Estrogen, Predominantly via Estrogen Receptor α, Attenuates Postpartum-Induced Anxiety- and Depression-Like Behaviors in Female Rats

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Contributions from estrogen receptor (ER) subtypes (ERα and ERβ) to postpartum anxiogenic and depressive responses remain unresolved in rats. Using the elevated-plus maze (EPM) and forced swim (FS) tests, we confirmed that primiparous rats exhibited anxiogenic and depressive responses 3 weeks postpartum, improved 5 weeks postpartum (EPM), and recovered at 5 (FS) or 10 weeks postpartum (EPM) compared with diestrus nulliparous females. Immunohistochemistry suggested that these behavioral changes were temporally associated with decreased ERα but not ERβ expression in the medial amygdala (MEA). Additionally, ERα expression in the medial preoptic area (MPOA) significantly increased 10 weeks postpartum. Brain-derived neurotrophic factor (BDNF) expression was significantly elevated in the MEA 3 weeks postpartum. BDNF receptor tropomyosin-related kinase expression was significantly elevated in the MEA at 3 and 10 weeks but not at 5 weeks postpartum. The phosphorylation of ERK (pERK)-2 in the MEA, MPOA, and hippocampal CA1 region was significantly elevated 3 and 5 weeks postpartum. The effects of single daily sc injections of the ERα-selective agonist, propyl pyrazoletriol (PPT); ERβ-selective agonist, diarylpropionitrile; 17β-estradiol (E2); and vehicle for 6 days in primiparous rats were assessed. PPT and E2 significantly produced anxiolytic and antidepressant actions in the EPM and FS tests but PPT to a lesser degree than E2 in the EPM test. Diarylpropionitrile affected the EPM test but was not significantly different from vehicle. BDNF expression was significantly increased 3 weeks postpartum by all treatments in the MPOA but not the CA1 and MEA. E2 and PPT treatment significantly increased tropomyosin-related kinase and pERK1/2 expression in the MEA and MPOA and increased pERK1/2 expression in the CA1. The onset of anxiety- and depression-like behaviors in postpartum rats may be partly caused by a complex estrogen-mediated mechanism; nevertheless, changes in the ERα-related system, likely in the MEA, are predominantly involved.

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system is thought to be involved in anxiety-like behavior (13, 14) and depression (3, 15, 16). However, studies on mental health in humans have generally focused on estrogen receptor-α, the human ERα ortholog (17–21).

Brain-derived neurotrophic factor (BDNF) is widely expressed in the mammalian brain and is involved in a variety of brain functions (22). BDNF reportedly has effects similar to those of antidepressant drugs (23, 24), and there is a close relationship between BDNF signaling and animal depressive behavior caused by a variety of stress conditions (25, 26). Thus, it is easy to speculate that the down-regulation of BDNF is related to psychiatric disorders, such as depression. Although it is unclear whether BDNF is implicated in the pathophysiology of postpartum depression, most studies that have examined functional interactions between gonadal steroids and BDNF have demonstrated that estrogen up-regulates mRNA and/or protein expression of BDNF throughout the brain (27–29).

In the present study, we examined the effect of the ERα-selective agonist propyl pyrazoletriol (PPT), the ERβ-selective agonist diarylpropionitrile (DPN), and 17β-estra diol (E2) on postpartum anxiogenic and depressive responses in rats. We also determined changes in the expression levels of ERα and ERβ in primiparous rats. Furthermore, alterations in the expression of BDNF, its receptor tropomyosin-related kinase (TrkB), and the phosphorylation of ERK1/2, a downstream intracellular signaling molecule (30), were investigated in various brain regions of postpartum rats.

Materials and Methods

Animals

Female Long-Evans rats were purchased at 8.5 days of pregnancy, maintained in individual cages (276 x 445 x 204 mm), and allowed free access to water and food (MF; Oriental Yeast) under controlled lighting (12 h light, 1 h dark, lights on at 4:00 PM) and temperature (21–24°C). In the present study, the postpartum rats were used after the weaning of the offspring (day 21 after birth). All animal use procedures were in accordance with the Ethics Review Committee for Animal Experimentation of the National Institute of Neuroscience (Tokyo, Japan).

Experimental design

Experiment 1

Postpartum females (n = 8 per group) were used for behavioral tests starting on postpartum days 22 (3 week group), 36 (5 week group), or 71 (10 week group). Nulliparous females were used as controls and were studied during diestrus 1 (D1) of the estrous cycle. The estrous cycle was monitored daily by vaginal smear and D1 was judged as a smear containing predominantly leukocytes. Because the expression of ERα (31) and ERβ (32), as well as anxiety- and depression-like behaviors (13, 14, 16, 33), are affected by circulating estrogen levels, diestrus females were chosen as nulliparous controls in this study. Their plasma estrogen concentrations (8) are reportedly similar to those of 3-week-postpartum rats (9). In the behavioral tests, the same individual rat that experienced the elevated plus maze test on the particular postpartum day was subjected to the forced swim test and immunohistochemistry 2 days later. For immunoblotting experiments, another set of animals (n = 5 per group) was used with a time course similar to that of the elevated plus maze test.

Experiment 2

Animals were treated at 3 weeks postpartum (n = 6–8 per group) with a sc injection of dimethyl sulfoxide (DMSO; vehicle control), E2 (0.25 mg/kg), PPT (1.0 mg/kg), or DPN (1.0 mg/kg) once daily for 6 days using a total volume of 0.2 mL. PPT is selective for ERα, with a 400-fold preference for ERα compared with ERβ (34), whereas DPN has a 70-fold greater relative binding affinity and 170-fold greater relative potency in transcriptional assays for ERβ compared with ERα (35, 36). DPN and PPT were used at 1.0 mg/kg because this dose provided the same biological potency as 0.1 mg/kg of E2 (37, 38). Elevated plus maze and forced swim tests were conducted 30 minutes after the fourth and sixth injections, respectively. With the exception that the same individual rat that experienced the elevated plus maze test was subjected to the forced swim test 2 days later in this study, the doses, dosing regimen, and timing of the behavioral tests after treatment are the same as those in a previous report in ovariectomized rats (13), with the observation that within 30 minutes after peripheral administration, DPN was able to bind ER in the brain and that E2, PPT, and DPN each affected behaviors. For immunoblotting experiments, another set of animals (n = 5 per group) was used with a time course similar to that of the elevated plus maze test.

Behavioral testing

The elevated plus maze, which consisted of 2 open arms (100 x 100 cm) with 3-mm-high ledges and 2 equal-sized enclosed arms with 30-cm-high transparent walls (O’Hara & Co), was conducted as previously described (39). At the beginning of the test, the rats were placed at the junction of the open and closed arms facing one of the closed arms. Rodents generally avoided the open arms of the maze and preferred to remain in the closed (dark) arms (40). Thus, an increase in time spent on the open arms indicates antianxiety behavior.

In the forced swim experiments, the rats were tested in a chamber filled with 50 cm of water at 27°C as previously described (41). The time the rats spent immobile was recorded during the 5-minute task. The forced swim test is an animal model of despair, depression (42), and negative symptoms of schizophrenia (43). A decrease in the immobility time indicates antidepressant-like behavior (42).

Immunohistochemistry

After the behavioral assessments, the rats were deeply anesthetized by ip injection with 100 mg/kg sodium pentobarbital and transcardially perfused with cold PBS. The brains were rapidly collected, fixed by immersion for 24 hours in 4% paraformaldehyde in 0.1 M PBS, and soaked in 30% sucrose in PBS for 24 hours. The fixed brains were blocked, sectioned to 30-μm
thickesses exhaustively in the coronal plane using a cryostat, and processed for immunohistochemistry using a standard protocol (11). The sections were initially washed with 0.1 M PBS for 30 minutes at room temperature followed by incubation with antirabbit ERα (1:50 000, C13355; Millipore) and ERβ (1:500, PA1–310B; Thermo) antibodies for 60 minutes. The specificity of the antibodies for the ERs was verified previously by preabsorption with the peptide against which the antibody was produced and by ERα- or ERβ-transfected cells [(44, 45) for C13355 (46–48) for PA1–310B (Thermo)].

In this study, substitution of the primary antibodies with blocking solution revealed no immunoreactivity (data not shown). It should be kept in mind, however, that the specificity of PA1–310B for ERβ (Thermo) was controversial in a previous study (48) with ERβ knockout and ERβ null mice. As a secondary antibody, we used biotinylated antirabbit IgG diluted 1:200 (Vector Laboratories). To visualize the immunoreactivity, the brain tissues were incubated for 120 minutes at room temperature with an ABC kit (Vector Laboratories) diluted 1:800 with PBS. These tissues were reacted with 0.05% dianinobenzidine (Sigma), 8% nickel ammonium sulfate, and 0.003% H2O2 in PBS for 10 minutes; dehydrated with increasing grades of alcohol and xylene; and coverslipped using Entellan (Merck).

**Figure 1.** Schematic illustration of coronal brain sections, modified from the rat atlas of Paxinos and Watson (66), examined for ERα and ERβ immunoreactivity. ER-ir cells were counted in the regions outlined by thick gray squares (600 × 600 μm) of the MPOA, hippocampal CA1 region (CA1), MEA, and PVN (for ERβ only). ac, anterior commissure; Arc, arcuate hypothalamic nucleus; CA2 and CA3, hippocampal CA2 and CA3 regions; cc, corpus callosum; cg, cingulum; MoDG, molecular layer dentate gyrus; Och, optic chiasm; opt, optic tract; Pe, periventricular hypothalamic nucleus; PMCo, postero medial cortical amygdala nucleus; PS, parasitral nucleus; Shy, septohypothalamic nucleus; STIA, bed nucleus striatum intra amygdala; 3V, third ventricle; VMH, ventromedial hypothalamic nucleus.

**Determination of the number of ER-immunoreactive (ER-ir) cells**

The number of cells with a clear blue-black nucleus (which represents ER immunoreactivity) was counted by visual inspection performed by an investigator who was blinded to the experimental groups. The bilateral number of ER-ir cells in a square region (600 × 600 μm, as illustrated in Figure 1) on serial 30-μm-thick sections was determined, and an average value across all sections was calculated for each rat. Approximately 5 sections per rat were counted in the medial preoptic area (MPOA), hippocampus (CA1 region), and medial amygdala (MEA). The MPOA is thought to be involved in fear/anxiety (49) and reproductive behaviors (50), whereas the hippocampus (51, 52) and MEA (53–55) are reportedly important for anxiety and depressive behaviors. The paraventricular nucleus (PVN), known to be important for stress response (15), was also included but only in the analysis of ERβ-ir cells because ERβ is abundant in the nucleus (11).

**Immunoblotting**

While under light anesthesia with carbon dioxide, the rats were decapitated. The brains were rapidly collected, frozen on dry ice, and sectioned to 500-μm thickness exhaustively in the coronal plane using a cryostat. A single micropunch sample (1 mm in diameter) was bilaterally dissected from the MPOA, CA1 region, and MEA on each of 2 serial sections. Tissue micropunch samples were homogenized and lysed in sodium dodecyl sulfate lysis buffer containing 1% sodium dodecyl sulfate, 20 mM Tris-HCl (pH 7.4), 5 mM EDTA (pH 8.0), 10 mM NaF, 2 mM Na3VO4, 0.5 mM phenylarsine oxide, and 1 mM phenylmethylsulfonyl fluoride. The protein concentration was determined using a BCA protein assay kit (Pierce) and equivalent proteins were loaded during each immunoblot. The primary antibodies used were anti-BDNF (1:250; Santa Cruz Biotechnology), anti-TrkB (1:1000; BD Biosciences), anti-ERK (1:1000; Cell Signaling Technology), anti-phosphorylated ERK (pERK; 1:1000; Cell Signaling Technology), and anti-β-actin (1:5000; Sigma). Immunoreactivity was quantified using the Lane and Spot analyzer software (ATTO Corp). Data were normalized to each control group.

**Measurement of plasma estrogen concentrations**

After decapitation (see Immunohistochemistry), trunk blood was collected from D1 nulliparous rats and from 3-week postpartum rats treated with vehicle, E2, PPT, and DPN. Plasma was isolated by centrifugation. Estradiol was measured in duplicate 50-μL aliquots of the plasma using an estradiol enzyme immunoassay kit (Cayman Chemicals).
Statistical analysis

Data are expressed as the mean ± SEM. For each group, all data from the behavioral, immunohistochemical, and endocrinological studies were analyzed using an ANOVA followed by Fisher’s protected least significant difference (LSD) post hoc test. Differences were considered significant at \( P < .05 \). The statistical significance of the results of the Western blotting analysis was determined using a nonparametric Mann-Whitney \( U \) test \( (P < .05) \).

Results

Postpartum changes in behavioral tests

In the elevated plus maze, 3-week-postpartum rats spent a significantly less time in the open arms than D1 nulliparous control rats \( (F_{3,22} = 13.90; P < .05; \) Figure 2A). The time spent in the open arms in the 10-week-postpartum rats was not different from that of the D1 nulliparous controls. The time spent in the open arms in the 5-week-postpartum rats was between those of 3- and 10-week-postpartum rats and was not significantly different from other groups, suggesting that the anxiogenic response revealed by the elevated plus maze task peaked at 3 weeks, improved at 5 weeks, and resolved at 10 weeks after the delivery compared with the D1 nulliparous controls within the time course we examined.

Three weeks postpartum, the rats also exhibited a significantly longer immobility time during the forced swim test \( (F_{3,21} = 5.56; P < .05 \) vs D1 nulliparous controls, 5 and 10 weeks postpartum rats; Figure 2B). The different recovery times between the elevated plus maze and forced swim test suggest a distinct control mechanism for these behaviors.

Postpartum changes in ER\( \alpha \) distribution

An immunohistochemical study was performed to investigate the involvement of different ER subtypes in postpartum anxiogenic and depressive responses. We selected several brain regions associated with anxiety- and depression-like behaviors as well as with the reproductive system.

In the MPOA, a significant increase in the number of ER\( \alpha \)-positive cells was observed in the 10-week-postpartum rats compared with the D1 nulliparous controls \( (MPOA, F_{3,12} = 4.26; P < 0.05; \) Figure 3, A and B). The prevalence of ER\( \alpha \)-positive cells in the 3- and 5-week-postpartum rats was not different from the D1 nulliparous controls, nor was the prevalence different between any postpartum groups. Differences among the 4 groups were not observed in the distribution of ER\( \alpha \) immunoreactivity in the CA1 region \( (F_{3,12} = 0.69; \) Figure 3, A and B). In the MEA, the 3-week-postpartum
rats exhibited a significant decrease in the number of ER\(\alpha\)-positive cells compared with the D1 nulliparous controls (MEA, \(F_{3,13} = 5.58; P < 0.05; \) Figure 3, A and B). Ten weeks postpartum, the rats exhibited a significant increase in the number of ER\(\alpha\)-positive cells. These results suggest that postpartum changes in the ER\(\alpha\) mechanism vary among the brain regions.

In ER\(\beta\)-positive cells, no evident differences in expression levels were observed in any of the tested brain regions (MPOA, \(F_{3,21} = 0.61; \) PVN, \(F_{3,21} = 0.48; \) CA1, \(F_{3,21} = 0.32; \) and MEA, \(F_{3,18} = 0.19\)) in rats during any of the postpartum periods compared with the D1 nulliparous controls (Figure 4, A and B). These results led us to speculate that an ER\(\alpha\)-related mechanism was involved in postpartum-induced anxiety- and depression-like behaviors in rats.

**Postpartum changes in BDNF and TrkB**

To clarify the possible involvement of BDNF/TrkB signaling in postpartum anxiogenic and depressive responses, we performed Western blotting using samples obtained from the MPOA, CA1, and MEA of 3-, 5-, and 10-week-postpartum and D1 nulliparous controls. No significant change was observed in BDNF expression in the MPOA or CA1 in each postpartum group compared with the D1 nulliparous controls (Figure 5A). However, BDNF levels in the MEA significantly increased only in the 3-week-postpartum rats \((P < .05)\), whereas no obvious

![Figure 4. A, ER\(\beta\) expression in the MPOA, PVN, CA1 region, and MEA of 3-, 5-, and 10-week-postpartum rats and diestrus nulliparous rats, D1. Data represent mean ± SEM. Scale bar, 30 µm. opt, optic tract; 3V, third ventricle. B, The number of ER\(\beta\)-ir cells in the MPOA, PVN, CA1 region, and MEA of 3-, 5-, and 10-week-postpartum rats and diestrus nulliparous rats, D1 \((n = 8\) animals per treatment group). Different letters indicate significant differences among the groups \((P < .05)\) by Fisher’s protected LSD test.]
changes in BDNF levels were observed in the MEA of the 5-week or 10-week-postpartum rats. We observed significant increases in the expression of the TrkB receptor for BDNF in the MEA of 3-week and 10-week-postpartum rats compared with the D1 nulliparous controls ($P < 0.05$; Figure 5B). No significant change was observed in the MEA of 5-week-postpartum rats. There was a trend toward increased TrkB expression detected in the MPOA and CA1 region of 3-week-postpartum rats, although these results were not statistically significant. These results imply that the BDNF system was associated with postpartum-induced anxiety- and depression-like behaviors but that the mechanism was rather complicated.

**Postpartum changes in pERK1/2**

We examined changes in the phosphorylation (activation) of ERK1/2 in postpartum rats (Figure 5C) because ERK1/2 is known to be stimulated through BDNF/TrkB signaling (30). The level of pERK1/2 significantly increased in the MPOA and CA1 region in 3- and 5-week-postpartum rats compared with the D1 nulliparous controls ($P < 0.05$), whereas no corresponding change was observed in 10-week-postpartum rats. Furthermore, the 3- and 5-week-postpartum rats exhibited a significant increase in pERK2, but not pERK1, in the MEA compared with the D1 nulliparous controls ($P < 0.05$). In all of the regions tested, the levels of ERK1 and ERK2 expression in the 3 postpartum groups were comparable with those of the D1 nulliparous controls.

**Effect of ER agonists on postpartum anxiety- and depression-like behaviors**

We tested the hypothesis that estradiol exerts antidepressant and anxiolytic effects through ER$\alpha$ and/or ER$\beta$ in primiparous 3-week-postpartum rats based on 2 behavioral paradigms for investigating anxiety- and depression-like behaviors. Three-week-postpartum rats treated with E2 and PPT spent significantly longer times in the open arms of the elevated plus maze than vehicle-treated 3-week-postpartum rats ($F_{3,16} = 8.38; P < 0.05$; Figure 6A), but the effects of E2 were stronger than those of PPT because the time spent in the open arms of the maze in the PPT-treated rats was between that of E2-treated and the control rats. Although there was no significant difference in the time spent in the open arms of the maze in the DPN-treated group compared with the control treatment, times in the DPN-treated group were between those of the PPT-treated and control groups. These results suggest that the ER$\alpha$-mediated mechanism was predominantly involved in postpartum-induced anxiety- and depression-like behaviors, but another mechanism, such as an ER$\beta$-mediated mechanism, was also involved.

Three-week-postpartum rats treated with E2 and PPT equally displayed a significantly shorter immobility time than control or DPN-treated animals during the forced swim test ($F_{3,16} = 3.48; P < 0.05$; Figure 6B). These dif-
Effects of ER agonists on postpartum BDNF, TrkB, and intracellular signaling

We investigated the possibility that changes in ERα-mediated neuronal function in 3-week-postpartum rats affected BDNF, TrkB, and ERK1/2 signaling (Figure 7, A–C). We observed a significant increase in BDNF expression in the MPOA after treatment with E2, PPT, and DPN ($P < 0.05$ for all vs vehicle treated controls; Figure 7A). There were trends toward increased BDNF expression in the CA1 region and MEA with the E2, PPT, and DPN treatments, but these differences were not statistically significant. We also observed a significant increase in the TrkB expression in the MPOA and MEA after treatment with E2 and PPT but not with DPN (Figure 7B). There was a trend toward increased TrkB expression in the CA1 region with E2 treatment, but the difference was not statistically significant. The phosphorylation of ERK1/2 in the MPOA, CA1 region, and MEA was significantly up-regulated by E2 and PPT ($P < 0.05$ vs vehicle treated controls; Figure 7C) but not by DPN. In all of the regions tested, no change in the expression of ERK1 or ERK2 was observed after the administration of E2, the ERα agonist, or the ERβ agonist.

Plasma estrogen levels

Plasma estrogen concentrations differed significantly among D1 nulliparous rats and 3-week-postpartum rats treated with vehicle, E2, PPT, and DPN ($F_{4,21} = 3.37; P < 0.05$). Treatment with E2 caused a significant increase in the plasma estrogen concentrations compared with the other treatment groups ($E_2, 23.53 \pm 7.20$ pg (mean ± SEM); D1, $11.88 \pm 1.04$ pg; vehicle, $9.52 \pm 2.84$ pg; PPT, $13.78 \pm 7.83$ pg; and DPN, $10.74 \pm 3.31$ pg). No significant difference was observed between other groups.

Discussion

In this study, postpartum primiparous female rats exhibited anxiogenic and depressive responses in the elevated plus maze and forced swim tests, respectively, compared with nulliparous female rats. Because the time course of the changes in these behavioral tests were different, distinct mechanisms may be involved in controlling these behaviors. Nonetheless, this result was in accordance with previous studies (4, 5, 56), which reported that a decline in circulating ovarian steroids caused postpartum symptoms using a hormone-simulated pseudopregnant rat model that mimicked the high estrogen and progesterone levels in late pregnancy and their rapid decline after delivery.

In the present study, treatment of 3-week-postpartum rats with E2 and an ERα agonist attenuated anxiety- and depression-like behaviors; this finding suggests the importance of ERα-mediated signals in these behaviors. In support of this view, it was recently reported that the change in ERα activity is involved in the regulation of anxiety-like behavior, depending on the reproductive experience (57). The idea is further supported by the immunohistochemical analysis in the present study. We have shown alterations in the expression of ERα but not ERβ in several brain regions in 3-week-postpartum rats, including a significant reduction in the MEA, which is an important structure for emotional responses related to anxiety and depressive behavior (53–55). The present result disagrees with previous reports showing that ERβ-selective but not ERα-selective agonists produced anxiolytic and antidepressant effects in nulliparous ovariectomized rats (13, 14, 16, 33). However, the results should be interpreted with caution. Because PPT did not improve anxiety-like behavior in postpartum rats in the elevated plus maze test to the extent achieved by E2, and DPN seemed to be effective, although not statistically significant compared with vehicle treatment, we cannot dismiss the possibility that an ERβ-mediated mechanism was involved as well. In addition, other data such as swim and climbing time, which would affect the overall interpretation of the results, may be needed to fully determine any antidepressant effect in the forced swim test.

The ERα gene has been a main focus in human mental health studies (17, 19). For example, some studies provide evidence of a relationship between the ERα gene $ESR1$ variants and the risk of certain mood disorders, including
anxiety, depression, premenstrual dysphoric disorder, and postmenopausal depression (18, 20). In addition, reduced ERα mRNA levels were also reported in the baso-medial nucleus of the amygdala in women with major depressive and bipolar disorders (58). Not only in human but also in rats, in agreement with our results showing that ERα has a role in postpartum anxiogenic and depressive responses, the expression of ERα reportedly varied between nulliparous and primiparous conditions (59). Basically, it is not clear what might underlie the distinctly different responses to a certain ER agonist in ovariectomized rats vs 3-week-postpartum rats. This study used a case design equivalent to that of a previous study (13) in ovariectomized rats (see Experimental design in Materials and Methods). Therefore, a possibility that pregnancy and/or a subsequent series of maternal behaviors in this study affected neural plasticity cannot be ruled out. Maternal experience and the associated hormonal changes reportedly influence various aspects of the neural system in numerous brain regions (60–62). In particular, the anterodorsal portion of the MEA exhibits more dendritic spines in postpartum rats compared with their nulliparous counterparts (63). Further studies are warranted to determine whether such changes after maternal experiences have functional consequences toward the distinctive estrogen responsiveness. Overall, we hypothesize that the MEA is involved in causing postpartum anxiety- and depression-like behaviors via an ERα mechanism. Additionally, postpartum symptoms are mediated through ERα; anxiety and depression, which are not related to gestation, are regulated through ERβ.

Several studies have shown that emotional stress induces c-Fos expression in the MEA (54, 55), suggesting that these types of stressors activate the MEA. In agreement with this finding, ERK2 was phosphorylated in the MEA of primiparous rats that exhibited anxiogenic and depressive responses in the present study. The results of the present study are complicated, but the first immunohistochemical study leads us to speculate that the responsible site is the MEA; the change in the expression of BDNF only in the MEA further supports this speculation (see Figure 5). BDNF in the MEA is critically involved with alcohol-related anxiety-like behaviors in rats and acts as an anxiolytic (64, 65). Therefore, it is conceivable that decreased BDNF/TrkB signaling induces postpartum anxiogenic and depressive responses. However, the result was just the opposite; BDNF in the MEA was increased in association with anxiogenic and depressive responses in postpartum primiparous female rats. We can only assume that a larger increase in BDNF/TrkB signaling above a certain thresh-
old may be indispensable for reinstating postpartum anxious and depressive responses because the antidepressant actions of BDNF are reportedly dose dependent (24). In experiment 2, the injection of E2 and PPT further in-

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