Behavioural Brain Research 343 (2018) 95–101

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Incorporation of new neurons in the olfactory bulb after paced mating in the female rat

R. Alvarado-Martínez, R.G. Paredes*

Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus UNAM-Juriquilla, Blvd Juriquilla 3001, 76230

ARTICLE INFO

Keywords:
Neurogenesis
Paced mating (PM)
Non-paced mating (NPM)
Accessory olfactory bulb (AOB)
Main olfactory bulb (MOB)

ABSTRACT

One of the regions that constantly produces neurogenesis in the adult brain is the subventricular zone (SVZ), whose new cells migrate to the olfactory bulbs (OB). When the females regulate the copulatory events (paced mating) the number of new cells in the SVZ increases, as well as those observed in the OB 15 days later. However, no changes were observed in the number of cells 45 days after the females paced the sexual interaction. Constant sensory stimulation is an important promoter of cell survival in the OB circuit. Hence, we increased the number of mating sessions in this study to cover the period where stimulation of the new cells is critical for their incorporation into pre-existing circuits in the OB. Ovariectomized female Wistar rats, were injected with the mitotic marker 5-bromo-2′-deoxyuridine (BrdU, 100 mg/kg, per injection) before, at the end and one hour after mating. Sexual behavior was recorded for 1 h in 10 weekly sessions. After the last mating session, brain sections were processed to determine BrdU immunoreactivity. Our results indicate that females that paced the sexual interaction for 10 sessions had a higher number of cells in the glomerular layer (GL) of the accessory olfactory bulb (AOB) and a higher number of neurons in the granular layer (GrL) of the main olfactory bulb (MOB) in comparison to the control group. These results indicate that continued sexual interaction contributes to the integration of new cells and neurons, induced in the first sexual experience, into pre-exiting circuits of the OB.

1. Introduction

In natural, semi-natural and laboratory conditions, female rats can control the frequency of copulatory events they received in a sexual interaction [1,2], a condition known as paced mating (PM). Thus, in the laboratory it is possible to study female sexual behavior in two modalities, PM and non-paced mating (NPM) [1,3,4]. Both copulatory paradigms promote different behavioral responses in the female. When the females control, or pace, the copulatory contacts, they receive fewer intromissions and ejaculations and show a higher inter-intromission interval than females that mate without pacing the sexual interaction [3,5]. It has been reported that PM induces several physiological changes that favor reproduction [6], and also induces a positive reward state, evaluated by the conditioned place preference paradigm [7,8]. As well, PM increases the release of luteinizing hormone, oxytocin and prolactin, hormones associated with ovulation and pregnancy [9,10]. It also increases the expression of c-Fos in several nuclei relevant for sexual behavior such as the medial amygdala (MeAD), the medial preoptic area (MPOA) and the nucleus accumbens (NAcc) [11].

PM also induces plastic changes such as neurogenesis in the subventricular zone, rostral migratory stream (RMS) olfactory bulb (OB) system [3,4]. One PM session induces a higher number of cells in the RMS 2 days after mating [12] and in the granular layer (GrL) of the AOB 15 days after the sexual interaction [3]. If the stimulus is repeated and females mate 4 times in PM conditions a higher number of cells and neurons is observed in comparison to the females that PM in one session. Moreover, a significantly higher number of cells and neurons is observed in other layers of the AOB and MOB [4]. Subsequent experiments evaluated the survival of the new cells and neurons in the OB 45 days after 1 PM session. No differences were found in the number of cells, but a significant increase in the number of neurons in the GrL of the AOB was observed after 1 PM session [12]. Thus, these results suggest that the cellular increase observed 15 days after 1 PM session is not sufficient to maintain the survival of the new cells 45 days after mating.

It is well established that olfactory input modulates neurogenesis in different brain regions, including the olfactory bulbs, reviewed in [13]. For example, enrich olfactory environments enhance the survival of new neurons that reach the OB [14]. Exposure to pheromones enhances the survival of new neurons in the AOB, indicating that the survival of

* Corresponding author at: Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus UNAM-Juriquilla, Blvd Juriquilla 3001, 76230.
E-mail address: rparedes@unam.mx (R.G. Paredes).

https://doi.org/10.1016/j.bbr.2018.02.006
Received 21 November 2017; Received in revised form 3 February 2018; Accepted 5 February 2018
Available online 06 February 2018
0166-4328/

the new cells is regulated by olfactory input [15]. On the other hand, naris occlusion reduces BrdU-IR neurons that reach different layers of the MOB. In the present study, we extended the number of PM sessions to 10 (PM10s), covering the critical period of cell incorporation and survival described for the OB, in order to determine if the repetition of the stimulus that originated the cellular increase in the first mating session, promotes cellular survival 45 days later. We also evaluated possible changes in neuronal differentiation associated with mating in the MOB and AOB.

2. Experimental procedures

2.1. Animals

Female Wistar rats (200–250 g) without sexual experience and sexually experienced males (300–350 g) of the same strain, were obtained from the local colony at the Instituto de Neurobiología of the UNAM, and were maintained at a temperature of 25 °C. The females were anesthetized with a mixture of 30% Ketamine (95 mg/kg, Procin) and 70% Xylazine (12 mg/kg, Cheminova) before they were ovariectomized (OVX). One week after their recovery, and to induce sexual receptivity, female rats were supplemented with estradiol benzoate (EB, 25 µg/kg, Sigma Aldrich) and progesterone (P, 1 mg/kg,), 48 and 4 h respectively, before eachbehavioral session. Female rats were randomly assigned to one of the following five groups: (1) Control group (Females placed alone in a mating cage with clean sawdust, N = 11); (2) Exposed: Females placed in a mating cage exposed to a sexually experienced male without the possibility of physical contact (N = 7); (3) Paced mating in 5 sessions, females that mated in five sessions in a 16-day interval controlling the sexual interaction (PM5s, N = 11); (4) Paced mating in 10 sessions, females that mated in ten sessions in a 45 day interval (PM10s, N = 11); (5) Females that mated without the possibility of pacing the sexual interaction for 10 sessions in a 45-day interval (NPM10s, N = 11). All 51 experimental females were sacrificed 45 days after the first sexual behavior test and administration of 5-bromo-2′-deoxyuridine (BrdU) (Fig. 1A). All experiments were carried out in accordance with the "Reglamento de la Ley General de Salud en Materia de Investigación para la Salud" of the Mexican Health Ministry, which follows NIH guidelines and were approved by the Bioethics Committee of the Instituto de Neurobiología.

2.2. Administration of BrdU

To identify the new cells originated by sexual behavior, we used the nucleoside analogue of thymine, BrdU, which is incorporated into the DNA of the cells during the synthesis phase of cell division. In the first behavioral session, BrdU (Sigma Aldrich) was injected intraperitoneally three times at a concentration of 100 mg/kg in a 0.9% NaCl solution [3]. The BrdU administration protocol started with the first injection one hour before the first behavioral test, the second injection was applied immediately after the behavioral test and the last dose was applied one hour later for a total dose of 300 mg/kg (Fig. 1B). For the PM5s group, females were housed in their home cage immediately after the fourth mating test until day 45 when the last PM session was done, thus having five PM sessions in total. All animals were sacrificed on day 45 after the first behavioral test and BrdU injection.

2.3. Mating tests

For the behavioral tests, an acrylic box (40 cm × 60 cm × 40 cm) was used. In the case of the PM tests, an acrylic barrier was placed in the middle of the box with a hole large enough (7 cm in diameter) to allow the female, not the male, to go back and forth. The female rat was able to move between both compartments, thus controlling the sexual interaction with the male. For the NPM tests the animals mated without the barrier, allowing the male, not the female, to control the frequency and number of sexual contacts [1,3].

The following parameters were recorded: number of mounts, number of intromissions, number of ejaculations; latency to mount, intromit and ejaculate, defined as the time required by the male to display the first sexual event of each type. We also calculated the inter-intromission interval (III), which results from dividing the latency of ejaculation by the number of intromissions received in each ejaculatory series. To determine the level of receptivity of the female, a modified classification of dorsiflexion was used [16]. A score of 0 was assigned when the female showed no change in posture in response to a mount by the male; 1 when the dorsiflexion was moderate, forming a line parallel to the ground; and 2 when the dorsiflexion was pronounced and the head was raised backward. Two parameters were obtained: the lordosis intensity (IL) and the lordosis quotient (LQ). The latencies to return to the male side and the percentages of exits after a mount, an intromission or an ejaculation were recorded. Each behavioral test lasted 1 h.

Forty-five days after the administration of BrdU and 90 min after the last behavioral test (PM5s, PM10s, NPM10s), all groups were administered a lethal dose of sodium pentobarbital (100 mg/kg, Cheminova) and subsequently perfused intracardially with a phosphate-buffered solution (PBS 0.1 M, 250 ml, pH 7.42) followed by 4% paraformaldehyde (300 ml, Sigma Aldrich) in a 0.1 M PBS solution. The brains were extracted and postfixed for one hour in paraformaldehyde,

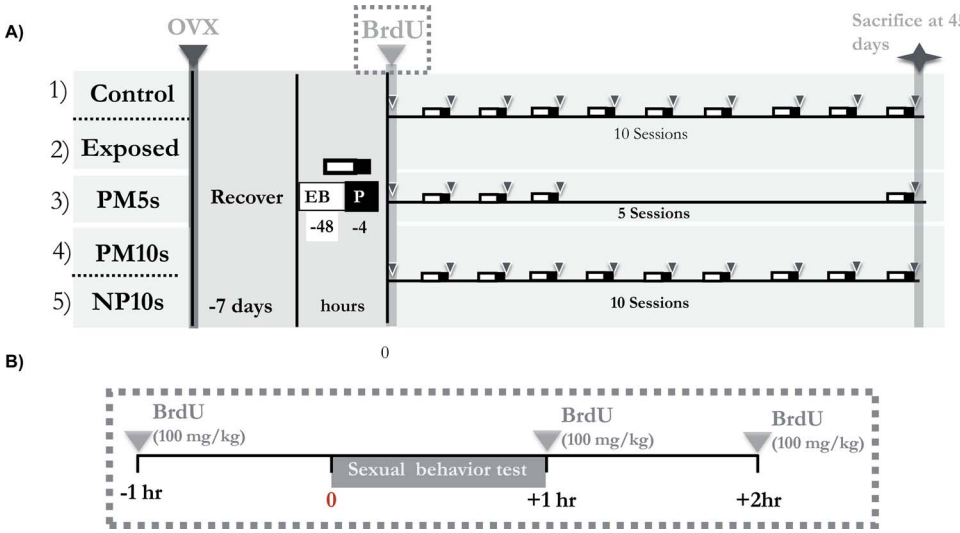

Fig. 1. Schematic representation of the procedure for the different groups of animals. (A) All subjects were ovariectomized (OVX) at least 7 days before the experiment. They were injected with BrdU in the first test and sacrificed 45 days later. (B) BrdU was injected 1 h before the behavioral test (100 mg/kg), at the end of the test (100 mg/kg) and 1 h after the test (100 mg/kg).