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Original Article

Changes in prefrontal cerebral hemodynamics during intermittent pain stimulation to gingiva: Preliminary study using functional near infrared spectroscopy



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Abstract *Background/purpose:* Elucidating the transmission mechanism of pain signals from the orofacial area and the corresponding modification mechanism will not only aid in the understanding of pain mechanisms but also provide useful information regarding the development of pain mitigation methods. In this study, the involvement of the pain suppression system in the trigeminal area was investigated through an analysis of the activation status over time in the prefrontal cortex using functional near-infrared spectroscopy (fNIRS).

Materials and methods: In 28 healthy, right-handed male volunteers (average age, 30.1 ± 4.2 years) as subjects, a mild, intermittent, acute pain stimulus was administered through the implementation of pocket probing of the gingiva surrounding the right maxillary central incisor. In the prefrontal cortex, the levels of hemoglobin (Hb) were measured using the fNIRS measurement system. Average values of both oxy-Hb and deoxy-Hb were calculated at four stages: rest stage, 20 s prior to the pain stimulus application, and three stages at 20-s intervals within 1 min of stimulation. One-way analysis of variance and multiple comparisons were used to compare representative values to investigate the changes due to pain.

Results: Oxy-Hb levels decreased the most during the 20 s stage directly after stimulus application. This change was seen mainly on the contralateral side, after which it returned to the resting baseline level before the stimulus application.

Conclusion: Our data demonstrate that in healthy males, a mechanism exists to mitigate pain involving the pain suppression system in the 20 s after feeling mild pain to the gingiva.

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Introduction

Pain information in the orofacial area is transmitted to the upper central nervous system. This transmission employs both the lateral and medial systems after peripheral input to the pons via the trigeminal ganglion. The lateral system is a path that conducts the sensory aspects of pain (the quality, intensity, and localization of the pain) and enters the somatosensory area of the cerebral cortex via the thalamus. The medial system is a path that conducts the emotional and cognitive aspects of pain, which enters the prefrontal cortex (PFC), and limbic system via the thalamus.¹ The PFC is involved in the integration of the emotion, cognition, and memory of pain and promotes the activity of regions related to pain based on traces of experience and memory.² Apkarian et al.³ conducted a meta-analysis based on functional brain imaging research and reported that PFC activity increases in healthy individuals upon painful stimulation. However, in studies with healthy individuals in addition to patients with pain after tooth extraction and patients with rheumatoid arthritis, reports have stated that PFC activity decreased with pain stimulus.^{4–6} Thus, a standard opinion on the status of PFC activity due to a pain stimulus has not been established.

Functional magnetic resonance imaging (fMRI) was used in research investigating brain activity when a pain stimulus is given to the oral cavity.^{7–10} However, for fMRI, a fixed head position is necessary, unlike in functional near-infrared spectroscopy (fNIRS). fMRI is also inconvenient for investigations of changes in blood flow over time as the temporal resolution is low.¹¹ On the contrary, fNIRS is desirable for investigating PFC activity in real-time and over time when pain stimuli are administered in various postures.¹² The current study investigated PFC activity at the time of a mechanical pain stimulation to the gingiva using fNIRS in healthy volunteers. There was a significant decrease in oxygenated hemoglobin (oxy-Hb) in the frontal gyrus on the side opposite to the gingiva from the pain stimulus; i.e., PFC activity was found to be decreased.¹³

In addition to being an area involved in attention and working memory, the dorsolateral prefrontal cortex (DLPFC), which corresponds to the superior frontal and middle frontal gyri—areas in which the activity decreases due to pain stimulus—is an area that regulates pain through the descending pain suppression system (DPSS).¹⁴ Even if a pain stimulus is intermittently administered, the pain does not persist; instead, the pain is said to recede.¹⁵ The first relay nucleus for pain signal in the trigeminal area is the spinal trigeminal nucleus. The sensation of pain at that time may be suppressed by DPSS. However, there is no report investigating whether DPSS works by focusing on brain activity when a painful stimulus is applied to the periodontal tissues. If brain activity is used as an index and

the timing of the onset of the pain suppression system is clarified, it will provide useful information not only for understanding the mechanism of pain but also for the development of acute pain relief methods.

In this study, fNIRS was used to measure the time course of hemodynamics in the PFC when acute and intermittent pain stimuli were applied to the gingiva, a study was conducted on the involvement of the pain suppression system in the trigeminal nerve area.

Materials and methods

Subjects

Past research has indicated the possibility of an interrelationship with sex hormones and the relationship between pain and analgesic response.¹⁶ It has been reported that there may also be gender differences regarding susceptibility to experimental pain stimuli.¹⁷ Consequently, in the present study, the investigation was limited to male subjects. Subjects were $n = 28$ (average age 30.1 ± 4.2 years) healthy, right-handed volunteers. The summary of the study was explained, and written consent for participation was obtained. This study was conducted after receiving approval from the ethics committee of the Aichi Gakuin University School of Dentistry (Approval Number: 163).

Pain stimulation to the gingiva

Periodontal pain stimulation (PPS) was achieved through the use of pocket probing, a method used for measuring periodontal pockets.¹⁸ Note that when PPS was administered, rather than using the usual periodontal probe, with consideration to safety, an accessory point, used in root canal fillings, was used (Gutta percha points accessory, GC Corporation, Tokyo, Japan). The accessory point for the PPS was inserted six times over 60 s at different locations and was focused on the right maxillary central incisor. To prevent the influence of artifacts accompanying head tilting and muscle activity of the stomatognathic system, subjects were seated in a chair in a relaxed position and instructed to keep their eyes and mouth slightly open when PPS was performed.¹³ When PPS was conducted, subjects experienced an intermittent, prickling pain. When the degree of pain from PPS was assessed using the visual analog scale (VAS) (range: 0–100 mm),¹⁹ the VAS value showed an average of 28 ± 4 mm. According to this measure, the present study presented a mild pain stimulus.²⁰

fNIRS measurement system

A multi-channel fNIRS measurement system (ETG-4000, Hitachi Medical Corporation, Tokyo, Japan) and the

attached 20-channel probes were used to measure the hemodynamics (Fig. 1). The probes were centered on the PFC, with the lowest line established to correspond to the

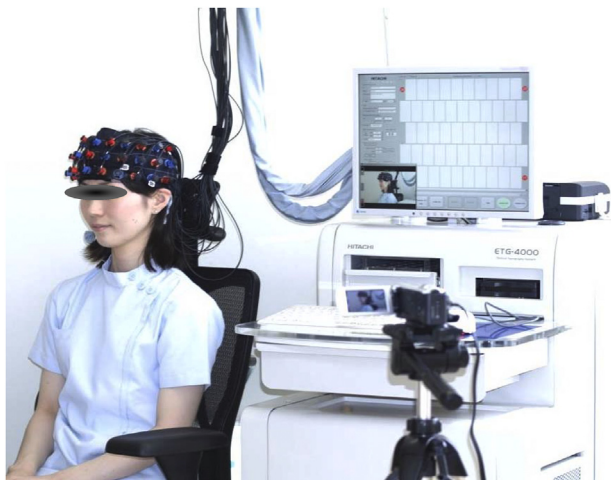


Fig. 1 Multi-channel fNIRS measurement system. The fNIRS measuring device measures the concentration changes of oxy-Hb and deoxy-Hb in the capillaries of the brain with near-infrared light of two wavelengths (695 and 830 nm) based on the modified Beer–Lambert method at a sampling time of 0.1 s. The distance between the light source and detector was 3 cm for the measurement probes and a 3×5 array of optical fibers was used.

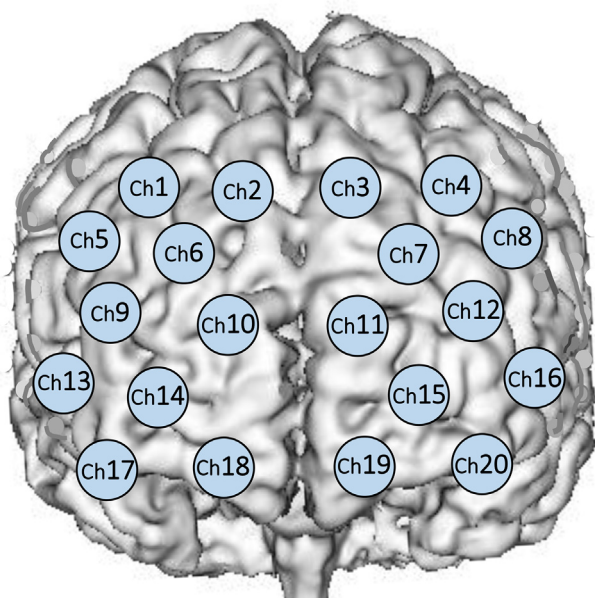


Fig. 2 Anatomical brain region corresponding to 20 channels located in the PFC. In the present study, the anatomical brain regions corresponding to the 20-channel probes marked with virtual registration were the superior frontal gyrus (Ch2, Ch3, Ch10, Ch11), middle frontal gyrus (Ch1, Ch4–Ch9, Ch12, Ch14, Ch15, Ch18, Ch19), and inferior frontal gyrus (Ch13, Ch16, Ch17, Ch20). The numbers in the figure represent the probe channels. A brain surface diagram was processed, which was provided by Hitachi Medical Corporation.

T3-Fpz-T4 line used in brain wave measurement (International 10–20 System Standard).²¹ During fNIRS measurement, heart rate, respiratory rate, and Mayer waves become artifacts that mix into the fNIRS signal, which adversely affects data analysis. Therefore, a 0.2 Hz low-pass filter was applied during the measurement.

The brain regions directly beneath each channel were identified using the virtual registration method.^{22,23} In this method, by registering information on the position of the probe and channel into a standard brain in the database obtained from MRI, the position of the probes can be corrected for each subject, and the anatomical brain region corresponding to each channel can be marked using numerical analysis software (MATLAB R2012b, MathWorks, Inc., Natick, Massachusetts, USA) (Fig. 2).

Measurement time schedule and data analysis

After 3 min of rest, PPS was implemented for 1 min, followed by 1.5 min rest upon cessation. In the present study, the oxy-Hb and deoxygenated hemoglobin (deoxy-Hb) obtained at a sampling time of 0.1 s were used for data analysis (Fig. 3).^{24,25}

When conducting data analysis, four analysis sections were established: the 20 s at rest directly before PPS (Rest) and 1 min of PPS divided into three 20 s intervals—directly after the start of PPS until 20 s after (20 s), 20 s–40 s after the start of PPS (40 s), and from 40 s to 60 s after the start of PPS (60 s). Next, an average value was calculated for each analysis section of data from each subject, and representative values (oxy-Hb value and deoxy-Hb value) were obtained. The question of whether a change in the value from before PPS and accompanying PPS was present, was investigated using the representative values obtained from all participants for each channel using a one-way ANOVA when the data had a normal distribution and a Friedman test when the data did not have a normal distribution. Multiple comparisons (Scheffe's method) were conducted for the channels for which significant differences were observed and the change due to PPS was investigated. Statistical significance was determined at a significance level of less than 5% ($p < 0.05$).

Results

The average values and standard deviations for the overall oxy-Hb levels for the 20-channels used were: Rest: 0.053 ± 0.200 mM·mm, 20 s: 0.011 ± 0.191 mM·mm, 40 s: 0.031 ± 0.190 mM·mm, 60 s: 0.060 ± 0.191 mM·mm. A significant decrease in oxy-Hb levels due to the pain stimulus was confirmed in thirteen channels (Ch4, Ch6–Ch8, Ch10–Ch12, Ch14–Ch16, Ch18–Ch20) (Table 1). Further, these channels decreased the most during 20 s, after which, they demonstrated a change of returning to the resting baseline from before PPS (Fig. 4).

The average values and standard deviations for the overall deoxy-Hb level for the 20-channels were: Rest: -0.051 ± 0.112 mM·mm, 20 s: -0.051 ± 0.113 mM mm, 40 s: -0.048 ± 0.110 mM·mm, 60 s: -0.036 ± 0.105 mM·mm.

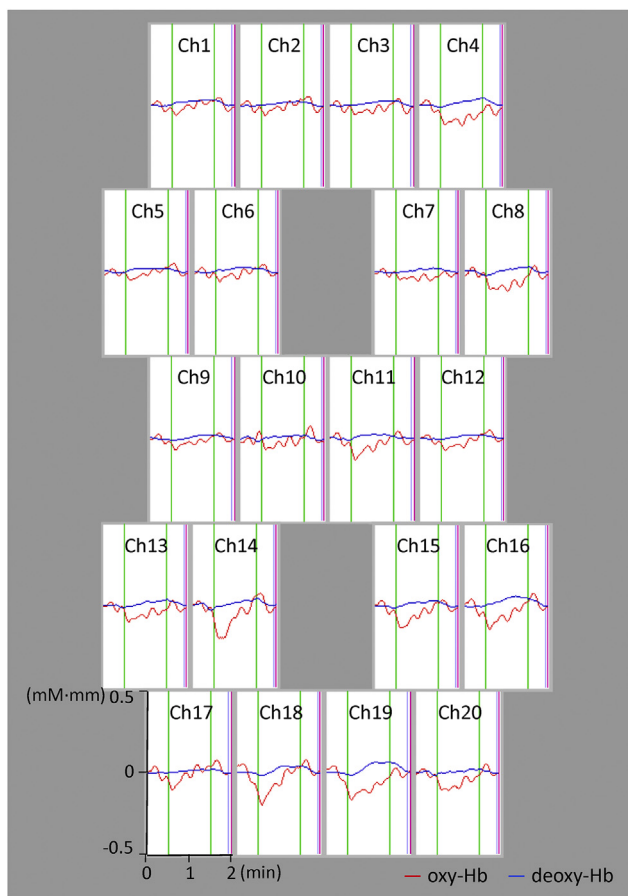


Fig. 3 Measurement example of time-dependent changes in Oxy-Hb and deoxy-Hb levels due to pain stimulus to the gingiva. The red line shows the oxy-Hb level and the blue line shows the deoxy-Hb level. The vertical axis is the measured value (mM·mm), and the horizontal axis is the time (unit: min). In this study, Hb levels obtained from 20 channels located in the prefrontal cortex were analyzed. The displayed number shows the channel numbers.

There were no channels for which a change due to PPS was observed for the deoxy-Hb level (Table 2).

Discussion

In this study, if the timing of changes in brain activity was clarified by mild pain, it was considered unnecessary to give the subject a moderate or higher pain load, and only a mild pain stimulus was applied. In addition, a study focused on acute pain was conducted to obtain information on the development of acute pain relief methods.

Oxy-Hb levels were significantly reduced by PPS, primarily in the left channel, opposite to that of the stimulus. Pain is transmitted through two types of sensory nerves: A δ fibers and C fibers. A δ fibers convey sharp pain with a high transmission speed, short duration, and clear localization, while C fibers convey dull pain with a slow transmission speed, long duration, and unclear localization to the cerebral cortex. With a stimulus, there is only an increase in the activity of A δ fibers in the anterior cingulate cortex

Table 1 Changes in oxy-Hb level due to pain stimulation.

Channels	Rest	20s	40s	60s	
Ch1	0.066 (0.270)	0.066 (0.270)	0.065 (0.268)	0.089 (0.251)	
Ch2	0.054 (0.164)	0.033 (0.161)	0.044 (0.167)	0.062 (0.182)	
Ch3	0.068 (0.146)	0.038 (0.125)	0.055 (0.140)	0.075 (0.143)	
Ch4	0.089 (0.172)	0.051 (0.145)	0.079 (0.152)	0.109 (0.139)	*
Ch5	0.104 (0.398)	0.063 (0.412)	0.070 (0.407)	0.099 (0.386)	
Ch6	0.001 (0.122)	-0.028 (0.129)	-0.009 (0.134)	0.011 (0.139)	*
Ch7	0.065 (0.187)	0.030 (0.173)	0.053 (0.177)	0.074 (0.173)	*
Ch8	0.101 (0.177)	0.057 (0.175)	0.072 (0.186)	0.106 (0.187)	*
Ch9	-0.032 (0.225)	-0.027 (0.154)	-0.010 (0.148)	0.023 (0.165)	
Ch10	0.049 (0.168)	-0.008 (0.170)	0.006 (0.169)	0.037 (0.169)	**
Ch11	0.032 (0.179)	-0.010 (0.181)	0.005 (0.179)	0.030 (0.181)	**
Ch12	0.098 (0.156)	0.051 (0.153)	0.068 (0.151)	0.104 (0.151)	**
Ch13	-0.031 (0.220)	-0.060 (0.232)	-0.041 (0.223)	-0.005 (0.234)	
Ch14	0.028 (0.183)	-0.024 (0.175)	0.007 (0.171)	0.035 (0.174)	**
Ch15	0.060 (0.194)	-0.003 (0.209)	0.027 (0.205)	0.065 (0.199)	**
Ch16	0.047 (0.197)	0.023 (0.205)	0.047 (0.201)	0.083 (0.198)	**
Ch17	0.055 (0.223)	0.013 (0.199)	0.043 (0.209)	0.059 (0.228)	
Ch18	0.083 (0.233)	-0.009 (0.172)	0.017 (0.166)	0.061 (0.175)	**
Ch19	0.087 (0.230)	0.012 (0.211)	0.035 (0.195)	0.078 (0.185)	**
Ch20	0.059 (0.194)	0.021 (0.192)	0.055 (0.188)	0.083 (0.198)	**

(Unit: mM·mm).

The oxygenated-hemoglobin (oxy-Hb) level average and standard deviation for each channel when the pain stimulus was applied are shown (top: mean, bottom inside (): standard deviation). PPS: periodontal pain stimulation Rest: 20 s of rest directly before PPS, 20s; 20 s from the start of PPS, 40s; 20–40 s of PPS, 60s; 40–60 s of PPS. *: $p < 0.05$, **: $p < 0.01$.

contralateral to the stimulus.²⁶ Additionally, when both A δ fibers and C fibers are stimulated, the anterior cingulate gyrus, secondary somatosensory cortex, and bilateral thalamus are activated.^{27,28} In the present study, a mild, intermittent, acute pain stimulus was applied to the right gingiva. It is hypothesized—regarding the reason why many channels existed in which a decrease in blood flow was confirmed in the PFC on the side opposite the stimulus—that because both C fibers and A δ fibers on the right

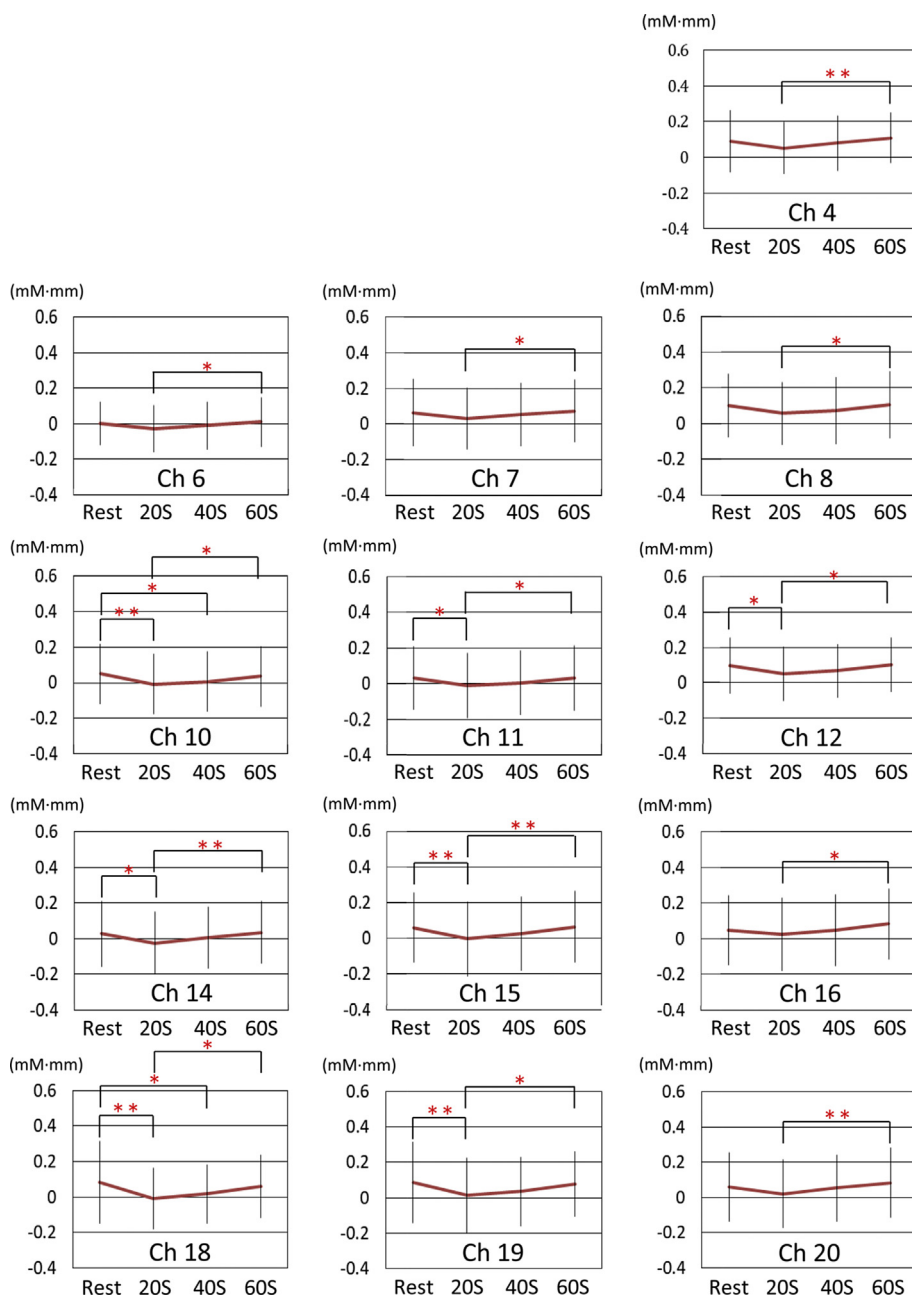


Fig. 4 Oxygenated-hemoglobin (oxy-Hb) level changes due to pain stimulation. Multiple comparisons were conducted for channels that demonstrated changes in oxy-Hb levels along with periodontal pain stimulation (PPS) compared to directly before the start of PPS, and the change characteristics were examined. Oxy-Hb levels decreased the most in the PPS 0–20s section. This demonstrated a characteristic change of returning to the baseline from before PPS. Rest: state of rest 20 s directly before PPS, 20s: 20 s from the start of PPS, 40s: 20–40 s of PPS, 60s: 40–60 s of PPS. *: $p < 0.05$, **: $p < 0.01$, unit: mM·mm.

side (the stimulus side) were being predominately stimulated, neural activity of the anterior cingulate cortex on the opposite side was activated. Because this area required cerebral blood flow, this blood flow came from the PFC, an area adjacent to the anterior cingulate cortex. Therefore, blood flow decreased mainly in the left PFC (the contralateral side).¹³

It was revealed that during 1 min of intermittent pain stimulation, the oxy-Hb decrease demonstrated a peak at 20s after the start of stimulation (20s), after which

regardless of continued stimulation, the oxy-Hb level increased and tended to return to the baseline value before the start of pain stimulation. The central gray substance, rostral ventromedial medulla, and posterolateral pontine tegmentum play an important role in the regulation of the transmission of pain information at the spinal cord level. In particular, the central gray substance receives input from cortical regions such as the PFC, anterior cingulate cortex, and insula. The hypothalamus, amygdala, reticular formation, and the locus coeruleus send descending fibers to the

Table 2 Changes in deoxy-Hb level due to pain stimulation.

Channels	Rest	20s	40s	60s
Ch1	-0.025 (0.196)	-0.014 (0.198)	0.002 (0.185)	0.041 (0.161)
Ch2	0.017 (0.180)	0.029 (0.178)	0.037 (0.166)	0.035 (0.096)
Ch3	-0.016 (0.124)	-0.006 (0.116)	0.003 (0.113)	0.001 (0.075)
Ch4	-0.061 (0.088)	-0.045 (0.086)	-0.034 (0.073)	-0.020 (0.069)
Ch5	-0.044 (0.170)	-0.036 (0.177)	-0.019 (0.162)	0.028 (0.162)
Ch6	-0.046 (0.100)	-0.038 (0.103)	-0.025 (0.089)	-0.010 (0.083)
Ch7	-0.052 (0.086)	-0.049 (0.090)	-0.047 (0.083)	-0.032 (0.081)
Ch8	-0.075 (0.119)	-0.071 (0.118)	-0.062 (0.113)	-0.046 (0.089)
Ch9	-0.088 (0.120)	-0.089 (0.123)	-0.077 (0.123)	-0.070 (0.123)
Ch10	-0.044 (0.082)	-0.053 (0.087)	-0.051 (0.089)	-0.042 (0.084)
Ch11	-0.047 (0.084)	-0.054 (0.086)	-0.055 (0.090)	-0.042 (0.090)
Ch12	-0.056 (0.091)	-0.056 (0.090)	-0.050 (0.078)	-0.045 (0.081)
Ch13	-0.093 (0.161)	-0.056 (0.090)	-0.078 (0.164)	-0.073 (0.187)
Ch14	-0.054 (0.067)	-0.056 (0.074)	-0.058 (0.076)	-0.051 (0.081)
Ch15	-0.058 (0.096)	-0.059 (0.091)	-0.070 (0.099)	-0.056 (0.101)
Ch16	-0.063 (0.129)	-0.067 (0.128)	-0.061 (0.124)	-0.055 (0.122)
Ch17	-0.058 (0.094)	-0.060 (0.097)	-0.066 (0.092)	-0.066 (0.095)
Ch18	-0.045 (0.090)	-0.063 (0.099)	-0.072 (0.109)	-0.068 (0.113)
Ch19	-0.056 (0.096)	-0.068 (0.097)	-0.075 (0.114)	-0.063 (0.114)
Ch20	-0.055 (0.095)	-0.059 (0.097)	-0.063 (0.100)	-0.054 (0.102)

(Unit: mM·mm).

The deoxygenated hemoglobin (deoxy-Hb) level average and standard deviation for each channel when the pain stimulus was applied are shown (top: mean, bottom inside (): standard deviation). Rest: state of rest 20 s directly before periodontal pain stimulation (PPS); 20s: 20s from the start of PPS; 40s: 20–40 s of PPS; 60s: 40–60 s of PPS.

nociceptive neurons in the dorsal horn of the spinal cord and directly or indirectly regulate signal transmission from primary neurons.²⁹ In the region innervated by the trigeminal nerve, the descending path that leaves the central gray substance, the nucleus raphe magnus of the medulla oblongata, and the magnocellular reticular nucleus of the medulla oblongata act on the dorsal horn of the spinal cord, the caudal subnucleus of the spinal nucleus of the

trigeminal nerve, and the associated lateral reticular nucleus of the medulla oblongata, respectively, blocking neurotransmission between the primary neurons and secondary neurons of the trigeminal nerve.^{30,31} Consequently, under the conditions of the present study in which a mild, intermittent pain stimulus was applied to the gingiva, the involvement of the DPSS, which mitigates pain—roughly 20 s after the start of stimulation was observed.

Melzack³² classified pain into sensory, emotional, and cognitive aspects and reported that different areas of the brain are involved with each. Even so, the PFC is involved in the integration of emotion, cognition, and memory, and is also reported to be involved in both acute and chronic pain.³ Further, Wager et al.,¹⁴ who investigated the analgesic effect of a placebo, reported that the PFC and central gray substance are associated with diffuse noxious inhibitory controls, which regulate pain. Investigations regarding familiarity with pain have been conducted. When a pain stimulus is applied to healthy individuals, decreases in the activity of brain areas related to pain and in the subjective intensity of the pain are observed.^{33,34} Moreover, the prefrontal cortex is activated when one consciously tries to suppress pain.³⁵ Apkarian et al.³⁶ reported that the DLPFC, which corresponds to the middle frontal gyrus and the inferior frontal gyrus, is involved in acute pain, while the medial prefrontal cortex is involved in chronic pain. According to this report, the DLPFC is largely involved with working memory, which focuses attention toward acute, noxious stimuli and the same regions are activated by awareness of pain. The pain stimulus in the present study can be considered acute. It was hypothesized that the activity of the prefrontal cortex shows suppression directly after becoming aware of pain; however, after this, attention is directed to the pain and as a result of consciously trying to alleviate the pain, due to activation of the DLPFC, oxy-Hb levels start increasing and tend to return to the baseline value.

On the other hand, deoxy-Hb did not change after PPS was performed. The typical hemodynamics associated with brain activation in healthy subjects show a pattern in which oxy-Hb increases and deoxy-Hb slightly decreases during task performance.³⁷ This phenomenon is reflected by the increase in blood flow to activated brain capillaries and the accompanying rapid inflow of blood flow into veins (washout effect) as an increase in oxy-Hb and a decrease in deoxy-Hb. Since the subjects of this study were healthy volunteers, it was speculated that deoxy-Hb did not decrease or increase due to the washout effect on the vein.

From the present study, it was possible to obtain an index for the hemodynamics in the prefrontal cortex when healthy males are aware of intermittent, mild pain in the gingiva.

When examining the hemodynamics of female, it is necessary to control the administration of oral contraceptives and the sexual cycle. Therefore, it was difficult to control these factors in healthy subjects. In the future, we will expand the target to painful patients and conduct a survey targeting females. In addition, we plan to examine these conditions as well as the involvement of emotional systems such as anxiety.

Taken together, our results suggest that the pain suppression system is involved 20 s after awareness of a mild,

acute pain in the gingiva, and that there are mechanisms that mitigate pain. These findings not only provide an index for objectively assessing pain but also provide useful information for the development of pain relief methods for acute pain that can be focused on the activation of the prefrontal cortex.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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