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Use of PVC Conduits by Rats of Various Strains and Ages Housed Singly and in Pairs

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This study observed the frequency with which laboratory rats (*Rattus norvegicus*) entered polyvinylchloride (PVC) conduits placed in their cages to provide environmental enrichment. The study found that use of PVC conduits by Norway rats varied with subjects' strain, age, sex, and housing condition. Adult male Long–Evans rats rarely entered PVC conduits unless housed in pairs, in which case the lighter (presumably subordinate) pair member frequently used the conduit, possibly to avoid contact with his dominant partner. Adult male Sprague–Dawley rats entered PVC conduits only during the illuminated portion of the day–night cycle and only if housed on shelves exposed to direct overhead illumination. Both juvenile rats and female rats made extensive use of PVC conduits throughout the day–night cycle. This article discusses implications of these findings for determining how best to enrich environments in which laboratory rodents are maintained.

Providing a place of refuge in the otherwise barren cages in which laboratory animals normally are housed is a frequent type of environmental enrichment, not only for Norway rats (*Rattus norvegicus*), the subject species in the studies reported here, but also for other species of laboratory rodent (Canadian Council on Animal Care [CCAC], 1993; National Research Council [NRC], 1996). In North America, we often provide shelter for laboratory rodents by introducing a section of inexpensive, easily cleaned, polyvinylchloride (PVC) conduit into a cage housing one or more animals.

Despite widespread use of PVC conduits for environmental enrichment, the effect of providing such shelters on the behavior of laboratory rats remains unexplored. In related studies, Manser, Broom, Overend, and Morris (1998a) and Townsend (1997) reported that albino laboratory rats prefer a cage containing a

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nest box to one lacking such shelter. Manser, Broom, Overend, and Morris (1998b) showed that albino rats also will work harder to reach a cage containing a nest box than to reach an empty cage. In experiments with rodents other than laboratory rats, Sherwin (1996) showed that if there is sawdust on the cage floor, individually housed laboratory mice prefer to sleep outside plastic tubes. Van de Weerd, Van Loo, Van Zutphen, Koolhaus, and Baumans (1998) found that individually tested mice prefer enclosures containing nest boxes to those without them. Clark and Galef (1977) found that access to shelter increased frequency of stress responses in Mongolian gerbils (*Meriones unguiculatus*).

Research on consequences of placing PVC shelters in rat cages is so preliminary that not even the frequency with which animals of different sizes and ages enter such shelters has been determined. Information about frequency of shelter use is important because it provides a preliminary indication of the potential of shelters to contribute to the psychological well-being of laboratory animals (Dawkins, 1977, 1990, 1998). If, for example, laboratory rats avoid contact with PVC conduits placed in their cages, it would be difficult to maintain that access to such shelters contributes to psychological health.

Informal observation of 125 individually housed, adult male Long–Evans rats maintained in standard laboratory cages provided with PVC conduits indicated that these animals rarely were seen in conduits and, thus, appeared to find conduits aversive rather than attractive (Galef, 1999). These experiments—undertaken to extend these preliminary observations—determine conditions under which rats of various ages, strains, and conditions of maintenance took shelter in PVC conduits present in their cages.

GENERAL METHODS

Subjects

Subjects were Long–Evans and Sprague–Dawley (*Rattus norvegicus*) purchased from Charles River Canada (St. Constant, Quebec) and maintained in a large colony room, part of the vivarium of the McMaster University Psychology Department. All subjects in these experiments had been purchased for use in other experiments and were being held in the vivarium until they reached the appropriate age for participation in those experiments. Requirements of the experiments in which subjects were to participate determined the age, sex, strain, and housing condition of subjects available to us for observation.

Apparatus

The study took place in a humidity-controlled colony room maintained at 22–23 °C and illuminated by overhead fluorescent fixtures on a 12:12 light–dark schedule (light onset 7 a.m.). Subjects were housed in transparent polycarbonate

shoebox cages, measuring 16.8 × 30 × 33 cm and closed with a lid of stainless steel rods. Wood-chip bedding (Beta-Chips, Warrensberg, NY) covered the solid floor of each cage.

The lid of each cage held both a water bottle and a supply of Purina Rodent Laboratory Chow 5001 (Ralston-Purina, Woodstock, Ontario). Each cage also contained a single piece of PVC tubing 7.5 cm inside diameter (9 cm outside diameter) with an average length of 14.8 cm that, on average, occupied 133.2 cm^2 or 13.5% of the total floor area (990 cm^2) of the cage in which it was placed. We selected these particular conduits for study because they are the ones provided our facility to meet the CCAC's requirements to provide environmental enrichment for laboratory-housed rats. The conduits commonly are used for that purpose throughout Canada.

Procedure

To begin the experiment, we recorded the weight or birth date of each subject. We also marked the tails of those rats living in cages with more than one occupant with colored ink so that we could identify individual cage occupants.

An observer visited the colony room twice each day (once during the light and once during the dark portion of the illumination cycle) and determined whether the residents of each cage were inside the PVC conduit available to them. We purposely varied the time of visits to include all times of the day and night. Subjects were considered to be inside a conduit if any portion of their head or body was within it. During the night portion of the illumination cycle, the observer used a dim flashlight to determine the position of subjects relative to conduits. The experimenter quietly entered the colony room and inspected—one after another—each cage, recording whether cage occupants were in the conduit.

Frequent intrusion of caretakers and experimenters into the colony room ensured that all subjects were habituated to the presence of humans, and cage occupants did not respond to an observer entering or present in the colony room by either entering or leaving conduits. Indeed, during several thousand observations, rats were seen entering or leaving conduits on fewer than 10 occasions.

Data Analyses

We analyzed the data using 2 × 2 or repeated-measures analyses of variance (ANOVAs), as appropriate, and used an alpha level of .05 throughout.

EXPERIMENT 1: USE OF PVC TUBING BY ADULT RATS HOUSED INDIVIDUALLY

Experiment 1 was undertaken both to formalize the observations of Galef (1999), indicating that adult male Long–Evans rats rarely enter PVC conduits

present in their cages, and to extend observations of conduit use to both adult female Long–Evans and adult male Sprague–Dawley rats.

Comparisons between Long–Evans and Sprague–Dawley rats are of potential interest because lack of pigmentation in the eyes of Sprague–Dawley rats might render them more sensitive to light than pigmented Long–Evans rats.

Methods

Subjects. A total of 100 male Long–Evans rats weighing an average (± 1 SEM) of 669.7 ± 5.7 g, 24 male Sprague–Dawley rats (477.2 ± 2.3 g), and 23 female Long–Evans rats (301.1 ± 2.3 g) served as subjects. We assigned the 100 male Long–Evans rats to two studies; we used 50 of these males for comparison with Long–Evans female rats in Study 1 and the remaining 50 male Long–Evans rats in Study 2 for comparison with male Sprague–Dawley rats.

Procedure. We observed each cage once during the illuminated and once during the dark portion on each of 5 consecutive days and awarded each subject two scores ranging from 1 to 5, one score based on daytime and one on nighttime observations. A score indicated how many times out of five we observed each subject with any part of the head or body within a conduit.

Results

The main results of Experiment 1 are presented in the two panels of Figure 1 that show the percentage of observations during both the day and night portions of the illumination cycle when subjects were seen with any part of their heads or bodies inside the PVC conduits in their cages.

Male and female Long–Evans rats. As can be seen in the upper panel of Figure 1, there was a pronounced sex difference in utilization of PVC conduits by male and female Long–Evans rats (2 × 2 ANOVA), $F(1, 142) = 60.24, p < .0001$, as well as (a) a significant main effect of time of day, $F(1, 142) = 5.43, p < .02$, and (b) a significant sex by time of day interaction, $F(1, 142) = 5.85, p < .02$. In brief, male Long–Evans rats rarely were seen inside conduits, whereas females of the same strain used conduits both regularly and more frequently during the day than during the night. Thirteen of the 16 female rats who differed in the frequency with which they were seen in conduits during day and night inspections were in the conduit more frequently during the day (Sign test, $p < .04$).

Male Long–Evans and male Sprague–Dawley rats. As can be seen in the lower panel of Figure 1, there was also a marked strain difference in utilization of PVC conduits by male Long–Evans and male Sprague–Dawley rats. Across the

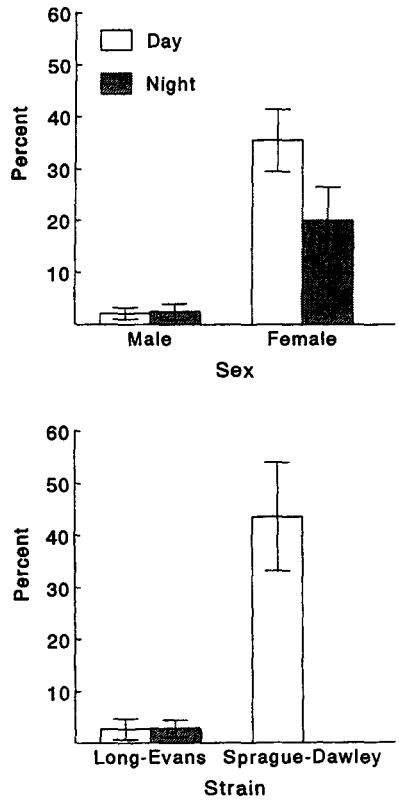

FIGURE 1 Mean percentage of observation periods (± 1 SEM) during day and night portions of the illumination cycle when (top panel) male and female Long–Evans rats, and (bottom panel) male Long–Evans and Sprague–Dawley rats were inside conduits.

entire day, male Sprague–Dawley rats were (a) inside PVC conduits significantly more frequently than were male Long-Evans rats, $F(1, 122) = 26.89, p < .0001$, and (b) used conduits more frequently during the day than at night, $F(1,122) = 35.01, p < .0001$. There was a significant interaction between the effects of strain and of time of day resulting from more frequent use of PVC conduits during hours of illumination than during hours of darkness by Sprague–Dawley but not Long–Evans rats, $F(1, 122) = 35.38, p < .0001$.

As can be seen in the bottom panel of Figure 1, male Sprague–Dawley rats never entered conduits during the night but did so frequently during the day, whereas male Long–Evans rats entered conduits both rarely and equally often during day and night.

Discussion

The results of Experiment 1 suggest that adult male Long–Evans rats infrequently enter PVC conduits. On the other hand, both adult male Sprague–Dawley rats and adult female Long–Evans rats used PVC tubes more frequently when the colony room was illuminated than when it was dark.

It is possible that male Long–Evans rats rarely were seen in conduits because their relatively large body size made it physically impossible for them to enter the conduits. Several observations make such an interpretation of the data unlikely. First, even the largest males occasionally were seen inside conduits. Second, it was extremely easy for a handler to induce the largest of the rats whom we studied to enter a conduit, even if the rat had failed to do so spontaneously. Last but not least, adult female Long–Evans rats in the last days of pregnancy, and therefore of considerably greater girth than even the largest males in our study, frequently are seen in conduits (L. Honey, personal communication, July 13, 2000).

EXPERIMENT 2: EFFECTS OF ILLUMINATION LEVEL ON USE OF PVC CONDUITS

A post-hoc analysis of data collected in Experiment 1 indicated that Sprague–Dawley males housed in cages on the top row of shelving ($n = 7$), and therefore exposed directly to illumination by fluorescent lights mounted in ceiling fixtures in the colony room, were seen in conduits far more frequently than were males of the same strain living in cages housed on lower shelves and therefore shaded from direct overhead illumination. The former animals were seen in conduits on 72% and the latter on only 16% of observations. Use of PVC conduits by female Long–Evans rats was not similarly affected by cage placement. Both animals on top and bottom shelves were seen in conduits on 32% of daytime observations.

We undertook Experiment 2 to examine directly effects of cage placement and consequent degree of exposure to direct overhead illumination on frequency of use of PVC conduits by male Sprague–Dawley and female Long–Evans rats. Male Sprague–Dawley and female Long–Evans rats were the subjects in Experiment 1 who differed in their use of conduits during day and night. They were therefore the subjects most likely to be using conduits to escape from aversive levels of illumination. In Experiment 2, we systematically moved cages between top and bottom shelves of cage racks to examine effects of cage location and consequent exposure to direct overhead illumination on use of PVC conduits.

Methods

Subjects. Twelve adult male Sprague–Dawley rats weighing 410.3 ± 5.7 g and 12 adult female Long–Evans rats weighing 298.4 ± 6.2 g served as subjects.

Procedure. The procedure was the same as that previously described except that we moved cages housing subjects. To start the experiment, we placed cages containing six subjects of each strain on the top shelves of racks and cages containing the remaining six subjects of each strain on the bottom shelves of the same racks. We left all cages undisturbed for 3 days, then reversed the positions of the cages containing rats of each strain, waited 1 day to allow cage occupants to habituate to their new locations, then once again examined the use of conduits by cage occupants for 3 consecutive days. To complete the experiment, we again reversed the locations of all cages, waited 1 day, and again examined use of conduits by subjects for 3 consecutive days.

We measured illumination on the floors of cages placed on top and bottom shelves with a luminance meter (Minolta model LS–100) with a 40.5-mm lens (Minolta Model 122). Light intensity was determined three times at each of four locations on top and bottom shelves, highest and lowest readings were discarded, and a mean and standard error of measurement were calculated for cages on top and bottom shelves.

Results

Illumination levels of top and bottom shelves were 20.4 ± .5 and 11.2 ± .4 cd/m^2, respectively, $t(18) = 14.15$, $p < .0001$. The main results of Experiment 2 are presented in the two panels of Figure 2 that show the percentage of observations made during day and night portions of the illumination cycle when subjects on top and bottom shelves were seen inside PVC conduits.

Male Sprague–Dawley rats. A 2 × 2 ANOVA of the data presented in the top panel of Figure 2 revealed significant main effects of both time of day and shelf location on conduit use by male Sprague–Dawley rats, both Fs$(1, 44) > 36.00$, both ps $< .0001$, as well as a significant interaction between main effects, $F(1, 44) = 36.10$, $p < .0001$. Males housed on top shelves used conduits more frequently than did males housed on bottom shelves during the illuminated but not the dark portion of the day–night cycle.

Female Long–Evans rats. As can be seen in the lower panel of Figure 2, time of day, $F(1, 44) = 6.62$, $p < .02$, but not location, $F(1, 44) = .01$, *ns*, affected frequency of conduit use by female Long–Evans rats, and there was no interaction between these main effects, $F(1, 44) = .14$, *ns*. Female Long–Evans rats simply used the conduits more frequently during the day than at night, regardless of their shelf location.

Discussion

The results of Experiment 2 confirm the finding in Experiment 1 that adult male Sprague–Dawley rats use PVC conduits only during the illuminated portion of

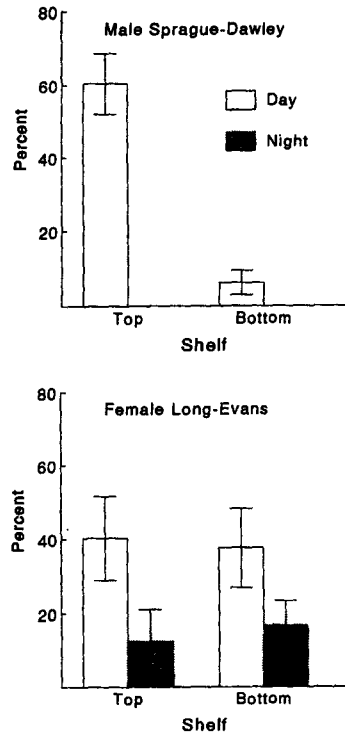

FIGURE 2 Mean percentage of observation periods (± 1 SEM) during day and night portions of the illumination cycle when (top panel) male Sprague–Dawley and (bottom panel) female Long–Evans rats whose cages were located on top and bottom shelves were inside conduits.

the day–night cycle. Taken together, the finding that these males used conduits only during daylight hours and the finding that the shelf on which their cages were kept affected males' frequencies of conduit use suggest that male Sprague–Dawley rats found direct exposure to overhead fluorescent illumination aversive and used conduits to escape photic stimulation. The results are consistent with the findings of Blom, Van Tintelen, Baumans, Van Den Brock, and Beynen (1996) and Schlingmann, de Rijk, Pereboom, and Remie (1994), indicating that the intensity of illumination in conventional facilities housing albino rats is aversive to the animals.

The finding that use of conduits by female Long–Evans rats during the day portion of the illumination cycle was not affected by the placement of their

cages on racks suggests that something other than avoidance of light motivated their use of conduits.

EXPERIMENT 3: USE OF PVC CONDUITS BY RATS OF DIFFERENT AGES

In Experiment 3, we examined use of PVC conduits by Long–Evans rats of both sexes as a function of age.

Methods

Subjects. Thirteen male and 13 female Long–Evans rats approximately 28 days old—weighing, respectively, 116.8 ± 2.3 g and 108.5 ± 1.1 g at the start of the experiment—served as subjects.

Procedure. We housed subjects individually and, starting when they were 28 days old, observed them—as previously described—for 3 days a week for 5 consecutive weeks as well as for 3 days a week when subjects were both 12 and 13 weeks old. We chose weeks for observation to maximize our collection of data during the period of maximum growth of our subjects and terminated the experiment when subjects in this experiment were behaving as did adults in Experiment 1.

Results and Discussion

The main results of Experiment 3 are presented in Figure 3, which shows the frequency of use of PVC conduits by male and female Long–Evans rats as they

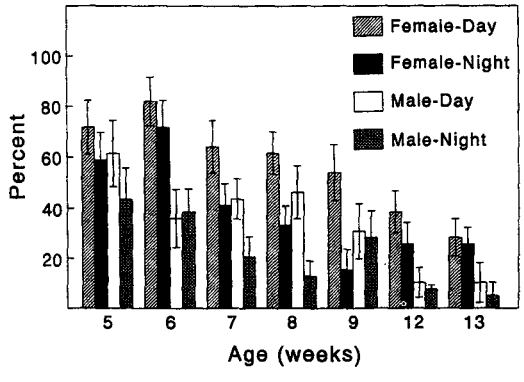

FIGURE 3 Mean percentage of observation periods (± 1 SEM) during day and night portions of the illumination cycle when male and female Long–Evans rats of different ages were inside conduits.

matured from 5 to 13 weeks of age. Examining Figure 3, we see that as subjects aged their use of PVC conduits decreased, $F(1, 48) = 78.18$, $p < .0001$. In addition, as expected from the results of Experiment 1, we found significant main effects on conduit use of both sex of subject, $F(1, 48) = 8.14$, $p < 0.01$, and time of day, $F(1, 48) = 4.69$, $p < .04$; females used conduits more frequently than did males, and conduits were used more frequently during the day than at night. There was no significant interaction between main effects, $F(1, 48) = .23$, *ns*.

EXPERIMENT 4: USE OF PVC CONDUITS BY PAIRS OF MALE LONG–EVANS RATS

Free-living wild rats are social animals. Consequently, their domesticated conspecifics are often housed in the laboratory in pairs or groups so as to provide more natural levels of social stimulation (CCAC, 1993; NRC, 1996). In Experiment 4, we examined use of PVC conduits by adult male and female Long–Evans rats housed in pairs in cages, each of which contained a single PVC conduit.

Methods

Subjects. Twenty-eight adult male and 28 adult female Long–Evans rats housed in same-sex pairs served as subjects. The heavier members of male pairs weighed an average 319.6 ± 3.9 g, and the lighter members weighed 296.4 ± 4.9 g. The heavier members of female pairs weighed an average 242.7 ± 1.4 g, and the lighter members weighed 229.9 ± 1.4 g.

Procedure. Each cage was observed over a period of 3 days, as previously described.

Results and Discussion

The main results of Experiment 4 are presented in the two panels of Figure 4 that show the mean percentage of observations during which heavier and lighter members of each pair of subjects were seen inside a PVC conduit.

Pairs of male rats. In 13 of the 14 pairs of males in which one pair member was seen in a conduit more frequently than the other, the lighter animal made greater use of the conduit (Sign test; $N = 14, x = 1, p = .001$). Further, comparison of the frequency of use of PVC conduits by male pair members in this experiment with that of individually housed male Long–Evans rats in Experiment 1 (Figure 1, upper

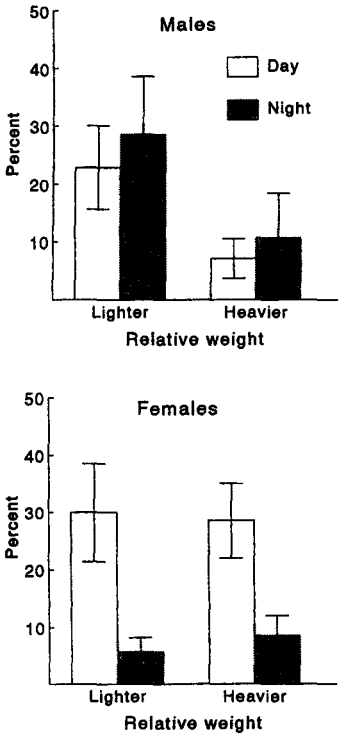

FIGURE 4 Mean percentage of observation periods (± 1 SEM) during day and night portions of the illumination cycle when (top panel) lighter and heavier members of pairs of Long–Evans male, and (bottom panel) Long–Evans female rats were inside conduits.

panel) indicated that lighter, but not heavier, members of pairs in this experiment showed elevated daily use of PVC conduits, $F(2, 99) = 17.91$, $p < .0001$; Tukey–Kramer multiple comparison tests, lighter versus heavier, $p < .01$; lighter versus Experiment 1, $p < .001$; heavier versus Experiment 1, *ns*. Although this experiment provides no direct evidence that dominance relations were involved in differential use of PVC conduits by members of pairs of male rats, we speculate that lighter, submissive animals (Barnett, 1975) might have used conduits to reduce contact with their heavier, dominant cage mates. We intend to further investigate the possibility.

Pairs of female rats. As can be seen in the lower panel of Figure 4, there was no difference in conduit use by lighter and heavier members of female pairs. One pair member was seen in a conduit more frequently than the other in 16 pairs of female subjects, and in only 8 of those 16 cases did the lighter pair member use the