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Transport of animals between rooms: A little-noted aspect of laboratory procedure that may interfere with memory

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ABSTRACT

This study investigated the effects of transporting animals from the experimental room to the animal facility in between experimental sessions, a procedure routinely employed in experimental research, on long-term social recognition memory. By using the intruder–resident paradigm, independent groups of Wistar rats exposed to a 2-h encounter with an adult intruder were transported from the experimental room to the animal facility either 0.5 or 6 h after the encounter. The following day, residents were exposed to a second encounter with either the same or a different (unfamiliar) intruder. Resident's social and non-social behaviors were carefully scored and subjected to Principal Component Analysis, thus allowing to parcel out variance and relatedness among these behaviors. Resident rats transported 6 h after the first encounter exhibited reduced amount of social investigation towards familiar intruders, but an increase of social investigation when exposed to a different intruder as compared to the first encounter. These effects revealed a consistent long-lasting (24 h) social recognition memory in rats. In contrast, resident rats transported 0.5 h after the first encounter did not exhibit social recognition memory. These results indicate that this common, little-noted, laboratory procedure disturbs long-term social recognition memory.

1. Introduction

Many studies on sociability and social recognition memory make use of the intruder–resident paradigm (Bannerman et al., 2001; Burman and Mendl, 2000; Dantzer et al., 1987; Ferguson et al., 2000, 2001; Popik and van Ree, 1998; Richter et al., 2005; Sekiguchi et al., 1991b). In this behavioral task, a juvenile conspecific intruder is typically exposed to a 5-min encounter within the cage with an adult resident animal; typically, the adult resident exhibits intense social investigation of the juvenile intruder. A second 5-min encounter with the same intruder with the resident animal elicits far less social investigation as compared to: (1) that observed during the first encounter and (2) that observed during the presentation of a different juvenile intruder. In rats, these effects are consistently observed when the inter-encounter interval is 30–60 min; however, these behaviors significantly decrease when the inter-encounter interval is increased to 2 h (Burman and Mendl, 2000; Castner et al., 2004; Dantzer et al., 1987, 1988; Ferguson et al., 2001; Garau et al., 2000; Prediger et al., 2004; Prediger and Takahashi, 2003; Young, 2002). These temporal effects suggest that social recognition memory in rats is a form of short-term memory (Dantzer et al., 1987; Kogan et al., 2000; Richter et al., 2005).

Moura et al. (2010) showed that social recognition memory in rats persists at least 24 h when the duration of the first encounter with an adult intruder was 2 h or longer, in contrast to prior studies that attempted to reveal long-term social recognition memory in rats related to memory consolidation.

Peptides such as oxytocin and vasopressin modulate social recognition memory in rats and mice (Dantzer et al., 1988, 1994; Dluzen et al., 1998; Ferguson et al., 2001; Le Moal et al., 1987); for instance, either systemic administration or intracerebroventricular, septal and olfactory bulb injections of vasopressin increases social recognition memory in rats and mice (Dantzer et al., 1988, 1994; Dluzen et al., 1998; Ferguson et al., 2001; Le Moal et al., 1987).

Abbreviations: AGR, mild aggression; ANO, sniffing the anogenital region; BOD, sniffing the body; CREB, cyclic AMP responsible element binding protein; DOM, dominance behavior; ENV, sniffing the environment; ERK, Extracellular-Signal-Regulated kinase; FOL, following the conspecific; GRO, self-grooming; HEA, sniffing the head; PCA, Principal Component Analysis; PKA, cAMP-dependent protein kinase; REA, rearing.

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While oxytocin knockout mice show social recognition memory impairment (Ferguson et al., 2000).

Housing conditions may also influence social recognition memory. For example, Kogan et al. (2000) showed that while group-housed mice retain social memory for at least 7 days after a single 2-min encounter with a juvenile, a 3-week period of social isolation prior to the start of the experiment disrupts long-term (24 h to 7 days), but not short-term (up to 30 min) social recognition memory. These authors also report that a single 24-h period of social isolation prior to the start of the experiment interferes with long-term social recognition memory and that this form of memory in mice is dependent on protein synthesis and cyclic AMP responsible element binding protein (CREB) function (Kogan et al., 2000). In addition, Richter et al. (2005) showed that social recognition memory in mice is blocked by the administration of anisomycin (an protein synthesis inhibitor) 20 min before, immediately, or 6 h after the first encounter; interestingly, no memory disruption with administration 3 h or 18 h after the first encounter, suggesting that the consolidation of social recognition memory requires at least two stages of protein synthesis, the first occurring immediately after and the second about 6 h after the acquisition training.

Other studies indicate that one-trial learning inhibitory avoidance in rodents is disrupted by exposure of the animals to novel experiences after task acquisition, including the open field (Izquierdo et al., 1999), the "Y" maze (Cahill et al., 1986) and habituation to a tone (Netto et al., 1985). These retrograde amnesic effects seem to be related to novelty-induced biochemical changes in brain regions related to memory processes, including the hippocampus (Cahill et al., 1986; Izquierdo and Netto, 1985; Izquierdo et al., 1999). Further, this novelty-induced amnesic seems to occur when novelty is presented up to 3 h but not 6 h after inhibitory avoidance training (Cahill et al., 1986) suggesting that it is related to the biochemical processes required for the consolidation of long-term memory (Grecksch and Matthies, 1980; Quevedo et al., 1999).

The present study investigated whether a procedure as common as an animal's transportation between the experimental room and the animal facility might interfere with long-term social recognition memory. Either 0.5 or 6 h after a 2-h first encounter with an adult conspecific, animals were transported to the animal facility where they were maintained undisturbed. On the following day they were again transported to the experimental room for the second encounter; thus, the inter-encounter interval was 24 h. Half of the resident rats of each group was exposed in the second encounter to the same (familiar) conspecific, while the other half was exposed to a different (unfamiliar) conspecific. Social and non-social behaviors of both the resident and intruder rats were individually scored.

If transportation represents a novel experience that interferes with social recognition memory, it seems more likely to disturb social recognition in rats transported to the animal facilities 0.5 h, but not 6 h, after the first encounter. Therefore, resident rats transported to the animal facilities 6 h after the first encounter should exhibit a typical decrease in social investigation towards the familiar intruder as compared to social investigation towards a different (unfamiliar) intruder. In contrast, transportation may disrupt the consolidation of social memories in resident rats transported 0.5 h after the first encounter, resulting in no decrease in social investigation towards a familiar intruder.

2. Material and methods

2.1. Animals

Fifty-four naïve, male, Wistar rats (*Rattus norvegicus*), 3-months-old, were purchased (Charles River Laboratories, Wilmington, MA) two weeks before the study began. The rats arrived at the laboratory in groups of 3–4 rats per cage, and were maintained within the same home cage. Light was provided from 7:00 to 19:00 h and temperature was set at 21 ± 3 °C. Food and water were available *ad libitum*. The animals were individually handled for 5 min per day for 2 days before the beginning of the study to habituate them to handling. Because rats usually exhibit greater social investigation of an intruder when tested during their inactive phase (Moura et al., 2009), all experiments were run from 9:00 to 11:30 h. Animals were randomly assigned to: (1) groups transported from the experimental room to the animal facilities either 0.5 or 6 h after the first encounter, (2) groups exposed either to the familiar or to a different intruder during the second encounter, and (3) the role of either resident or intruder (see below). Residents and intruders in these experiments were never taken from the same home cage. All procedures were conducted in accordance to the NIH Guide for the Care and Use of Experimental Animals.

2.2. Test chamber

Standard transparent polypropylene cages used for behavioral testing were positioned on a shelf located within an open field chamber (Med Associates Inc., St. Albans, VT) to attenuate external cues that could distract the animals. The chamber was located in a quiet experimental environment for testing. The test chamber was illuminated by fluorescent lamps (200–300 lx). A JVC camera (GZ-MG37) positioned on a tripod 40 cm apart from one side of the polypropylene testing cage was used to record social interactions in each encounter. There was no visual or physical contact among animals placed in different testing cages during testing.

2.3. Behavioral procedure for "resident" and "intruder" rats

Rats were transported from the animal facility to the experimental room 90 min before the beginning of the experiments. Social recognition memory testing was evaluated by using a modified version of the intruder–resident paradigm described by Thor and Holloway (1982), as adapted by Moura et al. (2010).

Animals assigned to the "resident" groups were individually placed into polypropylene cages 20 min before the introduction of an adult "intruder" rat of similar weight and age (see Moura et al., 2010). This first encounter lasted for 2 h (see Moura et al., 2010). The initial 10 min of each encounter was videotaped for later behavioral data analysis. At the end of the first encounter, the intruder rat was removed and individually housed in a new cage containing fresh bedding in order to avoid mixing their scents with those of other conspecifics until the second encounter. The resident rat was maintained within the same testing cage until the second encounter. Thirteen resident rats were transported back to the animal facilities 0.5 h after the first encounter, and fourteen resident rats were transported 6 h after the first encounter. During the time period prior to the transportation, referred to as "time undisturbed", the animals remained in a quiet experimental room. The interval between the end of the first encounter and the beginning of the second encounter was 24 h. At all times, animals were maintained within their individual cages located at opposite sides of the well-ventilated animal facility.

Ninety minutes before the second encounter, animals were transported from the animal facility to the experimental room. The resident rats remained within their individual cages when placed on the shelf in the open field chamber for their second encounter with an intruder rat. Among resident rats transported to the animal facilities 0.5 h after the first encounter, six were exposed to the familiar intruder and seven to a different intruder rat. Among those twelve residents transported 6 h after the first encounter, half were exposed the familiar and the other half to a different intruder rat.