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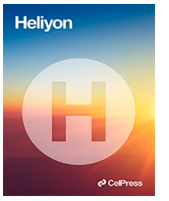

Protocol guide for food foraging behavior test: Assessment of decision making in rodents

Muhammad Jahangir [a], NanXi Zheng [a], S. Mudasser Shah [b], Ying Huang [c], Bing Lang [a,*], Xiao-Ping Wang [a,**]

[a] Department of Psychiatry, National Clinical Research Center for Mental Disorders, National Center for Mental Disorders, The Second Xiangya Hospital of Central South University, Changsha, 410011, China
[b] Department of Psychiatry and Psychosomatics, ZhongDa Hospital, Southeast University Nanjing, Jiangsu, China
[c] Department of Laboratory Animal Science, Fudan University, Shanghai, China

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ABSTRACT

Food foraging behavior requires higher cognitive function like investing efforts in decision making. Hoarding food for the future consumption in adverse climatic conditions or to avoid predatory threats needs precise perception and potential of decision making to overcome challenges in the time of need. The brain areas and neural circuitry responsible for such cognitive skills are poorly understood. Previously available animal models are trained prior to test, which makes it difficult to understand the true nature of animals, and hoard the food from external source into the cage. The new food foraging behavior test, recently developed and evidenced by Li et al., relies on untrained rats and test the competitive ability and hoarding from source within the test box. It can be used to study decision making potentials and underlying neural bases in laboratory settings. Multiple aspects like food quality/flavor preference, competitive nature can be assessed within the test box and the paradigm is conveniently customizable according the hypothesis. However, a detailed protocol guide, to be followed in the laboratory setting, for food foraging behavior test is not available. Therefore, it is urged to produce an elaborated guide for scientists to conduct food foraging behavior test in convenient and precise manner.

1. Introduction

Food removal, hoarding, and competitive predation on other animals are all aspects of food foraging behavior. It is linked to high cognitive abilities like making deliberate decisions as well as the choices that animals make [1]. Foraging depends on first determining where to look for food based on a quality assessment and a prediction of food availability. Afterward, actions might be designed to maximize food procurement throughout time [2,3]. Hoarding, which is particularly common in rodents, is a crucial adaptation tactic in times of food scarcity, predator risks, bad weather and reproduction [4]. Rodent species may have developed new foraging strategies to improve their evaluation of stimuli in their more proximate habitats with short time horizons or the frontal cortex's Evaluation functions [2]. Planning and resource assessment tasks are closely related to the frontal cortex function [3]. In both rodent and primate species, adaptation to foraging contexts shapes interactions between the frontal cortex and sensorimotor systems, which are necessary

* Corresponding author.
** Corresponding author.
E-mail addresses: xiaop6@csu.edu.cn (B. Lang), bing.lang@csu.edu.cn (X.-P. Wang).

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for action selection [5]. However, decision making is a complex cognitive process, entails assessing risk, reward, and cost. There is strong evidence that the anterior cingulate cortex (ACC) is involved in decision-making and the evaluation of social information [6]. According to a recent fMRI study, the human anterior cingulate cortex (ACC) can encode environmental signals as estimations of the foraging environment's richness and cost [7]. However, the brain parts that control decision making, cognitive abilities are poorly identified.

Food foraging behavior test (FFT) paradigm is used to assess the decision-making capacity and evaluation of risk-cost in proportion to reward in rodents. Moreover, the underlying brain regions for the decision making can be traced via FF test. Previous models merely measure the amount of hoarded food transferred from an external source into the home cage [8,9] and trained/exposed repetitively [10], but this is inaccurate because the rats used in their studies were trained prior to test, a complicating factor that affects our understanding of the true nature of animal behavior [1]. Winter et al. used the paradigm which tests the animals' ability to maintain spatial awareness in their testing area which is important for food hoarding behavior. This refers to the animal's ability to navigate from an established home base to a food source, and back to home base, frequently making dynamic modifications along the way to maximize efficiency. For detailed information regarding development of food hoarding tasks, one may see a review by winter et al., [11]. However, a small laboratory animal model of food foraging without prior training or manipulation is necessary to mimic the natural animal behavior. The food foraging behavior test with established novel laboratory model including competitive, non-competitive, and no-hurdle food foraging paradigms is recently developed by Ref. [1]. Therefore, it is urged to provide a protocol guide elaborated substantially. It will help the scientists across the globe to conduct food foraging test in a precise and controlled manner.

2. Materials and equipment

1. Protective wears (Mask, Gloves and lab coat)
2. Wooden box (Black in color)
3. *Sprague Dawley (SD) rats
4. Rodents bedding (wood chips)
5. Rodents food
6. Wire topped cage
7. Weight balance
8. Ethanol (for cleansing purpose)
9. Calm and dark room
10. Camera (Fig. 1)

* It is a well-established animal model in research and has been extensively characterized in terms of its genetics, behavior and physiology. However, this protocol is nascent and is handy to be exploited by the scientific community to test it with other strains of rats.

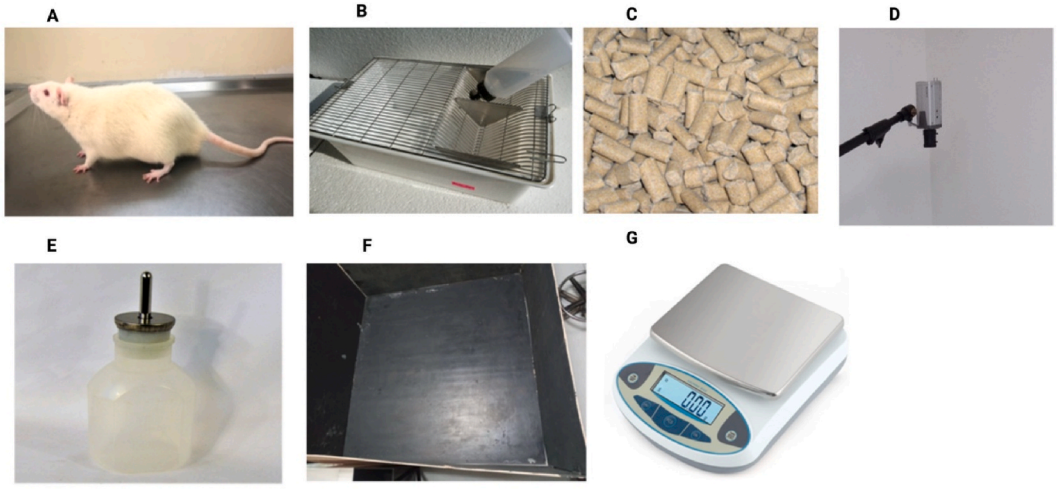

Fig. 1. Materials and equipment required for food foraging test. A: SD (Sprague-Dawley) rat, B: Rodents housing cage, C: Standard rodents' food, D: Camera, E: Water bottle; The rat remained food-deprived for 12 h, therefore, water bottle cork with a metallic covering should be used to avoid nibbling by the rat, F: Wooden open field box, G: Digital balance.

2.1. Animal acclimatization

All experimental SD-rats (250 g, 7 weeks) are housed two per cage in the wire-topped plastic home base with removable wire cover (30 cm × 18 cm × 16 cm) and *ad libitum* access to food and water at room temperature (23 °C ± 1), for seven days to acclimatize. Cage size can be selected as per the age and size of rat. At least once in a day, the animal should be placed on palm and groom gently to alleviate the fear of being handled and habituate with gloves smell, usually shocking colors of hand gloves, lab coats and surrounding in the test room.

Note: SD rats have been used to perform the test. However, it is warranted to test rest of the types/strains of animals to produce results for future guidance.

2.2. Test room condition

Test should be performed in a room free of noise, odor, and sound with maintained temperature. The test room should be away from busy corridors in the building and machines' noise in the Lab. Glass windows or any point allowing even dim light from surrounding should be covered to create dark environment. Drug administration, if is the part of experiment, should be done 30 minutes before the test in separate room, not in the test room. Test boxes should be cleaned with ethanol, let them dry, prior to execution of test.

2.3. Box size and shape: rectangular and circular

The test is performed in open field box placed in a sound free environment. The open field box is constructed from black wooden planks (Plexiglas recommended to avoid nibbling by rats) with dimensions of 150 cm × 150 cm × 50 cm shaped into rectangular box (Figs. 1 and 2). The open field should be cleaned with ethanol prior to conduct test and should be free of debris, fecal pellets and urine drops from previous test. Circular open field can be an alternate option for the test, however, it is being suggested to use rectangular box for stable results.

2.4. Food deprivation and test duration

The test rats are kept deprived of food, but access to water, for 12 h (7:00 a.m. to 7:00 p.m.) prior to test. AT 7:00 p.m., test rat is placed in open field for 2 h to habituate. Rats have free access to water during the test in open field. After habituating for 2 h (7:00 p.m. to 9:00 p.m.) in the field, a wired top cage is placed near the center in the open field with 250 gm food on top (a rat inside the cage, same gender and age, only in competitive test). The cage can be placed near the walls but should not be too close to the walls of box, otherwise, the rats can climb the cage and then over the wall of box to escape. The test rat is allowed to navigate the cage and foraged food freely from 9:00 p.m. to 7:00 a.m. (Fig. 2). The amount of food hoarded to the open field and the amount left in the food containers are separately weighed at 7:00 a.m. Rats in the cages during test are used once so that no animal experienced more than one session.

2.5. Cautions

- After 12 h of food deprivation, rats may show agitated behavior, therefore, careful handling is suggested.

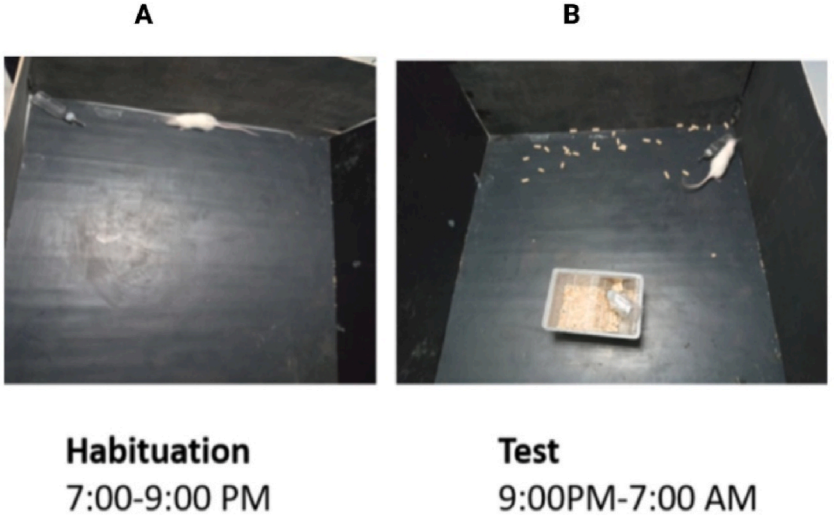

Fig. 2. Food foraging test open field. A: habituation phase (7:00 to 9:00 p.m.) only with a water bottle and rat, B: the test phase (9:00 to 7:00 a.m.) shows the end of the test session, in the morning, with food pellets hoarded in the open field.

- The wire top of cage should be checked to avoid any sharp edge, as it can cause injury to rats while climbing the cage for food foraging, which ultimately affects the behavior. Rats have to make many trips to forage food, so, injuries can affect the results and also increase the chances of infection.
- The wire top should be tightly closed over cage to avoid sliding away of wire top during test.

2.6. Test types

Food foraging test is customizable according to the hypothesis, maintaining the basic principles. The essence of the model in the competitive and non-competitive food foraging paradigms is that food is offered in a wire mesh container (the cage lid) on a 16-cm-high cage, with the same gender residing rat, which forces test rats to make an effort energetically or psychologically to overcome the hurdle and compete with residing rats to forage food. Here are few basic types elaborated:

- **Competitive food foraging test**. One rat is placed in a small plastic home cage (30 cm × 18 cm × 16 cm: cage size customizable) with a metal wire cover and 250 g of standard food pellets on the wire mesh for competitive food foraging activity, and a test rat is placed in the open field The rat in the home cage and the test rat should be from different litters and should had never interacted before the test. After that, the test rat is free to access the cage and freely forage for food, after habituation, from 9:00 p.m. to 7:00 a.m.

Note: The rat in the cage during test should be provided with separate water bottle and test rat can be provide with water bottle placed in the open field.

- **Non-competitive food foraging test.** The test rat is placed in the open field to test the noncompetitive food foraging activity and is free to navigate to the cage without any residing rat in the cage, and the rat foraged food freely from 9:00 p.m. to 7:00 a.m. (with 2 h of acclimatization from 7:00 p.m. to 9:00 p.m.) next day. The other steps of the process are carried out in the same way as the competitive food foraging paradigm test session.

2.7. Videotape recording of behavior

We recommend using video recording (with camera) to meticulously investigate the impact of foraging motor behavior on various ethological measurements related to anxiety-like behavior. This will allow for precise tracking and analysis of their foraging motor behavior, enabling a thorough examination of how competitive interactions may influence their food-seeking strategies and its impact on foraging decision. Moreover, ethological measurements of anxiety like behaviors; grooming and rearing activities, time spent in the center versus periphery can be observed and quantified, to gain valuable understanding about the anxiety-like behavior of the test animal during the experiments.

2.8. Analyses

Next morning, at 7:00 a.m., test rat to be removed from open field and returned to home cage. The hoarded food pellets scattered in the open field are collected to weigh and food pellets left over the wire top are weighed separately to quantify the amount of hoarded food. Few grams of food will be found missing, as the rats would consume some. It is recommended that the food hoarded to the open field should not be served to the rats again, as it could be contaminated with urine, feces and pheromones during the test. If interested in estimating anxiety level from the number of fecal pellets and urine drops, usually it is hard to find urine drops till morning due to evaporation, however, fecal pellets can be counted to estimate anxiety level.

Results can be presented in Mean ± SEM and statistical differences can be measured by applying ONE-WAY or TWO-WAY ANOVA followed by Tukey post hoc multiple comparison test or post hoc Dunnett test, according to the data available. If using only two groups of rats, then two tailed student's t-test can be applied.

3. Discussion

Food foraging behavior test (FFT) is a paradigm to evaluate the decision-making capacity of animal, hoarding food and the range of risk or challenge to overcome estimating risk-cost and reward ratio. The FFT has been developed by Li et al. [12] to quantify the cognitive function and determine the brain areas involved in decision making in a laboratory setting, without any artificial intervention or training. This paradigm reflect distinct situations: expend a greater psychological effort as well as a physical effort to earn a reward (competitive), expend a physical effort to obtain food (non-competitive). In the natural environment, animals engage in competitive interactions for food resources. To replicate this competitive scenario, the protocol was designed with a competitive paradigm, incorporating a recommended difficulty level of a 16 cm high hurdle presented to the animals. Interestingly, when the difficulty level was increased to a higher hurdle of 36 cm, the food foraging activity was significantly reduced, but this reduction was not statistically significant in non-competitive food foraging tests. This observation suggests a potential interaction between the physical hurdle and the presence of a competitor, indicating that these factors may jointly influence the foraging behavior of the animals [12]. In another study conducted by Bardgett et al. [13], animals displayed a preference for lower reward options when faced with a choice between a larger reward with a higher barrier and a lower reward with an easier access route. This finding suggests that

foraging decisions are primarily influenced by safety needs rather than solely being driven by food availability, especially when the animals are not severely food deprived [13,14].

Studies have demonstrated that cost/benefit decisions regarding relative effort are mediated by distributed dopaminergic and glutaminergic neural circuits [15]. The dopamine receptor antagonist haloperidol and the NMDA receptor antagonist MK-801 were utilized in both competitive and non-competitive food foraging tests. In both competitive and non-competitive food foraging tests, rats displayed a significant decrease in foraged food after haloperidol administration, indicating the essential role of D2 receptors in effort-based decision-making [12,13,15]. For haloperidol, a lower dose of 0.1 mg/kg was used to avoid locomotive deficits associated with higher doses [16,17]. On the other hand, MK-801 (an NMDA receptor antagonist) reduced the quantity of foraged food in competitive food foraging tests but had no impact on foraged food in non-competitive food foraging tests. This suggests the potential involvement of NMDA receptors in regulating social competitive activity, while appetitive behavior remained unaffected [12].

There had been no difference between male and female rats in the amount of foraged food in the competitive, non-competitive test [12]. The amount of foraged food found consistent each day for five consecutive days with a slight increase in following days. However, no significant difference was found in the amount of food foraged in the presence or absence of bedding materials [12]. Moreover, the preference for quality or taste of food (sweet and normal) and preferences during stress have been quantified via FFT with reasonable differences (Fig. 2) [18]. The test paradigm is novel and can be customized according to hypothesis. To make it more challenging for rat, the height of cage placed in the open field box can be increased. Furthermore, Tu et al., have reported variation in the test paradigm while presenting the acute restraint stress (ARS) rats with sweet and standard food in combination with competitive and non-competitive aspects (Figs. 3(1(A–E) and 2(A–E)) [18].

Several factors can affect the food foraging capacity, such as stress/anxiety, infections, lesions, pharmacological interventions or unstable noisy surrounding. Moreover, rodents are nocturnal and purely rely on olfaction for food foraging. Therefore, any fragrance, odor of chemicals or fecal/urine traces from the previously tested rats either in the open field arena or in the test room can disturb the food foraging process. For precision, it is suggested to perform olfaction test [19] before food foraging behavior test to confirm the sense of olfaction is intact. Moreover, the sense of taste can be tested using a gustometer for quick assay or a 2-bottle preference test, in which two bottles of fluid are placed on an animal's cage for a specified period of time, usually 24 h, with one bottle containing a taste solution or water and the other containing a different taste solution (as per the requirement of hypothesis) [20]. At the end of the experiment, the animal subjects' sense of smell (olfactory cortex) and taste cells (Gustatory cortex), which process gustatory and olfactory signals, could be histologically examined for further confirmation. In addition, the food fragrance or flavor may cause preference, thus, if testing preference for the food flavors is not the part of the experiment, then food of similar quality and flavor should be presented.

Furthermore, the rat will stay for 12 h in the open field, make sure the room temperature is maintained and stable. Open field is a

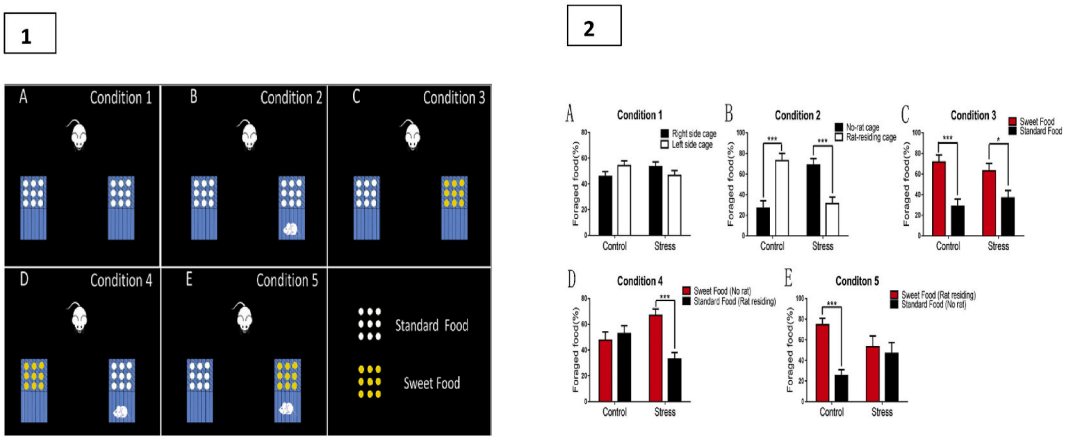

Fig. 3. (1) "Schematic illustration of the food foraging testing paradigms used in the current study. A Condition #1; B: Condition #2; C: Condition #3; D: Condition #4, and E; Condition #5. Two wire-topped plastic home cages (30 cm × 18 cm × 16 cm) are placed in parallel in an open field. In Condition #2, #4 and #5, a residential rat is housed in one of the cages for at least 1 week before the experiment. On each trial, the rat in the open field is allowed to forage the loaded regular or sugared food from the two cages as indicated" (2) "Effects of ARS on the food foraging decision making behaviors in different value-based and social-base contexts. A–E: The percentage of foraged food from the two-small cages in Conditions as indicated. Results indicated that both control and stress animals showed no significant difference in the amount of foraged food between two cages in the condition #1 (A). In Condition #2, foraged standard food is significantly more in the rat residing cage compared with no-rat cage for control group, whereas for stressed animals, food foraged from the rat residing cage is less than the no-rat cage (B). In condition #3, both the control and stressed group prefer to forage sweet food pellets (C). In condition #4, there is no significant difference between foraged standard food from the rat residing cage and foraged sweet food from no-rat cage for control group. For stressed rats, they tend to forage sweet food from the no-rat cage than forage standard food from the rat residing cage (D). In Condition #5, the percentage of sweet food foraged by the control animals from the rat residing cage is higher than the percentage of the standard food foraged from the no-rat cage, while there is no significant difference between the percent of sweet food pellets foraged from rat residing cage and the percentage of standard sweet food pellets foraged from no-rat cage for stress group (E). n = 9 for each group. ***p < 0.001; *p < 0.05". Adopted from Tu et al. [18].

challenging ground for test animal, therefore, anxiety can affect the results. For the reason, to eliminate the confounding anxiety factor and filter the exact results, an anxiety test [21] is suggested to perform before food foraging behavior test to evaluate the anxiety between groups. Sharp edges in the open field or in the cage wired top causes injury to the rats, can affect animal health and ultimately the results.

4. Recommendations

It is recommended to the scientific community investigating the brain and brain illnesses that there is still room to test this paradigm in the following domains:

1. Aging studies
2. Sociability/isolation effect (Group housed or isolated animals)
3. Animal models of Psychiatric disorders
4. Neuropathology studies

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Ethics approval

This work was approved by Institutional Animal Care and Use Committee (IACUC), The Second Xiangya Hospital, Central South University, China (Approval number: 2023890).

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Author contribution

MJ and **NXZ** wrote the paper and performed the experiments, **SMS** and **YH**: analyzed and interpreted the data, **BL** and **XPW**: Conceived and designed the experiments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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