Stress-like Responses to Common Procedures in Male Rats Housed Alone or with Other Rats

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The objective of this study was to assess the cardiovascular function and behavior of male Sprague-Dawley rats housed individually or with one or three cagemates during resting conditions and when subjected to common husbandry and experimental procedures and potentially stressful olfactory stimuli. Heart rate (HR), mean arterial blood pressure (MAP), and movement in the cage were collected by using radiotransmitters for 24 h on an experiment-free day and for 2 h before and 3 h after the following acute procedures: cage change, restraint and subcutaneous injection, restraint and tail-vein injection, exposure to the odor of urine and feces from stressed rats, and exposure to the odor of dried rat blood. Home cage behaviors (sleeping, awake, moving, rearing, and grooming) were scored once each minute for 15 min before and 45 min after the acute procedures. Resting HR and MAP values consistently were lower in rats housed four per cage than animals housed alone or with one cage mate. Compared to that of animals housed individually, general activity was higher during the light phase and lower during the dark phase in rats housed four per cage. Rats housed four per cage showed significantly lower HR and MAP in response to acute husbandry and experimental procedures than rats housed alone, and the HR and MAP of rats housed in pairs were not consistently lower than those of rats housed alone. Procedure-induced arousal behaviors were observed in all housing groups after the acute husbandry and experimental procedures, but rats housed four per cage returned to sleeping behavior more quickly than did rats in the other housing groups. In light of these results, we concluded that under resting conditions, rats housed four per cage were less stressed than were rats housed alone, that common procedures induce noteworthy stress-like responses in male rats, and that the magnitude and duration of these responses are reduced by group housing.

Numerous reports in the literature have documented the physiologic and pathophysiologic status of rats housed individually with that of those housed in groups (1-15). The observations made prior to the late 1970s have been summarized in an excellent review by Brain and Benton (16). By using indices of stress such as increased adrenal weight and elevated serum levels of corticosterone, some of these earlier studies suggested that individual housing was more stressful to rats than was group housing whereas other studies showed no difference. These variable outcomes might have resulted from confounding factors such as prior housing conditions, duration of housing, numbers of rats allocated to group housing, gender, and strain. In the two decades since this review was published, the contributions of some of these confounding variables have been clarified. Regarding gender, Brown and Grunberg (17) reported that male rats appear more stressed than do female animals in crowded conditions whereas when housed alone, female rats are more stressed than are male animals. Controversy remains as to the effects of housing on stress responses. For example, Giralt and Armario (2) reported no differences in the responses of male rats housed individually or four per cage whereas Gambardella and colleagues (1) observed that male rats housed individually had higher levels of corticosterone and prolactin than did those housed six per cage.

In addition to the confounding variables mentioned, the method used to monitor for stress may contribute to the variation in the results of previous reports. Radiotransmission offers a means to continuously monitor cardiovascular parameters in unrestrained animals, thus allowing accurate determinations of blood pressure and heart rate under resting conditions (18, 19) as well as a method for continuously assessing the acute changes induced by potentially stressful stimuli. Therefore, the objectives of our study were to use radiotransmission to evaluate the cardiovascular parameters of individually and group-housed male rats and to assess the home cage activity and behavior of these animals during resting conditions and after common husbandry and experimental procedures. Our hypotheses were that group-housed rats would show less evidence of stress than would individually housed rats, that common husbandry and experimental procedures would induce stress-like responses, and that group-housing would reduce these responses.

Materials and Methods

Animals. Adult male Holtzman Sprague-Dawley rats were purchased from Harlan Sprague-Dawley (Indianapolis, Ind.) at 200 to 224 g body weight and allowed to acclimate to the animal room conditions and husbandry procedures for 2 weeks prior to surgical implantation of radiotransmitters (described below). The environmental conditions in the animal room were: lighting, 300 to 1000 lumens/m² at cage level and lights on from 0700 to 1900; temperature, 20 to 28°C; and relative humidity, 30% to 60%. Animals were housed individually or with one or three cagemates of the same sex and strain in solid-bottom polycarbonate cages (nominal floor area, 930 cm²) with standard stainless-steel lids and hardwood chip bedding (Sanichip, P.J. Murphy Forest Products Corp., Montville, N.J.).

Cages were changed once weekly (Mondays) for rats housed individually or with one cagemate and twice weekly (Mondays and Thursdays) for animals housed with three cagemates. All cages were moved daily to a workbench to measure food and water. To facilitate this daily determination of food and water consumption, powdered rat chow (Purina #5001, Purina LabDiet, St. Louis, Mo.) was provided within the cage in a small (depth, 6.5 cm; diameter, 7.5 cm) ceramic jar with a stainless-steel cover, and tap water was provided in a water bottle with a sipper tube. For 2 weeks before and 2 weeks after surgery, the rats were implanted with the telemetry transmitter were weighed on Mondays, Wednesdays, and Fridays to monitor for surgical recovery and to acclimate the animals to the frequency of handling that would exist dur-
ing the experimental period.

**Surgical procedures.** Rats that were to receive the transmitters were selected randomly upon arrival from the vendor and marked by ear punch or by a smear of red food coloring on the back. In preparation for abdominal and femoral incisions, these areas were shaved, scrubbed with a 7.5% povidone-iodine solution (Betadine, Purdue Frederick Company, Norwalk, Conn.), and rinsed with sterile 0.9% NaCl. A radiotelemetry transmitter (model TA11PA-C40, Data Sciences Corporation, St. Paul, Minn.) was implanted aseptically via a ventral midline incision in the abdominal cavity of one rat in each cage while the animal was under ketamine (80 mg/kg intraperitoneally [i.p.] and xylazine (7 mg/kg i.p.) anesthesia. The attached catheter was tunneled through the abdominal wall to a point over an incision in the left femoral triangle and inserted centrally into the femoral artery to a depth of 3.5 cm. The catheter was secured in the femoral artery, and the incisions were closed. All animals were given 20 cc of sterile 5% dextrose subcutaneously (s.c.) immediately after surgery to prevent dehydration. In addition, as part of a companion study to be reported elsewhere, half of the animals in each of the three housing groups received postoperative analgesics. Rats housed alone or with one cagemate received Turbogesic (0.1 mg/kg s.c.; Fort Dodge Animal Health, Fort Dodge, Iowa) at 4, 12, and 20 h after surgery; rats housed with three cagemates were given Ketofen (Fort Dodge Animal Health; 16 mg/kg s.c. added to the 5% dextrose just after surgery).

Postoperative recovery was monitored by daily visual examination, daily food and water intake, body weight gain, and blood pressure and heart rate (measured every 5 min by telemetry). According to these parameters, rats were fully recovered in 7 to 10 days. All housing, surgical, and experimental procedures used were approved by the Wayne State University Animal Investigation Committee.

**Experimental procedures.** We conducted three separate sets of experiments, each at a different housing density (1, 2, or 4 rats per cage). For each set of experiments, a total of eight rats bearing radiotelemetry transmitters were subjected serially to several common husbandry and experimental procedures and potentially stressful olfactory stimuli: Mondays, Wednesdays, and Fridays. A separate group of eight rats was present in the room for each set of experiments and were housed in an identical fashion but did not undergo the experimental manipulations; the results obtained from these “witnessing” rats are presented elsewhere. Preliminary studies showed equivalent cardiovascular responses to a procedure repeated on Monday, Wednesday, and Friday, indicating no significant carryover effects. The procedures, in the order conducted, were: routine cage change; restraint and s.c. injection; restraint and tail-vein injection; exposure to the odors of urine and feces from stressed rats; and restraint and s.c. injection; restraint and tail-vein injection; exposure to the odors of urine and feces from stressed rats; and restraint and tail-vein injection; exposure to the odor of dried rat blood. The details of each procedure follow.

(i) Cage change. The cage was removed from the cage rack and placed on a workbench. The water bottle, cage lid, and food cup were removed. The rats then were gently transferred to the clean cage containing fresh woodchip bedding; the food cup, cage lid and water bottle were replaced; and the cage returned to the cage rack. The procedure required 20 to 30 sec per cage.

(ii) Restraint and s.c. injection. The cage was removed from the cage rack and placed on a workbench. The water bottle and cage lid were removed, and only the rat bearing the transmitter was gently removed from the cage to the workbench surface. The technician held the rat with one hand and lifted the loose skin at the nape of the neck. An injection of 0.2 cc sterile saline was made into this loose skin pocket by using a 1-cc tuberculin syringe and a 26-gauge needle. The rat was returned to its cage; the cage lid and water bottle were replaced, and the cage returned to the cage rack. The procedure required 20 to 30 sec per cage.

(iii) Restraint and tail-vein injection. The cage was removed from the cage rack and placed on a workbench. The water bottle and cage lid were removed, and only the rat with the transmitter was gently removed from the cage and placed on the workbench surface adjacent to the opening of an appropriately sized standard plastic rodent restrainer. If the rat did not voluntarily enter the open end of the restrainer, the technician gently pushed the rat into the restrainer and prevented the animal from backing out by using one hand. The rat’s tail was immersed in warm tap water for 30 sec to induce vasodilation, and then the injection site was swabbed with 70% ethanol. An injection of 0.2 cc sterile saline was made into the tail vein by using a 25-gauge butterfly infusion cannula. No more than two attempts to insert the cannula were made. The rat was returned to its cage; the cage lid and water bottle were replaced, and the cage returned to the cage rack. The procedure required approximately 2 minutes per cage.

(iv) Odor of urine and feces from stressed rats. A paper towel, collected from the floor of a large Plexiglas chamber in which six male rats (previously housed two or three per cage) had been placed together for 5 min, was positioned on the cage lid of each cage the evening before the experiment. A clean paper towel was applied in the same way to the cage lids of a separate set of eight rats, which served as the control group. The towel remained on the cage lid for 15 min. That the rats placed in the Plexiglas chamber were stressed is supported by their marked exploratory behavior and frequent urination and defecation and by previous unpublished results in which rats bearing telemetry transmitters showed pronounced elevations in heart rate (~100 beats per minute above resting levels) and mean arterial blood pressure (~20 mm Hg above resting levels) of within 30 sec of being placed in the same chamber.

(v) Odor of dried rat blood. A paper towel containing dried rat blood collected at decapitation of male rats was placed on the cage lid of each animal room visually observed the behavior of each transmitter-bearing rat in its home cage once each minute beginning 15 min before and continuing for 45 min after each acute experimental procedure. The following behaviors were scored: sleeping, awake but not moving, moving, rearing, grooming, feeding, and drinking. The sum of the number of times each behavior was observed in each 15-min period was the parameter compared across time and between housing conditions. For the sake of brevity, only sleeping behavior is reported in the Results section.

**Statistical analysis.** The data are reported as mean ± standard error of the mean (SEM). The effects of time and housing conditions were analyzed by using general linear model analyses of variance with repeated measures and post hoc contrasts (SPSS, Chicago, Ill.). Means were declared statistically significant at P ≤ 0.05.
Results

The mean arterial blood pressure (MAP) and heart rate (HR) of rats housed one, two, or four rats per cage averaged over the 12-h light (0700 to 1900 h) and dark (1900 to 0700 h) periods of an experiment-free day (Sunday) are shown in Figs. 1A (MAP) and B (HR). The MAP during the light period was significantly lower in rats housed four per cage than in the other two housing groups ($P < 0.05$); however, HR in the 4-rats-per-cage group was not different from that of those housed alone and was lower than that of those housed two per cage. Although the MAP was lower during the light period in rats housed four per cage compared with that those housed alone, activity in the home cage was significantly greater in the former than the latter group ($P < 0.05$) (Fig. 1C). Both HR and MAP increased significantly during the dark period of the light-dark cycle under all housing conditions, however, these increases were significantly less in animals housed four per cage than in those housed alone ($P < 0.05$) or with one cage mate ($P < 0.05$). These differences in nocturnal MAP and HR between housing groups were paralleled by the pattern of home cage activity (Fig. 1C).

The cardiovascular responses of rats subjected to a routine cage change are shown in Fig. 2A (HR) and B (MAP). Rats housed alone and with one cage mate showed significant increases ($P < 0.05$) were observed for 90 to 120 min after the movement to a new cage. Rats housed with three cage mates also showed significant increases in HR ($P < 0.05$) that initially were of the same magnitude as those of the rats in the other two housing conditions, but HR in the four-to-a-cage rats decreased more rapidly, returning to baseline values within 30 min. Changes in MAP after a cage change showed the same patterns as HR (Fig. 2B) except that the MAP of rats housed four per cage decreased significantly ($P < 0.05$) below baseline values from 90 to 180 min after the cage change.

Figure 3 provides data collected from animals subjected to hand restraint and a s.c. injection of saline. Rats housed under all three conditions had significant increases ($P < 0.05$) in HR (Fig. 3A) that did not return to baseline until 60 to 100 min after the procedure, but animals housed alone had significantly greater increases than did rats housed with one ($P < 0.05$) or three ($P < 0.05$) cage mates. This pattern also was observed in the changes in MAP (Fig. 3B).
When rats were held in a rodent restrainer and given a tail-vein injection, HR increased significantly in all housing groups, returning to baseline after 75 to 90 min (Fig. 4A). However, in contrast to what occurred after other procedures, rats housed two per cage showed greater responses than did animals housed alone or with three cagemates ($P < 0.05$). MAP also increased significantly after this procedure, but had returned to baseline by 60 min postinjection. In addition, there were no significant differences between housing groups until late in the recovery period, when the MAP of animals housed alone or with one cagemate was significantly greater than that of rats housed four per cage ($P < 0.05$).

Exposure of rats to the odors of urine and feces from stressed rats induced large increases in HR (Fig. 5A) and MAP (Fig. 6A), whereas exposure to a clean paper towel induced lesser responses in a separate set of control animals (Fig. 5B and 6B). The odor-induced HR responses decreased to baseline within 15 to 90 min, whereas MAP returned to baseline by 15 to 45 min after exposure. The magnitude and duration of these responses varied depending on the housing condition, with animals housed two or four per cage showing significantly lesser HR and MAP responses ($P < 0.05$) which were of shorter duration than those of rats housed alone. In addition, the responses to the odors of urine and feces of rats housed with one or three cagemates were only slightly greater than those of animals exposed to the clean paper towels.

When rats were exposed to the odor of dried rat blood, HR increased significantly, but the magnitude and duration varied with the housing condition (Fig. 7A). Rats housed alone showed the greatest change in HR, which did not return to baseline until approximately 100 min after exposure. Rats housed two per cage had increases in HR that were significantly less than those of animals housed alone ($P < 0.05$), and the responses returned to baseline in less than 30 min. Rats housed four per cage exhibited small and very transient increases in HR that were of the same magnitude and duration as those seen in similarly housed rats exposed to a clean paper towel (Fig. 7B). MAP responses (Fig. 8A) were similar to those of HR, except that the MAP of rats exposed to the blood-impregnated towel returned to baseline values sooner than did HR, and the responses of rats housed two per cage did not significantly differ from those of animals housed four per cage. The MAP of rats housed alone or four per cage and exposed to the clean towel increased significantly ($P < 0.05$) but returned to baseline quickly (Fig. 8B), whereas rats housed 2 per cage did not respond to the clean paper towel.

All the manipulations to which rats were exposed reduced sleeping behavior (Table 1), but rats housed alone showed significantly less sleeping behavior over the 45-min observation period than did animals housed with one ($P < 0.05$) or three ($P < 0.05$) cagemates.
cagemates. In addition, rats housed with three cage mates generally showed more sleeping behavior than did rats housed in pairs. The manipulations increased number of occurrences of the arousal behaviors (moving, rearing, grooming) during the observation periods about equally, and the increases were more prevalent in individually housed than group-housed rats (data not shown).

**Discussion**

This study, in which radiotelemetry was used to continuously monitor the cardiovascular system, demonstrates that unrestrained male Sprague-Dawley rats housed with one or three cagemates of the same age, strain, and gender generally have lower HR and MAP under resting conditions and after common acute husbandry and experimental procedures than do similar rats housed alone. These differences in cardiovascular parameters between individually and group-housed rats were paralleled by differences in general activity during the light and dark phases of the daily photoperiod and in home cage behaviors before and after the acute procedures. Finally, there was no evidence of crowding stress in rats housed four per cage.

Even though the floor space per rat was one half that stipulated in the U.S. federal guidelines governing research animals (24), there was no evidence that housing four rats per cage produced crowding stress, as resting HR and MAP were less in this group than either of the other two housing groups (Fig. 1), and the cardiovascular responses to the acute procedures were always the lowest in rats housed with three cagemates (Figs. 2-8). The only indication of a husbandry problem in the rats housed four per cage was that HR and MAP decreased below the baseline after a routine cage change (Fig. 2). This situation suggests that stressful than is housing them individually (1, 5, 12-15, 20-23), our observations also indicate that paired housing was not always as effective in reducing stress-like responses as was housing in groups of four. For example, subjecting rats to a routine cage change induced equivalent changes in HR and MAP in rats housed individually or in pairs (Fig. 2), and restraint and tail-vein injection (Fig. 4) induced the greatest increases in HR in rats housed two per cage compared with that of the other housing groups. Therefore, it appears that the responses observed under various housing conditions are dependent on the nature of the procedures to which rats are subjected. This variation could explain some of the controversial observations in the literature on the effects of housing density.

Although much of the data in our study supports the concept established by earlier reports that housing rats in groups is less stressful than housing them individually (1, 5, 12-15, 20-23), our observations also indicate that paired housing was not always as effective in reducing stress-like responses as was housing in groups of four. For example, subjecting rats to a routine cage change induced equivalent changes in HR and MAP in rats housed individually or in pairs (Fig. 2), and restraint and tail-vein injection (Fig. 4) induced the greatest increases in HR in rats housed two per cage compared with that of the other housing groups. Therefore, it appears that the responses observed under various housing conditions are dependent on the nature of the procedures to which rats are subjected. This variation could explain some of the controversial observations in the literature on the effects of housing density.

**Figure 5.** Effect of the odor of urine and feces from stressed rats on the heart rate of male Sprague-Dawley rats housed alone or with one or three cagemates. (A) Rats exposed to a paper towel saturated with urine and feces and placed on the cage lid. (B) Rats exposed to a clean paper towel placed on the cage lid. Data are expressed as the mean ± standard error of the mean across animals. The data from which group means were calculated were the means of five 10-sec samples collected from each animal at 1-min intervals. *, Significantly different from control period. The different superscripted letters indicate means for the different housing conditions at a given time point that are statistically different (P < 0.05).

**Figure 6.** Effect of the odor of urine and feces from stressed rats on the mean arterial pressure of male Sprague-Dawley rats housed alone or with one or three cagemates. (A) Rats exposed to a paper towel saturated with urine and feces and placed on the cage lid. (B) Rats exposed to a clean paper towel placed on the cage lid. Data are expressed as the mean ± standard error of the mean across animals. The data from which group means were calculated were the means of five 10-sec samples collected from each animal at 1-min intervals. *, Significantly different from control period. The different superscripted letters indicate means for the different housing conditions at a given time point that are statistically different (P < 0.05).
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Table 1. Effect of housing density on home cage sleeping behavior of male Sprague-Dawley rats before and after various manipulations

<table>
<thead>
<tr>
<th>Manipulation</th>
<th>Time</th>
<th>1 Rat per cage</th>
<th>2 Rats per cage</th>
<th>4 Rats per cage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cage change</td>
<td>15-0 min</td>
<td>8.8 ± 2.5</td>
<td>15.3 ± 0.9</td>
<td>6.8 ± 2.2</td>
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<tr>
<td></td>
<td>0-15 min</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
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</tr>
<tr>
<td></td>
<td>15-30 min</td>
<td>0.4 ± 0.0</td>
<td>0.0 ± 0.0</td>
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</tr>
<tr>
<td>Odor of urine and feces</td>
<td>30-45 min</td>
<td>0.3 ± 0.3</td>
<td>0.5 ± 0.3</td>
<td>6.4 ± 2.7</td>
</tr>
<tr>
<td>S.C. injection</td>
<td>15-0 min</td>
<td>7.6 ± 2.5</td>
<td>10.9 ± 1.0</td>
<td>7.3 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>0-15 min</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.1</td>
<td>4.1 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>15-30 min</td>
<td>0.9 ± 0.7</td>
<td>1.7 ± 0.9</td>
<td>11.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>30-45 min</td>
<td>1.0 ± 0.7</td>
<td>7.1 ± 2.2</td>
<td>11.9 ± 1.5</td>
</tr>
<tr>
<td>Tail vein injection</td>
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<td>12.1 ± 1.8</td>
<td>12.9 ± 0.8</td>
<td>13.9 ± 0.6</td>
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<tr>
<td></td>
<td>0-15 min</td>
<td>0.3 ± 0.3</td>
<td>1.6 ± 1.4</td>
<td>4.1 ± 1.9</td>
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<tr>
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<td>15-30 min</td>
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<td>3.6 ± 2.3</td>
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<tr>
<td></td>
<td>30-45 min</td>
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<td>6.7 ± 2.3</td>
<td>14.8 ± 0.2</td>
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<tr>
<td>Clean paper towel (no odors)</td>
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<td>10.9 ± 2.4</td>
<td>14.0 ± 0.6</td>
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<td>9.8 ± 1.4</td>
<td>7.1 ± 2.4</td>
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<tr>
<td></td>
<td>15-30 min</td>
<td>4.1 ± 0.9</td>
<td>11.8 ± 1.7</td>
<td>12.0 ± 1.8</td>
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<tr>
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<td>30-45 min</td>
<td>8.9 ± 1.9</td>
<td>13.0 ± 1.0</td>
<td>12.8 ± 1.8</td>
</tr>
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<td>Odor of urine and feces</td>
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<td>8.9 ± 1.7</td>
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<tr>
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<td>5.4 ± 1.6</td>
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<td>14.7 ± 0.2</td>
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<tr>
<td>Odor of rat blood</td>
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<tr>
<td></td>
<td>30-45 min</td>
<td>1.5 ± 0.6</td>
<td>13.4 ± 0.6</td>
<td>15.0 ± 0.0</td>
</tr>
</tbody>
</table>

*Mean number of times sleeping behavior was observed in 15 one-minute periods ± standard error of the mean (n = 8)

**Significantly less than behavior during control period (-15-0 min) (P < 0.05)

Means with different letters are significantly different across housing groups (P < 0.05)
HR and MAP were somewhat elevated during the control period, which may have been induced by the odor of ammonia from the soiled bedding that had accumulated in the cage during the 4 days since the cage was last changed. That the odor of urine and feces acutely induced increases in HR and MAP (Fig. 5) suggests that the accumulation of soiled bedding could have produced this effect. It is of interest to note that the data in Fig. 1 were obtained during the 24-h period just prior to the cage-change experiment. If ammonia accumulation was stressful to the rats housed four per cage, then resting HR and MAP values in the animals may have been even lower than those reported in Fig. 1 if the level of ammonia had been reduced by more frequent cage changing. The confirmation of this hypothesis awaits other experiments on cage-change frequency or the effects of various levels of ammonia on cardiovascular parameters.

That a common husbandry procedure, a cage change, induced increases in HR and MAP equivalent to or greater than the increases after procedures expected to induce stress (e.g., restraint and injections and the odor of rat blood) suggests that care should be exercised in dismissing a procedure as nonstressful just because it is routine. Although it is not clear from our study whether rats were stressed or merely aroused by being placed in a different cage with fresh bedding, the responses were induced by a procedure that was not novel—the animals had been subjected to cage changes once or twice weekly for 4 weeks prior to the experiment reported in this study. Further, we previously reported (19) that HR was increased to the same degree in rats moved to a cage containing fresh bedding mixed with one cup of soiled bedding from their previous cages as in animals placed in a cage with totally fresh bedding. These findings suggested that familiar odors did not diminish the cardiovascular responses to the cage-change procedure. Our cage-change results agree well with those reported recently by Harkin and colleagues (25). The current and previous observations also suggest that, for studies in which resting cardiovascular parameters are to be determined, data should not be obtained for at least 2 h after common procedures such as cage changing.

Our current results support our hypothesis that some common procedures evoke stress-like cardiovascular responses, which are significantly reduced when rats are housed in groups of four compared with those responses in rats housed alone. Housing rats in pairs did not always reduce these cardiovascular responses to the same degree as did four-per-cage housing.

Acknowledgments

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References