Expression and Function of Estrogen Receptor Beta in the Neural Networks for Social Behavior in Mice

マウスの社会行動神経ネットワークにおける
エストロゲン受容体ベータの発現と機能

（要約）

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17β-estradiol (E2) regulates various social behaviors, including sexual, aggressive, and parental, as well as social recognition and social anxiety. E2 acts through two types of nuclear estrogen receptors (ER), ERα and ERβ, which are expressed in a number of brain areas known to be part of neural networks for social behaviors. Two types of ERs have different roles in the regulation of social behaviors. However due to a lack of tools for identifying ERβ expression in the brain, detailed anatomical distribution and neurochemical characteristics of ERβ expressing cells, and cellular co-expression with ERα remain unclear. Revealing their distribution should be essential to understand the functions played by ERα and ERβ. In the current study, ERβ-*RFP*^{tg} mice, in which red fluorescent protein (RFP) was inserted downstream of ERβ BAC promotor, were used for the investigation of ERβ expressing cells and their possible functions.

The validity of RFP signals as ERβ in ERβ-*RFP*^{tg} mice was confirmed in three separate experiments: high ERβ mRNA levels in RFP-expressing cells collected by fluorescence-activated cell sorting, strong positive correlation between ERβ and RFP mRNA in hypothalamic tissues, and co-localization of ERβ mRNA and RFP proteins in the paraventricular nucleus (PVN).

In Chapter 1, the distribution of ERβ-RFP signals in social neural networks, and sex difference and effect of circulating gonadal steroids on expression of ERβ, were examined. Strong ERβ-RFP signals were found in the PVN, medial preoptic area (MPOA), bed nucleus of the stria terminalis (BNST), medial amygdala (MeA), and dorsal raphe nucleus (DRN) as consistent with the findings reported in previous works. There were male dominant sex differences in the posterior part of posterodorsal MeA (MeAPD) and BNST.

In Chapter 2, distribution of ERα and ERβ was compared. ERα and ERβ-RFP were co-localized in the MPOA, BNST and MeAPD. On the other hand, the majority of the PVN and DRN cells expressed only ERβ-RFP. Further analysis in Chapter 3 revealed that ERβ-RFP was expressed in oxytocin (OT) neurons in the PVN whereas ERβ-RFP was rarely co-localized in oxytocin receptors (OTR) expressing neurons through the brain areas except the posterior part of MeAPD (MeAPDp). These findings supported the hypothesis about the existence of social behavioral network consist of estrogenic and oxytocinergic systems; E2 action through ERβ regulates OT neurons in the PVN, and through ERα regulates OTR expression in the MeA. In addition, present study provides new evidence that E2 action through ERβ in the MeAPDp might be involved as well.

In Chapter 4, it was found that most of progesterone receptor (PR) positive cells expressed ERβ-RFP signals in the DRN. In addition, consistent with the previous findings, it was found that a large proportion of ERβ-RFP positive cells expressed tryptophan hydroxylase 2 (Tph2) immunoreactivity in the DRN as well. These results strongly suggested that estrogenic regulation of PR in the DRN is mediated by ERβ. Previous pharmacological inhibition or site-specific gene knockdown works have revealed that ERβ in the DRN may mediate anxiolytic effects of E2 and the decline of lordosis behavior on the day after behavioral estrus. The present results provide anatomical evidence of these E2 actions.

In the present study, it was revealed that the distribution of ERβ differs from it of ERα. Furthermore, co-expression of ERβ with several proteins, such as ERα, PR, Tph2, and OTR, was also varied depending on brain areas. Thus, these characteristics of ERβ expressing cells may underlie sex-, age-, and/or brain area specific regulation of social behavior by steroid hormones. I believe findings presented in this dissertation greatly contribute to design and interpretation for these future studies, and better and complete understanding of role and mechanisms of action ERβ in neural networks for social behavior.