



Research article

Decrease in neuronal spine density in the postpartum period in the amygdala and bed nucleus of the stria terminalis in rat



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HIGHLIGHTS

- Spine density in the (extended) amygdala was investigated in the peripartum period in rat.
- The spine density was significantly decreased at 4 days after delivery.
- The presence of pups after delivery influenced the spine density in the BNST.

ARTICLE INFO

Article history:

Received 8 December 2016

Received in revised form

28 December 2016

Accepted 17 January 2017

Available online 20 January 2017

Keywords:

Pregnancy

Postparturition

Spine density

Amygdala

Bed nucleus of the stria terminalis

ABSTRACT

In pregnancy and the postpartum period, many women have emotional instability and some suffer from depression. The ovarian steroid hormone milieu is markedly changed during these periods, and this hormonal change may be an important cause of peripartum emotional instability. The amygdala is a central region of emotion, and the bed nucleus of the stria terminalis (BNST), which is considered to be the extended amygdala, is also involved in the emotional response. The amygdala and BNST are well characterized as target brain regions for ovarian steroid hormones, and this suggests that the functional response of neurons in these regions to hormonal fluctuation is affected in the peripartum period. In this study, we investigated the neuronal morphology in the central (CeA) and basolateral (BLA) nucleus of the amygdala and BNST on gestational days 15 (G15) (mid-gestation) and 20 (G20) (late gestation) and 4 days after delivery (P4) (early postpartum) in rat. Golgi staining showed that the dendritic spine density, and particularly the number of mature mushroom-type spines, in the CeA, BLA and BNST was significantly decreased at P4, compared with G15 and G20 and with virgin females in the estrous phase in the normal estrous cycle (Est). Interestingly, the presence of pups after delivery influenced the spine density in the BNST. The density was significantly decreased with pup presence compared with pup absence at P4, and compared with G15, G20 and Est. These results provide fundamental insights into the neuronal basis underlying emotional instability during pregnancy and postpartum.

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1. Introduction

During pregnancy, women may have emotional sensitivity, increased anxiety, and emotional instability, such as mood, irritability and anxiety, from the first trimester to a few months after

delivery [1,2]. This emotional instability frequently deviates from the healthy range and falls into a pathological state. The postpartum period is the time of highest risk for development of depression, and the rate of postpartum depression is estimated to be 15% [3–5]. There is also evidence that depression is as common during pregnancy as postpartum [6–8], whereas anxiety generally tends to decrease throughout pregnancy [2,9,10].

Reproduction is associated with alterations in hormonal secretion [11]. Pregnancy, parturition and lactation result in dramatic changes in the neuroendocrine axis [2,12]. Ovarian steroid hormones (estrogen and progesterones) play important roles in

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maintenance of pregnancy, and the concentrations of these hormones in blood are increased by the developing placenta and reach much higher levels than in the normal sexual cycle [13]. After parturition, loss of the placenta results in a sudden decrease in circulating hormone levels. Estrogen and progesterone influence emotions and contribute to sex differences and variations in behavior in the sexual cycle [14,15]. Several studies have shown significant changes in anxiety-like behavior upon administration of estrogen and/or progesterone in rodent models [16,17].

Sex steroid fluctuations in the peripartum period are believed to play an important role in establishment of depressive symptoms due to changes in neural function and synaptic connectivity in brain regions regulating emotions [2,9,10,18,19]. The amygdala is an important nuclear complex that regulates emotional responses [20,21]. Neuroanatomically, the central nucleus (CeA) and basolateral nucleus (BLA) of the amygdala are particularly concerned with stress, fear and anxiety responses, as well as depressive symptoms [22–24]. The bed nucleus of the stria terminalis (BNST), which receives strong projections from the basolateral amygdala and is considered to be the extended amygdala, is also involved in anxiety and stress responses, including depressive-like behaviors, in rodent models [23,25–27]. The BLA, CeA and BNST express ovarian steroid hormone receptors [28,29] and contribute to regulation of sexually differentiated brain functions. These findings suggest that neurons in the BLA, CeA and BNST may be affected by changes in the peripartum hormonal milieu. In this study, we examined neuronal spine density during pregnancy and early postpartum in the amygdala and BNST of rat.

2. Materials and methods

2.1. Animals

Nulliparous female Wistar rats aged 12 weeks and primiparous pregnant Wistar rats were purchased from Shimizu Laboratory Supplies Co (Kyoto, Japan) and housed in plastic cages with standard bedding and continuous access to food and water. The temperature was maintained at 22 °C with a 12-h light/dark cycle.

Vaginal smears were taken from the nulliparous female rats to determine the ovarian cycle. The committee for Animal Research of Kyoto Prefectural University of Medicine authorized all animal experimental procedures, and the study conformed to international guidelines on the ethical use of animals.

Brains of dams on gestational days 15 (G15) and 20 (G20), and 4 days after delivery (P4) were used as samples. Brains from rats in the estrous phase (Est) in the normal estrous cycle were used as controls. To evaluate the effect of maternal care and lactation, we divided rats at P4 into two groups, in which dams were caged with their pups (P4(+)) or separated from the pups just after parturition (P4(-)).

2.2. Tissue preparation and Golgi staining

Rats were deeply anesthetized by intraperitoneal injection of sodium pentobarbital (100 mg/kg body weight). Fresh brains were removed and stained using a FD Rapid Golgi Stain Kit (FD NeuroTechnologies, Elliot City, MD, USA). The brain was sectioned coronally at 150 μm using a Cryostat (CM3050S; Leica, Nussloch, Germany). Stained tissues were observed with a digital biological microscope (DN-107T, AS ONE, Osaka, Japan). Pyramidal neurons in the amygdala (Fig. 1) and cerebral cortex (M1 and M2 areas), and bipolar neurons in the anterior division and the principal nucleus in the posterior division of the BNST were selected. Images were captured by a CCD camera on the microscope. Dendritic spines were counted along the first branch of the apical dendrite of pyramidal neurons or the primary dendrite of bipolar neurons, and spine density was determined by counting the number of spines in 10 μm . Fifteen neurons (N = 15) from 3 rats (5 neurons/rat) were analyzed for each condition and brain area. To count spines accurately, we captured three sequential dendrite images of near neighbor focal planes and observed carefully comparing each images. Spines were classified into mushroom-type, defined as those with enlarged head regions and a constricted neck; stubby-type, which lack neck regions; and thin-type, which have a length greater than the neck diameter, using previous definitions of the diameters of the head and neck of spines [30–32].

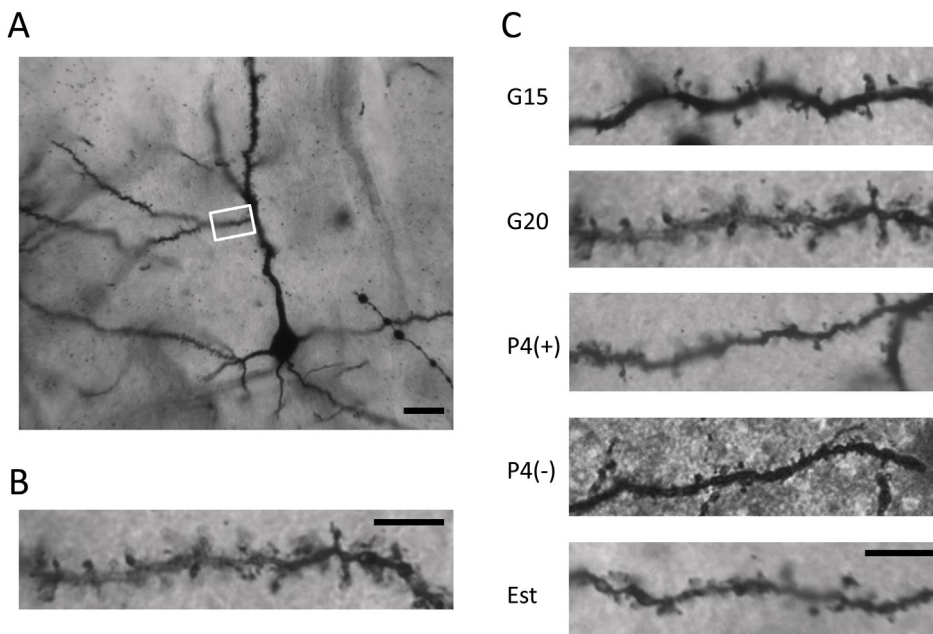


Fig. 1. (A) Photomicrograph of representative Golgi-impregnated pyramidal neurons from the CeA at G20. (B) Higher magnification of the first branch of the apical dendrite indicated by the white rectangle in (A). (C) Representative photomicrograph images of dendrite from the CeA of each group. Scale bars: 25 μm (A); 2 μm (B, C).

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