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Author(s)

Kentaro Matsuzaki & Masanori Katakura & Toshiko Hara & Guanghua Li & Michio Hashimoto & Osamu Shido

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INTEGRATIVE PHYSIOLOGY

Proliferation of neuronal progenitor cells and neuronal differentiation in the hypothalamus are enhanced in heat-acclimated rats

Kentaro Matsuzaki · Masanori Katakura · Toshiko Hara · Guanghua Li · Michio Hashimoto · Osamu Shido

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Abstract Male Wistar rats, 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) was intraperitoneally injected daily for five consecutive days after commencing heat exposure. On the 6th, 13th, 23rd, 33rd, 43rd, and 53rd day of heat exposure, rats' brains were removed. Immunohistochemical analysis showed that the numbers of BrdU-positive cells in the hypothalamus of HE were significantly and consistently greater than those of CN. In HE, the number of BrdU-positive cells double-stained by a mature neuron marker increased abruptly after 33 days of heat exposure by about seven times. This was not the case in CN. The results suggest that heat exposure facilitates proliferation of neuronal progenitor cells in the hypothalamus and promotes differentiation to neurons, which might have certain relation to establishing long-term heat acclimation in rats.

Keywords Thermoregulation · Hypothalamus · Heat-exposure · Progenitor cells

Introduction

For animals, repeated exposure to moderate heat has been well-known to result in the development of heat acclimation that improves heat tolerance [11, 42]. 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 [18]. For the peripheral mechanism, functional and morphological changes of thermoeffectors have been well shown [16, 24, 31, 39, 48]. In rat vascular system, for instance, heat acclimation enhanced arterial and venous distensibility [16, 24] and increased the density of arteriovenous anastomoses in acral parts of the body [8]. This resulted in a high capability for maintaining nonevaporative heat loss in the heat environment. 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 [23, 41] and morphological changes in synaptic structures, e.g., number, thickness, curvature, and complexity [2]. These studies reveal 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.

The heat acclimation process has two forms, namely short-term and long-term heat acclimation, depending on the length of the heat-exposed term [15, 17]. Briefly, thermoregulatory changes of short-term heat acclimation are lost rapidly after the end of heat exposure [40], while those of long-term heat acclimation are stable and sustained [15]. Thus, especially in long-term heat acclimation, persisting functional and/or morphological changes may be expected in the central thermoregulatory system. In adult mammals, it has been well-recognized that neuronal progenitor cells in the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the

K. Matsuzaki (✉) · M. Katakura · T. Hara · G. Li · M. Hashimoto · O. Shido
Department of Environmental Physiology, Faculty of Medicine, Shimane University,
89-1 Enya-cho,
Izumo 693-8501, Japan
e-mail: matuzaki@med.shimane-u.ac.jp

O. Shido
e-mail: o-shido@med.shimane-u.ac.jp

hippocampus formation [12, 28] proliferate and then differentiate into neurons, with the newly generated neurons having a significant role in acquisition and maintenance of brain function [25]. In addition to these brain regions, recent reports have clearly shown neurogenesis in the hypothalamus [30, 37, 49]. In adult rat brain, neuronal progenitor cells exist in the ependymal layer of the third ventricle and they migrate into the hypothalamic parenchyma where they differentiate into neurons [30, 49]. The new neurons could be functionally integrated into neuronal networks by forming synapses and producing neuropeptides [49]. The preoptic area of anterior hypothalamus (POA/AH) is known to harbor neurons involved in at least some afferent thermoregulatory pathways [34], as well as thermosensitive first efferent neurons involved in all autonomic thermoeffector pathways [33, 38]. Thus, neurogenesis and associated reconstructions of neuronal networks in the hypothalamic area might have a pivotal role in modulating thermoregulatory function even in adult rats.

On the basis of the foregoing findings, we hypothesized that long-lasting heat exposure might generate hypothalamic neurons which would then be integrated in neuronal networks in heat-acclimated animals. The present study investigated how heat exposure affects proliferation of hypothalamic neuronal progenitor cells using a proliferation marker and how newborn cells differentiate into neurons using neuronal and glial markers.

Materials and methods

All animal experiments were performed in accordance with the Guidelines for Animal Experimentation of Shimane University Faculty of Medicine, which were compiled from the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science.

Animals

Male Wistar rats (5 weeks of age) were housed individually in transparent plastic cages (width, 270 mm; length, 440 mm; height, 187 mm) with wood chippings and were initially maintained at an ambient temperature (T_a) of 24.0±0.1°C and relative humidity of 54±5% under a 12:12-h light–dark cycle (lights on at 1600 hours). Since the rats were kept in plastic cages, air velocity inside the cages was negligible. Rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and implanted in the intraperitoneal cavity with a temperature transmitter (TA10TA-F40; Data Sciences, St. Paul, MN, USA). After a 2-week recovery period, rats for heat acclimation (HE) were subjected to a constant T_a of 32.0±0.2°C and relative humidity of 35±8%, while control rats (CN) were continuously kept at 24.0±0.1°C. The T_a for CN (also HE before heat exposure) was selected, since freely moving Wistar rats have been reported to prefer about 24–26°C [43]. During the experiment, cages were cleaned and food and water were replaced every 2 or 3 days at random times of the day.

Experimental protocol

In HE, bromodeoxyuridine (BrdU; Sigma, St Louis, MO, USA), the most widely used marker to identify newly born cells in adult brain, was injected into the rats' abdominal cavity daily (50 mg/kg/day) for five consecutive days after starting heat exposure. Then, the rats were divided into six subgroups (n=4 in each group). On the 6th (HE6), 13th (HE13), 23rd (HE23), 33rd (HE33), 43rd (HE43), and 53rd (HE53) day of the heat exposure period, the animals were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and were perfused transcardially with 4% formaldehyde. Brains were removed, fixed overnight at 4°C in 4% formaldehyde, and then immersed in 20% (w/v) sucrose solution. The brain samples were used for immunohistochemical studies. After the brain sampling, both adrenal glands were removed from each rat and their wet weights were measured. The same procedure was applied to CN without heat exposure, i.e., the brains and adrenal glands 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 (n=4 in each group).

In a different series of study, three groups of rats (n=4 in each group) were used. They were exposed to heat as in the main experiment. Between the 11th and 15th day, the 21st and 25th day, and the 31st and 35th day of the heat exposure schedule, BrdU (50 mg/kg/day, i.p.) was injected daily into the rats. Four weeks after the end of BrdU injection, the rats were anesthetized and the brains were removed. The same samplings were made in CN (n=4 in each group).

Furthermore, an additional study was conduced in eight rats (SHE53). Rats were exposed to a constant T_a of 32°C for only six consecutive days and then kept at T_a of 24°C. BrdU was injected into the rats' abdominal cavity (50 mg/kg/day) daily for the first five consecutive days of heat exposure. On the 53rd day after starting heat exposure, rats were anesthetized and the brains were removed. Then, brain samples were used for immunohistochemical analyses.

Measurements of intra-abdominal temperature

Intra-abdominal temperature (T_{ab}) of the rats was measured using a biotelemetry system for 1 day before the onset and on the last day of heat exposure. All data were processed every minute with a computer logging system (Data quest, Data Sciences International, St Paul, MN, USA), and data

were separately analyzed in the light and dark phases of day.

Immunohistochemistry

Immunohistochemical analysis was carried out as described previously [21]. Brain sections (40 μm thickness) were prepared using a cryostat and collected as free-floating sections. For detection of BrdU incorporation, brain sections were incubated in 50% formamide/2× standard sodium citrate for 2 h at 65°C, incubated in 2 N HCl for 30 min at 37°C, rinsed in 100 mM boric acid (pH 8.5) for 10 min at 25°C, and then washed with 0.25% Triton X-100 in Tris-buffered saline (pH 7.4). For multiplex immunoassaying, coronal sections were incubated with several primary antibodies for 12 h at 4°C. The primary antibodies used in this study were monoclonal rat anti-BrdU IgG (1:10; Oxford Biotechnology, Oxford, UK), polyclonal mouse antineuronal nuclei (NeuN) IgG (1:500; Chemicon, Newcastle, UK), polyclonal goat antidoublecortin (Dcx) IgG (1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA), polyclonal rabbit anti-adenomatosis polyposis coli (APC) IgG (1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA), polyclonal rabbit antiglial fibrillary acidic protein (GFAP) IgG (1:100; Sigma, St Louis, MO, USA), and monoclonal antisynaptophysin (SYN) antibody (1:200; Sigma, St Louis, MO, USA). To identify localization of BrdU-immunopositive (BrdU+) cells colabeled with NeuN, Dcx, APC, GFAP, and SYN, Alexa Fluor 633 antirat IgG with Alexa Fluor 488 antimouse IgG, Alexa Fluor 488 antirabbit IgG, and Alexa Fluor 488 antigoat IgG (1:500, Molecular Probes, Eugene, OR, USA) were used as the secondary antibody. After staining, sections were mounted on glass slides and covered with 80% glycerol. All of sections were visualized under ×20 or ×40 magnifications using a confocal microscope (Olympus FV-300, Tokyo, Japan) and imaging software (Olympus Fluoview, Tokyo, Japan). BrdU+ cells were observed by a Cy5 filter, while other colabeled cells were detected by a fluorescein isothiocyanate filter.

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling staining

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining was carried out with an ApopTag Red In Situ Apoptosis Detection Kit (Chemicon, Newcastle, UK) according to the manufacturer's protocol. First, the brain sections were washed in phosphate buffered saline (PBS; Nissui, Tokyo, Japan). The sections were partially digested with proteinase K (20 μg/ml; Merck, NJ, USA) at room temperature for 15 min and washed twice in PBS. Thereafter, the sections were incubated at room temperature in an equilibration buffer. Moreover, the tissue sections were incubated at 37°C for 1 h with TUNEL reaction mixture (70% reaction buffer/30% terminal deoxynucleotidyl transferase enzyme) in a humidified chamber in the dark. The slides were agitated and incubated in stop/wash buffer for 10 min at room temperature. After incubation, the sections were washed three times with PBS. Then rhodamine-conjugated antidigoxigenin antibody diluted with blocking solution (47:53 *v/v*; Chemicon, Newcastle, UK) was applied to the sections, which were then incubated in a humidified chamber for 30 min at room temperature. Then, sections were mounted on glass slides and covered with 80% glycerol. All of sections were visualized under ×20 magnifications using a confocal microscope (Olympus FV-300, Tokyo, Japan) and imaging software (Olympus Fluoview, Tokyo, Japan), using standard rhodamine excitation and emission filters.

Data quantification and statistical analysis

For the hypothalamic area, brain sections (between −0.26 and −4.80 mm from bregma) were obtained according to the Paxinos and Watson atlas [36]. BrdU+ cells were counted at 12 sections per animal. Because the BrdU-labeled nuclei were counted at one sixth interval sections, the possibility of counting split cells on different sections was minimized to less than 10%, according to the equation of Abercrombie [14]. Individual BrdU+ cells stained with NeuN, Dcx, GFAP, or APC were also counted. TUNEL-labeled cells also were observed under a confocal laser microscope. The cell counts are shown as the total number of twelve sections.

The results are presented as means ± standard errors of the mean (SEMs). The parameters obtained were analyzed by two-way (the lapse of day and heat exposure) analysis of variance and the effects of heat exposure on parameters were evaluated using Student's *t* test. A level of $P<0.05$ was considered significant.

Results

The mean T_{ab} in the light and dark phases of day in CN and HE is summarized in Table 1. Before starting heat exposure, T_{ab} levels in both phases of CN did not differ from those of HE. Heat exposure significantly increased T_{ab} both in the light ($F_{(1,\ 36)}=45.61$, $P<0.0001$) and dark phases ($F_{(1,\ 36)}=39.92$, $P<0.0001$) in all subgroups. Body weights ($F_{(5,\ 36)}=381.73$, $P<0.0001$) and adrenal weights ($F_{(5,\ 36)}=36.12$, $P=0.0092$) significantly increased with the lapse of days in both CN and HE (Table 2). The adrenal weights were measured as one of markers of stress level. However, heat exposure had no significant effects on these weights, although body weight of HE6 was slightly lower than that of CN6.

t1.1 **Table 1** Mean intra-abdominal temperature (T_{ab}) on specific days in control and heat-acclimated rats

t1.2 Day	CN		HE		CN		HE	
t1.3	Light phase	Dark phase	Light phase	Dark phase	Light phase	Dark phase	Light phase	Dark phase
t1.4 6	37.21±0.06	37.82±0.05	37.20±0.07	37.82±0.05	37.22±0.09	37.81±0.07	37.76±0.10	38.62±0.12
t1.5 13	37.19±0.07	37.79±0.09	37.21±0.06	37.80±0.08	37.23±0.06	37.79±0.06	37.82±0.12	38.69±0.09
t1.6 23	37.21±0.08	37.84±0.07	37.20±0.05	37.82±0.07	37.21±0.08	37.82±0.08	37.81±0.11	38.66±0.11
t1.7 33	37.22±0.09	37.86±0.06	37.22±0.08	37.83±0.06	37.25±0.07	37.84±0.06	37.83±0.13	38.69±0.10
t1.8 43	37.21±0.11	37.86±0.07	37.21±0.09	37.84±0.07	37.24±0.10	37.84±0.07	37.89±0.12	38.68±0.12
t1.9 53	37.24±0.07	37.92±0.06	37.22±0.08	37.85±0.06	37.26±0.09	37.88±0.06	37.96±0.13	38.72±0.14

Values are the means ± SEMs (n=4 in each day of the heat exposure period)

Before a day before starting the heat exposure period, *During* the last day of the heat exposure period, *Day* days after starting the heat exposure period, *CN* control rats, *HE* heat-exposed rats

Proliferation of progenitor cells

In both CN and HE, BrdU+ cells were detectable in each field of the hypothalamus on all days examined (Fig. 1a). The total number of BrdU+ cells in HE was significantly greater than that of CN ($F_{(1,\ 36)}$=51.99, P<0.0001; Fig. 1b). The number of BrdU+ cells decreased over time ($F_{(5,\ 36)}$= 3.34, P=0.0139), as incorporated BrdU is known to be gradually lost from the cells [22]. However, the number of HE constantly maintained a higher level than CN. In HE6, a high density of BrdU+ cells was observed in the ependymal layer of the third ventricle (Fig. 1a, middle). In the other HE subgroups, in contrast, BrdU+ cells were broadly expressed in the parenchyma of the hypothalamic area (Fig. 1a, middle; photos of HE23, HE33, and HE43 not shown). The number of BrdU+ cells in HE6, HE13, HE23, HE33, and HE43 was significantly greater than that of CN6 (P=0.02), CN13 (P<0.01), CN23 (P<0.05), CN33 (P<0.05), CN43 (P<0.05), and CN53 (P<0.05), respectively.

In an additional study, when BrdU was injected into rats between the 11th and 15th days and the 21st and 25th days of heat exposure period, the numbers of BrdU+ cells of HE in the hypothalamus were significantly greater than those of CN (in both periods, P<0.05; Fig. 2). However, in heat-exposed rats injected with BrdU between the 31st and 35th days of heat exposure period, the number of BrdU+ cells in the hypothalamus did not differ from that of CN (P=0.09; Fig. 2).

Because constitutive birth of new neurons has been unambiguously demonstrated within the SVZ of the lateral ventricles and the SGZ of the dentate gyrus, BrdU+ cells of these areas were also inspected. However, heat exposure did not increase the number of BrdU+ cells in the SVZ (Fig. 3a, c) or the SGZ (Fig. 3b, d) on any days of heat exposure (only HE6 and HE53 are shown).

t2.1 **Table 2** Body and adrenal glands weighs on specific days in control and heat-acclimated rats

t2.2 Day	CN	HE	CN	HE
t2.3 6	250±8	230±13	41.6±1.2	41.1±0.8
t2.4 13	276±8	266±12	42.0±4.2	43.4±1.5
t2.5 23	315±9	305±12	45.2±3.2	44.8±3.1
t2.6 33	349±13	345±12	47.8±3.1	47.2±2.5
t2.7 43	384±10	374±14	51.1±2.1	50.1±3.2
t2.8 53	411±13	408±14	52.9±2.1	50.9±4.0

Values are the means ± SEMs (n=4 in each day of the heat exposure period)

Day days after starting the heat exposure period, *CN* control rats, *HE* heat-exposed rats

Neuronal phenotypes of hypothalamic newborn cells

Brain sections were immunolabeled with an anti-BrdU antibody together with an anti-NeuN antibody, which labels mature neuronal nuclei with lighter staining in the cytoplasm. BrdU and NeuN double-labeled (BrdU+/NeuN+) cells were detected in the hypothalamus in both CN and HE (Fig. 4a). The number of BrdU+/NeuN+ cells and percentage of BrdU+/NeuN+ cells to BrdU+ cells in the hypothalamic area significantly increased with the lapse of days (number; $F_{(5,\ 36)}$=30.43, P<0.0001, percentage; $F_{(5,\ 36)}$=26.53, P< 0.0001; Fig. 4c). In addition, heat exposure significantly elevated the number and percentages of BrdU+/NeuN+ cells to BrdU+ cells (number; $F_{(1,\ 36)}$=35.43, P<0.0001, percentage; $F_{(1,\ 36)}$=36.1, P<0.0001). In HE6, HE13, HE23, and HE33, only a small number of BrdU+/NeuN+ cells were observed in the hypothalamus, i.e., 0.2±0.2%, 1.2±0.3%, 2.3±1.3%, and 3.4±1.8% of the number of BrdU+ cells, respectively (Fig. 4c). HE43 and HE53, however, conspicuously exhibited increased numbers of BrdU+/NeuN+ cells in the hypothalamic area, i.e., 26.6±11.8% and 34.2±9.2% of the number of BrdU+ cells, respectively. Then, the percentage of BrdU+/NeuN+ cells to BrdU+ cells in HE43 and HE53 was significantly higher than that of CN43 (P< 0.03) and CN53 (P<0.001), respectively.

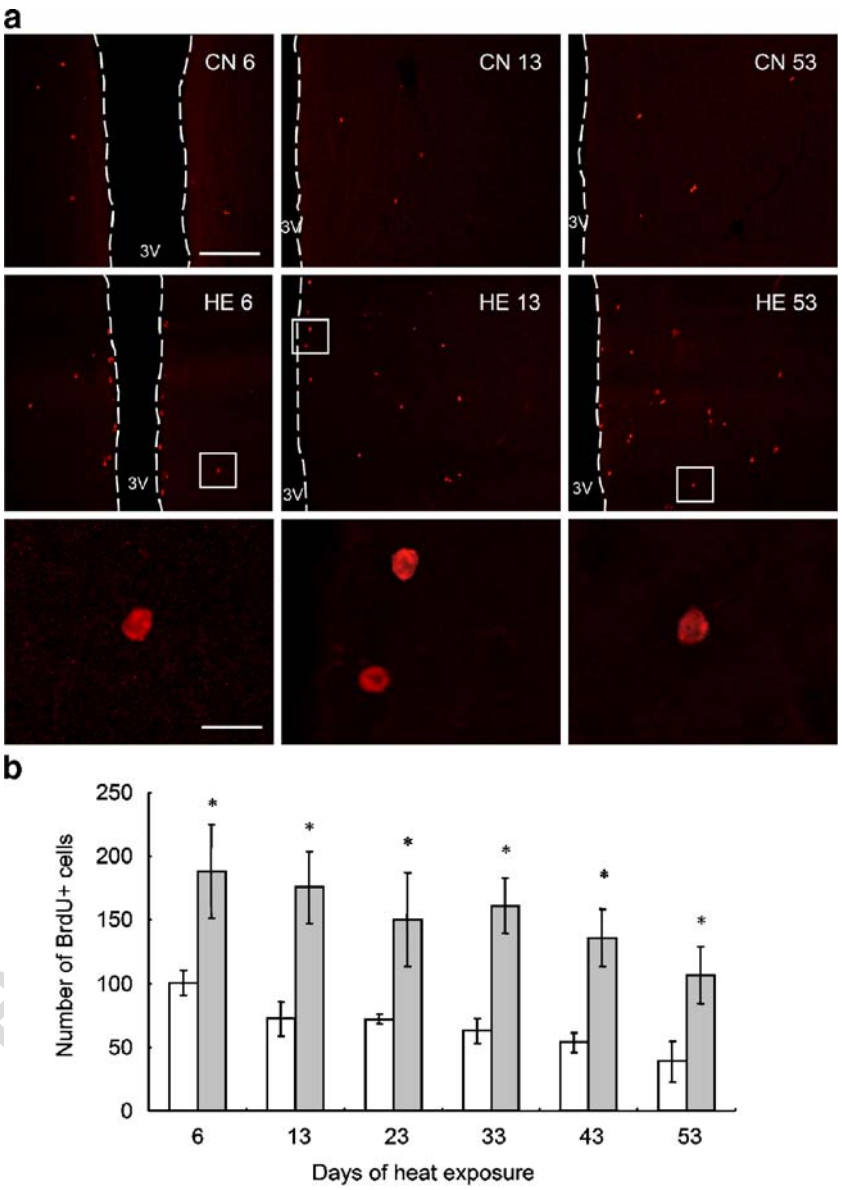

Fig. 1 Progenitor cell proliferation and migration in the hypothalamus. **a** Representative BrdU-labeled (*red*) sections of the hypothalamus inspected by laser-scanning confocal microscopy in CN (*top*) and HE (*middle*) rats; scale bar 100 μm. *Bottom*, BrdU+ cells of area boxed in HE6 (*left*) and HE53 (*right*); scale bar 10 μm. CN6, CN13, and CN53 show samples on the 6th, 13th, and 53rd day of the experiment, respectively. HE6, HE13, and HE53 show samples on the 6th, 13th, and 53rd day of heat exposure, respectively. *3V* third ventricle; scale bar 100 μm. **b** Total number of BrdU+ cells in the hypothalamus of CN (*open bars*) and HE (*gray bars*). *Numbers in the abscissa* indicate days after starting heat exposure (n=4 in each subgroup). Values are the means ± SEMs. Heat exposure significantly increased the numbers of BrdU+ cells. *Asterisk* indicates significant difference between CN and HE

Figure 5 shows localization of BrdU+/NeuN+ cells in the hypothalamus in HE. The numbers of BrdU+/NeuN+ cells in the POA/AH, ventromedial nuclei (VMH), dorsomedial nuclei (DMH), and posterior hypothalamic area (posterior hy) tended to increase with the lapse of days. As in the total count of hypothalamic BrdU+/NeuN+ cells, however, the numbers abruptly increased in HE43 an HE53. In the paraventricular nuclei (PVH) and lateral hypothalamic area, such rises in the number of double-stained cells were not seen. Among the six regions tested, the number of BrdU+/NeuN+ cells appeared to be the largest in the POA/AH, e.g., in HE53, 23.2±9.2% of BrdU+/NeuN+ cells to BrdU+ cells were located in the POA/AH, while 11.3±3.0%, 16.9±7.8%, 4.7±2.9%, and 17.3±6.9% were detected in the VMH, DMH, PVH, and posterior hy, respectively. The POA/AH includes several structures, most notably the medial preoptic area (MPO) and the median preoptic nucleus (MnPO) [34]. Our estimation in HE53 showed that in 23.2% of BrdU+/NeuN+ cells to BrdU+ cells of the POA/AH, 6.0% and 3.8% were found in MPO and MnPO, respectively.

We additionally examined whether heat exposure-induced newborn cells in the hypothalamus could differentiate to mature neurons without heat exposure. The number of BrdU+/NeuN+ cells in SHE53 was significantly larger than that of CN53 (P<0.05), while the number was significantly smaller than that of HE53 (P<0.05; Fig. 6a). The percentage of BrdU+/NeuN+ cells to BrdU+ cells in the hypothalamus was significantly depressed without heat exposure (comparison between HE53 and SHE53, P<0.05; Fig. 6b), and then, the percentage of SHE53 did not differ from that of CN53.

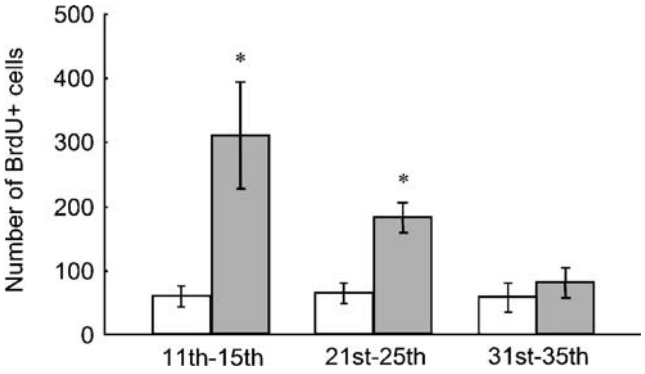

Fig. 2 Total number of BrdU+ cells in the hypothalamus of CN (*open bars*) and HE (*gray bars*). BrdU was injected between the 11th and 15th day, the 21st and 25th day, and the 31st and 35th day after commencing heat exposure. *Numbers in the abscissa* indicate days after starting heat exposure (n=4 in each subgroup). Values are the means ± SEMs. *Asterisk* indicates significant difference between CN and HE

Dcx expression in hypothalamic newborn cells

Brain sections were double-stained with antibodies against BrdU and the developmentally regulated marker Dcx. It has been shown that Dcx is a microtubule-associated protein found in the soma and processes of newborn and migrating neurons [5]. The soma and projections in part of the hypothalamic cells were immunohistochemically visualized in each sample (Fig. 7a, b). The total counts of BrdU+ cells labeled with Dcx in the hypothalamic area in HE were significantly larger than that of CN ($F_{(2,\ 36)}$= 21.36, P<0.0001), since the number of BrdU+ cells of HE was far greater than that of CN (Fig. 7c). It seemed that the number of BrdU+/Dcx+ cells deteriorated in HE53 when the number of BrdU+/NeuN+ cells increased. The ratio of BrdU and Dcx double-positive cells to BrdU+ cells did not differ between CN and HE in the hypothalamic area, e.g., 39.2±9.8% in HE53 and 34.7±12.6% in CN53.

Glial phenotypes of newborn hypothalamic cells

BrdU+ cells were double-labeled with glial markers, APC, an oligodendrocyte marker, and GFAP, an astrocyte marker, in CN and HE. In both groups, BrdU+ cells stained with APC were detectable in the hypothalamus (Fig. 8a, b). Heat exposure led to a marked increase in the total number of BrdU and APC double-labeled cells over CN (P<0.05). However, the percentage of colabeled cells to BrdU+ cells in the hypothalamus did not differ between CN and HE, e.g., that of CN53 was 3.7±1.2% and that of HE53 was 3.9±1.6%. In contrast, BrdU+ cells expressing GFAP were rarely detected in both CN and HE (Fig. 8c).

Synaptic formation of newly generated cells in HE

In the hypothalamus of HE, some of the BrdU+ cells appeared to be double-labeled with SYN, i.e., the confocal microscopic images clearly demonstrated synaptic vesicle membrane proteins surrounding a part of BrdU+ cells (Fig. 9). Because of the unique staining pattern of SYN (Fig. 9, left), accurate quantitative analysis (counting BrdU and SYN double-labeled cells) could not be made.

TUNEL-positive cells in the hypothalamus

It is well known that apoptotic signal enhances progenitor cell proliferation in central nerves system [44]. To determine whether heat exposure induces apoptosis, the number of TUNEL-positive cells in the hypothalamus was measured. TUNEL-positive cells in the hypothalamus were detectable in CN and HE in all samples. The numbers of TUNEL-positive cells were counted in CN (CN6, CN13, and CN23) and HE (HE6, HE13, and HE23) during the period when progenitor cell proliferation was facilitated in HE (Fig. 10a). There were no significant differences in the numbers of TUNEL-positive cells between two groups during the heat exposure period (Fig. 10b).

Discussion

The present study showed that in HE6, the number of BrdU-labeled cells in the hypothalamic area was significantly greater than that in CN6 (Fig. 1). The results strongly suggest that moderate heat exposure promoted proliferation of the progenitor cells within 5 days after starting heat exposure. A large number of the BrdU+ cells were detected in the ependymal layer of the third ventricle, where progenitor cells are shown to exist. In the other HE subgroups (HE13–HE53), BrdU+ cells were broadly expressed in the parenchyma of the hypothalamus. Similarly to the previous report in the hypothalamus of rats stimulated with basic fibroblast growth factor [49], heat exposure-induced newborn cells in the ependymal layer of the third ventricle may have migrated into the hypothalamic parenchyma. In addition, the enhancement of progenitor cells proliferation in response to heat exposure appears to last for until at least 25 days, since the additional study showed that the number of BrdU+ cells in HE was significantly increased compared with CN when BrdU was injected into rats between 11 and 15 days or between 21 and 25 days after the onset of heat exposure (Fig. 2). However, this is not the case after 31-day heat exposure.

To determine whether heat exposure affects the proportion of newborn cells developing a cell type and a neuronal phenotype, we also tested hypothalamic BrdU+ cells for the expression of NeuN, Dcx, APC, and GFAP. In HE6, HE13,

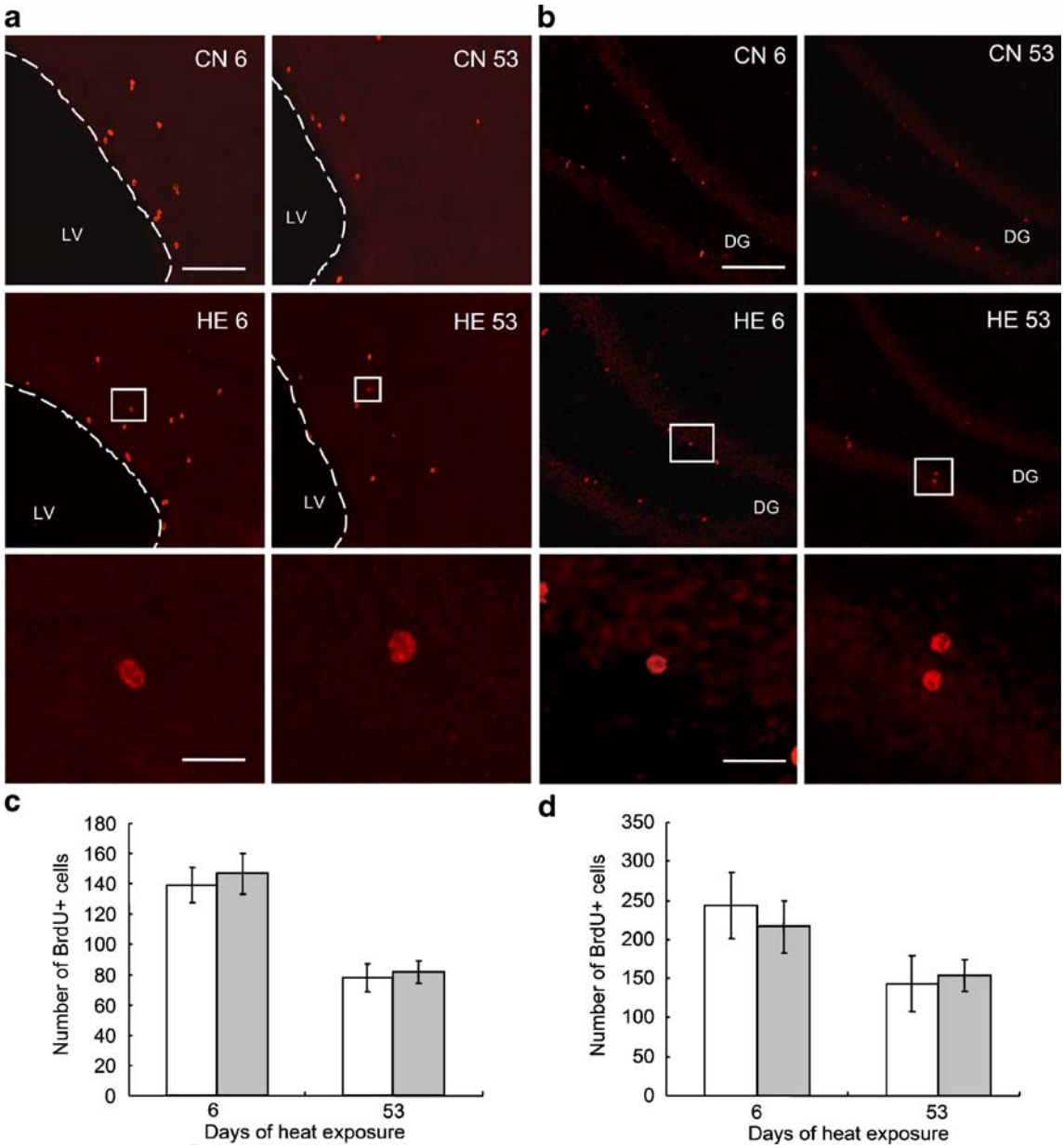

Fig. 3 Newborn cells in the SVZ and SGZ. Representative BrdU-labeled sections of the SVZ of the lateral ventricles (**a**) and the SGZ of the hippocampus formation (**b**) analyzed by laser-scanning confocal microscopy in CN (*left*) and HE (*right*). CN6 and CN53 show samples on the 6th and 53rd day of the experiment, respectively. HE6 and HE53 show samples on the 6th and 53rd day of heat exposure, respectively. *LV* lateral ventricle, *DG* dentate gyrus; scale bar 100 μm. *Bottom*, BrdU+ cells of area boxed in HE6 (*left*) and HE53 (*right*); scale bar 10 μm. **c**, **d** total numbers of BrdU+ cells in the SVZ and SGZ of CN (*open bars*) and HE (*gray bars*). Values are the means ± SEMs. Heat exposure did not affect the numbers of BrdU+ cells

HE22, and HE33, the percentages of BrdU+/NeuN+ cells number to BrdU+ cells number in the hypothalamus were below 4%. Interestingly, the percentage abruptly increased to more than 26% in HE43 and HE53 (Fig. 4c). In CN, the ratio of BrdU+/NeuN+ cells to BrdU+ cells gradually increased with passage of days, but vigorous neurogenesis was not seen even in CN43 and CN53. The observations certified that the proliferated cells in the hypothalamus following heat exposure differentiated to mature neurons when the term of heat exposure was more than 43 days. Furthermore, in HE, a large number of BrdU+ cells, e.g., nearly 30% in HE53, in the hypothalamus were expressing Dcx, a marker of immature neurons. In contrast, a small number of hypothalamic newborn cells were stained with APC, a marker of oligodendrocyte, and BrdU+ cells rarely expressed GFAP, a marker of astrocyte (Fig. 8). The results clearly suggest that a majority of hypothalamic newborn cells induced by 6-day heat exposure took on a neuronal

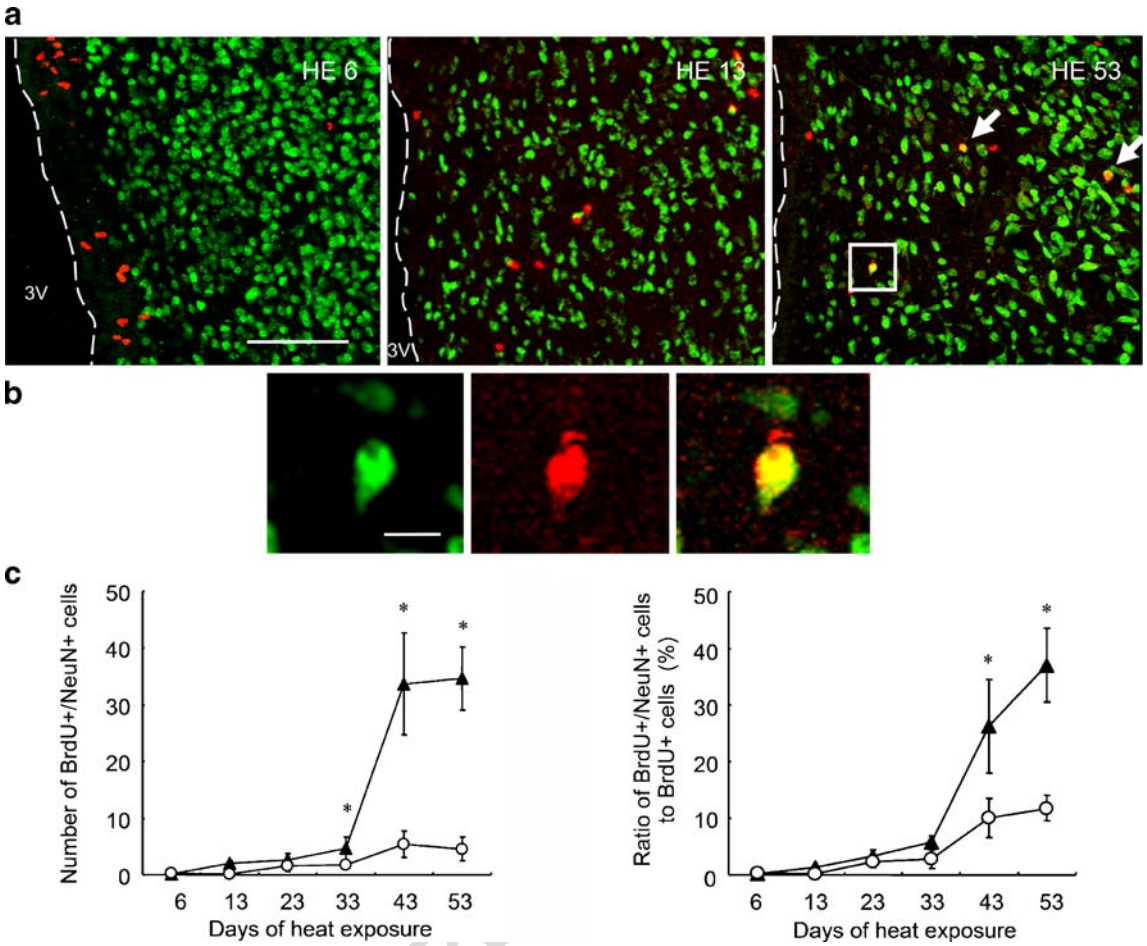

Fig. 4 Time-dependent changes of NeuN expressions of hypothalamic newborn cells. Colabeling of BrdU (*red*) with neuronal marker, NeuN (*green*), by confocal optical sectioning. **a** BrdU/NeuN-labeled sections of the hypothalamus in heat-exposed rats. HE6, HE13, and HE53 show samples on 6th, 13th, and 53rd day of heat exposure, respectively. *Arrows* denote double-labeled cells (*yellow*). *3V* third ventricle; scale bar 100 μm. **b** Confocal reconstruction of area boxed in **a**. *Left*, NeuN; *middle*, BrdU; *right*, merge. Scale bar 10 μm. **c** The numbers of BrdU+/NeuN+ cells (*left*) and percentages of BrdU+/NeuN+ cells number to BrdU+ cells numbers (*right*) in CN (*open circles*) and HE (*closed triangle*). *Numbers in the abscissa* indicate days after starting heat exposure. Heat exposure significantly increased the ratio. Values are the means ± SEMs. *Asterisk* indicates significant difference between CN and HE

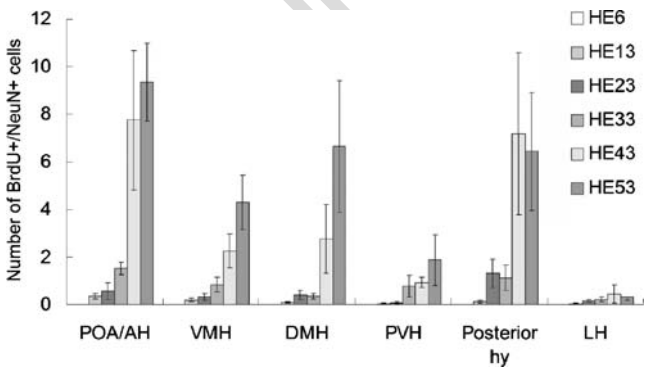

Fig. 5 Localization of BrdU+/NeuN+ cells in the preoptic area of anterior hypothalamus (*POA/AH*), ventromedial nuclei (*VMH*), dorsomedial nuclei (*DMH*), paraventricular nuclei (*PVH*), posterior hypothalamic area (*posterior hy*), and lateral hypothalamicarea (*LH*) of HE (n=4 in each subgroup). HE6, HE13, HE23, HE33, HE43, and HE53 show samples on the 6th, 13th, 23rd, 33rd, 43rd, and 53rd day of heat exposure, respectively. BrdU+/NeuN+ cell in HE6 were rarely detected in all regions. Values are the means ± SEMs

fate. Since the progenitor cell proliferation was shown between 11 and 15 days and between 21 and 25 days after the onset of heat exposure (Fig. 2), it may also be interesting to examine the fate of those newborn cells.

There might be a possibility that heat exposure-induced newborn cells could be automatically differentiate to neurons without any temperature stimuli. In the additional study, therefore, rats were exposed to heat for only 6 days to facilitate hypothalamic progenitor cell proliferation and then kept at a control temperature. As shown in Fig. 6, both the number of BrdU+/NeuN+ cells and the percentage of BrdU+/NeuN+ cells to BrdU+ cells in SHE53 were significantly smaller than those of HE53. Especially in the percentage of BrdU+/NeuN+ cells to BrdU+ cells, the value of SHE53 did not differ from that of CN53. Thus, constant heat exposure is required for promoting differentiation of newborn cells to mature neuron in the rat hypothalamus.

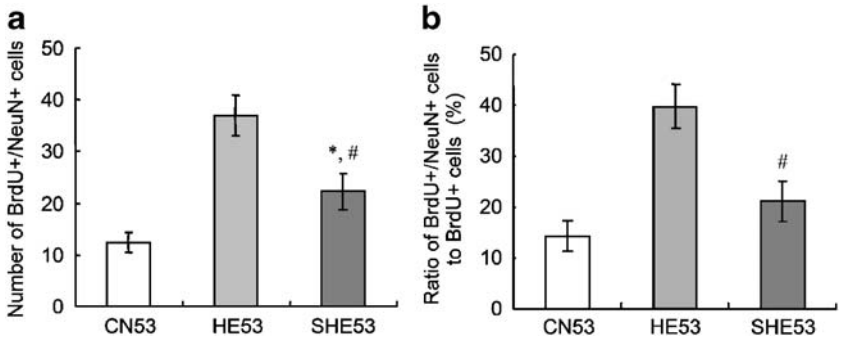

Fig. 6 Differentiation of newly born cells to mature neurons on 53rd day after starting heat exposure schedule. The number of BrdU+/NeuN+ cells (*left*) and percentage of BrdU+/NeuN+ cells number to BrdU+ cells number (*right*). *CN53* control rats constantly kept at 24°C, *HE53* rats constantly exposed to heat (32°C), *SHE53* rats exposed to heat for only 6 days and then transferred to control temperature. Values are the means ± SEMs (n=4 in each subgroup). *Asterisk* indicates significant difference between CN and SHE; *number sign* denotes significant difference between HE and SHE

It is noteworthy that the number of BrdU+/NeuN+ cells in the hypothalamus of HE was drastically increased after 33 days of heat exposure (Fig. 4c). This suggests that vigorous neuronal differentiation initiated between 33 and 43 days after commencing heat exposure in the hypothalamus. Recently, an arrest of cell proliferation has been shown to stimulate neuronal differentiation [3, 27] by for instance, inhibiting cycline-dependent kinases [7, 10]. Our additional study in HE showed that after 31-day heat exposure, progenitor cell proliferation was depressed (Fig. 2), whereas differentiation to neurons was markedly facilitated after 33-day heat exposure (Fig. 4c). Thus, these results may be consistent with those forgoing reports regarding the relationship between neuronal proliferation and differentiation. As described, the process of heat acclimation can be short term and long term, depending

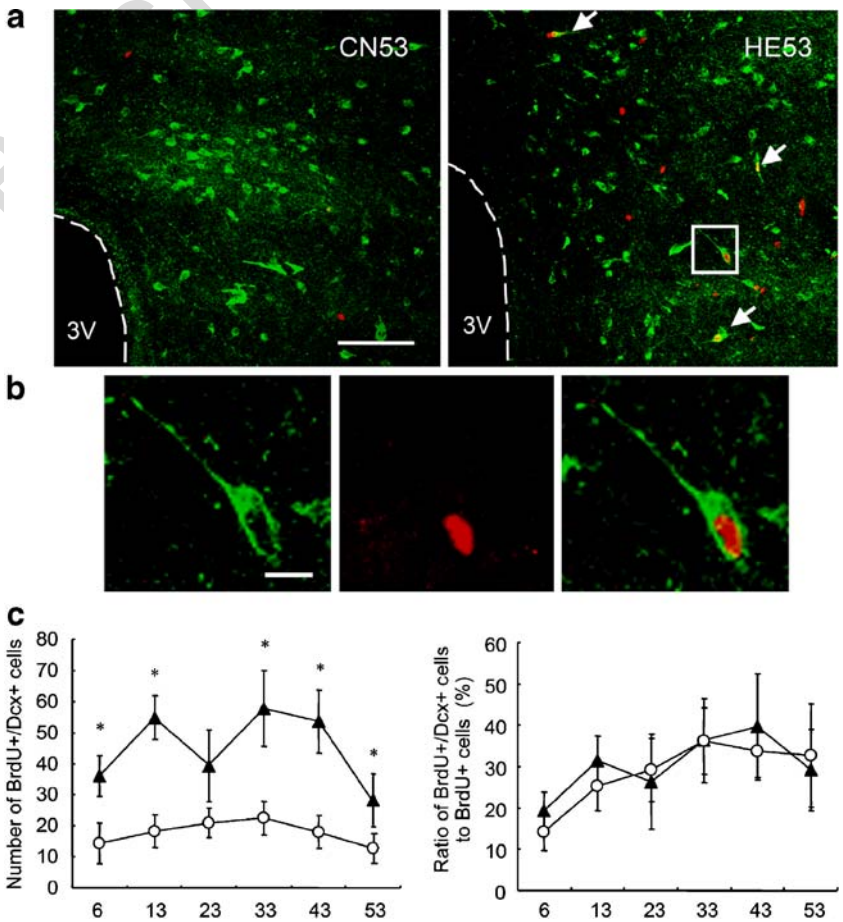

Fig. 7 Dcx expression of hypothalamic newborn cells. Colabeling of BrdU (*red*) with immature neuron maker, Dcx (*green*), by confocal optical sectioning. **a** BrdU- and Dcx-labeled sections of the hypothalamus in control (*left*) and heat-exposed (*right*) rats. *Arrows* denote double-labeled cells (*yellow*). CN53 and HE53 show samples of control and heat-exposed rats, respectively, on the 53rd day of the heat exposure period. Scale bar 100 μm. **b** Confocal reconstruction of area boxed in (**a**). *Left*, Dcx; *middle*, BrdU; *right*, merge. Scale bar 10 μm. **c** The number of BrdU+/Dcx+ cells (*left*) and percentages of BrdU+/Dcx+ cells number to BrdU+ cells number (*right*) in CN (*open circles*) and HE (*closed triangle*). *Numbers in the abscissa* indicate days after starting heat exposure. Heat exposure significantly increased the number of BrdU+/Dcx+ cells. Values are the means ± SEMs (n=4 in each subgroup). *Asterisk* indicates significant difference between CN and HE

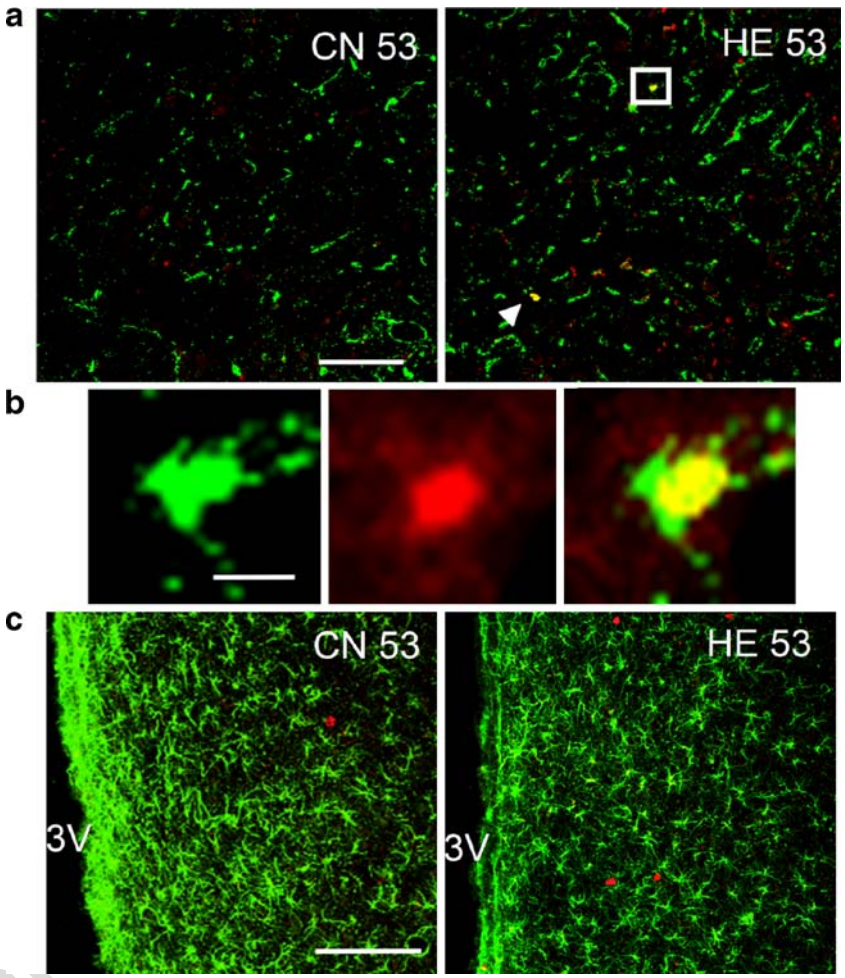

Fig. 8 Glial expressions of hypothalamic newborn cells. Colabeling of BrdU (*red*) with each glial markers (*green*) by confocal optical sectioning. CN53 and HE53 show samples of control and heat-exposed rats, respectively, on the 53rd day of the heat exposure period. **a** BrdU- and APC-labeled sections of the hypothalamus. *Arrows* denote double-labeled cells (*yellow*). Scale bar 100 μm. **b** Reconstruction of area boxed in (**a**). *Left*, APC; *middle*, BrdU; *right*, merge. Scale bar 10 μm. **c** BrdU- and GFAP-labeled sections in the hypothalamus. *3V* third ventricle; scale bars 100 μm

on term of heat exposure [17]. It is thought that in rats, conversion from short-term to long-term heat acclimation occurs, at a rough estimate, after around 4 weeks of exposure to moderate heat [18]. The critical period appears to be close to the period when differentiation of newborn cells to mature neurons (not proliferation) in the hypothalamic area is energetically promoted (Fig. 4c). Thus, heat exposure-induced neurogenesis might have certain relationship to acquisition of a new thermoregulatory function in long-term heat acclimation in rats.

There have been plenty of studies showing the anatomical localization of neurons involved in thermal afferent pathway, thermal sensitivity, and/or thermoeffector efferent pathway in the hypothalamus [4, 33, 34, 50]. The POA/AH

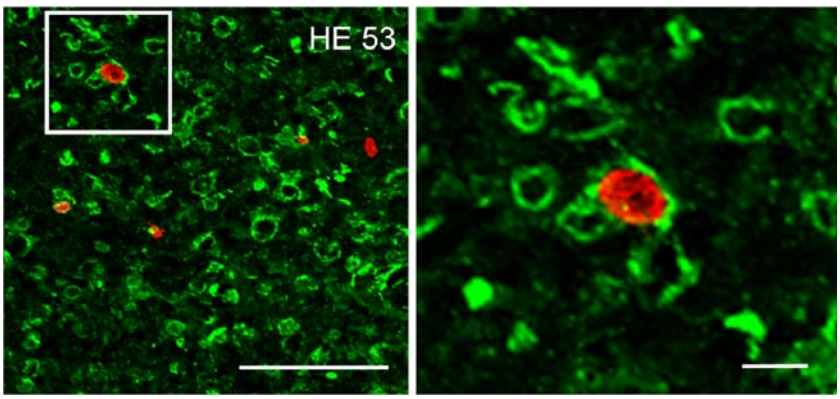

Fig. 9 SYN expression of hypothalamic newborn cells. Colabeling of BrdU (*red*) with SYN (*green*) by laser-scanning confocal microscopy in the hypothalamus of heat-exposed rats. The sample was obtained from a rat on the 53rd day of the heat exposure period. *Left panel*, scale bar 100 μm. *Right panel*, confocal reconstruction of area boxed in the *left panel*, scale bar 10 μm

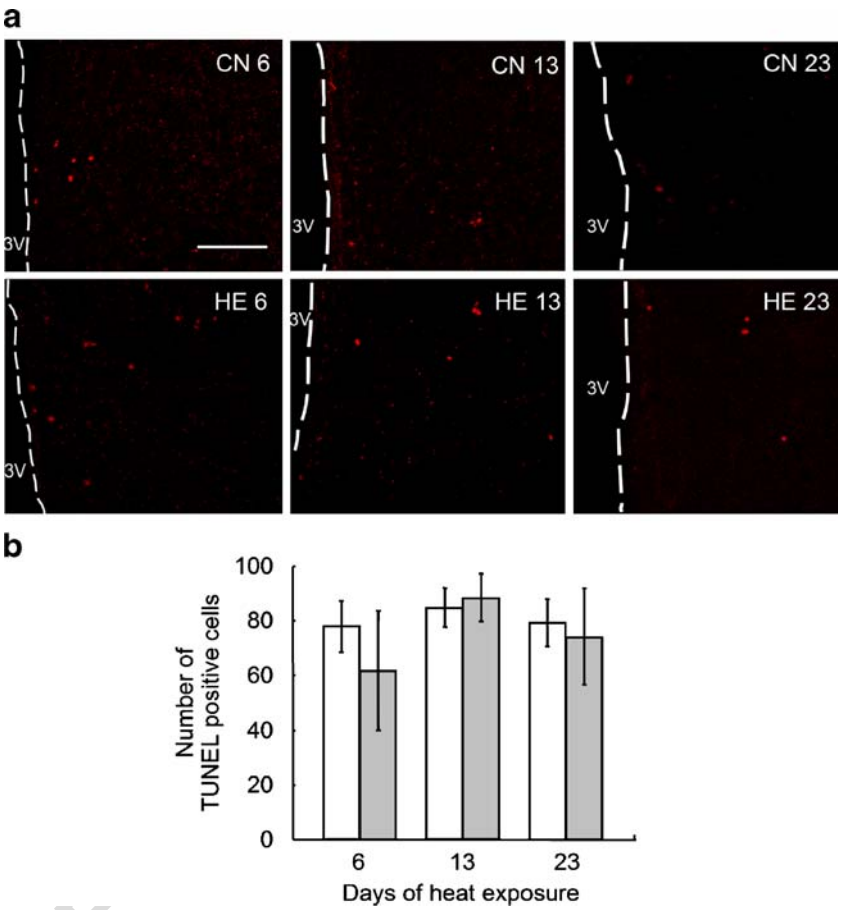

Fig. 10 TUNEL-positive cells in the hypothalamus. **a** Representative TUNEL-labeled (*red*) sections of the hypothalamus by confocal optical sectioning in CN (*upper panels*) and HE (*lower panels*). CN6, CN13, and CN23 show samples on the 6th, 13th, and 23rd day of the experiment, respectively. HE6, HE13, and HE23 show samples on the 6th, 13th, and 23rd day of the heat exposure period, respectively. *3V* third ventricle; scale bar 100 μm. **b** Total number of TUNEL-positive cells of CN (*open bars*) and HE (*gray bars*) in the hypothalamus. *Numbers in the abscissa* indicate days after starting heat exposure (n=4 in each subgroup). Values are the means ±SEMs. Heat exposure did not affect the numbers of TUNEL-positive cells

is well known to be one of the most important regions involved mainly in afferent thermoregulatory pathway [20, 33, 35, 38]. Interestingly, the percentage of newborn neurons of the POA/AH in the hypothalamus appeared to be the highest (Fig. 5) among regions examined especially in HE43 and HE53, e.g., in HE53, 23.2% of BrdU+/NeuN+ cells were located in the POA/AH. Other areas, such as the PVH, DMH, and VMH and posterior hy, are also known to participate significantly in thermoregulation [6, 26, 45], e.g., neurons in the DMH have a role in controlling thermoregulatory cutaneous vasomotion [9]. In HE53, a large number of BrdU+/NeuN+ cells were also located in the VMH, DMH, and the posterior hy. Newborn neurons in these regions may be involved in heat acclimation-induced changes in autonomic thermoregulatory function. Different from autonomic thermoregulation, it has been shown that the POA/AH does not have a significant role in selecting preferred ambient temperature, one of behavioral thermoregulations [1]. Since heat acclimation may alter preferred temperature in rats [43], studies of progenitor cell proliferation and neurogenesis of brain regions related to behavior in heat-acclimated rats may be needed.

Recent studies have suggested that newborn neurons generated from the adult hypothalamic progenitor cells can be integrated into neural networks by forming synapses and functionally working in, for instance, a feeding control system [22, 30]. In association with this study, we attempted to double-stain BrdU+ cells with a marker of synaptic vesicle membrane proteins, synaptophysin [29] in HE. The confocal microimages clearly demonstrated a part of BrdU+ cells were surrounded by synaptophysin (Fig. 9). Thus, heat exposure-generated newborn cells might have established synaptic connections with existing neurons of the hypothalamus. In addition, a large number of heat exposure-induced newborn cells were stained with Dcx. Since Dcx is a microtubule-associated protein present in neuronal projections [5], antibody to Dcx could allow visualization of dendritic arborization in immature neurons. In HE, some of BrdU and Dcx double-labeled cells exhibited fusiform shapes with a single process extending from their somata (Fig. 7). Others displayed more complex morphologies with many often-arborized projections. Thus, migrating and/or differentiating immature neurons might have been in the process of integrating into the hypothalamic circuitry. Nevertheless, these morphological observations may provide a good possibility that heat exposure-induced newborn cells in the hypothalamus have a capability to contribute to constructing a new neuronal network in rats.

Heat exposure significantly elevated core body temperature (Table 1). A high temperature physically facilitates biological reactions due to Q_{10} effect and then might accelerate cell proliferation. There are some brain regions which show a strong proliferative potency, e.g., the SVZ and SGZ. The SVZ gives neuronal precursors that migrate to the olfactory bulb through the rostral migratory stream, and the SGZ supplies the granular layer of the DG with new neurons [12]. We therefore examined BrdU+ cells in the two regions in HE and CN. However, heat exposure and an associated rise in core temperature had a minimum influence on cell proliferations in both the SVZ and SGZ (Fig. 3). The rise in cell temperature may not directly lead to proliferation of progenitor cells. The influence of heat exposure on vigorous proliferation of progenitor cells appears to be limited in the hypothalamic area where the thermoregulatory centers exist. Since heat exposure greatly elevates skin temperature, thermal inputs from the cutaneous thermoreceptors to the thermoregulatory centers may be one of key factors to promote progenitor cell proliferation in the hypothalamic area.

In studies in vitro, intense heat stress was reported to induce apoptotic cell death [46]. Neuronal cell death could be subsequently apoptotic stimulation and then facilitates neurogenesis in the central nervous system of rodents [44]. The present results, however, showed that the number of TUNEL-positive cells was not increased by exposure to moderate heat during the period when progenitor cell proliferation was vigorously induced in the hypothalamus (Fig. 10). Again, direct temperature effects on heat exposure-induced neurogenesis may be ruled out. For neurogenesis, a certain process of central adaptation has been known to be attained by apoptosis of neurons [44]. Obviously, this is not the case in the central mechanism of heat acclimation and this adaptation may be attributable not to loss of neurons but to neurogenesis.

Continuous exposure to heat could be a chronic stress on rats. Indeed, body weight of HE6 tended to decrease. Chronic stress has been known to activate hypothalamic–pituitary–adrenal axis (HPA) which induces hypertrophy of adrenal weights [47]. However, the adrenal wet weights of HE did not differ from those in CN (Table 2). In addition, activation of the HPA has been reported to interfere with proliferation of progenitor cells in the central nervous system [13, 19, 32]. The augmented cell proliferation and neurogenesis in the hypothalamus of heat-acclimated rats may not be caused by chronic stress.

In summary, constant exposure to moderate heat facilitated proliferation of progenitor cells in the ependymal layer of the third ventricle in rats. The proliferation started within the first 5 days of the heat exposure period and appeared to persist at least for the following 20 days. The newborn cells seemed to migrate into the hypothalamic parenchyma and dominantly differentiated to mature and immature neurons. Differentiation to mature neurons was significantly augmented after 33 days of heat exposure. Taken together, heat exposure promoted proliferation of progenitor cells in the hypothalamus for the first 30 days of heat exposure and then strongly facilitated neurogenesis thereafter. Some newly generated neurons appeared to be integrated in a neural network in the hypothalamus. Changes of thermoregulatory function in long-term heat-acclimated rats may possibly be at least in part attributable to generations of neurons in the hypothalamus.

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References

1. Almeida MC, Steiner AA, Branco LG, Romanovsky AA (2006) Neural substrate of cold-seeking behavior in endotoxin shock. PLoS ONE 1:e1
2. Armstrong LE, Stoppani J (2002) Central nervous system control of heat acclimation adaptations: an emerging paradigm. Rev Neurosci 13:271–285
3. Baldassarre G, Boccia A, Bruni P, Sandomenico C, Barone MV, Pepe S, Angrisano T, Belletti B, Motti ML, Fusco A, Viglietto G (2000) Retinoic acid induces neuronal differentiation of embryonal carcinoma cells by reducing proteasome-dependent proteolysis of the cyclin-dependent inhibitor p27. Cell Growth Differ 11:517–526
4. Bratincsák A, Palkovits M (2004) Activation of brain areas in rat following warm and cold ambient exposure. Neuroscience 127:385–397
5. Brown JP, Couillard-Després S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG (2003) Transient expression of doublecortin during adult neurogenesis. J Comp Neurol 467:1–10
6. Cano G, Passerin AM, Schiltz JC, Card JP, Morrison SF, Sved AF (2003) Anatomical substrates for the central control of sympathetic outflow to interscapular adipose tissue during cold exposure. J Comp Neurol 460:303–326
7. Cunningham JJ, Roussel M (2001) Cyclin-dependent kinase inhibitors in the development of the central nervous system. Cell Growth Differ 12:387–396
8. Demicka A, Caputa M (1993) Effect of warm rearing on the development of thermolytic effectors in rats. J Therm Biol 18:257–262
9. Dimicco JA, Zaretsky DV (2007) The dorsomedial hypothalamus: a new player in thermoregulation. Am J Physiol 292:R47–R63
10. Dyer MA, Cepko CL (2001) $p27^{kip1}$ and $p57^{kip2}$ regulate proliferation in distinct retinal progenitor cell populations. J Neurosci 21:4259–4271
11. Eynan M, Knubuvetz T, Meiri U, Navon G, Gerstenblith G, Bromberg Z, Hasin Y, Horowitz M (2002) Heat acclimation-induced elevated glycogen, glycolysis, and low thyroxine improve heart ischemic tolerance. J Appl Physiol 93:2095–2104
12. Gage FH (2000) Mammalian neural stem cells. Science 287:1433–1438
13. Gould E, Cameron HA, Daniels DC, Woolley CS, McEwen BS (1992) Adrenal hormones suppress cell division in the adult rat dentate gyrus. J Neurosci 12:3642–3650

14. Guillery RW, Herrup K (1997) Quantification without pontification: choosing a method for counting objects in sectioned tissues. J Comp Neurol 386:2–7
15. Horowitz M, Meiri U (1985) Thermoregulatory activity in the rat: effects of hypohydration, hypovolemia and hypertonicity and their interaction with short term heat acclimation. Comp Biochem Physiol 82:577–582
16. Horowitz M, Sugimoto E, Okuno T, Morimoto T (1998) Changes in blood volume and vascular compliance during body heating in rats. Pflugers Arch 412:354–358
17. Horowitz M, Kaspler P, Simon E, Gerstberger R (1999) Heat acclimation and hypohydration: involvement of central angiotensin II receptors in thermoregulation. Am J Physiol 277:47–55
18. Horowitz M (2002) From molecular and cellular to integrative heat defense during exposure to chronic heat. Comp Biochem Physiol A Mol Integr Physiol 131:475–483
19. Joëls M, Karst H, Alfarez D, Heine VM, Qin Y, van Riel E, Verkuyl M, Lucassen PJ, Krugers HJ (2004) Effects of chronic stress on structure and cell function in rat hippocampus and hypothalamus. Stress 7:221–231
20. Kanosue K, Yanase-Fujiwara M, Hosono T (1994) Hypothalamic network for thermoregulatory vasomotor control. Am J Physiol Regul Integr Comp Physiol 267:R283–R288
21. Kawakita E, Hashimoto M, Shido O (2006) Docosahexaenoic acid promotes neurogenesis in vitro and in vivo. Neuroscience 139:991–997
22. Kokoeva MV, Yin H, Flier JS (2005) Neurogenesis in the hypothalamus of adult mice: potential role in energy balance. Science 310:679–683
23. Labunskay G, Meiri N (2006) R-Ras3/(M-Ras) is involved in thermal adaptation in the critical period of thermal control establishment. J Neurobiol 66:56–70
24. Li GH, Katakura M, Maruyama M, Enhkjargal B, Matsuzaki K, Hashimoto M, Shido O (2008) Changes of noradrenaline-induced contractility and gene expression in aorta of rats acclimated to heat in two different modes. Eur J Appl Physiol 104:29–40
25. Lledo PM, Alonso M, Grubb MS (2006) Adult neurogenesis and functional plasticity in neuronal circuits. Nat Rev Neurosci 7:179–193
26. Lu J, Zhang YH, Chou TC, Gaus SE, Elmquist JK, Shiromani P, Saper CB (2001) Contrasting effects of ibotenate lesions of the paraventricular nucleus and subparaventricular zone on sleep-wake cycle and temperature regulation. J Neurosci 21:4864–4874
27. Lukaszewicz A, Savatier P, Cortay V, Kennedy H, Dehay C (2002) Contrasting effects of basic fibroblast growth factor and neurotrophin 3 on cell cycle kinetics of mouse cortical stem cells. J Neurosci 22:6610–6622
28. Magavi SS, Leavitt BR, Macklis JD (2000) Induction of neurogenesis in the neocortex of adult mice. Nature 405:951–955
29. Markakis EA, Gage FH (1999) Adult-generated neurons in the dentate gyrus send axonal projections to field CA3 and are surrounded by synaptic vesicles. J Comp Neurol 406:449–460
30. Markakis EA, Palmer TD, Randolph-Moore L, Rakic P, Gage FH (2004) Novel neuronal phenotypes from neural progenitor cells. J Neurosci 24:2886–2897
31. Maruyama M, Hara T, Hashimoto M, Koga M, Shido O (2006) Alterations of calf venous and arterial compliance following acclimation to heat administered at a fixed daily time in humans. Int J Biometeorol 50:269–274
32. McEwen BS (1999) Stress and hippocampal plasticity. Annu Rev Neurosci 22:105–122
33. Nagashima K, Nakai S, Tanaka M, Kanosue K (2000) Neuronal circuitries involved in thermoregulation. Auton Neurosci 85:18–25
34. Nakamura K, Morrison SF (2008) A thermosensory pathway that controls body temperature. Nat Neurosci 11:62–71
35. Nakayama T (1985) Thermosensitive neurons in the brain. Jpn J Physiol 35:375–389
36. Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates, 4th edition. Academic, New York
37. Pencea V, Bingaman KD, Wiegand SJ, Luskin MB (2001) Infusion of brain-derived neurotrophic factor into the lateral ventricle of the adult rat leads to new neurons in the parenchyma of the striatum, septum, thalamus, and hypothalamus. J Neurosci 21:6706–6717
38. Romanovsky AA (2007) Thermoregulation: some concepts have changed. Functional architecture of the thermoregulatory system. Am J Physiol 292:R37–R46
39. Saha SK, Ohno T, Tsuchiya K, Kuroshima A (2000) Adaptive modification of membrane phospholipid fatty acid composition and metabolic thermosuppression of brown adipose tissue in heat-acclimated rats. Int J Biometeorol 43:163–168
40. Sakurada S, Shido O, Sugimoto N, Fujikake K, Nagasaka T (1994) Changes in hypothalamic temperature of rats after daily exposure to heat at a fixed time. Pflügers Arch 429:291–293
41. Schwimmer H, Eli-Berchoer L, Horowitz M (2006) Acclimatory-phase specificity of gene expression during the course of heat acclimation and superimposed hypohydration in the rat hypothalamus. J Appl Physiol 100:1992–2003
42. Shein NA, Doron H, Horowitz M, Trembovler V, Alexandrovich AG, Shohami E (2007) Altered cytokine expression and sustained hypothermia following traumatic brain injury in heat acclimated mice. Brain Res 1185:313–320
43. Sugimoto N, Sakurada S, Shido O (1999) Selected ambient temperatures of rats acclimated to heat given on various schedules. Pflügers Arch 438:766–770
44. Thomaidou D, Mione MC, Cavanagh JF, Parnavelas JG (1997) Apoptosis and its relation to the cell cycle in the developing cerebral cortex. J Neurosci 17:1075–1085
45. Thornhill JA, Halvorson I (1994) Electrical stimulation of the posterior and ventromedial hypothalamic nuclei causes specific activation of shivering and nonshivering thermogenesis. Can J Physiol Pharmacol 72:89–96
46. Vogel P, Dux E, Wiessner C (1997) Evidence of apoptosis in primary neuronal cultures after heat shock. Brain Res 764:205–213
47. Watanabe T, Fujioka T, Hashimoto M, Nakamura S (1998) Stress and brain angiotensin II receptors. Crit Rev Neurobiol 12:305–317
48. Wyndham CH (1967) Effect of acclimatization on the sweat rate-rectal temperature relationship. J Appl Physiol 22:27–30
49. Xu Y, Tamamaki N, Noda T, Kimura K, Itokazu Y, Matsumoto N, Dezawa M, Ide C (2005) Neurogenesis in the ependymal layer of the adult rat 3rd ventricle. Exp Neurol 192:251–264
50. Yoshida K, Konishi M, Nagashima K, Saper CB, Kanosue K (2005) Fos activation in hypothalamic neurons during cold or warm exposure: projections to periaqueductal gray matter. Neuroscience 133:1039–1046