The Effects of Maternal Deprivation and Early Social Isolation on Emotionality and Discriminated Avoidance Learning in the Rat

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THE EFFECTS OF MATERNAL DEPRIVATION
AND EARLY SOCIAL ISOLATION ON EMOTIONALITY
AND DISCRIMINATED AVOIDANCE LEARNING IN THE RAT

by

Edward N. Schneider

A Thesis
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in partial fulfillment
of the
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Edward Norman Schneider
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INTRODUCTION

An ever increasing amount of effort is being expended in investigating the effects of early experience of organisms on later behavior patterns. For the past few years investigators have been observing the relation between early experience and later behavior in a variety of laboratory animals. These studies have been undertaken in an effort to understand more fully the processes of development, particularly during the early and formative stages, and to bring them under empirical control. Generally these studies follow either (a) an enrichment paradigm whereby the organism is reared in an environment designed to provide increased social, sensory, or locomotor stimulation above that which is usually encountered, or (b) a deprivation paradigm whereby the organism is reared in an environment designed to provide reduced amounts of such stimulation.

Laboratory Studies of Early Experience

Enrichment, supranormal stimulation

Numerous investigators in the field of early experience contend that animals which have been stimulated in infancy are 'better' animals in later life. Hebb (Scott, 1962) found that pet rats reared in the rich environment of the home performed much better on
learning tasks than rats reared in barren laboratory cages. Forgays and Forgays (1952) observed similar effects when they compared rats reared in an environment enriched with playthings to rats reared without such playthings.

Hymovitch (1952) in assessing the beneficial effects of a varied early environment, employed a large cage as a complex environment in which playthings were available. One group of rats was reared in the complex environment and had access to the playthings. A second group was reared in smaller mesh cages which were placed inside the larger cage and moved about periodically. As adults, the mesh-cage reared rats were indistinguishable from those given free run of the complex environment on problem solving tasks. Hymovitch was able then to conclude that the beneficial effects of a varied early environment are not due merely to unequal opportunities for motor experience nor to differential motivation.

Gibson and Walk (1964) sought to determine the effects of early and continued exposure to certain visually presented forms on ease of learning in a later discrimination test. Rats reared in cages where the forms were mounted on walls reached criterion significantly sooner and made fewer errors than a control group which saw the forms for the first time at testing. Early visual experience with the forms to be discriminated, even in the absence of differential reinforcement had obviously facilitated the discrimina-
tion learning.

Studies involving the direct physical stimulation of the organism also confirm the importance of stimulation in infancy as a favorable modifier of later behavior in situations dealing with learning, problem solving, stress, and exploration (Denenberg, 1964). Methods of infantile stimulation include mechanical shaking (Levine & Lewis, 1959); temperature variations (Hutchings, 1965); and olfactory stimulation (Gard, Hard, Larsson, & Petersson, 1967). Electric shock and handling are the two most commonly used stimulation methods.

Handling usually consists of removing the young subject from the nest and placing him in a container such as a can containing wood shavings, small wooden compartments, boxes with grid floors, etc. and leaving him there for a short period of time, generally two to three minutes. Stimulated subjects are then compared to a control group of nonstimulated or less stimulated subjects on some later task(s) to determine the effects of stimulation.

Tests may be made within a few days of stimulation or months afterward. Candland and Campbell (1962) found the age of the subject at testing to be an important variable. Fearfulness in rats ranging in age from 18 to 200 days increased with age when they were placed in a novel environment and reached an asymptote at approximately 54 days of age. Until the variable of age is more fully explored, it is not reasonable to speak of the effects of early experience...
upon later behavior, but only upon behavior at some later age (King, 1958).

A theory of infantile stimulation:
Denenberg's Monotonicity Hypothesis

In an attempt to explain how the organism profits behaviorally as a result of infantile stimulation, Denenberg (1964) has hypothesized that stimulation in infancy reduces the organism's emotional reactivity. The reduction then serves to enhance his adaptivity to a noxious or stressful situation resulting in increased performance. Denenberg's Monotonicity Hypothesis as it is called states that "emotional reactivity is reduced as a monotonic function of amount of stimulus input in infancy" (Denenberg, 1964, p. 338). That is, the greater the stimulation in infancy, the less emotional an organism will be sometime later in adulthood. The concept of animal emotionality is based on the observation that the presentation of a sudden or intense stimulation, or the placement of an organism in a novel environment, results in increased defecation and urination.

Research in rodent emotionality may be traced back to Yoshioka (Tobach & Schneirla, 1962), who in 1932, observed that when food-deprived rats were placed in an enclosure with food accessible, they often defecated and urinated, but would not eat. However, when the animals were gradually introduced to the same
situation, most of them began to eat and ceased eliminating. Hall called such behavior "emotional" and defined emotionality as "a convenient concept for describing a complex of factors...a general upset or excited condition of the animal" (Tobach & Scheirla, 1962, pa. 211-212).

The most commonly used technique for assessing rodent emotionality is the Open Field Test (Hall, 1941; Whimbey & Denenber, 1967). The Open Field Test uses the variables of movement and defecation rate as indices of emotionality. The Open Field Test consists of a circular or square-walled enclosure whose floor is marked off in area units by lines. The animal is placed inside the field and the number of units he enters plus the number of fecal boluses dropped are recorded to comprise an index of emotional reactivity.

Another test used to determine the emotional results of early stimulation involves consummatory behavior. Studies (Amsel, 1950; Siegal & Siegal, 1949) have demonstrated that emotional disturbance can affect the amount of water consumed after a period of deprivation. Levine (1957) has hypothesized:

"that since the deprivation constitutes a novel internal stimulus complex for nonhandled subjects, the novelty should result in greater emotional disturbance and produce reduced water consumption following a period of deprivation" (Levine, 1957, p. 609).

Levine (1957; 1958) and Spence and Maher (1962) have supported this
hypothesis: handled rats consume significantly more water after a period of deprivation than nonhandled controls.

In addition to emotionality and learning variables affected by stimulation in infancy, DeNelsky and Denenberg (1967a; 1967b) have demonstrated that the amount of visual and tactual stimulation sought by rats can also be influenced by infantile stimulation. Rats were given the opportunity to explore compartments of a Greek-cross apparatus painted white, grey, or black, and in another experiment, rats were allowed to explore compartments with floors of smooth fibreboard or sandpaper. In both experiments rats handled in infancy spent more time in environments designed to give greater stimulus input—white or grey compartments and sandpaper-floored compartments than nonhandled controls who chose black compartments and smooth floors.

Organisms receiving stimulation in infancy also differ markedly in their physiological responses to later environmental conditions other than defecation. Levine (1962) reviewed a number of infantile stimulation studies that dealt with:

1. The maturation of the hypothalmo-hypophysial system using the onset of adrenal ascorbic acid depletions as a measure of the functional status of the system. Manipulated (handled) subjects showed a significant earlier depletion of adrenal ascorbic acid than the nonmanipulated subjects.

2. The appearance of body hair and eye opening
occurred earlier by several days in stimulated subjects.

3. The process of myelination of the central nervous system appeared significantly earlier in stimulated subjects.

4. Stimulated rats showed significantly less mortality following 120 hours of total food and water deprivation than nonstimulated rats.

5. Adrenal hypertrophy, 24 hours after an injection of hypertonic glucose, was significantly greater in nonstimulated subjects.

In contrast to Denenberg's position that a stimulated rat reacts less to stress, Levine (1962) has demonstrated that a stimulated rat showed a greater and more rapid rise in corticosterone output following one minute of electric shock in adulthood. He also found that stimulated rats show a significantly higher percentage of full tonic-clonic seizures than nonstimulated rats when given 200 milliamperes of electro convulsive shock. Levine has concluded that:

"stimulated animals are more reactive to distinctly noxious and threatening situations, but that the nonstimulated animal appears to react to a greater variety of environmental changes. The nonstimulated subject seems to require less extreme changes in the environment to elicit a physiological response, and it is in this sense that they are hyperreactors. The nonstimulated subjects are hyperreactors in another sense. These animals appear to show a more sustained reaction to chronic stress.... The stimulated animal appears to have the capacity for responding rapidly and for returning to normal levels more quickly and persists longer in its response to stress" (Levine, 1962, p. 250).
Deprivation, subnormal stimulation

The other investigative paradigm followed by investigators in
the area of early experience is that of deprivation, or subnormal
stimulation in infancy. Among the laboratory studies following a
depprivation paradigm, primate studies have yielded some very dram-
atic results to confirm the importance of early experience and dem-
onstrate the deleterious effects of maternal deprivation and early
social isolation.

Harlow's famous studies of maternally deprived and socially
isolated monkeys (1962; 1968) showed that mothering and social
contact with other monkeys were necessary in order for later suc-
cessful development of grooming responses, normal sexual behavior,
and adequate infant rearing. Female monkeys separated from their
mothers and reared in isolation during infancy became very inade-
quate mothers later on, abusing and even rejecting their young.

Harlow describes these deprived monkeys:

"As month after month has passed, these monkeys have
appeared less and less normal. We have seen them sit-
ting in their cages strangely mute, staring fixedly into
space, relatively indifferent to people and other mon-
keys. Some clutch their heads in both hands and rock
back and forth....others, when approached or even
left alone, go into violent frenzies of rage, grasping
and tearing at their legs with such fury that they some-
times require medical care. Eventually we realized
we had a laboratory full of neurotic monkeys" (Harlow,
In an investigation of the temporal parameters of total social isolation in monkeys, Harlow (1965) found that monkeys can withstand at least 3 months of total social isolation starting at birth, or 6 months of total social isolation starting at 6 months of age, but that their social potentialities are destroyed if isolation from birth persists for 6 months or 12 months.

Melzack and Scott (1957) reared Scottish terriers in a restricted environment which permitted daylight through a vent, but prevented them from seeing outside. When compared to home reared controls on three tests of pain stimuli (shock, burn, and pinprick), the restricted dogs required many shocks to learn to avoid the shock apparatus. Similar results also occurred with the flaming match and pinprick. Melzack and Scott concluded that the extreme subnormal stimulation prevented the dogs from perceiving and responding appropriately to painful stimuli.

Thompson and Heron (1964) also used Scottish terriers to determine the effects of early social isolation upon later problem solving. On all tests the isolated dogs proved inferior to nonisolated controls, leading the authors to conclude that restriction of early perceptual experience has definite and fairly permanent retarding effects on dog intelligence.

Beach (1937) isolated rat pups at 21 days of age to study the effects of social deprivation on later maternal behavior. Later, when
these isolated subjects were mated and cast their litters, all pups were cleaned, suckled, and adequately cared for. Beach concluded that maternal behavior is innately organized.

Guze (Thoman, 1965) obtained somewhat different results in a similar study involving early social isolation. He removed rat pups from the home nest to separate cages, singly and in groups at seven days of age. Thereafter, he allowed them 3-1/2 hours of nursing each day with the mother. Guze found that when these subjects were mated, the grouped animals became the poorest mothers.

Denenberg (1963) found that female rats judged to be high in emotionality reared offspring also high in emotionality as measured by the Open Field Test. He also reported that when emotional females reared pups from normal mothers and vice versa, the emotional state of the rearing female was more powerful in determining the pups' emotionality than was the emotional classification of the biological mother. Denenberg concluded that:

"Genetic constitution is not without influence on later emotional behavior. Independant of such influence however, the mother in her interaction with the young between birth and weaning brings about a relatively permanent change in the emotional behavior of the offspring" (Denenberg, 1963, p. 139).

The Use of Incubator Reared Rats in Social Isolation Studies

In spite of the increasing interest in the area of early experience
and the growing number of laboratory studies, a potentially serious
deficiency exists in rat studies of social isolation and maternal de-
privation. The deficiency stems from the paucity of studies using
rats maternally and socially deprived from birth or very shortly
thereafter, through weaning and beyond. With few exceptions rat
studies of social isolation involve animals isolated after weaning,
or at best, animals weaned a few days earlier than the usual 21 days.
During this time, however, the rat pup has shared the nest with an
average number of seven crawling, squirming litter-mates and has
also been the recipient of its mother's stimulation and care.

Denenberg (1964) cites the preweaning period as the time
when stimulation will have maximum effectiveness on later emotional
behavior, not because of any clinical interpretation of mother-young
separation, but because all of the rat pup's senses are functioning
at weaning. The preweaning period is also important because it is
only after weaning that there is any evidence of long term retention
of a learned fear response. Campbell and Campbell (1962), meas-
ured the persistence of conditioned fear in rats of different ages and
found that retention of fear varied directly as a function of the age
of the rat at conditioning. Rats conditioned at 18 days of age showed
little or no retention of fear 21 days after conditioning, while rats
conditioned at 100 days of age showed nearly perfect retention after
a 42 day interval. Denenberg states that,
"The post weanling rat is a very different organism, behaviorally and biologically, from the preweanling animal, and it is not logical to assume that stimulation administered to the immature, recently born rat has the same consequences as the same stimulation administered to a weanling" (Denenberg, 1964, p. 346).

As a result of such evidence, the lack of preweaning isolation and maternal deprivation studies becomes more noteworthy. The need becomes clear for data on rats maternally deprived and socially isolated during the entire preweaning period, or certainly throughout as much as possible of this important period.

Undoubtedly the major reason for the lack of adequate preweaning isolation studies of rats has been the difficulty of rearing rat pups away from the mother. Rat pups weigh about a quarter-ounce at birth and grow to about three-quarters of an ounce at 10 days, and they require around-the-clock care for the first two weeks of life.

The greatest impetus for the hand rearing of rat pups has come from attempts to produce germ free animals for use in pharmaceutical studies. The hand rearing of rat pups was first reported by Gustaffson in 1946 (Pleasants, 1959). He inserted a thin rubber tube halfway down the esophagus and forced a sterilized milk formula into the stomach. The rat pup is taken from the mother just before birth, or after the first spontaneous birth of a pup by Caesarian section and is then hand reared under very strict and elaborate patho-
gen free conditions in isolators. At Lobund Institute, University of Notre Dame, Gustaffson's pioneering efforts have been refined to a more consistently reproducible technique involving forced feeding with a short, tapered rubber nipple and the use of a relatively simple milk formula (Pleasants, 1959). Similar techniques have been used at the Upjohn Company's animal laboratories in Kalamazoo, Michigan where they maintain a large colony of hand reared, germ free rats of several generations.¹

Until Thoman (1963) developed a less elaborate yet successful method for the incubator rearing of rat pups, no study had been able to provide an adequately controlled behavioral analysis of the effects of maternal deprivation and social isolation in the rat from shortly after birth. In a later study, Thoman (1968) sought to determine if Albino rat pups would exhibit maternal aberrations following social and maternal deprivation during infancy. When she compared the deprived rats to mother-reared grouped and mother-reared individual controls, the deprived rats did equally well on nest building, parturition, and pup retrieving tests.

However, Thoman did find large differences between deprived and mother-reared subjects as reflected in the development of their offspring on such measures as eye opening and mortality rate. Pups from the deprived (incubator-reared) Ss had a higher mortality rate

¹Personal communication.
than pups from mother-reared controls. Also, pups from Ss deprived of both mother and peers opened their eyes later than pups from Ss deprived only of mother and later than mother-reared controls.

These results may have been due to some physiological deficit in the deprived mothers caused by early incubator rearing and passed on to their pups. If pups from deprived mothers were physiologically inferior, they might have been more likely to die, and they might have opened their eyes later than normal pups from mother-reared Ss. A physiological explanation of these results was rejected by Thoman because of little variation in growth curves between incubator- and mother-reared Ss, similar litter sizes of Ss' offspring, and no differences in the general appearance of Ss' offspring at parturition.

One of the explanations discussed by Thoman was that the effects of early maternal and social deprivation as reflected in the development of Ss' offspring may have been mediated by differential levels of emotionality among the Ss. Evidence for this interpretation was found in the fact that the greatest losses of offspring coincided with the pup retrieval tests, a situation known to be disturbing to rats. Rosenblatt and Lehrman (1963) state that

"After parturition, retrieving appears regularly to the general stimulus of a pup located outside the nest... Where a female cannot establish and maintain a stable nest site, carrying of pups is often stimulated in excess but the female fails to deposit her young at
any one nest site. The young are therefore deposited
at many different places, to their detriment, for
often they are left to die there without the benefits
of maternal care" (Rosenblatt & Lehrman, 1963,
pp. 22-23).

Perhaps removing the young from the nest, or a change in
environmental conditions surrounding the pup retrieval tests was
more disturbing for the deprived Ss than for the mother-reared
controls due to greater emotionality, thus resulting in higher
mortality among their offspring. Denenberg's study (1963) has
demonstrated that early rearing conditions can lead to dif-
ferential levels of emotionality in rats. It is possible, then, that
differences in emotional reactivity resulting from maternal
deprivation and social isolation in infancy could have led to
differential maternal care during a stressful situation, causing
a higher mortality rate among the deprived Ss' offspring.

As stated earlier, there have been few studies investigating
the effects of early maternal deprivation and social isolation on
rats that have had adequate controls for these variables during
the important preweaning period. Using the incubator rearing
techniques now available, the present study will attempt to pro-
vide data on rats deprived of normal maternal care and peer inter-
action shortly after birth and continued throughout the preweaning
period. More specifically, this study will attempt to answer two
questions. The first question was raised by Thoman's 1968 study:
(a) does the absence of maternal care and peer interaction lead to increased emotionality later in life when a deprived animal encounters a novel situation designed to elicit emotional reactivity as measured by exploratory activity and defecation rate in the open field, and (b) does deprivation also affect later learning ability in a discriminated avoidance learning situation, as this task has been shown to be sensitive to the effects of early experience (Denenberg, 1964) and is widely used in studies of this nature?

Before proceeding any further, an apparent confounding that seems to be inherent in incubator rearing studies of social deprivation must be pointed out. The confounding is embedded in the incubator rearing process itself as the rat pup must be picked up and handled approximately six times a day for mechanical feeding, taking about one minute to complete. Thus, while the present study is designed to assess the effects of maternal and social isolation under a deprivation paradigm, the animals are also stimulated by handling under an unintentional stimulation paradigm. As pointed out earlier, stimulation serves to enhance later performance on certain dependant variables such as emotionality and learning, whereas deprivation serves to decrease later performance on these same dependant variables.
METHOD

Subjects

**Incubator-reared**

Twenty-four male and female Albino rat pups from ten litters were used. The litters were reduced to eight pups at birth. At 3 days of age, the pups were divided into experimental (incubator-reared) and control (mother-reared) groups using the split litter technique so that each incubator-reared pup had a corresponding littermate control of the same sex. The experimental pups were placed in an incubator and reared under conditions of maternal deprivation and social isolation.¹ The control pups were left with the mother and reared by her. Four additional rat pups were each reared by a mother as a safeguard against expected losses due to premature cessation of lactation when mother rats nurse only one pup. The remaining rat pups were placed with foster mothers.

Each incubator-reared (IR) rat pup was reared in an individual

¹Social isolation is defined as being separated from littermates and other subjects as well as being prevented from seeing them.
compartment of an incubator containing San-i-cel bedding material. Fig. 1 shows the incubator, and Fig. 2 shows the IR pups in their individual compartments. The IR pups were weaned (taken out of the incubator) at 22 days of age, weighed, and placed in individual living cages until testing and maintained on an ad-lib food and water supply.

Mother-reared

Each mother-reared (MR) rat pup was reared alone by its mother in an individual plastic rearing cage, measuring 19" long x 10-1/2" wide x 5" high, located in another room. These cages also contained San-i-cel bedding material which was not changed during the rearing period to avoid disturbing the mother rat who might have rejected or killed the pup. At 22 days of age the MR pups were weaned, weighed, and placed in individual living cages until testing. They were also maintained on an ad-lib food and water supply.

Apparatus

The incubator was constructed of wood and measured 72"

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1San-i-cel is a commercial bedding product made of ground corn cobs. It is sold by Paxton Processing Co., Inc., Paxton, Illinois.
Fig. 1. The Incubator.
Fig. 2. The incubator with the plastic cover pulled back, showing the incubator reared subjects.
long x 12" wide x 12" high. The individual rearing compartments measured 12" long x 6" wide x 4" high. The incubator was located in a separate lab room away from the MR animals. Every effort was made to keep the room clean and as pathogen free as possible to protect the IR animals from disease. The nest floor of the incubator was constructed of window screening and suspended over thermostatically-controlled, heated water to provide warmth and humidity. The temperature was kept at 92°F. for the first week and then lowered in stages to about room temperature at weaning time. A relative humidity of 60 to 80 per cent was maintained in the incubator at all times with the use of a plastic cover which captured moisture from the heated water (see Fig. 1).

Hand rearing

A four centimeter long, silicone nipple\(^1\) was used to feed the IR pups from a two milliliter capacity microsyringe\(^2\) clamped to a stand at a 45° angle. The microsyringe with its screw type plunger allowed maximum control of the rate at which the formula

\(^1\)Redar-S10029 Infant Rodent Feeder. Manufactured by R. E Darling Co., Inc., 16021 Industrial Drive, Gaithersburg, Maryland.

was administered. Every four hours for as long as the hand feeding process was necessary, measured amounts of a pasteurized\(^1\) formula were fed to the IR pups. The feeding process involved inserting the nipple, first lubricated with sterile corn oil, into the pup's mouth and down the esophagus, coordinating the pressure on the nipple with swallowing by the pup. Fig. 3 shows the feeding procedure. Feeding was accomplished as quickly as possible to avoid impairing S's breathing, yet with great care to avoid getting milk in the lungs and nasal passages. Feeding usually took less than thirty seconds to complete. After each feeding the IR Ss were perineally stroked with a corn oil-moistened brush to facilitate defecation. Fig. 4 shows this procedure.

A measured amount of .30cc. was given on the first day of feeding. On each subsequent day the amount was increased by .05cc., except when difficulty was encountered, then the dosage was not increased. At 16 days of age small pieces of commercial rat pellets dipped in formula were placed in small dishes inside of IR Ss' incubator compartments to supplement nipple feeding.

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\(^1\)I would like to thank the Upjohn Co. of Kalamazoo, Mich. for its suggestion of the formula: 108 gms. Esbilac, 1.2 gms. dextrose, 0.6 gms. D L Methionine, 1.2 cc. Zymatinic drops, 0.6 cc. Zyma Drops, 75 USP units of Tocopherols, plus three volumes of distilled water. The formula was pasteurize at 223° F. for ten minutes and kept at 98° F. while feeding.
Fig. 3. Feeding Procedure.
Fig. 4. Post-feeding anal stimulation.
At 18 days of age the IR Ss were taken off nipple feeding and given a diet of Gerber's strained beef, egg, and liver baby food also placed in small dishes inside of the incubator. The baby food was replaced by rat pellets and water at 21 days of age and continued thereafter.

Testing Schedule

Testing began at 79 days of age for each S and lasted 11 days. On days one through four Ss were placed in the open field test for 3 minutes. There was no testing on Day Five while Ss remained in their home cages. This was done in an attempt to keep the open field testing from influencing Ss' avoidance learning. On Day Six Ss were placed in the discriminated avoidance learning apparatus with no CS or UCS present (pseudo conditioning test). On days seven through eleven each S received 10 trials of discriminated avoidance learning.

Pretest defecation baseline

Before being tested in the open field, a pretest defecation baseline was obtained for each S while in the isolated home cage. The total number of boluses dropped by each S during the seven consecutive days immediately prior to the Open Field Test was recorded. The purpose of this measure was to determine if there
was any difference in normal defecation that could account for
differences displayed in the open field test.

Open field testing

The Open Field Test consisted of a plywood base, 45" square, with 18" high walls painted flat black except for thin
white lines which divided the floor into 9" squares (see Fig. 5). A 9" by 9" wooden "L" placed in one corner of the field formed a
starting compartment. Starting at 79 days of age, for four
successive days, each S was placed into the starting square;
after 10 seconds the "L" was removed giving S access to the rest
of the field. Each S remained in the field for 3 minutes during
which time the following measures were recorded:

(a) the total number of boluses dropped, (b) the number of
center squares entered, (c) the total number of center and outside
squares entered.

Discriminated avoidance testing

The discriminated avoidance learning apparatus consisted
of a chamber measuring 21.5" long by 9.5" wide by 11" high with
clear plexiglass walls and a grid floor of 3/16" stainless steel
rods set 3/4" apart. The chamber was divided in half by a
1-1/2" high plexiglass hurdle. A scrambler provided 0.5 ma.
of A.C. current through a 2.5 megohm resister wired in series
Fig. 5. Subject in the open field.
with the rat. The CS consisted of a buzzer located behind the middle of the rear wall and a light situated in each half of the ceiling. The chamber was positioned on a pivot device with the ends of the chamber resting on micro-switches. When a hurdle jump was made, $S$'s weight on that side of the chamber tripped the micro-switch and automatically terminated the CS when $S$ avoided, and the CS and UCS when $S$ escaped. The chamber was placed inside a sound attenuated box; white noise and fresh air was provided by an exhaust fan.

At the start of each trial $S$ was in one-half of the chamber. A buzzer sounded and the light on that side of the chamber went on. Five seconds later that side of the grid floor was electrified. If $S$ jumped to the other side of the chamber within 5 seconds it avoided the shock. Fig. 6 shows a $S$ making a hurdle jump response. If $S$ did not jump, it continued to receive shock until it escaped to the other side, except that the trial was automatically ended after 30 seconds. The total number of avoidance responses summed across the fifty trials was recorded. The $S$s received 10 trials per day with a 50 second interval separating each trial. Testing lasted five consecutive days.

To summarize the procedure, rat pups were reared during the preweaning period under one of two conditions: (a) with the mother, but without littermates ($N = 14$); (b) without the mother
Fig. 6. A subject in the discriminated avoidance apparatus making a hurdle jump response.
and without littermates in an incubator (N = 10). Following the rearing period the Ss were individually isolated until testing at 79 days of age. Measures of emotionality in the open field and the ability to learn a discriminated avoidance task were recorded. In addition to these behavioral measures the time S opened both eyes and weight at weaning (22 days) were recorded.

RESULTS

Eight of the Ss (two IR and six MR Ss) died before being tested in the open field and one more MR S died just prior to being tested on discriminated avoidance. As a result of these deaths the two groups did not contain equal numbers of the same sex. The IR group contained four males and two females (N = 8) for all tests, while the MR group contained six males and two females for all tests (N = 8), except for the avoidance test (N = 7). A non parametric statistical test, the Mann-Whitney U Test (Siegal, 1956), was used to analyze the data at the .05 level (two tailed).

The results and treatment of data for each of the measures will be presented in the following order:

(a) eye opening time, (b) weight at weaning (22 days), (c) pretest defecation baseline, (d) open field defecation, (e) open field exploration, (f) discriminated avoidance learning.
Eye Opening

Table 1 shows the age in days that each S opened both eyes. On the average, MR Ss opened both eyes a day earlier (14.5 days) than IR Ss (15.6 days), however this difference was not significant (U = 21, p(U = 21) = 0.278).

Weight At Weaning Time

The weight of each S at weaning is shown in Table 2. It became apparent shortly after the start of the experiment that the IR Ss were not developing as well as their MR counterparts. As a result of this disparity, the weight differences at age 22 days were found to be highly significant (U = 1, p(U = 1) = 0.000).

Pretest Defecation Baseline

The total number of boluses dropped by each S during the seven consecutive days prior to open field testing as shown in Table 3. The two groups did not differ significantly on this measure (U = 29, p(U = 29) = 0.798). Appendix A reflects the day-to-day recording by S of boluses dropped.

Open Field Defecation

The total number of boluses dropped by each S in the Open
### Table 1

*Age in Days of S's with Both Eyes Open*

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Sex</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR 1</td>
<td>M</td>
<td>16</td>
<td>MR 1</td>
</tr>
<tr>
<td>IR 2</td>
<td>F</td>
<td>12</td>
<td>MR 2</td>
</tr>
<tr>
<td>IR 3</td>
<td>M</td>
<td>13</td>
<td>MR 3</td>
</tr>
<tr>
<td>IR 4</td>
<td>F</td>
<td>17</td>
<td>MR 4</td>
</tr>
<tr>
<td>IR 5</td>
<td>M</td>
<td>18</td>
<td>MR 5</td>
</tr>
<tr>
<td>IR 6</td>
<td>F</td>
<td>14</td>
<td>MR 6</td>
</tr>
<tr>
<td>IR 7</td>
<td>M</td>
<td>18</td>
<td>MR 7</td>
</tr>
<tr>
<td>IR 8</td>
<td>F</td>
<td>17</td>
<td>MR 8</td>
</tr>
</tbody>
</table>

Mean = 15.6

Mean = 14.5
Table 2

Weight in Grams of each S at 22 Days of Age

<table>
<thead>
<tr>
<th>S</th>
<th>Sex</th>
<th>Weight</th>
<th>S</th>
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<th>Weight</th>
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<tr>
<td>IR 1</td>
<td>M</td>
<td>26.4</td>
<td>MR 1</td>
<td>M</td>
<td>38.1</td>
</tr>
<tr>
<td>IR 2</td>
<td>F</td>
<td>26.4</td>
<td>MR 2</td>
<td>M</td>
<td>55.8</td>
</tr>
<tr>
<td>IR 3</td>
<td>M</td>
<td>27.3</td>
<td>MR 3</td>
<td>M</td>
<td>42.6</td>
</tr>
<tr>
<td>IR 4</td>
<td>F</td>
<td>27.5</td>
<td>MR 4</td>
<td>F</td>
<td>40.5</td>
</tr>
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<td>IR 5</td>
<td>M</td>
<td>27.6</td>
<td>MR 5</td>
<td>M</td>
<td>47.7</td>
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<td>IR 6</td>
<td>F</td>
<td>27.6</td>
<td>MR 6</td>
<td>M</td>
<td>45.5</td>
</tr>
<tr>
<td>IR 7</td>
<td>M</td>
<td>33.8</td>
<td>MR 7</td>
<td>F</td>
<td>33.1</td>
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<tr>
<td>IR 8</td>
<td>F</td>
<td>22.6</td>
<td>MR 8</td>
<td>M</td>
<td>58.8</td>
</tr>
</tbody>
</table>

Mean = 26.5

Mean = 45.2
Table 3

Total Number of Boluses Deposited in the Home Cage During 7 Consecutive Days Prior to Open Field Testing

<table>
<thead>
<tr>
<th>S</th>
<th>Sex</th>
<th>Boluses</th>
<th>S</th>
<th>Sex</th>
<th>Boluses</th>
</tr>
</thead>
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<td>MR 1</td>
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<td>F</td>
<td>179</td>
<td>MR 2</td>
<td>M</td>
<td>219</td>
</tr>
<tr>
<td>IR 3</td>
<td>M</td>
<td>194</td>
<td>MR 3</td>
<td>M</td>
<td>190</td>
</tr>
<tr>
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<td>IR 6</td>
<td>F</td>
<td>217</td>
<td>MR 6</td>
<td>M</td>
<td>253</td>
</tr>
<tr>
<td>IR 7</td>
<td>M</td>
<td>203</td>
<td>MR 7</td>
<td>F</td>
<td>204</td>
</tr>
<tr>
<td>IR 8</td>
<td>F</td>
<td>257</td>
<td>MR 8</td>
<td>M</td>
<td>144</td>
</tr>
</tbody>
</table>

Total = 1,658  Total = 1,742

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Field Test is shown in Table 4. No significant difference between the two groups was found on this measure ($U = 21$, $p(U = 21) = .278$). A day by day comparison, however, resulted in a significant difference between the groups on Day One ($U = 8$, $p(U = 8) = .005$). A graph of these data showing the number of boluses dropped by each group on the four consecutive days of the open field test is presented in Fig. 7.

Open Field Exploration

The total number of squares entered by each $S$ is shown in Table 5. The two groups differed significantly on this measure ($U = 9$, $p(U = 9) = .014$). A graph of these data showing the total number of squares entered by each group on the four consecutive days of the open field test is presented in Fig. 8.

Discriminated Avoidance Learning

The total number of avoidance responses made by each $S$ is shown in Table 6. The $U$ Test failed to show a significant difference between the two groups on this measure ($U = 24$, $p(U = 24) = .694$). However, a day by day comparison revealed a significant difference between the groups on Day One ($U = 6$, $p(U = 6) = .015$). A graph of these data showing the average number of avoidance responses made by each group is presented in Fig. 9. The number of avoidance
Table 4

Number of Boluses Dropped by each S in the Open Field

<table>
<thead>
<tr>
<th>S</th>
<th>Sex</th>
<th>Boluses</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>Days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
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<td>0</td>
</tr>
<tr>
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<tr>
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<td>F</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

| MR 1 | M   | 2      | 2      | 0      | 0      | 4       |
| MR 2 | M   | 3      | 2      | 3      | 5      | 13      |
| MR 3 | M   | 2      | 4      | 2      | 6      | 14      |
| MR 4 | F   | 5      | 3      | 3      | 3      | 14      |
| MR 5 | M   | 4      | 2      | 0      | 1      | 7       |
| MR 6 | M   | 7      | 3      | 3      | 4      | 17      |
| MR 7 | F   | 1      | 2      | 1      | 2      | 6       |
| MR 8 | M   | 0      | 2      | 0      | 0      | 2       |
| Total|     | 24     | 20     | 12     | 21     | 77      |

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Fig. 7. Open Field Defecation Scores.
<table>
<thead>
<tr>
<th>S</th>
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<th>Squares</th>
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</thead>
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</tr>
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<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
</tbody>
</table>

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Fig. 8. Open Field Activity Scores.
Table 6

Total Number of Avoidance Responses for each S During 5 Test Sessions (10 Trials/Day)

<table>
<thead>
<tr>
<th></th>
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<th>Responses</th>
<th></th>
<th>Sex</th>
<th>Responses</th>
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<td>MR 3</td>
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<td>19</td>
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<tr>
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<td>35</td>
<td>MR 4</td>
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<tr>
<td>IR 6</td>
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<td>40</td>
<td>MR 6</td>
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<td>M</td>
<td>35</td>
<td>MR 7</td>
<td>F</td>
<td>15</td>
</tr>
<tr>
<td>IR 8</td>
<td>F</td>
<td>13</td>
<td>MR 8</td>
<td>M</td>
<td>**</td>
</tr>
</tbody>
</table>

Total 199                  Total 153
Mean = 24.9                Mean = 21.8

**S MR 8 died on day 2 of avoidance testing.
Fig. 9. Average number of avoidance responses.
responses made by each $S$ on each day of avoidance testing is shown in appendix B.

DISCUSSION

A relatively new procedure was used in this study to provide infant rats with minimal maternal care and peer interaction. Previous attempts have been made to deprive rats of normal social interaction by separating them from their mothers and littermates. This study however, was among the first to provide continuous isolation starting within 72 hours after birth. Isolating the $S$s so soon after birth was made possible through the use of newly developed hand rearing techniques with the aid of an incubator.

Hand rearing rat pups in an incubator has proven to be an effective method for producing socially isolated rat pups. Using these techniques, $S$s can be given a tightly controlled, limited social history. The effects of reduced social interaction can now be observed on many dependant variables with rats as subjects. Hand rearing rats however, does present a unique problem in studies of social isolation. The problem, as explained earlier, is that hand reared $S$s receive supranormal stimulation as a natural consequence of hand rearing. This unwanted stimulation occurs when the $S$s are picked up and fed--four times each day until weaning at approximately 22 days. Furthermore, it has
been amply demonstrated that social isolation and physical stimulation produce opposite behavior patterns with respect to emotionality. Social isolation has been shown to produce a timid, emotional S with impaired learning ability. Physical stimulation, to the contrary, yields a more hardy, less emotional S with increased learning ability. The problem, the intrusion of an unwanted variable, is presently unsolvable in hand rearing studies.

The effects of social isolation were observed on three types of measures in this study: (a) physiological--the number of days after birth when Ss' eyes opened and Ss' weight at weaning; (b) emotional--the number of boluses dropped and number of squares entered in an open field situation; and (c) learning--the number of avoidance responses made by Ss on a discriminated avoidance test.

The eye opening data and particularly Ss' 22 day weight reflect the IR Ss' retarded development. It became apparent after only a few days of incubator rearing that IR Ss were not developing as well as MR Ss. Also, IR Ss' body hair appeared several days later than on MR Ss. The manner in which the IR pups were fed, rather than any nutritional deficit in the formula

1 The subjects' weights were not recorded at the time of testing in order to minimize extraneous emotionality.
is believed responsible for these differences. The hand rearing techniques developed in earlier studies (Pleasants, 1965; Thoman et al., 1968) were followed yet modified to the particular needs of this study.

The fragile appearance of the new born IR Ss in addition to feeding difficulties encountered in previous studies produced a tendency to underfeed these Ss. This modification in procedure was accepted over the possibility of a stomach rupture or suffocation among IR Ss by overfeeding them. There is a great risk, especially during the first few days of rearing, of getting formula into the lungs and nasal passages. In this event, death usually occurs within minutes. As S gets older and stronger, he is more successful in expelling formula from his breathing passages.

The IR Ss appear to have demonstrated less emotional behavior in the open field inspite of their social deprivation than did MR Ss. The difference in the open field defecation rate between groups, while not significant for all 4 days combined, was highly significant for Day One. Only two IR Ss defecated in the open field on the first day of testing, while seven of the eight MR Ss dropped boluses.

The IR Ss also appeared less emotional according to their exploratory behavior. They entered twice as many squares as
did the MR Ss for the whole test. In addition, no MR S entered an inside square, those in the middle of the field not bordered by walls. Theoretically, entrance into these squares should create maximum emotionality levels. This would be due to the rat's thigmotaxic preference to be under or next to an object. In contrast, three IR Ss entered a total of 30 inside squares.

A closer inspection of the open field exploration results reveals that on Day One of testing MR Ss entered more squares than IR Ss. The difference was not significant. Such results on the initial day of open field testing are not considered unusual according to Whimbey and Denenberg (1967). They report that while high activity by Ss in the open field has usually been interpreted as an indication of low emotionality, it may not be a valid interpretation. The high activity—low emotionality interpretation of open field performance has been based on the assumption that activity in a novel situation is exploration and that Ss which explore are not fearful. It may be, however, that the increased activity of rats placed in a novel situation represents attempts to escape rather than exploration due to curiosity.

Welker (Whimbey & Denenberg, 1967) used an open field which had a small dark enclosed box attached to it. He allowed differently treated Ss to spend 4 min. in the field before opening a sliding door which gave S an opportunity to escape into the box.
After exhibiting heightened activity when first placed into the field, Ss still escaped into the dark box as soon as the open doorway was encountered. Welker's data, then, indicate that an active rat may be seeking escape from fear.

After administering different forms of stimulation to infant rats, Salama and Hunt (Whimbey & Denenberg, 1967) measured emotionality in adulthood by recording activity, urination, and defecation in a T maze on 2 successive days. The stimulated Ss defecated and urinated significantly less than controls on the first test day, and were less active than controls (not significantly so). However, on the second test day, the stimulated Ss were significantly more active than controls.

Whimbey and Denenberg (1967) stimulated rats in infancy and measured their emotionality in adulthood by placing them in the open field on four consecutive days and once 9 days later (14 days after the first day). They found that rats which were highly emotional had high defecation scores on Days 1, 2, 3, 4, and 14, but were more active on Day One and less active on the remaining days. Whimbey and Denenberg state that

"Our own experiences with open field testing have led us to conclude that the first day's activity score is not a very meaningful datum. On some occasions we found no activity differences between groups stimulated in infancy and nonstimulated controls, or we have found differences in the opposite direction...the defecation
index has consistently held up, showing a greater frequency or higher rate of defecation in the control group on the first test day (Whimbey & Denenberg, 1967, pp. 500-501).

The open field testing results of the present study correspond with both the Salama and Hunt study and the Whimbey and Denenberg study. The MR Ss had a significantly higher defecation score than IR Ss on Day One—which Whimbey and Denenberg cite as a consistently reliable indicator of emotionality. In addition, the MR Ss were more active on Day One than IR Ss, but significantly less active on days 2-4. High activity—attempts to escape from fear—on Day One followed by a reduction on succeeding days can be explained in terms of reinforcement theory principles. Attempts by MR Ss to escape were not rewarded by actual escape from the field and were consequently extinguished. The curve in Fig. 8 showing MR Ss' open field activity follows that of a classic extinction curve—MR Ss entered fewer squares on each successive day. The IR Ss whose open field activity was not motivated by fear, would be expected to explore more squares each day which they did. These results then lead to the conclusion that MR Ss were more emotional in the open field.

The discriminated avoidance learning data did not show a significant difference between the two groups for the test as a whole. Both groups reached the same level of avoidance responses
on Day Four with MR Ss making slightly more avoidance responses than IR Ss (not significant) on the last day of testing. The data do reveal, however, that IR Ss made more avoidance responses sooner than MR Ss. More avoidance responses were made by IR Ss on days 1, 2, and 3 of testing than by MR Ss with a significant difference between the groups on Day One.

Both groups of Ss had to receive at least one shock, usually a number of them, before learning to anticipate it and avoid it. There is little doubt that this is a highly emotional and disturbing experience for a rat. The Ss, when receiving shock, jumped about while squealing and defecating. It appears that IR Ss were able to profit more than MR Ss from initial exposure to this learning situation. The interpretation here is that IR Ss were again less emotional than MR Ss enabling IR Ss to withstand the shock and quickly adjust its stresses. Thus IR Ss were able to learn the correct response earlier than MR Ss.

The present study shows that the absence of maternal care and peer interaction during infancy (a) did not lead to increased emotionality in an open field situation, and (b) did not affect learning ability on a discriminated avoidance task. However, it is conceivable that the physical stimulation received by the IR Ss from hand rearing procedures might have produced the significant differences that were observed. The effects of stimulation
proved beneficial to IR Ss, resulting in lower emotionality and increased ability to meet stress. These findings coincide with the results of previous studies in which Ss were stimulated during infancy (Denenberg, 1964). The present study was not designed to test the variable of stimulation through handling of IR Ss. This question could be studied by employing a group of self-fed, incubator reared Ss.

An explanation for the results of the present study lies in the possibility that the effects of stimulation were stronger than the effects of isolation. In this event, the rather intense stimulation inherent in incubator rearing procedures could have masked the effects of a relatively weak isolation variable. Great concern was shown for the survival of the IR Ss and did result in their underfeeding. It is likely that this same concern also produced additional stimulation for IR Ss in the form of extra attention—picking them up for inspection, etc. This additional stimulation in conjunction with hand feeding very likely weakened the effects of isolation or masked them completely.

Previous studies which have employed incubator rearing techniques (Thoman, 1963, 1965; Thoman et al., 1968) were either unaware of the confounding effects of stimulation or chose not to mention them. This is surprising as the literature is full of examples demonstrating the beneficial effects of stimulation.
In view of the present findings, future studies employing incubator rearing techniques must become aware of possible stimulation effects inherent in these techniques. Also, steps must be taken to reduce these effects by minimizing the stimulation in hand feeding and related procedures.

An ideal method to achieve this goal would be the development and use of a self feeding apparatus. This device would serve two main purposes: First, it would allow each incubator reared S to feed at will. This would preclude force feeding and the danger of getting formula in Ss' lungs. Second, it would also reduce the number of times each S is picked up and the length of time he is held. It is also recommended that future studies limit the visual field of incubator Ss with an opaque screen over each incubator compartment. This measure would further reduce the stimulus input to other Ss when E has to inspect an S or service the incubator.

SUMMARY

This study attempted to provide data on rats deprived of normal maternal care and peer interaction. This deprivation started shortly after birth and was maintained throughout the preweaning period. Answers were sought for two questions: (a) does the absence of maternal care and peer interaction lead
to increased emotionality in a novel situation designed to elicit emotional reactivity? and (b) does such deprivation also effect later learning ability?

Rat pups were reared for the first 22 days of life under two conditions: (a) without the mother and without littermates in an incubator (IR S), (b) with the mother, but without littermates in rearing cages (MR S). At 22 days of age all Ss were removed to individual living cages and isolated until testing at 79 days of age. Measures of emotionality in the open field and discriminated avoidance learning were taken. In addition to these behavioral measures, the time S opened both eyes and S's weight at weaning were recorded.

Differences between the two groups were found on all measures. The IR Ss were found to be physiologically inferior to MR Ss. IR Ss opened their eyes later than MR Ss and weighed significantly less at 22 days of age. Reductions in the amounts of formula fed to IR Ss were held responsible for these results.

The results of the open field test and avoidance learning task showed that IR Ss were less emotional and able to adjust more rapidly to stress than MR Ss. These results coincided with those found in studies in which Ss had been given supra-normal stimulation during infancy. It was concluded that the physical stimulation of incubator rearing methods had produced
these results. The possibility that this heightened stimulation had masked the effects of isolation was discussed.

The development and use of a self feeding device was suggested as a means to minimize the confounding effects of stimulation in future incubator rearing studies.
REFERENCES


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APPENDIX A

Boluses Deposited by All Ss During the Pretest Defecation Baseline
Boluses Deposited by IR Ss During the Pretest Defecation Baseline

<table>
<thead>
<tr>
<th>S</th>
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<th>3</th>
<th>4</th>
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Totals: 246 221 260 235 206 221 269 1,658
Boluses Deposited by MR Ss During the Pretest Defecation Baseline

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Totals 251 249 269 265 228 235 245 1,742

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APPENDIX B

Avoidance Responses for All Ss on
All Five Test Sessions
### Avoidance Responses for IR Ss on All Five Test Sessions

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### Avoidance Responses for MR Ss on All Five Test Sessions

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**Totals** | **3** | **25** | **34** | **43** | **49** | **153**

*S MR 8 died on day 2 of avoidance testing.*