論文審査の要旨

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Activation of neurons in the insular cortex and lateral hypothalamus during food anticipatory period caused by food restriction in mice

（食餌制限による食物予測期におけるマウスの島皮質と外側視床下部ニューロンの活性化）

Mice that only eat one meal at a fixed time per day exhibit food anticipatory activity (FAA), which begins 2-3 hours before food acquisition. According to reports, the insular cortex (IC) plays an essential role in food anticipation, and orexin neurons in the lateral hypothalamus (LH) regulate the expression of FAA. However, it is still unclear whether and how neurons in IC and LH including orexin neurons are activated during the development of FAA. Therefore, the degree applicant first conducted a behavioral activity study to determine whether male adult C57BL/6 mice show FAA under scheduled daily 4 hours (from Zeitgeber Time (ZT) 4-ZT8) food restriction for 1 day, 8 days, and 15 days. Then, fluorescence immunohistochemistry techniques were applied to measure neuronal activity by c-Fos expression of neurons in the bilateral IC (including the anterior (AI), middle (MI) and posterior (PI) regions) and LH including orexin neurons during the food anticipatory period on the day-1, 8, and 15 of the restricted feeding (RF) to clarify the temporal and spatial activation pattern of these neurons and their role in FAA establishment.

As a result, this study identified the following insights.

1) 1 day RF did not affect the locomotor activity of mice during ZT2-ZT4 and did not result in FAA formation. 8 days or 15 days RF significantly increased the locomotor activity after 2 days or 3 days of RF and result in FAA formation. 2) 8 days and 15 days RF had no effect on 24 hours total locomotor activity of mice. 3) The number of c-Fos-positive neurons in the bilateral AI, MI, PI, and LH, including orexin neurons of mice during the food anticipatory period increased gradually from the 1st day to 15th day of RF, however, peaked at a different point in the food restriction procedure. 4) RF-mice showed a significant positive correlation between the number of c-Fos-positive neurons in AI or MI or PI or total IC and the number of c-Fos-positive orexin neurons in LH during the FAA. 5) In addition, 8 days and 15 days RF decreased significantly the daily food intake and body weight of mice only at the early phase of the scheduled RF protocol.

The current study demonstrates that the FAA development requires periodic, predictable feeding restriction stimulation and is a gradual process. The neurons in the bilateral IC and LH, including orexin neurons, are activated during FAA. There is an interaction between the activation of neurons in the IC (including AI, MI, PI) and orexin neurons in LH during FAA. The temporal patterns of neuronal activation in several subregions of the IC during the development of FAA are different, and those of neuronal activation between the IC and LH are also different, suggesting that the IC and LH are differently involved in the neural network for FAA production.

This study represents a significant contribution to the field as it quantifies the extent of temporal neuronal activation in IC and LH during the development of FAA through appropriate experimental methods and statistical analysis. Therefore, it is judged that this study has sufficient value as a degree thesis.