The Social versus Food Preference Test: A behavioral paradigm for studying competing motivated behaviors in rodents

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Method Article

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Abstract

Behavior is influenced by a combination of factors, with the expression of the appropriate behavior dependent on an individual's current motivational state and the presence of stimuli in their surrounding environment. Thus far, most laboratory studies have focused on uncovering the peripheral and central systems that regulate the expression of a single behavior or the expression of a suite of behaviors associated with a single motivational state. In natural settings, however, an individual can be simultaneously experiencing multiple motivational states with multiple choices of how to act. Yet, the direct assessment of the roles of peripheral and central systems in coordinating motivated behavioral choice is largely understudied. This may be due to a lack of behavioral tests that are suitable for such investigations. Here, we describe a recently developed behavioral paradigm, hereafter called the Social versus Food Preference Test. This behavioral paradigm was validated in both rats and mice and is highly flexible, which will allow addressing of a wide range of research questions concerning the peripheral and central systems that coordinate the choice to seek social interaction versus the choice to seek food.

Background

The Social versus Food Preference Test was developed to assess the preference of rats and mice to investigate a social stimulus versus a food stimulus, and was based on a two-chamber social interaction assay used to determine the effects of hunger signals on social interest in mice [1]. In our adaptation of this paradigm, we examined social versus food preference using a three-chamber apparatus where the social stimulus and the food stimulus were placed on opposite ends (Fig 1) [2]. This configuration allows for a neutral middle chamber zone instead of a forced choice that two-chamber configurations elicit, and its use was based on our previous experiences with social novelty preference [3] and opposite sex preference [4] tests in rats, and well-characterized sociability and social novelty preference tests in mice [5].

Method Details

Apparatus

Two sizes of the three-chamber apparatus were custom-constructed (Fig 1A, Fig 7), one for rats (Scientific Instrumental and Machining Services, Boston College) and one for mice (Physics and Astronomy Machine Shop, Michigan State University). The exterior of the apparatus was composed of Plexiglas (rats) or PVC (mice), and each chamber (rats: 40 cm x 40 cm x 27 cm; mice: 30 cm x 30 cm x 20 cm) was separated by a translucent Plexiglas (rats) or acrylic (mice) partition with an opening (rats: 10 cm x 10.2 cm; mice: 5 cm x 5 cm) to allow passage between chambers. When possible, the walls of the apparatus should be constructed of opaque materials to reduce visual distractions during testing. Alternatively, paint or opaque Con-Tact paper can be applied to the exterior walls. If automated tracking software will be used, the color of the apparatus should contrast to the color of the test subjects. Commercially available three-chamber apparatuses would also be well-suited for this test.
Custom-constructed corrals were used to hold the social and food stimuli (Fig 1B). These corrals allow for olfactory, visual, and auditory contact, but restricted tactile contact of the stimuli by the experimental subject. For rats, rectangular corrals (18 cm x W 10 cm D x 21 H cm) were composed of a solid translucent Plexiglas top/bottom/back and translucent Plexiglas bars (0.6 cm diameter, spaced 1.75 cm apart) on the other three sides. For mice, cylindrical corrals (8.5 cm ID, 10.5 cm OD x 17cm H) were composed of solid translucent Plexiglas top/bottom connected by translucent Plexiglas bars (0.6 cm diameter, spaced 1.5 cm apart). Commercially available corrals or pencil cup holders (for mice only) would also be well-suited for this test.

The apparatus should be wiped down with 70% ethanol and corrals should be wiped down with a dilute cleaning solution at the start and end of each day, as well as between subjects.

**Procedure**

**Habituation**

Experimental subjects and stimulus animals should be habituated to the testing procedures 1–2 days prior to their first test in order to acclimate them to the procedures and apparatus. Experimental subjects are placed into the center chamber and allowed to freely explore the apparatus and investigate empty corrals located on opposite ends for 10 min before being returned to their homecage. Habituation of experimental subjects should be video-recorded and the time spent in each chamber measured (see Behavioral Scoring, below). While individual subjects may spend more time in one chamber than others, as a cohort there should be no difference in the time spent in the two end chambers of the three-chamber apparatus. In separate trials, stimulus animals are habituated to confinement within a corral for 10 min before being returned to their homecage.

**Behavioral Testing**

1. To reduce the amount of food-related sensory cues present on the social stimuli, remove food from the cages of stimulus animals 2 hrs prior to the start of testing [1].

2. If testing occurs in a room separate from the housing room, move subjects to the test room at least 1 hr before the test to allow them to acclimatize.

3. Clean apparatus and corrals.

4. Set-up camera for video-recording of the tests.

   1. Direct overhead placement is ideal for subsequent videos analyses.

   2. Make sure the apparatus is equally illuminated, and glare minimized.

      1. Overhead white light should be used for testing during the light phase.
2. Infrared illuminators and indirect dim red light should be used for testing during the dark phase.

3. A camera that is connected to a computer in an adjoining room or outside the test room is recommended, so that tests can be monitored remotely and the animals experience no disturbance from the experimenter.

5. Place the selected social stimulus into one corral and selected food stimulus into a second corral, then put the two corrals on opposite ends of the three-chamber apparatus.

1. Corrals should be put into a designated location within each chamber (e.g., middle of back wall or back corner).

2. Because the Social versus Food Preference Test assesses real-time place preference, the location of the social and food stimuli (i.e., left chamber or right chamber) is independent of chamber preference during habituation.

3. However, the location of the social and food stimuli should be counterbalanced between subjects each test day.

4. When applicable, the location of the social and food stimuli should also be counterbalanced within subjects across test days to prevent the development of a conditioned place preference.

5. The specific social and food stimuli should be selected based on the research question.

   1. Potential considerations for the social stimulus: novelty/familiarity, age, sex
   2. Potential considerations for the food stimulus: novelty/familiarity, palatability, previously devalued
   6. Food should be moved away from the accessible edges of the corral to prevent consumption.

6. Start the video recording, then place the experimental subject into the center chamber and allow free exploration of the apparatus for 10 min.

   1. The experimenter should monitor the test, but remain out-of-sight, preferably outside the test room to minimize any disturbances to the animals.

   2. If an experimental subject climbs up and sits on top of the corral or the edge of the apparatus, allow ~10 sec for the subject to climb or jump down on their own. If they do not, gently pick up and place the subject on the floor of the chamber they climbed out of (not the center chamber).

7. Stop the video recording, then remove the experimental subject from the apparatus and return it to its homecage.

8. Remove the social stimulus from the corral and return it to its homecage.

9. Remove the food stimulus from the corral and discard.
10. Clean the apparatus and corrals, and reset (starting at item 5) for the next experimental subject (if applicable).

Behavioral Scoring

1. Automated tracking software can be used to quantify a wide range of behaviors.

   1. At a minimum, it is recommended to quantify the time spent in each of the three chambers, and the time spent in a designated “investigation zone” around each of the two corrals (e.g., rats: head placement within 6 cm of corral edge, mice: center mass placement within 5 cm of corral edge; Fig 1A).

   2. If issues arise with the automated tracking, it could be that there is too much glare, the camera was not positioned directly overhead the apparatus, the software is unable to distinguish between the experiment subject and the social stimulus, the experimental subject climbs the corrals, or the experimental subject did not contrast well enough against the apparatus.

2. Manual scoring can be used instead of, or in addition to, automated tracking software.

   1. At a minimum, it is recommended to quantify the time spent in each of the three chambers (especially if automated tracking is unavailable or technical difficulties arise), as well as the time experimental subjects spent actively investigating each of the two stimuli. Investigation is defined as when the experimental subject’s attention is directed towards the stimulus inside of the corral as indicated by head position/gaze orientation, and the subject is engaged with the corral (e.g., sticking nose between bars, pawing, sniffing).

   2. To reduce potential bias, experimenters scoring videos should be unaware of the characteristics and/or test conditions for experimental subjects (e.g., age, sex, homeostatic manipulation, drug condition) and stimuli (e.g., familiar, novel). However, blinding to the stimulus category is difficult and should be avoided if possible, since investigation directed towards the social and food stimuli is qualitatively different.

      1. For the food stimulus, investigation is almost exclusively around the bottom portion of the corral. Rearing and sniffing the top portion of the corral is not scored as investigation since the experimental subject’s attention is not directed at the food placed on the floor of the corral.

      2. For the social stimulus, rearing and sniffing the top portion of the corral by the experimental subject can be scored as investigation if the stimulus animal is also rearing and it is clear that the experimental subject’s attention is directed towards the social stimulus inside.

3. Data can be analyzed to assess both the absolute (i.e., time in sec) and the relative (i.e., preference) interest of experimental subjects to investigate the stimuli.
1. “Social over food preferences scores” can be computed as \([(\text{social time})/(\text{social time} + \text{food time}))\times100\], where values > 50% indicate that subjects spent more time with the social stimulus, and values < 50% indicate that subjects spent more time with the food stimulus. Alternatively, the inverse can be calculated to determine “food over social preferences scores”, depending on the research question.

1. These scores can be computed for time spent in the social chamber versus the food chamber, time spent in the social investigation zone versus time spent in the food investigation zone, and/or time spent investigating the social stimulus versus time spent investigating the food stimulus.

2. Chamber preference can be calculated taking the middle chamber into account, where preference for the social chamber is computed as \([(\text{social chamber time/length of test})\times100\] and preference for the food chamber computed as \([(\text{food chamber time/length of test})\times100\].

Validation

The Social versus Food Preference Test was validated by examining how acute food deprivation would alter stimulus preference, under the assumption that food deprivation would increase motivation for food and thus bias preference more towards the food stimulus [1, 6, 7]. Experimental subjects were individually housed adult (13–14 week old) Wistar rats (n = 7 males, n = 5 females) and C57BL/6J mice (n = 8 males, n = 6 females) [2]. Experimental subjects were first habituated to the testing apparatus as described above, and then tested in the Social versus Food Preference Test on two occasions each 48 hrs apart using a within-subjects counterbalanced design (sated x food-deprived). The length of food deprivation was 24 hrs for rats and 18 hrs for mice (per IACUC recommendation and pilot testing to ensure subjects would not lose more than 15% of their body weight). The social stimulus was an unfamiliar age, sex-, and species-matched conspecific, and each social stimulus was used twice per day in non-successive tests to reduce the number of animals used. The food stimulus was standard laboratory chow (Teklad Irradiated 22/5 Rodent Diet, 8940; ~8 pellets for mice, ~20 pellets for rats). A webcam (Logitech HD Pro C910) was attached to the ceiling and connected to a PC computer in an adjoining room to record the habituation and test sessions. All other methods were as described above.

Automated behavioral tracking software (AnyMaze, Stoelting) was used to quantify the amount of time experimental subjects spent in each of the three chambers, and the amount of time experimental subjects spent in designated investigation zones around the corrals (rats: head placement within 6 cm of corral edge, mice: center mass placement within 5 cm of corral edge; Fig 1A). Experimenters, who were unaware of sex and testing conditions, manually scored recorded videos using a freely-available behavioral coding program (Solomon Coder, https://solomon.andraspeter.com/) to quantify the amount of time the experimental subjects spent investigating each of the two stimuli. Social over food preference scores were then computed for all three of these measurements (i.e., chamber, investigation zone, active investigation). Data were analyzed using mixed-model omnibus ANOVAs [hunger condition (sated, food-deprived; within-subjects factor) x species (rats, mice; between-subjects factor)], and *post hoc* simple
effect F-tests were conducted to clarify significant interactions. One-sample t-Tests with a reference value of 50% were used to determine chamber preference during habituation and stimulus preference during tests. Estimates of effect sizes were assessed by partial eta squared ($\eta^2$) or Cohen’s d ($d$). All data were analyzed using IBM SPSS Statistics 26, and statistical significance was set at $p < 0.05$.

Neither rats (44.5 ± 3.6, $t_{(11)} = 1.51, p = 0.16$) nor mice (54.8 ± 3.93, $t_{(13)} = 1.22, p = 0.24$) exhibited a preference for the left chamber versus the right chamber during the habitation session.

As expected, food deprivation significantly increased the amount of time experimental subjects spent in the investigation zone around the corral containing the food stimulus and the amount of time subjects spent actively investigating the food stimulus, however the amount time spent in the chamber containing the food stimulus was similar between sated and food-deprived conditions (Table 1, Fig 2A-C). There was a significant hunger condition by species interaction on the time spent investigating the food stimulus (Table 1). Post hoc F tests indicated this was because the effect size for the increase in investigation time between sated and food-deprived conditions was larger in mice ($F_{(1, 24)} = 49.0, p < 0.001, \eta^2 = 0.67$) than in rats ($F_{(1, 24)} = 6.74, p = 0.016, \eta^2 = 0.22$). Mice spent more time than rats in the chamber containing the food stimulus and actively investigating the food stimulus, but time in the investigation zone around the corral containing the food stimulus was similar between rats and mice (Table 1, Fig 2A-C).

Across all measurements, the amount of time experimental subjects spent with the social stimulus was similar between sated and food-deprived conditions, but rats spent significantly more time than mice with the social stimulus (Table 1, Fig 2A-C).

Social over food preference scores as measured by automated tracking of chamber time or investigation zone time were similar between sated and food-deprived conditions (Table 1, Fig 2D-E). However, when preference was measured by manually scoring investigation time, food deprivation significantly decreased social over food preference scores in rats and mice (Table 1, Fig 2F). Across all measurements, rats had greater social over food preference scores than mice (Table 1, Fig 2D-F). Under both sated and food-deprived conditions rats exhibited an equal preference for the social chamber and the food chamber (Fig 2D), and a significant preference for the social stimulus as measured by investigation zone time or active investigation time (Table 2, Fig 2E-F). In contrast, for all measurements, mice had no stimulus preference when sated and a food preference when food-deprived (Table 2, Fig 2D-E).

To summarize, while there were robust differences between Wistar rats and C57BL/6J mice in stimulus investigation patterns, how their investigation patterns changed in response to the food deprivation manipulation was similar. Specifically, food deprivation increased the time spent with the food stimulus, and this decreased social over food preference scores. Importantly, the data presented here illustrate the benefits of conducting manual scoring for active investigation time, since this measurement typically had
larger effect sizes than those observed for automated tracking of chamber time or investigation zone time.

**Table 1.** ANOVA statistics and partial eta squared ($\eta^2$) effect sizes; significant effects shown in **bold**.

|                          | **Hunger Condition** | **Species** | **Interaction** |
|--------------------------|----------------------|-------------|-----------------|
| Social Chamber [sec]     | $F_{(1,24)} = 0.62, p = 0.484,$ | $F_{(1,24)} = 7.23, p = 0.013,$ | $F_{(1,24)} = 0.54, p = 0.47,$ |
|                          | $\eta^2 = 0.025$     | $\eta^2 = 0.23$ | $\eta^2 = 0.022$ |
| Food Chamber [sec]       | $F_{(1,24)} = 2.76, p = 0.11,$ | $F_{(1,24)} = 22.5, p < 0.001,$ | $F_{(1,24)} = 0.90, p = 0.35,$ |
|                          | $\eta^2 = 0.10$      | $\eta^2 = 0.48$ | $\eta^2 = 0.036$ |
| Chamber:                 | $F_{(1,24)} = 1.32, p = 0.26,$ | $F_{(1,24)} = 14.3, p < 0.001,$ | $F_{(1,24)} = 0.60, p = 0.45,$ |
| Social over Food Preference [%] | $\eta^2 = 0.052$ | $\eta^2 = 0.37$ | $\eta^2 = 0.025$ |
| Social Zone [sec]        | $F_{(1,24)} = 0.36, p = 0.55,$ | $F_{(1,24)} = 30.1, p < 0.001,$ | $F_{(1,24)} = 3.13, p = 0.090,$ |
|                          | $\eta^2 = 0.015$     | $\eta^2 = 0.56$ | $\eta^2 = 0.12$ |
| Food Zone [sec]          | $F_{(1,24)} = 5.34, p = 0.048,$ | $F_{(1,24)} = 1.90, p = 0.18,$ | $F_{(1,24)} = 0.41, p = 0.53,$ |
|                          | $\eta^2 = 0.15$      | $\eta^2 = 0.073$ | $\eta^2 = 0.017$ |
| Zone:                    | $F_{(1,24)} = 0.95, p = 0.34,$ | $F_{(1,24)} = 18.5, p < 0.001,$ | $F_{(1,24)} = 0.94, p = 0.34,$ |
| Social over Food Preference [%] | $\eta^2 = 0.038$ | $\eta^2 = 0.44$ | $\eta^2 = 0.038$ |
| Social Investigation [sec] | $F_{(1,24)} = 3.34, p = 0.080,$ | $F_{(1,24)} = 20.2, p < 0.001,$ | $F_{(1,24)} = 1.59, p = 0.22,$ |
|                          | $\eta^2 = 0.122$     | $\eta^2 = 0.46$ | $\eta^2 = 0.062$ |
| Food Investigation [sec] | $F_{(1,24)} = 44.34, p < 0.001,$ | $F_{(1,24)} = 24.3, p < 0.001,$ | $F_{(1,24)} = 8.12, p = 0.009,$ |
|                          | $\eta^2 = 0.65$      | $\eta^2 = 0.50$ | $\eta^2 = 0.25$ |
| Investigation:           | $F_{(1,24)} = 17.22, p < 0.001,$ | $F_{(1,24)} = 54.4, p < 0.001,$ | $F_{(1,24)} = 0.25, p = 0.62,$ |
| Social over Food Preference [%] | $\eta^2 = 0.41$ | $\eta^2 = 0.69$ | $\eta^2 = 0.01$ |
Table 2. One-sample t-Test statistics and Cohen’s $d$ effect sizes; *significant preference for social, ^significant preference for food.

|            | Rats                        | Mice                        |
|------------|-----------------------------|-----------------------------|
|            | Sated                      | Food-Deprived| Sated                      | Food Deprived |
| Chamber:   | $t_{(11)} = 1.31, p = 0.22,$ | $t_{(11)} = 1.48, p = 0.17,$ | $t_{(13)} = 1.33, p = 0.21,$ | $^\text{^}{t_{(13)} = 3.05, p = 0.009},$ |
| Social over Food Preference [%] | $d = 0.38$                | $d = 0.43$                | $d = 0.36$                | $d = 0.82$                |
| Zone:      | $^\text{*}{t_{(11)} = 2.68, p = 0.022},$ | $^\text{*}{t_{(11)} = 2.97, p = 0.013},$ | $t_{(13)} = 0.94, p = 0.37,$ | $^\text{^}{t_{(13)} = 2.79, p = 0.015},$ |
| Social over Food Preference [%] | $d = 0.77$                | $d = 0.86$                | $d = 0.25$                | $d = 0.75$                |
| Investigation: | $^\text{*}{t_{(11)} = 7.57, p < 0.001},$ | $^\text{*}{t_{(11)} = 4.00, p = 0.022},$ | $t_{(13)} = 1.26, p = 0.23,$ | $^\text{^}{t_{(13)} = 4.92, p < 0.001},$ |
| Social over Food Preference [%] | $d = 2.19$                | $d = 1.16$                | $d = 0.34$                | $d = 1.31$                |

Declarations

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Declaration of interests:

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.

All applicable international, national, and institutional guidelines for the care and use of animals were followed. All procedures involving animals were in accordance with the National Institute of Health Guidelines for Care and Use of Laboratory Animals and the Michigan State University Institutional Animal Care and Use Committee.

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Figures
Figure 1

Food deprivation biases preference more towards the food stimulus in rats and mice. Food deprivation increased the time spent with the food stimulus (B & C, right), but did not alter the amount of time spent with the social stimulus (A-C, left). Stimulus preference, as measured by automated tracking of chamber time or investigation zone time, was unchanged by food deprivation in rats or mice (D & E). However, when preference was measured by manually scoring investigation time, food deprivation significantly decreased social over food preference scores (F). Specifically, food deprivation attenuated preference for the social stimulus in rats (F, left), and produced a preference for the food stimulus in mice (F, right). Rats spent more time with the social stimulus than mice (A-C, left) and mice spent more time with food stimulus than rats (A & C, right), which resulted in higher social over food preference scores in rats compared to mice (D-F). Bar graphs display mean ± SEM; * p < 0.05, ANOVA main effect of hunger condition; # p < 0.05, ANOVA main effect of species; ^ p < 0.05, one-sample t-Test from 50% (gray dashed line).
Figure 2

Rats (A, top) and mice (A, bottom) are placed into the center of a three-chamber apparatus and then allowed to freely investigate a social stimulus and a food stimulus, which are each placed in corrals located on opposite ends (B, rats: left, mice: right), for a period of 10 min. Orange lines in A indicate chambers and investigation zones defined in AnyMaze for automated behavioral tracking (middle chamber borders were drawn on the apparatus floor).

Supplementary Files

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