EFFECTS OF HOUSING CONDITIONS ON STRESS RESPONSES, FEEDING, AND DRINKING IN MALE AND FEMALE RATS

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BROWN

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799

APPROVAL SHEET

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Name of Candidate: Kelly J. Brown
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Thesis and Abstract Approved:

Jerome E. Singer, Ph.D.
Committee Chairperson

20 March '95
Date

Neil E. Grunberg, Ph.D.
Committee Member

2/7/95
Date

David S. Krantz, Ph.D.
Committee Member

3/20/95
Date

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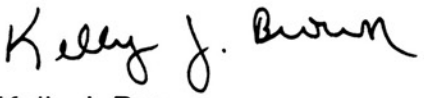

Kelly J. Brown
Department of Medical and Clinical Psychology
Uniformed Services University of the Health Sciences

ABSTRACT

Title of Thesis: Effects of Housing Conditions on Stress Responses, Feeding, and Drinking in Male and Female Rats

Kelly J. Brown, Master of Science, 1995

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

Professor

Department of Medical and Clinical Psychology

Housing animals under particular environmental conditions alters animals' behavior, physiological and biochemical status, and immune system functioning. If housing conditions prior to experimental treatments affects male and female animals differently, then these differences must be identified and accounted for to avoid reaching false conclusions regarding sex differences in response to other variables such as drugs, infections, and diets. Effects of differential housing conditions were examined in male and female rats to identify possible sex differences. Rats were assigned to same sex grouped, crowded, or individually housed conditions in two experiments. Experiment 1 examined the effects of individual versus crowded housing conditions on corticosterone, a biochemical index of stress, in male and female rats. Experiment 2 extended the findings of Experiment 1 by separately manipulating spatial and population aspects of housing with male and female rats. Male rats had a greater stress response, as indexed by an increase in

corticosterone, under crowded conditions. In contrast, female rats had a greater stress response when individually housed. Grouped conditions were relatively ineffective in producing a stress response in male rats, but had an effect on females similar to the crowded conditions. Spatial crowding was the key variable for males, whereas the number of other animals was more important for females. Taken together, these findings indicate that male and female rats are differentially affected by their environments and that housing conditions alone can affect biochemical stress responses. In addition, housing conditions affected food and water consumption independent of stress responding and differently for males and females. Specifically, crowded males consumed more bland food than did grouped males. Individually housed males consumed more sweet food than did grouped males. In contrast, the individually housed females consumed more bland food than did grouped or crowded females and these treatment conditions did not differ in sweet food consumption. Crowded males and females consumed the greatest amounts of water. These studies establish the use of housing conditions as a non-physical, non-painful animal model of stress that can be used to investigate further differences in stress and gender interactions.

Effects of Housing Conditions on Stress Responses, Feeding, and Drinking in Male and Female Rats

by

Kelly J. Brown

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INTRODUCTION

Previous studies have reported that stress may affect or interact with appetitive behaviors such as eating, and licit and illicit drug use and abuse. It has been reported that stress changes particular food preferences and increases nicotine and opiate self-administration. In addition, many findings have suggested that differences commonly are found between sexes in these variables (Grunberg, Winders, & Wewers, 1991; Lex, 1991).

Much of the literature on stress and appetitive behaviors is based on human self-report and, consequently, show correlation rather that causation. Reasons for this trend center around practical and ethical considerations that make it impossible to generate stress reactions in human subjects that are equivalent in intensity and duration to real life. In addition, it is unethical to present drug naive human subjects with addictive drugs over a long period of time.

Animal models provide a valuable tool to carefully examine effects of stress on behaviors and on physiological responses (Straub, Singer, & Grunberg, 1986; Surwit, Feinglos, Livingston, Kuhn, & McCubbin, 1984; Manuck, Kaplan, & Clarkson, 1983). The most commonly used stressors in animal studies include immobilization, electric footshock, and forced running (Raygada, Shaham, Nespor, Kant, & Grunberg, 1992; Kant, Mougey, & Meyerhoff, 1989; Meyerhoff, Kant, Nielsen, & Mougey, 1984). Although effective and useful, these stressors are physical, potentially painful, and they offer little face validity compared to the social and psychological stressors that are of particular interest in humans. A

non-painful stressor that could minimize discomfort but still maximize stress responses with animals would provide an important and useful paradigm for the study of stress and stressors' effects on other behaviors. Crowding is a stressor that can fulfil these requirements.

Housing animals together under particular environmental conditions results in behavioral, immunological, and biochemical changes indicative of a stress response (Singh, D'Souza, & Singh, 1991; Peng, Lang, Drozdowicz, & Ohlsson-Wilhelm, 1989; Armario, Garcia-Marquez, & Jolin, 1987; Gamallo, Villanua, & Beato, 1986; Calhoun, 1962). Social conditions have been manipulated by crowding a small number of animals in a small space or grouping a large numbers of animals in a large space. These previous studies are valuable but they have several major limitations. Specifically, these studies typically examine males and they have not examined whether sex differences exist. In addition these studies usually manipulate the environment for roughly 24 hours a day and, therefore, do not allow time for individual behavioral assessments (e.g., individual feeding, drug self-administration, activity) within the paradigm.

The present work included two experiments that were designed to examine the effects of differential housing on stress responses, feeding, and drinking in male and female rats. Experiment 1 tested the hypothesis that crowding males and females in same-sex cages would produce a stress response and change feeding and drinking patterns. It was hypothesized that both sexes would show a stress response under crowded conditions as indexed

by an increase in biochemical markers (e.g., corticosterone, adrenocorticotrophin hormone). In addition, it was hypothesized that males would decrease food but increase water consumption, whereas females would show an increase in both these appetitive behaviors. These hypotheses were based on research findings reporting that different housing conditions produce behavioral, immunological, and biochemical changes indicative of stress (Peng et al., 1989; Armario et al., 1987; Gamallo et al., 1986; Armario, Ortiz, & Balasch, 1984; Calhoun, 1962). Although no previous studies has examined the direct effects of crowding on the appetitive behaviors of female rats, Singh and Singh (1991) reports that crowded female rats do not show weight decreases. Furthermore, human data show that women increase food consumption under stressful conditions (Grunberg & Straub, 1992).

Experiment 2 was designed to independently manipulate spatial and population dimensions of crowding in male and female rats. It was hypothesized that males would be stressed by spatial restrictions, whereas females would be stressed by the number of potential interactions. It also was hypothesized that crowded (5-crowded and 10-crowded) males would consume more food and water relative to grouped (5-grouped and 10-grouped) males and that males in both the crowded or grouped conditions would consume more than individually housed males. In contrast, individually housed females were predicted to consume more food and water than females housed in numbers of 10 (10-grouped and 10-crowded) that would consume more than females housed in numbers of 5 (5-grouped and 5-crowded). These hypotheses were based on the

results of Experiment 1 and on sex differences reported in the human crowding literature (Baum & Koman, 1976; Epstein & Karlin, 1975; Ross, Layton, Erickson, & Schopler, 1973; Stokols, Rall, Pinner, & Schopler, 1973; Freedman, Levy, Buchanan, & Price, 1972). Specifically, males are found to be more reactive to spatial restrictions, whereas males and females negatively respond to conditions involving too many uncontrollable interactions (Baum & Koman, 1976).

Experiment 1

Overview

The purpose of this experiment was to examine the effects of spatial crowding in male and female rats. The experiment consisted of two phases with between and within subject components in each phase. Phase 2 was added to the design of the experiment to make comparisons between grouped and crowded conditions that were not examined in Phase 1 and to account for size differences between male and female rats by providing them with different size cages. These additions allowed for space and population comparisons to be made in relation to the individually housed condition. It was hypothesized that male and female rats would exhibit similar stress response patterns, as indexed by increases in biochemical markers, under the crowded condition compared with the individually housed condition.

Subjects included male and female Wistar rats. Housing rooms were maintained at 23°C and 50% relative humidity on a 12 hour light/dark cycle. All animals had continuous access to food and water.

During Phase I, all subjects were individually housed in standard rat cages (43 X 20 X 20 cm) for 5 days; crowded in same-sex numbers of 4 per cage (27 X 15 X 13 cm) or individually housed for 4 days; individually housed for 5 days; and again crowded or individually housed for 9 days. All subjects were kept in their experimental conditions for 18 hours/day and were transferred to individual rat cages for 6 hours/day during which time food and water consumption were

measured and behavioral observations were made. All animals were individually housed again at the end of Phase 1 for six weeks. During this time subjects were left undisturbed except for standard care. This procedure was adopted to maintain experimental control and to minimize any between-subject differences prior to the start of Phase 2.

During Phase 2, subjects were group housed in numbers of 3 and 4 per cage for 4 days. Male rats were group housed in 47 X 37 X 19 cm cages and females were group housed in 43 X 20 X 20 cm cages. Different cages were used to account for male and female size differences. Next, all animals were crowded for 5 days in same-sex numbers of three or four. Male rats were crowded in 32 X 20 X 18 cm cages and female rats were crowded in 27 X 15 X 13 cm cages. Rats then were group housed for another 3 days, individually housed for 3 days, and left undisturbed for 2 days. During the last 9 days of Phase 2, 4 rats that were originally individually housed in Phase I were crowded and the remaining 3 rats that were originally crowded were individually housed. Throughout the experiment, all rats were kept in their experimental conditions for 18 hours/day and were transferred to individual rat cages for 6 hours/day during which time food and water consumption was measured and behavioral observations were made. All rats were sacrificed and plasma was assayed for corticosterone and adrenocorticotrophin hormone to assess stress responses.

Hypotheses

Hypothesis 1. It was hypothesized that crowded male and female rats

would have a greater stress response, as indexed by biochemical markers (increases in corticosterone and adrenocorticotrophin hormone), than would the individually housed rats.

Rationale: Various physical stressors increase corticosterone and Adrenocorticotrophin hormone in rats (Raygada et al., 1992; Kant et al., 1989). In addition, housing animals together under particular environmental conditions can result in behavioral, immunological, and biochemical changes indicative of stress (Peng et al., 1989; Armario et al., 1987; Gamallo et al., 1986; Calhoun, 1962).

Hypothesis 2. It was hypothesized that male rats would decrease food consumption when housed under crowded conditions compared with their individually housed counterparts and compared with their behavior during group housing.

Rationale: Armario et al. (1984) reported that crowding 10 male rats per cage (48 X 23 X 14 cm) resulted in decreased food consumption compared with control male rats housed 3 rats per cage (48 X 23 X 14 cm). Gamallo et al. (1986) showed no difference in food intake between males crowded 10 per cage and control males grouped 5 per cage.

Hypothesis 3. It was hypothesized that female rats would consume more food under the crowded condition compared with their individually housed counterparts and compared with their behavior during group housing.

Rationale: No previous studies have examined the direct effects of crowding on female rats. Following the hypothesis that crowded rats should be more stressed than individual rats and human data that stress increases food

consumption in women (Grunberg & Straub, 1992), it follows that crowded female rats should consume more food than individually housed or grouped females.

Hypothesis 4. It was hypothesized that male rats would increase water consumption when housed under crowded conditions as compared with their individually housed counterparts or their own behavior during group housing.

Rationale: Armario et al. (1984) showed that crowding 10 male rats per cage (48 X 23 X 14 cm) resulted in increased water consumption compared with male control rats housed 3 rats per cage (48 X 23 X 14 cm).

Hypothesis 5. It was hypothesized that crowded female rats would consume more water in comparison to their individually housed counterparts and as compared with their own behavior during group housing.

Rationale: No previous studies have examined the direct effects of crowding on female rats. Because food and water consumption are highly correlated in rat populations and because it was hypothesized that female rats would consume more food under crowded conditions, it follows that crowded female rats also should consume more water than individually housed or grouped females.

Hypothesis 6: It was hypothesized that crowded male rats would not gain weight as quickly or would lose weight compared with their individually housed counterparts and compared with themselves during group housing.

Rationale: Earlier studies have reported (Armario et al., 1987; Gamallo et al., 1987; Armario et al., 1984) that crowding 10 male rats per cage (48 X 23 X

14 cm) results in decreased body weight compared with male control rats housed 3 or 5 rats per cage.

Hypothesis 7: It was hypothesized that crowded female rats would gain weight more quickly than would their individually housed counterparts and compared with themselves during group housing.

Rationale: No previous studies have examined the direct effects of crowding on female rats. However, based on the previous hypotheses that crowded female rats would consume more food and water than individually or grouped housed female rats, it follows that they should gain weight more quickly.

Methods

Subjects

Subjects included 8 male and 8 female Wistar rats (Charles River, Wilmington, MA). All animals were individually housed in standard polypropylene shoebox cages (44 X 23 X 20 cm) on hardwood chip contact bedding (Pine-Dri) for two weeks after their arrival. During this time animals had continuous access to standard rodent pellets (Agway Prolab 3500) and water. Housing rooms were maintained at 23°C at 50% relative humidity on a 12 hour light/dark cycle. At the start of the experiment male and female rats weighed an average of 305 g and 239 g and the average ages were approximately 9 and 11 weeks, respectively.

Materials and Equipment

Food cups

Animals had access to standard powdered rat chow (Agway Prolab 3200) for 6 hours a day. Food was provided to the animals in metal food cups with lids that had a 2-inch diameter opening. Food cups were designed to hang over the edge of the cage and sit 1-2 inches off the cage bottom.

Activity chambers

Animals were placed in individual electronic physical activity monitoring chambers (Omnitech Electronics, Columbus, Ohio) to measure activity and record vertical and horizontal movement via a grid of infrared light beams. Equally spaced beams traversed the plastic chambers (42 X 42 X 30 cm) from front to back and left to right. The body of the animal placed in the chambers broke the beams revealing any horizontal or vertical movement.

Procedure

Phase 1

Baseline measurements were taken for 5 days. During this time all animals were individually housed in standard rat cages (44 X 23 X 20 cm) for 18 hours (1630-1030) where they had continuous access to food pellets and water. Rats then were transferred to another standard rat cage with grid floors for 6 hours (1030-1630) where they were again individually housed and had continuous access to standard powdered rat chow and water. Grid floors were used during the 6-hour period so rats could not eat their feces.

Body weight, 6-hour water consumption, and 6-hour powdered chow

consumption were measured. Animals were weighed before and after being placed in the 18-hour housing condition. Food cups and water bottles were weighed before and after each 6-hour housing phase on an electronic balance (Sartorius). Amount consumed was calculated as the pre-housing condition minus the post-housing condition.

For one hour during the 6-hour housing condition, animals were placed in individual activity chambers to record horizontal and vertical activity and observe specific behaviors. Four rats were observed at one time and groups were rotated to control for naturally occurring changes in activity levels. Occurrences of sniffing, rearing, trembling, freezing, teeth chattering, and grooming were recorded by two raters for twenty minutes at the beginning of the hour, then for one minute every ten minutes. Raters observed one rat at a time for 1 second and recorded the behaviors. The rat's position in the chamber was recorded every 5 minutes, horizontal and vertical readings were recorded every 10 minutes, and number of feces was recorded at the end of the hour. Chambers were cleaned with a mild cleanser and rinsed with water between subjects.

After the baseline period, half the rats were crowded in same-sex numbers of 4 in standard mouse cages (27 X 15 X 13 cm) for 18 hours where they again had continuous access to food and water. At the end of the 18 hours, all subjects were transferred to standard rat cages and individually housed for 6 hours. The other 8 rats continued to be individually housed for the 18-hour housing condition and then were transferred to identical 6-hour housing conditions. This testing phases continued for 4 days after which time, animals

were individually housed for 4 days. Then a second testing phase was performed that was identical to the first testing phase and lasted for 9 days. The same dependent measures recorded during baseline were assessed throughout the remainder of Phase 1 except that rats were placed in the activity monitors and observed only every other day, alternating between male and female rats. Next, subjects were individually housed and left undisturbed for 6 weeks except for normal care.

Phase 2

Phase 2 consisted of grouped and crowded phases. First, all subjects were group housed in same-sex numbers of 4. Male rats were group housed in 47 X 37 X 19 cm cages and females were group housed in 43 X 20 X 20 cm cages. Different cages were used to account for male and female size differences that were not controlled for in Phase 1.

On the first 18 hour housing day, one male rat was attacked by his group and had to be removed from the experiment. To keep conditions comparable between males and females, a female rat also was removed. The remaining 7 rats were group housed in same-sex numbers of 3 and 4 in cages with the same dimensions as specified above. This period lasted for 4 days.

All animals then were crowded for 5 days in same-sex numbers of 3 and 4. Male rats were crowded in 32 X 20 X 18 cm cages and female rats were crowded in 27 X 15 X 13 cm cages. Rats then were grouped housed for another 3 days, individually housed for 3 days, and left undisturbed for 2 days. Nine days before the animals were sacrificed, the four rats that were originally individually

housed in Phase 1 were crowded and the remaining three rats that were originally crowded were individually housed. Animals were put in these conditions so that individual housing and crowded conditions could be compared based on their biochemical indices. In addition, the conditions were crossed to minimize any carry over effects from the previous manipulations.

Throughout the experiment, all rats were kept in their experimental conditions for 18 hours/day and were transferred to individual rat cages for 6 hours/day during which time powdered food and water consumption were measured. Observations were made for 20 minutes by two independent raters every other day when rats were initially placed in their 18-hour and 6-hour home cages. Group observations included chasing, approach, avoidance, sniffing, fighting, community grooming, community eating, and community drinking. Raters observed each cage of subjects for 5 seconds and recorded the behaviors for each rat in the cage. Individual recorded behaviors included eating food, eating feces, drinking, grooming, trembling, hopping, abnormal posture, teeth chattering, sniffing, rearing, rocking, freezing, sleeping, and nonmovement. Raters observed one rat at a time for 1 second and recorded the behaviors.

At the completion of Experiment 1, animals were sacrificed without anesthesia and trunk blood was collected in tubes containing ethylenediamine tetra-acetic acid (.07 ml of 15% EDTA solution). Blood was centrifuged (1500 x g) for 20 minutes at 4°C and serum or plasma was stored and frozen at -70°C in 3 separate micro tubes until assayed. Aprotinin (40 µg/ml of plasma) was added to 1 micro tube of plasma prior to storage to inhibit enzymatic degradation. Plasma

was assayed for corticosterone and adrenocorticotrophin hormone using standard radioimmunoassay kits (ICN Biomedicals and Incstar, respectively).

Results

PHASE 1

Food consumption

Figure 1 presents the amount of powdered chow consumed in six hours when the male and female rats were taken out of their experimental condition and transferred to a separate feeding cage. A repeated measures ANCOVA, using the last baseline day of powdered chow consumption as a covariate, was conducted using time as a within subject factor and sex and group as between subjects factors. These analyses revealed a significant main effect for time [$F(4,48)=32.77$, $p<.05$] with most rats generally increasing their consumption over time, and significant interactions between sex and time [$F(4,48)=3.56$, $p<.05$], group and time [$F(4,48)=3.20$, $p<.05$] and sex and group and time [$F(4,48)=6.77$, $p<.05$]. These interactions reflect the general increase in food consumption over time for the crowded males and individually housed females, in contrast to the relatively stable consumption of food for the individually housed males and crowded females (see Figure 1). The between subjects analyses revealed a significant sex by group interaction [$F(1,11)=6.11$, $p<.05$]. There was no overall effect for sex [$F(1,11)=1.56$, n.s.], or group condition F[(1,11)=2.53, n.s.].

To examine the effects of time more thoroughly, separate repeated measures ANCOVAs, using the last baseline day of powdered chow

consumption as a covariate, were conducted on both the individually housed and the crowded rats. Using the last day of baseline as a covariate, the repeated measures ANCOVAs revealed significant main effects for time for both the crowded [$F(4,24)=15.63$, $p<.05$] and for the individually housed animals [$F(4,24)=21.09$, $p<.05$]. The crowded rats, however, also exhibited a significant sex by time interaction [$F(4,24)=8.60$, $p<.05$], whereas the individually housed rats did not [$F(4,24)=.64$, n.s.]. This sex by time interaction reflects the relatively stable consumption of food for the crowded females in contrast to the overall increased food consumption by the crowded males (see Figure 1 and analyses reported below). Both the individually housed males and females showed some general increase in food consumption over time.

To determine which days accounted for the overall group by sex interaction and for the various time effects, an ANCOVA was conducted at each individual time point. ANCOVA revealed a significant group effect for the mean of the first three stress days [$F(1,11)=15.88$, $p<.05$] with the individual rats consuming more than the crowded rats. Significant sex by group interactions on the mean of the first 5 days of repeated stress [$F(1,11)=5.95$, $p<.05$] and the mean of the last four days [$F(1,11)=13.72$, $p<.05$] also were found with the individual females and the crowded males consuming more than the crowded females and the individual males.

Simple one-way ANOVAs on the males and females separately showed that the group effect during the first stress period was a result of the males' behavior [$F(1,6)=10.83$, $p<.05$] and not to the females' [$F(1,6)=1.61$, n.s.] with the

individually housed males increasing their consumption in comparison to the crowded males. Additional one-way ANCOVAs (with the last baseline day of powdered chow consumption as a covariate) on the crowded rats and ANOVAs on the individual rats revealed that the significant sex by group interactions found on the two repeated stress periods were due to the effects of crowding during these periods, [$F(1,5)=4.86$, $p=.08$]; [$F(1,5)=14.53$, $p<.05$] respectively, and were not due to individual housing [$F(1,6)=.503$, n.s.]; [$F(1,6)=1.4$, n.s.]. Again, this effect occurred for the males [$F(1,6)=15.16$, $p<.05$] and not the females [$F(1,6)=.910$, n.s.] during the last stress period.

Water consumption

Figure 2 presents the amount of water consumed in six hours after the male and female rats were taken from their experimental condition and transferred to a separate drinking cage. A repeated measures ANCOVA, using the last day of baseline water consumption as a covariate, was conducted using time as a within subject factor and sex and group as between subjects factors. There was a trend for time [$F(4,48)=2.11$, $p=.09$] and no time interactions with sex [$F(4,48)=1.38$, n.s.], group [$F(4,48)=1.18$, n.s.], or sex and group [$F(4,48)=1.73$, n.s.]. The between subjects analyses revealed no main effect for sex [$F(1,11)=.29$, n.s.], group [$F(1,11)=.00$, n.s.], or a sex by group interaction [$F(1,11)=1.49$, n.s.].

Body weight

Figure 3 presents the body weights of the male and female rats under the individual and crowded conditions. A repeated measures ANCOVA, using the

last day of baseline body weight as a covariate, was conducted using time as a within subject factor and sex and group as between subjects factors. Within subject analyses revealed a significant main effect for time [$F(4,48)=528.46$, $p<.05$] with all groups generally increasing in body weight over time, and significant interactions between sex and time [$F(4,48)=150.06$, $p<.05$], and group and time [$F(4,48)=6.99$, $p<.05$]. There was no sex by group by time interaction [$F(4,48)=1.95$, n.s.]. The between subjects analysis revealed a significant main effect for group [$F(1,11)=9.11$, $p<.05$]. In addition there was a trend for sex [$F(1,11)=3.5$, $p=.09$] and a trend for a sex by group interaction [$F(1,11)=3.39$, $p=.09$].

To determine which days accounted for the overall group effect and the various time effects an ANCOVA (with the last day a baseline body weight as a covariate) was conducted at each individual time point. The ANCOVA revealed a significant group effect for the mean of the first three stress days [$F(1,11)=6.09$, $p<.05$]. There were significant main effects for sex [$F(1,11)=9.47$, $p<.05$] and group [$F(1,11)=12.15$, $p<.05$] and a significant sex by group interaction [$F(1,11)=8.1$, $p<.05$] found on the mean of the first 5 days of repeated stress. There also were significant main effects for sex [$F(1,11)=14.66$, $p<.05$] and group [$F(1,11)=10.56$, $p<.05$] for the last four days of repeated stress.

PHASE 2

Biochemical measures

Plasma corticosterone

Plasma corticosterone (CCS) was used as a biochemical index of stress.

Figure 4 presents the mean plasma corticosterone values for males and females under crowded and individual housing conditions. There was a significant main effect for sex [$F(1,10)=50.56$, $p<.05$] with the females having higher corticosterone levels than the males. In addition, there was a significant sex by housing interaction [$F(1,10)=9.00$, $p<.05$] with crowding increasing corticosterone for males but decreasing it for females. One-way ANOVAs conducted on males and females separately indicated a significant main effect for housing condition for females [$F(1,5)=2.57$, $p>.05$].

Adrenocorticotrophin hormone

Adrenocorticotrophin hormone (ACTH) was used as another biochemical index of stress. Figure 5 presents the mean plasma ACTH values for males and females under crowded and individual housing conditions. Significant main effects for sex [$F(1,10)=17.56$, $p<.05$] and for housing condition [$F(1,10)=9.07$, $p<.05$] were found. In addition, the significant sex by housing interaction [$F(1,10)=30.50$, $p<.05$] revealed that crowding increased ACTH in males but decreased it in females. One-way ANOVAs conducted on males and females separately indicated a trend in the housing effect for males [$F(1,5)=3.83$, $p=.11$] and a significant housing effect for females, [$F(1,5)=30.96$, $p<.05$].

Food consumption

Part A. Figure 6 presents the amount of the powdered chow consumed in six hours when the male and female rats were taken out of their experimental condition and transferred to a separate feeding cage. A repeated measures ANCOVA, using the mean of 4 days of group housing food consumption as a

covariate, was conducted using time as a within subject factor and sex and number of conspecifics per cage as between subjects factors. This analysis revealed a significant main effect for time [$F(3,30)=12.67$, $p<.05$]. There were no significant interactions.

Because there was no a priori reason to suspect that the difference between three and four conspecifics per cage would have an effect on food consumption, a one-way repeated measures ANCOVA (with the mean of 4 days of group housing food consumption as a covariate) was conducted between males and females collapsing across the number of conspecifics per cage. These means are shown in Figure 7. In addition to a significant main effect for time [$F(3,36)=14.24$, $p<.05$], these analyses also found a trend for a sex by time interaction [$F(3,36)=2.34$, $p=.09$]. Baseline food consumption (group housing) was significantly different from crowded ($t=3.32$, $df=13$),repeated grouped ($t=4.33$, $df=13$), and individual housing conditions ($t=3.95$, $df=13$). The repeated group housing condition also was significantly different from the individual housing condition ($t=3.83$, $df=13$). The crowded and individually housed conditions did not differ.

Part B. The second half of phase 2 was a repeated measures between subjects design with half the male and female rats being crowded or individually housed for 18 hours a day for 8 consecutive days. Figure 8 presents the amount of the powdered chow consumed during the six hours the male and female rats were taken out of their experimental condition and transferred to a separate feeding cage.

A repeated measures ANOVA was conducted using time as a within subject factor and sex and housing condition as between subjects factors. These analyses revealed a significant main effect for time [$F(8,80)=2.48$, $p<.05$] and a significant housing by time interaction [$F(8,80)=2.04$, $p=.05$]. Baseline food consumption (individual housing) was significantly higher than on days 3 ($t=2.42$, $df=13$) and 6 ($t=2.82$, $df=13$) of repeated housing. By days 7 and 8, however, food consumption was higher than on days 3 ($t=2.26$, $df=13$) and 6 ($t=2.75$, $df=13$) and days 3 ($t=2.43$, $df=13$), 4($t=2.62$, $df=13$), and 6($t=2.75$, $df=13$), respectively. Differences in food consumption occurred between day 7 and days 3 ($t=2.46$, $df=13$) and 6 ($t=2.91$, $df=13$) and day 8 and days 3 ($t=2.97$, $df=13$), 4 ($t=3.22$, $df=13$), 5 ($t=2.63$, $df=13$), and 6 ($t=3.12$, $df=13$).

Water consumption

Part A. During the first half of this phase, the between subjects variable was the number of conspecifics per cage when not individually housed and the within subject variable was the type of housing condition. Figure 9 presents the amount of water consumed in six hours when the male and female rats were taken out of their 18-hour condition and transferred to a separate feeding cage.

A repeated measures ANCOVA, using the mean of 4 days of group housing water consumption as a covariate, was conducted using time as a within subject factor and sex and number of conspecifics per cage as between subjects factors. This analyses revealed a significant main effect for time [$F(3,30)=8.98$, $p<.05$], and significant interactions between sex and time [$F(3,30)=3.05$, $p<.05$] and number and time [$F(3,30)=4.12$, $p<.05$]. The between subjects analyses

revealed main effects for sex [$F(1,9)=5.58$, $p<.05$] and number [$F(1,9)=6.62$, $p<.05$].

A one-way repeated measures ANCOVA (with the mean of 4 days of group housing water consumption as a covariate) also was conducted between groups with three and four conspecifics collapsing across sex because of the between subjects main effect for number of conspecifics per cage. This analysis revealed a significant between subjects effect for number [$F(1,11)=4.95$, $p<.05$], a significant within-subject effect for time [$F(3,36)=8.04$, $p<.05$] and a significant number by time interaction [$F(3,36)=3.69$, $p<.05$]. Baseline water intake during group housing was significantly lower than consumption during the crowded ($t=3.78$, $df=13$), repeated grouped ($t=3.31$, $df=13$), and individual housing phases ($t=2.25$, $df=13$). Water consumption during the crowded period was significantly higher than during the individual housing period ($t=2.21$, $df=13$) as was water intake during the repeated group housing phase ($t=2.42$, $df=13$). The crowding and repeated grouping phases did not significantly differ ($t=.48$, $df=13$).

Part B. The second half of Phase 2 was a repeated measures between subjects design with half the male and female rats being crowded or individually housed for 18 hours a day for 8 consecutive days. Figure 10 presents the amount of the water consumed during the six hours the male and female rats were taken out of their experimental condition and transferred to a separate feeding cage.

A repeated measures ANOVA was conducted using time as a within subject factor and sex and housing condition as between subjects factors. This

analyses revealed a significant main effect for time [F(8,80)=2.39, p<.05] and a trend for a sex by housing by time interaction [F(8,80)=2.00, p=.06]. Water consumption was higher on day 1 than on days 2 (t=2.70, df=13), 4(t=2.20, df=13), 5(t=3.00, df=13), and 6(t=4.35, df=13) but was not different from baseline water consumption (t=1.69, df=13) which was measured after all animals were individually housed for 18-hours.

Body weight

Part A. During the first half of this phase, the between subjects variable was the number of conspecifics per cage when not individually housed and the within subject variable was being either grouped, crowded, or individually housed for 18-hours a day. Figure 11 presents the body weights of males and females each day when they were transferred to their 18-hour experimental conditions.

A repeated measures ANCOVA, using the last day of body weight prior to the beginning of Phase 2 as a covariate, was conducted using time as a within subject factor and sex and group as between subjects factors. Within subject analyses revealed a significant main effect for time [F(3,30)=76.95, p<.05] with all groups generally increasing in body weight over time, and a significant sex by time interaction [F(3,30)=43.29, p<.05]. The between subjects analyses revealed a marginally significant effect for sex [F(1,9)=4.93, p=.05]. Body weight during baseline was significantly lower than during any other phase and the body weight during each phase that preceded the next also was lower. These data indicate that all rats continued to gain weight over time regardless of their housing condition.

Part B. The second half of Phase 2 was a repeated measures between subjects design manipulating housing condition between groups. Figure 12 presents the body weights of males and females each day before they were transferred to their 18-hour experimental conditions.

A repeated measures ANCOVA was conducted, using the last day of body weight prior to the beginning of Phase 2 as a covariate, and using sex and housing condition as between subjects factors. The between subjects analysis revealed a significant main effect for housing [$F(1,9)=15.96$, $p<.05$] and a trend for a sex by housing interaction [$F(1,9)=4.84$, $p=.06$]. The within subject analyses revealed a significant main effect for time [$F(8,80)=9.75$, $p<.05$], and significant sex by time [$F(8,80)=10.09$, $p<.05$], housing by time [$F(8,80)=10.15$, $p<.05$] and sex by housing by time [$F(8,80)=4.01$, $p<.05$] interactions.

Because of the between subjects main effect for housing, a one-way repeated measures ANCOVA (the last day of body weight prior to the beginning of Phase 2 as a covariate) also was conducted between housing conditions collapsing across sex. This analysis revealed a significant main effect for housing [$F(1,11)=12.14$, $p<.05$]. A subsequent one-way ANOVA was conducted to determine during which days the groups differed in their body weight. The groups significantly differed on days 3 [$F(1,11)=7.89$, $p<.05$], 4 [$F(1,11)=11.56$, $p<.05$], 5 [$F(1,11)=7.18$, $p<.05$], 6 [$F(1,11)=9.03$, $p<.05$], 7 [$F(1,11)=11.05$, $p<.05$], 8 [$F(1,11)=54.05$, $p<.05$] and 9 [$F(1,11)=14.12$, $p<.05$]. These differences were a result of the individually housed animals gaining weight more quickly than the crowded animals.

Discussion

Confirmation of hypotheses

Hypothesis 1. The hypothesis that crowded male and female rats would have greater stress responses, as indexed by corticosterone and ACTH, than would the individually housed rats was partially confirmed. Although crowded male rats had higher corticosterone and ACTH levels than individually housed male rats, female crowded rats had lower corticosterone and ACTH levels than individually housed female rats.

Hypothesis 2. Crowded male rats consumed more food than individually housed male rats, disconfirming Hypothesis 2.

Hypothesis 3. Individually housed female rats consumed more food than crowded female rats, disconfirming Hypothesis 3.

Hypothesis 4. The hypothesis that crowded male rats would consume more water than grouped or individually housed male rats was not confirmed.

Hypothesis 5. The hypothesis that crowded female rats would consume more water than grouped or individually housed female rats was not confirmed.

Hypothesis 6. Individually housed male rats gained weight at a faster rate than did crowded male rats, confirming Hypothesis 6.

Hypothesis 7. Individually housed female rats gained weight at a faster rate than did crowded female rats, disconfirming Hypothesis 7.

Experiment 1 was designed to examine crowding as a potential social stressor in male and female rats and its effects on food and water consumption

and body weight. Consistent with Hypothesis 1, 18 hours of crowding produced a stress response in male rats indexed by increased levels of corticosterone and ACTH as compared to individually housed males. Surprisingly, however, and in contrast to Hypothesis 1, female rats did not exhibit a stress response under conditions of crowding but instead had lower levels of corticosterone and ACTH in comparison with the individually housed females. Hypothesis 2 was not confirmed with data showing crowded male rats to consume more food than did individually housed males. Similarly, Hypothesis 3 also was not confirmed with the individually housed females consuming more food than the crowded females. Hypotheses 4 and 5 regarding water consumption were disconfirmed with no differences being found between housing conditions for male or female rats. Individually housed male and female rats gained weight more quickly than did crowded males and females confirming Hypothesis 6 but disconfirming Hypothesis 7.

These results are consistent with the findings of Singh et al. (1991) who reported that crowded conditions affected males more than females on physiological and behavioral measures. Unfortunately, Singh et al. (1991) did not control for the size difference between males and females, and consequently could not rule out the possibility that the males may have been more crowded than the females in same size cages.

In addition, these results are similar to findings in the human literature examining both males and females (Baum & Koman, 1976; Epstein & Karlin, 1975; Ross et al., 1973; Stokols et al., 1973; Freedman et al., 1972). The

differences reported for men and women have been explained through a complex interaction between density, social circumstances, and individual differences. According to the crowding literature, high spatial density is necessary but not sufficient for "crowding" to be experienced (Stokols, 1972). These conditions are only likely to induce stress when they are accompanied by frequent, unwanted, and uncontrollable interactions (Baum & Valins, 1977). In general, density, interactions, and crowding are all elements of a complex interpersonal process characterized by a syndrome of stress and related coping strategies (Baum & Koman, 1972).

The differences between spatial density and number of social interactions that occur are thought to account for the sex differences reported in acute human crowding studies (Baum & Koman, 1976; Epstein & Karlin, 1975; Ross, et al., 1973; Stokols, et al., 1973; Freedman et al., 1972). In situations where the primary source of crowding is from spatial cues, males and females get physiologically aroused but they cope with the situation differently. The males cope by responding more aggressively in order to maintain territory, whereas the females cope by sharing their distress with one another. When interactions are too frequent, however, males and females respond similarly and show negative interpersonal affect, active avoidance, and social withdrawal (Baum & Koman, 1976).

A second experiment was conducted to extend the findings of Experiment 1 and to manipulate independently spatial density and social interactions in male and female rats while taking the size of the animals into consideration. It was

hypothesized that males would be more stressed by spatial restrictions, whereas females would be stressed by number of potential interactions.

Experiment 2

Overview

The purpose of Experiment 2 was to manipulate spatial density and social interactions separately in male and female rats. It was hypothesized that males would be more stressed by spatial restrictions, whereas females would be stressed by number of animals.

Experiment 2 examined male and female Wistar rats. Animals of comparable weight were assigned to one of five same-sex housing conditions (N=10 per condition): (1) individual housing in 44 X 23 X 20 cm cages; (2) 5-grouped housing in 47 X 37 X 19 cm or 35 X 30 X 15 cm cages for males and females, respectively; (3) 10-grouped housing in 77 X 37 X 19 cm or 64 X 32 X 18 cm cages for males and females, respectively; (4) 5-crowded housing in 40 X 22 X 18 cm or 27 X 19 X 18 cm cages for males and females, respectively; and (5) 10-crowded housing in 47 X 37 X 19 cm or 35 X 30 X 15 cm cages for males and females, respectively. The housing room was maintained at 23°C, 50% relative humidity, and a 12 hour light/dark cycle (lights on at 0700 hours). Food and water were readily accessible at all times.

Number of interactions and spatial density were independently manipulated by housing animals in sets of 5 or 10 and providing each animal the U.S. Department of Health and Human Services recommended amount of floor area space (grouped) or half this amount (crowded). Cage dimensions were derived separately for each sex to maintain similar amounts of floor space per

subject across sex and were based on the number of rats per cage and their average body weight.

Rats remained in these conditions for 18 hours/day and then were transferred to individual housing for the remaining 6 hours during which time food and water consumption was measured. This cycle was repeated for 15 days. Animals were sacrificed and plasma was assayed for corticosterone.

Hypotheses

Hypothesis 1: It was hypothesized that male rats would be maximally affected by spatial restrictions and affected by population parameters only to the extent that larger numbers decrease space and force unwanted interactions.

Rationale: Experiment 1 found elevated levels of biochemical indices of stress in crowded versus individually housed conditions for males. This finding is consistent with the human literature (Baum & Koman, 1976; Epstein & Karlin, 1975; Ross et al., 1973; Stokols et al., 1973; Freedman et al., 1972) which suggested that males get physiologically aroused and aggressive when spatial cues are restricted. Similarly, human males show negative personal affect when interactions are too frequent which is an interaction between number and space.

Hypothesis 2: It was hypothesized that female rats would be minimally affected by spatial restrictions and maximally affected by population parameters.

Rationale: Experiment 1 found elevated levels of corticosterone, a biochemical index of stress, in the individually versus crowded housing conditions for females. This finding is consistent with the human literature (Baum & Koman,

1976; Epstein & Karlin, 1975; Ross et al., 1973; Stokols et al., 1973; Freedman et al., 1972) which suggested that females get physiologically aroused when spatial cues are restricted but cope better by sharing their distress. When interactions are non-existent or too frequent, however, females are unable to effectively cope.

Hypothesis 3: It was hypothesized that male 10-crowded rats would show the highest elevated levels of corticosterone followed by 5-crowded, 10-grouped, 5-grouped and individually housed male rats.

Rationale: Increases in corticosterone occur in stressed male rats (Raygada et al., 1992) compared with control groups. Based on Hypothesis 1, it follows that males rats should be most stressed by housing conditions where their space is restricted (crowded) and least stressed when they are given ample room (grouped or individual). In addition, male rats should be least stressed when they are with a smaller number of conspecifics (5 or 1) than with a larger number (10).

Hypothesis 4: It was hypothesized that individually housed females would show the highest elevated levels of corticosterone followed by 10-crowded, 10-grouped, 5-crowded and 5-grouped female rats.

Rationale: Increases in corticosterone occur in stressed male rats (Raygada et al., 1992) compared with control groups. Based on Hypothesis 2 and extrapolating from Raygada et al.(1992), female rats should be most stressed by housing conditions where they are alone (individually housed) or experience too many uncontrollable interactions (10) and least stressed when

they are able to interact by choice with other conspecifics (5). In addition, female rats should be least stressed when they are given room (group) to control their interactions than when they do not have room (crowded).

Hypothesis 5: It was hypothesized that male and female rats would have equal plasma insulin levels across housing conditions.

Rationale: Raygada et al. (1992) reported no differences in plasma insulin levels between stress groups and controls in male rats. This finding was extrapolated to include female rats in this study.

Hypothesis 6: It was hypothesized that male rats would increase bland and sweet food consumption following 18-hours of stress. Specifically, based on Hypothesis 3, 10-crowded rats should consume the most followed by 5-crowded, 10-grouped, 5-grouped and individually housed animals.

Rationale: Experiment 1, Phase 1, found that crowded males consumed more food than individually housed males during 9 days of repeated housing in these respective conditions. Because rats will normally eat more of a sweet food than a bland food, sweet food consumption should increase as well as bland food consumption under periods of stress.

Hypothesis 7: It was hypothesized that female rats would increase bland food consumption following 18-hours of stress. Specifically, based on Hypothesis 4, individually housed females should consume the most followed by 10-crowded, 10-grouped, 5-crowded and 5-grouped animals.

Rationale: Experiment 1, Phase 1, found that individually housed females consumed more food than crowded females during 9 days of repeated housing in

these respective conditions. Because rats will normally eat more of a sweet food than a bland food, sweet food consumption should increase as well as bland food consumption during periods of stress. In addition, human data have reported that females increase both bland and sweet food consumption during stress (Grunberg & Straub, 1992).

Hypothesis 8: It was hypothesized that male rats would increase water intake following 18-hours of stress. Specifically, based on Hypothesis 3, 10-crowded rats should consume the most water followed by 5-crowded, 10-grouped, 5-grouped and individually housed animals.

Rationale: Because food and water consumption are highly correlated behaviors it was predicted that if stressed males ate more (Hypothesis 6) than they would drink more.

Hypothesis 9: It was hypothesized that female rats would increase water consumption after 18-hours of stress. Specifically, based on Hypothesis 4, individually housed female rats should consume the most water followed by 10-crowded, 10-grouped, 5-crowded and 5-grouped animals.

Rationale: Because food and water consumption are highly correlated behaviors it was predicted that if stressed females ate more (Hypothesis 7) than they would drink more.

Hypothesis 10: It was hypothesized that male and female rats would gain the most weight in individually housed conditions, less in 5-grouped and crowded conditions, and least in 10-grouped and crowded conditions.

Rationale: Experiment 1, Phases 1 and 2 (Part B), found that both males

and females in individually housed conditions gained more weight than did their counterparts in crowded conditions. These data suggest that the effect on body weight is not related to stress as indicated by biochemical markers, but may be related to some other environmental factor.

Methods

Subjects

Subjects were 50 male and 50 female Wistar rats (Charles River, Wilmington, MA). All animals were housed in same-sex pairs in standard polypropylene shoebox cages (44 X 23 X 20 cm) on hardwood chip contact bedding (Pine-Dri) for two weeks after their arrival. During this time animals had continuous access to standard rodent pellets (Agway Prolab 3500) and water. Housing rooms were maintained at 23°C at 50% relative humidity on a 12 hour light/dark cycle. Male and female rats were individually housed 1 and 3 weeks prior to baseline measurements, respectively. At the beginning of the experiment, male and female rats weighed an average of 384 g and 267 g and the average age was approximately 14 and 16 weeks, respectively.

Materials

Animal housing consisted of both standard polypropylene cages (Lab Products, Inc., Rockville, MD) and specially built polycarbonate cages of various sizes and dimensions. Custom-built cages were made from 1/4 inch thick polycarbonate plastic sheets cut to size (Reed Plastics, Inc., Rockville, MD) and chemically fixed together with methylene dichloride (Reed Plastics, Inc.,

Rockville, MD). Polycarbonate was chosen for the home-built cages because it was most similar to the standard cages in terms of lucidness and durability to high temperature cleaning. Cages were fitted with stainless steel wire-bar lids with slotted feeders. In cases where the lid was too small for the cage, a 1/2 inch gauge hardware cloth (Strosnider's, Kemp Mill, MD) top was provided to support the steel lids. Holes were cut in the hardware cloth so that the slotted feeders were accessible to the rats.

Three standard polypropylene cages were used with the following dimensions: 47 X 37 X 19 cm, 35 X 30 X 15 cm, and 44 X 23 X 20 cm. Four polycarbonate cages were made to the following: 40 X 22 X 18 cm, 77 X 45 X 18 cm, 27 X 19 X 18 cm and 64 X 32 X 18 cm. Cage dimensions were calculated to create predetermined amounts of floor space availability. These amounts were derived separately for each sex and were based on the number of rats per cage and their average body weight. Minimum floor area space recommendations were taken from the U.S. Department of Health and Human Services "Guide for the Care and Use of Laboratory Animals" based on 100 g body weight ranges. Table 1 describes the cage dimensions, total available floor space, and available floor space per animal in each housing condition for males and females. Population density (number of animals per cage) and spatial density (space availability) were independently manipulated by housing animals in cages with 5 (low population) or 10 (high population) same sex conspecifics and providing per animal the recommended amount of floor area space (grouped) or half this amount (crowded). Table 2 provides a comparison of space availability within

the different housing conditions manipulating population density, spatial density or both.

Standard powdered rat chow (Agway Prolab 3200) and a 70% rat chow 30% alpha-D(+)-glucose (Sigma, St. Louis, MO) mixture were provided to the animals in metal food cups with lids that had a 2 inch diameter opening through which the animals could access the food. Food cups were designed to hang over the edge of the cage and sit 1-2 inches off the cage bottom.

Procedure

Animals were assigned within sex to either one of four conditions in which rats were housed together (5-grouped, 10-grouped, 5-crowded, and 10-crowded) or an individually housed condition (see Tables 1 and 2 for descriptions of conditions). Individual housing was added as a comparative condition because some experiments require individual housing in order to conduct experimental procedures.

Baseline measurements began for each sex when the group average body weight was great enough to achieve the predetermined floor space area per animal. During baseline all animals were individually housed in standard rat cages (44 X 23 X 20 cm) for 18 hours (1630-1030) where they had continuous access to food pellets and water. Rats then were transferred to another standard rat cage for 6 hours (1030-1630) where they were again individually housed and had continuous access to standard powdered rat chow, a chow/glucose mixture, and water. Baseline procedures continued for 5 days.

Dependent variables included: body weight, 18-hour water consumption,

18-hour pellet consumption, 6-hour water consumption, 6-hour powdered chow consumption, and 6-hour chow/glucose mixture consumption. Animals, food pellets, filled food cups, and filled water bottles were measured in grams before and after each housing phase on an electronic balance. Amount consumed was calculated as the pre-housing condition minus the post-housing condition.

The same daily measures were made during the testing phase that lasted 14 days. During this phase, animals assigned to 1 of 5 treatment groups based on average body weight, 6-hour powdered chow consumption, and 6-hour mixture consumption taken during baseline days 2-4. Animals were housed in their assigned treatment condition for 18 hours (1630-1030) where they had continuous access to food pellets and water.

At the end of 18 hours, all rats were transferred to standard rat cages and individually housed for 6 hours (1030-1630). For the first seven days, animals received continuous access to powdered rat chow, a chow/glucose mixture, and water. After this time, the chow/glucose mixture was no longer made available to the rats. Animals continued to have access to powdered chow and water for the remaining 7 days.

At the completion of the experiment, animals were sacrificed without anesthesia and trunk blood was collected in tubes containing ethylenediamine tetra-acetic acid (.07 ml of 15% EDTA solution). Blood was centrifuged (1500 x g) for 20 minutes at 4°C and 1600 µl of plasma was stored and frozen at -70°C in 4 separate micro tubes until assayed. Aprotinin (40 µg/ml of plasma) was added to 1 micro tube prior to storage to inhibit enzymatic degradation. Blood plasma

was assayed for corticosterone using standard radioimmunoassay kits (ICN Biomedicals).

Results

Biochemical measures

Plasma Corticosterone

Plasma corticosterone (CCS) was used as a biochemical index of stress. Figure 13 presents the mean plasma corticosterone values for males and female under five different housing conditions. Significant main effects for sex [$F(1,90)=54.51$, $p<.05$] and housing condition [$F(4,90)=2.70$, $p<.05$] were found. In addition, the significant sex by housing interaction [$F(4,90)=11.33$, $p<.05$] showed that females and males were dissimilarly affected by their housing conditions.

One-way ANOVAs indicated significant main effects for housing conditions for males [$F(4,45)=12.34$, $p<.05$] and females [$F(4,45)=4.27$, $p<.05$]. Post-hoc analyses (SNK, $p<.05$) on the male CCS means revealed that the 10-crowded condition produced significantly higher CCS levels than did any other housing condition. CCS levels in the 5-crowded condition were significantly greater than the individually housed and the 10-grouped conditions, but were not significantly greater than the 5-grouped condition. Post-hoc analyses (SNK, $p<.05$) on the female mean CCS levels revealed that the individually housed conditions produced significantly higher levels of CCS than did the 5-crowded, 10-crowded, and 5-grouped conditions. CCS levels in the 10-grouped condition were not

significantly different from any other housing condition.

Because housing conditions were manipulated by number of animals per cage and average space per animal, housing conditions were either collapsed across population (5 or 10) or space (grouped or crowded) and separate analyses were conducted on both variables. The individually housed condition was treated as a separate condition in both analyses.

Figure 14 presents the CCS means for males and females in conditions of 5 or 10 conspecifics per cage collapsed across spacial density conditions. One-way ANOVAs indicated significant main effects for population conditions for males [$F(2,47)=3.63$, $p<.05$] and females [$F(2,47)=6.95$, $p<.05$]. Post-hoc analyses (SNK, $p<.05$) conducted on the male CCS means revealed that the 5 and 10 population conditions had significantly higher means than did the individually housed condition. The 5 and 10 population conditions were not significantly different. Post-hoc analyses (SNK, $p<.05$) performed on the female CCS means revealed that the individually housed condition had a significantly higher CCS mean than did the 5 or 10 population conditions which did not differ from one another.

Figure 15 presents the CCS means for males and females in grouped or crowded conditions collapsed across population density conditions. One-way ANOVAs indicated significant main effects for spatial conditions for males [$F(2,47)=14.08$, $p<.05$] and females [$F(2,47)=6.34$, $p<.05$]. Post-hoc analyses (SNK, $p<.05$) conducted on the male CCS means revealed that the crowded condition had a significantly higher mean CCS value than did the grouped or

individually housed conditions. The grouped and individually housed conditions did not differ significantly. Post-hoc analyses (SNK, $p<.05$) on the female CCS means revealed that the individually housed condition had a significantly higher CCS mean than did the grouped or crowded conditions which did not differ from one another.

Physiological measures

Plasma Insulin

Plasma insulin was measured to examine the relationship between housing conditions and physiological functioning in male and female rats. Figure 16 presents the mean plasma insulin values for males and females under five different housing conditions. Significant main effects for sex [$F(1,89)=9.92$, $p<.05$] and housing condition [$F(4,89)=4.70$, $p<.05$] were found. In addition, the significant sex by housing interaction [$F(4,89)=9.98$, $p<.05$] showed that males and females were dissimilarly affected by their housing conditions.

One-way ANOVAs indicated a significant main effect for housing on plasma insulin values of the female rats [$F(4,44)=9.75$, $p<.05$]. Post-hoc analyses (SNK, $p<.05$) on the female mean plasma insulin values showed that females in the 5-crowded and 10-crowded housing conditions had significantly higher levels of plasma insulin than did the females in the 5-grouped, individually housed, and 10-grouped conditions. Plasma insulin levels in the 5-crowded and 10-crowded conditions were not significantly different from one another. Similarly, the plasma insulin levels of the females in the 5-grouped, individually housed and 10-grouped conditions were not significantly different (Student Newman Keuls).

Because housing conditions were manipulated by number of animals per cage and average space per animal, housing conditions were collapsed across population (5 or 10) and space (grouped or crowded) and separate analyses were conducted on both variables. The individually housed condition was treated as a separate condition in both analyses.

Figure 17 presents the mean plasma insulin values for males and females in conditions of 5 or 10 conspecifics per cage collapsed across spacial density conditions. An ANOVA conducted on these means collapsed across spatial conditions revealed a significant effect for sex [$F(1,93)=9.75$, $p<.05$] but not for population [$F(2,93)=1.25$, $p>.05$]. There was a significant sex by population interaction [$F(2,93)=3.12$, $p<.05$]. Post-hoc analyses (SNK, $p<.05$) on the plasma insulin levels of the individually housed rats revealed significant differences between males and females.

Figure 18 shows the mean plasma insulin levels for males and females in grouped, crowded, and individually housed conditions collapsed across population density conditions. ANOVA revealed significant main effects for sex [$F(1,93)=13.75$, $p<.05$] and space [$F(2,93)=7.66$, $p<.05$] and a significant sex by space interaction [$F(2,93)=17.57$, $p<.05$]. One-way ANOVAs indicated a significant main effect for spatial condition for the females [$F(2,47)=1.58$, $p<.05$]. A post-hoc analysis (SNK, $p<.05$) on the female plasma insulin means revealed that the females in the crowded conditions had significantly higher plasma insulin levels than did the grouped or individually housed females. Females in the individually housed and grouped housed conditions did not differ from one

another.

Food consumption

Glucose/chow mixture

Figures 19 and 20 present the amount of the glucose/chow mixture consumed under all 5 housing conditions for males and females, respectively. A repeated measures ANCOVA, using mean mixture consumption during baseline days 2-5 as a covariate, revealed a main effect for sex [$F(1,87)=23.74$, $p<.05$]. There was no overall housing effect [$F(4,87)=1.83$, $p>.05$], or sex by housing interaction $F[(4,87)=.59$, $p>.05]$. Because of the significant main effect for sex, separate ANCOVAs were conducted on males and females alone. A main effect for housing [$F(4,42)=2.97$, $p<.05$] was found for the males.

To determine when the housing effect occurred for the males, oneway ANOVAs were conducted at each day. Days 1 [$F(4,42)=3.33$, $p<.05$], 2 [$F(4,42)=4.66$, $p<.05$], 3 [$F(4,42)=6.01$, $p<.05$], and 7 [$F(4,42)=2.64$, $p<.05$] were significant. Based on these results, a mean for days 1-3 was taken to create an "early" period and days 4-7 were collapsed to create a "late" period. Subsequent ANOVAs at these two different phases, revealed a significant main effect for housing at the early phase [$F(4,45)=3.23$, $p<.05$] but not at the late phase. A post-hoc analysis (SNK, $p<.05$) at the early phase indicated that males in the individually housed condition consumed significantly more of the sweet food mixture than the males in the 5-grouped condition. No other groups differed from one another.

Sweet food consumption also was analyzed for population and spatial condition effects. Figure 21 shows the amount of glucose/chow mixture consumed for males and females under the different population conditions. A repeated measures ANCOVA, using mean mixture consumption during baseline days as a covariate, collapsed across spatial conditions revealed a main effect for sex [$F(1,91)=20.67$, $p<.05$] and population [$F(2,91)=3.23$, $p<.05$]. Separate ANCOVAs revealed that only the males' sweet food consumption was affected significantly by population differences [$F(2,44)=3.58$, $p<.05$]. Subsequent ANOVAs on the male means at each time point revealed significant population effects to occur at days 1 [$F(2,44)=3.89$, $p<.05$], 2 [$F(2,44)=3.90$, $p<.05$], 3 [$F(2,44)=7.15$, $p<.05$], 6 [$F(2,44)=4.46$, $p<.05$] and 7 [$F(2,44)=4.41$, $p<.05$]. Days 1-3 and 4-7 were collapsed to create early and late period means, respectively. ANOVAs at these two different periods, revealed a significant population effect in the early phase [$F(2,47)=3.50$, $p<.05$] but not at the late phase. A post-hoc analysis (SNK, $p<.05$) at the early phase indicated that males in the individually housed condition consumed significantly more of the sweet food mixture than did the males in the 5 or 10 grouped conditions.

Figure 22 presents the amount of glucose/chow mixture consumed by males and females in the grouped, crowded, and individually housed conditions. A repeated measures ANCOVA, using mean mixture consumption during baseline days as a covariate, collapsing across population conditions revealed a main effect for sex [$F(1,91)=20.21$, $p<.05$]. Separate ANCOVAs revealed that only the males' sweet food consumption was significantly affected by spatial

differences [$F(2,44)=4.26$, $p<.05$]. Subsequent ANOVAs on the male means at each time point showed significant space effects to occur at days 1 [$F(2,44)=6.01$, $p<.05$], 2 [$F(2,44)=9.25$, $p<.05$], and 3 [$F(2,44)=7.46$, $p<.05$]. Days 1-3 and 4-7 were collapsed to create early and late phase means, respectively. ANOVAs at these two different periods, revealed a significant space effect for the early phase [$F(2,47)=6.19$, $p<.05$] but not at the late phase [$F(2,47)=1.09$, $p>.05$]. A post-hoc analysis (SNK, $p<.05$) at the early phase indicated that males in the grouped condition consumed significantly less sweet food mixture than the males in the crowded or individually housed conditions.

Chow Consumption

Six-hour standard powdered chow consumption was measured throughout the study for a total of 14 days. For the first 7 days, the rats were presented with a choice between bland powdered chow and the sweet food mixture. During the last 7 days, the standard powdered chow was given alone. Separate analyses were conducted on these two phases. The mean of baseline chow consumption during days 2-5 was used as a covariate to analyze the first 7 days.

Baseline - Test Day 7. Figures 23 and 24 present the amount of standard powdered rat chow consumed during the mixture-chow choice phase for females and males, respectively, under the different housing conditions. A repeated measures ANCOVA, using the mean of baseline chow consumption during days 2-5 as a covariate, revealed a main effect for sex [$F(1,87)=53.40$, $p<.05$].

Bland powdered chow consumption also was analyzed for population and spatial condition effects. Figure 25 shows the amount of powdered chow

consumed for males and females under the different population conditions. A repeated measures ANCOVA, using the mean of baseline chow consumption days as a covariate, collapsed across spatial conditions revealed a main effect for sex [$F(1,91)=51.39$, $p<.05$] and population [$F(2,91)=3.80$, $p<.05$]. Separate ANCOVAs revealed that only the females' bland food consumption was significantly affected by the population manipulation [$F(2,46)=4.24$, $p<.05$]. Subsequent ANOVAs on the female means at each time point revealed significant population effects to occur at days 5 [$F(2,46)=3.63$, $p<.05$] and 6 [$F(2,46)=4.24$, $p<.05$]. Based on these results, a collapsed mean was calculated for Days 1-4 and Days 5-7. Subsequent ANOVAs at these two different time blocks revealed that there was no significant population effect during the first 4 days [$F(2,47)=2.50$, $p>.05$]. A significant housing effect, however was found during the last 3 days [$F(2,47)=5.06$, $p<.05$]. A post-hoc analysis (SNK, $p<.05$) at the later period indicated that females in the individually housed condition consumed significantly more of the bland food than the females in the 5 or 10 housed conditions.

Figure 26 presents the amount of bland powdered chow consumed by males and females in the grouped, crowded, and individually housed conditions. A repeated measures ANCOVA collapsing across population conditions and using mean baseline chow consumption days as a covariate revealed a significant main effect for sex [$F(1,91)=52.98$, $p<.05$] and space [$F(2,91)=3.31$, $p<.05$]. Separate ANCOVAs revealed that only the females' bland food consumption was significantly affected by spatial differences [$F(2,46)=3.19$,

$p<.05$]. Subsequent ANOVAs on the female means at each time point showed significant space effects to occur only at day 6 [$F(2,46)=4.84$ $p<.05$]. A post-hoc analysis (SNK, $p<.05$) at Day 6 indicated that individually housed females consumed significantly more bland food than females in the crowded or grouped housed conditions.

Test Day 8 - Test Day 13. Figures 27 and 28 present the amount of standard powdered rat chow consumed in 6 hours during the last 8 days of stress, for males and females, respectively, under different housing conditions. There was no sweet food choice given during this phase. A repeated measures ANOVA revealed a main effect for sex [$F(1,87)=28.81$, $p<.05$] and housing [$F(4,87)=3.58$, $p<.05$]. Separate ANOVAs revealed a main effect for housing for the males [$F(4,44)=2.99$, $p<.05$] but not for the females. Subsequent ANOVAs on the male means at each time point showed significant housing effects to occur at days 10 [$F(4,44)=3.43$, $p<.05$], 12 [$F(4,44)=2.72$, $p<.05$], and 13 [$F(4,44)=3.57$, $p<.05$]. Days 8-10 and 11-13 were collapsed to create early and late period means, respectively. The overall housing effect remained significant [$F(4,44)=2.65$, $p<.05$], and the separate ANOVAs at these two different periods revealed a significant housing effect in the early phase [$F(4,44)=2.49$, $p<.05$] but not at the late phase. A post-hoc analysis (SNK, $p<.05$) at the early phase indicated that males in the 10-crowded condition consumed significantly more of the bland food than did the males in the 5 grouped conditions. No other housing conditions differed significantly.

Bland powdered chow consumption also was analyzed for population and

spatial condition effects. Figure 29 shows the amount of powdered chow consumed for females and males under the different population conditions. A repeated measures ANOVA collapsed across spatial conditions revealed a main effect for sex [$F(1,91)=22.57$, $p<.05$] and population [$F(2,91)=3.95$, $p<.05$]. Separate ANOVAs revealed that males' bland food consumption was significantly affected by the population manipulation [$F(2,46)=3.62$, $p<.05$]. Subsequent ANOVAs on the male means at each time point revealed significant population effects at days 10 [$F(2,46)=4.32$, $p<.05$], 12 [$F(2,46)=4.14$, $p<.05$], and 13 [$F(4,44)=4.70$, $p<.05$]. Based on these results, a collapsed mean was calculated for Days 8-10 and Days 11-13. The overall main effect for population remained [$F(2,46)=3.62$, $p<.05$] and the subsequent ANOVAs at these two different time blocks revealed that there were significant population effects during both periods; [$F(2,46)=3.32$, $p<.05$] and [$F(2,47)=3.23$, $p<.05$], respectively. A post-hoc analysis (SNK, $p<.05$) at both periods indicated that no housing condition was significantly different from any other housing condition.

Figure 30 presents the amount of bland powdered chow consumed by females and males in the grouped, crowded, and individually housed conditions. A repeated measures ANOVA collapsing across population conditions revealed a main effect for sex [$F(1,91)=22.50$, $p<.05$] and a trend for space [$F(2,91)=2.59$, $p=.08$].

Water Consumption

Figures 31 and 32 present the amount of water consumed under all 5 housing conditions for males and females, respectively. A repeated measures

ANCOVA, using the mean of baseline water consumed during days 2-5 as a covariate, revealed significant main effects for sex [$F(1,81)=18.06$, $p<.05$] and housing [$F(4,81)=3.95$, $p<.05$], and a significant sex by housing interaction $F[(4,81)=3.57$, $p<.05$]. Separate ANCOVAs revealed significant main effects for housing for males [$(4,44)=3.47$, $p<.05$] and for females [$F(4,36)=4.17$, $p<.05$].

To determine when the housing effect occurred for the males alone, one-way ANOVA's were conducted at each day. Days 1 [$F(4,44)=3.29$, $p<.05$], 2 [$F(4,44)=3.78$, $p<.05$], 6 [$F(4,44)=3.00$, $p<.05$], 7 [$F(4,44)=3.02$, $p<.05$], 8 [$F(4,44)=3.27$, $p<.05$], 10 [$F(4,44)=5.85$, $p<.05$] and 13 [$F(4,44)=3.68$, $p<.05$] were significant. Based on these results, means for days 1-4, 5-8, 9-13 were calculated to create "early," "intermediate," and "late" housing periods. The main effect for housing reached significance during the late phase [[$F(4,45)=3.73$, $p<.05$]. A post-hoc analysis (SNK, $p<.05$) at the late phase indicated that males in the 10-crowded condition consumed significantly more water than the males in the 5-crowded condition. No other groups were significantly different.

To determine when the housing effect occurred for the females, one-way ANOVA's were conducted at each day. Days 4 [$F(4,36)=3.42$, $p<.05$], 6 [$F(4,36)=5.05$, $p<.05$], 7 [$F(4,36)=4.93$, $p<.05$], 8 [$F(4,36)=2.82$, $p<.05$], 10 [$F(4,36)=3.48$, $p<.05$], 11 [$F(4,36)=3.68$, $p<.05$], 12 [$F(4,36)=8.43$, $p<.05$] and 13 [$F(4,36)=5.42$, $p<.05$] were significant. Means for days 1-4, 5-8, 9-13 were calculated to create "early," "intermediate," and "late" housing periods. Subsequent ANOVAs at these three different phases revealed a trend for housing at the early phase [$F(4,39)=2.46$, $p=.06$] that was significant during the

intermediate [$F(4,40)=3.62$, $p<.05$] and late [$F(4,45)=3.62$, $p<.05$] time periods. Post-hoc analyses (SNK, $p<.05$) at the intermediate and late phases indicated that females in the 10-crowded condition consumed significantly more water than the females in the 5-grouped condition. No other groups were significantly different.

Water consumption also was analyzed for population and spatial condition effects. Figure 33 shows the amount of water consumed for males and females under the different population conditions. A repeated measures ANCOVA, using the mean of baseline water consumed during days 2-5 as a covariate, collapsed across spatial conditions, revealed a main effect for sex [$F(1,85)=10.10$, $p<.05$] and a significant sex by population interaction [$F(2,85)=4.57$, $p<.05$]. Separate ANCOVAs revealed that only the males' water consumption was significantly affected by population differences [$F(2,46)=5.00$, $p<.05$]. Subsequent ANOVAs on the male means at each time point showed significant population effects to occur at days 2 [$F(2,46)=6.72$, $p<.05$], 6 [$F(2,46)=3.46$, $p<.05$], 7 [$F(2,46)=4.17$, $p<.05$], 8 [$F(2,46)=3.50$, $p<.05$], 12 [$F(2,46)=4.84$, $p<.05$], and 13 [$F(2,46)=4.58$, $p<.05$]. Days 1-4, 5-8, and 9-13 were collapsed to create early, intermediate and late period means, respectively. ANOVAs at these three different periods, revealed significant population effects in the early [$F(2,47)=4.18$, $p<.05$], intermediate, [$F(2,47)=3.71$, $p<.05$] and late [$F(2,47)=3.78$, $p<.05$] phases. A post-hoc analysis (SNK, $p<.05$) at the early phase indicated that males in the individually housed condition consumed significantly more water than the males in the 5 or 10 number conditions. Additional post-hoc analyses (SNK, $p<.05$)

during the intermediate and late phases showed individually housed males to consume still more water than the males housed in numbers of 5 but not the males housed in numbers of 10. Males housed in numbers of 5 or 10 did not significantly differ from one another.

Figure 34 presents the amount of water consumed by females and males in the grouped, crowded, and individually housed conditions. A repeated measures ANCOVA, using a mean of baseline water consumption days as a covariate, collapsing across population conditions, revealed significant main effects for sex [$F(1,85)=9.70$, $p<.05$], space [$F(2,85)=6.59$, $p<.05$], and a significant sex by space interaction [$F(2,85)=6.47$, $p<.05$]. Separate ANCOVAs revealed that both the males' and females' water consumption were significantly affected by spatial differences [$F(2,46)=5.23$, $p<.05$ and [$F(2,38)=7.82$, $p<.05$], respectively.

Subsequent ANOVAs for the male means at each time point showed significant space effects to occur at days 1 [$F(2,46)=5.12$, $p<.05$], 2 [$F(2,46)=7.09$, $p<.05$], 3 [$F(2,46)=4.30$, $p<.05$], 4 [$F(2,46)=3.38$, $p<.05$], 6 [$F(2,46)=5.96$, $p<.05$], 7 [$F(2,46)=4.81$, $p<.05$], 8 [$F(2,46)=3.69$, $p<.05$], 10 [$F(2,46)=6.53$, $p<.05$], 12 [$F(2,46)=3.35$, $p<.05$], and 13 [$F(2,46)=4.42$, $p<.05$]. Days 1-4, 5-8 and 9-13 were collapsed to create early, intermediate, and late phase means, respectively. ANOVAs for these three different periods revealed significant space effects for the early [$F(2,47)=3.78$, $p<.05$] and intermediate phases [$F(2,47)=3.23$, $p<.05$]. A post-hoc analysis (SNK, $p<.05$) at the early phase indicated that males in the individually housed condition consumed

significantly more water than the males in the grouped or crowded conditions. During the intermediate phase, the individually housed males were still drinking significantly more than the grouped males but neither the individually housed or grouped males differed from the crowded males.

ANOVAs for the female water consumption means at each day showed significant space effects on days 6 [$F(2,38)=7.79$, $p<.05$], 7 [$F(2,38)=7.32$, $p<.05$], 8 [$F(2,38)=4.39$, $p<.05$], 9 [$F(2,38)=3.51$, $p<.05$], 10 [$F(2,38)=7.18$, $p<.05$], 11 [$F(2,38)=7.38$, $p<.05$], 12 [$F(2,38)=17.78$, $p<.05$], and 13 [$F(2,38)=11.06$, $p<.05$]. Days 1-4, 5-8 and 9-13 were collapsed to create early, intermediate, late phase means, respectively. ANOVAs at these three different periods revealed significant space effects during the intermediate [$F(2,42)=3.45$, $p<.05$] and late [$F(2,47)=6.35$, $p>.05$] phases. A post-hoc analysis (SNK, $p<.05$) revealed that during the late phase, the crowded females consumed significantly more water than the grouped or individually housed females. The grouped females did not differ from the individually housed females.

Body Weight

Figures 35 and 36 present the body weights of males and females each day before they were transferred to their 18-hour experimental conditions. A repeated measures ANCOVA, using the last day of baseline body weight as a covariate, revealed significant main effects for sex [$F(1,89)=4.63$, $p<.05$] and housing [$F(4,89)=5.52$, $p<.05$]. Separate ANCOVAs revealed a significant main effect for housing for males [$(4,44)=3.90$, $p<.05$] but not for females.

To determine when the housing effect occurred for the males, oneway

ANOVAs were conducted for each day. Days 2 [$F(4,44)=6.83$, $p<.05$], 3 [$F(4,44)=7.36$, $p<.05$], 4 [$F(4,44)=8.49$, $p<.05$], 5 [$F(4,44)=5.83$, $p<.05$], 6 [$F(4,44)=7.92$, $p<.05$], 7 [$F(4,44)=4.45$, $p<.05$], 8 [$F(4,44)=2.88$, $p<.05$], 9 [$F(4,44)=2.68$, $p<.05$], and 10 [$F(4,44)=3.31$, $p<.05$] were significant. Means for days 1-4, 5-9, 10-14 were taken to create "early," "intermediate," and "late" housing periods. Subsequent ANCOVAs (with last day of baseline body weight as a covariate) at these three different phases, revealed significant main effects for housing at the early [$F(4,44)=6.37$, $p<.05$] and intermediate [$F(4,44)=4.77$, $p<.05$] time periods. Post-hoc analyses (SNK, $p<.05$) at the early and intermediate phases indicated that males in the individually housed condition weighed significantly more than males in any other housing condition. No other conditions were significantly different.

Body weight also was analyzed for population and spatial condition effects. Figure 37 shows the body weight of males and females under the different population conditions. A repeated measures ANCOVA, using the last day of baseline body weight as a covariate, collapsed across spatial conditions, revealed main effects for sex [$F(1,93)=5.24$, $p<.05$] and for population [$F(2,93)=9.05$, $p<.05$]. Separate ANCOVAs revealed that both the males' and females' body weights were affected by population manipulations, [$F(2,46)=6.24$, $p<.05$] and [$F(2,46)=3.15$, $p=.05$], respectively.

Subsequent ANOVAs on the male body weight means for each time point showed significant population effects on days 2 [$F(2,46)=6.24$, $p<.05$], 3 [$F(2,46)=9.80$, $p<.05$], 4 [$F(2,46)=10.66$, $p<.05$], 5 [$F(2,46)=8.21$, $p<.05$], 6

[F(2,46)=11.52, p<.05], 7 [F(2,46)=6.46, p<.05], 8 [F(2,46)=4.44, p<.05], 9 [F(2,46)=4.45, p<.05], 10 [F(2,46)=5.99, p<.05], 11 [F(2,46)=3.74, p<.05], and 12 [F(2,46)=4.13, p<.05]. Days 1-4, 5-9, and 10-14 were collapsed to create early, intermediate, and late period means, respectively. ANCOVAs (with last day of baseline body weight as a covariate) at these three different periods revealed significant population effects in the early [F(2,47)=7.24, p<.05], intermediate [F(2,47)=7.16, p<.05], and late [F(2,47)=3.71, p<.05] phases. Post-hoc analyses (SNK, p<.05) at all three phases indicated that males in the individually housed condition weighed significantly more than did the males in the 5 or 10 number conditions. Males housed in numbers of 5 or 10 did not significantly differ from one another.

Subsequent ANOVAs on the female body weight means for each time point revealed significant population effects at days 10 [F(2,46)=4.27, p<.05], 11 [F(2,46)=6.07, p<.05], and 12 [F(2,46)=4.10, p<.05]. Days 1-4, 5-9, and 10-14 were collapsed to create early, intermediate, and late period means, respectively. The population manipulation significantly affected body weight during the late phase [F(2,46)=3.93, p<.05]. A post-hoc analysis (SNK, p<.05) at the late phase indicated that females in the individually housed condition weighed more than the females in the 5 number conditions but did not differ from the females in the 10 number condition. Females housed in numbers of 5 or 10 did not significantly differ from one another.

Figure 38 presents the male and female body weights means in the grouped, crowded, and individually housed conditions. A repeated measures

ANCOVA, using the last day of baseline body weight as a covariate, collapsing across population conditions revealed significant main effects for sex [$F(1,93)=5.39$, $p<.05$] and space [$F(2,93)=9.77$, $p<.05$]. Separate ANCOVAs revealed that the males' body weight was significantly affected by the spatial manipulation differences [$F(2,46)=7.50$, $p<.05$].

Subsequent ANOVAs on the male body weight means at each time point showed significant space effects on days 2 [$F(2,46)=11.33$, $p<.05$], 3 [$F(2,46)=14.16$, $p<.05$], 4 [$F(2,46)=15.15$, $p<.05$], 5 [$F(2,46)=11.91$, $p<.05$], 6 [$F(2,46)=15.98$, $p<.05$], 7 [$F(2,46)=9.03$, $p<.05$], 8 [$F(2,46)=5.72$, $p<.05$], 9 [$F(2,46)=5.42$, $p<.05$], 10 [$F(2,46)=6.60$, $p<.05$], 11 [$F(2,46)=4.10$, $p<.05$], and 12 [$F(2,46)=4.27$, $p<.05$]. Days 1-4, 5-9, and 10-14 were collapsed to create early, intermediate, and late phase means, respectively. ANOVAs at these three time periods revealed significant space effects during all three phases [$F(2,47)=3.78$, $p<.05$], [$F(2,47)=3.23$, $p<.05$] and [$F(2,47)=2.48$, $p=.09$], respectively. Post-hoc analyses (SNK, $p<.05$) conducted at the early and intermediate phases indicated that males in the individually housed condition weighed significantly more than the males in the crowded or grouped conditions. Additionally, the crowded males weighed more than the grouped males. During the late phase, the individually housed males still weighed significantly more than the grouped and crowded males but the grouped males did not differ significantly from the crowded males.

Discussion

Confirmation of hypotheses

Hypothesis 1. The hypothesis that males rats would be maximally

affected by spatial restrictions and affected by population parameters only to the extent that larger numbers decrease space and force unwanted interactions was confirmed.

Hypothesis 2. The hypothesis that female rats would be minimally affected by spatial restrictions and maximally affected by population parameters was partially confirmed.

Hypothesis 3. The mean corticosterone level of the 10-crowded male rats was higher than the mean of any other housing condition. In addition, the mean corticosterone level of the male rats in the 5-crowded condition was greater than the mean of the males in the individually housed and 10-grouped conditions but not the 5-grouped condition, partially confirming Hypothesis 3.

Hypothesis 4. The mean corticosterone level of the individually housed female rats was higher than the mean of the female rats in 5-crowded, 10-crowded, and 5-grouped conditions. In addition, the mean corticosterone level of the female rats in the 10-grouped condition did not differ from the mean of the females in any other housing condition, partially confirming Hypothesis 4.

Hypothesis 5. The plasma insulin levels of the female rats in the 5-crowded and 10-crowded housing conditions were greater than the mean plasma insulin levels of the female rats in the 5-grouped, individually housed, and 10-grouped conditions, partially disconfirming Hypothesis 5.

Hypothesis 6. Male rats in the individually housed condition consumed more sweet food than did male rats in the 5-grouped condition, partially disconfirming Hypothesis 6, whereas male rats in the 10-crowded condition

consumed more bland food than did male rats in the 5-grouped condition, partially confirming Hypothesis 6.

Hypothesis 7. The hypothesis that individually housed females would consume more sweet food than female rats in any other housing condition was not confirmed. However, female rats in the individually housed condition consumed more bland food than did female rats in the 5 and 10 housed conditions or the grouped and crowded conditions when spatial and population conditions were collapsed, partially confirming Hypothesis 7.

Hypothesis 8. Male rats in the 10-crowded condition consumed more water than did male rats in the 5-crowded condition, partially confirming Hypothesis 8. However, male rats in the individually housed condition consumed more water than did male rats in the 5 and 10 housed conditions or the grouped and crowded conditions when spatial and population conditions were collapsed, disconfirming Hypothesis 7.

Hypothesis 9. Female rats in the 10-crowded condition consumed more water than did female rats in the 5-crowded condition, disconfirming Hypothesis 9. In addition, female rats in the 10-crowded condition consumed more water than did female rats in the grouped and individually housed conditions when spatial conditions were collapsed, disconfirming Hypothesis 9.

Hypothesis 10. Male and female rats in the individually housed condition weighed more than did male and female rats in the other housing conditions, confirming Hypothesis 10.

General Discussion

These two experiments examined the effects of differential housing conditions on biochemical, physiological, and behavioral functioning of male and female rats. Housing conditions were manipulated both numerically (number of animals per cage) and spatially (size of cage). Experiment 1 examined the effects of spatial crowding. Experiment 2 independently manipulated both numeric and spatial aspects of the housing environment. Plasma corticosterone and insulin were measured to assess biochemical stress levels. Food and water consumption and body weight were examined to determine whether appetitive behaviors would be affected by the environmental manipulations.

Both experiments found that male and female rats are differentially affected by their environments based on biochemical changes. Specifically, crowded males had higher levels of corticosterone than did the individually housed males, in contrast to individually housed females that had higher corticosterone levels than did the crowded females. Because females had higher or equal levels of corticosterone than did males in all five housing conditions, there are alternative explanations for the female data. The individually housing of females may actually increase corticosterone responding or, alteratively, crowded females may exhibit decreased corticosterone responding. These findings are consistent with other animal studies that have reported that crowded males show greater behavioral changes, indicative of stress, than do females under similar conditions (Singh et al., 1991).

In addition to confirming the results of Experiment 1, Experiment 2 also

revealed sex differences in response to the population and to spatial manipulations as indicated by corticosterone levels. Whereas females were similarly affected by both housing manipulations, males were more affected by spatial restrictions. In general, the females had lower levels of corticosterone responses when they were with other females compared to being alone, regardless of the spatial restrictions. Males, in contrast, exhibited higher levels of corticosterone when housed with other animals compared with their individually housed counterparts. When spatial restrictions were considered, the crowded males had the highest corticosterone levels comparison with the grouped and individually housed males.

A reasonable explanation for the differential responding of the male and female rats to the crowded conditions is that male rodents are instinctively territorial and form hierarchies to establish positions of dominance (Brain, 1971). Dominance-subordination relationships in male rats appear to be stressful to the subordinates as indicated by chronically increased defensive behaviors and increased corticosterone levels (Blanchard, Yudko, & Blanchard, 1993). No such hierarchies appear to be created among female rats (Blanchard, Flannelly, & Blanchard, 1988). Future studies manipulating the number of dominant and subordinate male rats in a given cage could provide additional information regarding the differential biochemical stress responding of male and female rats.

Housing manipulations also affected indices of physiological functioning and, again, differences were found between the male and female rats. Specifically, crowded females had greater plasma insulin levels than did grouped

or individually housed females. In contrast, male plasma insulin levels were not different between housing conditions.

It is reasonable to suspect that environmental manipulations powerful enough to change biochemical and physiological functioning also can influence behavior. Other experiments have reported that housing conditions influence food and water consumption (Armario et al., 1984), alcohol (Hannon & Bolter, 1980; Hannon & Donlon-Bantz, 1976), and morphine (Alexander, Coambs, & Hadaway, 1978) self-administration, and general motor activity (Singh et al., 1991). The two present experiments also found that differences occur in food and water consumption and in body weight across different environmental conditions.

In Experiment 1, crowded males consumed more bland food than did individual males and, in Experiment 2, 10-crowded males consumed more bland food than did the 5-grouped males. This finding is inconsistent with previous studies which have reported that crowded males decrease food consumption in comparison to control males that are housed three to a cage (Armario et al., 1984). When males were given a choice between sweet food and bland food, the housing condition effect changed and the individual males consumed more sweet food than did the 5-grouped males. In addition, when the sweet food was available, no differences among housing conditions were found for bland food consumption. It can be speculated that the increase in bland food consumption may be a response to stress, as indicated by the biochemical markers. This explanation, however, is inconsistent with the human literature which has found

decreases in consumption of all food stuffs among stressed males (Grunberg & Straub, 1992). Furthermore, it does not explain the differences in sweet consumption among the individual and the grouped males. It is likely that the milieu of the environment, including the number of animals, the amount of space available, and the resources interacts with the organism at a different level (i.e., motivation, hedonism) and that there is no simple one-to-one relationship between stress and behavior.

Sex differences in both bland and sweet food consumption also were found with the females. Whereas the crowded males consumed more bland food than did the individually housed males, the individually housed females consumed more bland food than did the grouped or the crowded females. Although this finding is consistent with the hypothesis that stress increases food consumption, this was not true when sweet food was available. According to Grunberg and Straub (1992), human females increase the consumption of all food stuffs, especially sweet foods under conditions of stress. The present experiments, however, found no differences in sweet food consumption between females in different housing conditions.

Water consumption was highest in crowded males and in crowded females during the intermediate and late phases Experiment 2. Specifically, 10-crowded males consumed more water than did 5-crowded males and 10-crowded females consumed more water than did 5-grouped females. When population and spatial parameters were collapsed, individual males actually consumed more water than did males in 5- or 10-housed conditions, grouped,

and the crowded conditions. No other differences were found among the females. Once again, there was no trend to suggest a direct relationship between stress and water consumption. However, because both crowded males and crowded females consumed the highest amounts of water, it is likely that the environment plays a strong and common role in this appetitive drive for both sexes.

Because body weight is correlated with food and water consumption, body weight was examined as an indirect measure of behavior differences. Consistent with other reports (Singh et al., 1991; Armario et al., 1987; Gamallo et al., 1987, Armario et al., 1984) the present experiments found that individually housed males and individually housed females weigh more than same sex animals that are in any other housing condition. It is possible that this difference in body weight is a reflection of the individual males' consumption of high amounts of sweet food and the individual females' consumption of high amounts of bland food. Alternative explanations for these differences may include differential metabolic rates or activity levels, changes in sleeping patterns, overall health functioning, or just having the physical space to grow. Similar to the other physiological and behavioral measures, however, there does not appear to be a direct link between stress and body weight across sexes. It is possible that stress interacts with sex and that this interaction leads to a decrease in body weight in males and to an increase in body weight in females through some mechanism other than food consumption.

The two present experiments confirm that crowding increases biochemical

indices of stress in males and that this effect is a result of spatial restrictions. In contrast, however, the individually housing of females leads to elevated biochemical indices of stress or the crowding of females leads to decreased biochemical indices of stress. In general, these findings establish different housing conditions as models of social or psychological stressors in male and female rats. In addition, these experiments parallel previous human studies that have examined behavioral, cognitive, and physiological measures of the stress response under different population and spatial parameters (Baum & Koman, 1976). Consequently, the manipulation of different housing conditions is a useful, face valid, and non-painful animal model of stress as indexed by biochemical markers. In addition, because of the sex differences involved with this manipulation, it is now possible to distinguish between environment and sex interactions in contrast to stress and sex differences.

These experiments provide evidence which suggest that the environment plays a major role in changing the behaviors of animals and that there are major interactions between the environment and the sex of the animal. To date, most experiments have been designed to house both male and female animals under similar conditions in order to maintain consistency and increase experimental control. Unfortunately, by housing males and females under the same environmental conditions, a major confound is being introduced into the equation. Based on the results of Experiment 2, both males and females should be housed in either 10-crowded or 5-grouped conditions in order to maintain similar biochemical profiles, or the males should be housed individually and the females

should be housed with other females to produce the lowest within sex baseline biochemical levels.

Depending on the research variable of interest, researchers should be aware of the sex differences produced by differential housing conditions. If the intention is to affect biochemical or molecular changes within an organism, it would be beneficial to house male and female rats differently according to the desired effect. If the research question involves the examination of different environmental conditions on a desired measure, then it is important to be aware of these additional underlying mechanisms.

TABLES

Table 1. Cage dimensions, total available floor space, and available floor space per animal in each housing condition for males and females.

SEX	HOUSING CONDITION	DIMENSIONS (cm)	TOTAL SPACE (cm^2)	SPACE PER ANIMAL (cm^2)
MALE	INDIVIDUAL	44 x 23 x 20	1012	1012
MALE	5-CROWDED	40 x 22 x 18	880	176
MALE	5-GROUPED	47 x 37 x 19	1739	348
MALE	10-CROWDED	47 x 37 x 19	1739	174
MALE	10-GROUPED	77 x 37 x 19	3465	347
FEMALE	INDIVIDUAL	44 x 23 x 20	1012	1012
FEMALE	5-CROWDED	27 x 19 x 18	513	103
FEMALE	5-GROUPED	35 x 30 x 15	1050	210
FEMALE	10-CROWDED	35 x 30 x 15	1050	105
FEMALE	10-GROUPED	64 x 32 x 18	2048	205

Table 2. Comparison of space availability within different housing conditions manipulating population density, spatial density or both.

MANIPULATION	HOUSING CONDITION	DESCRIPTOR	COMPARATIVE HOUSING CONDITION
POPULATION	5-GROUPED	HALF	10-GROUPED
POPULATION	5-CROWDED	HALF	10-CROWDED
SPATIAL	5-CROWDED	HALF	5-GROUPED
SPATIAL	10-CROWDED	HALF	10-GROUPED
POPULATION X SPATIAL	5-GROUPED	HALF/DOUBLE	10-CROWDED
POPULATION X SPATIAL	5-CROWDED	HALF/HALF	10-GROUPED

FIGURES

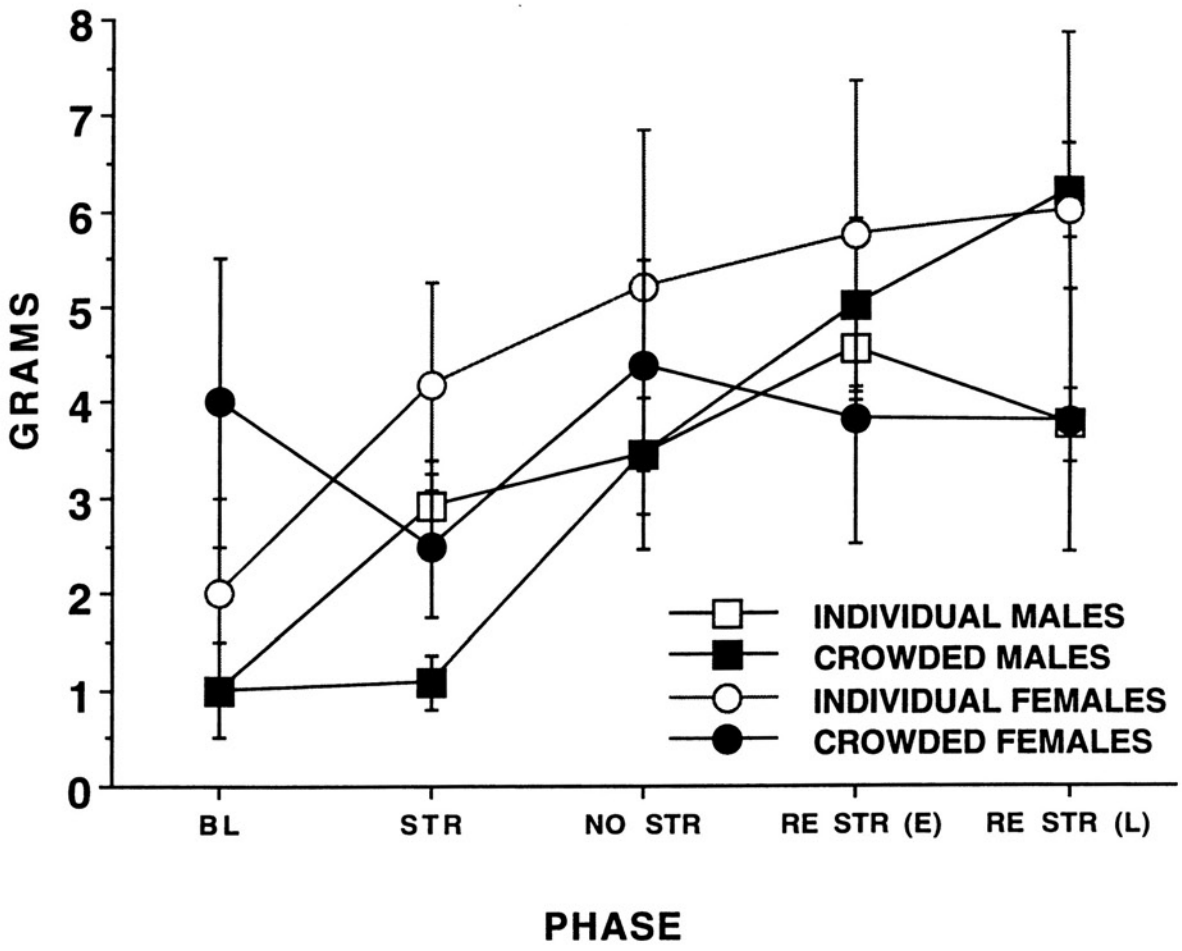

Figure 1.
Experiment 1. Amount of bland powdered food consumed by male and female rats in 6 hours following 18 hours of crowding or individual housing (means and standard errors).

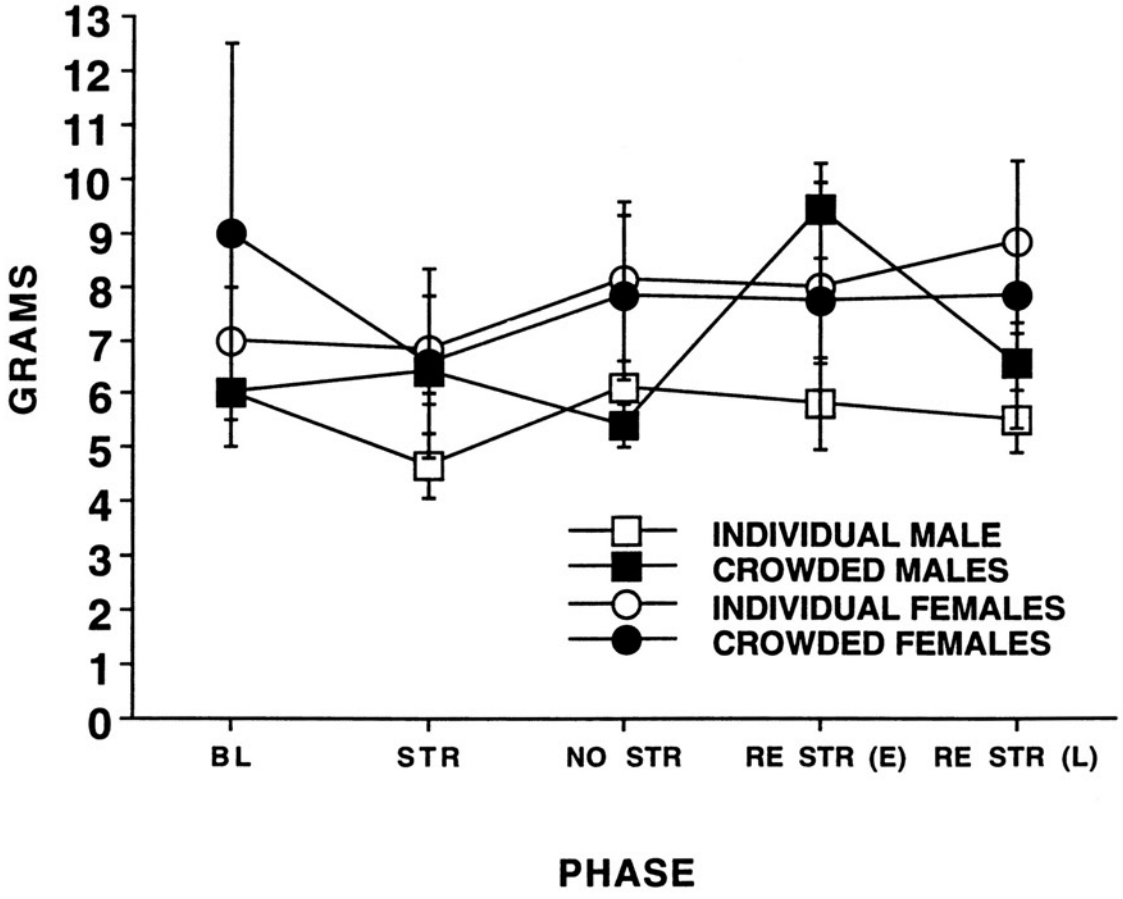

Figure 2.
Experiment 1. Amount of water consumed by male and female rats in 6 hours following 18 hours of crowding or individual housing (means and standard errors).

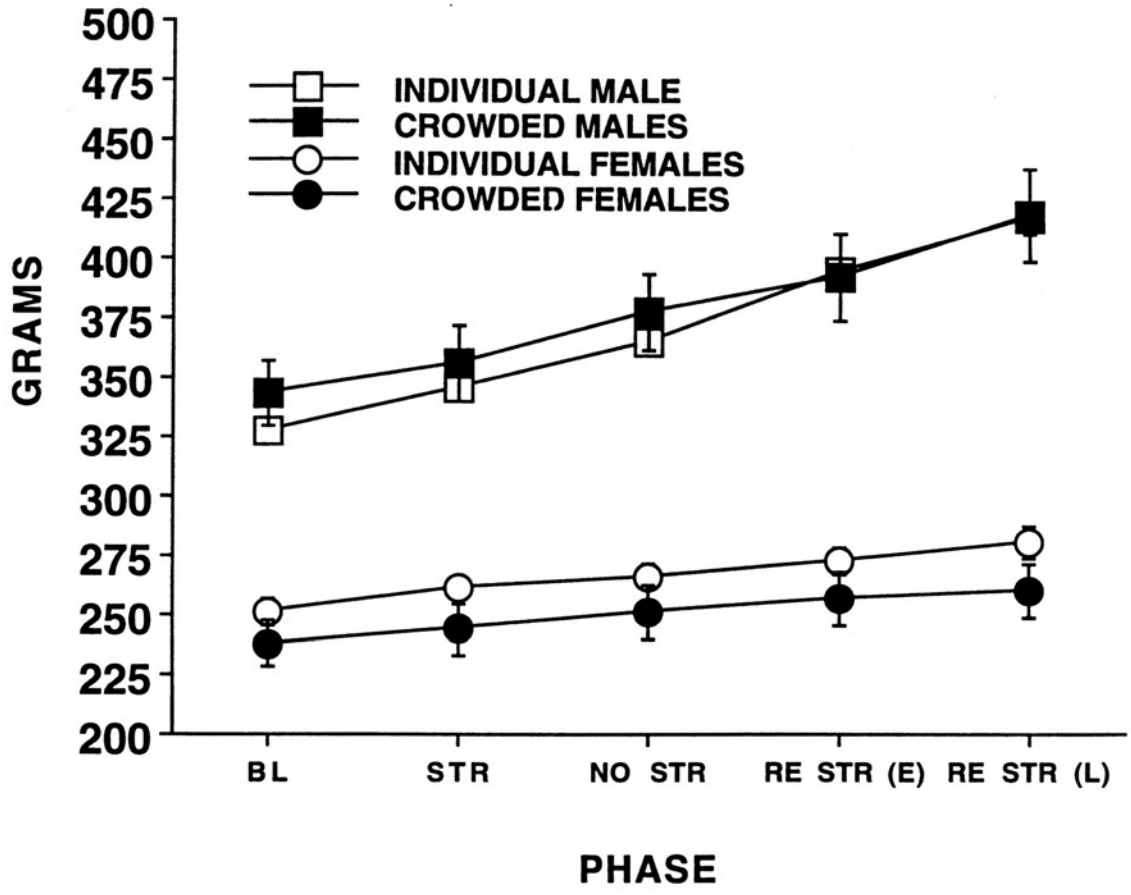

Figure 3.
Experiment 1. Body weight of male and female rats following 18 hours of crowding or individual housing (means and standard errors).

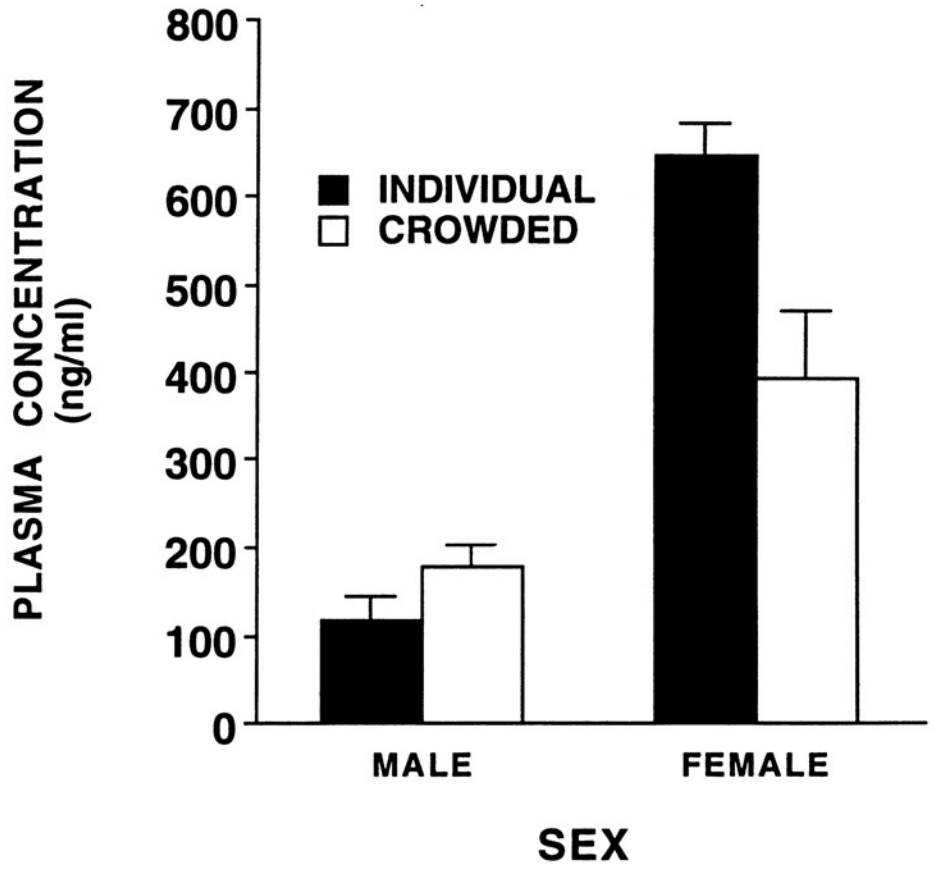

Figure 4.
Experiment 1. Effects of crowding or individual housing on plasma corticosterone levels of male and female rats (means and standard errors).

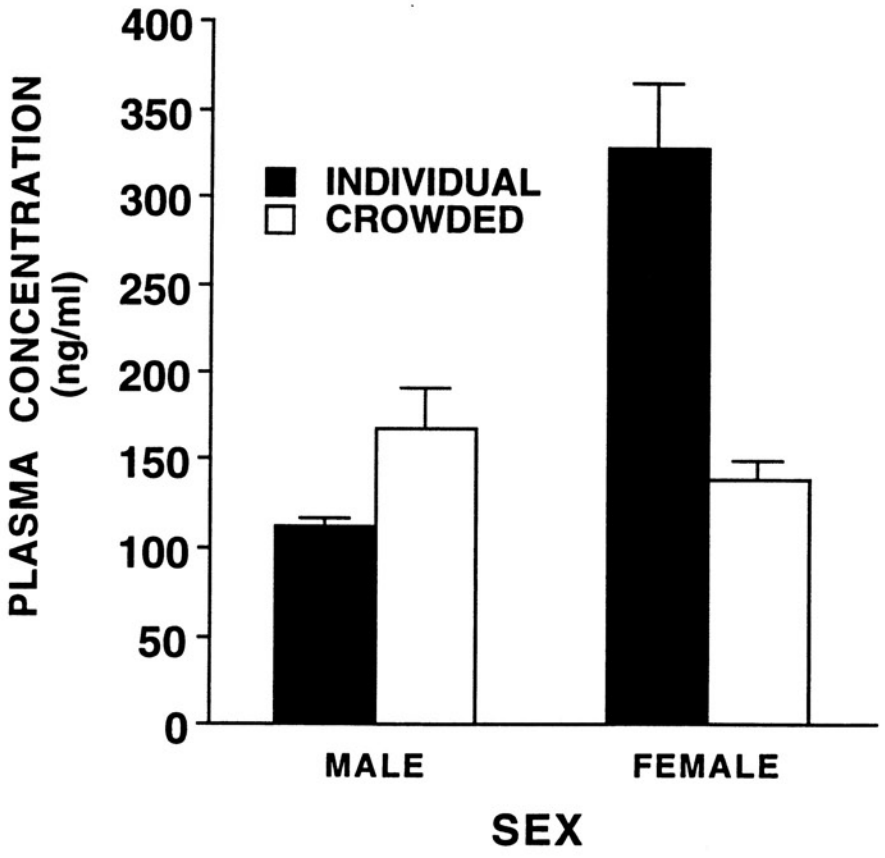

Figure 5.
Experiment 1. Effects of crowding or individual housing on plasma adrenocorticotrophin hormone levels of male and female rats (means and standard errors).

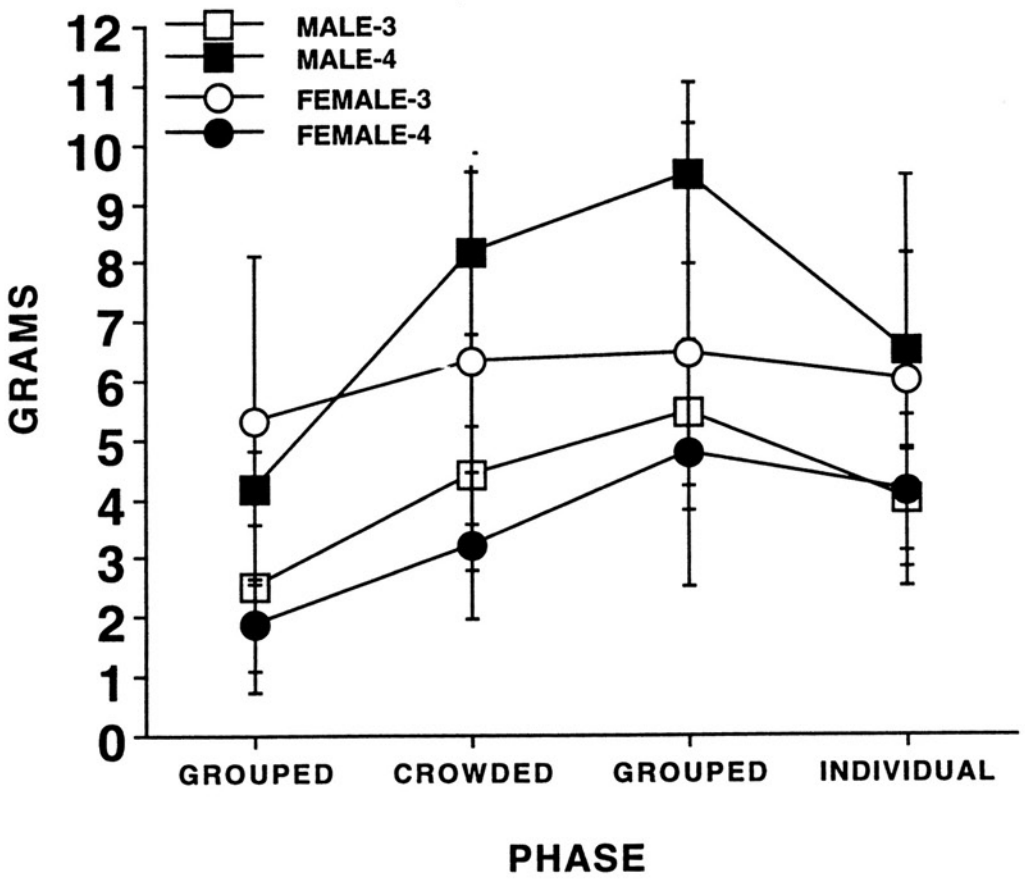

Figure 6.
Experiment 1. Amount of bland powdered food consumed by male and female rats in 6 hours following 18 hours of grouping or crowding with different number of conspecifics or individual housing (means and standard errors).

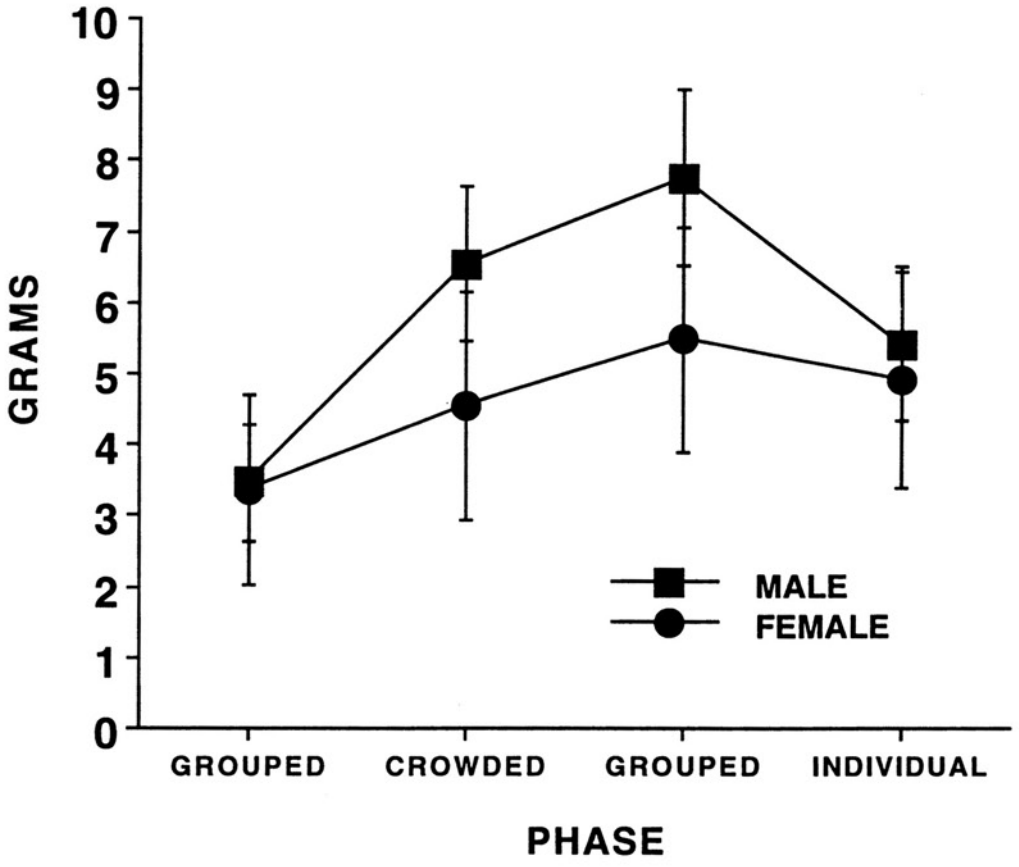

Figure 7. Amount of bland powdered food consumed by male and female rats in 6 hours following 18 hours of grouping, crowding, or individual housing (means and standard errors).

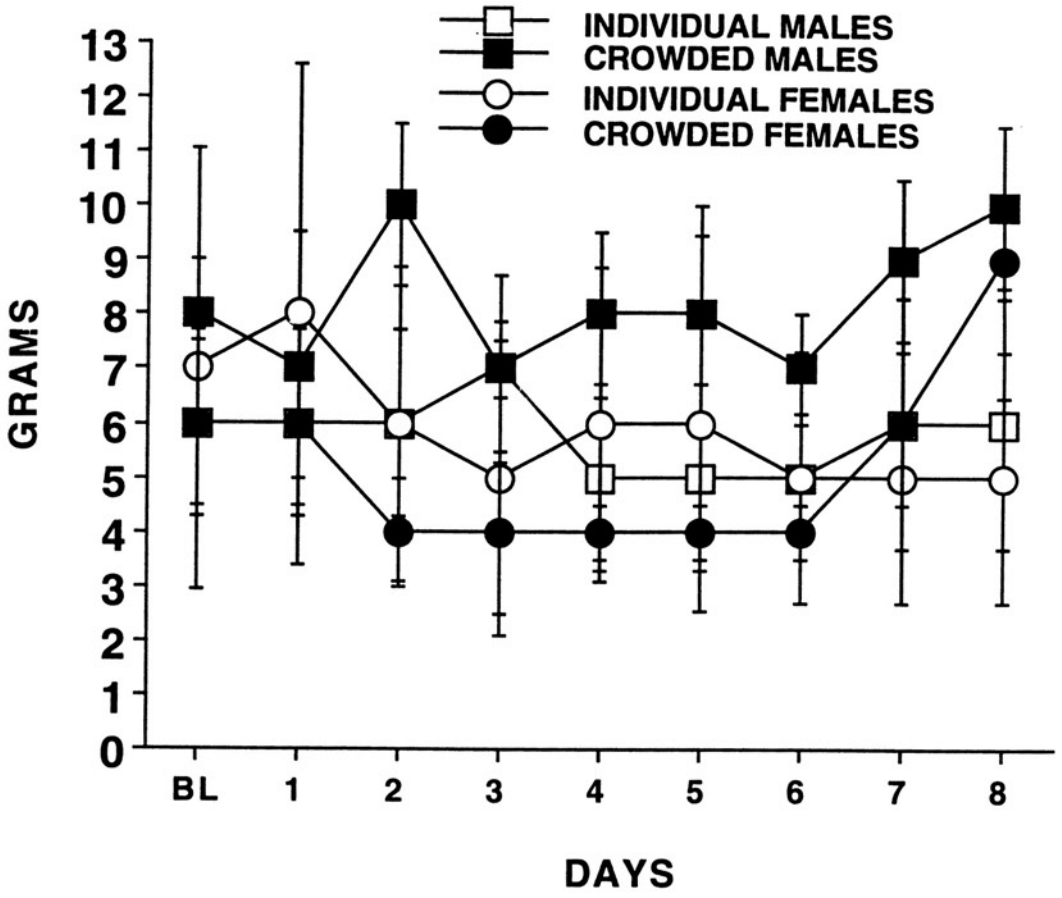

Figure 8.
Experiment 1. Amount of bland powdered food consumed by male and female rats in 6 hours following 18 hours of crowding or individual housing (means and standard errors).

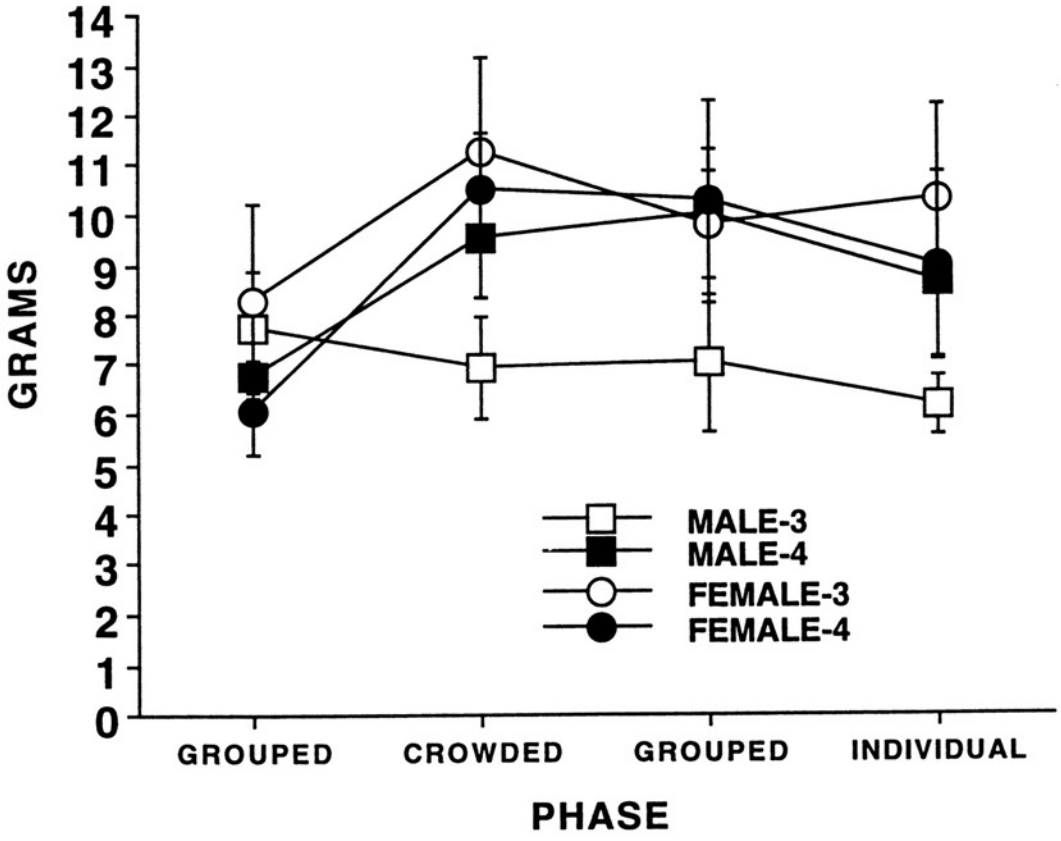

Figure 9.
Experiment 1. Amount of water consumed by male and female rats in 6 hours following 18 hours of grouping or crowing with different number of conspecifics or individual housing (means and standard errors).

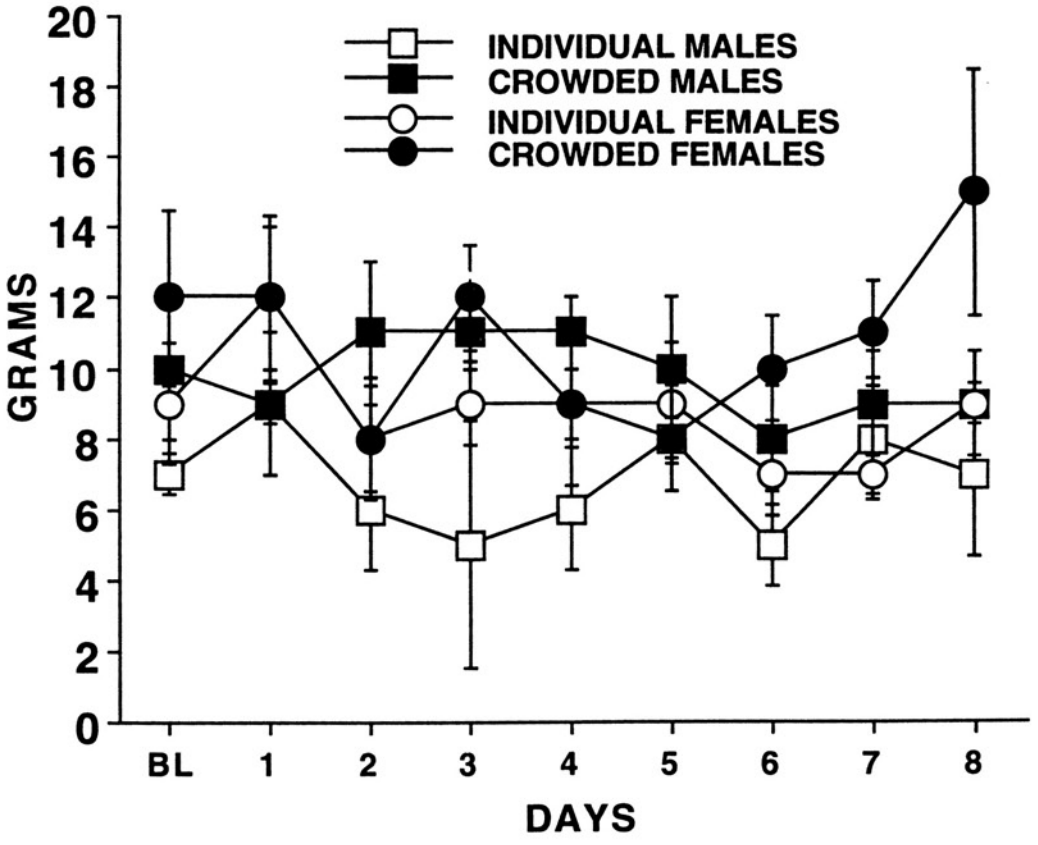

Figure 10.
Experiment 1. Amount of water consumed by male and female rats in 6 hours following 18 hours of crowding or individual housing (means and standard errors).

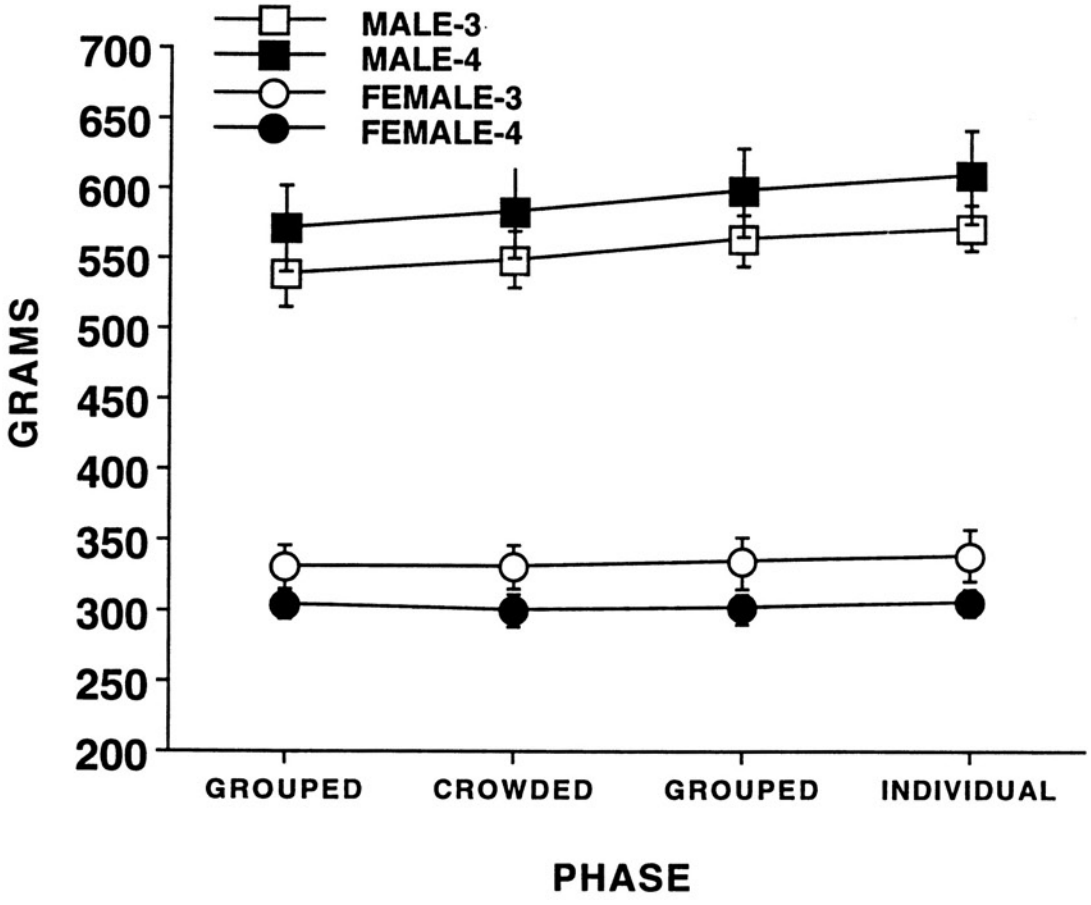

Figure 11.
Experiment 1. Body weight of male and female rats following 18 hours of grouping or crowding with different number of conspecifics or individual housing (means and standard errors).

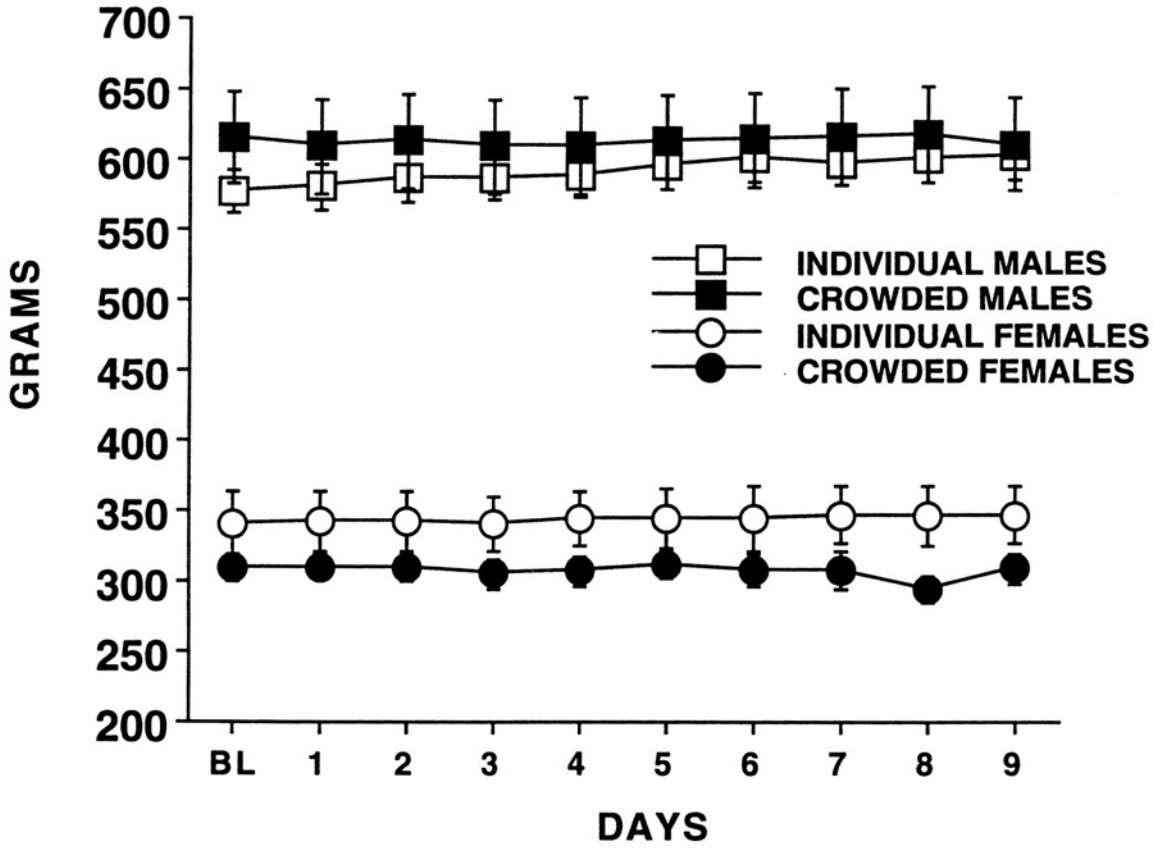

Figure 12.
Experiment 1. Body weight of male and female rats following 18 hours of crowding or individual housing (means and standard errors).

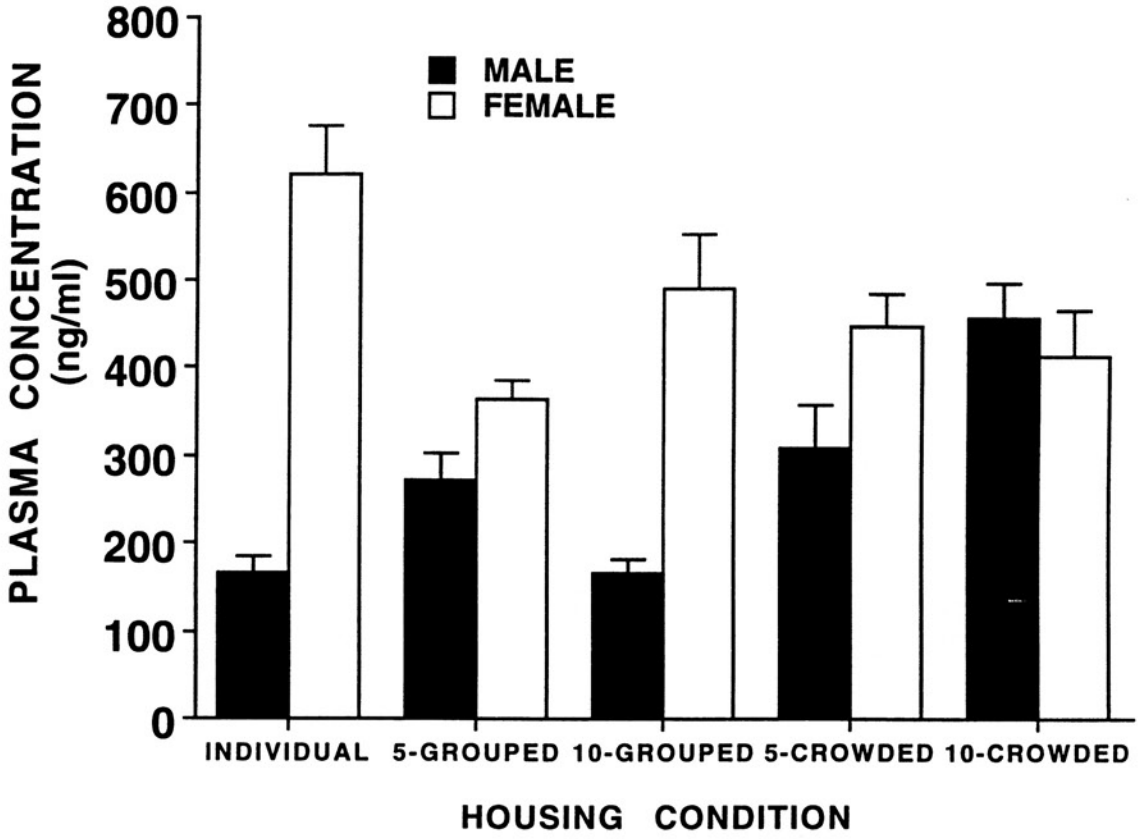

Figure 13.
Experiment 2. Effects of differential housing on plasma corticosterone levels of male and female rats (means and standard errors).

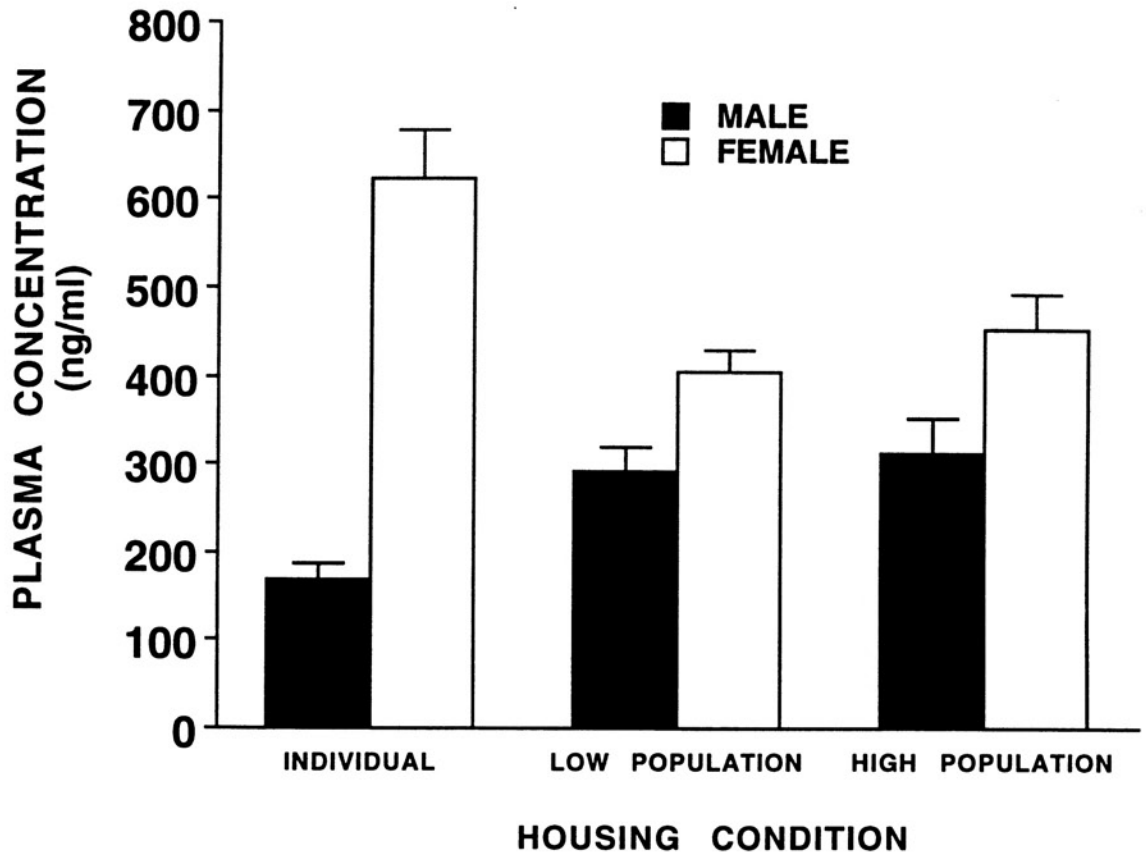

Figure 14.
Experiment 2. Effects of individual, low population, or high population housing conditions on plasma corticosterone levels of male and female rats (means and standard errors).

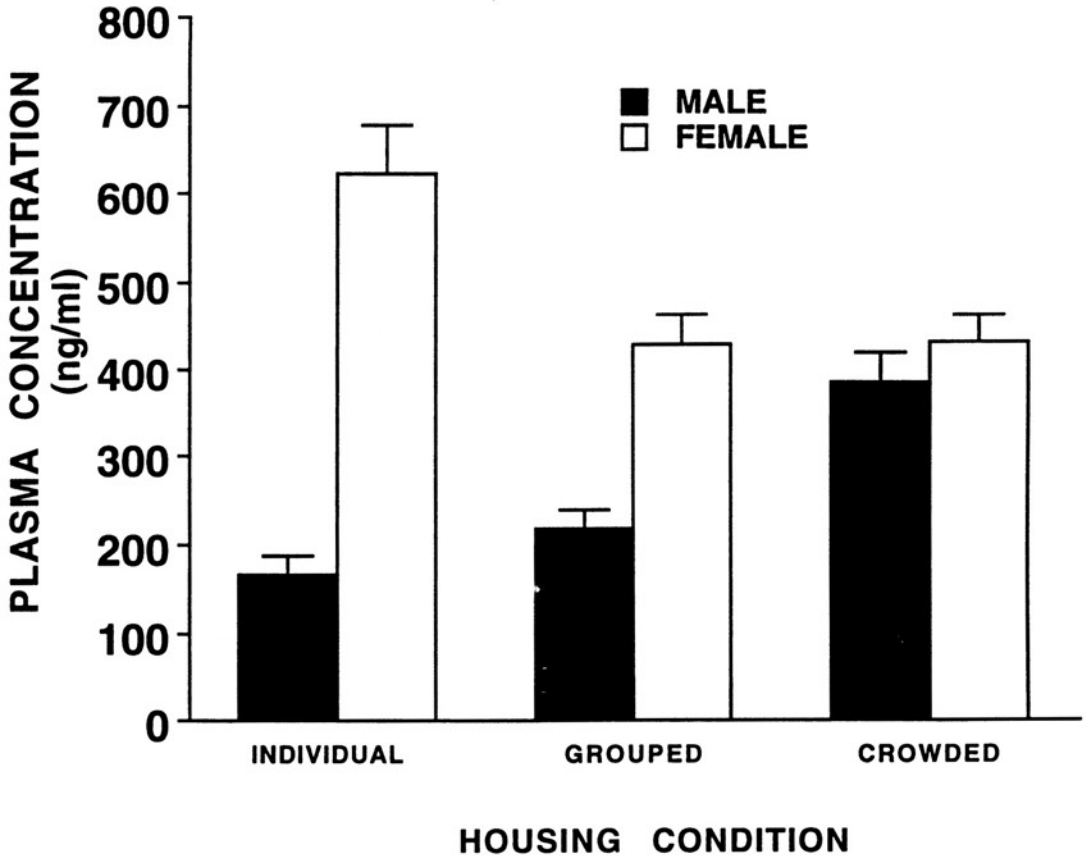

Figure 15.
Experiment 2. Effects of individual housing, grouping, or crowing on plasma corticosterone levels of male and female rats (means and standard errors).

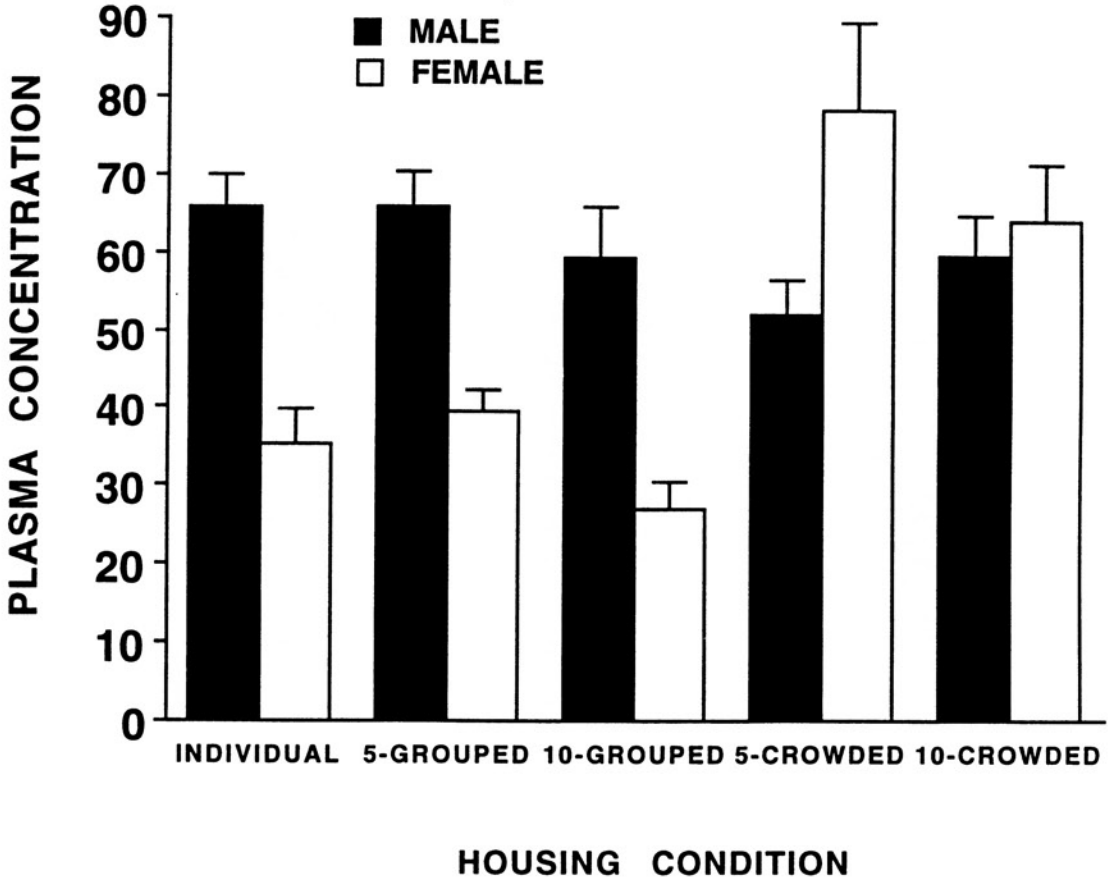

Figure 16.
Experiment 2. Effects of differential housing on plasma insulin levels of male and female rats (means and standard errors).

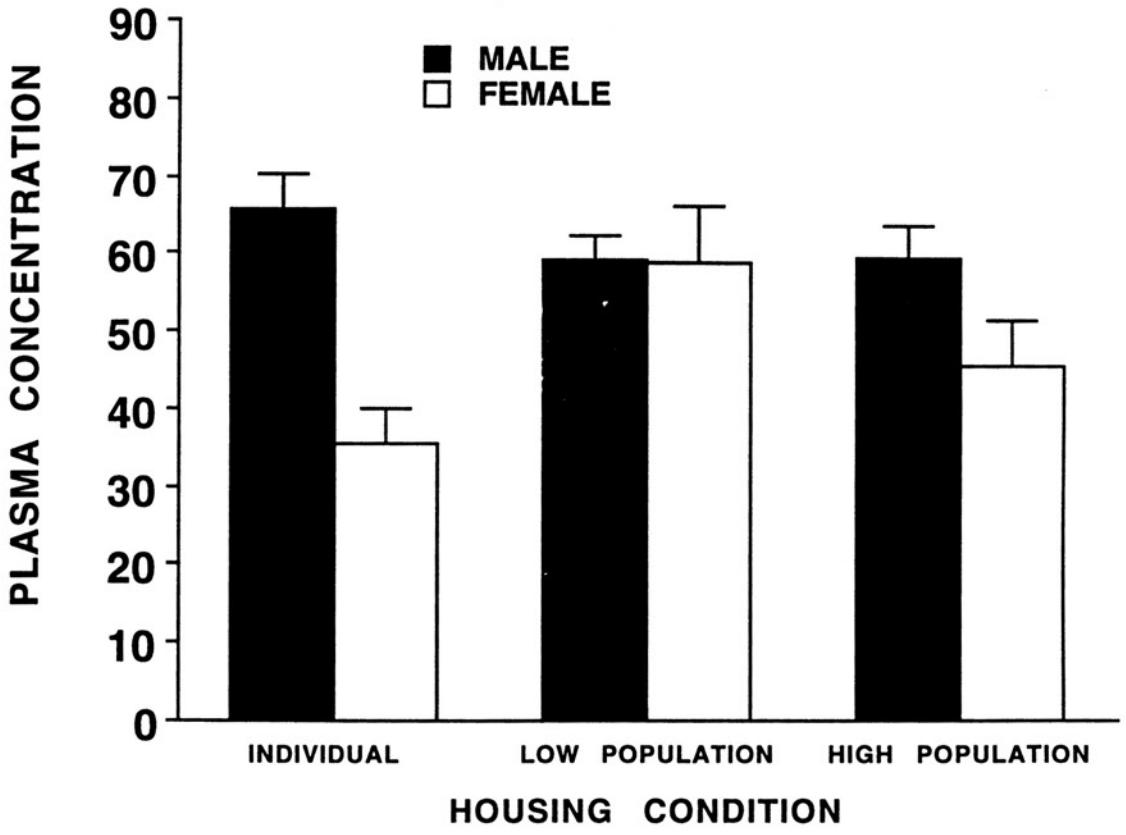

Figure 17.
Experiment 2. Effects of individual, low population, or high population housing conditions on plasma insulin levels of male and female rats (means and standard errors).

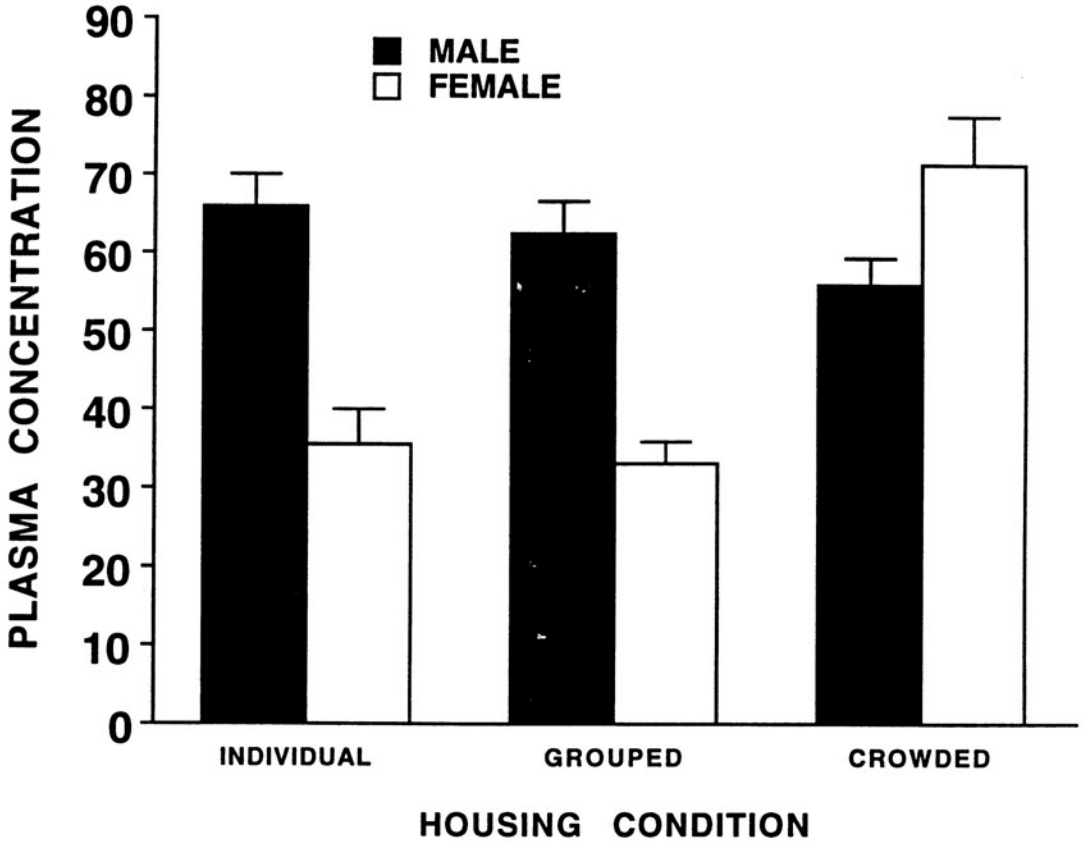

Figure 18.
Experiment 2. Effects of individual housing, grouping, or crowding on plasma insulin levels of male and female rats (means and standard errors).

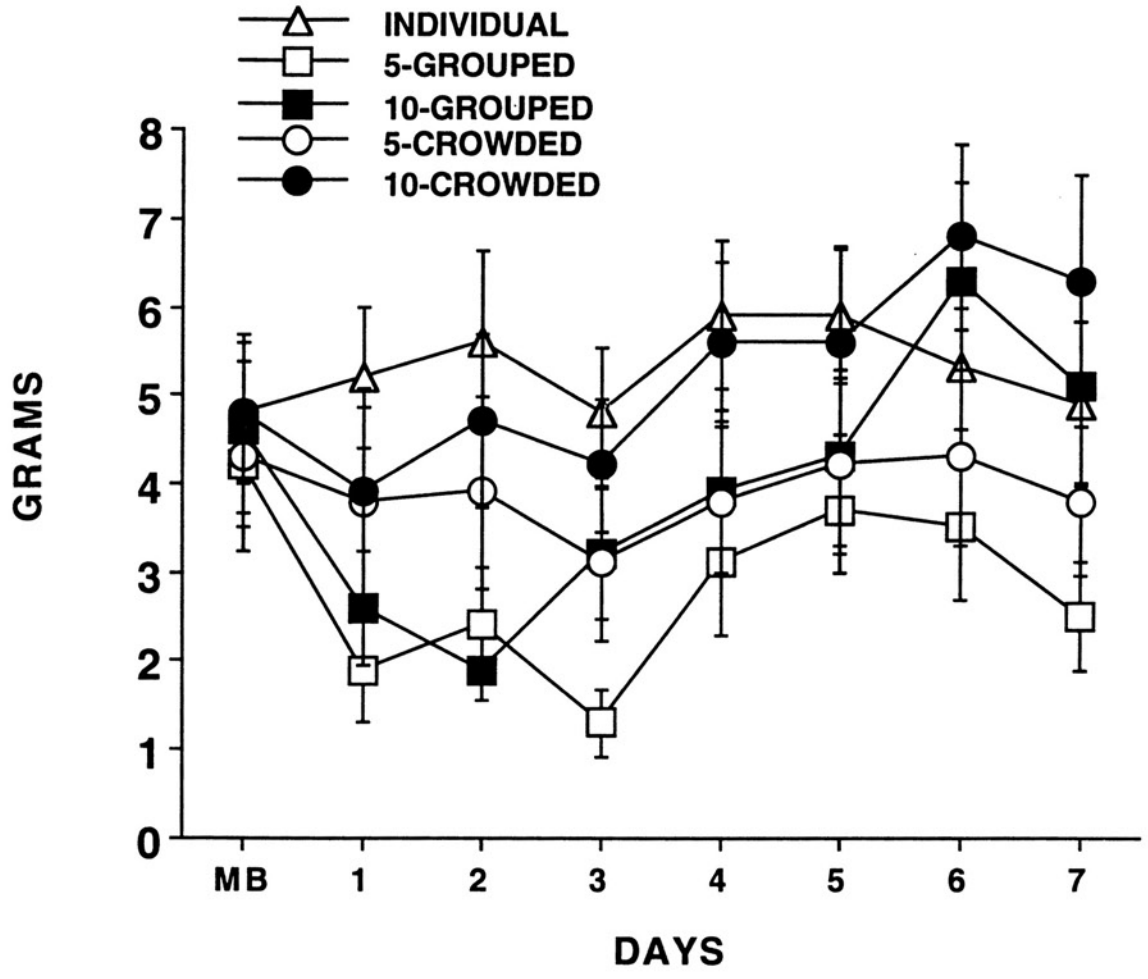

Figure 19.
Experiment 2. Amount of sweet food consumed by male rats in 6 hours following 18 hours of housing in 5-crowded, 10-crowded, 5-grouped, 10-grouped, or individual conditions (means and standard errors).

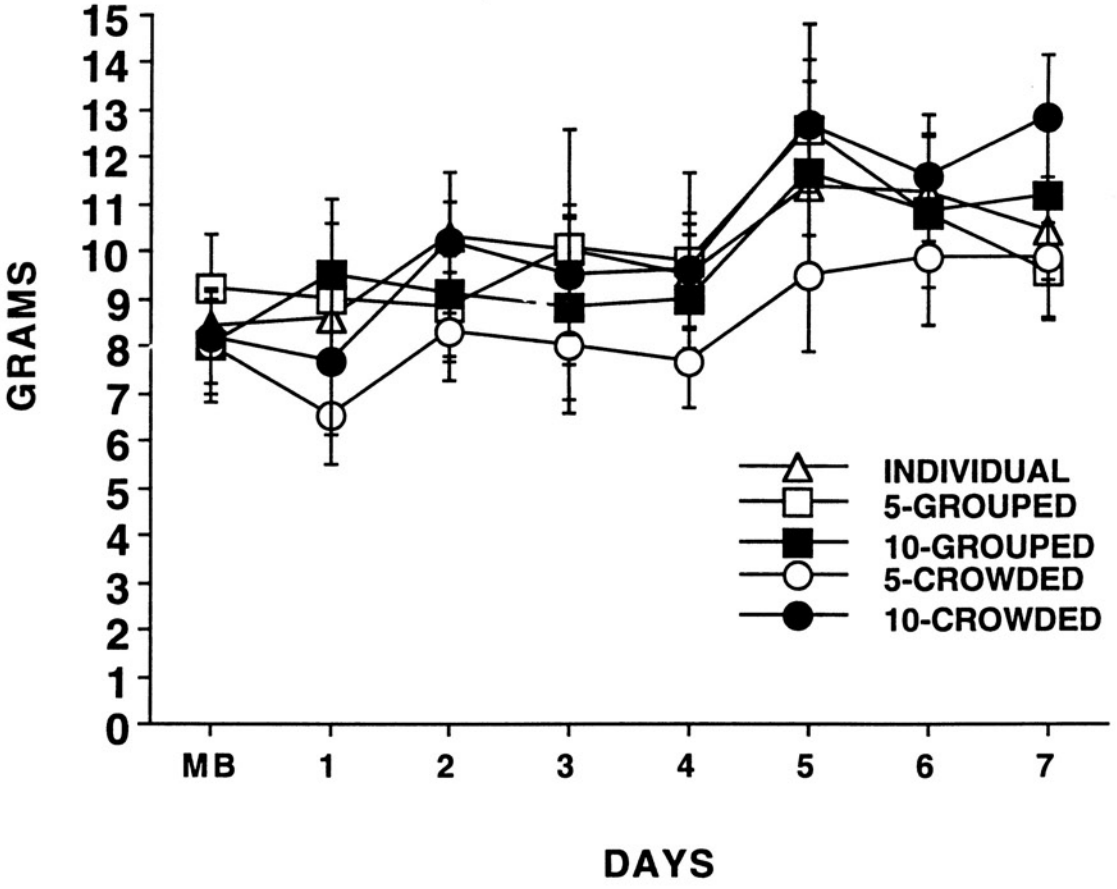

Figure 20.
Experiment 2. Amount of sweet food consumed by female rats in 6 hours following 18 hours of housing in 5-crowded, 10-crowded, 5-grouped, 10-grouped, or individual conditions (means and standard errors).

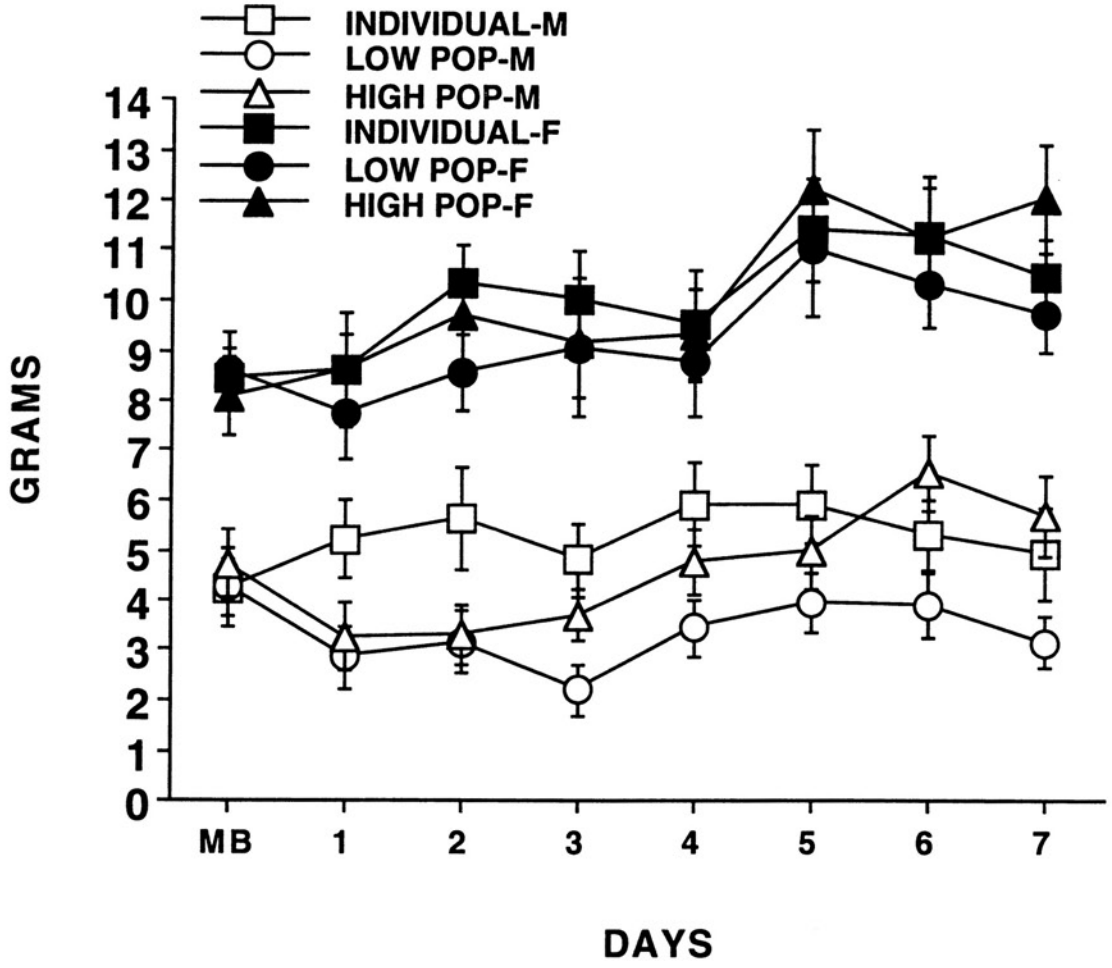

Figure 21.
Experiment 2. Amount of sweet food consumed by male and female rats in 6 hours following 18 hours in individual, low population, or high population housing conditions (means and standard errors).

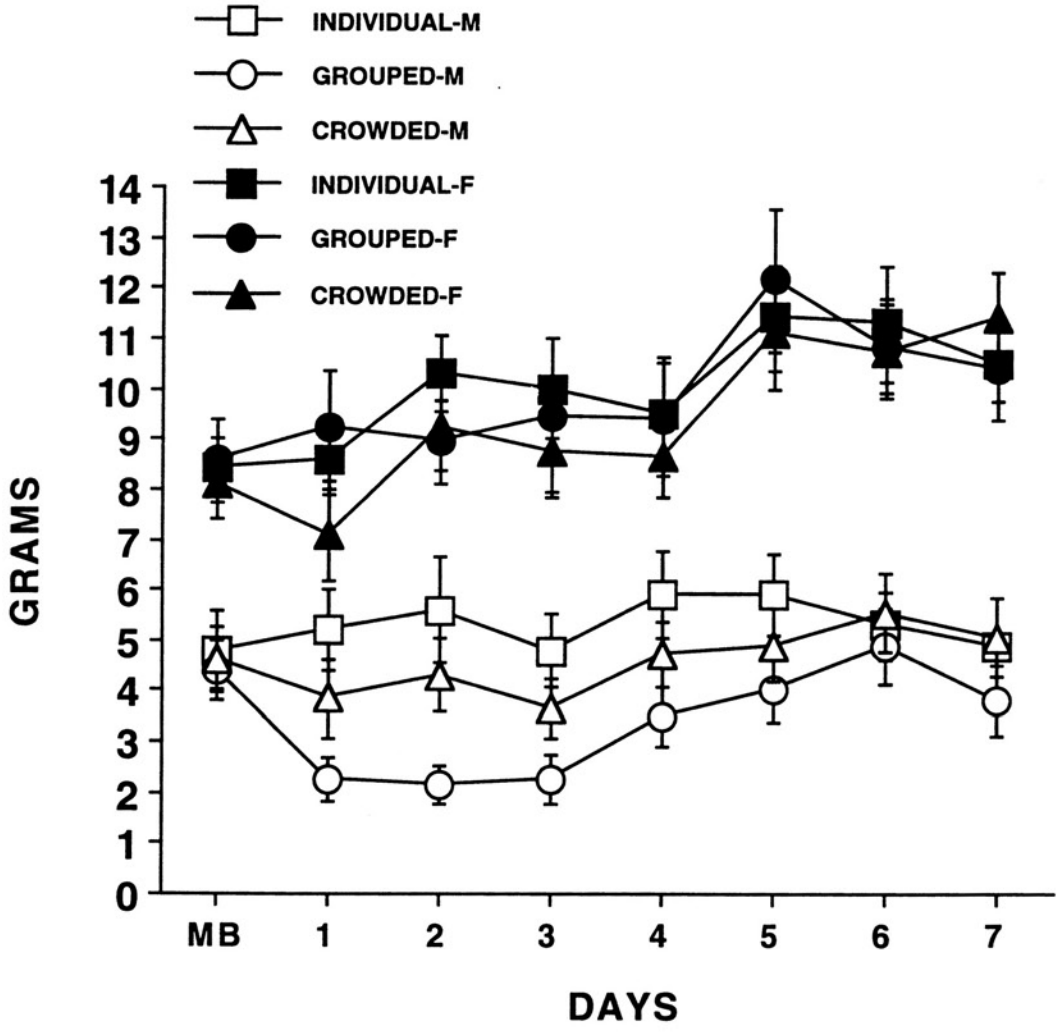

Figure 22.
Experiment 2. Amount of sweet food consumed by male and female rats in 6 hours following 18 hours of individual housing, grouping, or crowding (means and standard errors).

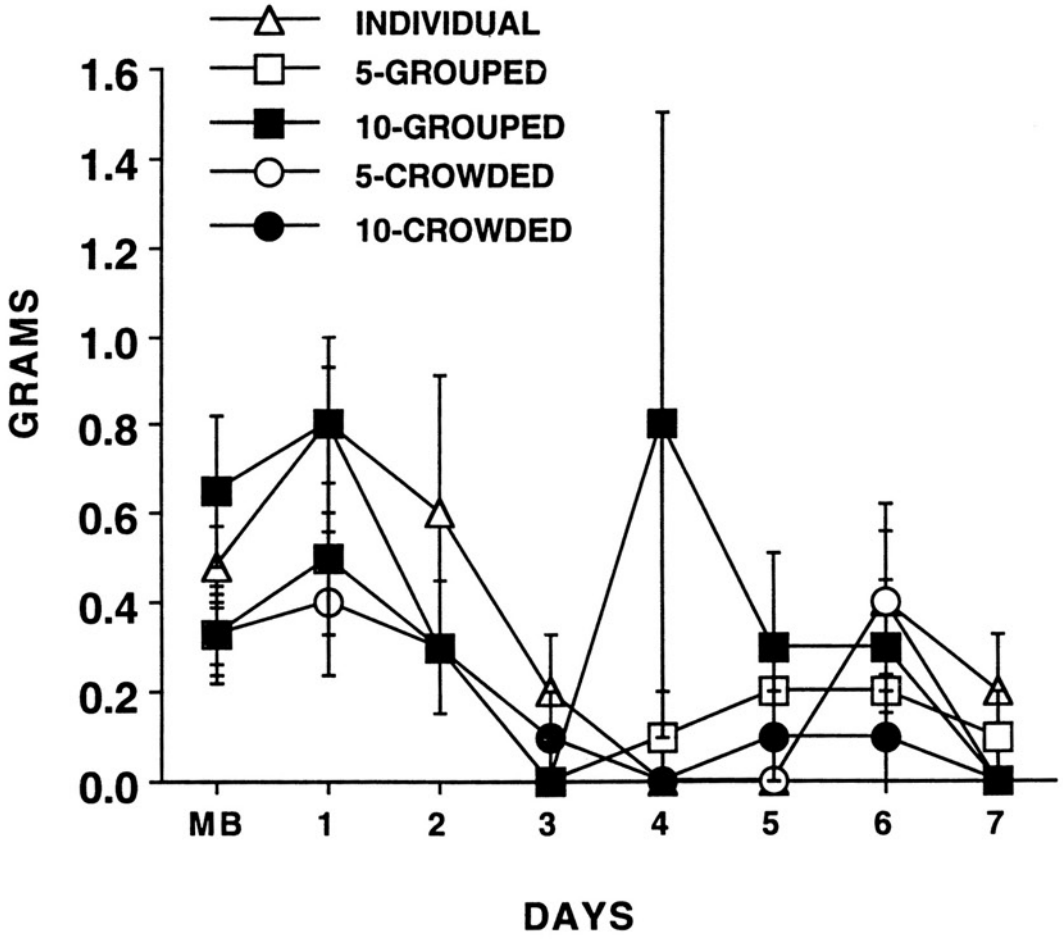

Figure 23.
Experiment 2. Amount of bland food consumed by male rats in 6 hours following 18 hours of housing in 5-crowded, 10-crowded, 5-grouped, 10-grouped, or individual conditions (means and standard errors).

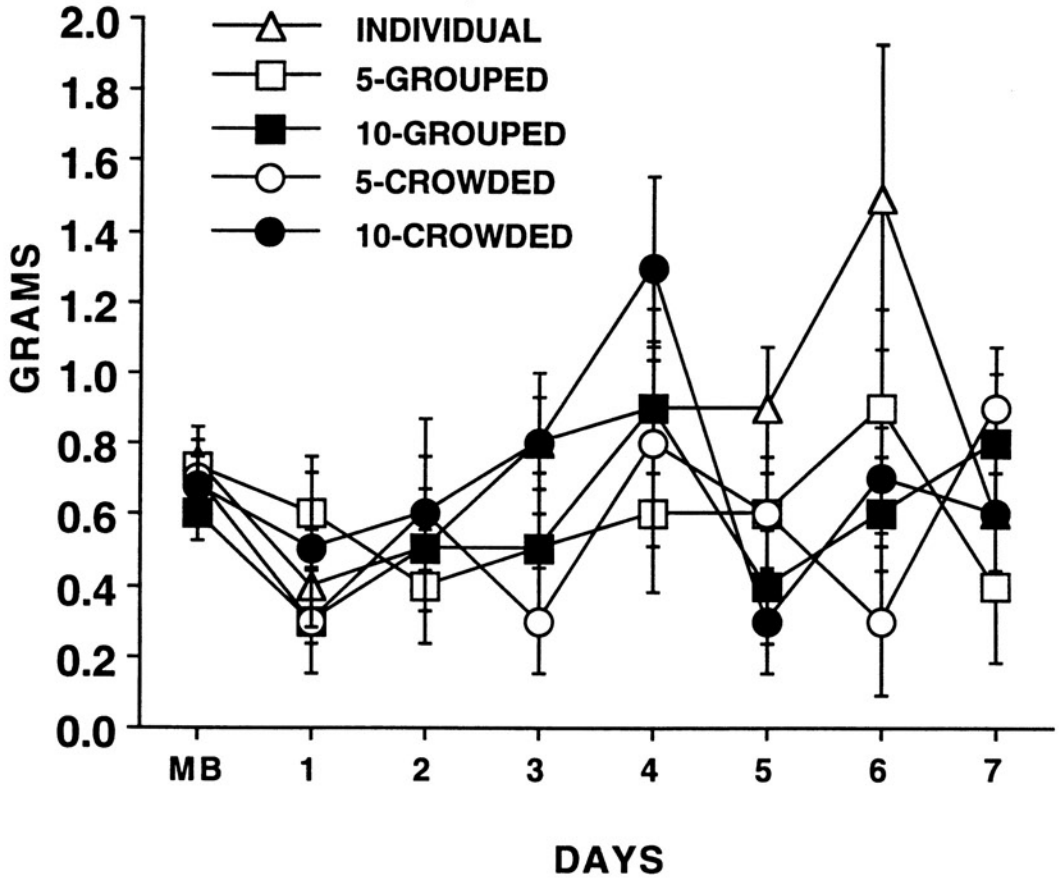

Figure 24.
Experiment 2. Amount of bland food consumed by female rats in 6 hours following 18 hours of housing in 5-crowded, 10-crowded, 5-grouped, 10-grouped, or individual conditions (means and standard errors).

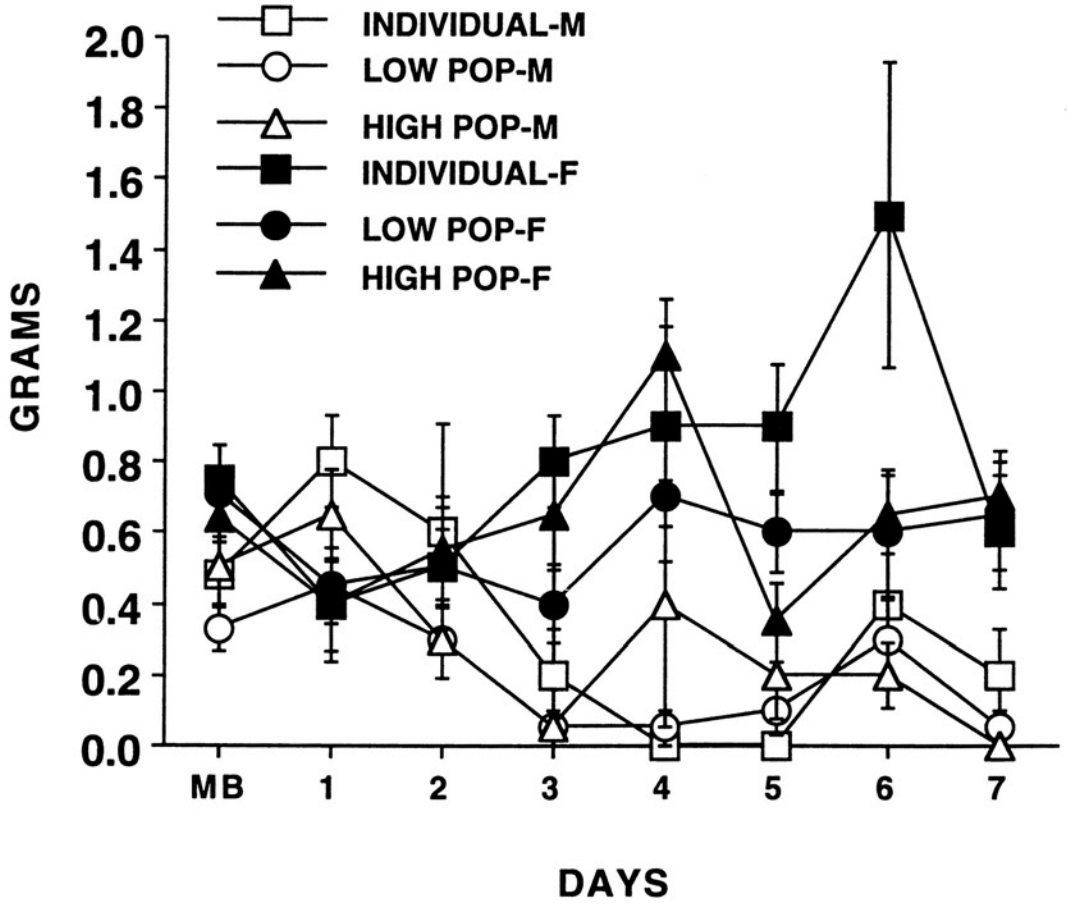

Figure 25.
Experiment 2. Amount of bland food consumed by male and female rats in 6 hours following 18 hours in individual, low population, or high population housing conditions (means and standard errors).

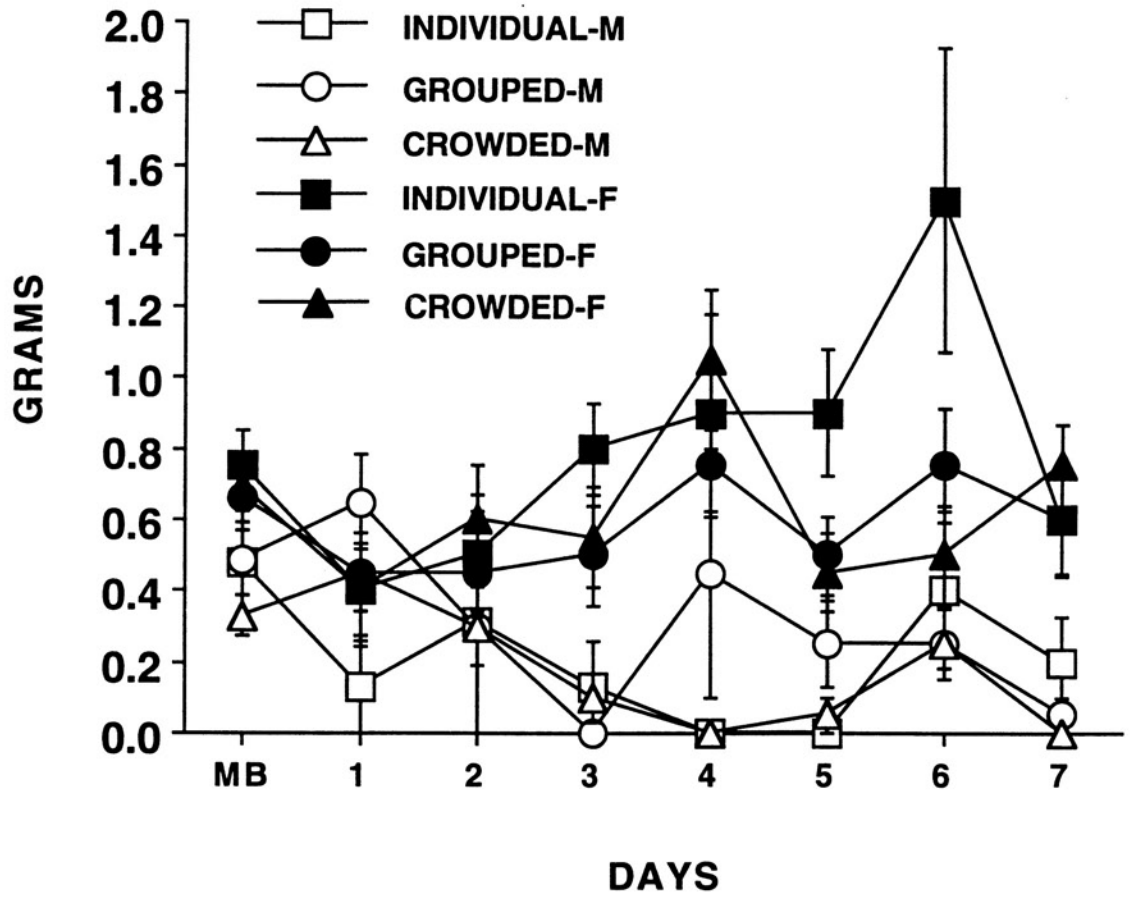

Figure 26.
Experiment 2. Amount of bland food consumed by male and female rats in 6 hours following 18 hours of individual housing, grouping, or crowding (means and standard errors).

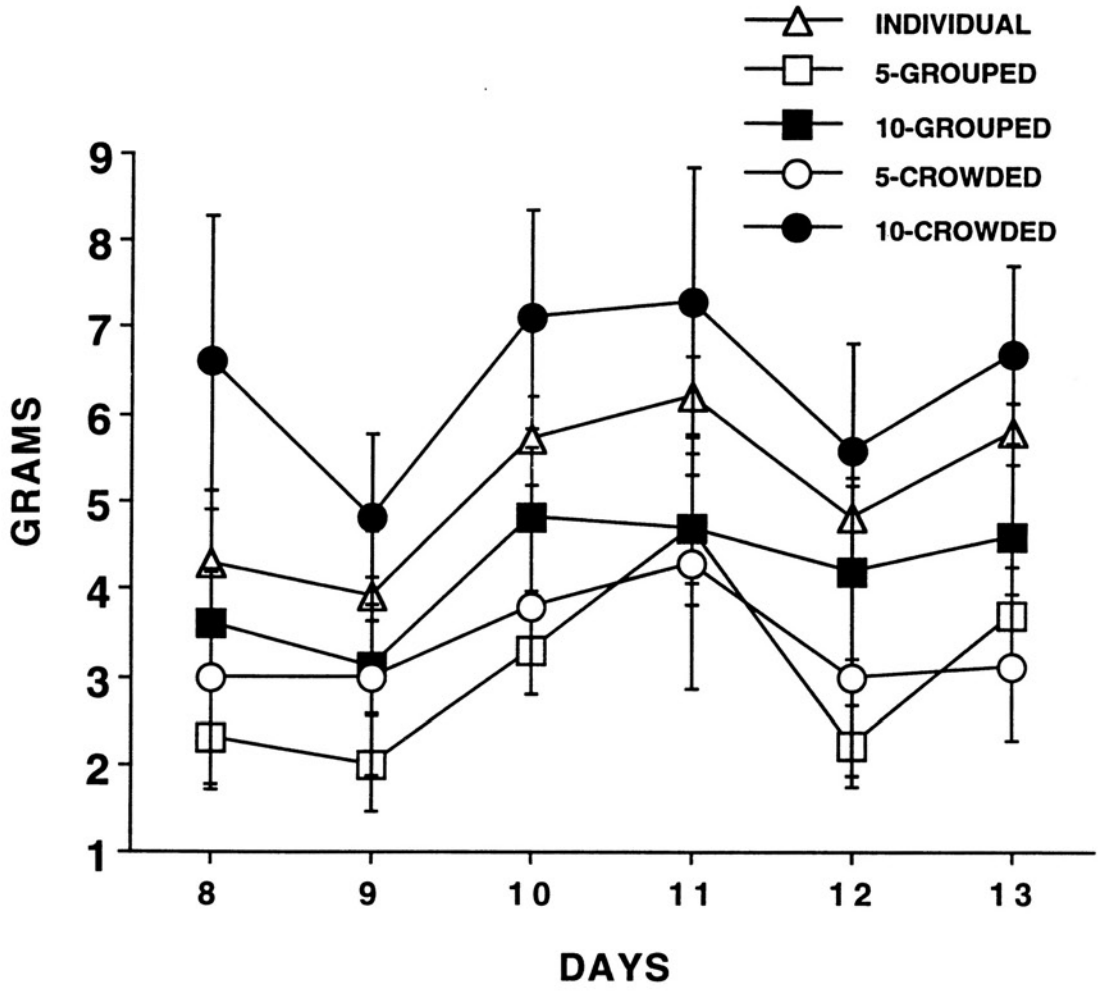

Figure 27.
Experiment 2. Amount of bland food consumed by male rats in 6 hours without sweet food availability following 18 hours of housing in 5-crowded, 10-crowded, 5-grouped, 10-grouped, or individual housing (means and standard errors).

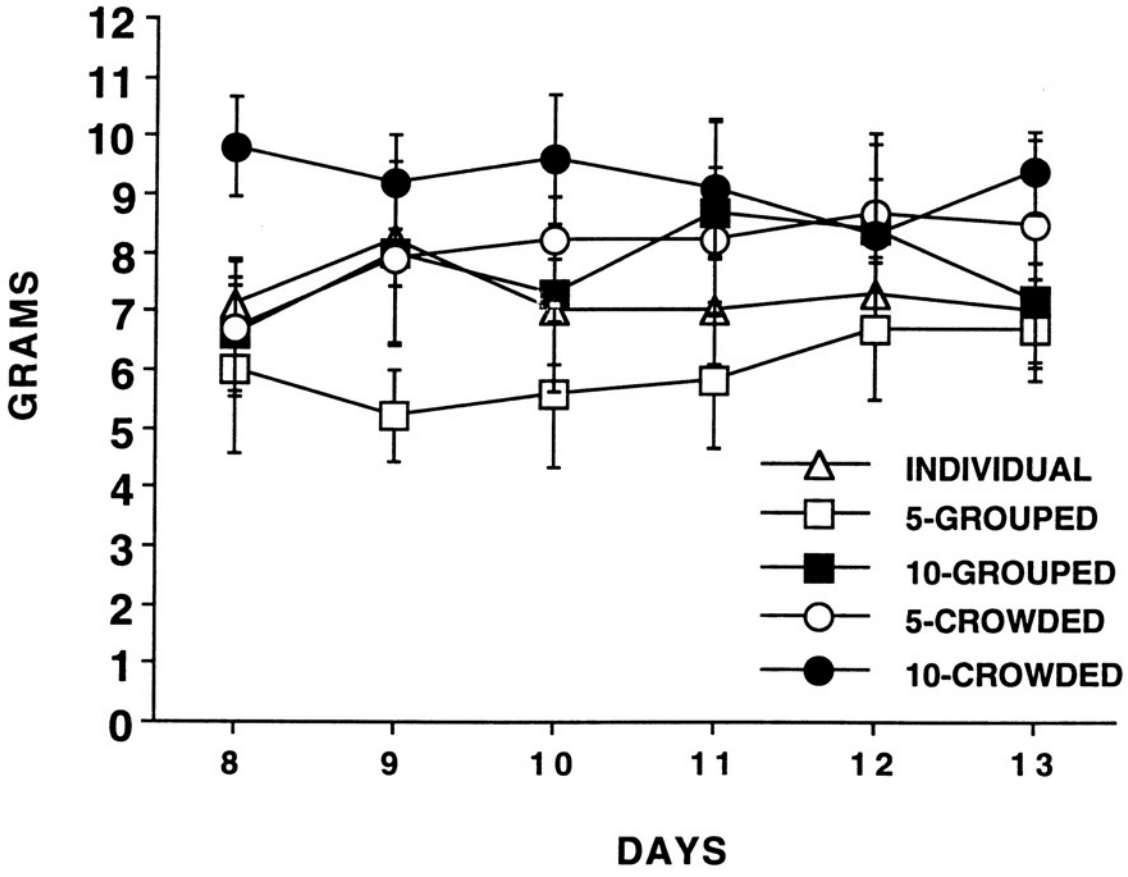

Figure 28.
Experiment 2. Amount of bland food consumed by female rats in 6 hours without sweet food availability following 18 hours of housing in 5-crowded, 10-crowded, 5-grouped, 10-grouped, or individual housing (means and standard errors).

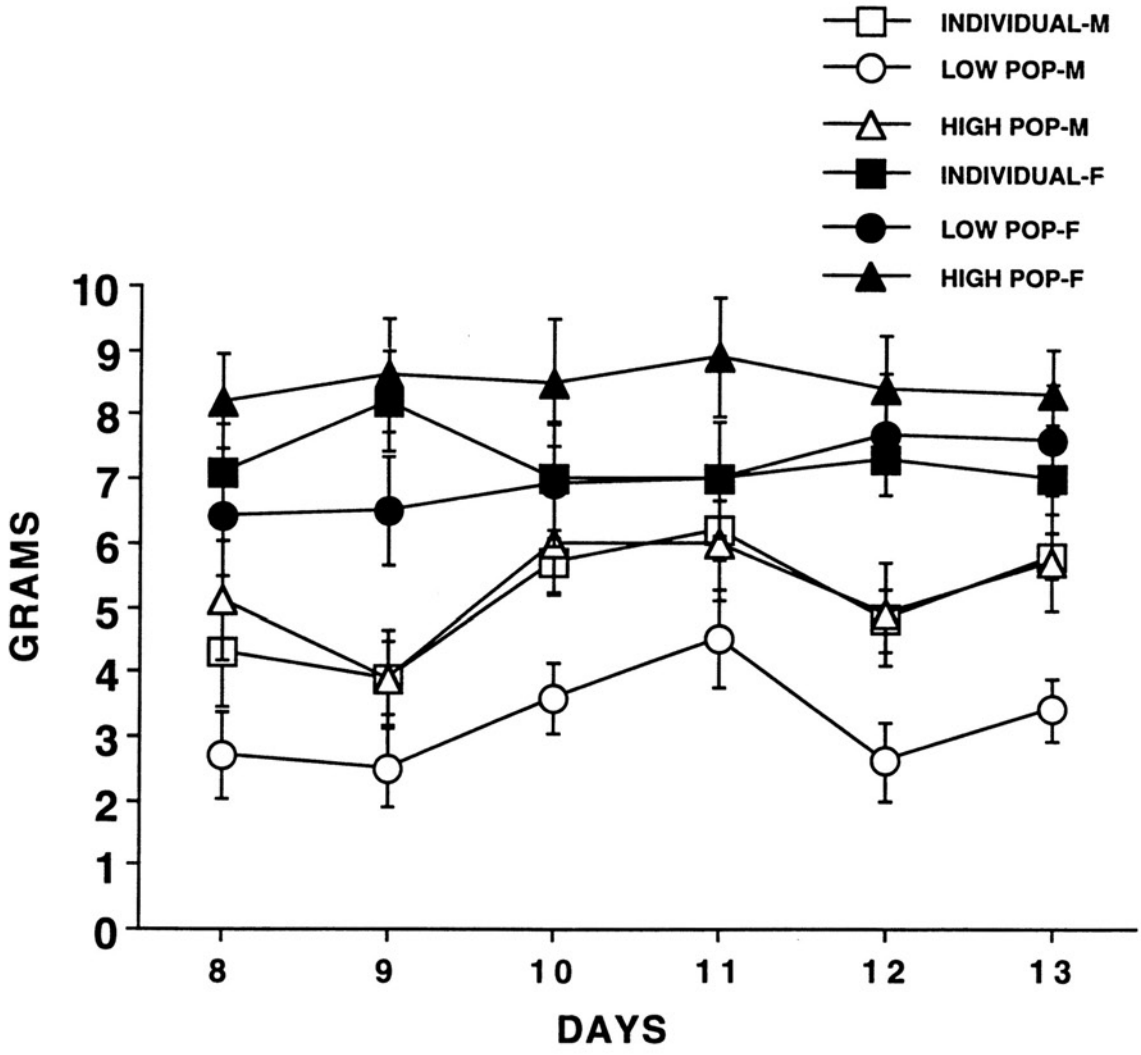

Figure 29.
Experiment 2. Amount of bland food consumed by male and female rats in 6 hours without sweet food availability following 18 hours in individual, low population, or high population housing conditions (means and standard errors).

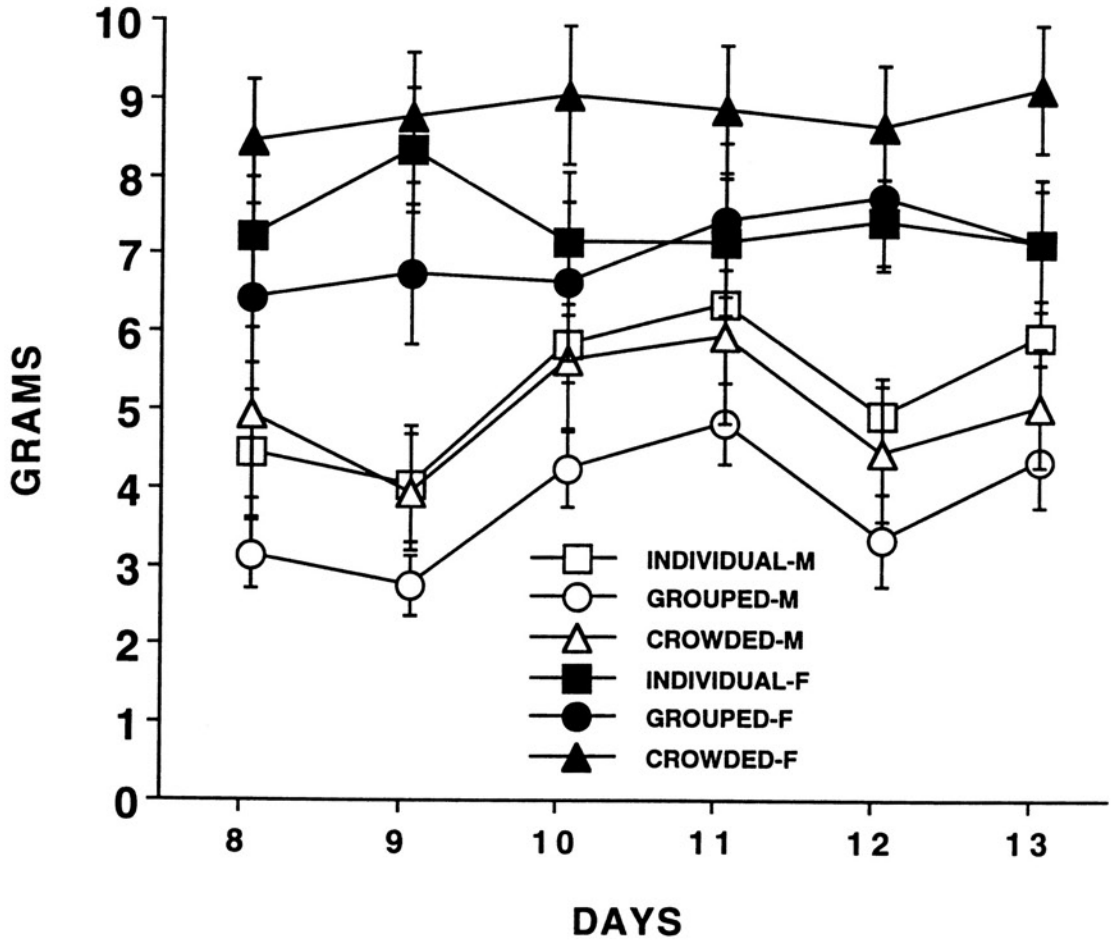

Figure 30.
Experiment 2. Amount of bland food consumed by male and female in 6 hours without sweet food availability following 18 hours of individual housing, grouping, or crowding (means and standard errors).

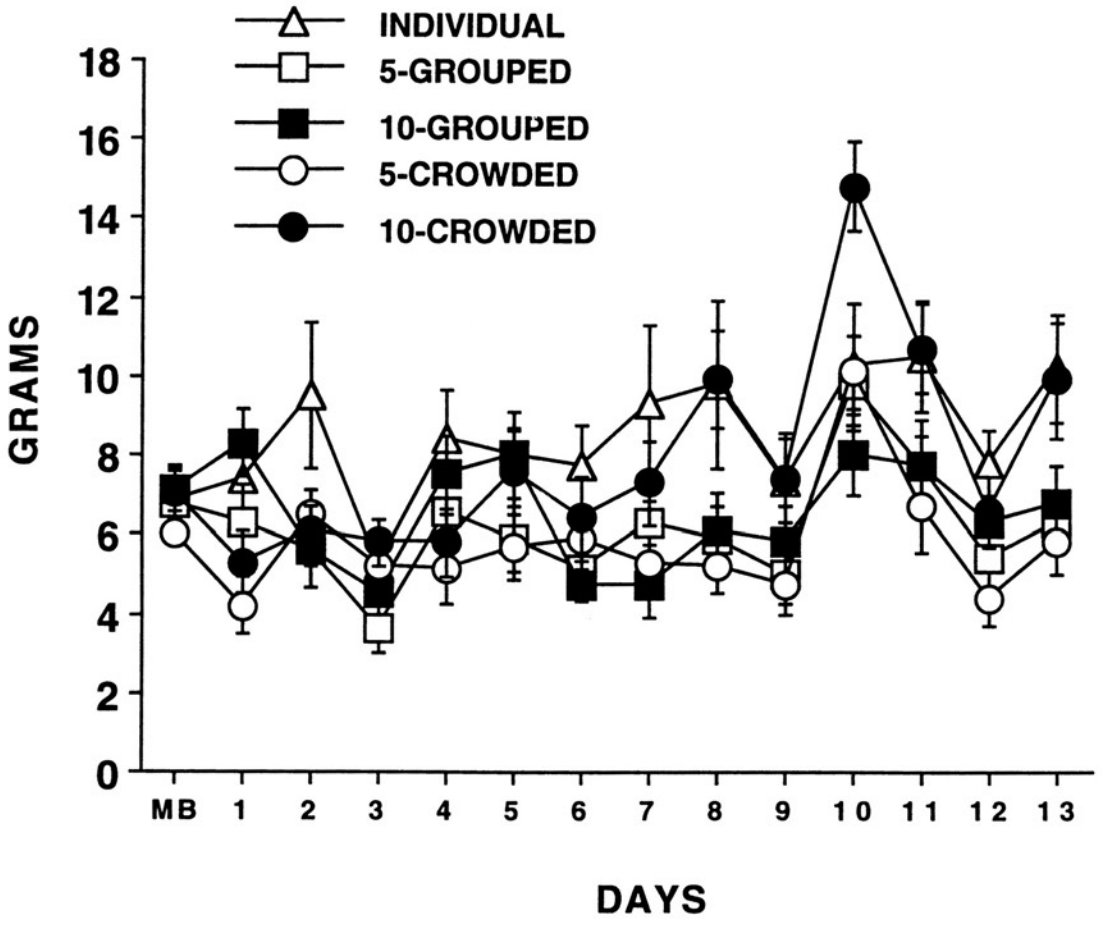

Figure 31.
Experiment 2. Amount of water consumed by male rats in 6 hours following 18 hours of housing in 5-crowded, 10-crowded, 5-grouped, 10-grouped, or individual conditions (means and standard errors).

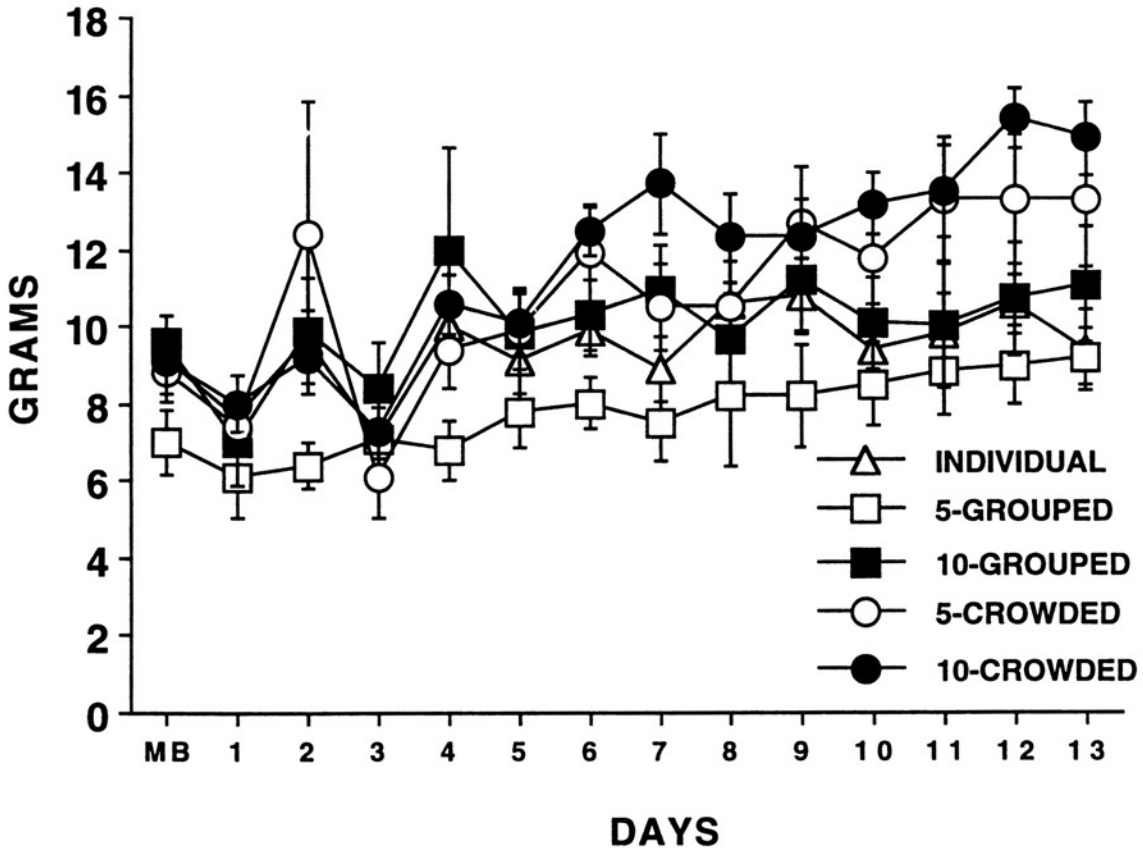

Figure 32.
Experiment 2. Amount of water consumed by female rats in 6 hours following 18 hours of housing in 5-crowded, 10-crowded, 5-grouped, 10-grouped, or individual conditions (means and standard errors).

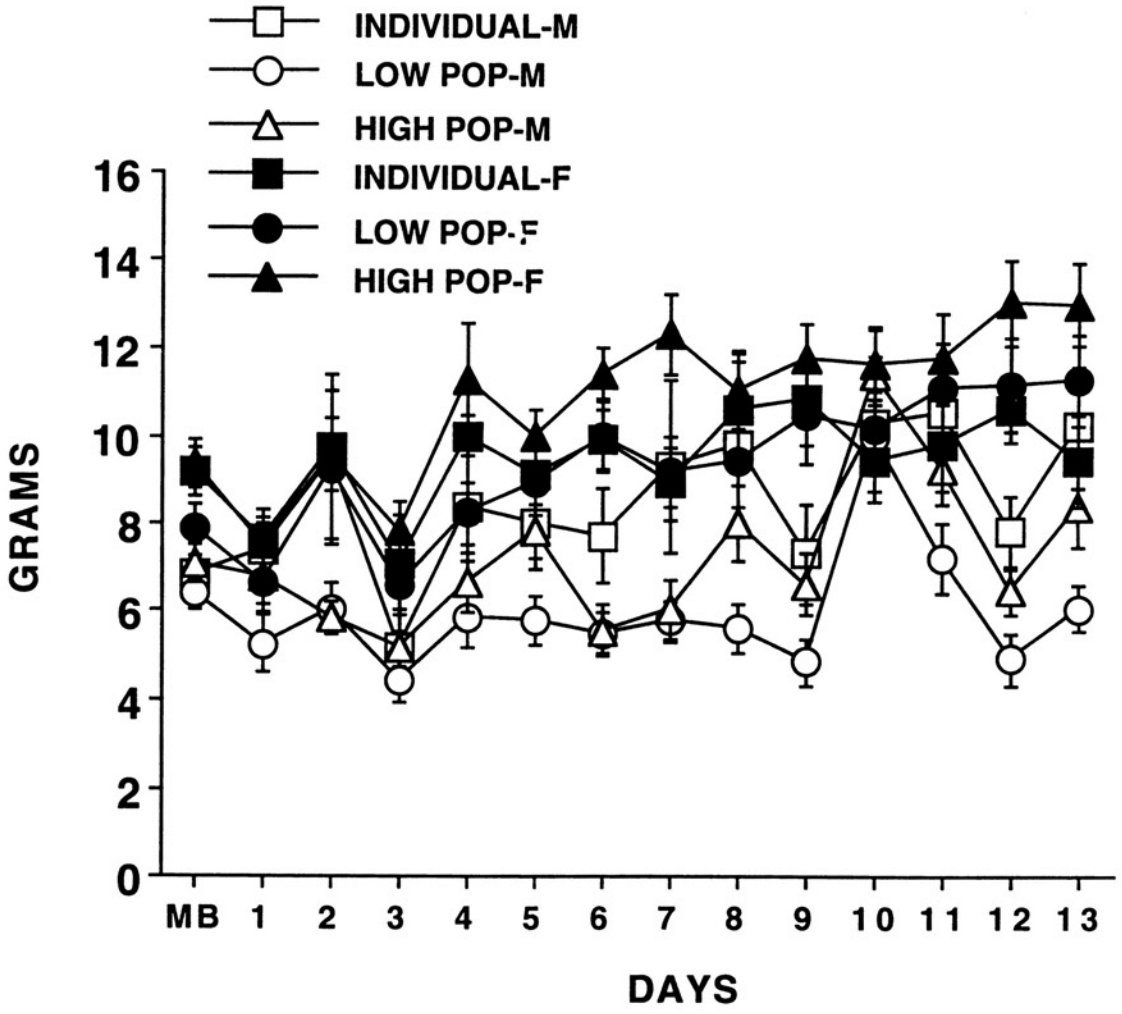

Figure 33.
Experiment 2. Amount of water consumed by male and female rats in 6 hours following 18 hours in individual, low population, or high population housing conditions (means and standard errors).

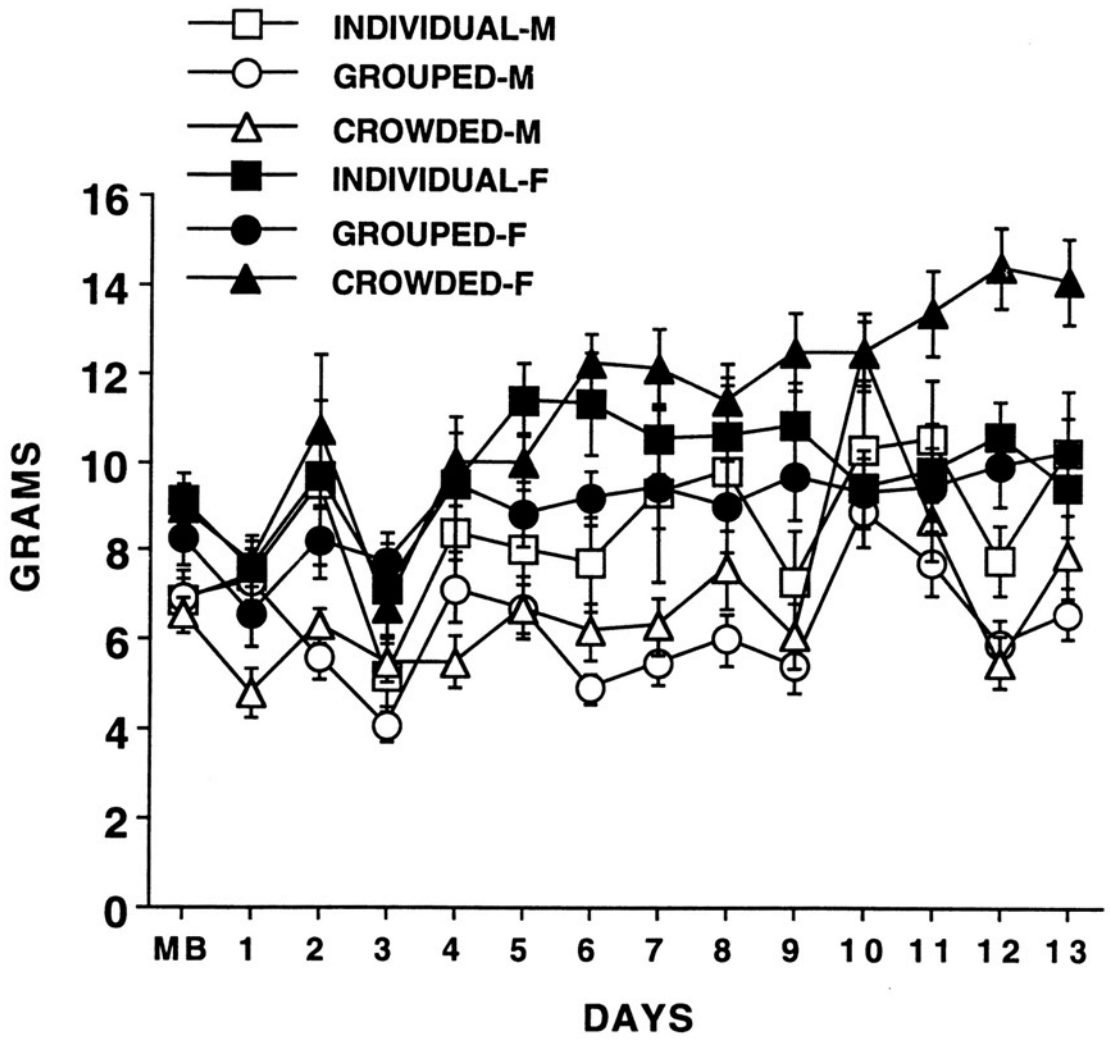

Figure 34.
Experiment 2. Amount of water consumed by male and female rats in 6 hours following 18 hours of individual housing, grouping, or crowding (means and standard errors).

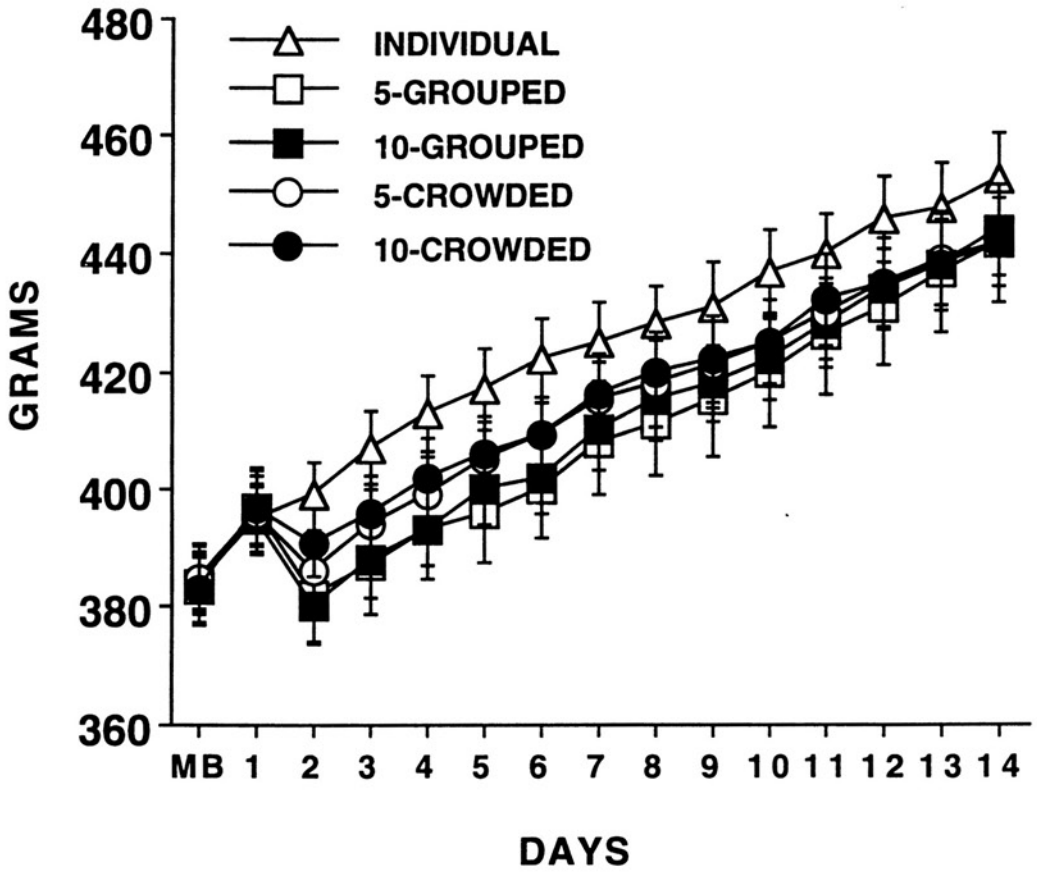

Figure 35.
Experiment 2. Body weight of male rats following 18 hours of housing in 5-crowded, 10-crowded, 5-grouped, 10-grouped, or individual conditions (means and standard errors).

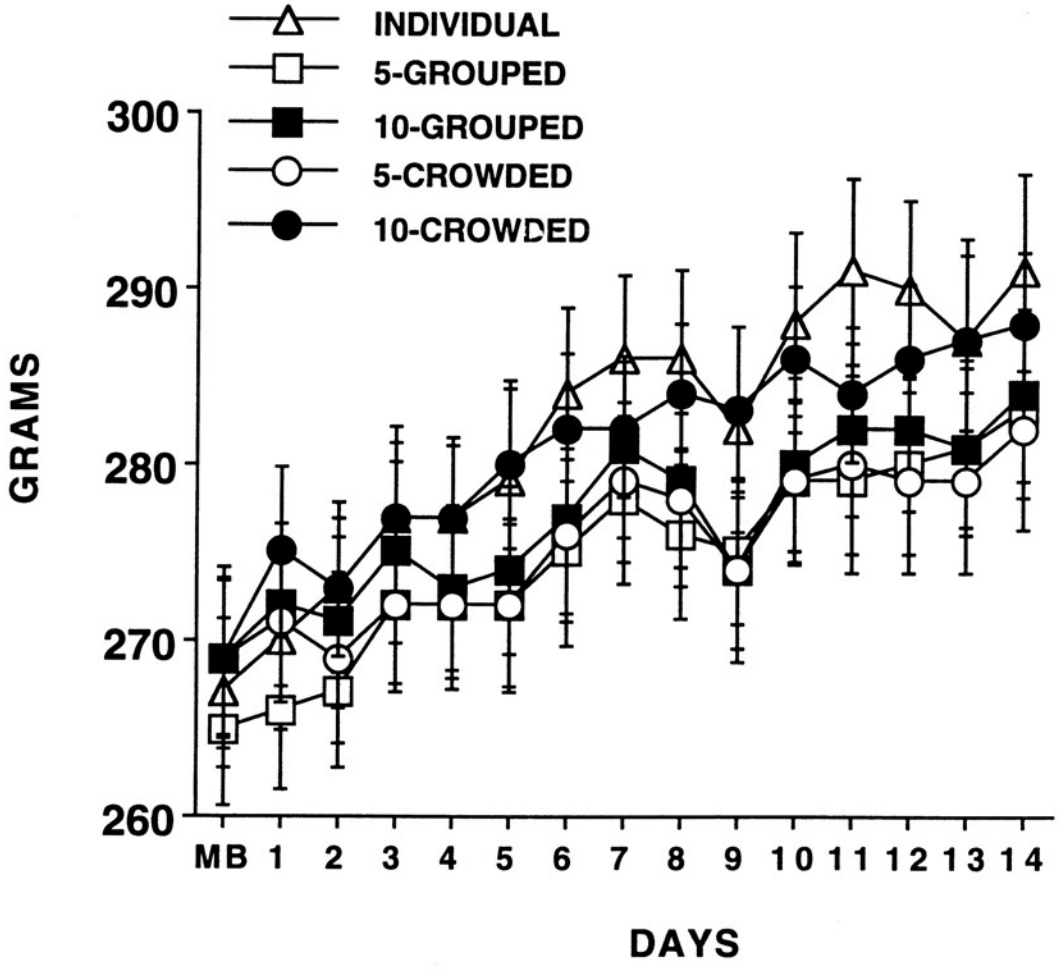

Figure 36.
Experiment 2. Body weight of female rats following 18 hours of housing in 5-crowded, 10-crowded, 5-grouped, 10-grouped, or individual conditions (means and standard errors).

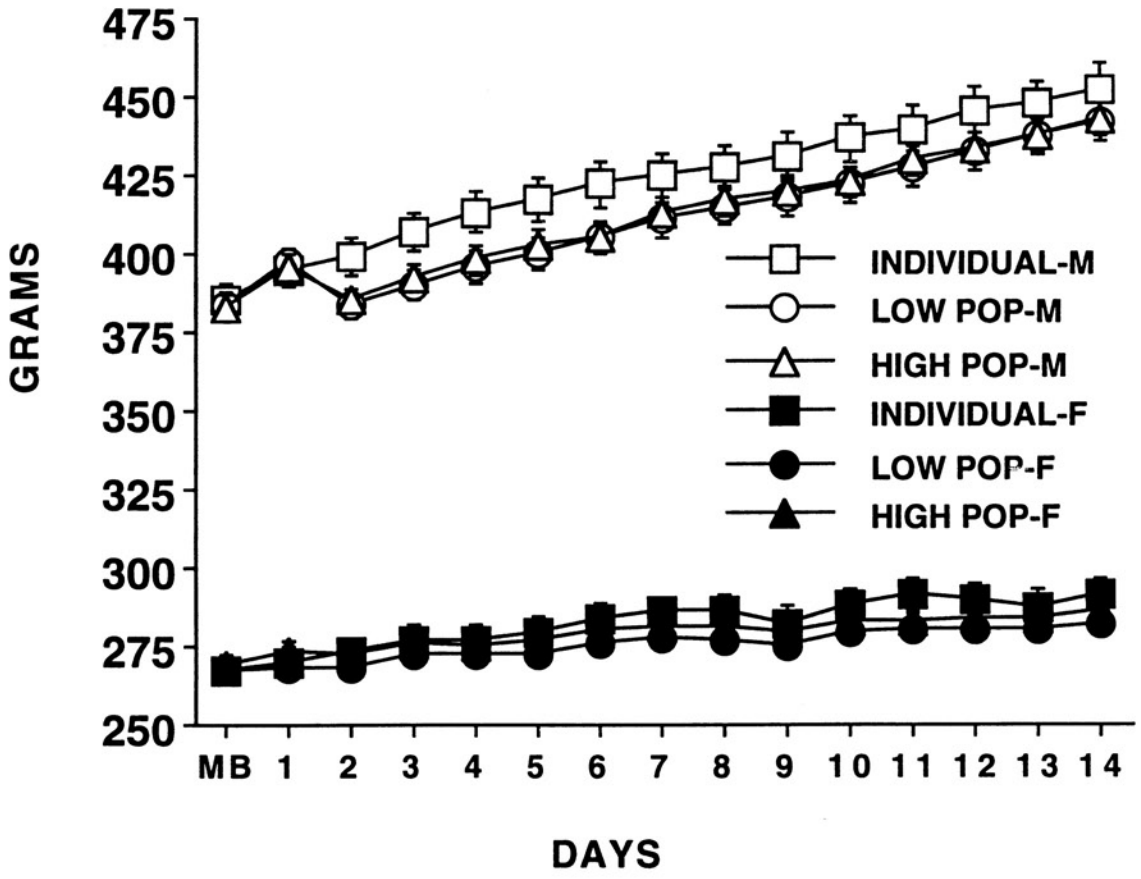

Figure 37.
Experiment 2. Body weight of male and female rats following 18 hours in individual, low population, or high population housing conditions (means and standard errors).

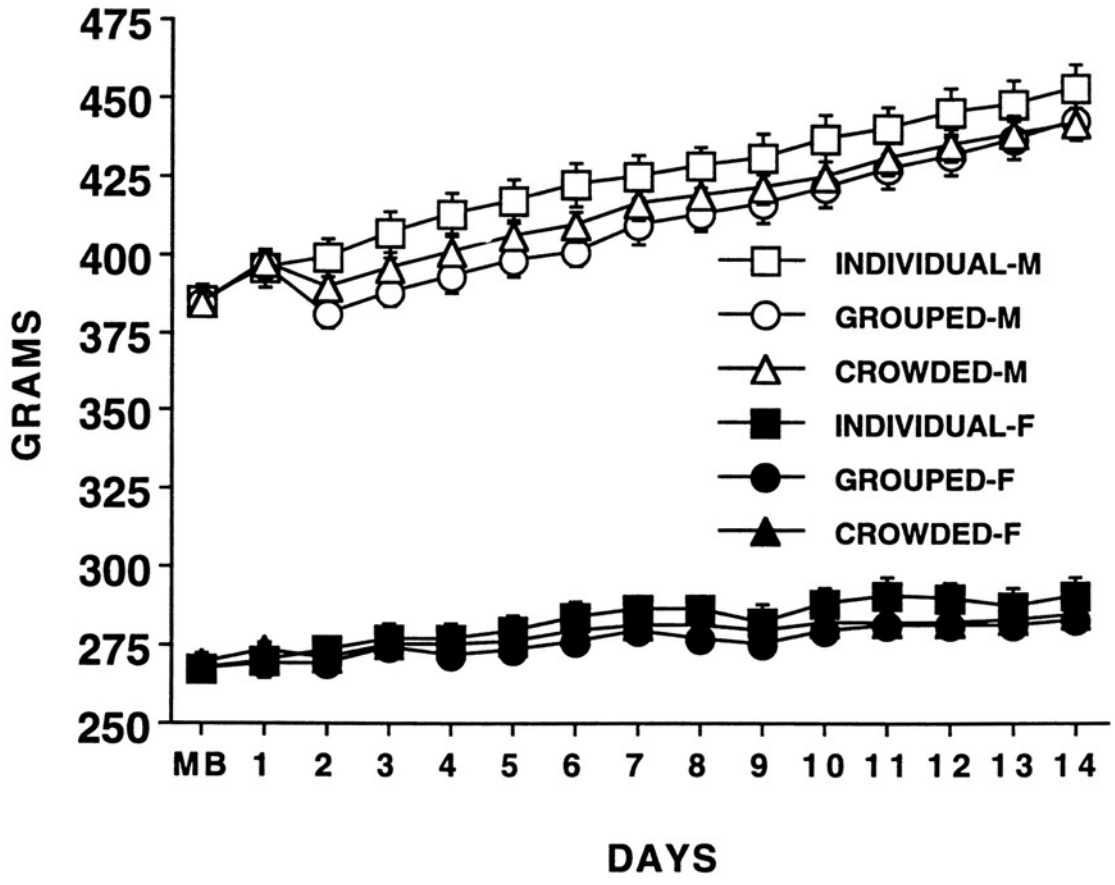

Figure 38.
Experiment 2. Body weight of male and female rats following 18 hours of individual housing, grouping, or crowding (means and standard errors).

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