Original

Amygdala Response During Anticipatory Anxiety
in Patients with Tension-type Headache

Kanako Satoh[1, 2], Yuri Masaoka*[1], Hideyo Kasai[2],
Hironori Satoh[2], Setsurou Tsukada[1, 2], Taketoshi Seino[1],
Mitsuru Kawamura[2], Ikuo Homma[1] and Masahiko Izumizaki[1]

Abstract : Tension-type headache (TTH) is the most prevalent primary headache disorder, affecting 0.5%～4.8% of the population worldwide. Psychological factors play an important role in the pathogenesis of TTH. For instance, depression and anxiety are thought to enhance central sensitization, and thus increase the frequency of headaches. In this study, we used the Minnesota Multiphasic Personality Inventory (MMPI) and measures of anxiety to analyze personality traits associated with TTH. Specifically, we were interested in the relationship between these variables, respiratory responses, and brain activity. Our results showed that individuals with TTH had significantly higher state anxiety scores compared with healthy controls. In addition, individuals with TTH showed a greater increase in RR during a stressful task involving anticipation of an electrical stimulation. During anticipatory anxiety, there was bilateral amygdala (AMG) activation in TTH patients, while only the right AMG was activated in healthy controls. Interestingly, patients in the TTH group with high levels of state anxiety and high scores on schizophrenia scales had the following MMPI personality traits : peculiar perception, poor familial relationship, difficulties concentrating, and lack of deep interest. We suggest that the psychological factors associated with the above-mentioned brain activation might induce peripheral muscle pressure, which then triggers headaches.

Key words : tension-type headache, anxiety, respiration, electroencephalogram, amygdala

Introduction

Tension-type headache (TTH) is the most prevalent primary headache disorder, affecting 0.5% ～ 4.8% of the population worldwide[1]. However, TTH has been relatively neglected in terms of scientific research, receiving less attention than migraine headaches due to TTH being regarded as a normal part of life. Another reason may be the fact that single episodes of TTH are less severe than migraine attacks. As a result, the mechanisms underlying TTH remain unclear. There is some controversy in the literature regarding whether TTH is associated with central

[1] *Department of Physiology, Showa University School of Medicine,* 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan.
[2] *Division of Neurology, Department of Medicine, Showa University School of Medicine.*
* To whom corresponding should be addressed.

or peripheral neural mechanisms[2, 3]. For instance, increased pericranial muscle tenderness and generalized pressure pain hypersensitivity have been associated with TTH[3]. In addition to these peripheral mechanisms, some research indicates that central sensitization, owing to continuous nociceptive input from peripheral muscles, might play a role in the pathogenesis of TTH[4]. It appears that the contributions of peripheral and central mechanisms are intermingled, thus, the cause-effect relationship between pericranial tenderness and central sensitization remains unclear.

In addition to physiological mechanisms, psychological factors play an important role in the pathogenesis of TTH. In patients with TTH, depression and anxiety may enhance central sensitization and thus increase the frequency of headaches[5]. Psychological stress is reportedly the most common trigger of TTH[6].

In this study, we examined the effects of psychological stress on the physiological responses of patients with TTH. We exposed patients with pre-existing TTH to a situation intended to induce anticipatory anxiety, and assessed how anxiety levels correlated with respiratory responses, which we used as an index of the level of emotional arousal[7]. In addition, we simultaneously collected electroencephalogram (EEG) and respiration data with the aim of finding an anxiety-related potential[8]. We used these potentials to estimate source generators, which enabled us to predict whether the amygdala (AMG) was activated in individuals with TTH.

Respiratory activity has previously been used to evaluate emotional levels in humans[7]. An increased respiratory rate (RR) has been correlated with increased levels of anxiety, suggesting that respiratory change and anxiety might be modulated by a common brain region, namely the AMG[7]. Indeed, a previous study estimated the AMG to be the source generator of respiration-related anxiety potentials (RAPs) during anticipatory anxiety[8].

In this study, we compared measures of anticipatory anxiety in TTH patients with those of normal controls. Specifically, we tested anxiety levels, personality traits, respiratory responses, and AMG activation while patients anticipated painful stimuli to investigate whether there were differences between patients with TTH and healthy controls for any of the factors inducing TTH.

Subjects and methods

Six TTH patients (mean age, 30 ± 13 years ; all women) and eight age-matched healthy normal individuals (mean age, 34 ± 13 years ; all women) participated in this study. All participants gave informed consent and the study was approved by the Ethics Committee of Showa University School of Medicine. None of the patients were currently receiving pharmaceutical treatment. All participants were tested during their post-menstrual period.

The patients with TTH had been diagnosed according to the criteria of the International Classification of Headache Disorders (second edition)[9]. TTH is distinguished from migraine by its bilateral location, mild-to-moderate pain intensity, and non-pulsatile pain (pressing, tightening, and band-like pain). TTH is not associated with significant nausea or vomiting, and can be subclassified based on the presence or absence of tenderness of the pericranial muscles. Our TTH patients reported episodes lasting $1 \sim 2$ hr per day, and ranging in frequency from 1 to 14 days per month.

We used Spielberger's State-Trait Anxiety Inventory (STAI) [10] to measure anxiety levels and the Minnesota Multiphasic Personality Inventory (MMPI) to assess personality traits in all participants [11]. The MMPI is a globally accepted tool for assessing personality types. To reduce the length of the procedural session, we used the short version of the MMPI, which consists of 383 questions and 13 domains (four validity scales and nine clinical scales). The MMPI represents a standardized and quantitative measure of personality traits. The validity scales include the "cannot say" scale (?), the lie scale (L), the frequency scale (F), and the correction scale (K). The clinical scales detect the presence of psychopathological features, namely, hypochondriasis, depression, hysteria, psychopathic deviate, paranoia, psychasthenia, schizophrenia, hypomania, and social introversion.

Measurement of inspiration-related potential

Our method of observing inspiration-related potentials was first described in a study by Masaoka and Homma [7, 8]. We informed the participants that an electrical pain stimulation would be delivered through a needle attached to the dorsum of their left hand (as described below) at various times during the experiment. We referred to the time that elapsed while the participant awaited electrical stimuli as "anticipatory anxiety." We simultaneously recorded EEG and respiration data throughout the experiment.

RAP refers to the average of the inspiration-related potentials triggered by the onset of inspiration during emotional and olfactory stimuli. Specifically, a RAP is observed when an emotional change and a respiratory change occur simultaneously in an emotional situation. This calculation is based on the assumption that averaging a set of EEG signals based on the onset of inspiration events elicited by emotional stimuli will produce RAPs.

Pain stimuli

Before the experiment, we tested the thresholds for pain detection (the level where "the stimulation is small, but can be felt") and pain tolerance (the level where "the stimulation is painful, but can be endured") in the TTH and control participants.

We delivered the electrical stimuli according to the method described by Kasai *et al* [12]. The cathode consisted of a plastic plate, a soft stop device, and a stainless steel needle (0.5 mm in diameter). The soft stop device protruded 1.0 mm from the plate and the tip of the needle, in turn, protruded 0.2 mm from the soft device. By pressing the electrode plate gently against the skin, the needle tip was inserted adjacent to the nerve endings of the thin myelinated fibers in the epidermis and superficial part of the dermis. The anode was a surface electrode 1.0 cm in diameter placed on the skin at a distance of 4 cm from the needle electrode. The stimulation produced a well-defined pricking pain without a definite tactile sensation. The needle was set on the dorsum of the left hand between the first and second metacarpal bones.

The stimuli were delivered via an isolator connected to an electrical stimulation unit (Nihon Kohden, Tokyo, Japan). After obtaining threshold levels of stimulation, the TTH and control participants were instructed to rate the pain intensity and pain-induced discomfort using a visual

analog scale (VAS) ranging from 0% (no pain at all) to 100% (worst imaginable pain). We also measured VAS scores at the completion of each experiment.

Measurement of EEG and respiration

We attached 19 electrodes to the scalp according to the International 10 ∼ 20 system, with the reference electrode on the right earlobe. EEG and electro-oculogram data were recorded and stored in a digital EEG analyzer (DAE-2100; Nihon Kohden). EEG data were sampled at 500 Hz through a 0.016- to 30-Hz bandpass filter. Impedances were kept below 10 KΩ. Subjects wore a face mask with transducer to measure respiratory flow using a respiratory flow monitor (Minato, Osaka, Japan) and respiratory flow data were also stored in the EEG analyzer. The experimental apparatus is shown in Fig. 1. Inspirations were measured as flows that moved downwards from the 0 level, and expirations were flows that moved upwards. We used the onset of inspiration (onset of flow moving downwards from the 0 level) as a trigger for averaging potentials (number of potential averaged, mean 60.3 ± 4.5). We excluded all sniffing activity to reduce the impact of artifacts caused by mechanical movements of the mandibular muscles on the EEG data. We also excluded eye blinks and artifactual activity exceeding $\pm 50 \mu$ V. We averaged the potentials collected during the time that elapsed while the participants awaited the electrical pain stimuli.

Dipole modeling analysis

To estimate the location of source generators, the averaged EEG potentials were transferred to dipole tracing software (Brain Space Navigator [BS-navi]; Brain Research and Development, Tokyo, Japan)[8]. The details of the dipole tracing method using the scalp-skull-brain head model from the Montreal Neurological Institute (MNI) are reported elsewhere[8]. The accuracy of the generator locations estimated via the MNI standard head model was confirmed by comparison with models generated using individual head models. Previous reports that established the locations of generators, estimated via grand-averaged potentials across participants, indicated typical dipole localization of movement-related potentials[13], auditory-related potentials[14], and olfactory-related potentials[15]. In this study, we estimated the locations of dipoles using both grand-averaged potentials and individual-averaged potentials, which were based on the MNI model.

Statistical analysis

Data are expressed as the mean ± standard deviation. All statistical analyses were performed using a commercially available statistical package (SPSS, Ver. 22; IBM, Tokyo, Japan). We used Wilcoxon's signed rank tests to compare the age, pain threshold level, maximum pain levels, STAI scores, RR during rest, and RR during pain in the TTH participants with those in the control participants. *P* values < 0.05 were considered significant.

In dipole analysis, the degree of source concentration can be calculated in terms of goodness of fit. While a goodness of fit of 100% is ideal, in practice, it is usually less than 100% because of noise, electrode misalignment, or interference from the non-dipole components of the
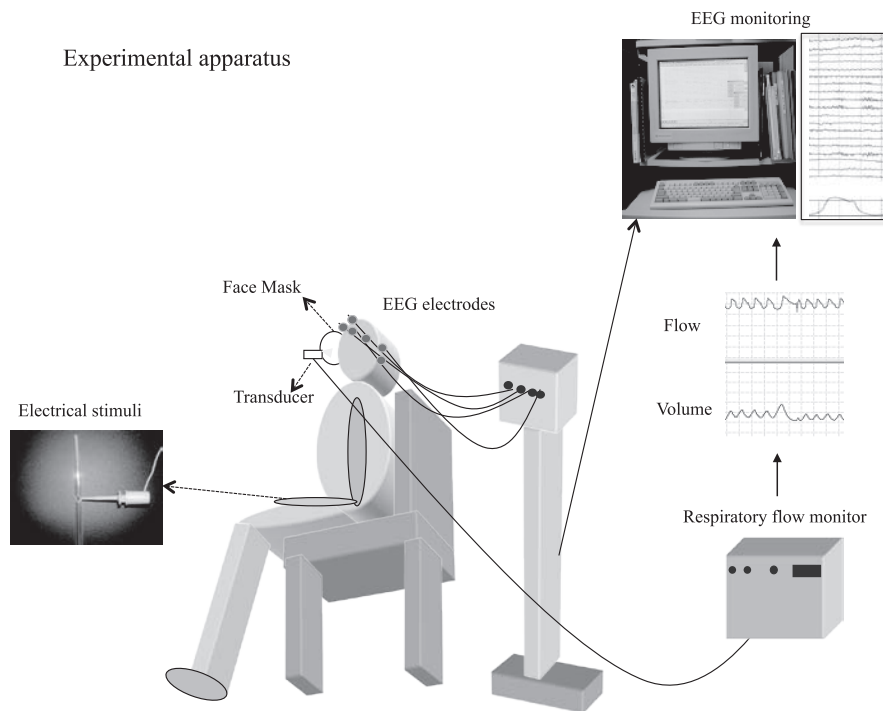
Experimental apparatus

Fig. 1. Experimental apparatus for the measurement of electroencephalogram (EEG) and respiration during anticipation of electrical stimuli.

electricity sources. In the present study, a goodness of fit greater than 98% was considered to indicate a concentrated source [8, 13-15].

Results

Pain thresholds, maximum pain levels, anxiety, and respiration

Table 1 shows the pain threshold level, maximum pain level, VAS score for level of pain, state anxiety and trait anxiety scores, and increase in RR, i.e., RR during anticipatory anxiety minus RR during rest. We found a significant difference in pain thresholds and maximum pain levels between the healthy controls and the TTH group $(P < 0.05)$. State anxiety was significantly higher in the TTH group $(P < 0.001)$ while there was no difference in trait anxiety between the two groups. During anticipatory anxiety, the patients with TTH showed a greater increase in RR compared with the healthy controls $(P < 0.05)$.

We used the MMPI to investigate the relationships between variables related to anxiety in people with TTH. We found no significant differences between the TTH group and healthy controls on the following scales : L scale $(44.1 \pm 7.1$ vs. $43.2 \pm 6.7)$; F scale $(50.6 \pm 7.4$ vs. $49.2 \pm 6.8)$; K scale $(50.3 \pm 8.2$ vs. $49.2 \pm 7.8)$; hypochondriasis $(48.5 \pm 9.8$ vs. $47.0 \pm 8.8)$; depression $(46.8 \pm 12$ vs. $45.6 \pm 9.8)$; hysteria $(48.1 \pm 12.0$ vs. $47.6 \pm 11.0)$; psychopathic device $(45.1 \pm 9.2$ vs. $44.6 \pm 8.7)$; paranoia $(48.33 \pm 6.5$ vs. $47.8 \pm 7.7)$; psychasthenia $(38.8 \pm 14.6$ vs. $40.1 \pm 13.9)$; schizophrenia $(44.0 \pm 9.8$ vs. $43.5 \pm 8.8)$; hypomania $(41.0 \pm 15.2$ vs. $40.3 \pm 14.5)$; and social introversion $(52.3$

Table 1. Comparison of pain perception, anxiety and respiratory rate（RR）between healthy controls and patients with tension-type headache

	Healthy control	Tension-type headache
Age（years）	30.3±11.8	30±13.0
Pain threshold（mV）	0.1±0.1	0.4±0.3 *
Maximum pain level（mV）	1.0±0.8	2.7±3.3 *
VAS for painfulness（%）	62.5±8.7	63.3±11.0
State anxiety score	40.0±9.5	44.0±9.8 **
Trait anxiety score	46.2±5.8	46.5±5.6
Increase in RR during anticipatory anxiety	2.0±1.3	3.5±2.9 *

*$P < 0.05$, **$P < 0.001$, compared to control. VAS, visual analog scale

± 11.0 vs. 51,2 ± 12.0, for TTH vs healthy control, respectively ; all $P > 0.05$）． However, we did find a significant negative correlation between the schizophrenia（MMPI Sc ＋ 1K）scores and the state anxiety scores in the TTH group（r ＝ −0.86 ; $P < 0.05$; Fig. 2）．

RAPs and dipole localizations

Fig. 3A shows the grand-averaged EEG data from 19 electrodes triggered by the onset of inspiration in the healthy controls（left panel）and the patients with TTH（right panel）． We observed RAPs with a characteristic 9– to 12–Hz frequency in both groups, as confirmed via a power spectrum analysis. Fig. 3B shows the estimated grand-averaged typical dipole localizations for the normal controls（left）and the patients with TTH（right）． The dipole locations, which were estimated from individual RAP data during a 400–msec period, are summarized in Table 2（i.e., the number of dipoles in each anatomical region）． Based on the RAP data, the dipoles converged in the right AMG in the normal controls（Fig. 3B, left）, and in the AMG bilaterally in the patients with TTH（Fig. 3B, right）． The dipoles in the AMG are shown in coronal and horizontal sections and the MNI coordinates are shown at the bottom of each coronal section in Fig. 3B.

Discussion

In this study, we tested the effect of psychological stress, specifically anticipatory anxiety, on the respiratory response and brain activity in healthy controls and individuals with TTH. We also investigated the differences in responses between the two groups, taking into account personality traits.

The TTH group had significantly higher state anxiety scores compared with the healthy controls. Additionally, the TTH group exhibited a greater increase in RR during anticipation of an electrical stimulation. A previous study tested in healthy subjects reported that anticipatory anxiety increases RR, and that this increase in RR is directly associated with individual anxiety levels[7]. This phenomenon was also observed not only in normal healthy subjects but also in highly anxious patients[16]. In this study, we also found that TTH with high anxiety showed a

MMPI (Sc+1K)

State anxiety

r = -0.86

Fig. 2. Correlation between schizophrenia-type Minnesota Multiphasic Personality Inventory (MMPI) scores and state anxiety scores in patients with tension-type headache.

（A）　Control　　　　　　　　　　TTH

（B）

Fig. 3. （A）Grand-averaged electroencephalograms （EEGs）from 19 electrodes triggered by the onset of inspiration during anticipatory anxiety, showing respiration-related anxiety potentials （RAPs）in normal participants （left panel）and in patients with tension-type headache （TTH）（right panel）. （B）Dipoles converged in the right amygdala （AMG） in healthy controls （left panel）and in both the left and right AMG in patients with TTH （right panel）during the interval from 100–200 msec of RAPs （indicated by white arrows）. Only dipoles with a goodness of fit greater than 98% were accepted.

Table 2. The dipole locations estimated from individual respiration-related anxiety potential data during a 400-msec period after inspiration onset

	Temporal lobe												Parietal lobe								Other			
	Fusi		ITG		MTG		STG		Para HC		AMG		OcG		AG		Precune		A cingulate		Retro		Cbll	
	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
Healthy controls	0	2	1	4	2	1	2	3	3	4	8	12*	0	2	1	0	2	1	1	0	1	1	0	1
Tension-type headache	0	1	2	2	1	1	2	2	4	4	20*	16*	1	2	0	0	1	0	4	3	2	1	2	1

Fusi, fusiform gyrus; ITG, inferior temporal gyrus; MTG, middle temporal gyrus; STG, superior temporal gyrus; Para HC, parahippocampal gyrus; AMG, amygdala; OcG, occipital gyrus; AG, angular gyrus; Precune, precuneus; A cingulate, anterior cingulate gyrus; Retro, retrosplenial cortex; Cbll, cerebellum; L, left; R, right
*Significant anatomical regions estimated by one dipole analysis.

high increase of RR.

We were surprised to find that there were no differences in trait anxiety scores between TTH and healthy individuals. The state and trait anxiety scales can be defined as follows. The state anxiety scale measures anxiety that is experienced under specific conditions and times, and changes according to external factors. In contrast, the trait anxiety scale measures the general feelings of the individual and reflects their general predisposition to anxiety. In this sense, state anxiety levels in individuals with TTH might depend on the specific characteristics of the stressful situation. Additionally, state anxiety might be easily influenced by anticipation of anxiety, for instance, in the conditions used in this study. Previous reports have indicated that stress and anxiety might trigger headaches in people with TTH[5, 6]. Thus, the tendency of people with TTH to be in a higher state of anxiety might contribute to their increased frequency of headaches. In the patients with TTH in our study, a high state of anxiety with an increasing RR was associated with bilateral activation in the AMG.

The AMG plays a role in the reaction element of emotions, especially fear and anxiety[17]. Source generators associated with RAP have been observed in the right AMG in healthy controls, indicating that the right AMG is involved in strong emotional reactions[8]. The left AMG is thought to be involved in the semantic processing of emotions, as it plays a role in cognitive and resonance processing[8, 15]. Dolan and Morris[18] suggested that the right AMG might depend on triggers associated with the internal state, while the left AMG might be related to conscious processing of external stimulation. However, both the left and right AMG interact ; for instance, strong activity in the right AMG can activate the left AMG[8]. Thus, our patients with TTH may have been more sensitive to internal anxiety, and therefore, evaluated the external stimuli more consciously compared with the healthy controls.

Interestingly, we found a correlation between state anxiety and schizophrenia scores in the patients with TTH. The schizophrenia scale measures bizarre thoughts, peculiar perceptions, poor familial relationships, difficulty concentrating, and lack of deep interest[11]. Thus, the greater the state of anxiety, the more abnormal the thought patterns in patients with TTH. In terms of

brain site, the participants with the highest levels of state anxiety exhibited multiple dipoles in both sides of the AMG.

The psychological factors associated with the above-mentioned brain activation might induce peripheral muscle pressure, thus triggering headaches. Previous reports have described muscle tension and sustained muscle contractions around the neck and scalp of patients with TTH[2]. In fact, this is the case in many highly anxious individuals, who often exhibit tightness in peripheral muscles. For instance, patients with hyperventilation syndrome often present with chest wall tightness and neck tightness[16]. Stress and anxiety affect muscle tone by increasing gamma-motor input at the muscle spindle, as well as increasing respiration-related muscle activity[16].

Although the participants with TTH exhibited a high state of anxiety, these individuals had a pain threshold and maximum pain tolerance that was higher than that of healthy controls. However, Bezov *et al*[19] found that patients with TTH had a decreased electrical pain threshold and pain tolerance threshold, suggesting that TTH is accompanied by non-specific hypersensitivity. This means that general pain sensitivity is altered in individuals with TTH. A previous imaging study found a decrease in the volume of gray matter brain structures implicated in pain processing in patients with TTH. This decrease was associated with prolonged nociceptive input from pericranial myofascial structures[20]. We did not directly evaluate morphological changes in our participants with TTH ; however, this analysis might be worth investigating with respect to the relationship between anxiety-AMG responses and pain sensitization.

Recently, behavioral treatments (relaxation training, EEG biofeedback training, and cognitive therapy) have been validated in patients with TTH. Relaxation training therapy may reduce headache activity by nearly 50%[2]. Cognitive behavioral therapy is an effective treatment for patients with low severity TTH. Future research should test these treatments to investigate any associated reduction in state anxiety in individuals with TTH. Additionally, future studies could assess whether decreases in stress and/or anxiety lead to a reduction in symptoms, potentially inhibiting AMG activation.

Conflict of interest disclosure

The authors have declared no conflict of interest.

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