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Preferences of Minipigs for Environmental Enrichment Objects

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The minipig is an increasingly popular species for use in behavioral and toxicologic studies. As a result, quantification of environmental enrichment preferences for this species is especially important. We exposed 6 individually housed prepubertal female Yucatan minipigs to 1 of 3 different objects on a rotating schedule: 2 sessions with a hard plastic ball (diameter, 21.0 cm), and 3 sessions each with a large plastic apple (diameter, 22.5 cm) and a soft rubber cone (height, 48.0 cm). Objects were changed every 4 to 5 d. The initial 15 min after each object change was recorded, and duration of object interaction and other behaviors (activity and interaction with the food bowl) were measured. Results indicated significantly longer interactions with the cone (mean ± SE, 282 ± 54 s) than the ball (14 ± 3 s). Interactions with the apple (66 ± 18 s) and ball did not differ significantly. Interactions with the apple decreased across the 3 sessions, whereas interaction with the cone remained high for most minipigs over the 3 sessions. Duration of activity appeared to be inversely correlated with duration of object interaction (that is, the longer the subject interacted with the object, the less it engaged in nonobject activity). These results provide valuable and practical information on the features of objects that minipigs appear to prefer and offer suggestions for future studies evaluating environmental enrichment paradigms with individually housed minipigs.

An important and recommended component of laboratory animal studies is the provision of environmental enrichment. Extensive research for appropriate environmental enrichment types for rodents and nonhuman primates has been conducted.[12,15] The increasing use of the minipig in behavioral and toxicologic studies (for example, reference 7) as well as drug development[6] led us to investigate what types of enrichment can successfully be used with this animal model. Certain types of environmental enrichment for laboratory swine may satisfy the need to chew and root, especially if bedding is not provided.[18]

Environmental enrichment has been defined as "any measure which promotes expression of natural, species-specific behaviors and a decrease in, if not disappearance of, abnormal behaviors."[5] A common behavior of all swine is rooting, and mini- and full-size pigs typically explore their environment through this behavior.[4,18] To encourage rooting behavior, bedding materials (for example, hay, straw, and pine shavings) have been provided for enrichment, traction for walking, and sleeping. Some studies have used concealed treats, such as jelly beans and dog biscuits, to encourage rooting further.[4]Specially designed playrooms and 'stimulating' environments have been used with swine to test the effects of enrichment;[4,10] but many researchers place enrichment objects directly into the home cage.[8,9,18] Those objects can be suspended from a ceiling (for example, large rope, chain, hose) or placed on the floor (for example, milk crate, small garbage can). Soft pliable objects appear preferable to hard, less pliable objects and are thought to reduce excitability.[3,8,9,11] Minipigs appear to avoid soiled or dirty objects;[10,18] therefore, for practical purposes, objects must be easy to clean. Further, object novelty is important, because minipigs tend to lose interest in familiar objects.[10,14,16]

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A recent study at our institution (the National Center for Toxicological Research) provided the opportunity to examine the environmental enrichment preferences of prepubertal female Yucatan minipigs. We tested a limited selection of distinct objects with different attributes. Behavioral interactions with the objects were recorded and quantified as an indication of preference or interest.

Materials and Methods

Subjects. Six female prepubertal Yucatan minipigs (*Sus scrofa domestica*) were obtained from Sinclair Research Center (Columbia, MO). According to the Herd Health Profile provided by the vendor, there were no cases of common domestic swine diseases (for example, *Brucella*, leptospirosis, pseudorabies, transmissible gastroenteritis, porcine reproductive respiratory syndrome virus, vesicular stomatitis) during October 2005 through October 2006 (these subjects were born at the vendor facility in May and June 2005). Further, random evaluations for ecto- and endoparasites were negative. On arrival, the minipigs averaged 149 d of age (range = 136 to 158 d), weighed 18.0 ± 0.9 kg (mean ± SE), and were individually housed in aluminum cages (252 × 150 × 185 cm). The study population included 2 pairs of littermates: PF8370 and PF8369 were siblings, as were PF8331 and PF8332. Water was provided by using lixit watering systems attached to 1 wall (43.2 cm above the floor). Cage floors were painted concrete and covered with kiln-dried pine shavings (Northeastern Products, Warrensburg, NY). Cages were arranged such that each pig had visual, auditory, and olfactory contact with other pigs. Feeding (Certified Laboratory Minipig Grower/Maintenance Diet, 5K99, PMI Nutrition International, St Louis, MO) occurred twice daily, with the afternoon feeding occurring after videorecording was completed. The housing room was programmed on a 12:12-h light:dark cycle. However, because pigs prefer dim light to complete darkness,[1,2] lights in a neighboring room were left on 24 h a day to provide dim lighting through a window into the housing room. Temperature

was maintained at 20 to 24 °C with humidity levels of 40% to 60%. All animal procedures were approved in advance by the Institutional Animal Care and Use Committee of the National Center for Toxicological Research. These subjects were part of a larger dermatologic study, and those results will be reported separately. The coupling of these behavioral endpoints to the dermatologic study was done in an effort to maximize the use of animal resources.

Procedure. Before their purchase and arrival, the pigs were provided with hanging chains in their cages but no other environmental enrichment objects[17]After 1 wk for habituation, each pig was provided with 1 of 3 objects in its cage (an apple, a ball, or a cone). The plastic apple (Giant Scented Apple Horse Toy, discontinued item 821, PetSmart, Phoenix, AZ) was 22.5 cm in diameter, had a small hole in the bottom, and (according to the manufacturer) was apple-scented. The ball (diameter, 21 cm; Best Balls K3128, Bio-Serv, Frenchtown, NJ) was composed of inflexible polyethylene plastic. The cone (height, 48 cm; Training Cone, discontinued item 310379, PetSmart) was composed of soft pliable plastic with a hollow core and small rubber feet on the bottom. The apple and cone are currently available from State Line Tack (Hazelton, PA).

Test sessions occurred on Tuesdays and Fridays between 1400 and 1500 and began with removal of the previous object (except on the first session, when no object was in the cage) and placement of the new object into the cage. Thus, each subject was allowed 4 to 5 d with each object. The initial 15 min after the addition of a new object was videorecorded. Two camcorders mounted above the cages were used; thus, 2 cages could be recorded simultaneously. After the 15-min test session, the cameras were moved to the next 2 cages, old objects were removed and new objects placed into the cages, and videorecording of those 2 cages began. This procedure was repeated for the last 2 cages, such that all videorecording was done within 1 h of placement of the new objects into the first 2 cages. Videotaping order (and therefore object placement) into pairs of cages was random. On each test session, all pigs received the same object. Order of object presentation was: apple, cone, ball.

Figure 1 shows these 3 enrichment objects and the food bowl. On each test session, the previous object was removed and cleaned in the cage–rack washer. The new object remained in each pig's cage until the subsequent test session, when it was replaced with the next object in the sequence.

Scoring. There were 3 test sessions each with the cone and apple, and 2 test sessions were recorded with the ball. According to previous methods,[19] behavior during each 15-min session was categorized as object interaction, food bowl interaction, activity, and other. These 4 behaviors were mutually exclusive. Object interactions varied depending on the object but generally consisted of chewing, pushing, pulling, and sniffing. Food bowl interactions were similar, given that the food bowl was not fixed in any location. Activity included walking, rooting, and other movement that did not involve interactions with the object or food bowl. Other behaviors consisted of rare instances when the minipig was not fully visible in the videotaped portion (to categorize a behavior, more than half of the minipig's body had to be visible), lay on the floor, or was quiet or still. Each behavior had to last at least 0.5 s to be categorized. A single tester (MES) scored all videotaped sessions, which were scored within 6 wk to minimize tester drift.

Analysis. Duration of each of the 4 behaviors (object interaction, food bowl interaction, activity, and other) was analyzed individually by repeated-measures ANOVA (JMP version 7.0, SAS Institute, Cary, NC) with factors of object type and session.

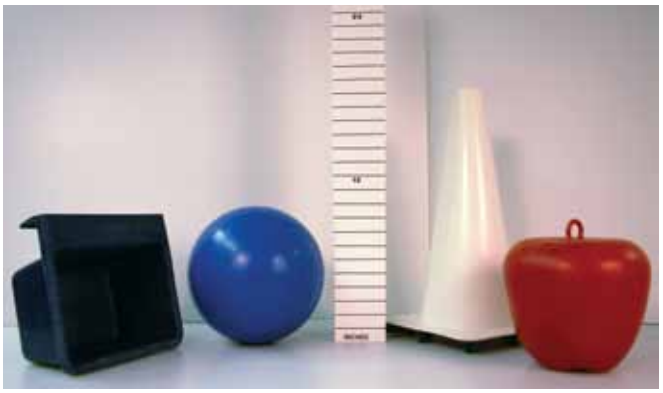

Figure 1. From left to right, the food bowl and the 3 enrichment objects (ruler indicates approximate sizes of objects).

Significant main effects of object type or interactions of object type with session were analyzed further by using Tukey HSD tests; a P of less than 0.05 was considered significant.

Results

Each minipig had 2 sessions with the ball and 3 sessions each with the cone and apple. Duration of interaction with the apple (Figure 2 A) and cone (Figure 2 C) were variable across both animals and sessions, whereas duration of interaction with the ball (Figure 2 B) was consistently low. None of the 4 formal tests of normality (Shapiro–Wilk, Kolmogorov–Smirnov, Cramer–von Mises, and Anderson–Darling) rejected normality. Analysis of duration of object interaction indicated a significant main effect of object type [$F(2, 2) = 4.07$, $P < 0.03$], and post hoc tests indicated that interaction with the cone was significantly ($P < 0.05$) longer than that with the ball. Durations of interaction with the cone, apple, and ball (averaged over sessions; mean ± SEM) were 282 ± 54, 66 ± 18, and 14 ± 3 s, respectively. The interaction of object type with session ($P < 0.08$) showed a trend toward statistically significant difference.

Analysis of duration of activity revealed a significant interaction of object type × session [$F(3, 38)=3.04$, $P < 0.05$]. Post hoc tests indicated that duration of activity with the cone during session 3 was lower than that during session 1 with the cone or session 3 with the apple ($P < 0.05$; Figure 3); that is, the minipigs exhibited less nonobject-directed activity during session 3 with the cone than the other objects. Analysis of duration of interaction with the food bowl did not indicate any significant effects; duration of food bowl interactions averaged less than 30 s per session. Similarly, analysis of duration of the behavioral category 'other' did not indicate any significant effects; duration of other behaviors averaged 20 s per session.

Discussion

Duration of interaction with objects provided in the cages of Yucatan minipigs was measured as a quantitative indication of environmental enrichment. Increasing use of this species in neurobehavioral and toxicologic research requires such information about object preferences. The objects differed in composition, shape, and color. The minipigs markedly preferred the pliable object (small cone) relative to the plastic ball, which were inflexible. In general, interactions with the cone remained high over 3 sessions, whereas interactions with the apple declined substantially. These data indicate that the type of environmental enrichment object is important to preference. Factors such as composition, shape or hollow structure may be important, and interactions with nonpreferred objects declined rapidly.

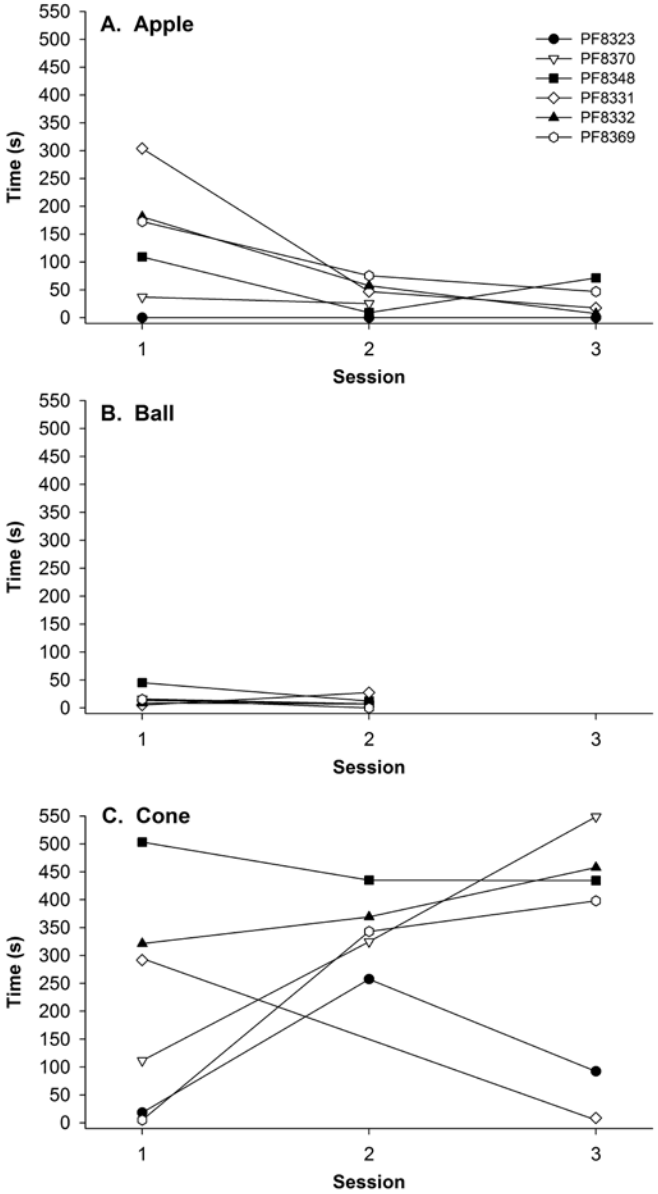

Figure 2. Duration of object interaction for each of the 6 minipigs by session. (A) Duration of interaction with the apple. (B) Duration of interaction with the ball. Each subject had 2 sessions with the ball. (C) Duration of interaction with the cone. Due to camera malfunction, no data are available from session 2 for subject PF8331.

Previous studies[14,16] have indicated that pigs explore novel objects more than those that are familiar; our current results are somewhat supportive of this. Durations of interaction with the apple and ball were longest during the first session. Compared with those from later sessions with the apple and ball, average durations of interaction with the cone were longer on the second and third exposure to this object. This difference may have been due to the greater complexity of the cone relative to the other objects. The cone was hollow, with holes at both ends and small rubber feet on the bottom. These attributes enabled the minipigs to interact with the cone in different ways relative to the other objects.

Consistent with our findings, previous work has indicated that swine prefer soft, pliable objects.[9] Pliable rubber toys often are suggested as a source of enrichment for confined pigs.[3] Although the apple was not pliable, durations of interaction with this object were relatively high during the first session, perhaps

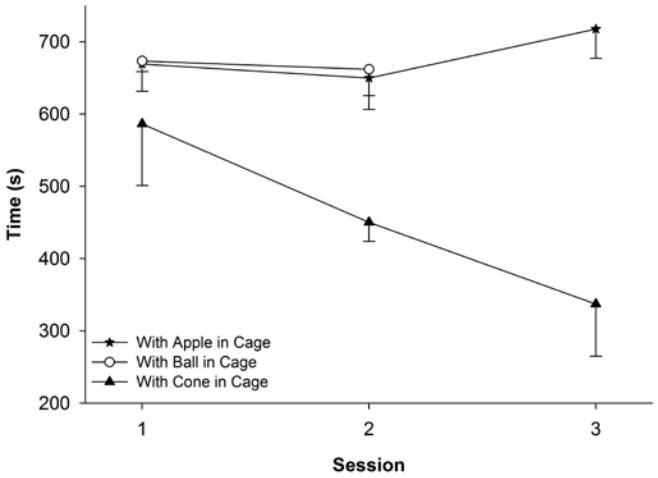

Figure 3. Duration of activity (mean ± SEM) during each session with each object. Activity was defined as walking, rooting, and other movement not directed at the object or food bowl.

because of its scent and pigs' superior olfactory sensitivity.[14] However, the scent likely diminished with cleaning and time. In 1 study,[19] characteristics of objects most preferred by large pigs included "odorous," "chewable," and "deformable," and the authors suggest that the ability to manipulate the object is critically important. Similarly here, the cone was the most pliable (that is, deformable and chewable) and most preferred object.

The 3 objects differed in color, but color was probably not an important factor in the interaction durations. Pigs are capable of dichromatic vision, but their visual capabilities are inferior to those of primates.[14] The ball used here was bright blue, a color that pigs can distinguish from gray.[14] However, the ball, which had a smooth and hard surface, was the least preferred of the objects and elicited the shortest interaction durations.

The behaviors our pigs demonstrated with the objects were similar to those described by others. Studies using novel objects have reported nosing, biting, and manipulation of the object[9,13,16] and jerking or shaking of the object:[9] we observed all of these behaviors in the current study as well. Interactions with the cone were varied, and the behaviors displayed included some that would not be possible with other objects, such as placing the head inside the cone, shaking it against cage walls, and using the mouth to turn the cone end over end. Interactions with the apple were not as diverse and consisted mainly of pushing it on the floor, sniffing the scented area, and chewing on the small top loop. Interactions with the ball consisted of pushing the ball on the cage floor.

Quantitative work on environmental enrichment for laboratory pigs is a small but growing field. Still, most studies have examined the enrichment needs or preferences of full-sized swine.[9-11,18] Here, preferences of the Yucatan minipig were measured, and the preferred objects were inexpensive and easily cleaned, making use of such objects practical. These results add to the growing database of knowledge about minipigs in general and will help to serve as a guideline for future enrichment work with other breeds of minipigs.

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