Stereotype of mouse social competency and status revealed by a novel competition paradigm in combination with available paradigms

Meiqiu Liu[1], Yue Chen[1], Rongqing Chen[2,3]

[1]Guangdong Province Key Laboratory of Psychiatric Disorders, Department of Neurobiology, School of Basic Medical Sciences, Southern Medical University, 510515, Guangzhou, China.

[2]The National Key Clinic Specialty, The Engineering Technology Research Center of Education Ministry of China, Guangdong Provincial Key Laboratory on Brain Function Repair and Regeneration, Department of Neurosurgery, Zhujiang Hospital, Southern Medical University, 510282, Guangzhou, China.

[3]Key Laboratory of Mental Health of the Ministry of Education, Guangdong-Hong Kong-Macao Greater Bay Area Center for Brain Science and Brain-Inspired Intelligence, Department of Neurobiology, School of Basic Medical Sciences, Southern Medical University, 510515, Guangzhou, China.

Correspondence:

creatego@hotmail.com (R.C.)

Keywords：

Social competition; Social hierarchy, Food pellet competition test, Tube test.

Stereotype of mouse social competency and status revealed by a novel competition paradigm in combination with available paradigms

Abstract

With the acceleration of urbanization process, psychological, behavioral and biological studies on social organization and competition are boosting. The mouse has been recognized as valuable and economic model animal for biomedical research in social behaviors, but the application of reliable, valid and easily executable social competition paradigm for mouse is still limited. Moreover, discrepant paradigms containing different competitive factors such as muscular confrontation, threatening level, boldness or timidity tendency might lead to task-specific win-or-lose outcomes and confusing rankings. Here, we developed a convenient mouse competition behavioral paradigm in which contenders were a pair of mice eager to take over the same food pellet hidden under a movable block in the middle of a narrow chamber where they were separated to the either right or left side. Our design mentality of this food pellet competition test (FPCT) avoids the direct violent competition between mice and facilitates to expose the psychological motivation of the contenders. Application of FPCT in combination with typically available paradigms, tube test and warm spot test (WST), discovered a stereotypic property of mouse social organization and competitivity in a given society of either males or females that were raised in an either 2- or 3-member cage, indicating that hierarchical sense of animals might be part of a comprehensive identify of self-recognition of individuals in an established society. More importantly, FPCT may largely facilitate the researches regarding the social organization and competition due to its reliability, validity and easy operability.

Introduction

For gregarious animals and human beings, both competition and cooperation between individuals or populations are fundamental social behaviors important for the survival and evolution of the collective strains and species. Social competitions occur naturally to determine the ownership or priority of living territory, food, water, mates, other resources

and non-resource requirements [1,2]. Through these competitions, social hierarchies from the dominant to the inferior are established within a living group, where individuals at higher social ranks are granted with corresponding priorities of resources and non-resource requirements like living space, food availability, reproduction and safeguard [1,2]. The establishment of dominance hierarchies reduces the intensity and frequency of mutual aggression within groups and strains, so to maintain their inner stability and fulfil outer assignments [3]. Disability in social recognition and unsatisfied social status are associated with brain diseases such as autism, depression and schizophrenia [4,5].

While the human beings and primates exhibit complex social interactions relatively easier to be observed and detected, the rodent mouse and rat have been recognized as valuable and economic models for researches investigating biological insights of cognitive, mood and social behaviors [4,6,7]. Among the social competing objects, resources of living space and food are fundamentally essential for survival of animate beings [2,8]. Therefore, living space and food competitions are frequently used to investigate social competitive behaviors and hierarchical ranking of rats or mice. The tube test is a simple and reliable method for assessing social hierarchy by simulating the competition for living space among animals. In the tube test, the dominant mice largely consistently squeeze out the weaker ones from the narrow tube, indicative of an overwhelming space demand of mice at higher rank [9]. Warm spot test is another space competition paradigm where cagemate mice compete for pitiful small warm corner in a freezing cage [7,10]. Some food competition tests are already available for animals with larger body size, such as rats, chickens, and pigs, which make detecting behavioral patterns easier by the experimenters or monitoring video [11-15], but those procedures are difficult to be applied to mice as they have a much smaller size. Although both mice and rats are rodents and mostly used experimental animals, rats are more socially tolerant and less hierarchical than mice [16,17]. Until recently, only a couple of food competition tests have been designed for mice [18,19]. Some issues are significant in those food competition test which include the instability of hierarchies, complex calculation, difficulty in observing and interpreting animal behaviors from videos, and the occurrence of aggressive behaviors during food competition.

Moreover, those social competition tasks had not been conceived to know mice's

winning/losing the competition via physical strength, psychological motivation or other factors. In other words, the outcomes of individual's social competency and status might be not stereotypic in a given social group, but rather variable in different contexts where individuals can exert their specific expertise and advantages such as muscular strength, boldness, focused motivation and versatile skills. Thus, it is more likely expected that different paradigms to weigh the social competency and status may lead to diverse readouts, given that competitive factors are included in competition paradigms.

Therefore, on one hand, to generate a convenient, easily operative and peaceful competition paradigms, we developed a food pellet competition test (FPCT) for a pair of mice competing for a food pellet after calorie restriction without involvement of physical contact. On the other hand, to characterize the mouse social hierarchical organization, we ranked the mice in a 2 or 3-member society of either males or females according to the winning/losing outcomes defined by ascription of the food pellet, using FPCT in combination with tube test and WST.

Results

Ranking 2-cagemate male mice based on measurements of winning/losing in FPCT and verification by tube test

Social hierarchy of many animals is largely arranged as the outcomes of winning or losing social competitions, manifesting as dominant or subordinate behaviors in a well-established society. To see the efficacy of FPCT in examining the competitive behaviors, we conducted 4 trials (1 trial per day) for 16 pairs of cagemate male mice in test 1 (direct win-lose test via analyzing food occupation). In each trial of the test 1, the mouse which obtained the food pellet was credited a score of 1 (time of winning), while the mouse which failed to obtain the food pellet was credited a score of 0 (time of winning). In the final trial of test 1, the mouse in a pair obtaining the food pellet was ranked #1 and claimed as winner. On the contrary, the mouse in a pair failing to get the food pellet was rank #2 and claimed as loser. Statistics of the all 4 trials showed that only one 1 pair exhibited alternating winning/losing outcomes with an inter-trial consistency at 50% (Figure 4A) and all the other 15 pairs kept 100% congruent winning/losing readouts (Figure 4B), returning minimal

fluctuation over through complete trials (Figure 4C) of all pairs and a rate of overall consistency at 96.88% (Figure 4D). In test 2 (indirect win-lose test via analyzing mouse's attempt to gain the food), we observed that the winners claimed in test 1 tried harder to get the food pellet by spending more time to push the block within the single 2-minute test, a limited time to avoid distinction of attempt (Figure 4E). Thus, these data demonstrate that FPCT are effective to rank the established society of mice, and that consecutive 4 trials (one trial daily) might be least required for FPCT to achieve readout consistency and reliability.

Many factors may contribute to the competitive outcomes in mice, such as age, sex, physical strength, training level, and intensity of psychological motivation. We were interesting to understand which factor determines the outcomes of winning/losing of mice in the FPCT match. First, effects of age and sex on wining/losing outcomes were excluded as the mice were age-matched and solely males. Second, it was also excluded that the weight of mice determining competency by providing physical strength of pushing, as the weight difference between paired contender mice before training was controlled in less than 10% and after food competition tests, the weight of the mice was measured again and no difference in weight was found between the winners and losers (Figure 4F). Third, to see whether alternate entry from the left and right might produce distinct likelihood of harvesting the food pellet, we compared the difference of latency to win when the mice entered the chamber from the left and right entries during test, but similar latency to win between the left and right entering was observed (Figure 4G). Forth, we considered that inadequate training might produce distinct likelihood of winning and losing, so we retrospectively compared the performance of winner and loser mice (test 1) during their training to find the hidden food pellet (step 4 of training) by analyzing duration they took to obtained the hidden food pellets without competition. This analysis reported no difference in the latency to obtain the food pellet between the winners and losers (Figure 4H) during training, indicating that the mice were well and equally trained. Fifth, direct violence depending on fierce aggressiveness was not possible to be exerted on this competitive scenario due to separation of the mice by the block. Thus, winning/losing outcomes in this paradigm rely on mice's intensity of psychological motivation.

To see whether social ranking revealed by FPCT is consistent with the tube test that represents the simple and robust behavioral assay for space competition [9], we continued the experiments to rank the mice using tube test following FPCT (Figures 4I and 4J). We found that consistency of outcomes between FPCT and tube test were 100 percent, as the winners and losers in the last trial of FPCT continued consecutively to be the winners and losers during the 4 consecutive trials of tube tests, respectively (Figure 4K). Therefore, ranking established social status with FPCT is fully verified by tube test.

Social ranking of 2-cagemate female mice using FPCT, tube test and warm spot test

While it is widely accepted that males, including human beings and animals, are evolutionarily more eager to be dominant, more aggressive and more hierarchical, evidence is little regarding whether females have less competition and looser social organization[20,21]. Taking use of this newly designed mouse competition task in which dependence of competency on physical power is minimized, we examined whether intrasexual social hierarchies exist between the two female mice housed in a cage. Notably, we found that FPCT test 1 showed that 3 in 10 pairs of cagemate female mice exhibited alternating winning/losing outcomes (Figures 5A-5C), while the majority of cagemates (7 in 10 pairs) showed fully congruent grades (Figure 5D). Averagely, the 10 female pairs displayed stable rankings over through all trials (Figure 5E) with an overall rate of inter-trial consistency at 90% (Figure 5F). In FPCT test 2, we observed that winner mice of test 1 paid more efforts trying to get the food pellet (Figure 5G). The body size and training level were likely to contribute to the competency (Figures 5H-5J). Thus, these FPCT results suggested that female mice society were also stratified.

In addition to the tube test, warm spot test (WST), the regional urine marker and the courtship ultrasound vocalization test are established behavioral methods for determining social rank in mice [7,10,11,22-29]. We assumed that different competition paradigms may result in different social ranking readouts of the same society, as tested animals in different tasks pursue specific goals which induce different level of contending motivation and they can prioritize using their specific expertise, strength and skill. Aiming to examine this assumption, we continued to rank these female mice using tube test and WST following

FPCT (Figure 5K). Unexpectedly, in most of the trials the mice preserved the winner or loser identity acquired in FPCT into tube test and WST (Figures 5L-5O). No difference was found when comparing the rate of consistency between FPCT and tube test, FPCT and WST, as well as tube test and WST (Figure 5P). These data illustrate that mouse social competency and status are stereotypic so that a well competitive subject exhibit dominance not just in a single, but in a variety of contexts such as food and space or aspects such as strength and motivation.

Social ranking of multiple cagemate mice using FPCT, tube test and WST

Social organization of the bigger crowd could be much more complicated than the simplest 2-member society. It is unknown whether the dominate and subordinate roles in a larger group of mice tend to be mobile or rigorous in variety of tasks. To address this issue, we raised 3 male mice in a cage and then following adequate habituation and training, we ranked their hierarchies using FPCT, tube test and warm spot test sequentially (Figure 6A). In the FPCT and tube test, the 3 cagemate mice contested in a round-robin style as the both were designated for two contenders in a time. Despite that 6 in 9 groups of mice display some extent of flipped ranking (Figures 6B-6G) and only 3 in 9 groups displaying continuously unaltered ranking (Figure 6H) during totally 9 trials consisting of 3 trials of FPCT, 3 trials of tube test and 1 trial of WST, an obvious stable linear intragroup hierarchy was observed throughout all the trials and tasks (Figures 6I and 6J). Comparison of inter-task consistency revealed that the ranks evaluated by FPCT, tube test and WST did not differ from each other (FPCT vs tube test, 77.78 $\pm$ 11.11; FPCT vs WST, 81.48 $\pm$ 12.56; tube test vs WST, 70.37 $\pm$ 15.16 $P > 0.05$, one-way ANOVA test. Figure 6K). These results, together with the results of female mice tested by FPCT, tube test and WST, illustrate that the dominate and subordinate roles in a society of mice are rigorously linearly stratified in various circumstances.

Discussion

Social hierarchy established following social competition is an innate property of gregarious animals. Among a variety of contents for social competition, resources of living space and

food are instinctively critical for animals [2,8]. Here, we invented and validated a food competition assay, a novel tool designed to provide a means of assessing hierarchies of mice with easy-going procedures, favoring the growing scientific interest in understanding neurobiological insights of social hierarchy.

The procedure of FPCT paradigm is easily operated and conveniently applicable. At first, the matched mice were housed together to habituate each other and establish social hierarchy. Then, the mice were trained individually to accommodate the chamber arena and learn to push away the block above the food pellet to get the hidden food. Importantly, stable social hierarchy relies on adequate habituation and reliable ranking test is based on well training. Next, in the ranking test, the mice were allowed to go into the either right or left side of the chamber separated by the movable block, where they competed for the same food pellet hidden under the block.

In recent years, mice have been designated to participate in food competition tests. In those food competition tests, the total amount of food consumed by each rat or mouse competing for food with the other one in the same chamber or cage are used to determine their social rank by assigning behavior scores, along with analysis of bodily aggressive acts mice [18,19]. Common issues in these food competition tests include the instability of hierarchies, the occurrence of aggressive behaviors during food competition, requirement of prolonged food deprivation, complex calculation formulas, long video recording duration and difficulty in observing and interpreting animal behaviors from videos [19,23,30-34]. In rodents, being physically aggressed and prolonged food deprivation can influence social competitive behaviors via disturbing the internal state and triggering robust stress responses of the tested animals. These situations are minimized in our task, where the mice only underwent a 24-hour food deprivation period at the beginning of training and were separated into either side of chamber during ranking test [35-37].

Although body weight has been regarded as an indicator of social hierarchy only in rats but not in mice, it is hard to attribute winning a competition in a shared space to stronger motivation rather than muscular superiority for mice [7,16,17]. By avoiding the direst violent competition between mice separated in either side of the chamber, controlling body weight of paired mice and conducting adequate unbiased training, the winning/losing outcomes of

the matches of our designed paradigm is oriented by the psychological motivation of the contenders. Our FPCT results are highly replicable with tube test, where the mice compete for right-of-way type of space, suggesting that dominance-driven motivation commonly determines the competency in the two different types of competitive behavioral paradigms. Thus, FPCT is a valid, practical and motivationally tailored social competition paradigm for mice.

Via this novel social task in combination with typically available paradigms including tube test and WST, we addressed whether female mice are socially hierarchical, whether FPCT is effective to discover hierarchy rankings across multiple cagemates, as well as whether different social tasks may result in discrepant hierarchy rankings considering that diverse competitive factors lying in specific tasks. First, we found that female mice expressed hierarchy ranks consistently, suggesting that the stratified relationship between mice is not matter of gender. However, processes establishing social hierarchy may be specific to males or females [38]. Second, by means of round-robin across multiple cagemates, FPCT was able to detect a stable linear intragroup hierarchy, in line with tube test conducted by us and previously by Fan et al. [9]. Third, the hierarchical organization between paired females and among 3-cagemate males were highly consistent each other among FPCT, tube test and WST, i.e., hardly associated with examining tasks containing different competitive factors, indicative of hierarchical sense of animals might be part of a comprehensive identify of self-recognition of individuals within an established society. Hopefully, FPCT will facilitates future studies to reveal more detailed properties of social organization, social competition and their underlying neurobiological mechanisms.

Methods and materials

Animals

All animal experiments were conducted according to the Regulations for the Administration of Affairs Concerning Experimental Animals (China) and were approved by the Southern Medical University Animal Ethics Committee. The mice used in this study were 6-8 weeks old C57/BL6J purchased from SPF (Beijing) Biotechnology Company (Beijing, China) and were raised in the environment with the relative humidity 50-75%, temperature 22-24°C

and 12-12-h light-dark cycle. The mice were allowed to freely access to food and water unless undergoing food restriction requirement during experiments.

Food pellet competition test (FPCT) setup

The experimental setup consists of a food competition arena (Figure 1) and a camera. The arena includes a base, a chamber, two entries, two doors, a movable block, a pulley system, a food trough and the roof (Figure 2) made up of a batch of wheels and a wheel track for the pulley system, as well as 15 pieces of acrylic plates for other parts listed with dimensions in Figure 2F. The movable block hung up under the track section separates the arena into the left and right compartments. It is necessary to apply some paraffin oil to the track to reduce the friction between the pulley and the track (Supplementary figure 1). The food trough is positioned in the middle of the chamber floor and is covered under the movable block unless the block is pushed away by mice. Food pellets are tiny delicious milk crackers. Mouse behaviors are monitored by a side view cameral with PlotPlayer software.

FPCT procedure

The experimental procedure consists of habituation, training and test (Figure 3A). The details of the procedure are described below.

Habituation

Contender pair of C57BL/6 mice with similar age and weight (the weight difference was less than 10%) were housed in the same cage for at least 3 days under a 12-12-h light-dark cycle. Before the formal training of mice, each mouse was handled by experimenters for 3-5 minutes per day for approximately 3 days. Overall, the habituation period lasts about 6-8 days.

Training

Step 1, food restriction. To enhance the appeal of the food pellet to the mice, the mice were deprived of food for 24 hours while water consumption remained normally. After 24 hours of food deprivation, each cage of mice was given 10 g of food every morning to meet their daily food requirements until the end of the test.

Step 2, contextual familiarization (Figure 3B). At each time after mice homecage was translocated from animal facility to behavioral room, the cage lid was removed to allow the mice to freely explore the cage for about 1-2 minutes to become accustomed to the open roof. Before training to find the food pellet (step 3), each mouse was allowed to enter the arena chamber from the left side where the mouse was kept for 3 minutes before been gently driven out from the right side of the chamber to be back to homecage to have a 2-minute rest. Then, the mouse would enter the arena chamber from the right side and 3 minutes later was gently driven out from the left side. Arena familiarization was conducted 1 round per day for 2-3 days.

Step 3, training to find the food pellet (Figure 3C). The experimenter placed a small food pellet in the trough in the middle of the floor of chamber. Let one mouse enter the chamber from the left side and stay there until it found and ate the pellet. After that, the mouse was gently driven out of the chamber from the right side to have a 2-minute rest in its homecage. Then, the mouse would enter the chamber from the right side and stay there until it found and ate food. The training was repeated 1 round daily until each mouse directly went to the trough and ate the small pellet after entering the chamber (Supplementary video1).

Step 4, learning to get the hidden food pellet (Figure 3D). In this step, the small food pellet in the trough was hidden under the vertical block. As the block was transparent and had holes at lower portions facing to the entries, and it was movable thanks to the sliding wheels and the track at the top of the block attached to the roof, the mouse was able to learn to push away the block to get the pellet. The roof attached with track and block was lifted up after the mouse ate the pellet to allow the mouse go out of the chamber from the side opposite to the entry side. Each mouse was repeatedly trained to get the hidden food pellet alternatively from left and right entry until they were able to harvest the pellet directly and easily without competition (Supplementary video 2).

Test

Test 1, direct win-lose test via analyzing food occupation (Figure 3E). Test 1 required several trials, in the first of which the paired contenders entered the chamber from the opposite sides simultaneously. The mice entered the chamber from left or right entry alternatively in two consecutive trials. As there was only one small pellet hidden under the

movable block in each trial, the mouse that obtained the pellet was deemed as winner, while the other one was the loser (Supplementary video 3). To calculate the latency of food harvesting of mice, a starting state was marked when its four legs just entered the chamber arena (Supplementary figure 2). The time spent from starting state to food-getting for a mouse was calculated as latency of food harvesting. One competition test comprised at least 4 consecutive trials.

Test 2, indirect win-lose test via analyzing mouse's attempt to gain the food (Figure 3F). In this test, a slightly larger pellet was placed in the inner bottom of the block, rather than in the trough under the block, so that the mice could see, smell but not access it. Once the mice entered the chamber, they would push the block and attempted to gain the food. The winner or loser was determined by times of pushing the block in 2 minutes (Supplementary video 4).

Tube test

The detailed steps of tube test were described previously by Fan et al. [9]. It consists of the following three steps: adaptation, training and test. The adaptation phase had been completed at FPCT, referred to as the habituation step of FPCT. In the training phase, the tail of the mouse was gently lifted and placed at one end of the tube, and when the mouse entered the tube, the tail was released and the mouse was allowed to pass through the tube. A plastic rod was used, when the mouse retreated or stagnated for a long time, to gently touched the tail so that the mouse could continue to move until it passed through the tube. The test phase required four consecutive days, and the social rank of the mice was ranked for four consecutive days. During the experiment, the camera was placed directly in front of the tube, and the whole process of the mouse experiment could be recorded.

Warm spot test

The experimental apparatus utilized for the WST consisted of a rectangular behavior box with dimensions of 28 cm in length, 20 cm in width, and 40 cm in height. Prior to the commencement of the experiment, the bottom of the box was placed on ice to ensure that

its temperature was approximately 0°C. A heating sheet measuring 2 cm by 2 cm was positioned in one of the inner corners of the box, providing sufficient space for only one mouse. The temperature of the heating sheet was maintained at 34°C, and this temperature was monitored using a temperature measuring gun. During the experiment, three mice were introduced into the box simultaneously, allowing them to move freely for a duration of 15 minutes. The entire experimental procedure was recorded via video, and the time each mouse spent on the heating sheet was subsequently quantified from the recorded footage.

Statistical analysis

The experimental data were statistically analyzed using *Prism* 9 or origin 9.0 software and presented as mean ± SEM. ANOVA, unpaired or paired Student's t-test were used to compare the difference between groups. Statistical significance level (*P* value) was set at 0.05.

Author contributions

M.L. and Y.C. acquired the experimental data. M.L. analyzed the data. M.L. and R.C. established methodology and wrote the manuscript. R.C. conceived the project and supervised the research. All authors contributed to the finalization and approved the content of the manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (NSFC) (82271859 and 31771127) and the grant from the Natural Science Foundation of Guangdong Province (2023A1515010639).

Availability of data and materials

The data and supplementary information supporting the findings of this study are within the paper and other information helping to understand this study could be available upon request from the corresponding authors.

Declaration of interests

The authors declare no competing interests.

References

1. Shimamoto, A. (2018). Social Defeat Stress, Sex, and Addiction-Like Behaviors. Int Rev Neurobiol *140*, 271-313. 10.1016/bs.irn.2018.07.009.
2. Korzan, W.J., and Summers, C.H. (2021). Evolution of stress responses refine mechanisms of social rank. Neurobiol Stress *14*, 100328. 10.1016/j.ynstr.2021.100328.
3. Guo, S.T., He, S.J., Zhang, H., Bai, R.F., Zhang, S.M., Hou, R., Grueter, C.C., Chapman, C.A., Dunn, D.W., and Li, B.G. (2020). Male social rank and food competition in a primate multi-level society. Am J Phys Anthropol *173*, 630-642. 10.1002/ajpa.24141.
4. Fan, Z., Chang, J., Liang, Y., Zhu, H., Zhang, C., Zheng, D., Wang, J., Xu, Y., Li, Q.-J., and Hu, H. (2023). Neural mechanism underlying depressive-like state associated with social status loss. Cell *186*, 560-576.e517.
5. Sato, M., Nakai, N., Fujima, S., Choe, K.Y., and Takumi, T. (2023). Social circuits and their dysfunction in autism spectrum disorder. Mol Psychiatry *28*, 3194-3206. 10.1038/s41380-023-02201-0.
6. Wang, F., Zhu, J., Zhu, H., Zhang, Q., Lin, Z., and Hu, H. (2011). Bidirectional control of social hierarchy by synaptic efficacy in medial prefrontal cortex. Science *334*, 693-697. 10.1126/science.1209951.
7. Zhou, T., Zhu, H., Fan, Z., Wang, F., Chen, Y., Liang, H., Yang, Z., Zhang, L., Lin, L., Zhan, Y., et al. (2017). History of winning remodels thalamo-PFC circuit to reinforce social dominance. Science *357*, 162-168. 10.1126/science.aak9726.
8. Goodman, M., Kneafsey, M., Maye, D., and Holloway, L. (2021). Geographies of Food: An Introduction 10.5040/9781474204392.
9. Fan, Z., Zhu, H., Zhou, T., Wang, S., Wu, Y., and Hu, H. (2019). Using the tube test to measure social hierarchy in mice. Nat Protoc *14*, 819-831. 10.1038/s41596-018-0116-4.
10. Zhu, H., and Hu, H. (2018). Brain's neural switch for social dominance in animals. Sci China Life Sci *61*, 113-114. 10.1007/s11427-017-9181-1.
11. Cordero, M.I., and Sandi, C. (2007). Stress amplifies memory for social hierarchy. Front Neurosci *1*, 175-184. 10.3389/neuro.01.1.1.013.2007.
12. Lee, Y.-P., Craig, J.V., and Dayton, A.D. (1982). The social rank index as a measure of social status and its association with egg production in White Leghorn pullets. Applied Animal Ethology *8*, 377-390.
13. Hessing, M.J., and Tielen, M.J.M. (1994). The effect of climatic environment and relocating and mixing on health status and productivity of pigs. Animal production *59*, 131-139.
14. Jupp, B., Murray, J.E., Jordan, E.R., Xia, J., Fluharty, M.E., Shrestha, S., Robbins, T.W., and Dalley, J.W. (2015). Social dominance in rats: effects on cocaine self-administration, novelty reactivity and dopamine receptor binding and content in the striatum. Psychopharmacology *233*, 579 - 589.

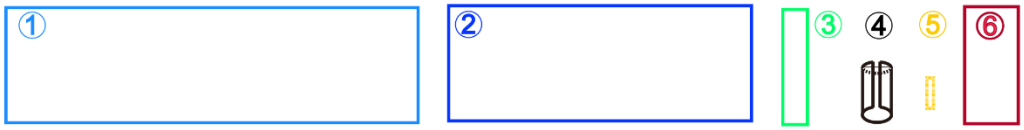
15. Timmer, M., and Sandi, C. (2010). A role for glucocorticoids in the long-term establishment of a social hierarchy. Psychoneuroendocrinology *35*, 1543-1552.
16. Kim, B., Colon, E., Chawla, S., Vandenberg, L.N., and Suvorov, A. (2015). Endocrine disruptors alter social behaviors and indirectly influence social hierarchies via changes in body weight. Environ Health *14*, 64. 10.1186/s12940-015-0051-6.
17. Bartos, L., and Brain, P. (1994). Influence of body weight on dominance and aggression in groups of male Swiss strain mice. Animal Technology *45*, 161-168.
18. Yoon, Y., Shin, H., Byun, D., Woo, J., Cho, Y., Choi, N., and Cho, I.J. (2022). Neural probe system for behavioral neuropharmacology by bi-directional wireless drug delivery and electrophysiology in socially interacting mice. Nat Commun *13*, 5521. 10.1038/s41467-022-33296-8.
19. Shin, H., Byun, J., Roh, D., Choi, N., Shin, H.S., and Cho, I.J. (2022). Interference-free, lightweight wireless neural probe system for investigating brain activity during natural competition. Biosens Bioelectron *195*, 113665. 10.1016/j.bios.2021.113665.
20. Ivan, S., Daniela, O., and Jaroslava, B.D. (2023). Sex differences matter: Males and females are equal but not the same. Physiol Behav *259*, 114038. 10.1016/j.physbeh.2022.114038.
21. Stockley, P., and Campbell, A. (2013). Female competition and aggression: interdisciplinary perspectives. Philos Trans R Soc Lond B Biol Sci *368*, 20130073. 10.1098/rstb.2013.0073.
22. Arrant, A.E., Filiano, A.J., Warmus, B.A., Hall, A.M., and Roberson, E.D. (2016). Progranulin haploinsufficiency causes biphasic social dominance abnormalities in the tube test. Genes Brain Behav *15*, 588-603. 10.1111/gbb.12300.
23. Timmer, M., and Sandi, C. (2010). A role for glucocorticoids in the long-term establishment of a social hierarchy. Psychoneuroendocrinology *35*, 1543-1552. 10.1016/j.psyneuen.2010.05.011.
24. Ujita, W., Kohyama-Koganeya, A., Endo, N., Saito, T., and Oyama, H. (2018). Mice lacking a functional NMDA receptor exhibit social subordination in a group-housed environment. Febs j *285*, 188-196. 10.1111/febs.14334.
25. Lucion, A., and Vogel, W.H. (1994). Effects of stress on defensive aggression and dominance in a water competition test. Integr Physiol Behav Sci *29*, 415-422. 10.1007/bf02691361.
26. Desjardins, C., Maruniak, J.A., and Bronson, F.H. (1973). Social rank in house mice: differentiation revealed by ultraviolet visualization of urinary marking patterns. Science *182*, 939-941. 10.1126/science.182.4115.939.
27. Long, S.Y. (1972). Hair-nibbling and whisker-trimming as indicators of social hierarchy in mice. Anim Behav *20*, 10-12. 10.1016/s0003-3472(72)80167-2.
28. Kalueff, A.V., Minasyan, A., Keisala, T., Shah, Z.H., and Tuohimaa, P. (2006). Hair barbering in mice: implications for neurobehavioural research. Behav Processes *71*, 8-15. 10.1016/j.beproc.2005.09.004.
29. Dizinno, G., Whitney, G., and Nyby, J. (1978). Ultrasonic vocalizations by male mice (Mus musculus) to female sex pheromone: Experiential determinants. Behavioral Biology *22*, 104-113. https://doi.org/10.1016/S0091-6773(78)92094-1.
30. Merlot, E., Moze, E., Bartolomucci, A., Dantzer, R., and Neveu, P.J. (2004). The rank assessed in a food competition test influences subsequent reactivity to immune and social challenges in mice. Brain Behav Immun *18*, 468-475. 10.1016/j.bbi.2003.11.007.

31. Li, F.H., Zhong, W.Q., Wang, Z., and Wang, D.H. (2007). Rank in a food competition test and humoral immune functions in male Brandt's voles (Lasiopodomys brandtii). Physiol Behav *90*, 490-495. 10.1016/j.physbeh.2006.10.009.
32. Weger, M., Sevelinges, Y., Grosse, J., de Suduiraut, I.G., Zanoletti, O., and Sandi, C. (2018). Increased brain glucocorticoid actions following social defeat in rats facilitates the long-term establishment of social subordination. Physiol Behav *186*, 31-36. 10.1016/j.physbeh.2018.01.008.
33. Löfgren, M., Bengtsson, S.K., Johansson, M., and Bäckström, T. (2013). Allopregnanolone promotes success in food competition in subordinate male rats. Neuropsychobiology *68*, 15-23. 10.1159/000350478.
34. Costa, D.F., Moita, M.A., and Márquez, C. (2021). Novel competition test for food rewards reveals stable dominance status in adult male rats. Sci Rep *11*, 14599. 10.1038/s41598-021-93818-0.
35. Márquez, C., Poirier, G.L., Cordero, M.I., Larsen, M.H., Groner, A., Marquis, J., Magistretti, P.J., Trono, D., and Sandi, C. (2013). Peripuberty stress leads to abnormal aggression, altered amygdala and orbitofrontal reactivity and increased prefrontal MAOA gene expression. Transl Psychiatry *3*, e216. 10.1038/tp.2012.144.
36. Kennedy, P.J., and Shapiro, M.L. (2009). Motivational states activate distinct hippocampal representations to guide goal-directed behaviors. Proc Natl Acad Sci U S A *106*, 10805-10810. 10.1073/pnas.0903259106.
37. Reppucci, C.J., Brown, L.A., Chambers, A.Q., and Veenema, A.H. (2020). Wistar rats and C57BL/6 mice differ in their motivation to seek social interaction versus food in the Social versus Food Preference Test. Physiol Behav *227*, 113162. 10.1016/j.physbeh.2020.113162.
38. Williamson, C.M., Lee, W., DeCasien, A.R., Lanham, A., Romeo, R.D., and Curley, J.P. (2019). Social hierarchy position in female mice is associated with plasma corticosterone levels and hypothalamic gene expression. Sci Rep *9*, 7324. 10.1038/s41598-019-43747-w.

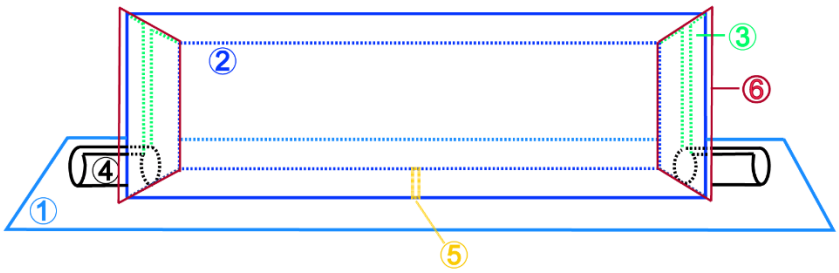
Figures and legends

Figure 1. A photograph of food pellet competition test (FPCT) setup.

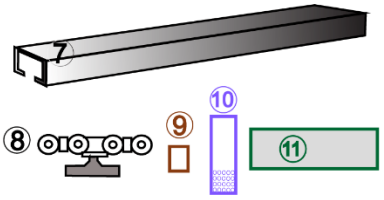
A



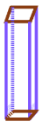
B



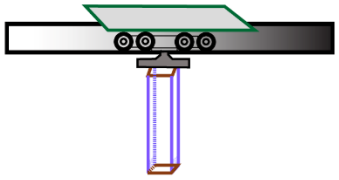
C



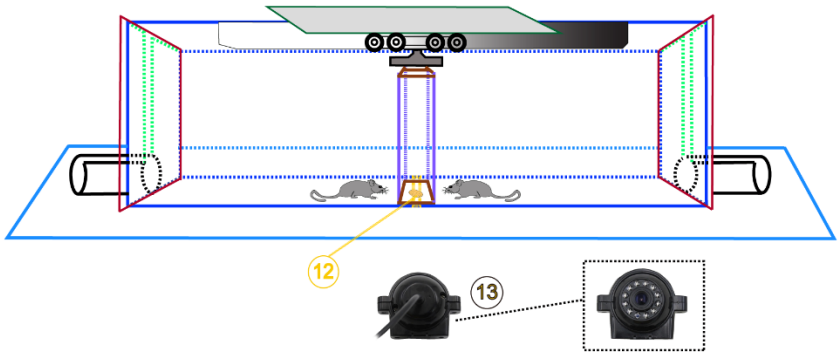
D



E



F



G

Name of parts	I.D. number of pieces	Number of pieces	Material and size (length × width/diameter in cm)	Thickness (mm)
Base	①	1	acrylic plate; 75 × 15	4
Chamber	②	2	acrylic plate; 50 × 15	4
	③	4	acrylic plate; 15 × 2	4
Entry	④	2	acrylic plate; 8 × 3 (slit width: 1.5)	4
Food trough	⑤	1	acrylic plate; 4 × 0.5	4
Door	⑥	2	acrylic plate; 15 × 6.8	4
Pulley system	⑦	1	metal track; 45 in length	2
	⑧	4	wheel; 2.5 in diameter	/
Movable block	⑨	2	acrylic plate; 3.2 × 2.4	2
	⑩	2	acrylic plate with holes at lower portion; 10 × 3.2	2
Roof	⑪	1	acrylic plate; 20 × 9	4
Food pellet	⑫	1	/	/
Camera	⑬	1	/	/

Figure 2. Schematic illustration of the assembling of the FPCT setup.

(A-B) Depiction of the work pieces (A) to make up the main body of the arena (B).

(C-E) Depiction of the work pieces (C) to make up the movable block (D) attached to the pulley system and the roof (E).

(F) Cartoon demonstrating the FPCT setup and working model with the existence of food pellet and camera.

(G) Detailed list of the work pieces and parts of the FPCT setup.

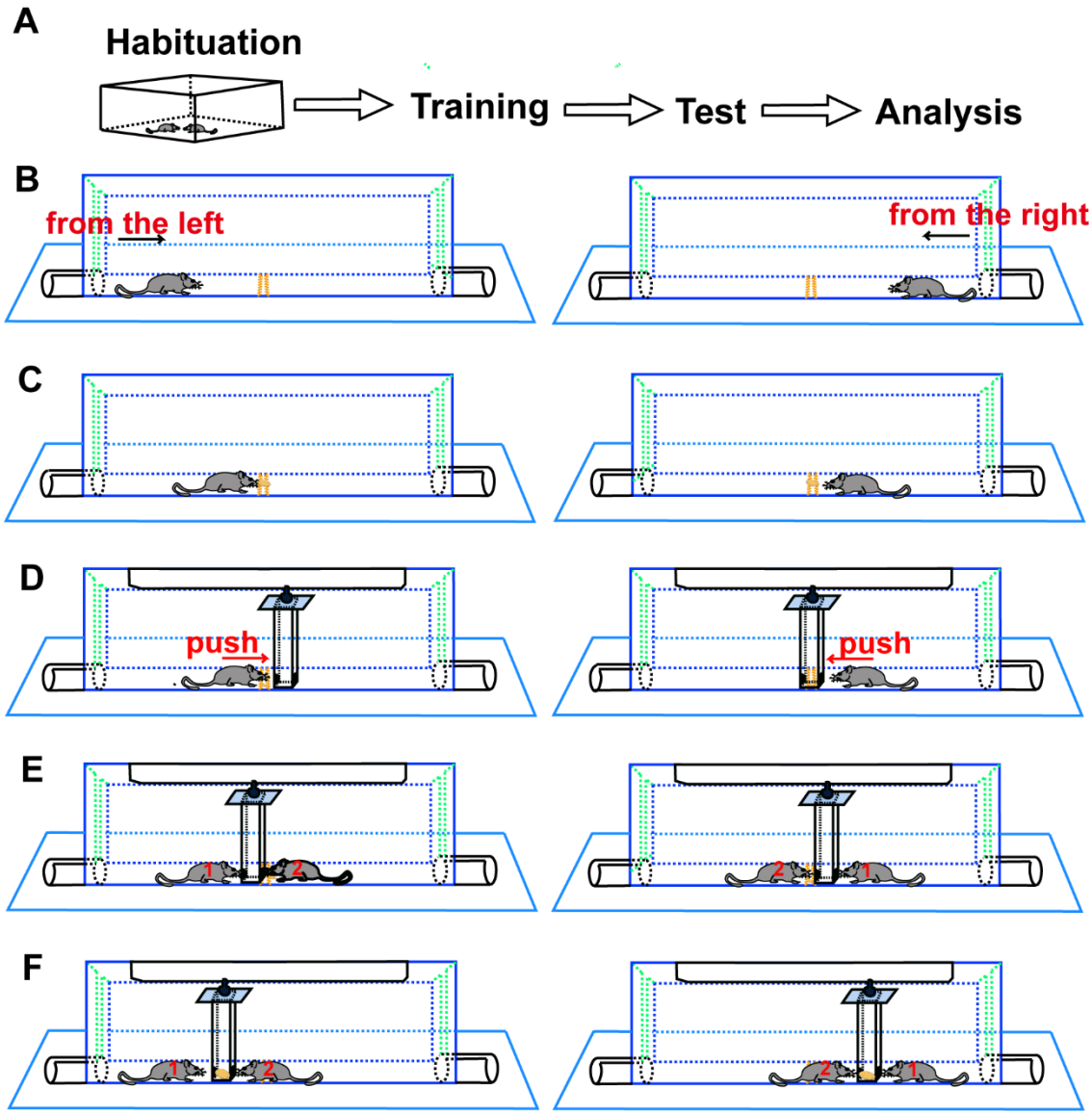

A
Habituation
Training
Test
Analysis
B
from the left
from the right
C
D
push
push
E
1
2
2
1
F
1
2
2
1

Figure 3. Schematic illustration of the procedure of FPCT experiment.

(A) Overall procedure of FPCT experiment.

(B) Habituation to the arena where the mice entered alternately from left and right sides.

(C) Training to find food pellet without the existence of the movable block.

(D) Training to get the hidden food pellet under the movable block.

(E) Direct win-lose test via analyzing food occupation (test 1).

(F) Indirect win-lose test via analyzing mouse's attempt to gain the food (test 2).

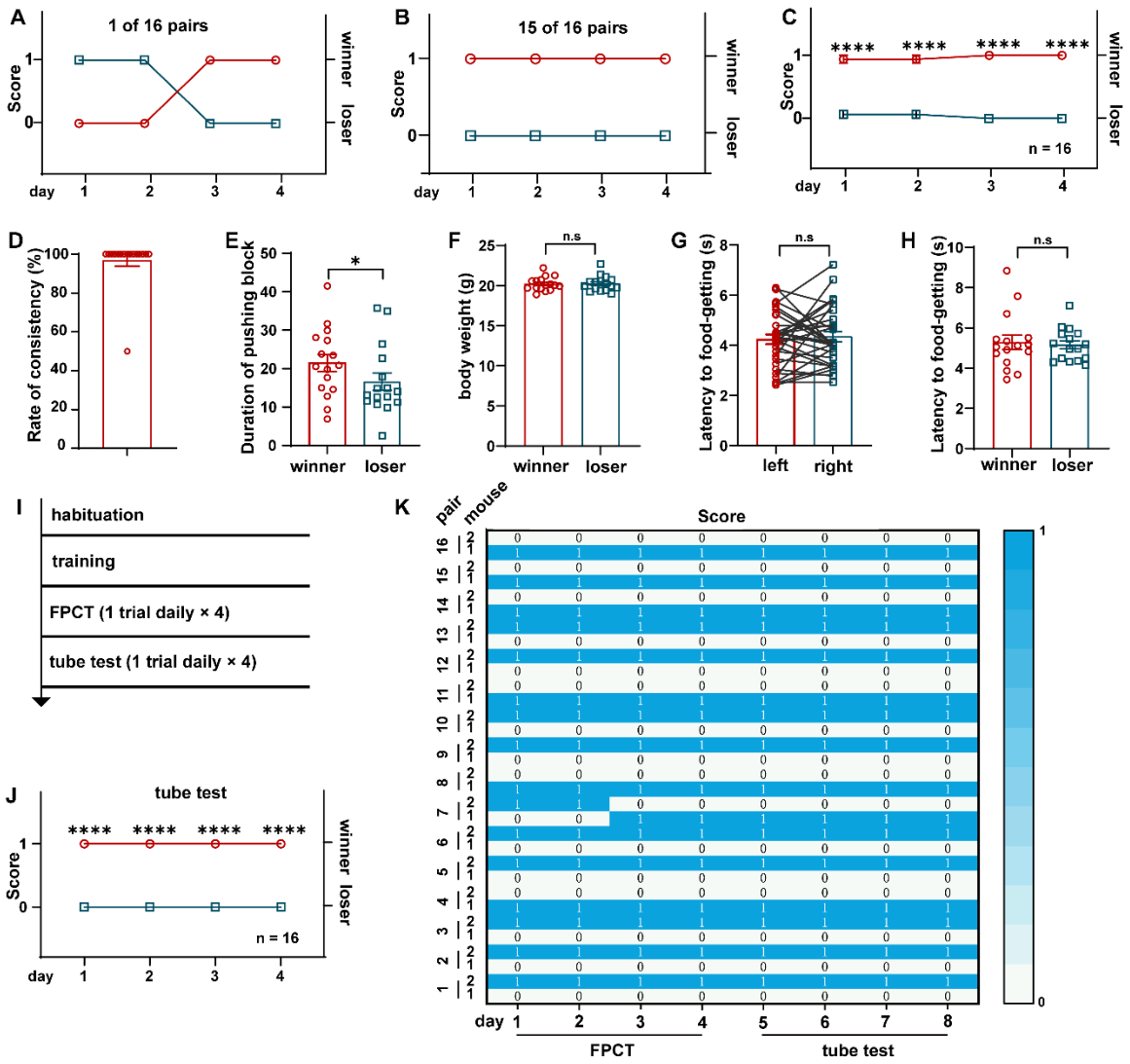

A
1 of 16 pairs
Score
winner
loser
day
B
15 of 16 pairs
C
****
n = 16
D
Rate of consistency (%)
E
Duration of pushing block
*
winner
loser
F
body weight (g)
n.s
G
Latency to food-getting (s)
left
right
H
I
habituation
training
FPCT (1 trial daily × 4)
tube test (1 trial daily × 4)
J
tube test
K
pair
mouse
Score
day
FPCT
tube test

Figure 4. Ranking the 2-cagemate male mice based on measurements of winning/losing outcome of FPCT and verification of the results of FPCT using tube test.

(A) 1 of the 16 pairs of cagemate male mice exhibited alternating winning/losing outcome in FPCT test 1.

(B) 15 of the 16 pairs of male mice exhibited fully congruent winning/losing outcome in FPCT test 1.

(C) Statistics of ranking of male mice over through 4 trials (1 trial daily) of FPCT test 1. Two-way ANOVA, n = 16, ****$P < 0.0001$.

(D) Average rate of consistency within trials. For each pair, rate of consistency was calculated as the percentage of the number of trials (in all 4 trials) resulting in the outcome same as the 4th trial (n = 16).

(E) Statistics of duration spent on pushing block in FPCT test 2. Paired Student's t test, *$P < 0.05$, n = 16.

(F) Body weight of male mice measured after FPCT. Unpaired Student's t test, n.s. stands for non-significant difference, n = 16.

(G) Comparison of the latency to food-getting in training when the male mice entered the arena from the left vs from the right. Paired Student's t test, n = 32, n.s. stands for non-significant difference.

(H) Comparison of the latency to food-getting of winner vs loser male mice in training. Unpaired Student's t test, n = 16, n.s. stands for non-significant difference.

(I) Timeline of experiments showing tube test was conducted after FPCT.

(J) Statistics of ranking of male mice over through 4 trials (1 trial daily) of tube test. Two-way ANOVA, n = 16, ****$P < 0.0001$

(K) Heatmap showing the outcomes of social competition of paired male mice ranked with FPCT and tube test.

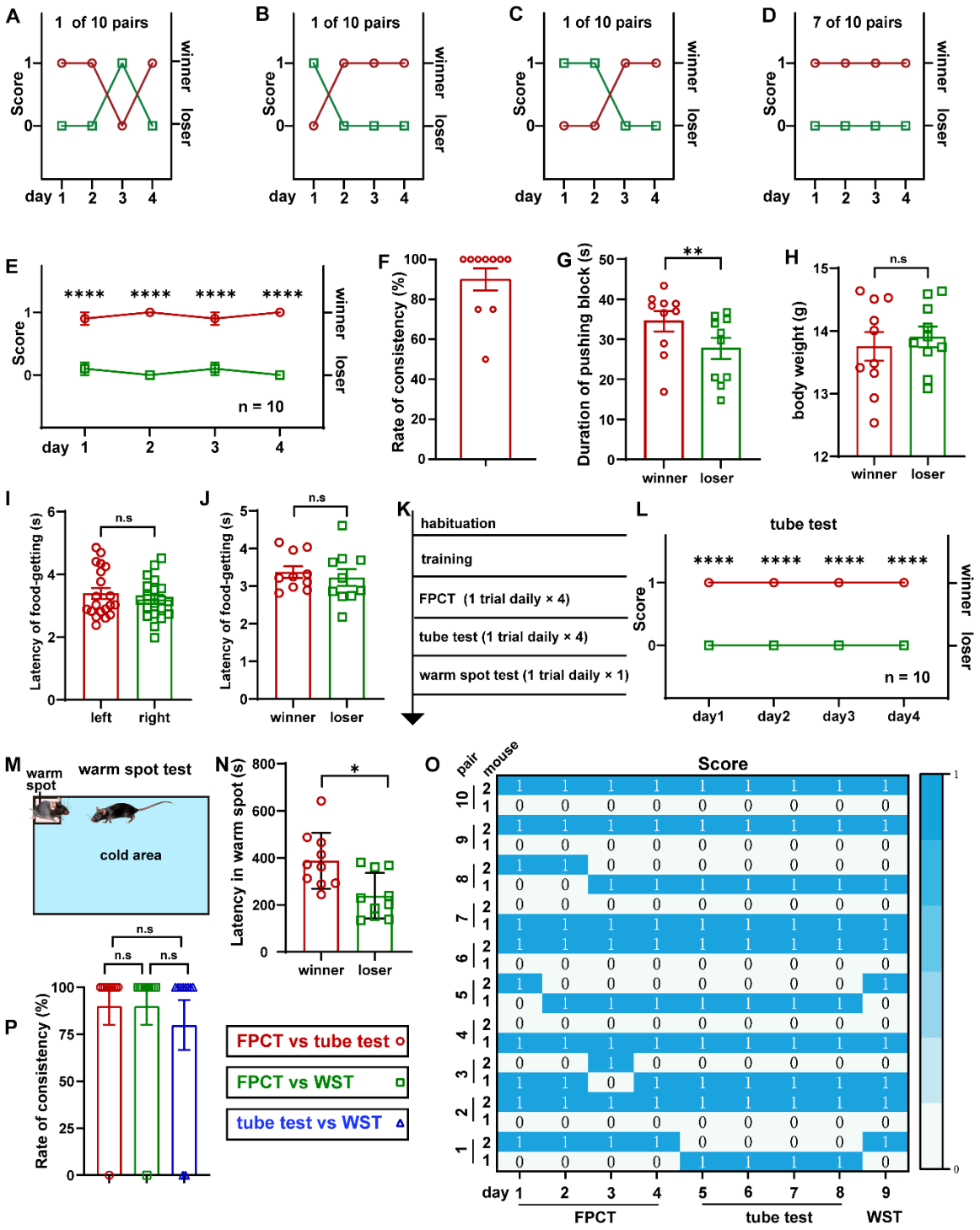

A
1 of 10 pairs
Score
winner
loser
day 1 2 3 4
B
1 of 10 pairs
C
1 of 10 pairs
D
7 of 10 pairs
E
****
n = 10
F
Rate of consistency (%)
G
Duration of pushing block (s)
**
winner
loser
H
body weight (g)
n.s
I
Latency of food-getting (s)
left
right
J
K
habituation
training
FPCT (1 trial daily × 4)
tube test (1 trial daily × 4)
warm spot test (1 trial daily × 1)
L
tube test
day1 day2 day3 day4
M
warm spot test
warm spot
cold area
N
Latency in warm spot (s)
*
P
Rate of consistency (%)
FPCT vs tube test
FPCT vs WST
tube test vs WST
O
pair
mouse
Score
FPCT
tube test
WST

Figure 5. Ranking the 2-cagemate female mice using FPCT, tube test and warm spot test (WST).

(A-C) 3 of the 10 pairs of cagemate female mice exhibited alternating winning/losing outcome in FPCT test 1.

(D) 7 of the 10 pairs of female mice exhibited fully congruent winning/losing outcome in FPCT test 1.

(E) Statistics of ranking of female mice over through 4 trials (1 trial daily) of FPCT test 1. Two-way ANOVA, n = 10, ****$P < 0.0001$.

(F) Average rate of consistency within trials. For each pair, rate of consistency was calculated as the percentage of the number of trials (in all 4 trials) resulting the outcome same as 4th trial (n = 10).

(G) Statistics of duration spent on pushing block in FPCT test 2. Paired Student's t test, *$P < 0.05$, n = 10.

(H) Body weight of female mice measured after FPCT. Unpaired Student's t test, n.s. stands for non-significant difference, n = 10.

(I) Comparison of the latency to food-getting in training when the female mice entered the arena from the left vs from the right. Paired Student's t test, n = 20, n.s. stands for non-significant difference.

(J) Comparison of the latency to food-getting of winner vs loser female mice in training. Unpaired Student's t test, n = 10, n.s. stands for non-significant difference.

(K) Timeline of experiments showing tube test and warm spot test were conducted after FPCT.

(L) Statistics of ranking of female mice over through 4 trials (1 trial daily) of tube test. Two-way ANOVA, n = 10, ****$P < 0.0001$.

(M) Schematic of the warm spot test.

(N) Cumulative duration of mice occupying the warm spot. The winner or loser identity was determined by FPCT. Paired Student's t test, n = 10, *$P < 0.05$.

(O) Heatmap showing the outcomes of social competition of paired female mice ranked with FPCT, tube test and WST.

(P) Rate of consistency between FPCT and tube test (day 4 vs day 5), FPCT and WST (day 4 vs day 9), as well as tube test and WST (day 8 vs day 9). One-way ANOVA test, n = 10, n.s. stands for non-significant difference.

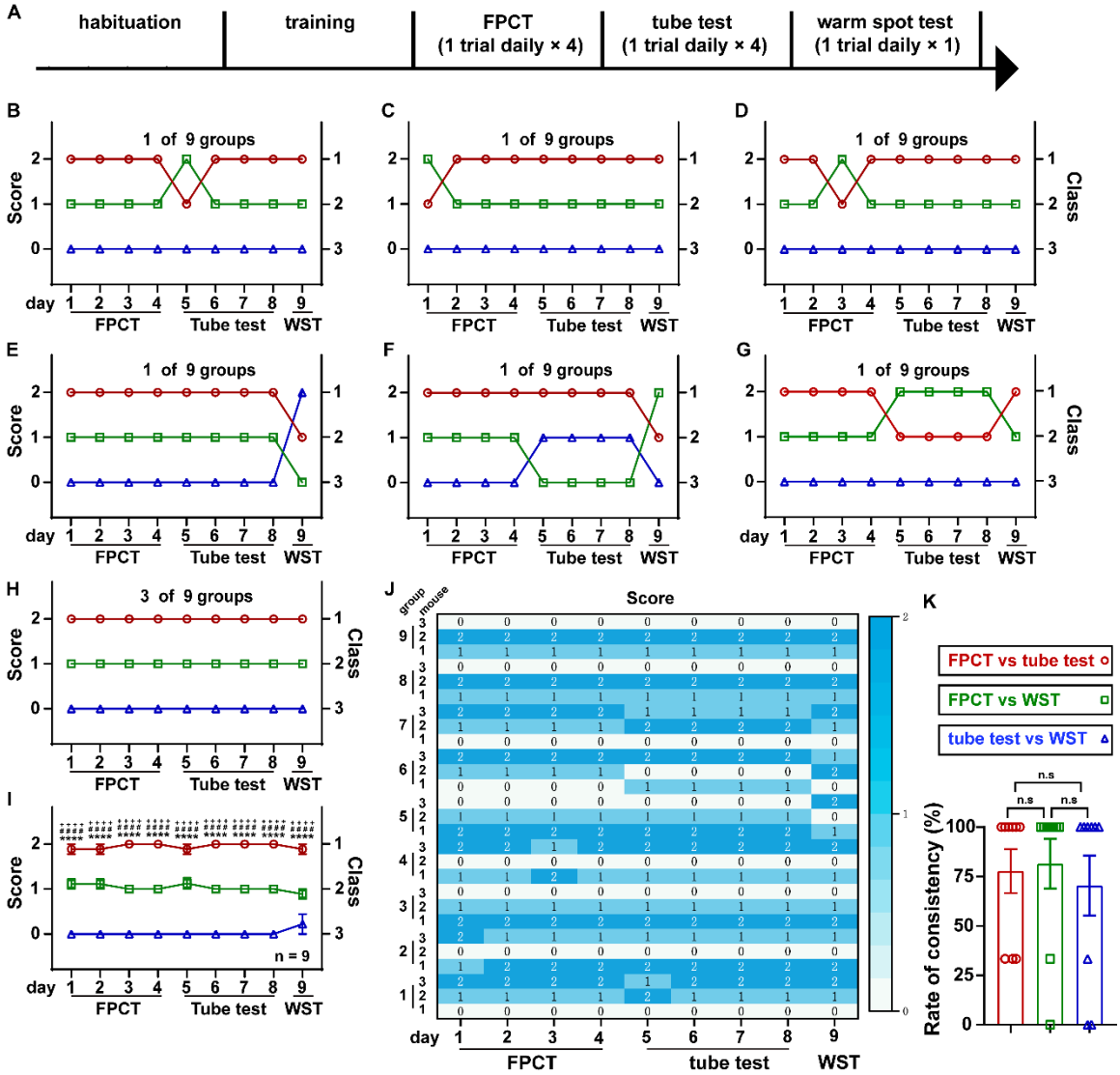

A
habituation
training
FPCT
(1 trial daily × 4)
tube test
(1 trial daily × 4)
warm spot test
(1 trial daily × 1)
B
C
D
E
F
G
H
I
J
K
1 of 9 groups
3 of 9 groups
Score
Class
day
FPCT
Tube test
WST
n = 9
group
mouse
tube test
FPCT vs tube test
FPCT vs WST
tube test vs WST
n.s
Rate of consistency (%)

Figure 6. Ranking the grouped male mice using FPCT, tube test and WST.

(A) Timeline of experiments to rank 3-cagemate male mice using FPCT, tube test and WST sequentially. In the FPCT and tube test, the mice contested in a round-robin style within the each 3-cagemate group.

(B-G) 7 of the 10 groups of male mice exhibited alternating winning/losing outcome during the whole competition tasks.

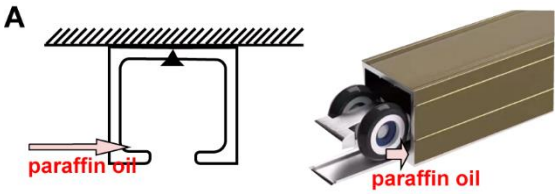
(H) 3 of the 10 groups of male mice exhibited fully congruent winning/losing outcome during the whole competition tasks.

(I) Statistics of ranking of mice over through the whole competition tasks. Two-way ANOVA test, n = 10, $^{++++}P < 0.0001$ comparing class 1 and class 2, $^{****}P < 0.0001$ comparing class 1 and class 3, $^{\#\#\#\#}P < 0.0001$ comparing class 2 and class 3.

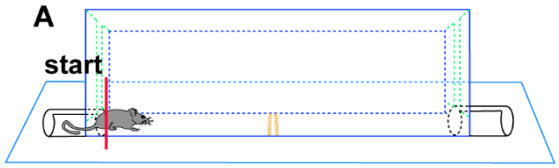
(J) Heatmap showing the ranking outcomes of grouped male mice during the whole competition tasks.

(K) Rate of consistency between FPCT and tube test (day 4 vs day 5), FPCT and WST (day 4 vs day 9), as well as tube test and WST (day 8 vs day 9). One-way ANOVA test, n = 10, n.s. stands for non-significant difference.

Supplementary figures and legends



Supplementary figure 1. Schematic drawing to show a tip to reduce the friction between the pulley and the track.



Supplementary figure 2. Schematic drawing to show the marking of the starting state when the mouse's four legs just entered the chamber arena.

Supplementary videos

Supplementary video 1. In step 3 of training, the mouse was trained to know the position of the food pellet in the absence of the movable block.

Supplementary video 2. In step 4 of training, the mouse was learning to get the hidden food pellet in trough of the chamber floor under the movable block.

Supplementary video 3. In FPCT test 1, the mice were competing for the hidden food pellet.

Supplementary video 4. In FPCT test 2, the mice were attempting to get the food pellet in the inner bottom of the block.