早稲田大学審査学位論文

博士（スポーツ科学）

概要書

# The effect of daily exercise on behavioral thermoregulation in mice

# 日常運動が行動性体温調節におよぼす影響

２０１３年１月

早稲田大学大学院　スポーツ科学研究科

林　政賢

Lin, Cheng-Hsien

研究指導教員：　彼末 一之　教授

## II. Abstract

The aims of my study were to develop an experimental system to assess behavioral thermoregulation, and establish animal model to clarify the influence of dehydration on it. Moreover, I planned to evaluate the effect of chronic exercise on behavioral thermoregulation and the neural mechanisms. I conducted three studies to fulfill the aims.

Study 1. Hyperosmolality in the plasma modulates behavioral thermoregulation in mice: the quantitative and multilateral assessment using a experimental system. We have developed a new system to evaluate behavioral thermoregulation and the effect of plasma hyperosmolality on the behavioral responses to heat in mice. The system consisted of Plexiglas box (dimensions: 50×12×19 cm) with five computer-controlled Peltier boards (dimensions: 10×10 cm) at the bottom. We set the system to two different settings by changing the operating program as follows: a) an operant behavior setting, in which the temperature of each board was first set at 39°C, and the right-end board was changed to 20°C within 1 min of a mouse moving on the left-end board; and b) a temperature gradient setting, in which each board was randomly set at 15°C, 22°C, 28°C, 35°C, or 39°C with a 6-min interval. Mice received subcutaneously (s.c.) injection of isotonic or hypertonic saline (154 mM (IS group) or 2,500 mM (HS group), 10 ml/kg body wt). The mice were exposed to each setting for 90 min. In the operant setting, the HS group had fewer operant behavior counts than the IS group (11 ± 5 and 25 ± 4 counts, respectively; $P<0.05$) and an increase in body temperature (1.6 ± 0.4°C vs. 0.0 ± 0.2°C, respectively; $P< 0.05$). In the gradient setting, both groups had greater counts at a board temperature of 35°C. The system enabled us to evaluate and compare the behavioral responses to the two different settings. In addition, plasma hyperosmolality might be a signal to change thermal preferences and maintain a higher body temperature.

Study 2. Effect of daily exercise on heat-escape/cold-seeking behavior and thermal preference in mice: exercise training modulates feeling of hot? We tested chronic exercise in mice augments heat tolerance by modulating behavioral thermoregulatory responses.

Mice housed with or without a running-wheel for 8 weeks (WR and NWR groups, n=47 and 40, respectively) were used. Implanted a body temperature ($T_b$) measurement device, the mice received s.c. injection of isotonic- or hypertonic-saline (1 ml/100 g of body wt; 154 or 2,500 mM, IS or HS subgroup) and were placed in a box with 5 Peltier boards at the bottom. Three experiments were conducted for 90 min, using different controlling programs of the board temperatures: 1) constant board temperatures of 28°C or 39°C; 2) an operant-behavior setting: each board was set at 39°C and the right-end board was changed to 20°C within 60 s only when the mouse moved to the left-end board; and 3) a thermal mosaic setting: each board was set at either 15°C, 22°C, 28°C, 35°C, or 39°C with a 6-min interval. In *Experiment 1*, $T_b$ in both subgroups of the WR group became higher than that in the NWR group. In *Experiment 2*, the NWR group showed smaller operant counts in the HS subgroup than the IS subgroup; however, the WR group did not. In *Experiment 3*, the WR group preferred lower temperatures than the NWR group without any differences between the subgroups (e.g., 33.4 ± 0.3°C and 34.7 ± 0.1°C in IS groups). Exercise training may alter thermal preference and behavioral responses, thereby increases thermal tolerance, diminishing the effect of dehydration.

Study 3. Facilitating c-Fos expression in the POAH of the mouse brain by heat stress or hyperosmolality can be potentiated by chronic exercise. Recent studies have used the expression of immediate early gene such as c-Fos to demonstrate that exercise increases the neural activity in the hippocampal neurons of mouse brain. It is currently unknown whether the basal c-Fos expression as well as the increased c-Fos expression caused by heat exposure and/or salt loading in the brain can be affected by chronic exercise. The purpose of this study was to test the hypothesis in mice by using a newly developed device. Mice were divided into two groups: one group had free access to running wheel for 8 weeks (WR) and the other had no access (NWR). After subcutaneous injection (1 ml/100 g of body weight) of either normal (154 mM, IS) or hypertonic saline (2,500 mM, HS), each mouse was placed in a behavior box with 5 peltier boards at the bottom, where a) thermal

mosaic (temperature of each board was randomly chosen among set at 15°C, 22°C, 28°C, 35°C, or 39°C; mice could select preferable position while the temperature setting was changed each 6 min) or b) operant behavior available (the temperature of each was set at 39°C; one board temperature was changed to 20°C within 60 second when a mouse moved to a specific board). In the result, when the operant heat escape/cool seeking behavioral setting was unavailable, we could assess overall autonomic response to heat. Both the basal c-Fos expression and the increased c-Fos expressions caused by heat exposure and/or salt loading in the preoptic are/anterior hypothalamus (POAH), but not in the paraventricular nucleus (PVN) or in the central amygdaloid nucleus (CeM), of WR mice were significantly higher than those of the NWR mice. Additionally, when the heat escape/cool seeking behavioral setting was available, both the basal c-Fos expression and the c-Fos overexpression induced by heat exposure and/or salt loading in both the POAH and the PVN, but not in the CeM, of the exercise mice were significantly higher than those of the sedentary mice. In experiment 3, during a thermal mosaic setting, the same results as those of the operant behavioral setting were observed. It can be derived from the foregoing results that (i) chronic exercise (voluntary wheel running) is able to induce an upward shift in the basal c-Fos expression in the POAH, but not in the PVN or in the CeM, of the mouse brain; (ii) when the operant behavior setting is unavailable, heat-induced and/or salt loading-induced c-Fos overexpressions in the POAH, but not in the PVN or in the CeM, are significantly potentiated by chronic exercise; and (III) when an operant behavior setting or a thermal mosaic setting is available, heat exposure-induced and/or salt loading-induced c-Fos overexpressions in both the POAH and the PVN, but not in the CeM, of mouse brain are significantly enhanced by chronic exercise. Thus, it appears that the POAH is involved in autonomic thermoregulation, whereas both POAH and PVN are involved in behavioral thermoregulation. Chronic exercise induces an upward shift in the basal c-Fos expression and enhances c-Fos overexpression caused by heat or salt loading in the mouse hypothalamus.