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ORIGINAL ARTICLE

# Hyperthermia combined with ethanol administration induces c-fos expression in the central amygdaloid nucleus of the mouse brain. A possible mechanism of heatstroke under the influence of ethanol intake

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**Abstract** Heatstroke is defined as a core body temperature that rises above 40.6°C and is accompanied by mental status abnormalities such as delirium, convulsions, or coma resulting from exposure to environmental heat. There is fairly wide agreement that ethanol intake is a predisposing factor in heatstroke. This study was performed to identify the brain changes induced by heatstroke, using a mouse hyperthermia model with and without preceding ethanol administration. Exposure to heat of 42°C until the core temperature reached to 43°C followed by exposure to 37°C for 15 min decreased the levels of partial pressures of $O_2$ in blood. Preceding ethanol administration and heat exposure induced hypotension, severe metabolic acidosis and respiratory failure, and, accordingly, produced heatstroke. Immunohistochemistry of the brains showed that preceding ethanol administration increased the number of c-fos-immunoreactive neurons, as a marker of neuronal activation, in the central amygdaloid nucleus, which is involved in thermoregulation. These results indicate that combined effects of ethanol and heat exposure induce heatstroke that is associated with activation of the central amygdaloid nucleus, implicating the pathophysiology and mechanisms of heatstroke under the influence of ethanol intake.

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## Introduction

Heatstroke is defined clinically as a core body temperature that rises above 40.6°C and is accompanied by hot, dry skin and mental status abnormalities such as delirium, convulsions, or coma resulting from exposure to environmental heat or strenuous physical exercise [1, 2]. Pathophysiologically, heatstroke is a form of hyperthermia associated with a systemic inflammatory response leading to a syndrome of multiorgan dysfunction in which encephalopathy predominates [2]. Despite adequate lowering of the body temperature and aggressive treatment, heatstroke is often fatal, and those who survive may sustain substantial neurological impairments [3]. Because heatstroke causes brain dysfunction, the identification of brain changes induced by heatstroke is essential to understand its pathophysiology and to establish therapeutic strategies.

Disabled elderly people who live alone and infants who are left unattended are at risk of heatstroke because of an inability to care for themselves and to avoid hot environments. Ethanol intake is also a predisposing factor in heatstroke [4, 5]. Impaired judgment due to acute ethanol intoxication in hot environments can cause heatstroke [6]. Drinking ethanol while bathing in a sauna of 80−90°C can create serious health risks and lead to death from hyperthermia [7]. Ethanol intake produces acute effects such as sedation, anesthesia, and a hypnotic state [8]. The acute depressive effects and addiction produced by ethanol are hypothesized to be related to altered signal transduction and increased fluidity of neuronal cell membranes [9]. Because both environmental heat and ethanol intake affect

the brain, analysis of the combined effects of both exposures on the brain might lead to the discovery of the mechanisms of brain dysfunctions in heatstroke.

The principal objective of this study was to identify the brain changes induced by heatstroke using a mouse hyperthermia model. Hyperthermia was induced in mice with and without preceding intraperitoneal (ip) administration of ethanol by exposing the mice to a temperature of 42°C until the core temperature reached 43°C; they were subsequently exposed to a temperature of 37°C for 15 min. Immunohistochemistry for c-fos was used to identify the brain regions activated by hyperthermia, ethanol administration, or both. C-fos is an immediate early gene product and is widely used as a marker of neuronal activation in specific brain regions by noxious stimuli in laboratory animals [10, 11]. C-fos immunoreactivity in the brain was compared between mice of control, ethanol, hyperthermia, and ethanol–hyperthermia groups. The present results show that combined effects of ethanol and heat exposure activate the central amygdaloid nucleus (CeA) which is involved in thermoregulation, implicating the pathophysiology and mechanisms of heatstroke under the influence of ethanol intake.

## Materials and methods

### Experimental animals

Procedures using animals were approved by the ethical committee on animal experiments in Saga University. Female ddY mice (Kyudo, Japan) aged 5–6 weeks (20–27 g) were used after at least 1 week for acclimation to the housing conditions. The mice were housed in a humidity- and temperature-controlled environment on a 12:12-h light–dark schedule and had free access to food and water. Prior to the experiment, the mice were fasted overnight (12 h) to stabilize blood glucose levels but retained free access to water. All efforts were made to minimize suffering and the number of mice used. A total of 76 mice were randomly assigned to four groups: control, ethanol, hyperthermia, and ethanol–hyperthermia (Table 1). Mice in the ethanol and ethanol–hyperthermia groups were administered a single dose of ethanol (13% weight/volume; 2 g/kg, ip) as described by Herring et al. [12]. Ethanol for ip injection was diluted with saline. Mice in the control and hyperthermia groups received an injection of 0.9% saline equivalent to the volume of a 2-g/kg dose of 13% ethanol. After being left for 15 min in a room at a temperature of 27°C, mice were anesthetized with pentobarbital (50 mg/kg, ip). Core temperature was monitored continuously using a digital thermometer (BAT-12) with a rectal probe for mice (RET-3; Physitemp Instruments, Clifton, NJ, USA). Hyperthermia was induced in mice according to the modified method for heat-shocked rat model described by Papasozomenos [13]. Mice in the hyperthermia and ethanol–hyperthermia groups were exposed to a temperature of 42°C in a temperature-controlled fan-ventilated chamber until the core temperature reached 43°C. They were subsequently exposed to a temperature of 37°C for 15 min in another temperature-controlled chamber. Mice in the control and ethanol groups were kept at room temperature throughout the entire experiment.

### Assessment of physiological parameters

Immediately before and after the period of incubation, heart rate and mean blood pressure (MBP) of the mice ($n$=5 per group) were measured from their tails by a noninvasive blood pressure monitor (MK-1030; Muromachi Kikai, Japan). A significant decrease of MBP from the level before incubation period was taken as the onset of heatstroke [14].

### Assessment of blood parameters

Immediately before and after the period of incubation, 60 μl of blood was taken from the cut edge of the mouse tails and collected in a heparinized tube ($n$=5 per group). Blood levels of pH, $HCO_3^-$, base excess (BE), partial pressures of $O_2$ ($pO_2$) and $CO_2$ ($pCO_2$), $O_2$ saturation ($SO_2$), sodium (Na), potassium (K), ionized calcium (iCa), hematocrit (Hct), hemoglobin (Hb), and glucose were measured by an i-STAT® Portable Clinical Analyzer with cartridge $CG8^+$ (i-STAT Corporation, East Windsor, NJ, USA), the performance of which is not affected by the presence of ethanol in blood [15].

**Table 1** The number of mice in groups used for each analysis

| Groups | Physiological and blood parameters | Immunohistochemistry on whole mount | Immunohistochemistry on section |
|---|---|---|---|
| Control ($n$=19) | 5 | 2 | 12 |
| Ethanol administration ($n$=19) | 5 | 2 | 12 |
| Hyperthermia ($n$=19) | 5 | 2 | 12 |
| Ethanol administration ($n$=19) and hyperthermia | 5 | 2 | 12 |

### Tissue preparation

After the experiment, mice were kept at room temperature and had free access to food and water for 1, 3, or 6 h. Mice were then reanesthetized with pentobarbital and perfused transcardially with 0.01 M phosphate-buffered saline (PBS; pH 7.4), followed by 4% paraformaldehyde–0.1 M phosphate buffer (PFA–PB; pH 7.4). Brains were removed, cut coronally into 2-mm-thick sections using a mouse brain slicer (Muromachi Kikai, Japan) and postfixed at 4°C overnight in PFA–PB.

### Immunohistochemistry on whole mounts

Coronal brain sections of the mice that were kept for 6 h after the experiment were used for whole-mount immunostaining ($n$=2 per group). The brain sections were washed with PBS (3×5 min) at 4°C and immersed in methanol at −20°C for 20 min. Sections were subsequently washed with Tris–HCl-buffered saline (TBST; 25 mM Tris–HCl, pH 7.4, 137 mM NaCl, 2.7 mM KCl, 0.1% Triton-X100; 3×10 min) and treated with 2% hydrogen peroxide in TBST at 4°C for 1 h to block endogenous peroxidase activity. After washing with TBST (3×30 min), sections were blocked with TBST blocking solution containing 2% bovine serum albumin at 4°C for 1 h and then incubated with a c-fos rabbit polyclonal antibody (1:10,000, Ab-5, Calbiochem, San Diego, CA, USA) in blocking solution at 4°C overnight. Following washing with TBST (8×30 min), sections were blocked with blocking solution at 4°C for 1 h and incubated with horseradish-peroxidase-conjugated goat antirabbit immunoglobulin antiserum (1:500, ALI3404, Biosource, Camarillo, CA, USA) in blocking solution at 4°C overnight. Sections were then washed with TBST (12×30 min) and preincubated with TBST containing 500 μg/ml 3,3′-diaminobenzidine tetrahydrochloride at 4°C for 30 min. Hydrogen peroxide was added to a final concentration of 0.02%, which resulted in a brown reaction product. The reaction was stopped by washing the sections with TBS (3×10 min). Sections were postfixed with 4% PFA in PB at 4°C for 3 h. To obtain transparency, sections were transferred through a graded series of up to 80% glycerol in distilled water. Control experiments were performed with the omission of the primary antibody yielding immunonegative results.

### Immunohistochemistry on sections

Coronal brain sections of the mice that were kept for 1, 3, and 6 h after the experiment were used for immunohistochemical detection of c-fos protein ($n$=4 per group at each time point). Vibratome sections (50 μm thick) were obtained from the coronal section at the bregma level (−1.58 mm) and placed in 0.01 M PBS ($n$=4 per group). Immunohistochemistry for c-fos was performed on free-floating vibratome sections according to the method for the whole-mount immunostaining. Immunostained sections were mounted on silane-coated slides, air-dried, counterstained with hematoxylin, dehydrated through a graded series of alcohol, cleared with xylene and coverslipped with the mounting medium Permount (Fisher Scientific, Fair Lawn, NJ, USA). Control experiments were performed with the omission of the primary antibody yielding immunonegative results.

### Quantification of c-fos immunolabeled cells

The CeA was identified in the c-fos immunolabeled vibratome brain sections according to the mouse stereotaxic atlas of Paxinos and Franklin [16]. Serial sections stained with cresyl violet were used for the exact neuroanatomical determination of the nuclear boundary. Digital images of CeA were acquired using a ×10 objective on a light microscope equipped with a digital camera interfaced with a personal computer. All images were stored and analyzed blindly to group assignment. After printing the digital images, the observers counted cells expressing nuclear c-fos staining within the left and right CeA. An average number for bilateral nuclei was obtained, and group averages were subsequently calculated.

### Data analysis

Data are expressed as the mean±standard deviation (SD). Two-way repeated-measures analysis of variance (ANOVA) was used to compare the physiological and blood data among the groups. One-way ANOVA was used to compare the numbers of c-fos immunolabeled cells among the groups. Both analyses were followed by Tukey's post hoc test (SPSS, Version 11.0; SPSS Inc., Chicago, IL, USA). $p<0.05$ was interpreted as a statistically significant difference.

## Results

### Core temperature

Core temperatures of mice in the control, ethanol, hyperthermia, and ethanol–hyperthermia groups before the period of incubation were within the physiological range ($n$=5 per group, Table 2). The core temperatures of mice in the hyperthermia group reached 43°C in 22.6±1.2 min after the initiation of exposure to 42°C and were 40.6±0.2°C at the end of the 15 min exposure to 37°C. The core temperatures of mice in the ethanol–hyperthermia group reached 43°C in 22.6±2.3 min after the initiation of

**Table 2** Core temperature, heart rate, and mean arterial pressure of the experimental groups

| | CT (˚C) | HR (beats/min) | MAP (mmHg) |
|---|---|---|---|
| Control | | | |
| Before incubation at 27°C | 36.7±0.9 | 455.5±26.9 | 35.5±5.4 |
| After incubation at 27°C | 32.3±1.5 | 251.0±21.7 | 42.1±6.0 |
| Ethanol administration | | | |
| Before incubation at 27°C | 36.9±0.3 | 516.7±66.6 | 34.3±3.5 |
| After incubation at 27°C | 32.4±1.2 | 387.5±34.4* | 36.7±9.7 |
| Hyperthermia | | | |
| Before incubation at 42°C and 37°C | 36.4±0.4 | 441.5±50.2 | 34.4±4.2 |
| After incubation at 42°C and 37°C | 40.6±0.2**, **** | 647.0±55.5**, *** | 34.3±3.9 |
| Ethanol administration and hyperthermia | | | |
| Before incubation at 42°C and 37°C | 36.7±0.7 | 471.7±20.6 | 35.1±4.0 |
| After incubation at 42°C and 37°C | 41.2±0.2**, **** | 571.8±102.5** | 25.7±3.3* |

Data represent means±standard deviation (SD) of five mice per group.
*CT* Core temperature, *HR* heart rate, *MAP* mean arterial pressure
*$p<0.05$; **$p<0.01$, differences between groups were regarded statistically significant at these levels when compared to control; ***$p<0.05$; ****$p<0.01$, differences between groups were regarded statistically significant at these levels when compared to ethanol administration

exposure to 42°C and were 41.2±0.2°C at the end of 15-min exposure to 37°C. No significant differences were observed in the length of time required to reach 43°C and in the core temperature at the end of 15-min exposure to 37°C between the hyperthermia and ethanol–hyperthermia groups.

### Physiological parameters

The values of physiological parameters in the four groups ($n$=5 per group) measured before and after the period of incubation are shown in Table 2. In mice in the ethanol–hyperthermia group, MBP after the period of heat incubation was 25.7±3.3 mmHg and was significantly lower than those of other three groups, indicating the onset of heatstroke.

### Blood parameters

The values of parameters in blood samples in the four groups ($n$=5 per group) taken before and after the period of incubation are shown in Table 3. In mice in the ethanol group, $HCO_3^-$ and BE were significantly decreased, indicating metabolic acidosis. In mice in the hyperthermia group, in addition to $HCO_3^-$ and BE, $pO_2$ was significantly decreased after the period of heat incubation. In mice in the ethanol–hyperthermia group, all of the above parameters and $SO_2$ were significantly decreased after the period of heat incubation, indicating metabolic acidosis and respiratory failure.

In mice in the ethanol group, the levels of iCa were significantly decreased after the period of incubation; mice in the hyperthermia and ethanol–hyperthermia groups additionally showed a significant increase in K levels. Blood glucose levels were significantly increased in mice in the hyperthermia group after the period of heat incubation.

### Immunohistochemistry on whole mounts

Whole-mount immunostaining with an anti-c-fos rabbit polyclonal antibody showed c-fos immunoreactivity in bilateral amygdala regions, in coronal sections at the bregma level (−1.58 mm) of the cerebra of mice in the hyperthermia and ethanol–hyperthermia groups. Immunostaining in the amygdaloid regions was not observed in mice in the control and ethanol groups. A comparison of coronal sections from mice in each of the four groups showed no additional differences in c-fos immunoreactivity. Increased immunostaining could not be observed in the hypothalamic regions in mice in the hyperthermia and ethanol–hyperthermia groups ($n$=2 per group, Fig. 1).

### Immunohistochemistry on sections

The CeA was identified as a site of increased c-fos expression in the amygdaloid region of coronal sections at the bregma level (−1.58 mm; Fig. 2). In mice of hyperthermia group, the number of CeA neurons expressing c-fos was increased at 6 h after heat incubation, but this increase was not statistically significant. In mice of ethanol–hyperthermia group, the number of CeA neurons expressing c-fos was significantly increased at 3 and 6 h after heat incubation, as compared with those in other groups at each time point ($n$=4 per group at each time point, Figs. 3 and 4).

## Discussion

In this animal study, administration of ethanol induced metabolic acidosis. Exposure to heat of 42°C until the core

**Table 3** Blood parameters of the experimental groups

| | pH | $HCO_3^-$ (mM) | BE (mM) | $pO_2$ (mmHg) | $pCO_2$ (mmHg) | $SO_2$ (%) | Na (mM) | K (mM) | iCa (mM) | Hct (%) | Hb (g/dl) | Glucose (mg/dl) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | | | | | | | | | | | | |
| Before incubation at 27°C | 7.3±0.1 | 16.2±1.1 | −9.6±1.1 | 80.8±10.3 | 31.1±5.3 | 94.6±2.5 | 147.0±2.1 | 3.7±0.3 | 1.2±0.0 | 42.4±2.7 | 14.4±0.9 | 110.4±19.5 |
| After incubation at 27°C | 7.3±0.1 | 20.6±1.1 | −6.2±1.1 | 105.0±11.9 | 46.2±9.4 | 97.0±0.7 | 147.0±2.1 | 3.4±0.9 | 1.4±0.1 | 40.4±1.7 | 13.8±0.4 | 114.0±10.3 |
| Ethanol administration | | | | | | | | | | | | |
| Before incubation at 27°C | 7.3±0.0 | 14.8±2.2 | −12.2±1.5 | 74.6±7.1 | 32.9±4.9 | 92.4±1.8 | 148.8±1.1 | 4.1±0.7 | 1.1±0.0 | 41.4±1.8 | 14.0±0.7 | 129.2±5.9 |
| After incubation at 27°C | 7.1±0.0** | 16.8±1.6* | −13.0±2.0** | 95.0±15.3 | 54.9±4.4 | 92.6±4.4 | 149.2±1.9 | 3.8±0.4 | 1.2±0.0** | 41.2±1.8 | 14.2±0.8 | 123.0±7.6 |
| Hyperthermia | | | | | | | | | | | | |
| Before incubation at 42°C and 37°C | 7.3±0.0 | 16.0±1.4 | −9.8±1.3 | 77.4±7.8 | 30.7±3.7 | 94.4±1.5 | 146.0±1.9 | 4.0±0.7 | 1.2±0.1 | 40.6±2.1 | 13.8±0.8 | 107.0±10.6 |
| After incubation at 42°C and 37°C | 7.3±0.0**** | 14.0±0.7** | −13.0±1.0** | 68.4±8.4**, *** | 31.4±1.9**** | 90.4±3.4 | 145.0±1.6*** | 5.8±0.4* | 1.1±0.0** | 42.0±2.4 | 14.2±0.8 | 171.4±16.5* |
| Ethanol administration and hyperthermia | | | | | | | | | | | | |
| Before incubation at 42°C and 37°C | 7.3±0.0 | 14.2±0.8 | −12.6±0.9 | 72.4±5.9 | 31.1±3.4 | 92.0±2.3 | 148.0±1.2 | 4.1±0.6 | 1.1±0.0 | 42.4±2.7 | 14.4±1.1 | 128.6±24.9 |
| After incubation at 42°C and 37°C | 7.2±0.0*, ***** | 11.2±1.3**, ****, ***** | −17.0±1.6**, ***, ****** | 54.4±4.0**, **** | 31.0±3.0**** | 79.6±3.6**, ****, ****** | 146.4±3.0 | 6.8±1.4**, **** | 1.0±0.1**, ****, ***** | 46.2±1.8 | 15.6±0.5 | 86.6±17.4***** |

Data represent means±standard deviation (SD) of five animals per group.

*$p<0.05$; **$p<0.01$ differences between groups were regarded statistically significant at these levels when compared to control; ***$p<0.05$; ****$p<0.01$ differences between groups were regarded statistically significant at these levels when compared to ethanol administration; *****$p<0.05$; ******$p<0.01$ differences between groups were regarded statistically significant at these levels when compared to hypothermia

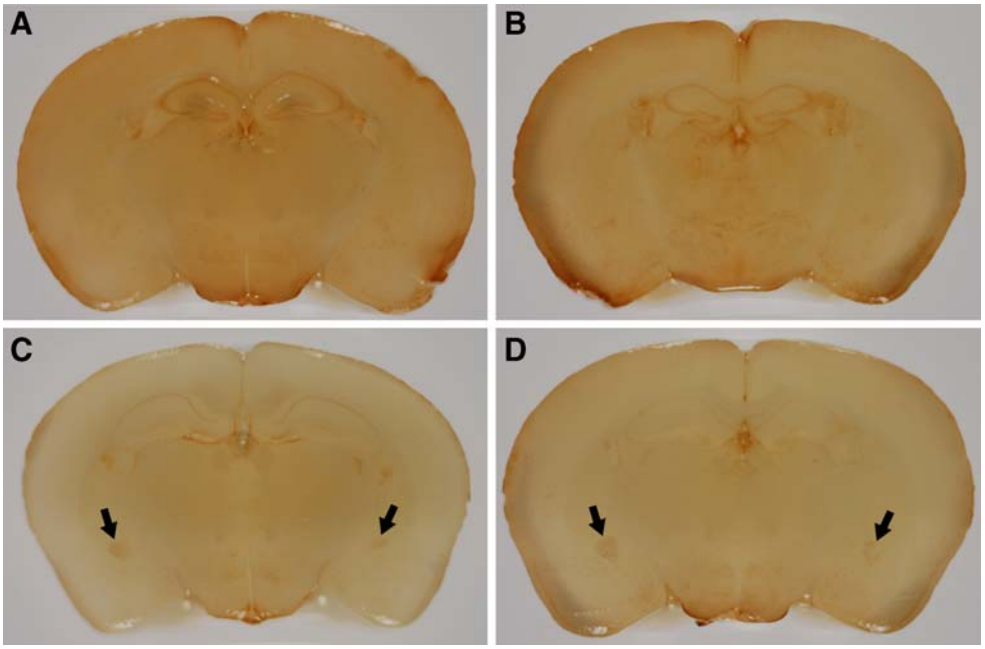


**Fig. 1** Whole-mount immunostaining with an anti-c-fos antibody of coronal brain sections at the bregma level (−1.58 mm) of mice at 6 h after the experiment. As compared with control (**a**) and ethanol-administered (**b**) mice, hyperthermia (**b**) and ethanol-preadministered hyperthermia (**d**) mice show immunostaining in the bilateral amygdala regions (*arrows*)

temperature reached to 43°C followed by exposure to 37°C for 15 min decreased the levels of $pO_2$ in blood. Preceding ethanol administration and heat exposure induced hypotension, metabolic acidosis, and respiratory failure and, accordingly, produced heatstroke. In addition, preceding ethanol administration increased the number of c-fos-positive neurons in the CeA, a center of thermoregulation, in hyperthermia. These results indicate that combined effects of ethanol and heat exposure induce heatstroke that is associated with activation of the CeA, implicating the pathophysiology and mechanisms of heatstroke under the influence of ethanol intake.

The results of analyses of blood parameters in this study show that hyperthermia is accompanied by electrolyte disturbances and hyperglycemia. In addition, ethanol-pretreated mice developed severe acidosis with respiratory failure and severe hyperkalemia during heatstroke, indicating the involvement of these mechanisms in heatstroke under the influence of ethanol. Ethanol administration decreases the levels of iCa in blood [15]. Heatstroke decreases the levels of iCa in patients with rhabdomyolysis and acute renal failure due to altered calcium metabolism [17]. The severe decrease of iCa in ethanol-preadministrated heatstroke mice supports

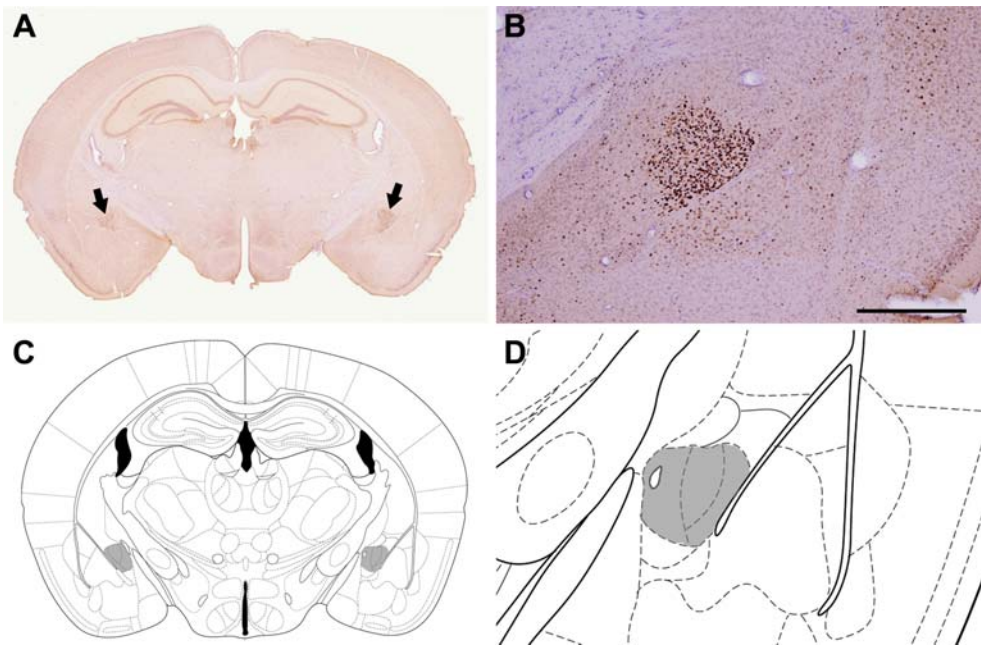


**Fig. 2** Immunohistochemistry with an anti-c-fos antibody of a vibratome section of the coronal brain section at the bregma level (−1.58 mm) of an ethanol-preadministered hyperthermia mouse at 6 h after the experiment. **a** Immunostaining in the bilateral amygdala regions (*arrows*). **b** Immunostaining of the central amygdaloid nucleus of the amygdala region (*bar*=1 mm). **c** Schematic coronal sections of the mouse brain obtained from a mouse brain atlas. *Gray areas* indicate CeA (reprinted from [16] and modified with permission from Elsevier). **d** The amygdala region of C

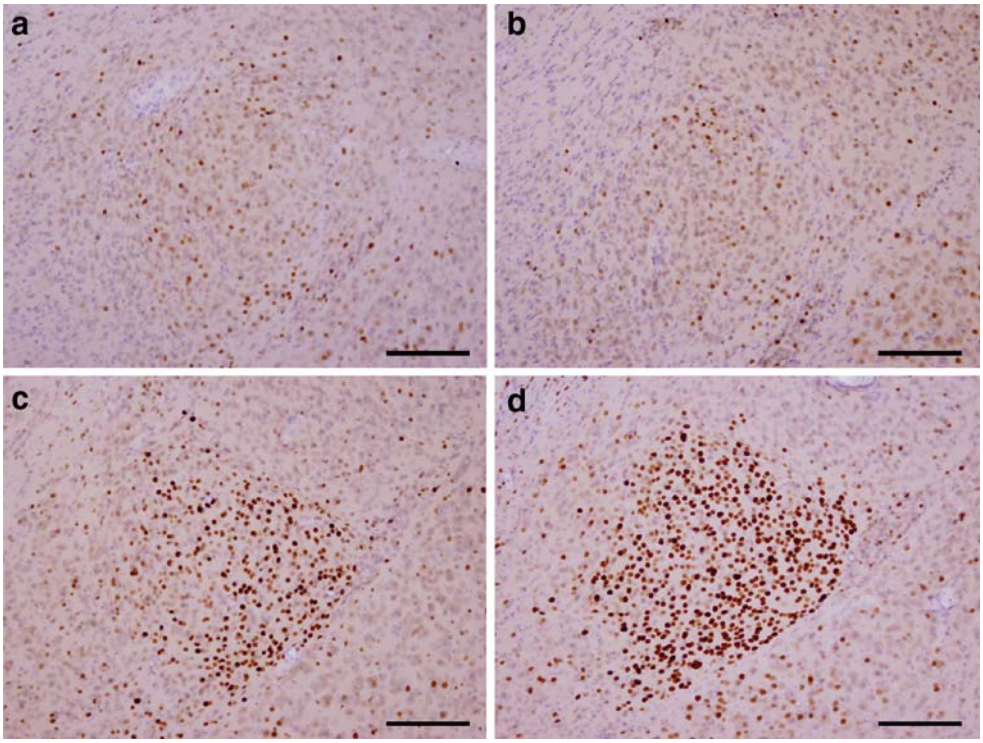


**Fig. 3** C-fos-immunoreactive neurons in the central amygdaloid nucleus of mice brain at 6 h after the experiment. **a** Control mouse. **b** Ethanol-administered mouse. **c** Hyperthermia mouse. **d** Ethanol-preadministered hyperthermia mouse. *Bar*=300 μm

the hypothesis that ethanol intake and heat exposure mutually enhance their effects on the body.

Because heat exposure raises core temperature and causes dehydration, previous studies on the relationship between heat exposure and c-fos expression in the brain have focused on the hypothalamic region, which is involved in thermoregulation and fluid regulation. Exposure of rats to heat of 42°C for 80 min induces c-fos expression in the paraventricular, supraoptic, and preoptic hypothalamic nuclei [18]. Exposure of mice to heat of 38°C for 60 min also induces c-fos expression in these nuclei, as well as the lateral septal nucleus, medial preoptic area, lateral hypothalamic area, and zona incerta [19]. In these two studies, immunohistochemistry on sections is used to observe c-fos expression in the brain. We have employed whole-mount immunohistochemistry to take a global view of c-fos expression in the brain and identified the CeA as a site of strong c-fos expression in heatstroke under the influence of ethanol. Increased immunostaining could not be observed in the hypothalamic regions in hyperthermia mice in this study. This finding may be explained by the low sensitivity of whole-mount immunohistochemistry.

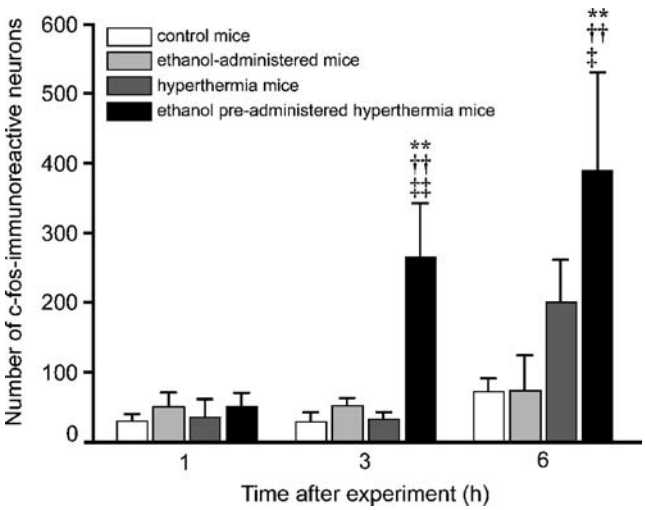


**Fig. 4** Numbers of c-fos-immunoreactive neurons in the central amygdaloid nuclei of mice brains at 1, 3, and 6 h after the experiment. Mean values±standard deviation (SD) are shown for four mice per group. Comparisons among groups were made at the same time point. $^{**}p<0.01$ compared to control mice group; $^{††}p<0.01$ compared to ethanol-administered mice group; $^{‡}p<0.05$ and $^{‡‡}p<0.01$ compared to hyperthermia mice group

The CeA plays a major role in the integration of autonomic and behavioral responses to fear and anxiety-provoking conditions [20]. In addition, several reports have provided evidence that the CeA is involved in thermoregulation. First, systemic administration of pyrogens causes activation of the CeA in rats [21]. Second, a bilateral electrolytic lesion of central amygdaloid nuclei attenuates the fever induced by herpes simplex virus-1 encephalitis in rats [22]. Third, the CeA has direct projections to nuclei in the hypothalamus and brainstem that are involved in the genesis of fever [23, 24]. In this study, the core temperature of hyperthermia mice exceeded the temperature of the exposing heat, suggesting that physiological responses elicited by heat exposure elevate core temperature. We also show here that heatstroke is associated with increased activity of the CeA in mice with preadministration of

ethanol. Activated CeA might contribute to the elevation of core temperature in heatstroke under the influence of ethanol intake. Studies on attenuation of the hyperactivity of the CeA might lead to the development of effective strategies for treating clinical heatstroke.

Ethanol administration activates the CeA. A study using rats shows that ip injection of a 2-g/kg dose of ethanol increases the number of c-fos-immunoreactive neurons in the CeA [12]. Studies with mice show a role for the CeA in mediating the locomotor-stimulating actions of ethanol [25]. In this study, although a 2-g/kg dose of ethanol alone did not induce c-fos immunoreactivity in the CeA, a 2-g/kg dose of ethanol followed by heat exposure significantly increased the number of c-fos-immunoreactive neurons in the CeA as compared to heat exposure alone. This finding indicates that the combined effects of ethanol and heat exposure strongly activate the CeA. Activation of the CeA due to the combined effects of ethanol and heat exposure might contribute to the prolonged elevation of core temperature in clinical heatstroke.

Hypotension alone has been reported to induce c-fos expression in the CeA [26]. Heatstroke can cause hypotension; a study using rats shows that exposure to heat of 40°C for 100 min significantly decreases mean arterial pressure and cerebral blood flow [27]. Therefore, arterial hypotension and cerebral hypoperfusion associated with heatstroke might contribute to the activation of the CeA in heatstroke. Bratincsák and Palkovits [28] observed an increase in c-fos expression in the CeA of rats following warm exposure of 37°C for 60 min and reported that core temperature elevation induced a nonspecific stress reaction. Although the CeA is involved in the regulation of stress-related responses [23], studies using a variety of stressors, including restraint and footshock, show minimal c-fos induction in CeA neurons and do not support the possibility that nonspecific stress invariably results in a CeA c-fos response [20, 21].

In forensic autopsy, the diagnosis of heatstroke is often difficult because there are no pathognomonic features of hyperthermia, and it is often too late for meaningful rectal temperature measurement [4]. Presence or absence of ethanol intake of the deceased is determined by the examination of antemortem or postmortem blood samples [29, 30]. We show here that heatstroke is associated with increased activity of the CeA in ethanol-preadministrated mice. This study suggests that identification of neuronal activation in CeA may be helpful for the autopsy diagnosis of heatstroke in individuals under the influence of ethanol. We also observed the increase of neuronal activity of the CeA in heatstroke mice without preadministration of ethanol, although statistically not significant. This finding suggests that neuronal activation in CeA is a possible indicator for the autopsy diagnosis of heatstroke in persons without the influence of ethanol as well.

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