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African elephants (*Loxodonta africana*) display remarkable olfactory acuity in human scent matching to sample performance

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ABSTRACT

This paper presents data on the success rate of African elephants in human scent matching to sample performance. Working with equipment and protocols similar to those used in the training of forensic canine units in Europe, scent samples were collected on cotton squares from twenty-six humans of differing ethnic groups, sexes and ages, and stored in glass jars. Three African elephants were trained to match human body scent to the corresponding sample. In total, four hundred and seventy trials, during which the elephant handlers were blind to the experiment details, were conducted. Each trial consisted of one scent that served as the starting (target) sample to which the elephant then systematically determined a potential match in any of the nine glass jars presented. Elephants matched target and sample at levels significantly higher than indicated by random chance, displayed no loss of working memory, and successfully discriminated target odours. They also discriminated between related human individuals spanning three generations and including sibling pairs. In addition to demonstrating scent matching capabilities, this experiment supported the elephants' significant ability to perform well at operant conditioning tasks.

1. Introduction

Elephants have the largest absolute brain in terrestrial mammals, with a large amount of cerebral cortex, especially in the temporal lobe, and a well-developed olfactory system. These structures are linked to complex learning and behavioral functions in humans, while elephant brain size itself may be associated with memory storage and intelligence (Shoshani et al., 2006). The enlarged neocortex is shared by other species such as humans, apes and certain dolphins; species that have been attributed with higher intelligence and cognitive abilities.

The elephant brain is considered a macrosmatic brain, since the rhinencephalon, or olfactory bulb, is very large. The enlarged rhinencephalon is associated with a keen sense of smell (Shoshani et al., 2006). An elephant's sense of smell may be five times as sensitive as that of a bloodhound, with neuroanatomical studies indicating a highly convoluted cerebral cortex and a series of 32 large olfactory turbinals, primary endoturbinals (Rasmussen et al., 2005) and an unusually extensive bilobed vomeronasal organ (VNO). Recent studies confirmed that elephants have an astonishingly large repertoire of olfactory receptor (OR) genes (Niimura et al., 2014). These ~ 2000 OR genes (humans have 400) may be linked to the elephant's heavy reliance on olfaction for fundamental life-strategy decisions during foraging, reproduction and social interaction.

Elephants also use their sense of smell to find food (Plotnik et al., 2014, Santiapillai and Read, 2010), with olfactory cues in the physical environment influencing elephant behaviour and migration patterns (Douglas-Hamilton and Douglas-Hamilton, 1975; Sukumar, 2003). According to Rasmussen and Krishnamurthy (2000), chemical memories of landscapes, pathways, mineral sources, waterholes, coming rains or flooding rivers, and tree odours signifying seasons are key to elephant survival. When foraging near human settlement areas, elephants also use olfaction to detect and classify human threats into subcategories (Bates et al., 2007a, 2007b; McComb et al., 2014).

Dung and urine deposits on elephant pathways connecting core areas within home ranges (Von Gerhardt et al., 2012) are used to assess reproductive and fitness status of conspecifics (Sukumar, 2003). Female elephants can detect when a conspecific is in oestrus from urine (Meyer et al., 2008), while males use the flehmen response in response to the

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oestrus pheromone (urine or genitalia) and assess musth/non-musth status from temporal gland secretions and urine in other males. Studies have shown that elephants can detect pheromones in urine, breath and temporal glands (Rasmussen and Krishnamurthy, 2000; Rasmussen 1988, 1995, 1998; Rasmussen and Schulte, 1998).

Chemical signals and olfaction are also fundamental to the preservation of familial and non-familial relationships. Elephants live in complex fission-fusion societies, and the maintenance of social cohesion necessitates the ability to identify a large number of individuals. This is especially relevant as dominance hierarchies emerge along matrilineal lines when competition for resources is high (Wittemyer et al., 2007). Auditory evidence suggests that African elephants can recognize the contact calls of up to 100 individuals (McComb et al., 2014), while olfactory research by Bates et al. (2007a, 2007b) suggests that elephants keep track of their family members by monitoring chemical cues in urine. Elephant bond groups rejoin when conditions are favourable, and the greeting ceremony among African elephants is marked by intense urination, defecation, and temporal gland secretions. Although no empirical studies have explained the function of these chemical emissions, it is likely that these individual odour signatures underlie kin recognition in elephants (Wyatt, 2003) and promote herd stability.

Experimental studies on habituated elephants allow for unique opportunities to further our understanding of the significance of olfaction to elephant behaviour: chemical communication in Asian elephants and the significance of musth and oestrus signals in particular were elucidated by extensive research into Asian elephants (Rasmussen, 1988, 1995), with the subsequent identification and verification of a female-to-male sex pheromone ((*Z*)-7-dodecenyl-acetate) (Rasmussen et al., 1996, 2005). Evidence suggests that elephants display olfactory learning capabilities (Arvidsson et al., 2012; Slotnick et al., 1991), have excellent long-term olfactory memory (Markowitz et al., 1975), and excel at olfactory discrimination tasks (Rizvanovic et al., 2013) among structurally related odorants. In this study, we hypothesize that African elephants are not only able to classify human ethnic groups into subgroups (Bates et al., 2007a, 2007b), but are also able to discriminate between individual humans. Human beings have unique odour profiles which are stable and constant over time, which canines can recognize and use to discriminate among individuals (Curran et al., 2005). The aim of this study was to test olfactory discrimination in African elephants in a controlled field setting, and to collect data on olfactory discrimination performance using equipment and protocols similar to those used by the police working dog units in Europe (Schoon and de Bruin, 1994; Marchal et al., 2016). The MTS procedure using a line-up addresses not only the elephants olfactory acuity, but their ability to respond correctly to operant conditioning and the systematic execution of tasks over a given time period.

2. Materials and methods

2.1. Subjects

This research used three elephants (*L. africana*) from Adventures with Elephants (AWE) in BelaBela, South Africa (Table 1) to test MTS capability in African elephants. AWE controlled all aspects of husbandry, enrichment, handling and training. Three out of six elephants were selected to participate in the research as Shan, the other female was with calf, while Nuanedi (another mature female) would not separate easily from the mother and calf, and was therefore not selected for training in order to minimise stress. Elephants are housed together at night in 9 m x 9 m enclosures. The elephants are fed bana grass (*Pennisetum purpureum*), oat hay and lucerne (*Medicago sativa*) at night, and game pellets, cut branches and citrus during interactions with tourists. During the day, in between tourist interactions, elephants are let out into the 3000 ha reserve to roam and feed freely. The elephants are kept in a hands-on system, in which handlers have full access to the animals and they are therefore accustomed to follow commands. Elephant handlers have been in AWE's employ since 2008. Elephants are well habituated to people, and have been successfully utilised for scientific research on elephant morphology, scent detection, hormones and biochemistry, and communication infrasound communication (Baotic and Stoeger, 2017; Panagiotopoulou et al., 2016; Miller, 2015; Zeppelzauer et al., 2014; Stoeger et al., 2012). Training was conducted at the facility and disruptions to elephants' daily routine were minimised in order to limit stress (Stellenbosch University Ethics Approval Protocol Number: SU-ACUM15-00002).

Table 1
Age and sex related details of the three elephants used in human scent matching to sample trials.

Elephant	Age	Sex
Mussina	13	Female
Chishuru	18	Male
Chova	18	Male

2.2. Experimental design

Elephants were trained to walk along a line-up of mounted glass jars in a free search, without direction from a handler riding the elephant, to smell the provided scent samples, and to alert if the target odour was encountered. Training procedures were adapted from Miller (2015), where AWE elephants were trained to alert to a target scent by operant conditioning, and food was given as a reward for correct alerts. The line-up consisted of nine glass jars (80 cm apart) in metal holders mounted on a 7 m horizontal wooden pole at 1.6 m above the ground (Fig. 1). Although up to seven jars are routinely used for training of highly specialised forensic canines in olfactory matching to sample tasks (Marchal et al., 2016; Pinc et al., 2011), we used nine jars for the MTS procedure given the elephant's superior olfactory and cognitive ability (Byrne and Bates, 2009). Glass jars had a diameter of 8.5 cm, and were sealed with a metal lid, punctuated by holes. This allowed elephants to sniff the sample without soiling it. Between each session, glass jars were removed, rinsed with unscented soap and sundried to avoid any possible contamination. Elephant alerts differed markedly – Mussina alerted with a high foot salute and ears forward, Chova alerted by "freezing", with his trunk curled on the scent target and remaining immobile, while Chishuru alerted by bringing his ears forward and raising his head (Fig. 2).

2.3. Odour sample preparation and collection

For target samples, body odour from unspecified body parts was collected from 26 humans of four ethnic groups (Shona, Xhosa, Coloured and Caucasian), sexes and ages around the town of BelaBela. The scent donors consisted of 17 men, and 9 women, including two young children. Each individual wore four woven cotton fabric cloths (20 cm squares) on their body for a minimum of 15 min. Cloths were then sealed and stored in sterilized glass jars with removable metal caps. Cloths were used within two days of collection. All samples were handled by the same individual wearing latex gloves in order to avoid contamination, and cloths were indistinct from each other in colour. Cotton was used as it yields the greatest number of volatile compounds and the highest scent mass amount among textiles (Prada et al., 2011).

2.4. Training and testing procedure

2.4.1. Training

Elephants were first trained to detect a single scent. During training, elephants were presented with a cloth containing the target scent, and the same cloth was then placed on top of a jar in the line-up in an established training area. As soon as the elephant regularly retrieved