due to surgery or chronic electrical stimulation, consistent with our previous study.[27]

Discussion

The present study showed the changes in astrocytes following continuous electrical stimulation of SMC in rats. As shown in our previous study by c-Fos histochemistry,[24] the present study found that astrocytes were activated by chronic electrical stimulation in rats without damage to the neural structures. Most previous studies showed that neurons are activated following short-term electrical stimulation ranging from 3 hours to 2 weeks,[4,10,11,16,25,31] but the present study found changes in astrocytes following chronic electrical stimulation up to 8 weeks.

Our in vivo study showed that the astrocytes were activated in a delayed manner as the activity indicated by the number of c-Fos/GFAP-double-immunopositive cells peaked following 1 week of continuous stimulation. Moreover, the astrocytes become larger following activation by continuous stimulation as the area of astrocytes peaked after 4 weeks. Other in vitro studies have also demonstrated that electrical stimulation activated and enlarged astrocytes.[10,11]

Recent studies have shown that astrocytes are important in synaptic activity, and that structures consisting of astrocytes and neurons are known as "tripartite synapses."[2,8] In this context, astrocytes and neurons may influence each other under MCS. High-frequency electrical stimulation facilitates the release of brain-derived neurotrophic factor (BDNF) from neurons,[2] and the morphology of astrocytes is regulated via a BDNF-specific receptor.[18] In addition, BDNF is derived from neurons not astrocytes, and astrocytes regulate synaptic formation via BDNF-specific receptors.[5] Together with these previous reports, the present study indicates that MCS activates both neurons and astrocytes, and then the astrocytes are morphologically changed via neurotrophic factors such as BDNF released by neurons. Furthermore, local contacts of astrocytes with neurons enhance synaptogenesis.[9] We speculate that the number of contacts of astrocytes with neurons is increased by the enlargement of astrocytes in a tripartite synapse, and that this increase may result in the enhancement of synaptogenesis in the electrically stimulated brain.

Concerning chronological changes in a clinical setting, patients with chronic pain usually experience gradual improvement in pain over hours or days following MCS.[6] In addition, some patients with thalamic pain experience functional recovery following chronic MCS,[30] and MCS may enhance the effect of rehabilitation.[3] Our findings that chronic MCS activated and enlarged astrocytes over weeks in rats may shed light on the mechanism underlying the effect of chronic MCS in practice. We speculate that MCS activates astrocytes and modulates the function of neurotransmitters, which may result in pain relief and functional recovery from stroke or movement disorders in humans. On the other hand, our results may implicate the decreased number of reactive astrocytes after electrical stimulation is continuously applied for a long period. A phenomenon called "tolerance" or "habituation" occurs in patients with pain or movement disorders following long-term neurostimulation therapy including MCS and deep brain stimulation.[17,19] The decrease in the number of c-Fos/GFAP-double-immunopositive cells and the mean area of astrocytes following 8 weeks may be related to the mechanisms of tolerance/habituation.

In our study, the CG, TH, and SMC were investigated as the motor cortex has projections to and from different brain areas such as the thalamocortical projections, corticocortical projections, and local cortical connections in parallel to the cortical layers.[12] A recent positron emission tomography study in humans has demonstrated increased cerebral blood flow following MCS in neuronal structures such as the ventrolateral nucleus of the thalamus, medial thalamus, insula, orbitofrontal area, CG, and upper brain stem.[16] In particular, the anterior cingulate cortex is considered to be important in moods: for example, the number and density of glial cells in depressed or bipolar patients are reduced in the anterior cingulate cortex, which is associated with the affective components of pain.[21] Our study showed that the astrocytes in the CG were both activated and enlarged, suggesting that these changes may contribute to the pain relief mechanism induced by MCS in human patients.

In this study, the chronological changes in astrocytes were observed up to 8 weeks. Further study will be necessary to determine the more detailed reciprocal relationship between neurons and astrocytes, and the mechanism of modulation of the clinical symptoms in humans.

Changes in GFAP-immunopositive astrocytes were observed over time in this study. Activation and morphological changes of astrocytes may contribute to the mechanisms underlying pain relief or functional recovery from stroke or movement disorders. MCS may induce neuroplasticity through the activation of astrocytes.

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Alexithymia and Chronic Pain
The Role of Negative Affectivity

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Objectives: Alexithymia has been shown to be associated with key pain-related variables in persons with chronic pain from western countries, but the generalizability of these findings across cultures has not been examined adequately. Also, there remain questions regarding the importance of alexithymia to patient functioning over and above the effects of the general negative affectivity.

Methods: Alexithymia, pain intensity, pain interference, depression, anxiety, and pain catastrophizing were measured in 128 Japanese patients with chronic pain. Because of the low internal consistency coefficients for 2 of the alexithymia scales (measuring difficulty describing feelings and externally oriented feelings) in our sample, we limited our analyses to a scale assessing difficulty identifying feelings and the total alexithymia scale score.

Results: Although the 20-item Toronto Alexithymia Scale total and the Difficulty Identifying Feelings scale scores were not significantly associated with pain intensity, these scales were associated with pain interference, catastrophizing, and negative affectivity in our sample. However, these associations became nonsignificant when measures of negative affectivity were controlled.

Discussion: The findings support the cross-cultural generalizability of significant associations between alexithymia and both pain interference and catastrophizing. However, whether (1) alexithymia influences patient functioning indirectly by its effects on negative affect or (2) the univariate associations found between alexithymia and measures of patient functioning are a byproduct of both being influenced by negative affect needs to be tested using longitudinal and experimental research.

Key Words: alexithymia, chronic pain, pain interference, pain catastrophizing, negative affectivity

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Alexithymia is a term used to describe a reduced emotional awareness and inability to describe feelings.[1] Sifneos noted that alexithymic patients commonly complain of anxiety and depression. However, when questioned further about their anxiety, patients with alexithymia talk "...only about nervousness, agitation, restlessness, irritability and tension." Also, when asked about depression, they tend to talk about "sensations of emptiness, void, boredom, and pain."[2] Thus, paradoxically, although individuals with alexithymia struggle with describing their feelings, they also report more distress than individuals who do not have alexithymia, perhaps in part because a common method of coping with negative feelings (ie, talking about feelings) is not available to them. Alexithymia also tends to be more common in men than in women, perhaps because of cultural factors that inhibit emotional expression among men.[3]

Alexithymia has also been shown to be associated with pain and pain-related functioning in individuals with chronic pain from western countries,[4–7] although the associations found between alexithymia and pain intensity is less consistent than those between alexithymia and a negative mood. Regarding associations with pain intensity, for example, some investigators have not found significant associations,[8–10] some have found significant positive associations,[4,11] and others have found both significant and nonsignificant associations.[6,12] However, when significant associations are found, alexithymia is always associated positively with pain intensity. Although the findings regarding alexithymia and pain intensity are inconsistent, researchers have consistently found positive associations between measures of alexithymia and both pain interference[7,13–15] and depression.[7,16]

It is possible that cultural background and ethnicity could influence alexithymia and its associations with symptom reporting.[6,17] Japan has unique cultural characteristics that differ from western countries in many ways, including in the areas of religion,[18] ideas about virtue,[19] and styles of communication.[21] Although there is some limited research suggesting that the concept of alexithymia may translate to Japanese samples who do not have chronic pain,[21,22] it is not known if the findings regarding the associations between alexithymia and pain-related variables found in western samples have cross-cultural generalizability. We were able to identify only 1 study that has examined the relationship between alexithymia and pain in Japanese people.[23] This study found that healthy subjects with high alexithymia scores undergoing colonic distension stimulation reported a stronger response to stimulation—including more pain—than subjects with low alexithymia scores. However, we were not able to identify any research that has examined the associations between alexithymia and pain-related variables in Japanese patients with chronic pain.

The 20-item Toronto Alexithymia Scale (TAS-20)[24,25] assesses global alexithymia and 3 alexithymia subdomains: (1) difficulty identifying feelings (DIF); (2) difficulty describing feelings (DDF); and (3) externally oriented thinking (EOT). Although, as mentioned above, research does

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not always find significant associations between TAS-20 scale scores and pain intensity,[8–10] when significant associations are found, they are positive and are most often found with the TAS-20 Difficulty Identifying Feelings (TAS-DIF) scale.[5,6,15]

Recently, we reported that the TAS-DIF and TAS-20 DDF (TAS-DDF) scales were significantly associated with reports of more pain intensity and pain interference in a sample of predominantly white individuals with neuromuscular disease and pain.[15] We also found that the associations between alexithymia and both pain intensity and pain interference became nonsignificant after controlling for a measure of psychological distress, consistent with the possibilities that general negative affectivity may drive the significant associations found between alexithymia and patient functioning[7,15] or that the effects of alexithymia on patient functioning are mediated by its effects on negative affect.

One key pain-related factor linked to both pain intensity and negative affect is catastrophizing.[26] Given previous findings that alexithymia is associated with "emotional constriction" and experiential avoidance,[27] it is reasonable to hypothesize that individuals reporting higher levels of alexithymia may be more likely to catastrophize about their pain. This hypothesis has been supported in 2 studies of patients from western cultures.[7,28] In the first of these, alexithymia was moderately associated with pain catastrophizing ($r = 0.47$),[7] and had a strong positive association with depression ($r = 0.71$).[7] In the second study, measures of alexithymia, especially the TAS-DIF scale, showed a moderate association with pain catastrophizing ($r = 0.43$),[28] and demonstrated a moderate association with anxiety sensitivity ($r = 0.35$).[29] However, the extent to which these associations generalize to patients with chronic pain from other cultures, including the Japanese culture, has not yet been tested. In addition, findings have supported that the decreasing of catastrophizing may be a potential factor to improve pain outcomes.[30,31] However, the studies that examined the association between catastrophizing and alexithymia are limited, and there has been no study that has examined the associations between alexithymia and pain catastrophizing when controlling for the negative affectivity (eg, measures of depression and anxiety) in patients with chronic pain.

People with alexithymia tend to show lower empathy[31] with others' beliefs, emotions, and desires,[32] perhaps because of their lack of knowledge of their own emotional experience.[31] This lack of empathy may also contribute to the higher rates of interpersonal problems reported by persons with alexithymia.[33] Because Japanese people place a high priority on harmony,[19] they may be less likely to directly express their negative feelings and intent, relative to people from western cultures. In addition, to succeed in the Japanese society, it is necessary to have a well-developed ability to empathize with others and understand others' intentions without verbal communication. Given these considerations, alexithymic individuals in Japan may experience even more distress in daily life than those living in western cultures, which could result in alexithymia having a greater (negative) impact on all outcomes.

Given these considerations, we hypothesized that alexithymia would be more strongly associated with pain intensity, pain interference, and pain catastrophizing in our sample of patients from Japan than has been found in patients from western countries. Also, given the possibility that (1) alexithymia might impact functioning by its negative impact on negative emotions or that (2) the negative affect might drive both alexithymia and negative outcomes in both cultures, we hypothesized that, similar to what we found in a study of alexithymia and pain in patients from the United States, the significant associations between alexithymia and pain and functioning will disappear when negative affectivity is controlled.

METHODS

Participants

Participants were consecutive Japanese outpatients with chronic pain (pain of at least 3-month duration) seen in the outpatient clinic of the department of psychosomatic medicine at Kyushu University Hospital in Japan from November 2006 to August 2008. To participate in the study, patients must have been at least 18 years old and not have symptoms of dementia or other significant cognitive deficits or severe psychopathology (ie, suicidal patients, severe depression, and psychosis). All of the 142 eligible patients seen in the clinic during this time period agreed to participate. However, 14 of these did not provide complete responses to the study questionnaires. This left a final sample size of 128 (90% response rate) participants. All participants did not have a history of psychiatric disorders except 2 patients who had a history of bipolar affect disorder, although symptoms of this disorder were not present at the time of the participation in this study.

The sample contained 95 women (74.2%), and the mean age of the participants was 52.31 years (SD = 16.30 y; range, 22 to 83 y). The mean duration of pain was 5.1 years (SD = 6.84 y; range, 3 mo to 36 y). The educational level of the participants varied widely; 15 participants (11.7%) graduated from junior high school, 62 (48.4%) graduated from high school, 25 (19.5%) completed junior college or vocational school, 24 (18.8%) graduated from a 4-year university, and 2 (1.6%) held a postgraduate degree. Seventy patients (54.7%) were married/living with significant other, 14 (10.9%) were divorced, 11 (8.6%) were widowed, and 33 (25.8%) were never married. Primary pain sites included the abdomen in 21 (16.4%), the lower back in 18 (14.1%), shoulder or arm in 13 (10.2%), the upper back in 14 (10.9%), head in 14 (10.9%), the lower limb in 12 (9.4%), neck pain in 9 (7.0%), chest pain in 6 (4.7%), and other sites in 21 (16.4%) participants. The percentage of patients taking prescribed medications for pain relief at the time of assessment was 21.1%, including nonopioid analgesics (17.9%), opioid analgesics (1.6%), antiepileptics (7.0%), and antidepressants (16.4%).

Procedures

The study participants were asked to complete the measures described below and also provide demographic and medical history information while waiting to be examined in the clinic. The study procedures were approved by the Kyushu University IRB.

Predictors and Covariate Measures

TAS

Alexithymia was assessed using the TAS-20.[24,25] The TAS-20 has 3 scales assessing 3 components of alexithymia: the TAS-DIF scale, the TAS-DDF scale, and the TAS-EOT scale. The TAS-20 items are rated on 5-point Likert scales, ranging from 1 (strongly disagree) to 5 (strongly agree). The

TAS-20 scales have demonstrated strong psychometric properties including good test-retest reliability and criterion validity.[24] The original 3-factor model of the TAS-20 items has been supported in the Japanese community and clinical samples.[22] Good internal consistency has been demonstrated in previous patient samples for total TAS-20 scale and 2 of the TAS-20 scales (TAS-DIF and TAS-DDF). However, the TAS-EOT scale has not demonstrated adequate internal consistency in some studies.[34] Consistent with previous research, the TAS-DIF scale in our sample evidenced adequate internal consistency (Cronbach $\alpha = 0.75$), whereas the TAS-DDF and TAS-EOT scales did not evidence acceptable reliability (Cronbach $\alpha = 0.63$ and 39, respectively). The total TAS-20 scale also evidenced adequate internal consistency (Cronbach $\alpha = 0.74$). Because of the low internal consistency coefficients for TAS-DDF and TAS-EOT scales in our sample, we limited our analyses to TAS-DIF scale and the total TAS-20.

Hospital Anxiety and Depression Scale (HADS)

Negative affectivity was assessed using the HADS.[35] The 14 HADS items ask respondents to indicate the frequency or severity of different symptoms of depression and anxiety during the past week on 4-point (0 to 3) scales. Optimal balance between sensitivity and specificity for HADS as a screening instrument was achieved most frequently at a cut-off score of 8 + for both HADS-anxiety (HADS-A) and HADS-depression (HADS-D), yielding sensitivities and specificities for both subscales of approximately 0.80.[36] A great deal of evidence supports the validity of the HADS for assessing depression and anxiety in patients with various medical conditions.[35] The Japanese version of HADS has been shown to have adequate validity and reliability.[37] The internal consistencies of both the HADS-D and the HADS-A scales in our sample were adequate (Cronbach $\alpha = 0.78$ and 0.77, respectively).

Criterion Measures

Pain Catastrophizing Scale (PCS)

Pain catastrophizing was assessed using a Japanese version of the PCS.[38] The PCS is a 13-item scale measuring 3 domains of pain-catastrophizing cognitions, including rumination, magnification, and helplessness. Respondents indicate the frequency with which they experience each catastrophizing item on a 0 to 4 scale. The PCS does not ask respondents to consider a specific time period when rating pain-catastrophizing responses, but research supports the conclusion that pain catastrophizing is relatively stable over time in individuals with chronic pain.[39] Evidence supports the reliability and validity of the PCS total score and the individuals' PCS scale scores as measures of pain catastrophizing in samples of individuals with chronic pain.[40] The Japanese version of PCS has been shown to have adequate validity and reliability.[41] The reliability of the PCS total scale score in our sample was excellent (Cronbach $\alpha = 0.84$).

Numerical Rating Scales (NRS)

Pain intensity was assessed by asking participants to rate the intensity of their pain in the last 24 hours on 0 to 10 NRSs assessing average, least, worst, and current pain. These 4 ratings were then averaged to create a composite score of the usual pain intensity. A 24-hour recall period was chosen over a 7-day recall period because evidence suggests that a 24-hour recall for pain intensity is more accurate than a 7-day recall.[42] NRS have been shown to be reliable and valid measures of pain intensity in numerous patient samples,[43] and the 0 to 10 NRS has been recommended as the pain intensity scale with the most psychometric strengths and the fewest psychometric weaknesses of all available pain scales.[43]

Brief Pain Inventory (BPI)

Pain interference was assessed using the pain interference scale of the BPI.[44] This measure asks respondents to indicate the amount of interference in 7 daily activities (general activity, walk, work, relations with others, sleep, enjoyment of life) caused by pain during the past 24 hours on 0 (no interference) to 10 (complete interference) NRSs. The BPI interference scale has evidenced high levels of reliability and validity in numerous samples of patients with chronic pain. The reliability and validity of the Japanese version of the BPI is established in cancer pain.[45] In the current sample, the internal consistency was high (Cronbach $\alpha = 0.88$), indicating excellent reliability.

Data Analysis

Before examining the study questions, the predictor, covariates, and criterion measures were examined for outliers and normality. All variables met requirements for analysis and so no transformations were necessary. To examine the hypothesized associations between alexithymia and pain intensity, pain interference, and pain catastrophizing, we first computed the correlation coefficients between the total TAS-20 and the TAS-DIF scale scores and the criterion variables assessing pain intensity, interference, and catastrophizing. We then divided the sample into alexithymic and nonalexithymic groups on the basis of their total TAS-20 scores (total TAS-20 score ≥ 61 and total TAS-20 score < 61, respectively),[2] and used t tests to compare the 2 groups on the criterion variables. We used regression analyses to test our hypothesis that any significant univariate associations found between alexithymia and the study criterion variables would become nonsignificant when measures of negative affectivity were controlled. The criterion variables that evidenced significant univariate associations with alexithymia were the dependent variables in these analyses. Previous research has shown higher alexithymia in men than in women[3] and also the possibility that lower educational level is associated with higher alexithymia.[46] Research also indicates that the pain site is associated with the level of patient functioning.[47] We performed 4 linear regression analyses (1 for each criterion variable) with the TAS-DIF scale scores and the total TAS-20 scores as the predictor variables separately while controlling for demographic variables [age, sex, education, and pain site (coded as low-back pain vs. other pain site)] by entering them in the first steps of the regression analyses to control for their possible confounding effects. For each model, 2 separate steps 3 and 3′ are presented, 1 using the TAS-DIF scale score as a predictor and the other using the total TAS-20 score as a predictor. The measures of negative affect (HADS-D and HADS-A) were entered in the second step. Finally, the TAS-DIF scale score or the total TAS-20 score was entered (in separate regression analyses) to determine whether they remain as the significant predictors when the demographic and negative affect variables were controlled. We set α at .01 for all analyses to balance the need to control for type I errors (detecting a significant

TABLE 1. Means and SDs for Each of the Measures (N = 128)

Measures	Mean	SD	Range
TAS-20 total	53.20	10.64	31-79
TAS-DIF	18.32	6.19	7-34
Anxiety (HADS-A)	7.98	4.79	0-21
Depression (HADS-D)	9.99	4.82	0-21
Catastrophizing (PCS)	34.37	10.16	5-52
Pain intensity (NRS)	5.84	2.06	1-10
Pain interference (BPI-mean)	5.85	2.56	0-10

BPI indicates Brief Pain Inventory; HADS, Hospital Anxiety and Depression Scale; NRS, Numerical Rating Scale; PCS, Pain Catastrophizing Scale; TAS-20, the 20-item Toronto Alexithymia Scale; TAS-DIF, Toronto Alexithymia Scale Difficulty Identifying Feelings scale.

effect in the sample when it is not present in the population) while also avoiding type II errors (determining that an effect is not significant in the sample when in fact it is present in the population). SPSS 17.0 was used for all analyses.

RESULTS

Descriptive Statistics

Table 1 lists the means and SDs for each of the study variables. The average score for the total TAS-20 was consistent with other (non-Japanese) samples of persons with chronic pain (mean ± SD: 53.5 ± 16.4),[48] and slightly higher than normative Japanese scores (48.3 ± 8.9)[22] (50.5 ± 9.5).[49] The average score for HADS-D was consistent with other non-Japanese samples of persons with chronic pain HADS-D (9.67 ± 3.86),[50] although the average score for HADS-A scales was somewhat lower than that reported in a sample of non-Japanese patients with chronic pain (11.18 ± 4.34).[50] The average score for pain catastrophizing (PCS score) was higher than that reported in a sample of non-Japanese patients with chronic pain (25.63 ± 12.4)[51] (16.76 ± 10.04).[52] The average usual pain intensity over the 24 hours was similar to the average pain intensity rating from other samples of patients with chronic pain. The mean pain interference (BPI interference scale) was slightly lower than those from a sample of non-Japanese patients with chronic pain (6.04 ± 2.26).[53]

Correlation Analyses

Table 2 presents the correlation coefficients between the total TAS-20 and TAS-DIF scale scores and the study criterion variables. Neither of the TAS-20 scales was significantly associated with pain intensity in our sample. However, the total TAS-20 and TAS-DIF scores were both associated significantly (and moderately) with pain interference and pain catastrophizing. The total TAS-20 and TAS-DIF scores were also associated significantly and positively with both anxiety and depression.

Differences Between Alexithymic and Nonalexithymic Groups

We examined whether or not there were differences between the alexithymic and nonalexithymic groups in pain-related outcomes. The alexithymic group showed significantly higher scores on pain catastrophizing (alexithymic group vs. nonalexithymic group, mean ± SD: 38.86 ± 8.19 vs. 32.81 ± 10.34; $t = -3.41$, $P = 0.001$) and anxiety (10.33 ± 4.08 vs. 7.16 ± 4.76; $t = -3.68$, $P < 0.001$), and showed a nonsignificant trend to report higher levels of pain interference (6.64 ± 1.96 vs. 5.57 ± 2.69; $t = -2.43$, $P = 0.02$) and depression (11.95 ± 5.06 vs. 9.31 ± 4.57; $t = -2.65$, $P = 0.01$). However, alexithymic group membership was not significantly related to pain intensity (5.69 ± 1.77 vs. 5.89 ± 2.16; $t = 0.53$, $P = 0.60$).

Predictors of Pain Interference and Catastrophizing, Controlling for Negative Affect

The results of the regression analyses predicting pain interference and catastrophizing from the total TAS-20 scale or the TAS-DIF scale (controlling for age, gender, education, pain site, and negative affect) are presented in Tables 3 and 4. As can be seen, neither TAS scale was a significant predictor of either criterion variable once the demographic variables and negative affect were controlled.

DISCUSSION

There are 3 primary findings from this study. First, we found that alexithymia was not significantly associated with pain intensity in our sample. Second, we found that alexithymia was moderately associated with pain interference and catastrophizing, although these associations became nonsignificant when demographic variables and measures of negative affectivity were controlled. Finally, the associations between alexithymia and both pain interference and

TABLE 2. Zero-Order Correlations Among Measures of Alexithymia (TAS-20 total, TAS-DIF), Anxiety, Depression, Catastrophizing, Pain Intensity, and Pain Interference

Measures	1	2	3	4	5	6
1. TAS-20 total						
2. TAS-DIF	0.85**					
3. Anxiety (HADS-A)	0.35**	0.49**				
4. Depression (HADS-D)	0.34**	0.35**	0.53**			
5. Catastrophizing (PCS)	0.32**	0.35**	0.49**	0.35**		
6. Pain intensity (NRS)	−0.02	0.04	0.20	0.15	0.31**	
7. Pain interference (BPI-mean)	0.26*	0.29*	0.38**	0.53**	0.38**	0.52**

*$P < 0.01$.
**$P < 0.001$.
BPI indicates Brief Pain Inventory; HADS, Hospital Anxiety and Depression Scale; NRS, Numerical Rating Scale; PCS, Pain Catastrophizing Scale; TAS-20, the 20-item Toronto Alexithymia Scale; TAS-DIF, Toronto Alexithymia Scale Difficulty Identifying Feelings scale.

TABLE 3. Regression Analysis Results Predicting Pain Interference

Steps	Total R^2	ΔR^2	*F*-change	β to Enter	*t*
Criterion: pain interference (BPI interference score)					
Step 1: demographics	0.11	0.11	3.87*		
Age				0.01	0.09
Sex				0.04	0.51
Education				−0.21	−2.30
Pain site				−0.25*	−2.92
Step 2: anxiety and depression	0.39	0.27	26.92**		
HADS-A				0.15	1.79
HADS-D				0.44**	5.15
Step 3: alexithymia	0.39	0.01	1.02		
TAS-DIF				0.09	1.01
Step 3′: alexithymia.	0.39	0.00	0.70		
TAS-20 total				0.07	0.84

Each criterion variable was predicted with TAS-DIF scale and TAS-20 total entered into the final step (steps 3 or 3′, respectively).

*$P < 0.01$.

**$P < 0.001$.

BPI indicates Brief Pain Inventory; HADS, Hospital Anxiety and Depression Scale; PCS, Pain Catastrophizing Scale; TAS-20, the 20-item Toronto Alexithymia Scale; TAS-DIF, Toronto Alexithymia Scale Difficulty Identifying Feelings scale.

catastrophizing were moderate and consistent with findings from western countries.[7,15,28]

Not all studies—including ours—find support for a significant association between pain intensity and alexithymia in individuals with chronic pain. This inconsistency suggests the possibility that there may be contextual factors that influence the strength of the associations between alexithymia and pain intensity. Although it is not clear what those factors are, 1 possibility is the diagnosis or the type of the pain. The studies showing significant associations appear to include patients who have a clear medical diagnosis associated with their pain, such as neuromuscular disease and cancer,[15,54] whereas the studies not showing significant associations tend to include patients with nonspecific pain problems (eg, the current study and others).[48,55] Also, research has shown that among patients with nonspecific pain problems, the association between alexithymia and the affective component of pain is stronger than the association between alexithymia and the sensory component of pain, consistent with the possibility and alexithymia might influence pain intensity (when it does) by factors related to the processing and expression of emotions.[5,7,56] Consistent with this idea, previous findings have shown that chronic pain that is not secondary to a specific organic disease was associated with a number of psychosocial factors that are likely associated with emotional functioning, such as a history of sexual and physical abuse,[57,58] personality types,[59] and psychological trauma.[60] Interestingly, these factors also appear to contribute to secondary alexithymia, and may increase affective distress, which could then contribute to an increase in the affective or suffering component of pain.[61–63] Future research should specifically test the moderating effects of pain type (ie, pain associated with a specific illness/diagnosis vs. pain not associated with a specific illness/diagnosis) on the association between alexithymia and pain intensity, and the extent to which such association may be due to the effects of alexithymia on the affective (vs. the sensory) components of pain.

TABLE 4. Regression Analysis Results Predicting Pain Catastrophizing

Steps	Total R^2	ΔR^2	*F*-change	β to Enter	*t*
Criterion: pain catastrophizing (PCS score)					
Step 1: demographics	0.01	0.01	0.18		
Age				0.06	0.66
Sex				−0.04	−0.46
Education				0.01	0.14
Pain site				0.02	0.24
Step 2: anxiety and depression	0.27	0.27	22.01**		
HADS-A				0.43**	4.72
HADS-D				0.14	1.53
Step 3: alexithymia	0.29	0.02	3.54		
TAS-DIF				0.17	1.88
Step 3′: alexithymia	0.31	0.04	6.15		
TAS-20 total				0.22	2.48

Each criterion variable was predicted with TAS-DIF scale and TAS-20 total entered into the final step (steps 3 or 3′, respectively).

**$P < 0.001$.

HADS indicates Hospital Anxiety and Depression Scale; PCS, Pain Catastrophizing Scale; TAS-20, the 20-item Toronto Alexithymia Scale; TAS-DIF, Toronto Alexithymia Scale Difficulty Identifying Feelings scale.

The results of this study replicate the findings showing significant associations between alexithymia and both pain interference[7,15] and catastrophizing[7,28] that have been found in patients with chronic pain from western countries. However, inconsistent with our hypothesis, these associations were not stronger in our sample than those found in samples of patients from western cultures.[7,28] These findings suggest that the concept of alexithymia for pain interference and catastrophizing translates well across cultures, and whatever influence alexithymia may have appears to be similar in both the cultures.

A previous finding that examined the association between alexithymia and pain disability in patients with rheumatoid arthritis among individuals with 2 different ethnicities showed that the total TAS-20, the TAS-DIF, and the TAS-DDF scores were correlated with disability for the African Americans but not for the whites. However, these differences between ethnicities were eliminated after controlling for other demographic variables.[6] These researchers also found that the total TAS-20 score was correlated with disability for the whites but not for African Americans with migraine headache, although these differences were also eliminated after controlling for other demographic variables.[6] In addition, previous findings have suggested that alexithymia is associated with pain interference in different cultures (ie, Finland,[14] France,[13] and the US[7,15]). As a group, these findings suggest that the influence of alexithymia on the pain interference generalize well across different ethnicities and cultures. Further support for this conclusion comes from research showing that the association between alexithymia and negative affect is consistent across different western countries (ie, Turkey and[8,16] Finland[64]). However, research has shown that this association—like that between alexithymia and pain interference—becomes nonsignificant when negative affect is controlled.[7,15] These findings are consistent with previous research in patients with chronic pain from western

cultures,[15] and the possibility that negative affectivity might be a third (confounding) variable that explains the significant associations found between alexithymia and measures of patient functioning[7] beyond the cultural differences.

To date, no other researchers have published findings regarding the associations between alexithymia and pain catastrophizing when controlling for negative affect (eg, measures of depression and anxiety) in patients with chronic pain. Our finding that anxiety (but not depression) made a unique contribution to the prediction of catastrophizing raises the possibility that anxiety plays a more important role than depression in the associations between negative affect and alexithymia. Future researchers should examine this possibility further by including measures of both depression and anxiety when examining the role that negative affect plays in contributing to (or being influenced by) alexithymia.

Although catastrophizing is reported to have the function of distraction,[65] previous studies reported that alexithymic students used significantly more distraction than nonalexithymic students.[66] In addition, alexithymic individuals have problems in interpersonal relationships,[33] and report reduced social support.[67] It is possible that catastrophizing plays a maladaptive role in eliciting social support in alexithymic patients with chronic pain.

Because Japanese people place such a high priority on harmony,[19] they may be less likely to express their negative feelings and intents directly, relative to individuals from western cultures. This may make it more difficult for alexithymic individuals in Japan to obtain support from other people and result in greater anxiety. If this is the case, and considering the social context of pain catastrophizing,[39] it might be possible that catastrophizing may tend to work more as a strategy to get support from others and reduce anxiety among alexithymic individuals. Future research is needed to determine whether our findings replicate in other Japanese samples and other samples from different cultures.

A competing hypothesis to viewing catastrophizing as a coping strategy used by alexithymic individuals with chronic pain is that alexithymia is a form of "emotional constriction" that contributes to experiential and emotional avoidance.[27] In stressful situations, people with more alexithymia may be less able to simply be aware of or even just "feel" the unpleasant emotions associated with the stressful situation, but cope by trying to "think" cognitively about the event.

The possibility that time spent being aware of one's emotions is adaptive—or at least leads to an attenuation of perceived stress—is supported by a recent neuroimaging study in which healthy patients were asked to self-reflect (a cognitive task) or be aware of their emotions and feelings.[68] These investigators found less activity in the amygdala (thought to be associated with emotional arousal) during the emotional awareness condition than during the cognitive condition.[68] Depending on the extent to which persons with alexithymia are unable to be aware of their emotions, they may experience more arousal, as they may be unable to use effective strategies (ie, reappraisal and affect labeling) for processing negative emotions.[69,70] As emotional arousal escalates, this could contribute to an increase in cognitions and thoughts that are linked to negative emotions, that is, catastrophizing cognitions. This hypothesis is consistent with the findings from the current study and with the findings from other studies, showing that alexithymia is associated with measures reflecting catastrophizing, distress, and negative affectivity.[7,16] With research showing that depressed individuals with chronic pain exhibit significantly more negative automatic thoughts than nondepressed individuals with chronic pain or healthy controls,[71] it is possible that the greater negative affect reported by individuals with alexithymia may be due to a limited ability to process emotions, which could lead to increased catastrophizing, and ultimately contribute to emotional distress.[39] Consistent with this possibility, in our sample, the alexithymic group showed significantly higher scores on the measures of pain catastrophizing and anxiety, and a nonsignificant trend to be positively associated with depression. These findings are also consistent with past findings suggesting an association between alexithymia and negative affect.[5,7]

The limitations of this study include the exclusive use of self-report measures, which may enhance the strength of associations found among the variables because of shared method variance. Although self-report measures are appropriate when assessing subjective domains such as alexithymia, pain, and affect, observational measures could be used to assess physical functioning. Future research should include observational measures of patient functioning, when possible. A second limitation is that the sample came from patients seen at a medical clinic that tends to treat patients presenting with more severe pain problems, which may affect the distribution of our data and it may be a confounding factor for our results. Therefore, the extent to which the findings generalize to other Japanese patients—especially perhaps other Japanese patients with less severe pain problems—is not clear. Research using samples from other pain clinics is needed to help determine the generalizability of the current findings. A third limitation is that we excluded both TAS-DDF and TAS-EOT scales from analyses because of their low internal consistency in our sample. Previous findings suggest that the TAS-DIF scale tends to be more strongly associated with pain outcomes than the TAS-DDF and the TAS-EOT scales, and so excluding the latter scales from our analyses may not have excluded the most important (to pain) alexithymia domain from our analyses.[6,7] Still, it would be useful to determine the associations between the difficulty describing feelings and externally oriented thinking domains of alexithymia and pain-related outcomes in other Japanese samples of patients to better understand their potential role.

Finally, because the data collected in this study were cross-sectional, it is not possible to draw conclusions about causal associations among the study variables. Using these data, we cannot determine, for example, whether alexithymia contributes to negative outcomes (by its effects on negative affect) or whether negative affect influences alexithymia and the other outcomes. Both possibilities remain viable. Research is needed to determine whether a treatment that reduces alexithymia results in subsequent improvements in catastrophizing and emotional functioning. The findings do suggest, however, that such research is warranted.

Despite the study's limitations, the findings confirm the cross-cultural importance of alexithymia as being associated with pain interference and indicate that, at least in our sample of Japanese patients with chronic pain, alexithymia is also significantly associated with depression, anxiety, and catastrophizing. Longitudinal and in particular experimental research in which alexithymia is altered in 1 group but not in another is needed to help tease out the

relative influence of the variables examined in this study on the other variables.

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REVIEW

Pain perception in humans: use of intraepidermal electrical stimulation

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ABSTRACT

The choice of a system specific stimulus is difficult when investigating the human nociceptive system, in contrast with the tactile, auditory and visual systems, because it should be noxious but not actually damage the tissue. The discomfort accompanying system specific stimulation must be kept to a minimum for ethical reasons. In this review, recent progress made in the study of human pain perception using intraepidermal electrical stimulation (IES) is described. Also, whether IES is a viable alternative to laser stimulation is discussed. IES selectively activates Aδ nociceptors, elicits a sharp pricking sensation with minimal discomfort and evokes cortical responses almost identical to those produced by laser stimulation. As IES does not require expensive equipment, and is easy to control, it would seem useful for pain research as well as clinical tests.

INTRODUCTION

Pain, particularly its emotional component, is essential for survival. However, excessive pain is distressful. Therefore, pain research in humans is important for uncovering the underlying mechanisms of this essential function as well as for establishing treatment for pain relief. The recent development of non-invasive techniques has enabled us to examine directly the human brain, and the number of reports on pain perception using functional brain imaging techniques has progressively increased in the past 20 years. In general, studies using non-invasive techniques, such as electroencephalography, magnetoencephalography (MEG), positron emission tomography and functional MRI (fMRI) have found that noxious stimuli activate several areas of the brain, including the thalamus, basal ganglia, primary (S1) and secondary (S2) somatosensory cortex, insula and cingulate cortex (figure 1A).

The choice of an appropriate stimulus is another important aspect of pain research in humans because research into the human nociceptive system is limited by ethical constrains because of possible tissue damage and the discomfort evoked by a noxious stimulus. There are various ways to activate the nociceptive system, including chemical, thermal, electrical and mechanical stimulation. Each method has its own advantages and disadvantages but, ideally, the stimulation should be safe, reproducible and quantifiable.[1] In addition, it should stimulate Aδ or C nociceptors selectively if one wants to specifically investigate activation of the nociceptive system. For research or clinical testing that requires precise information of latency, such as evoked potentials, a steep rise in the intensity of the stimulus is also important. From an ethical point of view, the discomfort accompanying system specific stimulation should be as weak as possible.

Electrical stimuli fulfil many of these requirements but lack selectivity. Because mechanoreceptors have a lower electrical threshold than nociceptors, electrical stimuli always coactivate mechanoreceptors of the tactile system at a noxious intensity. Mechanical stimuli, such as pinpricks, which are often used for clinical tests, lack selectivity as well as the steepness. For a similar reason, the usefulness of chemicals for pain research is limited.[2]

Laser stimuli delivered as a brief pulse with a steep rise in intensity can activate cutaneous nociceptors without the concomitant activation of mechanoreceptors.[3] Therefore, laser stimulation is the best means of activating the human nociceptive system at present. In fact, lasers are used in research as well as clinical testing.[4 5] One problem with laser stimulation however is that the equipment needed is expensive.

Here we review studies using intraepidermal electrical stimulation (IES) developed for the selective activation of cutaneous nociceptors. An electrical method that can selectively stimulate nociceptors would clearly be useful for pain research or clinical tests.

INTRAEPIDERMAL ELECTRICAL STIMULATION

Electrode

This method is based on the fact that nociceptive fibre terminals are located in the epidermis and superficial layer of the dermis, while other fibres end deep in the dermis. When the superficial layer of the skin is electrically stimulated, the localised current is expected to selectively activate nociceptors. For this purpose, we made a pushpin-like electrode with a stainless steel needle, 0.2 mm in length.[6] Although it successfully stimulated cutaneous Aδ nociceptors,[6 7] the range of current at which there was no concomitant activation of Aβ mechanoreceptors was narrow—that is, as the current increased in intensity, it reached far enough to activate mechanoreceptors located deeper than nociceptors. Then we improved the method by employing a concentric bipolar configuration (figure 1B). The cathode used was an outer ring, 1.2 mm in diameter, and the anode was an inner needle that protruded 0.1 mm from the outer ring.[8] The effective range for the selective activation of nociceptors widened because less current spread to undesired skin layers (figure 1C). We confirmed the effectiveness of the concentric configuration at reducing undesired loop current in rats.[9]

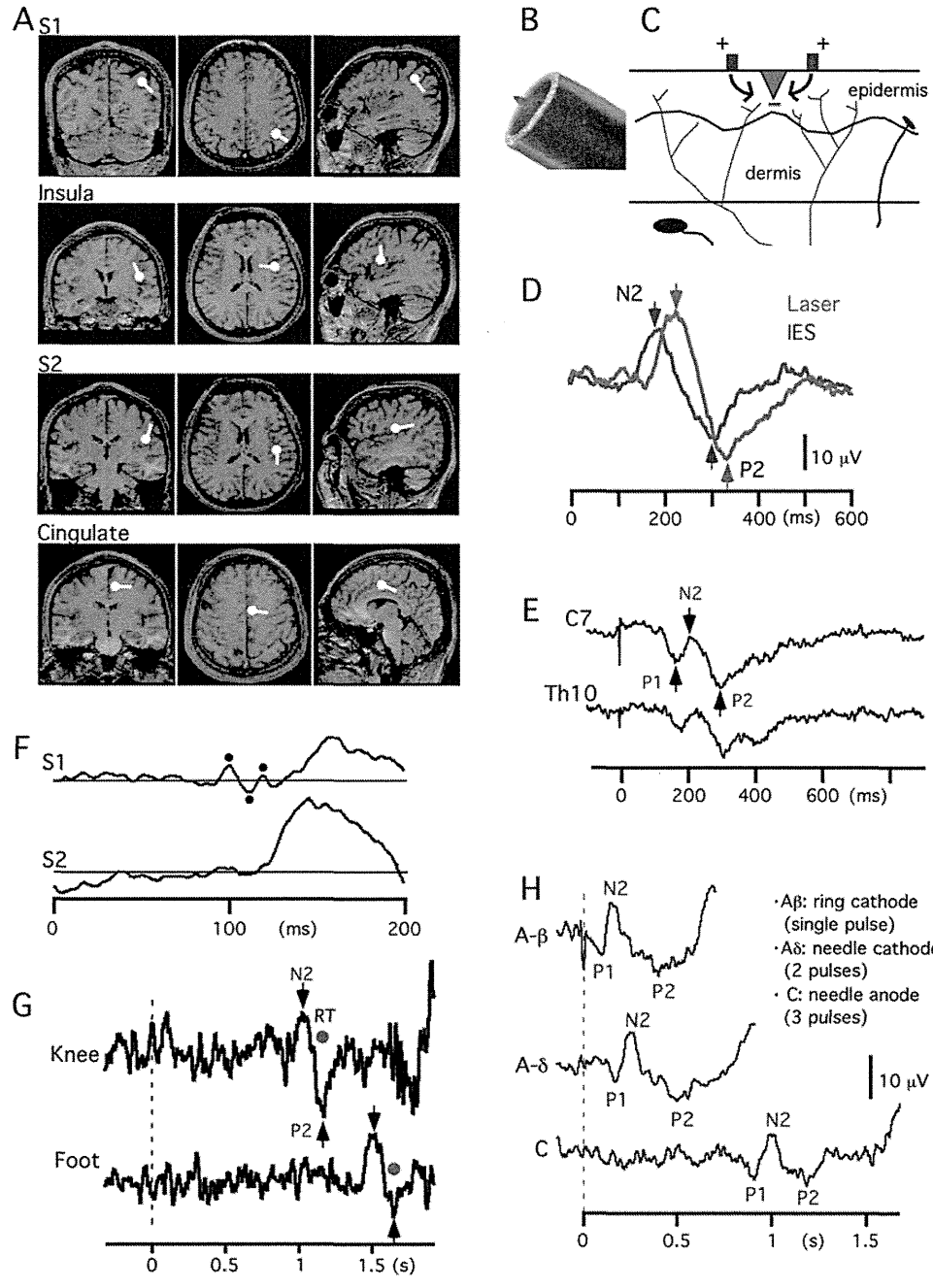

Figure 1 Use of intraepidermal electrical stimulation (IES) for studies on human pain perception. (A) Cortical activity detected by magnetoencephalography (MEG) in the primary somatosensory cortex (S1), secondary somatosensory cortex (S2), insula and cingulate cortex following IES to the left hand. (B) Photograph of the concentric bipolar needle electrode for IES. (C) The current passing through the electrode is spatially restricted to the superficial part of the skin where nociceptive free nerve endings are located. (D) Comparison of evoked potentials following stimulation of the hand between IES (blue) and laser (red) stimulation. Note the similar waveform and a 40 ms delay for laser stimulation. (E) Estimation of conduction velocity (CV) in the spinal cord. Very similar waveforms are evoked by IES to the back midline at the C7 and Th10 levels. (F) Primary responses to IES in S1 recorded by MEG. Note the triphasic waveform of the early S1 activity with polarity reversals at a 10 ms interval. (G) Estimation of CV of C fibres of the lower limb. The calculated CV was 1～1.1 m/s for N2, P2 and RT (reaction time). (H) Activation of Aβ, Aδ and C receptors by one electrode. By using different parameters, different receptors can be stimulated at the same site.

Stimulation

When the electrode is gently pressed against the skin, the needle tip is inserted adjacent to the nerve endings of the thin myelinated fibres in the epidermis and superficial part of the dermis. As there is no blood in the epidermis, the IES electrode cannot cause bleeding. Although we have never had an infection due to insertion of the electrode, the skin is first disinfected with alcohol and the electrode is for single use only. Unlike with laser stimulation, there is no undesired skin effect, such as heat burn or erythema. The electric stimulus can be a conventional square wave pulse of 0.5～1.0 ms but a slowly rising pulse, such as a triangular wave,[10] is better. Double pulses with a 10～25 ms interval are usually used to obtain clear responses but a single pulse is also used when a precise response latency is necessary (eg, see Inui *et al*[11]). The current is of an intensity that produces a definite sensation of pain, 2～6 on the visual analogue scale (0～10). IES can be applied to any area of the body. To augment the response, two or three electrodes, 10 mm apart, are used. In recent studies, a triple electrode type (NM-980W; Nihon Kohden, Tokyo, Japan) has been used.

Sensations

When a weak current, of approximately 0.1～0.5 mA, is applied by IES, a sharp pricking sensation, an indication of Aδ nociceptor activation, is elicited without any other sensations. The magnitude of the pricking sensations increases with an increase in stimulus intensity, number of pulses and pulse duration. The intensity of the painful sensation increases slightly with the use of multiple electrodes. These results suggest that for painful sensations, the contribution of temporal summation is greater than that of spatial summation. The pricking sensation is abolished by the local application of lidocaine.[12]

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Cortical responses to IES and conduction velocity

Cortical responses to IES are recorded using an evoked potential (EP) as large vertex potentials consisting of a negativity (N2) and a positivity (P2),[6] sometimes preceded by an earlier positivity (P1).[13] Figure 1D compares N2/P2 following IES of the hand and following stimulation of Aδ nociceptors by a CO_2 laser at the same site. The waveform is very similar but with a latency difference of 40 ms due to the temperature conduction time in the skin for laser stimulation.[3] Therefore, IES and laser evoked potentials are almost the same. As the latency of the somatosensory vertex potentials depends on the time taken for the signals to reach the brain, the type of peripheral nerve can be roughly estimated based on N2/P2 latency. For example, in the hand area, stimulation of Aβ fibres results in N2 peaking at 140 ms while N2 for Aδ and C nociceptor stimulation peaks at about 200 ms and 800~900 ms, respectively.

Responses in the somatosensory cortex are also very similar between IES and laser stimulation—that is, in studies using MEG, S1 in the hemisphere contralateral to the stimulation and S2 of both hemispheres are activated.[11 14 15] When the time delay for laser beams due to temperature conduction is taken into consideration, the response latency of each cortical activity is almost the same. In addition, the temporal profile of the IES induced cortical response is similar to that evoked by high intensity electrical stimulation in a patient who has no Aβ fibres due to sensory neuropathy.[16]

As the latency of EP components is longer following IES of a distal rather than a proximal site due to the distance travelled, peripheral conduction velocity (CV) can be calculated by dividing the difference in latency between the EP components by the distance between the two sites. With this method, mean CV was 15.1 m/s using EPs (hand and upper arm)[6] and 15.6 m/s using MEG (hand and elbow).[7] Both values are within the range for Aδ fibres (4~30 m/s), as measured by microneurographic studies.[17 18]

In summary, IES elicits a sharp pricking sensation without other sensations via peripheral signals ascending through Aδ fibres, and produces cortical responses that are almost the same as those evoked by laser stimulation. Both the painful pricking sensation and evoked brain responses (Aδ fibre latency) are abolished by local application of lidocaine. These findings suggest that IES selectively activates Aδ nociceptors. Recently, a European group verified this by showing that: (1) after the selective denervation of capsaicin sensitive nociceptors by 72 h application of a capsaicin cream, IES evoked cortical responses were almost abolished, and the threshold for detecting IES increased markedly (0.09 vs 0.6 mA) compared with controls; (2) when the conduction of myelinated nerve fibres was selectively blocked by compression, the time course of the blockade of responses to IES followed closely the time course of the blockade of Aδ fibre responses to laser stimulation; and (3) IES with a high current (2.5 mA) coactivated Aβ-fibres.[19] In some studies, a similar concentric bipolar electrode without a needle was used to elicit a painful sensation.[20] Stimulation with this electrode is easy to control and less invasive than IES but it activates Aβ fibres.

MERITS OF IES

IES has several advantages over other noxious stimuli and should contribute to progress in pain studies. We next present some of the studies taking advantage of IES.

Selective activation of Aδ nociceptors without the need for expensive equipment

The stimulus is easy to control and requires no specialised skills. In certain clinical patients, the assessment of small fibre function is important. However, because few hospitals have a laser stimulator, mechanical stimuli are often used even though they lack nociceptive selectivity. As IES is very simple, it seems useful for clinical tests. A new portable stimulator weighing just 290 g and specialised for IES (PNS-7000; Nihon Kohden) should enhance its use in clinics. When starting IES with a current of 0.01 mA and increasing the stimulus in steps of 0.01 mA, a weak pricking sensation occurs at the threshold. As there is no other sensation below the threshold, the threshold for Aδ nociceptor activation can be easily assessed. Therefore, IES is expected to detect functional changes in peripheral small fibres. To test this possibility, we examined the effects of lidocaine tape on pain threshold and EPs.[12] As expected, local application of lidocaine significantly elevated the pain threshold, and almost abolished EPs while effects on tactile sensation and tactile EPs were very small.

One possible use of IES is for so-called small fibre neuropathy. The diagnosis of small fibre neuropathy is often difficult because small fibres are invisible in routine nerve conduction studies. For example, in diabetic patients, disturbances begin in small fibres in the distal part of the limb. Our recent study[21] confirmed the usefulness of IES for evaluating small fibre function in diabetic patients. As another example, Obayashi *et al*[22] recently reported a case of domino liver transplantation induced amyloid neuropathy. The patient, due to sclerosing cholangitis, underwent a domino liver transplantation reusing a resected liver from a patient with familial amyloid polyneuropathy. When thermohypesthesia and hypoalgesia appeared 7 years after the transplantation, results of neurological examinations, including tests of tendon reflexes, vibration sense, proprioception and nerve conduction, were all normal but the Aδ nociceptor pain threshold by IES was elevated. This report suggests that a follow-up examination of small fibre function is important for such patients and IES can serve this purpose.

IES can be applied to any part of the body

With most MEG or fMRI machines, applying laser beams to areas other than the limbs is difficult. Therefore, if we want to stimulate these cutaneous sites and record brain responses, IES is useful. A recent study showed that cortical magnetic responses are clearly recorded following IES to various areas, including the neck, face and back (Omori and Isose, unpublished data). The use of IES would stimulate pain studies using fMRI. Yoshino *et al*[23] reported that IES could be used safely in an fMRI room and evoked clear brain activity detectable as haemodynamic changes.

By recording EPs following stimulation of two cutaneous sites, we can measure the CV of the periphery as well as in the spinal cord. This information may be useful for certain clinical cases such as demyelinating diseases. The CV in the spinothalamic tract can be estimated by stimulating two different levels of the back midline. As the peripheral conduction distance is short and similar between two sites, the latency difference is due to the conduction time difference in the spinal cord. However, because N2/P2 is an endogenous EP component, its latency and amplitude are affected by the subject's internal state. To reduce this undesired effect, random stimulation of the two sites is useful. Figure 1E shows an EP recording following stimulation of the back at the C7 and Th10 vertebral spinous process levels. In this case, very similar waveforms were evoked although that for the distal site had a longer response latency. The calculated CV is 13.5 m/s for P1 and 13.0 m/s for N2. The results indicate that the CV is similar between the periphery and spinal cord, which is consistent with the results of studies using laser stimulation.[24–26] Although N2/P2 is an endogenous component

common to all sensory modalities,[14 27] it is easily recorded and useful for estimating CV or task related EP components.[28]

Steep rise in stimulus intensity

As IES is an electrical method, it provides a good time locked stimulus, which is important when analysing responses in the order of milliseconds. Several studies have taken advantage of this. In an MEG study investigating early responses to IES in S1,[11] the early S1 activity was a sharp transient of approximately 80~100 ms following IES and reversed its polarity once or twice after a 10 ms interval similar to the 20/30 ms component evoked by tactile stimulation of the hand (figure 1F), a common feature of the primary response among sensory modalities (for visual and auditory systems, see Inui *et al*[29 30]). Because of the polarity reversing nature of primary cortical responses with a 10 ms interval, a small latency jittering of 10 ms of peripheral activation among each trial is enough to cancel out the response. In fact, no studies using laser stimulation have detected early cortical activation except one by Wang *et al*[31] in which jittering of the response was corrected for each trial. As another example, there are two studies using a pair stimulation paradigm.[8 32] When one wants to deliver two different stimuli at various conditioning test intervals, the timing of the onset of peripheral activation is particularly important. In an MEG study by Inui *et al*,[8] cortical responses to paired noxious (IES) and innocuous (conventional transcutaneous electrical stimulation) stimuli applied to the back at 11 conditioning test intervals of −500~500 ms were recorded to reveal cortical mechanisms underlying pain relief by tactile inputs. Results showed that IES induced responses were markedly inhibited when transcutaneous electrical stimulation was applied 20~60 ms later and 0~500 ms earlier than IES. Based on the time taken for each signal to reach the spinal cord and the cortex, we concluded that cortical responses to IES can be inhibited by innocuous tactile stimuli at the cortex without a contribution at the spinal level.

Minimal discomfort and possible use in animal studies

IES evokes clear cortical responses at a weak current around twice the threshold. However, at this intensity, some subjects report that the stimulus is not painful at all (visual analogue scale 0~1). This means that the IES evoked sensation is a pure noxious sensation with minimal discomfort—that is, pain. Indeed, IES evoked sensations are very weak compared with the uncomfortable feeling caused by conventional transcutaneous electrical stimulation at a painful intensity. Unless the aim of the study is discomfort, a less uncomfortable stimulus is better with respect to the ethical restrictions of studies on the nociceptive system. In an MEG study by Wang *et al*[33] investigating the effects of sleep on IES induced cortical responses, subjects were rarely awakened by IES at an intensity high enough to obtain clear cortical responses before sleep.

In animal studies, the use of mechanical stimuli, such as pinching the tail, is common. However, such a stimulus inevitably coactivates Aβ mechanoreceptors. Therefore, if possible, selective stimulation is desirable. In spite of its usefulness for pain studies in humans, laser stimulation is rare in animal studies. One reason is the cost of laser machines but another may be the difficulty of applying laser beams to freely moving animals. In addition, adjusting the laser energy to an appropriate strength is difficult. IES can be applied at any time without immobilising the animal once the electrode is attached to the skin.

For research into the animal nociceptive system, one Australian group used IES. In awake dogs, van Oostrom *et al*[34] recorded EPs following IES to the hind paw. Results showed that: (1) the amplitude of the N2/P2 components increased with an increase in stimulus intensity (0.2~1.0 mA); (2) CV was 5~20 m/s; and (3) when the stimulus intensity was increased, there were mild behavioural reactions, withdrawal of the stimulated hind paw and lip licking. It is worth noting that a clear EP recording is possible in awake animals and, in addition, the behavioural response is mild when IES evokes clear cortical responses.

WEAK POINTS OF IES

As nociceptive free nerve endings are located in the epidermis while the other thicker fibres run more deeply in the dermis, the current passing through the electrode should be spatially restricted to the superficial layer of the skin. In other words, IES activates tactile mechanoreceptors in the dermis when the current is too strong. In fact, results of a study by Mouraux *et al*[19] using a nerve conduction blockade showed that IES at 2.5 mA activates Aβ mechanoreceptors in addition to Aδ nociceptors. Therefore, one cannot use a strong current even when intense sensations of pain are necessary. Usually, the threshold for stimulation of Aδ nociceptors by IES with double pulses is below 0.1 mA, and 2~3 times the threshold is enough to obtain clear cortical responses. At around this intensity, IES selectively activates Aδ nociceptors. However, one would consider the painful sensation to be too weak at this intensity. For a stronger sensation, spatial summation by use of multiple electrodes or temporal summation by a long duration pulse or pulse train should be considered instead of an increase in intensity.

STIMULATION OF C FIBRE BY IES

Now we shall describe our recent attempt to selectively stimulate C nociceptors by IES. Because of the very high electrical threshold, it is difficult to stimulate C nociceptors selectively by conventional transcutaneous electrical stimulation. Although in isolated nerves of animals a specific method such as anodal blocking[35] can be used for this purpose, such an invasive technique cannot be applied to humans. Based on differences in the distribution of C and Aδ nociceptors (Aδ <C), Plaghki's group reported the successful stimulation of C nociceptors by laser beams for the first time.[36] They stimulated a tiny area of the skin with a laser beam that is expected to hit C nociceptors exclusively, and the results supported this. The difference in the threshold of the response to thermal stimuli between C (40°C) and Aδ (46°C) nociceptors is also useful for the selective activation of C nociceptors by laser beams. Tran *et al*[37] successfully stimulated C nociceptors by employing a low energy laser beam to a tiny skin area.

As for electrical stimulation, the selective activation of C nociceptors seems impossible because of the high electrical threshold. However, the higher density of C nociceptors might be an advantage if the current passing through the concentric electrode is limited to a very small area. In addition, there are several reports of factors that are effective at activating C fibres: a pulse of long duration, a slowly rising pulse, temporal and spatial summation, and anodal stimulation (see Otsuru *et al*[10]). Based on these reports, we tested IES for the selective stimulation of C nociceptors under the following conditions: (1) the anode was the inner needle and the cathode was the outer ring; (2) the electric pulse was a triangular wave with a rise and fall time of 1 ms; (3) the stimulus was a train of three pulses at 50 Hz; and (4) three electrodes 10 mm apart were used.[10]

Sensations

IES elicits weak sensations with a reaction time of approximately 1 s following stimulation of the hand area. The sensory threshold is approximately 0.04 mA. The sensation evoked varies among subjects or among penetrations but is usually the feeling of a light touch and sometimes pricking or slight burning, which is similar to the sensations evoked by a low intensity laser beam applied to a tiny area of the skin.[37 38] A warm or itchy sensation is very rare. Under these conditions, there is no clear axonal reflex flare reaction mediated by mechanoinsensitive C nociceptors.[39] We consider IES to activate polymodal C nociceptors.[40] Therefore, the electrical threshold for the stimulation of C nociceptors is not as high as generally considered.

Conduction velocity

Similar to Aδ nociceptors, CV can be measured by recording EPs. Because of the long reaction time (RT) for C nociceptor stimulation, RT can be also used to estimate CV. Figure 1G shows an example of EPs following stimulation of the foot and knee. Note the very late N2/P2 component compared with Aδ stimulation. The distance between the two stimulated sites was 43 cm and the latency difference was 392 ms for N2, 404 ms for P2 and 440 ms for RT, which yielded a conduction velocity of 1.0∼1.1 m/s. The mean CV following stimulation of the hand and forearm among eight subjects was 1.5 m/s.[10]

Stimulation of Aβ, Aδ and C fibres through the same electrode

As IES stimulates Aδ nociceptors when the inner needle is the cathode, Aδ and C nociceptors can be stimulated with one electrode by switching the polarity. Figure 1H shows an example of hand stimulation. First, we recorded C responses with anodal stimulation. The peak latency of N2 was about 1 s. Then the polarity was switched. The cathodal stimulation now evoked Aδ responses with N2 peaking at 200 ms.

As the outer ring of the IES electrode is attached to the skin, Aβ mechanoreceptors can be activated by standard monopolar stimulation through the outer ring. The waveform in response to Aβ stimulation in figure 1H has a well known N2 component peaking at 140 ms. In this example, each type of stimulation evoked very similar potentials, consisting of P1, N2 and P2 components. If we want to stimulate different nerve fibres at the same cutaneous site, this method seems useful and easy.

FUTURE PERSPECTIVES

We have reviewed studies using IES. We believe that it is useful for basic research as well as clinical tests, and will help us to better understand the physiology of the nociceptive system, pathology of pain related disorders or mechanisms of the analgesic effects of drugs. To validate the usefulness of IES and to establish normative data, however, studies using a large group of normal subjects are necessary. For clinical testing, IES can be used for pain disorders such as fibromyalgia as well as small fibre neuropathies such as diabetic neuropathy. IES seems particularly suitable as a screening test because it can be used easily in a clinical setting. As for the stimulation of C nociceptors by IES, however, we feel that there is still room for improvement to obtain responses good enough for clinical testing.

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Competing interests None.

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Neuropharmacology and analgesia

Increased brain monoaminergic tone after the NMDA receptor GluN2A subunit gene knockout is responsible for resistance to the hypnotic effect of nitrous oxide

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ABSTRACT

N-methyl-D-aspartate (NMDA) receptors can be inhibited by inhalational anesthetics in vitro at clinically relevant concentrations. Here, to clarify the role of NMDA receptors in anesthetic-induced unconsciousness, we examined the hypnotic properties of isoflurane, sevoflurane and nitrous oxide in NMDA receptor GluN2A subunit knockout mice. The hypnotic properties of inhalational anesthetics were evaluated in mice in the loss of righting reflex (LORR) assay by measuring the 50% concentration for LORR (LORR ED_{50}). Knockout mice displayed isoflurane and sevoflurane LORR ED_{50} values similar to wild-type controls, indicating no significant contribution of these receptors to the hypnotic action of halogenated anesthetics. However, compared with wild-type controls, mutant mice displayed larger isoflurane LORR ED_{50} values in the presence of nitrous oxide, indicating a resistance to this gaseous anesthetic. Knockout mice have enhanced brain monoaminergic activity which occurs secondary to NMDA receptor dysfunction, and the observed resistance to the isoflurane LORR ED_{50}-sparing effect of nitrous oxide could be abolished by pretreatment with the dopamine D_2 receptor antagonist droperidol or with the serotonin 5-HT $_{2A}$ receptor antagonist ketanserin. Thus, resistance to nitrous oxide in knockout mice appears to be a secondary phenomenon of monoaminergic origin and not a direct result of impaired NMDA receptor function. Our results indicate that NMDA receptors are not critically involved in the hypnotic action of conventionally-used inhalational anesthetics. Also, they suggest that increased brain monoaminergic tone can diminish the effects of general anesthesia. Finally, they provide further evidence that changes secondary to genetic manipulation can explain the results obtained in global knockouts.

1. Introduction

Anesthetic-induced loss of consciousness and immobility to noxious stimulation are two major determinants of the anesthetized state. In animal experiments, selective blockade of excitatory synaptic transmission in the central nervous system can induce both loss of righting reflex (LORR, a correlate for a loss of consciousness in humans) and immobility in response to painful stimuli (Irifune et al., 2007). *N*-methyl-D-aspartate (NMDA) receptors constitute a major component of excitatory synaptic transmission and were previously regarded as promising candidate mediators of anesthetic-induced immobility (Sonner et al., 2003). However, we have recently shown that NMDA receptors are not critically involved in mediating immobility (Petrenko et al., 2010), which confirmed the current point of view that their role in this anesthetic phenomenon is probably only indirect and modulatory (Eger et al., 2008).

In contrast to the immobilizing action, the molecular mechanisms by which inhalational anesthetics produce loss of consciousness (hypnosis) have received less focused research attention. Sevoflurane and isoflurane can inhibit recombinant NMDA receptors at clinically relevant concentrations (by 14 and 28%, respectively, at 1 MAC (minimum alveolar anesthetic concentration)) (Solt et al., 2006). Such inhibition is greater in the case of nitrous oxide (by 31% at 0.5 MAC) (Yamakura and Harris, 2000), a gaseous anesthetic often used to potentiate inhalational anesthesia. Thus, following from these and other related observations (Sato et al., 2005), there is still a possibility that NMDA receptors may directly mediate the hypnotic action of at least some of the inhalational anesthetics.

Functional NMDA receptors are composed of essential channel-forming GluN1 and at least one of four GluN2 (GluN2A-D) subunits (Mori and Mishina, 1995). Among the latter, GluN2A subunits show the widest expression in the adult animal central

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nervous system (CNS) and are found throughout the cerebral cortex and thalamus (Watanabe et al., 1993). It should be noted that the corticothalamic networks are now considered to be essential for the maintenance of consciousness and thus represent a proper target for anesthesia (Alkire et al., 2008). In fact, anesthetics such as isoflurane can block somatosensory information transfer in the thalamus by a reduction of glutamatergic excitation, and application of NMDA can restore the information flow to the cortex (Vahle-Hinz et al., 2007). This suggests the possibility that NMDA receptors containing GluN2A subunits could mediate the neurotransmission-interrupting effects of inhalational anesthetics in corticothalamic circuits and, hence, loss of consciousness.

Here, we decided to examine the role of NMDA receptors in producing unconsciousness using the NMDA receptor GluN2A subunit knockout mice that have established dysfunction of the NMDA receptors (Sakimura et al., 1995). Provided that possible confounding influences intrinsic to global knockouts are carefully excluded, the reduced ability of inhalational anesthetics to produce LORR in knockout animals would suggest their likely in vivo role as molecular targets determining loss of consciousness. Otherwise, and combined with our previous results, anesthetic potencies in knockout mice not different from those in wild-type controls would suggest that NMDA receptor GluN2A subunits are not the major contributors to the anesthetic state produced by conventional inhalational agents.

2. Materials and methods

2.1. Animals

This study was approved by the Committee for the Guidelines on Animal Experimentation of Niigata University (Niigata, Japan). All procedures were performed on male adult (aged 8–14 weeks) C57BL/6 and NMDA receptor GluN2A subunit knockout mice, hereafter referred to as wild-type and mutant mice, respectively. Mutant mice were produced as described previously (Sakimura et al., 1995) and had more than 99.99% homogeneity with wild-type animals because of a series of backcrosses. They exhibit increased spontaneous locomotor activity in a novel environment (Miyamoto et al., 2001) and impairment of several forms of learning (Mori and Mishina, 2003). Nevertheless, they show normal responses to painful stimuli (Petrenko et al., 2006, 2003), and have no alterations in gross motor abilities under control conditions. Animals were housed 4–6 per cage under a standard 12-h light-dark cycle; water and food were available ad libitum. The temperature of the testing room was kept at 24 °C, and experiments were conducted between 09:00 and 17:00.

2.2. Studies of sensitivity to inhalational anesthetics

To conduct anesthetic experiments, mice were individually placed in small plastic chambers connected to a vaporizer and oxygen source. Measurements were performed on two wild-type and two mutant mice in each session. Isoflurane (Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan) and sevoflurane (Maruishi Pharmaceutical Co., Ltd., Osaka, Japan) were vaporized in 1 l/min oxygen. The concentrations of anesthetics were continually monitored using an infrared gas analyzer (Capnomac Ultima, Instrumentarium Corp., Helsinki, Finland). Each concentration of anesthetic was maintained for a minimum equilibration period of 20 min. Rectal temperatures were determined using a digital thermometer (TD-300; Shibaura Electronics, Saitama, Japan) before and after each measurement, and body temperature was actively maintained between 36° and 38 °C by warming with a plastic plate filled with circulating hot water placed below the chambers.

To investigate the hypnotic properties of inhalational anesthetics in mice, the behavioral endpoint of LORR was used (Petrenko et al., 2007). Isoflurane or sevoflurane was administered to each chamber at initial concentrations of 1.0 and 1.8%, respectively. After the equilibration period, the chamber was gently rotated to place the mouse on its back, and the righting reflex was monitored for up to 15 s. All mice experienced LORR at these concentrations. The concentration of each anesthetic was decreased by 0.1% for another equilibration period and the response was tested again. The 50% concentration for LORR (LORR ED_{50}) was calculated by averaging the two concentrations at which the mouse either retained LORR or regained the righting reflex.

To determine the isoflurane LORR ED_{50}-sparing effect of nitrous oxide, the latter was administered at one of three concentrations of 25, 50 and 75%, which were kept constant throughout the experiment. Isoflurane was co-administered to each chamber at initial concentrations of 0.8, 0.6 and 0.4%, which corresponded to three above-mentioned concentrations of nitrous oxide; all mice experienced LORR at these concentrations. The concentration of isoflurane was decreased by 0.1% for another equilibration period and the response was tested again. The 50% concentration for LORR (LORR ED_{50}) was calculated by averaging the two concentrations at which the mouse either retained LORR or regained the righting reflex.

2.3. Drug pretreatment experiments

Two series of experiments included pretreatment with droperidol or ketanserin. Droperidol (Droleptan, Daiichi Sankyo Co., Ltd., Tokyo, Japan) was used in the form of 2.5 mg/ml ready-to-use solution. Ketanserin tartrate (Tocris Cookson Inc., Ellisville, MO, USA) was dissolved in distilled water and its concentration was adjusted so that the injected volume was 10 ml/kg body weight. Drugs were injected intraperitoneally following the induction of anesthesia. Control animals in pretreatment experiments were injected with the same volume of normal saline. Their LORR ED_{50} values were not different from those of animals not pretreated exposed to isoflurane in the presence of 50% nitrous oxide and hence were pooled with the latter. Isoflurane and sevoflurane LORR ED_{50} values, the LORR ED_{50}-sparing effect of nitrous oxide, and the effects of ketanserin and droperidol on the LORR ED_{50}-sparing effect of 50% nitrous oxide were measured in different sets of animals. All mice were used in several experiments and at least 1 week was allowed after each experiment to provide sufficient time for recovery. An outline of the experiment schedule is shown in Fig. 1.

2.4. Statistical analysis

For statistical analysis of differences in isoflurane and sevoflurane LORR ED_{50} values and isoflurane LORR ED_{50} values in the presence of 75% nitrous oxide between wild-type and mutant mice, we used the unpaired Student's *t*-test or Mann–Whitney *U*-test when the data were not normally distributed. Differences in the isoflurane LORR ED_{50} values in the presence of various concentrations of nitrous oxide between the two genotypes were compared by 2-way analysis of variance (ANOVA) followed by Bonferroni's test. For analysis of the effect of droperidol or ketanserin on the isoflurane LORR ED_{50}-sparing effect of 50% nitrous oxide in each genotype, we used 1-way ANOVA followed by Dunnett's test. Differences in the responses to different doses of droperidol or ketanserin between the two genotypes were compared by 2-way ANOVA followed by Bonferroni's test. The data are presented as means $\pm$ S.D. In all cases, $P < 0.05$ was considered significant.

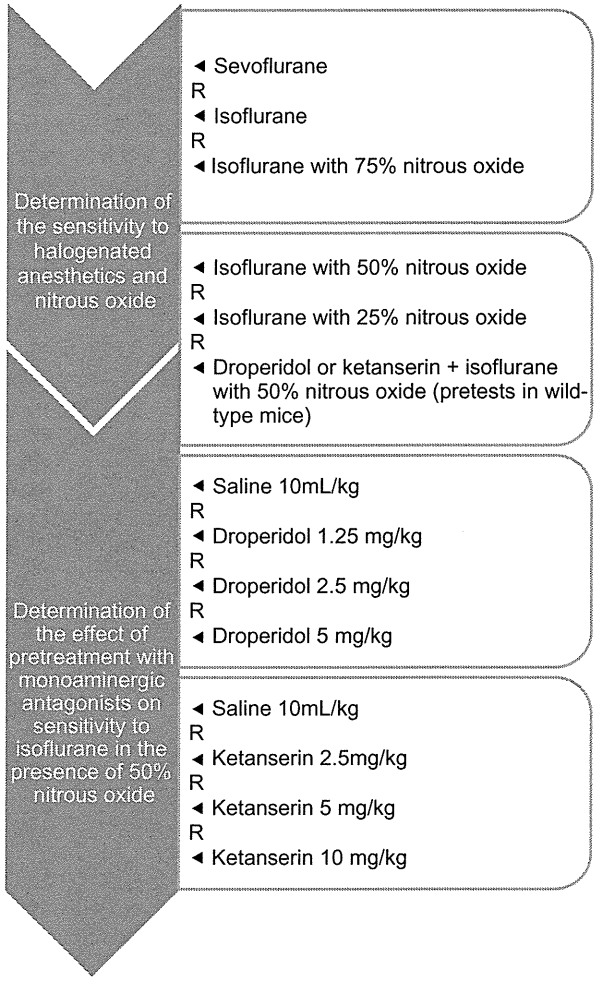

Fig. 1. Experiment schedule. Text bars on the right encompass four separate sets of wild-type and mutant mice. In each set, animals were used in 3–4 experiments. "R"s indicate ≥1-week periods of rest between successive experiments. In pretreatment experiments, normal saline was injected into 2–3 control animals of each genotype.

3. Results

3.1. Sensitivity to halogenated anesthetics is unaltered after the NMDA receptor GluN2A subunit gene knockout

First, we conducted the LORR assay to determine whether the hypnotic effects of halogenated anesthetics were altered after GluN2A subunit gene knockout. As shown in Fig. 2, we found that in mutant mice the righting reflex was inhibited at concentrations of isoflurane and sevoflurane similar to those in wild-type controls (isoflurane LORR ED_{50} values of 0.64 ± 0.11, $n=9$, in mutant mice versus 0.66 ± 0.12, $n=9$, in wild-type mice; $P=0.8448$, unpaired t-test; sevoflurane LORR ED_{50} values of 1.30 ± 0.14, $n=9$, in mutant mice versus 1.22 ± 0.19, $n=10$, in wild-type mice; $P=0.3223$, unpaired t-test). This suggested that NMDA receptor GluN2A subunits do not play a critical role in mediating the LORR produced by halogenated anesthetics.

3.2. Sensitivity to nitrous oxide is reduced after the NMDA receptor GluN2A subunit gene knockout

Since nitrous oxide does not produce LORR under normoxic conditions, to evaluate the contribution of NMDA receptors to the hypnotic effect of this gaseous anesthetic, we examined the ability of 75% nitrous oxide to reduce isoflurane LORR ED_{50} requirements

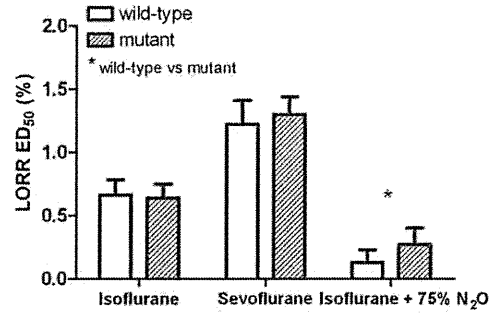

Fig. 2. Isoflurane and sevoflurane LORR ED_{50} values and isoflurane LORR ED_{50} values in the presence of 75% nitrous oxide measured in wild-type and mutant mice. Values are means ± S.D. ($n=9$–12 per group). LORR ED_{50}=the 50% effective dose for the loss of righting reflex. *$P=0.0312$, mutant vs wild-type, Mann–Whitney U-test.

(the isoflurane LORR ED_{50}-sparing effect) in wild-type and mutant animals. As shown in Fig. 2, mutant mice appeared resistant to the potentiating effect of nitrous oxide on the isoflurane hypnotic effect (isoflurane LORR ED_{50} values in the presence of 75% nitrous oxide of 0.13 ± 0.1, $n=11$, in wild-type mice versus 0.27 ± 0.13, $n=12$, in mutant mice; $P=0.0312$, Mann–Whitney U-test). This suggested that NMDA receptor GluN2A subunits may be involved in the hypnotic effect of nitrous oxide.

3.3. Pretreatment with monoaminergic antagonists normalizes the reduced sensitivity to nitrous oxide after the NMDA receptor GluN2A subunit gene knockout

The NMDA receptor GluRε1 subunit knockout mice exhibit hyperfunction of brain dopaminergic and serotonergic monoaminergic systems (Miyamoto et al., 2001). Drugs like amphetamines that act by increasing brain dopamine and serotonin levels are known to have analeptic properties and can shorten the duration of LORR caused by anesthetics such as pentobarbital (Horita and Carino, 1991; Horita et al., 1994). Therefore, we had to exclude the possibility that an increase in monoaminergic tone, and not NMDA receptor dysfunction, was a reason for resistance to the potentiating effect of nitrous oxide on isoflurane LORR ED_{50} values in mutant animals.

3.3.1. Selection of suitable concentration of nitrous oxide for pretreatment experiments

Our preliminary experiments indicated that the monoaminergic antagonists droperidol and ketanserin reduce isoflurane LORR ED_{50} values. Accordingly, owing to the fact that isoflurane LORR ED_{50} values were already diminished in the presence of 75% nitrous oxide (Fig. 1), we had to confirm whether the resistance to nitrous oxide in mutant mice would be retained at its lesser concentrations. As shown in Fig. 3, in the presence of 50% nitrous oxide mutant mice also exhibited greater isoflurane LORR ED_{50} values compared with wild-type controls, but the difference between the two genotypes became insignificant in the case of 25% nitrous oxide ($P<0.05$ and $P>0.05$, respectively, Bonferroni's test following 2-way ANOVA). Therefore, in the following experiments with monoaminergic antagonists, the 50% concentration of nitrous oxide was used.

3.3.2. Pretreatment with droperidol

We began by evaluating the effects of the D_2/D_1 receptor antagonist droperidol on isoflurane LORR ED_{50} values in the presence of 50% nitrous oxide in both groups of animals. As shown in Fig. 4, the administration of droperidol resulted in a