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Effects of an Enrichment Device on Voluntary Alcohol Consumption on Single-Housed Rats

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We evaluated the effect of an enrichment device (that is, a polyurethane bone) on the voluntary consumption of ethanol-containing gel by single-housed rats. Male Sprague–Dawley rats (n = 5 per group) were exposed for 4 d to each of the following 3 treatments: access to a new synthetic bone and ethanol gel for 1 h daily (treatment 1); a new bone was left in the cage for 24 h, with access to ethanol gel for 1 h daily (treatment 2); and both the bone and ethanol gel remained in the cage for 24 h (treatment 3). Average alcohol consumption over 4 d was 0.86 ± 0.13, 0.99 ± 0.13, and 5.19 ± 0.37 g/kg in the absence of the bone for treatments 1, 2, and 3, respectively, and 1.00 ± 0.13, 0.620 ± 0.07, and 5.55 ± 0.38 g/kg with the bone for treatments 1, 2 and 3, respectively; none of these values differed significantly with regard to presence of the bone. During treatment 1, time spent with the synthetic bone was highest on the first 2 d, which altered the rate of ethanol consumption but not the total amount of ethanol consumed. During treatments 2 and 3, the rate and amount of ethanol consumption were comparable to basal levels. We conclude that adding an enrichment device that rats can chew and manipulate does not alter ethanol gel consumption. If used, environmental enrichment techniques should be evaluated during the research planning stages to avoid unintended alterations in the response to variables of interest.

Abbreviation: ANOVA, analysis of variance

The need to provide laboratory rodents with an environment in which they can perform species-specific behaviors has been a topic of discussion for the last 20 y.[15,29] Laboratory rats and mice account for more than 90% of the animals used in biomedical research. The shoe-box style caging used to house laboratory rodents have been optimized to ensure biosecurity, minimize environmental variables, and maximize cost-efficiency.[1,2,18] Although effective at minimizing contamination with infectious agents, this housing style offers the animal minimal opportunity to participate in species-typical behavior, such as gnawing, object manipulation, nest building, exploring, and hiding.[26,29] As a consequence, this housing style has been viewed as a potential animal welfare dilemma in that the environment fulfills the animal's basic needs yet does not provide the opportunity to perform species-typical behaviors.

The objective of providing environmental enrichment is to allow the animal to perform species-typical behavior in an environment they can control, thereby promoting normal behaviors and minimizing abnormal behavior.[8] Abnormal behavior can occur if an animal is maintained in an environment that does not permit innate normal behaviors.[14] Rats are a gregarious, typically nonaggressive species that dig complex burrows and manipulate and gnaw on small objects as part of their species-typical behavioral repertoire.[4] These behaviors are maintained in laboratory rats despite their extensive inbreeding and domestication.[4]

Common environmental enrichment practices used in the laboratory setting include: 1) provision for social contact with compatible conspecifics; 2) addition of various substrates, such as nesting material and hiding shelters; 3) use of objects that can be manipulated by the animal; and 4) provision of novel or preferred food items. Among these, social housing is considered the most important enrichment practice because it provides animals with continuous and unpredictable situations to which they must react.[3] Manipulanda increase exploratory behavior and promote other species-typical behaviors, such as gnawing and object manipulation. Rats show an increased interest for objects that they can easily manipulate and gnaw on.[5] Objects like nylon balls and wooden blocks provide rats with the opportunity to perform species-typical gnawing behaviors.

Although rats can easily be housed in pairs or groups, doing so is problematic if research variables include the activity level or amount of food and water consumed, if the animals are fitted with external devices such as head caps, or if the study design requires self-administration of intoxicating substances. In these situations, individual housing may be necessary. For such experiments, an alternate method of enrichment, such as manipulanda, may give the animal an avenue for performing a species-typical behavior.

In a rat model of long-term voluntary ethanol consumption, limited access to the ethanol in a palatable polycose vehicle resulted in high elective intake and substantial blood alcohol levels.[25] Rats permitted 24-h access to the ethanol–polycose mixture showed consistent caloric compensation of approximately 10% of the total calories consumed daily.[25] The rats in such studies must be housed individually so that individual alcohol consumption can be documented accurately. Because the effect of enrichment devices alone on ethanol self-administration is unknown, the purpose of this study was to evaluate the effect of an enrichment device (a synthetic bone) on self-administration of an ethanol-containing gel in singly housed rats used in alcohol consumption studies.

Materials and Methods

Test subjects and housing conditions. Five male Sprague–Dawley rats (SD:Crl; Charles River Laboratories, Wilmington,

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MA), 9 mo of age with an initial weight of 625 to 685 g at the beginning of the study, were singly housed in polycarbonate static microisolation cages (35 cm × 24 cm × 20.5 cm; Allentown Caging Equipment, Allentown, NJ) with corncob bedding (Harlan Teklad, Madison, WI). Standard rodent chow (Teklad irradiated LM485 rat/mouse diet, Harlan Teklad, Madison, WI) and purified reverse osmosis water were available ad libitum. Rats were antibody-negative for coronavirus (sialodacryoadenitis virus, rat coronavirus), Kilham rat virus, lymphocytic choriomeningitis virus, mouse adenovirus, *Mycoplasma pulmonis*, pneumonia virus of mice, rat minute virus, rat parvovirus, reovirus type 3, Sendai virus, Theiler murine encephalomyelitis virus, and Toolan H1 virus. They also were free of any external and internal parasites. The rats were housed in a temperature controlled room (21 ± 2 °C) on a 12:12-h light:dark cycle (lights on at 06:00). Facilities housing the animals were AAALAC-accredited at the time of the study. The experimental protocol was approved by the University of Florida Animal Care and Use Committee, and the procedures were in compliance with the *Guide for the Care and Use of Laboratory Animals*.[16]

Ethanol gel and synthetic bone. Rats were trained to self-administer ethanol by using the 'jello-shot' procedure,[16] through which they had free access to a small glass jar that was hung over the side of the cages and that contained a sweetened gel substrate with a given ethanol concentration (Figure 1). The rats were trained to consume the ethanol gel by allowing them free access to the ethanol for 24 h for 2 d, followed by 6 h for 2 d, 3 h for 2 d, and thereafter maintained at 1 h of free access daily for 35 wk. The ethanol gel consisted of 10% ethanol (w/w) sweetened with 10% polycose caloric supplement (Abbott Laboratories, Abbott Park, IL) and 0.25% nonflavored gelatinous substrate (Knox Type A, Kraft Foods, Northfield, IL). Small glass jars were filled with either 25 ml (for the 1-h exposure) or 45 ml (for the 24-h exposure) of the ethanol gel solution and left to solidify overnight in a refrigerated room. These containers were selected randomly and hung from holders on the sides of the cages. Synthetic bones (8.9 cm × 2.2 cm × 0.75 cm, Gumabone, Bio-Serv, Frenchtown, NJ) were made of flexible, nontoxic 100% polyurethane, contained no flavoring, and were autoclaved prior to use (Figure 1).

Experimental procedures. Rats were exposed to ethanol gel for 5 d prior to starting the experiment to allow alcohol gel consumption to stabilize. The experimental regimen consisted of 3 phases, each lasting 4 d, with a device-free rest period (access to alcohol gel but no synthetic bone) between phases. Phase I consisted of a 1-h free access session to both a new synthetic bone and ethanol gel. After a 2-d rest period, phase II began, during which the rats had overnight access to a new synthetic bone before and during their 1-h ethanol free access period to gauge the effects of prolonged exposure to the synthetic bone on ethanol consumption. Briefly, a new synthetic bone was placed in the cage every day after removal of the alcohol gel jar and left overnight until the following day's alcohol gel exposure. An exception to this procedure occurred on day 1, when a new synthetic bone was placed at the same time as the gel. Phase III followed a 4-d rest period and consisted of 24-h free access to both synthetic bone and ethanol gel; a new synthetic bone was placed in the cage daily each time the alcohol gel jar was placed in the cage. Experimental data were collected between 1000 and 1200; the 1-h exposure to ethanol gel occurred during this period as well (phases I and II). During phases I and II, data were collected by direct visual observation by the same person (LE), who sat on a chair placed in front of the cages at a distance of approximately 5 feet from the cages. Data for phase III were collected only at the beginning and end of, not during, the test period.

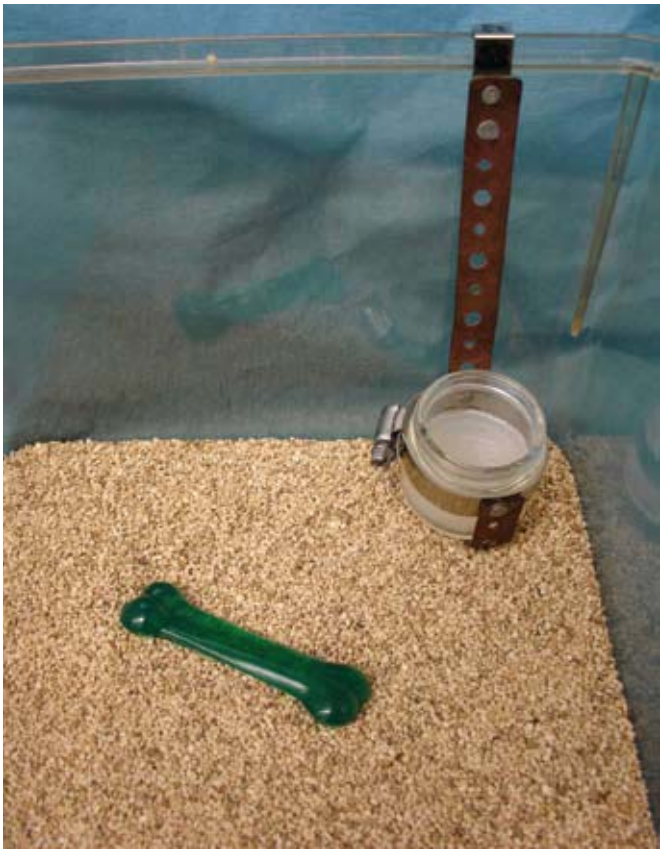

Figure 1. This picture depicts the glass jar containing ethanol gel provided and the placement of the synthetic bone in relation to the glass jar.

Behavioral measurements. The subjects' gel eating patterns were observed before access to the synthetic bone and during rest periods. For each 1-min interval, a 1 was recorded if the rat consumed alcohol gel during that period of time; otherwise, a 0 was recorded. At the end of the hour, scores for each rat were tallied and their temporal consumption patterns examined. Alcohol gel consumption and interaction with the synthetic bone were similarly scored during the 1-h ethanol access periods of phases I and II: if a rat consumed gel or gnawed, picked up, or otherwise actively interacted with the synthetic bone within any 60-s period, a score of 1 was given. These scores were summed for each 5-min stage of the hour. Two different marks were possible each minute, if a rat interacted with both the synthetic bone and the gel within the minute. During all experimental phases, ethanol consumption was determined by the difference between the initial and final weights of the gel filled jars. The initial and final synthetic bone weights were also compared as an indirect way of quantifying their use during phase 3.

All data were analyzed using 1-way and 2-way analysis of variance (ANOVA) with repeated measures including time per session, treatment days, and treatment as within-subject variables using an in-house computer statistical program.[11] A power analysis was not performed due to the fact that we did not know how the rats were going to respond to the synthetic bone. Follow-up 1-way analyses of variance were performed when significant interactions were found in order to determine the basis for the interaction.

Results

During a basal period before the rats were given access to synthetic bones, the amount of time they spent interacting with the gel was largely confined to the first 5 min, after which they then ignored it to groom or sleep. On the first and second day of synthetic bone exposure, the interval spent with the gel was low during minutes 0 through 4, occurring more evenly throughout the later intervals. On days 3 and 4, however, the interaction pattern resembled that during the basal period (Figure 2 A). These observations were supported by a significant day×time interaction ($F[44,176] = 4.68$, $P < 0.001$). When the basal day (day 0) and days 3 and 4 were analyzed separately from days 1 and 2, a significant day×time interaction was not detected.

Accordingly, the time spent with the synthetic bone was highest on the first and second days of exposure to the object, when the rats spent approximately 4.5 min of every 5-min interval interacting with the bone during the first 45 min of the session, after which the time spent with the bone fell to around 1-min per interval (Figure 2 B). On days 3 and 4, the time spent with the bone decreased, particularly during the first 5 min. These observations were supported by significant main effects of day ($F[3,12] = 8.03$; $P < 0.01$) and time ($F[11,44] = 19.10$; $P < 0.001$) and a significant day×time interaction ($F[33,132] = 1.92$; $P < 0.01$).

Examination of the baseline consumption data (average grams of ethanol consumed per kilogram body weight on each day without synthetic bone exposure) shows no significant differences compared with consumption when the synthetic bone was present (Figure 3). However, analysis of variance revealed a significant treatment×day interaction ($F[3,12] = 10.74$; $P < 0.01$), which was due to low consumption on day 3 in the absence of the synthetic bone and high consumption on day 3 when the device was present. When consumption was averaged over the 4 d of each treatment, rats ate 0.86 ± 0.13 g/kg without the synthetic bone and 1.00 ± 0.13 g/kg with the synthetic bone; these values do not differ significantly.

During phase II of the experiment, rats were allowed overnight access to a synthetic bone and then given 1-h free access to ethanol gel with the same synthetic bone. The time spent with the gel when the bone was present was compared with that during a basal period (in this case, a 2-d rest period prior to the exposure to the synthetic bone) when only gel was present. Throughout this 1-h access, the consumption pattern was similar to the basal pattern during phase I. Interaction with the gel occurred almost exclusively during the first 5-min interval (Figure 4 A). This observation was supported by a main effect of time ($F[11,44] = 67.59$; $P < 0.001$), with no effect of day effect or a day×time interaction. The rats' interaction with the synthetic bone during phase II was fairly limited, both in the total time spent with the bone and in the distribution of the interaction time. Days 1 and 2 showed similar patterns, consisting of no interaction with the bone during the first 5 min, about 1 to 2 min of total interaction for the following 20 min, and no interactions with the bone during the last 15 min (Figure 4 B). The apparent increase in synthetic bone interactions on day 3 of phase II was due to 1 rat interacting constantly with the bone. ANOVA showed a significant main effect of time ($F[11,44] = 4.26$; $P < 0.001$) but no effect of day or day×time interaction.

In general, there was no significant effect of 24-h synthetic bone access on consumption of gel during phase II (Figure 5), and ANOVA failed to reveal any significant effects of treatment or day. Average ethanol consumption during phase II was 0.99 ± 0.13 g/kg during the basal period and 0.620 ± 0.07 g/kg when the synthetic bone was present.

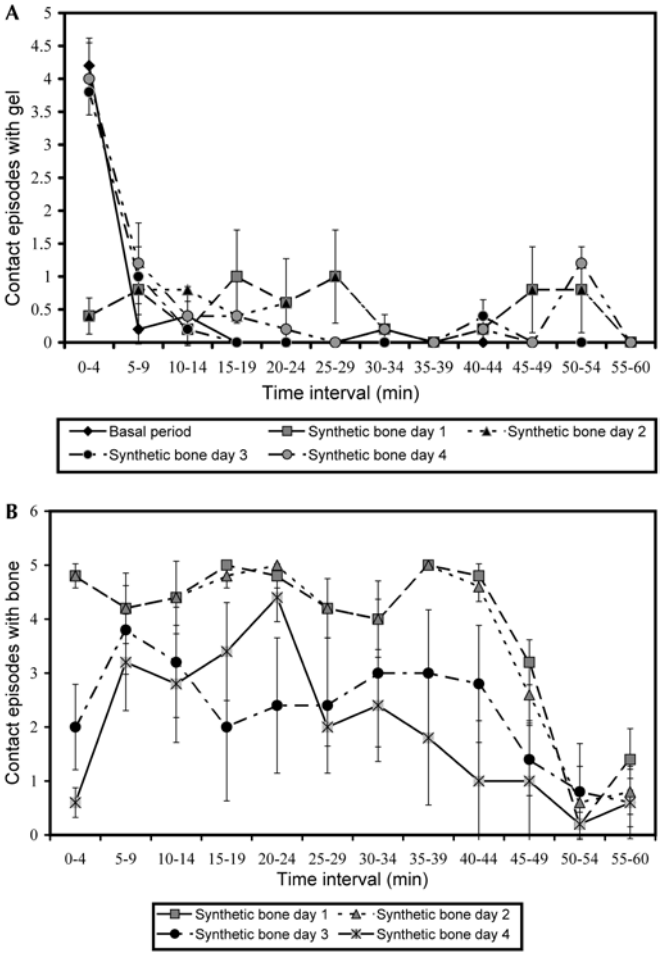

Figure 2. (A) Gel contact episodes per 5-min interval (phase I). Gel contact during basal day 1 included for comparison with days of synthetic bone exposure. (B) Synthetic bone contact time per 5-min interval (phase I). Time spent with the synthetic bone was highest on the first and second days of exposure to the synthetic bone and decreased on days 3 and 4, particularly during the first 5 min. Data are given as mean ± standard error.

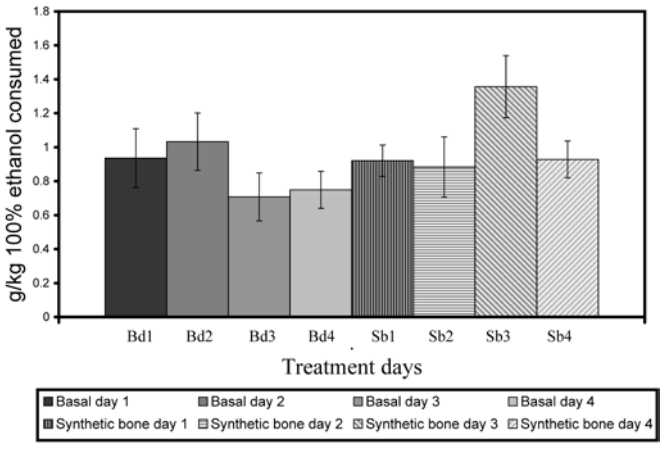

Figure 3. Daily ethanol consumption (g/kg per 1-h access period): comparison between basal and synthetic bone exposure periods (phase I). Data are given as mean ± standard error.

During phase III, interaction with the synthetic bone and gel was not recorded. The 24-h ethanol consumption of the rats during phase III (Figure 6) in the absence of the synthetic bone was not significantly different from that when it was present.

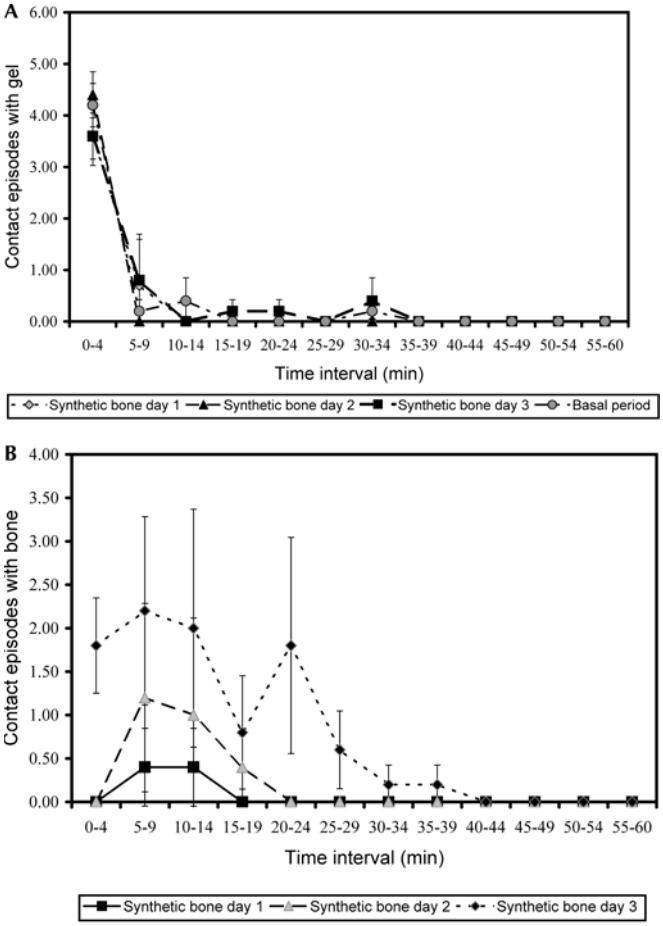

Figure 4. (A) Gel contact episodes per 5-min interval after overnight synthetic bone exposure: phase II. Gel contact during basal day 1 included for comparison with days of synthetic bone exposure. (B) Average synthetic bone contact time per 5-min interval (phase II). Data are given as mean ± standard error.

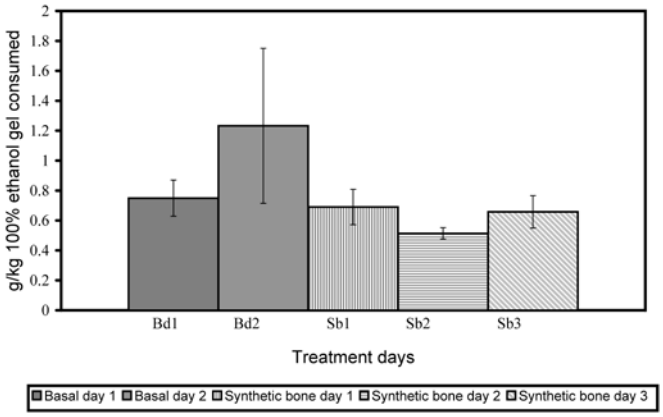

Figure 5. Daily ethanol consumption (g/kg per 1-h access period): comparison between basal and synthetic bone exposure periods (phase II). Data are given as mean ± standard error.

However, ANOVA indicated a significant treatment×day interaction ($F[3,12] = 4.51$; $P < 0.05$), which was due to high consumption on basal day 3 and low consumption on day 3 of synthetic bone exposure. Ethanol consumption averages were 5.19 ± 0.37 g/kg without the synthetic bone and 5.55 ± 0.38 g/kg with the synthetic bone.

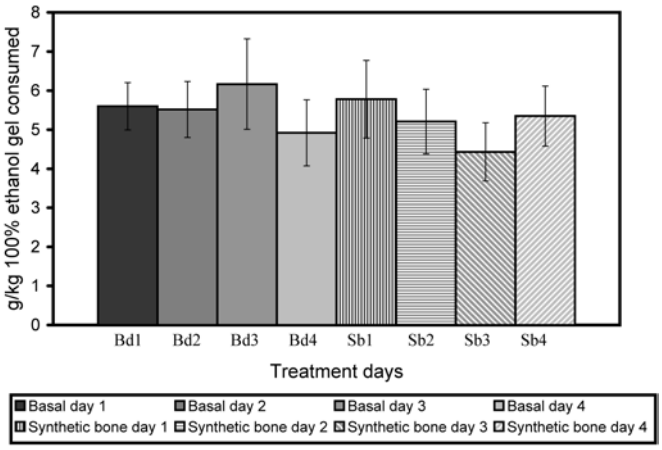

Figure 6. Daily ethanol consumption (g/kg per 24-h access period; phase III). Data are given as mean ± standard error.

Discussion

Our results show that providing an enrichment device (a polyurethane bone) does not alter total alcohol gel consumption in a rat model of voluntary alcohol consumption. These results were consistent in all phases of this study, whether the device was added at the same time as the gel during restricted access to the alcohol gel, or if the device was left in the cage during 24-h access to the alcohol gel. Overall, the rats in this study consumed comparable amounts of alcohol gel whether they had a device in the cage or not.

One interesting finding was the increased attention the rats showed to the synthetic bone compared with the alcohol gel during the first and second day of exposure during phase 1. This increased attention to the synthetic bone affected the rate of alcohol consumption during the first and second days of exposure. When exposure to the alcohol gel was restricted, rats tended to consume the majority during the first few minutes after introduction of the gel. This behavior produces substantial blood alcohol levels consistently, making it an excellent model for alcohol consumption research.[17,19] Even though the overall amount of alcohol gel consumed was similar during all 4 d of phase I, rats consumed small amounts of alcohol gel throughout the entire hour, shifting their interest between the alcohol gel and the synthetic bone. This increased interest in the synthetic bone subsided by day 3 of exposure, when the rate of alcohol gel consumption returned to baseline levels (that is, the rats had familiarized to the synthetic bone in the cage). Therefore, blood alcohol levels might differ if animals are not sufficiently familiarized to the bone before introduction of the ethanol gel.

Manipulanda in the cage provide animals with the opportunity to engage in some forms of species-typical behaviors.[26] Behaviors that are expressed with intrinsically reinforcing enrichment, such as synthetic bones, are often short-lived, lasting between 2 and 4 d.[26]

As noted during phase I of this study, the synthetic bone encouraged the rats' exploratory behavior to the point that it affected the rate of alcohol consumption. The novelty of the synthetic bone subsided by day 3, as evidenced by the return of the expected rate of alcohol consumption.

The variation in the rate of alcohol consumption seen during days 1 and 2 of phase I was not evident when the rats had 24-h access to the synthetic bone (phases II and III). The daily alcohol consumption, whether the rats had 1 h or 24 h of access to ethanol gel, was not affected by the presence of the synthetic bone in the cage. On the contrary, rats seemed to interact with

the synthetic bone during scotophase, their active phase of the day.

The length of the study (4 d) was chosen in light of the fact that once the rats are trained to consume the gel, alcohol consumption in this animal model stabilizes within 4 d after having access to the gel base.[19] The rationale for using synthetic bones rather than other harder objects such as nylon bones was based on the assumption that a softer texture could provide a more robust method to estimate the amount of daily manipulation during the 4-d period of data collection for phases II and III of the study. However, synthetic bone manipulation could not be quantified in this study because the rats destroyed the bone overnight. All of the rats in our study interacted with every synthetic bone provided. In another study,[28] 85% of the nylon bones used weighed as much as 14% less after a 13-wk study. The apparently aggressive manipulation of the synthetic bone in our study might be attributed to the difference in texture of the objects used. In another study,[5] the palatability and hardness of various wooden objects were thought to have contributed to differences in preference. In addition, the ease of modification of the object (being able to change the shape of the object by removing pieces of it) may provide another form of enrichment. The ability to bite off pieces of various sizes rapidly might represent a form of extrinsic reinforcement.

Given the results of this study, we conclude that providing synthetic bone as a method of enrichment for singly housed rats in animal models of voluntary alcohol consumption does not adversely affect total alcohol consumption. Published studies evaluating the effects of environmental enrichment on alcohol consumption have conflicting results. For example, rats reared in an enriched environment consumed similar amounts of ethanol as rats reared individually.[10] Other studies[6,13] have indicated that rats reared in individual housing consume more ethanol than do rats reared in group-housed environments. Still other studies[21-24] have shown that rats reared in an enriched environment consume more ethanol than those reared in nonenriched environments. Even though the previously described studies compared 2 extremely different environments during rearing, alcohol consumption was not affected in single-housed rats that were reared in a 'standard cage' for 90 d before exposure to an enriched environment.[23] The results from this study are comparable with the results presented here.

Due to the complexity of the primary enclosure described in previous studies,[21-24] the enrichment conditions used may be difficult to implement as standard practices in a research facility. Our findings indicate that an enrichment device can be provided to single-housed adult rats in a voluntary alcohol consumption model without affecting alcohol consumption. The novelty gained by the addition of the synthetic bone did not influence the amount of ethanol gel consumed. The rats we used did not show any signs of abnormal repetitive behaviors while being maintained in single housing with only food and water. However, the *Guide* states that "Animals should be housed with a goal of maximizing species-specific behaviors and minimizing stress-induced behaviors."[16] Published preference studies have concluded that rats prefer to have access to chewing objects over objects that could not be either manipulated or used for hiding.[5] However, to our knowledge, no published studies document the impact that gnawing behavior has in maintaining a behaviorally normal rat. Given that our rats displayed normal species-typical behavior toward the synthetic bone, adding such a device can be considered an effective enrichment practice and consequently may improve animal well-being.

As briefly seen in phase 1 of this study, instituting environmental enrichment practices in the laboratory environment should be discussed during the planning stages of the study. This practice will provide the opportunity to assess the animal's behavioral response toward the enrichment device used, thus minimizing potential confounding effects.

Compared with nonenriched environments, housing rodents in enriched environments has been shown to cause physical, physiologic, and neurologic changes.[7,9,12,20,23,27,28] Due to their aggressive gnawing behavior, our rats could have ingested parts of the synthetic bone, which might have affected the rates of ethanol absorption and food and water consumption or even caused problems like gastrointestinal obstruction. Our current study does not address any potential effects of the enrichment device on food or water intake or on alcohol absorption if pieces of the enrichment object were ingested. In addition, our study does not address how the addition of environmental enrichment may change biochemical parameters studied in this particular animal model. Regardless, the reward value of the ethanol gel does not appear to be altered by the addition of the enrichment device.

Investigators and veterinarians should work together to develop an effective enrichment program whenever the model requires that the animal perform a specific task or limits social housing. Key aspects of developing an environmental enrichment plan for single-housed rats include: 1) analysis of the devices to be used during the research; 2) evaluation of behavior elicited (novelty or exploratory behavior) for each device used; and 3) assessment of the frequency and times of day at which objects may be added or exchanged relative to research data collection. An observation and acclimation period should be included before the start of the study to analyze any potential unforeseen effects that could be attributed to the environmental enrichment technique used.

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