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A Task for Assessing the Impact of a Partner on the Speed and Accuracy of Motor Performance in Rats

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To the Editors:

I, along with my coauthors, would like to re-submit the attached manuscript entitled "An experimental task for investigating the effect of a social situation on the speed and accuracy of motor performance in rats" as an original article. The manuscript ID is JoVE60176R1.

The manuscript has been carefully rechecked and appropriate changes have been made in accordance with the editors' suggestions. The responses to their comments have been prepared and attached herewith.

We revised the manuscript according to comments, and we replied to the comment in the revised manuscript. For your convenience, revisions in the present manuscript based on editors' comments are shown in blue text. We added a certificate of English proofreading as a supplemental file.

We thank you and the reviewers for your thoughtful suggestions and insights, which have enriched the manuscript and produced a more balanced and better account of the research. We hope that the revised manuscript is now suitable for publication in your journal.

I look forward to your reply.

Sincerely,

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TITLE:

A Task for Assessing the Impact of a Partner on the Speed and Accuracy of Motor Performance in Rats

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KEYWORDS:

rats, motor performance, social facilitation, mere presence, speed, accuracy

SUMMARY:

A procedure to measure the speed and accuracy of rats' motor performance in a social condition is described. The protocol enables us to investigate the effect of the mere presence of others on speed and accuracy of motor performance in one experiment.

ABSTRACT:

To our knowledge, no study has examined the effect of mere presence on accuracy of performance in animals. Therefore, we developed an experimental task to measure rats' motor performance (speed and accuracy) in a social condition. Rats were trained to run on a runway and pull down a lever at the end of the runway. In testing, rats performed the task solitarily (single) or in the presence of a confederate rat beyond the lever (pair or a social condition). As indices of the performance speed, we measured the time needed to start running, run through the runway, and pull down the lever. As the index of performance accuracy, we counted the number of trials in which rats could pull down the lever during their first attempt. One-way and two-way repeated-measure analyses of variance were used to analyze the data. This run-and-pull task enabled us to examine the effect of the presence of another conspecific on both speed and accuracy of motor performance in one experiment. The results showed that rats performed the task faster but less accurately in pair sessions than in single sessions. This protocol would be a valid animal model to examine the effect of mere presence on speed and accuracy of motor performance in rats.

INTRODUCTION:

The effect of social conditions on one's performance has been investigated in humans and animals for a long time, since Allport[1] referred to "social facilitation" as "an increase in

response merely from the sight or sound of others making the same movement"[2]. Although Allport[1] did not distinguish the social situation (co-action or the mere presence of another), it has been shown that the mere presence of other(s) affects one's performance speed or frequency[3-6]. Additionally, in animals, the mere presence of other conspecific results in a higher response rate or higher response speed during a lever-press task in rats[7,8] and higher response rate in rhesus monkeys during a simple cognitive task[9].

In humans, it has been shown that social situations affect not only response frequency or speed but also accuracy of performance[10]. Based on a meta-analysis by Bond and Titus[11], Strauss[12] argued that situations used in studies on social facilitation would have a different effect depending on features of the task used. Especially, decrement in the performance would be expected when the study used a task that placed high demands on one's ability to control his/her body precisely and to perform with some extent of speed, which tends to be scored by its qualitative aspects (e.g., accuracy of the performance)[13].

Except for a couple studies[14,15], however, most studies on social facilitation in animals have not focused on the accuracy of performance. For instance, Takano and Ukezono[16] investigated the effect of mere presence in rats using a skilled reaching task[17]. They required rats to turn around and then grasp a reward pellet on a shelf by using their forelimb. The authors reported only the performance speed, despite the fact that the task could provide an index of performance accuracy. Conversely, Ogura and Matsushima[14] examined the effect of co-action on the pecking accuracy as well as running speed in chicks. The result showed that the pecking accuracy was lower and running speed was higher in the co-action situation than in the solitary situation.

Although Ogura and Matsushima[14] focused on the qualitative aspect of an action for the first time, their study was about the effect of co-action. Most social conditions, including co-action, inevitably imply the presence of another. To examine the effect unique to co-action, it is indispensable to dissociate the effect of mere presence from that of co-action on an individual's performance. However, the study did not investigate the effect of mere presence. To our knowledge, no study has examined the effect of mere presence on the accuracy of performance in animals.

We modified the task used in Takano and Ukezono's study[16] to evaluate the effects of mere presence on both performance speed and accuracy. This method enables us to examine the effect of social conditions, especially the mere presence of a conspecific, on performance accuracy and performance speed in rats in one experiment.

PROTOCOL:

This experimental protocol was approved by the Doshisha Committee of Animal Experiment.

NOTE: Conduct all experimental sessions during the light period.

1. Animals

1.1. Use 15 experimentally naïve male albino Wistar rats that weighed 300−350 g. House them in individual cages of a controlled breeding room with the appropriate temperature and humidity (23 ± 2 °C, 70%) and *ad libitum* access to the water. Maintain the light/dark cycle at 12 h/12 h (the light period begins at 8:00 AM).

1.2. Assign 10 rats as subjects, and the remaining as confederate rats using randomization.

1.3. Maintain rats' weights at 85−90% of their free-feeding weights throughout the experiment by food deprivation.

NOTE: The assignment of confederate is to reduce the number of animals and to reduce the load of animals.

2. Apparatus

NOTE: The overview of the apparatus is shown in **Figure 1**. The apparatus was developed and modified in reference to previous studies[16,17].

2.1. Construct a clear acrylic box (19 cm x 110 cm x 20 cm) with a central partition (5 cm wide) and insert two guillotine doors. Place the guillotine doors 15 cm away from each end of the box. Mount a buzzer (400 Hz, 75 dB) on the partition, and set up a pellet dispenser for serving a reward pellet (45 mg) into a food receptacle on the subject's side of the box.

2.2. Set up a metal lever (grip bar: ϕ3 mm, 5 cm high) on a shelf inside the central partition, which has a slit (1.5 cm wide) on each wall facing the runway (**Figure 2**), in order to allow the subject rat to access the lever by using its forelimbs. On the confederate's side, however, insert a transparent wall in front of the partition's wall that prohibits the confederate from accessing the lever.

2.3. Place a switch for activating the dispenser under the lever that is pressed by the lever-pull movement of rats. Tie the proper length of gut to the lever, which is needed for the experimenter to pull up the lever.

2.4. Use Arduino Mega 2560 REV3 to control the guillotine doors, buzzer, and pellet dispenser, and to obtain the value from the infrared sensors and switch of the dispenser. Prepare an air compressor (25 L) to operate the air cylinder and open the guillotine door.

2.5. Place a video camera near the partition of the outside of the apparatus, in order to record lever-pull performance of the rats from a lateral view (60 frames per second [fps]). Use an adequate video playback software to conduct frame-by-frame analysis of the video recording.

[Place **Figure 1** and **Figure 2** here]

3. Procedure

3.1. Handling and habituation for the reward pellets

3.1.1. Before the training procedures, handle all rats for 10 min/day for 3 days by the experimenter.

3.1.2. As a habituation for the reward pellets, give each rat 3 g of reward pellets for 3 days after handling.

3.2. Habituation to the apparatus

3.2.1. Subject rats

3.2.1.1. On the third day of habituation for the reward pellets, before the experimenter gives daily pellets, place each rat in the runway of the subject's side of the apparatus for 15 min for habituation, with the guillotine door closed.

3.2.2. Confederate rats

NOTE: There are two habituation sessions.

3.2.2.1. At the first habituation, place each rat on the runway of the confederate's side of the apparatus for 15 min during the same period as step 3.2.1.1.

3.2.2.2. At the second habituation, follow the method in step 3.2.2.1 on the day after subject rats completed training for the run-and-pull sequence (as mentioned in section 3.6).

NOTE: Conduct habituation sessions for confederate rats and subject rats separately, i.e., one rat at a time. Do not let a rat meet another rat in the apparatus until the test phase starts.

3.3. Magazine training

NOTE: For the subject rats, conduct a magazine training session on the next day of the habituation to the apparatus as follows. If rats do not eat all the food pellets, then conduct the magazine training session again on the next day.

3.3.1. Put one reward pellet on the food receptacle on the confederate's side to exclude the effect of the olfactory property of the pellet, which the confederate eats in the test phase (refer to section 3.7).

3.3.2. Place a subject rat on the runway of the subject's side, with the guillotine door closed.

3.3.3. Deliver a reward pellet into the food receptacle 60 times using the pellet dispenser on a

variable time 30-s schedule. Ensure that the subject rat eats all the delivered pellets.

3.4. Shaping for the lever-pull action

NOTE: **Figure 3** is a flowchart of the experiment.

3.4.1. Put one reward pellet on the food receptacle on each subject's side and confederate's side. Do not place the confederate rat in shaping sessions.

3.4.2. Place the subject rat on the runway of the subject's side with the guillotine door closed. Then train the rats to pull down the lever. Shape the lever-pull behavior gradually with following five reinforcement criteria (A-E): (A) Approach the slit. (B) Touch the grip bar to the rat's muzzles or forelimbs, with the lever beforehand pulled down to the subject's side. (C) Touch the grip bar, with the lever inclined to the rat's side (at an angle of 60°−30°). (D) Grasp and pull down the bar to rat's side using the same condition as in criterion C. (E) Grasp and pull down the bar to rat's side with the lever standing upright.

3.4.3. Finish daily sessions when 60 rewards are given, or 30 min has elapsed. When a rat completes criterion E 40 times in a session, the lever-pull shaping phase is finished on that day.

NOTE: Almost all rats finish the daily shaping session within 20 min. Wistar rats need about 3 days to reach criterion E.

[Place **Figure 3** here]

3.5. Training for the lever-pull action

3.5.1. Place the subject rat on the start area. Do not place the confederate rat in the training sessions. Present the buzzer tone for 5 s, and then open the door. When the subject rat pulls down the lever, deliver a food pellet and pull the lever up using a gut tied to the lever.

3.5.2. After rats pull down the lever for 10 times (first 3 days of the phase) or 6 times (last 3 days of the phase) and consume all reward pellets, close the door and move them to the start area by the experimenter's hand.

3.5.3. After an inter-trial interval (ITI) of 20 s, repeat steps 3.5.1 and 3.5.2. Finish daily sessions when each rat has earned 60 pellets.

NOTE: This training also aims to habituate rats to the buzzer tone and door opening because Wistar rats ordinarily show freezing behavior at first in response to door movement. A daily session for each rat requires about 15 min. Six days are needed for this training phase.

3.6. Training phase for the run-and-pull sequence

3.6.1. Conduct the same procedure in step 3.5.1.

3.6.2. When rats pull down the lever one time and consume a reward pellet, close the door and move them to the start area by the experimenter's hand.

3.6.3. After an ITI of 20 s, start the next trial (steps 3.6.1 and 3.6.2). Finish the daily session when each rat has earned 30 pellets.

NOTE: A daily session for each rat requires about 20 min. About 10 days are needed for rats' scores to reach asymptote.

3.7. Test phases

[Place **Figure 4** here]

NOTE: Test phases include sessions in two conditions, single or pair (**Figure 4**). In the single condition, rats perform the task solitarily; that is, trials are identical to the session of the training for the run-and-pull sequence (section 3.6). In the pair condition, the confederate rat is present at the opposite side of the box. The confederate rat cannot access the lever because of the clear acrylic wall in front of the partition.

3.7.1. Conduct a single session that is identical to the sessions in the training phase for the run-and-pull sequence (section 3.6). Finish the daily session when each rat has earned 30 pellets. Put one reward pellet on the food receptacle on the confederate's side, especially during sessions in the single condition.

3.7.2. In pair sessions, give the confederate rat a reward pellet during the ITI of the subject rat in order to keep the confederate rat near the partition.

4. Data analysis

4.1. Index of performance accuracy

4.1.1. Record videos of rats' lever-pull movement using the video camera near the partition of the outside of the apparatus. After all sessions are completed, confirm the evaluation by frame-by-frame analysis of the video recordings using an adequate video playback software.

4.1.2. Evaluate whether the rat's pulling movement of a trial was the first-hit or not by visual observation of the experimenter during the experiment.

NOTE: The first-hit trial is defined as the trial in which rats could grasp and pull down the lever during their first attempt to the lever.

4.1.3. Calculate first-hit rates for each subject as the proportion of first-hit trials to all trials in

each session (for the run-and-pull training phase) or in each phase (for test phases) to use in the analysis.

4.2. Indices of performance speed

4.2.1. Calculate the time needed to complete a trial from the value of the switch of the pellet dispenser: the time needed to complete a trial = (the time when the switch is pressed) - (the time when the door is opened). Then divide the time needed for completion of a trial into three sections using values from the infrared sensors (**Figure 5**).

NOTE: Start latency (**Figure 5a**) is defined as the time from the door opening to the arrival of the rat at the first sensor. Similarly, running time (**Figure 5b**) is the time from the arrival at the first sensor to the arrival at the second sensor. Lever-pull latency (**Figure 5c**) is the time from the arrival at the second sensor to the time point when the switch of the dispenser is pressed.

4.2.2. Use only the durations from the first-hit trials for analysis. Calculate median values of each subject for each session (of the training sessions) and each phase (of the test phases) for analysis.

[Place **Figure 5** here]

4.3. Statistical analysis

4.3.1. For the indices in the training phase of the run-and-pull sequence, conduct one-way repeated-measure analysis of variance (ANOVA) with the number of sessions as the within-subject factor for each index.

4.3.2. For the indices in the test phase, conduct two-way repeated-measure ANOVA with the number of the phase (phase 1 or 2) and condition (pair or single) as the within-subject factors for each index in the test phases. Statistical significance was set at $\alpha = 0.05$.

REPRESENTATIVE RESULTS:

Training phase for the run-and-pull sequence

Figure 6 shows the mean ± standard error of the mean (SEM) scores from the training phase for the run-and-pull sequence. The mean first-hit rate (**Figure 6A**) increased gradually during the first half of the training phase and then stopped at about 85%. The ANOVA results showed that the main effect of the number of sessions was significant ($F(7,63) = 3.74$, $p = 0.002$, $\eta^2_G = 0.211$). Multiple comparisons revealed that there were no significant differences among the latter four sessions (all p values > 0.60).

[Place **Figure 6** here]

Similarly, indices of performance speed (**Figure 6B**; start latency, running time, and lever-pull latency) decreased continuously during the first four sessions, and all values stabilized at about 600 ms among the latter four sessions. For all indices, ANOVA showed that main effects of the number of sessions were significant (start latency: (F(7,63) = 6.21, p < 0.001, η^2_G = 0.279; running time: (F(7,63) = 3.98, p = 0.001, η^2_G = 0.170; lever-pull latency: (F(7,63) = 11.85, p < 0.001, η^2_G = 0.350). Multiple comparisons by sessions resulted in no significant difference among the latter four sessions for all measures (all p values > 0.12).

Figure 7 shows the mean ± SEM scores from sessions in the test phase. Regarding the index of performance accuracy, the first-hit rate (**Figure 7A**) in pair phases was lower than in the single phases. Additionally, the first-hit rate in the second phase was higher than in the first phase in the two conditions. The results of ANOVA showed significant main effects of the condition (F(1,9) = 6.25, p = 0.034, η^2_G = 0.114) and phases (F(1,9) = 14.1, p = 0.005, η^2_G = 0.147), but the interaction was not significant (F(1,9) = 0.15, p = 0.703, η^2_G = 0.002).

[Place **Figure 7** here]

Regarding indices of performance speed, the start latency in pair phases (**Figure 7B**) was shorter than in single phases. The ANOVA results showed that for the start latency, only the main effect of the condition was significant (F(1,9) = 23.1, p = 0.001, η^2_G = 0.065), while the main effect of phases and the interaction was not significant (phases: F(1,9) = 0.03, p = 0.878, η^2_G < 0.001; interaction: F(1,9) = 0.002, p = 0.970, η^2_G < 0.001). Similarly, a difference was observed between conditions for the lever-pull latency (**Figure 7D**). As with the start latency, for the lever-pull latency, ANOVA showed a significant main effect of the condition (F(1,9) = 23.3, p = 0.001, η^2_G = 0.183). There was no significant main effect of phases (F(1,9) = 2.72, p = 0.133, η^2_G = 0.028) and the interaction (F(1,9) = 1.07, p = 0.327, η^2_G = 0.002). For the running time, there was no significant effect (**Figure 7C**, condition: F(1,9) = 3.03, p = 0.116, η^2_G = 0.004; phases: F(1,9) = 4.46, p = 0.063, η^2_G = 0.010; interaction: F(1,9) = 0.29, p = 0.602, η^2_G < 0.001).

FIGURE LEGENDS:

Figure 1: A schematic of the apparatus used in this protocol. A central partition divides the box into two fields. There is a guillotine door on each side of the box, and the door divides the field into the start area and runway.

Figure 2: The central partition of the apparatus. Rats can grasp a bar and pull the lever through a slit of the partition. A switch of the pellet dispenser is set under the lever, and one lever-pull action results in one pellet delivery. This figure has been modified from Sekiguchi and Hata[12].

Figure 3: A flowchart of the experimental procedure. Subject rats go through the training phases and test phases in this order. This figure has been modified from Sekiguchi and Hata[12].

Figure 4: Description of each test condition. In single phases, the subject rat performed the task solitarily. In the pair phases, place the confederate rat in the runway on the opposite side

to the subject rat. A transparent wall in front of the partition precludes the confederate rat from accessing the lever. This figure has been modified from Sekiguchi and Hata[12].

Figure 5: Measurement of the indices of performance speed. (**a**) Start latency: the duration from the door opening to the arrival of the rat at the first sensor. (**b**) Running time: the duration from the arrival of the rat at the first sensor to its arrival at the second sensor. (**c**) Lever-pull latency: the duration from the arrival of the rat at the second sensor to the completion of a lever-pull response. This figure has been modified from Sekiguchi and Hata[12].

Figure 6: Mean ± SEM scores from sessions in the training phase for the run-and-pull sequence. (**A**) Index of performance accuracy. (**B**) Indices of performance speed. This figure has been modified from Sekiguchi and Hata[12].

Figure 7: Mean ± SEM scores from sessions in the test phase. Index of performance accuracy (**A**: first-hit rate) and indices of performance speed (**B**: start latency, **C**: running time, and **D**: lever-pull latency). *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. This figure has been modified from Sekiguchi and Hata[12].

DISCUSSION:

This task enables us to evaluate the effect of the mere presence of others on speed and accuracy of motor performance. The effect sizes reported herein would be large enough. We recalculated η^2 (there was not much difference between η^2 and η^2_G in this experiment), and these effect sizes are regarded as medium ($\eta^2 > 0.06$) or large ($\eta^2 > 0.14$) according to the criterion presented by Cohen[18]. For this reason, we considered that the differences seen in this study are meaningful and reliable. Results in the experiment were almost in line with those from studies in humans[4,10], and the result of Ogura and Matsushima's study[14], which investigated the effect of co-action on the speed and accuracy of running and pecking behavior in chicks. Although studies about social facilitation in humans investigated the effect of social conditions on both speed and accuracy of behavior, most previous studies in animals have not investigated the mere presence effect on performance accuracy. The protocol presented herein provides a better animal model for investigating the effect of mere presence on motor performance.

As a limitation, the difference between the first-hit rate in pair and single conditions could be interpreted as an effect of practice. Despite presumably sufficient training, there could be room for improvement in the performance of rats. There were no more changes in the indices of performance accuracy and performance speed in the last four sessions of the training phase for the run-and-pull sequence. However, in the test phases, the first-hit rate increased continuously. This increase might be interpreted as the effect of practice. Additionally, this experimental design (A-B-A-B design) cannot exclude the effect of practice from the effect of conditions. Future experiments should (1) use the A-B-B-A design or another appropriate experimental design to exclude the effect of practice, and (2) consider extending the training phase for the run-and-pull sequence.

This protocol can be used in a study with a between-subject design, although the answer to the questions “Which design is appropriate? Within subject or between subject?” would be on a case-by-case basis. Generally, a study using the between-subject design can exclude the practice effect that might be observed in this study. However, the between-subject design requires more animals and more time for one study (i.e., if using the protocol in this article, 7−8 h per day would be needed to test all the rats in 1 day using one apparatus). A study that uses the within-subject design can reduce the number of animals required and time for one study, but the experimenter must control the effect of practice. Time and cost should be carefully weighed before the experimenter choses the experimental design.

With minor modification, this task can be applied to investigate the effect of co-action or social facilitation by mirror[10] and other social situations that have been studied in humans on the performance speed and performance accuracy of rats. To investigate the effect of co-action, separate the lever in the central partition into two levers, and arrange the levers so that each lever can be pulled by one side of the box. To investigate the effect of using a mirror, change the acrylic clear wall on the confederate’s side of the box to the mirror. In the same manner, the effect of an invisible confederate behind the opaque wall could be investigated. Future studies using these modifications will contribute to comprehensively understanding social facilitation by multifaceted comparison of motor performance between species.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

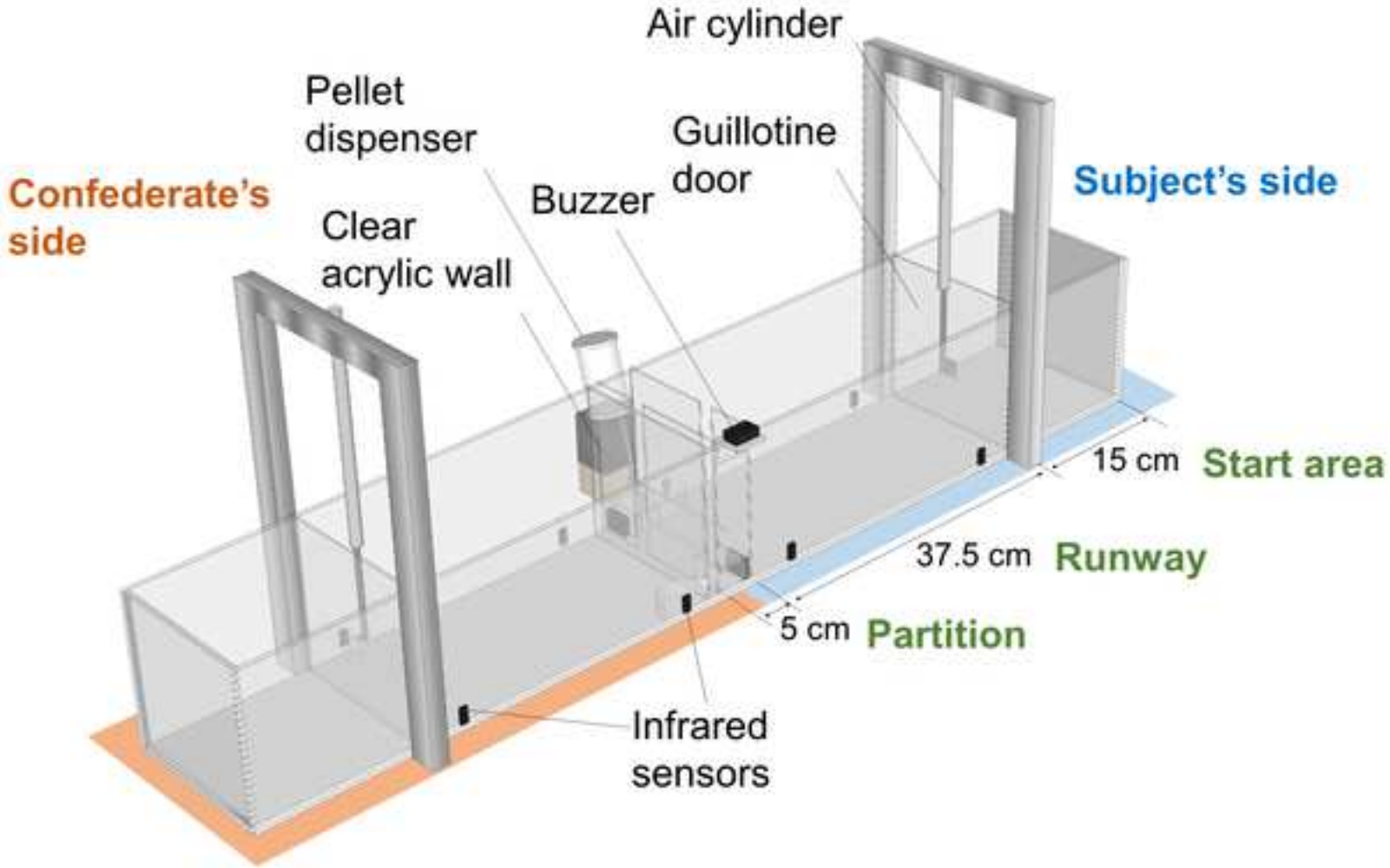


Figure 2

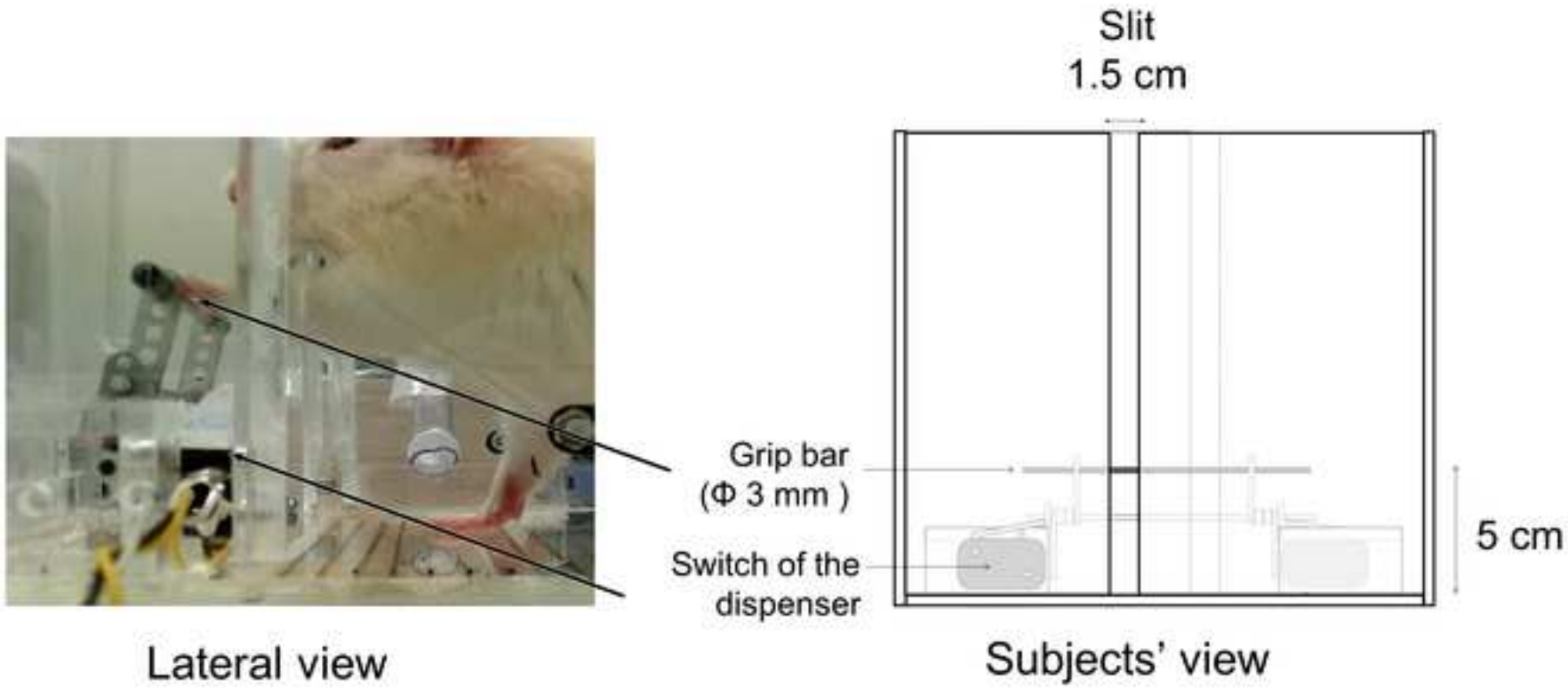


Figure 3

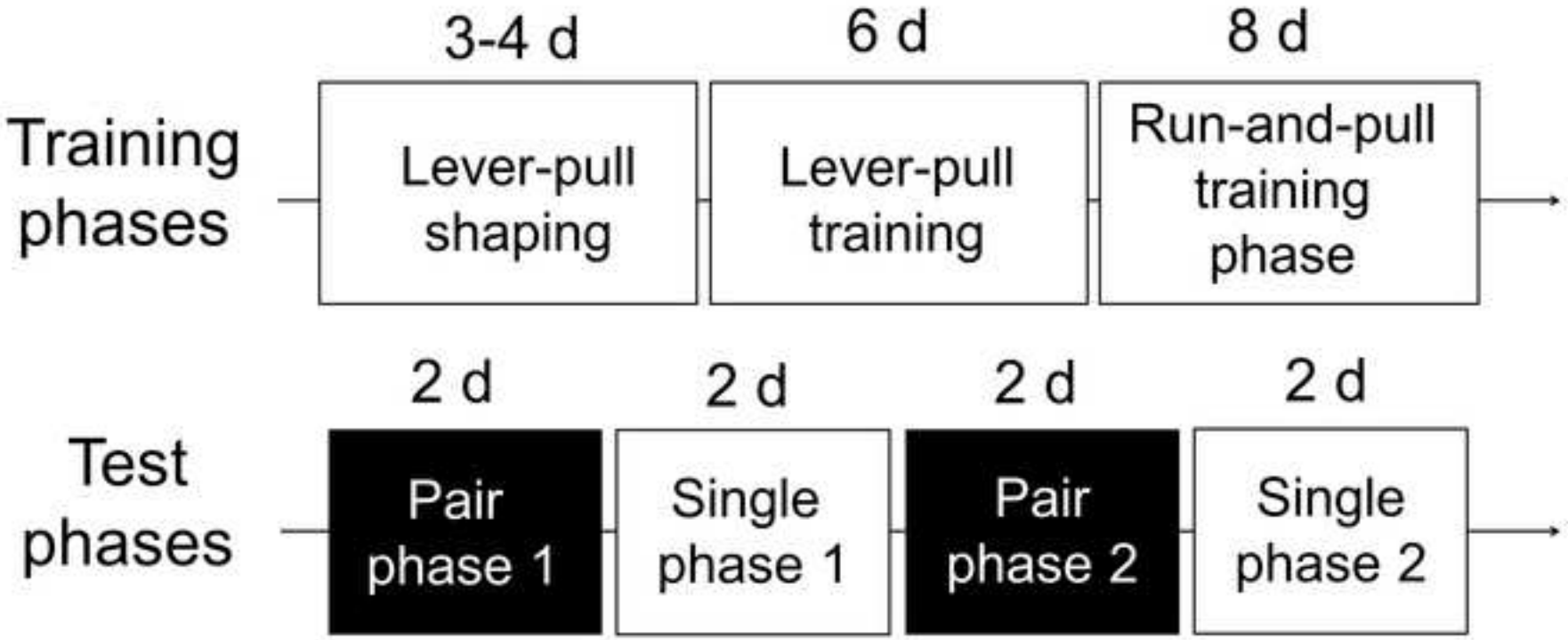


Figure 4

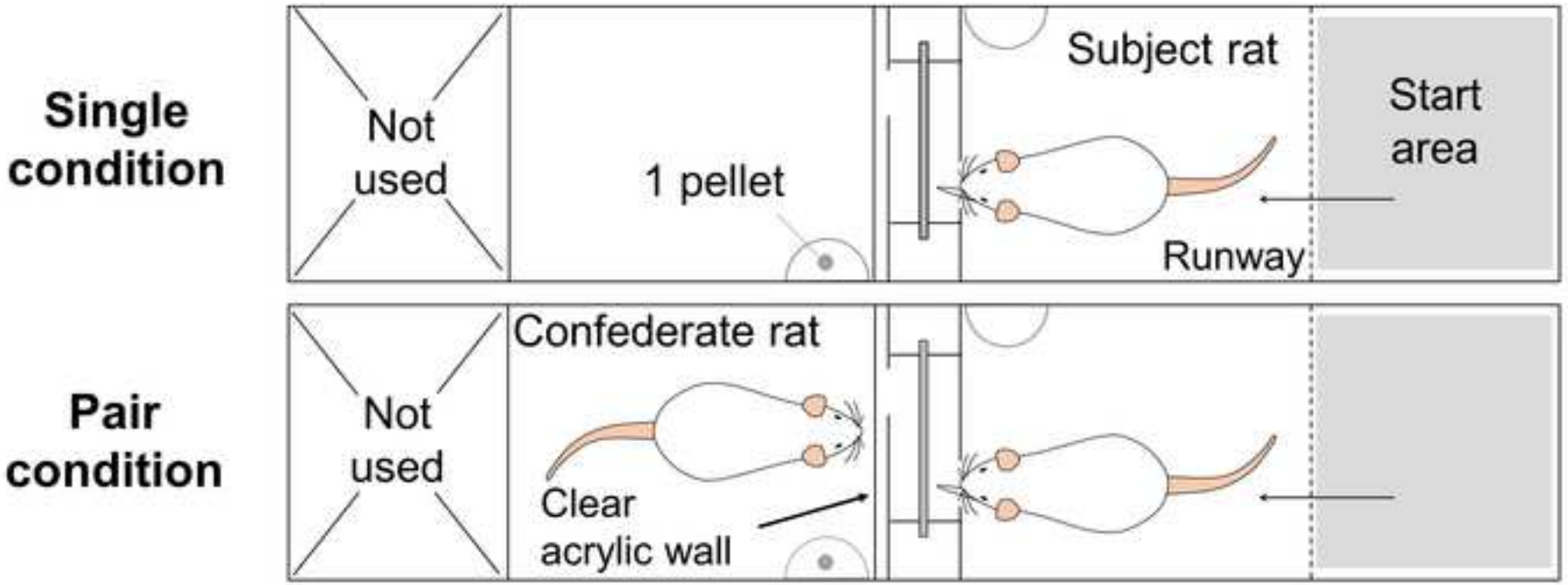


Figure 5

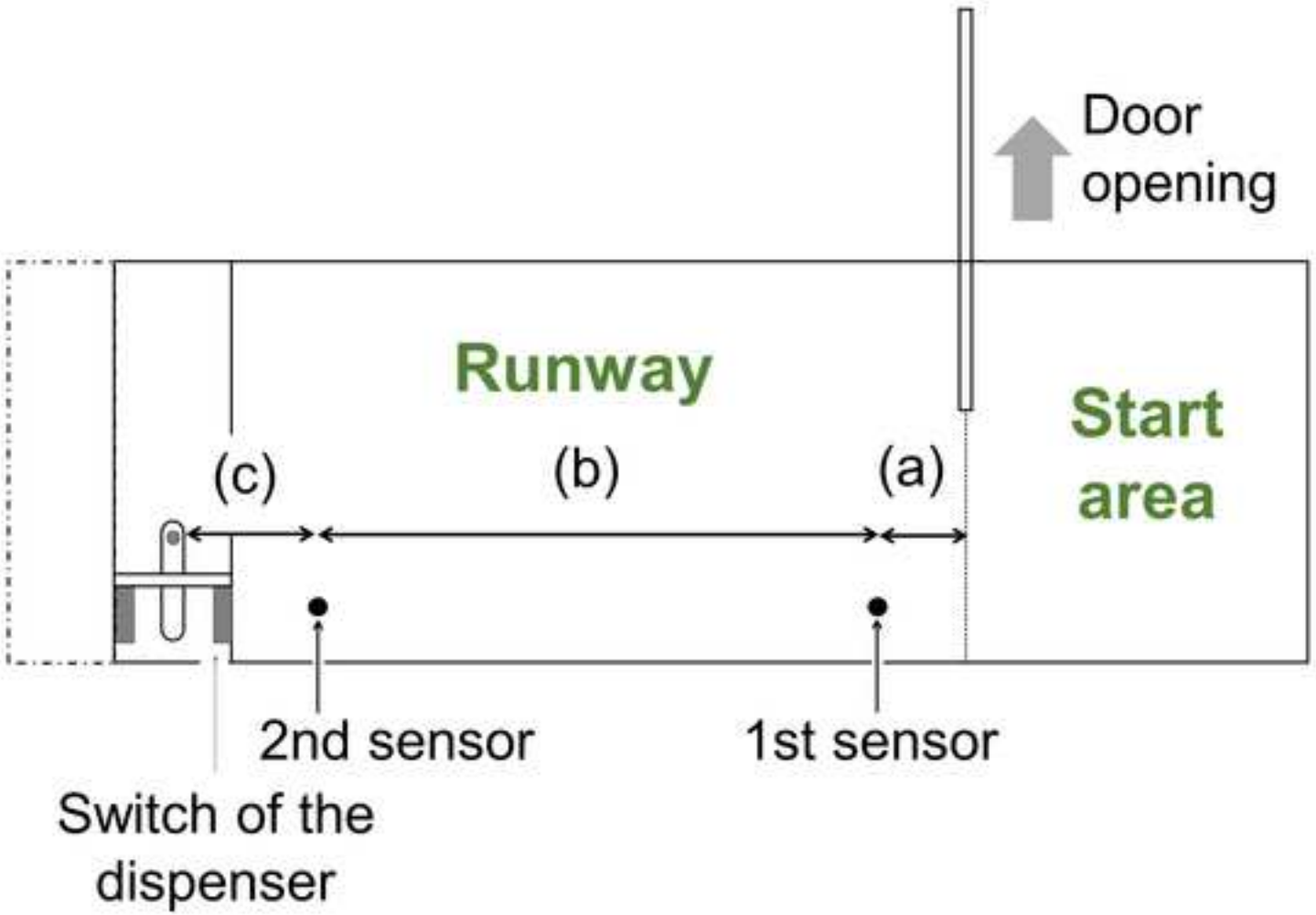


Figure 6

Click here to access/download;Figure;Figure 6.TIF

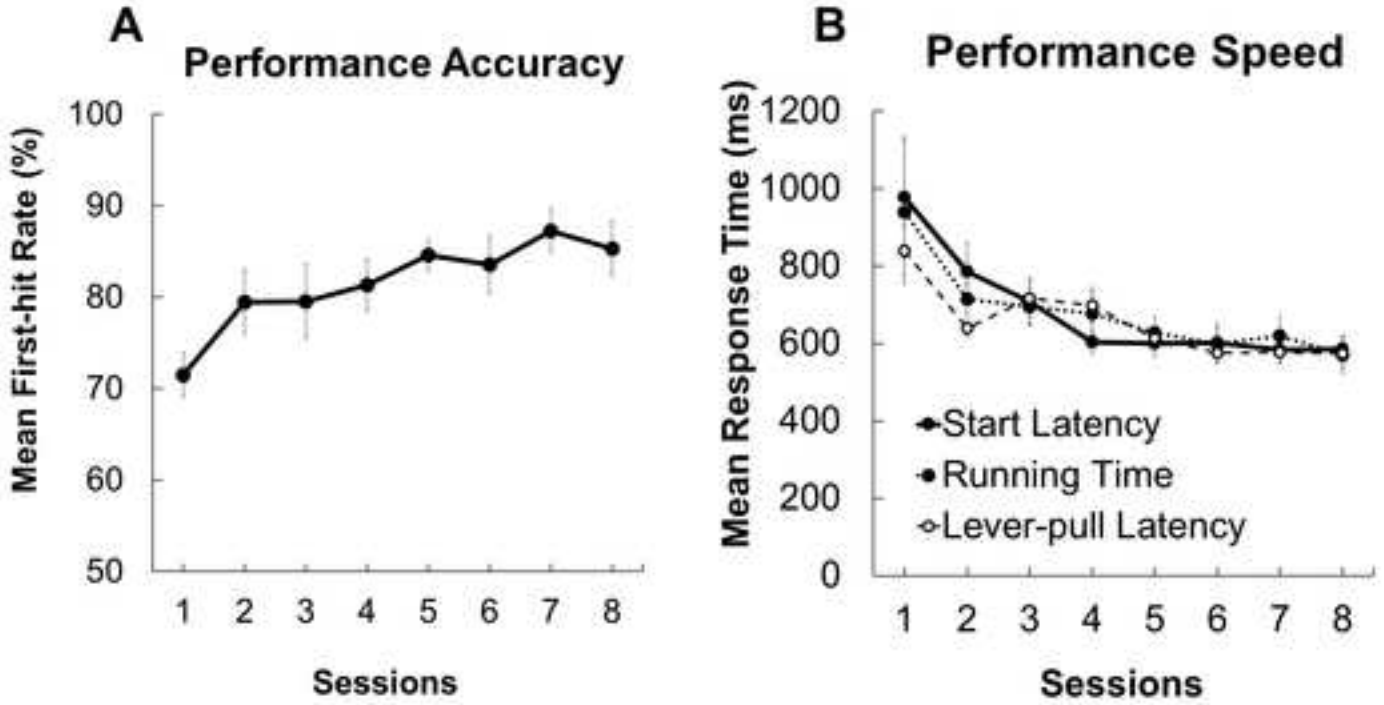
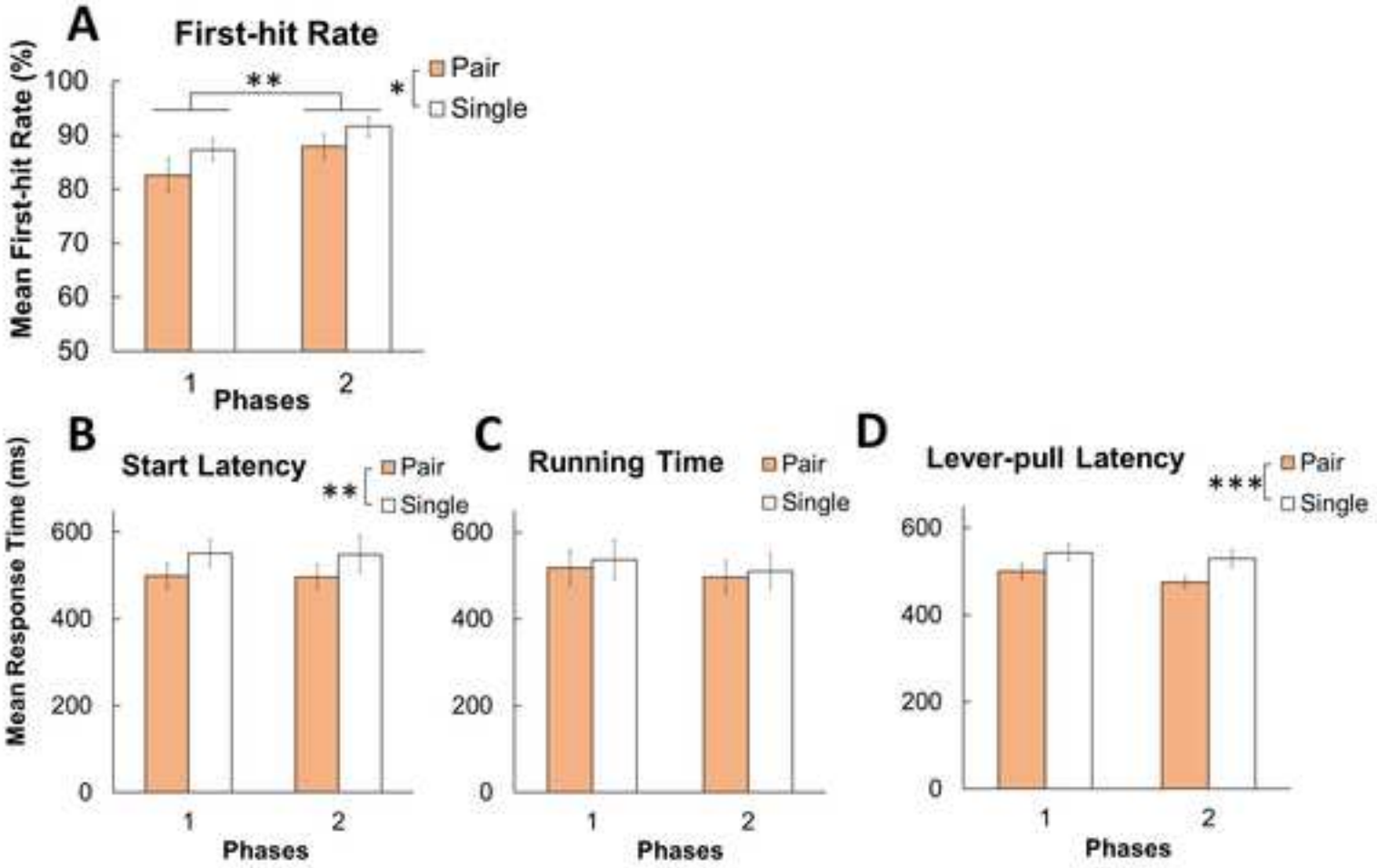


Figure 7



Name of Material/Equipment	Company	Catalog Number	Comments/Description
45 mg Dustless Precision Pellets Rodent, Purified	Bio-Serv.	F0021	
Arduino Mega 2560 REV3	Arduino S.r.l.	None	
Pellets Dispenser with Feeder (Rats)	Harvard Apparatus	76-0353	
Power DVD 14	CyberLink	None	Use an adequate video playback program which enables frame-by-frame playback.
Run-and-pull task apparatus	Bio Medica Corp.	Custom-made item	The set of apparatus (box), an air compressor, and a control device for air cylinders which receives inputs from Arduino.
Video camera	JVC	GZ-R300	

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Author(s): Yayoi Sekiguchi, Toshimichi Hata

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CORRESPONDING AUTHOR

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Department:	Graduate school of Psychology
Institution:	Doshisha University
Title:	Ph.D student
Signature:	Yayoi Sekiguchi
Date:	April 29, 2019

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Editorial comments:

Changes to be made by the author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

We rewrite some minor details and requested native speakers of English to proofread.

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Reply:

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3. Please revise lines 56-58, 230-233, 259-263, 267-271, 275-284 to avoid textual overlap with previously published work.

Reply:

We rewrite suggested sentences or words in the sentences.

4. Lines 89-118, 199-243: The Protocol should be made up almost entirely of discrete steps without large paragraphs of text. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary. Please use the active/imperative voice and complete sentences. Please move the discussion about the protocol to the Discussion.

5. Please combine some of the shorter Protocol steps so that individual steps contain 2-3 actions and maximum of 4 sentences per step.

6. Please include single line spacing between each numbered step or note in the protocol.

Reply to 4,5,6:

We reflected the suggested corrections.

7. After you have made all the recommended changes to your protocol section (listed above), please highlight in yellow up to 2.75 pages (no less than 1 page) of protocol text (including headers and spacing) to be featured in the video. Bear in mind the goal of the protocol and highlight the critical steps to be filmed. Our scriptwriters will derive the video script directly

from the highlighted text.

8. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. The highlighted text must include at least one action that is written in the imperative voice per step. Notes cannot usually be filmed and should be excluded from the highlighting.

9. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

Reply to 7,8,9:

About highlighting, we corrected according to instructions.

10. Table of Materials: Please ensure that it has information on all relevant supplies, reagents, equipment and software used, especially those mentioned in the Protocol. Please sort the materials alphabetically by material name.

Reply:

We sorted the materials alphabetically by material name.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

Description of a novel dyadic apparatus

Major Concerns:

The major concern is the message of the manuscript, that there are a few (if any) other studies on social facilitation in either humans or animals, with emphasis on studies regarding the effect of being watched (what here is termed 'mere presence'). First studies like these are mentioned in the text (L.48-L.51), standing in contrast with other places in the text where it is stated that there is only one such study (e.g.- the first sentence in the abstract; L.27). This must be corrected - here are a few examples: Dorfman et al, PLoS 1, 2016; Dorfman et al. Behav Brain Res, 2019.; several studies by M.Brown, A. Avital; B. Berger; R. Shuster - all deal with the performance in dyads, groups, etc. Other studies deal with the impact of observer on performance of the individual being watched (for example - Grutter et al. Current Biol., 2011)

Reply:

Thank you for your suggestion. We added Dorfman et al. (2016) and Grutter et al. (2011; is this Pinto et al.?) as a previous studies of social facilitation (reference No.15 and

No. 6, respectively: L.48).

However, we consider that the suggested studies are not about the effect of “mere presence” on “performance accuracy”. For example, Dorfman et al. (2016) is the study of c—action, although the study could be considered as investigating the performance accuracy. Dorfman et al. (2019) is the studies of “co-action” situation but they did not measure the accuracy of performance. Grutter et al. (2011) certainly studied the effect of mere presence of another in cleaner fishes (*Labroides dimidiatus),* but they did not measure the accuracy of performance.

Another concern is the qualitative-quantitative argument with is very faint L.38). It is almost inevitable to remain accurate when speed increases, and these two parameters could be linked, or, at least, their independence should be proved (by demonstrating that one could change without the other). Accordingly, the suggestion that these reflect two independent domains: qualitative and quantitative is not convincing.

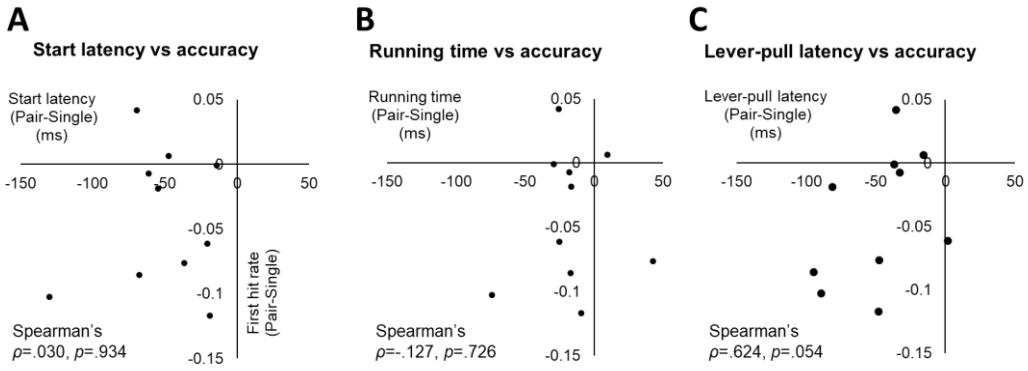
Reply:

We appreciate your comment. We changed the words “qualitative aspect” to “speed”, “quantitative aspect” to “accuracy” in revised paper, to clarify what we can investigate using this protocol. Along with this, some words are changed to appropriate words.

The reason that we used the “qualitative aspect” “quantitative aspect” in the original manuscript is below. We have recognized that accuracy is a qualitative aspect of motor performance, according to argument by Strauss (2002) which was based on the conversation in Bond and Titus (1987): “Important moderators are not only task complexity (‘simple’ vs ‘complex’) but also the type of performance. The latter can be differentiated according to whether a quantitative performance e.g., measured in terms of speed, latencies, response rates) or a qualitative performance e.g., measured in terms of accuracy, errors, number of attempts) is required. [sic] (p.244 in Strauss (2002))“.

However, we now consider that “speed” and “accuracy” is more appropriate in revised paper. So the changes were made.

As you have pointed out, performance speed and performance accuracy are not independent to each other. We did an additional correlation analysis in our previous study. At first, to extract changes between conditions, we calculated the difference between single and pair phases for each index of each rat, namely, start latency, running time, lever-pull latency and first hit rate. And then, the Spearman's rank correlation coefficients were calculated. The rank correlation analysis revealed a moderate positive correlation between the difference of lever-pull latency vs the difference of first hit rate (Fig. C, i.e., a rat performed the task faster will perform the task less accurately), although it was not statistically significant. The order correlation coefficient between differences of start latency vs first hit rate (Fig. A, ρ=-.030, p=.934), running time and first hit rate (Fig. B, ρ=-.127, p=.726) were very small and not



significant.

*This figure has been cited from the original study (Sekiguchi & Hata, 2019).

Minor Concerns:

Fig. 7: Why error bars are shown for both sides of the mean of the pair bars, but only for the mean + SEM of the single bar?

Reply:

Thank you for the comment. We corrected the figure 7 to show error bars both sides of the single bar.

Finally, it is much desired to get a hind on how this model could be implementation in future studies.

Having said all that - I wonder if a mirror would exert the same effect of the partner rat. Or - would an invisible partner (behind an opaque door) exert the same effect.

Reply:

We appreciate for your suggestion. We added a discussion about application of this protocol to the study about the effect of invisible partner [L.405-406].

Reviewer #2:

Manuscript Summary:

The article by Sekiguchi and Hata provides a description of a task that is useful for assessing the social psychology phenomenon known as "mere presence effect" in rats, showing mixed effects of the presence of a partner on speed and accuracy of instrumental responding.

Major Concerns:

In the abstract (and elsewhere in the manuscript), the authors suggest that accuracy is a qualitative aspect of performance. I must disagree; I think that this index is, in fact, a quantitative aspect of performance; it is nothing more than a percentage of trials in which the rat reaches a criterion (i.e., first-hit). The word "qualitative" could convey the wrong idea that the measure implies a 'how' element of lever pressing response, which is not the case. It would be better to remove the distinction between qualitative and quantitative measures throughout the article and stress in the distinction between speed and accuracy.

Reply:

We appreciate your advice. We changed the words “qualitative aspect” to “speed”, “quantitative aspect” to “accuracy” in this paper. Along with this, some words are changed to appropriate words.

The reason that we used the “qualitative aspect” “quantitative aspect” in the original manuscript is below. We have recognized that accuracy is a qualitative aspect of motor performance, according to argument by Strauss (2002) which was based on the conversation in Bond and Titus (1987): “Important moderators are not only task complexity (‘simple’ vs ‘complex’) but also the type of performance. The latter can be differentiated according to whether a quantitative performance e.g., measured in terms of speed, latencies, response rates) or a qualitative performance e.g., measured in terms of accuracy, errors, number of attempts) is required. [sic] (p.244 in Strauss (2002))“.

However, we now consider that “speed” and “accuracy” is more appropriate in revised paper. So the changes were made.

Minor Concerns:

The title would be more accurate if the authors remove the word "Experimental". While the proposed protocol could be used in experimental designs, it also might be used in different research designs in which there are no manipulated variables, such as comparisons between sexes, developmental stages, or metabolic rates. I believe that a more appropriate title could be "A task for assessing the impact of a partner on the speed and accuracy of motor performance in rats".

Reply:

We appreciate your suggestive comments and completely agree with your idea. We corrected the title as suggested.

Page 1, line 64: "They authors" should be "The authors".
Page 1, line 72: "inescapably contain" should be "inevitably imply".
Page 1, line 81: "on the performance accuracy" should be "on performance accuracy".
Page 2, line 129: "habituation for the apparatus" should be "habituation to the apparatus".
Page 2, line 131: "place each rat on the runway" should be "place each rat in the runway".
Page 3, line 136: "As the first habituation" should be "At the first habituation session".
Page 3, line 138: "As the second habituation" should be "At the second habituation session".
Page 3, line 143: "the habituation for the apparatus" should be "the habituation to the apparatus".
Page 3, line 159: "successive approximation method" citation needed.
Page 3, line 169: "Finish daily session" should be "Finish daily sessions".
Page 3, line 169: "30 minutes has passed" should be "30 minutes have elapsed".
Page 3, line 170: "completes criterion E for 40 times" should be "completes criterion E 40 times".
Page 3, line 170: "the lever-pull shaping is" should be "the lever-pull shaping phase is".
Page 4, line 180: "give a reward pellet" should be "deliver a food pellet".
Page 4, line 186: "finish the daily session" should be "finish daily sessions".
Page 5, line 249: "Results of the ANOVA revealed" should be "The ANOVA revealed".
Page 6, line 294: "pellet emission" should be "pellet delivery".
Page 6, line 301: "prohibits" should be "precludes".
Page 7, line 322: "The experimental task" should be "This task".
Page 7, line 329: "The experimental protocol" should be "The protocol presented here".

Reply:

We sincerely appreciate for your detailed corrections. We reflected your corrections, except the correction "Page 3, line 159: "successive approximation method" citation needed." Because we thought the term "successive approximation method" is not a common word, we deleted the "successive approximation method" and rewrote the details as below:

"3.4.2 ...Then train the rats to pull down the lever. Shape the lever-pull behavior gradually with following five reinforcement criteria (A-E)." [L.176]

Reviewer #3:

Manuscript Summary:

This manuscript describes a protocol designed to investigate the effect of the presence of a confederate animal on speed and accuracy performance in the rat. The manuscript generally describes the protocol clearly, and the implications of the protocol are clear.

Major Concerns:

There are several areas of the protocol that could be described with more detail to make application of the protocol easier for readers:

1. The description of habituation is unclear (3.2.1 and 3.2.2). Are confederate and subject rats habituated together? Why do confederate rats have two days of habituation, but subject rats only have 1? If rats are habituated together, why? If not, why not? What is the justification for this procedure?

Reply:

We appreciate for your helpful comments.

>Why do confederate rats have two days of habituation, but subject rats only have 1?

Because confederate rats have to wait the completion of shaping and training phases of subject rats, we conduct second habituation session for reminding confederates of the apparatus.

> Are confederate and subject rats habituated together?

The habituation process is to habituate rats to the apparatus, not to (the presence of) conspecifics, so we did not habituate them together.

We added the NOTE below to “3.2 Habituation to the apparatus” section [L.153-154].

“NOTE: Confederate rats and subject rats are habituated separately, i.e., one rat at a time. Do not let a rat meet another rat in the apparatus until the test phase starts.”

2. Although the figure is helpful in understanding how many days are required to complete the experiment, more detail in the methods would be useful to understand how to conduct daily trials more effectively. For example, in 3.4, how long does this take? If it requires a total of about 3 days to get through 5 shaping approximations, what does an individual daily trial look like, and how long is each daily trial per rat? In 3.5, how long is required for each animal's daily trial?

Reply:

We appreciate your helpful comment. We added the descriptions below:

“Almost all rats finish the daily shaping session within 20 minutes.” as NOTE of 3.4 Shaping for the lever-pull action [L.190].

“A daily session for each rat requires about 15 minutes” as NOTE of 3.5 the training for the lever-pull action [L.209-210].
“A daily session for each rat requires about 20 minutes.” as NOTE of 3.6 training phase for the run-and-pull sequence and test phase [L.222].

3. It is unclear in the methods if the study should be within subjects or between subjects. It is more clear in the discussion, but it should be discussed in the methods. What about practice effects impacting results? This is discussed briefly in the discussion, but it deserves more attention as it seems a major limitation to the current study.

Reply:

We appreciate your suggestive comment. We considered the design of the study, within or between subjects, should be discussed in this paper. We added a paragraph based on this argument into Discussion section [L.390-398].

We consider that this protocol can be used in the between-subjects design. However, the answer to the question “Which design is appropriate?” would be on a case by case basis. As you know, the study using between-subject design can exclude the practice effect which might be observed in this study. However, between-subject design needs more animals and more time for one study (i.e., if using the protocol in this paper, 7-8 hours per day will be needed if test all rats in one day using one apparatus). The study using within-subject design can reduce the animals and time for one study, but the experimenter has to control the effect of practice.

4. The results do not look overly convincing. Though differences might be significant, they do not look meaningful? What is the reproducibility of this protocol? How reliable are these results - if the effect size is small, is it at least consistent?

Reply:

Thank you for your comment. We discussed about effect sizes in “Discussion” section in revised manuscript [L.366-370]. We do not consider the effect size of each independent variable as small, according to the criterion presented by Cohen (1988). In the original manuscript, we reported the effect sizes as below.

For the first hit rate, the main effects of the condition ($F(1,9)=6.25$, $p=0.034$, $\eta^2_G=0.114$) and phases ($F(1,9)=14.1$, $p=0.005$, $\eta^2_G=0.147$) were significant. For the start latency, only the main effect of the condition was significant ($F(1,9)=23.1$, $p=0.001$, $\eta^2_G=0.065$) As with the start latency, for the lever-pull latency, ANOVA showed that the main effect of the condition was significant ($F(1,9)=23.3$, $p=0.001$, $\eta^2_G=0.183$).

In case, we recalculated η^2 (there are not much of differences between η^2 and η^2_G

in this study), and these effect sizes are regarded as medium (η^2>.06) or large (η^2>.14). For this reason, we considered the differences seen in this study is meaningful and reliable enough.

Minor Concerns:

The highlighting in the methods seems excessive and is not focused on only the critical steps.

Reply:

Thank you for your advice. We corrected the highlighting appropriately after this revision.

Reviewer #4:

The authors describe a useful protocol for examination of social-facilitation effects in rats. I have only a few minor comments/queries that could be worthwhile to address:

-Sample size planning. Is N = 10 subject rats a recommendation and if yes, on what basis? A power analysis (based on effect size estimates derived from the studies of T&U/O&M) could be helpful. It is likely that more subject rats will be needed for reasonable statistical power (P>=.80).

Reply:

Thank you for your comment. We did not do sample size planning before this experiments start, because studies of Takano and Ukezono, Ogura and Matsuhima did not report the effect size. However, a post hoc power analysis for all dependent variables showed that N=10 subjects are sufficient to ensure the reasonable statistical power (all (1-β)>0.9).

-I did not exactly understand the assignment of the 5 confederate rats to 10 subject rats. Why not use 10/10? Or a single confederate rat for all 10 subjects?

Reply:

This assignment of confederate is to reduce the number of animals and to reduce the load of animals. This experiment needs 4 hours per day, if the experimenter tests all subject rats in one day. We think it would be appropriate to avoid such a long constraint for animal(s) as possible.

We added the sentence below to “1.Animals” section according to this conversation [L.95-96].

“This assignment of confederate is to reduce the number of animals and to reduce the load of animals.”

-Data analysis: Any outlier analyses/truncations planned for RTs/MTs?

Reply:

Thank you for your comment. Since we used only RTs/MTs of first-hit trials in analysis, there were few outliers first. We used median values of RTs/MTs to minimize the effect of outliers.

Changes to be made by the author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Response:

We rewrite some minor details and after that we requested native speakers of English to proofread. We added a certificate of English proofreading as a supplemental file

2. Please revise lines 302-304, 309-313, and 317-325 to avoid textual overlap with previously published work.

Response:

We rewrite the text as possible. However, we do not delete or rewrite statistical results (e.g., F-values), for it is indispensable.

3. Please revise the Protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “NOTE.”

Response:

We checked our manuscript and revised the text, mainly the “apparatus” section.

4. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. The highlighted text must include at least one action that is written in the imperative voice per step. Notes cannot usually be filmed and should be excluded from the highlighting.

5. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

Response:

We checked and corrected the highlighting.

6. Table of Materials: Please ensure that it has information on all relevant supplies, reagents, equipment and software used, especially those mentioned in the Protocol. Please sort the materials alphabetically by material name.

Response:

We added two components (Pellets Dispenser with Feeder (Rats), Power DVD 14) to “Table of Materials”.

7. Please address specific comments marked in the attached manuscript.

Response:

Thank you for your detailed comments. We revised the manuscript according to comments, and we replied to the comment in the revised manuscript.

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AUTHORS

Yayoi Sekiguchi & Toshimichi Hata

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