様式 2-4-1

日米科学技術協力事業「脳研究」分野
グループ共同研究実施報告書 [研究分野：情動・記憶]

１．グループ共同研究代表者

日本医科大学・教授・佐久間康夫

２．研究課題名

性成熟と情動行動の調節に性腺刺激ホルモン放出ホルモンが果たす役割のコンディショナルジーンターゲティングによる解明

３．日本側グループ組織（代表者及び分担者の所属・職・氏名）

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分担者　ロックフェラー大学 助教授　Sonoko Ogawa

５．研究期間　平成１２年４月１日～平成１５年３月３１日

６．研究の概要，成果及び意義（１０００字）

生殖内分泌調節は前脳底部に散在し、正中隆起の下垂体門脈系に投射するゴナドトロピン放出ホルモン(GnRH)産生ニューロンを最終共通路として行われる。GnRH は下垂体前葉からゴナドトロピンの放出を起こす。一部の GnRH 産生ニューロンは中脳に投射し、排卵と生殖行動を同期させる。形態学的に総数 1000-2000 の GnRH ニューロンが前脳から視床下部の底部に散在することがラットやヒトで示されている。この部位は他の内分泌調節、睡眠・摂食・自律神経機能などに関わるニューロンも分布する極めてヘテロジーニアスな領域で、GnRH ニューロンを対象とする研究には大きな制約がある。GnRH 合成能を失った hpg マウスは不妊で、雌の生殖行動の一要素であるロードーシス反射を示さない。ヒトでも GnRH ニューロンの移動の障害による Kallman 症は不妊である。しかし、胎生 13 日には GnRH が合成され、性腺や脳の発生に関わることから、当初から GnRH ニューロンを欠くこれらの個体による研究にも限界がある。そこで、本研究計画ではコンディショナルジーンターゲティング法により、任意の時期に GnRH 遺伝子のノックアウト(KN)を行い、GnRH 作用の解析を目指した。日本側では KN マウスの作成に着手し、共同研究先のロックフェラー大学 Pfaff 研究室で、マウスの生殖行動の行動解析を実施した。これまで生殖行動の研究はラットが中心で、マウスの基礎データが存在しなかったためである。ジーンターゲティングについては研究期間中に LoxP 配列を GnRH 遺伝子エクソンの両端に導入した約 16Kb の targeting vector を作成し、ES 細胞にエレクトロポレイション法により導入した。ターゲティング遺伝子の導入された ES 細胞を PCR とサザンブロットで選抜、さらに PGK-neo 遺伝子を除去したクローンを選び、この ES 細胞を 8 細胞胚に注入して KN マウスの作成を 4 回試みたが、全て失敗しキメラマウスを得ることができなかった。煩瑣な操作が主因と考えている。一方、行動実験および並行して行った KN マウス脳の形態学的研究は着実に成果を生み、(1)エストロゲン受容体(ER)β KN マウスにおいて攻撃行動が亢進する、(2)マウスにおける ERβ 発現の性差がラットとは全く逆のパターンを示しこの性差が ERα を介する、(4)腹内側核の特定の出力を切断すると、エストロゲンにより雌ラットが雄型の行動を示すことを発見した。これらの成果は北米神経科学学会で発表し、３編の論文を作成した（うち一編は既に受理、印刷中）。

７．　その他（実施上の問題点，特記事項等）

特記事項なし

８．　参考資料

１．第３２回北米神経科学学会(2002 年 11 月)抄録４編

２．*J. Neuroendocrinology* 掲載受理レター

Form 2-4-2

Japan-US Brain Research Cooperation Program

Group Joint Study Report

[field: emotionality & memory]

1. The Representative of Group Joint Study:

Yasuo Sakuma, M.D., Ph.D., Professor, Nippon Medical School

2. Project Title:

Conditional knock-out of GnRH neurons and its effects on development, emotionality and behavior in mice

3. Japanese Investigator's Name, Title, Affiliation and Phone Number:

Chief: Yasuo Sakuma, Professor, Nippon Medical School, Tel: 03-3824-6640
Collaborators: Kunihiko Obata, Professor, National Institute for Physiological Sciences, Tel: 0564-55-7700
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Yukitoshi Nanasaki, Post Doctoral Fellow, Nippon Medical School, Tel: 03-3822-2131

4. U.S. Investigator's Name, Title, and Affiliation:

Chief: Donald W. Pfaff, Professor, The Rockefeller University
Collaborator: Sonoko Ogawa, Associate Professor, The Rockefeller University

5. The Term of Research: From 2000/4/1 To 2003/3/31 (3 Years)

6. Abstract, Result and Significance of Research (300 Words):

Detection of the odor of receptive females is a key step for the onset of male sexual behaviors. Previously, we have reported that estrogen receptor beta knockout (bERKO) mice exhibited normal levels of male sexual behaviors such as mounts and intromissions (Ogawa et al., 1999). However, it is not known whether they show any behavioral alterations in terms of sexual odor discrimination and preference. We tested gonadally intact male bERKO and wild type (WT) mice in a three-chamber apparatus for nose poking toward the odors of sexually receptive female mouse and gonadally intact male mouse. Odor preference indexes (PI) as responses toward the female odor over the male odor were calculated for each mouse in terms of cumulative duration (PID) and frequency (PIF) of nose poking during 20min test sessions. Each mouse was tested twice (one week apart) and between the two tests, a half of mice from each genotype were exposed overnight in their home cage to a sexually receptive female mouse through a perforated transparent plexiglass board. In the first test, both genotypes of mice showed similar levels of preference toward the female odor. However, overnight exposure to receptive female mice affected WT and bERKO mice differently. The two groups of WT mice showed similar preference toward the female odor in both tests. In bERKO mice, overnight exposure increased PIF and PID, while non-exposed males showed decreased preference to the female odor in the second tests. These findings suggest that ER-b activation may be responsible for fine-tuning of social recognition in male mice.

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7. The Others (Practical Issues, Special Mention Matters): NA

参考資料1

第 32回北米神経科学学会　(2002) 抄録

Program Number: 482.15 Day / Time: Tuesday, Nov. 5, 10:00 AM - 11:00 AM

FACILITATION OF MOUNT BEHAVIOR IN FEMALE RATS BY CAUDAL DEEFFERENTATION OF THE VENTROMEDIAL HYPOTHALAMUS

H.Ohnishi; Y.Kondo*; Y.Sakuma
Dept. of Physiology, Nippon Medical School, Tokyo, Japan

Exposure to sex steroids at the early stage produces sex differences, both functional and morphological, of mammalian brain. Testosterone makes brain masculinized (and defeminized), whereas a lack of sex steroids makes brain feminized. However, it has been reported that a destruction of the ventromedial hypothalamus (VMH) induced mount, a typical male sexual behavior, in female rats when ovariectomized and treated with testosterone. This suggests that female rat brain possesses the neural circuit for expressing male sexual behavior, and also has the inhibitory system for male sexual behavior, presumably in the VMH. In the present experiment, we examined the effect of a caudal deefferentation of the VMH on sexual behavior and partner preference in female rats. Ovariectomized Wistar rats were subjected to a coronal transection caudally located in the VMH with a razor blade (1 mm or 2 mm in width). After recovery, the partner preference, female sexual behavior and male sexual behavior were tested after a single injection of 2 g estradiol benzoate 48 hrs prior to the testing. The transection significantly enhanced mount behavior when placed with highly receptive females, while no effect was found in female sexual behavior, lordosis and soliciting behavior. It was also ineffective on the partner preference which is indicated by time spent to explore sexually active male odors over estrous female odors. The results indicate that the VMH may exert an inhibitory influence on neural circuits regulating masculine behavior, but not partner preference. After the disinhibition, a low dose of estrogen is sufficient to induce mount behavior in female rats. Citation: H.Ohnishi, Y.Kondo, Y.Sakuma. FACILITATION OF MOUNT BEHAVIOR IN FEMALE RATS BY CAUDAL DEEFFERENTATION OF THE VENTROMEDIAL HYPOTHALAMUS. Program No. 482.15. 2002 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience, 2002. CD-ROM

Program Number: 288.6 Day / Time: Monday, Nov. 4, 9:00 AM - 10:00 AM

SPECIES DIFFERENCES AND EFFECTS OF ESTROGEN RECEPTOR (Er)-alpha GENE DISRUPTION IN SEXUALLY DIMORPHIC EXPRESSION OF Er-beta IN THE PREOPTIC AREA

Y.Sakuma1*; Y.Nanasaki2; M.Nomura2; C.Orikasa1; D.W.Pfaff2; S.Ogawa2
1. Dept Physiology I, Nippon Medical School, Tokyo, Japan; 2. Lab. Neurobiology and Behavior, The Rockefeller University, New York, NY, USA

Female rats have a significantly larger number of estrogen receptor (ER)-beta positive cells than males in the anterior medial preoptic area (MPOA), particularly in the medial-most portion of the anteroventral periventricular nucleus (AVPV; Orikasa et al., 2002). In the present study, we examined whether a similar sex difference in the expression of ER-beta mRNA and protein might be detected in the mouse brain and whether it might be affected by ER-alpha gene disruption. In situ hybridization histochemistry (ISHH) revealed that unlike in rats, ER-beta gene expression in the MPOA was significantly higher in gonadectomized male mice than in females ($p<0.05$). Furthermore, in the MPOA, ER-alpha knockout male mice had significantly reduced levels of ER-beta mRNA ($p<0.05$ vs wild type). Consistent with the ISHH results, male mice had significantly higher number of ER-beta immunoreactive (IR) cells compared to female mice in the MPOA ($p<0.01$), whereas in the ventromedial nucleus of the hypothalamus, an opposite sex difference was found ($p<0.01$). Detailed immunocytochemical analyses performed in parallel in gonadectomized rats and mice revealed that the number of ER-beta IR cells was significantly higher in female rats compared to male rats ($p<0.01$) not only in the AVPV but also throughout the anterior MPOA. In mice, there was no apparent localization of ER-beta IR cells in the AVPV, regardless of sex. These findings suggest that there is a clear species difference in the sexually dimorphic expression of ER-beta in the MPOA, which may be modified by ER-alpha activation in mice. Supported by: MH 62147 to SO Citation: Y.Sakuma, Y.Nanasaki, M.Nomura, C.Orikasa, D.W.Pfaff, S.Ogawa. SPECIES DIFFERENCES AND EFFECTS OF ESTROGEN RECEPTOR (Er)-alpha GENE DISRUPTION IN SEXUALLY DIMORPHIC EXPRESSION OF Er-beta IN THE PREOPTIC AREA. Program No. 288.6. 2002 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience, 2002. CD-ROM.

Program Number: 383.1 Day / Time: Monday, Nov. 4, 1:00 PM - 2:00 PM

EFFECTS OF ESTROGEN RECEPTOR-beta GENE DISRUPTION ON SEXUAL ODOR PREFERENCE IN MALE MICE

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1. Lab. Neurobiol. & Behav., The Rockefeller Univ., New York, NY, USA; 2. Dept. Psychol., Univ. of Kagoshima,

Kagoshima, Japan
Detection of the odor of receptive females is a key step for the onset of male sexual behaviors. Previously, we have reported that estrogen receptor beta knockout (bERKO) mice exhibited normal levels of male sexual behaviors such as mounts and intromissions (Ogawa et al., 1999). However, it is not known whether they show any behavioral alterations in terms of sexual odor discrimination and preference. We tested gonadally intact male bERKO and wild type (WT) mice in a three-chamber apparatus for nose poking toward the odors of sexually receptive female mouse and gonadally intact male mouse. Odor preference indexes (PI) as responses toward the female odor over the male odor were calculated for each mouse in terms of cumulative duration (PID) and frequency (PIF) of nose poking during 20min test sessions. Each mouse was tested twice (one week apart) and between the two tests, a half of mice from each genotype were exposed overnight in their home cage to a sexually receptive female mouse through a perforated transparent plexiglass board. In the first test, both genotypes of mice showed similar levels of preference toward the female odor. However, overnight exposure to receptive female mice affected WT and bERKO mice differently. The two groups of WT mice showed similar preference toward the female odor in both tests. In bERKO mice, overnight exposure increased PIF and PID, while non-exposed males showed decreased preference to the female odor in the second tests. These findings suggest that ER-b activation may be responsible for fine-tuning of social recognition in male mice. Supported by: MH 62147 to SO Citation: Y.Nanasaki, K.Tomihara, D.W.Pfaff, S.Ogawa. EFFECTS OF ESTROGEN RECEPTOR-beta GENE DISRUPTION ON SEXUAL ODOR PREFERENCE IN MALE MICE. Program No. 383.1. 2002 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience, 2002. CD-ROM.

Program Number: 383.2 Day / Time: Monday, Nov. 4, 2:00 PM - 3:00 PM
SEXUAL ODOR PREFERENCE IN FEMALE WILD - TYPE AND ESTROGEN RECEPTOR KNOCKOUT MICE.
K.Tomihara1*; Y.Nanasaki2; D.W.Pfaff2; S.Ogawa2
1. Dept. Psychol., Kagoshima Univ, Kagoshima, Japan; 2. Lab. Neurobiol. & Behav., Rockefeller Univ., New York, NY, USA
We have previously demonstrated that female mice showed significantly higher lordosis quotient when they approached to a male mouse compared to the cases when the male mouse approached them (Tomihara and Makino, 1991). This finding suggests male olfactory cues may be important when female mice choose mating partners. In the present study, we examined the roles of two types of estrogen receptors (ER), alpha and beta, in the odor preference between intact and castrated male mice using a three-chamber apparatus, which was designed to deliver two kinds of odors separately. We found that unlike its strongly destructive effects on lordosis, ER-alpha gene disruption did not affect odor preference. Gonadally intact ER-alpha knockout (aERKO) and wild type (aWT) mice equally showed a strong preference toward castrated male odors as measured as frequency and duration of nose poking during 20min tests. Priming with estradiol benzoate (10ug/0.1ml) and progesterone (500ug/0.1ml) in gonadectomized mice reduced preference toward castrated male odors ($p<0.01$ vs oil treated control) in both aERKO and aWT mice. Preference toward castrated male odors over intact male odors was also observed in gonadectomized ER-beta knockout (bERKO) and their WT (bWT) control mice. However, steroid priming did not significantly affect the odor preference in bERKO mice, although it tended to decrease preference toward castrated male odors in bWT mice. These results suggest that ER-alpha activation may be crucial for the expression of lordosis but not for sexual odor preference by female mice. Supported by: MH 62147 to SO. Citation: K.Tomihara, Y.Nanasaki, D.W.Pfaff, S.Ogawa. SEXUAL ODOR PREFERENCE IN FEMALE WILD-TYPE AND ESTROGEN RECEPTOR KNOCKOUT MICE. Program No. 383.2. 2002 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience, 2002. CD-ROM.

参考資料2

*J. Neuroendocrinology*掲載受理レター

JOURNAL OF NEUROENDOCRINOLOGY

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26 February 2003

Ms E2K3-825
"Estrogen-induced vigorous mounting in female rats carrying hypothalamic knife cuts"
By H. Ohnishi, Y. Kondo, Y. Sakuma
Received in Edinburgh Office: 7th January 2003

Dear Dr Kondo,
Your paper was sent for review to two independent expert referees whose reports I enclose. I am pleased to inform you that both referees complement the authors on an interesting study and a very clearly presented paper. I have read your manuscript myself with interest and enclose an annotated copy with minor modifications to improve readability and to conform to Journal style.

In your resubmission please pay careful attention to the 'Instructions to Authors' and "Style Notes" (htp://www.blackwell-science.com/jne). I would be pleased if you could send three copies of a revised manuscript, together with a letter explaining how you have responded to each of the points raised by the referees. Could I also ask you to complete the enclosed copyright declaration form, and to enclose a disk copy of your revised manuscript, with a completed file description form. I must ask that you put the date of your final revision on the cover page of the disk version of your revised manuscript. If the top copy of your final manuscript does not bear the same date as that on the disk version, then the publishers will assume that the disk version is incorrect and will ask you to provide a new disk that does match the accepted copy, with a likely delay in publication.

Thank you for submitting your work to The Journal, and I hope that we may receive further contributions from you in the future.

Yours sincerely

Professor Gareth Leng
Editor-in-Chief