

(甲種)

論 文 要 旨

学 位 論 文
要 約

表 題 深層学習による保育行動の評価と V1b バソプレシン
ノックアウトマウスでの知見
Maternal behavior analysis in vasopressin V1b receptor knockout mouse
using deep learning)

申 請 者 氏 名 Sajjaviriya Chortip

担当指導教員氏名 興水 崇鏡 教授

所 属 自治医科大学大学院医学研究科
専攻 人間生物学系
専攻分野 生体防御医学
専攻科 分子薬理学

使用文字数 1128 字

論 文 要 旨

氏名 Sajjaviriya Chortip

表題

深層学習による保育行動の評価と V1b バソプレシンノックアウトマウスでの知見
(Maternal behavior analysis in vasopressin V1b receptor knockout mouse using deep learning)

1 研究目的

Maternal behavior is a crucial characteristic for promoting the survival of offspring and parents' large efforts made during childcare can affect mental and physical health. The neurohypophysial hormone, arginine vasopressin (AVP), has been known to regulate maternal behavior mainly through activation of vasopressin V1a subtype receptors in the central nervous system (CNS). According to recent studies, blockage of another vasopressin receptor subtype in CNS, V1b receptor, was implied in maternal care. However, our knowledge of V1b receptors on maternal behavior is very limited. Because the complex and long-lasting behavior observation generates a large scale of the dataset, therefore, this study aims to investigate the role of V1b receptor on the relationships between dam and pups in free moving and non-stressed conditions using deep learning methods for the first time. Moreover, we had investigated activities of the brain and V1b receptor expression in the maternal behavior-specific brain area, the median preoptic area (MPOA), to understand the efficiency of maternal care during the lactation period.

2 研究方法

2.1 Open-field test

Male and female of Wild-type (WT) and V1b knockout (V1bKO) mice at 8 – 12 weeks of age (N = 10 per genotype) were observed their movement. A mouse was put in the center of the new polymethylpentene (TPX) cage (18 x 26 x 13 cm) and recorded by video camera without human interference for 15-minute. The experiment was performed between 9.00 and 12.00 A.M.

2.2 Pup retrieval test

Pup retrieval test was used to study maternal behavior. WT and V1bKO mothers with their pups were used at the lactation day2 and day4. Four pups were placed in each corner of the cage prior to the test. Then mother was placed in the center of the cage and the number of retrieved pups by their mother in 15 minutes were counted by an investigator. During the observation, they were recorded by video.

2.3 Supervised training of deep learning model

We prepared a computer server and accessed the server from a personal computer for data transfer and analysis. Our machine learning models need to be trained by different sets of mother and pup images. Thousands of images and objects were boxed and annotated by an investigator. After the annotation, the initial model was trained using annotated images to make calculation models. The accuracy of the models was evaluated before further analysis by an investigator.

2.4 Object detection and analysis of mouse behavior

We used our model of deep learning algorithms to recognize mother and/or baby in the picture. Next, the x-y coordinates of the objects were extracted and distances between mother and baby during the test were calculated. The number of the objects became one after successful retrieval of all babies by a mother. The number of objects was compared between WT and V1bKO group. Images were extracted from video records of pup retrieval test and open-field test in the frequency of 1 image per second. About one thousand pictures generated from each 15 minutes test were analyzed. The model will recognize mother and baby and evaluate the distance of recognized objects throughout the observation period. Mother's nurturing behavior in terms of total distances among objects was plotted on the graph overtime.

2.5 *c-Fos* immunohistochemistry

Furthermore, the brain MPOA activities of dams were examined using an immunohistochemical technique to check *c-Fos* expression at MPOA. Brain tissue sections were processed with avidin-biotin peroxidase complex kit (Vectastain Elite Kit; Vector Laboratories) before stained with 3,3'-diaminobenzidine (DAB). Cells with positive *c-Fos* expression signals were compared between nursing dams of WT and V1bKO and evaluated by Image-J.

2.6 In situ hybridization

Virgin female and lactation day 2 mother of WT mice were sacrificed and the brain included MPOA was collected. Brain tissue sections were subjected to fluorescence in situ hybridization with ACDBio RNAscope® multiplex fluorescence assay. The positive target probe was a combination of ACD bio: Mm-Gal (#400961), -Mm-Avpr1b-C2 (#480141-C2), and -Mm-Fos-C3 (#316921-C3). DAPI staining was performed at the last step. A fluorescent confocal microscope was used to detect positive signals.

3 研究成果

3.1 Deep learning analysis of freely moving mice

After confirming the detection accuracy, we evaluated the effect of V1b receptor deletion on basal locomotion in an open-field test for 15 min. The deep learning models showed high accuracy percentage and detected the specific objects in images. The total moving distances of female V1bKO mice in the open-field environment were significantly reduced compared to WT mice. Especially, when the tracking movement showed V1bKO female mice were less exploring and moving through the center of cages than WT.

3.2 Manual measurement and deep learning analysis of pup retrieval test

To examine the role of V1b in maternal care during the early lactation period, pup retrieval test was performed on lactation days 2 and 4. Manual counting of pup retrieval showed that the V1bKO mother collected her pups faster than the WT mother. The deep learning models detected mother and baby. The accumulated total moving distances was reduced in V1bKO dams on lactation day 4.

3.3 Histological examination in MPOA

The results showed that the number of *c-Fos* positive nuclei was significantly lower in V1bKO dams.

In galanin-positive and -negative neurons in MPOA of virgin female mice and dams on lactation day 2, the number of V1b transcript-positive neurons was similar independent of the status of mother's pregnancy. V1b transcript was detected in both galanin-positive and -negative neurons

4 考察

V1bKO virgin female and postpartum dam entered less into center of the field, indicating anxiety-like phenotype. Our environment for pup retrieval test was set up in a new cage with small amount of new bedding. However, WT female and dam explored more by moving more distances. Our results of V1bKO mother on pup retrieval is in good agreement with a previous study, which reported that blockage of the V1b receptor by local administration of the antagonist into MPOA led to faster pup retrieval. Moreover, we showed, for the first time to our knowledge, that mother-infant relationship can be analyzed by deep neural network and computer vision. Therefore, deep learning analysis can be effectively applied to investigation of mother-pup interactions.

5 結論

In summary, the V1b receptor is involved in the exploration of new environment and collection of pups by lactation mothers. Moreover, knowledges from deep learning analysis are useful to help understand large efforts of parents to foster their infants during early phase of postpartum period.