Urinary, temporal gland, and breath odors from Asian elephants of Mudumalai National Park

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ABSTRACT *Chemical investigations, based on previously substantiated behavioral interactions, have identified specific compounds or combinations of compounds in emissions from captive Asian elephants,* Elephas maximus, *that are biologically active, eliciting either previously observed behaviors or new reactions. In addition, these emissions vary with the age, sex and hormonal state of a particular elephant; conversely responses by elephants vary according to their physiological status, gender and experience. Such chemical signals aid in the functioning of elephant society. This study is an initial effort to determine the chemical similarities/dissimilarities between temporal gland, breath and urinary emissions of Asian elephants living in their native Asia and conspecifics dwelling in the northern western hemisphere. This investigation reflects cooperative efforts between mahouts, the staff of Mudumalai Wildlife Sanctuary, veterinarians, and research scientists; its goal is information that ultimately will be useful in the management of Asian elephants in their native environment.*

Keywords: Asian elephants, *Elephas maximus*, chemical signals, breath, urine, temporal gland secretions, India.

Introduction

Previous studies have demonstrated a spectrum of characteristic volatile compounds in the exhaled air and in the airspace (headspace) above various elephant excretions and secretions, including urine and temporal gland secretions (Rasmussen *et al.*, 1997a, b; Rasmussen, 1998) Especially interesting are compounds that degas from the apparently dry orifice of male Asian elephants *(Elephas maximus)* just prior to overt musth (Rasmussen & Perrin, 1999). Since all these investigations were based on samples from captive populations in the northern western hemisphere, this study represents the first analyses of elephant-released volatiles from Asian elephants dwelling in their native land. Because of the inherent honesty that chemical signals represent, we are interested in the similarities/dissimilarities of the volatile compounds between nine samples (four urine, three breath, and two temporal gland) obtained from elephants at the Mudumalai Wildlife Sanctuary in south India (~12° north latitude) and samples from captive elephants in the northern western hemisphere (latitudes 29-48°) experiencing similar physiological states.

Materials and methods.

Sample Collection

Under the direction of Dr Krishnamurthy and the auspices of the Forestry Division of Mudumalai Wildlife Sanctuary, we were able to obtain volatiles directly from the orifice of the temporal gland and from the exhaled air, and indirectly by the collection of urine and subsequent headspace procedures.

For the collection of the TGS volatiles, a specially contoured open-bottom, stainless-steel, funnel-like device with a diameter of 7cm was gently placed directly on the cheek of the two male elephants so that the temporal gland orifice was centered within this primary collection device. The device was connected via ultraclean Nupro SS-4H4 bellow-stem valves to a 0.8-L internally summa-polished, stainless-steel receiving bottle evacuated to - 30 in Hg vacuum. After several seconds, the valve of the receiving bottle was opened, and volatiles emanating from the orifice were drawn into the evacuated receiving bottle from the primary collection device. The first bull sampled, Subramanian, a 47-year-old tusker, was in a premusth state. The samples were obtained on October 31, 1998; he came into musth on November 15, 1998. While Drs Krishnamurthy and Rasmussen took the sample, the mahout, Bomman, controlled Subramanian with the assistance of Forester Babu and the watcher, Sivakumar. The second male, Anna, 38-year-old tusker, had been in musth 4 months previously; therefore he was in very late postmusth or nonmusth condition. His mahout, Kalan, controlled him while the guard, Halan, and Dr Krishnamurthy took the sample.

From these two males, Subramanian and Anna, concurrent samples of exhalant breath were obtained by Dr Krishnamurthy and the guard, Halan. To obtain the breath samples, the narrow (1cm^2) orifice of the valve of the evacuated receiving bottle was placed in the end of the trunk. When moist, warm exhalant air was felt, the valve was opened and closed almost immediately to prevent the inclusion of inhaled air. Between 3 and 10 breaths were required to fill the canister. Subsequent analyses for CO_2 percentage content confirmed the breaths were exhalant.

In addition to the two adult males, on November 1, 1998, Drs Krishnamuruthy and Rasmussen obtained an exhalant breath sample from a 42-year-old, very-near-term pregnant female elephant, Kamatchi, who delivered her fifth calf, a male, on November 24, 1998. The mahout restraining Kamatchi was Bomman. In addition, during the evening, the mahouts had kindly collected in a glass jar 500-mL of fresh urine from Kamatchi. This sample was subdivided into two aliquots for subsequent collection of headspace volatiles. Each aliquot was placed in a 500-ml glass sample collection apparatus with a special lid equipped with a swaglock fitting. Under field conditions, we were unable to purge the system with zero air in order to reduce the concentration of background compounds to insignificant levels; however, in practice we have found that our modified system yields almost identical results provided that the surrounding air is relatively clean. We attached an evacuated bottle to the fitting on the jar top; the system was allowed to equilibrate for 30 min. Then the glass jar containing the urine aliquot was heated to 38^0 C while the receiving canister was attached at the lid region. The jar was maintained at this constant temperature for the duration of the headspace collection. After 30 min at 38°C headspace volatiles were drawn into the stainless-steel, evacuated receiving bottle when the valve of this bottle was opened. Because the system was deliberately not sealed, surrounding air slowly replenished the air supply in the jar. Again, a 30-min interval was observed before the next headspace sample was taken. For the first aliquot, the sample was maintained at 38^{0}C for 3h, and six headspace collections were added to the receiving canister. For the second aliquot, the sample was maintained at 38°C for 6h, and headspace collections were added to the receiving canister starting at 2 h, for a total of eight collections.

A 35-40-year-old, recently captured (4 months previously) makhna bull elephant was intermittently dribbling urine, and we were interested in comparing his urinary volatiles with those of musth bulls that were also urine dribbling. We obtained a 500-ml sample and subdivided it into two 250-ml samples; headspace collections were conduced similar to those described above for the female sample. For the first aliquot, during a 4-h period, we collected four headspace samples into one receiving canister; for the second aliquot, during the second half of an 8-h period, we collected three headspace samples into one receiving canister.

After transport of these now-ambient-pressure receiving bottles, the samples were pressurized with helium to 30 psig at the laboratory in Oregon to ensure long-term storage and to facilitate subsequent chromatographic analysis.

Gas Chromatography-Mass Spectrometry Analyses

All samples were analyzed for total non-methane hydrocarbons prior to analysis by gas chromatography-mass spectrometry (GC-MS), thus allowing optimal concentrations of each sample to be analyzed on the GC-MS. In addition, breath samples were analyzed for total CO_2 content to assess their validity as exhalant air samples. The sample introduction system involved loading measured mg amounts of the sample onto a tenax trap followed by desorption at temperatures reaching 50°C. Subsequently, the volatiles were cyro-trapped on a U-tube cryogenic trap (0.125 in. ODx9in.) containing 60/80 mesh glass beads. A Carle six-port valve was employed in line to achieve these maneuvers. GC-MS analysis was conducted using a Hewlett-Packhard 5890A GC and a Hewlett-Packard 5970B MS. The GC used a DB-1, 0.25 mm ID x 60 m x 1.0 µmfilm thickness, polymethyl silicone-coated capillary column (J&W Scientific, Inc.) The GC oven was temperature programmed from -60°C to 200°C at 4°C/min, with a 5-min initial hold at -60°C (Perrin et al., 1996). The mass spectrometer was programmed for a mass scan of 33-300, which allowed for identification of compounds from C3 through C14. The conditions allowed quantitation as low as 0.10 ppbv. Compounds were identified using an NBS 75 K Hewlett-Packard MS Chem Station library search and were manually rechecked with NIST/EPA/NIH Mass Spectral Data base Version 4.01.

Results

Temporal Gland Volatiles

Premusth

A striking characteristic of the volatiles of this sample taken above the dry orifice area of the temporal gland of a bull elephant who was nearing the time of his annual musth was the presence of a high concentration of 2-butanone. The corresponding aldehyde, butanal, another ketone, acetone, and isoprene (2-methyl-1,3-butadiene) were in high concentration (Table1)

Table 1 Volatiles in temporal gland emissions

Compound	*RT-min*	*PostM-TG-I*	*PreM-TG-I*	*PreM-TG-C*	*Skin-C*
acetaldehyde	18.10		x	x	
ethanol	26.20	x	x	x	x
acetone	26.75	x	x	x	x
propanal	27.60		X	X	
acetic acid	28.00	x			x
furan	29.20	x			x
isoprene	29.80		X	X	
2-methyl 2-propanal	31.20		x	x	
carbon disulfide	31.37	x			x
butanal	35.40			X	
2-butanone	35.70		X	X	
2-butanol	36.70		x	x	
tetrahydrofuran	38.10			x	
acetic acid	40.90	x	x	x	x
pentanal	41.58	x			x
hexanal	48.20	x	x	x	x
decenal	53.80	x			x
cyclohexanone	54.20				
2-butoxyethanol	54.30	x			
benzaldehyde	56.90	x	x	x	x
octanal	54.00	x			x
4-methyl phenol	63.00		x	x	
nonanal	64.10	x	x	x	x
2-nonanone	64.50				
azulene	68.70	x			

x,present; X,present in high concentration; PostM-TG-I, temporal gland emissions from male Asian elephant four months after musth at Mudumalai Wildlife Snctuary; PreM-TG-I, temporal gland emissions from male Asian elephant premusth at Mudumulai Wildlife Sanctuary; PreM-TG-C, temporal gland emissions from male Asian elephant premusth in captivity in the U.S.A.: Skin-C, temporal gland emissions from the skin of male Asian elephant.

Postmusth

In contrast, the volatiles of the dry orifice area of the 4-month postmusth bull closely resembled skin volatiles. This sample contained acetone, acetic acid and a number of aldehydes, including pentanal, hexanal, decenal, octanal, and nonanal (Table 1).

Breath Volatiles

The volatile compounds emanating from the breath of the two bulls were very similar to compounds from the breath of captive Asian male elephants in the U.S.A. who were in similar premusth and postmusth states. Aldehydes, furans and certain hydrocarbons and ketones predominated. These two samples were distinctly different from the breath of captive musth bulls. In particular, a high concentration of C-3 to C-9 ketones, which characteristically dominates musth breath, was not observed.

Premusth

The sample of premusth breath was characterized by a high 2-butanone concentration. Such high 2-butanone concentrations have been observed in numerous male Asian elephants in the U.S.A. (Rasmussen & Perrin, 1999) (Table 2)

Postmusth

Postmusth breath had a similar qualitative pattern and similar concentration ranges of hydrocarbons and aldehydes demonstrated in breath samples analyzed from Asian male elephants in nonmusth in the U.S.A. However, one unusual compound, 4-hexen-1-ol, was noted (Table 2).

Pregnant female

The breath of the pregnant female contained many hydrocarbons, aldehydes and furans. The most distinguishing components were relatively high concentrations of isoprene and 1,3 butanediol, and octylthioglycolate, a previously undetected compound (Table 2)

Table 2 Dominant volatiles in breath of Asian elephant during several physiological states

Compound	*RT-min*	*PerM-I*	*PreM-C*	*PostM-I*	*PostM-C*	*Preg-I*	*Preg-C*
carbonyl sulfide	9.70			x			
acetone	26.75	x	x	x	x	x	x
fural	29.20			x	x		
pentane	29.35	x	x			x	x
isoprene	29.80	X	X			X	X
1,2-pentadiene	30.88	x	x	x	x		
butanal	35.40	X	X				
2-butanone	35.70	X	X			x	x
2-butanol	36.70	x	x				
3-methyl-furan	37.10					x	x
3-buten-2-ol	37.30	X				x	
3-methyl butanal	39.40					x	
formic acid	39.40					x	
acetic acid	40.90	x	x	x	x		
pentanal	41.58			x	x		
2-ethyl furan	42.60					x	
hexanal	48.20			x	x		
2,3-butanediol						x	X
2,5-dihydrofuran	49.12			x	x	x	
benzaldehyde	56.90			x	x		X
a-pinene	56.95	x				x	
camphene	58.00	x				x	
β-pinene	59.23					x	
octanal	59.09					x	x
3-ethylphenol	59.20						X
4-hexan-1-ol	59.20			x			
limonene	61.49					x	
nonanal	64.10				x	x	x
decanal	68.60				x	x	x
octylrhioglycolate	72.00					x	
tritetracontane	73.10						x

PreM-1, breath from male Asian elephant during premusth at Mudumalai Wildlife Sancuary; PreM-C, breath from male Asian elephant during premusth in captive facility in the U.S.A.; PostM-I, breath from male Asian elephant four months after musth at Mudumalai Wildlife Sanctuary; PostM-C, breath from male Asian elephant four months after musth in captive facility in the U.S.A.; Preg-I, breath from pregnant female Asian elephant at Mudumalai Wildlife Sanctuary; Preg-C, breath from pregnant female Asian elephant in captive facility in the U.S.A.

Urine Volatiles

Pregnant female

The urine headspace sample from the pregnant female was very distinctive. In the first aliquot, there was a strikingly high concentration of 4-heptanone. In addition, many C5 and C6 ketonic compounds, such as 1-penten-3-one, 3-penten-2-one, 2-pentanone, 3-pentanone, 2 methyl 2 cyclopenten-1-one and E-3 methyl 2-pentanone, were evident (Table 1). Ketones clearly were the dominant chemical species (whereas aldehydes dominate in follicular urine).

The sulfer compounds 3-methyl thiophene, DMDS, isothiocyanate, and 2 phenols, 3-ethylphenol and 2-ethyl 4,5 dimethylphenol were detected, but no (Z)-7-dodecenly acetate, the estrous pheromone, was resolved. The second aliquot demonstrated a similar signal profile; the major difference was a higher percentage of acetones among the total volatiles (Table 3). Interestingly, both urine samples contained 4-hexan-1-ol, a compound that was also detected in the breath of the postmusth male elephant.

Recently captured makhna

The makhna male was recently captured from the wild and intermittently dribbled urine. The urinary headspace volatiles did not contain volatiles charateristic of musth urine. Trimethylamine, 2-nonanone, most of the spectrum of ketones, frontalin (1,5 dimethyl 6,8 dioxbicylo [3.2.1] octane) and cyclohexanone were lacking. Dimethly disulfide was in striklingly high concentration (Table 3).

Table 3 Volatiles in urine headspace

Compound	RT-min	Preg-I	Preg-C	Makhna-I
carbonyl sulfide	9.70			x
ethanol26.50				
acetone26.85	X	x	X	
carbon diulfide	31,37			X
2-butanone	34.90		x	x
3-methyl butanal	39.40	x		
formic acid	39.42	x		
acetic acid	40.90	x		
1-penten-3-one	40.87	x		
2-pentanone	41.03	x	x	
pentanal	41.58	x	x	
3-pentanone	41.69	x		
2-ethyl furan	42.60	x		
2,5-dimethyl furan	42.98	x		
2-methyl 2-cyclopenten-1-one	43.45	x		
E-3-methyl 2-pentanone	44.09	x	x	
2-methyl 1-penten-3-yne	44.40	x		
methyl isobutyl ketone	44.54	x		
dimethyl disulfide	45.00	x		X
3-methyl 2-pentanone	45.40	x		
3-hexanone	47.23	x		
3-methyl thiophene	47.40	x		
hexanal	48.20		x	
2,5- dihydro furan	49.12	x		
isopropyl isothiocyanate	49.75	x		
4-methyl 3-hexanone	50.70	x		
E-2-hexenal	50.89	X		
4-heptanone	51.70	x	x	
6-methyl 3, 5-heptadien-2-one	52.00	x		
allyl isothiocyanate	52.24			X
2,5-dihydrofuran	53.90	x		
heptanal	54.70		x	
an acetate	57.89	x		
3-octanone	58.20	x		
2-pentylfuran	59.01	x		
octanal	59.09	x	x	
4-hexen-1-ol	59.20	x		
B-pinene	59.23	X		
6-methyl bicyclopetan-2-one	59.90	x		
acetophenone	62.10	x		
nonanal	64.10	x	x	
2-ethyl 4,5-dimethyl phenol	64.90	x		
5-methyl hexanal	66.60	x		
decanal	68.60		x	

Preg U-I, volatile compounds in urine of near-term pregnant female Asian elephant at Mudumalai Wildlife Sanctuary; Preg U-C, volatile compounds in urine of near-term pregnant female Asian elephant in captive facility in the U.S.A.; MakU-I, urine from Makhna male elephant captured several months before at Mudumalai Wildlif Sanctuary.

Discussion

Chemical signals are known to be important in the lifestyle of the Asian elephant (Rasmussen, 1998). Such signals are relatively honest, being hard to disugise and often emitted involuntarily. We are especially interested in the variations of chemical signals between sexes and among elephants of differing physiological states and living in different locales and habitats, perhaps eating considerably different foods.

A striking characteristic of the volatiles of the sample taken above the dry orifice area of the temporal gland of a bull elephant who was nearing the time of his annual musth was the presence of a high concentration of the ketone, 2-butanone. Odoriferous 2-butanone is usually present among many other ketones in male TGS during musth, but during premusth has been demonstrated in a number of captive Asian male elephants to be the dominant component degasing from the apparently dry temporal orifice (Rasmussen & Perrin, 1999). In contrast, the volatiles from the postmusth temporal gland sample only contained compounds characteristic of skin volatiles. Such chemical information detected and quantified by GC-MS is presumably also readily detectable by conspecifics. In captivity in the U.S.A., we have seen females touch the apparently dry orifice of male elephants. In addition, 2-butanone is a low-molecular-weight, readily volatile, reasonably stable compound that has been demonstrated in other mammals to be readily detected by olfaction at low concentrations (Cometto-Muniz & Cain, 1995).

The sample of premusth breath was also characterized by a high 2-butanone concentration. Such high 2-butanone concentrations have been observed in numerous male Asian elephants in the U.S.A. (Rasmuseen, 1997b). Our current investigations are aimed at determining if this ketone is a functional chemosignal as a single or as part of a ketonic blend. In contrast, postmusth breath had normal ranges of hydrocarbons and aldehydes similar to numerous breath samples of nonmusth Asian male elephants previously analyzed. However, the alcohol 4-hexen-1-ol has not yet been previously identified in elephant emissions.

The predominance of aldehydes in the breath of the pregnant female was within the normal range for female elephants; such high aldehyde concentrations are especially evident during the follicular stage of the estrous cycle but may occur during the luteal phase (Rasmussen *et al.*, 1997b). The concentrations of furans were somewhat unusual. The relatively high concentration of isoprene is interesting in view of increases in isoprene levels in the blood during early musth and its release in high concentration in temporal gland secretions prior to musth (Rasmuseen & Perrin, 1999). The high concentration of isoprene was also observed in the breath of a captive female Asian elephant during her seventh month of pregnancy (Rasmuseen, unpublished). Isoprene is known to accumulate in fatty tissue (Dahl *et al*, 1987).

The most striking observation in both aliquots of the urine headspace of the pregnant female was the predominance (except for the omni-present acetone) of 4-heptanone. The synthetic and metabolic history of 4-heptanone is interesting. A considerable amount of information has been obtained from human studies: 4-heptanone is syntheized from ß-oxocarboxylic acid or from 2-ethyl-3-oxohexanoic acid via decarboxylation (Liebich, 1983). During diabetes mellitus the urinary excretion of 4-heptanone is often elevated fiv-fold (from 200-2500 micrograms/24 h to 1000 microgrms/24h) (Liebich, 1986). Especially interesting was the spectrum of C5 and C6 ketones in the urine of the pregnant female. These ketones were distinctive from the ketones characteristic of musth urine, 2-heptanone, cyclohexanone, 2-nonanone. Our limited knowledge of the metabolism of the Asian elephant makes further interpretation difficult. This suggests that further research on these aspects would be of value.

The lack of ketones in the volatiles of the urine from the recently captured adult male was surprising, considering his condition and age. With presumed changes in diet and certainly the stress of capture, metabolic perturbation would not have been unexpected. However, the extremely high dimethyl disulfide in the urine of this makhna could be metabolic or bacterial in origin.

These initial studies clearly demonstrate that we are just beginning to decipher the chemistry of elephant emissions and that we have barely begun to decipher their meanings to the elephants.

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