

## 最終試験の結果の要旨

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<p>主査および副査の5名は、令和6年3月12日、学位申請者 Ma Jihao 君に面接し、学位申請論文の内容について説明を求めると共に、関連事項について試問を行った。具体的には、以下のような質疑応答がなされ、いずれについても満足すべき回答を得ることができた。</p> <p>Question 1) Regarding influence of age to food anticipatory activity (FAA), how do younger or older mice show FAA?  Answer: Younger mice tend to exhibit more robust FAA compared to older mice. Previous study has showed that older rats expressed little food anticipation behavior and general hypoactivity, and both their food intake during the scheduled mealtime and their general activity throughout the day were significantly lower than those in younger rats. Therefore, the general hypoactivity and lower food requirement of older rats may result in a reduction of food anticipation.</p> <p>Question 2) What is the special reason to use urethane for anesthesin? Is it better to use urethane for immunohistochemistry study?  Answer: Urethane is commonly used as an anesthetic in animal experiments due to its stable anesthetic effect, no significant impact on cardiovascular and respiratory functions, and high safety. Especially, it is better to use urethane for immunohistochemistry study because it has been reported that the normal dose of urethane has no significant effect on c-Fos expression induced by the stimulus.</p> <p>Question 3) Fos is a marker for activating cell. Briefly explain how to activate gene in the activated cells.  Answer: Cells receive environmental stimuli through receptors and then activate intracellular pathways. This occurs through signaling pathways activating transcription factors, which bind to the gene's promoter, initiating transcription. Consequently, c-fos protein is produced. Thus, in active cells, c-fos activity is driven by the activation of its transcription via cellular signaling pathways stimulated by external cues.</p> <p>Question 4) There are no difference in locomotor activity and food intake, but the 15-day restricted feeding (RF) shows higher body weight. How do you explain this phenomenon?  Answer: One possibility is that the RF group may have adapted metabolically to the reduced calorie intake, leading to increase body weight compared to ad libitum (AL) group.</p> <p>Question 5) Food intake is not changed by the activation of orexin. How do you explain?  Answer: The activation of orexin neurons may compensate for the reduction of food intake during the early phase of RF, and lead to the gradual recovery to normal levels of food intake with the number of days of RF. The activation of orexin neurons remains at a stable level since the 8th day of RF, so the food intake in the late stage of RF remains at normal levels. Moreover, in addition to orexin, food intake is also influenced by multiple factors such as leptin.</p> <p>Question 6) Before increasing of the FAA, do you look at another time? Is the brain's activation continuous or just before starting?  Answer: In my experiment, I only observed the activation of the insular cortex (IC) and lateral hypothalamus (LH) caused by food restriction during FAA. But I think these brain regions are not continuously activated. Further experimental research will be conducted by observing whether these brain regions are activated by food restriction during periods other than FAA.</p> <p>Question 7) In the relationship between the LH and IC, what is the major neurotransmitter for orexin neurons?  Answer: The main neurotransmitters are orexin, acetylcholine, glutamate, GABA, etc.</p> <p>Question 8) What type of experiment can you imagine to further investigate the relationship between the LH and IC?  Answer: I will apply optogenetic techniques to inhibit neurons in the LH or IC, and observe the changes in the number of c-Fos positive neurons in IC or LH during FAA in order to explore the further relationship between the IC and LH during the formation of FAA.</p>			

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Question 9) Do mice usually take food during the dark time?

Answer: Yes, mice usually take food during the dark time.

Question 10) Locomotor activities increase not only during ZT2 to ZT4, but also at ZT 12 to ZT 16. Are there any differences in behavior between FAA period and the night active period?

Answer: The activity of mice during ZT2 to ZT4 is different from that during ZT12 to ZT16. The former is caused by RF. Only RF-mice exhibit this activity, while AL mice do not. The latter is not related to feeding, but rather the nocturnal activity of mice. There is no difference between RF-mice and AL-mice.

Question 11) How do you think of neuron activity in the IC by giving more RF days than 15 days?

Answer: As the number of RF days increases, the number of activated neurons in the anterior and middle IC will gradually increase and maintain peak levels, but there will be no significant change in the posterior IC.

Question 12) Explain functional difference between three subregions of the IC.

Answer: Anterior IC is mainly involved in attention/emotion/olfactory/gustatory processing. Middle IC is mainly involved in interoceptive sensation and olfactory/gustatory processing. Posterior IC is mainly involved in sensorimotor tasks and olfactory/gustatory processing.

Question 13) Why do you give food restrictions at 1 day, 8 days or 15 days?

Answer: It has been reported that rodents express FAA within 3-14 days under the scheduled RF paradigm. Some brain structures began significantly increasing c-Fos expression on the 8<sup>th</sup> day of palatable food entrainment. Therefore, we selected day 15 (14 days of RF and 20-hour fasting) and day 8 (7 days of RF and 20-hour fasting) of RF for locomotor activity and immunohistochemical studies of c-Fos. In addition, to determine whether 20-hour fasting can produce FAA and its effect on the expression of c-Fos in neurons of the IC and LH, we also observed the effect of 1-day RF (20-hour fasting) on locomotor activity and the expression of c-Fos in neurons of the IC and LH as a control for day 8 or day 15 of RF.

Question 14) Why are you using 8-week-old mice in this study?

Answer: Because I wanted to use adult mice, which refer to mice born 8-12 weeks after birth.

Question 15) Is there any difference of FAA between genders?

Answer: A few studies reported no significant differences in FAA between male and female mice. However, it has also been reported that male mice show significantly more FAA than female mice.

Question 16) Why did the 1-day RF mice only show a significant increase in c-Fos expression on the right anterior IC but not the left? What does this mean?

Answer: 1-day food restriction caused an increase in c-Fos expression of neurons in the bilateral anterior IC, and it was only significant on the right side, suggesting neurons in the right anterior IC can be more sensitive to the hunger signal. There may be some functional differences between the bilateral anterior IC.

Question 17) What is the significance of the first period LH activation compared to IC neurons activation?

Answer: We speculate that LH neurons, including orexin neurons, are first activated during the food anticipation period to awaken mice. As the number of RF days increases, the food intake and body weight of the mice return to normal levels to cope with changes in feeding patterns to adapt to RF. Then this information might be transmitted to the IC by the orexinergic nerve fibers projected from the LH and exciting neurons in the IC to be involved in FAA.

Question 18) Mice usually eat at night time but you give them food at daytime. If you don't eliminate the element of light in time, your experiment shows the adaptation of reversal light and darkness circle. What do you think of this point?

Answer: Previous studies have shown that mice can anticipate feeding time in artificial environments lacking any variation in light, temperature, or sound that may serve as a signal of imminent food access. FAA in mice can be expressed to mealtimes scheduled at any fixed circadian phase during the day or night. As long as a mouse is maintained by a single daily meal provided at a fixed time of day, it will, within a few days, exhibit increased arousal and activity during the hours immediately preceding feeding time. Thus, although mice prefer to eat at night, it will exhibit diurnal FAA if the food is supplied during the daytime.

Question 19) Why you didn't administer the orexin antibody in your locomotor experiment?

Answer: Because it has been reported that mice with orexin neurons ablated had a severe defect in showing expected food-anticipatory increases in locomotor activity under RF conditions, suggesting that orexin neurons in LH are required for the robust expression of FAA in mice during the food anticipatory period. As my further research, I will consider the use of orexin antibodies or receptor blockers to study the mechanism of FAA formation.

Question 20) Do you think ghrelin is also elevated as the same as orexin level?

Answer: Yes, ghrelin levels rise before an anticipated feeding bout.

以上の結果から、5名の審査委員は申請者が大学院博士課程修了者としての学力・識見を有しているものと認め、博士(医学)の学位を与えるに足る資格を有するものと認定した。