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Influence of Landmarks on Spatial Memory in Short-nosed Fruit Bat, *Cynopterus sphinx*

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Abstract: In order to study the relationship between landmarks and spatial memory in short-nosed fruit bat, *Cynopterus sphinx* (Megachiroptera, Pteropodidae), we simulated a foraging environment in the laboratory. Different landmarks were placed to gauge the spatial memory of *C. sphinx*. We changed the number of landmarks every day with 0 landmarks again on the fifth day (from 0, 2, 4, 8 to 0). Individuals from the control group were exposed to the identical artificial foraging environment, but without landmarks. The results indicated that there was significant correlation between the time of the first foraging and the experimental days in both groups (Pearson Correlation: experimental group: r=-0.593, P<0.01; control group: r=-0.581, P<0.01). There was no significant correlation between the success rates of foraging and the experimental days in experimental groups (Pearson Correlation: r=0.177, P>0.05), but there was significant correlation between the success rates of foraging and the experimental days in the control groups (Pearson Correlation: r=0.445, P<0.05). There was no significant difference for the first foraging time between experimental and control groups (GLM: $F_{0.05,1}$=4.703, P>0.05); also, there was no significant difference in success rates of foraging between these two groups (GLM: $F_{0.05,1}$=0.849, P>0.05). The results of our experiment suggest that spatial memory in *C. sphinx* was formed gradually and that the placed landmarks appeared to have no discernable effects on the memory of the foraging space.

Key words: *Cynopterus sphinx*; Spatial memory; Landmarks

地标对犬蝠空间记忆的影响

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摘要：为了研究地标（landmarks）是否影响犬蝠（*Cynopterus sphinx*）的空间记忆，我们通过室内模拟试验研究犬蝠和地标在觅食过程中空间记忆形成的关系。实验组按照每天地标数分别为 0、2、4、8、0 的数目连续进行 5 天实验，对照组不设地标进行相同条件的实验。结果显示，两组犬蝠第一次取食所用的时间与实验天数之间极显著相关（Pearson Correlations: 实验组 r=-0.593, P<0.01；对照组 r=-0.581, P<0.01）；实验组取食成功率与实验天数之间无明显相关性（Pearson Correlations: r=0.177, P>0.05）；对照组取食成功率与实验天数之间显著相关（Pearson Correlations: r=0.445, P<0.05）。实验组与对照组犬蝠第一次取食的时间差异不显著（GLM: $F_{0.05,1}$=4.703, P>0.05），两组间取食的成功率差异也不显著（GLM: $F_{0.05,1}$=0.849, P>0.05）。这些结果说明了随着时间增加，犬蝠对取食地的空间记忆逐渐形成，放置地标在犬蝠对取食地空间记忆形成的过程中无显著影响。

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The short-nosed fruit bat, *Cynopterus sphinx* (Pteropodidae, Megachiroptera), is a common fruit bat species in southern China. This bat species includes seven sub-species, with *C. s. angulatus* distributed in China (Mickleburgh et al, 1992). The short-nosed fruit bat mainly feeds on succulent berries (Ruby et al, 2000), and sometimes leaves and nectar (Ruby et al, 2000; Singaravelan & Marimuthu, 2004). Previous studies have shown that *C. sphinx* roosts under the large leaves of coconut, fan palm or other plants, as well as abandoned houses (Zhu et al, 2007), feeds on fruits (Tang et al, 2005; Wu et al, 2008) or occasionally young leaves and halms (Zhu et al, 2007).

Animals could return accurately to their nest after they went out for food, indicating that “the way back home” was retained in their memory. Animals finding their way back to places where they have visited before accurately was called idiothetic mechanisms (Muller et al, 1988). Does this memory mechanism has some relationships with the surrounding objects besides idiothetic mechanisms? In related studies of animal spatial memory, Ulf et al (2008) investigated the effect of local echo acoustic cues on the spatial memory of Pallas’s long-tongued bat, *Glossophaga soricina*. They found that increasing the density of local spatial cues would improve accuracy in re-locating rewarding feeders by helping bats identify profitable flowers on a small scale. As the distance between food and local spatial cues became shorter, the correct rate of *G. soricina* finding the reward nectar would increase gradually, which indicated that in a certain space, local echo acoustic cues were helpful to spatial memory of *G. soricina*. But Richard et al (2005) found that once the spatial location memory of Egyptian rousette bat (*Rousettus aegyptiacus*) was formed, moving the landmarks made no effect on their spatial memory. On the process of obtaining nectar, the nectar feeding bats of family Glossophaginae pay attention only to the location of the flowers in the space, instead of the shape and color (Winter & Stich, 2005; Thiele & Winter, 2005). The above studies have shown that landmarks or cues played different roles in the spatial memory of different animals when they were foraging. Landmarks or cues were useful for some species in their spatial memory, while for others no apparent assistance was shown. The aim of this study is to investigate whether the short-nosed fruit bat, *C. sphinx*, uses landmarks for orientation, and whether landmarks are helpful to the spatial memory.

1 Materials and Methods

1.1 Materials and study site

We captured twenty bats from Huanghuagang Park during daytime, and recorded the sex of all individuals. The experiments were conducted in the bat room of Guangdong Entomological Institute (4 m×3 m×3 m). In addition to a special wire door, red bricks surrounded the wall. Half of the roof of the room was covered with asbestos, others were covered with wire to provide fresh air to the bats. The average humidity in the bats room was (61.00±2.00)% and average temperature (26±0.5) ℃ (both mean±*SE*, *N*=30).

1.2 Distribution of bananas

A board (2.3 m×2.3 m) was put in the middle floor of bats’ room. Twenty five containers (18 cm×19 cm×16 cm) were put in equal distance on the board. On both face sides of the container, we opened a gate (14 cm×14 cm) to form a channel as the entrance for the short-nosed fruit bats feeding. We numbered each container (1−25 numbers, same color) and chose seven of the containers (number 01, 04, 08, 13, 15, 16, 24) in which we placed banana slices (5 mm thick, cut into four pieces), the remaining 18 containers were placed with plastic banana slices (plastic banana model, syncopation with the same shape as real bananas). In the containers with plastic banana slices, we placed an uncovered bottle containing banana juice (bottle mouth diameter: 10 mm-*C. sphinx* unable to access juice). In the real banana containers a bottle filled with fresh water was placed to exclude the odor effect. The rules of bananas’ distribution in the array are followed: (1) no more than three successive feeders in any row or column were rewarding; (2) no more than four rewarding feeders per quadrant of the array were allowed (Fig. 1).

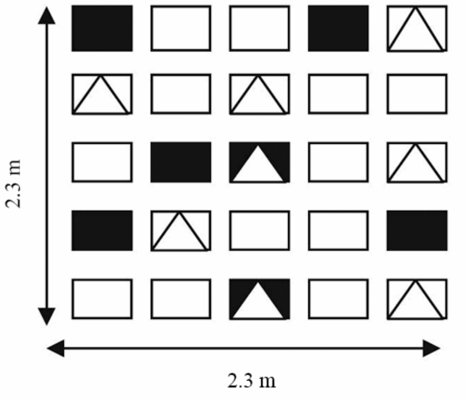

Fig. 1 Distribution of bananas (■) and landmanks (△)

1.3 Methods

Before the experiment, the short-nosed fruit bats were fed in large cages (90 cm×50 cm×55 cm). We trained the bats to feed in the containers which were filled with bananas. Once the bats learned to feed from the container, we chose twelve bats (6 females and 6 males) and divided them into two groups randomly. We selected six (3 males and 3 females) of them as experimental groups and other individuals as the control groups. Each short-nosed fruit bat was kept in a wire cage (45 cm×45 cm×45 cm). During experiment days, we put one bat into the bat room at 19:30 every day for 5 h continuously, and then we changed another individual at 00:30 for 5 h (until 05:30). We placed the distilled water bottles filled with water on the top of the container as landmarks. The landmarks were placed in the same principles as real bananas. We changed the number of landmarks every day with 0 landmarks again on the fifth day (from 0, 2, 4, 8 to 0). Individuals from the control group were exposed to the same foraging environment, but without landmarks. We observed the foraging behaviour of bats by using OS-20G9 series multimedia digital video monitor which connected to a computer with memory chip (AD-804V). The foraging behaviour and time were recorded in the computer, and analyzed the next day.

1.4 Statistical analysis

The time of the first foraging was recorded when the bat entered a container for the first time after release. We recorded the correct times of foraging that the bat entered the banana containers and fed successfully, and the errors times that the bat entered the false banana containers during the 5 hour experimental period. We then calculated the daily foraging success rates of each bat based on these data. The experimental data was analyzed by Spss11.0 for Windows. We used a GLM to analyze whether there were differences between the experimental and control groups in foraging behavior. We made parametric analyses (Pearson correlation) between the time of the first foraging, the foraging success rates of the experimental and the control groups and the days of experiment respectively. In addition, for the experimental group, One-way ANOVA analyzed the data of the fifth day and the other four days respectively. All mean values were expressed as mean ± standard error, and all tests were conducted at the 0.05 significance level.

2 Results

2.1 The foraging behavior of *C. sphinx*

The foraging peak of *C. sphinx* was at 20:00 to 22:00 every night and the next morning from 02:00 to 04:00. From 04:00 to 05:30, the bats' foraging frequency diminished. The bats foraged little after 05:30 am. During observation, we found that the foraging behaviour would not be affected by rain during evenings without thunder and lightning. The average foraging frequency was (7.13±1.12) times (n=7) during nights of rain without thunder and lightning, and 7.36±1.22 times (n=8) during nights of no rain, but there were no difference between these two weather conditions. However, during evenings of thunderstorms, the average foraging time of *C. sphinx* was delayed, its average foraging frequency also decreased to (4.24±1.86) (n=4). It showed that the foraging behavior of *C. sphinx* was affected by thunderstorms. In the experiment, we found that 83% (10/12) of bats began their foraging from the corner of the array (Fig 1), and interestingly, each bat began their foraging from the same container on five consecutive days of experiments. In addition, once the bats have formed a memory of the container with bananas, they would visit the same container every night. In the experiment, we found that the bats could rediscover the bananas which they had lost during flying. In addition, 11 in 12 bats we found to have a habit that they would look for new container until all bananas in the visited one were eaten.

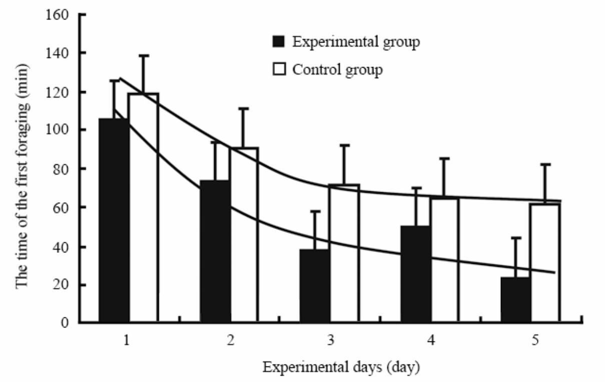

Fig. 2 The relationship between the time of the first foraging and the experimental days

The time gradually decreased from the second day in the whole experimental period. The curve-fitting equation: experimental group, y=131.766×0.6937^x, control group, y=387.250-198.01x+47.19x^2-4.2917x^3.

2.2 Landmarks and spatial memory of *C. sphinx*

For the experimental and control groups, the relation between the time of the first foraging and the days of experiment was analyzed by parametric analysis (Pearson correlations). The result showed that there were significant negative correlations (Pearson Correlations: experimental groups, r=-0.593, P<0.01; control groups, r=-0.581, P<0.01). The time of the first foraging was gradually shortened when the experiment day increased (Fig. 2). We use GLM to analyze whether there were differences between the experimental and control groups in the time of the first foraging. The result showed that there was no significant difference (GLM: $F_{0.05,1}$=4.703, P>0.05). The time of the first foraging between the fifth day (24.00±6.20 min) and the first day (106.16±24.53 min) in the experimental group showed significant difference, and the same as the second day (74.00±18.60 min) (ANOVA: $F_{0.05,1}$=10.539, P<0.05; $F_{0.05,1}$=6.494, P<0.05, respectively). However, there was no significant difference with the third day (38.33±5.10 min) and the fourth day (50.00±12.90 min) (ANOVA: $F_{0.05,1}$ = 3.181, P>0.05; $F_{0.05,1}$ = 3.270, P>0.05, respectively).

For the experimental and control groups, the relation between successful foraging rates and the days of experiment was analyzed by parametric analyses (Pearson correlation). The result showed that there was no significant correlation in the experimental groups (Pearson Correlations: experimental groups, r=0.177, P>0.05). However, positive correlation was found in the control groups (Pearson Correlations: control groups, r=0.445, P<0.05) (Fig. 3). The foraging success rates between the experimental and control groups were analyzed by GLM. The result showed that there was no significant difference between them (GLM: $F_{0.05,1}$=0.849, P>0.05). The successful foraging rates of the fifth days (0.82±0.02) and the first to fourth day (0.71±0.15; 0.88±0.39; 0.85±0.02; 0.88±0.03, respectively) in the experimental groups showed no significant difference (ANOVA: $F_{0.05,1}$=0.514, P>0.05; $F_{0.05,1}$=1.513, P>0.05; $F_{0.05,1}$ = 0.608, P>0.05; $F_{0.05,1}$ = 1.985, P>0.05, respectively).

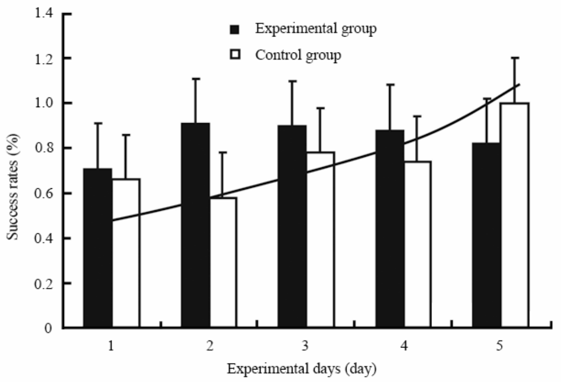

Fig. 3 The relationship between the success rates of foraging and the experimental days

The success rates of foraging in control groups are increasing when the experimental days are increasing. The curve-fitting equation of control group: y=0.1835+0.4101x-0.1207x^2+0.0142x^3. But there was no significant correlation between the success rates of foraging and the experimental days in experimental groups.

3 Discussion

3.1 The foraging behavior of *C. sphinx*

In the experiment, we found that the bats began their foraging from the corner of the array, which was similar with the foraging behaviour in the field. They chose food firstly from the edge, and then gradually moved to the centre of the orchards. The bats' habit was not affected by rain without thunder and lightning. However, during thunderstorms in the evening, the foraging time of *C. sphinx* was significantly delayed, and its foraging frequency was also decreased. Previous studies indicated that moonlight influences the foraging activity of *C. sphinx*. Cloud cover enhanced the foraging activity, but lightning and thunder suppressed it (Elangovan & Marimuthu, 2001). The first feeding time of the fourth day in the experimental group slightly elevated, possibly due to suppressing of foraging activity by lightning and thunder (Fig. 2).

3.2 Landmarks and spatial memory of *C. sphinx*

In this study, we found that bats could learn to find the rewarding locations (banana containers), but tried to avoid the un-rewarding locations (fake bananas containers). The performance was similar in experimental and control groups, in which the successful foraging rates were both very high. They rarely made a mistake, the success rates were up to 100% as the experimental days increased (Fig. 3). The time to first foraging became shorter when the experimental days increased (Fig. 2). There were significant negative correlations in both groups between the time of the first foraging and the experimental days, which suggested that spatial memory in *C. sphinx* was formed gradually. The time of the first foraging of the fifth day was shorter than the first days', which suggested that spatial memory in *C. sphinx* had formed (Fig. 2). If the landmarks placed in the experiment were helpful to spatial memory of *C. sphinx*, the foraging activity would be influenced in a certain extent after removing the landmarks (the fifth day), and the time of the first foraging would be enhanced when compared with the fourth day. But the average time of the first foraging of the fifth day was shorter than the fourth and the third days' (Fig. 2), so we can confirm that the landmarks do not contribute to the spatial memory of short-nosed fruit bats. In addition, after removing the landmarks, there were also no significant differences between the successful foraging rates of the fifth day and the fourth day in the experimental group (Fig. 3). These results suggest that the landmarks have no discernable effects on the memory of the foraging space.

In a previous study, Möhres et al (1949) reported that the microchiropteran bats *Rhinolophus ferrumequinum* and *Myotis myotis* would refuse to fly into space that was made available by the enlargement of a familiar room, because these bats had formed profound spatial memory of their roosts. Short-term changes in their living space did not affect their choice of roosts. Bats can accurately remember the nest, or a fixed food source (like fruits, nectar) position, using spatial information rather than shape or color information of food and nests (Höller, 1995; Thiele & Winter, 2005; Winter & Stich, 2005). Similar results were confirmed in pigeons (Strasser & Bingman, 1996) and hummingbirds (Hurly & Healy, 1996). In another similar study of spatial memory, Richard et al (2005) designed an experiment to test the memory of *R. aegyptiacus* to a perch location, the results demonstrated that once the location of a perch was learned, *R. aegyptiacus* was not influenced by the movement of local landmark cues in the vicinity of the perch under either light or dark conditions. When the surrounding environment was relative stable, and the *R. aegyptiacus* had learned the perch position in space, the moving cues placed had little effect on their spatial memory. It should be emphasized that the landmarks or cues associated with a location in space are not important in the process of learning the location, only that once learned, the memory is resistant to the movement of some of these landmarks or cues. Our study has validated the above conclusion. Once *C. sphinx* learned to feed in a certain space, the movement of local landmarks did not influence their activity. It meant that the bats only paid attention to the spatial position of food when they were foraging, and paid less attention to the objects around the food. *C. sphinx* is without echolocation ability, so olfaction plays a very important role in their feeding process. *C. sphinx* used olfaction combined with vision and spatial memory to locate food (Elangovan et al, 2006). And spatial memory was only formed in a stable environment. The stable landmarks may be helpful for their spatial memory (Beigler & Morris, 1993, 1996;Höller, 1995). Therefore, the changed number of landmarks in our experiment appeared to have no discernable effects on the space memory of *C. sphinx*.

In another study, the nectar-feeding bat *G. soricina* found 16 rewarding feeders out of 64 feeders (Ulf et al, 2008). Increasing density of local spatial cues improved accuracy in re-locating rewarding feeders by helping bats identify profitable flowers on a small scale. They believed that with the increased density of local echo acoustic cues there would be an increase in spatial accuracy because the decreasing average distance between cues and goals has increased the utility of cues. *G. soricina* used local echo acoustic cues to distinguish the 16 rewarding feeders from the 48 non rewarding feeders, so they can find rewarding feeders faster and more accurately. Our results are not consistent with the conclusion of Ulf et al (2008), which may be related to the difference in species. Many animals are able to find the place where they went to before. The ability is generally called “spatial memory”, but the underlying mechanism of them is different (Winter & Stich, 2005), mainly including echolocation (Ulf et al, 2008; Richard et al, 2005), vision (Strasser & Bingman, 1996) and olfaction (Richard et al, 2005). In the study mentioned above, *G. soricina* relys on echolocation to identify and remember the spatial location (Ulf et al, 2008), while the *R. aegyptiacus* is able to orient and navigate using both vision and echolocation (Richard et al, 2005), pigeons use vision to remember the spatial position of nests and returns to their nests accurately (Strasser & Bingman, 1996). *C. sphinx* uses olfaction combined with vision and spatial memory to locate food (Elangovan et al, 2006). We believe that *C. sphinx* uses olfaction combined with vision and spatial memory to form a stable spatial memory in our experiment. The results of our experiment suggest that spatial memory in *C. sphinx* was formed gradually and the placed landmarks appeared to have no discernable effects on the memory of the foraging space.

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