

## Brain activation during whole body cooling in humans studied with functional magnetic resonance imaging

Kazuyuki Kanosue<sup>a,\*</sup>, Norihiro Sadato<sup>b</sup>, Tomohisa Okada<sup>b</sup>, Tamae Yoda<sup>a</sup>,  
Sadamu Nakai<sup>a</sup>, Kyoko Yoshida<sup>a</sup>, Takayoshi Hosono<sup>a</sup>, Kei Nagashima<sup>a</sup>,  
Tomoko Yagishita<sup>a</sup>, Osamu Inoue<sup>c</sup>, Kaoru Kobayashi<sup>c</sup>, Yoshiharu Yonekura<sup>d</sup>

<sup>a</sup>Department of Physiology, School of Allied Health Sciences, Osaka University Faculty of Medicine, Yamadaoka 1-7, Suita, Osaka 565-0871, Japan

<sup>b</sup>Psychophysiology Section Department of Cerebral Research, National Institute of Physiological Sciences, Okazaki National Research Institutes, Myodaiji-cho, Okazaki 444-8585, Japan

<sup>c</sup>Department of Medical Physics, School of Allied Health Sciences, Osaka University Faculty of Medicine, Yamadaoka 1-7, Suita, Osaka 565-0871, Japan

<sup>d</sup>Biomedical Imaging Research Center, Fukui Medical University, Matsuoka, Yoshida, Fukui 910-1104, Japan

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### Abstract

Regional activation of the brain was studied in humans using functional magnetic resonance imaging during whole body cooling that produced thermal comfort/discomfort. Eight normal male subjects lay in a sleeping bag through which air was blown, exposing subjects to cold air (8°C) for 22 min. Each subject scored their degree of thermal comfort and discomfort every min. As the subjects reported more discomfort the blood oxygen level dependent response in the bilateral amygdala increased. There was no activation in the thalamus, somatosensory, cingulate, or insula cortices. This result suggests that the amygdala plays a role in the genesis of thermal discomfort due to cold. © 2002 Published by Elsevier Science Ireland Ltd.

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Sensations evoked by innocuous thermal stimulation can be divided into two categories, ‘temperature sensation’ and ‘thermal comfort/discomfort’ [9]. Temperature sensation is directed toward the objects outside the body, as expressed with a sentence like ‘this is cold’ or ‘this is hot’. This sensation is evoked by the signals from warm and cold receptors in the skin. Neurons responding to innocuous thermal stimulation of the skin are located in the lamina I of the spinal cord [5,7]. Signals from these neurons then reach the thalamus, mainly in the posterior part of the ventral medial nucleus ( $V_{mpo}$ ) in primates [2]. Recent studies on humans that utilized positron emission tomography (PET) or functional magnetic resonance imaging (fMRI) have shown that thermal signals from skin seem to reach several regions in the cerebral cortex, including the insula, primary and

secondary somatosensory (SI and SII), orbitofrontal, and cingulate cortices [1,3,4,15].

The other category of thermal sensation, thermal comfort/discomfort, is expressed with a sentence like ‘I feel cold’ or ‘I feel hot’, and is important for body temperature regulation in that it drives an individual to search for a better environment to maintain normal body temperature. Thermal comfort/discomfort depends not only on the thermal condition of the external environment but also on that of the body [11]. In contrast to temperature sensation the neuronal mechanism of thermal comfort/discomfort is poorly understood. Present study investigated brain regions activated in relation to thermal comfort/discomfort during whole body cooling in humans, using fMRI.

Eight normal male volunteers ( $28 \pm 8$  years old, mean  $\pm$  SD) gave written informed consent before participating in the experiment. The subjects were all right-handed by the Edinburgh’s handedness inventory [12]. There was no history of neurological or psychiatric illness in any of the

\* Corresponding author. Tel./fax: +81-6-6879-2582.  
E-mail address: kanosue@sahs.med.osaka-u.ac.jp  
(K. Kanosue).

subjects. The protocol was approved by the ethical committee of Fukui Medical University.

The subjects wore only short pants and lay in a sleeping bag. The bag had two openings, one at the chest and the other at the feet. Air was blown into the bag from the opening at the chest and out through the opening at the feet. The circulating air was supplied by an air supply unit (3 m<sup>3</sup>/min, CAU-210, TABAI ESPEC, Osaka, Japan) located outside the room. The temperature of the air at the outlet of the unit could be controlled in a range from 5 to 50°C. Wooden frames were placed around the subjects so that there was a space between the sleeping bag and the subject for air flow. Tight but comfortable foam padding was placed around the subject's head. The forehead and the chin were fixed with straps to minimize head motion. Throughout the MRI scan, the subjects were instructed to close their eyes. Room temperature was maintained at 25°C.

During a 72-min MRI scanning session, air of four different thermal conditions was supplied. Initially, as a baseline condition, air of neutral temperature (28°C) was circulated for 15 min. Then, air circulation was stopped for 15 min. Next, cool air (8°C) was circulated for 22 min, and finally warm air (32°C) for 20 min. Each min the subject reported affective sensation (comfort/discomfort) about the thermal condition of the body with a seven point scale (1, very uncomfortable (very cold); 3 uncomfortable (cold); 5, neutral; 7, comfortable (warm)) to the examiner by means of predetermined hand signs, through a plastic film window on the right side of the bag. It should be noted that in Japanese different words are used for 'warm' and 'cold' describing temperature sensation and for those describing thermal comfort/discomfort. So, subjects clearly recognized that they had to report thermal comfort/discomfort and not temperature sensation. Temperature at the air intake of the sleeping bag was measured with a Cu-Co thermocouple.

T2\*-weighted, gradient echo echo planar imaging (EPI) sequences were used for functional images using a 1.5 Tesla MR imager (Horizon, GE, WI, USA). Each volume consisted of 24 slices; the slice thickness was 5 mm with a 1 mm gap, thus covering the whole brain. The time interval between two successive acquisitions of the same image volume was 6000 ms, and the echo time was 40 ms. EPI images were continuously obtained for 35 min, followed by 2-min intermission and another 35-min imaging session followed, generating a total of 700 volumes.

The data were analyzed with SPM 99 (Wellcome Department of Cognitive Neurology, London, UK; [6]) implemented in Matlab (Mathworks Inc. Sherborn, MA, USA). Following realignment and anatomic normalization, the fMRI data were then filtered with a Gaussian kernel of 10 mm full width and half maximum in the *x*, *y*, and *z* axes. To represent blood oxygen level dependent (BOLD) response changes within the time-window of 1 min, ten consecutive volumes were averaged. Hence, 70 volumes were obtained from each subject. The general linear model [6] was applied on a voxel-by-voxel basis to explore the effect of subjective

thermal comfort scoring on the BOLD response changes in each subject. High-pass filtering was used to eliminate the effect of low-frequency signal drift. The hypotheses tested asked whether the slope fitted for the regressor was significantly less than 0. The resulting set of voxel values constituted a statistical parametric map of the *t* statistic SPM{Z} for each subject. The threshold for SPM{Z} was set at  $P < 0.05$  with a correction for multiple comparison at a voxel level. The areas that showed significant covariation in each and every subject were depicted as the intersection of the SPM{Z} maps of all subjects.

Changes in bag temperature and thermal comfort score are shown in Fig. 1. During the first 15 min application of 28°C air, the subjects reported 'neutral' (Fig. 1B). In the next 15 min, when the air flow was stopped, the comfort score changed to 'comfortable' (Fig. 1B). All the subjects explained that the 28°C air flow gave a weak cold discomfort and stopping the air flow produced a sudden comfort of warmth, even though the bag temperature increased only slightly in that period (Fig. 1A). With an application of cold air, the bag temperature dropped to 12°C. In response to this cold stimulation the comfort score dropped to 2 (Fig. 1B). Discomfort further became stronger during the cold stimulation. In the present study, we tried to obtain a sharp contrast in discomfort due to cold, because we thought this would help to detect activation of the brain. Therefore, we applied warm (32°C) instead of

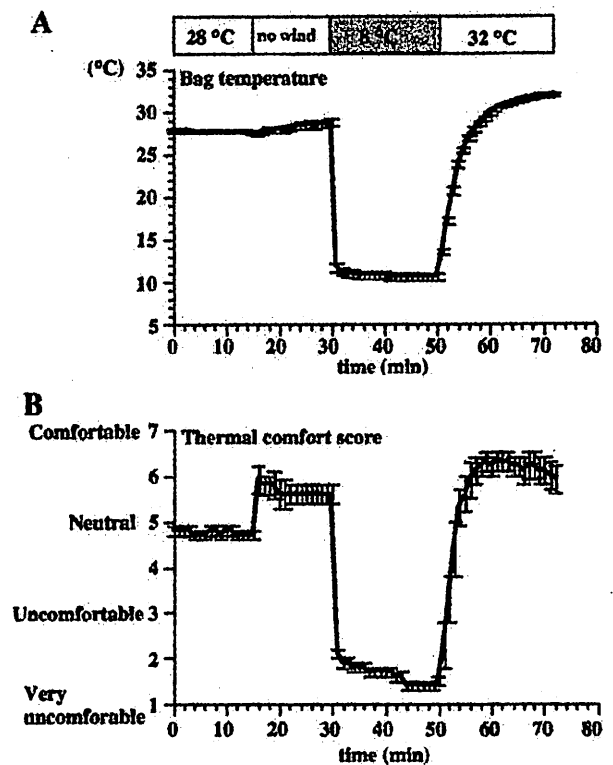


Fig. 1. Changes in temperature at the inlet of the bag (A); and thermal comfort score (B). Shown at the top of A is temperature at the outlet of the air supplier. Error bars indicate standard error.

Table 1  
Brain regions correlated with cold discomfort<sup>a</sup>

Area	Cluster size ( $\mu$ l)	Center of gravity*			Regression slope (mean $\pm$ SD, $n = 8$ )	T value**	P value
		x	y	z			
R amygdala	304	-15.5	1.7	-16.3	-0.335 $\pm$ 0.226	4.21	0.002
L amygdala	104	20.0	-1.5	-18.2	-0.268 $\pm$ 0.149	5.09	0.0014

<sup>a</sup> R: right, L: left. \*Talairach's coordinates; and \*\*one sample *t*-test.

neutral air (28°C) after the cold stimulation. The comfort score returned to neutral after 4 min and stayed at slightly 'comfortable' level until the end of the experiment. Seven out of eight subjects reported to have shivered, but had no pain during cold exposure.

We searched for the brain regions showing significant correlation between the BOLD response and thermal

comfort score in all subjects. Only the amygdala on both sides fulfilled this criteria (Table 1 and Fig. 2). Interestingly, the correlation was negative, i.e. neural activities increased as the subjects felt more cold discomfort. Fig. 2B shows the correlation between MR signal of the right amygdala and the comfort score in one subject. Other brain regions, including the thalamus, insula, SI, SII, orbitofrontal and cingulate cortices, showed no correlation between the BOLD response and thermal comfort score. There was no brain region that showed a positive correlation between these two parameters. Head motion, measured by the realignment parameters, was relatively small, subvoxel level. The correlation coefficient between comfort score and the head motion parameters was not significant and varied across the subjects.

The activation of the amygdala has never been reported in previous studies concerning temperature and sensation in humans where PET or fMRI was utilized [1,3,4,15]. On the other hand, in the present study, no activation was detected in previously reported areas such as the anterior and posterior insula, the anterior and posterior cingulate gyrus, the thalamus, and the SI and SII. The previous studies dealt with 'temperature sensation', while in the present study we searched for the regions responsible for 'thermal comfort/discomfort'. Therefore, it is not surprising that different brain regions were activated for these distinctive sensations. However, the cold stimulus in the present study should have evoked not only thermal comfort/discomfort, but also temperature sensation (feeling of coldness). Thus, we had expected that those brain regions previously reported to be activated in relation to cold temperature sensation would have also been activated in the present experimental conditions. But this was not the case. One possible reason could be differences in the duration of the cold stimulus. In previous studies, the stimuli lasted from one second to several minutes. In the present study the cold stimulus was applied for 22 min. With the wide stimulus area (the whole body) and temperature range below 20°C, temperature sensation in the present study should not have adapted completely and steady temperature sensations should have remained even at constant temperature during most of the cooling period. Nevertheless, the dynamic component of cold temperature sensation is stronger than its static component [9]. In contrast, thermal discomfort never shows adaptation and becomes rather stronger with time [8]. Thus, activation of the sites responsible for

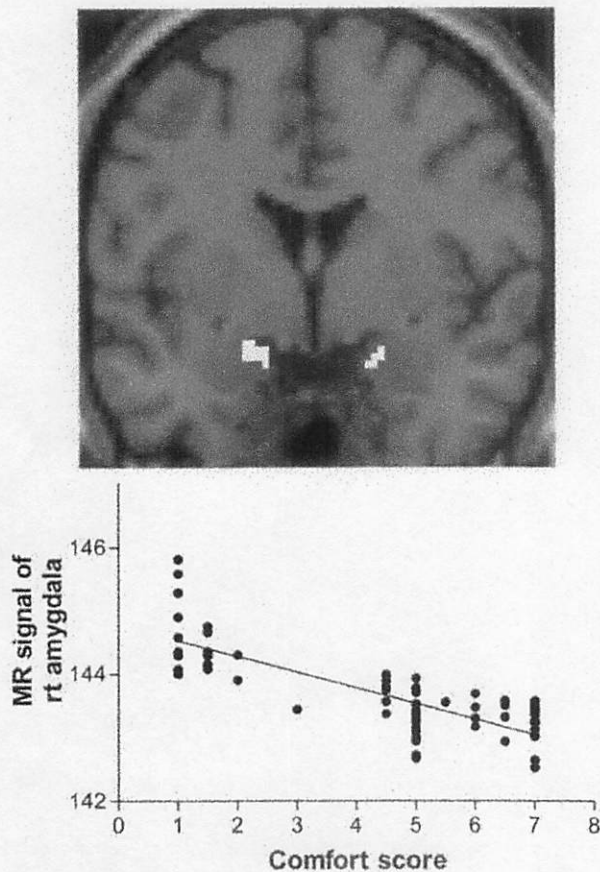


Fig. 2. (Top) The areas that showed significant covariation of BOLD signals with cold discomfort in each and every subject, superimposed on the high resolution MRI of the individual unrelated to the study. In all subjects, only bilateral amygdala showed negative correlation with the comfort score. (Bottom) Significant correlation of MR signal change by individual analysis. Normalized MR signal (relative to the global signal which was set to 100) in the right amygdala was plotted against the comfort score. The regression line was  $y = -2.46x + 144.8$ ,  $r^2 = 0.63$ ,  $F(1, 68) = 115.7$  ( $P < 0.0001$ , *F* test).

temperature sensation may not reach a significant level due to the long stimulus period utilized in the present study.

The amygdala is well known to be responsible for various kinds of emotion [14]. The amygdala receives sensory inputs from various modalities such as vision, audition, gustation, somatosensing, and pain [13]. It is likely that the amygdala receives projection of innocuous thermal input from the skin, although no study so far has analyzed the thermal responses of amygdaloid neurons. The amygdala receives input from the hypothalamus [14], which is the main receptive site of deep body temperature [10]. Therefore, the amygdala is quite suitable for the signal processing of thermal comfort/discomfort, which requires information both from the skin and the body core [11]. Innocuous thermal stimulation to a limited area for a short period, which was used in previous studies on temperature sensation, would not produce thermal comfort/discomfort, since it would not become a substantial load for thermoregulation. Thus, it is natural that no activation was reported in the amygdala in previous studies.

The cold stimulus in the present study would have evoked not only sensations, but also autonomic responses for thermoregulation. Indeed, most subjects reported that they had shivered during the cold stimulation. And skin blood vessels should have been strongly constricted in the present experimental condition. Therefore, the activation observed in the present study might be secondary to such autonomic thermoregulatory responses. The amygdala has never been reported to play a role in the control of autonomic thermoregulation against cold. Therefore, the BOLD response in the amygdala would reflect activity involved in the genesis of cold discomfort itself, rather than autonomic thermoregulation.

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