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Correspondence and requests for materials should be addressed to Y.T. (takano.yuji@lab.ntt.co.jp)

An experimental task to examine the mirror system in rats

Yuji Takano[1,2] & Masatoshi Ukezono[1,2,3]

[1]NTT Communication Science Laboratories, Nippon Telegraph and Telephone Corporation, [2]CREST, Japan Science and Technology Agency, [3]Department of Psychology, Meiji Gakuin University.

The mirror system in the brain is considered to be a neural basis of sociality, but previous studies have been limited to primates. Here we report an experimental task to examine the mirror system in rats. We show that a rat could reach to a pellet and grasp and eat it in front of another rat that was observing the reaching, which indicates that the task will enable us to start exploring the rat mirror system.

The mirror system in the brain is considered to be a neural basis of sociality[1]. However, all of the data collected so far is for monkeys and humans. No research on the mirror system has been conducted in other experimental animals because of a lack of an experimental task for doing so. Here we report the development of a new behavioral task to examine the mirror system in rats.

The discovery of mirror neurons in the ventral premotor cortex and inferior parietal lobe was based on neural recordings of a monkey reaching to a food reward and grasping and eating the food while an observing human experimenter reached to it in the same way. The recordings showed that some neurons were firing with the same pattern during the execution and observation, which the researchers called mirror neurons[2]. Given the situation in previous studies, in developing an experimental task for examining the mirror system in rodents, we had to determine whether rodents can reach to a piece of food and grasp and eat it in front of another rat, whether a rat actually observes the other rat's reaching and whether the social meaning of reaching to food in front of another is similar between rodents and humans.

Reaching behavior in rats has been examined in previous studies to devise models for rehabilitation after stroke[3,4]. Moreover, a previous study has shown kinetic similarities between reaching behaviors in rats, monkeys, and humans[5]. Therefore, using the reaching task in rats is not an issue.

When in the company of others, humans change their behavior. For example, our performance of a task in front of other people sometimes becomes better than when we do the task alone. This effect has been called social facilitation[6]. Social facilitation is observed across species – in insects[7], birds[8], rodents[9], and primates[10]. In this regards, we can check the social meaning of reaching to a food reward and grasping and eating it in front of others by comparing social facilitation effects between rats and humans.

On this account, we modified an apparatus used in a previous study of rat reaching[3] by adding an observational room opposite the reaching room. For ease in creating a "face to face" situation, we prepared a slit in the observational room for nose-poking. The slit in the observational room is too narrow for a rat to reach through it. We conducted two experiments. In the first one (exp. 1), we taught reaching behavior in front of another rat and examined whether rats paid attention to another's reaching behavior. In the second one (exp. 2), we compared reaching behaviors in an individual condition or in a pair condition using rats and human participants. Then, we compared the social facilitation effects in rats and humans using a similar social situation. In humans, we asked participants to reach and grasp and eat a piece of chocolate in front of another person who was observing or without the observer.

Results

In exp.1, we trained rats to reach for a pellet and grasp and eat it in front of the cage mate (pair condition) that was observing its reaching. One day of training consisted of 30 trials. After the rats had learned to reach for a pellet and grasp and eat it, we trained the rats to spin 360 degrees before reaching in order to reduce wasteful motions. We trained rats until they achieved an averaged success rate of 60%.

The rats learned the reaching to a pellet in front of their cage-mate (Fig. 1a, b), and then we conducted simple regression analysis. The results of simple regression analysis (Fig. 1b) showed that the success rate was positively related to the number of training sessions (i.e., days of training) ($R^2 = .756, F(1, 6) = 8.011, p < .05$). These results suggest that the rate of success increases with training.

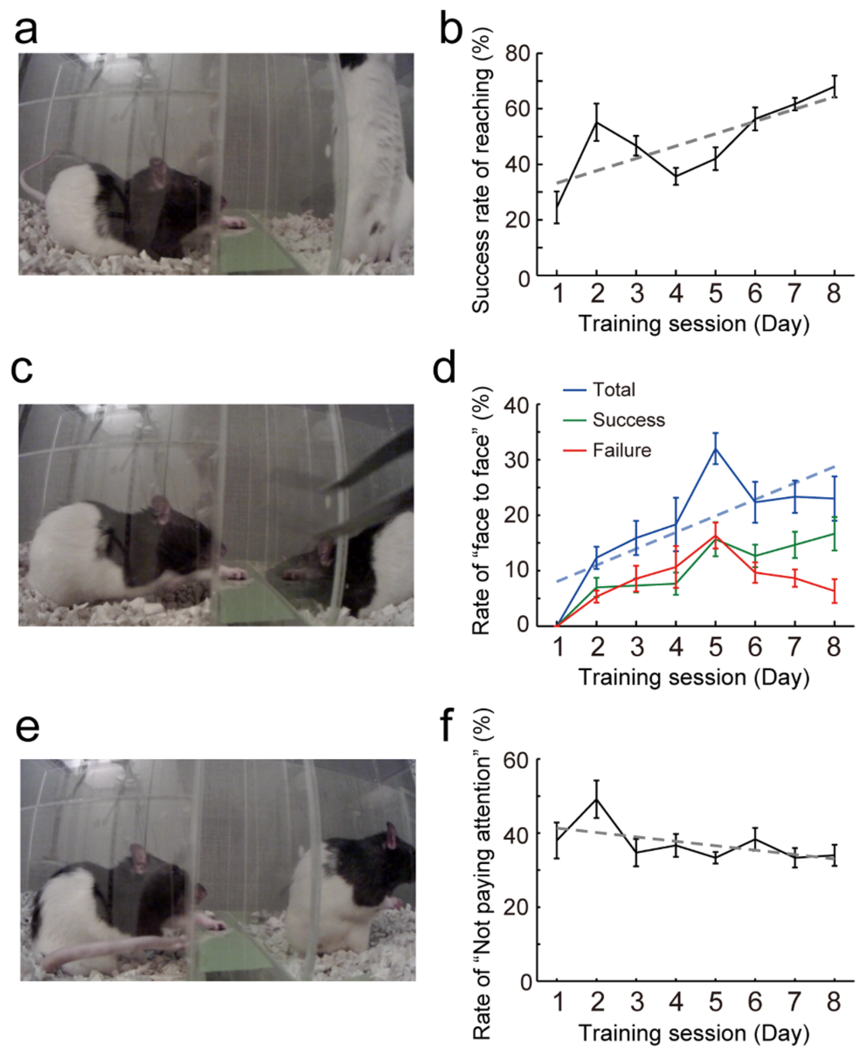

Figure 1 | Training for reaching, grasping, and eating a pellet in front of a cage mate. (a) Training scene. (b) Learning curve for reaching. Dashed line represents results of linear regression. (c) "Face to face" condition. (d) Occurrence rate for "face to face" condition (blue line). Blue dashed line represents results of linear regression. Green line represents occurrence rate of success-trials. Red line represents occurrence rate of failure-trials. (e) "Not paying attention" condition. (f) Occurrence rate for "not paying attention" condition. Dashed line represents results of linear regression. Error bars in all graphs indicate standard error.

The rats were "face to face" sometimes in the training (Fig. 1c, d and Movie S1), and then we conducted simple regression analysis. Simple regression analysis (Fig. 1d) showed that the success rate was positively related to the number of training sessions ($R^2 = .767$, F (1, 6) = 8.559, $p < .05$). These results suggest that the appearance rate of the "face to face" situation increases as the training continues.

The cage-mate was sometimes in the "not paying attention" condition during training (Fig. 1e), and the rate for this condition did not change during training (Fig. 1f). To confirm that the rate did not change, we conducted simple regression analysis. The results showed that the rate of "not paying attention" was not related to the number of training sessions ($R^2 = .556$, F (1, 6) = 0.152, *n.s.*).

In exp. 2 for rats, we tested the individual condition. After testing the first individual condition for two days, we retested the pair condition for two days. Moreover, we added the individual condition one more time to rule out the possibility of these conditions having turn effects. We found that the averaged speed of spinning before reaching in the pair condition was higher than that in the individual condition (t(9) = 6.47, $p < .001$) (Fig. 2c and Movie S2). Furthermore, we compared the speed time when the rat was in the "face to face" situation with the speed time when it was in the "not paying attention" situation. In result, this comparison was not significant (t(9) = 0.85, n.s.) (Fig. S1).

In exp. 2 for humans, participants were instructed to reach to a small piece of chocolate and grasp and eat it in front of another person (called a pair session) or alone (called an individual session). We counterbalanced for the flow of individual and pair sessions. Before reaching, the participants were requested to show gratitude for the food (In Japan, this custom is popular). We found that the gratitude in the pair condition was expressed faster than in the individual condition (t(11) = 5.00, $p < .001$).(Fig. 3b and Movie S2).

Discussion

We successfully made rats reach to a pellet and grasp and eat it in front of another rat that was observing the reaching rat. In addition, the social meaning of the experimental situation in rats would be similar to that in humans because the social facilitation appeared in rats and humans. These results indicate our developed experimental task could lead to a new field in neuroscience – the exploration of the mirror system in rats.

An advantage of the task is a short training period and test period. Training a rat until sufficient learning has occurred would take about ten days (We trained rats to reach or observe every other day). After that, approximately 30 trials of neural recording during execution and observation takes about ten days (Estimated under the assumption

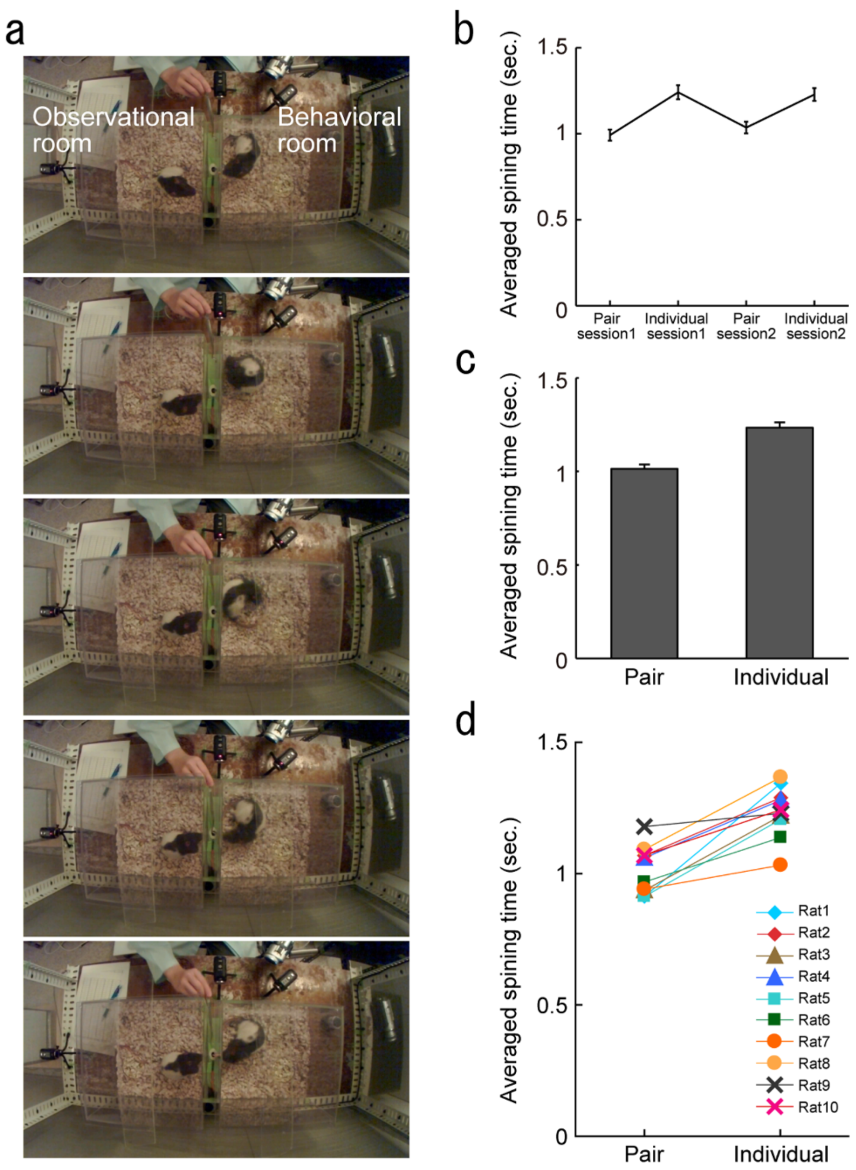

Figure 2 | Effects of other's presence on rat behavior. (a) Example of spin behavior before reaching. Spin behaviors were needed to control behavioral state before reaching. (b) Spinning time in pair and individual sessions. Data for pair session 1 were average time for sessions 7 and 8. Data for individual session 1 were average time for sessions 9 and 10. Data for pair session 2 were average time for sessions 11 and 12. Data for individual session were average time for sessions 13 and 14. (c) Average spinning times for pair and individual sessions. Average time for pair sessions was significantly less ($t(9) = 6.47$, $p < .001$). (d) Individual spinning times. Error bars in (b) and (c) represent standard error.

that the rate of "face to face" situations is 20%, with about six "face to face" situations per day). We believe the task is available for studies in neuroscience. In particular, single or multiple neuron recordings and local field or ECoG recordings would be effective methods. Some human EEG studies have shown that mu waves appear during a grasping action and are suppressed when the grasping action is observed. However, autistic people do not exhibit suppressed mu waves when they are watching it[11]. Because the suppression of mu waves would be related to the mirror system, the recording of mu waves in rat brain would be a potent approach. One more advantage of the task is ease of modification. In the present work, we completely separated the observational room from the behavioral room. One could change the accessibility to a pellet and thereby make the situation more competitive. That is, researchers can modify the task in accordance with the intended use.

In summary, our experimental task is well suited to examining the rat mirror system. We will now use it to examine whether there is actually a mirror system in the rat brain and, if there is one, to examine how the system functions in their social context. Note that the common ground of sociality based on social facilitation does not necessarily mean that there is a rat mirror system. Our first task is to conduct a neural recording study using the present experimental setup and task.

Methods

Experiment 1: Training period of behaviors. *Animals.* All animal procedures were performed in accordance with the guideline for animal research of NTT Communication Science Laboratories based on Helsinki Declaration. The experimental protocols were approved by the ethical review board of NTT Communication Science Laboratories (No.H22-010). Ten Male Long-Evans rats (SLC Inc.) with mean weight of 293 (SD: 9.27) at the time of experiment were housed double in a temperature-controlled (about 23°C) animal room under a 12-h light/dark cycle (light from 8:00 to 20:00). Before the experiments, the rats had free access to laboratory chow (CE-2, CREA Japan Inc.) and tap water in their home cages. Approximately 18 g of food per day was given to each rat after the day's experiment. Tap water was freely available in their home cages at all times.

Apparatus. The apparatus consisted a behavioral room and an observational room. Both compartments were 27(W) × 27(D) × 27(H) cm and were made of transparent acrylic. Between these two sides was a feeding table (3.5(W) × 27(D) × 5(H) cm). In

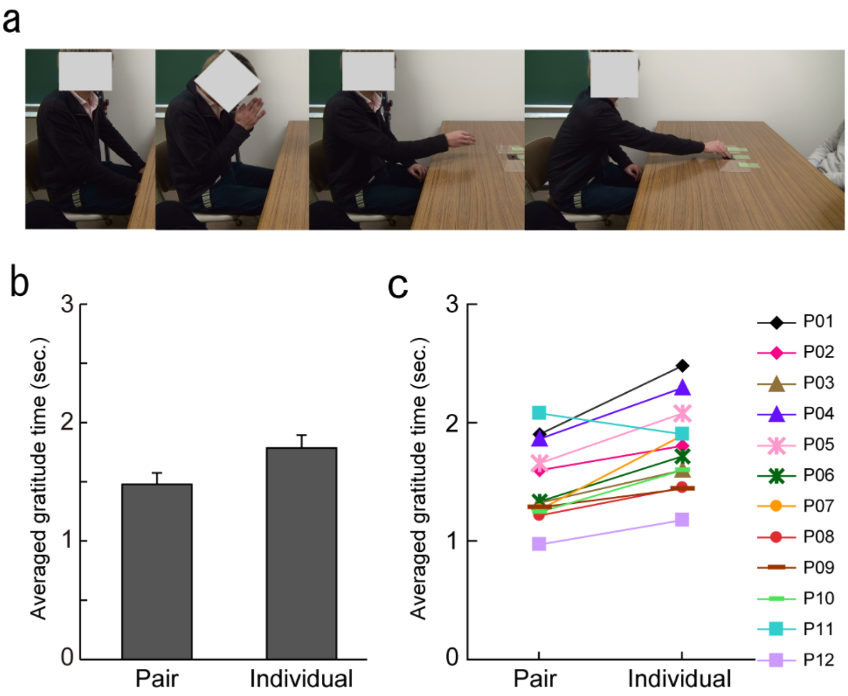

Figure 3 | Effects of other's presence on human behavior. (a) Example of expressing gratitude before reaching. Japanese people generally bow and say "いただきます(itadakimasu)" before eating. (b) Average time for showing gratitude in pair and individual sessions. Average time for pair sessions was significantly less ($t(11) = 5.00$, $p < .001$). (c) Individual gratitude times. Error bars in (b) represent standard error.

the behavioral room, a slit (1 cm) for reach to a pellet and grasp it, was prepared in the side of a feeding table. In the observational room, a slit (1 mm) for nose poking was prepared in the side of a feeding table. There was a set place for a pellet in front of the slits. The set place for a pellet was midway between the slits. We arranged four video cameras around the apparatus (Fig. S3 and Movie S1) (GC-XA1-B, JVC, Victor inc.) and recorded the animal behaviors (60 fps).

Procedure. Before the first training, we fed the rat's reward pellets (about 50 mg of Meiji Milk Chocolate). With respect to one pair in a cage, we assigned one rat to the behavioral room and the other to the observational room. We alternately trained them to reach or observe every other day. The number of reaches in a session was 30. We trained the rats to reach to a pellet and grasp with using their forepaws in the first and second sessions. In the second, third or fourth sessions, we did not put a pellet on the set-point when a rat was sitting in front of it, and put it in turning their back to the set-point. In the seventh and eighth sessions, all rats could reach to a pellet and grasp it after spinning, and the average rate of successful grasps was over 60%. That is, the rats could reach for, grasp, and eat a pellet in a sequence of actions. Failure occurred when a rat did not grasp the pellet correctly and dropped it. The inter-trial interval depended on the individual rat behaviors. As a reference for the individual differences in training time, the averaged daily session time is shown in Fig. S2.

We sorted the reaching situations into three different categories. The first was "face to face" based on the two heads being in a straight-line by checking 4 cameras (Movie S1). In the overview, we judged whether a "ruler" covering the width of the observing rat's head also covered the reaching rat's head. In the other three images, we judged whether the two rats were actually face-to-face. (Fig. S3). We judged the situation to be face-to-face only when the observer was right in front of the slit. Therefore, we assumed that the observer watched the actions of the other rat in "face to face" situations. The second was "ambiguous face to face", defined as the two heads were close to each other but not in a straight-line: the observer was in front of the slit but was standing or had its head down or the observer was facing the demonstrator but was not in front of the slit. We call these situations "ambiguous" because we could not assume the observation with certainty. The third was "not paying attention", in which the observer was looking away at over 90 degrees from the partner that was reaching to a reward independent of position, for example, when the observer was being in front of the slit or stepping away from it. We also assumed that the observer did not watch the action of the partner.

Analyze. We conducted simple regression analysis to examine the trend of the success rate of reaching, the appearance rate of "face to face" situations, and the appearance rate of "not paying attention" situations.

Experiment 2: Test period of social facilitation. *Rat study.* ***Animals and apparatus.*** Same as described above in Exp.1.

Procedure. After the session 8 in exp.1, we tested the rats in an individual-session for two days, and then tested them in a pair-session for two days. After that, we retested them in an individual session for two days again.

Analyze. We measured the spin time before reaching. In the rat experiment, the starting point was the first frame in the video (60 fps) in which the rat started spinning after sitting in front of the slit, and the ending point was the frame before the one in which the rat's front paws left the ground. We measured the time with a stopwatch and then we compared the average spin times for the pair sessions with those for the individual session using a paired t-test.

Human Study. ***Participants.*** Twelve normal healthy college students (six males; six females) participated in the experiment. They were told about the ethical considerations before entering the study, and each gave written informed consent. The experimental protocols were approved by the Meiji Gakuin University Ethical Board for experiments in psychology.

Apparatus. We put a feeding table on a desk. The feeding table was made of black acrylic [6 cm(W) × 6 cm(D)]. There was a set place for a pellet (Marble Chocolate, Meiji Inc.) in the center of the feeding table. We prepared a slit to guide the reaching for a pellet in front of the set-point on the feeding table. The slit was made of transparent acrylic and was the shape of a pair of bookends. The participants sit on a chair in front of the desk. The feeding table was put at the position that made the participants stretch out their arm. The slit was the size of the hand plus 1 cm. We recorded the behaviors with video camera (30 fps) (Handycam, Sony).

Procedure. The participants were requested to reach to a pellet and grasp and eat it on the feeding table. Before reaching, they were requested to show gratitude for the food by bowing and saying "いただきます(itadakimasu)". After five practices, they performed the tasks alone (single session; 15 times) and in front of an observer (pair session; 15 times). The order of single sessions and pair sessions was decided by the counterbalancing. The observers were cooperators in the experiment, who first met with the participants and were persons of the same sex as the participants. The observers did not talk during the experiment. We measured how long it took to express the gratitude. The gratitude time was defined as a time joining their hands after raising their hands from their knees.

Analyze. In human experiment, we measured the gratitude time before reaching. In the human experiment, the starting point was the frame (30 fps) in the video before the frame in which the hands started leaving the knees, and the ending point was the frame before the frame in which the hands were separated. We counted the times by a stop timer, and then we compared the averaged gratitude time in pair session with the time in individual session by paired t-test.

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Author contributions

Y.T. and M.U. designed the study, developed the apparatus, and conducted preliminary experiment. M.U. performed the research. Y.T. and M.U. analyzed the data. Y.T. wrote the paper.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

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