531.1	0.21
Error between Ss	60,635.6	24	2,526.4	
Within Subjects				
Measurement period(M)	12,731.2	1	12,731.2	18.97*
Stimulus presence(P)	3,139.2	1	3,139.2	4.68*
MxI	548.3	2	274.2	0.41
PxI	998.3	2	499.2	0.74
MxP within Ss	**	0		
MxPxI within Ss	**	0		
Error within Ss	16,108.0	24	671.2	

*p <.05

**The overall MxP and MxPxI interactions are partially confounded in this analysis. Specifically, information on the within subjects component of these interactions is unavailable, see text below.

A summary of the analysis of variance for simple effects on the measurement period factor (M) is presented in Table 10, below.

Table 10

Analysis of Variance for Simple Effects on HR Scores during First and Second Fifteen Minute Periods of the Test by Treatment Group (N=30)

Source of Variation	SS	df	MS	F
L-SFN	4,000.00	1	4,000.00	5.95*
L-SNF	2,890.90	1	2,890.90	4.30*
M-SFN	4,284.90	1	4,284.90	6.38*
M-SNF	25.60	1	25.60	0.04
H-SFN	6,100.90	1	6,100.90	9.09*
H-SNF	115.60	1	115.60	0.17
Error within Ss	16,108.00	24	671.20	

*p <.05

The data presented in Table 10 show that the L-SFN, L-SNF, M-SFN, and H-SFN subject groups experienced reliable reductions in heart rate from the first to the second 15 minutes of the test (Fs = 5.95, 4.30, 6.38, 9.09, p <.05). The M-SNF and H-SNF groups did not show reliable HR reduction. In summary, this analysis indicates that in all three groups where the stimuli were present during the second 15 minutes of the test there was a significant HR reduction during this period. For the three groups where the intense stimuli were removed during the second 15 min., only one showed a significant decrement; and this the group

previously exposed to the LOW stimulus intensity.

As was noted above in Table 9, the latin square analysis partly confounds the measurement period by stimulus presence (MxP), and the measurement period by stimulus presence by stimulus intensity (MxPxI) interactions. The manner in which this confounding occurs is detailed by Winer, 1962, p. 555. The between subjects component of these interactions was available, however, and partial information may be obtained using a pooled error term (sum of squares for error within plus sum of squares for error between divided by df for error within plus df for error between). The partial information available on the MxP and MxPxI interactions for heart rate is presented below in Table 11.

Table 11

Partial Information on MxP and MxPxI Interactions for Heart Rate (N=30)

Source of Variation	SS	df	MS	F
MxP between Ss	2,232.6	1	2,232.6	1.40*
MxPxI between Ss	1,062.1	2	531.1	0.33**
Error (pooled)	76,743.6	48	1,598.8	

*p >.10; critical value (p <.05) = 4.04
**p >.25; critical value (p <.05) = 3.19

It can be seen from Table 11 that the between subjects component of the overall measurement period by stimulus presence interaction was of moderate size (Factor MxP, F=1.40).

Were full information available, the measurement period by stimulus presence interaction may have been shown to have been reliable. Table 11 also shows that the between Ss component of the measurement period by stimulus presence by stimulus intensity interaction was quite small (Factor MxPxI, F=0.33). Were full information available on this factor, it is very unlikely that the interaction would have been reliable.

Figure 4, page 37, shows the mean heart rate, in beats per minute, of all SFN and SNF animals in successive three-minute blocks of the test session. It can be seen from Figure 4, and Table 5 above, that the heart rates of all treatment groups during the second 15 minutes were similar to their pre-test rates. A correlated samples t-test comparing each S's mean pre-test HR with its mean HR during the second 15 minutes showed that no difference existed ($t=1.91$, $p > .05$).

A record was kept of the total number of fecal boluses deposited by each rat during the 30 minute test session. This measure is used by some investigators as an index of the rat's level of fearfulness. A summary of the analysis of variance of the number of fecal boluses deposited is presented in Table 12, below. Table 12 shows that the treatment groups did not differ in the number of fecal boluses deposited during test ($F=1.06$, $p > .05$). The overall mean number of boluses deposited was 5.2 per rat.

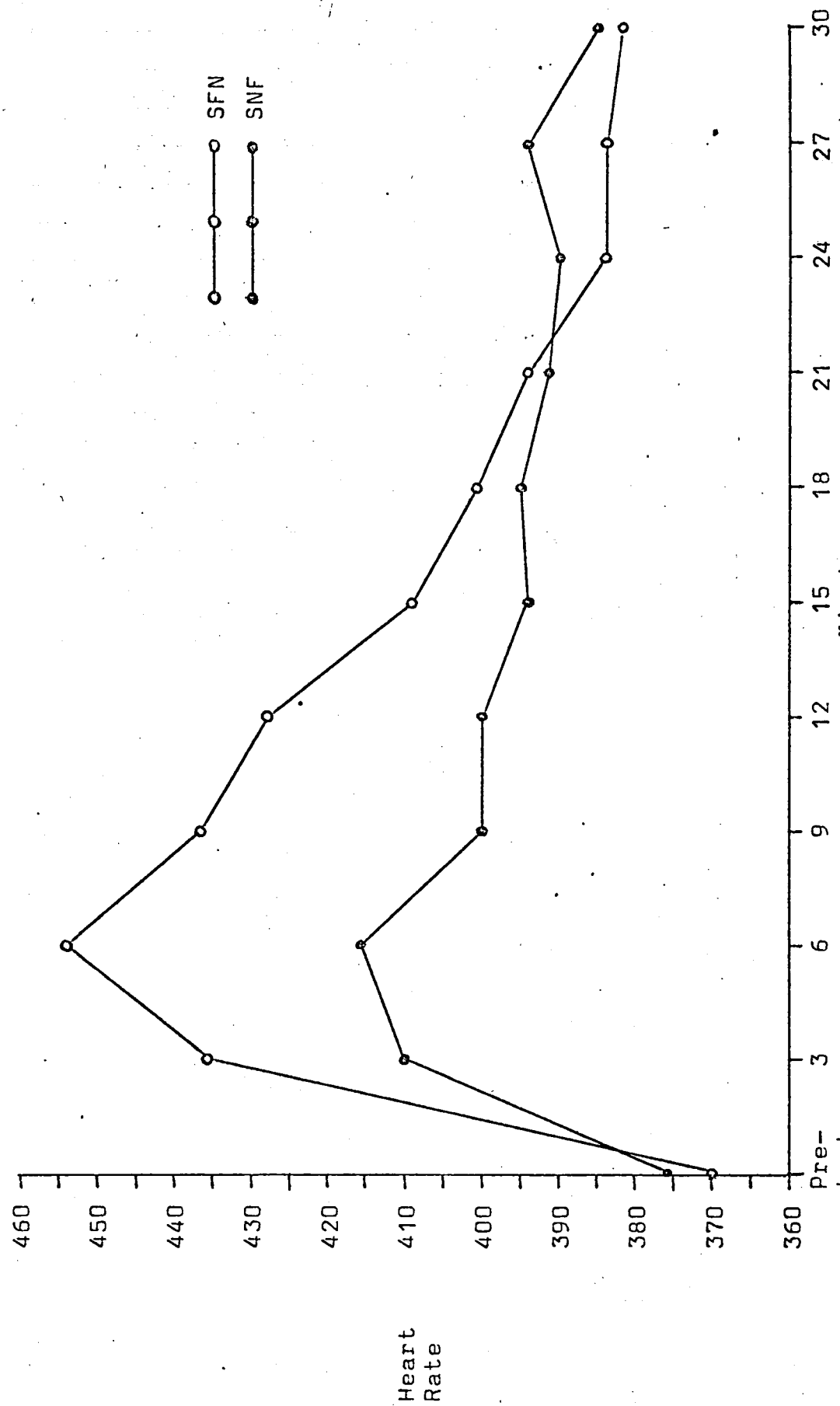


Figure 4. Mean Heart Rate in Beats per Minute of SFN and SNF Subjects in Successive Three-minute Blocks of the Test.

Table 12

Analysis of Variance of Number of Fecal Boluses Deposited during Test by Treatment Group (N=30)

Source of Variation	SS	df	MS	F
Treatment groups	25.00	5	5.00	1.06*
Error	113.20	24	4.71	

*p >.05

Grooming

The mean amount of grooming, in seconds, performed by each treatment group during the first and second 15 minutes of the test is presented in Table 13, below.

Table 13

Mean Seconds of Grooming, by Treatment Group, by Measurement Period (N=30)

Treatment Group	First 15 Min.	Second 15 Min.
L-SFN	78.2	122.8
L-SNF	101.4	85.2
M-SFN	44.2	99.6
M-SNF	61.8	141.8
H-SFN	113.4	138.2
H-SNF	51.8	62.2

The raw data of all of the main measures taken in this study were tested for homogeneity of variance using the F_{max} statistic (Winer, 1962). The grooming raw data

were found to not satisfy the assumptions underlying the analysis of variance (F_{max} = 92.77, $p < .05$). Therefore, the raw data were transformed using the transformation $\sqrt{x} + \sqrt{x+1}$ (Winer, 1962, p. 220). Unless otherwise noted, all subsequent presentation of grooming behaviour data refers to transformed scores.

A summary of the latin square analysis of variance of the total amount of grooming produced by the subjects during the first and second 15 minutes of the test is presented below in Table 14.

Table 14

Latin Square Analysis of Varinace of Seconds of Grooming ($\sqrt{x} + \sqrt{x+1}$) by Measurement Period by Treatment Group (N=30)

Source of Variation	SS	df	MS	F
Between Subjects				
Stimulus intensity(I)	184.07	2	92.04	0.10
MxP between Ss	323.50	1	323.50	0.34
MxPxI between Ss	1,985.67	2	992.84	1.05
Error between Ss	22,676.03	24	944.83	
Within Subjects				
Measurement period(M)	1,060.92	1	1,060.92	1.21
Stimulus presence(P)	40.44	1	40.44	0.05
MxI	1,326.18	2	663.09	0.75
PxI	748.72	2	374.36	0.43
MxP within Ss	**	0		
MxPxI within Ss	**	0		
Error within Ss	21,082.10	24	878.42	

**See footnote to Table 9, page 33, and text, page 35.

It can be seen from Table 14 that no differences in amount

of grooming were attributable to the effects of: measurement period (Factor M, F=1.21, p >.05); stimulus presence (Factor P, F=0.05, p >.05); or stimulus intensity (Factor I, F=0.10, p >.05). Similarly, neither the measurement period by stimulus intensity nor the stimulus presence by stimulus intensity interactions were reliable (Fs = 0.75, 0.43, p >.05). The partial information available on the MxP and MxPxI interactions is presented below in Table 15.

Table 15

Partial Information on MxP and MxPxI Interactions for Grooming (N=30)

Source of Variation	SS	df	MS	F
MxP between Ss	323.50	1	323.50	0.35*
MxPxI between Ss	1,985.67	2	992.84	1.10**
Error (pooled)	43,758.13	48	911.63	

*p >.25, critical value (p <.05) = 4.04
**p >.25, critical value (p <.05) = 3.19

Table 15 shows that the between subjects component of the measurement period by stimulus presence interaction was quite small (Factor MxP, F=0.35) indicating that the overall MxP interaction was probably not significant. The between subjects component of the MxPxI interaction was of moderate size (F=1.10), indicating that the overall measurement period by stimulus presence by stimulus intensity interaction may have been significant. Figure 5, page 41,

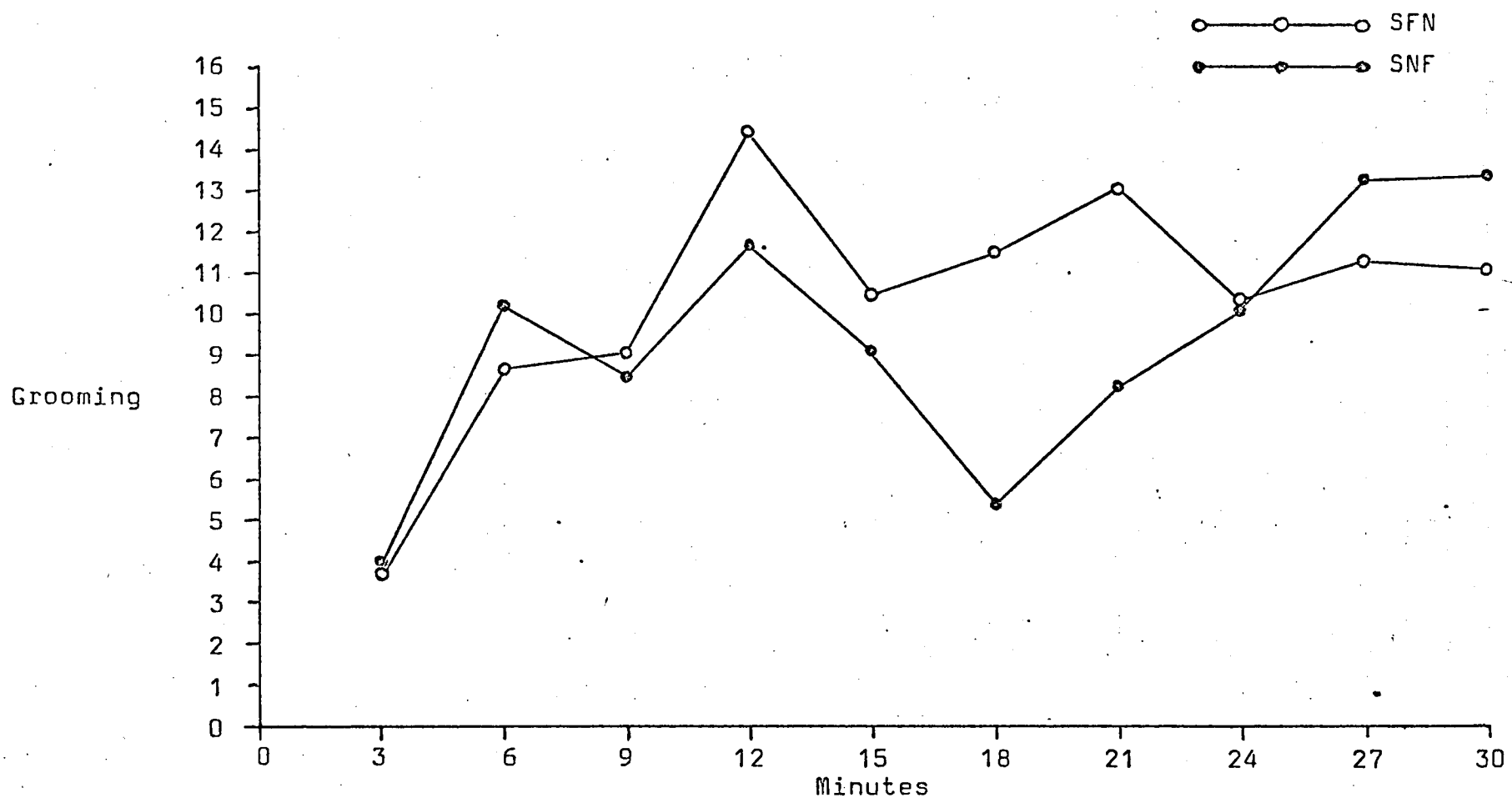


Figure 5. Mean Seconds of Grooming ($\sqrt{x} + \sqrt{x+1}$) of SFN and SNF Subjects in Successive Three-minute Blocks of the Test.

shows the mean amount of grooming ($\sqrt{x} + \sqrt{x+1}$) of SFN and SNF animals during successive three-minute blocks of the test. Figure 6, page 43, shows the mean amount of grooming ($\sqrt{x} + \sqrt{x+1}$) of each treatment group during the first and second 15 minutes of the test.

During a period of 15 minutes it is seldom the case that a rat grooms on only one occasion. A more typical observation is that the total amount of grooming will have occurred in several distinct grooming sessions, or episodes. Although no overall differences in amount of grooming existed, it was decided to examine the number of grooming episodes performed by each rat during the first and second 15 minutes of the test in order to assess the possibility that the subjects may have distributed their grooming differently under the various test conditions. A grooming episode was defined as a sequence of grooming responses unbroken by ambulation or by one full minute of non-grooming. The total number of grooming episodes performed by the treatment groups during the first and second halves of the test are presented below in Table 16.

A summary of the latin square analysis of variance of the number of grooming episodes is presented in Table 17, below. The data in Table 17 show that no differences in number of grooming episodes were attributable to the effects of: measurement period (Factor M, $F=3.00$, $p > .05$);

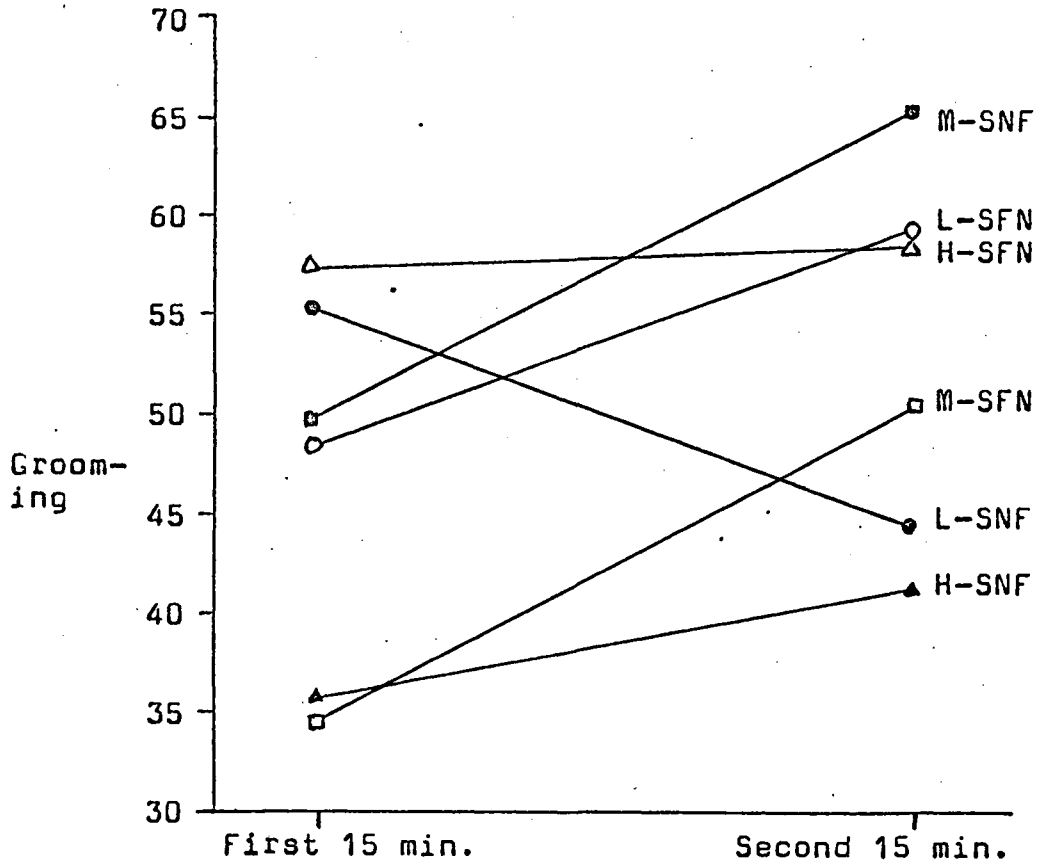


Figure 6. Mean Seconds of Grooming ($\sqrt{x} + \sqrt{x+1}$) by Measurement Period by Treatment Group.

Table 16

Number of Grooming Episodes, by Treatment Group, by Measurement Period (N=30)

Treatment Group	First 15 Min.	Second 15 Min.
L-SFN	24	20
L-SNF	27	9
M-SFN	12	16
M-SNF	13	26
H-SFN	24	10
H-SNF	14	19

Table 17

Latin Square Analysis of Variance of Total Number of Grooming Episodes by Measurement Period by Treatment Group (N=30)

Source of Variation	SS	df	MS	F
Between Subjects				
Stimulus intensity(I)	5.6	2	2.8	0.31
MxP between Ss	0.0	1	0.0	0.00
MxPxI between Ss	9.3	2	4.7	0.52
Error between Ss	216.8	24	9.0	
Within Subjects				
Measurement period(M)	13.2	1	13.2	3.00
Stimulus presence(P)	13.2	1	13.2	3.00
MxI	29.5	2	14.8	3.36
PxI	18.7	2	9.4	2.14
MxP within Ss	**	0		
MxPxI within Ss	**	0		
Error within Ss	106.4	24	4.4	

**See footnote to Table 9, page 33, and text, page 35.

stimulus presence (Factor P, F=3.00, p >.05); or stimulus intensity (Factor I, F=0.31, p >.05). Similarly, neither the MxI nor PxI interactions were significant (Fs = 3.36, 2.14, p >.05). The partial information available on the MxP and MxPxI interactions is presented below in Table 18.

Table 18

Partial Information on MxP and MxPxI Interactions for Number of Grooming Episodes (N=30)

Source of Variation	SS	df	MS	F
MxP between Ss	0.00	1	0.00	0.00*
MxPxI between Ss	9.30	2	4.65	0.69**
Error (pooled)	323.20	48	6.73	

*p >.25, critical value (p <.05) = 4.04
**p >.25, critical value (p <.05) = 3.19

The data in Table 18 show that the between subjects components of the measurement period by stimulus presence, and the measurement period by stimulus presence by stimulus intensity interactions were very small (Fs = 0.00, 0.69), indicating that these overall interactions were almost certainly not reliable.

To summarize, no grooming differences were observed, either in terms of amount of grooming, or distribution in time, between the treatment groups under the various test conditions.

Ambulation

The mean amount of ambulation, in inches, shown by each of the treatment groups during the first and second 15 minutes of the test is presented in Table 19, below.

Table 19

Mean Inches of Ambulation, by Treatment Group, by Measurement Period (N=30)

Treatment Group	First 15 Min.	Second 15 Min.
L-SFN	958.04	466.12
L-SNF	602.00	89.44
M-SFN	741.32	223.60
M-SNF	526.32	362.92
H-SFN	890.96	495.36
H-SNF	517.72	113.40

A summary of the latin square analysis of variance of the amount of ambulation shown by the treatment groups during the first and second halves of the test is presented below in Table 20. Table 20 shows that there was a highly reliable difference in the amount of ambulation between the first and second 15 minutes (Factor M, $F=34.87$, $p < .05$). Examination of the data in Table 19 indicates that all groups ambulated less during the second half of the test. The data in Table 20 indicate that no differences in amount of ambulation were attributable to the effects of: stimulus intensity (Factor I, $F=0.16$, $p > .05$); stimulus presence (Factor P, $F=0.66$, $p > .05$); the measurement period by sti-

mulus intensity interaction (Factor MxI, F=0.45, p >.05); or the stimulus presence by stimulus intensity interaction (Factor PxI, F=0.74, p >.05).

Table 20

Latin Square Analysis of Variance of Ambulation in Inches by Measurement Period by Treatment Group (N=30)

Source of Variation	SS	df	MS	F
Between Subjects				
Stimulus intensity(I)	589.8	2	589.8	0.16
MxP between Ss	16.0	1	16.0	0.01
MxPxI between Ss	18,790.5	2	9,395.3	5.06*
Error between Ss	44,545.2	24	1,856.1	
Within Subjects				
Measurement period(M)	34,800.4	1	34,800.4	34.87*
Stimulus presence(P)	660.0	1	660.0	0.66
MxI	904.3	2	452.2	0.45
PxI	1,470.1	2	735.1	0.74
MxP within Ss	**	0		
MxPxI within Ss	**	0		
Error within Ss	23,952.4	24	998.0	

*p <.05
**See footnote to Table 9, page 33, and text, page 35.

The mean inches of ambulation of all SFN and SNF animals during successive three-minute blocks of the test are shown in Figure 7, page 48. The partial information available on the measurement period by stimulus presence and the measurement period by stimulus presence by stimulus intensity interactions is presented in Table 21, below. The data in Table 21 show that the between Ss component of the MxP interaction was very small (F=0.01) in-

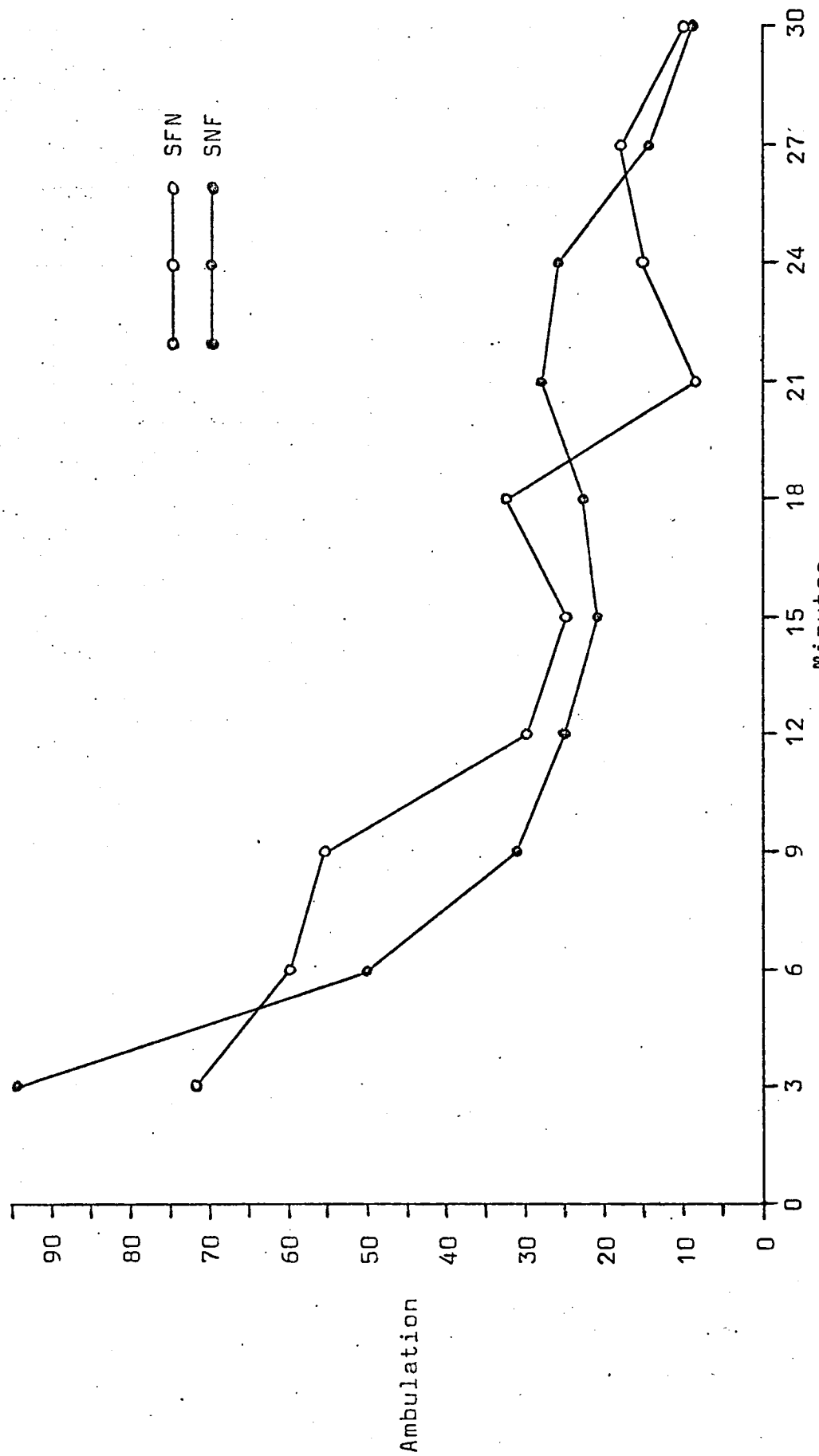


Figure 7. Mean Inches of Ambulation of SFN and SNF Subjects in Successive Three-minute Blocks of the Test.

dicating that, were full information available, this interaction would almost certainly be non-significant.

Table 21

Partial Information on MxP and MxPxI Interactions for Ambulation (N=30)

Source of Variation	SS	df	MS	F
MxP between Ss	16.0	1	16.0	0.01*
MxPxI between Ss	18,790.5	2	9,395.3	6.58**
Error (pooled)	68,497.6	48	1,427.0	

*p >.25, critical value (p <.05) = 4.04
**p <.05

The between Ss component of the MxPxI interaction was reliable (F=6.58, p <.05), indicating that the treatment groups differed in the relative ambulatory decrements shown during the second 15 minutes of the test. The MxPxI interaction is illustrated in Figure 8, page 50, which shows the mean ambulation of each of the treatment groups in the first and second halves of the test. Figure 8 indicates that all treatment groups showed similar reductions in ambulation during the second 15 minutes except the M-SNF group, which showed only a slight reduction.

A summary of the analysis of variance for simple effects on the measurement period factor is presented in Table 22, below. The data in Table 22 show that all subject groups, except the M-SNF group, ambulated reliably

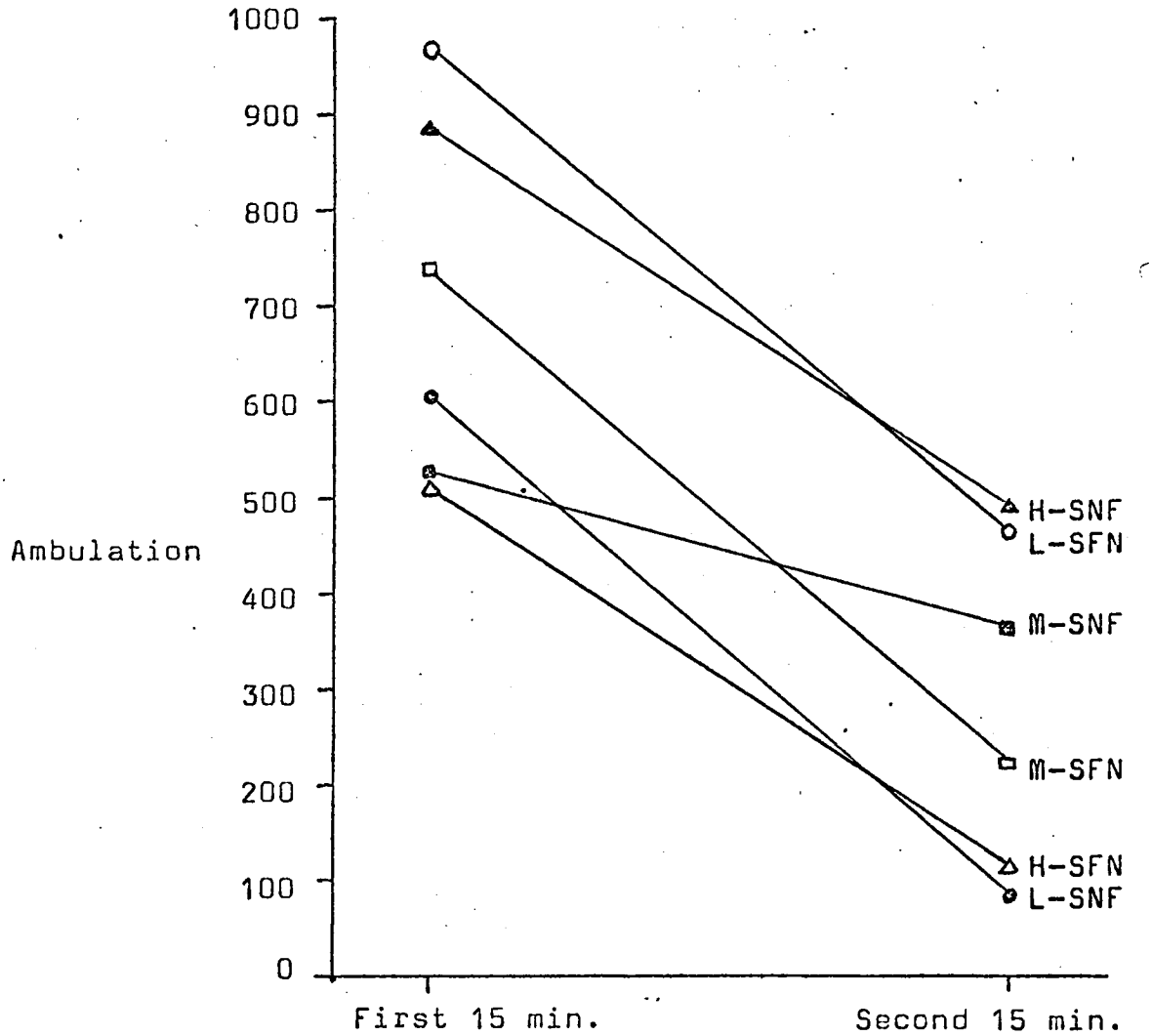


Figure 8. Mean Inches of Ambulation by Measurement Period by Treatment Group.

less during the second than during the first 15 minutes of the test session.

Table 22

Analysis of Variance for Simple Effects on Ambulation Scores during First and Second Fifteen Minutes of Test by Treatmemt Group (N=30)

Source of Variation	SS	df	MS	F
L-SFN	8,179.60	1	8,179.60	8.20*
L-SNF	8,880.40	1	8,880.40	8.90*
M-SFN	9,060.10	1	9,060.10	9.08*
M-SNF	902.50	1	902.50	0.90
H-SFN	5,522.50	1	5,522.50	5.53*
H-SNF	5,290.00	1	5,290.00	5.30*
Error within Ss	23,952.40	24	998.00	

*p <.05

In summary, the treatment groups showed fairly similar ambulatory behaviour. The major tendency was toward decreased ambulation as the test session progressed.

CHAPTER IV

DISCUSSION

As was pointed out earlier, two interpretations have been advanced to explain the occurrance of grooming in novel situations. Since there is evidence that novel stimulation is fear-inducing (Montgomery, 1955), and since the grooming response produces familiar self-stimulation, the rat may groom itself in an attempt to reduce fear or anxiety (Hinde, 1966; Hinde, personal communication). This position relates the occurrance of grooming to the experience of fear, and predicts that relatively more fearful situations will give rise to relatively more grooming.

An alternative explanation, put forth by Bindra and Spinner (1958) and Bindra (1961), is that grooming is entirely a function of novelty and is unrelated to fearfulness. This view states that when the environmental stimuli are novel the animal will not have any directly appropriate responses at its disposal. Its behaviour will therefore be a function of the response heirarchy which it brings into the situation. Under such circumstances grooming is likely to occur because it is a well-practised response in everyday life. This view predicts that situations differing in fearfulness, but identical in novelty, would give rise to stimilar amounts of grooming.

If a relative decrement in heart rate is a valid indicator of fear, (and evidence cited previously supports this view), then it was shown in the present study that rats were more fearful when exposed to the white noise and bright illumination than when observed under relative quiet and dim illumination (See Tables 5, 8, 9, and 10). However, no differences in amount of grooming were observed (See Tables 13 and 14).

The conclusion seems inescapable that, under the conditions of novelty and stress employed in this experiment, fear is not the major cause of grooming. The explanation of this phenomenon would seem to lie elsewhere, probably in the interpretation offered by Bindra. Since this conclusion hinges on the reliability of the measures taken, it is appropriate to examine these measures in greater detail. The remainder of this section is devoted to this exposition.

Main Measures

Heart Rate

A few comments would seem to be in order concerning the method by which HR data were collected. It was decided to use telemetry apparatus as a means of obtaining the heart rate measure because, inasmuch as the subject is not restricted by any tether, this technique seems to provide the least mechanical interference with normal motor behaviour. The principle difficulty associated with the use of telemetry with animals as small as the rat is the problem of

attaching the transmitter to the animal's body. The only published method is that by Longo and Pelligrino (1967). This technique involves securing a transmitter housing to the rat's skull and, subsequently, inserting the transmitter when the animal is to be tested. This attachment method was judged inappropriate for the present study because the entire weight of the transmitter and housing is placed on the subject's head. Hence, as rats normally adopt a rearing position to groom themselves, it was feared that such a positioning of the load might interfere with the rat's ability to adopt a grooming posture. Also, since part of the grooming activity consists of brushing the head with the forepaws, the presence of an object secured to the skull might interfere with this response. It was therefore decided to develop an alternative attachment, which is described above in the Methodology section. The transmitter attachment technique used in this study placed the load of the transmitter and its housing in a more convenient carrying position for the animal, and seemed not to inhibit ambulation or grooming. Pilot study data, which compared the amount of ambulation between transmitter prepared and non-prepared animals, showed that no reliable difference existed.

The method used to attach the telemetry transmitter to the subject in this experiment has two disadvantages

which limits its general use. First, it is suitable only for short-term experiments. Almost all animals prepared with the transmitter packet will make some attempt to remove it from their bodies, and eventually, all will succeed. In the present experiment, four subjects could not be tested because they had destroyed their transmitter packets during the two days which intervened between preparation and testing. Some animals carried their transmitter packets for several weeks without apparent concern; however, the average usable life of the packet was about five days after preparation. The second limitation of this technique concerns the clarity of the heart rate records obtained. If one is interested only in heart rate, as in the present case, then this method of transmitter attachment seems quite adequate, as only four of the 34 animals tested had to be discarded because of unscoreable HR records.

We may now proceed with a discussion of the heart rate data itself. The mean pre-test heart rates of the treatment groups were found to range between 367 and 388 beats per minute. These rates are at the lower end of the range identified by Word, Stern, Sines, and McDonald (1959) as representing resting heart rates in rats. It is evident then that the 90 minute interval between transmitter insertion and the initiation of testing was an adequate time

for the animals to recover from any trauma associated with the handling procedure. Analysis of the pre-test HR scores indicated that no differences existed between the treatment groups.

All treatment groups experienced some increment in mean heart rate during the first 15 minutes of the test, relative to pre-test rates. The heart rate increase from pre-test to first 15 minutes of test was found to be reliable at the .10 level for all three Stimulus OFF-ON groups, and reliable at the .05 level for the L-SFN and M-SFN groups. None of the Stimulus ON-OFF groups showed reliable HR increments during the first 15 minutes. The analysis of variance of the mean heart rate scores during the first and second 15 minutes of the test showed that a reliable overall difference in heart rate existed between Stimulus-ON and Stimulus-OFF periods. Inspection of the data indicated that heart rates were lower during Stimulus-ON periods.

These results are precisely what the literature on rats' heart rate response to novel and stressful situations, and the pilot study data, would lead one to expect. The SFN animals, when introduced into the Open Field in its Stimulus-OFF condition (i.e., quiet and dimly illuminated), responded to this novel but relatively non-stressful stiuation with heart rate acceleration. Candland, Pack, and

Matthews (1967) also report that rats responded to a novel, but non-stressful, situation with HR increment. Data reported by Word, Stern, Sines, and McDonald (1959), Boyles, Black, and Furchtyott (1965), Stern and Word (1961), Holdstock and Schwartzbaum (1965), Wenzell (1961), Snowdon, Bell, and Henderson (1964), and Harrington and Hanlon (1966) all show that stressful situations and stimuli result in heart rate decrements. In the present experiment, those subjects which were exposed to the intense illumination and white noise during the first 15 minutes did not show reliable (at the .10 level) heart rate increments. In the overall comparison between heart rate scores during Stimulus-ON and Stimulus-OFF periods of the test it was shown that heart rates were reliably lower during Stimulus-ON periods. Thus, it is clear that the presence of the intense stimulation depressed the heart rate response relative to Ss not exposed to this stress.

In the pilot study, owing probably to procedural inadequacies, no reliable difference was obtained between heart rates during Stimulus-ON and Stimulus-OFF periods. It was evident from inspection of the data, however, that Ss introduced into the Stimulus-ON condition showed less HR acceleration than those exposed to the Stimulus-OFF condition. It was also noticed that the presentation of the intense sound and illumination stimuli at the end of

15 minutes resulted in an immediate and marked HR decrement. It was concluded that the measure of relative heart rate decrement would probably be a useful measure of fear, given a refinement of the procedure by which Ss were introduced into the Open Field. It is evident that the data of the main experiment support this conclusion.

As in the pilot study, there was found to be a reliable overall decline in mean heart rate from the first to the second 15 minute period of the test. Pilot data led to the expectation that the sudden appearance of intense illumination and white noise stimulation at the end of the first 15 minute period would cause the heart rates of SFN animals to show decrements during the second half of the test. By contrast, it was expected that SNF rats, for which the stressful stimuli were removed during the second 15 minutes, would show relatively little HR decrement. A test for simple effects showed that all Stimulus OFF-ON groups experienced reliable HR decrements during the second 15 minutes, as expected. Also as expected, the H-SNF and M-SNF groups did not show reliable mean HR decrements during the second half of the test; however, the L-SNF group did show significant HR reaction. The M-SNF and L-SNF groups showed similar mean HR increments from pre-test to first 15 minutes of test (44 and 34 beats per min. respectively), but only the L-SNF animals showed heart rate reduction during the

second 15 minute period. Possibly the removal of the 100 ft.c. illumination and 60 db white noise for the L-SNF subjects did not represent as marked an alleviation of the stress of the situation as did the removal of the 550 ft.c. and 75 db for the M-SNF group, and the 1000 ft.c. and 90 db for the H-SNF group, so that the L-SNF rats behaved, in terms of their heart rates, as though they were under continued stress.

The partial information available on the measurement period by stimulus presence interaction indicates that this factor may have been shown to have been reliable, were full information available. Figure 4, page 38, shows that the mean heart rate of SNF animals was considerably lower than that of SFN subjects during the first half of the test; however, the heart rates of the two groups were quite similar during the second 15 minute period. These data indicate that the relatively depressing effects of the stressful stimulation on the HR response were largely confined to the first half of the test. This, in turn, suggests a reliable measurement period by stimulus presence interaction. This interaction was reliable in the pilot study data.

No differences in mean HR were attributable to the factor of the intensity of the stressful stimulation. It would appear that almost any illumination and sound stimulus

input, above the ambient levels to which the animals are accustomed, effectively produces heart rate decrement. The relative HR decrement produced by the 1000 ft.c. and 90 db in the H-SNF group during the first 15 minutes was greater than the decrements produced by the MEDIUM and LOW levels of the intense stimulation; however, no reliable differences in mean HR were attributable to any interactions with the stimulus intensity factor.

The analysis of the number of fecal boluses deposited by each animal during the test showed that no differences existed between the treatment groups. The conditions of observation did not permit the recording of the time of deposition of each bolus, so that a comparison of the defecation scores between Stimulus-ON and Stimulus-OFF periods is impossible. If the defecation score is related to rats' level of fearfulness, these data are in agreement with the heart rate results in that no differences are attributable to the levels of the intense stimulation.

Grooming

As was noted above in the Results, the grooming data were characterized by a high degree of variability. A transformation of the raw scores was required before data analysis could be performed. The analysis of the transformed grooming data showed that no differences in amount of grooming were attributable to the effects of stimulus

presence, stimulus intensity, measurement period, or to any of the first-order interactions. In short, the treatment groups did not differ in the amount of grooming produced during either the first or second half of the test; nor did an overall difference exist between the two halves of the test period.

The partial information available on the measurement period by stimulus presence by stimulus intensity interaction indicates that, were full information available, this interaction may have been shown to have been reliable. Figure 6, page 44, shows that all treatment groups showed an increased amount of grooming during the second 15 min. of the test except the L-SNF group, which exhibited a lesser amount of grooming. The reasons for this opposition to the general trend are not apparent.

An analysis of the number of grooming episodes performed by each animal during the first and second 15 min. of the test period indicated that no reliable differences were attributable to the effects of stimulus presence, stimulus intensity, measurement period, or to any of the interactions. It is clear that no differences existed between the treatment groups, or between the various test conditions, in the manner in which the subjects distributed their grooming responses.

It can be seen from Figure 5, page 42, that the SNF and SFN animals exhibited highly similar amounts of grooming

during the first half of the test even though the SNF animals were more frightened, i.e., showed relative HR decrement. Figure 5 also shows that all subjects exhibited, during the first 15 minutes, a generally increasing frequency of grooming until the 12th minute, after which grooming declined. This is precisely the observation reported by Bindra and Spinner (1958). The present data offer no support for the view that the occurrance of grooming in novel and fearful situations is a manifestation of fear. Instead, the present results support the view that such grooming represents the spontaneous elicitation of a routine, well-practised, everyday response. The present data are therefore in agreement with results reported by Bindra and Spinner (1958), Doyle and Pratt-Yule (1959), Singer (1963), and Woods (1962).

The present results do not agree fully with the pilot study data in which there was clear evidence that more grooming occurred during Stimulus-ON periods. There would appear to be two possible explanations for these divergent results, these being the temperature variation factor, and the strain of the subjects.

One might expect sudden changes in environmental temperature to affect the grooming response (see discussion of Open Field temperature below). No attempt was made during the collection of pilot study data to control the temperature variation in the Open Field caused by the

heat output from the intense illumination lamps. It is not known exactly how much temperature variation existed in the case of each S but it is thought to have been about 10 degrees Fahrenheit. It seems quite likely that a temperature change of this magnitude, occurring in the space of a few minutes, could cause changes in vasodialtion and sweating which might in turn act as grooming stimuli. It is interesting to note that those pilot study animals which were observed under relatively stable temperature conditions throughout the test, the Stimulus ON-ON and Stimulus OFF-OFF groups, yielded grooming data similar to that obtained in the main experiment, i.e., no change in amount of grooming from first to second 15 minute periods of the test.

The pilot study animals were Sprague-Dawley albino rats while the subjects in the main experiment were Long-Evans Hooded animals. Although this strain difference may account for the divergent grooming results, the highly similar heart rate and ambulation data obtained in the two studies make this seem unlikely. Furthermore, previous workers have used a variety of strains and, insofar as these studies are comparable, have reported consistent results. In particular, the Bindra and Spinner (1958) study, using Hooded rats, and the Woods (1962) experiment, using Sprague-Dawley rats, were very similar in design and reported identical grooming results. Thus, it would appear that the factor of strain differences is not a crucial one

in the investigation of grooming responses, and probably does not account for the divergent observations collected in the present pilot and main experiments.

Ambulation

All of the treatment groups showed highly similar ambulation responses during the test session. No differences in amount of ambulation were attributable to the effects of stimulus presence, stimulus intensity, or to any of the first-order interactions between these and the measurement period factor. The overall effect for measurement periods was highly reliable in the direction of less ambulation during the second half of the test. The decreasing frequency of activity as the test session progressed (See Figure 7, page 49) is a typical observation of rat behaviour in novel environments (Berlyne, 1955). The ambulation results of the main experiment agree with pilot study observations which also indicated that less ambulation occurred during the second 15 minutes of the test.

The partial information available on the measurement period by stimulus presence by stimulus intensity interaction shows this interaction to be reliable. It can be seen from Figure 8, page 51, that the MxPxI interaction is accounted for mainly by the M-SNF group, which exhibited a much smaller reduction in ambulation from the

first to the second 15 minute periods of the test than did the other treatment groups. A test for simple effects on the measurement period factor showed that all subject groups, except the M-SNF group, ambulated reliably less during the second 15 minutes. As both the L-SNF and the H-SNF animals showed a reliable reduction in ambulation during the second half of the test, no explanation for the failure of the M-SNF subjects to behave similarly is apparent.

The maintenance of a relatively high level of ambulatory activity during the second 15 minutes may partly account for the failure of the M-SNF group to show a reduced mean heart rate, relative to the first 15 minutes. However, it must be noted that the H-SNF group also did not experience HR reduction during the second 15 minutes, but ambulation was reduced. Therefore the maintenance of heart rate by the M-SNF animals during the second half of the test period cannot be attributed entirely to sustained ambulation. A more probable explanation might be that the M-SNF and H-SNF groups did not experience reliable heart rate decrements because their initial HR accelerations, during the first 15 minutes, were relatively slight.

Subsidiary Measures

Subjects' Weights

The collection of the heart rate data required each

animal to carry a packet weighing approximately 11 grams which consisted of the telemetry transmitter and its protective housing. This burden would require more physical exertion of smaller, and lighter, animals. Since physical exertion is known to affect heart rate (Langley, Cheraskin, and Sleeper, 1963), it is obviously imperative to know whether the various treatment groups differed in their relative ability to carry the weight of the transmitter and packet. Each animal was weighed on the day that it was prepared with a transmitter packet. The animals' weights were used as a measure of their size and hence their ability to bear the packet. Analysis of these data indicated that the treatment groups did not differ with respect to subjects' weights. Thus, it is reasonably assured that the effects of carrying the telemetry transmitter did not affect heart rates.

Open Field Temperature

In any experiment in which the heart rate measure is taken it is also important that temperature variation be controlled, both within any given test session and between sessions, as it has been shown that extreme environmental temperature fluctuations affect many physiological functions (Morgan, 1965). Yamauchi, Takahashi, and Nomura (1966) have reported that, in mice, the environmental temperature affects heart rate only when it departs considerably from an optimum range. Specifically, their results

indicate that mice exposed to an environmental temperature of 25°C (77°F) show no HR change during 60 minutes of observation. These authors describe the heart rate response to environmental temperatures of 20°C to 30°C (68°F to 86°F) as "generally stable". Their data also show that the optimum environmental temperature for mice is about 25°C (77°F), and that very small fluctuations in environmental temperature do not affect HR. As was noted above, the mean start temperature in the Open Field during this study was 76.0°F, and the mean finish temperature was 77.6°F. In no instance did the temperature change during the 30 minute test exceed three Fahrenheit degrees. It was further shown that no reliable differences existed between the treatment groups in start temperature in the Open Field. Although data comparable to that reported by Yamauchi, Takahashi, and Nomura (1966) for mice is not available for rats, it seems most unlikely that the factor of temperature variation affected the heart rate measures taken in this study.

It could also be expected that sudden and substantial changes in environmental temperature would have effects on grooming behaviour. The responses of all homeotherms to temperature changes in the environment include changes in vasodilation, sweating, and level of activity (Morgan, 1965). If grooming responses are elicited by stimuli acting on the surface of the body, then it is evident that

the vascular and sweating reflexes could influence, or themselves be, grooming stimuli. A shift in the animal's overall activity level would be, in effect, a shifting of the thresholds for various responses including grooming. Although published data on the relationship between grooming and environmental temperature is unavailable, some pilot study observations suggest that more grooming occurs in warmer situations. Indeed, it was this observation which led to the construction of the devices used to control temperature variation in this study. The correlation between the amount of temperature change during test in degrees Fahrenheit and the amount of increase in grooming from the first to the second 15 minutes (raw scores used), based upon all 30 Ss, was found to be $r = -0.01$. It is evident then, that environmental temperature variation of the amount which occurred in this study is not related to amount of grooming.

Time of Testing

All subjects were tested during the dark phase of their diurnal cycles. Since it is known that animals possess circadian rhythms of activity (Marler and Hamilton, 1966), and presumably responsivity, the question of time of testing is a vital one. Analysis of the time of testing data indicated that no differences existed between the treatment groups. Thus, it may be assumed that the factor of time of testing did not influence the data collected in this study in any systematic way.

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of this research was to establish whether the grooming produced by rats in novel and stressful situations is related to fear induced by the strange environment or represents merely the elicitation of a well-practised everyday behaviour. Subjects were observed in an Open Field apparatus under various levels of white noise and illumination stimulation.

Measures were taken of grooming behaviour, heart rate, and ambulation. Grooming and ambulation were recorded continuously by means of direct observation and the use of an Easterline-Angus event recorder. The HR measure was obtained through the use of biometric telemetry apparatus. The measure of relative heart rate decrement was used as an objective measure of fear.

The results showed that the subjects were more frightened, i.e., exhibited reliable HR decrement, when the intense stimuli were present than when these were absent from the testing arena. This result supports the findings of previous workers who have reported that rats respond to stressful situations and stimuli with heart

rate decrement. The HR measure did not, however, discriminate between the levels of intense stimulation used. It must be concluded that relatively mild levels of illumination and white noise stimulation are as frightening to the rat as are very intense levels, at least as measured by heart rate decrement.

The analysis of the amount of grooming produced, and the number of separate occasions of grooming, showed that no differences existed that were attributable to the effects of stimulus presence, stimulus intensity, or to the passage of time in the Open Field (measurement period factor). It is concluded that the experience of fear, as measured by relative HR decrement, is not related to grooming, either in terms of the amount produced or to the manner in which this behaviour is distributed in time. The present data support the view that the occurrence of grooming in novel situations represents the spontaneous production of a routine everyday response.

The analysis of the ambulation data indicated that no differences existed in the amount of ambulation which were attributable to the effects of stimulus presence or stimulus intensity. It must be concluded that, over periods of 15 minutes, different amounts of fear experienced by rats are not reflected in different amounts of ambulation. A highly reliable overall tendency did exist in the direction of less ambulation during the second half of the test

session. The finding that activity decreases as a function of time is a typical observation of the behaviour of rats in novel environments.

Recommendations for Further Research

The design of the present experiment was based on an attempt to observe subjects under all four of the possible combinations of fearful and novel stimulation. The second half of the test session was intended to examine the effects of the fearful stimuli upon animals in a relatively non-novel situation. It was felt that during the second 15 minutes the SNF subjects would be in a relatively non-novel and non-fearful situation. The SFN subjects, during the second 15 minutes, would be in a non-novel but relatively fearful environment. In none of the data gathered were there found to be reliable differences in behaviour between the treatment groups during the second half of the test. Thus, virtually no additional information was contributed by the second 15 minutes of observation. In addition, the use of the latin square design carries with it some difficulties in the interpretation of certain interactions. For these reasons, it is recommended that future experimenters avoid the cross-over design used in this study.

Some pilot study data suggested that more grooming occurs under conditions of higher environmental temperature. The possible influence of this variable on the groom-

ing response requires clarification.

Further research on the factors which elicit grooming behaviour must include an examination of the effects of the presence of other animals in the situation. How, for example, might the heart rate and grooming responses have been affected had two, or three, rats been released together in the Open Field?

Finally, the scope of grooming behaviour research should be extended to the rich mileau of social interactions in rat societies. The use of telemetry apparatus will enable such observations to take on a new dimension, and the potential rewards would seem to be substantial.

APPENDIX A

THE PILOT STUDY WORK

Considerable trial and error was required before a suitable technique was developed for using telemetry apparatus to obtain the heart rate measure. When this had been accomplished it was decided to test some animals in an Open Field apparatus under relatively stressful and non-stressful conditions. The following is a broad summary of this work. Details of apparatus and procedure are omitted here as these are presented fully in the methodology of the main experiment.

The non-stressful condition (Stimulus-OFF) consisted of a quiet and dimly illuminated (3 foot candles) Open Field. In the stressful condition the Open Field arena was illuminated with 700-900 foot candles (ft.c.) and bombarded with 90 db intensity white noise. Each subject was confined in a pre-test chamber for one hour prior to being lifted by hand and placed in the Open Field for a 30 minute observation period. There were four treatment conditions in all. The SNN group (Stimulus ON-ON) was exposed to the

intense illumination and white noise throughout the entire 30 minutes. The SFF group (Stimulus OFF-OFF) was observed throughout under quiet and dim illumination conditions. The SNF animals (Stimulus ON-OFF) received the intense stimulation during the first 15 minutes only, and the SFN group (Stimulus OFF-ON) during the second 15 minutes only. Six animals were observed under each treatment condition. Three of the six were experimental subjects (i.e., were prepared with the telemetry transmitter) and the remaining three were control (unprepared) animals. All 24 animals were naive adult Sprague-Dawley males ranging in age from 110 to 200 days of age. The entire compliment of treatment groups is summarized below in Table 23.

Table 23

Pilot Study Treatment Groups

Group	n	Treatment	Illumination and White Noise 1st 15 min.	2nd 15 min.
E-SNN	3	experimental	ON	ON
C-SNN	3	control	ON	ON
E-SNF	3	experimental	ON	OFF
C-SNF	3	control	ON	OFF
E-SFN	3	experimenatl	OFF	ON
C-SFN	3	control	OFF	ON
E-SFF	3	experimental	OFF	OFF
C-SFF	3	control	OFF	OFF

Heart rate, grooming, and ambulation measures were taken on all experimental subjects (Ss). The recording of heart rate began 10 minutes prior to introduction into the Open Field and continued uninterrupted throughout the test. For all Ss, control and experimental, the measurement of grooming and ambulation began with their release in the Open Field.

Results and Discussion

Heart Rate

The heart rate data were scored in beats per minute. The mean heart rates of the four experimental groups during the pre-test period, and the first and second 15 minutes of the test, are presented below in Table 24.

Table 24

Mean Heart Rate, by Treatment Group, by Measurement Period (Pilot Study Data, N=12)

Treatment Group	Pre-test	First 15 Min.	Second 15 Min.
E-SNN	382	409	393
E-SNF	413	447	417
E-SFN	377	452	384
E-SFF	359	414	386

An analysis of variance of the pre-test heart rate means yielded no reliable group differences. Analysis of the HR means of the first and second 15 minute periods of

the test indicated a highly reliable tendency for heart rates to be lower during the second 15 minutes. No differences in mean HR were attributable to the presence of the intense stimulation, but a significant groups by measurement periods interaction was obtained. It was found that the SFN group (for which the intense stimulation was turned on during the second 15 minutes) showed greater relative heart rate decrement during the second 15 minutes than did the other groups.

It had been expected on the basis of the literature reviewed that the heart rate response to the stressful stimulation would be that of a decrement relative to the heart rates of animals in the non-stress condition. Overall, the HR measure did not differentiate animals under stress from those exposed to the non-stress condition. However, as was noted above, when the intense stimulation was introduced after the end of 15 minutes a marked HR decrement was observed. It was thought that the solution to this paradox lay in the procedure by which animals were introduced into the Open Field. All of the subjects were handled in being transferred from the pre-test chamber to the Open Field. It is known from the work of Black, Fowler, and Kimbrell (1964) that rats respond to being handled with HR acceleration. Thus the effects of the handling experience may have obscured the effects of the illumination and white noise stimulation. It was decided, in the main ex-

periment, to transfer the animals from the pre-test compartment to the Open Field without handling them in any way.

Grooming

The mean amount of grooming, in seconds, shown by each of the treatment groups during the first and second 15 minutes of the test is presented in Table 25, below.

Table 25

Mean Seconds of Grooming, by Treatment Group, by Measurement Period (Pilot Study Data, N=24)

Treatment Group	First 15 Min.	Second 15 Min.	Total
E-SNN	110.7	79.7	190.4
C-SNN	38.3	51.7	90.0
E-SNF	25.0	0.0	25.0
C-SNF	105.3	0.0	105.3
E-SFN	2.0	65.0	67.0
C-SFN	13.7	113.7	127.4
E-SFF	56.7	18.0	74.7
C-SFF	3.7	8.7	12.4

A t-test for means (Ferguson, 1966) was conducted comparing the total grooming of experimental animals with that of control Ss. This analysis showed that no reliable difference existed ($t = 0.13$, $p > .05$). This result was interpreted to mean that the presence of the telemetry transmitter did not affect the grooming response.

A t-test comparing the mean seconds of grooming per minute produced in the presence of the intense illumination and white noise stimulation with the mean amount produced

per minute during Stimulus-OFF periods yielded a significant result ($t = 3.95$, $p < .05$). As can be seen from Table 25, more grooming occurred during Stimulus-ON periods. The interpretation of the grooming data was complicated by the fact that it was noticed that the lamps which were used to produce the intense illumination also produced considerable heat. No attempt was made during the pilot study to control this heat production. Consequently the temperature variation during and between test sessions may have affected any or all of the responses measured. This confounded factor made clear interpretation of the data impossible. It was decided that, in the main study, the temperature variation in the Open Field must somehow be controlled.

Ambulation

The mean amount of ambulation, in inches, shown by each of the treatment groups during the first and second 15 minutes of the test is presented below in Table 26. It can be seen from Table 26 that all groups tended to ambulate less during the second 15 minutes regardless of the presence or absence of the intense stimulation. A t-test comparing the total ambulation of the experimental animals with that of control Ss showed that no reliable difference existed ($t = 1.78$, $p > .05$). In addition, the ambulation curves plotted for the experimental and control Ss showed a high degree of similarity.

Table 26

Mean Inches of Ambulation, by Treatment Group, by Measurement Period (Pilot Study Data, N=24)

Treatment Group	First 15 Min.	Second 15 Min.	Total
E-SNN	398.2	111.8	510.0
C-SNN	544.4	105.8	650.2
E-SNF	885.8	215.0	1,100.8
C-SNF	1,304.6	802.4	2,107.0
E-SFN	352.6	275.2	627.8
C-SFN	926.2	255.4	1,181.6
E-SFF	367.2	169.4	536.6
C-SFF	814.4	378.4	1,192.8

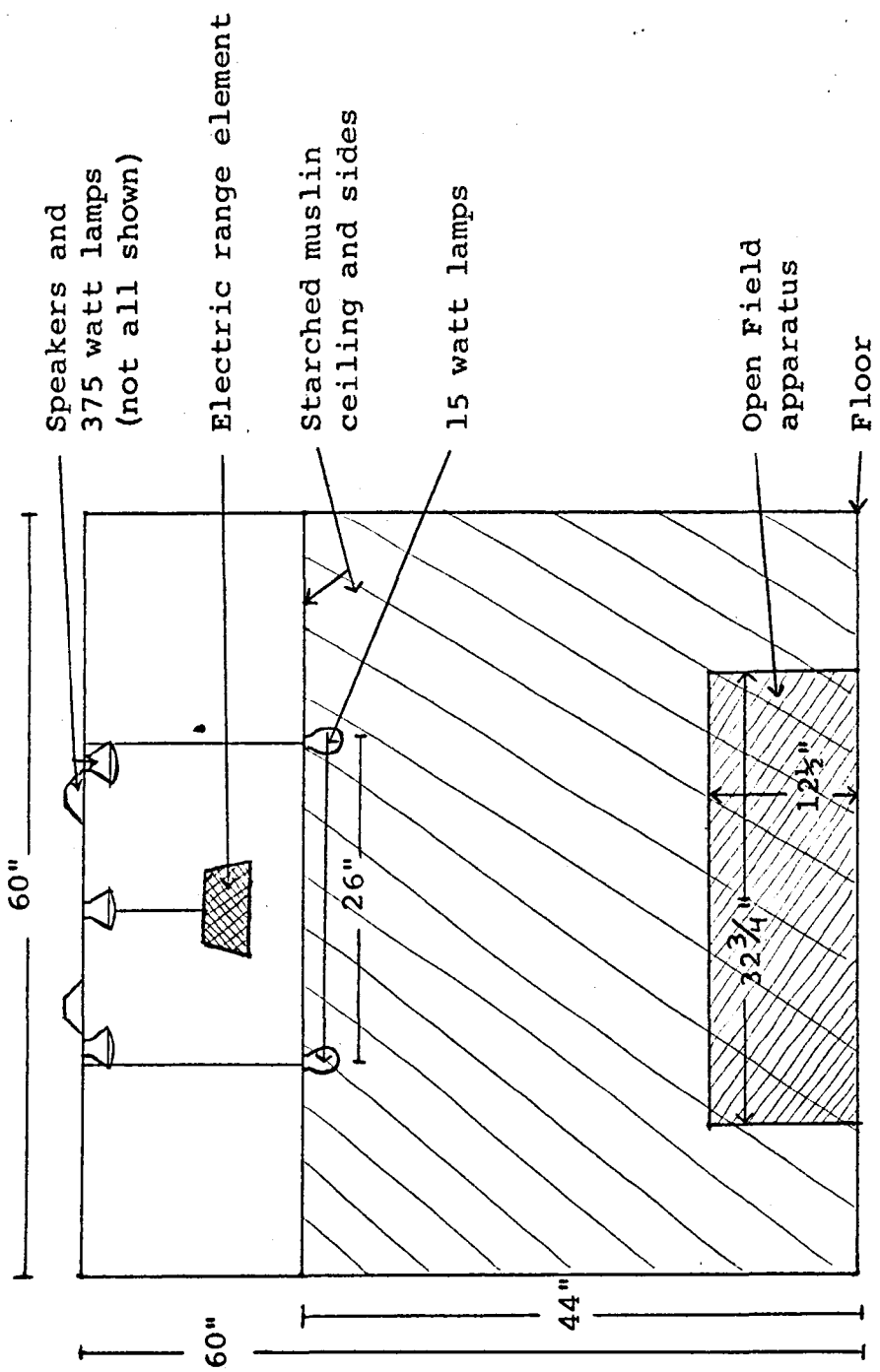
These ambulation data, plus the observation that no difference existed in the amount of grooming shown by control and experimental subjects, were the basis for the conclusion that the presence of the telemetry transmitter on the animal's body did not alter or interfere with normal behaviour. All of the measures indicated that the data collected under constant conditions for 30 minutes were superfluous. The behaviour of the SNN and SFF animals during the second 15 minutes was generally similar to their first period responses but with proportional reduction in magnitude. Thus, these groups did not contribute any additional information to that available from the SNF and SFN groups.

More refined statistical analyses of the data were not attempted because of the age differences of the Ss,

inconsistencies in time of testing, and procedural errors usually made in the collection of pilot data. In summary, despite certain procedural inadequacies and the confounding introduced by uncontrolled temperature variation, the following conclusions were made. It was concluded that the telemetric collection of heart rate data was technically feasable and that, given procedural refinements, the heart rate measure would probably serve as a useful measure for differentiating animals in stressful from those in non-stressful situations. It was also concluded that control subjects, i.e., those not outfitted with a telemetry transmitter, need not be tested since their behaviour had not differed from that of animals so prepared. Finally, it was concluded that the observation of animals under continuous stimulus conditions for the entire 30 minutes of the test need not be continued since no additional information had been contributed by such observations in the pilot study.

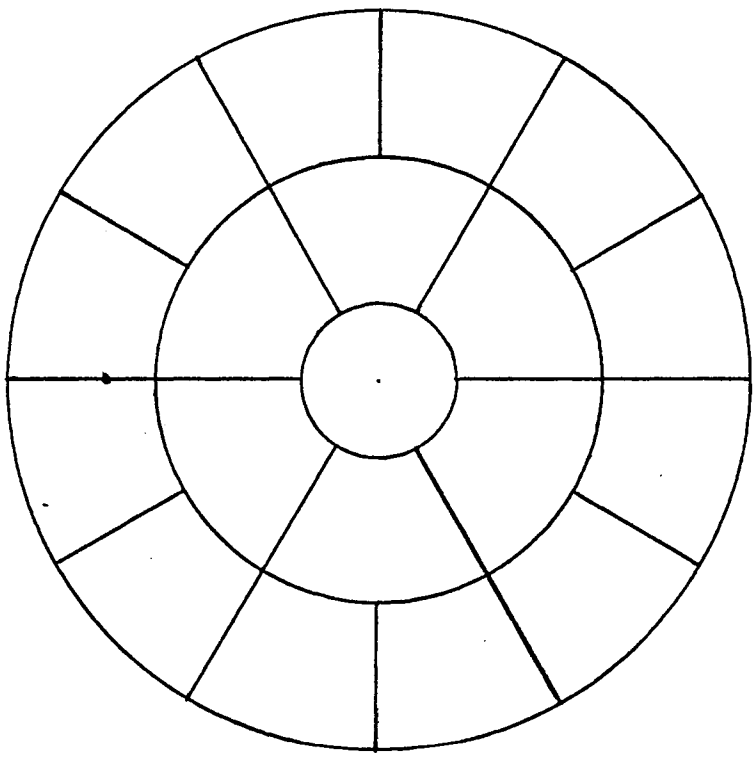
APPENDIX B

Diagram of Testing Apparatus



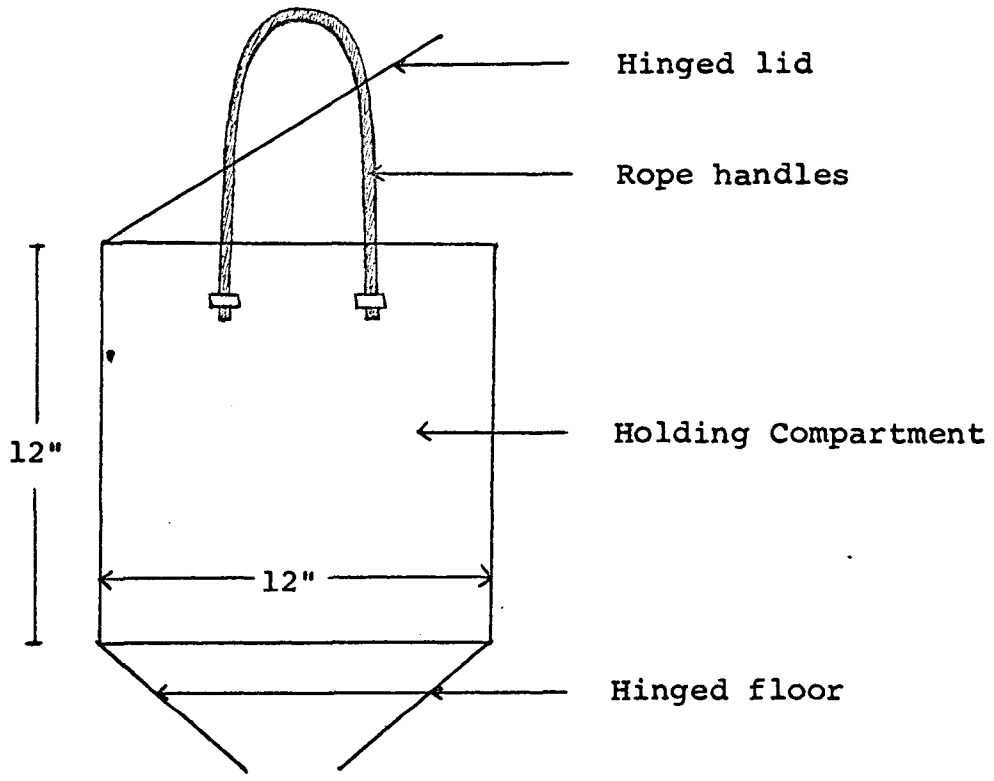
APPENDIX C

Grid Pattern on Floor of Open Field Arena



APPENDIX D

Diagram of Pre-test Holding Compartment



APPENDIX E

Testing Day and Time of Placement (military time) in Pre-test Compartment by Subject

Group	Subject No.	Testing Day	Time of Placement in Pre-test Compartment
L-SFN	1	1	12:00
	10	4	15:00
	21	6	11:55
	29	9	11:35
	31	9	18:00
L-SNF	2	1	15:00
	11	4	11:50
	12	4	18:00
	19	6	15:00
	30	9	14:40
M-SFN	4	2	14:55
	14	4	18:00
	25	7	18:00
	34	10	17:30
	38	12	18:15
M-SNF	5	2	18:05
	6	2	11:55
	15	4	15:00
	24	7	14:45
	32	10	14:30
H-SFN	7	3	12:05
	18	5	15:00
	27	8	17:30
	28	8	11:30
	40	12	11:30
H-SNF	16	5	11:55
	17	5	18:00
	26	8	14:30
	35	10	13:00
	39	12	14:30

APPENDIX F

Subjects' Weights in Grams on Day of Preparation with a Transmitter Packet

Group	Subject No.	Weight
L-SFN	1	283
	10	304
	21	331
	29	331
	31	389
L-SNF	2	373
	11	322
	12	294
	19	372
	30	339
M-SFN	4	305
	14	308
	25	315
	34	295
	38	332
M-SNF	5	358
	6	295
	15	280
	24	360
	32	385
H-SFN	7	351
	18	301
	27	346
	28	313
	40	340
H-SNF	16	345
	17	335
	26	381
	35	327
	39	385

APPENDIX G

Open Field Temperature (in degrees Fehrenheit) at Start and Finish of Test by Subject

Group	Subject No.	Start Temp.	Finish Temp.
	1	75	77
	10	75	77
L-SFN	21	76	78
	29	76	78
	31	77	78
	2	76	77
	11	73	74
L-SNF	12	76	77
	19	78	79
	30	78	78
	4	74	77
	14	77	78
M-SFN	25	77	78
	34	79	80
	38	78	81
	5	75	76
	6	73	74
M-SNF	15	75	76
	24	76	77
	32	78	79
	7	74	77
	18	75	78
H-SFN	27	77	80
	28	75	77
	40	78	80
	16	74	75
	17	74	76
H-SNF	26	75	77
	35	77	78
	39	78	80

APPENDIX H

Heart Rate Mean Scores by Measurement Period by Subject

Group	Subject No.	Pre-test	First 15 Minutes	Second 15 Minutes
	1	406	502	459
	10	469	418	380
L-SFN	21	293	414	392
	29	330	397	363
	31	333	459	396
	2	389	417	381
	11	373	478	435
L-SNF	12	327	447	347
	19	400	390	391
	30	450	380	388
	4	366	455	423
	14	417	391	358
M-SFN	25	407	420	404
	34	331	420	393
	38	324	461	362
	5	368	418	420
	6	397	448	404
M-SNF	15	371	319	378
	24	367	440	429
	32	323	418	396
	7	400	478	433
	18	424	374	403
H-SFN	27	378	418	346
	28	333	446	378
	40	344	434	343
	16	369	410	388
	17	459	488	437
H-SNF	26	344	362	363
	35	332	366	360
	39	381	306	350

APPENDIX I

Number of Fecal Boluses Deposited during Test by Subject

Group	Subject No.	Boluses
L-SFN	1	5
	10	5
	21	5
	29	8
	31	7
L-SNF	2	3
	11	4
	12	2
	19	9
	30	3
M-SFN	4	4
	14	9
	25	5
	34	9
	38	6
M-SNF	5	6
	6	5
	15	2
	24	4
	32	6
H-SFN	7	3
	18	4
	27	4
	28	8
	40	8
H-SNF	16	7
	17	5
	26	5
	35	2
	39	2

APPENDIX J

Total Seconds of Grooming (raw scores) by Measurement Period by Subject

Group	Subject No.	First 15 Min.	Second 15 Min.
	1	111	139
	10	4	26
L-SFN	21	218	275
	29	58	43
	31	0	126
	2	34	23
	11	69	27
L-SNF	12	314	0
	19	47	113
	30	43	263
	4	9	230
	14	0	44
M-SFN	25	0	20
	34	157	166
	38	55	38
	5	9	140
	6	109	72
M-SNF	15	28	355
	24	64	133
	32	99	9
	7	187	207
	18	75	351
H-SFN	27	91	0
	28	119	130
	40	95	3
	16	28	52
	17	59	18
H-SNF	26	62	164
	35	45	75
	39	65	2

APPENDIX K

Total Seconds of Grooming ($\sqrt{x} + \sqrt{x+1}$) by Measurement Period by Subject

Group	Subject No.	First 15 Min.	Second 15 Min.
	1	61.86	70.21
	10	18.23	25.51
L-SFN	21	97.99	106.64
	29	42.68	35.74
	31	15.00	61.51
	2	35.57	26.77
	11	44.97	26.93
L-SNF	12	121.72	15.00
	19	37.75	64.17
	30	36.95	91.56
	4	24.60	99.85
	14	15.00	34.90
M-SFN	25	15.00	23.05
	34	78.78	72.64
	38	39.74	29.51
	5	20.16	64.40
	6	59.47	48.62
M-SNF	15	29.79	133.86
	24	35.63	53.76
	32	50.38	24.83
	7	80.93	85.05
	18	43.24	120.02
H-SFN	27	53.90	15.00
	28	61.55	54.50
	40	52.58	17.73
	16	30.84	45.03
	17	36.83	28.84
H-SNF	26	37.47	67.51
	35	38.37	49.15
	39	40.15	17.14

APPENDIX L

Total Number of Grooming Episodes by Measurement Period by Subject

Group	Subject No.	First 15 Min.	Second 15 Min.
	1	3	4
	10	1	2
L-SFN	21	14	6
	29	6	5
	31	0	3
	2	3	1
	11	9	1
L-SNF	12	6	0
	19	5	5
	30	4	2
	4	4	8
	14	0	2
M-SFN	25	0	1
	34	4	4
	38	4	1
	5	1	4
	6	6	8
M-SNF	15	2	8
	24	1	2
	32	3	4
	7	6	4
	18	2	4
H-SFN	27	6	0
	28	7	1
	40	3	1
	16	4	7
	17	2	4
H-SNF	26	2	2
	35	3	3
	39	3	3

APPENDIX M

Total Inches of Ambulation by Measurement Period by Subject

Group	Subject No.	First 15 Min.	Second 15 Min.
	1	301.0	335.4
	10	782.6	8.6
L-SFN	21	954.6	318.2
	29	1,204.0	1.290.0
	31	1,548.0	378.4
	2	473.0	197.8
	11	1,118.0	68.8
L-SNF	12	111.8	0.0
	19	679.4	120.4
	30	627.8	60.2
	4	911.6	335.4
	14	610.6	189.2
M-SFN	25	989.0	464.4
	34	223.6	25.8
	38	971.8	103.2
	5	60.2	25.8
	6	129.0	516.0
M-SNF	15	369.8	240.8
	24	1,186.8	404.2
	32	885.8	627.8
	7	748.2	103.2
	18	344.0	283.8
H-SFN	27	713.8	137.6
	28	713.8	43.0
	40	68.8	0.0
	16	567.6	154.8
	17	1,057.8	593.4
H-SNF	26	1,186.8	404.2
	35	1,049.2	533.2
	39	593.4	791.2

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