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1 Baker: Olfactory stimulation in lemurs

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**The effects of olfactory stimulation on the behavior of captive ring-tailed lemurs (*Lemur catta*)**

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## **Abstract**

Ring-tailed lemurs reside in many animal collections worldwide. Lemur welfare may be a cause of concern due to some captive individuals exhibiting stereotypic behavior. Despite these concerns, there has been little exploration of methods of environmental enrichment for ring-tailed lemurs. Olfactory stimulation can enhance captive animal welfare by encouraging species-typical behaviors, enhancing behavioral diversity and decreasing stereotypic behaviors. We aimed to investigate the effects of olfactory stimulation via lavender, peppermint, coconut and prey odor upon the behavior of eight captive ring-tailed lemurs. We exposed the lemurs to six individual odor conditions (odor control, novel object control, lavender, peppermint, coconut and Morio worms) and observed them for 4 hours a day for 3 days with an intervening period of 4 days between conditions. We recorded the lemurs' behavior under each condition using instantaneous scan sampling. We found significant effects of olfactory stimulation on the ring-tailed lemurs' behavior in the initial analysis but these did not survive correction for multiple testing. Overall, whilst our findings are suggestive of a general effect of olfactory stimulation on the captive ring-tailed lemurs they did not indicate a marked influence of olfactory condition. However, further investigation with a larger sample size and more biologically relevant odors may be beneficial to fully examine potential effects of olfactory stimulation in captive lemurs.

**Key words:** environmental enrichment, odors, zoo welfare, lemur behavior

1 **Introduction**

2 Ring-tailed lemurs are the most common lemur in captivity with estimated numbers of 1869-2500  
3 ring-tailed lemurs kept in zoos around the world (Andriaholinirina et al. 2014; WAZA, n.d).  
4 Captive lemur welfare may be a cause of concern with some individuals exhibiting stress-related  
5 stereotypic behavior (Tarou, Bloomsmith & Maple, 2005). In addition, zoo visitors may cause  
6 stress in ring-tailed lemurs, with visitor presence being associated with increases in aggression  
7 and decreased grooming behavior (Chamove, Hosey & Schaetzel, 1988). The expression of  
8 stereotypic behavior in some individuals and visitor effects suggestive of stress highlights that  
9 exploring methods to enhance welfare in lemurs would be of value. Despite their popularity, and  
10 these concerns, there is minimal research into methods of enhancing welfare for captive ring-  
11 tailed lemurs, with a focus on exploring feeding enrichment in existing research (e.g. Dishman,  
12 Thomson & Karnovsky, 2009; Maloney, Meiers, White & Romano, 2006).

13 One common approach to enhancing welfare in captive animals is by environmental enrichment.  
14 Shepherdson (1998) defined environmental enrichment as “an animal husbandry principle that  
15 seeks to enhance the quality of captive animal care by providing the environmental stimuli  
16 necessary for optimal psychological and physiological well-being”. Environmental enrichment  
17 involves providing stimuli to animals in under-stimulating captive environments in order to  
18 enhance the expression of species-appropriate behavioral and mental activities (Reinhardt &  
19 Reinhardt, 2001). There are various types of environmental enrichment including occupational,  
20 physical, social, nutritional and sensory enrichment (Young 2003).

21 Olfactory stimulation is a form of sensory enrichment that aims to trigger the sense of olfaction  
22 by applying a variety of non-biologically (e.g. plant matter and essential oils) and biologically  
23 relevant odors (e.g. conspecifics, heterospecifics) (Wells, 2009). One area of increasing study is  
24 the use of non-biologically relevant odors, such as essential oils, due to their ease of access and  
25 ready availability, as well as these scents being potentially appealing to keepers and visitors.

26 Olfactory stimulation may have positive behavioral effects in a range of captive species. Olfactory  
27 stimulation has increased activity in chimpanzees, *Pan troglodytes* (Peppermint: Struthers &  
28 Campbell, 1996), African and Asiatic lions, *Panthera leo* (Peppermint, almond and rosemary:  
29 Pearson, 2002; Powell, 1995) and African wild dogs, *Lycaon pictus* (Prey feces: Rafacz &  
30 Santymire, 2014). Olfactory stimulation has also been found to increase foraging in Javan  
31 gibbons, *Hylobates moloch* (Ginger: Gronqvist, Kingston-Jones, May & Lehmann, 2013) and  
32 exploration in cheetahs, *Acinonyx jubatus* (prey feces: Quirke & O’Riordan 2011) and decrease  
33 stereotypic pacing in oncolla Cats, *Leopardus tigrinus* (Cinnamon: Resende et al. 2011).

34 Ring-tailed lemurs have highly developed scent glands and use olfactory cues extensively in their  
35 natural habitat (Drea & Scordato, 2008; Kappeler, 1998; Schilling, 1974). They use olfactory cues  
36 in a range of contexts such as territory marking, displaying dominance, and signaling reproductive  
37 status (Drea & Scordato, 2008; Jolly, 1996; Kappeler, 1990, 1998). Olfactory stimuli could  
38 therefore hold the potential to increase behavioral diversity and encourage species-typical  
39 behavior in ring-tailed lemurs in captive environments. To date, the effects of olfactory  
40 stimulation on the behavior of ring-tailed lemurs has received little attention with past research  
41 tending to focus upon the effects of feeding enrichment in captive ring-tailed lemurs. For example,  
42 the addition of browse was found to increase ring-tailed lemurs’ activity levels (Dishman,  
43 Thomson & Karnovsky, 2009) and food enrichment items were found to increase playing and  
44 grooming behavior (Maloney, Meiers, White & Romano, 2006). In this study we aimed to  
45 investigate the effects of olfactory stimulation via peppermint, coconut, lavender and prey odor  
46 on the behavior of captive ring-tailed lemurs. We chose these odors as lavender has been found  
47 to have relaxant effects in dogs (Graham, Wells & Hepper, 2005) and peppermint has increased  
48 activity in captive chimpanzees (Struthers & Campbell, 1996) and African lions (Powell, 1995).  
49 Coconut has also increased exploratory behaviors in wombats, *Lasiornhinus latifrons* (Hogan et  
50 al. 2010), and prey odor has increased activity in African wild dogs (Rafacz & Santymire, 2014)  
51 and increased exploratory behaviors in cheetahs (Quirke & O’Riordan 2011).

52

53 **Materials/Methods:**

54 *Study Site*

55 Eight captive-bred ring-tailed lemurs (six females; two males) aged between 10 months and 14  
56 years old (mean age= 47.5 months) were used within the study. All of the lemurs were housed at  
57 Birmingham Wildlife Conservation Park, Birmingham, UK. The lemurs resided in an enclosure  
58 consisting of indoor and outdoor aspects. The outside enclosure was 40m by 11m with an  
59 electrified wire barrier on three sides, and a brook as a natural barrier on the other side of the  
60 enclosure. The inside enclosure was 6m by 4.5m with the height of the inside enclosure varying  
61 from 2m to 3m. Both inside and outside enclosures contained terrestrial substrate and bark  
62 mulch as well as climbing facilities in the form of wooden logs. The lemurs' enclosure was spot  
63 cleaned once at 12:30 hours and once again at 16:30 hours. Keepers fed the lemurs once a day at  
64 12:30 hours by scattering food between four bowls and the floor within the inside enclosure.  
65 Daily each Lemur received 40g LowFE pellets, 120g apple/cucumber/tamarind/beans, 60g root  
66 vegetables, 60g leafy vegetables or fruit, plus a protein item which varied between 40g fruit/nut  
67 mix, chickpeas, half an egg or crickets. Keepers provided a fresh supply of water at 12:30 hours  
68 and 16:30 hours.

69

70 *Olfactory Conditions*

71 The lemurs experienced six olfactory conditions: two control conditions (odor control, novel  
72 object control) and four experimental odor conditions. In the experimental conditions, we  
73 exposed the lemurs to cloths scented with the essential oils, lavender, peppermint (Naissance  
74 Ltd, UK), and coconut (Freshskin Beauty Ltd, UK) or the prey odor, Morio worms. The odor

75 control condition provided a comparison for the effects of the odor conditions by the use of an  
76 unscented cloth, whilst the novel object control condition, where we utilized no cloth or odor,  
77 provided a control to ensure that any effects upon behavior were not due to the novel cloth  
78 stimulus within the lemurs' environment. We chose odors based on their previously reported  
79 positive effects on captive animal welfare. We provided the control conditions first (novel  
80 object control, odor control) followed by lavender, peppermint, coconut and prey odor. We  
81 randomly determined the order of exposure to experimental conditions. Due to the lemurs being  
82 group housed, we simultaneously exposed the lemurs to each olfactory condition.

83

84 *Procedure*

85 Our experimental design was based on that used in previous similar studies investigating effects  
86 of olfactory stimulation in captive animal collections (e.g. Myles & Montrose, 2015; Wells,  
87 Hepper, Coleman & Challis, 2007). For the four experimental conditions, we introduced odors  
88 (lavender, peppermint, coconut and prey) on eight sterilized 15cm x 15cm square cotton cloths.  
89 We randomly scattered the cloths around the inside enclosure, although an approximate two-  
90 meter distance was maintained between each cloth to ensure appropriate coverage of the lemurs'  
91 environment. For the lavender, peppermint and coconut conditions we impregnated the cloths  
92 with one of the odors 60 minutes before placing the cloths within the enclosure. We used twenty  
93 ml of each essential oil treatment with a 1:2 dilution of water. For the prey odor, we placed the  
94 cloths in the Morio Worms' enclosure for 60 minutes prior to placing the cloths within the  
95 lemur enclosure. We used the same scented cloths throughout all sessions in each daily  
96 condition but we provided fresh scented cloths each day for each odor. The experimenter wore  
97 plastic gloves whilst handling cloths and applying scents, and sealed cloths, post scenting, in  
98 plastic bags, to reduce risks of contamination with human and other odors. We applied the  
99 conditions over six weeks between the 13th June and 20th July 2016.

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100 We exposed the ring-tailed lemurs to each condition from 10:30 - 16:00 hours and observed  
101 them for 4 hours a day, in two 2 hour sessions (10:30- 12:30 hours and 14:00 – 16:00 hours).  
102 Before each observation period there was a 30 minute observer habituation period (10:00-10:30  
103 hours; 13:30-14:00 hours). This allowed the lemurs to habituate to the observer's presence.  
104 During the observer habituation period and observations, the observer stood in the same  
105 position in the inside enclosure. We chose these time periods to allow observations to occur  
106 around the animal collection's usual feeding and cleaning routines. The observer placed the  
107 cloths within the environment at the end of the observer habituation period in the morning (at  
108 10:30 hours) and they remained there till the observer removed them at the end of the day's  
109 observations (at 16:00 hours). Post placement of the cloths within the enclosure we began  
110 behavioral observations. Observations occurred three days a week (providing a total of 12 hours  
111 observations for each condition), with a four-day interval between olfactory conditions to allow  
112 time for previous odor treatments to disperse. During observation periods, the observer recorded  
113 the lemurs' behavior every 5 minutes using instantaneous scan-sampling providing 48  
114 observations of each lemur's behavior per day. The observer recorded behaviors using an  
115 ethogram adapted from previous work (Table 1; Ellwanger, 2002; Meredith, 2012; Shire, 2012)  
116 and when scan-sampling the group the observer ordered this based on individual. The observer  
117 individually identified the lemurs based on their knowledge of the lemurs' distinctive features  
118 due to their previous volunteering experience with the lemurs. Photographs of the lemurs were  
119 also available to support individual identification if required.

120

121

122

123



124 Table 1: Ethogram of behaviors utilized in this study (based on Ellwanger, 2002; Meredith, 2012;  
 125 Shire, 2012).

<b>Behavior</b>	<b>Description</b>
Resting/sleeping	Putting their head down and closing their eyes. The lemur is not engaged in any other behavior (e.g. feeding, foraging, grooming) whilst resting.
Sitting	Sitting with head up and eyes open. The lemur is not engaged in any other behavior (e.g. feeding, foraging, grooming) whilst sitting.
Locomotion	Moving within or between the enclosures, climbing facilities or on the ground. This includes all forms of movements such as climbing, walking and running. The lemur is not engaged in any other behavior (e.g. feeding, foraging, grooming) whilst moving.
Feeding	Placing a food item into their mouth and chewing the food item.
Foraging	Searching for food in the enclosure either by actively moving through the enclosure or by visually searching for food items. This also involves manipulation of food items without placing them in the mouth.
Drinking	Drinking from their water source.
Mutual grooming	Grooming another individual whilst being simultaneously groomed by another individual.

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One-way grooming	Grooming another individual (without being groomed by another individual).
Mating	Engaging in copulation with another individual.
Sitting in contact	Sitting in contact with another individual (e.g. bodies or limbs are in physical contact).
Stink fighting	Rubbing their tail with scent glands and directing their tail towards another individual.
Slapping	Hitting another individual with their hand.
Fighting	Engaging in an agonistic encounter with another individual where agonistic behaviors are actively reciprocated. Fighting could include aggressive behaviors such as slapping (hitting another individual with their hand), biting (sudden motion involving oral contact) and lunging (sudden aggressive movement toward another individual with the front of the body while the hindlimbs maintain their position on the substrate).
Chasing	Pursuing another individual that is simultaneously running away.
Self-grooming	Grooming self.
Scent marking	Marking an area of their environment with their scent glands.
Interaction with cloths	Interacting with the cloth for more than 5 seconds.
Sniffing cloths	Sniffing the cloth for more than 5 seconds.

Vocalizing	Opening mouth and emitting a vocalization (e.g. yap, mew, squeak, grunt, purr, squeal etc).
Vigilance	Looking intently at surroundings/into vacant space.
Pacing	Repeatedly traveling the same path.
Self-injuries	Using their claws or teeth to cause harm to themselves; such as biting, chewing or scratching.
Out of sight	Not visible to the observer.
Other	Behaviors are displayed by the focal individual not listed above.

126

127 *Data Analysis*

128 We summed the total frequency that we observed each lemur performing each behavior,  
129 providing an overall frequency count per lemur per behavior in each olfactory condition. We  
130 omitted behaviors exhibited at very low levels (mean occurrence < 1) from analysis as statistical  
131 analyses are not robust at such low levels. We used Friedman ANOVAs to determine whether  
132 olfactory condition significantly affected the ring-tailed lemurs' behavior. Where these tests  
133 found significant differences, we conducted post-hoc analysis with Wilcoxon signed-rank pair-  
134 wise tests to determine where these differences lay between olfactory conditions. We applied a  
135 Bonferroni correction to these results resulting in a Bonferroni-adjusted significance level of  $P <$   
136  $0.003$  to control for type I errors. We also performed Wilcoxon signed-rank tests to determine if  
137 there was a difference in lemur behavior between no-odor and odor conditions, with the former  
138 comprising a grouped mean for each behavior for both controls and the latter a grouped mean

139 for each behavior for the four olfactory treatments. We carried out all analyses using SPSS  
140 (version 23.0, SPSS Inc. 2016).

141

## 142 **Results**

### 143 *Effect of olfactory stimulation on lemur behavior*

144 From the Friedman ANOVA tests, we found that there was a significant effect of olfactory  
145 stimulation on resting/sleeping behavior ( $\chi^2(5) = 30.986$ ,  $P < 0.001$ ), locomotive behavior ( $\chi^2(5)$   
146  $= 30.735$ ,  $P < 0.001$ ), sitting behavior ( $\chi^2(5) = 16.884$ ,  $P = 0.005$ ), foraging behavior ( $\chi^2(5) =$   
147  $23.590$ ,  $P < 0.001$ ) and drinking behavior ( $\chi^2(5) = 11.295$ ,  $P = 0.046$ ). We also found a  
148 significant effect of olfactory stimulation for mutual grooming behavior ( $\chi^2(5) = 24.114$ ,  $P <$   
149  $0.001$ ), sitting in contact behavior ( $\chi^2(5) = 19.119$ ,  $P=0.002$ ) and chasing behavior ( $\chi^2(5) =$   
150  $18.345$ ,  $P = 0.003$ ). We also found a significant effect of olfactory condition on scent marking  
151 behavior ( $\chi^2(5) = 16.094$ ,  $P = 0.007$ ), self-grooming behavior ( $\chi^2(5) = 29.797$ ,  $P < 0.001$ ),  
152 vocalization behavior ( $\chi^2(5) = 26.752$ ,  $P < 0.001$ ) and on vigilance behavior ( $\chi^2(5) = 31.843$ ,  $P <$   
153  $0.001$ ). However, our post hoc pairwise analysis via Wilcoxon signed-rank tests found no  
154 significant differences in any of these behaviors between olfactory conditions (Figure 1). We  
155 also found no significant effect of olfactory stimulation on feeding behavior ( $\chi^2(5) = 9.065$ ,  
156  $P=0.107$ ) and one-way grooming behavior ( $\chi^2(5) = 8.419$ ,  $P = 0.135$ ). We observed no  
157 occurrences of pacing, self-injurious behavior, mating or stink fighting in any of the lemurs  
158 under any of the conditions, therefore we excluded this from the analysis. Slapping, fighting,  
159 sniffing cloths and cloth interaction occurred at very low levels and we omitted these behaviors  
160 from the statistical analyses.

161

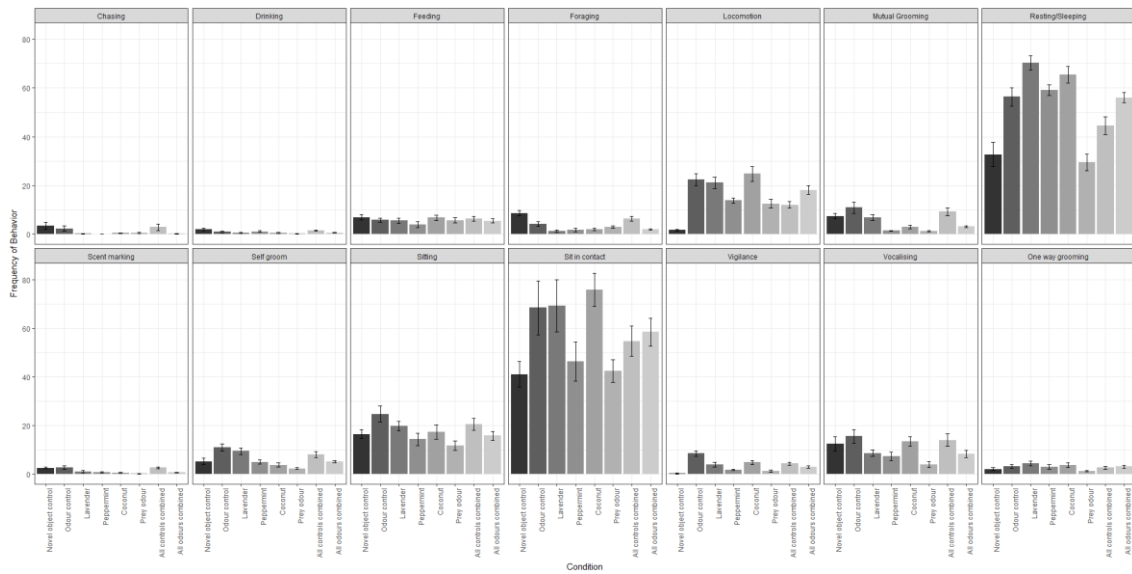
162 *Effect of odor versus no-odor conditions on lemur behavior*

163 We found a significant difference between odor and no-odor conditions for resting/sleeping  
164 behavior ( $Z = -2.243$ ,  $P = 0.025$ ) and locomotive behavior ( $Z = -2.100$ ,  $P = 0.036$ ). We found  
165 higher levels of resting/sleeping behavior and locomotion in the odor condition (Figure 1).

166 We also found a significant difference between odor and no-odor conditions for sitting behavior  
167 ( $Z = -1.965$ ,  $P = 0.049$ ), foraging behavior ( $Z = -2.524$ ,  $P = 0.012$ ), drinking behavior ( $Z = -2.038$ ,  
168  $P = 0.042$ ), self-grooming behavior ( $Z = -2.521$ ,  $P = 0.012$ ), mutual grooming behavior ( $Z = -$   
169  $2.371$ ,  $P = 0.018$ ) and chasing behavior ( $Z = -2.201$ ,  $P = 0.028$ ). We also found a significant  
170 difference between odor and no-odor conditions for scent marking behavior ( $Z = -2.521$ ,  $P =$   
171  $0.012$ ), vocalization behavior ( $Z = -2.521$ ,  $P = 0.012$ ) and vigilance behavior ( $Z = -2.033$ ,  $P =$   
172  $0.042$ ). We found higher levels of these behaviors in the no-odor condition (Figure 1).

173 We found no significant difference between odor and no-odor for feeding behavior ( $Z = -0.845$ ,  
174  $P = 0.398$ ), one-way grooming behavior ( $Z = -1.053$ ,  $P = 0.292$ ) and sitting in contact behavior  
175 ( $Z = -0.980$ ,  $P = 0.327$ ). Again, we recorded no occurrences of pacing, self-injurious behavior,  
176 mating or stink fighting in any of the lemurs under any of the conditions, therefore we excluded  
177 this from the analysis. Slapping, fighting, sniffing cloths and cloth interaction were exhibited at  
178 very low levels and we omitted these behaviors from the statistical analyses.

179



180

181 Figure 1: The mean ( $\pm$ S.E.) number of times the ring-tailed lemurs were recorded exhibiting each  
 182 behavior during the six conditions of olfactory stimulation and the two grouped odor and no-odor  
 183 conditions. The mean frequencies are presented for the three days of each olfactory condition.

184

185 **Discussion**

186 Our findings are suggestive of a general effect of olfactory stimulation on this group of captive  
 187 ring-tailed lemurs but did not indicate a marked influence of olfactory condition. Whilst we  
 188 found that the odor condition enhanced resting/sleeping behavior and locomotion when  
 189 considered broadly against the no-odor condition, the individual odor conditions had relatively  
 190 little effect on the behavior of the lemurs. Although we initially found significant effects of  
 191 olfactory stimulation for many of the behaviors, these effects did not survive correction for  
 192 multiple testing such that we found no significant differences in the posthoc pairwise  
 193 comparisons. The relatively small sample size used in this study and risk of type II errors  
 194 associated with use of the Bonferroni correction (Nakagawa, 2004; Perneger, 1998) may  
 195 contribute to these findings. Whilst further research repeating this study with a larger sample

196 size would be of value, we also need to consider other explanations for the lack of marked  
197 behavioral effects seen for the specific olfactory conditions.

198 Firstly, whilst the odors used in the study have been found to have beneficial effects in other  
199 species (e.g. Graham et al. 2005, Hogan et al. 2010; Rafacz & Santymire, 2014; Struthers &  
200 Campbell, 1996), the effects of olfactory stimulation are not consistent across all captive  
201 species. For instance, orange, almond, vanilla and peppermint had little effect on the behavior of  
202 Gorillas, *Gorilla gorilla gorilla* (Wells, et al. 2007) and lavender, rosemary, catnip and prey  
203 odor did not greatly influence the behavior of meerkats, *Suricata suricatta* (Myles & Montrose,  
204 2015).

205 Secondly, the odors used in this study, bar the prey odor, were not biologically relevant to the  
206 lemurs. Considering lemurs' extensive use of conspecific olfactory cues (Drea & Scordato,  
207 2008; Jolly, 1996; Kappeler, 1990, 1998), utilization of more biologically relevant cues such as  
208 fur, urine or feces from unfamiliar conspecifics may be of value in future studies. Consideration  
209 is needed though in the use of conspecific odor as this can induce anxiety under some  
210 conditions (Morgan & Tromborg, 2007), and exposure to female and male conspecific odors  
211 can result in differing behavioral effects (e.g. Descovich, Lisle, Johnston, Nicolson & Phillips,  
212 2012; Swaisgood, Lindburg, Zhou & Owen, 2000).

213 Finally, the method of odor presentation may not have been appropriate for use in this context  
214 or with this species. Within the field of olfactory stimulation, studies vary in their delivery of  
215 scents, for example, through use of scent impregnated cloths (e.g. Ellis & Wells, 2010; Myles &  
216 Montrose, 2015; Wells & Egli, 2004) or via dispersed scent presentation through vaporizers or  
217 oil burners (e.g. Graham et al. 2005; Spielman, 2000; Struthers & Campbell, 1996). Dispersed  
218 scent presentation is likely to provide wider coverage for the odor than use of cloths due to fully  
219 scenting indoor enclosures (Clark & King, 2008). This may be more effective as a method of  
220 odor presentation for the lemurs. Wells et al. (2007) made similar suggestions for odor

221 presentation in gorillas and this may be an important approach to consider in primate olfactory  
222 enrichment. There are constraints associated with scent diffusion though as this does not allow  
223 the animal to escape from the stimuli if it wishes to do so (Clark & King, 2008). Consideration  
224 could also occur of application of scents directly onto the substrate in the enclosure. This  
225 method of application could be more ecologically meaningful to the lemurs and may be an  
226 effective method of odor presentation.

227 Overall, our results, whilst suggestive of a general effect of olfactory stimulation on the  
228 behavior of captive lemurs in the group studied, did not indicate a marked influence of olfactory  
229 condition. However, considering lemurs' extensive use of olfactory cues in their natural  
230 environment (Drea & Scordato, 2008; Kappeler, 1990, 1998; Schilling, 1974), as well as the  
231 significant effects of olfactory stimulation found for many behaviors in the initial analysis, we  
232 believe that our findings warrant further study of the potential use of olfactory stimulation as  
233 environmental enrichment for lemurs. Future research using a larger sample size and continuous  
234 recording methods in order to enable scoring of longer duration behavioral patterns would be  
235 beneficial. In addition, further study utilizing conspecific odors and considering different  
236 methods of odor presentation may be of value.

237

## 238 **Conclusions**

239 1. There is a general effect of olfactory stimulation (e.g. odor versus no odor conditions) on the  
240 ring-tailed lemurs' behavior in the group studied.

241 2. Exposure to olfactory conditions of lavender, peppermint, coconut and prey odor affected the  
242 ring-tailed lemurs' behavior in the initial analysis but these did not survive correction for  
243 multiple testing.



244 3. Future research using larger sample sizes, more biologically relevant odors and different  
245 methods of odor presentation may be beneficial to fully explore the application of sensory  
246 stimulation as enrichment in this species.

247

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252

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