Effects of Olfactory Enrichment on the Stereotypic Behaviors of Owl Monkeys (*Aotus nancymaae*)

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Abstract:

The purpose of environmental enrichment is to improve the well-being of captive animals. Types of environmental enrichment include, but are not limited to, the introduction of music, toys, and scents. Animals given such enrichment often exhibit a reduction in stereotypic behaviors (e.g., pacing and self-scratching), which are often linked to elevated stress. Owl monkeys (*Aotus nancymaae*) are nocturnal, monogamous primates. In captivity, some owl monkeys exhibit repetitive flipping, pacing, and scratching. Despite reliance on chemical communication and use of olfactory cues in foraging and social interactions, their responses to olfactory enrichment have not been investigated. We examined the effects of olfactory enrichment on the behavior of captive owl monkeys (DuMond Conservancy, Miami, FL). Ten pairs of monkeys were systematically presented four different types of scents (cinnamon, nutmeg, rosemary, and sage). Male and female owl monkeys displayed the greatest interest to cinnamon (via sniffing and touching scent vials). Of the five females that flipped, four flipped less often when cinnamon was present than during control trials (empty spice vials). The rates of scratching and pacing were not affected by the presence of the scents. Our findings corroborate previous studies in mice, felids, and canids, which suggest that the presence of cinnamon decreases repetitive behaviors and improves welfare. Olfactory enrichment elicits interest and reduces repetitive flipping in owl monkeys and may offer similar benefits to other nocturnal primates.

Introduction:

Animal caretakers should strive to maximize the physical, mental and social health of captive animals (Maple & Bloomsmith, 2018). Unfortunately, captive animals face a plethora of potential stressors, including artificial lighting and sound, forced proximity to human visitors and/or unnatural social groups (Morgan & Tromborg, 2007), that may result in a reduction in animal wellness. The presence of a species' naturalistic behaviors and the lack of stress-related behaviors can be used to indicate positive wellness (Maple & Bloomsmith, 2018).

Stereotypic behaviors are repetitive motor patterns that occur at a higher frequency in captivity than normal and appear nonfunctional (Mason, 1991). Potential causes for the development of stereotypic behaviors in animals includes stress, a lack of behavioral opportunities, an absence of sensory stimulation, and frustration that results from being made to exhibit certain behaviors (Mason & Rushen, 2006). Substantial evidence from captive studies of animals suggests that stereotypic behaviors are linked to stress. Captive jungle cats (*Felis chaus*) were more likely to pace when they were housed in enclosures that lacked hideouts and when they were housed with unrelated conspecifics, factors that may induce stress (Marinath et al. 2019). Fecal glucocorticoids were highest in cats that did not have access to hideouts (Marinath et al. 2019). Interestingly, in tufted capuchins (*Cebus apella*), higher levels of head twirling are linked to both a negative bias while judging ambiguous stimuli and higher levels of fecal corticosteroids. On the other hand, pacing was not linked to higher levels of fecal corticosteroids (Pomerantz et al. 2012). Fortunately, animal caregivers can reduce the time captive animals spend engaged in these stereotypic behaviors by creating more naturalistic and complex environments, reducing stressors, and providing environmental enrichment (Mason & Rushen, 2006).

Environmental enrichment is a manipulation of an animal's environment that improves the biological functioning and welfare of captive animals (Young, 2003). Enrichment promotes the expression of more naturalistic behaviors in captive animals. When captive animals are provided with environmental enrichments, they tend to increase their behavioral repertoire and reduce their time spent engaged in stereotypic behaviors (Hoy et al., 2010). In practice, environmental enrichment often involves manipulating how food is presented (e.g., scattering, hiding, or using puzzle boxes), structural enrichment (e.g., physical alteration of enclosure and re-perching with artificial or natural structures), opportunities for social interactions (e.g., changes in group composition), and exposure to sensory stimuli (Hoy et. al. 2010). Auditory stimuli (e.g., sounds from the animal's natural environment and music), visual stimuli (e.g., television, color, and mirrors), tactile stimuli (e.g., novel objects such as balls or bags) olfactory stimuli (e.g., heterospecific scents and artificial scents like perfume) and any combination of these may be used as enrichment (Wells, 2009; Hoy et. al. 2010).

Environmental enrichment often results in measurable physiological benefits. For example, when zebrafish (*Danio rerio*) were exposed to music, they increased their levels of surface activity and appeared calmer than control fish that were not exposed to music (Barcellos et al., 2018). The zebrafish exposed to music also experienced positive effects on their immune systems (via a reduction in pro-inflammatory cytokinases) (Barcellos et al. 2018). Environmental enrichment can also positively impact sociality. For instance, the addition of nesting material to an otherwise barren enclosure decreases male aggression in laboratory mice (Van Loo et al., 2002). When live plants and a hollow log with a cricket dispenser were added to the enclosures of pottos (*Perodicticus potto*), they exhibited more time allogrooming and were more likely to engage in sexual behavior (Frederick & Fernandes, 1996).

Stereotypic behaviors in captive animals may decrease in the presence of environmental enrichment if there is a reduction in physiological stress (Mason & Rushen, 2006). Environmental enrichment reduces stereotypic behavior in captive primates. For example, monkeys spend less time pacing when provided with foraging puzzles (*Saimiri sciureus*; Sha et al. 2012) and toys (*Macaca nemestrum*; Kessel & Brent, 1998). Western lowland gorillas

(*Gorilla gorilla*) reduced their rates of yawning and self-picking when given the opportunity to engage in traditional or digital painting (Wells et al., 2007).

According to a survey conducted at 60 zoos across 13 countries, olfactory enrichment is the most important sensory enrichment and is one of the easiest forms of enrichment for animal caregivers to provide (Hoy et al. 2010). This finding is not surprising given that social mammals often rely on chemical signals for mate attraction, mate guarding, and/or territorial advertisement (Hurst et al. Ed. 2008). A wide range of olfactory natural and artificial stimuli is considered to be enriching, including scents from an animal's natural environment (e.g., heterospecific urine and fur), essential oils, and aromatic plant components (e.g., spices and herbs) (Wells, 2009). Olfactory enrichment has shown to have positive effects on captive animals. Giraffes *(Giraffa camelopardalis rothschildi*) spend more time being active and less time standing and resting when they are exposed to olfactory enrichment (Fay & Miller, 2015). Interestingly, individual giraffes had different preferences for specific scents (e.g., rose, vanilla, orange, or mint) (Fay & Miller, 2015). Sea lions *(Zaophus californianus)* not only increased their habitat use, but they also reduced their stereotypic circle swimming when they were introduced to scents from their natural habitat (e.g., kelp) and other unfamiliar scents (e.g., orange and cinnamon) (Samuelson et al. 2017). Spices and herbs such as cinnamon and nutmeg tend to have arousing effects in felids (Wells & Egli, 2004; Skibiel et al. 2007; Resende et al. 201l). Rosemary increases locomotion in mice (Kovar, et al., 1987) and alertness in people (Diego, et al., 1998). Sage improves memory in humans (Moss, et al., 2014), and, in mice, acts as an antidepressant and elicits anxiolytic activity (Mora et al., 2006). Despite these findings, there is a substantial discrepancy between the ranked importance of olfactory enrichment by animal caretakers and the frequency in which it is actually being provided at zoos (Hoy et al., 2010).

Data on olfactory enrichment in primates are limited. Most studies have focused primarily on diurnal primates, and the findings are mixed. Researchers have determined that experimentally introduced scents (orange, almond, vanilla, and peppermint) have no effect on the overall activity patterns or the autogrooming behavior of captive gorillas (*Gorilla gorilla*) (Wells et al., 2007). On the other hand, the Javan gibbons (*Hylobates moloch*) nearly doubled the amount of time they spent foraging (a naturalistic behavior) when exposed to olfactory enrichment (Gronquvist et al., 2013). Ring-tailed lemurs (*Lemur catta)* showed initial behavioral changes when exposed scents such ads lavender, peppermint, and coconut, but the findings were limited due to the small number of lemurs used in the study (Baker, et al., 2018). Given that nocturnal primates rely heavily on olfaction, it is likely that they may be more responsive to olfactory enrichment.

Nancy Ma's owl monkeys (*Aotus nancymaae*) are nocturnal New World primates that rely heavily on their olfactory senses for foraging and social communication (Hunter et al., 1984; Bolen & Green, 1997; Wolovich & Evans 2007). Previous experimental work suggests that owl monkeys are able to find hidden food using olfaction alone (Bolen & Green, 1997). Because owl monkeys have a functional vomeronasal organ (VNO) (Hunter et al., 1984; Smith et. al., 2011), they are likely responsive to both volatile and non-volatile chemical cues. Owl monkeys may facilitate the detection of these cues via a flehmen response that involves the retraction of an upper lip along with tongue protrusion and/or yawning (Wolovich & Evans 2007). Given that owl monkeys regularly use chemical communication and have a suite of behaviors associated with the detection of such cues, they are an ideal primate species to examine questions relating to olfactory enrichment. Despite the fact that there are a large number of owl monkeys in captive facilities (as they have been widely used in medical research) (Fitch, 1970; Nisole et al., 2004), and that the details of their social and chemosensory behaviors are described (Wolovich & Evans 2007), their stereotypic behaviors have not adequately been described. Furthermore, few studies have systematically studied their responses to environmental enrichment (Kondo et al., 2003) and their behavioral responses to olfactory enrichment remain unknown.

We aimed to examine the effects of olfactory enrichment on the behavior of captive owl monkeys (*Aotus nancymaae*) by introducing spices (cinnamon and nutmeg) and herbs (rosemary and sage) into their enclosures. Because most olfactory enrichment studies have utilized physical enrichment (e.g., cloth) in addition to the scents, it is difficult to determine the effect of an olfactory cue alone. We purposely chose to isolate the olfactory cues in order to reduce the impact of any potential confounding effects associated with tactile or visual stimulation.

Methods:

<u>Study Site and Subjects:</u>

We conducted this study at the DuMond Conservancy for Primates and Tropical Forests, Inc. (Miami, FL, USA) between June and August 2019 using 15 male-female pairs of captive owl monkeys (*Aotus nancymaae*). None of these pairs had offspring in their enclosures at the time of the study. The monkeys' origins (either wild caught or captive born; laboratory exposure [CDC]) and ages were known for most subjects (minimum estimates for age was used for wild caught individuals). The owl monkeys at the DuMond Conservancy are housed outdoors in large wire mesh enclosures (> 2.4 m width; $\geq$ 2.4 m height) that each contain a nest box, various flexible PVC poles, and fixed wooden perches. The monkeys have access to naturally growing vegetation as well as arthropods, lizards and other wildlife. For the purpose of this study, a small Ziploc® container (4 oz.) with a removable lid was attached to the outside of each enclosure using cable ties. We strategically placed each container near a perch, but attempted to place them at least one meter away from the food tray and the nest box to ensure that they were easily accessible to the monkeys while minimizing the chances that the monkeys were near them simply to access food or shelter.

Olfactory Enrichment

We used two types of dried herbs (sage [*Salvia officinalis*] and rosemary [*Salvia rosmarinus*]) and two types of spices (nutmeg [*Myristica fragrans*] and cinnamon [*Cinnamomum cassia*]) as the olfactory enrichment during experimental trials. Both spices and the dried sage and rosemary leaves were pre-ground prior to presentation. We added two grams of each of the spices and herbs into separate spice vials (5.08 x 4.45 cm cylindrical container). To prevent olfactory contamination, we wore gloves when filling the vials. We used separate vials for each spice, and each group of owl monkeys had its own set of vials.

Experimental Design

Nine groups of owl monkeys received a series of control and olfactory enrichment trials over the course of nine weeks. We introduced one scent at a time to each of the nine experimental groups of owl monkeys. Each group of owl monkeys received only one type of olfactory enrichment per night, with presentations of each of the four scents occurring approximately once per week for a period of five weeks. We presented each group with each type of scent on three separate evenings, for a total of 120 experimental trials. The order of presentation of the spices and herbs was systematically rotated across the groups to create a balanced schedule. This method of presentation minimized the chance that the monkeys habituated to any of the scents. In addition, all nine experimental groups and an additional six control groups of owl monkeys received four control trials that consisted of the presentation of an empty vial.

Behavioral Observations

All trials began within two hours following sunset, a time when owl monkeys are most active. For nighttime observations, we used flashlights covered in red cellophane because owl monkeys are least sensitive to light in the red spectrum (Jacobs, 1977).

Each trial began with an initial three-minute habituation period. We then removed the outer lid of the vial to expose a perforated lid. Next, we placed the vial into the Ziploc® container on the outside of the enclosure. Vials were placed into the containers such that the small holes of the vials were exposed and adjacent to the wire mesh of the monkeys' enclosures. We then sealed the lid onto the larger Ziploc® container so that the monkeys could not physically move the vials. We began scoring behavioral data immediately after the vials were secured for a total of 10 minutes. Two observers recorded data simultaneously, with each observer scoring the behavior of a different focal monkey (either the male or female).

We scored the following behaviors: 1) chemoreception (lip smacking and sneezing), 2) exploratory behavior [time spent near the vial, touching the vial with hands, moving nose within three centimeters of the vial (sniffing)], 3) stereotypic behaviors (pacing, flipping, and scratching), 4) scent marking (subcaudal marking, partner marking, muzzle rubbing, urinating, urine washing) and 5) foraging behavior (see Table 1). We used a combination of all-occurrences sampling for most event behaviors, but used one-zero sampling with 30-second intervals (Martin & Bateson, 2007) to score bouts of pacing and 'chirping' [a commonly used high-frequency vocalization that may indicate arousal] (Wright, 1981). We used instantaneous sampling to score the proximity of the monkeys to the vial (in contact; not in contact, but < 1 meter to the vial; > 1 meter to the vial) and to record state behaviors (alert, foraging, moving). Vials were removed at the end of each 10-minute trial.

Table 1: Description of Behaviors Scored in this Study

Behavior	**Description**
Allogrooming	Touch another individual with added movement of the mouth or fingers (Wolovich et al., 2017)
Body Scratch	Rapid movements of hands and feet along fur (Case, 2013)
Flipping	Repeated circular motion of body around a central point using surroundings to push off (this study) (pers. obs.)
Foraging	Searching for food, including ground digging, scanning the environment for insects or pieces of food, and eating (Dufour et al., 2011)
Lip-smack	Quick, repeated opening and closing of mouth without food in mouth (Wolovich and Evans, 2007)
Manipulate Spice	In contact with spice vial
Mouth/Nose Touch	Within 3 cm of spice with nose
Out of Sight	Not able to observe
Pacing	Monkey goes from point A to point B and returned to A at least two times without stopping for more than five seconds (this study) (pers. obs.)
Scent Marking	Subcaudal: rubs subcaudal gland (at base of tail) against a substrate Face: rubs cheek region against a substrate (muzzle rubbing) Sternal: rubs sternal region against substrate (Moynihan, 1964)
Sneezing	Exhaling larger than normal amount of air from nose (this study)
Alert	Sitting or standing, with eyes actively scanning the surroundings (Dufour et al., 2011)
Urinating	At least one drop of urine released; may appear to be a few droplets or a stream of urine expelled (this study) (pers. obs.)
Urine drinking	Licks urine from a substrate or directly midstream (Wolovich and Evans, 2007)

Analysis:

We calculated the mean rates (per hour) for all event behaviors and the mean proportion of time spent engaged in each of the state behaviors for each monkey for each experimental condition and for the control trials. We used Mann-Whitney U tests to determine if there were any sex differences in their stereotypic behaviors. We compared the rates of each behavior and the mean proportion of time spent foraging and in contact with the vials across experimental all scent conditions and control trials using Wilcoxon's Matched Pairs Signed Ranks tests. Post hoc analyses was done using both non-parametric Friedman's two-way analysis of variance by ranks and Wilcoxon's Matched Pairs Signed Ranks tests across all scent conditions and the control trials. We used SPSS 26.0 for all statistical analyses.

Results:

Repetitive Behaviors

Over half of the owl monkeys observed during the study exhibited stereotypic behaviors at least once during the experiment (n = 6 males; n = 6 females). Males and females displayed the same types of stereotypic behaviors. For example, of the ten monkeys that paced, four were females and six were males. Of the seven monkeys that flipped, five were females and three were males. In general the rates of pacing were low and were not significantly different for females (median = 0.00 /hr, range = 0 – 6.0/hr) and males (median = 0.00/hr, range = 0 – 1.50/hr) ($n_1 = 9$; $n_2 = 9$; U =30.5; p = 0.225). Flipping appeared to be more frequent than pacing. The rates of flipping did not differ between females (median = 1.5 flip/hr, range = 0.00 – 111.0 /hr) and males (median = 0.00 flip/hr, range = 0.00 – 6.00/hr) ($n_1 = 9$; $n_2 = 9$; U= 24.5; p = 0.108).

Behavioral Responses to Olfactory Enrichment

When monkeys received the olfactory enrichment, all but one male interacted with the vials during at least one trial. Monkeys often approached the spice vial immediately after it was introduced. When a monkey approached the vial, it usually first sniffed the vial and then either touched it using its hands or simply moved away from it. Interactions with the vial were often short (< 30 sec); however, some monkeys approached the container more than once during a trial. One monkey did not approach the spice vial at all (n = 1 male). When monkeys engaged in longer bouts of sniffing, they tended to return to the vials and sniffed them again. The rates of sniffing the vial for females (median = 5.5 sniffs/hr, range = 0–23.5 hr) and males (median = 8.5 sniffs/hr, range = 0.0–24.0/hr) were not significantly different ($n_1 = 9$; $n_2 = 9$; $U = 31.0$; $p = 0.40$).

Eight of the nine females and seven of the nine males reached their fingers through the wire mesh of the enclosure and touched the spice vials. Monkeys only ever used their hands to touch the vial when they had also sniffed it. The monkeys usually touched the spice vials for less than 10 seconds. The rates of touching the vials for females (median = 2.5 touches/hr, range = 0.0–25.0/hr) and for males (median = 2.5 touches /hr, range = 0.0–10.5 /hr) were similar ($n_1 = 9$; $n_2 = 9$; $U = 28.0$; $p = 0.269$).

Males and females responded to some spices and herbs more so than others. The rates of sniffing by females varied significantly across treatments ($x^2 = 9.93$, df = 4, $p = 0.042$). Females sniffed the vials significantly more often when cinnamon (median = 8 sniffs/hr, range = 0.0–42/hr) and nutmeg (n = median = 4 sniffs/hr, range = 0.0–38/hr) were present than when the vials were empty (median = 1.5 sniffs/hr, range = 0.0–6/hr)(post-hoc comparisons: cinnamon vs. control: $Z = -2.38$, $p = 0.017$;nutmeg vs. control: $Z = -2.374$, $p = 0.018$) (Figure 1).

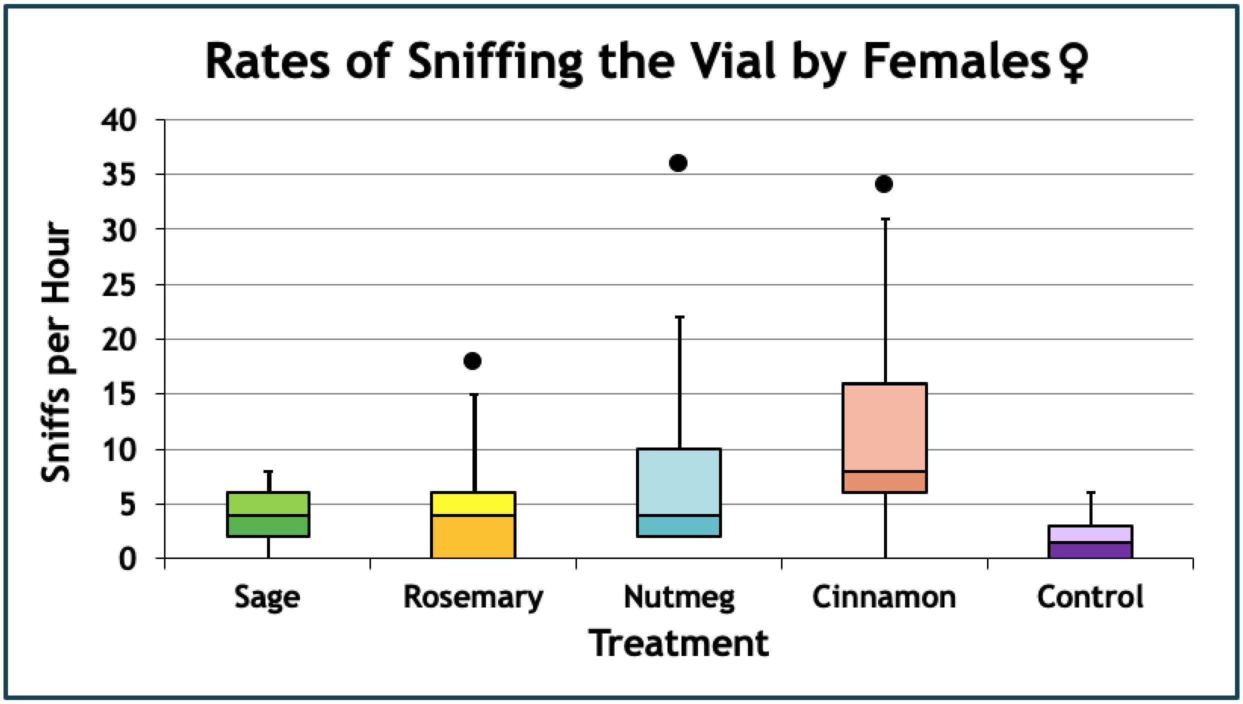

Figure 1: A comparison of female sniffing rates across treatments. Horizontal lines within the box plots represent median values and box edges represent interquartile ranges. Whiskers are the minimum and maximum values.

The rates of sniffing by males also varied significantly across treatments (x^2=11.02, df = 4, p = 0.026). Males sniffed the vials significantly more often when cinnamon (median = 10 sniffs/hr, range = 0.0–36/hr) was present than when rosemary (median = 0 sniffs/hr, range = 0.0–/12hr) or the empty vial (median = 8.5 sniffs/hr, range = 0.0–24/hr) were present (post hoc comparisons: cinnamon vs rosemary: Z = -2.524, p = 0.012; cinnamon vs. control: Z = -2.254, p = 0.024)(Figure 2).

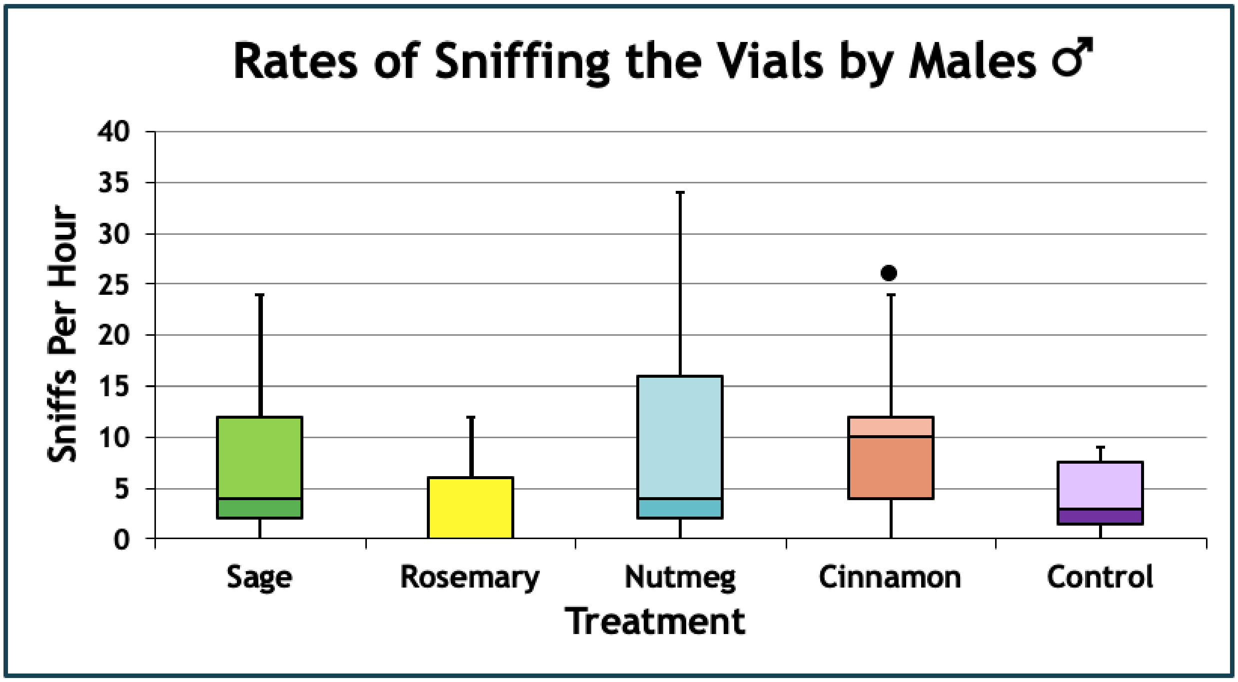

Figure 2: A comparison of male sniffing rates across treatments. Horizontal lines within the box plots represent median values and box edges represent interquartile ranges. Whiskers are the minimum and maximum values.

The rates that females touched the vials with their hands varied significantly across treatments (x^2= 18.25, df = 4, p = 0.001). Females touched the vials significantly more often when cinnamon (median = 10 touches/hr, range = 0.0–24/hr) and nutmeg (median = 4 touchese/hr, range = 0.0–24/hr) were present than when an empty vial was present (median = 0 touches/hr, range = 0.0–1.5/hr) (post hoc comparisons: cinnamon vs control: Z = -2.530, p = 0.011; nutmeg vs control: Z = -2.384, p = 0.017)(Figure 3). The rates in which males touched the vials did not vary across treatments (x^2 = 7.516, df = 4, p =0.111).

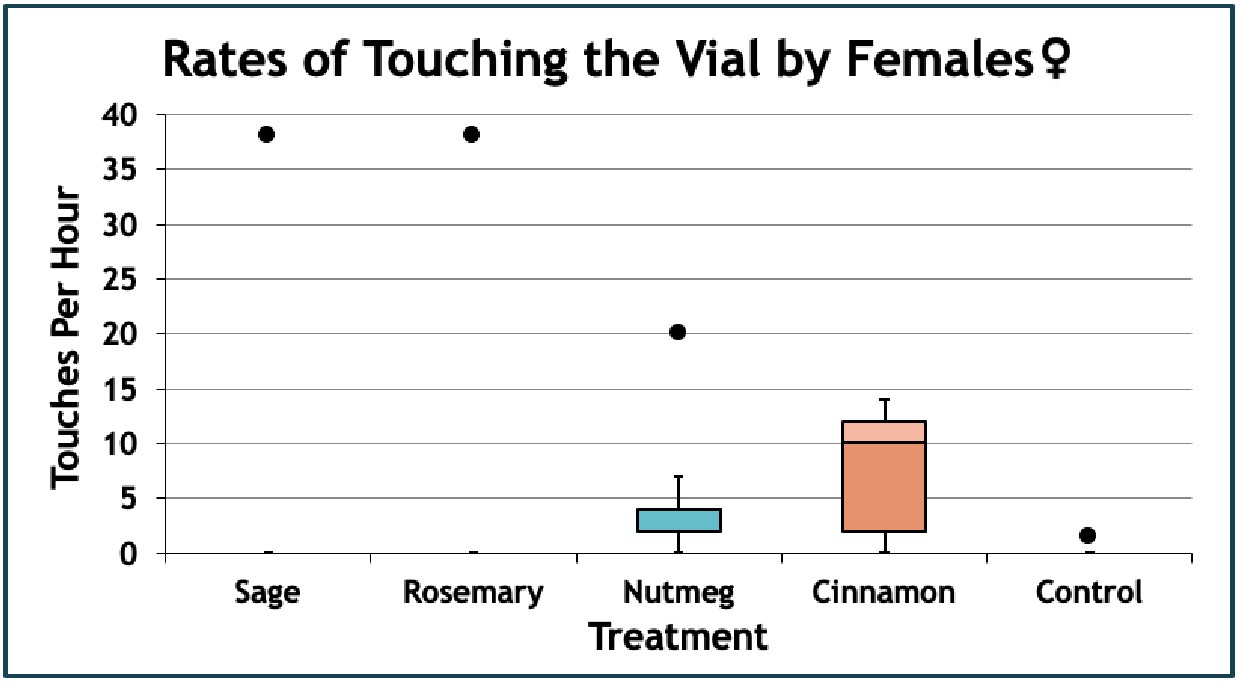

Figure 3: A comparison of female touching rates across treatments. Horizontal lines within the box plots represent median values and box edges represent interquartile ranges. Whiskers are the minimum and maximum values.

<u>Pacing</u>

Pacing was highly variable among the monkeys. Sometimes a monkey would pace on a particular perch while other monkeys paced along a series of horizontal and slanted perches. We did not observe the monkeys pacing while only moving vertically. The rates of pacing by females (median = 0.00 paces/hr, range = 0 – 6.0/hr) or by males (median = 0.00 paces/hr, range = 0.00-1.5/hr) did not vary across treatments (n = 9 females, x^2= 2.22, df = 4, p = 0.695) (n = 9 males, x^2= 4.30, df = 4, p = 0.367). None of the males ever paced during any of the rosemary or cinnamon trials. Sometimes monkeys would pace to one end of a perch, flip and then turn and continue their pacing bout. In general, flipping was typically more frequent than pacing.

Flipping

Flipping most often occurred in short bouts (usually < 15 sec, but up to 45 sec), and when monkeys flipped, they tended to do so in specific locations within their enclosures. These locations were almost always on a perch that was both near the top of the enclosure as well as near the side. Monkeys often used the ceiling and/or the wall to push off during the flip.

The rates of flipping tended to vary across treatments for females (x^2= 9.053, df = 4, p = 0.06). Females exhibited significantly lower rates of flipping in the presence of cinnamon (median = 0 flips/hr, range = 0 – 48/hr) than in the presence of either an empty vial (median = 0 flips/hr, range = 0 – 30/hr), (Z = -2.374, p= 0.018) or in the presence of rosemary (median = 2 flips/hr, range = 0 – 78/hr), (Z = -2.023, p = 0.043) (Figure 4). Females also flipped less often in the presence of sage (median = 0.00/hr, range = 0-4.00/hr) than in the presence of rosemary (median = 2.00/hr, range = 0-78.00/hr) (Z = -2.023[b], p = 0.043) (Figure 4). The rates of flipping by males (median = 0.00, range = 0.00-6.0/hr) did not vary across treatments (x^2= 6.595, df = 4, p = 0.159).

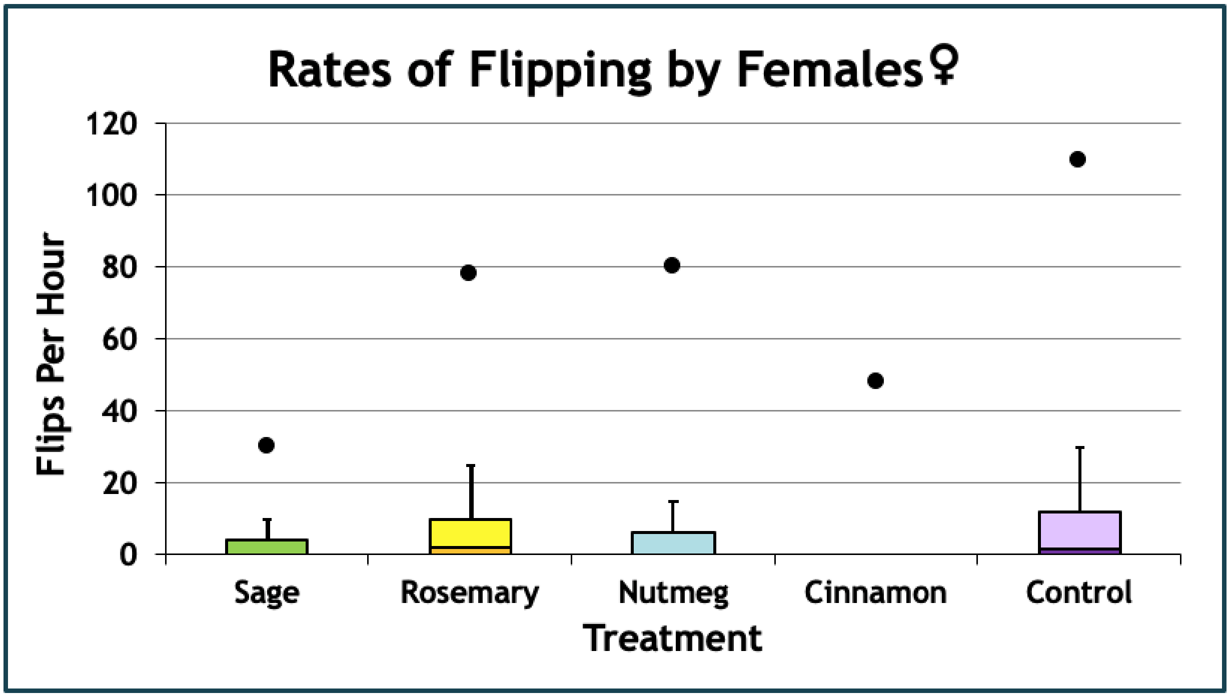

Fig 4: A comparison of female flipping rates across scent treatments. Horizontal lines within the box plots represent median values and box edges represent interquartile ranges. Whiskers are the minimum and maximum values.

Chemoreception and General Activity Patterns

We did not observe any differences in the rates of foraging, lip smacking, partner marking, time spent near the vial, scratching, sneezing, subcaudal scent marking, urinating, and urine washing across trials (Table 2).

Table 2. A summary of descriptive statistics for all behaviors that did not differ between olfactory enrichment trials and control trials. Partner mark and urine washing were not analyzed due to the infrequency of observing the behaviors. Males did spend more time near the vial with scents present than the control; however, post hoc analysis found no significance comparing the different treatments.

Behavior	**Olfactory Enrichment Trials**	**Control Trials**	**Z-value**	**p-value**
Females				
Foraging	median = 0.117, range = 0.021 – 0.237/hr	median = 0.138, range = 0.00 – 0.186/hr	-0.059	0.953
Lip Smacking	median = 1.0, range = 0.00 – 7.0/hr	median = 1.5, range = 0 .00– 7.5/hr	-0.169	0.866
Partner Mark	median = 0.5, range = 0.00 – 0.5/hr	median = 0.00, range = 0.00 – 0.00 /hr		
Time Spent Near Vial	median = 0.004, range = 0.00 – 0.03/hr	median = 0.00, range = 0.00 – 0.0125/hr	-1.781	0.075
Scratching	median = 9.0, range = 2.0 – 15.5/hr	median = 7.5, range = 1.5 – 13.5/hr	-0.356	0.722
Sneeze	median = 2.5, range = 0.00 – 9.0/hr	median = 0.00, range = 0.00 – 10.5/hr	-1.364	0.173
Subcaudal Mark	median = 2.0, range = 0.00 – 4.5/hr	median = 1.5, range = 0.00 – 6/hr	-0.210	0.833
Urinating	median = 1.0, range = 0.00 – 4.0/hr	median = 3.0, range = 0.00 – 6.0/hr	-1.757	0.079
Urine Washing	median = 0.00, range = 0.00 – 0.5/hr	median = 0.00, range = 0.00 – 0.00 /hr		
Males				
Foraging	median = 0.0926, range = 0.055 – 0.242/hr	median = 0.15, range = 0.052 – 0.255/hr	-1.481	0.139
Lip Smacking	median = 1.0, range = 0.00 – 4.0/hr	median = 0.00, range = 0.00 – 4.5/hr	-0.962	0.336
Partner Mark	median = 0.00, range = 0.00 – 5.0/hr	median = 0.00, range = 0.00 – 0.00 /hr		
Time Spent Near Vial	median = 0.008, range = 0.00 – 0.021/hr	median = 0.00, range = 0.00 – 0.0125/hr	2.375	0.018
Scratching	median = 10.0, range = 2.0 – 22.75/hr	median = 6.0, range = 3.0 – 12.0/hr	-0.890	0.373
Sneeze	median = 5.0, range = 0.5 – 87.5/hr	median = 4.5, range = 0.00 – 36.0/hr	-1.069	0.285
Subcaudal Mark	median = 14.0, range = 0.00 – 65.0/hr	median = 7.5, range = 0.00 – 61.5/hr	-0.912	0.362
Urinating	median = 2.0, range = 0.00 – 11.5/hr	median = 0.00, range = 0.00 – 4.5/hr	-1.872	0.058
Urine Washing	median = 0.5, range = 0.00 – 1.5/hr	median = 0.00, range = 0.00 – 0.00/hr		

Discussion:

Owl monkeys responded positively to the presentation of olfactory enrichment, and there were no adverse reactions to any of the spices or herbs. The monkeys investigated the spices and herbs by approaching, sniffing, and touching the vials. In most cases, the monkeys moved toward the scent vial immediately after the lid was removed. There appeared to be some behavioral synchrony between the male and female within each pair. For example, when one monkey approached the vial, the other monkey almost always approached as well, especially if the first monkey remained by the vial for more than a few seconds. Several factors may have contributed to the fact that monkeys did not spend much time interacting with the vials. On several occasions during the habituation period, the monkeys approached the empty Ziplock® containers, and then did not return and approach the spice vial during the actual trial. If the monkeys detected the scents, they may have become desensitized to the them over the course of the trials or, as time passed, they may have been better able to detect the volatile odors from a greater distance. Finally, the monkeys were unable to actually manipulate the container given that we purposely designed the set up to eliminate the potential role of tactile or physical enrichment on their behavioral responses.

Males and females showed similar rates of stereotypic behaviors. This finding may not be surprising given the fact that owl monkeys exhibit similar rates of other behaviors such as scent-marking (Wolovich & Evans 2007) and food sharing to their partners (Wolovich, et al., 2006). Some stereotypic behaviors may not be independent of one another. Monkeys that flipped also tended to pace. Sometimes flipping was even incorporated within a pacing bout. Pacing, however, was extremely difficult to score given our empirical definition. Some monkeys would repeatedly walk back and forth on one perch, but would then pause for extended periods of time and sit still while moving their heads around (possibly insect foraging) before they resumed pacing. These more extended pauses precluded us from scoring the behavior as pacing. It was also very challenging to score pacing during these nocturnal observations as the owl monkeys often ran and leaped up, down and around their enclosures rapidly. Their movement paths appear more complex than those typical of larger mammals (e.g., big cats and bears) housed in captivity.

Behavioral Responses to Spices

Cinnamon and nutmeg elicited the greatest amount of exploratory behaviors by both the female and male owl monkeys. Both sexes investigated cinnamon and nutmeg the most, while males investigated rosemary the least. Furthermore, we discovered that females, but not males, flipped less often during the trials in which cinnamon was present. However, because only two males flipped during this study, it would be difficult if not impossible to detect any differences in this behavior. Although we expected rates of pacing to vary across treatments, we did not find any differences. While more male monkeys exhibited pacing (5 males versus 3 females), the monkeys that did pace did so relatively infrequently (mean = 2 bouts/hr), making it difficult to determine if pacing was impacted by the experimental conditions. Despite our initial predictions that prosocial behaviors would increase in the presence of olfactory enrichment, we found no differences in the rates of scent marking or allogrooming across treatments.

Cinnamon has positive effects on many species, including a reduction in the rates of stereotypic behaviors in sea lions *(Zaophus californianus)* and felids (Samuelson et al. 2017; Skibiel et al., 2007; Resende et al., 2011). Cinnamon also elicits movement in felids (*Panthera tigris, Leopardus pardalis, Panthera onca, Puma concolor, Acinonyx jubatus, Panthera leo*;

Skibiel et al. 2007). In male lab-reared mice, cinnamon increases levels of LH, FSH and testosterone which can lead to increased fertility (Vahid, et al., 2012). Cinnamon is likely to provide health benefits in humans as it acts as an antioxidant, anti-inflammatory, and antimicrobial compound (Rao & Gan, 2014). People with type-2 diabetes show lower levels of serum glucose, LDL cholesterol, triglyceride, and total cholesterol when given cinnamon (Khan, 2003). Extracts of Ceylon cinnamon (*C. zeylanicum*) inhibits Tau aggregation and filament formation, two traits linked with Alzheimer's disease (Peterson, 2009). With the plethora of health benefits associated with cinnamon, owl monkeys may also gain positive physiological benefits from the spice.

While monkeys explored vials with cinnamon more than the other three scents, there was a great deal of variation among the monkeys. For example, one female monkey actually spent about one minute sniffing and touching a vial containing rosemary, eventually removing its lid. However, the mean rate of interest in rosemary was lower than those for the other scents.

Because of the greater use of olfactory cues in nocturnal primates and the positive effects of olfactory enrichment found in owl monkeys, olfactory enrichment should be considered for other nocturnal primates such as lemurs (*Cheirogaleidae*) and the aye-aye (*Daubentonia madagascariensis*) as well as other nocturnal mammals found in captivity [e.g., kinkajous (*Potos flavus*), sloth bears (*Melursus ursinus*), porcupines (*Chordata*), and two-toed sloths (*Choloepus spp.*)]. Future studies should examine the use of other scents that are known to reduce anxiety (e.g., lavender) or act as a stimulant (e.g., peppermint) as potential scents for use as olfactory enrichment (Wells, 2009). Ultimately, a reduction in anxiety could improve animal wellness if stereotypic behaviors and/or lethargy decreased or if movement patterns increased.

The well-being of captive owl monkeys needs to be examined holistically. The presence of stereotypic behaviors are often a signal of poor animal welfare, but they should not be considered to be the sole indicator of health and wellness (Mason & Latham, 2004). Sometimes these stereotypic behaviors may develop earlier in life as coping mechanisms, but may still be retained and expressed later in life despite improved captive conditions (Mason & Rushen, 2006). Because offspring typically acquire behaviors similar to that of their parents (Lonsdorf, 2006; Fragaszy et al., 2017), monkeys may acquire a stereotypic behavior through observational learning if a parent or group mate exhibits that behavior. Three monkeys used in our study arrived at the DuMond Conservancy after being retired from a government laboratory where they had been used in medical research. Interestingly, four of the five monkeys that either were obtained from a laboratory environment or had a parent that experienced the laboratory environment paced or flipped. Further investigation into the potential intergenerational transmission of stereotypic behavior is warranted.

Summary:

It is important to identify the most suitable types of enrichment for captive animals in order to improve their wellness. We recommend that cinnamon be used as olfactory enrichment for owl monkeys because it elicits interest and reduces stereotypic behavior. Nutmeg and sage should also be considered as the monkeys actively responded to these scents with no adverse effects. Olfactory enrichment should be offered to other captive nocturnal animals.

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