Journal of the American Association for Laboratory Animal Science
Copyright 2014
by the American Association for Laboratory Animal Science

Vol 53, No 1
January 2014
Pages 52–60

Effects of a Complex Housing Environment on Heart Rate and Blood Pressure of Rats at Rest and after Stressful Challenges

Jody Sharp, Toni Azar, and David Lawson*

Housing enrichment for rodents continues to be a discussion topic within the animal care community. The objective of this study was to determine the extent to which a complex housing environment affects heart rate, blood pressure, and activity of rats when undisturbed and after exposure to stressful challenges and whether autonomic controls of heart rate would be affected. Male and female Sprague–Dawley and Wistar rats with radiotelemetry transmitters were evaluated under nonenriched single-housing conditions and after acclimation to a complex environment of dim light and cohabitation with 3 conspecifics in large cages with hiding, food foraging, and nesting items. Telemetry data were collected when rats were undisturbed, after acute challenges (cage change, intraperitoneal injections, restraint), during a forced running protocol, and after cholinergic or adrenergic blockade. The complex environment reduced heart rate and increased activity in undisturbed rats but did not affect blood pressure. Heart rate responses to challenges were unaffected, decreased, or increased by complex housing, depending on the stock and sex of rats. Forced running was either unaffected or decreased, depending on the stock and sex of rats. Heart rate responses to cholinergic or β1-adrenergic blockade were not affected. We conclude that the complex housing did not reduce indices of stress (for example, heart rate) as compared with simpler housing. However, the possibility that some environmental elements interact negatively with each other must be considered in future studies.

The inclusion of environmental enrichment for individually housed rats is generally supported by a sizable volume of literature that shows positive effects on behavior, cognitive function, and recovery from induced neural deficits[5,7,10,12,13,17-20,22-25,31,32] and on some parameters of wellbeing and various stress responses.[1,6,9,14] A recent review[21] summarized the effects of enrichment and physical activity on cognitive function.

One problem encountered when comparing the results in the various reports on environmental enrichment is that its specific elements are inconsistent across studies, and its effects have been variable depending on the type, onset, and duration of the enrichment; the age, strain, and sex of the animals; and the physiologic parameters examined. These issues have been reviewed recently.[29] Such inconsistencies of design and variable results complicate the decision of whether enrichment should be adopted and, if so, which type of enrichment is most effective. One approach is to adopt or recommend a simple program to minimize possible confounding effects on experimental outcomes. An alternative approach is to select a complex enrichment plan in the attempt to achieve maximal effect.

The objective of the current study was to determine the extent to which a complex housing environment affects the heart rate, blood pressure, and activity of rats when undisturbed and the heart rate after exposure to stressful challenges and whether autonomic controls of heart rate would be affected. The underlying premise for these experiments was that rats housed individually without any form of environmental enrichment are believed to experience chronic stress, leading to increased heart rate and blood pressure due to altered autonomic drive to the heart and blood vessels, and that a complex environment can reduce or alleviate this chronic stress. Many of the elements that we combined to form the complex environment (10 lx room lighting,[3] group housing,[2,27,28] and addition of inanimate hiding, food foraging, and nesting items[4,26]) are those that we have shown previously to significantly reduce heart rate. The hypotheses were that the combined program would have larger effects than those previously reported for the separate environmental elements and would increase parasympathetic or decrease sympathetic input to the heart.

Received: 26 Mar 2013. Revision requested: 06 May 2013. Accepted: 28 Jun 2013.
Department of Physiology, Wayne State University School of Medicine, Detroit, Michigan.
Corresponding author. Email: dlawson@med.wayne.edu

Materials and Methods

Routine husbandry. Young (age, 7 to 12 wk) adult male and female outbred Sprague–Dawley (Hsd:Hot) and Wistar (Hsd:WI) rats were purchased from Harlan Laboratories (Indianapolis, IN) at a body weight of 200 to 225 g. All rats were obtained from colonies reported by the vendor to be free from adventitious viruses, *Mycoplasma*, respiratory and enteric bacteria (except several strains of *Helicobacter*), and ecto- and endoparasites (except a nonpathogenic commensal protozoa).

Male rats of both stocks (12 Sprague–Dawley and 12 Wistar) were housed in the same room at the same time. The female rats (12 of each stock), obtained immediately after the experiments with male rats were completed, were housed concurrently in the room where the male rats had been housed. All rats were allowed to acclimate to the animal room conditions and husbandry procedures for 1 wk prior to surgical implantation of radiotelemetry transmitters. Environmental conditions in the animal room were: temperature, 22 to 26 °C; relative humidity, 30% to 60%; lighting, 200 lx at cage level; and lights on, 0700 to 1900. During this presurgical acclimation period, rats were housed individually in conventional solid-bottom polycarbonate cages (nominal floor area of 930 cm^2; model 121C; Ancare, Bellmore, NY) with standard stainless steel lids and hardwood chip bedding (depth, 3 to 5 cm; Sanichip, PJ Murphy Forest

Products, Montville, NJ). Cages were changed once weekly (Mondays). Pelleted rat chow (no. 5001, Purina Mills, Richmond, IN) was provided ad libitum, and tap water was provided in a glass water bottle with a stainless steel sipper tube.

Implantation of radiotelemetry transmitters. A radiotelemetry transmitter (model TA11PA-C40; Data Sciences International, St Paul, MN) was implanted aseptically in the abdominal cavity of each rat, with the pressure-sensing catheter inserted into the descending aorta through the left femoral artery as described previously.[26] The anesthetic was a mixture of ketamine (80 mg/kg; Ketaset, Ft Dodge, Overland Park, KS) and xylazine (10 mg/kg; Rompun, Bayer Animal Health, Toronto, Ontario, Canada) given intraperitoneally. The analgesic treatment was ketoprofen (Ketofen, Ft Dodge) diluted in sterile 0.9% saline and given once at 16 mg/kg SC immediately after surgery. Monitoring during the postsurgical recovery period included daily visual examination of the animal's condition and the status of the abdominal incision, daily food and water intakes, and measurement of blood pressure, heart rate, and activity at 5-min intervals by using radiotelemetry. On the basis of these observations and data, rats were judged to be fully recovered by 10 d after the telemetry surgery.

Experimental sequence and enrichment scheme. After 10 to 11 d of recovery from surgery, the rats, which continued to be individually housed as outlined earlier, underwent a series of experimental manipulations (described in the next section). Once this series was complete, the rats were transferred to the complex environment and, after a 2-wk adaptation period, again underwent the same series of experimental manipulations. A crossover design was not used because we thought that exposure to the complex environment might result in carryover effects in experimental arms where rats were exposed to the complex environment before the nonenriched housing.

The complex environment consisted of all of the following for every cage: dim illumination in the animal room during the light phase of the photocycle (10 lx at cage level); a large cage (2000 cm^2 of floor space; model 2000P; Tecniplast, Bugugglate, Italy); continuous presence of 3 noninstrumented rats of the same stock, sex, and body weight which were group-housed (3 per cage) for 1 wk prior to introduction to the experimental cages; continuous presence of 2 simulated burrows occupying a total of approximately 550 cm^2 of cage floor space, each consisting of 2 red rectangular Rat Retreats (Bio-Serv, Frenchtown, NJ) with the smaller end of one inserted into the larger end of the other; once-weekly (at 1300 on Wednesdays) addition of a food-foraging object consisting of a 150-g size Nestpak filled with a mixture of corncob and wood chips (WF Fisher and Son, Watertown, TN), into which was placed five 1-g chocolate-flavored rodent treats (SupremeTreats, Bio-Serv,); and addition of a shredding and nesting item (150-g size Nestpak filled with a mixture of corncob and wood chips without treats) placed in the cage once each week (on Fridays at 1300). Both Nestpaks usually were completely shredded or consumed in 1 to 3 d; when destroyed, they were not replaced until the next scheduled addition. The simulated burrows were replaced with clean ones when the cages were changed at 1300 on Mondays. The selection of inanimate items was based on their probable stimulation of species-specific behaviors (for example, hiding, food foraging, shredding, nesting), their commercial availability, and their known composition and suitability for Good Laboratory Practices studies.

Data collection and experimental challenges. After rats had recovered from surgery, we collected heart rate, blood pressure, and activity data at times (0800 to 0900 and 1300 to 0700) when the rats were undisturbed (that is, no humans were present in the animal room). In addition, heart rate and blood pressure data were collected during and for 3 h after (1000 to 1300) exposure to several acute challenges: cage change, to determine effects on responses to a routine husbandry procedure; 2 intraperitoneal injections of saline 15 min apart, to determine effects on responses to a slightly stressful experimental procedure and to serve as controls for the drug protocols; intraperitoneal injection of methylatropine followed 15 min later by intraperitoneal injection of hexamethonium, to determine the effect on the status of cholingeric drive to the heart; intraperitoneal injection of metoprolol followed 15 min later by intraperitoneal injection of hexamethonium, to determine the effects on the status of β-adrenergic drive to the heart; physical restraint for 10 min, to determine the effect on the responses to a moderately stressful procedure; physical restraint for 60 min, to determine the effect on the responses to an intensely stressful procedure; and exposure to a stepped exercise protocol in a motorized running-wheel apparatus, to determine the effects on the outcome of a physiologic experiment involving forced exercise. There were short periods of time that elapsed at the beginning of challenges when the rats were off the telemetry receiver plate and data were not recorded: no more than 30 s for the cage change and injections and about 1 to 3 min as the rats were placed in the restraining device or into the running wheel apparatus. Each of these acute challenges is described in detail in the following sections.

Cage change. The rat's cage was removed from the cage rack and placed on a workbench. The water bottle and cage lid were removed, and the rat was grasped gently at the base of its tail and transferred to the clean cage containing fresh woodchip bedding. A clean cage lid with fresh rat chow and clean water bottle were placed on the cage, and it was returned to the cage rack. The procedure required 20 to 30 s per cage.

Intraperitoneal injection of saline. The rat's cage was removed from the cage rack and placed on a workbench. The water bottle and cage lid were removed, and the first research assistant gently grasped the rat at the base of its tail and placed it on the workbench. The rat then was restrained and turned on its back to expose the abdomen. The second research assistant then injected 0.2 mL sterile saline into the lower left or right quadrant of the abdomen by using a 1-cc tuberculin syringe and a 26-gauge needle. The rat was returned to its cage, which was replaced on the cage rack. The procedure required 20 to 30 s per cage. This procedure was repeated 15 min later.

Intraperitoneal injection of drugs. This procedure was done as outlined for intraperitoneal injection of saline except that the muscarinic cholinergic antagonist methylatropine (3 mg/kg) or the β1-adrenergic antagonist metoprolol (10 mg/kg) was substituted for the first saline injection and the autonomic ganglionic antagonist hexomethonium (20 mg/kg) was substituted for the second saline injection. The doses of methylatropine, metoprolol, and hexamethonium were those described previously[17] and were used to define parasympathetic and sympathetic tonus, with heart rate as the measured parameter.

Physical restraint. The cage was removed from the cage rack and placed on a workbench. The water bottle and cage lid were removed, and the rat grasped gently at the base of its tail and placed into a restrainer, which had been fabricated locally by gluing a transparent acrylic cylinder (diameter, 7.6 cm; length, 17.8 cm) horizontally to a 15.2 × 15.2 × 5.1 cm acrylic riser (S and S Acrylics, Norcross, GA) with multiple 6-mm holes drilled in the top and front of the acrylic cylinder for ventilation. The open end of the restrainer was closed with a disposable plastic lid that had a 13-mm hole in the center for the rat's tail and was secured to the acrylic cylinder with duct tape. The restrainer containing

the rat then was placed in the animal's home cage, which was returned to the cage rack for 10 or 60 min. The rat then was removed from the restrainer and returned to its home cage.

Stepped exercise in a motorized running wheel apparatus. For 3 d prior to the experiment, each rat was removed from its home cage and placed in one of the 6 running wheels of the forced running wheel apparatus (model 80805, Lafayette Instrument, West Lafayette, IN). After 3 min of exposure to the wheel without movement, the apparatus was operated for 9 min at a speed of 8 m/min to familiarize the rats with rotation of the wheel. After familiarization, the rats were removed from the apparatus and returned to their home cages. On the day of the experiment, each rat was removed from its home cage, placed in one of the running wheels of the apparatus and allowed to acclimate for 3 min without movement of the wheel. The wheel then was operated for 3 min at 8 m/min. This stage was followed by 3 min at 12 m/min and finally 3 min at 16 m/min. If the rat did not run at any point, the wheel was lifted off the drive rollers briefly to allow the rat to reorient to the bottom of the wheel; the wheel then was placed back on the apparatus. After 3 min at 16 m/min, the apparatus was stopped, the rats remained in the stopped wheels for another 3 min, and then which they were transferred back to their home cages. The duration and speed were chosen to replicate a recently reported forced treadmill exercise protocol.[16]

The rats underwent the above procedures on Mondays, Wednesdays, and Fridays (Figure 1). Over the course of the study, each rat experienced every procedure twice (once when housed in the nonenriched environment and once after being adapted to the complex environment). The every-other-day schedule was established to reduce any carryover effects that may exist from one procedure to the next. There were no indications that the rats became conditioned to procedures being conducted on this schedule (that is, there were no changes in heart rate at 1000 on intervening nonexperimental days in the current study and no differences in heart rate responses to some of the same procedures applied on the Monday–Wednesday–Friday schedule for 2 consecutive weeks in a separate study). To reduce experimental error due to personnel, all routine animal care and experimental procedures were performed by the same 2 persons, with care taken to ensure that both used the same techniques.

All procedures were approved by the Wayne State University IACUC.

Data analysis. All telemetry data were downloaded to spreadsheets (Excel, Microsoft, Redmond, WA) and summarized as follows: Heart rate and blood pressure data collected at 5-min intervals while rats were undisturbed were averaged across each reported time period (that is, 0800 to 0900, 1300 to 1700, and 1700 to 0700) for each rat on each experimental day. These values then were averaged for each rat across all experimental days. These time-averaged data were used to calculate group means and standard errors and for statistical analysis. Activity data collected at 5-min intervals while rats were undisturbed were summed for each rat for the reported period (for example, 1300 to 1700 or 1700 to 0700) on each experimental day. These daily sums were averaged for each rat over all the experimental days, and these time-averaged data were used to calculate group means and standard errors and for statistical analysis.

For the heart rate data collected at 1-min intervals after the acute challenges (cage change, saline injections, restraint), each data point was corrected for the average undisturbed value for 0800 to 0900 for that rat, and these corrected values were summed across time from the point the challenge was initiated until the response returned to the 0800 to 0900 control value for that rat. This summed value was designated the area under the response curve. These individual sums then were used to calculate group means and standard errors and for statistical analysis.

For the heart rate data collected at 1-min intervals after the injection of drugs, the values at 14 min after methylatropine or metoprolol injection were determined to be the maximal responses and were corrected for the value at 14 min after the first saline injection on the control day for each rat. The responses to hexamethonium were taken as the maximal change in heart rate relative to the 14-min value after methylatropine or metoprolol. These corrected values were used to calculate group means and standard errors and for statistical analysis.

For the heart rate data collected at 1-min intervals before, during, and after the forced-running experiment, the absolute values were averaged for each rat over each of the 3 time periods. Basal values were the means of the 0800 to 0900 values collected on the morning the running experiment was conducted. The time-averaged values were used to calculate group means and standard errors and for statistics.

Data from nonenriched control rats and from the same rats housed in the complex environment were compared within times, stocks, and sexes by using one-factor ANOVA (SigmaStat, Systat Software, Chicago, IL). The criterion for a statistically significant difference was a P of 0.05 or less. The variation in the reported number of rats per group (8 to 10) resulted from the loss of a few animals because surgical complications (no more than one per group) or incomplete data collection due transient telemetry signal failure (the remainder of excluded rats).

Results

Heart rates in undisturbed male rats of both stocks were significantly ($P < 0.05$) lower at all times of the day and night when the rats were housed under complex as compared with control conditions (Figure 2 A, C, and F). In addition, activity in the cage was significantly ($P < 0.05$) greater in both stocks during the afternoon and at night under complex conditions (Figure 2 E and H). However, systolic blood pressure was not affected by complex housing in either stock (Figure 2 B, D, and G).

The effects of complex environment on the heart rate, systolic blood pressure and cage activity of undisturbed Sprague–Dawley and Wistar female rats were similar to those in male rats, except that Sprague–Dawley female rats showed no significant effect on cage activity at night (Figure 3).

When rats were exposed to potentially stressful procedures, heart rate responses varied by stock and sex (Figure 4). The complex environment significantly ($P < 0.05$) reduced the heart rate response of Sprague–Dawley male rats to 60 min of restraint but not to cage change, intraperitoneal injections of saline, or 10 min of restraint (Figure 4 A). In contrast, complex housing significantly ($P < 0.05$) reduced the heart rate response of Wistar male rats to intraperitoneal injections of saline but did not alter responses to the other challenges (Figure 4 B).

In Sprague–Dawley female rats, the complex environment significantly ($P < 0.05$) reduced the heart rate response to cage change but not to the other challenges (Figure 4 C). In contrast, complex housing significantly ($P < 0.05$) decreased the heart rate responses of Wistar females to 10 and 60 min of restraint but significantly ($P < 0.05$) increased the heart rate response to cage change (Figure 4 D).

When rats were subjected to a forced running challenge in a motorized running wheel apparatus, heart rates of Sprague–Dawley male rats were not significantly affected by the complex environment, whereas the heart rates of Wistar male rats were

Week	Monday	Tuesday	Wednesday	Thursday	Friday
1	Cage change		Two intraperitoneal saline injections, 15 min apart		Methylatropine + hexamethonium
2	Cage change		10 min of restraint		60 min of restraint
3	Cage change		Two intraperitoneal saline injections, 15 min apart		Metoprolol + hexamethonium
4	Cage change	Running adaptation	Running adaptation	Running adaptation	Forced-running experiment
5	Cage change; transfer to complex environment		No experimental manipulations		No experimental manipulation
6	Cage change		No experimental manipulations		No experimental manipulation
7	Cage change		Two intraperitoneal saline injections, 15 min apart		Methylatropine + hexamethonium
8	Cage change		10 min of restraint		60 min of restraint
9	Cage change		Two intraperitoneal saline injections, 15 min apart		Metoprolol + hexamethonium
10	Cage change	Running adaptation	Running adaptation	Running adaptation	Forced-running experiment

Figure 1. Schedule of acute challenges to which each rat was exposed. Prior to week 1, the rats had been adapted for 1 wk to individual housing under standard, nonenriched, conditions after arrival from the vendor and then for 10 or 11 additional days under the same housing conditions to recover from the surgical implantation of the telemetry transmitter.

significantly ($P < 0.05$) lower at wheel speeds of 8, 12, and 16 m/min (Figure 5). In contrast, the heart rates of Sprague–Dawley and Wistar female rats to the running challenge were not altered by complex housing (Figure 6). Wistar female rats exhibited significantly ($P < 0.05$) higher resting heart rates in complex housing than when living in nonenriched conditions. This finding was at variance with the values obtained under undisturbed conditions (Figure 3), and the reason for this difference is unknown.

Housing rats in the complex environment did not alter the responses to muscarinic cholinergic, β1 adrenergic, or ganglionic blockade when compared with the responses of rats housed in nonenriched conditions (Table 1).

Discussion

In summary, the present data show that housing rats in a complex housing environment had significant effects on heart rate and cage activity but not on systolic blood pressure when the rats were undisturbed (that is, no humans were present in the room). Heart rates throughout the day were lower, whereas activity in the cage in the afternoon and night was higher. Heart rate responses to experimental challenges were variable and not consistently affected by complex housing; stock- and sex-associated differences were noted. Pharmacologic treatments to determine the status of cholinergic and β-adrenergic drives on heart rate showed that the drug-induced heart rate responses of rats living in a complex environment were not different from those observed in the same rats living in nonenriched conditions.

One hypothesis of the current study was that housing rats in a complex environment, consisting of several elements each previously shown to significantly reduce heart rate, would have additive effects compared with what was previously reported for the individual elements.[2-4,26,27] This hypothesis was not supported. Although individual enrichment elements were not compared directly with the combined set owing to insufficient telemetry instrumentation, we previously reported on each of the enrichment elements, except large cages, in the same facility with the same personnel and with at least one stock of rats in common (Sprague–Dawley). In addition, undisturbed rats were evaluated at the same times, and at least 2 of the challenges (cage change and 60 min of restraint) were common to both the complex and the previous simple enrichment studies.

The sequential experimental design (that is, exposure of rats to challenges more than once or at 2 different ages) could have affected the outcome. Statistical analysis of the heart rate responses to the 8 weekly cage changes showed no significant difference across the 4 wk prior to transfer to the complex environment or across the 4 wk after transfer in either sex or stock (data not shown). These observations suggest that rats did not adapt to this weekly procedure and that aging by at least 1 mo did not alter the response to the same challenge.

Within these limitations, at least 2 explanations may explain the lack of support for the hypothesis. One possible explanation is that one of the environmental elements may have maximized changes in the dependent measures (for example, heart rate), preventing detection of additive effects caused by other elements. This possibility seems unlikely, given that undisturbed heart rates were 15 to 25 bpm lower in the complex environment (Figures 2 and 3) and 50 to 90 bpm lower after β1-adrenergic blockade (Table 1). Although the magnitude of the decreases noted with adrenergic blockade were not expected in the complex

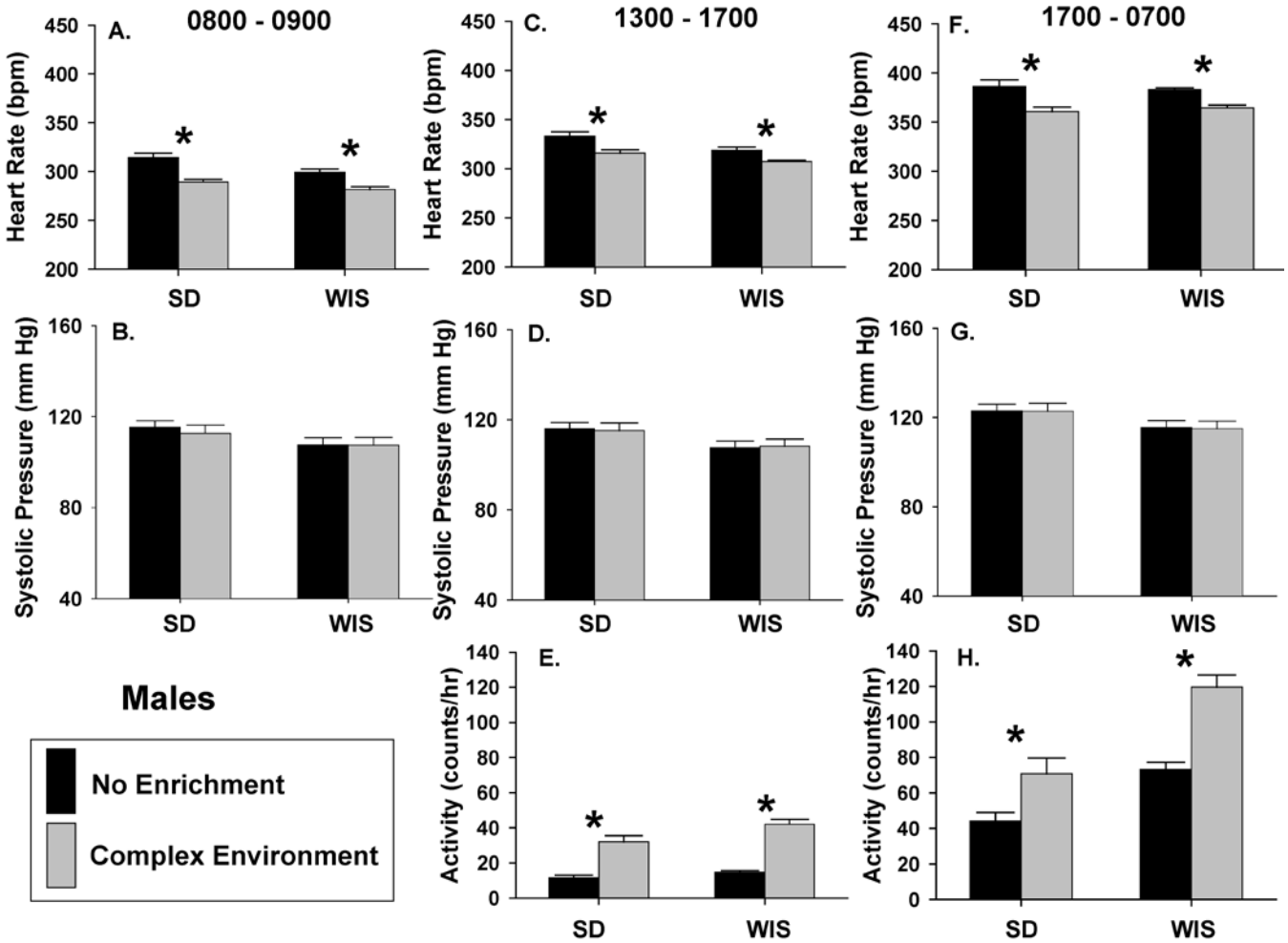

Figure 2. Effect of a complex environment on (A, C, and F) heart rate, (B, D, and G) systolic pressure and (E and H) activity of undisturbed male Sprague–Dawley (SD) and Wistar (WIS) rats at different times of the day and night. Data are reported as mean ± SEM. *, Value for complex environment significantly ($P < 0.05$, 1-factor ANOVA, $n = 8$ [Sprague–Dawley] or 10 [Wistar]) different from that for nonenriched environment.

environment, the effect of the complex environment was small relative to the overall magnitude of the measurable change.

Another possible explanation is the potential for negative interactions between some of the combined enrichment elements. For example, cagemates competing for the hiding, foraging, and nesting elements in the cage may create stress that countered, to some degree, the decreases in heart rate produced by either of these elements alone or by other of the enrichment elements (for example, dim light). Group housing and the presence of inanimate objects in the cage are known to interact with body weight gain, feeding, and activity in male Sprague–Dawley rats.[33] Interactions between conspecifics and inanimate items in the cage may differ in female rats or across stocks or strains. Furthermore, the social hierarchy of the 4 rats in the cage could affect the outcome. If the instrumented rat was submissive to the others, it may have experienced greater stress, and the effects of enrichment elements may have been reduced. If the instrumented animal was the dominant rat, stress effects perhaps would have been less, and the effects of enrichment elements may have been more pronounced. We did not determine the social hierarchy among the rats in our current study. Additional studies testing possible interactions between enrichment elements, particularly when group housing is involved, are needed to resolve this issue. Particularly important to such future studies is the evaluation of interacting behaviors among rats in the group.

Negative interactions between enrichment elements may also have contributed to the lack of effect of complex housing on parasympathetic and sympathetic blockade on heart rate. We hypothesized that the complex housing environment would decrease heart rate by increasing parasympathetic input or by decreasing sympathetic input to the heart. The results did not support this hypothesis. However, the experiments with pharmacologic blockade were limited in that the drugs could not be administered to undisturbed rats via chronic indwelling catheters, used by others to determine parasympathetic and sympathetic tone,[8] owing to the presence of cagemates in the complex housing condition. Therefore, the drugs were administered by acute intraperitoneal injections which themselves induced stress responses (for example, increases in heart rate [Figure 4]) and probably affected parasympathetic and sympathetic neural activity. In an attempt to control for the injection effect, we reported the changes in heart rate after methylatropine or metoprolol relative to the responses to acute intraperitoneal injections of saline. However, this mathematical correction may not have removed the confounding stress effects of acute injections.

That responses to acute challenges in the current study (Figures 4 through 6) were either not affected, were decreased,

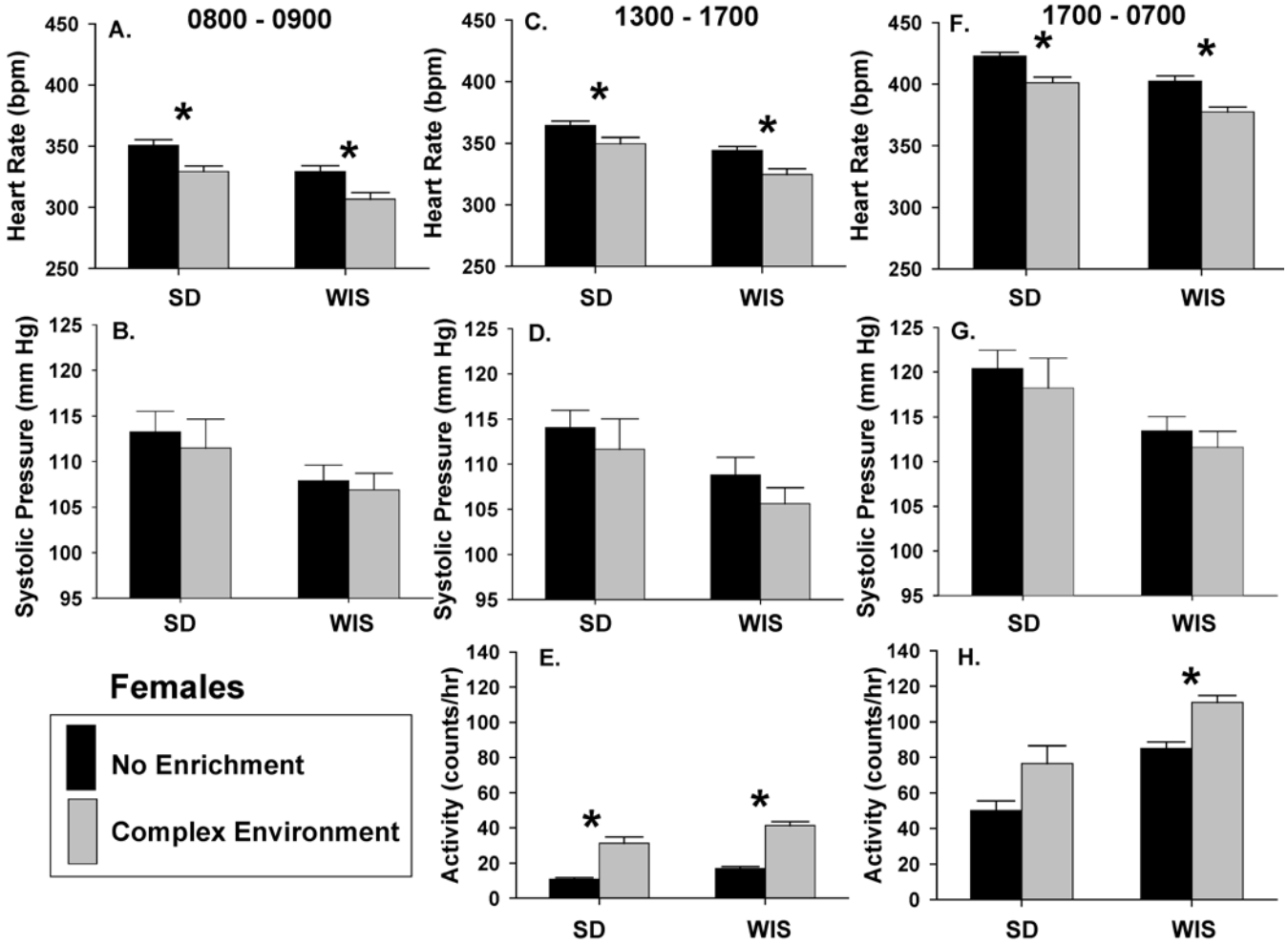

Figure 3. Effect of a complex environment on (A, C, and F) heart rate, (B, D, and G) systolic pressure, and (E and H) activity of undisturbed female Sprague–Dawley (SD) and Wistar (WIS) rats at different times of the day and night. Data are reported as mean ± SEM. *, Value for complex environment significantly ($P < 0.05$, 1-factor ANOVA, $n = 8$ [Sprague–Dawley] or 9 [Wistar]) different from that for nonenriched environment.

or were increased by the complex environment is similar to what we observed previously with individual enrichment elements (group housing,[2] dim light[3] and addition of inanimate objects to the cage[4,26]). Stock, sex, and the type of challenge may have influenced the previous and current results. For example, in the present study, responses of Wistar rats appeared to be affected more often by the complex environment than were those of the Sprague–Dawley stock. Specifically, the heart rate responses of Wistar male rats to intraperitoneal injection and running challenge and those of Wistar female rats to 10 and 60 min of restraint were significantly reduced by the complex environment, whereas the response of Wistar female rats to cage change was significantly increased. In contrast, the only response of Sprague–Dawley male rats that was affected significantly (decreased) was to 60 min of restraint, and the only response of Sprague–Dawley female rats that was affected significantly (decreased) was to cage change. Why Wistar rats appeared to be more affected by the complex environment than were Sprague–Dawley is unclear. Perhaps Wistar rats were more compatible or less competitive with their cagemates than were Sprague–Dawley rats. Another possibility is that some of the other environmental elements were more effective in Wistar than in Sprague–Dawley rats. Our previous studies evaluated Sprague–Dawley and spontaneously hypertensive rats.[2,23] In those studies, responses to acute challenges under dim light conditions were greater in Sprague–Dawley than in spontaneously hypertensive rats,[2] but responses to challenge when inanimate objects were present in the cage were less in Sprague–Dawley than in spontaneously hypertensive rats.[23] Although we deemed it important to design the experiments so that both sexes of the 2 stocks were exposed to multiple types of challenges to obtain a more comprehensive description of the possible effects of complex housing, the mixed results make it difficult to conclude that the complex housing environment used in this study should be recommended to reduce acute stress in rats. Furthermore, the observation that the complex environment, which included group housing, did not have the same outcome in both stocks or sexes is important to the general discussion of environmental enrichment for rats. Because regulatory agencies are now requiring social housing for rats, additional studies in which social (group) housing is compared with social housing coupled with addition of other enrichment items are particularly important. These studies should include both sexes and as many strains or stocks as possible. The ambient temperature in the animal room (or, better yet, the core temperature of the rats) is another important consideration for future experiments that study the effects of enrichment in group compared with individually housed rats. In this regard, significant changes in both heart rate and blood pressure occurred in individually housed rats as ambient temperature decreased over a range of 30 to 18 °C; [30] this range includes the mandated acceptable range for animal rooms. In

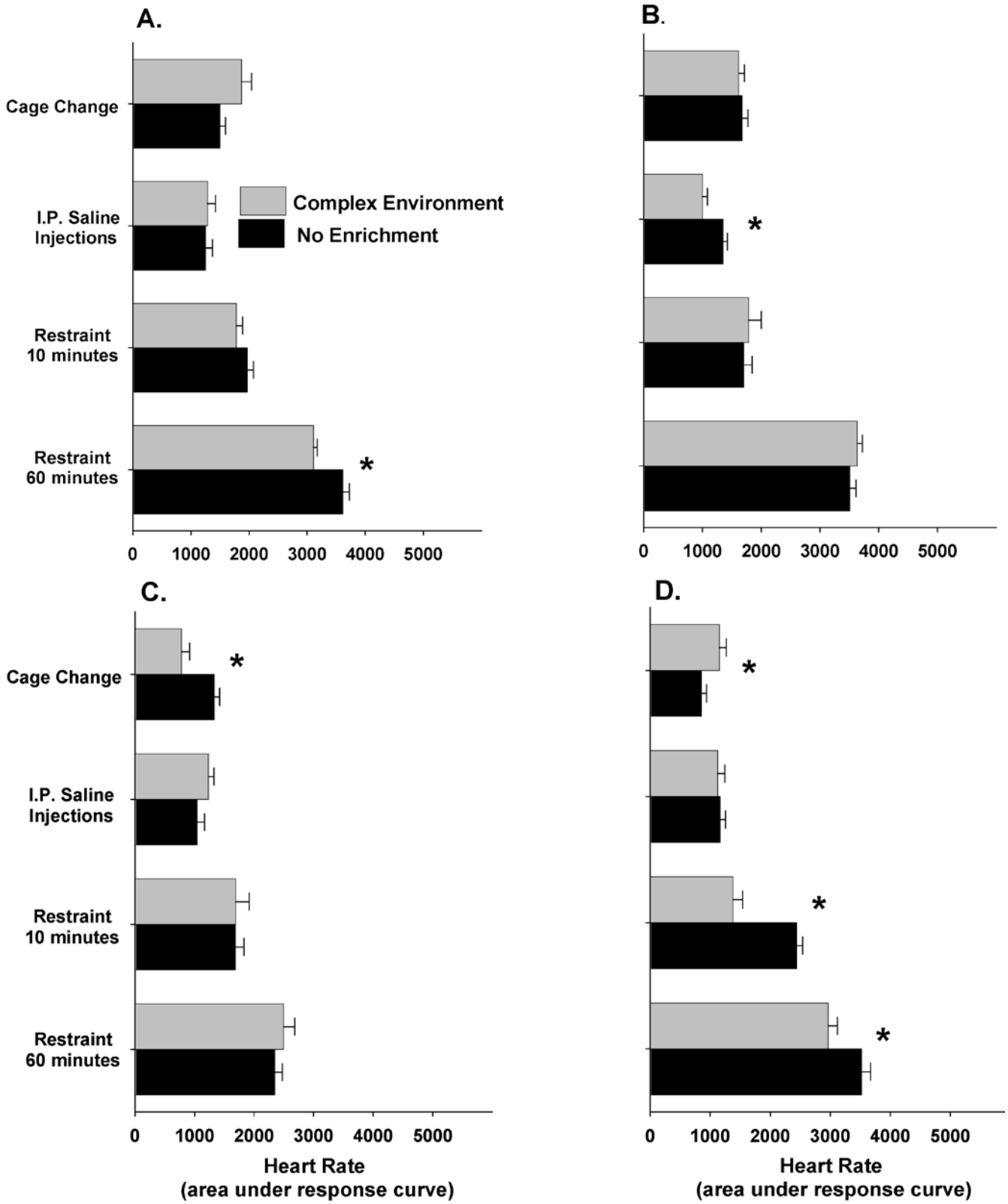

Figure 4. Effect of a complex environment on heart rate responses to acute challenges in male (A and B) and female (C and D) Sprague–Dawley (A and C) and Wistar (B and D) Rats. Values represent mean ± SEM. *, Value for complex environment significantly ($P < 0.05$, 1-factor ANOVA, $n = 9$ [Sprague–Dawley] or 10 [Wistar]) different from that for nonenriched environment.

addition, because of huddling behavior, rats housed in groups likely have higher body temperatures than do singly housed rats, especially in cooler animal room environments.

Regarding recommendations for simple or complex environments, the current results suggest that the particular complex environment that we used was no more effective than were simpler forms when the measure was decreased heart rate or increased activity in the cage. Whether other physiologic or behavior parameters of wellbeing were modified more by the complex environment than by simpler programs is unknown.

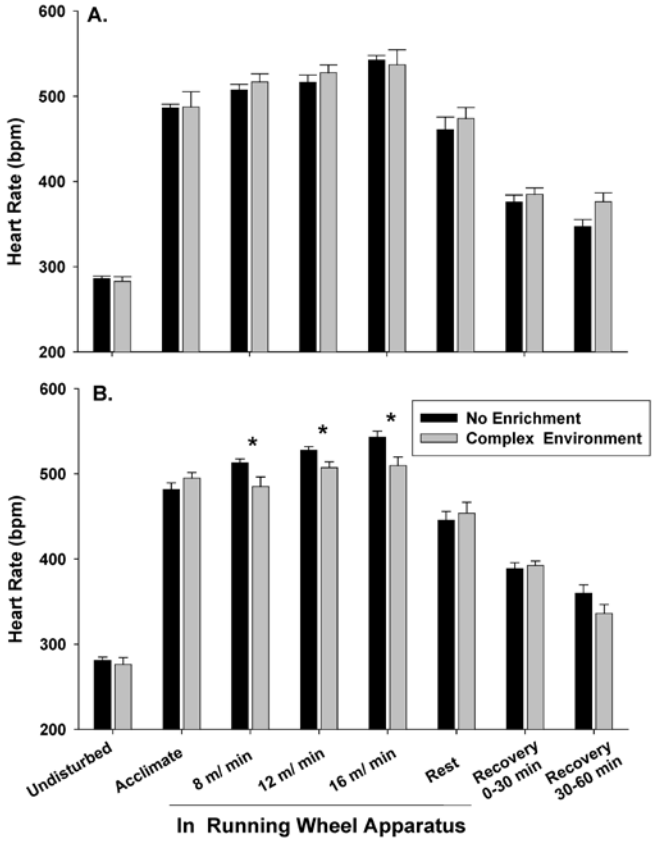

Figure 5. Effect of a complex environment on heart rate responses in male (A) Sprague–Dawley and (B) Wistar rats to a stepped forced-running challenge in a motorized running-wheel apparatus. Values for basal and recovery periods were obtained in animal's home cage; other values represent the mean and SEM over consecutive 3-min periods in the apparatus. *, Value for complex environment significantly ($P < 0.05$, 1-factor ANOVA, $n = 8$ [Sprague–Dawley] or 10 [Wistar]) different from that for nonenriched environment.

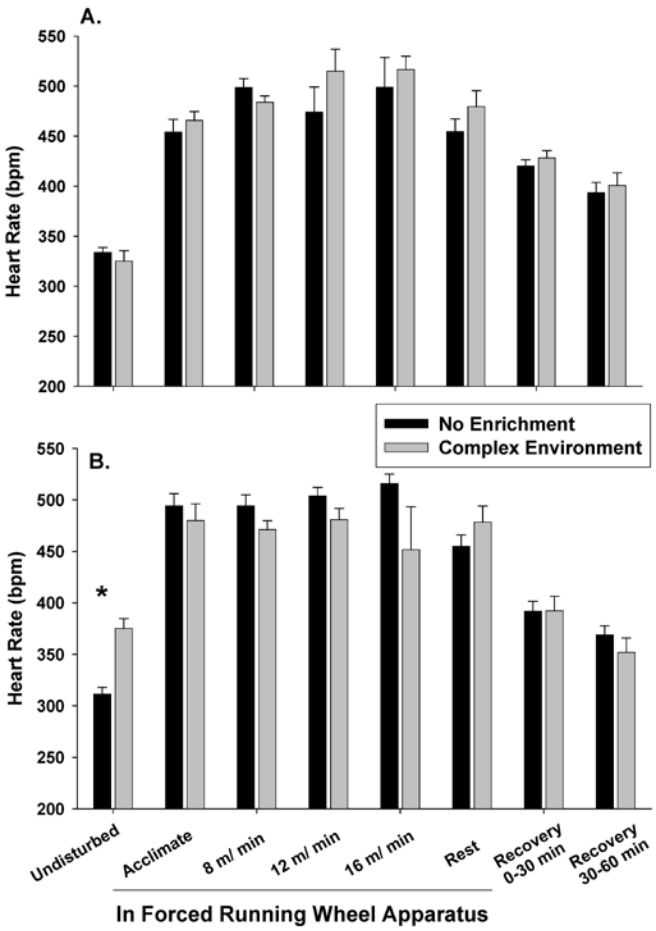

Figure 6. Effect of a complex environment on heart rate responses in female (A) Sprague–Dawley and (B) Wistar rats to a stepped forced-running challenge in a motorized running-wheel apparatus. Values for basal and recovery periods were obtained in animal's home cage; other values represent the mean and SEM over consecutive 3-min periods in the apparatus. *, Value for complex environment significantly ($P < 0.05$, 1-factor ANOVA, $n = 8$ [Sprague–Dawley] or 10 [Wistar]) different from that for nonenriched environment.

Table 1. Effect of complex housing on the maximal heart rate change (bpm; mean ± SEM) after muscarinic cholingeric blockade with methylatropine (MA), β1-adrenergic blockade with metoprolol (MT), or ganglionic blockade with hexamethonium (Hex) after MA or MT

Mice	Housing	MA	Hex after MA[a]	MT	Hex after MT[b]
Sprague–Dawley, male	Nonenriched	95 ± 14	−150 ± 21	−76 ± 14	−10 ± 9
	Complex	59 ± 17	−114 ± 20	−61 ± 8	−16 ± 9
Wistar, male	Nonenriched	130 ± 18	−174 ± 18	−93 ± 17	−23 ± 4
	Complex	89 ± 9	−157 ± 20	−59 ± 12	−28 ± 13
Sprague–Dawley, female	Nonenriched	42 ± 12	−89 ± 18	−51 ± 17	−25 ± 7
	Complex	62 ± 14	−80 ± 18	−74 ± 7	−12 ± 17
Wistar, female	Nonenriched	66 ± 25	−125 ± 23	−53 ± 17	−5 ± 9
	Complex	104 ± 18	−162 ± 18	−62 ± 13	−26 ± 11

There was no significant difference between values for nonenriched compared with complex housing environment for any strain, sex, or treatment.

[a]Data represent the maximal change in heart rate induced by hexamethonium from the peak heart rate response induced by MA.

[b]Data represent the maximal change in heart rate induced by hexamethonium from the maximal reduction in heart rate induced by MT.

In addition, complex environments that include other elements of enrichment (for example, running, exploration, or problem-solving activities such as negotiation of a maze) may be more effective than was the program we selected for the current study. For example, running is an effective enrichment element for neurogenesis—at least in mice[15]—and rats that are group-housed in a standardized complex environment that includes a maze and running wheels achieve a quicker recovery of glucocorticoid

secretion after acute stress and greater neural plasticity than do those group-housed in nonenriched conventional cages.[11] Resolution of these issues requires additional experimentation. From a practical perspective, providing a complex environment involves more logistical support, animal care staff time, and expense than do simpler programs, particularly when the complex programs have to be tailored differently for male and female mice and for different strains or stocks. Clearly, environmental enrichment for rats is not a closed issue.

Acknowledgment

Supported by NIH grant RR13600.

References

1. **Abou-Ismail UA, Mahboub HD.** 2011. The effects of enriching laboratory cages using various physical structures on multiple measures of welfare in singly housed rats. Lab Anim **45:**145–153.
2. **Azar T, Sharp J, Lawson D.** 2011. Heart rates of male and female Sprague–Dawley and spontaneously hypertensive rats housed singly or in groups. J Am Assoc Lab Anim Sci **50:**175–184.
3. **Azar TA, Sharp JL, Lawson DM.** 2008. Effect of housing rats in dim light or long nights on heart rate. J Am Assoc Lab Anim Sci **47:**25–34.
4. **Azar TA, Sharp JL, Lawson DM.** 2012. Effects of cage enrichment on heart rate, blood pressure, and activity of female Sprague–Dawley and spontaneously hypertensive rats at rest and after acute challenges. J Am Assoc Lab Anim Sci **51:**339–344.
5. **Bell JA, Livesey PJ, Meyer JF.** 2009. Environmental enrichment influences survival rate and enhances exploration and learning but produces variable responses to the radial maze in old rats. Dev Psychobiol **51:**564–578.
6. **Belz EE, Kennell JS, Czambel RK, Rubin RT, Rhodes ME.** 2003. Environmental enrichment lowers stress-responsive hormones in singly housed male and female rats. Pharmacol Biochem Behav **76:**481–486.
7. **Brillaud E, Morillion D, de Seze R.** 2005. Modest environmental enrichment: effect on a radial maze validation and wellbeing of rats. Brain Res **1054:**174–182.
8. **Chandler MP, DiCarlo SE.** 1998. Acute exercise and gender alter cardiac autonomic tonus differently in hypertensive and normotensive rats. Am J Physiol **274:**R510–R516.
9. **Del Arco A, Segovia G, Garrido P, De Blas M, Mora F.** 2007. Stress, prefrontal cortex, and environmental enrichment: studies on dopamine and acetylcholine release and working memory performance in rats. Behav Brain Res **176:**267–273.
10. **Elliott BM, Grunberg NE.** 2005. Effects of social and physical enrichment on open-field activity differ in male and female Sprague–Dawley rats. Behav Brain Res **165:**187–196.
11. **Fares RP, Belmeguenai A, Sanchez PE, Kouchi HY, Bodennec J, Morales A, Georges B, Bonnet C, Bouvard S, Sloviter RS, Bezin L.** 2013. Standardized environmental enrichment supports enhanced brain plasticity in healthy rats and prevents cognitive impairment in epileptic rats. PLoS ONE **8:**e53888.
12. **Gelfo F, Cutuli D, Foti F, Laricchiuta D, De Bartolo P, Caltagirone C, Petrosini L, Angelucci F.** 2011. Enriched environment improves motor function and increases neurotrophins in hemicerebellar lesioned rats. Neurorehabil Neural Repair **25:**243–252.
13. **Gobbo OL, O'Mara SM.** 2004. Impact of enriched-environment housing on brain-derived neurotrophic factor and on cognitive performance after a transient global ischemia. Behav Brain Res **152:**231–241.
14. **Klein SL, Lambert KG, Durr D, Schaefer T, Waring RE.** 1994. Influence of environmental enrichment and sex on predator stress response in rats. Physiol Behav **56:**291–297.
15. **Kobilo T, Liu QR, Gandhi K, Mughal M, Shaham Y, van Praag H.** 2011. Running is the neurogenic and neurotrophic stimulus in environmental enrichment. Learn Mem **18:**605–609.
16. **Koch LG, Britton SL, Barbato JC, Rodenbaugh DW, DiCarlo SE.** 1999. Phenotypic differences in cardiovascular regulation in inbred rat models of aerobic capacity. Physiol Genomics **1:**63–69.
17. **Leggio MG, Mandolesi L, Federico F, Spirito F, Ricci B, Gelfo F, Petrosini L.** 2005. Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. Behav Brain Res **163:**78–90.
18. **Matter AM, Folweiler KA, Curatolo LM, Kline AE.** 2011. Temporal effects of environmental enrichment-mediated functional improvement after experimental traumatic brain injury in rats. Neurorehabil Neural Repair **25:**558–564.
19. **Paban V, Jaffard M, Chambon C, Malafosse M, Alescio-Lautier B.** 2005. Time course of behavioral changes following basal forebrain cholinergic damage in rats: environmental enrichment as a therapeutic intervention. Neuroscience **132:**13–32.
20. **Pamplona FA, Pandolfo P, Savoldi R, Prediger RD, Takahashi RN.** 2009. Environmental enrichment improves cognitive deficits in spontaneously hypertensive rats (SHR): relevance for attention deficit/hyperactivity disorder (ADHD). Prog Neuropsychopharmacol Biol Psychiatry **33:**1153–1160.
21. **Pang TY, Hannan AJ.** 2013. Enhancement of cognitive function in models of brain disease through environmental enrichment and physical activity. Neuropharmacology **64:**515–528.
22. **Passineau MJ, Green EJ, Dietrich WD.** 2001. Therapeutic effects of environmental enrichment on cognitive function and tissue integrity following severe traumatic brain injury in rats. Exp Neurol **168:**373–384.
23. **Pham TM, Soderstrom S, Winblad B, Mohammed AH.** 1999. Effects of environmental enrichment on cognitive function and hippocampal NGF in nonhandled rats. Behav Brain Res **103:**63–70.
24. **Saland SK, Rodefer JS.** 2011. Environmental enrichment ameliorates phencyclidine-induced cognitive deficits. Pharmacol Biochem Behav **98:**455–461.
25. **Schrijver NC, Bahr NI, Weiss IC, Wurbel H.** 2002. Dissociable effects of isolation rearing and environmental enrichment on exploration, spatial learning, and HPA activity in adult rats. Pharmacol Biochem Behav **73:**209–224.
26. **Sharp J, Azar T, Lawson D.** 2005. Effects of a cage enrichment program on heart rate, blood pressure, and activity of male Sprague–Dawley and spontaneously hypertensive rats monitored by radiotelemetry. Contemp Top Lab Anim Sci **44:**32–40.
27. **Sharp J, Zammit T, Azar T, Lawson D.** 2003. Stress-like responses to common procedures in individually and group-housed female rats. Contemp Top Lab Anim Sci **42:**9–18.
28. **Sharp JL, Zammit TG, Azar TA, Lawson DM.** 2002. Stress-like responses to common procedures in male rats housed alone or with other rats. Contemp Top Lab Anim Sci **41:**8–14.
29. **Simpson J, Kelly JP.** 2011. The impact of environmental enrichment in laboratory rats—behavioural and neurochemical aspects. Behav Brain Res **222:**246–264.
30. **Swoap SJ, Overton JM, Garber G.** 2004. Effect of ambient temperature on cardiovascular parameters in rats and mice: a comparative approach. Am J Physiol Regul Integr Comp Physiol **287:**R391–R396.
31. **Wagner AK, Kline AE, Sokoloski J, Zafonte RD, Capulong E, Dixon CE.** 2002. Intervention with environmental enrichment after experimental brain trauma enhances cognitive recovery in male but not female rats. Neurosci Lett **334:**165–168.
32. **Woodcock EA, Richardson R.** 2000. Effects of environmental enrichment on rate of contextual processing and discriminative ability in adult rats. Neurobiol Learn Mem **73:**1–10.
33. **Zaias J, Queeney TJ, Kelley JB, Zakharova ES, Izenwasser S.** 2008. Social and physical environmental enrichment differentially affect growth and activity of preadolescent and adolescent male rats. J Am Assoc Lab Anim Sci **47:**30–34.