Putrescine--a chemical cue of death—is aversive to chimpanzees

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Abstract

As in many other species of nonhuman primates, chimpanzee mothers with a dead infant may continue to care for and transport the infant for days, weeks, or even longer. The bereaved females do this despite what humans perceive as the foul odour from the putrefying corpse. Putrescine is a major contributor to the "smell of death," and it elicits behaviours aimed at getting rid of the source of the smell, or escape responses in mammals including humans. However, it has never been shown that the odour of putrescine is aversive to chimpanzees. To address this question, we visually presented six adult chimpanzees with the corpse of a small bird, or a stuffed glove, in association with putrescine, ammonia, or water, and recorded the chimpanzees' reactions. The apes spent significantly less time near the object when it was paired with putrescine than the other substances, although they showed no signs of increased arousal or anxiety. We interpret the findings as evidence of an aversion to the smell of death in chimpanzees, discuss the implications for understanding the continued maternal-like behaviour of bereaved female chimpanzees, and suggest future research directions for the field of comparative evolutionary thanatology.

Keywords: Death, mother-infant bond, olfaction, *Pan troglodtyes*, Thanatology

1. Introduction

Necrophobia, the fear of or aversion toward death, corpses, or associated stimuli, is an adaptive trait in many animal species (including humans), as it promotes avoidance of potentially dangerous situations. Paradoxically, however, in many nonhuman primate species, mothers continue to carry and care for their dead infants, sometimes for weeks or even months, despite the strong stench from the decaying corpse, an odour that humans find repugnant (Biro et al., 2010; Wilson and Matsuzawa, 2018; Lonsdorf et al., 2020). The existence of maternal post-mortem infant care and transport (PICT) of dead infants raises questions about motivational factors (Biro et al., 2010; Gonçalves and Carvalho, 2019; Anderson, 2021). One unaddressed issue surrounding the phenomenon concerns nonhuman primates' sensitivity to the "smell of death" (see Gonçalves and Biro, 2018). Putrescine, one of many volatile organic compounds that emanate from decaying corpses, is a major contributor to this odour (Izquierdo et al., 2018). The avoidance of such substances, reflecting sensory and behavioural responses such as disgust and necrophobia, is probably an evolutionarily ancient trait (Yao et al., 2009), advantageous for avoiding risks of predation and illness. In an experimental study on three species of monkeys (squirrel monkeys, *Saimiri sciureus*; spider monkeys, *Ateles geoffroyi*; pigtail macaques, *Macaca nemestrina*), Laska et al. (2007) used conditioning methods to assess olfactory sensitivity to thiols and indols – substances associated with putrefaction, but as pointed out by Anderson (2020), there are no focused studies of nonhuman primates' spontaneous behavioural responses to putrescine or any other necromones, the generic term for chemical cues of death.

Putrescine is an attractant for invertebrates that deposit eggs or feed on corpses, and possibly for vertebrate scavengers (DeVault et al., 2003; Hussein et al., 2013), but for other species the smell is aversive, eliciting actions to dispose of or escape from the source. For example, when an anesthetized rat or a wooden dowel was sprinkled with putrescine, other rats responded as they might to a dead rat, by burying it under bedding material (Pinel et al., 1981), which the authors considered to be a defensive behaviour. That was a laboratory study; in free-ranging animals, where conditions permit, simple avoidance might be more likely (see Prounis and Shields, 2013). In humans, the repugnant smell of a decaying corpse typically activates behaviours to stifle or get rid of the smell, such as covering one's nose, disposing of the corpse (e.g., by covering, burying, or burning) or moving away. In one experimental study even very brief exposure to putrescine reportedly hastened leaving the area compared to other odours (Wisman and Shrira, 2015).

Although chimpanzees kill and eat other animals, and sometimes engage in cannibalistic acts, corpses discovered by chance are usually avoided, or explored only briefly (van Lawick-Goodall, 1968; Anderson, 2018; Watts, 2020). The role of the pungent odour in triggering avoidance of corpses remains unclear, as some individuals show little in the way of avoidance (see Anderson, 2020, and Gonçalves and Carvalho, 2019 for examples of contrasting responses in primates, and Peterson and Fuentes, 2021 for a recent report in monkeys). To clarify whether they are sensitive to putrescine, we investigated chimpanzees' reactions to a dead animal paired with putrescine or with control substances. We made two predictions: (1) If the odour of putrescine is repugnant to chimpanzees, then they will avoid an object paired with putrescine more than an object paired with another odour. (2) If they associate the smell of putrescine specifically with death, then any avoidance will be stronger when it is paired with a dead animal than with a control object.

2. Methods

2.1. Subjects and apparatus

The study was conducted at Kyoto University's Kumamoto Sanctu- ary. Six group-living, adult chimpanzees (*Pan troglodytes*, ages: 24–48 years; two females, four males, including two paternal half-brothers) were tested in familiar indoor rooms (approximately 2 m x 2 m x 3.5 m) that contained platforms and bars for resting and climbing, and browse and jute sack pieces for enrichment. Experimental sessions were conducted during early evening feeding periods, when the chimpanzees were brought inside from their outdoor areas and temporarily separated from their groupmates for the night. Each chimpanzee participated in six different conditions, presented in random order and on separate days, with at least one week between trials.

In each session putrescine, ammonia, or fresh water was paired with either a taxidermically prepared small bird (*Emberiza spondocephala*) lying supine with its wings spread, or a similar-sized, stuffed gardening glove (Fig. 1). The chimpanzee's reaction to each substance-object pair was video recorded for 5 min on two tripod-mounted digital cameras. To diffuse the putrescine, 3.5 g were soaked in a cotton pad which was then heated by an electronic hand warmer to 45–65 °C and placed in a plastic bucket with holes drilled in the side. A small USB-powered fan inside the bucket wafted the odour through the holes toward the subject, 0.5 m from the bucket. The water and ammonia were diffused identically but without being heated. The bird or glove was clearly visible inside a cardboard box which had one removeable side and which sat on top of the bucket (Fig. 1).

2.2. Procedure

To start a trial the experimenter switched on the cameras, and then after at least 2 min she removed pieces of duct tape that covered the holes in the bucket and stepped behind it. If required, she operated one of the cameras by hand to follow the chimpanzee if it moved around. After 5 min the bucket and box were removed; the cameras continued to record for at least another 2 min

After all trials were completed, the videos were imported into BORIS [12], without identifying information such as time and test condition. Coding of the videos was done blind, as neither the odour nor the object presented was known to the coder. Distance between chimpanzee and apparatus was coded as "near" (chimpanzee on ground, < 1 m away), "far" (on ground, > 1 m away) or "up" (climbing or immobile on the cage mesh or on an elevated platform). Frequency and duration of looks towards the apparatus were coded only when chimpanzees were near the apparatus, as gaze direction was relatively easy to detect in this location. Statistical comparisons of the chimpanzees' reactions across conditions were made using nonparametric tests (Friedman and exact Wilcoxon signed-rank tests), with alpha set at 0.05.

3. Results

Following a study on humans' defensive reactions to putrescine [12], we first compared adult humans' ratings of ammonia, putrescine and water on the dimensions of familiarity, intensity, and repugnance, to determine the appropriate concentrations of putrescine and ammonia to use with chimpanzees. Two grammes of each substance were soaked in a cotton pad inside an airtight glass jar, and each of eight participants was asked to remove the lid, sniff inside each jar (presented in random order), and rate the odour on each dimension on a scale from 1 (low) to 9 (high). There were no differences between the substances in odour familiarity ($\chi2 = 1.28$, df = 2, $p = 0.53$), but they differed in intensity ($\chi2 = 15.0$, df = 2, $p < 0.001$), and repugnance ($\chi2 = 12.3$, df = 2, $p = 0.002$). Post-hoc sign tests indicated that water received the lowest rating on each dimension; there were significant differences between putrescine and water ($p = 0.008$) and ammonia and water ($p = 0.008$) for intensity. For repugnance, there was no significant difference between

putrescine and water ($p = 0.07$), but there was a significant difference between ammonia and water ($p = 0.008$). Importantly, post-hoc sign tests indicated no difference between ammonia and putrescine in terms of either intensity ($p = 0.062$) or repugnance ($p = 0.12$). Thus, the aversive qualities of both ammonia and putrescine for humans were confirmed (Table 1).

Analyses of the chimpanzees' looking time and distance during the odour-object pair presentations revealed no differences between the bird and the glove (Fig. 2: data for all chimpanzees are available in electronic supplementary material, Table 1). However, there was a significant effect of odour (Friedman test: $\chi2 = 9.33$, df = 2, $p < 0.01$). Pairwise Wilcoxon tests showed that chimpanzees remained near the object for significantly less time in the putrescine condition than in ammonia ($Z = 2.28$ N = 6, $p = 0.02$) and water ($Z = 2.85$, N = 6, $p < 0.01$) conditions, with no significant difference between the latter two ($Z = 0.16$, N = 6, $p = 0.91$).

Apart from relatively brief peering at the objects, the chimpanzees showed little sign of curiosity; attempts to contact the object were seen in only three of 36 trials. Finally, we assessed whether the chimpanzees' showed behavioural signs of arousal or anxiety when the objects were associated with putrescine. The absolute frequencies of the behaviours scratch, rock, display, spit, and urinate were combined to form a composite arousal/anxiety score, which was then summed across the two objects. The mean scores in the ammonia, putrescine and water conditions were 4.7, 4.2, and 4.3, respectively, with no significant variation (Friedman test: $\chi2 = 0.58$, df = 2, $p = 0.75$).

Discussion

Chimpanzees were less likely to remain near an object paired with the odour of putrescine than an object paired with ammonia or water. This finding supports our first prediction: the odour of putrescine, one component of the characteristic "smell of death" is indeed aversive to chimpanzees. However, as the chimpanzees' behaviour was not influenced by whether putrescine was paired with a stuffed bird or a stuffed glove, our second prediction was not supported: the aversive reaction was not stronger in the presence of a dead animal compared to a non- animal object. We interpret these results as evidence that chimpanzees show a particular aversion to the odour of putrescine, even though for humans the putrescine odour was not ranked as more intense or repugnant than ammonia, which is also known to accumulate in putrefying corpses (Vass, 2001; Donaldson and Lamont, 2013).

Relating our results to PICT by bereaved chimpanzee mothers (van Lawick-Goodall, 1968; Biro et al., 2010; Wilson and Matsuzawa, 2018; Lonsdorf et al., 2020), we can now be more confident that mothers (and others) are aware of the pungent smell from the dead infant, but the mother's motivational state is strong enough for her to overcome aversion to the smell and perform PICT. Another possibility is olfactory adaptation (or fatigue; see Cometto-Muniz and Cain, 1995): the mother's almost constant exposure to the odour from the corpse reduces her sensitivity to it. It also conceivable that continuing to transport and care for the dead infant reduces the mother's stress levels, in other words, it is a way of coping with the psychological trauma of the loss (Nicolson, 1991; Takeshita et al., 2019). Future studies might be able to compare stress profiles of mothers who show PICT and those who do not or who cannot, due to the newly dead infant being taken by a predator or by another (possibly cannibalistic) chimpanzee.

We did not test bereaved mothers, but our results demonstrate aversion to putrescine in both adult male and non-mother female chimpanzees. One factor that might contribute to PICT is lowered ol- factory sensitivity in early postpartum females, a change reported in human mothers (Ochsenbein-Kolbe et al., 2007). However, if they are not cannibalized soon after death, in the wild most chimpanzee infant corpses are abandoned within a few days (Lonsdorf et al., 2020) and it has been suggested that the strong odour from the corpse may deter cannibalism (Kooriyama, 2009). We tentatively propose the existence of an odour intensity-PICT relationship in which likelihood of abandonment of the corpse increases at around the

time when the odour is strongest. Gonçalves and Carvalho (2019) stated something similar, noting that abandonments appear to occur when the infant corpse changes from bloating to active decay.

Another aspect of aversion to necromones that merits further investigation is a possible age- related variation in sensitivity. In several reports, immature chimpanzees are described as exploring and playing with carcasses despite the strong odour (Biro et al., 2010; Lonsdorf et al., 2020). Casual inspection of our data suggested that the two oldest subjects in our sample (46 and 48 years old) were the least repulsed by putrescine. Although diminished olfactory function is reported in at least half of people older than 65 years (Attems et al., 2015), in nonhuman primates the question has barely been addressed.

We found no evidence of an interactive effect of the odour of putrescine and the type of visual object, and no overt signs of heightened arousal or anxiety in the apes during the tests. One possibility is that chimpanzees do not process this odour specifically as a cue of death. Alternatively, the body of small bird might be a weak elicitor of death- related responses compared to larger carcasses, as reported by Kortlandt (1967), who presented dead and stuffed specimens of several species to free-ranging chimpanzees. There are clear opportunities for future naturalistic observations and careful experiments to contribute to the growing field of comparative evolutionary thanatology (Anderson et al., 2018; Anderson, 2020). In addition to comparisons of behavioural and other reactions to the myriad biochemical changes in corpses, exposure to death cues in other modalities and across developmental stages and species can help elucidate biological mechanisms and psychological correlates of reactions to death in other animals, including our nearest evolutionary neighbours.

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Fig. 1. Photos (not to scale) of the objects (bird, glove) presented as visual stimuli. Top: on "glove" trials the glove lay in the presentation box on the bucket from which the odours were diffused. Bottom: the stuffed bird, which replaced the glove in the box on "bird" trials.

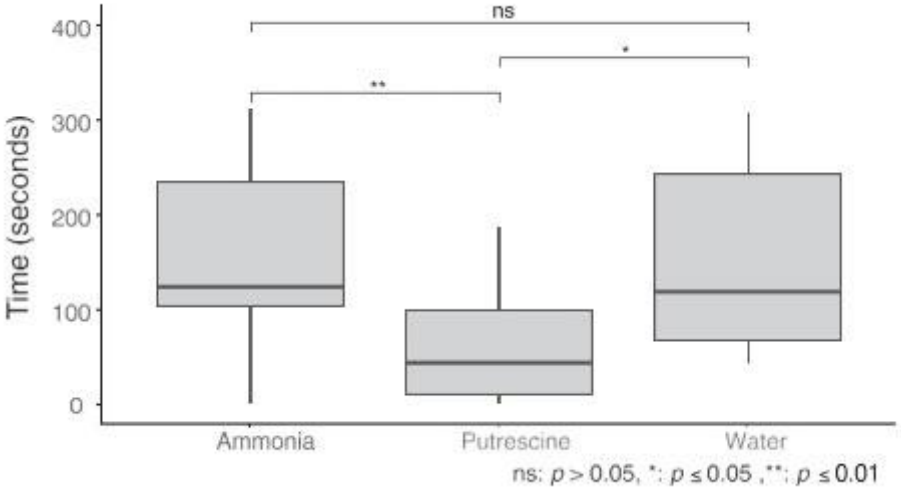

Fig. 2. Amount of time chimpanzees spent near the object when it was asso- ciated with ammonia, putrescine, and water.

Table 1
Mean ratings of odours for the three substances by humans (N = 8).

Scent	Water	Ammonia	Putrescine
Intensity			
M	1.00	8.75	6.00
SD	0.00	0.71	2.67
Familiarity			
M	2.86	4.38	3.00
SD	2.48	2.92	2.33
Repugnance			
M	1.50	8.50	6.75
SD	1.41	0.76	2.19