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# Use of an Aquarium as a Novel Enrichment Item for Singly Housed Rhesus Macaques (*Macaca mulatta*)

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**Locomotor stereotypies are behaviors often seen in singly housed rhesus macaques (*Macaca mulatta*) and are considered to represent a maladaptive response to captive environments. Active and passive enrichment items are commonly used to mitigate these and other abnormal behaviors. Active enrichment items allow physical manipulation and may be temporarily successful in reducing stereotypies, but their beneficial effects usually are confined to relatively short periods of active use. Passive enrichment items that do not involve physical manipulation are less well studied, and the results are mixed. This study evaluated an aquarium with live fish for use as a novel passive enrichment item in a common facility setting as a means to decrease locomotor stereotypy. We hypothesized that the introduction of the aquarium would decrease the frequency of locomotor stereotypy in a group of singly housed rhesus macaques (*n* = 11) with a known history of abnormal behaviors. Unexpectedly, locomotor stereotypy increased with the introduction of the aquarium and then decreased over time. Furthermore, when the aquarium was removed, the frequency of stereotypy decreased to below baseline levels. These unexpected results are best explained by neophobia, a common phenomenon documented in many animal species. The increase in abnormal behavior is likely to result from the addition of a novel object within the environment. This study demonstrates that, in the context of reducing abnormal behavior, presumably innocuous enrichment items may have unexpected effects and should be evaluated critically after their introduction to a captive population.**

Locomotor stereotypy is a commonly observed behavior in singly housed rhesus macaques (*Macaca mulatta*) and has been defined as any repetitive, ritualized behavior that is idiosyncratic and serves no obvious function.[23] Social housing is considered the most effective means for decreasing these abnormal behaviors[21,27,28] but is often constrained by experimental requirements in research facilities. Environmental enrichment items therefore are commonly used as a means to mitigate locomotor stereotypy as well as other abnormal behaviors in singly housed animals. Furthermore, federal law mandates that research facilities provide for the "psychological well-being" of nonhuman primates, with particular attention required for animals exhibiting abnormal behavior.[1] Consequently, most institutions have developed extensive environmental enrichment programs for nonhuman primates.

Environmental enrichment is a provision of resources that promotes psychologic well-being by either facilitating species-typical behavior or eliminating abnormal behavior.[21] Enrichment therefore is used as a means to enhance a normal animal's surroundings and as a treatment for a behaviorally abnormal animal. Enrichment items can be broadly categorized into 2 distinct categories—active and passive. In general, active enrichment allows a means of physical interaction between the animal and an inanimate item, whereas passive enrichment provides sensory stimulation but no direct contact or control.[21] Very similar enrichments can be active or passive depending on their implementation. Playing videotapes on a television typically would be passive enrichment, whereas allowing the animal to control some aspect of the video, such as volume or type of program, would instead be considered active enrichment.

Although passive enrichments are alluring because of their potential to benefit several animals simultaneously and their durability due to limited direct animal contact, controlled studies evaluating them have produced inconsistent results. In normal animals, passive enrichments have been found to increase activity[31] or have equivocal effects[6] after exposure. Similarly, studies specifically evaluating passive enrichments as a means to reduce abnormal behaviors have produced ambiguous conclusions. Several authors have demonstrated that daily television or videotape viewing by singly housed rhesus macaques did not influence abnormal behavior.[34,35] However, a more recent study found that individually housed Japanese macaques (*Macaca fuscata*) showed decreased displays of abnormal behaviors in conjunction with regular videotape viewing.[29] Furthermore, giving the animals some degree of control over the video (thereby making this an active enrichment item) did not significantly affect the frequency of abnormal behavior as compared with simple, passive viewing. Increased novelty in the more recent study[29] may explain the positive outcome, whereas the more commonly reported equivocal results have generally been explained by the innate repetitive nature of passive enrichment items, which induces habituation over time.[6,34] According to these findings, it may be that the introduction of variability to a passive enrichment item is key to its efficacy.

Various macaque species have been reported to play or fish in bodies of water.[32] The use of water is therefore a potential choice for creating variety and novelty in an animal's environment. Appealing to the natural behavior of playing in water, some authors found that providing troughs of water as forms of active enrichment increased exploratory behaviors without affecting levels of aggression in socially housed rhesus macaques.[30] For singly housed rhesus macaques, providing

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large receptacles of water may not be practical, but given the natural aquatic hunting and foraging behaviors of macaques, we suspected that an aquarium filled with fish would promote attention. Furthermore, the variable activity of the fish within the aquarium may command continued interest, unlike other passive enrichments.

Indeed, the effects of an aquarium have been investigated with human patients, and studies suggest that its use provides a beneficial physiologic effect.[10-12,17] Dental patients typically experienced a decrease in anxiety as evidenced by decreased blood pressure and heart rate prior to procedures.[17] An aquarium also provided stress relief for elderly observers, according to decreases in heart rate and muscle tension.[10] Only one study with nonhuman primates has attempted to evaluate a similar object.[19] Goldfish in individual aquaria were presented to singly housed chimpanzees, with limited success. Although no noticeable change in any behavior was noted, the study[19] suffered from practical issues, including complications keeping the goldfish alive, that make generalization difficult. To our knowledge, an aquarium containing live fish has not been evaluated systematically as a passive enrichment item for rhesus macaques.

Here we used an aquarium as enrichment for a group of singly housed rhesus macaques with a known history of abnormal behavior. Our intended goal was to evaluate this unique enrichment item for use in a common facility setting and incorporate it into the standard rotation for enrichment items. We expected that the beneficial effects seen in humans would translate to captive macaques and hypothesized that abnormal behavior, namely locomotor stereotypy, would decrease after the introduction of the aquarium. We expected that this item would provide a novel addition to the environment, produce a positive behavioral benefit for this particular group of animals, and offer a simple addition to many rotating environmental enrichment programs.

## Materials and Methods

**Animals.** Subjects were 12 male rhesus macaques (*Macaca mulatta*; age, 8 to 9 y; year of birth, 2004) of Chinese origin with preexisting self-injurious behaviors, which had been identified by trained behavior staff as requiring special attention to address their locomotor stereotypies. Subjects were imported from a facility in China, which provided minimal records regarding their rearing and social environment prior to shipment. We know that these animals were imported between 458 to 600 d of age and have been housed individually since that time, conditions that previously have been associated with the development of abnormal behaviors.[3,37] Previous research with this group involved use of experimental drugs,[36] including amphetamines. Although amphetamines have been shown to cause stereotyped behaviors in a variety of animal models, these psychoses typically are limited to the time during which the animal remains on the drug;[33] these macaques had been exposed 5 y prior to the study described herein. Furthermore, amphetamine administration in this group of macaques has shown no effects of long-term exposure,[36] and our group of high-stereotypy animals included subjects from the control condition that received no amphetamines. In addition to the stereotypic behaviors, every macaque in this study had previously been observed to have at least one occurrence of self-directed biting. Although these animals may require pharmacologic intervention intermittently, none was on medications during the course of the study. Only one animal caused wounds that required treatment during the study, which was initiated during the B2 phase of the study. The treatment included potentially psychoactive therapies that may affect levels of stereotypy, and the macaque therefore was excluded from the study 2 d before removal of the aquarium.

A within-subjects design was used because we specifically intended to explore the effect of our chosen novel enrichment on abnormal behavior. An alternative experimental design using a control set of animals was not feasible due to the lack of a suitable group that both exhibited similar high levels of abnormal behavior and had similarly controlled housing conditions.

All subjects were housed in 6.0 $ft^2$ individual cages and weighed an average of 10.7 kg (range, 8.3 to 14.9 kg). These cages were arranged in blocks of 4 (quads) within a windowless 715-$ft^2$ room maintained on a 10:14-h dark:light cycle. All macaques had been housed in this room for more than a year prior to this study and were part of a separate, ongoing research project with an IACUC-approved exemption from social housing. No experimental manipulations related to this separate study occurred in the month leading up to or during our evaluation of the fish tank. Housing quads were arranged along 3 sides of the room (Figure 1 A). In addition to the study subjects, one additional housing quad of rhesus macaques was present in the room. These animals had a similar history to the study subjects but were excluded due to treatment for self-injurious behavior at the time. All Johns Hopkins University facilities are AAALAC-accredited and are maintained in accordance with the *Guide for the Care and Use of Laboratory Animals*[16] and the Animal Welfare Act.[1]

Throughout the study, previous enrichment items remained in place. Music was played in the room, and all animals had identical mirrors as well as a soft and a hard toy within the cage. Toy types were rotated on a 2-wk schedule, according to standard operating procedure. These toys represent the only variability between individuals. This pattern of alteration in toy type as well as all other provided enrichments was consistent throughout the life of these animals since their arrival in our facilities. Furthermore, these macaques had never experienced passive visual enrichment, such as a TV, prior to introduction of the aquarium. To our knowledge, no external sources of novelty were introduced in addition to the aquarium; the study group did not experience new caretakers, staff, animals, enrichment items, or food items throughout the duration of the study. Although every effort was made to avoid external influences upon social structure (that is, no animals were moved so that they gained visual access to a new individual), it was impossible to rule out naturally occurring social fluctuations.

Previous research suggests that abnormal behaviors increase in frequency during husbandry procedures.[24,35] Therefore, this study was performed between 0830 and 1030 Monday through Friday when we were able to exclude husbandry procedures including cleaning, daily feeding, and rotation of enrichment, which occurred in the afternoons. No additional food treats or human interactions were provided to study animals throughout the experiment.

**Aquarium.** The aquarium (0.91 m long × 0.2 m deep × 0.64 m tall) was custom built with polycarbonate panels (thickness, 12.7 mm) and silicone adhesive and was secured to an industrial-strength wheeled cart (Rubbermaid, United States Plastic, Lima, OH). It was brightly illuminated via a canopy-mounted high-intensity discharge lighting system (Aquatic Life, Burbank, CA) and filtered via an Eheim 2217 canister (Eheim GMbH, Diezaisau, Germany). The light illuminating the aquarium was turned off at night and for entire weekends, and the filter did not produce any appreciable sound over typical ambient noise. The aquarium contained 181 L of reverse-osmosis–filtered water and 5 ornamental goldfish (*Carassius auratus auratus*) with

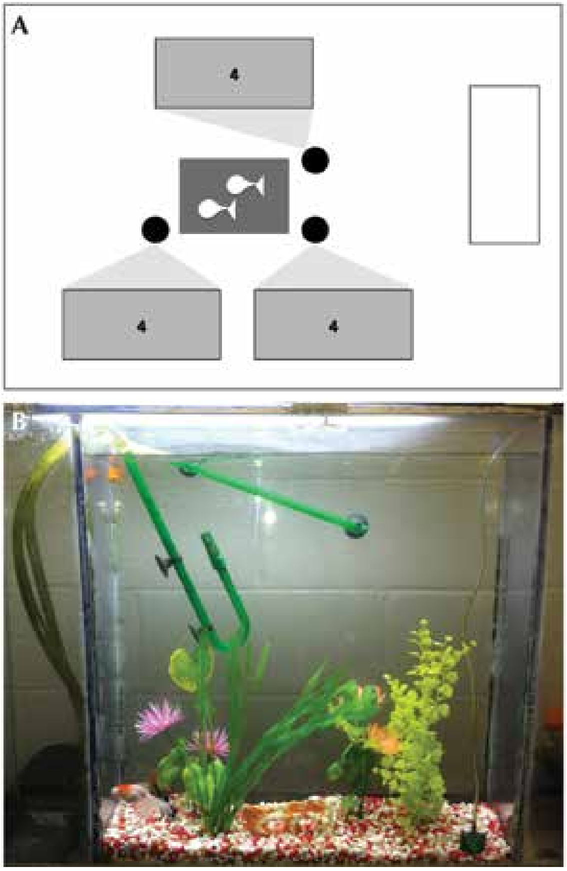


**Figure 1.** (A) Physical setup of the experimental area with the recording equipment (•) and the aquarium and (B) a photograph (taken separate from the study) of the aquarium with ornamental fish. The gray boxes labeled with '4' indicate housing quads, each of which held 4 macaques. Note that all macaques had a direct frontal view of the aquarium.

5-centimeter length decorative fins (Figure 1 B). Fish were fed daily after behavioral recording.

**Experimental protocol.** The video setup consisted of 3 cameras connected to Argus Surveillance software (Argus Surveillance, Toronto, Canada). Two cameras were mounted on a cart, and a third video camera was mounted on a tripod. The position of cameras did not change after the introduction of the aquarium into the room. Macaques were recorded for 5 d each week, starting at 0830, for a period of 45 min. The study followed an ABBA design. Video equipment was placed in the room for 2 wk prior to baseline data collection. Baseline locomotor stereotypy was documented for 2 wk prior to introduction of the fish tank (A1). This phase was followed by 2 sequential 2-wk blocks of recording with the aquarium in the room (B1, B2). Finally, the aquarium was taken from the room, and 2 wk of recording occurred after its removal (A2). Cleaning and enrichment rotation schedules took place on the same 2-wk cycle. The aquarium remained in place throughout the duration of the experimental period (B1, B2). The Johns Hopkins University IACUC approved all procedures prior to animal use.

**Locomotor stereotypy.** In this study, locomotor stereotypy was defined as any repetitive, ritualized behavior that is idiosyncratic and serves no obvious function (as compared with play, grooming, or dominance displays). This definition was adapted from Mason and has been used in several other studies of abnormal behavior.[15,18] Behaviors included in this definition were rocking, spinning, pacing, flipping, head tossing, and jumping (Figure 2). Pacing and spinning were scored as stereotypical only after 1 full repetition of the locomotor pattern (for example, one full revolution around the cage) to distinguish from normal locomotion. Jumping and rocking were considered stereotypical after demonstration of the behavior for 3 consecutive seconds, to distinguish from dominance displays. Flipping and head tossing were considered abnormal on first observation. The type of locomotor stereotypy was not scored, but rather the presence or absence of this group of behaviors.

**Behavioral scoring.** Video segments, each 45 min in length, were scored from a maximum of 10 d during every experimental phase, yielding between 255 and 450 min (mean, 297.5 min) of focal animal sampling per subject during each phase. These segments occurred during the same time period for all macaques. Variation in total duration of video recording between macaques was a result of camera failures, which were distributed randomly throughout the experiment; acquired video sufficiently represented all time blocks and animals. Total scores were adjusted for videos that were less than 45 min or for missing days.

Locomotor stereotypy was quantified by using 15-s fixed interval scoring and analyzed with the PinPoint Digital Video Coding and Analysis System (www.UDiscovering.org). If a single instance of locomotor stereotypy occurred at any point within the fixed interval, the interval was scored as positive. If the behavior was absent within the 15-s time block, it was scored as negative. The proportion of 15-s intervals within which locomotor stereotypy occurred was calculated for each subject during each experimental phase. Self-injurious behavior was not scored due to its relative rarity and because of previous reports that enrichment items have little effect on self-injurious behavior.[26]

Other than for the one-animal pilot study, the order in which videos were scored was randomized within and between macaques. A single blinded author (CK) scored the behavior in all videos. Interobserver reliability was calculated by using an additional observer (TM) to ensure internal consistency as well as accurate interpretation of the behavioral definition. A Spearman rank correlation coefficient was calculated on the basis of 2 series of recording sessions. Behavioral scoring was positively correlated between observers ($r_s = 0.98$, $P < 0.01$).

**Data analysis.** Taking into account our experimental model, with subject and experimental phase as independent variables, data were normally distributed according to a Shapiro–Wilk test of normality ($z = 0.215$; $P = 0.4148$). A repeated-measures ANOVA then was used to compare average frequencies between subjects and experimental phases. Posthoc tests consisted of pairwise comparisons according to the Tukey Honestly Significant Difference test. Significance was defined as a $P$ value of less than 0.05. Data were analyzed by using the Stata 11.2 data analysis and statistical software package (www.stata.com).

## Results

Repeated-measures ANOVA revealed that frequency of stereotypy varied significantly over the course of the study ($F_{22,43} = 7.98$, $P < 0.00005$; Figure 3) as an effect of subject ($F_{10,43}$) = 8.48, $P < 0.00005$) and experimental phase ($F_{3,43} = 20.64$, $P < 0.00005$). We tested whether the position in the quad (that is, top compared with bottom row and straight-on compared with angled view) affected the rate of locomotor stereotypy throughout the experiment, and there was no primary effect ($F_{3,43} = 1.52$; $P = 0.29$) or interaction with experimental phase ($F_{9,43} = 1.04$, $P = 0.44$). Tukey posthoc comparisons showed that locomotor stereotypy significantly increased compared with baseline on

| | |
|---|---|
| Flip | Repetitive or single somersaulting motion |
| Head toss | Angular movement of the head to one side either alone or in combination with locomotor behaviors |
| Jump | More than 3 repetitive up-and-down movements off the ground and in the same location |
| Pace | More than one full repetition of the same traveled path |
| Rock | More than 3 repetitive rhythmic motions of the body starting from a stationary position |
| Spin | More than one repetition of a 360 degree motion in the same location |
| Other | Any repetitive, ritualized behavior that is idiosyncratic and serves no obvious function (as compared with play, grooming, and dominance displays) |

**Figure 2.** Operational definitions of locomotor stereotypy (adapted from reference 12).

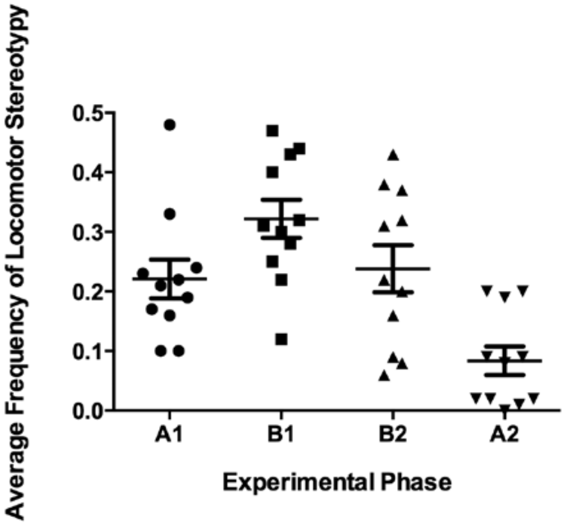


**Figure 3.** Average frequency of locomotor stereotypy by experimental phase. All comparisons significant at $P < 0.05$, except for A1 compared with B2.

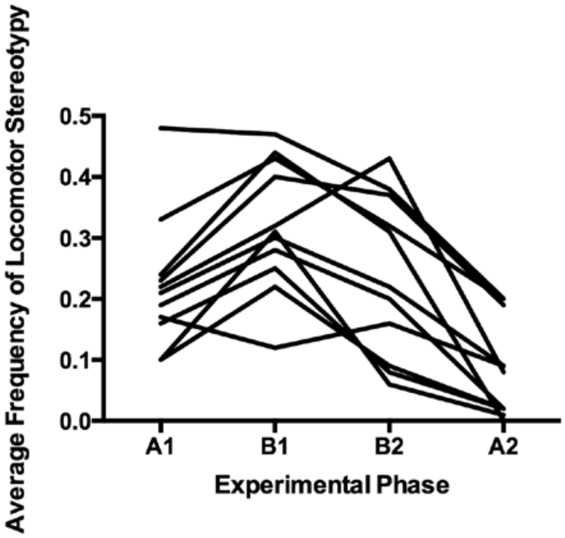


**Figure 4.** Average frequency of locomotor stereotypy by subject. All macaques followed the same overall trend, except for 2 animals that continued to have elevated locomotor stereotypy levels after continued exposure to the aquarium.

introduction of the aquarium (A1 compared with B1, $P < 0.05$). This frequency significantly decreased during the second 2 wk of exposure to the aquarium (B1 compared with B2, $P < 0.05$) and did not differ significantly from baseline (B2 compared with A1, $P > 0.05$). With removal of the aquarium, locomotor stereotypy dropped to significantly lower frequencies as compared with baseline (A2 compared with A1, $P < 0.05$) and aquarium exposure (A2 compared with B1, $P < 0.05$; A2 compared with B2, $P < 0.05$). Nine of the 11 macaques exhibited frequencies of locomotor stereotypy that increased with introduction of the aquarium and then decreased below baseline with removal of the aquarium (Figure 4).

## Discussion

We scored locomotor stereotypy in a group of singly housed rhesus macaques as a means to evaluate the efficacy of a novel passive enrichment item, namely an aquarium filled with decorative fish, to decrease levels of this abnormal behavior. The mechanisms linking stereotypic behavior and the contexts in which it frequently occurs, such as frustration, anxiety, and boredom, are unknown. Despite the lack of a known mechanism, stereotypic behavior has long been thought to reflect compromised well-being of an animal[9,23,25] and has been shown to increase after the administration of angiogenic drugs.[22] When used in human settings, aquariums alleviate some of the physiologic changes associated with stress[10] and reduce anxiety.[17] We therefore hypothesized that an aquarium used as one component of a standard enrichment rotation schedule would provide similar beneficial effects to a group of rhesus macaques, increasing their welfare as reflected by decreased locomotor stereotypy. However, instead of the hypothesized decrease in locomotor stereotypy, there was an increase in this abnormal behavior after the introduction of the aquarium, followed by a decrease in the behavior after the removal of the aquarium, thereby invalidating our hypothesis. Not only was the response to the aquarium unexpected, the response was highly consistent within the group. Nine of 11 macaques reacted with this pattern of an increase in stereotypy after the aquarium's introduction and a decrease from baseline after its removal. These results were unexpected and contrary to our proposed hypothesis.

Neophobia, or a fear of novelty, may cause unexpected reactions to enrichment. Mice with a documented fear of previously unseen objects used enrichment items less than did nonneophobic conspecifics.[39] Amazon parrots exhibiting high levels of neophobia showed increased fearfulness when new enrichment items were introduced at high rates.[13] Although the use of novelty has been a key element of many nonhuman primate environmental enrichment plans since the mandated establishment of such programs in the Animal Welfare Regulations in 1991,[2] there are some suggestions in the literature that novelty is not uniformly beneficial. A recent study evaluating music as a passive enrichment item with group-housed gibbons found no significant change in the behaviors recorded but noted increases in behaviors typically associated with stress.[40] Recent work looking at temperament testing of nonhuman primates further demonstrates that individual nonhuman primates vary remarkably in their reaction to a novel stimulus even as nonthreatening as a food item, with reactions ranging from immediate approach to sustained avoidance.[8] Research in cotton-top tamarins also suggests that temperament can predict how a subject interacts with different enrichment objects within the environment.[14] Neophobia could reasonably explain the increased stereotypy seen with the introduction of the aquarium; novelty within a consistent environment likely caused anxiety for our macaques.

Not only has recent literature demonstrated the capacity for nonhuman primates to react negatively to novel stimuli, it also suggests that our particular cohort may have been at an increased risk to display this effect. Abnormal behavior in captive nonhuman primates is common and has been documented at many facilities.[4,7,20] Our group, however, has higher than reported levels of behavioral abnormalities, which include locomotor stereotypy and self-injurious behavior. Other studies of macaques have found that some animals engage in locomotor stereotypy at much lower frequencies than do ours; in one study,[4] animals in a general laboratory population spent 1.7% of their time engaged in stereotypy, whereas other authors[7] reported the higher rates of 17.6% and 39.7% in groups that had already been identified as displaying abnormal behavior. During the current study, our animals engaged in stereotypy at an average rate of 76% of the time observed. These high levels of abnormal behavior likely result from a myriad of factors, one of which is undoubtedly their single housing between 1 and 2 y of age. Abnormal behaviors in captivity are often associated with abnormal rearing conditions at an early age.[5,37] Furthermore, abnormal rearing specifically leads to anxiety in the face of novelty.[38] We aimed to evaluate this particular enrichment item in the context of an abnormal behavioral repertoire and therefore selected macaques with unusually high levels of stereotypy for the study. Their reaction, however, may not have been representative of a more general population, and evaluation in a different group of animals with lower rates of behavioral abnormalities may be beneficial as a much-needed counterpoint to the present study.

In this study, we found a paradoxical effect in which stereotypy levels decreased below baseline levels after removal of the enrichment item. We believe that there are 2 possible interpretations of this unusual trend. One explanation is a positive carryover effect of enrichment itself, that is, after an initial increase, the aquarium caused a steady decrease in stereotypy encompassing the 2 weeks after its removal. Furthermore, a similar effect has been seen with pharmacologic treatment for abnormal behaviors.[18] The second interpretation suggests that the decrease in stereotypy after aquarium removal was a 'relief effect," that is, after an initial increase in stereotypy and return to baseline brought about by habituation, the removal of the novel stimulus led to a decrease in abnormal behavior after removal of the stressor.

Nevertheless, this study was intended to mimic standard practices and therefore was designed with the applicability of results in mind; our 2-wk ABBA blocks mirror a typical enrichment 'rotation.' One weakness of this focus is that we were unable to fully explore our unexpected results. After an initial increase, the macaques demonstrated a return to baseline levels of stereotypy with continued exposure to the fish tank and may have shown further decreases in the abnormal behavior, as hypothesized originally. Similarly, the reduction in abnormal behavior after aquarium removal may not have persisted with further follow-up. These results serve to inform typical facility practices: the use of novel enrichment items may have undesired effects, especially on their initial introduction, and these effects may depend on individual characteristics of the animals themselves.

Although there is a regulatory mandate for extra consideration to nonhuman primates that display abnormal behavior in captivity, careful attention must be paid to the specific interactions between an animal and its enriched environment. Neophobia combined with an extensive history of an abnormal behavioral repertoire reversed the expected beneficial effects of environmental enrichment in our experimental population. This study provides just one example in which an enrichment item, namely an aquarium with live fish, unexpectedly increased the occurrence of abnormal behaviors within a colony of rhesus macaques. We therefore recommend critical assessment of both the enrichment item (no matter how simple) and the intended group prior to introduction. Even more importantly, the effects of new items must be monitored after introduction. Results for animals with abnormal behavior may be unpredictable, and any novel proposed enrichment item, whether passive or active, should be evaluated before and during its intended use in an enrichment program.

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