Training of spider monkeys in a food-rewarded two-choice olfactory discrimination paradigm and assessment of olfactory learning and memory performance

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Titel
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Författare
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Sammanfattning
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Nyckelord
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1 Abstract

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Keywords: *Ateles geoffroyi*, behavioral testing, discrimination learning, odor memory, olfaction, olfactory discrimination, olfactory learning, spider monkey

2 Introduction

Among mammals, primates are traditionally considered to have a reduced sense of smell (Rouquier et al. 2000) and to rely more on other senses such as vision (Dominy and Lucas 2001). This view is supported by genetic and neuroanatomical features such as the number of functional olfactory receptor genes (Roquier et al. 2000) and the relative size of olfactory brain structures (Brown 2001) rather than by behavioral studies. However, primates have been shown to use olfactory cues in a number of behaviors including food identification and selection (Bolen and Green 1997, Nevo et al. 2015), territorial defense (Mertl-Millhollen 1988, Garber et al. 1993, Klailova and Lee 2014), communication of social and reproductive status (Converse et al. 1995, Heymann 1998, Kappeler 1998, Smith and Abbott 1998, Klailova and Lee 2014) and recognition of conspecifics and group members (Epple 1970, Epple et al. 1988, Smith et al. 1997). Thus, it

should be assumed that olfaction in fact has behavioral relevance for primates and that they are also capable of olfactory learning.

Spider monkeys (*Ateles geoffroyi*) are a frugivorous, arboreal and relatively large species of New World primates, with a geographic range from southern Mexico to Panama (Campbell 2008). They live high in the canopy in groups of varying sizes, they are among the few primate species possessing a fully prehensile tail and they lack a thumb (Campbell 2008). In 2003, Laska et al. introduced a behavioral test which allowed, for the first time, the investigation of olfactory performance in spider monkeys. Briefly, animals are trained to discriminate between two simultaneously presented odorants, one of which indicates the presence of a food reward and the other one indicates the absence of a food reward. After the initial shaping, animals are subjected to so-called intramodal transfer tasks, where the odorants are exchanged for novel ones. Using this method, Laska et al. (2003) showed that spider monkeys can learn to discriminate between odors and that they can remember the reward values of previously learnt odors even after a 4-week break. Further, they showed that the same method is suitable for investigating olfactory sensitivity in spider monkeys. Since then, using the same method, several studies assessed olfactory sensitivity in spider monkeys (see for example Laska et al. 2005, Laska et al. 2009, Løtvedt et al. 2012, Eliasson et al. 2015). Spider monkeys have now been shown to possess a highly developed sensitivity for structurally related monomolecular substances such as aliphatic esters (Salazar et al. 2003), carboxylic acids (Laska et al. 2004) and monoterpenes (Joshi et al. 2006) and for other compounds such as amino acids (Wallén et al. 2012). In some cases, spider monkeys outperform even dogs and rats who are generally considered to possess a well-developed sense of smell (Moulton 1960, Passe and Walker 1985, Salazar et al. 2003, Laska et al. 2004), questioning the notion that the relative size of olfactory brain structures or the number of functional olfactory receptor genes would correlate positively with olfactory performance and that primates in general would possess only poor olfactory capabilities.

As demonstrated by the studies just mentioned, primates are clearly able to use their sense of smell in discriminating between objects. Further, they are prepared to do so even on the basis of relatively weak odors and after considerably long retention intervals. However, there are so far only few studies considering olfactory learning in primates (Laska and Hudson 1993, Hübener and Laska 1998, Hübener and Laska 2001, Laska et al. 2003). The testing paradigm that was

developed for assessing olfactory discrimination ability, sensitivity and odor memory in spider monkeys is also suitable for studying olfactory learning in this species of New World primates.

Thus, the first aim of the present study was to train five adult, experimentally naïve spider monkeys and to collect data on their learning speed for acquiring the olfactory discrimination paradigm developed by Laska et al. The second aim was to collect data on the spider monkeys' performance in a series of intramodal transfer tasks. More specifically, I assessed if they can form a learning set (Harlow 1949), that is, if they can "learn to learn" using odor stimuli. The concept of learning sets states that initially, an animal learns to solve a problem using trial and error, and later, when confronted with a problem of the same kind but different stimuli, it applies the previously gained knowledge and needs fewer and fewer trials to solve new problems (Harlow 1949). The third aim was to assess the spider monkeys' olfactory memory performance by presenting the animals with familiar odor pairs after different retention periods and finally, to compare the spider monkeys' olfactory learning and memory performance to that of other species tested previously on comparable tasks.

To summarize, the four main aims of the present study were 1) to train five adult spider monkeys in a food-rewarded two-choice olfactory discrimination paradigm and to assess their initial olfactory learning performance, 2) to assess their learning performance in a series of intramodal transfer tasks, 3) to assess their olfactory memory performance and 4) to compare their olfactory learning and memory performance to that of other species.

3 Materials and Methods

3.1 Subjects

The study was conducted using five adult spider monkeys (*Ateles geoffroyi*), two males (Gruñón, eleven years and Neto, seven years) and three females (Margarita, Mary and Paulina, all eight years). The animals were maintained at the field station Pipiapan of the Universidad Veracruzana, near Catemaco, Veracruz, Mexico. They were fed fresh fruits and vegetables ad libitum once a day. The data were collected between May 2018 and October 2018, during 108 working days.

3.2 Test apparatus

The test apparatus was a 50-cm long and 6-cm wide metal bar with two cube-shaped PVC-boxes (side length 5.5 cm) attached to the metal bar at a distance of 22 cm (Figure 1). The boxes were equipped with metal clips that allow the attachment of odorized filter paper strips and with lids that the spider monkeys are able to open. The filter paper strips used (Schleicher & Schuell, Einbeck, Germany) were about 5 cm long and they were impregnated with 20 µl of a given odorant. Inside each box was a shallow deepening, allowing the animals to retrieve a food reward in the case of a correct decision. The food reward used in the present study was mainly Honey Cheerios®. On rare occasions, when the animals needed further motivation, other rewards, such as raisins, other cereals, peanuts and pieces of cookies were used as food reward. At the beginning and at the end of each session the animals were given a piece of a Maria cookie, presented together with a filter paper strip bearing the rewarded odor (see chapter 3.3).

Figure 1 The test apparatus for the food-rewarded two-choice olfactory discrimination paradigm. The lids of both boxes have a metal clip that allows the attachment of odorized filter paper strips.

3.3 The behavioral test

The method used in the present study has previously been used with minor adjustments with spider monkeys and has been described in detail by Laska et al. (2003). It is a food-rewarded two-choice olfactory discrimination paradigm, and it is based on an instrumental conditioning procedure in which animals learn to discriminate between two simultaneously presented odor

stimuli: a rewarded odor, and a non-rewarded odor. The animals were trained to sniff at two odorized filter paper strips attached to the apparatus (see chapter 3.2) and to use these odor stimuli as cues to identify the rewarded odor. They were allowed to sniff the papers as many times as they wished before making the decision, which they indicated by opening the corresponding box of the apparatus. In the case of a correct decision, the animals retrieved a food reward from the apparatus and in the case of an incorrect decision, they were not allowed to correct themselves (and they stayed without food reward). After each decision the apparatus was removed and, out of sight of the animal, prepared for the next trial. Usually ten trials were conducted per session per animal and each animal performed three sessions a day. The learning criterion was set at 24 correct decisions out of 30 daily trials (80 %), corresponding to $p < 0.01$ in a two-tailed binomial test. To assess learning speed with a given odor pair, the number of trials an animal needed to reach the criterion was recorded.

The method described here was used in all experiments of the present study: in initial shaping, in intramodal transfer tasks and in assessment of long-term odor memory (see chapter 3.5). Once an animal had learnt to discriminate between the initial odor pair, it was subjected to intramodal transfer tasks where the rewarded odor (positive transfer), the non-rewarded odor (negative transfer) or both the rewarded and the non-rewarded odors (double transfer) were exchanged for novel odorants. Odor memory assessment consisted of presenting an animal after a certain period of time with an odor pair it had previously learnt to the criterion.

3.4 Odor stimuli

The odorants and dilutions used in the present study are listed in the Appendix 1. The rationale for choosing these odorants was to present the animals with stimuli that are readily discriminable, that is, qualitatively different from each other based on human perception. In addition, some odorants used in the present study had already been used successfully in previous studies on odor discrimination learning in primates and in non-primate mammals (see for example Laska 1990, Laska et al. 1996, Hübener and Laska 2001, Laska et al. 2003, McBride and Slotnick 2006, Arvidsson et al. 2012). The odorants were diluted by using the nearly-odorless solvent diethyl phthalate (CAS# 84-66-2). The dilutions were chosen so that human subjects perceived all odorants at approximately equal subjective intensities. The dilutions ranged between 1:50 and 1:500.

3.5 Experimental design

The present study consisted of three main parts: 1) initial shaping, which included the training of five spider monkeys in a food-rewarded two-choice olfactory discrimination paradigm, 2) intramodal transfer tasks, where the animals were presented with novel odorants in the same paradigm and 3) the assessment of long-term odor memory by presenting the animals with previously learnt odor pairs after different time periods, using the same paradigm. In each part, the number of trials needed to reach the pre-set learning criterion (24/30 correct decisions) was recorded.

3.5.1 Initial shaping

The initial odor pair that was used to get the animals familiar with the paradigm was n-pentyl acetate (banana-like odorant, designated as A+) as rewarded odor and 1,8-cineole (eucalyptus-like odorant, designated as B-) as non-rewarded odor. Both were diluted to concentrations of 1:100. The animals were trained with these two odorants until they had learnt the method and when they had reached the learning criterion at least three times in total and at least twice in a row.

3.5.2 Intramodal transfer tasks

After an animal had learnt the initial odor pair to criterion, it was subjected to a series of intramodal transfer tasks. First a positive transfer was conducted: the first rewarded odor, the banana-like n-pentyl acetate (A+), was replaced by the anise-like anethole (designated as C+) while keeping the non-rewarded odor, the eucalyptus-like 1,8-cineole (B-), constant. Once an animal had learnt to distinguish between these two odorants i.e. when it had reached the learning criterion at least twice in total and when it scored at least 75 % of correct decisions during the latest six sessions (60 trials), it was subjected to the first negative transfer: now already learnt rewarded odor, the anise-like anethole (C+), was kept the same and the non-rewarded odor, the eucalyptus-like 1,8-cineole (B-) was replaced by the citrus-like (+)-limonene (designated as D-). The additional criterion of 75 % of correct decisions during the latest six sessions was used in order to make sure that an animal had really learned to discriminate between the odor pair in question before moving on to further transfer tasks. After an animal had mastered the first negative transfer (again, when it had reached the learning criterion at least twice in total and when it scored at least 75 % of correct decisions during the latest six sessions (60 trials)), it

moved on to the second positive transfer and then to the second negative transfer task. After mastering the second negative transfer task, an animal was subjected to several double transfer tasks where both odorants were exchanged for novel ones at the same time. Depending on the learning speed of each individual, the animals went through 0-6 double transfers. Table 1 shows the complete sequence of all the transfer tasks.

Table 1 Sequence of the transfer tasks. Each letter represents a different odorant (for letter abbreviations, see Appendix 1).

Rewarded odor	Non-rewarded odor	
A+	B-	initial odor pair
C+	B-	1st positive transfer
C+	D-	1st negative transfer
E+	D-	2nd positive transfer
E+	F-	2nd negative transfer
G+	H-	1st double transfer
I+	J-	2nd double transfer
K+	L-	3rd double transfer
M+	N-	4th double transfer
O+	P-	5th double transfer
Q+	R-	6th double transfer

3.5.3 Assessment of long-term odor memory

Long-term odor memory performance was investigated by presenting an animal with odor pairs that it had previously learnt to the criterion after different time periods had passed since a certain odor pair had been presented the last time. Memory tasks were conducted the same way as other tasks, with the exception that during the memory tasks the animals were not presented with a filter paper strip bearing a rewarded odor at the beginning and at the end of each session. The number of trials needed to reach the learning criterion again was then compared to the number of trials needed to reach the learning criterion when the animal was presented with a certain odor

pair the first time, that is, during initial learning. It was a requirement that the same period of time had passed since the presentation of both the rewarded odor and the non-rewarded odor in question. Thus, only the individuals that had mastered several double transfer tasks participated in this test. Time periods and odor pairs used for each individual's memory tasks varied: there were memory tasks of two, three, four, five and six weeks, considering odor pairs E+F-, G+H-, K+L- and M+N-.

3.6 Training Procedure

Usually each animal performed three sessions of ten trials each day and the training (and also all further experiments) took place in the morning before feeding time, between 7.30 am and 1.30 pm. However, to speed up the learning process, some afternoon sessions between 2 pm and 5 pm were also conducted. Sometimes the animals were not motivated to work, and, on those days, it was not possible to finish all 30 trials. Most working weeks were from Monday to Friday but sometimes data were also collected during weekends. The longest break the animals had without working with the method used in this study was three days.

3.6.1 First step

The training was started by presenting the animals with a filter paper strip bearing the first rewarded odor, the banana-like odorant n-pentyl acetate (A+), together with a food reward. This was done manually, holding the paper strip close to the animal's nose using forceps and giving the reward using another forceps. Little by little a time gap between presenting of the odorized filter paper strip and giving the food reward was added. The animals were now presented only with the filter paper strip bearing the rewarded odor and as soon as they approached it closely with their face, they were rewarded. At this point the animals were still allowed to bite or lick the paper, but little by little they were required to approach the filter paper strip without direct physical contact. If they tried to bite the filter paper strip, it was withdrawn a little bit and held close to their nose again while they took the food reward. An animal moved to the next phase of training after it had started to reliably sniff or at least approach (depending on every individual's personality) the odorized filter paper strip without seeing the food reward first.

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3.6.2 Introduction of the test apparatus

At the next step of the training procedure the experimental apparatus was introduced. A filter paper strip bearing the first rewarded odor, the banana-like n-pentyl acetate (A+) was attached to one of the apparatus' two boxes and as soon as an animal approached the box (with its face) where the filter paper strip was attached, it was manually rewarded, using forceps. If an animal only investigated the box without the filter paper strip bearing the rewarded odor, the apparatus was occasionally removed and prepared for the next trial, without rewarding the animal. More often, however, to promote the animals to learn the required behavior, they were encouraged to investigate also the box bearing the odorized filter paper strip. This was done by moving the box bearing the filter paper strip with the rewarded odor close to the animal's nose and then immediately rewarding the animal. From this phase onwards, during one session (ten trials) five times the left and five times the right box was equipped with the paper strip bearing the rewarded odor. The presentation of a rewarded odor on the left or right box was pseudorandomized and the same box was never equipped with a rewarded odor more than three times in a row. This was done in order to prevent an animal from developing a side preference. An animal moved to the next phase of training as soon as it had reliably started to approach the filter paper strip attached to the apparatus and to retrieve the food reward from the forceps.

3.6.3 Baiting the apparatus

The third step of the training procedure was exactly the same as the previous step, with the exception that now the food reward was put inside the box bearing the filter paper strip with the rewarded odor, instead of giving it manually using forceps. The box bearing the odorized filter paper strip was baited with a food reward and when an animal approached it, the experimenter opened, (or allowed an animal to open) the box and let an animal retrieve the food reward. The animals that took part in the present study had previously been working with a similar apparatus and thus all of them already knew how to open the boxes. The lids of the boxes were held closed by the experimenter so that it was not possible for the animals to open the boxes prior to olfactory investigation. This was also the case for all further steps. Occasionally, if an animal only approached the box that was not bearing the odorized filter paper strip, it was allowed to open the lid of the corresponding box. It was shown that there was no reward inside that box and then the apparatus was removed and prepared for the next trial, without allowing an animal to investigate and open the baited box bearing the odorized filter paper. However, like in the previous step, in

most trials an animal was encouraged to investigate also the box bearing the filter paper strip with the rewarded odor. Upon doing this, the lid was opened, and an animal was allowed to retrieve the food reward. Thus, in some trials the animals were allowed to open the first box they investigated but, in most trials, they were required to sniff also the box with odorized filter paper strip. Once an animal had started to reliably approach the box bearing the filter paper strip impregnated with the rewarded odor and opening the corresponding box, it moved to the next step of training.

3.6.4 Initial shaping

In this phase the first non-rewarded odor, the eucalyptus-like odorant 1,8-cineole (B-) was introduced. The apparatus was now equipped with two filter paper strips bearing both the rewarded odor, and the non-rewarded odor, respectively, each attached to one of the apparatus' two boxes. In most trials the animals were encouraged to sniff both filter paper strips before making the decision but occasionally they were allowed to open the first box they investigated. Upon choosing the box bearing the rewarded odor A+, they opened the corresponding box and retrieved the food reward from the box. Upon an incorrect decision, i.e. upon choosing and opening the box bearing the non-rewarded odor B-, the apparatus was removed without rewarding the animals. During this phase of training the method was adjusted for each animal's personality. Some individuals, for example, adopted the strategy to always open the first box they happened to investigate while others tended to work more systematically, investigating both boxes before making the decision. Thus, for the systematically working individuals, I simply presented the apparatus and let them make their decision, no matter whether they investigated both boxes or not. The rationale for this was that it would not make sense to always require an animal to sniff also the other filter paper if it had already recognized the rewarded odor. For the individuals who tended to open the first box they investigated, I adjusted the method so that I presented them the apparatus one box at a time. More precisely, I placed the apparatus diagonally in front of the animal, one of the two boxes being close to its face, and, when it had sniffed the first box, I placed the other box in front of its face. Then, when it had also sniffed the other box, I withdrew the apparatus and let the animal indicate which box it had chosen. They did this by reaching towards one of the two boxes. Then I let them open the box they had chosen and, in the case of a correct decision, retrieve the food reward. In some trials the baited box bearing the rewarded odor was presented first while in others the box bearing the non-rewarded odor was

presented first. From this stage on, the number of trials needed to reach the pre-set learning criterion was recorded (24 correct decisions out of 30 trials, corresponding to 80 % correct decisions). The animals were required to reach the criterion with the initial odor pair at least three times in total and at least twice in a row before moving on to intramodal transfer tasks.

In most trials the apparatus was presented to an animal so that each of the apparatus' two boxes was at an equal distance from the animal's head, allowing it to have an equal opportunity to investigate both boxes. During the first trials the animals were considered to have made a decision as soon as they approached one of the two boxes: they were rewarded or left without the reward, depending on whether the box they investigated was bearing the rewarded odor or not. Little by little the animals were required to investigate both boxes in most trials (in the case of some individuals, by presenting the animals one box at a time) and then indicating their decision.

3.7 Data analysis

In each trial, the animals had two options: to choose correctly, i.e. open the box bearing a rewarded odor, or to choose incorrectly, i.e. open the box bearing a non-rewarded odor. The learning criterion was set at 80 % correct decisions over 30 daily trials (at least 24 correct decisions) for each individual ($p<0.01$, two-tailed binomial test). Assessment of learning set formation, that is, the correlations between the number of trials needed to reach the learning criterion in each task and the number of tasks completed were evaluated using the Spearman rank correlation coefficient.

4 Results

4.1 Pre-training

Three out of five individuals (Mary, Neto and Paulina) required ten days of pre-training before starting with the actual data collection period with the initial odor pair. Pre-training included presentation of the odorized filter paper, introduction of the test apparatus and finally, baiting of the test apparatus with a food reward. One male, Gruñón, only spent five days with pre-training whereas one female, Margarita, was pre-trained 27 days before she moved on to the data collection period.

4.2 Initial shaping

The number of trials that the animals required to reach the learning criterion with the initial olfactory discrimination between the banana-like n-pentyl acetate (A+) and the eucalyptus-like 1,8-cineole (B-) ranged between 530 trials with Gruñón and 1102 trials with Paulina (Figure 2). Mary needed 661 trials, Margarita 809 trials and Neto 928 trials to reach the learning criterion with the initial odor pair. After reaching the criterion for the first time, all animals continued to score on average above 70 % correct decisions. Only occasionally there was a drop to 60 % of correct decisions. The animals moved on to intramodal transfer tasks once they had reached the learning criterion at least three times in total and at least twice in a row. They also had to learn the required behavior, that is, to sniff the odorized filter papers attached to the apparatus, in this phase. Thus, the training continued with the initial odor pair A+B- for 2-20 days after an animal had reached the criterion for the first time and only then they continued to intramodal transfer tasks. Neto reached the learning criterion three times in a row, Gruñón continued the training with the initial odor pair for seven days, Mary for 20 days and Paulina and Margarita for five days after they had reached the criterion for the first time.
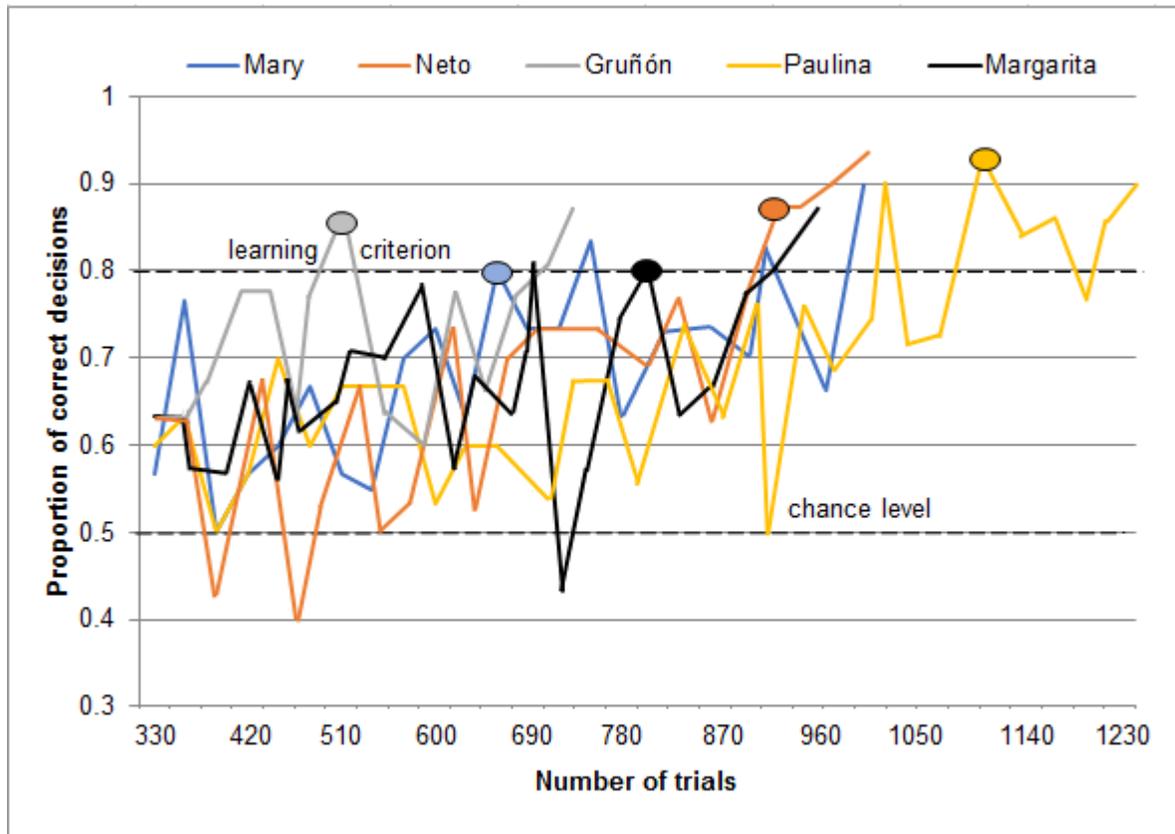
Figure 2 Performance during initial shaping. In this phase, the animals had to learn to open the box bearing the banana-like odorant, n-pentyl acetate (A+), and ignore the box bearing the eucalyptus-like odorant, 1,8-cineole (B-). Each data point represents the proportion of correct decisions on one day (usually 30 trials/day). For each individual, the oval shape indicates the number of trials needed to reach the learning criterion (24/30 correct decisions, according to p<0.01, two-tailed binomial test) for the first time.

4.3 Intramodal transfer tasks

The number of trials that the animals required to reach the learning criterion (24 correct decisions out of 30) during the intramodal transfer tasks varied between 30 and 510 trials. In all transfer tasks, the animals were required to reach the learning criterion at least twice in total and to score at least 75 % of correct decisions during the latest six sessions (60 trials) before moving on to the next transfer task. There was one exception to these rules: Gruñón moved on to fourth double transfer even though he had only reached the learning criterion for once in the third double transfer. This was because of a free-ranging monkey who disturbed Gruñón and it was clear that Gruñón would have scored above criterion if he could have worked without interference by a

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conspecific (he scored 23/30 that day and 29/30 the next day). The time period the animals stayed with a certain odor pair was thus 2-16 working days, depending on the individual and on the odor pair in question.

4.3.1 First positive transfer

The number of trials that the animals required to reach the learning criterion during the first positive transfer, where the banana-like n-pentyl acetate (A+) was replaced by the anise-like anethole (C+) while keeping the already learnt non-rewarded odor, the eucalyptus-like 1,8-cineole (B-) constant, ranged between 30 trials with Paulina and 250 trials with Mary (Figure 3). Gruñón reached the learning criterion with this task after 60 trials, Neto after 73 trials and Margarita after 207 trials.
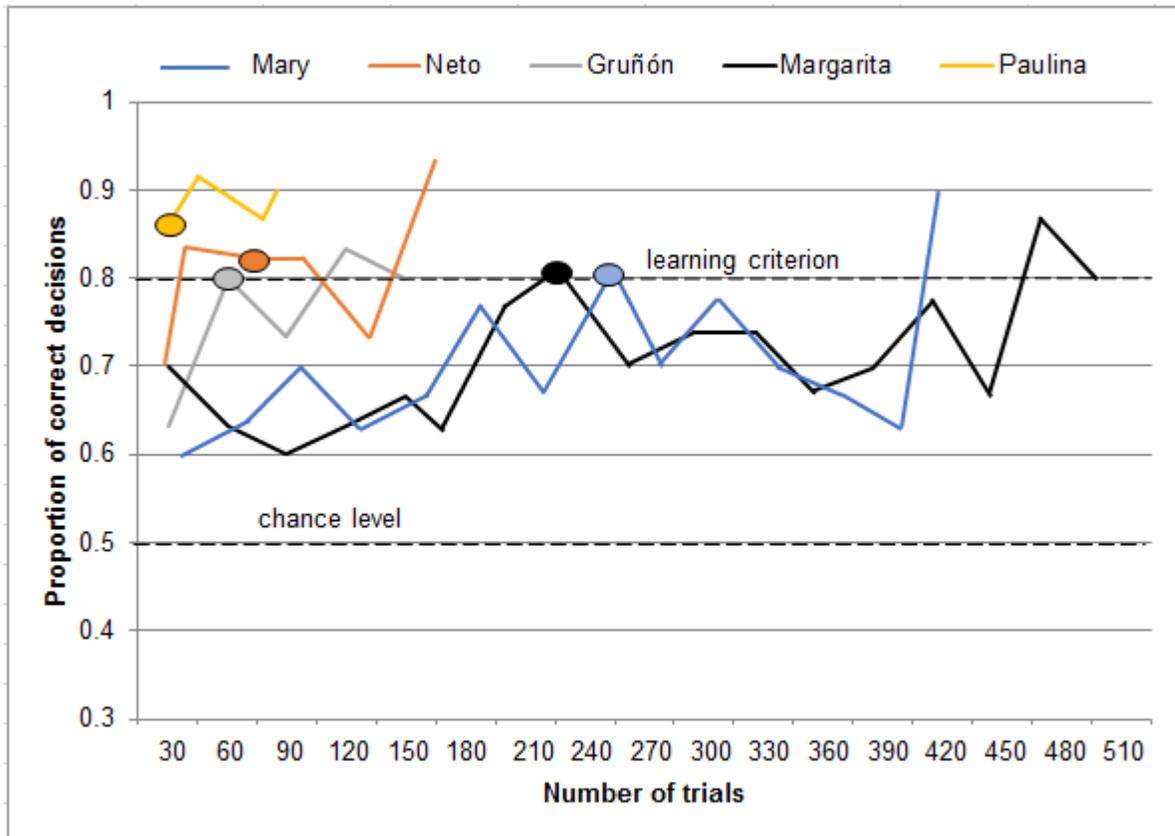
Figure 3 Performance during the first positive transfer where the banana-like n-pentyl acetate (A+) was replaced by the anise-like anethole (C+) while keeping the already learnt non-rewarded odor, the eucalyptus-like 1,8-cineole (B-) constant. Each data point represents the proportion of correct decisions on one day (usually 30 trials/day). For each individual, the oval shape indicates the number of trials needed to reach the learning criterion (24/30 correct decisions, according to p<0.01, two-tailed binomial test) for the first time.

4.3.2 First negative transfer

The number of trials that the animals required to reach the learning criterion during the first negative transfer where the eucalyptus-like 1,8-cineole (B-) was replaced by the citrus-like (+)-limonene (D-) while keeping the already learnt rewarded odor, the anise-like anethole (C+) constant, ranged between 60 trials with Neto and Margarita, and 146 trials with Paulina (Figure 4). Gruñón reached the learning criterion in this task after 80 trials and Mary after 90 trials.
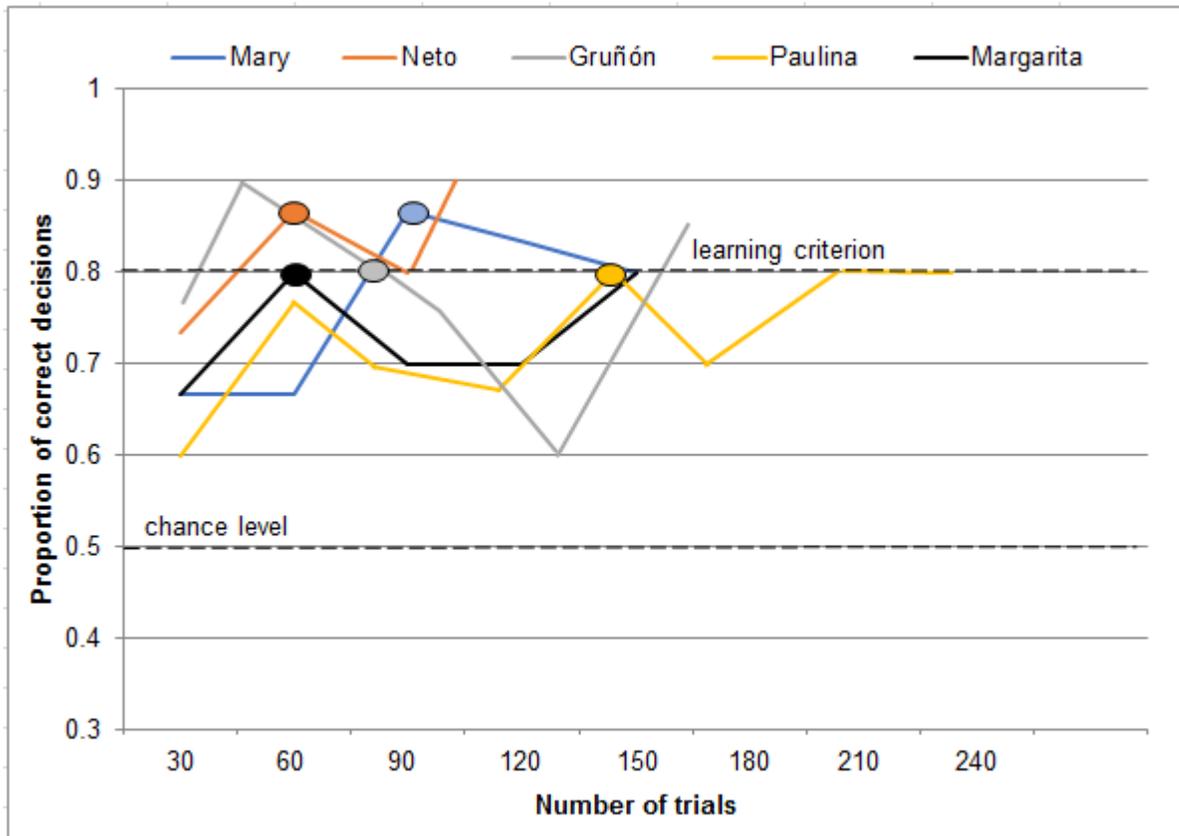
Figure 4 Performance during the first negative transfer where the eucalyptus-like 1,8-cineole (B-) was replaced by the citrus-like (+)-limonene (D-) while keeping the already learnt rewarded odor, the anise-like anethole (C+) constant. Each data point represents the proportion of correct decisions on one day (usually 30 trials/day). For each individual, the oval shape indicates the number of trials needed to reach the learning criterion (24/30 correct decisions, according to $p<0.01$, two-tailed binomial test) for the first time.

4.3.3 Second positive transfer

The number of trials that the animals required to reach the learning criterion during the second positive transfer where the anise-like anethole (C+) was replaced by the clove-like eugenol (E+) while keeping the already learnt non-rewarded odor, the citrus-like (+)-limonene (D-) constant, ranged between 90 trials with Neto and 540 trials with Mary (Figure 5). Margarita reached the learning criterion in this task after 120 trials, Gruñón after 224 trials and Paulina after 420 trials.
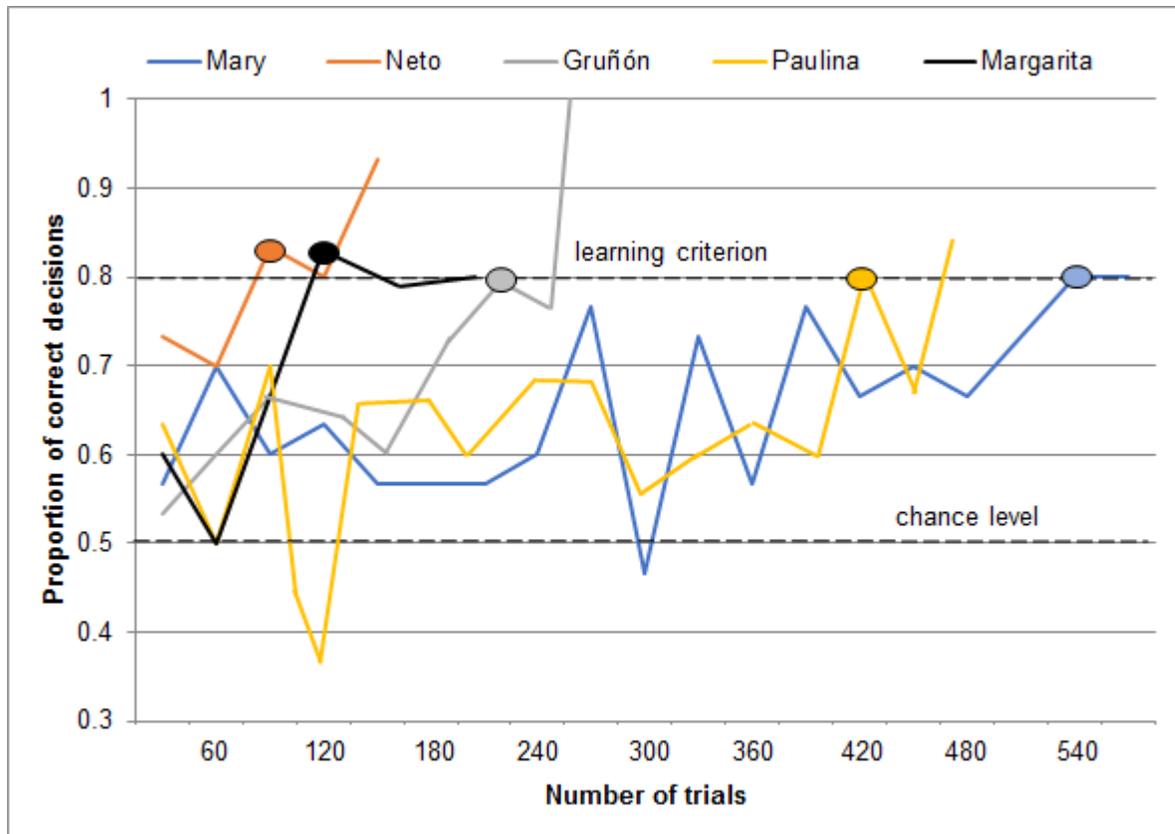
Figure 5 Performance during the second positive transfer where the anise-like anethole (C+) was replaced by the clove-like eugenol (E+) while keeping the already learnt non-rewarded odor, the citrus-like (+)-limonene (D-) constant. Each data point represents the proportion of correct decisions on one day (usually 30 trials/day). For each individual, the oval shape indicates the number of trials needed to reach the learning criterion (24/30 correct decisions, according to p<0.01, two-tailed binomial test) for the first time.

4.3.4 Second negative transfer

The number of trials that the animals required to reach the learning criterion during the second negative transfer where the citrus-like (+)-limonene (D-) was replaced by the peppermint-like (-)-carvone (F-) while keeping the already learnt rewarded odor, the clove-like eugenol (E+) constant, ranged between 30 with Neto and 406 trials with Margarita (Figure 6). Gruñón reached the learning criterion in this task after 120 trials, Mary after 150 trials and Paulina after 390 trials.
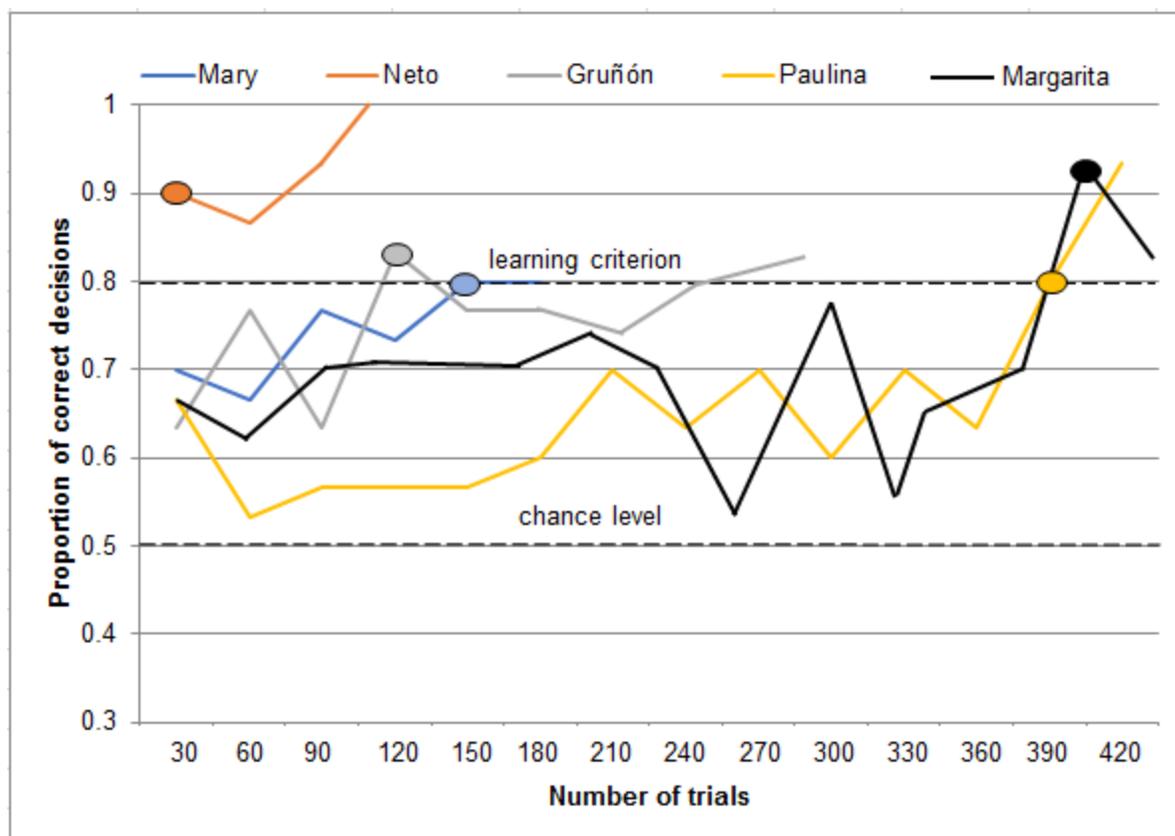
Figure 6 Performance during the second negative transfer where the citrus-like (+)-limonene (D-) was replaced by the peppermint-like (-)-carvone (F-) while keeping the already learnt rewarded odor, the clove-like eugenol (E+) constant. Each data point represents the proportion of correct decisions on one day (usually 30 trials/day). For each individual, the oval shape indicates the number of trials needed to reach the learning criterion (24/30 correct decisions, according to $p<0.01$, two-tailed binomial test) for the first time.

4.3.5 First double transfer

The number of trials that the animals required to reach the learning criterion during the first double transfer where the rewarded odor, the clove-like eugenol (E+) was replaced by the parmesan-like pentanoic acid (G+) and the non-rewarded odor, the peppermint-like (-)-carvone (F-) was replaced by the plastic-like 1-octanol (H-), ranged between 60 trials with Gruñón and Paulina, and 150 trials with Neto (Figure 7). Mary had not reached the criterion after 260 trials (Figure 7) and Margarita did not proceed to double transfer tasks.
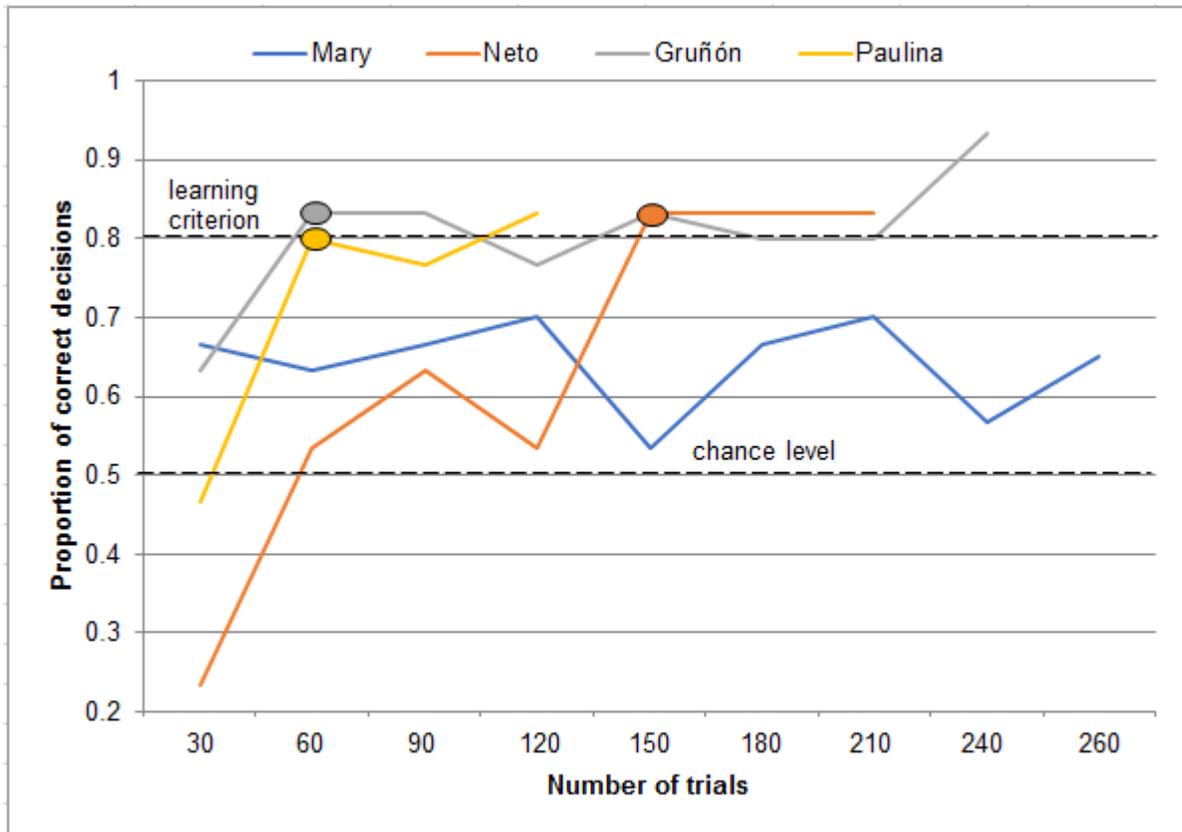
Figure 7 Performance during the first double transfer where the rewarded odor, the clove-like eugenol (E+) was replaced by the parmesan-like pentanoic acid (G+) and the non-rewarded odor, the peppermint-like (-)-carvone (F-) was replaced by the plastic-like 1-octanol (H-). Each data point represents the proportion of correct decisions during 30 trials. For each individual, the oval shape indicates the number of trials needed to reach the learning criterion (24/30 correct decisions, according to p<0.01, two-tailed binomial test) for the first time.

4.3.6 Second double transfer

The number of trials that the animals required to reach the learning criterion during the second double transfer where the rewarded odor, the parmesan-like pentanoic acid (G+) was replaced by the pineapple-like ethyl butyrate (I+) and the non-rewarded odor, the plastic-like 1-octanol (H-) was replaced by the almond-like benzaldehyde (J-), ranged between 30 trials with Gruñón and 240 trials with Neto (Figure 8). Paulina reached the learning criterion in this task after 60 trials.
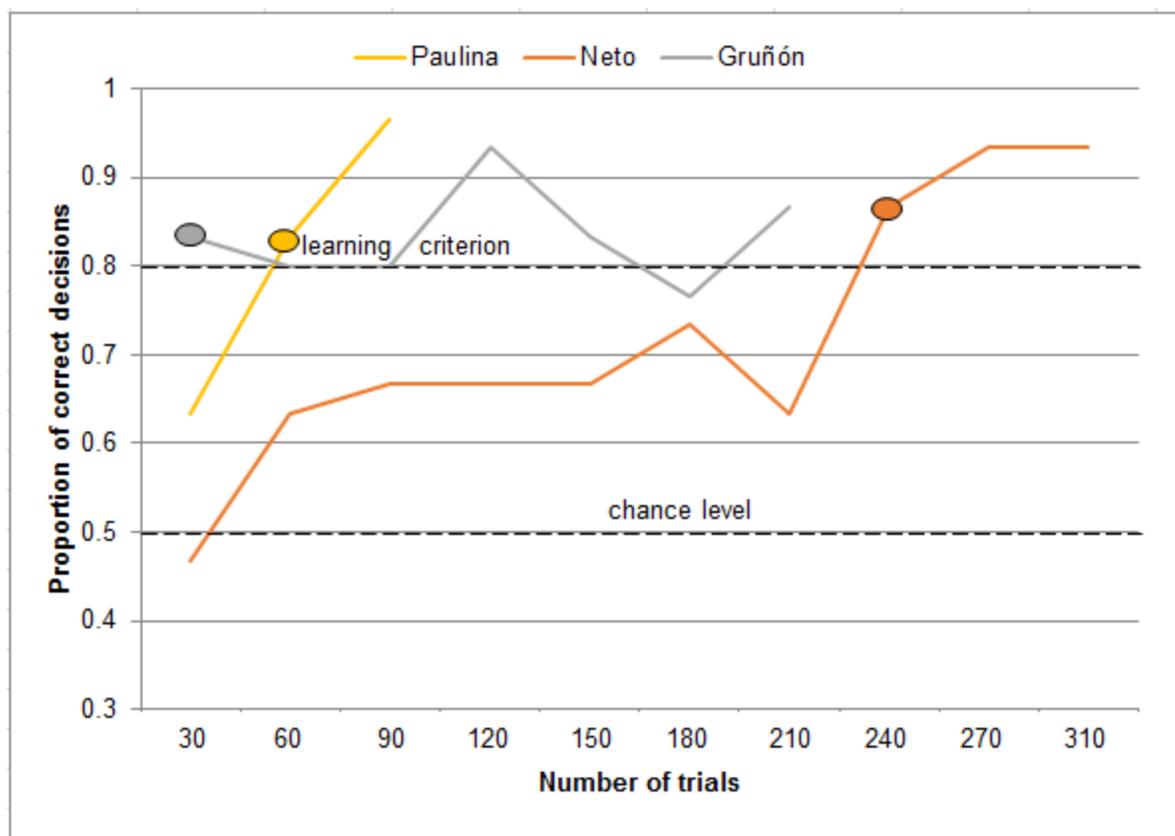
Figure 8 Performance during the second double transfer where the rewarded odor, the parmesan-like pentanoic acid (G+) was replaced by the pineapple-like ethyl butyrate (I+) and the non-rewarded odor, the plastic-like 1-octanol (H-) was replaced by the almond-like benzaldehyde (J-). Each data point represents the proportion of correct decisions during 30 trials. For each individual, the oval shape indicates the number of trials needed to reach the learning criterion (24/30 correct decisions, according to p<0.01, two-tailed binomial test) for the first time.

4.3.7 Third double transfer

The number of trials that the animals required to reach the learning criterion during the third double transfer where the rewarded odor, the pineapple-like ethyl butyrate (I+) was replaced by the turpentine-like butyraldehyde (K+) and the non-rewarded odor, the almond-like benzaldehyde (J-) was replaced by the lavender-like (±)-lavandulol (L-), ranged between 30 trials with Neto and Paulina, and 60 trials with Gruñón (Figure 9).
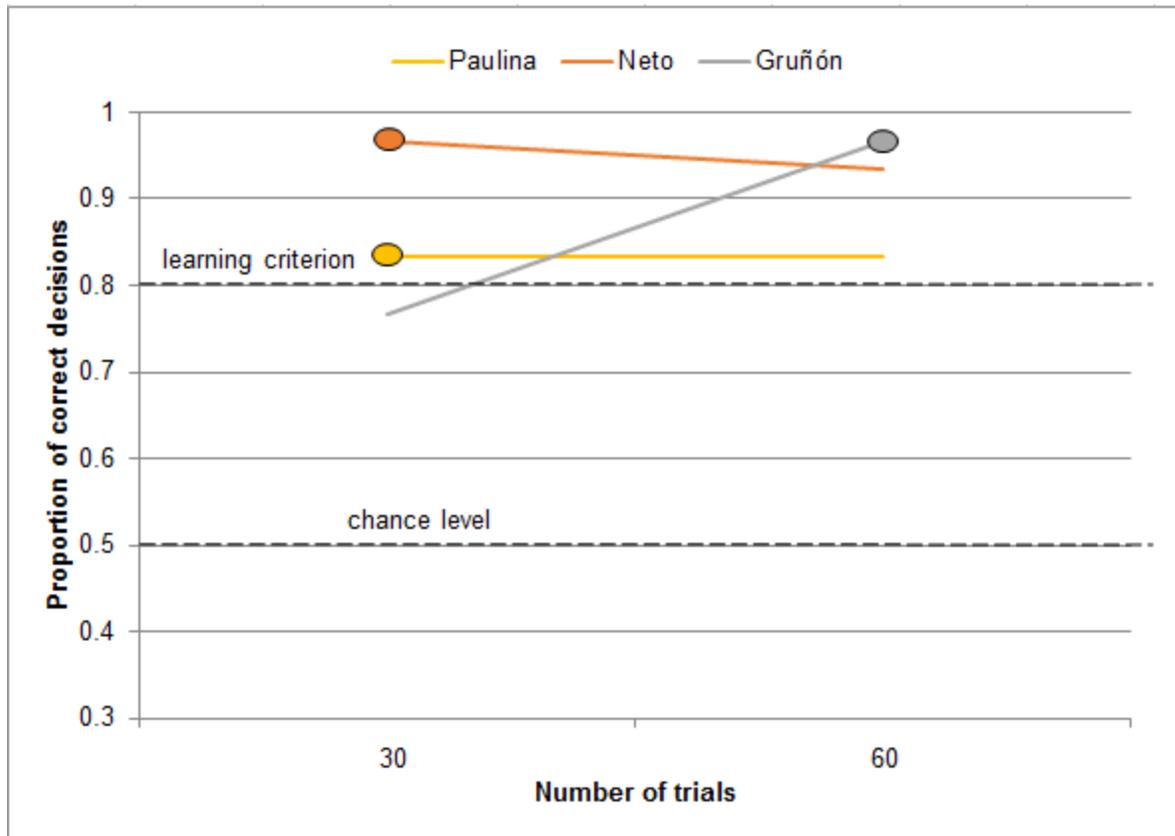
Figure 9 Performance during the third double transfer where the rewarded odor, the pineapple-like ethyl butyrate (I+) was replaced by the turpentine-like butyraldehyde (K+) and the non-rewarded odor, the almond-like benzaldehyde (J-) was replaced by the lavender-like (±)-lavandulol (L-). Each data point represents the proportion of correct decisions during 30 trials. For each individual, the oval shape indicates the number of trials needed to reach the learning criterion (24/30 correct decisions, according to $p<0.01$, two-tailed binomial test) for the first time.

4.3.8 Fourth double transfer

The number of trials that the animals required to reach the learning criterion during the fourth double transfer where the rewarded odor, the turpentine-like butyraldehyde (K+) was replaced by the rose-like 2-phenylethanol (M+) and the non-rewarded odor, the lavender-like (±)-lavandulol (L-) was replaced by the lemongrass-like citral (N-), ranged between 60 trials with Neto and Paulina, and 239 trials with Gruñón (Figure 10).
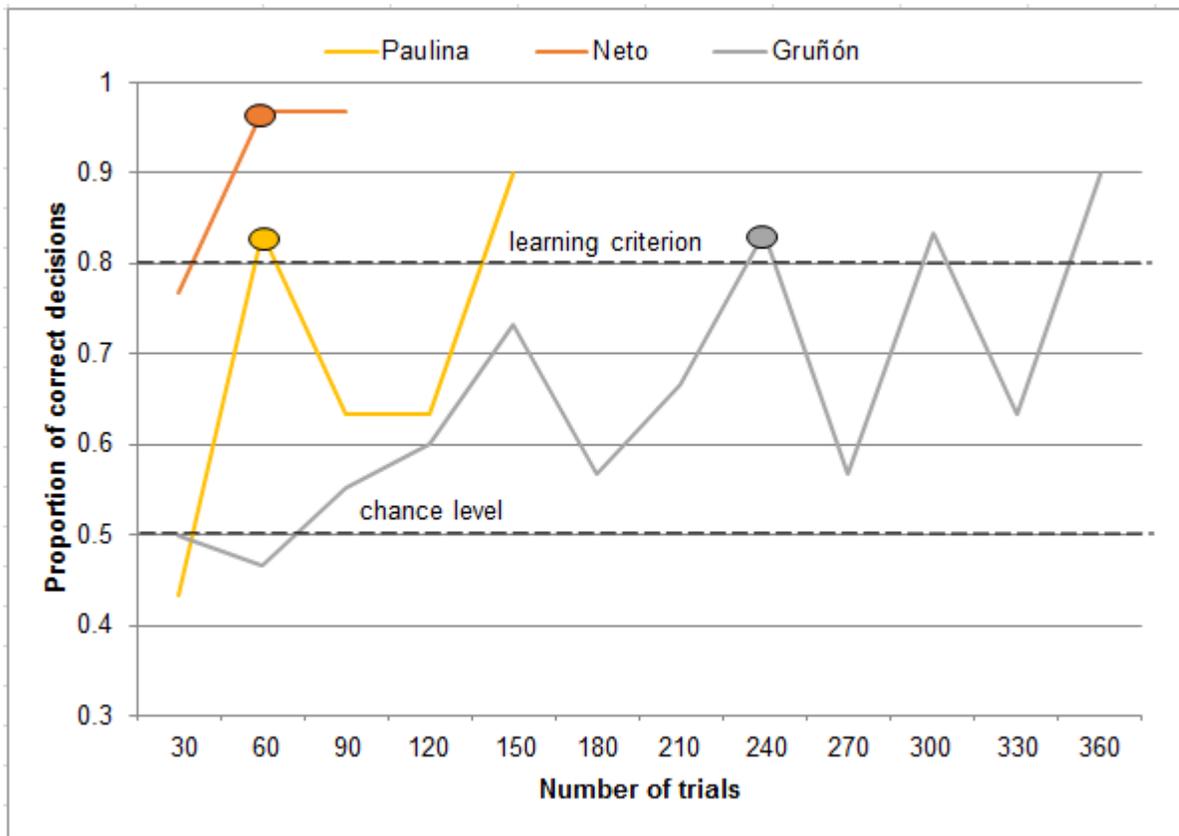
Figure 10 Performance during the fourth double transfer where the rewarded odor, the turpentine-like butyraldehyde (K+) was replaced by the rose-like 2-phenylethanol (M+) and the non-rewarded odor, the lavender-like (±)-lavandulol (L-) was replaced by the lemongrass-like citral (N-). Each data point represents the proportion of correct decisions one day (usually 30 trials/day). For each individual, the oval shape indicates the number of trials needed to reach the learning criterion (24/30 correct decisions, according to $p<0.01$, two-tailed binomial test) for the first time.

4.3.9 Fifth double transfer

Neto, the only animal who completed the fifth double transfer where the rewarded odor, the rose-like 2-phenylethanol (M+) was replaced by the cut flower stem-like (-)-rose oxide (O+) and the non-rewarded odor, the lemongrass-like citral (N-) was replaced by the mandarin-like decanal (P-), required 330 trials to reach the learning criterion (Figure 11). Gruñón had not reached the learning criterion after 547 trials (Figure 11) and Paulina did not proceed to this transfer task.
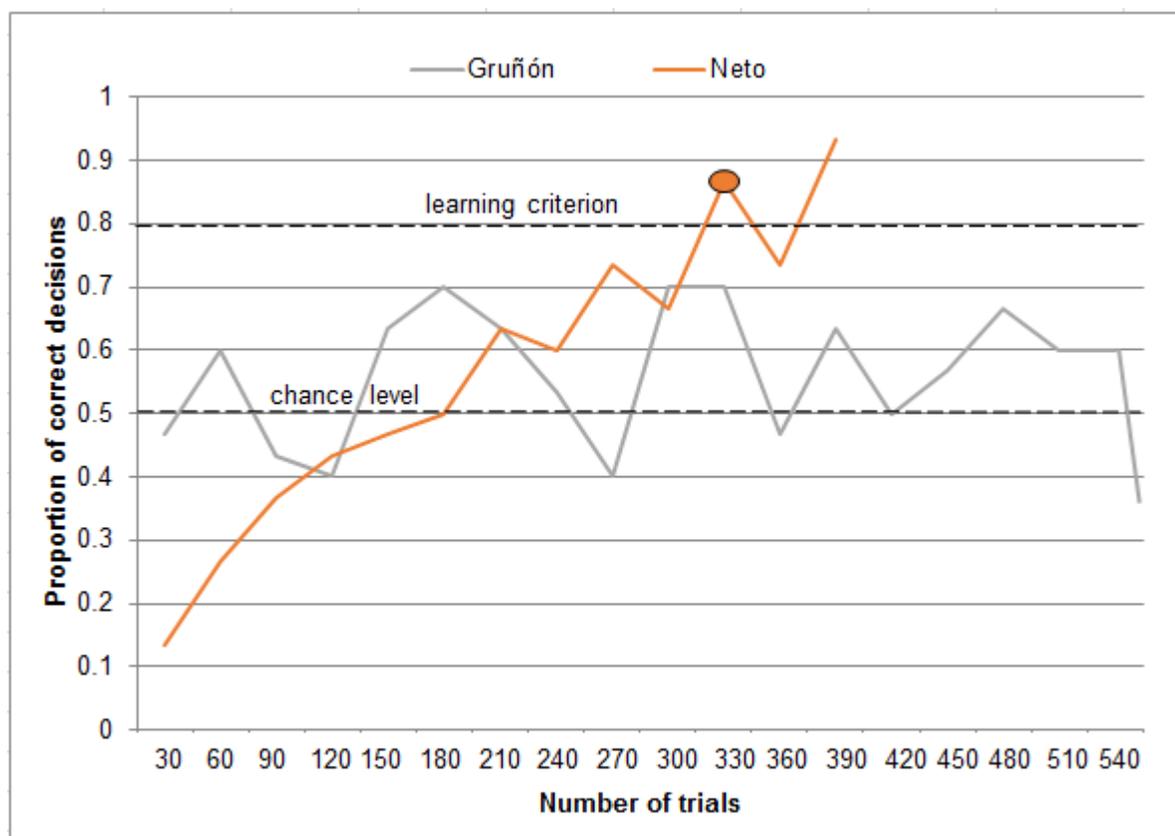
Figure 11 Performance during the fifth double transfer where the rewarded odor, the rose-like 2-phenylethanol (M+) was replaced by the cut flower stem-like (-)-rose oxide (O+) and the non-rewarded odor, the lemongrass-like citral (N-) was replaced by the mandarin-like decanal (P-). Each data point represents the proportion of correct decisions on one day (usually 30 trials/day). The oval shape indicates the number of trials needed to reach the learning criterion (24/30 correct decisions, according to p<0.01, two-tailed binomial test) for the first time.

4.3.10 Sixth double transfer

Neto, the only animal who proceeded to the sixth double transfer where the rewarded odor, the cut flower stem-like (-)-rose oxide (O+) was replaced by the cardamom-like linalyl acetate (Q+) and the non-rewarded odor, the mandarin-like decanal (P-) was replaced by the geranium-like geraniol (R-), required 57 trials to reach the learning criterion (Figure 12).
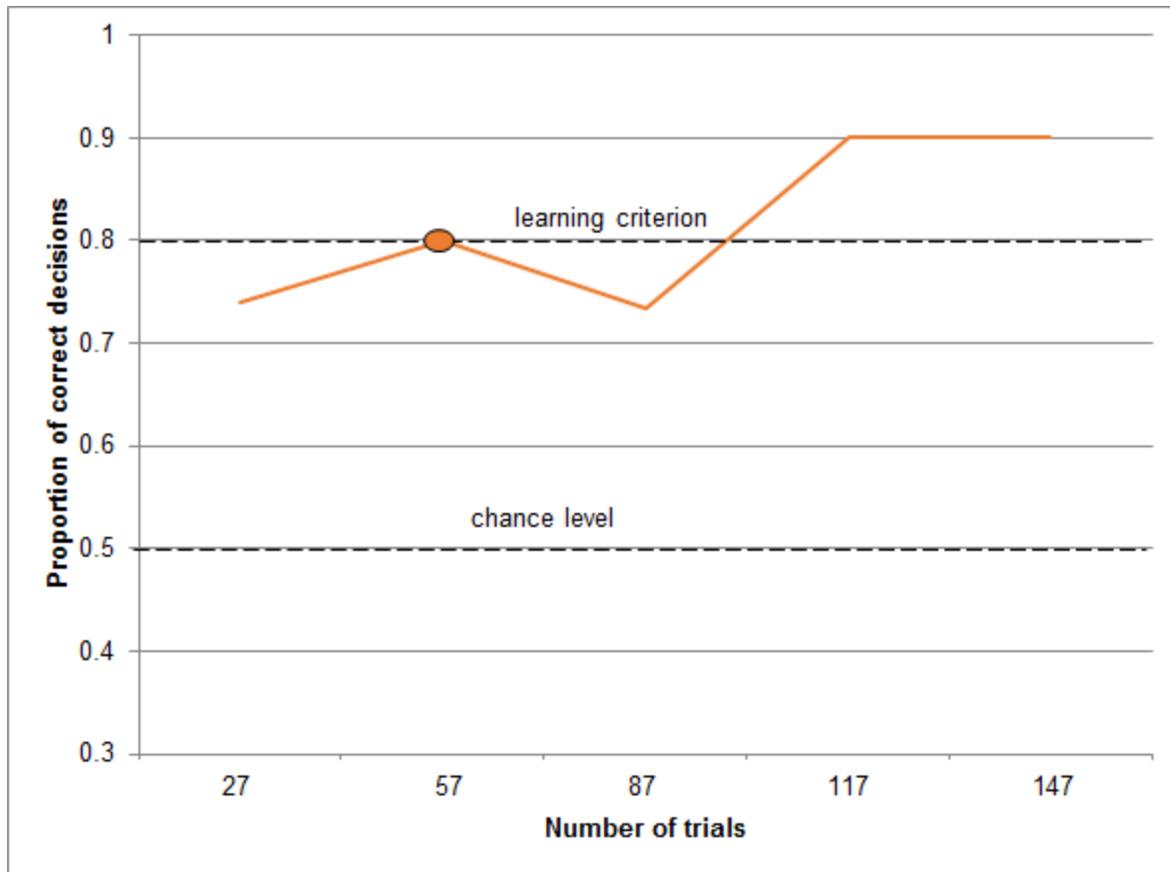
Figure 12 Neto's performance during the sixth double transfer where the rewarded odor, the cut flower stem-like (-)-rose oxide (O+) was replaced by the cardamom-like linalyl acetate (Q+) and the non-rewarded odor, the mandarin-like decanal (P-) was replaced by the geranium-like geraniol (R-). Each data point represents the proportion of correct decisions on one day (usually 30 trials/day). The oval shape indicates the number of trials needed to reach the learning criterion (24/30 correct decisions, according to $p < 0.01$, two-tailed binomial test) for the first time.

4.4 Assessment of learning set formation

All five animals needed fewer trials to reach the learning criterion in the intramodal transfer tasks compared to the number of trials needed to reach the criterion with the initial odor pair. This was true for positive, negative and double transfer tasks. There was one exception to this: Gruñón had not reached the learning criterion in the fifth double transfer even after 547 trials, compared to the 530 trials he needed to reach the learning criterion with the initial odor pair. There was a significant negative correlation between the number of trials needed to reach the learning criterion and the number of transfer tasks completed ($r_s = -0.473$, $p < 0.01$). Thus, as a group the

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animals showed a tendency towards learning set formation (Figure 13). On an individual level, there was no significant correlation with any of the five animals. Some odor pairs seemed to be more difficult for the animals to learn to distinguish than others and each individual had different odor pairs that were difficult to learn. For example Neto, the only individual who went through all transfer tasks, needed 330 trials to reach the learning criterion during the fifth double transfer task where the rose-like 2-phenylethanol (M+) was replaced by the cut flower stem-like (-)-rose oxide (O+) and the non-rewarded odor, the lemongrass-like citral (N-) was replaced by the mandarin-like decanal (P-). This is about 3.8 times more than the number of trials that he needed on average to reach the learning criterion with other odor pairs. Likewise, Gruñón had not reached the learning criterion in the fifth double transfer task after 547 trials, which is even more trials that he needed to reach the learning criterion with the initial odor pair.
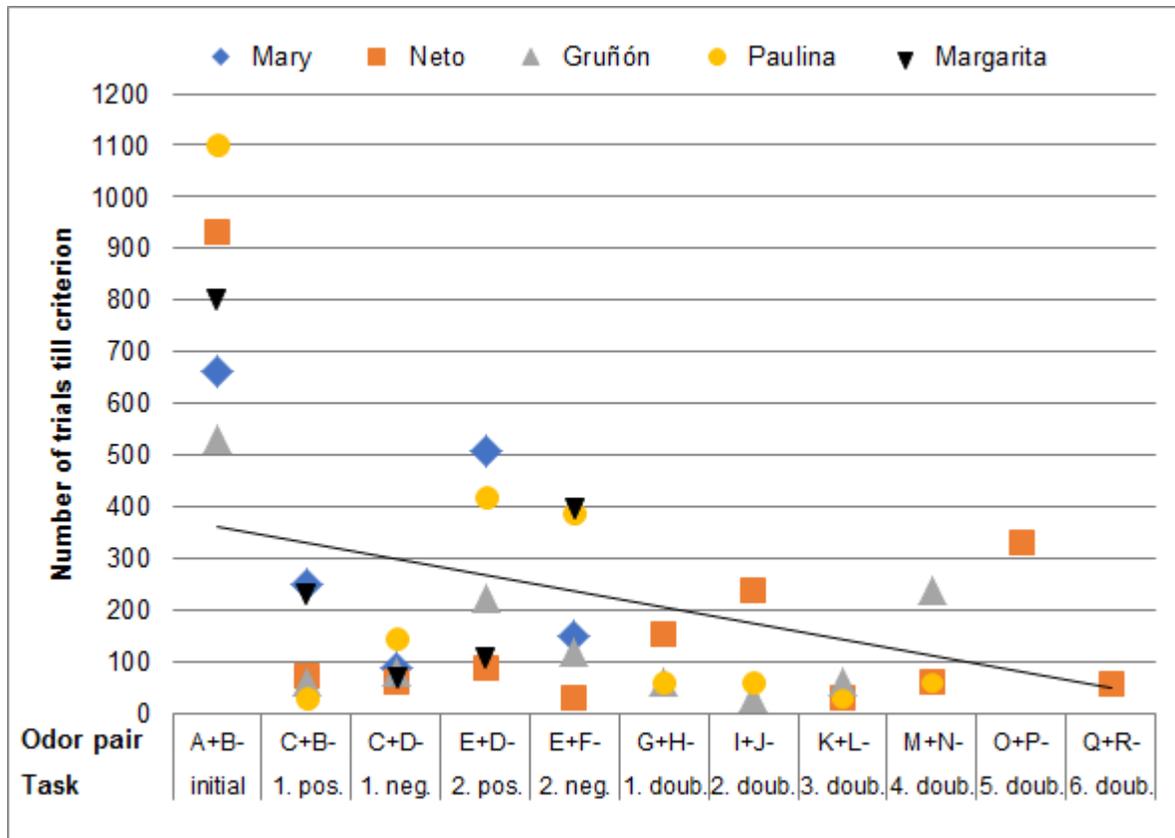
Figure 13 Assessment of learning set formation. In positive transfer tasks (pos.) the rewarded odor was replaced by a novel odor stimulus while keeping the already learnt non-rewarded odor stimulus constant and in negative transfer tasks (neg.) the non-rewarded odor was replaced by a novel odor stimulus while keeping the already learnt rewarded odor stimulus constant. In double transfer tasks (doub.) both odor stimuli were exchanged for novel ones at the same time.

4.5 Assessment of long-term odor memory

All three animals (Neto, Gruñón and Paulina) who participated in the long-term odor memory tasks, scored above the learning criterion straight on the first day after a retention interval, i.e. during the first 30 trials. The retention intervals tested were two weeks, considering the odor pair G+H- for Neto and Gruñón (Figure 14.1); three weeks, considering the odor pair E+F- for Paulina (Figure 14.2); four weeks, considering the odor pairs K+L- and M+N- for Neto (Figure 14.3); and five weeks, considering the odor pair G+H- for Neto (Figure 14.4). The longest retention interval tested was six weeks (performed by Neto, considering the odor pair E+F-), and there, too, the animal scored 24/30 correct responses straight (Figure 14.4).

Figure 14.1 Performance in the odor memory task where the animals were presented with the odor pair G+H- after a 2-week retention interval. Each data point represents the proportion of correct decisions during 30 trials. For each individual, the oval shape indicates the number of trials needed to reach the learning criterion (24/30 correct decisions, according to p<0.01, two-tailed binomial test) for the first time.
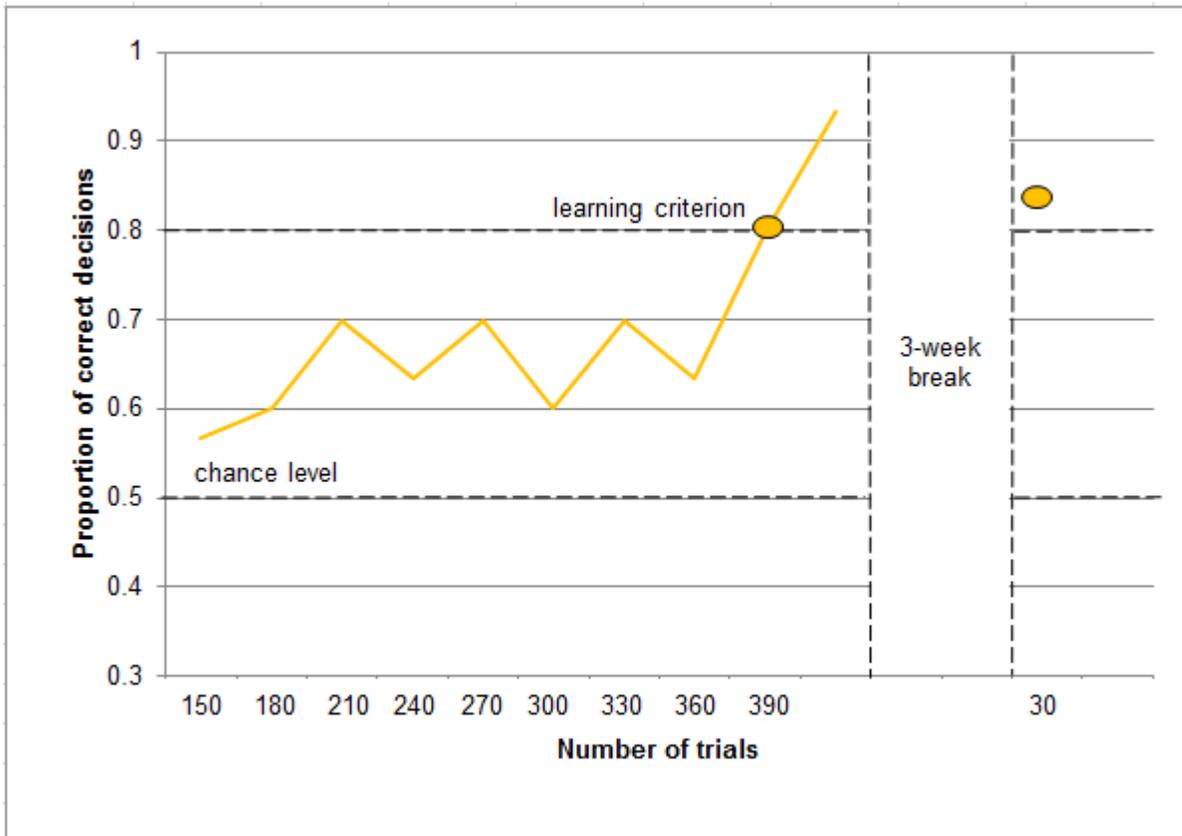
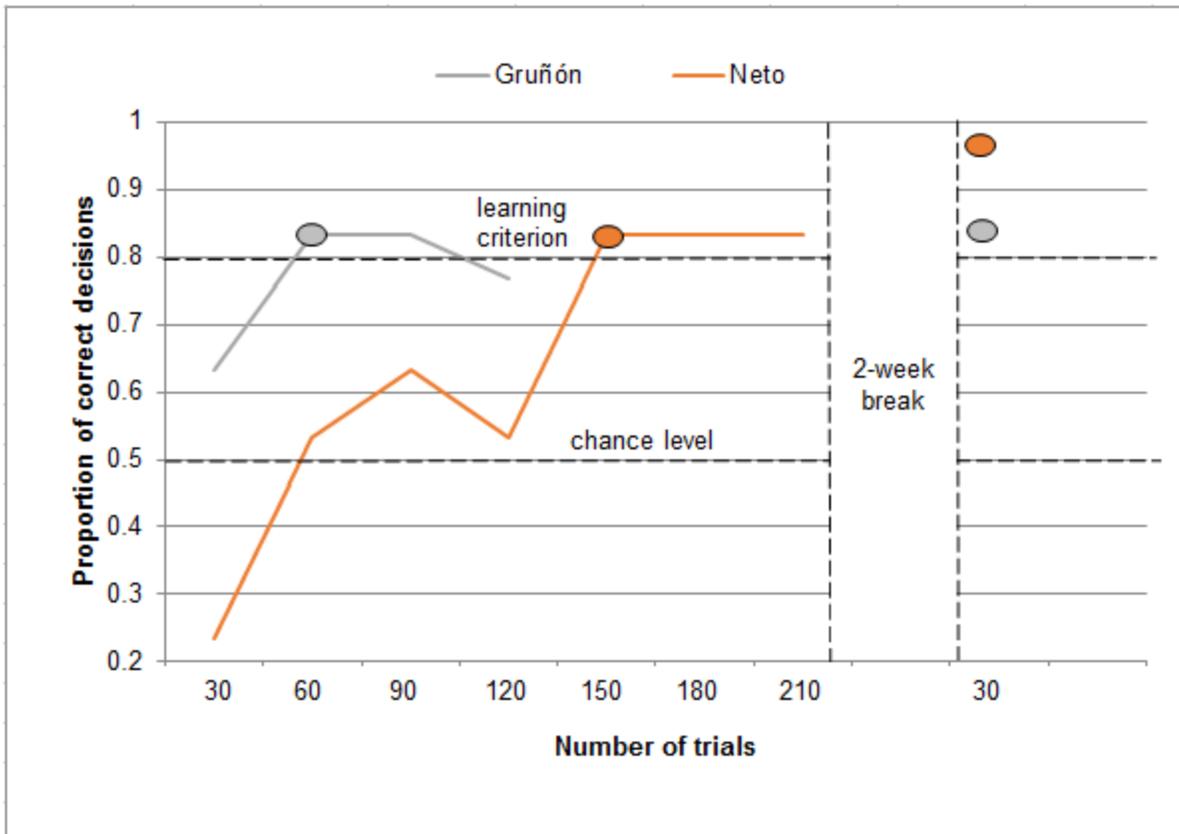Figure 14.2 Paulina's performance in the odor memory task where the animal was presented with the odor pair E+F- after a 3-week retention interval. Each data point represents the proportion of correct decisions during 30 trials. The oval shape indicates the number of trials needed to reach the learning criterion (24/30 correct decisions, according to p<0.01, two-tailed binomial test) for the first time.
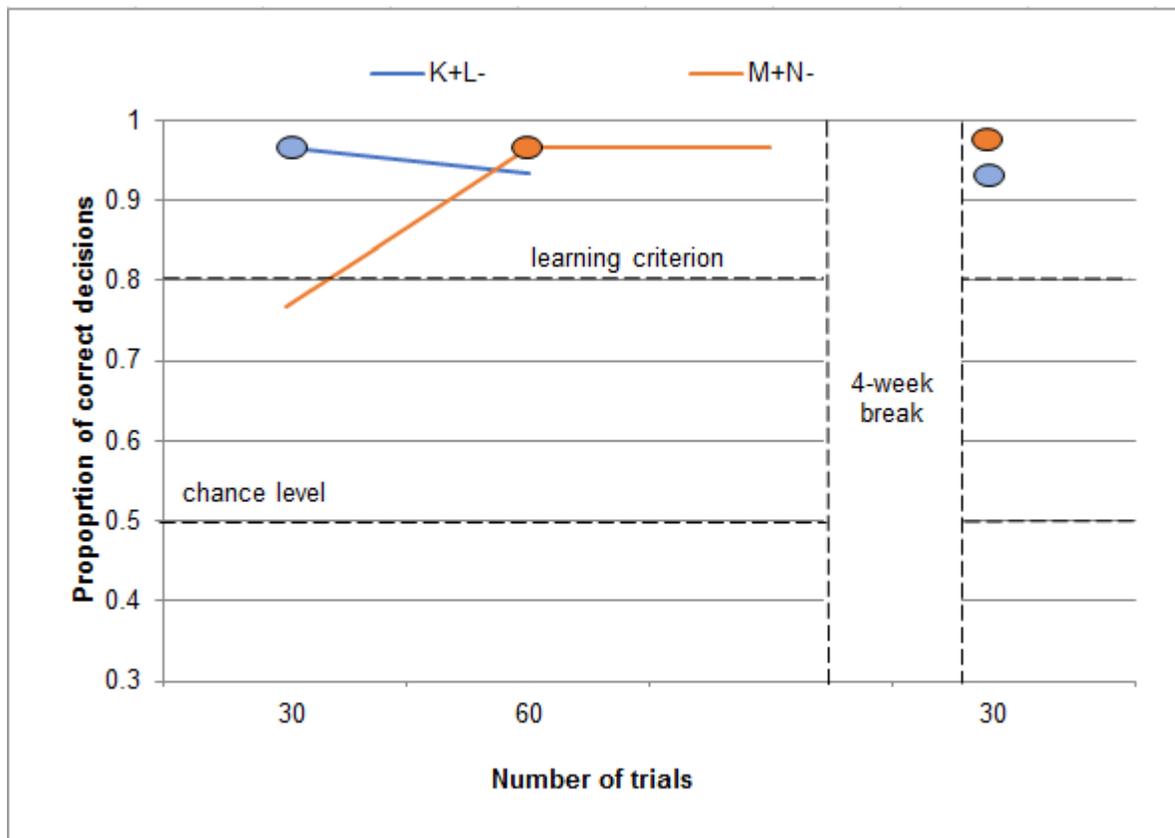
Figure 14.3 Neto's performance in the odor memory tasks where the animal was presented with the odor pair K+L- (blue) and M+N- (orange) after 4-week retention intervals. Each data point represents the proportion of correct during 30 trials. The oval shape indicates the number of trials needed to reach the learning criterion (24/30 correct decisions, according to $p<0.01$, two-tailed binomial test) for the first time.
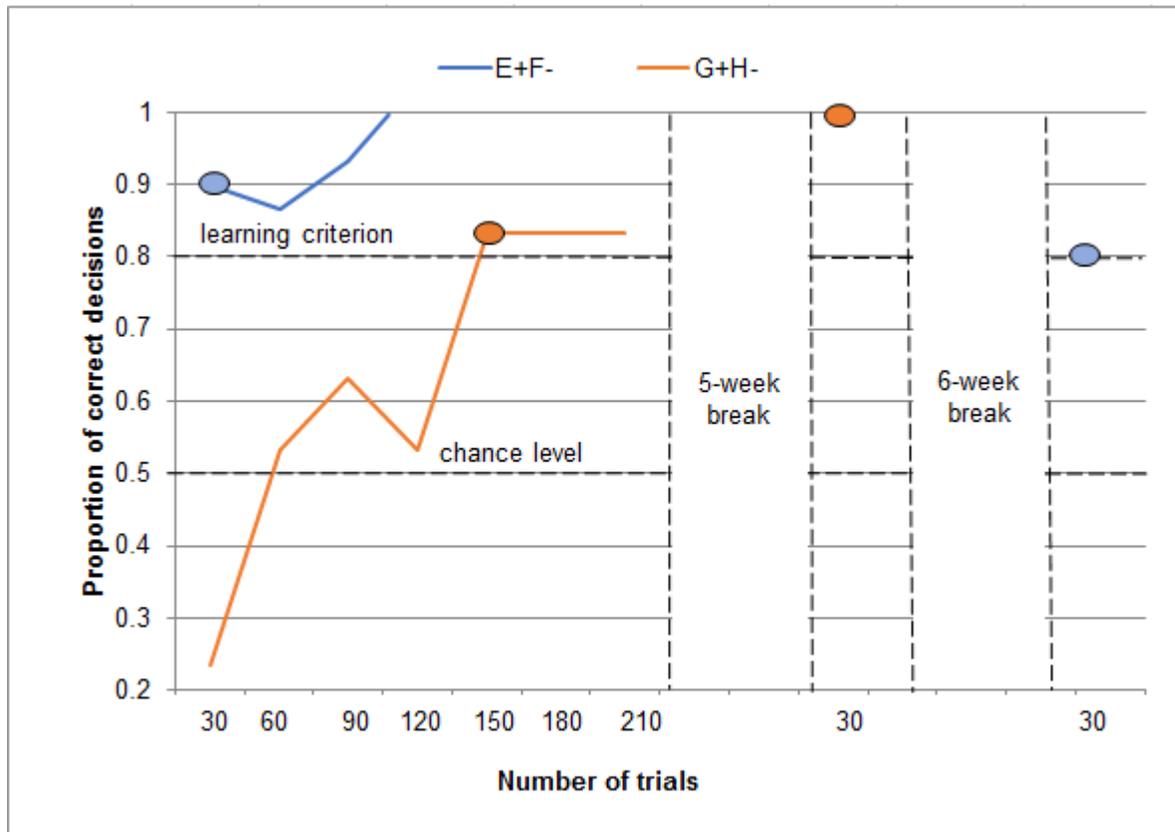
Figure 14.4 Neto's performance in the odor memory tasks where the animal was presented with the odor pair G+H- after a 5-week retention interval and with the odor pair E+F- after a 6-week retention interval. Each data point represents the proportion of correct decisions during 30 trials. The oval shape indicates the number of trials needed to reach the learning criterion (24/30 correct decisions, according to p<0.01, two-tailed binomial test) for the first time.

## 5 Discussion

5.1 Initial odor discrimination

5.1.1 Within-species comparisons

In the present study, the spider monkeys needed between 530 and 1102 trials to reach the learning criterion in the food-rewarded two-choice olfactory discrimination paradigm. An earlier study by Laska et al. (2003) reported that spider monkeys tested with the same method needed between 330 and 360 trials to reach the learning criterion of 24 correct decisions in 30 trials. The numbers obtained in the present study are thus about 1.6-3.2 times higher than the numbers reported in the previous study. Possible reasons for this difference in learning speed include the previous

experience of the experimenter, the odor stimuli used, a slightly different experimental set-up and the set of individuals that took part in the experiments. Firstly, the previous study was conducted by experienced scientists while for me the current project was the very first one in animal training. Thus, I might not have known some animal training techniques that can promote animals to learn faster. Secondly, the different odor stimuli used in the current and in the previous study may also have contributed to the different learning speed of the animals between the two studies because as demonstrated by the intramodal transfer tasks (see chapter 5.2), the nature of the odor stimuli used may play a role in how fast animals learn to discriminate between two odors. In the current study, the first rewarded odor was the banana-like n-pentyl acetate and the first non-rewarded odor was the eucalyptus-like 1,8-cineole, whereas in the previous study the peanut-like commercial aroma was used as the first rewarded odor and the anise-like anethole was used as the first non-rewarded odor. Thirdly, the experimental set-ups of the current and the previous study were slightly different because in the previous study the animals were tested in a test cage while in the present study the animals were tested in their home enclosures. In the previous study the animals could not leave the test cage before completing the session while in the present study the spider monkeys could easily leave for other parts of their home enclosure whenever they wished. Thus, the sessions were probably interrupted more often during the current than during the previous study by Laska et al. (2003). Further, the circumstances during the present study were probably less favorable for the monkeys to learn in comparison with the previous study. There were several distracting factors for the animals of the present study, such as several free-ranging monkeys and the occasional presence of unfamiliar people right around the enclosures. The kitchen where the monkeys' daily feed was prepared was also visible for the animals, which had not been the case in the previous study. These factors may have contributed to the fact that the animals that took part in the present experiments were occasionally distracted and did not concentrate on the task or even refused to participate. Finally, the set of individuals that took part in the experiments may have affected the differences between the studies because individuals differ considerably in their learning speed for acquiring the paradigm. It was evident that the personality of the animals affected the learning performance as the "least shy" animals, Neto and Gruñón, proceeded faster than the more reserved individuals. Mary was an exception to this as she usually completed the daily 30 trials without a problem but was a slow learner and only proceeded to the first double transfer. Margarita was very shy in the beginning and it took

about two months before she started to reliably cooperate with me. Likewise, Paulina was not willing to work in the beginning and she became easily frustrated and then stopped participating. Once she started to understand what the training was about and getting used to me, she started to perform better and completed the daily trials.

5.1.2 Between-species comparisons

Pigtail macaques (*Macaca nemestrina*), the other primate species tested in a very similar food-rewarded two-choice olfactory discrimination paradigm (Hübener and Laska 2001) as used in the present study, needed 480-900 trials to reach the learning criterion of 18 correct decisions in 20 trials. These numbers are similar to the ones obtained with the spider monkeys in the present study, suggesting similar olfactory learning speed between New World and Old World primates when presented with the first odor discrimination problem. It was previously reported that New World primates might be more prepared to use olfactory cues in learning a task than Old World primates (Laska et al. 2003). Based on the present study this cannot be concluded but more species need to be tested to determine this. In comparison, pigtail macaques tested in a multiple-choice olfactory discrimination paradigm reached the learning criterion (8/10 correct decisions) after 140-280 trials (Hübener and Laska 1998), and squirrel monkeys (*Saimiri sciureus*) tested with a very similar multiple-choice method reached the learning criterion (20/25 correct decisions) between 75 and 125 trials (Laska and Hudson 1993). (Please note that the experimental set-ups of a two-choice versus multiple choice discrimination paradigms are different, and the results are thus not directly comparable. In the case of squirrel monkeys, for example, 75-125 trials equal about 450-750 stimulus contacts.)

To compare with non-primate mammals tested on a similar two-choice discrimination paradigm as used with the spider monkeys in the present study, Asian elephants (*Elephas maximus*) reached the learning criterion (42/60 correct decisions) after only 60 trials (Arvidsson et al. 2012) and South African fur seals (*Arctocephalus pusillus*) needed 240-440 trials to reach the learning criterion (30/40 correct decisions, Laska et al. 2008). Other species tested on somehow comparable odor discrimination tasks (i.e. in tasks that use operant conditioning) include mice (Bodyak and Slotnick 1999), rats (Eichembaum et al. 1986, Lu et al. 1990, Slotnick et al. 1991, Setzer and Slotnick 1998, Yoder et al. 2014, Yoder et al. 2017) and dogs (Hall et al. 2015). These species needed less than 200 trials, 40-320 trials, and 60-120 trials, respectively, to reach at least

80 % correct decisions. Even though only rough between-species comparisons of acquiring the initial olfactory discrimination can be done as the experimental set-ups as well as the learning criteria used vary between studies, primates seem to learn odor cues on average slower than the other species tested.

5.2 Intramodal transfer tasks

In the present study, the number of trials needed to reach the learning criterion during the intramodal transfer tasks varied between 30 and 510 trials while the spider monkeys tested in the previous study reached the learning criterion in intramodal transfer tasks straight during the first 30 trials (Laska et al. 2003). These different results between the two studies, again (as demonstrated by the results of initial odor discrimination), suggest that the circumstances during the previous study and the current study differed and that interindividual variation in olfactory learning speed is considerably large in spider monkeys. However, in the previous study the animals were subjected to only two intramodal transfer tasks compared to 4-10 transfer tasks in the present study. It can thus only be speculated whether the animals of the previous study would have reached the learning criterion also in all other intramodal transfer tasks during the first 30 trials because the nature of the odor stimuli used may affect the learning performance (see chapter 5.2.1). Pigtail macaques and squirrel monkeys needed between 20 and 100 trials, South African fur seals 40-120 trials, Asian elephants 60-300 trials and mice usually less than 200 trials to reach the learning criterion in intramodal transfer tasks (Laska and Hudson 1993, Hübener and Laska 1998, Bodyak and Slotnick 1999, Laska et al. 2008, Arvidsson et al. 2012). As the number of trials that the spider monkeys needed to reach the learning criterion during the intramodal transfer tasks ranged from 30 to 510 trials, the results of the other species tested fall into this range. Therefore, the spider monkeys' learning speed in intramodal transfer tasks is comparable to that of pigtail macaques, squirrel monkeys, South African fur seals, African elephants and mice. Primates might thus be less prepared to use olfactory cues in the initial solving of a problem compared to non-primate mammals, but the efficiency of their olfactory system in discriminating between odors is not necessarily inferior (Hübener and Laska 2001, Laska et al. 2003). In visual discrimination tasks primates outperform for example rats, suggesting that primates are more prepared to use visual, rather than olfactory cues (Mustafar et al. 2018).

5.2.1 Learning set formation

At the group level, the spider monkeys in the present study showed a significant negative correlation between the number of trials needed to reach the learning criterion and the number of transfer tasks completed. Based on this finding spider monkeys are able to form an olfactory learning set, similar to visual and spatial learning sets that primates have been reported to be able to acquire (Lepoivre and Pallaud 1986, Yokoyama et al. 2004, Yokoyama et al. 2005, Takemoto et al. 2015). The finding that the spider monkeys learned to discriminate between several odor pairs and even acquired a learning set, questions the traditional view that primates would have a poor sense of smell. Rats have also been shown to need fewer and fewer trials to reach the learning criterion in consecutive olfactory transfer tasks and then reaching near-errorless learning, but some authors argue whether this can be taken as an evidence of an olfactory learning set formation or if other mechanisms can explain the gradual improvement in the performance (Eichenbaum et al. 1986, Lu et al. 1990, Slotnick et al. 1991, Reid and Morris 1992, Slotnick et al. 2000, Bailey 2006). The number of trials that Asian elephants, South African fur seals and mice needed to reach the learning criterion with a certain transfer task did not drop systematically as the animals went through more transfer tasks (Bodyak and Slotnick 1999, Laska et al. 2008, Arvidsson et al. 2012). Thus, currently there is no evidence of olfactory learning set formation in these species.

5.2.1 About the nature of the stimuli

The fact that the number of trials needed to reach the learning criterion differed considerably between different transfer tasks suggests that the nature of the stimuli used may have played a role in how well the spider monkeys performed in the olfactory discrimination tasks. Ueno (1994) demonstrated that tufted capuchin monkeys (*Cebus apella*) performed better in odor discrimination tasks when the odor stimuli were "aroma/fruity" rather than "fishy", and that they tended to innately avoid "fishy" odors. Thus, it seems reasonable to assume that the ecological relevance of certain odor stimuli can affect olfactory performance. This notion is also supported by studies on olfactory sensitivity. Hübener and Laska (2001) showed that pigtail macaques (*Macaca nemestrina*) had lower olfactory detection thresholds for the banana-like iso-amyl acetate than for the parmesan-/sweaty feet-like pentanoic acid. They then compared the thresholds with other species tested previously and showed that the thresholds for iso-amyl acetate, an odor that is present in a variety of fruits, are lower for frugivorous species whereas

carnivorous species have lower thresholds for pentanoic acid, a typical body-borne prey odor. Because there is little knowledge about the natural occurrence of the odorants used in the present study, it cannot be concluded that the behavioral relevance of a given odorant would have affected the discrimination performance of the spider monkeys in the same way as it affects their detection thresholds. However, differences in the biological relevance of certain odor stimuli remain as a possible explanation for why some transfer tasks were more difficult for the animals than others. Another feasible explanation could be that the spider monkeys may have perceived one of the two odor stimuli used as qualitatively similar to an odor stimulus they had encountered in one of the previous transfer tasks. Moreover, as the animals at the field station Pipiapan come from different backgrounds, some animals may have encountered some of the odorants used in their earlier life. Previous experience of the animals may thus also have affected the odor discrimination performance. It is, however, challenging to determine the reason for inconstant performance of the animals across tasks because each individual had different odor pairs that were difficult to learn and because of the low number of individuals that took part in the experiments.

In most cases, when presented for the first time with a novel odor pair, the animals scored around or above chance level straight, suggesting that they did not have a spontaneous aversion for the odorants used. It is, however, worthwhile to mention that in the fifth transfer task where the rewarded odor was the rose-like 2-phenylethanol and the non-rewarded odor was the mandarin-like decanal, Neto (the only animal who completed this task) had a surprisingly high proportion of incorrect decisions in the beginning. For one week he scored less than 50 % correct decisions, starting from only 13 % correct decisions and then proceeding quite steadily towards the learning criterion which he reached after 330 trials. Also Gruñón, the other animal who proceeded to the fifth double transfer (but did not complete it even after 547 trials), had in general lower number of correct decisions in this than in other transfer tasks, scoring below 50 % in several sessions. It seemed that in this case the animals indeed initially avoided 2-phenylethanol and/or preferred decanal, but whether this was because of mandarin-like decanal is more biologically relevant for spider monkeys than the rose-like odorant 2-phenylethanol (both compounds are present in fruits), cannot be concluded. Yet, if the time had allowed, it would have been interesting to assess how the other animals would have performed in the transfer task in question and to test with other individuals whether the performance had been different if the mandarin-like decanal was

the rewarded odor and the rose-like 2-phenylethanol the non-rewarded odor. Likewise, the clove-like eugenol was, on average, difficult for the animals to learn as a rewarded odor, which is in accordance with previous experience with spider monkeys (Hernández Salazar, personal communication). Taken together, these findings suggest that, despite the fact that the animals had clearly understood the method, the nature of the odor stimuli can affect the learning performance. Similar results have been reported from Asian elephants (Arvidsson et al. 2012) and South African fur seals (Laska et al. 2008), where some individuals initially avoided certain rewarded odors.

5.3 Long-term odor memory

The fact that all animals reached the learning criterion in all memory tasks straight during the first 30 trials (which is faster than they reached the criterion on average during the initial learning) suggests that their good performance was indeed due to memory rather than due to a quick relearning. Neto even scored 100% correct decisions in the 5-week memory task and thus did not make a single mistake. The spider monkeys' performance in long-term odor memory tasks is comparable with that of other species such as Asian elephants (Arvidsson et al. 2012), dogs (Lubow et al. 1973), mice (Bodyak and Slotnick 1999, Schellinck et al. 2001), pigtail macaques (Hübener and Laska 1998), South African fur seals (Laska et al. 2008) and squirrel monkeys (Laska et al. 1996), who also remembered the reward values of previously learnt odors after several weeks or even months. Similar long-term olfactory memory capacities across different mammalian species suggest that long-term odor memory is an important cognitive ability not only for non-primate mammals, but also for primates.

To conclude, the present study shows that spider monkeys (*Ateles geoffroyi*) can be trained on a food-rewarded two-choice olfactory discrimination paradigm and that their learning speed in acquiring the initial odor discrimination is comparable to that of other primate species tested with similar methods. Spider monkeys acquire the initial olfactory discrimination slower than non-primate mammals tested on comparable tasks, but their learning speed in intramodal transfer tasks, at least with certain odorants, is comparable to that of non-primate mammals tested. As a group, the spider monkeys even showed acquisition of an olfactory learning set, that is, the animals needed fewer and fewer trials in consecutive tasks to reach the learning criterion. This suggests that primates may be more prepared to use other than olfactory cues in the initial solving

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of a problem but that once they learn the concept, their learning speed with novel odor discrimination problems is not generally slower than that of non-primate mammals. The nature of the odor stimuli used seemed to affect the learning performance as with certain odorants a surprisingly high number of trials was needed to reach the learning criterion. Finally, the spider monkeys showed no signs of forgetting the reward values of previously learnt odors even after a 6-week retention interval. Thus, the long-term odor memory of spider monkeys is comparable to that of other mammals such as dogs, mice and rats.

6 Societal & ethical considerations

The experiments reported here comply with the *American Society of Primatologists' Principles for the Ethical Treatment of Primates*, and also with current Swedish and Mexican laws. The experiments were performed according to a protocol approved by the ethical board of the Federal Government of Mexico's Secretariat of Environment and Natural Resources (SEMARNAT; Official permits no. 09/GS-2132/05/10).

The spider monkeys that participated in the study were not forced in any way to take part in the experiments. Instead, the experiments were based on the animals' willingness to voluntarily participate. The animals had the opportunity to leave the testing area at any moment of a session and thus choose not to participate if they wanted to. However, as the animals were usually willing and even eager to participate, it is likely that the experiments served as environmental enrichment. That, in turn, can contribute positively to the welfare of the animals as cognitive and sensory enrichment provides different kinds of mental stimuli for captive animals living in a rather barren and constant environment. Besides that, the study of cognitive and sensory abilities is also important because it can help to better understand the behavior of animals. Increasing the knowledge about an animal's behavior, in turn, is crucial for example in conservation of endangered species.

Even though food was used as a reward during the experiments, the animals were not deprived from food at any moment of the experiments (there was always food left from the previous day when they were given their fresh, daily feed). This supports the notion that the animals may have perceived the experiments themselves as rewarding and that they thus served as enrichment. No negative reinforcement (physical punishment) was used but all experiments were carried out using only positive reinforcement (food-reward). The odor stimuli and the solvent used were

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mostly compounds that are used in artificial flavoring agents and in cosmetic industry and thus safe for humans. They were also considered harmless for spider monkeys as the animals only smelled the odorized filter papers instead of ingesting the chemicals and the quantities used were very small (only 20 µl of diluted odorant applied on a filter paper).

7 Acknowledgements

8 References

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Appendix

Appendix 1. Odorants and dilutions

Abbreviation	Odorant's name	CAS#	Description	Dilution
A+	n-pentyl acetate	628-63-7	banana	1:100
B-	1,8-cineole	470-82-6	eucalyptus	1:100
C+	anethole	104-46-1	anise	1:100
D-	(+)-limonene	5989-27-5	citrus	1:50
E+	eugenol	97-53-0	clove	1:100
F-	(-)-carvone	6485-40-1	peppermint	1:100
G+	pentanoic acid	109-52-4	parmesan, sweaty feet	1:500
H-	1-octanol	111-87-5	spa, plastic and a bit fruity	1:200
I+	ethyl butyrate	105-54-4	very ripe pineapple/pineapple candy	1:100
J-	benzaldehyde	100-52-7	marzipan, almond	1:100
K+	butyraldehyde	123-72-8	light turpentine	1:200
L-	(±)-lavandulol	58461-27-1	lavender	1:50
M+	2-phenylethanol	60-12-8	rose perfume/soap	1:50
N-	citral (cis, trans)	5392-40-5	lemongrass	1:100
O+	(-)-rose oxide	3033-23-6	cut flower stem (not the flower itself)	1:100
P-	decanal	112-31-2	mandarin candy	1:100
Q+	linalyl acetate	115-95-7	cardamom	1:100
R-	geraniol	106-24-1	geranium	1:100