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Mirror Perception in Mice: Preference For and Stress Reduction by Mirrors

Shigeru Watanabe

Keio University, Tokyo, Japan

The amount of time mice spent in a compartment with either a mirror or an opaque screen was measured and mice stayed longer in the compartment with the mirror. This finding suggests that mice prefer mirrors. They also showed a preference for the mirror over unfamiliar live mice but did not show a differential preference for the mirror over a familiar live mouse (cage mate). Restraint stress caused hyperthermia (known as stress-induced hyperthermia) in the mice. When cage mates received the restraint stress together, the hyperthermia was reduced. Placement of mirrors instead of the cage mates also showed stress-reducing effects, while restraint with unfamiliar mice did not reduce the hyperthermia. These results suggest that mirrors have familiar cage mate-like social effects in mice.

Visual stimulation via a mirror image has been examined in a variety of species. Monkeys (*Macacca mulata,* Gallup & Suarez, 1991; *Macaca arctoides,* Straumann & Anderson, 1991, nocturnal prosimians (*Otolemir garnettii*, Becker, Watson, & Ward, 1999), cheetahs (*Acinonyx jubatus*, Wielebnowski, 1999, hermaphroditic fish (*Rivulus marmoratus,* Earley, Hsu, & Wolf, 2000), and lizards (*Anolis carolinesis,* Korzan, Summers, Ronan, & Summers, 2000) display social behavior in front of a mirror. Jungle crows (*Corvus macrorhynchos*) attack a mirror image (Kusayama & Watanabe, 2000); flamingos (*Phoeniconais minor)* perform a marching display in front of a large mirror (Pickering & Duverge, 1992). African parrots (*Psittacus erithacus*) attempt to beak wrestle a mirror image (Pepperberg, Garcia, Jackson, & Marconi, 1995), and pigeons (*Columbidae* sp) exhibit a schedule-induced attack towards a mirror image (Cohen & Looney, 1973). Finches (*Passer demesticus domesticus*) and parakeets (*Melopsittacus unduluatus*) prefer a mirror image to a live animal (Gallup & Capper, 19, and Siamese fighting fish (*Betta splendens*) fight a mirror image (Thompson, 1963). These results indicate that animals perceive self-mirror images as conspecific and suggest the possible use of mirrors as a tool for social enrichment (for example, McAfee, Mills, & Cooper, 2002).

Mice (*Mus* sp) display slight mirror aversion (Fuss et al., 2013; Sherwin, 2004), and a chamber constructed of mirrored glass has been used to test the effects of anti-anxiety drugs in mice (Kliethermes, Finn, & Vrabbe, 2003; Lamberty, 1998; Toubas, Abla, Cao, Logan, & Seale, 1990). These results indicate that mice are exceptional animals showing mirror aversion.

The purpose of the present experiment was to examine the effect of mirror exposure in mice in two different situations, preference and stress reduction/enhancement. In both situations, mirror exposure was compared with exposure to an unfamiliar mouse and a familiar mouse (cage mate). In Experiment 1, mirror preference in choice tests between a mirror and an opaque screen, between a mirror and an unfamiliar mouse, and between a mirror and a familiar mouse (cage mate) were examined. In Experiment 2, the effect of mirror exposure on stress-induced hyperthermia (SIH) was compared with that of unfamiliar and familiar mice.

Please send correspondence to Shigeru Watanabe, Keio University, Department of Psychology, Mita 2-15-45, Minato-ku, Tokyo, Japan. (Email: swat@flet.keio.ac.jp)

Experiment 1: Mirror Preference Test

Experimental design similar to one used for Java sparrows (Watanabe, 2002) was employed. Animals were placed in a test chamber consisting of two compartments. One chamber had a mirror and one had another non-mirror stimulus, and the time spent in each chamber was measured as an index of preference. Because mice usually have never seen their self-image in a mirror, the image could be interpreted as an “unfamiliar mouse”. Unfamiliar conspecifics could be intruders and thus possibly aversive. Mirror preference/aversion was examined under three conditions: mirror versus non-mirror (opaque screen), mirror versus an unfamiliar mouse, and mirror versus a familiar mouse (cage mate) using a conventional preference test procedure. One problem of the preference test with two choice is confusion of preference and aversion, because avoiding one alternative directly results in preference for the other alternative. Here, a three choice procedure was employed to reduce this confusion.

Method

Subjects. Forty-eight male C57/BL6 mice were used as test subjects. Another 12 mice were used in the experiment as the unfamiliar mice. Cages were placed on a shelf separated from the subject mice in an animal keeping room. Mice were obtained from the Nihon Biomaterial Company and were 8-weeks-old when acquired. Mice were housed under reversed 12/12 h light/dark conditions with the temperature maintained at 24°C. Food and water were freely available. Each cage contained a group of four mice, and each group lived together for more than two weeks before the start of the experiment. All mice were treated in accordance with the guidelines of the Japanese Society for Animal Psychology, and the protocol was approved by the Animal Care and Use Committee of Keio University (no. 08011).

Apparatus. The apparatus used for the mirror preference tests was a conventional conditioned place preference apparatus (MED ENV3015; Med Associates Inc., Fairfax, VT, USA) with three compartments: two side compartments (16 x 13 x 12 cm) and a center compartment (6 x 13 x 12 cm). The center compartment was connected to the two side compartments by guillotine doors. Each compartment had a ceiling lamp. Luminance in the compartments was 11.1 lux. A box made of a grey acrylic plate was placed in each side chamber so that they provided identical environments. The boxes had an opening connected to the guillotine door. In each box, a transparent acrylic partition (11.5 x 11.5 cm) was placed 5 cm from the end wall to create a stimulus area. For the mirror versus non-mirror test, an acrylic mirror was placed on the partition in one side compartment and a white acrylic plate was placed in the partition in the other side compartment. For the mirror versus cage mate test, the opaque partition was changed to a transparent one and a stimulus mouse (cage mate) was placed in the stimulus area. An unfamiliar mouse was placed in the stimulus area in the mirror versus unfamiliar mouse test. The MED-SKED system (Med-Con Technologies, LLC, Clinton, NJ, USA) was used to control the experiment. White noise (75 dB) was broadcast throughout the experiment.

Procedure. The subjects were divided into two groups of 24. Each group received mirror versus non-mirror test, and, in addition, group 1 received the mirror versus unfamiliar mouse test and group 2 received the mirror versus familiar mouse test.

Mirror versus non-mirror test. In the mirror versus non-mirror test, one side compartment of the apparatus contained a mirror, and the other side compartment contained a white opaque partition. The subject was placed in the central compartment, and after 5 min, the doors to the side compartments were opened and the mouse was free to move around the chamber for 15 min. Time spent in each compartment was measured by photo-sensors attached to the apparatus. The next day, the side of the mirror and the opaque partition were reversed, and the subject was tested again. Average of the two tests was used for statistical analysis.

Mirror versus unfamiliar mouse test. The procedure was identical to that of the mirror versus non-mirror test except that an unfamiliar mouse was placed in a stimulus area that was separated from the rest of the compartment by a transparent partition.

Mirror versus familiar mouse test. The procedure was identical to that of the mirror versus unfamiliar mouse test except that one cage mate was placed in the stimulus area instead of an unfamiliar mouse.

Statistical analysis. In addition to paired t-tests to analyze differences in the time spent between the two compartments, the χ^2 test was used to analyze the numbers of subjects that stayed in each compartment for a longer duration than the other compartment.

Results and Discussion

Figure 1 presents results of the preference tests. In the mirror versus non-mirror test for group 1 (Figure 1a), time spent in the compartment with the mirror was greater than in the compartment with the white opaque partition, $t(23) = 3.35$, $p = 0.003$ (two tailed paired *t*-test), indicating a preference for the mirror. Twenty-one of 24 mice stayed longer in the compartment with the mirror (χ^2 test, $p < 0.0005$) than in the compartment with the opaque partition. Group 2 also showed mirror preference in the mirror versus non-mirror test (Figure 1b). There was a statistically significant difference in the time spent between the mirror and non-mirror stimulus, $t(23) = 3.35$, $p = 0.003$ (two-tailed paired *t*-test). Seventeen of the 24 mice preferred the mirror. The χ^2 test also showed a significant difference ($p = 0.04$).

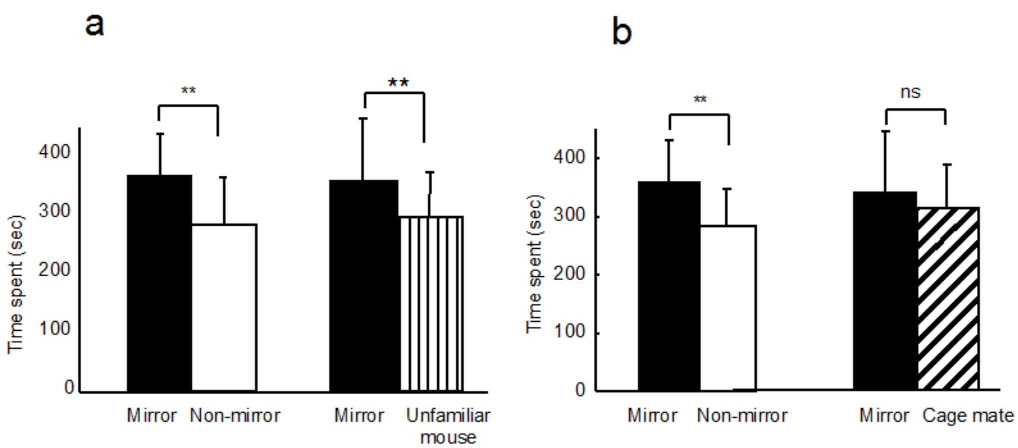

Figure 1. **Results of Experiment 1.** Group 1 received the mirror versus non-mirror and mirror versus unfamiliar mouse tests (a), and group 2 received the mirror versus non-mirror and mirror versus familiar mouse (cage mate) tests (b). **$p < .05$, error bars indicate *SD*.

The preference for the mirror over the white opaque partition was similar to that observed in Java sparrows (*Lonchura oryzivora*, Watanabe, 2002) despite the fact that mice are not visual animals. These results do not agree with those of a previous experiment by Fuss et al. (2013) that showed mice had no preference for the mirror. There are several procedural differences between the two experiments that may explain this discrepancy. In the present experiment, the mice were exposed to the mirror for the first time during the test, whereas Fuss et al. (2013) exposed the mice to the mirror for five weeks before the test. In the present experiment, the mice lived in groups of four, whereas in the experiment of Fuss et al. (2013), the mice lived alone. Furthermore, in the present experiment, the preference was measured twice with each measurement lasting 15 min, whereas in the experiment of Fuss et al. (2013), the preference was measured once for 5 min. The results of the present study also contrast those of Sherwin (2004) who studied C57BL/6J mice who had been living with siblings and observed a slight aversion to the chamber with a mirror (47.6% vs. 52.4%). Procedural differences may also account for this discrepancy. Sherwin (2004) studied 45-week-old mice in contrast to the 8-week-old mice in the present study, and Sherwin (2004) measured preference every 10 min for 24 h. In addition, the illumination used in the present experiment was rather dark (11.0 lux). The luminance of the apparatus used by Fuss et al. (2013) and Sherwin (2004) is not known, but mice are averse to bright light and may have masked slight differences in mirror preference in these studies.

The mirror versus unfamiliar test (Figure 1a) demonstrated that mice preferred the mirror to the unfamiliar mouse, $t(23) = 2.22$, $p = 0.04$. Fifteen of 24 mice stayed in the compartment with the mirror longer than in the compartment with the opaque partition (χ^2 test, $p = 0.22$). Thus, there was a discrepancy between parametric and non-parametric analyses. Since unfamiliar conspecifics have an aversive property, the results may indicate an aversion to unfamiliar mice rather than mirror preference.

In the mirror versus familiar mouse test (Figure 1b), time spent in the compartment with the mirror was similar to in the compartment with the familiar mouse, $t(23) = 0.84$ (two-tailed paired *t*-test), indicating no preference for the mirror or the live cage mate. Thirteen of the 24 mice showed a preference for the mirror, and 15 showed a preference for the live cage mate (χ^2 test, $p = 0.68$).

These results contrast the findings in Java sparrows, which displayed a strong preference for live familiar conspecifics over a mirror (Watanabe, 2002). The mirror reflection provides only visual information, whereas live animals provide multi-sensory information. Thus, the live animal is a richer stimulus than the mirror. Mirror preference over live mice suggests unfamiliar conspecific aversion rather than mirror preference. Familiar mice are not aversive, and the subjects did not show a selective preference for the mirror over familiar mice.

Experiment 1 showed 1) mirror preference in mice, 2) mirror preference over unfamiliar conspecifics, and 3) mirror preference is approximately equal to the preference for familiar mice. Finally, “preference” here is relative preference, thus, relative aversion might affect the relative preference.

Experiment 2: Effects of Mirror Exposure on SIH

The results of Experiment 1 suggest that a mirror has social stimulus-like effects in mice. Stress causes several autonomic responses, including changes in heart rate, blood pressure, and respiration rate. Stress also raises body temperature, a phenomenon known as SIH (see Bouwknecht, Oliver, & Paylor, 2007 for review). The author previously reported social effects on SIH in mice using a thermograph to measure body surface temperature (Watanabe, 2015). The infrared thermograph is a suitable tool to measure body temperature in such a social experiment because it is completely non-invasive and allows the assessment of several individuals simultaneously. Mice restrained in acrylic holders for blood sampling showed SIH. Mice restrained at the same time as similarly restrained cage mates (familiar mice to the subjects) showed a reduction in SIH. Thus, the presence of conspecifics under similar stress conditions reduced stress. These results agree with a previous study measuring levels of corticosterone after restraint stress (Watanabe, 2011).

If a mirror has effects similar to images of conspecifics, mirror exposure should reduce SIH. If mice perceive self-mirror images as unfamiliar conspecifics, the effect of mirror exposure should be similar to exposure to unfamiliar mice. The SIH in four conditions, namely single restraint, restraint with similarly restrained cage mates, restraint with mirrors, and restraint with similarly restrained unfamiliar mice were compared.

Method

Subjects. Thirty-two male C57BL/6J mice were used. All mice were 8-weeks-old when the experiments were started. The mice were housed four per cage (30 x 20 x 13 cm) with food and water freely available for more than 2 weeks at the start of the experiments. The temperature was kept at 24°C, and the light-dark cycle was reversed (12/12 h light/dark). All animals were treated in accordance with the guidelines of the Japanese Society of Animal Psychology, and the protocol was approved by the Animal Care and Use Committee of Keio University (no. 08011).

Apparatus. A mouse holder for blood sampling (ASONE, To-12B-508-02) was used to restrain each animal. The cylindrical holder (diameter, 30 mm; length, 100 mm) was made of transparent acrylic and was small enough so that a mouse could not turn around in it. The upper part of the holder contained a slit (5 x 50 mm) through which the body surface temperature was measured. The bottom part had a slit (5 x 100 mm), and the rostral part had a vertical slit (5 x 30 mm). A cubicle consisting of four acrylic mirrors (14 x 10 cm) was used for mirror presentation.

The relationship between infrared thermal radiation energy and the surface temperature of an object is expressed by the Stephen-Boltzmann formula using an ideal object (also known as a blackbody). The radiation ratio of the blackbody is 1.0. As a mouse is not a blackbody, the radiation ratio of the body surface of the mouse was estimated by matching the temperature obtained by an infrared thermometer to that obtained by a contact thermometer. It was estimated the radiation ratio of the body surface of the mice to be 0.98 using nine mice. The thermal image of each animal was recorded using an infrared thermal imaging camera (G100, NEC Avio, San Fernando, CA, USA). The distance between the camera and subjects was 60 cm. Body surface temperature was obtained from the thermal images using InfRecAnalyzer NS9500 Lite software (NS9500LT; NEC Avio). The highest temperature in a particular area was obtained by drawing a rectangle around the slit of the holder on the thermal image. In free-moving animals, approximately the same area on the back was used for measurement. The room temperature was maintained at 23 ± 2°C with 60-75% humidity.

Procedure. All animals were handled for approximately five min per day for seven days before the start of the experiment. Then the animals were divided into two groups of 16, the mirror and unfamiliar conspecific groups. The animals in each group were treated under the two test conditions (single and cage mate conditions) in a quasi-random order. In addition to the two tests, animals in the mirror group received a test with the mirror, and those in the unfamiliar conspecific group received a test with unfamiliar mice.

In the single stress condition, mice were placed in single-animal cages (20 x 15 x 13 cm) and their body temperatures were measured. These body temperatures were used as the baseline. Each mouse was then inserted into a holder, and a lid was fixed in place. The holder was placed in the single-animal cage, and the body surface temperature was measured 20, 40, and 60 min after the insertion of the mouse into the holder. The mean of the three measurements was used for analysis. After the sampling period, the mice were removed from the holders and returned to their group living cages.

In the cage mate condition, restraint stress occurred in the living cage. All four animals in each cage were restrained in individual holders at the same time, and the holders were placed in a radial arrangement facing the center of the cage so that each animal could see all other animals in its cage.

In the mirror condition, only one mouse in each living cage was restrained in a holder and placed in a mirror cubicle (10 cm in width, 13.8 cm in length and 10 cm in height). Each wall of the mirror cubicle was acryl mirror and the mouse was placed to face the smaller wall. The unfamiliar conspecific condition was the same as the cage mates condition except that three free-moving mice were unfamiliar to the one restrained mouse. The unfamiliar mice were housed in the same animal room as the subject, but their cages were placed on distant sides of the room.

Statistical analysis. The average of the three temperature measurements after restraint was used as the body surface temperature during stress. Because the SIH means increase of body temperature from baseline, the difference between the baseline body temperature and the averaged body temperature under the stress was used as an index of the SIH. One-way ANOVA of the SIH was carried out followed by multiple comparisons using paired *t*-tests after Holm's correction.

Results and Discussion

Figure 2a presents the results of the mirror group. The vertical axis indicates change of body temperature from the baseline (SIH). In the mirror test group, one-way ANOVA revealed a significant effect of test conditions, $F(2,47) = 3.67$, $p = 0.03$). There was a significant difference between the single condition and the cage mates condition, $t(15) = 3.10$, $p = 0.02$ after Holm's correction, and a tendency towards a difference between the single and mirror conditions, $t(15) = 2.04$, $p = 0.06$ after Holm's correction. There was no significant difference between the cage mates condition and the mirror condition, $t(15) = 0.20$, $p = 0.84$. The stress reducing effect in the group condition corroborated a previous experiment (Watanabe, 2015). The mirrors also showed stress-reducing effects. This finding suggests that the mirror had social stimuli-like effect in reducing SIH.

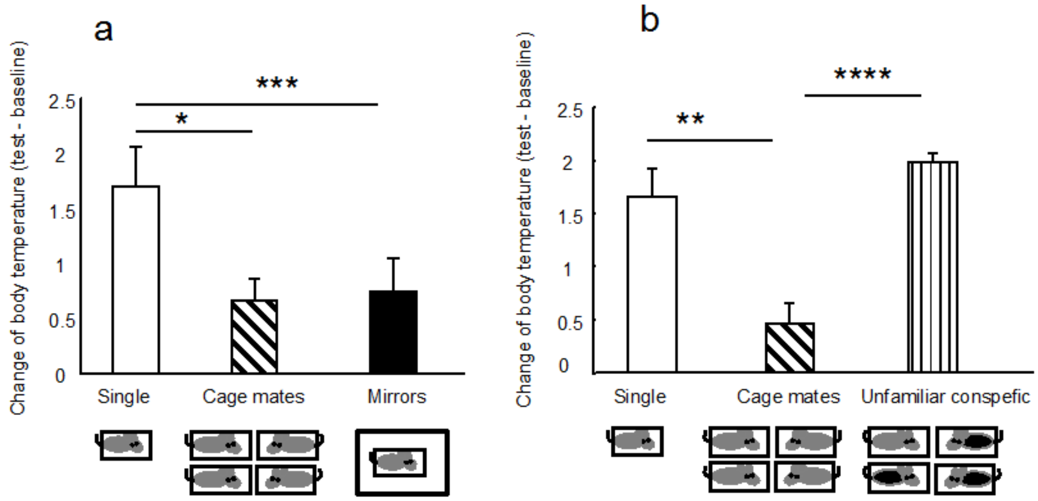

*Figure 2***. Results of Experiment 2.** The mirror group (a) displayed cage mate-like SIH-reducing effects. The unfamiliar conspecific group (b) showed no SIH-reducing effects by unfamiliar mice. $^*p < 0.10$, $^{**}p < 0.05$, $^{***}p < 0.01$, $p < 0.001$. Error bars indicate *SE*.

Figure 2b presents the results of the unfamiliar conspecific test group. One-way ANOVA revealed a significant effect of test conditions, $F(2, 47) = 15.45$, $p < 0.0001$. There was a significant difference between the single condition and the cage mates condition, $t(15) = 3.08$, $p = 0.04$ after Holm's correction, and between the cage mates and unfamiliar conspecific conditions, $t(15) = 6.05$, $p < 0.0001$ after Holm's correction. There was no significant difference between the single condition and the unfamiliar conspecific condition, $t(15) = 1.06$, $p = 0.30$. The stress-reducing effect of the group condition was confirmed. The unfamiliar conspecific condition did not reduce SIH, suggesting that familiarity was crucial to induce the stress-reducing group effect.

Reduction of SIH in the group stress condition is consistent with a previous experiment with ICR mice (Watanabe, 2015) and also agrees with measurements of corticosterone levels and stress-induced memory-enhancing effects (Watanabe, 2011).

A comparison of the mirror condition and the unfamiliar mouse condition suggests a difference between the mirror and unfamiliar conspecifics. Cross-group comparisons of the mirror and the unfamiliar conspecific conditions revealed a significant difference, $t(30) = 3.39$, $p < 0.001$, in SIH. Thus, the mirrors had effects different from unfamiliar conspecifics.

There are several differences between the mirror and unfamiliar conspecifics. First, the mirror had just visual cues, not auditory or olfactory cues. Movement of the image in the mirror was synchronized to the subject, but movement from the unfamiliar mice was not synchronized. The results of Experiment 1 showed positive rather than aversive properties of the mirror. The stress-reducing effect of the mirror in Experiment 2 was consistent with the mirror preference in mice demonstrated in Experiment 1. Indifferent preference between the mirror and the cage mate in Experiment 1 suggests a positive emotional effect of the mirror similar to familiar conspecifics. This positive property might reduce stress. Generally, animals are averse to unknown conspecifics. The results for the unfamiliar mice condition showed that unfamiliar mice did not reduce stress even though the subjects were surrounded by similarly restrained conspecifics. The present results suggest the possibility of mirror use for social enrichment. However, it must be pointed out that mirror reflection has several possible effects in addition to giving self-image. It may increase brightness and apparent largeness of the environment. Employment of a video monitor can separate these different aspects of a mirror.

Conclusions

C57/BL6/J mice preferred a mirror to an opaque screen or unfamiliar live mouse. They displayed indifferent preference between the mirror and familiar cage mates. Similar restraint with familiar cage mates reduced SIH, and mirrors had stress-reducing effects similar to the familiar cage mates. On the other hand, unfamiliar mice did not have stress-reducing effects. Thus, the mirror had cage mate-like effects in reducing SIH.

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