Fluvoxamine reverses estrogen-dependent decline in voluntary activities and decreased amygdala levels of serotonin in ovariectomized rats

（フルボキサミンは、卵巣摘出ラットのエストロゲン消失による自発運動量の抑制と扁桃体セロトニン遊離量の減少を回復する）

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Research and reports

Fluvoxamine reverses estrogen-dependent decline in voluntary activities and decreased amygdala levels of serotonin in ovariectomized rats

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Abstract

Studies suggest that increased expression of brain-derived neurotrophic factor (BDNF) could mediate the antidepressant effects of drugs. We analyzed the effects of fluvoxamine on locomotor activities, serotonin levels in the amygdala, and hippocampal expression of BDNF mRNA in ovariectomized (OVX) rats. Female Wistar rats (8 weeks, 180–200 g) were divided into four groups: sham; OVX; OVX with estrogen; and OVX with fluvoxamine. Six weeks after ovariectomy, rats were assessed according to spontaneous locomotor activity, forced-swimming test (FST), and microdialyses experiments. Body and uterine weight of OVX rats 6 weeks after surgery were significantly increased and decreased, respectively, compared with those of the sham group, but these changes were returned to sham-group levels upon chronic administration of estrogen and fluvoxamine. More potent decreases in voluntary activities were observed in OVX rats compared with rats in the sham group, but were increased markedly upon administration of estrogen and fluvoxamine. In the FST, immobility time and beat counts were increased and decreased significantly by ovariectomy compared with those of the sham group, respectively, but estrogen and fluvoxamine treatment reversed these changes significantly. More potent

decreases in serotonin release in the amygdala were observed in OVX rats compared with those of sham rats, but were reversed upon estrogen replacement. Similar recovery was observed in OVX rats upon fluvoxamine treatment. These data suggest that, in OVX rats, chronic administration of fluvoxamine can recover estrogen-dependent changes in behaviors, decreased serotonin release in the amygdala, and reduced expression of BDNF mRNA.

(245 words)

Keywords: Amygdala, Serotonin, Estrogen, Fluvoxamine, OVX

Abbreviations

BDNF, brain-derived neurotrophic factor; ER, endoplasmic reticulum; OVX, ovariectmized; RT-PCR, reverse transcription-polymerase chain reaction; SSRI, selective serotonin reuptake inhibitor; XBP-1, X-box binding protein-1; mRNA, messenger ribonucleic acid

1. Introduction

The female hormone estrogen has an important role in bone physiology (reviewed in [15, 16]). Moreover, there is a close relationship between estrogen and memory, learning, and emotion in the brain (reviewed in [16]). Also, it has been reported that replenishment with estrogen can improve recognition, learning and memory (reviewed in [3, 5, 14]).

We reported that, in female rats 6 weeks after their ovaries had been removed, depression-like symptoms (as manifested by decreases in spontaneous locomotor activities and serotonin levels in the amygdala) could be observed [9].

Depression is a complex disorder brought about by genetic and environmental conditions. Depression involves brain abnormalities as well as dysfunction of the endocrine system, inflammation, altered glucose metabolism and, in some cases, coronary artery disease [8, 18]. Selective serotonin reuptake inhibitors (SSRIs) are first-line treatment for depression and depression-like symptoms. However, resistance to the effects of antidepressants has been documented, so development of new agents with new mechanisms of action is needed [13].

Studies have suggested that brain-derived neurotrophic factor (BDNF) signaling is necessary and sufficient for the action of antidepressant drugs [20]. BDNF signaling is associated with cyclic adenosine monophosphate responsive element binding protein, which induces neurogenesis [20]. Moreover, high levels of BDNF have been observed in *post mortem* hippocampal samples from individuals suffering from depression [20]. Such findings suggest a correlation between decreased expression of BDNF and the onset of depression. Furthermore, a recent report demonstrated that the SSRI fluvoxamine reversed the reduced expression of BDNF messenger ribonucleic acid (mRNA) by chronic infusion of dexamethasone in mice exhibiting depression-like behaviors [17]. Therefore, the therapeutic effects of antidepressants could be mediated by increased expression of BDNF.

In the present study, we analyzed the chronic (6-week) effects of the antidepressant fluvoxamine on locomotor activities, serotonin levels in the amygdala, and expression of BDNF mRNA in the hippocampus in ovariectomized (OVX) rats.

2. Materials and methods

2.1 Ethical approval of the study protocol

The study protocol was approved by the Ethics Committee of Yokohama College of Pharmacy (Kanagawa, Japan). All experiments using animals were carried out based on *Guidelines for the Care and Use of Laboratory Animals* (National Institutes of Health, Bethesda, MD, USA) as approved by the Japanese Pharmacological Society.

2.2 Animals, ovariectomy, and drug administration

Animal care and ovariectomy were as described previously with minor modification [9]. Briefly, 36 female Wistar rats (8 weeks, 180–200 g) were divided into four groups of nine. Rats were allowed to acclimatize for ≥1 week to their surroundings before the start of experimentation (12-h light–dark cycle; lights on at 7 am and off at 7 pm). After 1 week, OVX and sham operations were undertaken as described previously (Fukushima et al., 2000). One week after ovariectomy, fluvoxamine (50 mg/kg body weight; Wako Pure Chemical Industries, Osaka, Japan) and β-estradiol (50 μg/kg body weight; Sigma–Aldrich,

Saint Louis, MO, USA) were administered (p.o. and i.p., respectively) five times per week for 6 weeks.

2.3 Voluntary momentum, forced-swim test (FST), and microdialyses

Six weeks after ovariectomy, rats were assessed according to spontaneous locomotor activity, FST, and microdialyses experiments, as described previously [9, 17].

2.4 Quantitative reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR was carried out as described previously [17].

2.5 Statistical analyses

Statistical analyses were undertaken as described previously [17].

3. Results

Body weight and uterine weight of OVX rats 6 weeks after surgery were increased and decreased significantly, respectively, compared with that of the sham group (Fig. 1a and b; sham and OVX groups). The increased body weight and decreased uterine weight of OVX rats returned to sham-group levels upon chronic administration of estrogen (Fig. 1a and b; sham and estrogen groups). A tendency for recovery was observed upon chronic administration of fluvoxamine in terms of increases and decreases of body weight and uterine weight, but the differences were not significant (Fig 1a and b; sham and fluvoxamine groups).

Figure 2a shows spontaneous locomotor activity for 12 h in the dark phase (7 pm to 7 am) for the four groups 6 weeks after ovariectomy. More potent decreases in voluntary activities were observed in OVX rats compared with rats in the sham group (Fig. 2b). These data were similar to those of our previous report [9]. These decreases in locomotor activities in OVX rats in the dark phase were increased markedly upon administration of estrogen and fluvoxamine (Fig. 2b).

Next, we analyzed the immobility time and beat counts on FST of the four rat

groups (Fig. 3). Immobility time and beat counts were increased and decreased significantly by ovariectomy compared with those of the sham group, respectively (Fig. 3a and b; sham and OVX groups). These data were consistent with our previous report [9]. Treatment with estrogen and fluvoxamine reversed these changes significantly to levels seen in control rats (Figs. 3a and b).

Next, we analyzed serotonin levels in the amygdala under identical conditions (Fig. 4). More potent decreases in serotonin release in the amygdala were observed in OVX rats compared with those of sham rats (Fig. 4a). These observations were consistent with our previous report [9]. These decreases in serotonin levels in the amygdala were reversed upon estrogen replacement (Fig. 4a; OVX and estrogen), suggesting that decreases in serotonin level by ovariectomy were dependent upon the disappearance of estrogen. A similar recovery was observed in OVX rats upon fluvoxamine treatment (Fig. 4a; OVX and fluvoxamine). However, this recovery in serotonin release was not detected in the case of dopamine release in the amygdala (Fig. 4b). This result was expected because SSRIs have a mechanism of action specific for serotonin.

Next, we analyzed hippocampal BDNF expression of sham rats and OVX rats using RT-PCR (Fig. 5). No significant changes were observed in samples from any group obtained from the cerebral cortex or brainstem (Fig. 5; middle and lower panels). More potent decreases in hippocampal BDNF expression were observed in the OVX group compared with that in sham groups (Fig. 5; upper panel). This elevation of BDNF expression was recovered markedly upon estrogen treatment (Fig. 5; upper panel). Hippocampal BDNF expression in OVX rats was recovered significantly upon fluvoxamine treatment (Fig. 5; upper panel). Hippocampal expression of the estrogen receptor-1 gene was not changed in all groups (data not shown).

4. Discussion

The present study showed that the body weight and uterine weight of OVX rats 6 weeks after ovariectomy were increased and decreased significantly compared with that of the sham group, respectively (Fig. 1), and that this phenomenon was consistent with previous reports [1,4,9]. These results suggest that we carried out ovariectomy successfully. Such increases in body weight and decreases in uterine weight in OVX rats were returned to sham-group levels upon chronic administration of estrogen (Fig. 1), suggesting that the increased weight of OVX rats was significantly dependent upon disappearance of estrogen. Moreover, this tendency for recovery in increases and decreases in body weight and uterine weight was also observed upon chronic administration of fluvoxamine except that the differences were not significant (Fig. 1).

The present study demonstrated that BDNF expression was decreased significantly in the hippocampus, but not the cerebral cortex, of OVX rats, and these effects were dependent upon estrogen (Fig. 5). Marked recovery of decreases in hippocampal BDNF expression was observed in OVX rats upon fluvoxamine administration, but not in the cerebral cortex. Hippocampal BDNF

and its neurogenic effects are closely related to depression and depression-like behaviors in animals. Therefore, it is strongly suggested that increased expression of BDNF causes replacement of depression-like symptoms in OVX rats. Similar results have been observed in the hippocampi of OVX rats undergoing exercise [10] and in the whole brains of depressed mice (induced by chronic infusion of dexamethasone) treated with fluvoxamine [17]. Our data support reports suggesting that the hippocampus is an important component of the limbic system, is closely related to depression, and a putative area for estrogens [11,19].

The affinity of fluvoxamine to the sigma-1 receptor (which binds to fluvoxamine and has neuroprotective activities) as a “molecular chaperone” under endoplasmic reticulum (ER) stress is higher than that observed for other antidepressants [12]. Unfolded protein responses and ER stress responses are also associated with depression. These responses are activated and required for neurite outgrowth by BDNF signaling due to increases in expression of X-box binding protein-1 (XBP-1) [6,7]. However, the depression-like behavior due to ovariectomy and recovery upon fluvoxamine administration shown in the present

study may be dependent upon another BDNF signal because the sigma-1 receptor and ER stress-related gene XBP1, in addition to the BDNF signal, have been linked to depression-like behavior due to dexamethasone infusion [17].

Our previous study suggested that OVX rats could become models of depression because ovariectomy elicits depression-like symptoms, such as a decline in spontaneous movement and decreases in serotonin release in the amygdala [9]. In the present study, in OVX rats, chronic administration of fluvoxamine recovered: estrogen-dependent changes in behaviors (Fig. 2 and 3); decreased serotonin release in the amygdala (Fig. 4); reduced expression of BDNF mRNA (Fig. 5). We are the first research team to show such data, though our experimental setup may be different to that of other scholars [2]. Nevertheless, the observations in the present study strongly support those in our previous work [9].

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Conflicts of interest

The authors have no conflicts of interest to declare

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Figure legends

Fig. 1. Body weight (a) and uterine weight (b) of rats in sham, OVX, OVX with estrogen, and OVX with fluvoxamine groups. **P<0.01.

Fig. 2. Spontaneous locomotor activities of rats in sham, OVX, OVX with estrogen, and OVX with fluvoxamine groups 6 weeks after ovariectomy. Data are the number of counts (a) and summed counts over 12 h in the dark phase (7 pm to 7 am) (b). *P<0.05, **P<0.01.

Fig. 3. Forced-swimming tests in rats of the sham, OVX, OVX with estrogen, and OVX with fluvoxamine groups 6 weeks after ovariectomy. Data are the immobility time (a) and summed counts of the number of beats (b). *P<0.05, **P<0.01.

Fig. 4. Release of serotonin and dopamine in the amygdala of rats in sham, OVX, OVX with estrogen, and OVX with fluvoxamine groups 6 weeks after ovariectomy. Data are the summed amounts (pg) of serotonin (a) and dopamine (b) over 12 h in the dark phase (7 pm to 7 am). **P<0.01.

Fig. 5. Quantitative RT-PCR of BDNF mRNA in the hippocampus (upper panel), cerebral cortex (middle panel) and brainstem (lower panel) of rats in sham, OVX, OVX with estrogen, and OVX with fluvoxamine groups. **P<0.01.

Fig. 1

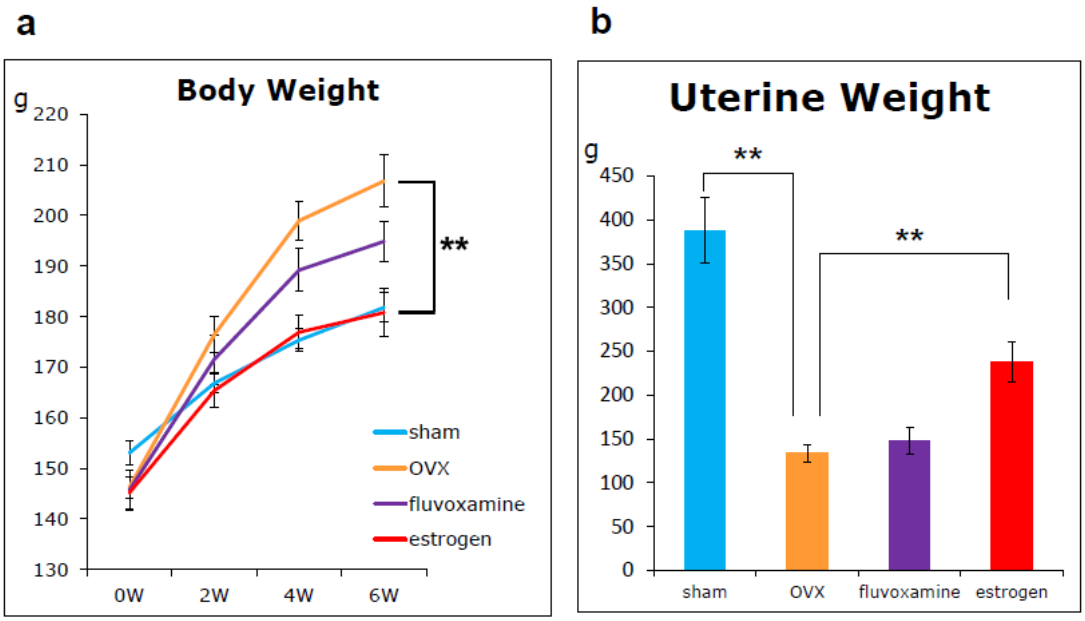
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Fig. 2

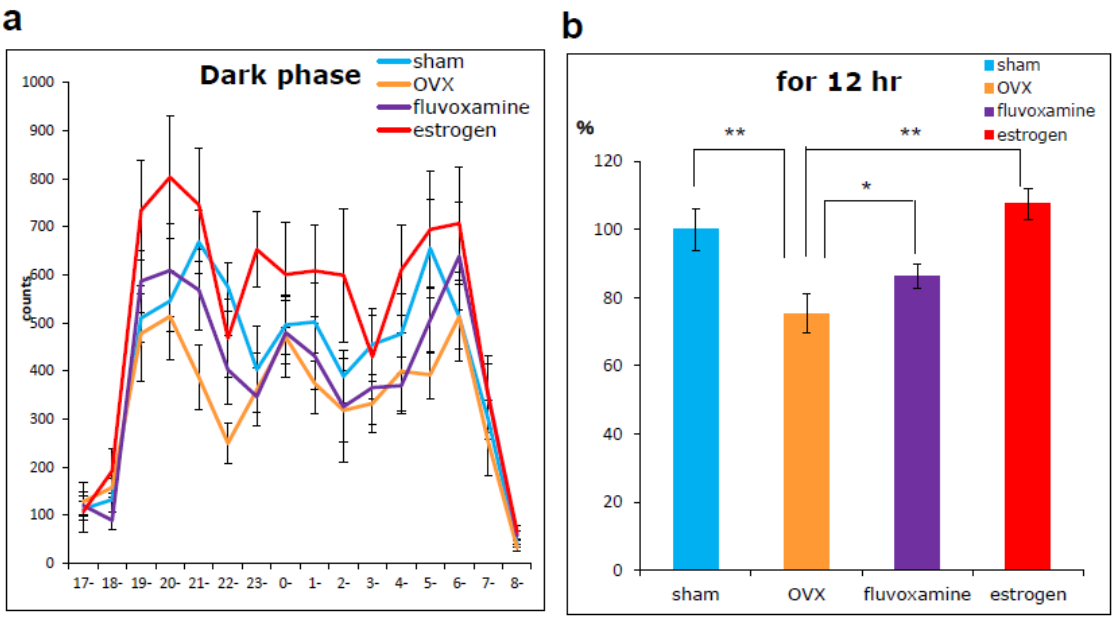
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Fig. 3

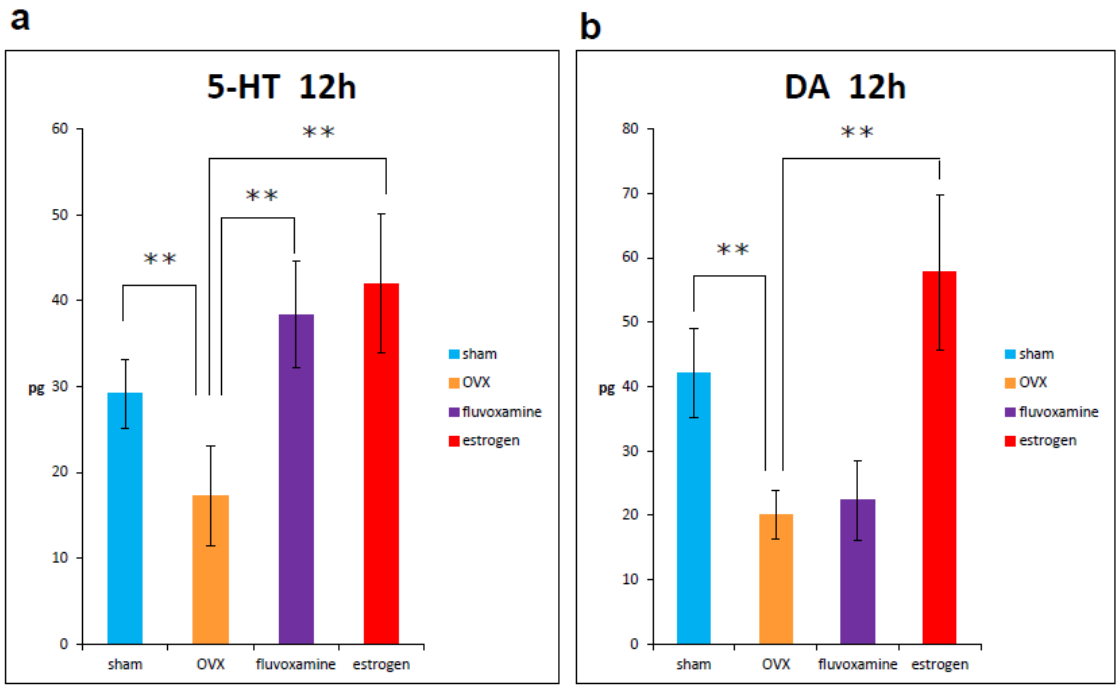
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Fig. 4

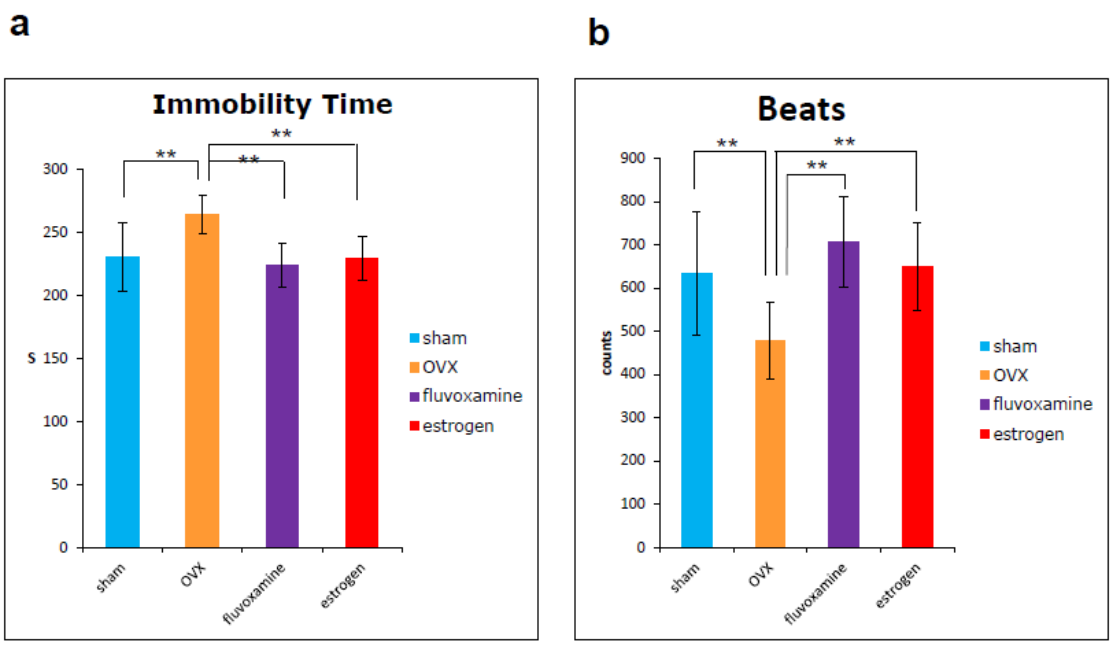
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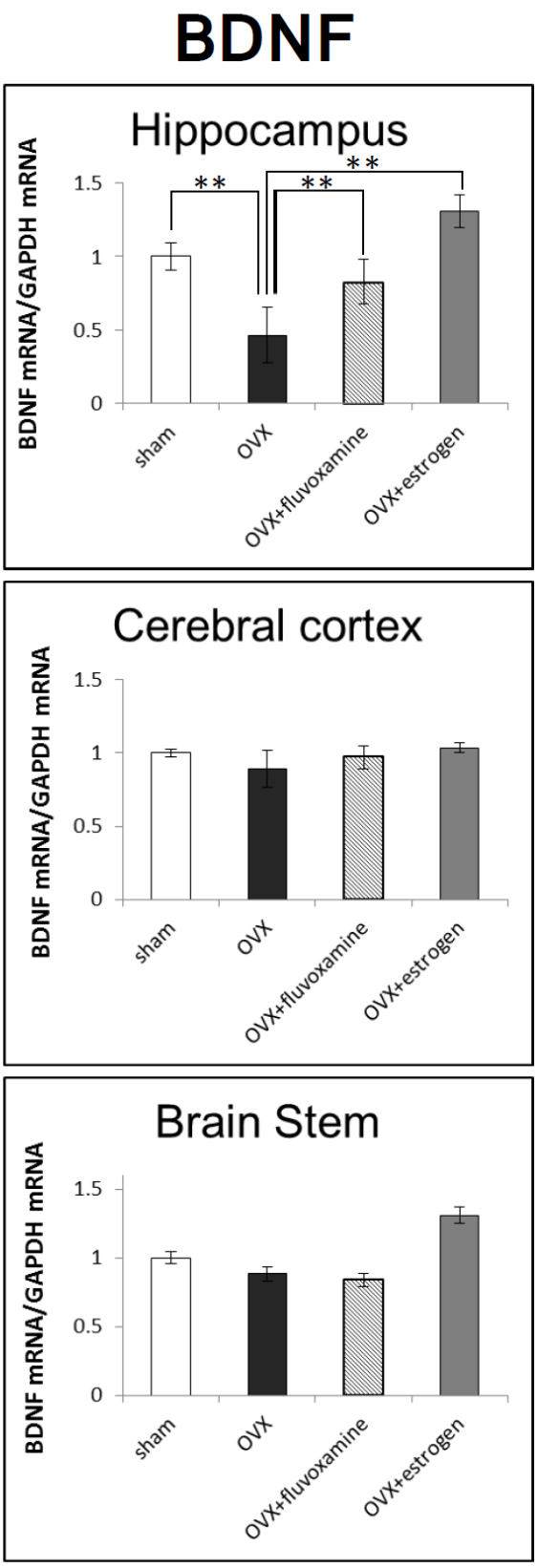
Fig. 5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Cerebral cortex
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OVX
OVX+fluvoxamine
OVX+estrogen
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BDNF mRNA/GAPDH mRNA
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