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Research report

Odor-enriched environment rescues long-term social memory, but does not improve olfaction in social isolated adult mice

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ABSTRACT

Prolonged permanence of animals under social isolation (SI) arouses a variety of psychological symptoms like aggression, stress, anxiety and depression. However, short-term SI is commonly used to evaluate social memory. Interestingly, the social memory cannot be accessed with delays higher than 30 min in SI mice. Our hypothesis is that SI with intermediate duration, like one week (1 w), impairs the long-term storage of new social information (S-LTM), without affecting anxiety or other types of memories, because the SI compromises the olfactory function of the animal. Our results demonstrated that SI impaired S-LTM, without affecting other kinds of memory or anxiety. In addition, the SI increased the latency in the buried-food finding task, but did not affect the habituation or the discrimination of odors. Next, we postulated that if continuous input to the olfactory system is fundamental for the maintenance of the olfactory function and social memory persistence, isolated mice under odor-enriched environment (OEE) should behave like group-housed (GH) animals. In fact, the OEE prevented the S-LTM deficit imposed by the SI. However, OEE did not restore the SI mice olfaction to the GH mice level. Our results suggest that SI modulates olfaction and social memory persistence, probably, by independent mechanisms. We also showed for the first time that OEE rescued S-LTM in SI mice through a mechanism not necessarily involved with olfaction.

1. Introduction

It is well known that environmental conditions modulate neuronal plasticity, as well as behavior, in several species [1–6]. The social isolation (SI) is one of the paradigms used to alter the environmental conditions. SI arouses a variety of psychological symptoms like aggression [7], stress [8], anxiety [9,10] and depression [11].

The majority of studies about SI effects on behavior exploit a model of isolation rearing early in life, either as pups or adolescents [12], which is a different model than adulthood SI. Prolonged SI in the adulthood induces stress [13], promotes anxiety-like phenotype [9,10], increases alcohol intake [14] and impairs memory [15]. Conversely, social isolation, for hours or few days, is commonly used to increase territorial behavior and exacerbate social memory measurement [16–18].

Social memory can be defined as the ability of individuals to recognize co-specifics [16]. This kind of memory is modulated by the olfactory system [20] and can be considered a hippocampus-dependent memory [21]. Interestingly, social long-term memory (S-LTM) can be accessed only if the animals are maintained grouped, since SI, even during short periods, impaired the expression of S-LTM [21].

Considering the dual effect of SI on cognition, we hypothesized that SI with intermediate duration, like one week (1 w), exposes animals to an olfactory monotony, which is detrimental to olfaction and long-term storage of new social information. Moreover, we predicted that mice under 1 w of SI have no anxiety-like phenotype and behave like group-housed mice in memory tasks, which encoding did not relies on olfactory cues. We also tested the hypothesis that odor-enriched environment (OEE) allows the persistence of social memory, even under SI, through maintenance of the olfactory function.

2. Materials and methods

2.1. Animals

Adult male C57BL/6J mice (eight to nine weeks old) and juvenile male swiss mice (25–30 days old), used as intruder in the social memory experiments, were purchase from the Animal Facility of Federal University of Minas Gerais (Brazil). They

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all were maintained in opaque polypropyl (28 cm × 17 cm × 12 cm) cages, equally illuminated, inside a climate-controlled (light, temperature and ventilation) animal housing unit (Alesco, Brazil) on a 12 h light/12 h dark cycle. Ambient temperature was maintained at 22 ± 2 °C and relative humidity was 55 ± 10%. Unless otherwise stated, food and tap water were available *ad libitum*. All experimental procedures were carried out in the light phase.

All the procedures were conducted in accordance with NIH guidelines for the care and use of animals. Experiments were performed accordingly to approve animal protocols from the Institutional Animal Care and Use Committees at the Federal University of Minas Gerais (Protocol 168/2008).

2.2. Environmental conditions

2.2.1. Group-housed (GH)

Mice were maintained in groups of five per cage.

2.2.2. Social isolation

SI mice were individually housed during one week (1 w) for acute and four weeks (4 w) for chronic social isolation. Cages were cleaned twice a week. Animals were not handled during this period. The probability of visual contact was minimal. There was no control to reduce social odors between one cage and another. However, there was no physical contact with co-specifics during the social isolation.

2.2.3. Odor-enriched environment

The OEE was established through the addition of social or non-social odor cues in the mice's home cage. The odor cues were renewed daily and its presence lasts during the whole period of isolation (1 w).

The non-social stimuli odors group (NSSO) was presented to three non-social odor cues, which were pure essences of strawberry, passion fruit or tutti-frutti (a mixture of several fruits) (Dr. Oetker, Brazil). The same essence was used in set of three animals to ensure that results were not specific to any given odor. The mixture of clean bedding (100 g) and pure extract essence (1 mL) was prepared daily, in a separate room, and after 15 min the animal was introduced to the cage containing the mixture.

The animals from social-stimuli odors group (SSO) were exposed to male-soiled bedding obtained from three cages containing five C57BL/6J adult male mice each. The mixture of clean bedding (50 g) and male-soiled bedding (50 g) was prepared daily, in a separate room, and after 15 min the animal was introduced to the cage containing the mixture.

The control groups were equally isolated during 1 w, though one group (NS: no-stimuli) remained untouchable during the whole period of isolation and the other one (HS: handling-stimuli) has its bedding changed daily by a clean one.

2.3. Behavioral evaluations

2.3.1. Social recognition (SR)

To assess social memory, we followed a modification of the method originally described by Thor et al. [16]. Swiss juvenile male mice were used as intruder and were presented to the resident subject inside a transparent acrylic cylinder (10 cm diameter) containing 60 holes equally distributed [18,22]. The holes in the cylinder allow physical contact between the nose and/or whiskers of the adult mice and the juvenile.

The resident mouse was habituated to the presence of the empty cylinder in their home cage during 30 min. During the last 5 min of the habituation, the intruder juvenile mouse was placed inside another clean cylinder, in its home cage, to habituate as well. The training session started when the cylinder containing the intruder replaced the empty cylinder, in the resident mouse's cage. Training session (TR) lasted 5 min and there was no baseline for social recognition test. Social investigation was quantified every time the resident animal introduced its nose and/or whiskers inside any of the cylinder's holes. The position of the cylinder, inside the resident's cage, was kept constant throughout the experiment and was cleaned with 70% alcohol between animals and trials.

The test session (TT) consisted in reintroducing the intruder juvenile mouse into the resident's cage during 5 min. The inter-trial period for social short-term memory (S-STM) was 30 min and during this period the empty cage remained into the resident's cage. To assess social long-term memory (S-LTM), 24 h after TR, mice were habituated exactly as describe above and the social investigation was scored. Animals were used only once, or to access S-STM or S-LTM. None of the juvenile mouse was used more than three times. Apart from the main role of the sense of smell in the social recognition memory, we cannot rule out that vision might be involved, since the cylinder where the juvenile was presented to the adult mice was transparent.

2.3.2. Social discrimination (SD)

To evaluate social discrimination, the animals were habituated during 30 min into a plastic box (50 cm × 40 cm × 20 cm) containing clean bedding and two transparent acrylic cylinders, like the one used for the SR test. Next, two swiss juveniles were introduced in each cylinder and during 5 min the social investigation was measured (day 1). Twenty-four hours later, the animal returned to the same box with one cylinder containing a familiar juvenile and the other one a new juvenile. The social investigation was measured during 5 min (day 2) [adapted from 21, 23, 24]. Data were expressed as discrimination index, calculated using the following formulae: time exploring the new juvenile/total time of social investigation.

2.3.3. Novel object recognition (NOR)

All animals were given a single 20 min habituation, with no objects, in a white plastic cage (50 cm × 40 cm × 20 cm), which was equally illuminated. Twenty-four hours later, in the training session, animals were allowed to explore two copies of an identical object during 10 min. Memory retention was evaluated during the test session (TT) carried out 24 h after TR. In the TT, with duration of 10 min, one object was identical from TR and the second was a new one. All objects (available in duplicate) presented similar material and size, but distinct color and shape. The objects had been selected from a large pool of objects on the criterion that mice would spend approximately equal amounts of time exploring each of them (data not shown). Between animals, box and objects were cleaned with 70% alcohol and air-dried [25]. Exploration time was defined as sniffing or touching the object with the nose and was quantified by the software Debut Video Captura®.

Data are expressed as recognition index, calculated according to the following formulae: time exploring the new object/time exploring the familiar object + time exploring the new object.

2.3.4. Inhibitory avoidance (IA)

The apparatus was a Plexiglas box (21 cm × 22.5 cm × 22.5 cm) with a 10 cm^2 platform on the left end of a series of steel bars, spaced 1 cm apart, that made up the floor of the box (Insight Equipamentos, Ribeirão Preto, Brazil). For training, animals were gently placed on the platform facing the left rear corner of the box. When they stepped down and placed their four paws on the grid, they received a 2 s, 0.3 mA scrambled footshock [26,27]. Memory retention was evaluated in a non-reinforced test session (TT) carried out 24 h after TR.

2.3.5. Elevated plus maze (EPM)

The apparatus consists of two open arms (30 cm × 6 cm) and two closed arms (30 cm × 6 cm × 16 cm) extended from a common central platform (6 cm × 6 cm). The whole apparatus is elevated 30 cm above the floor. Mice were allowed to move freely between the arms during 5 min. The number of entries into the open arms and the time spent in the open arms were used as indices of open space-induced anxiety in mice. Since animals have innate fear of elevated open places, they stay for a shorter time in the open arms as compared to the closed arms when allowed to freely explore the maze [28].

2.3.6. Buried food-finding test

Each mouse had contact with one peace of chocolate (7 g) per day, during 3 days. Twelve hours before the olfaction test, the food and chocolate were withdraw. The olfaction test consisted in introducing the animal in a clean cage (28 cm × 17 cm × 12 cm) containing a peace of chocolate hidden under the clean bedding (3 cm layer). The latency to find the candy was measured [29].

2.3.7. Olfactory habituation/dishabituation test

The olfactory habituation/dishabituation paradigm is commonly used to test whether the animal can detect and discriminate distinctive odors, including both non-social and social odors [30,31].

Each animal was placed in the test cage (40 cm × 33 cm × 16 cm) with two peaces of filter paper (2 cm × 2 cm) containing 15 μL of mineral oil each (Farmax, Brazil), for 10 min habituation. The filter papers were fixed at the bottom of the box with sticky tape. After, one filter paper was removed and replaced by the habituation odorant (15 uL of OVX-female urine). The habituation odorant was presented during four successive 2 min trials (H1–H4), separated by 5 min intervals. To evaluate the odor discrimination, after habituation the mouse was exposed to a test odorant (15 uL of 1:100 Vanilla essence, Dr. Oetker, Brazil) during 2 min. Then, a fifth presentation of the habituation odorant was performed (H5) to ensure that mice were still habituated to this odorant. During each trial we measured the time the mouse spent sniffing the filter paper. During the intervals, the mouse remained in the test cage with two filter papers containing mineral oil. The filter papers were changed place in all sessions to maintained their localization new to the mouse.

2.4. Experimental design

Each animal was tested only in one paradigm, to avoid maintaining animals isolated for a period longer than 9 days, except the chronic social isolation group, which was isolated for four weeks.

2.5. Data analysis

Social recognition data (Figs. 1A and B and 4) were analyzed by three distinct tests. The paired *t*-test was used to within-group comparison. Two-way ANOVA followed by Bonferroni's multiple comparison test was used to evaluate interaction between factors. To evaluate differences between groups, first we calculated the recognition index (time of social investigation during TT/sum of the time of social investigation during TR and TT) and then we compared groups by independent *t*-test or one-way ANOVA followed by Bonferroni's multiple comparison test.