

Department of Physics, Chemistry and Biology

Master Thesis

Development and application of an olfactory
discrimination paradigm for Asian elephants
(Elephas maximus)

Josefin Arvidsson

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Supervisor: Matthias Laska, Linköpings universitet

Examiner: Mats Amundin, Linköpings universitet



Linköpings universitet

Department of Physics, Chemistry and Biology

Linköpings universitet

SE-581 83 Linköping, Sweden

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1 Abstract

The sense of smell plays an important role in regulating the behavior of Asian elephants but until now, no behavioral test to systematically assess the olfactory capabilities of this species existed. Using a voluntary, food-rewarded two-alternative operant conditioning procedure, three female Asian elephants were successfully taught to discriminate between rewarded and unrewarded odors and also succeeded in intramodal stimulus transfer tasks in which either the rewarded odor, or the unrewarded odor, or both odors were exchanged simultaneously for new odors. The animals readily mastered the initial task within only 120 stimulus contacts, demonstrating rapid olfactory learning and performing at least as good as rodents and dogs and even better than other species, including nonhuman primates, tested in similar studies before. When presented with pairs of structurally related odorants, the discrimination performance of the elephants decreased with increasing structural similarity of the odorants, but the animals still significantly discriminated between aliphatic acetic esters even when they only differed by one carbon chain length. The elephants also demonstrated an excellent long-term odor memory and successfully remembered the reward value of previously learned odor stimuli after two, four, eight and even 16 weeks of recess in testing. The paradigm developed and applied in the present study proved to be useful to assess the olfactory capabilities in Asian elephants.

Key words: Asian elephant; Behavioral testing; *Elephas maximus*; Odor learning; Olfactory discrimination; Olfactory memory

2 Introduction

Behavioral evidence suggests that Asian elephants (*Elephas maximus*) strongly rely on their sense of smell in a variety of contexts such as foraging (Santiapillai and Read, 2010) and social communication (Langbauer, 2000; Rasmussen and Krishnamurthy, 2000; Scott and Rasmussen, 2005). Chemical communication in elephants has been thoroughly studied and is considered an important mechanism in regulating the behavior of elephants (Rasmussen 1998; Rasmussen, 1999; Rasmussen and Krishnamurthy, 2000) and the long-term memory of elephants for social odors seems to be excellent (Rasmussen, 1995). In fact, the Asian elephant is one of the few mammal species so far for which a sex pheromone has been chemically identified and functionally verified (Rasmussen et al., 1997, Rasmussen et al., 2005).

The long-term stability and permanence of Asian elephant social groups depends on effective intraspecific communication, operational at both short and long distances (Rasmussen, 1999; Langbauer, 2000). The interactions are facilitated through vocalization (McComb et al., 2000; Nair et al., 2009), social odors (Rasmussen, 1998) and tactile and visual displays (Langbauer, 2000; Sukumar, 2003). However, long-distance communication demands signals that can be emitted and received over a larger distance such as infrasonic vocalization (Langbauer, 2000) and stable chemical signals (Rasmussen, 1998; Rasmussen et al., 2000), where the chemical signals have the advantage of being accessible over both time and space (Schulte et al., 2007). Elephants can perceive these chemical signals via the main olfactory and vomeronasal systems but also via the trigeminal system (Sukumar, 2003; Rasmussen, 2006). Anatomical evidence of well-developed olfactory and vomeronasal systems (Johnson and Rasmussen, 2002; Göbbel et al., 2004; Shoshani et al., 2006) as well as of specialized skin glands (Wheeler et al., 1982; Lamps et al., 2001) further supports the idea that the sense of smell plays a crucial role for elephants.

Of all land animals, elephants have the largest absolute brain size (Cozzi et al., 2001; Hart et al., 2001; Shoshani et al., 2006; Bates et al., 2008b) and accordingly the largest cerebral cortex which is involved in higher-order brain functions. The ratio of brain volume to body mass in Asian elephants is comparable to that of chimpanzees (Shoshani et al., 2006; Hart and Hart, 2007) which suggests that elephants may possess the capacity for complex cognitive processes. The cognitive abilities of elephants are considered extraordinary by some authors (Bates et al., 2008b; Hart et al., 2008; Byrne and Bates, 2009) but most of the evidence is anecdotal, insufficient or inconsistent (Plotnik et al., 2011). Asian elephants have been trained successfully in two-choice visual (Rensch, 1957; Savage et al., 1994; Nissani et al., 2005), auditory (Rensch, 1957; Heffner and Heffner, 1982), and tactile (Dehnhardt et al., 1997) discrimination tasks and were found to readily learn such tasks. However, no studies so far assessed the olfactory discrimination ability in this species, nor the learning speed of elephants with odor stimuli. A comparison of the number of stimulus contacts needed until reaching criterion in different discrimination tasks would offer an opportunity to evaluate which senses the elephants may use more readily when solving a learning problem.

So far, no behavioral test to systematically assess the olfactory capabilities of elephants exists and it was therefore the aim of the present study to develop and apply an olfactory discrimination paradigm for Asian elephants. A second aim was to collect first data on olfactory learning speed, memory and discrimination performance in this species. The behavioral test is based on a voluntary, food-rewarded two-alternative operant conditioning procedure. The animals were taught to sample two odor ports and were food-rewarded when they performed an operant response (putting the trunk at a certain position of the experimental set-up) upon correctly identifying the rewarded odor. Similar operant conditioning procedures to assess olfactory learning, memory and discrimination capabilities have been employed with other mammals such as squirrel monkeys (Laska and Hudson, 1993), spider monkeys (Laska et al., 2003), pigtail macaques (Hübener and Laska, 2001), South African fur seals (Laska et al., 2008), mice (Bodyak and Slotnick, 1999), rats (Slotnick et al., 1991), and dogs (Lubow et al., 1973). This allowed for direct comparisons of the speed of initial task acquisition, the ability to master intramodal stimulus transfer tasks, and olfactory memory performance between species. Also, by using a set of structurally related odorants that has been used with squirrel monkeys (Laska and Freyer, 1997), humans (Laska and Hübener, 2001) and South African fur seals (Laska et al., 2010), the discrimination performance of the elephants for these odors was assessed and compared to that of the other species. The olfactory discrimination paradigm developed and applied in the present study will enable a better understanding of the basic olfactory abilities of Asian elephants and allow for direct comparisons of olfactory discrimination, long-term memory and learning competence between species.

2.1 Aim of the project

The aim of the present study was to train three female Asian elephants to cooperate in an olfactory discrimination paradigm and to collect first data on their olfactory learning speed, olfactory discrimination performance and olfactory memory and to compare these data to those collected in other species. The hypotheses were that:

1. Asian elephants can be trained to cooperate in an olfactory discrimination paradigm,
2. the olfactory learning speed, olfactory memory, and olfactory discrimination performance of Asian elephants are at least as good as that of other mammals.

3 Material and methods

3.1 Animals and management

The study was conducted using three adult female Asian elephants (*Elephas maximus*) housed at Kolmården Wildlife Park. Saba (born 1968) was transferred to Kolmården Wildlife Park from Zoo Le Pal, France, at the end of 2007. Saonoi and Bua (born 1996 and 1997, respectively) were both born at work camps in Thailand and have been housed at the Swedish zoo since 2004. The animals were kept as a group in two indoor enclosures (approximately 150 m² and 250 m²) but were let outside into an outdoor back enclosure (750 m²) or an outdoor exhibit (3000 m²) for a larger part of the day or at least once a day and when the weather was appropriate. The elephants were fed pellets in the morning and roughage and branches were provided ad libitum. Environmental enrichment in the form of scattered and hidden fruits and vegetables throughout the enclosure was provided at least once a day and no food-deprivation was required during the study. The elephants were kept in a hands-on system in which the keepers have full access to the animals and they were therefore trained to follow commands and perform certain motor patterns upon demands.

3.2 Odor stimuli

For the initial acquisition of the olfactory discrimination paradigm a set of eight odorants was used (amyl acetate, anethole, carvone, cineol, ethyl butyrate, limonene, pinene and 2-phenylethanol). For the assessment of odor discrimination capability with structurally related odorants a set of four acetic esters was used (amyl acetate, butyl acetate, propyl acetate and ethyl acetate).

All substances were obtained from Sigma-Aldrich (St. Louis, MO) and had a nominal purity of at least 99 %. The odorless solvent diethyl phthalate (Sigma-Aldrich) was used to dilute the odorants at approximately equal subjective intensities. The odor concentrations were prepared at the laboratory at Linköping University by the supervisor, and evaluated by the trainer at Kolmården Wildlife Park, before presented to the animals. The condition was that these two persons could discriminate between the given odor combination and that the two odors were perceived as equally intense to the human subjects. The concentrations of the odors are shown in table 1.

Table 1: The odors used and their concentrations.

| <i>Odorant</i> | <i>CAS number</i> | <i>Concentration</i> |
|-----------------------|-------------------|----------------------|
| n-Amyl acetate | 628-63-7 | 1:5 |
| Anethole | 104-46-1 | 1:10 |
| 1,8-Cineol | 470-82-6 | 1:10 |
| (+)-limonene | 5989-27-5 | 1:3 |
| (+)- α -pinene | 7785-70-8 | 1:5 |
| (-)-carvone | 6485-40-1 | 1:3 |
| Ethyl butyrate | 105-54-4 | 1:3 |
| 2-phenylethanol | 60-12-8 | 1:3 |
| Ethyl acetate | 141-78-6 | 1:10 |
| n-Propyl acetate | 109-60-4 | 1:10 |
| n-Butyl acetate | 123-86-4 | 1:5 |

3.3 Experimental set-up

For the presentation of odorants two high density polyethylene (HDPE) boxes with removable lids (Rubbermaid Cooling Bags, 35 cm high x 35 cm wide x 20 cm deep) were used (Figure 1). The tight fitted lid of each container was equipped with a ventilator (6 cm in diameter) powered by a lead accumulator (Clas Ohlson, power: 12V, 1.3Ah), which provided an ingoing airflow of approximately $0.58 \text{ m}^3 \text{ min}^{-1}$. A total of 130 holes of 3 mm diameter placed in intervals of even distance forming a filled circle with a diameter of 7.5 cm were drilled in an exact pattern in the middle of one of the front sides of each odor box, serving as an outlet for the airflow provided by the ventilator.

In order to present the odorant, a circular filter paper of 9 cm diameter (Grycksbo Pappersbruk AB) was placed into an open Petri dish and 1 ml of the odorant was pipetted onto it. The petri dish, in turn, was placed into an open white plastic HDPE box (12 cm high x 20 cm wide x 12 cm deep) inside the odor box to avoid contaminating the box. The odor boxes were cleansed with warm water after the completion of each session.



Figure 1: The experimental set-up. The picture on the left shows the odor boxes, with an airflow outlet visible in the background, the plastic boxes and the petri dish to the front left and the lid with the battery powered ventilator at the front right. The picture on the right shows the experimental set-up from the trainer's side with the odor boxes being presented to the animal. The service door covered with a grid separates the trainer from the elephant while allowing for presenting the odorant and the reward.

The testing was carried out in a separate indoor enclosure in which the animals could be trained individually. The trainer was positioned in the experimental room on the second floor where an opening in the wall was fitted with a service door (106 cm high x 90 cm wide x 5 cm deep) made of steel. The door was modified to hold a window (96 cm high and 75 cm wide) in its upper part which was covered with a steel grid (with a mesh width of 4 x 4 cm) that physically separated the trainer from the animals while allowing the trainer to observe and interact with the animals and to present the food-reward (Figure 1). The grid also served as a barrier that kept the elephants from reaching and grabbing the odor boxes. The location of the door allowed the trainer to observe the animals while the animals had a very limited opportunity to see the trainer.

The service door was located approximately 3 meters above the ground of the testing enclosure and it was divided by a vertical bar in the middle into a left and a right section. Each section contained an odor port (a round opening with a diameter of 21 cm) at the lower half part of the door. Above the odor ports was the rectangular grid-covered window where the animals could get access to their reward (Figure 2). A pallet and a wooden platform on the

floor inside the experimental room (Figure 1), ensured that the odor boxes were placed with their outlets congruent with the odor ports of the experimental set-up.



Figure 2: The experimental set-up shown from the animal's side. The picture on the left shows an elephant sampling one of the two round odor ports at the lower part of the experimental set-up, and the picture on the right shows the same elephant performing the operant response, that is, putting its trunk onto one of the two rectangular grids upon correctly identifying the rewarded odor. The vertical bar in the middle served as a divider to make the animal's decision unequivocal.

3.4 Training and testing procedure

The training and testing took place between the 6th of September 2010 and the 17th of March 2011. All training was carried out in the indoor enclosure in which the animals could be separated and trained individually. The animals had no visual contact with each other but were still within auditory distance. The training took place five days a week twice a day, one session in the morning and one in the afternoon. The time of day varied to some degree but the sessions were at least 3 hours apart. During each session approximately 30 trials were completed. Each animal participated in a total number of 146 sessions.

The elephants were brought individually, in a predetermined and fixed order, into the testing enclosure by the keepers. Once the elephant was brought into the enclosure, the keepers locked the door and left the enclosure.

Each session started with the removal of a wooden board covering the trainer side of the experimental set-up which made the animals voluntarily approach the set-up. Each trial started with the trainer presenting the odor boxes with their outlets facing the odor ports. After a verbal command – the loudly spoken word “now” – the elephants were allowed to sample the odor ports and to indicate their decision by putting the tip of their trunk onto the grid above the corresponding odor port. Since the animals were not restrained they could continuously explore the set-up with their trunks but any exploring of the set-up before the verbal command was ignored. No reward was given before the verbal command, regardless if the elephant had lifted its trunk into the correct position or not. Only decisions made after the odor boxes were in place and the command had been given, were considered as actual decisions.

The odor boxes were presented in a pseudo randomized order either to the left or to the right. However, the same side was not used more than three times in a row. The decision (correct or incorrect) of the animals was recorded after each trial. Correct decisions were

rewarded with a bridge (a whistle) followed by a food-reward (a carrot). When an animal made an incorrect decision, the odor boxes were simply removed and no reward was given.

When the elephants had completed the 30 trials, the end of the session was signalled by a verbal command – the loudly spoken words “now you’re done” - followed by a few carrots being distributed through the grid onto the enclosure floor. This made the animals move away from the experimental set-up and the wooden board was again placed to cover the inside of the set-up. The elephant was then led out from the testing enclosure by the keepers.

3.5 Initial training

The behavioral test was based on a food-rewarded two-alternative instrumental conditioning paradigm. The animals were taught to sample (by sniffing at) two odor ports and were food-rewarded when they performed an operant response (putting the trunk at a certain position of the experimental set-up) upon correctly identifying the rewarded odor (Figure 2). The training method was based on a voluntary participation of the animals and only positive reinforcement was used as a tool to shape the desired behavior. Since the training method was completely new to the animals, they first had to learn the reward value of a bridge signal (a whistle) which could then serve as a conditioned reinforcer whenever the animals showed the correct behavior (or behaviors leading up to the correct behavior). When the animals had made the association that bridge equals reward, the trainer could guide the animals in the right direction without using any additional tools (such as body language or voice). The rationale for using a bridge as a training tool was that the trainer could not handle the animals hands-on, but was required to direct the animals and their behavior from a distance (beyond the grid-door). Under such circumstances, the bridge served as a guide to show the animals when they were performing a desired behavior or not.

Table 2 shows the different stages of the training in detail. The training was carried out step-wise, where every new stage required a little bit more from the animals in order to gain access to the food-reward. For example: during the initial training, it was enough for the elephant just to explore the experimental set-up in order to get a bridge followed by a food-reward. The final stage (stage 9, Table 2) was the aim of the training, where the animal would make its own decision and perform the operant response to demonstrate its decision. Only if the animal performed the complete behavior sequence and made the correct decision, it would result in a bridge and a food-reward.

Table 2: The training of the elephants in the olfactory discrimination paradigm. The table shows the different stages of the training in order to shape the final operant response, that is, putting the trunk at a certain position of the experimental set-up upon correctly identifying the rewarded odor.

| | <i>Stages of training</i> |
|----|---|
| 1. | No odor boxes present. Establishing bridge signal (the whistle) as a reward. When the elephant is approaching (any part of) the experimental set-up, bridge and offer food- reward. |
| 2. | No odor boxes present. Continuing establishing bridge signal. When the elephant is approaching the randomly chosen part (left or right side) of the experimental set- up, bridge and offer food-reward. |
| 3. | No odor boxes present. When the elephant is approaching any of the ports, bridge and offer food-reward through the grid (over the odor port) on the same side. |
| 4. | No odor boxes present. When the elephant is approaching the randomly chosen port (left or right side), bridge and offer food-reward through the grid on the same side. |

| | |
|----|--|
| 5. | No odor boxes present. Petri dish with odorant presented at the port and when the elephant is showing interest or sniffing at the randomly chosen port, bridge and offer food-reward trough the grid on the same side. |
| 6. | No odor boxes present. Petri dish with odorant presented at the port and when the elephant sniffs at the port and stations (for about a second), bridge and offer food-reward trough the grid on the same side. |
| 7. | Odor boxes with ventilator on (Box A: S+ and Box B: empty). The animal is guided (with the first bridge) to make the right decision and to lift its trunk into position. When the animal performs the operant response (lifting the trunk over the correct odor port onto the grid), bridge a second time and offer food-reward. |
| 8. | Full setting (Box A: S+ and Box B: S-). The elephant is guided (with the first bridge) to make the right decision and to lift its trunk into position. The first bridge is slowly faded and the animal is asked to make its own decision from time to time. Only correct decision plus operant response is rewarded with a second bridge, followed by a food-reward. |
| 9. | Full setting (Box A: S+ and Box B: S-). No first bridge is given and the elephant makes its own decision by lifting its trunk into position. Only correct decision plus operant response is rewarded by the second bridge, followed by a food-reward. |

3.6 Experimental design

A total of 10 experiments was conducted (see table 3). Experiments 1-7 were performed to assess the elephant's ability to perform intramodal stimulus transfer tasks and the learning speed of the animals in such tasks. Experiment 8 was conducted to assess the ability of the animals to discriminate between odors that are structurally related to each other (only differ in carbon chain length). Experiment 9 was conducted to evaluate the long-term odor memory of the animals for a given odor combination after a recess in training for a given period of time. Finally, a control experiment (10) was conducted in which the lids and ventilators of the odor boxes were switched.

Table 3: Experimental design. The table shows the different odor combinations used.

| <i>Experiment</i> | <i>Rewarded odor (S+)</i> | <i>vs.</i> | <i>Unrewarded odor (S-)</i> |
|--------------------------------------|---------------------------|------------|-----------------------------|
| 1. Initial training | n-Amyl acetate | vs. | Blank (empty odor box) |
| 2. Initial odor discrimination | n-Amyl acetate | vs. | Anethole |
| 3. First negative stimulus transfer | n-Amyl acetate | vs. | 1,8-Cineol |
| 4. First positive stimulus transfer | (+)-limonene | vs. | 1,8-Cineol |
| 5. Second negative stimulus transfer | (+)-limonene | vs. | (+)- α -pinene |
| 6. Second positive stimulus transfer | (-)-carvone | vs. | (+)- α -pinene |

| | | | |
|---|----------------------|-----|-----------------------|
| 7. Double stimulus transfer task | Ethyl butyrate | vs. | 2-phenylethanol |
| 8. Odor discrimination with structurally related odorants | | | |
| C7 vs. C4 | n-Amyl acetate | vs. | Ethyl acetate |
| C7 vs. C5 | n-Amyl acetate | vs. | n-Propyl acetate |
| C7 vs. C6 | n-Amyl acetate | vs. | n-Butyl acetate |
| 9. Odor memory | | | |
| 2 weeks recess | Ethyl butyrate | vs. | 2-phenylethanol |
| 4 weeks recess | (+)-limonene | vs. | (+)- α -pinene |
| 8 weeks recess | n-Amyl acetate | vs. | Anethole |
| 16 weeks recess | (-)-carvone | vs. | (+)- α -pinene |
| 10. Control | Change of ventilator | | |

Experiment 1 was performed to demonstrate that the elephants can be trained to respond to a given odor. During the initial training the odor was presented by hand through the odor port on a petri dish. The petri dish was later placed in an odor box with a ventilator on and presented together with an empty odor box (for the different stages of training see table 2).

Experiment 2 was performed to demonstrate that elephants can be trained to discriminate between two given odors. The rewarded odor from experiment 1 was presented together with an unrewarded odor and the animals were to identify the correct odor and perform the operant response.

Experiments 3 and 5 were performed to demonstrate that the elephants can successfully make negative stimulus transfers. During a negative transfer the rewarded odor (S+) is kept constant while a new S- is introduced as the unrewarded odor.

Experiments 4 and 6 were performed to demonstrate that the elephants can successfully make positive stimulus transfers. During a positive transfer the unrewarded odor (S-) is kept constant while a new S+ is introduced as the rewarded odor.

Experiment 7 was performed to demonstrate that the elephants can successfully make a double-transfer. During a double-transfer both odors are replaced by a new rewarded odor (S+) and a new unrewarded odor (S-).

Experiment 8 was performed to assess the discrimination ability of the animals with structurally related odorants. One of the odorants (C7) from the chemical class of aliphatic acetic esters was chosen as the rewarded odor (S+) and during the initial phase the animals were allowed to become familiar with this S+ by using cineol as the unrewarded odor. Once familiarized with the S+, the cineol was replaced by one of the other odorants from the same chemical class and each of the critical odor combinations (C7 vs. C4, C7 vs. C5, and C7 vs. C6) were presented during two consecutive sessions. To avoid a possible order effect, the animals were assigned to different treatments. One elephant was assigned C4 as the first unrewarded odor, followed by C5 and C6 as the second and third S- while the second elephant was assigned C6 as the first unrewarded odor (followed by C5 and C4). Consequently the third elephant was assigned C5 as the first unrewarded odor (followed by C6 and C4). Between the different test combinations, two sessions with cineol as S- was implemented in order to boost the animal's confidence and to refresh its memory for the reward value of the C7.

Experiment 9 was performed to assess the long-term odor memory of the animals. After two, four, eight and 16 weeks of recess in testing, a previously used odor combination was presented to the animals during two consecutive sessions.

Experiment 10 was performed to affirm that the animals were actually responding to the odors and not to cues provided by the lids or ventilators of the odor boxes. During one session the lids of the odor boxes were switched so that the ventilator that was regularly used to present the rewarded odor, was placed onto the box that was used to present the unrewarded odor and vice versa.

3.7 Statistics

In the method applied here, the animal had two options: (1) to correctly respond to the correct odor (hit), and (2) to falsely respond to the incorrect odor (false alarm). To measure performance the percentage of correct decisions per session was calculated for each individual and each session.

In the initial learning tasks the criterion was set at 70 % hits, which corresponds to 21 correct out of 30 decisions in two consecutive sessions (corresponding to $p < 0.01$ two-tailed binomial test). In the discrimination tasks with structurally related odorants the criterion was set to 66.7 % correct in two consecutive sessions of 30 decisions each (corresponding to $p < 0.05$ two-tailed binomial test). The rationale for choosing these criterion levels were that similar standards have been used in previous olfactory studies, which allowed for comparisons of performance across species.

Correlations between discrimination performance and structural similarity of odorants in terms of differences in carbon chain length were evaluated using the Spearman rank correlation coefficient. Comparisons of performance across individuals were made using the Mann-Whitney U-test for independent samples. Comparisons of individual performance in the first and second transfer (negative and positive) as well as before and after recess in training were made using Wilcoxon Signed Ranks Test for related samples.

4 Results

4.1 Initial training and initial odor discrimination: Experiments 1 and 2

A total of twelve sessions were completed before all animals were ready for stage 7 of training (see table 2 for the different stages of training) in which the odor boxes were introduced. During stages 1-6, the animals had successfully learned to voluntarily approach the experimental set up and had made the association between bridge signal and food-reward. They had also learned to sample the two odor ports and search for the odor being presented through one of them. Upon identifying the odor and receiving a bridge signal, all elephants also reliably collected the food-reward through the grid above the odor port where the odor was presented.

From stage 7 and forward (see table 2), the animals had to perform the operant response, that is, lifting their trunk into position when identifying the rewarded odor, before being food-rewarded. Figure 3 shows the performance of the animals after the odor boxes were introduced. All three individuals reached the learning criterion (70 % correct decisions in two consecutive sessions) within two sessions ($p < 0.01$). This corresponds to 60 trials and 120 stimulus contacts. They all performed stable during the six sessions carried out (Figure 3) before the second odor was introduced as the S- during the initial odor discrimination task. All three individuals also mastered this task within two sessions ($p < 0.01$). Both Saba and Bua performed stable during the following 11 sessions while Saonoi performed below the

criterion during the fourth session when the first bridge was removed. However, the performance of Saonoi was well above chance level and rapidly improved during the following sessions.

A comparison of performance across individuals during the initial training and odor discrimination showed a difference between Saba and Saonoi ($z=-2.635$; $p < 0.01$) with Saba performing superior. No significant difference was found when comparing the performance of Saba to that of Bua ($z=-1.828$; $p=0.068$), or the performance of Saonoi to that of Bua ($z=-1.000$; $p=0.317$).

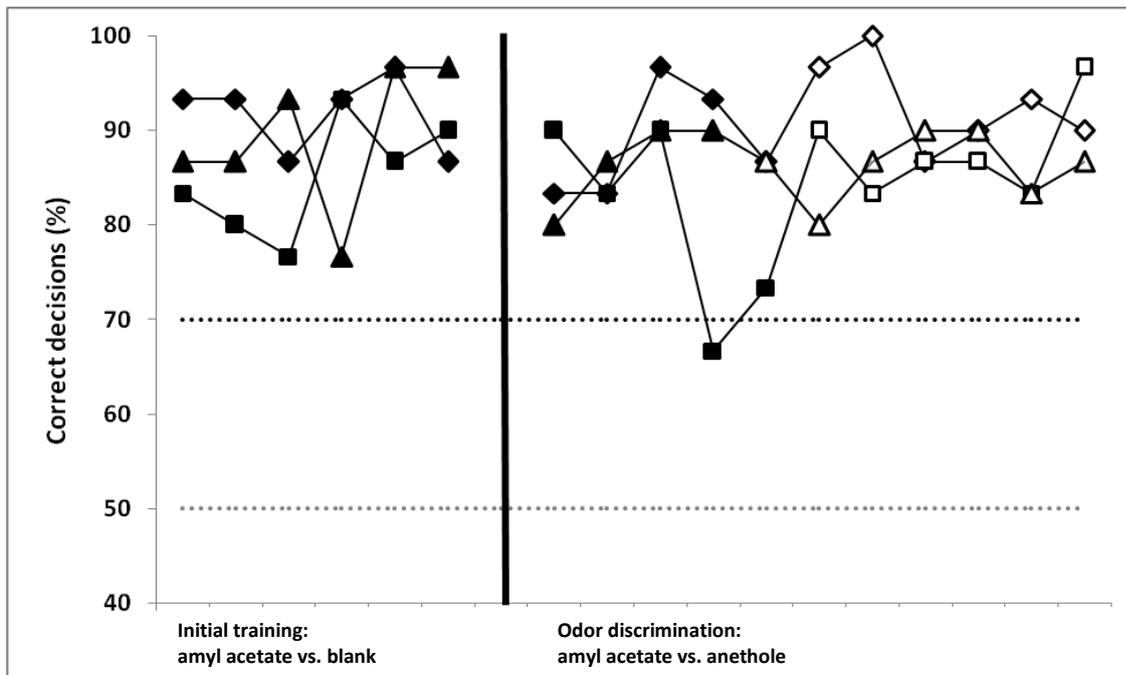


Figure 3: The performance of all three individuals (Saba: diamond, Saonoi: square, Bua: triangle) shown as percentage of correct decisions per session during the initial training and odor discrimination task. Each symbol represents one session of 30 trials. The learning criterion (70 % correct) is indicated by the horizontal black dotted line. The horizontal grey dotted line represents chance level and the black vertical line illustrates when a second odor was introduced as the (S-). The black symbols indicate when both the first and the second bridge were used during training.

4.2 Intramodal transfer tasks

The results from the five intramodal tasks are shown in figures 4 (Saba) and 5 (Saonoi and Bua) since the number of sessions were different for the two groups of individuals. A comparison of performance across individuals during the intramodal transfer tasks showed that Saonoi performed superior to Saba ($z=-3.265$; $p < 0.01$) and to Bua ($z=-3.372$; $p < 0.01$). No difference in performance was found between Saba and Bua ($z=-0.039$; $p=0.969$).

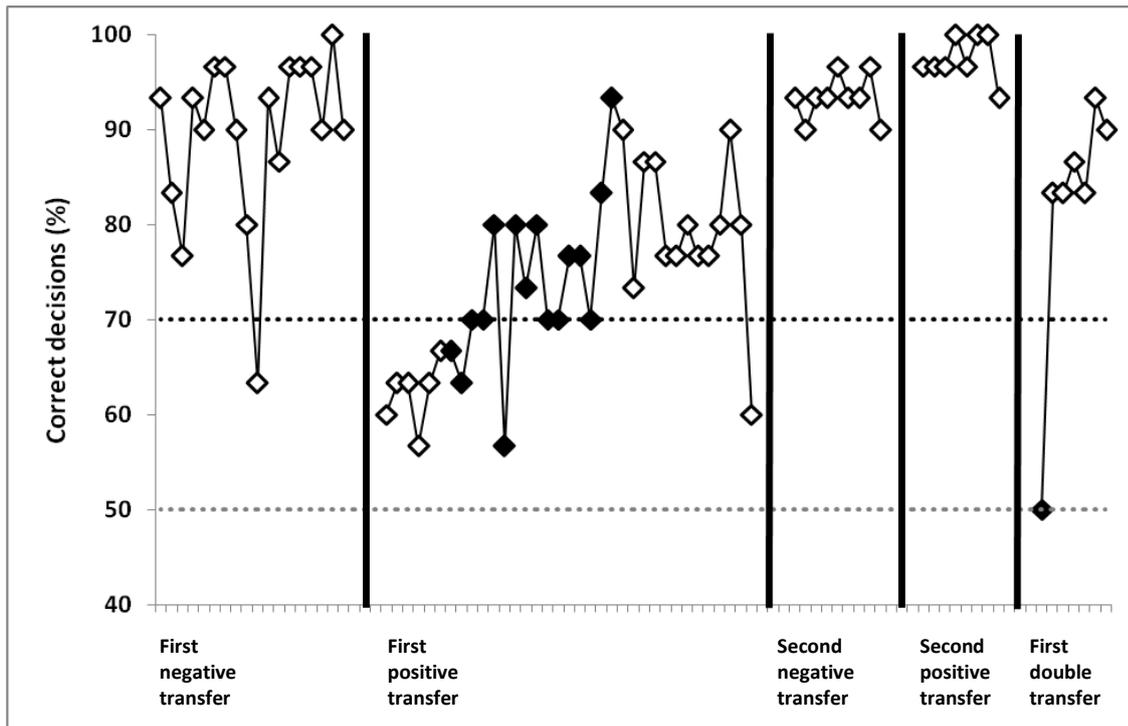


Figure 4: The performance of Saba during the five intramodal transfer tasks. The horizontal black dotted line represents learning criterion (70 % correct) and the horizontal grey dotted line represents chance level. The black vertical lines show when a new task was initiated. Each symbol represents one session of 30 trials and the black symbols indicate when both the first and the second bridge were used during training.

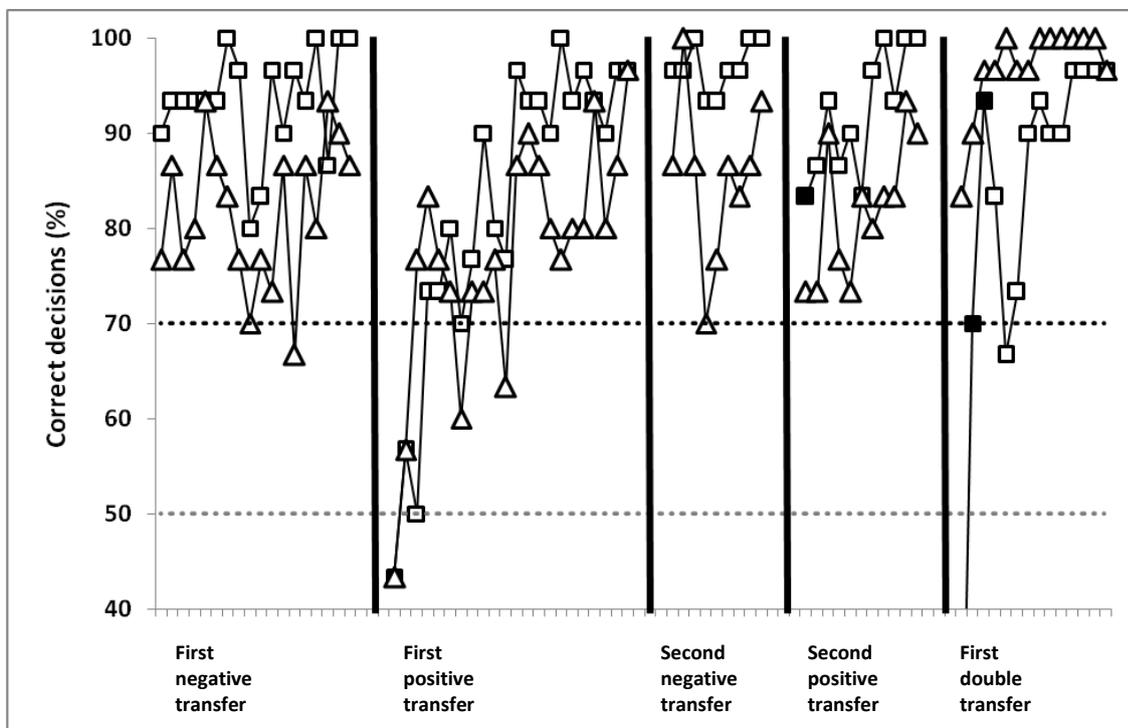


Figure 5: The performance of Saonoi (square) and Bua (triangle) during the five intramodal transfer tasks. The horizontal black dotted line represents learning criterion (70 % correct) and the horizontal grey dotted line chance level. The black vertical lines show when a new task was initiated. Each symbol represents one session of 30 trials and the black symbols indicate when both the first and the second bridge were used during training.

4.2.1 Negative stimulus transfers: Experiments 3 and 5

All individuals successfully mastered the two negative stimulus transfer tasks and all individuals reached learning criterion within two sessions for both transfers ($p < 0.01$). Figure 4 shows that Saba performed below criterion (but well above chance level) during one session (number 10) in the first negative transfer. During the second transfer she performed more stable and never scored less than 90 % correct decisions per session. Figure 5 shows that Bua also performed below criterion during one session (number 13) in the first negative transfer task. However, she was still performing well above chance level and in the second task, Bua never scored below criterion level. Saonoi performed stable during both negative transfers, scoring 100 % correct in four out of 18 sessions in the first transfer and 100 % correct in three out of nine sessions in the second transfer. The performance of Saba in both transfer tasks was superior to that of Bua (first transfer: $z=-2.051$; $p < 0.05$, second transfer: $z=-3.630$; $p < 0.01$) and so was also the performance of Saonoi compared to the performance of Bua (first transfer: $z=-2.933$; $p < 0.01$, second transfer: $z=-2.820$; $p < 0.01$). There was no significant difference in the performance of Saba compared to that of Saonoi in either tasks (first transfer: $z=-1.097$; $p=0.273$, second transfer: $z=-1.740$; $p=0.082$).

Even though the learning speed of the animals did not differ between the first and the second negative transfer, the performance level improved for Saonoi ($z=-2.124$; $p < 0.05$) in the second task when comparing the performance of the nine first sessions. No such significant improvement was evident for Saba ($z=-1.876$; $p=0.061$) or Bua ($z=-0.947$; $p=0.406$).

4.2.2 Positive stimulus transfers: Experiments 4 and 6

Figure 4 shows that it took Saba 10 sessions to reach the learning criterion ($p < 0.01$) in the first positive stimulus transfer task. Her performance was unstable and therefore both the first and the second bridge were used during sessions 7-22. However, in the second positive transfer task, Saba improved and reached the criterion within only two sessions ($p < 0.01$). In this second task, she performed stable and scored above 90 % correct during all sessions. Saonoi and Bua also reached criterion within two sessions in the second positive transfer task (see figure 5) while the first positive transfer took Saonoi five sessions and Bua four sessions to master ($p < 0.01$).

In the first positive transfer, Saonoi performed better than Saba ($z=-2.709$; $p < 0.01$) but otherwise no difference between individuals was found. In the second positive transfer, Saonoi performed better than Bua ($z=-2.820$; $p < 0.01$) as did Saba compared with Bua ($z=-3.630$; $p < 0.01$). There was no significant difference in the performance between Saba and Saonoi ($z=-1.740$; $p=0.082$).

When comparing the performance of Saba for the eight first sessions of each positive transfer task, her performance level improved from the first transfer to the second ($z=-2.536$; $p < 0.05$). The performance level of Saonoi ($z=-2.937$; $p < 0.01$) and Bua ($z=-2.674$; $p < 0.01$) also improved from the first positive transfer to the second when comparing the performance from the 11 first sessions of each transfer task.

4.2.3 Double stimulus transfer: Experiment 7

In the double transfer task, both Saba and Saonoi reached the learning criterion within three sessions while Bua already mastered the task within two sessions ($p < 0.01$). In the very first session when the two new odors were introduced, the performance of Saba was just about

chance level (see figure 4) but her performance quickly improved and she never scored less than 80% after this first session. Saonoi actively avoided the new S+ during the same first session (see figure 5) and scored only 16.7 % correct, but already during the next session her performance was above the criterion level again. Bua performed stable and above 80 % correct from the very first session. In seven out of 14 sessions she even scored 100 % correct during this transfer task.

The performance of Bua during the double transfer task was superior to that of Saba ($z=-3.303$; $p < 0.01$) and to that of Saonoi ($z=-3.350$; $p < 0.01$). There was no significant difference in the performance between Saba and Saonoi ($z=-1.056$; $p=0.291$).

4.3 Odor discrimination with structurally related odorants: Experiment 8

In this task, the elephants were presented with three different odor combinations (see table 3) for two sessions each. The S+ (amyl acetate) was kept constant during the entire experiment while the S- (ethyl acetate, propyl acetate and butyl acetate) was altered. Figure 6 shows the performance of the animals during the first session with each odor combination. Ethyl acetate (C4) was structurally the least similar to the S+ (C7) and butyl acetate (C6) was structurally the most similar to the S+. All three individuals performed above criterion level (66.7 % correct in two consecutive sessions) and were successful in discriminating between all of the three odor combinations ($p < 0.05$). A comparison of performances across individuals during the odor discrimination task showed no significant difference (Saba compared to Saonoi: $z=-1.750$; $p=0.091$, Saba compared to Bua: $z=-0.905$, $p=0.426$, Saonoi compared to Bua: $z=-1.032$, $p=0.405$).

The results showed a significant negative correlation between discrimination performance and structural similarity in terms of differences in carbon chain length ($r_s=0.905$, $p < 0.05$) with a decrease in performance with increased structural similarity.

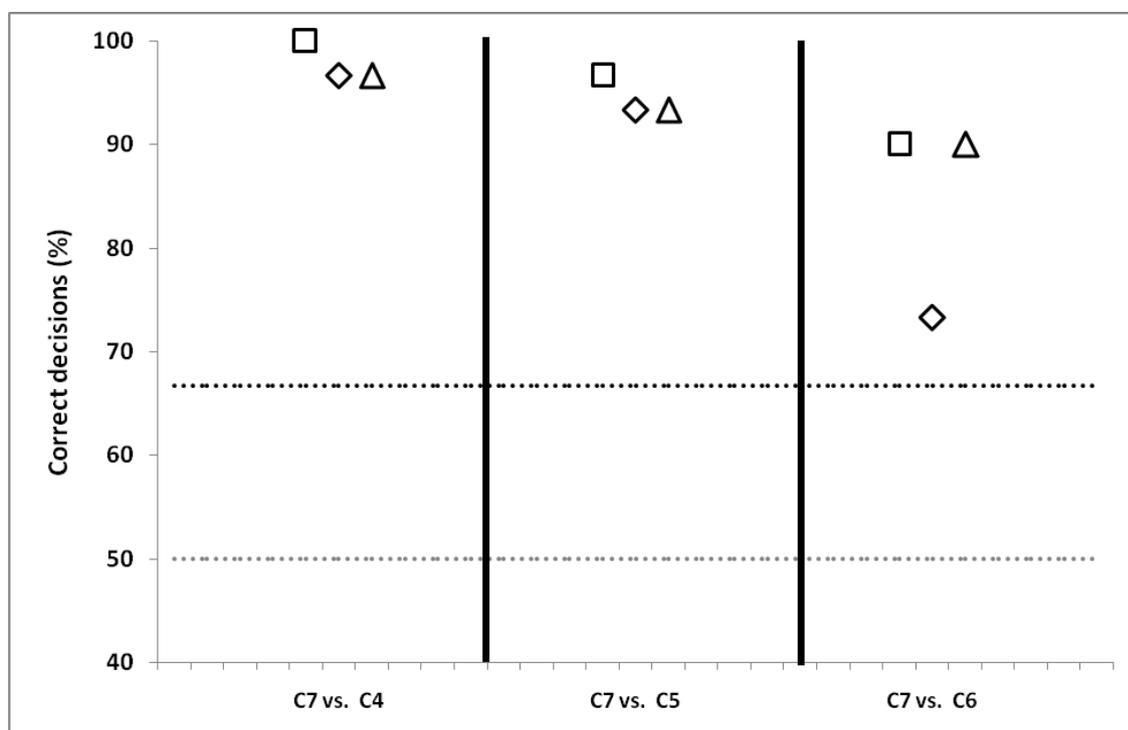


Figure 6: The performance of all three individuals (Saba: diamond, Saonoi: square, Bua: triangle) during the first session with each of the critical odor combinations in the odor discrimination with structurally related odorants. The black vertical lines show when a new

task was initiated. The horizontal black dotted line represents learning criterion (66.7 % correct) and the horizontal grey dotted line chance level.

4.4 Long-term odor memory: Experiment 9

After a given period of recess in testing, the elephants were presented with a previously learned odor combination to assess the long-term odor memory of the animals (see figure 7). After a two weeks recess, all three individuals were successful in remembering the reward value of both the S+ and the S- (in the odor combination ethyl butyrate vs. 2-phenylethanol) and their performance showed no significant difference between before and after the recess ($z=-0.816$; $p=0.750$). Bua even scored 100 % correct in the first session after the recess, while Sanoi and Saba only made one and two incorrect responses, respectively. Neither did the performance of the elephants differ when another odor combination (limonene vs. pinene) was presented after four weeks of recess ($z=-0.447$; $p=1.000$). All three individuals scored 90 % correct in the first session after the recess.

When a recess of eight weeks was implemented for a third odor combination (amyl acetate vs. anethole), it showed that the animals did not only remember the reward value of both the S+ and the S-, but even improved in performance after the recess relative to before the recess ($z=-2.214$; $p < 0.05$). Both Saba and Saonoi scored 100 % correct in the first session after the recess, while Bua made only one incorrect response. Finally, after a recess of 16 weeks for a fourth odor combination (carvone vs. pinene), the high performance of the elephants was still not affected ($z=-1.604$; $p=0.109$).

Comparisons of performances across individuals after the recesses showed no significant difference (Saba compared to Saonoi: $z=-1.825$; $p=0.068$, Saba compared to Bua: $z=-1.662$; $p=0.096$, Saonoi compared to Bua: $z=-0.269$; $p=0.788$).

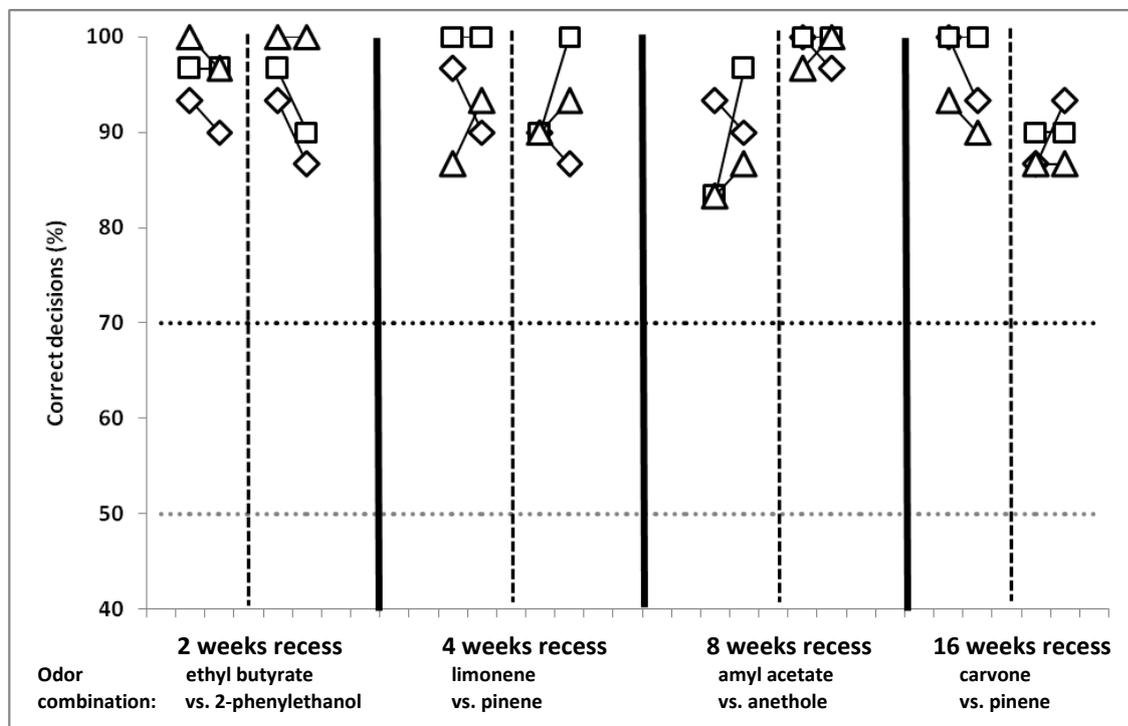


Figure 7: The performance of all three individuals (Saba: diamond, Saonoi: square, Bua: triangle) during the long-term memory tests. The figure shows the performance of the animals in the two last sessions before the recess in training, followed by the performance after two, four, eight and 16 weeks of recess (the recess is indicated by the vertical black dotted line),

for the given odor combination. The black vertical lines show when a new task was initiated. The horizontal black dotted line represents learning criterion (70 % correct) and the horizontal grey dotted line chance level.

4.5 Control: Experiment 10

When the lids and ventilators were switched during one session, the elephants showed no signs of being affected by the change. The performances of the animals during this session did not differ from the performances during the two sessions before ($z=-0.816$, $p=0.750$).

5 Discussion

The present study shows that Asian elephants can be trained to respond to a given odor and to discriminate between different odors. The animals readily mastered intramodal stimulus transfer tasks in which either the rewarded odor, or the unrewarded odor, or both odors were exchanged simultaneously for new odors. They were also able to discriminate between structurally related odorants belonging to the same chemical class, even when the S+ and the S- only differed by one carbon chain length. The long-term odor memory of the elephants proved to be excellent and the animals did not only remember the reward value of an odor combination after an eight week recess, but they even improved in performance after such a break.

5.1 The training and the method

The elephants used in this study were kept in a hands-on system, raised and handled with the traditional elephant training technique that involves a combination of negative reinforcement (i.e. physical punishment) and positive reinforcement (i.e. food-reward) (Whittaker and Laule, 2008). During this study the training was carried out in a protected contact manner and therefore only positive reinforcement (i.e. food-reward or bridge-signal) was used to shape the behavior of the animals (Laule, 2003). Even though this training method was new to the animals, they soon became accustomed to the training and testing procedure. From day one, all animals willingly approached the experimental set-up once they had been brought into the testing enclosure. Since the elephants were not restrained, they could move freely in the enclosure. In the very beginning of the training, the animals would sometimes lose interest or get distracted from outside disturbance (from other elephants vocalizing or making noise, workers and machines or visitors at the zoo) and thereby leave the experimental set-up for shorter or longer breaks. However, within two weeks of training, all individuals had learnt to maintain in position and readily worked the complete session without breaks. The only exception was when Saonoi was first introduced with the odor combination during the double stimulus transfer. During this session she avoided approaching the set-up and especially the new rewarded odor (ethyl butyrate), but already the next session, she performed stable again (see figure 5). For some unknown reason, she initially seemed to find this odor aversive. During the initial training, a session would last up to 30-40 minutes (depending on how many and how long breaks the animal would take), while during the later part of the study, a session would normally be completed within 5-6 minutes.

Even though the elephants were fed roughage ad-libitum, their motivation to earn the food-reward was never an issue. Once the training procedure had been established, the elephants would approach the experimental set-up immediately, ignoring food lying on the floor of the testing enclosure and work through the entire session before attempting to feed off these other items, suggesting the training itself to be rewarding for the animals. No signs of

satiation or loss of motivation to earn the food-reward was ever observed during training or testing.

Most elephants used in previous discrimination studies have been relatively young, but ranging in age from 5 to 47 years (Markowitz et al., 1975; Heffner and Heffner, 1982; Savage et al., 1994; Nissani et al., 2005; Shyan-Norwalt et al., 2010). In studies including older elephants, such individuals did not perform well (Markowitz et al., 1975; Nissani et al., 2005) and the expectations on Saba (43 years old) were therefore not very high. Saba was also separated early from her mother and kept isolated from other elephants during her upbringing which has resulted in abnormal behaviors such as extensive head-bobbing and weaving. Stress or disturbance during early development has been shown to affect elephants, both in terms of neurological development and behavior (Bradshaw and Schore, 2007). For successful development and learning, a young elephant is dependent on members from its family or social cohort in almost every situation (Lee and Moss, 2008). Early social context parameters influence the HPA-axis (which in turn affects and regulates behavior) so if the calf is removed from the mother during infant rearing, it can negatively affect the maturation of the brain and its plasticity which, in turn, may affect cognitive functions (Bradshaw and Schore, 2007). The kind of stereotypic behaviors that Saba exhibited is prevalent in captive elephants (Clubb et al., 2008) and was most often observed when routines were changed or when Saba was awaiting something (as being moved from one enclosure to another, having a shower etc.). As a result of the rather flexible time of training, no increase of stereotypic behaviors was observed in Saba, prior to sessions. Besides from Saba having a very brief episode of head-bobbing during one of the first training sessions, no such stereotypic behaviors were ever observed again during training or testing in this study, suggesting that the method was not stressful in any way to the animals.

During the initial training, before the odor boxes were introduced, it is possible that the animals were responding to both visual and olfactory cues since the odors were presented on a Petri dish by hand and at one side at a time. However, the opportunity for the animals to observe the trainer or to locate the Petri dish only by using their sense of vision, was still very limited due to the location of the experimental set-up. There was also a risk that the odor being presented in one port, would spread to the other port, making the decision of the animals somewhat more difficult. The rationale for still presenting the odor this way initially, despite these apparent disadvantages, was to make sure that the elephants actually sampled the odor ports before making the operant response. When the odor boxes were in place, they covered the odor ports and the trainer could no longer observe or control the elephant's odor sampling procedure and it was therefore crucial that this behavior was established in advance. However, once the odor boxes were introduced, the risk of mixing of odors was eliminated and visual cues were no longer available for the elephants. Given the elephant's limited visual acuity and rather poor eyesight (Shoshani and Eisenberg, 1982; Sukumar, 2003) and the fact that the elephants in the present study immediately responded to the correct odor once the odor boxes were presented, suggest that the animals primarily used olfactory cues rather than visual cues also during the initial part of the training.

5.2 Initial training, initial odor discrimination and intramodal transfer tasks

A comparison of performance of the elephants in the present study to that of other species trained in similar two-choice odor discrimination tasks shows that the elephants performed at least equally good or better than other species tested before. The speed of initial task acquisition of the elephants in the present study (120 stimulus contacts until reaching the criterion) is comparable to that of dogs (Lubow et al., 1973), rats (Slotnik et al., 1991) and mice (Bodyak and Slotnick, 1999), which all have been shown to need less than 150 stimulus

contacts to acquire an olfactory discrimination task. The speed of initial task acquisition of the elephants was clearly superior to that of South African fur seals (*Arctocephalus pusillus*), which needed 480-880 stimulus contacts to reach criterion (Laska et al., 2008). It was also superior to that found with three different species of primates: squirrel monkeys (*Saimiri sciureus*) (Laska and Hudson, 1993), spider monkeys (*Ateles geoffroyi*) (Laska et al., 2003), and pigtailed macaques (*Macaca nemestrina*) (Hübener and Laska, 1998) which needed 450-750, 660-720, and 960-1800 stimulus contacts, respectively, before mastering the initial task.

When comparing the learning speed of initial task acquisition for different species, one must keep in mind that the specific methods used in two-choice odor discrimination tasks might differ to some extent and the numbers should not be taken as absolute measurements. However, the performance of the elephants in the initial task acquisition shows that the learning speed of elephants is excellent and that they readily use olfactory cues when solving a learning task. When Rensch (1957) taught a young female Asian elephant to discriminate between two visual objects it took the elephant 330 trials, that is, 660 stimulus contacts, before mastering the task. Savage et al. (1994) also taught three female African elephants (*Loxodonta africana*) to perform a two choice visual discrimination task and these individuals needed 150-700 stimulus contacts before reaching criterion in the initial task acquisition. Another young female Asian elephant, trained in a two-choice auditory discrimination task by Heffner and Heffner (1982) reached criterion within two sessions. During each session, the elephant usually completed about 1000 trials, which show that more than 2000 stimulus presentations were needed before the individual could master the task. These results suggest that elephants use olfactory cues more readily than visual or auditory cues to solve a learning problem.

The negative stimulus transfer tasks presented no problems for the elephants and they all immediately performed above 70 % correct in the first session of each transfer. The change of the unrewarded odor had little or no effect on their high level of performance and these results are similar to those obtained for pigtailed macaques (Hübener and Laska, 1998) and spider monkeys (Laska et al., 2003). However, for both squirrel monkeys (Laska and Hudson, 1993) and South African fur seals (Laska et al., 2008), a similar change of the S- initially led to a decrease in performance level. This effect was not seen in the elephants in the present study. The elephants readily mastered the transfer of the S- within two sessions, that is, the minimum number of sessions needed to reach criterion and equals 120 stimulus contacts.

The first positive transfer, however, proved to be more of a challenge and while Saonoi and Bua needed five and four sessions, respectively, Saba completed ten sessions before reaching the learning criterion. Thus, the elephants needed between 240-600 stimulus contacts before mastering a first change of the rewarded odor. The performance of the elephants was comparable to the results obtained for the pigtailed macaques (Hübener and Laska, 1998), who needed 270-900 stimulus contacts, to master a first change of the rewarded odor. However, the performance was slightly inferior to that of the South African fur seals, who needed 80-240 stimulus contacts until reaching criterion (Laska et al., 2008), and clearly inferior to the performance of spider monkeys, who reached criterion immediately after the first positive transfer (Laska et al., 2003). This rather high number of stimulus contacts needed until understanding the task, might partially be explained by the rigidity of the elephant's learned behavior (Nissani, 2008) and the strict training method by which elephants are usually trained and kept (Nissani, 2006). When training elephants in a hands-on system, establishing the trainer as being dominant is essential for human safety and therefore the animals are taught to listen to commands and perform certain behavior sequences (Whittaker and Laule, 2008). Taking own initiatives or breaking these sequences are usually punished which might affect the way that animals approach a problem even in other situations (Nissani, 2006). In the present study, the animals had to explore, take initiative and make their own

decisions without being commanded to do so. Such a change in training method might at first have been confusing to the animals, especially when the exchange of the rewarded odor required the animals to decide for a new odor and change their response. Nissani (2006) assessed the ability of Asian elephants to apply causal reasoning in a tool-use task, and found that the animals easily learned to perform a motor pattern, but were reluctant in breaking the behavior sequence once it had been established. Even though these animals were Burmese logging elephants and kept under much more strict and harsh conditions than the elephants used in the present study, the training method is quite the same and could affect the way a problem is approached by the animals.

The comparatively high number of stimulus contacts before reaching the criterion and the rather inconsistent performance of Saba during the first positive transfer, was first believed to be due to a lack of understanding of the task itself. Given the problematic background and the rather high age of Saba, such a failure would not have been improbable. In two experiments exploring the behavior of 20 Asian elephants in simultaneous visual discrimination tasks, Nissani et al. (2005) found that age strongly affected the ability of the animals to acquire the discrimination tasks. Older elephants (20-30 years of age) failed to reach criterion in both tasks and were unable to learn the task in the number of sessions given. However, for Saba, once the unrewarded odor (cineol) was replaced by another S- (pinene), her performance immediately (i.e. during the first session) improved from 60 % correct to above 90 % correct. Her performance also instantly became much more stable, suggesting a difficulty in discriminating between the odors (limonene vs. cineol), rather than a lack of understanding of the task. However, none of the other individuals showed any signs of such difficulty regarding this odor combination.

In the second positive transfer, all individuals needed fewer stimulus contacts before reaching the learning criterion (i.e. two sessions) than during the first positive transfer. Similarly, in the double-transfer task, only two (Bua) or three (Saonoi and Saba) sessions were needed before the animals mastered the task. The decrease in the number of sessions and stimulus contacts needed before reaching criterion over time, suggests that the elephants showed a gradual learning of the task. Comparable results were obtained by Rensch (1957) when training a young Asian elephant to perform a visual object discrimination task. For the first object combination, the elephant needed more than 300 trials to reach criterion, while only ten trials were required by the fourth object combination. Another study of three African elephants and Californian sea lions (*Zalophus californianus*) in a two-choice object discrimination task showed that the sea lions needed fewer trials to reach criterion than the elephants did. However, once the elephants understood the concept, they needed fewer trials for mastering the task (Savage et al., 1994). The animals were presented with 24 object combinations and they were required to make ten correct responses consecutively before proceeding with the next object combination. In the beginning of the study, the elephants required significantly more trials before reaching criterion compared to the number of trials needed towards the end of the study. This was not the case for the sea lions whose performance did not change significantly over time. Even though the mice in the study of Bodyak and Slotnick (1999) readily mastered olfactory transfer tasks, this gradual learning of the task was not observed for these animals, suggesting that this specific feature might be exclusive for elephants.

There is an ongoing debate about the cognitive abilities of elephants. For example, several reports confirm that elephants readily use tools (Chevalier-Skolnikoff and Liska, 1993; Hart and Hart, 1994; Hart et al., 2001; Nissani, 2006), but the actual cognitive ability underlying this tool-use is sometimes questioned (Nissani, 2006). Some argue that elephants do possess extraordinary cognitive abilities such as, for example, mirror self-recognition (Plotnik et al., 2010), ability to show empathy (Bates et al., 2008a), understanding of invisible

displacement and person permanence (Bates et al., 2008c), understanding of cooperation (Plotnik et al., 2011), ability to make relative quantity judgment (Irie-Sugimoto et al., 2009) and show evidence of mean to an end behavior (Irie-Sugimoto et al., 2008). However, Nissani (2006) reported that elephants could not apply causal reasoning in a tool-use task and Povinelli (1989) found that elephants would readily use mirrors to find hidden food, but failed to show mirror self-recognition. These contradictive results obtained from different studies might more reflect both differences in the tasks and their set-ups, as well as large individual differences between animals and the relative few numbers of elephants often available when conducting a study, than an actual ability (or lack of ability) for a given task. In the present study, the cognitive ability of elephants was measured in form of the learning speed for mastering the given olfactory discrimination paradigm. The animals were to sample the odor ports and make their decision. If they sampled the correct port at first, they would often make the operant response directly. When sampling the incorrect odor port at first, they would move on to the correct port, sample this port and then make their decision. However, towards the end of the study, Saonoi was frequently observed shifting directly from the incorrect odor port, to lifting her trunk over the other (the correct) port, not having to sample the odor before making her decision. This suggests that she was able to make a more complex association between the stimuli and the operant response. This flexibility in her problem-solving strategy, that is, not simply performing a fixed behavior sequence but actually changing the sequence in response to a certain situation, would be an interesting subject to further investigate.

5.3 Odor discrimination with structurally related odorants

The results from the present study show that elephants can discriminate between structurally related odorants belonging to the same chemical class, even when these are structurally very similar and only differ by one carbon chain length. A significant negative correlation between discrimination performance and structural similarity of the acetic esters in terms of differences in carbon chain length was evident in the present study, and in accordance with the results from other studies with squirrel monkeys and humans (Laska and Freyer, 1997; Laska and Hübener, 2001). Esters constitute a major part of the odors of a variety of fruits (Sun Pan and Kuo, 1994) and even though the Asian elephant is a mixed feeder that mainly feeds off browse and grass (Steinheim et al., 2005) it also selectively feeds on seeds and fruits when available (Kitamura et al., 2007). The ability of the elephants in the present study to discriminate between the structurally related acetic esters therefore makes sense from an ecological point of view.

Esters have also been identified in urine from African elephants, where the urine from young non-musth males contained a higher concentration of esters and acids than that from males in musth (Rasmussen and Wittemyer, 2002) suggesting a role for this class of odorants in assessing the reproductive status of an individual. For a social species as the Asian elephants which strongly rely on the sense of smell for individual recognition and social status assessment through chemical signals (Schulte and Rasmussen, 1999; Greenwood et al., 2005; Bagley et al., 2006; Bates et al., 2008c), the ability of the elephants in the present study to discriminate between structurally related acetic esters was therefore not unexpected. Further tests are required to assess the capability of elephants to differentiate among other structurally related odorants and to assess whether the discrimination ability shown for the acetic esters is restricted to this chemical class or is also true for a broader array of odorants.

5.4 Long-term odor memory

After two-, four- eight and even 16 weeks of recess in testing, the elephants in the present study showed excellent retention of the reward value of both the rewarded and the unrewarded odor. This finding of an excellent long-term odor memory is in agreement with the results from other studies assessing the same capability in species such as South African fur seals (Laska et al., 2008), squirrel monkey (Laska and Hudson, 1993), spider monkeys (Laska et al., 2003), and pigtailed macaques (Hübener and Laska, 1998; Hübener and Laska, 2001). After the eight weeks recess, the elephants even improved in performance relative to before the recess. The odor combination used to assess the long-term odor memory after the eight weeks recess was first introduced to the animals in the very beginning of the study, when the method was still new to the elephants. Once the odor combination was reintroduced towards the end of the study the animals were more confident and certain in their decisions which might also explain the improvement in performance that was evident for all individuals.

The results from the present study are also in accordance with the only previous study examining the odor memory of elephants. Rasmussen (1995) found evidence of long-term odor memory in Asian elephants when examining the response of males to female urine. When elephants examine urine from other individuals, they often use the trunk to sample the substance and transfer it via the trunk tip to the roof of the mouth where the vomeronasal organ is located (Rasmussen and Munger, 1996). This is called a flehmen response and allows the individual to detect and evaluate the chemical signals in the urine (Sukumar, 2003). Elephant bulls show a high frequency of flehmen responses to preovulatory female urine, but the males in the Rasmussen study (1995) did not respond in this way when presented with preovulatory maternal urine. The fact that they had been physically separated from their mother from two and up to 27 years suggests that elephants possess a long-term odor memory that allow filial-to-maternal recognition and serves to avoid inbreeding between closely related individuals (Rasmussen, 1995).

The long-term memory of elephants is often said to be outstanding (Hart et al., 2008; Byrne and Bates, 2009) but few studies have scientifically tested this statement (Irie and Hasegawa, 2009). However, the auditory memory of elephants has been shown to last over an extended period of time. In a study by McComb et al. (2000), family groups of wild African elephants were shown to recognize the calls of members that were not longer present and had been absent for two to up to twelve years. The calls from these absent family members elicited the same response in the family group as other calls from known group members. The elephants showed no such response to the call of individuals from unrelated or unknown family groups, suggesting that elephants possess an excellent long-term auditory memory.

The memory tests for the two-, four- and eight-weeks of recess in testing in this study, were implemented in between other tasks, while the 16-weeks memory test was conducted after a total recess in testing for eleven weeks. During this period of time, the elephants were not subjected to any olfactory training or testing. Still, after this longer recess, there was no change in either the behavioral response or the discrimination performance in any of the individuals. This demonstrates that the animals did not only show excellent retention of an odor combination, but that they also remembered how to execute the task they had learned to perform even after a total recess of almost three months. Another memory-test performed on a 20 year old captive Asian elephant which was retested in a visual discrimination task eight years after she had first been trained to perform the task showed excellent retention. Only six minutes was needed before the elephant reached the criterion of achieving 20 correct responses in a row and she made only two errors, showing no problem in remembering the task she had learned years ago (Markowitz et al., 1975). The results of the present study strengthen the evidence for an outstanding long-term memory in elephants.

5.5 Behavioural relevance of the sense of smell in the Asian elephant

The ability of the elephants in the present study to discriminate between odors and structurally related acetic esters was found to be excellent. Since esters are emitted by a variety of fruits (Sun Pan and Kuo, 1994) it seems reasonable to assume that elephants have a sensitive olfactory system that responds also to other chemical signals emitted by desirable food such as grass and seeds. To what extent, is yet to be evaluated. Wild Asian elephants seem to use olfactory cues when deciding where and when to forage and seem to be able to time the arrival in certain areas with the time of ripening of preferred food (Santiapillai and Read, 2010). Human-elephant conflicts often arise in areas where elephants raid crop-fields during the time of ripening, which coincides with harvest. This causes danger to the farmers as well as an increase in the number of elephants killed by humans (Chelliah et al., 2010; Hedges and Gunaryadi, 2010; Santiapillai and Read, 2010), which might be detrimental to the already highly endangered elephant population (Dale, 2010). Using chemical signals as a repellent against wild elephant populations could aid in solving the human-elephant conflict in such areas (Schulte and Rasmussen, 1999) and has proven effective at least in short-term (Osborn and Rasmussen, 1995; Chelliah et al., 2010; Hedges and Gunaryadi, 2010). The development of more long-term and reliable methods with effective chemical signals would be useful in modifying the behavior of the elephants and assist in successful conservation (Schulte and Rasmussen, 1999). However, for the development of such methods, the olfactory abilities of elephants need to be further examined, especially regarding discrimination ability and sensitivity for behaviorally relevant odors.

Even though a well developed sense of smell is essential in foraging, the most prominent area of usage for elephant olfaction seems to be in a social context. Chemical communication in elephants has been thoroughly examined and is considered an essential mechanism in regulating the behavior of elephants (Rasmussen, 1998; Rasmussen, 1999; Rasmussen and Krishnamurthy, 2000). Female Asian elephants live in closely related family groups while the young males disperse from their natal group when reaching adolescence (Fernando and Lande, 2000; Vidya and Sukumar, 2005). This complex society requires a well functioning communication system to maintain group cohesion and to locate reproductive partners, and the long-distance communication is made possible through infrasonic vocalizations and chemical signals (Rasmussen 1999; Langbauer, 2000, Sukumar, 2003). Chemical signals are released in breath and urine as well as through secretions from numerous glands (Rasmussen and Krishnamurthy, 2000), and are thoroughly investigated by other individuals and processed by the olfactory and vomeronasal systems. The signals are important in individual recognition, and tell the receiver whether the emitter belongs to the same or to a different family group (Bates et al., 2008c). The signals also tell the receiver about the social status of the emitter and are useful for determining the reproductive state of individuals of the opposite or the same sex (Schulte and Rasmussen, 1999; Greenwood et al., 2005; Bagley et al., 2006; Meyer et al., 2008). This form of communication is precise, and Asian elephants have been shown to be able to discriminate between, for example, different enantiomers of the pheromone frontalol. This pheromone in combination with testosterone is released by male Asian elephants in a specific ratio dependent on age and stage of musth, and the behavior of the receiver changes in response to altered ratios (Schulte and Rasmussen, 1999; Greenwood et al., 2005). In captivity, the reproduction of elephants is rather poor (Mason and Veasey, 2010; Dale, 2010) and for successful conservation of the species, a more successful breeding program is necessary. Given the importance of chemical communication among elephants and its role in reproduction (Rasmussen and Schulte, 1998; Rasmussen et al., 2005; Schulte et al., 2007) a deeper knowledge of the biologically relevant chemicals could lead to a more successful reproduction of captive Asian elephants.

Another reason for the poor reproduction and survival of captive elephants (Mason and Veasey, 2010) is probably related to the captive environment and is most obviously evident in the high frequency of stereotypic behaviors that the captive elephant population exhibits (Clubb et al., 2008). Even with the best intentions, the zoos cannot create such a complex environment as the wild would offer. At Kolmården Wildlife Park, environmental enrichment is part of the daily routines for the elephant keepers, but still, there is a limitation in, for example, food-enrichment, which needs to be weighed against nutritional value and risk of obesity. Since the movements of elephants are restricted, and inactivity is a common problem in captive environment (Stoinsky et al., 2000; Rees, 2009), food might not be the sole solution in such situations. Food-enrichment for captive elephants has been little studied but inactivity has been found to be reduced when replacing hay with browse (Stoinsky et al., 2000) and a flexible feeding schedule has been proposed as a tool to reduce stereotypic behaviors and increase the activity level of elephants (Rees, 2009). Also auditory enrichment has been found to reduce stereotypic behavior in captive Asian elephants (Wells and Irwin, 2008). As a complement to food-enrichment, stimulation by odors could provide the elephants with a more complex environment (Schulte et al., 2007) that would enhance, for example, exploratory behavior, which, in turn, would increase the amount of exercise for the animals. However, one must be cautious when applying odors as environmental enrichment, since different chemicals may elicit different responses. For example, elephants have few potential predators except for humans, and among humans, only some ethnic groups in specific areas, pose a real threat to the immediate survival of the elephants. In an evolutionary perspective, it would be adaptive for the animals to be able to distinguish between these subgroups. A study conducted on African elephants in Amboseli National Park, Kenya, showed that elephants could classify humans into separate subgroups dependent on the level of threat these subgroups posed to the animals, and that this classification was based on olfactory and visual cues independently (Bates et al., 2007). The fearful reaction of the animals towards a specific odor or odor combination, in this case emitted by a group of people, shows that the past experience of an odor by the animals affects the reaction towards the stimulus. The reaction of elephants towards a biological relevant odor is also likely to be dependent on the physiological state of the individual (Schulte and Rasmussen, 1999; Schulte et al., 2007). For example, pheromones present in preovulatory female urine, bring forth a strong interest in conspecific males but little interest from other female elephants (Schulte et al., 2005) while the pheromone frontalin, secreted by males exhibiting musth, repels non-musth males and pregnant females but seems to attract oestrous females (Schulte et al., 2007). If odors are to be used as environmental enrichment, it is important to examine which kind of odors might elicit fearful reactions and which odors might serve as positive signals that will promote natural behaviors in the elephants.

Conclusions

The present study shows that Asian elephants can be trained to respond to a given odor and that their olfactory learning performance is equally good or even better, than other species tested in similar studies before. The discrimination performance of the elephants regarding structurally related odorants, decreased with increasing structural similarity of the odorants, but the animals could still discriminate between aliphatic acetic esters even when they only differed by one carbon chain length. The long-term odor memory of the elephants also proved to be excellent in that they successfully remembered the reward value of previously learned odor stimuli after up to 16 weeks of recess in testing. The method applied in this study has been shown to be an effective technique to assess the discrimination ability of elephants for a variety of odorants. The method involved no compulsion or punishment, which is beneficial

from both an animal welfare perspective and from public concern. The method can easily be used to further investigate the olfactory capabilities of elephants and to gain knowledge about the importance of olfaction in a wide variety of contexts, such as foraging, social, or human-elephant associations. To better understand the chemical world of elephants, the sensitivity and discrimination ability for other biologically relevant odors should first of all be further examined.

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