

# 学位論文の要旨

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学位論文名 Proliferation of Neuronal Progenitor Cells and Neuronal Differentiation in the Hypothalamus Are Enhanced in Heat-Acclimated Rats.

発表雑誌名  
(巻, 初頁～終頁, 年) } 別紙の通り

著者名 }

## 論文内容の要旨

### INTRODUCTION

In mammals, repeated exposure to moderate heat has been well-known to result in the development of heat acclimation that improves heat-tolerance. Such thermoregulatory changes in heat-acclimated subjects are known to be attributable to both the peripheral thermoeffector efficiency at a given level of central thermoregulatory drive and to changes in a gain of the thermoregulatory centers. The heat acclimation process has two forms, namely short-term and long-term heat acclimation, depending on a length of the heat-exposed term. Briefly, thermoregulatory changes of short-term heat acclimation are lost rapidly after the end of heat exposure, while those of long-term heat acclimation are stable and sustained. Thus, especially in long-term heat acclimation, persisting functional and/or morphological changes may be expected in the central thermoregulatory system. For the central mechanism of heat acclimation, several investigations have been made in the anterior hypothalamus from various points of views, regarding gene expression profiles and morphological changes in synaptic structures, e.g. number, thickness, curvature and complexity. These studies suggest repetitive heat exposure-induced neuronal plasticity in the thermoregulatory center and suggest a possible contribution of such hypothalamic neuronal modifications to the establishment of heat

acclimation. However, the central mechanism of heat acclimation has not been fully elucidated. In these studies, we examined the effect of heat exposure on proliferation and differentiation of neuronal progenitor cells in the rat hypothalamus.

### **MATERIALS AND METHODS**

Male Wistar rats (5 weeks old), initially maintained at an ambient temperature ( $T_a$ ) of 24°C, were subjected to a constant high  $T_a$  of 32°C (HE) or were constantly kept at 24°C (controls, CN). Bromodeoxyuridine (BrdU; 50 mg/kg/day) was intraperitoneally injected daily for 5 consecutive days after commencing heat exposure. On the 6th (HE6), 13th (HE13), 23rd (HE23), 33rd (HE33), 43rd (HE43) and 53rd (HE53) day of the heat exposure period, the brain samples were used for immunohistochemical studies. The same procedure was applied to CN without heat exposure, i.e. the brains were removed on the 6th (CN6), 13th (CN13), 23rd (CN23), 33rd (CN33), 43rd (CN43) and 53rd (CN53) days corresponding to the heat exposure period in HE. Intra-abdominal temperature ( $T_{ab}$ ) of the rats was measured using a biotelemetry system. All experiments with animals in this study were approved by the Ethics Committee for Animal Experimentation of Shimane University and they were handled according to our institutional guidelines.

### **RESULTS AND DISCUSSION**

Immunohistochemical analysis showed that the numbers of BrdU-positive (BrdU+) cells in the hypothalamus of HE were significantly and consistently greater than those of CN. In HE6, a high density of BrdU+ cells was observed in the ependymal layer of the third ventricle. In the other HE subgroups, in contrast, BrdU+ cells were broadly expressed in the parenchyma of the hypothalamic area. These results suggest that heat exposure promoted cell proliferation in the ependymal layer of the third ventricle and these cells migrated into the hypothalamic parenchyma thereafter. In HE, the number of BrdU+ cells double-stained by Neuronal nuclei (NeuN), a mature neuron marker, (BrdU+/NeuN+ cells) increased abruptly after 33 days of heat exposure by about 7 times. In HE6, HE13, HE23 and HE33, only a small number of

BrdU+/NeuN+ cells were observed in the hypothalamus. In HE43 and HE53, however, conspicuously exhibited increased numbers of BrdU+/NeuN+ cells in the hypothalamic area. This result suggest that hypothalamic newborn cells differentiated to mature neurons 33-day after commencing heat exposure. Moreover, the total counts of BrdU+ cells labeled with doublecortin (Dcx), an immature neuron marker, in the hypothalamic area in HE were significantly larger than that of CN. In contrast, BrdU+ cells expressing glial markers were rarely detected in the hypothalamus of both CN and HE. These results clearly suggest that a majority of hypothalamic newborn cells induced by heat exposure took on a neuronal fate. We additionally investigated age-dependent changes in heat exposure-induced hypothalamic neurogenesis and acquired heat tolerance in rats. In old rats (22-25 month of age), heat exposure did not promote cell proliferation and neural differentiation in the hypothalamus. Also, old rats could not improve heat tolerance by 40-day heat exposure.

### **CONCLUSION**

Heat exposure facilitates proliferation of neuronal progenitor cells in the hypothalamus and promotes neural differentiation of newly generated cells, which may have a potential role in functional changes in thermoregulatory center in heat-acclimated rats. Also, aging may interfere with heat exposure-induced hypothalamic neurogenesis and acquired heat tolerance in rats.

別紙

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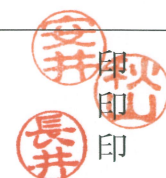
論 文 名 1. Proliferation of Neuronal Progenitor Cells and Neuronal Differentiation  
in the Hypothalamus Are Enhanced in Heat-Acclimated Rats.  
2. Aging Attenuates Acquired Heat Tolerance and Hypothalamic  
Neurogenesis in Rats.

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論文審査及び最終試験又は学力の確認の結果の要旨

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論文審査の結果の要旨

動物では、暑熱環境への暴露により体温調節に関与する末梢効果器の機能的・器質的变化が誘導され、耐暑熱性が亢進する（暑熱馴化の形成）。しかし、その中枢機序は不明であった。本研究では、暑熱暴露されたラットにおいて、体温調節中枢が存在する視床下部を中心として神経前駆細胞の増殖と分化を解析した。Wistar 系雄性ラット(5 週齢)を環境温 24°C で 2 週間飼育した後、32°C の高温環境に暴露した。暴露開始直後から細胞分裂のマーカーである Bromodeoxyuridine (BrdU; 50 mg/kg/day) を腹腔内へ 5 日間連続投与した。暑熱暴露開始から 6～53 日目にラットの脳を摘出し、免疫組織化学的に解析した。暑熱暴露により、ラット視床下部における BrdU 陽性細胞数が増加した。さらに、BrdU 陽性細胞の一部は抗 NeuN 抗体（成熟神経細胞のマーカー）により二重に染色され、その数は暑熱暴露開始後 33 日から 43 日の間に著増した。これらの結果は、長期の暑熱暴露によりラット視床下部の神経前駆細胞の増殖が促進され、新生細胞の多くは成熟神経細胞に分化することを示唆する。老齢ラットでは長期の暑熱暴露により耐暑熱性がほとんど亢進せず、また、視床下部における神経新生も誘導されなかった。以上の結果から、申請者は長期の暑熱暴露がラット視床下部における神経細胞新生を誘導し、暑熱馴化を形成する可能性を考えた。本研究結果は視床下部神経新生が暑熱馴化形成に寄与する可能性を初めて示した独創的な内容であり、さらなる体温調節機構の解析に繋がる知見となるため、学術的意義を有する。

最終試験又は学力の確認の結果の要旨

申請者は、暑熱馴化の中枢機序に暑熱暴露によるニューロン新生が関与するのではないかとという斬新なアイデアで研究を進め、その可能性を細胞分裂や分化に関する緻密な実験系を組むことによって明確に示した。考察力や関連領域の知識も十分であり、学位授与に値すると判断した。

(主査：安井 幸彦)

申請者は、長期の暑熱暴露が第 3 脳室周囲領域において神経前駆細胞の新生を誘導し、神経系細胞に分化しつつ視床下部へ遊走する現象をラットの実験系を用いて免疫組織学的に見出した。中枢神経における暑熱馴化形成機構の 1 つを明らかにする発見であり、学位に値すると判断した。

(副査：秋山 恭彦)

申請者は、暑熱馴化の過程においてラット視床下部で神経新生と分化遊走が生じることを見出し、分化した神経細胞の馴化への寄与を免疫組織学的分析で明示した。暑熱馴化形成機序を示唆する極めて有用な研究で、申請者は関連領域の知識も十分であり、学位授与に値すると判断した。

(副査：長井 篤)

(備考) 要旨は、それぞれ 400 字以内とする。