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REPEATED PACED MATING PROMOTES THE ARRIVAL OF MORE NEWBORN NEURONS IN THE MAIN AND ACCESSORY OLFACTORY BULBS OF ADULT FEMALE RATS

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Abstract—We have previously shown that the first-paced mating encounter increases the number of newborn cells in the granule cell layer (Gra; also known as internal cell layer, ICL) of the accessory olfactory bulb (AOB) in the adult female rat (Corona et al., 2011). In the present study we evaluated if repetition of the stimulus (paced mating) could increase the arrival of more newborn neurons in the olfactory bulb generated during the first session of paced sexual contact. Sexually naive female rats were bilaterally ovariectomized, hormonally supplemented with estradiol (E2) and progesterone (P) and randomly assigned to one of four groups: (1) without sexual contact, (2) one session of paced mating, (3) four sessions of paced mating, and (4) four sessions of non-paced mating. We also included a group of gonadally intact females. On the first day of the experiment, all females were i.p. injected with the marker of DNA synthesis bromodeoxyuridine and were killed 16 days later. Blood was collected at sacrifice to determine the plasma levels of E2 and P. The number of newborn neurons that arrived at the ICL of the AOB and the Gra of the main olfactory bulb (MOB) increased, relative to all other groups, only in the group that repeatedly mated under pacing conditions. No differences were found in E2 and P levels between supplemented groups indicating that our results are not influenced by changes in hormone concentrations. We suggest that repeated paced mating promotes the arrival of more newborn neurons in the AOB and MOB.

Key words: olfactory bulb, neurogenesis, paced mating, granule cells.

INTRODUCTION

There are two neurogenic niches broadly accepted in the adult mammalian brain: the subgranular zone of the hippocampus and the subventricular zone in the lateral ventricle wall (Alvarez-Buylla and Lois, 1995; Vukovic et al., 2011). The latter contains neural stem cells that differentiate and give rise to new olfactory interneurons (Lois and Alvarez-Buylla, 1994; Whitman and Greer, 2009). The olfactory bulb (OB) is composed of the main OB (MOB) and accessory OB (AOB). The MOB has three layers: the glomerular layer (Glo), the mitral cell layer (Mi), and the granule cell layer (Gra). The AOB, according to the classification suggested by Larriva-Sahd (2008), also contains three layers: the Glo, the external cell layer (ECL), and the internal cell layer (ICL). The ICL, Glo and Gra incorporate newborn interneurons during adulthood (Alvarez-Buylla and Garcia-Verdugo, 2002). These interneurons regulate the activity of projection neurons, mitral and tufted cells, that reside in the Mi and external plexiform layer, respectively (Shepherd, 1972). The OB plays an important role in rodent sexual behavior, both the main and accessory olfactory systems modulate receptivity and proceptivity in the female rodent (Mackay-Sim and Rose, 1986; Saito and Moltz, 1986; Keller et al., 2006a). Moreover, Oboti et al. (2011) reported that newborn granule cells in AOB are essential for mating and partner recognition in the female mouse. Indeed, disruption of OB neurogenesis by focal radiation of the subventricular zone in adult female mice alters social interactions with males, but not with females (Feierstein et al., 2010).

Sexual behavior in female rodents has both appetitive and aversive consequences. A key component that reduces the aversive consequences and enhances the appetitive components is the female's ability to control or pace the sexual interaction (Erskine et al., 1989; Paredes, 2009, 2010). Studies in natural and naturalistic settings have shown that both males and females control or pace the sexual interaction (Robitaille and Bouvet, 1976; McClintock and Adler, 1978; McClintock and Anisko, 1982; McClintock et al., 1982). Paced mating induces physiological and behavioral changes in the female rat that enhance reproductive success (Erskine and Baum, 1982; Erskine et al., 1989). It has been demonstrated that there is a difference in the amount and timing of vaginocervical (VC) stimulation that the female receives during paced mating in comparison to non-paced mating (Coopersmith et al., 1996). This VC stimulation activates noradrenergic

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Abbreviations: AOB, accessory olfactory bulb; BNST, bed nucleus of the stria terminallis; BrdU, 5-bromo-2′-deoxyuridine; E2, estradiol; ER, estrogen receptor; Glo, glomerular layer; Gra, granule cell layer; ICL, internal cell layer; III, inter-intromission interval; LQ, lordosis quotient; MePD, posterodorsal medial amygdala; Mi, mitral cell layer; MLI, mean lordosis intensity; MOB, main olfactory bulb; mPOA, medial preoptic area; OB, olfactory bulb; P, progesterone; PBS, phosphate-buffered saline; RMS, rostral migratory stream; SGZ, subgranularzone; SVZ, subventricularzone; VC, vaginocervical; VMH, ventromedial nucleus of the hypothalamus.

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nuclei, that project to the posterodorsal medial amygdala (MePD) and the ventromedial nucleus of the hypothalamus (VMH) (Northrop et al., 2010) which may differentially activate other structures that respond to mating activity, such as the medial preoptic area (mPOA) and the bed nucleus of the stria terminallis (BNST) (Rowe and Erskine, 1993; Polston and Erskine, 1995).

We and others have repeatedly shown that sexual behavior is rewarding for rats of both sexes when they are allowed to pace the sexual interaction (Agmo and Berenfeld, 1990; Paredes and Vazquez, 1999; Arzate et al., 2011), and this state is modulated by opioids (Agmo and Gomez, 1993; Garcia-Horsman et al., 2008). We have also shown that paced mating induces plastic changes in the OB of adult females after one paced session. Specifically, there is an increase in the number of newborn cells that arrive to the granule (internal) cell layer of the AOB 15 days later (Corona et al., 2011). During this critical period, the survival of newborn neurons is activity dependent (Yamaguchi and Mori, 2005). The aim of the present study was to evaluate the effect of repeating the paced mating stimulation during the first 2 weeks of age of the new cells upon the maintenance of newborn neurons in the OB. We also determined plasma levels of estradiol (E2) and progesterone (P) among supplemented groups and a naturally cycling group of intact females, in order to determine if the increase in neurogenesis could be associated with different levels of these hormones.

EXPERIMENTAL PROCEDURES

Animals

Sexually naive Wistar female rats weighting 200–250 g were bilaterally ovariectomized after the administration of a mixture of ketamine (95 mg/kg) and xylazine (12 mg/kg) and randomly assigned to one of four groups ($n = 7$ for each group): (1) females without sexual contact (control), (2) females that were allowed to pace the sexual interaction during one session (pacing-1), (3) females that were allowed to pace the sexual interaction during four sessions (pacing-4), and (4) females that did not pace the sexual interaction during four sessions (non-pacing-4). A group of intact females without sexual contact was also included (intact, $n = 7$).

Starting a week after surgery, all ovariectomized females received a subcutaneous injection of E2 benzoate (25 μg/rat; Sigma St. Lous, Missouri, USA) and P (1 mg/rat; Sigma), 48 h and 4 h, respectively, before each behavioral test. These doses were chosen because they consistently induce sexual receptivity (Whalen, 1974; Ydstebo and Sodersten, 1977; Paredes and Martinez, 2001; Arzate et al., 2011). Only males with a high level of sexual experience were used as stimulus. All animals were maintained under a reversed light/dark cycle (12-h light/12-h dark; lights off at 09:00) with food and water *ad libitum*. The 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 local animal care committee.

Sexual behavior tests

The sexual behavior tests (1 h) began on day 1. The pacing-1 group mated only on day 1. The groups of females that mated repeatedly, pacing-4 and non-pacing-4, mated in four different tests every 5 days (days 1, 6, 11 and 16; see Fig. 1). We chose this interval because a natural cycling female rat enters in receptivity every 5 days (LeFevre and McClintock, 1988). All behavioral tests were performed in mating cages (40 cm × 60 cm × 40 cm) made of transparent acrylic. For the females that were allowed to pace the sexual interaction, a barrier with a hole in the base was placed in the middle of the cage, as a result, only the female could move freely from one side of the cage to the other controlling the rate of sexual interaction. The females were placed in the left side of the cage, whereas the male was always confined to the right side. When males were allowed to control the sexual interaction they mated without the removable partition, having free and continual access to the female and therefore females were not able to pace the sexual interaction (Paredes, 2009).

The following parameters of sexual behavior were registered: number of mounts, intromissions, and ejaculations; mount, intromission and ejaculation latency as well as the interintromission interval (III, ejaculation latency divided by the number of intromissions before ejaculation). Lordosis intensity was rated as follows: 0, no lordosis displayed; 1, when the female presented a medium lordosis after a sexual stimulation; and 2, when a full lordosis was displayed. In this way, the mean lordosis intensity (MLI, the sum of points per test divided by the number of lordotic responses) and the lordosis quotient (LQ, total number of lordosis responses divided by the total number of mounts plus intromissions multiplied by 100) were calculated.

5-Bromo-2′-deoxyuridine (BrdU) administration

During the first mating test (day 1), female groups were i.p. injected with the marker of DNA synthesis, BrdU. BrdU was diluted in saline and i.p. injected in three equal doses of 100 mg/kg: 1 h before the mating test, at the end of the test, and 1 h after the test to maintain BrdU available for labeling in the brain (Taupin, 2007). The intact group received the same doses of BrdU in the proestrus phase of the estrus cycle determined by the presence of many cornified epithelial cells and abundant nucleated epithelial cells in vaginal smears (LeFevre and McClintock, 1988; Hubscher et al., 2005).

Tissue preparation

Sixteen days after BrdU administration all groups were killed with an overdose of pentobarbital and underwent transcardiac perfusion with 0.1 M phosphate-buffered saline (PBS, pH 7.2) followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). Ovary-intact females were at proestrus stage before sacrifice. Before perfusion, blood samples were obtained by cardiac puncture and centrifuged during 15 min at 1300*g*. Plasma samples were stored at −80°C for posterior E2 and P determination. All brains were removed and cryopreserved in a 30% sucrose solution.

Immunodetection and quantification of BrdU-labeled cells

Brain sagittal sections of 30 μm were obtained using a cryostat and stored free-floating in 0.05 M Tris-buffered saline (TBS), pH 7.6 until immunolabeling. To determine the number of BrdU-positive cells in the different layers of the OB, every third section was selected from each animal and processed for immunohistochemistry. This technique was performed using the method previously described by Corona et al. (2011). We selected six tissue sections per animal that contained both the AOB and MOB and were incubated in 2 N HCl for 1 h at 37°C followed by incubation in 0.5% sodium borohydride during 15 min. To visualize the BrdU-labeled cells, sections were