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Research report

Mutual mother-infant recognition in mice: The role of pup ultrasonic vocalizations

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HIGHLIGHTS

- Mouse pups showed a preference for their own mother over an alien mother.
- Mouse mothers located their own pups faster than alien pups.
- Mouse mothers preferred pup USVs of their own pups over those of alien pups.
- There is a mutual recognition between mother and infant in mice.

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ABSTRACT

The importance of the mother-infant bond for the development of offspring health and sociality has been studied not only in primate species but also in rodent species. A social bond is defined as affiliative behaviors toward a specific partner. However, controversy remains concerning whether mouse pups can distinguish between their own mother and an alien mother, and whether mothers can differentiate their own pups from alien pups. In this study, we investigated whether mutual recognition exists between mother and infant in ICR mice. Furthermore, we studied pup ultrasonic vocalizations (USVs), which are emitted by pups when isolated from their mothers, to determine whether they constituted an individual signature used by the mother for pup recognition. We conducted a variety of two-choice tests and selective-retrieving tests. In a two-choice test for mother recognition by the pup, pups between the ages of 17 and 21 days preferred their own mothers to alien mothers. In a two-choice test for pup recognition by its mother, the mothers located their own pups faster than alien pups at the beginning of the test, yet displayed similar retrieving activity for both their own and alien pups in the subsequent selective-retrieving test. Furthermore, after recording USVs from pups from subject and alien mothers, then playing them simultaneously, subject mothers displayed a preference for pup USVs emitted by their own pups. Overall, our findings support the existence of mother-infant bonding in mice and suggest that pup USVs contribute to pup recognition by mothers.

1. Introduction

Within social bonds, the mother-infant bond is particularly important for the development of an offspring's health and sociality. This concept was first demonstrated in humans by Bowlby's attachment theory [1] and in non-human primates through a series of experiments conducted by Harlow and his colleagues [2]. Thereafter, many clinical and experimental studies on humans have indicated that child abuse or neglect is correlated with severe long-term effects on the child's cognitive and socio-emotional development, and such children carry a greater risk for future psychiatric illness [3–5]. On the other hand, intensive mother-offspring interactions are universal to all mammalian species. Previously, we revealed that an impairment of the mother-infant relationship influences cognitive and/or socio-emotional development of

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offspring in rodent species [6–11], thereby suggesting that mother-infant bonds not only occur in primates but also in rodent species [12]. We are especially interested in the developmental consequences of mother-infant bonding in the mouse, a mammalian organism that has been well studied from a neurochemical perspective. This knowledge will help us understand the underlying neural mechanism of social bonds and psychiatric disorders related to child abuse or neglect.

Social bonds are defined as affiliative behaviors toward a specific partner. However, the question concerning whether rodent pups can distinguish their own mothers from alien mothers remains controversial [13–17]. With regard to pup recognition by mouse mothers, it has been reported that female mice could distinguish their own pups from alien pups in a habituation/dishabituation paradigm [18]. It was also shown that mothers retrieved their own pups before other pups when the retrieving tests were conducted by presenting the mother with her own pups and alien pups simultaneously [19]. In addition, it was suggested that mouse mothers could recognize their own pups using olfactory cues [19], which is similar to the recognition observed in other adult conspecifics [20]. On the other hand, it is known that mouse pups emit ultrasonic vocalizations (USVs) when they are isolated from their mother and/or littermates [21]. Previously, we demonstrated that mouse mothers were attracted by pup USVs reproduced by a nanocrystalline silicon thermo-acoustic emitter (nc-Si emitter), but they did not respond to other synthesized sounds [22]. In addition, we also found that the response to pup USVs was enhanced by social experiences, such as mating or alloparenting, and a similar result was observed for retrieving behavior [23]. However, it is not yet known whether pup USVs contribute to individual recognition by mouse mothers.

The present study was conducted to examine mutual recognition in mice between mothers and their own pups. First, we examined mother recognition by pups aged between 17 and 21 days using a two-choice test in which pups were presented with their own mother and an alien mother simultaneously. We then examined pup recognition by mothers using a variety of two-choice tests or selective-retrieving tests, in which mothers were presented with their own pups alongside alien pups aged between 5 and 6 days. Finally, we examined the influence of pup USVs on pup recognition during two-choice tests that reproduced USVs from the mothers' pups and alien pups simultaneously.

2. Materials and methods

2.1. Animals

ICR mice from Japan Clea Co., Ltd. (Yokohama, Japan) were used in all of our experiments. Mice of each sex were housed in groups of 3–5 individuals per cage. The environment was maintained at a constant temperature (24 ± 1 °C) and humidity (50 ± 5%) under a 12-h light-dark cycle (lights were switched on at 0800). Food and water were supplied ad libitum. To synchronize the delivery in females, a male was introduced into a female cage for a period of two days and removed thereafter. Two weeks after removal of the male, pregnant females were individually housed in breeding cages (24.5-cm long × 17.5-cm wide × 12.5-cm tall), and checked daily in the morning until parturition. For each litter, the date of birth date was designated as postnatal day 0 (PD0). Between PD0 and PD1, each litter was culled to a standard size of 10 pups, with five pups of each sex. For Experiment 3, each litter was culled to a size of three pups with a random mix sexes. For each experiment, care was taken to avoid disturbing the animals throughout the nursing period, except for a brief weekly cage cleaning. All animal experiments were approved by the Ethical Committee of Azabu University (#080214-5).

2.2. Apparatuses used for the two-choice test

The test cage used in Experiments 1 and 2 was an acrylic rectangular box (35-cm long × 20.5-cm wide × 20-cm tall), modified for assessing a pup's preference for mothers (Fig. 1a) or mother's preference for pups (Fig. 1d). There were no lids on either cage types. In Experiment 1 (Fig. 1a), pups could move into each dam area (14-cm long), which were separated by a partition from the adjacent middle area, and accessible by a narrow entrance (1.5-cm wide × 1.5-cm tall) through which mothers could not pass. These entrances could be closed by lids. Between each middle and neutral area (neutral area, 14-cm long), there was also a gate (5-cm wide × 5-cm tall) that could be shut by a mesh door. Airflow from the dam area to the neutral area circulated through holes (8 cm in diameter) in the short wall of the neutral area and holes (4 cm in diameter) in every dam area; all airflow holes were covered by mesh. During the habituation period, air was drawn by a fan attached above the mesh on the short wall of the neutral area. The test cage of Experiment 2 was similar to that of Experiment 1, apart from the fact that there were no middle areas and therefore no narrow entrances (Fig. 1d). In Experiment 2, mothers could move into each pup area (29-cm long) from the neutral area through each gate. The pups were placed inside clear acrylic bottles (6.5 cm in diameter, 20 cm in length) at the end of each pup area. Both bottles had multiple small holes to allow air exchange and allow auditory, visual, and olfactory information to be exchanged between the pups in the bottle and the mother outside. These test cages and bottles were washed and wiped with 70% alcohol between trials.

The test cage used in Experiments 3 and 4 was made of clear Plexiglas to assess the mother's preference for pups (Fig. 2b) or preference for pup USVs (Fig. 2e), and was the same size as the breeding cage. A hole (4 cm in diameter, the center of which was 6 cm from the left corner and 3.5 cm from the bottom) and 12 slits (8 × 12 mm, 4 mm between slits, 2 cm from the right corner, and 1 cm from the bottom) were cut in each long wall to reduce sound echo. Tubes (4 cm outer diameter, 15-cm long, covered with mesh on the external end) were attached to the holes of the test cage. To present the pups in Experiment 3 (Fig. 2b), a mesh divider was inserted 5 cm from the external end of each tube. Pups could be placed between this mesh and the mesh at the external end of the tubes. In Experiment 3 and 4, an nc-Si emitter, which was used in our previous studies [22–25], was attached immediately outside the mesh on the end of each tube (Fig. 2e). All test cages were placed in a soundproof chamber during the experiment.

2.3. Ultrasound recording and reproduction

Ultrasound recording was performed using a condenser microphone (UltraSoundGate CM16/CMPA, Avisoft Bioacoustics, Berlin, Germany) as previously described [23,24]. Ultrasonic sounds were recorded for 3 min when a 5- or 6-day-old pup was isolated from its mother and littermates, and a 1-min-long recorded file (from the moment the pup began to cry) was prepared for each pup. Considering that the reproduction of pup USVs from a subject's own (biological) pups and from alien pups in each experiment was done simultaneously, the recorded files were selected in a way to ensure the number of calls was almost identical between the pup groups. The files were repeated for a period of 5 min in Experiments 3 and 4. For the reproduction of pup USVs by the nc-Si emitter, both sexes of pups were randomly assigned in Experiment 3. In Experiment 4, only male pups were assigned to exclude a possibility that the preference for pup USVs was influenced by call features with sex difference. In a previous report [26], it was suggested that

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