in SNL rats. These rates and magnitudes of decompression are quite near to what are observed while a LP system is approaching: in the case of a typhoon of which the pressure change at the authors' institute was shown in Fig. 2b, the atmospheric pressure decreased by as much as 50.7 hPa in 12 h (average ca. 4 hPa/h) was reported in the Kyusyu area (about 900 km away from Nagoya where the present experiments were taking place) where the typhoon directly attacked. Thus, the minimum pressure change needed to induce aggravation of pain behavior in neuropathic rats is within the range of naturally occurring pressure changes. Therefore, the presently observed aggravation of pain-related behavior by LP in SNL rats simulates to some extent the climatic change-induced aggravation of chronic pain in humans. There appears to be a notable absence of a stimulus–response relationship, namely, the smallest barometric pressure decrease (by 5 hPa at 5 hPa/h) induced almost the full effect, whereas a more intense pressure decrease (e.g., by 10 hPa at 5 hPa/h) induced no further augmentation in pain-related behaviors except for additional responses to the weaker VFH(s). This might reflect the characteristics of either a pressure-sensing system or efferent system (possibly the sympathetic nerve activities described below).

We observed that a faster and higher level of decompression were required to induce pain-related behaviors in the CCI rats than in the SNL rats. The observation is in good agreement with the report that signs of 'mechanical allodynia' (foot withdrawal in response to normally innocuous mechanical stimuli) in SNL rats were stronger than that in CCI rats, i.e., in the former, evoked pain-related behavior of greater magnitude was regularly observed (Kim et al. 1997). We also confirmed it (Fig. 3a, b). In addition, the present results also exhibited a tendency toward stronger augmentation of pain-related behavior during LP exposure in SNL rats than in CCI rats. The more sensitive response to LP in the SNL rats may be related to the pronounced changes in their withdrawal response. We observed in the previous experiment that, although sympathectomized CCI rats showed no aggravation of their pain-related behavior in response to LP exposure (Sato et al. 1999), they did exhibit an augmentation of such behavior under low-temperature exposure (Sato et al. 2000). These results suggest that sympathetic nerve activity makes an important contribution to the LP effect, while it appears to be of little importance to the low-temperature effect. In addition, activation of the sympathetic nervous system in response to LP exposure has been demonstrated by increased blood pressure and heart rate in both normal and CCI rats (Sato et al. 2001). It has also been reported that the contribution of sympathetic nerve activity to pain-related behavior is greater in SNL rats than in CCI rats (Kim et al. 1997). Taken together, these results suggest that the mechanism for a higher sensitivity of SNL rats to LP exposure might be related to a more significant contribution of sympathetic nerve activity to the aggravation of pain-related behavior in this model.

We have recently found that when the inner ear-lesioned SNL or CCI rats were exposed to LP, they showed no augmentation of pain-related behavior (Funakubo et al. 2010). We also observed, though preliminary, that some neurons in the rat vestibular nucleus responded to lowering barometric pressure by 40 hPa within 8 min (Funakubo et al. 2008). These results may suggest that the barometric sensor/sensing system influencing nociceptive behavior during LP in rats is located in the inner ear. Further studies are needed to clarify the neural mechanism of weather-induced aggravation of pain.

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References

Bennett GJ, Xie YK (1988) A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 33:87–107, PMID: 2837713

Funakubo M, Sato J, Mizumura K (2008) Search for barometric pressure-sensitive neurons in the vestibular nuclei. J Physiol Sci 57:S194, http://www.jstage.jst.go.jp/article/psjproc/2007/0/2007_194_4/_article

Funakubo M, Sato J, Honda T, Mizumura K (2010) The inner ear is involved in the aggravation of nociceptive behavior induced by lowering barometric pressure of nerve injured rats. Eur J Pain 14:32–39, PMID: 19318284

Guedj D, Weinberger A (1990) Effect of weather conditions on rheumatic patients. Ann Rheum Dis 49:158–159. doi:10.1136/ard.49.3.158, http://www.ard.bmj.com/cgi/reprint/49/3/158

Hendler NH, Jamison RN, Morrison CH, Jenepher KP, Kahn Z (1995) The relationship of diagnoses and weather sensitivity in chronic pain patients. J Neuromusculoskel Syst 3:10–15

Hollander JL, Yeostros SY (1963) The effect of simultaneous variations of humidity and barometric pressure on arthritis. Bull Am Meteorol Soc 44:389–393, PMID: 13961319

Jamison RN, Anderson KO, Slater MA (1995) Weather changes and pain: perceived influence of local climate on pain complaint in chronic pain patients. Pain 61:309–315, PMID: 7659442

Jin Y, Sato J, Yamazaki M, Omura S, Funakubo M, Senoo S, Aoyama M, Mizumura K (2008) Changes in cardiovascular parameters and plasma norepinephrine level in rats after chronic constriction injury on the sciatic nerve. Pain 135:221–231, PMID: 17611035

Kim SH, Chung JM (1992) An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 50:355–363, PMID: 1333581

Kim KJ, Yoon YW, Chung JM (1997) Comparison of three rodent neuropathic pain models. Exp Brain Res 113:200–206, PMID: 9063706

Kranzl B (1977) Trigeminal neuralgia—effect of external and internal influences on the disease as well as studies on infiltration treatment of pain. Österr Z Stomatol 74:246–261, PMID: 362339

Mitchell SW (1877) The relations of pain to weather, being a study of the natural history of a case of traumatic neuralgia. Am J Med Sci April 305–329

Sato J (2003) Weather change and pain: a behavioral animal study of the influences of simulated meteorological changes on chronic pain. Int J Biometeorol 47:55–61, PMID: 12647091

Sato J, Morimae H, Seino Y, Kobayashi T, Suzuki N, Mizumura K (1999) Lowering barometric pressure aggravates mechanical allodynia and hyperalgesia in a rat model of neuropathic pain. Neurosci Lett 266:21–24, PMID: 10336174

Sato J, Morimae H, Takanari K, Seino Y, Okada T, Suzuki M, Mizumura K (2000) Effects of lowering ambient temperature on pain-related behaviors in a rat model of neuropathic pain. Exp Brain Res 133:442–449, PMID: 10985679

Sato J, Takanari K, Omura S, Mizumura K (2001) Effects of lowering barometric pressure on guarding behavior, heart rate and blood pressure in a rat model of neuropathic pain. Neurosci Lett 299:17–20, PMID: 11166927

Sato J, Aoyama M, Yamazaki M, Okumura S, Takahashi K, Funakubo M, Mizumura K (2004) Artificially produced meteorological changes aggravate pain in adjuvant-induced arthritic rats. Neurosci Lett 354:46–49

Ta LE, Dionne RA, Fricton JR, Hodges JS, Kajander KC (2000) SYM-2081 a kainate receptor antagonist reduces allodynia and hyperalgesia in a freeze injury model of neuropathic pain. Brain Res 858:106–120, PMID: 10700603

Verges J, Montell E, Tomas E, Cumelles G, Castaneda G, Marti N, Moller I (2004) Weather conditions can influence rheumatic diseases. Proc West Pharmacol Soc 47:134–136, PMID: 15633634

Zhou S, Bonasera L, Carlton SM (1996) Peripheral administration of NMDA, AMPA or KA results in pain behaviors in rats. NeuroReport 7:895–900, PMID: 8724668

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The inner ear is involved in the aggravation of nociceptive behavior induced by lowering barometric pressure of nerve injured rats

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ABSTRACT

Patients suffering from neuropathic pain often complain of pain aggravation when the weather is changing. The exact mechanism for weather change-induced pain has not been clarified. We have previously demonstrated that experimentally lowering barometric pressure (LP) intensifies pain-related behaviors in rats with chronic constriction injury (CCI). In the present experiment we examined whether this pain aggravating effect of LP exposure in nerve injured rats is still present after lesioning of the inner ear. We used both CCI and spinal nerve ligation (SNL) models for this study. We injected into the middle ear sodium arsanilate solution (100 mg/ml, 50 μl/ear), which is known to degenerate vestibular hair cells, under anesthesia the day before surgery. Rats were exposed to LP (27 hPa decrease over 8 min) 7–9 days after CCI or 5–8 days after SNL surgery, and pain-related behavior (number of paw lifts induced by von Frey hair stimuli) was measured. When the inner ear lesioned SNL or CCI rats were exposed to LP, they showed no augmentation of pain-related behavior. On the other hand, the pain aggravating effect of a temperature decrease (from 24 to 17 °C) was maintained in both SNL and CCI rats. These results suggest that the barometric sensor/sensing system influencing nociceptive behavior during LP in rats is located in the inner ear.

1. Introduction

There are many patients with chronic pain from such conditions as neuropathic pain, low back pain and rheumatoid arthritis who complain that their condition is aggravated by changes of the weather (Mitchell, 1877; Kranzl, 1977; Guedj and Weinberger, 1990; Hendler et al., 1995; Jamison et al., 1995; Verges et al., 2004). This symptom is called "weather-sensitive pain". Regarding meteorological factors that influence pain, a variety of causes have been claimed, e.g., barometric pressure, humidity and temperature (Hollander and Yeostros, 1963; Verges et al., 2004).

Detection of "weather-sensitive pain" in human studies (Mitchell, 1877; Hollander and Yeostros, 1963; Kranzl, 1977; Guedj and Weinberger, 1990; Hendler et al., 1995; Jamison et al., 1995; Verges et al., 2004) is always complicated by psychosocial factors, which is considered to be minimal, if not lacking, in animal experiments (Sato, 2003). We have therefore previously examined the effects of lowering barometric pressure (LP) on pain-related behaviors in animals and have demonstrated that LP (27 hPa decrease in 8 min) augments pain-related behaviors of chronic constriction injury rats (Sato et al., 1999) and monoarthritic rats (Sato et al., 2004). In normal animals no aggravation of pain-related behaviors was detected (Sato et al., 1999, 2004).

The finding of an LP-induced aggravation of pain suggests the existence of a specific organ or system that senses atmospheric pressure. In birds, sensitivity to small barometric pressure changes as low as 1–2 hPa has been reported (Kreithen and Keeton, 1974). A paratympanic organ in birds innervated by the facial nerve and projecting to the vestibular brainstem nuclei (von Bartheld, 1990) may mediate barometric perception (von Bartheld, 1994). Among mammals, such an organ possibly exists only in bats (von Bartheld, 1990), and no other system for sensing small barometric pressure changes is presently known. However, in addition to pain aggravation by LP, evidence for a barometric pressure sensing system comes from different observations. Activities of mice have been described to change along with natural barometric fluctuations (Sprott, 1967), and rapid and large pressure changes during flight or diving have occasionally been found to induce transient and reversible vertigo (alternobaric vertigo) in humans (Lundgren, 1965; Lundgren and Malm, 1966). In analogy to the birds, we assumed that the pressure sensing system may be located somewhere inside the ear. Since we have previously shown that tympanectomy does not influence the augmentation of neuro-

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pathic pain induced by LP (Takanari et al., 1999), we hypothesized here that such a sensor may be located in the middle or inner ear and examined the effect of inner ear lesions on LP-induced aggravation of pain in neuropathic pain models (spinal nerve ligation (SNL, Kim and Chung, 1992) and chronic constriction injury (CCI, Bennett and Xie, 1988). We used these two models because contribution of sympathetic nerve activities is reportedly different in these models (Kim et al., 1997), thus effect of inner ear lesion might be different.

Preliminary results have been published as an abstract (Funakubo et al., 2005).

2. Methods

Forty-three male Sprague-Dawley (SD) rats, 250–300 g (Japan SLC), were used. The animals were housed two to three per cage under controlled temperature (24 °C) and on a 12 h light/dark cycle, and were given free access to food and water. All surgical procedures described below were performed under surgically clean conditions and sodium pentobarbital anesthesia (50 mg/kg, i.p.). All the experiments were conducted according to the Regulations for Animal Experiments in Nagoya University, and the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions in Japan.

2.1. SNL surgery

The left L5 spinal nerve was tightly ligated with silk thread according to the method described by Kim and Chung (1992), except that only the L5 spinal nerve was ligated in this experiment.

2.2. CCI surgery

CCI surgery was performed according to the method previously described (Bennett and Xie, 1988). Briefly, the left sciatic nerve was exposed at the mid-thigh level, and was then constricted with four loose ligatures using chromic gut (4/0), each spaced about 1 mm apart.

2.3. Inner ear lesion

The bilateral inner ears of the SD rats were lesioned one or two days before CCI or SNL surgery by an injection of sodium arsanilate solution conducted according to the method previously described (Hunt et al., 1987). This solution is known to degenerate the vestibular hair cells when injected into the middle ear cavity (Anniko and Wersall, 1977). Under pentobarbital anesthesia (50 mg/kg, i.p.), a disposable needle (25 G) was inserted through an otoscope and advanced into the middle ear through the tympanic membrane. Through this needle the solution of sodium 4-aminophenylarsonate (100 mg/ml saline, 50 μl/ear, Tokyo Kasei Kogyo Co., Ltd, Tokyo, Japan) was then slowly injected into the middle ear, which was then packed with a piece of gelatin sponge to prevent the solution from flowing back. The effect of the inner ear lesion was confirmed 24–48 h after injection by a contact-righting test (Ossenkopp et al., 1990): the rats were put in a supine position on a horizontal surface and an acrylic plate was lightly placed in contact with the plantar side of the animal's feet. In this situation normal animals quickly turn upright (normal righting response). Animals with inner ear lesion do not turn upright by themselves, instead they remain in the supine position, with their soles in contact with the plastic plate and 'walk' about under the plate (abnormal righting response). Therefore, when rats showed this abnormal righting response, it was concluded that their inner ear was lesioned. In addition these animals tended to move back and showed exaggerated head dorsiflexion, similar to surgically labyrinthectomized rats (Tang and Wu, 1937 requoted from Hunt et al., 1987). If the unilateral inner ear was lesioned, the animals tilted their head on lesioned side, turned heavily to lesioned side when they were suspended by their tails. These rats were omitted from the experiment. There was no change in appetite and body weight after inner ear lesion.

The vestibular lesion was histologically examined in three animals. The rats 8 days after arsanilate injection into bilateral inner ears were perfused with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PFA, pH 7.4) under deep pentobarbital anesthesia. The temporal bone was removed, post-fixed with PFA for 3 days, and then immersed in a solution of 10% EDTA (pH 7.4, Wako, Japan) for 7 days at 4 °C. After decalcification, the temporal bone was embedded in paraffin and then sliced on a microtome into serial 5-μm sections. Every section was stained with hematoxylin-eosin (Mayer's hematoxylin solution followed by Mayer's method and 1% eosin in ethanol, Sakura, Japan) and observed with a light microscope (Eclipse E800, Nikon, Japan). The cochlea-vestibule was photographed with a computer-aided CCD-camera (KP-C231, Nikon, Japan). For comparison, histological examination of the inner ear of a control rat without arsanilate injection was done using the same method.

2.4. Measurement of nociceptive behaviors

2.4.1. Measurement of punctate hyperalgesia

Each rat was individually placed beneath an inverted transparent plastic cage (11 × 17 × 11 cm) with a wire mesh bottom. Pain-related behaviors induced by mechanical stimulation were measured with home-made von Frey hairs (VFHs, diameter: 0.5 mm, bending forces 34.3, 92.2, and 197.2 mN). Each VFH was applied ten times (once every 2–3 s) to the plantar surface of the nerve-injured paw, and the number of foot withdrawals was then counted. Stimulation of normal human skin with the weak (34.3 mN) and then the stronger (92.2 and 197.2 mN) VFHs elicits a sensation of pressure and painful pricking, respectively. A significant increase in the frequency of foot withdrawals in response to these mechanical stimuli was interpreted as punctate hyperalgesia (Zhou et al., 1996; Ta et al., 2000). Because of typical abnormal behaviors of inner ear lesioned rats (Hunt et al., 1987), it was impossible to blind the experimenter to which group animals belonged.

2.4.2. Low-pressure exposure

We examined the effects of inner ear lesions on the low pressure-induced aggravation of nociceptive behavior in a climate-controlled room installed in our Institute, which was used in previous experiments (Sato et al., 1999, 2001, 2004). The rats of this experimental series received an injection of sodium arsanilate one or two days before CCI or SNL surgery. Seven to nine days after CCI or 5–8 days after SNL, they were exposed to low-pressure. The barometric pressure of the climate-controlled room was lowered by 27 hPa below the atmospheric pressure, which is the type of change often observed when a typhoon passes. This was accomplished over 8 min. The pressure was maintained at this level for 80 min, and then returned to the baseline pressure over 8 min. The ambient temperature was controlled at 24 °C, with relative humidity at 50%. Behavioral tests were carried out five times; 60 min before exposure (Pre), twice at the lowered pressure (just after (Mid 1), and 60 min (Mid 2) after reaching the pre-set low-pressure level), and 60 (Post 1) and 120 min (Post 2) after exposure (Fig. 1, upper panel).

2.4.3. Low-temperature exposure

To confirm that the effects of inner ear lesion were distinctive to pressure change-induced effects, inner ear lesioned rats were

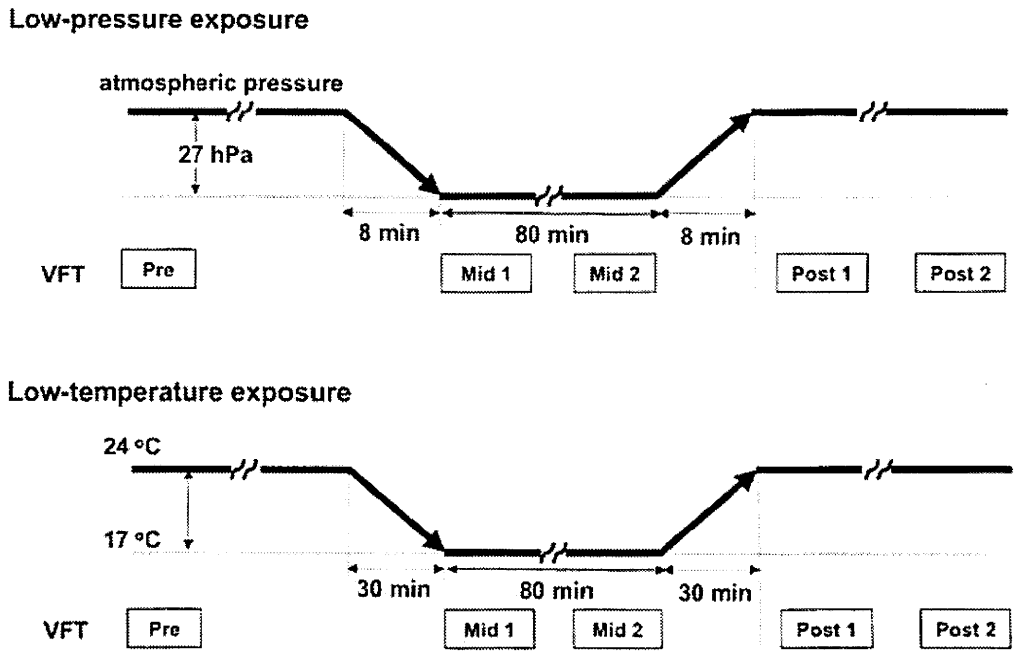

Fig. 1. Time schedule of low-pressure (upper panel) or low-temperature exposure (lower panel). Behavioral tests (von Frey test; VFT) were carried out 5 times on the day of low-pressure or low-temperature exposure: 60 min before exposure (Pre), twice at the lowest pressure or temperature (just after (Mid 1), and 60 min (Mid 2) after reaching the pre-set low-pressure or temperature level), and 60 (Post 1) and 120 min (Post 2) after exposure.

also exposed to low temperature between 5 and 11 days after CCI or 3 and 7 days after SNL surgery. The ambient temperature was lowered by 7 °C, which is a typical daily temperature drop in Japan: the drop, from 24 to 17 °C, took 30 min. The temperature was then maintained at 17 °C for 80 min, and it took another 30 min to return to the baseline. Relative humidity was kept at 50%, and barometric pressure was left at its natural level. Experiments were done on days when no abrupt barometric pressure change was forecast. Behavioral nociceptive tests were carried out on the same time schedule as that during LP exposure (Fig. 1, lower panel).

2.4.4. Statistical analysis

The results of LP and LT exposures were expressed as the mean and standard error of the mean (SEM), and the difference from the Pre value was analysed with non-parametric one way analysis of variance with repeated measures on ranks followed by the Student–Newman–Keuls method. Differences were considered significant at the $P < 0.05$ level in both experiments.

3. Results

A group of ten rats received a sodium arsanilate injection into the middle ear cavity. None of the injected rats showed normal contact-righting response, instead, they remained supine and 'walked' with their soles in contact with the lower surface of the plate that was put on them, thus confirming inner ear lesion. For further confirmation, a histological examination was carried out on three rats (Fig. 2). Except one ear, hair cells were enlarged and vacuolar degeneration or complete degeneration was observed in crista ampullaris, macula sacculi and macula utricli. Sample photographs of vacuolar degeneration are shown in Fig. 2B. Even in the animal with remained hair cells in one ear (Fig. 2C), contact-righting reflex was abnormal and no unilateral tilting of the head was observed, thus it is considered that inner ears of all rats examined lost their function. The inner ear lesion itself induced slight increase in number of paw withdrawals but it was not significant (Fig. 3A).

We carried out VFT on SNL (Fig. 3B) and CCI (Fig. 3C) rats with inner ear lesioned, with the same procedures as LP or LT experiment but without changing pressure or temperature, and confirmed that placing animals in the climate chamber and repeating VFT induced no change in VFT.

We also confirmed that rats with inner ear lesions became hyperalgesic after SNL surgery (Fig. 3B, Pre), and on days of low-pressure exposure they remained so (Fig. 4B, Pre). The intact inner ear SNL rats showed an increase in the number of paw lifts in response to noxious stimuli immediately after reaching the low-pressure level (Fig. 4A, Mid 1). This increase, however, was transient, and the number of paw lifts had already returned to baseline levels on the second measurement during low-pressure exposure 60 min later (Fig. 4A, Mid 2), confirming the previous result (Sato et al, 1999). In contrast, the SNL rats with inner ear lesions showed neither an increase nor a decrease in the number of paw lifts during the entire period of low-pressure exposure (Fig. 4B).

Next, we examined the effect of low-temperature exposure to inner ear lesioned SNL rats to see whether the inhibitory effect of inner ear lesions was not due to motor deficit, and whether inner ear is distinctively needed for low-pressure effects. Confirming the previous result (Sato et al., 2000), low-temperature exposure augmented the responses of inner ear intact SNL rats to noxious mechanical stimuli, and this augmentation not only lasted during exposure period but tended to be further intensified at Mid 2, showing a slower time course than low-pressure exposure (Fig. 5A, Mid 1 and 2). Inner ear lesioned SNL rats showed almost the same augmentation of nociceptive behavior upon exposure to low temperature as inner ear intact rats (Fig. 5B). These results demonstrated that the inner ear was distinctively needed for low-pressure effects.

When we also examined low-pressure effects in inner ear lesioned CCI rats, they showed mechanical hyperalgesia after CCI surgery (Pre in Figs. 6 and 7), but the change tended to be less than

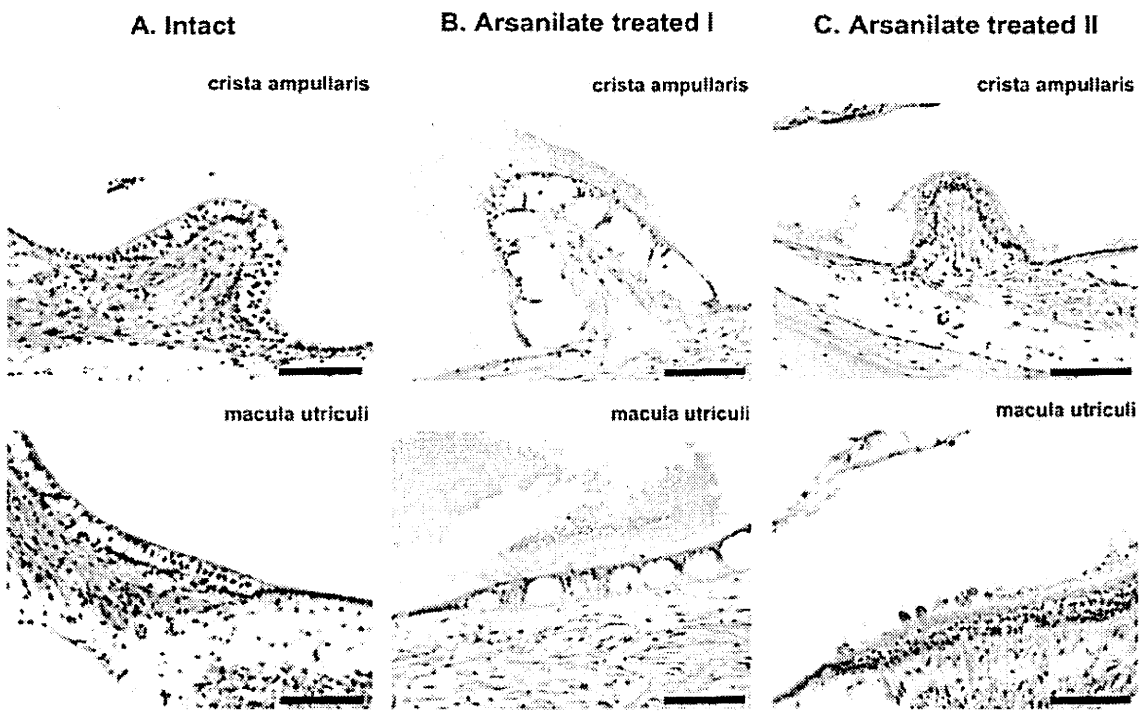

Fig. 2. Histological sections of the inner ear. A: Inner ear of intact rat without treatment. B: Completely lesioned inner ear after arsenilate treatment. C: An ear less injured after arsanilate injection. Arsanilate injection into the middle ear was done 8 days before. The hair cells of crista ampullaris and macula sacculi had vacuolar degeneration in (B), but they are still left in (C) although their shapes are irregular and atrophic. Despite some hair cells remained in one ear (C), this animal showed abnormal contact-righting reflex. Scale: 500 μm.

that in SNL rats. Again, quite similar to SNL rats, inner ear lesioned CCI rats ($n = 11$) showed no augmentation of nociceptive behavior upon exposure to low-pressure (Fig. 6A and B), leaving the nociception aggravating effect of low temperature intact (Fig. 7A and B).

4. Discussion

The present experiments clearly demonstrate that the nociception aggravating effects of low-pressure exposure were lost following an inner ear lesion. The nociception aggravating effects of low-temperature exposure, on the other hand, were not altered at all in these rats, proving that inner ear was needed for low-pressure effects. This result suggests that a barometric sensor and/or sensing system relating with the low-pressure effect on nociceptive behavior is located in the inner ear of rats.

It is known that changes in ambient pressure (though a major pressure change is required) can elicit vertigo and bodily disequilibrium, known clinically as alternobaric vertigo (Lundgren, 1965; Lundgren and Malm, 1966). In their neurophysiological study addressing the underlying mechanism of this vertigo, Suzuki et al. (1998) have reported that vestibular activities were altered by changes in middle ear pressure, though they used a much more rapid pressure change (100 mmH_2O/s) than we did in the present experiment. We also observed, though preliminary, that some neurons in the rat vestibular nucleus responded to lowering barometric pressure by 40 hPa within 8 min (Funakubo et al., 2007). Such a pressure reduction temporarily renders pressure in the middle ear relatively positive to barometric pressure, that of the perilymph relatively negative to the middle ear and that of the endolymph relatively positive to the perilymph, thus producing a transient pressure difference between peri- and endolymph. These authors have suggested that this pressure difference between the peri- and endolymph induced vestibular nerve activity. However, positive middle ear pressure does not seem necessary for nociception aggravating effects of low-pressure, since a previous report from our lab showed that a tympanectomy had no influence on the pain aggravating effect of low-pressure (Takanari et al., 1999). Even in that case, temporary pressure difference between the peri- and endolymph can be produced by lowering the barometric pressure, though the pressure difference would be opposite. For the moment, though the exact location and mechanism of pressure sensing remain unknown, the importance of the inner ear in barometric pressure detection relating with nociception was clearly demonstrated in this experiment.

In this experiment we found no noticeable difference in the effect of inner ear lesion on LP-induced aggravation of hyperalgesia in SNL and CCI rats, although contribution of sympathetic nerve activities were reported to be different (Kim et al, 1997). Usage of a rather large and quick pressure change (27 hPa/8 min) might have hindered from detecting difference. In fact, using slower and smaller pressure change we have observed different magnitude and speed threshold of pressure change in inducing aggravation of hyperalgesia in SNL and CCI models (Funakubo et al., 2008).

How could LP aggravate mechanical hyperalgesia? Since LP-induced aggravation of nociception was observed only in animals with chronic pain, and not in normal animals (Sato et al, 1999), some mechanism involved in mechanical hyperalgesia might be influenced by inner ear activities. Many factors and mechanisms are involved in nerve injury-induced mechanical hyperalgesia (Devor 2005 for review), e.g. ectopic novel mechanosensitivity along injured nerves, in neuromas and DRGs, novel sensitivity to noradrenaline (Sato and Perl, 1991) and adrenaline (Khasar et al, 1999; Chen and Levine, 2005), changed expression/trafficking of channels (Lindia et al, 2005) and changed expression of neuropeptides (Noguchi et al., 1995). Of these many factors and mechanisms: (1) hormonal changes, (2) descending control system of nociception and (3) sympathetic activities could be modified

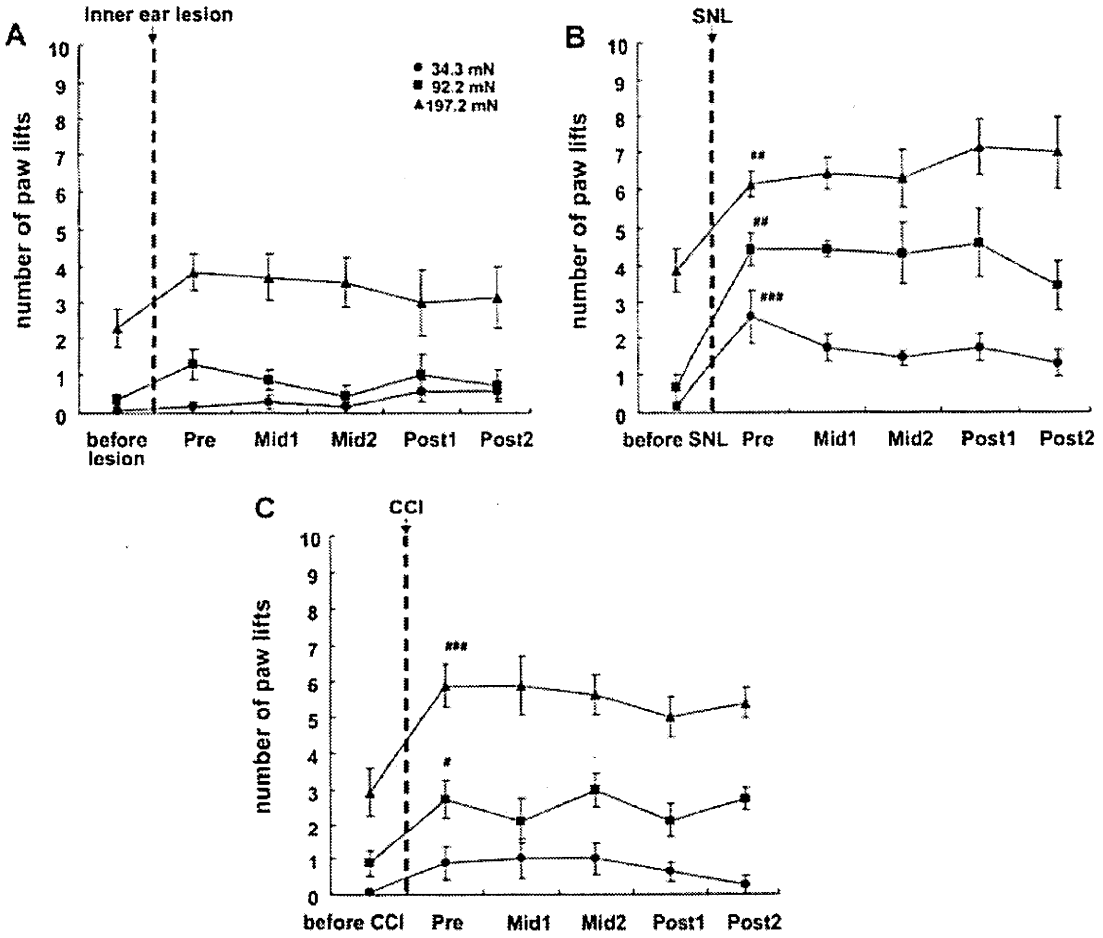

Fig. 3. Control experiment with repeated VFTs in the climate chamber without LP or LT exposure. A: results of the lesioned inner ear group ($n = 7$). VFTs were carried out 3–9 days after inner ear lesion with the same time schedule as LP or LT exposure but under atmospheric pressure and 24 °C level. B and C: result of the SNL rats (B) with lesioned inner ear ($n = 7$), and that of the CCI rats (C) with lesioned inner ear ($n = 8$). SNL or CCI surgery was carried out the next day after inner ear lesion and VFT was carried out 6–9 days after SNL or CCI surgery. B: Data are presented as mean ± SEM. Vertical axis: number of paw lifts, horizontal axis: order of the test. Broken line shows the time point of treatment (A: inner ear lesion, B: SNL surgery, and C: CCI surgery). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with intact condition (before lesion, SNL, CCI) (Student–Newman–Keuls Method).

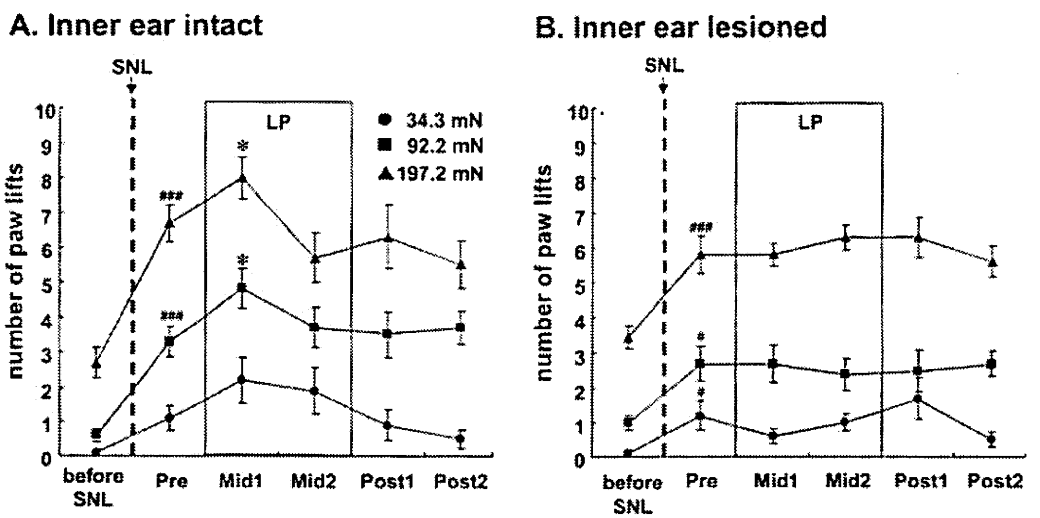

Fig. 4. Nociception-aggravating effect of low-pressure (LP) exposure was eliminated in the SNL rats with lesioned inner ears. A: results of the intact inner ear group and B: result of the lesioned inner ear group. Data are presented as mean ± SEM ($n = 10$). Vertical axis: number of paw lifts, horizontal axis: order of the test. Broken line shows the time point of SNL surgery. $^{*}P < 0.05$, $^{***}P < 0.001$ compared with 'before SNL', $^{\dagger}P < 0.05$ compared with 'Pre' (Student–Newman–Keuls Method).

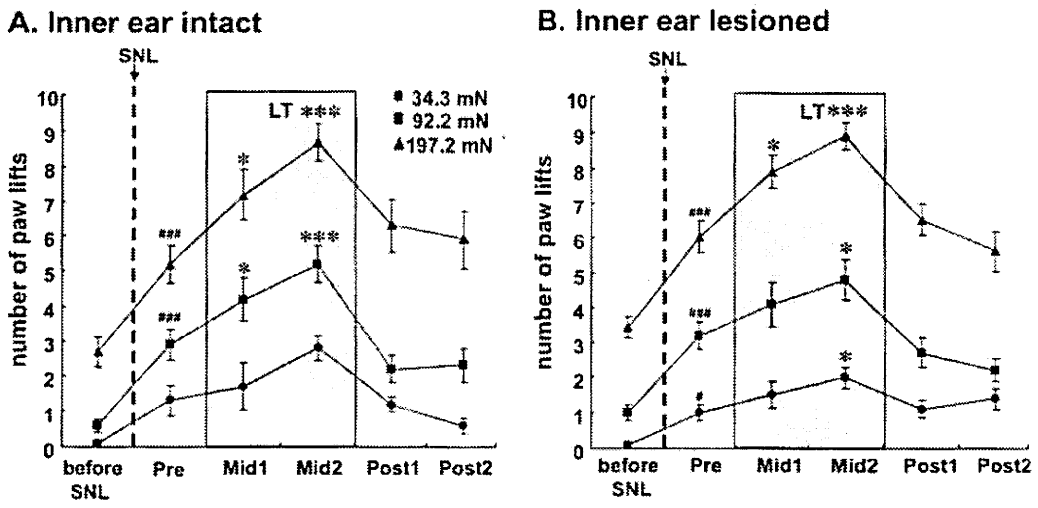

Fig. 5. Nociception-aggravating effect of low-temperature (LT) exposure was maintained in the inner ear lesioned SNL rats. The form of presentation is the same as in Fig. 4 ($n = 10$ for both A and B). $^{***}P < 0.001$ compared with 'Pre' (Student–Newman–Keuls Method).

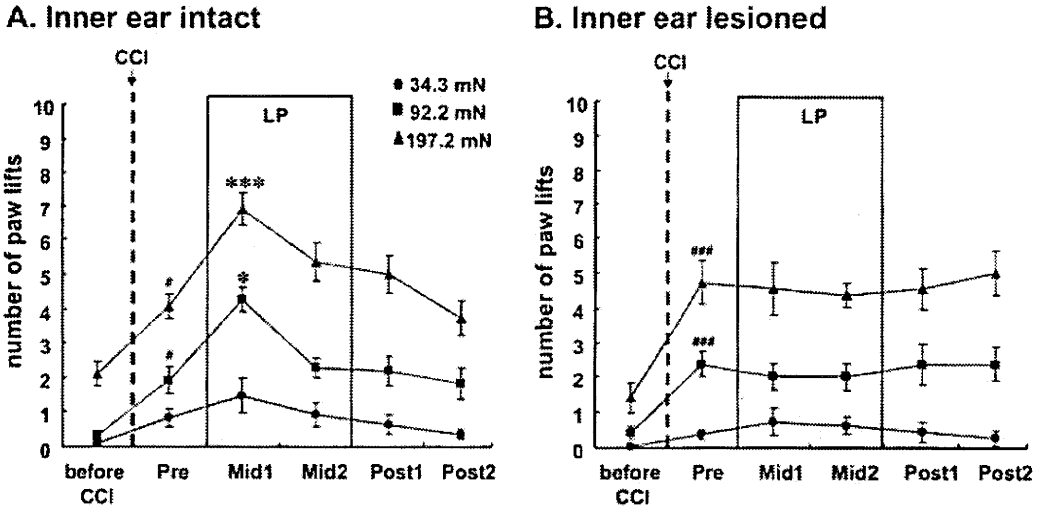

Fig. 6. Nociception-aggravating effect of LP exposure was eliminated in inner ear lesioned CCI rats. The form of presentation is the same as in Fig. 4 ($n = 11$). $^{*}P < 0.05$, $^{***}P < 0.001$, compared with 'before CCI' value, $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ compared with 'Pre' value (Student–Newman–Keuls Method).

within several minutes of low-pressure exposure, while others might require too much time to explain LP-induced hyperalgesia. The first possibility, hormonal changes, is based on the report that neurons in vestibular nuclei project to the hypothalamus (Matsuyama et al., 1996; Markia et al., 2008), thus possibly modulating the environment of hormones such as adrenaline. The second possibility is that descending inhibitory control system might be modified by inner ear inputs because the vestibular nuclei that receive inner ear inputs also project to the nuclei (raphe magnus, locus ceruleus, periaqueductal gray matter, etc.) that are involved in descending inhibitory control. The third possibility is that the profound influence of vestibular stimulation on autonomic functions (resulting in pale face, nausea, vomiting, sweating, etc.), which occurs through modification of autonomic centers in brain stem, also affects sympathetic and vagal nerve activities (Yates et al., 1995; Porter and Balaban, 1997). The influence of sympathetic nerve activities on cutaneous thin-fiber afferents after nerve injury but not in normal animals has been well documented (Sato and Perl, 1991; Devor et al., 1994; Bossut and Perl, 1995). In addition, our group showed that there was no LP-induced aggravation of mechanical hyperalgesia after sympathectomy, suggesting involvement of sympathetic nerve activity in this phenomenon (Sato et al., 1999). We also showed elevation of blood pressure and heart rate during exposure to LP, which also suggest sympathetic activation during LP (Sato et al, 2000). Thus linkage from the inner ear through sympathetic activation to increased nociceptor activities in LP has been the most addressed of these three possibilities. Yet all are worthy of future study.

Maintaining the homeostasis of the physical conditions is essential for organisms, and organisms are able to respond also to minute changes in barometric pressure induced by climatic change. This mechanism may exist also in human beings, although such responses may not be detectable by those in good health as concluded from the absence of effects on nociception in normal rats (Sato et al, 1999, 2004). The present results, by elucidating the aggravation of nociception in pathological conditions, indicate the existence of such responses and the important role of the inner ear in barometric pressure detection.

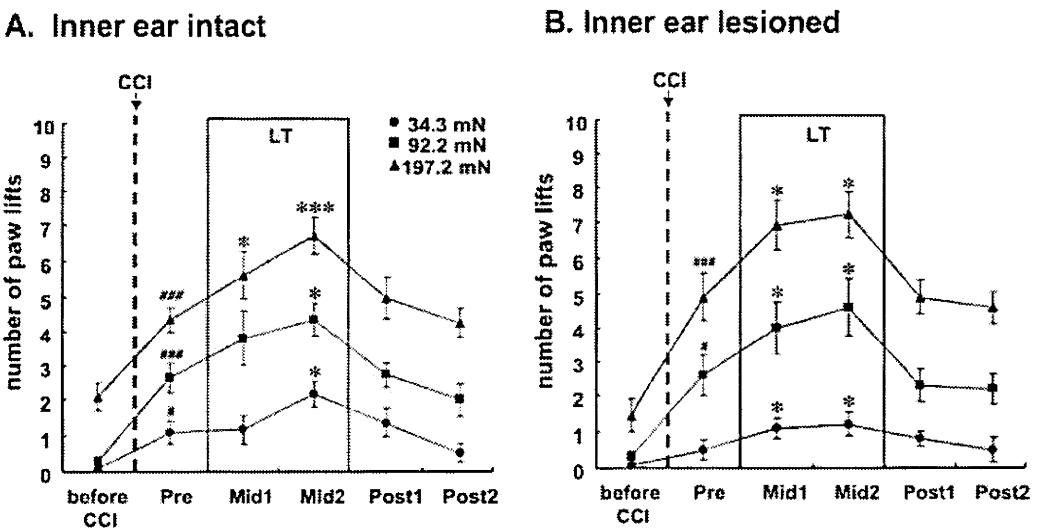

Fig. 7. Nociception-aggravating effect of LT exposure was maintained in the inner ear lesioned CCI rats. The form of presentation is the same as in Fig. 6. Number of animals is 11 (A) and 10 (B).

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References

Anniko M, Wersall J. Experimentally (atoxyl) induced ampullar degeneration and damage to the maculae utriculi. Acta Otolaryngol 1977;83:429–40.
Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988;33:87–107.
Bossut DF, Perl ER. Effects of nerve injury on sympathetic excitation of A delta mechanical nociceptors. J Neurophysiol 1995;73:1721–3.
Chen X, Levine JD. Epinephrine-induced excitation and sensitization of rat C-fiber nociceptors. J Pain 2005;6:439–46.
Devor M. Response of nerves to injury in relation to neuropathic pain. In: McMahon SB, Koltzenburg M, editors. Textbook of pain. 5th ed. Amsterdam: Elsevier; 2005. p. 905–27.
Devor M, Janig W, Michaelis M. Modulation of activity in dorsal root ganglion neurons by sympathetic activation in nerve-injured rats. J Neurophysiol 1994;71:38–47.
Funakubo M, Sato J, Mizumura K. Inner ear destruction inhibits the augmentation of mechanical hyperalgesia in nerve-injured rats induced by lowering barometric pressure but not lowering ambient temperature. In: 11th World congress on pain; 2005 [Abstracts: 163-P140].
Funakubo M, Sato J, Mizumura K. Search for barometric pressure-sensitive neurons in the vestibular nuclei. J Physiol Sci 2007;57:S194.
Funakubo M, Sato J, Mizumura K. Lowering of barometric pressure at slower rates aggravates neuropathic pain-related behaviors in rats. In: 12th World congress on pain; 2008 [Abstracts: PM 041].
Guedj D, Weinberger A. Effect of weather conditions on rheumatic patients. Ann Rheum Dis 1990;49:158–9.
Hendler NH, Jamison RN, Morrison CH, Jenepher KP, Kahn Z. The relationship of diagnoses and weather sensitivity in chronic pain patients. J Neuromusc Syst 1995;3:10–5.
Hollander JL, Yeostros SY. The effect of simultaneous variations of humidity and barometric pressure on arthritis. Bull Am Meteorol Soc 1963;44: 389–93.
Hunt MA, Miller S, Nielson H, Horn K. Intratympanic injection of sodium arsanilate (atoxyl) solution results in postural changes consistent with changes described for labyrinthectomized rats. Behav Neurosci 1987;101:427–8.
Jamison RN, Anderson KO, Slater MA. Weather changes and pain: perceived influence of local climate on pain complaint in chronic pain patients. Pain 1995;61:309–15.
Khasar SG, McCarter G, Levine JD. Epinephrine produces a beta-adrenergic receptor-mediated mechanical hyperalgesia and in vitro sensitization of rat nociceptors. J Neurophysiol 1999;81:1104–12.
Kim KJ, Yoon YW, Chung JM. Comparison of three rodent neuropathic pain models. Exp Brain Res 1997;113:200–6.
Kim SH, Chung JM. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 1992;50:355–63.
Kranzl B. Trigeminal neuralgia – effect of external and internal influences on the disease as well as studies on infiltration treatment of pain. Osterr Z Stomatol 1977;74:246–61.
Kreithen ML, Keeton WT. Detection of changes in atmospheric pressure by the homing pigeon, *Columba livia*. J Comp Physiol 1974;89:73–82.
Lindia JA, Kohler MG, Martin WJ, Abbadie C. Relationship between sodium channel NaV1.3 expression and neuropathic pain behavior in rats. Pain 2005;117:145–53.
Lundgren CE. Alternobaric vertigo – a diving hazard. Br Med J 1965;2:511–3.
Lundgren CE, Malm LU. Alternobaric vertigo among pilots. Aerosp Med 1966;37: 178–80.
Matsuyama T, Kayahara T, Nomura J, Nakano K. Direct projections from the medial vestibular nucleus to the posterior hypothalamic area in the monkey (*Macaca fuscata*). Neurosci Lett 1996;219:199–202.
Markia B, Kovacs ZI, Palkovits M. Projections from the vestibular nuclei to the hypothalamic paraventricular nucleus: morphological evidence for the existence of a vestibular stress pathway in the rat brain. Brain Struct Funct 2008;213:239–45.
Mitchell SW. The relations of pain to weather, being a study of the natural history of a case of Traumatic Neuralgia. Am J Med Sci 1877(April):305–29.
Noguchi K, Kawai Y, Fukuoka T, Senba E, Miki K. Substance P induced by peripheral nerve injury in primary afferent sensory neurons and its effect on dorsal column nucleus. J Neurosci 1995;15:7633–43.
Ossenkopp KP, Prkacin A, Hargreaves EL. Sodium arsanilate-induced vestibular dysfunction in rats: effects on open-field behavior and spontaneous activity in the automated digiscan monitoring system. Pharmacol Biochem Behav 1990;36:875–81.
Porter JD, Balaban CD. Connections between the vestibular nuclei and brain stem regions that mediate autonomic function in the rat. J Vestib Res 1997;7: 63–76.
Sato J. Weather change and pain: a behavioral animal study of the influences of simulated meteorological changes on chronic pain. Int J Biometeorol 2003;47: 55–61.
Sato J, Perl ER. Adrenergic excitation of cutaneous pain receptors induced by peripheral nerve injury. Science 1991;251:1608–10.
Sato J, Morimae H, Seino Y, Kobayashi T, Suzuki N, Mizumura K. Lowering barometric pressure aggravates mechanical allodynia and hyperalgesia in a rat model of neuropathic pain. Neurosci Lett 1999;266:21–4.
Sato J, Morimae H, Takanari K, Seino Y, Okada T, Suzuki M, et al. Effects of lowering ambient temperature on pain-related behaviors in a rat model of neuropathic pain. Exp Brain Res 2000;133:442–9.
Sato J, Takanari K, Omura S, Mizumura K. Effects of lowering barometric pressure on guarding behavior, heart rate and blood pressure in a rat model of neuropathic pain. Neurosci Lett 2001;299:17–20.
Sato J, Aoyama M, Yamazaki M, Okumura S, Takahashi K, Funakubo M, et al. Artificially produced meteorological changes aggravate pain in adjuvant-induced arthritic rats. Neurosci Lett 2004;354:46–9.

Sprott RL. Barometric pressure fluctuations: effects on the activity of laboratory mice. Science 1967;157:1206–7.
Suzuki M, Kitano H, Yazawa Y, Kitajima K. Involvement of round and oval windows in the vestibular response to pressure changes in the middle ear of guinea pigs. Acta Otolaryngol 1998;118:712–6.
Ta LE, Dionne RA, Fricton JR, Hodges JS, Kajander KC. SYM-2081 a kainate receptor antagonist reduces allodynia and hyperalgesia in a freeze injury model of neuropathic pain. Brain Res 2000;858:106–20.
Takanari K, Sato J, Mizumura K. Influence of tympanectomy on the augmenting effect of exposure to low barometric pressure on the mechanical allodynia in a neuropathic rat. Environ Med 1999;43:10–2.
Tang Y, Wu CF. The effects of bilateral labyrinthectomy in albino rats. Proc Chinese Physiol Soc 1937;10:33–4.
Verges J, Montell E, Tomas E, Cumelles G, Castaneda G, Marti N, et al. Weather conditions can influence rheumatic diseases. Proc West Pharmacol Soc 2004;47:134–6.
von Bartheld CS. Development and innervation of the paratympanic organ (Vitali organ) in chick embryos. Brain Behav Evol 1990;35:1–15.
von Bartheld CS. Functional morphology of the paratympanic organ in the middle ear of birds. Brain Behav Evol 1994;44:61–73.
Yates BJ, Balaban CD, Miller AD. Endo K, Yamaguchi Y. Vestibular inputs to the lateral tegmental field of the cat: potential role in autonomic control. Brain Res 1995;689:197–206.
Zhou S, Bonasera L, Carlton SM. Peripheral administration of NMDA, AMPA or KA results in pain behaviors in rats. Neuroreport 1996;7:895–900.

Web references

Anniko, Wersall, 1977. PMID: 888679.
Bennett, Xie, 1988. PMID: 2837713.
Bossut, Perl, 1995. <http://www.jn.physiology.org/cgi/reprint/73/4/1721>.
Chen, Levine, 2005. 10.1016/j.jpain.2005.02.004 <http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WKH-4GHS9V5-C&_user=119029 &_rdoc=1&_fmt=&_orig=search&_sort=d&view=c &_acct=C000009318&_version=1&_urlVersion=0&_userid=119029 &md5=b66aa4756751d636bff097678ee4868f>.
Devor et al., 1994. <http://www.jn.physiology.org/cgi/reprint/71/1/38>.
Funakubo et al., 2007. <http://www.jstage.jst.go.jp/article/pjsjproc/2007/0/2007_194_4/_article>.
Guedj, Weinberger, 1990. 10.1136/ard.49.3.158 http://www.ard.bmj.com/cgi/reprint/49/3/158.
Hollander, Yeostros SY, 1963. PMID: 13961319.
Hunt et al., 1987. PMID: 3606813.
Jamison et al., 1995. 10.1016/0304-3959(94)00215-Z <http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T0K-3YYV2KN-1B&_user=119029 &_rdoc=1&_fmt=&_orig=search &_sort=d&view=c&_acct=C000009318&_version=1&_urlVersion=0 &_userid=119029&md5=bcff2eee0380eebf6fa995f3d461f061>.
Khasar SG et al., 1999. <http://www.jn.physiology.org/cgi/reprint/81/3/1104>.
Kim KJ et al., 1997. <http://www.springerlink.com/content/7wqg4empyhxep5lu/fulltext.pdf>.
Kim, Chung, 1992. PMID: 1333581.
Lindia et al., 2005. 10.1016/j.pain.2005.05.027 <http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T0K-4GSJRC8-5&_user=119029 &_rdoc=1&_fmt=&_orig=search &_sort=d&view=c&_acct=C000009318&_version=1 &_urlVersion=0&_userid=119029 &md5=9500e67c84f95ddd1b69038badccfbe0>.
Lundgren, 1965. PMID: 14321224 <http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&pubmedid=14321224>.
Lundgren, Malm, 1966. PMID: 5906039.
Lundgren and Malm, 1966. PMID: 5906039.
Matsuyama T et al., 1996. <http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T0G-3P92KR8-15&_user=119029 &_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000009318&_version=1&_urlVersion=0 &_userid=119029&md5=c6e928014e065fbd9b66670694fa30c3>.
Noguchi K et al., 1995. <http://www.jneurosci.org/cgi/reprint/15/11/7633>.
Ossenkopp KP et al., 1990. <http://www.ncbi.nlm.nih.gov/pubmed/22175177?ordinalpos= &itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.SmartSearch &log$=citationsensor>.
Porter and Balaban, 1997. <http://www.ncbi.nlm.nih.gov/pubmed/9057160?ordinalpos=3 &itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ ResultsPanel.Pubmed_DefaultReportPanel.Pubmed_RVDocSum>.
Sato 2003. 10.1007/s00484-002-0156-9 <http://www.springerlink.com/content/bq2wt4rmga9p2d0n/fulltext.pdf>.
Sato, Perl, 1991. PMID: 2011742 <http://www.sciencemag.org/cgi/reprint/251/5001/1608.pdf>.
Sato et al., 1999. 10.1016/S0304-3940(99)00260-8 <http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T0G-3WRJPMH-6&_user=119029&_rdoc=1&_fmt=&_orig=search &_sort=d&view=c&_acct=C000009318 &_version=1&_urlVersion=0 &_userid=119029&md5=75cfaedfb5976cb08fb9f632d6277613>.
Sato et al., 2000. 10.1007/s002210000451 <http://www.springerlink.com/content/8bhpnxtd1ptu7rxg/fulltext.pdf>.
Sato et al., 2001. 10.1016/S0304-3940(00)01769-9 <http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T0G-42991Y9-5&_user=119029&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000009318&_version=1 &_urlVersion=0&_userid=119029 &md5=48fd1027929271ae54abe2f46c65caee>.
Sato et al., 2004. 10.1016/j.neulet.2003.09.057 <http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T0G-49Y3KJT-4&_user=119029&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c &_acct=C000009318&_version=1&_urlVersion=0 &_userid=119029&md5=3ea7530e63f758d47ba16934dce0fdf7>.
Sprott, 1967. PMID: 6038695 <http://www.sciencemag.org/cgi/reprint/157/3793/1206.pdf>.
Suzuki et al., 1998. PMID: 9840510.
Ta et al., 2000. 10.1016/S0006-8993(99)02437-3 <http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6SYR-3YNYOSR-21&_user=119029&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000009318&_version=1 &_urlVersion=0&_userid=119029 &md5=852aace377a5afde9652249324fea141>.
Verges et al., 2004. PMID: 15633634 <http://www.ncbi.nlm.nih.gov/pubmed/15633634?ordinalpos=&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.SmartSearch &log$=citationsensor>.
von Bartheld CS, 1990. PMID: 2340410.
von Bartheld CS, 1994. PMID: 7953609.
Yates et al., 1995. 10.1016/0006-8993(95)00569-C <http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6SYR-4007503-4C&_user=119029&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000009318&_version=1&_urlVersion=0&_userid=119029 &md5=cec1dc5765e51ad713c7b7ea7f5c7de7>.
Zhou S, Bonasera L, Carlton SM. Peripheral administration of NMDA, AMPA or KA results in pain behaviors in rats. Neuroreport 1996;7:895–900. PMID: 8724668.

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

The Impact of a Genome-Wide Supported Psychosis Variant in the *ZNF804A* Gene on Memory Function in Schizophrenia

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A recent genome-wide association study showed that a variant (rs1344706) in the *ZNF804A* gene was associated with schizophrenia and bipolar disorder. Replication studies supported the evidence for association between this variant in the *ZNF804A* gene and schizophrenia and that this variant is the most likely susceptibility variant. Subsequent functional magnetic resonance imaging studies in healthy subjects demonstrated the association of the high-risk *ZNF804A* variant with neural activation during a memory task and a theory of mind task. As these cognitive performances are disturbed in patients with schizophrenia, this gene may play a role in cognitive dysfunction in schizophrenia. The aim of the current study was to investigate the potential relationship between this *ZNF804A* polymorphism and memory function. The effects of the high-risk *ZNF804A* genotype, diagnosis, and genotype–diagnosis interaction on verbal memory, visual memory (VisM), attention/concentration, and delayed recall (measured by the Wechsler Memory Scale-Revised) were analyzed by two-way analysis of covariance in 113 patients with schizophrenia and 184 healthy subjects. Consistent with previous studies, patients with schizophrenia exhibited poorer performance on all indices as compared to healthy control subjects ($P < 0.001$). A significant *ZNF804A* genotype–diagnosis interaction was found for VisM performance ($P = 0.0012$). Patients with the high-risk T/T genotype scored significantly lower on VisM than G carriers did ($P = 0.018$). In contrast, there was no genotype effect for any index in the healthy control subjects ($P > 0.05$). Our data suggest that rs1344706 may be related to memory dysfunction in schizophrenia. © 2010 Wiley-Liss, Inc.

Key words: ZNF804A; memory; schizophrenia; polymorphism; rs1344706

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INTRODUCTION

Schizophrenia (OMIM: 181500) is a common complex psychiatric disease with a lifetime risk of approximately 1%. There are strong genetic components of this disease, with an estimated heritability of approximately 80% [Cardno and Gottesman, 2000; Tsuang, 2000]. In a genome-wide association study and follow-up studies, a single nucleotide polymorphism (SNP) in the *ZNF804A* gene (rs1344706) was found to be associated with schizophrenia and bipolar disorder [O'Donovan et al., 2008]. Subsequent replication studies demonstrated the association between schizophrenia and the *ZNF804A* gene and that rs1344706 remained the most strongly associated marker in the gene after fine mapping of *ZNF804* locus [Riley et al., 2010; Steinberg et al., 2010; Williams et al., 2010; Zhang et al., 2010].

The *ZNF804A* gene (OMIM: 612282) is located on chromosome 2q32.1 and consists of four exons and three introns spanning 341 kb. Although little is known about the encoded protein and its function, the sequence contains predicted zinc ion and DNA-binding domains, suggesting a role in the regulation of gene expression. Two imaging genetics studies using functional magnetic resonance imaging (fMRI) have demonstrated associations between the high-risk *ZNF804A* variant and neural activation during a memory task and a theory of mind task in healthy subjects [Esslinger et al., 2009; Walter et al., 2010]. The high-risk *ZNF804A* variant had impact on brain functional dysconnectivity between dorsolateral prefrontal cortex (DLPFC) and hippocampal formation during an N-back memory task in healthy subjects [Esslinger et al., 2009]. This altered connectivity between DLPFC and hippocampal formation might be a basis of human memory function.

Patients with schizophrenia have pronounced deficits in aspects of neurocognitive function such as speed of processing, attention/vigilance, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving, and social cognition [Nuechterlein et al., 2004]. Cognitive impairments are strongly related to functioning in areas such as work, social relationships, and independent living in schizophrenia. The lack of marked cognitive benefit of present antipsychotics has led to the investigation of alternative drugs and mechanisms for the treatment of these impairments [Buchanan et al., 2007]. Intermediate phenotypes/endophenotypes represent simpler clues to genetic underpinnings than the disease syndrome itself, promoting the view that psychiatric diagnoses can be decomposed or deconstructed, which can result in more straightforward and successful genetic analysis [Gottesman and Gould, 2003; Preston and Weinberger, 2005]. Memory deficits are prominent trait markers of schizophrenia, with impairments also observed in first-degree relatives [Snitz et al., 2006]. Genetic risk for schizophrenia could affect functional activity in the brain; such changes have been shown to mediate disturbed memory function [Meyer-Lindenberg and Weinberger, 2006]. In the present study, we examined the effect of the genome-wide supported variant in the *ZNF804A* gene on memory functions in patients with schizophrenia.

MATERIALS AND METHODS

Sample Description

The subjects of this study consisted of 113 patients with schizophrenia [53.1% males, mean age ± standard deviation: 38.3 ± 12.1 years] and 184 healthy control subjects [47.8% males, 36.2 ± 11.5 years]. The sex ratio and mean age did not differ significantly between patients and control subjects ($P > 0.05$), whereas the years of education were significantly lower among patients with schizophrenia (14.2 ± 2.4) than among control subjects (15.4 ± 2.4) [$z = -4.20$, $P < 0.001$]. All subjects were biologically unrelated Japanese individuals. Subjects were excluded from this study if they had neurological or medical conditions that could affect the central nervous system, such as atypical headache, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, cancer in an active stage, cerebrovascular disease, epilepsy, seizures, substance-related disorders, or mental retardation. Cases were both outpatients and inpatients at Osaka University Hospital. Each patient with schizophrenia had been diagnosed by a trained psychiatrist according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria based on the Structured Clinical Interview for DSM-IV (SCID) for schizophrenia. Healthy control subjects were recruited through local advertisements at Osaka University. Psychiatrically, medically, and neurologically healthy control subjects were evaluated using the DSM-IV-Non-Patient version of the Structured Clinical Interview to exclude individuals who had current or past contact with psychiatric services or had received psychiatric medication [Ohi et al., 2009]. Written informed consent was obtained for all subjects after the procedures had been fully explained. This study was carried out in accordance with the World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University.

Genotyping

We selected rs1344706 in the *ZNF804A* gene because this SNP has been found to be associated with schizophrenia and bipolar disorder in genome-wide association and follow-up studies [O'Donovan et al., 2008] and the four replication studies confirmed the association [Riley et al., 2010; Steinberg et al., 2010; Williams et al., 2010; Zhang et al., 2010]. Furthermore, this SNP was related to functional brain activity in healthy subjects [Esslinger et al., 2009; Walter et al., 2010]. Venous blood was collected from the subjects, and genomic DNA was extracted from whole blood according to standard procedures. The SNP was genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, CA) as described previously [Hashimoto et al., 2006, 2007]. Detailed information on the PCR conditions is available upon request.

Phenotype Measures

A full version of the Wechsler Memory Scale-Revised (WMS-R) [Sugishita, 2001], which is generally used to measure memory functions, was administered to the subjects. The four indices of the WMS-R, that is, verbal memory (VerM), visual memory (VisM), attention/concentration (AC), and delayed recall (DR), were used for the analysis. Psychiatric symptoms in patients with schizophrenia were evaluated using the positive and negative syndrome scale (PANSS) [Kay et al., 1987].

TABLE I. Demographic and Clinical Characteristics of Patients with Schizophrenia and Controls

	Schizophrenia (n = 113)						Control (n = 184)					
	T/T (n = 21)		G carrier (n = 92)				T/T (n = 44)		G carrier (n = 140)			
Variables	**Mean**	**SD**	**Mean**	**SD**	***P*-value**	**z**	**Mean**	**SD**	**Mean**	**SD**	***P*-value**	**z**
Age (years)	38.1	11.2	38.4	12.4	0.99	0.01	36.5	10.8	36.1	11.8	0.68	0.42
Sex (male/female)[a]	10/11		49/43		0.94	0.01	24/20		64/76		0.31	1.05
Education (years)	14.2	2.2	14.2	2.4	0.80	0.25	14.7	1.9	15.6	2.5	**0.05**	1.99
CPZeq (mg/day)	586.2	518.6	535.9	443.1	0.95	0.06	—	—	—	—	—	—
Age at onset (years)	23.7	10.2	24.2	8.6	0.76	0.31	—	—	—	—	—	—
Duration of illness (years)	14.4	9.7	14.2	11.1	0.74	0.33	—	—	—	—	—	—
Positive symptoms[b,c]	16.0	7.9	18.2	5.5	0.10	1.62	—	—	—	—	—	—
Negative symptoms[b,c]	18.6	7.5	18.6	7.0	0.89	0.14	—	—	—	—	—	—

CPZeq, chlorpromazine equivalents of total antipsychotics; [b]PANSS, positive and negative syndrome scale; SD, standard deviation. T/T: individuals with T/T genotype of rs1344706. G carriers: individuals with G/G and G/T genotypes of rs1344706. Differences in clinical characteristics between genotype groups were analyzed using the Mann–Whitney *U*-test, except for [a]χ^2 test. [c]T/T: n = 18; G carrier: n = 84. A significant *P*-value is shown as bold face and underlined.

Statistical Analyses

Statistical analyses were performed using SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan) and PASW Statistics 18.0 software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and control subjects as well as between genotype groups were analyzed using χ^2 tests for categorical variables and the Mann–Whitney *U*-test for continuous variables. The presence of Hardy–Weinberg equilibrium was examined using the χ^2 test for goodness of fit. No deviation from Hardy–Weinberg equilibrium was detected in cases or in controls ($P > 0.05$). To examine the effect of ZNF804A rs1344706 genotype on memory function, the effects of *ZNF804A* genotype, diagnosis, and genotype–diagnosis interactions on four memory domains were analyzed by a two-way analysis of variance (ANOVA). In further analysis to control for confounding factors, the genotype effects, diagnosis effects, and genotype–diagnosis interactions on the memory functions were adjusted by a two-way analysis of covariance (ANCOVA) with sex and years of education as covariates (the scores of indices were previously corrected by age). When genotype–diagnosis interaction was found, cases and controls were separately analyzed by ANOVA and ANCOVA. The Bonferroni correction was applied for multiple testing on four indices of the WMS-R to avoid type I error. Standardized effect sizes were calculated using Cohen's *d* method (http:www.uccs.edu/faculty/lbecker). The significance level for statistical tests was set at two-tailed $P < 0.05$.

RESULTS

The Effect of the High-Risk *ZNF804A* Polymorphism on Memory Functions

We examined potential associations between the *ZNF804A* genotype and memory functions in patients with schizophrenia and healthy controls. There was no difference in age, sex, chlorpromazine equivalents of total antipsychotics, age at onset, duration of illness, or PANSS scores between genotype groups. The only difference in demographic variables was a significantly greater number of years of education in the control groups ($z = 1.99$, $P = 0.05$; Table I). The *ZNF804A* genotype effects, diagnosis effects, and genotype–diagnosis interactions on memory functions are shown in Table II. We found significant effects of diagnosis (VerM: $F_{1,293} = 146.91$, $P < 0.001$; adjusted $F_{1,291} = 133.70$, $P < 0.001$, VisM: $F_{1,293} = 114.30$, $P < 0.001$; adjusted $F_{1,291} = 103.87$, $P < 0.001$, AC: $F_{1,293} = 53.46$, $P < 0.001$; adjusted $F_{1,291} = 48.59$; $P < 0.001$, DR: $F_{1,293} = 200.36$, $P < 0.001$; adjusted $F_{1,291} = 186.09$, $P < 0.001$) and genotype–diagnosis interaction (VisM: $F_{1,293} = 8.21$, $P = 0.0045$, adjusted $F_{1,291} = 10.76$, $P = 0.0012$). Significant genotype effects were only found for VisM ($F_{1,293} = 4.46$, $P = 0.036$, adjusted $F_{1,291} = 3.40$, $P = 0.066$). The effect of diagnosis and the diagnosis–genotype interaction remained positive after correction for multiple tests (corrected *P*-values, VerM: $P < 0.001$, VisM: $P < 0.001$, AC: $P < 0.001$, DR: $P < 0.001$, interaction in VisM: $P = 0.0048$), whereas the genotype effect on VisM did not remain after correction for multiple tests ($P > 0.14$). Patients with schizophrenia displayed lower scores on all memory indices than did controls, and the effect sizes of VerM, VisM, AC, and DR were −1.72, −1.21, −1.17, and −1.89, respectively. As a genotype–diagnosis interaction was found for VisM, we separately analyzed the effects of genotype on VisM in patients and controls (Fig. 1). There was a significant genotype effect in patients with schizophrenia ($F_{1,111} = 5.05$, $P = 0.027$; adjusted $F_{1,109} = 5.82$, $P = 0.018$), whereas there was no genotype effect in controls ($F_{1,182} = 0.88$, $P = 0.35$; adjusted $F_{1,180} = 1.43$, $P = 0.23$). The patients with the high-risk T/T genotype scored significantly lower on VisM than did those who carry a G genotype (effect size: −0.56).

When the two genotypes were divided into three genotype groups (patients with T/T genotype, T/G genotype, and G/G genotype), the patients with the high-risk T/T genotype scored significantly lower on VisM than patients with the T/G genotype (adjusted $F_{1,68} = 8.59$, $P = 0.0046$) and marginally lower than patients with the G/G genotype (adjusted $F_{1,58} = 2.89$, $P = 0.09$;

TABLE II. Effects of the *ZNF804A* Genotype on Memory Function Determined Using WMS-R

	Schizophrenia (n = 113)				Control (n = 184)				ANOVA						ANCOVA (adjusted)					
	T/T (n = 21)		G carrier (n = 92)		T/T (n = 44)		G carrier (n = 140)		Diagnosis effect		Genotype effect		Interaction		Diagnosis effect		Genotype effect		Interaction	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*-value	$F_{1,293}$	*P*-value	$F_{1,293}$	*P*-value	$F_{1,293}$	*P*-value	$F_{1,291}$	*P*-value	$F_{1,291}$	*P*-value	$F_{1,291}$
VerM	82.6	14.6	84.6	18.3	110.2	14.1	111.3	13.0	$<10^{-3}$	**146.9**	0.50	0.45	0.83	0.04	$<10^{-3}$	**133.7**	0.78	0.08	0.47	0.52
VisM	81.7	18.2	92.3	19.7	110.5	8.3	108.9	10.3	$<10^{-3}$	**114.3**	**0.036**	**4.46**	**0.0045**	**8.21**	$<10^{-3}$	**103.9**	0.066	3.40	**0.0012**	**10.8**
AC	92.0	16.5	90.9	15.2	105.1	13.6	109.2	13.9	$<10^{-3}$	**53.5**	0.48	0.50	0.23	1.44	$<10^{-3}$	**48.6**	0.54	0.37	0.28	1.17
DR	77.1	18.4	82.6	19.5	111.7	12.7	112.0	11.8	$<10^{-3}$	**200.4**	0.20	1.65	0.25	1.31	$<10^{-3}$	**186.1**	0.35	0.88	0.10	2.71

WMS-R, Wechsler Memory Scale-Revised; VerM, verbal memory; VisM, visual memory; AC, attention/concentration; DR, delayed recall; SD, standard deviation. T/T: individuals with T/T genotype of rs1344706. G carriers: individuals with G/G or G/T genotype of rs1344706. The effects of the ZNF804A genotype and the effects of diagnosis on the memory function were analyzed by a two-way analysis of variance (ANOVA). Adjusted effects of genotype were analyzed by a two-way analysis of covariance (ANCOVA) with sex and years of education as covariates. Significant *P*-values are shown as bold face and underlined.

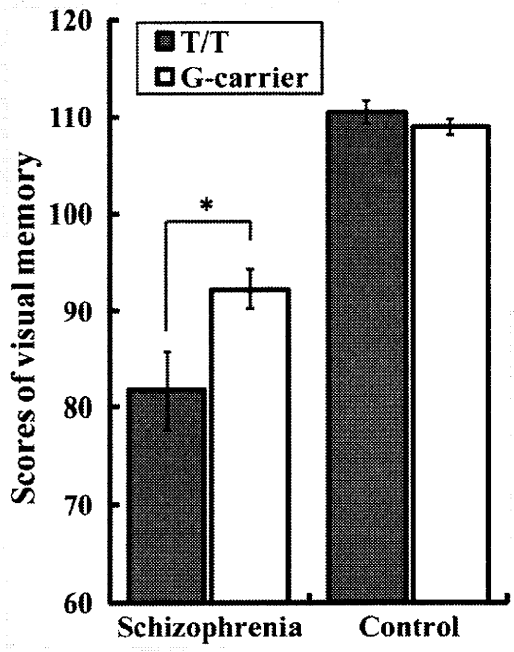

FIG. 1. The association between the high-risk *ZNF804A* genotype and visual memory in patients with schizophrenia. X-axis: gray bars, individuals with T/T genotype of rs1344706; white bars, individuals with a G allele (G/T and G/G genotypes) of rs1344706. Y-axis: scores of visual memory from the WMS-R. Error bars represent standard errors of the mean. *$P < 0.05$, compared with patients with a G allele.

Table III). However, there was no significant difference in scores between patients with the T/G genotype and G/G genotype ($F_{1,88} = 1.39$, $P = 0.24$).

DISCUSSION

In the present study, we first demonstrated an association between the high-risk *ZNF804A* SNP and memory performance in patients with schizophrenia. We provided evidence that patients with the high-risk T/T genotype had lower performance on VisM than patients who carry a G allele. The effect size of the difference in VisM scores between patients with the T/T genotype and G carriers was −0.56; this effect is typically considered a medium-sized effect. We do not know why we found the genotype effect on only VisM. A possible explanation is that a previous study reported suggestive linkage evidence for the VisM on 2q36 near the locus of the *ZNF804A* gene [Paunio et al., 2004]. Another possibility is that this SNP is associated with connectivity during N-back memory task, which is an fMRI task using visual cue [Esslinger et al., 2009]. This study showed no effect of genotype on a memory task in healthy subjects, which is consistent with our data [Esslinger et al., 2009].

A linear genotype effect on connectivity in DLPFC and hippocampal formation during a memory task was found in healthy control subjects in an fMRI study [Esslinger et al., 2009]. These data

TABLE III. Effects of the *ZNF804A* Genotype on Memory Performance

	Schizophrenia (n = 113)						Control (n = 184)						ANCOVA (adjusted)					
	T/T (n = 21)		T/G (n = 51)		G/G (n = 41)		T/T (n = 44)		T/G (n = 85)		G/G (n = 55)		Diagnosis effect		Genotype effect		Interaction	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*-value	$F_{2,289}$	*P*-value	$F_{2,289}$	*P*-value	$F_{2,289}$
VerM	82.6	14.6	83.9	19.1	85.5	17.3	110.2	14.1	112.5	12.8	109.4	13.2	**<$\underline{10^{-3}}$**	**168.5**	0.61	0.49	0.52	0.66
VisM	81.7	18.2	93.2	20.2	91.1	19.3	110.5	8.3	108.4	10.0	109.7	10.9	**<$\underline{10^{-3}}$**	**111.6**	0.15	1.94	**0.0028**	**5.99**
AC	92.0	16.5	89.9	14.6	92.1	15.9	105.1	13.6	109.8	14.8	108.2	12.4	**<$\underline{10^{-3}}$**	**70.7**	0.84	0.18	0.45	0.81
DR	77.1	18.4	83.1	20.8	82.0	18.1	111.7	12.7	113.2	11.6	110.2	12.0	**<$\underline{10^{-3}}$**	**227.5**	0.18	1.71	0.23	1.47

WMS-R, Wechsler Memory Scale-Revised; VerM, verbal memory; VisM, visual memory; AC, attention/concentration: DR, delayed recall; SD, standard deviation. T/T, T/G, G/G: individuals with three genotypes of rs1344706. Adjusted effects of three genotypes were analyzed by a two-way analysis of covariance (ANCOVA) with sex and years of education as covariates. Significant *P*-values are shown as bold face and underlined.

might indicate that quantitative traits (i.e., brain physiological activity measured by fMRI) are closer to the genetic substrate than behavioral traits, such as neuropsychological functions and psychiatric disorders, and should be observable in genetically at-risk but behaviorally unaffected individuals [Meyer-Lindenberg and Weinberger, 2006]. Such physiological quantitative traits are likely to influence a neuropsychological trait, memory performance, in patients with schizophrenia, however, they might not affect memory performance in healthy subjects. This phenomena suggests that the high-risk SNP in the *ZNF804A* gene might be related to the neuropsychological disturbance in schizophrenia.

There were several limitations to this study. Although the sample was moderate in size, it might not be representative of the schizophrenic population. A false-positive association cannot be excluded as a possibility in our study, despite the precautions of ethnic matching and correction for multiple testing. The effects of the ZNF804A gene on VisM could be an epiphenomenon of the severity of the disease and/or medication. In conclusion, we found an effect of the high-risk *ZNF804A* SNP on VisM in schizophrenia. Further research will be required to clarify the role of the high-risk *ZNF804A* SNP in the pathophysiology of schizophrenia.

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REFERENCES

Buchanan RW, Freedman R, Javitt DC, Abi-Dargham A, Lieberman JA. 2007. Recent advances in the development of novel pharmacological agents for the treatment of cognitive impairments in schizophrenia. Schizophr Bull 33(5):1120–1130.

Cardno AG, Gottesman II. 2000. Twin studies of schizophrenia: From bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet 97(1):12–17.

Esslinger C, Walter H, Kirsch P, Erk S, Schnell K, Arnold C, Haddad L, Mier D, Opitz von Boberfeld C, Raab K, Witt SH, Rietschel M, Cichon S, Meyer -Lindenberg A. 2009. Neural mechanisms of a genome-wide supported psychosis variant. Science 324(5927):605.

Gottesman II, Gould TD. 2003. The endophenotype concept in psychiatry: Etymology and strategic intentions. Am J Psychiatry 160(4):636–645.

Hashimoto R, Numakawa T, Ohnishi T, Kumamaru E, Yagasaki Y, Ishimoto T, Mori T, Nemoto K, Adachi N, Izumi A, Chiba S, Noguchi H, Suzuki T, Iwata N, Ozaki N, Taguchi T, Kamiya A, Kosuga A, Tatsumi M, Kamijima K, Weinberger DR, Sawa A, Kunugi H. 2006. Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology and ERK signaling. Hum Mol Genet 15(20): 3024–3033.

Hashimoto R, Hashimoto H, Shintani N, Chiba S, Hattori S, Okada T, Nakajima M, Tanaka K, Kawagishi N, Nemoto K, Mori T, Ohnishi T, Noguchi H, Hori H, Suzuki T, Iwata N, Ozaki N, Nakabayashi T, Saitoh O, Kosuga A, Tatsumi M, Kamijima K, Weinberger DR, Kunugi H, Baba A. 2007. Pituitary adenylate cyclase-activating polypeptide is associated with schizophrenia. Mol Psychiatry 12(11):1026–1032.

Kay SR, Fiszbein A, Opler LA. 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 13(2):261–276.

Meyer-Lindenberg A, Weinberger DR. 2006. Intermediate phenotypes and genetic mechanisms of psychiatric disorders. Nat Rev Neurosci 7(10):818–827.

Nuechterlein KH, Barch DM, Gold JM, Goldberg TE, Green MF, Heaton RK. 2004. Identification of separable cognitive factors in schizophrenia. Schizophr Res 72(1):29–39.

O'Donovan MC, Craddock N, Norton N, Williams H, Peirce T, Moskvina V, Nikolov I, Hamshere M, Carroll L, Georgieva L, Dwyer S, Holmans P, Marchini JL, Spencer CC, Howie B, Leung HT, Hartmann AM, Möller HJ, Morris DW, Shi Y, Feng G, Hoffmann P, Propping P, Vasilescu C, Maier W, Rietschel M, Zammit S, Schumacher J, Quinn EM, Schulze TG, Williams NM, Giegling I, Iwata N, Ikeda M, Darvasi A, Shifman S, He L, Duan J, Sanders AR, Levinson DF, Gejman PV, Cichon S, Nöthen MM, Gill M, Corvin A, Rujescu D, Kirov G, Owen MJ, Buccola NG, Mowry BJ, Freedman R, Amin F, Black DW, Silverman JM, Byerley WF, Cloninger CR, Molecular Genetics of Schizophrenia Collaboration. 2008. Identification of loci associated with schizophrenia by genome-wide association and follow-up. Nat Genet 40(9):1053–1055.

Ohi K, Hashimoto R, Yasuda Y, Kiribayashi M, Iike N, Yoshida T, Azechi M, Ikezawa K, Takahashi H, Morihara T, Ishii R, Tagami S, Iwase M, Okochi M, Kamino K, Kazui H, Tanaka T, Kudo T, Takeda M. 2009. TATA box-binding protein gene is associated with risk for schizophrenia, age at onset and prefrontal function. Genes Brain Behav 8(4):473–480.

Paunio T, Tuulio-Henriksson A, Hiekkalinna T, Perola M, Varilo T, Partonen T, Cannon TD, Lonnqvist J, Peltonen L. 2004. Search for cognitive trait components of schizophrenia reveals a locus for verbal learning and memory on 4q and for visual working memory on 2q. Hum Mol Genet 13(16):1693–1702.

Preston GA, Weinberger DR. 2005. Intermediate phenotypes in schizophrenia: A selective review. Dialogues Clin Neurosci 7(2):165–179.

Riley B, Thiselton D, Maher BS, Bigdeli T, Wormley B, McMichael GO, Fanous AH, Vladimirov V, O'Neill FA, Walsh D, Kendler KS. 2010. Replication of association between schizophrenia and ZNF804A in the Irish case–control study of schizophrenia sample. Mol Psychiatry 15(1):29–37.

Snitz BE, Macdonald AW III, Carter CS. 2006. Cognitive deficits in unaffected first-degree relatives of schizophrenia patients: A meta-analytic review of putative endophenotypes. Schizophr Bull 32(1): 179–194.

Steinberg S, Mors O, Børglum AD, Gustafsson O, Werge T, Mortensen PB, Andreassen OA, Sigurdsson E, Thorgeirsson TE, Böttcher Y, Olason P, Ophoff RA, Cichon S, Gudjonsdottir IH, Pietiläinen OP, Nyegaard M, Tuulio-Henriksson A, Ingason A, Hansen T, Athanasiu L, Suvisaari J, Lonnqvist J, Paunio T, Hartmann A, Jürgens G, Nordentoft M, Hougaard D, Norgaard-Pedersen B, Breuer R, Möller HJ, Giegling I, Glenthøj B, Rasmussen HB, Mattheisen M, Bitter I, Réthelyi JM, Sigmundsson T, Fossdal R, Thorsteinsdottir U, Ruggeri M, Tosato S, Strengman E, Kiemeney LA, Melle I, Djurovic S, Abramova L, Kaleda V, Walshe M, Bramon E, Vassos E, Li T, Fraser G, Walker N, Toulopoulou T, Yoon J, Freimer NB, Cantor RM, Murray R, Kong A, Golimbet V, Jönsson EG, Terenius L, Agartz I, Petursson H, Nöthen MM, Rietschel M, Peltonen L, Rujescu D, Collier DA, Stefansson H, St Clair D, Stefansson K. 2010. Expanding the range of ZNF804A variants conferring risk of psychosis. Mol Psychiatry (online publication).

Sugishita M. 2001. Japanese Wechsler Memory Scale-Revised. Tokyo: Nihonbunkakagakusha.

Tsuang M. 2000. Schizophrenia: Genes and environment. Biol Psychiatry 47(3):210–220.

Walter H, Schnell K, Erk S, Arnold C, Kirsch P, Esslinger C, Mier D, Schmitgen MM, Rietschel M, Witt SH, Nothen MM, Cichon S, Meyer-Lindenberg A. 2010. Effects of a genome-wide supported psychosis risk variant on neural activation during a theory-of-mind task. Mol Psychiatry (online publication).

Williams HJ, Norton N, Dwyer S, Moskvina V, Nikolov I, Carroll L, Georgieva L, Williams NM, Morris DW, Quinn EM, Giegling I, Ikeda M, Wood J, Lencz T, Hultman C, Lichtenstein P, Thiselton D, Maher BS, Malhotra AK, Riley B, Kendler KS, Gill M, Sullivan P, Sklar P, Purcell S, Nimgaonkar VL, Kirov G, Holmans P, Corvin A, Rujescu D, Craddock N, Owen MJ, O'Donovan MC, Molecular Genetics of Schizophrenia Collaboration (MGS) International Schizophrenia Consortium (ISC), SGENE-plus, GROUP. 2010. Fine mapping of ZNF804A and genome-wide significant evidence for its involvement in schizophrenia and bipolar disorder. Mol Psychiatry (online publication).

Zhang R, Lu SM, Qiu C, Liu XG, Gao CG, Guo TW, Valenzuela RK, Deng HW, Ma J. 2010. Population-based and family-based association studies of ZNF804A locus and schizophrenia. Mol Psychiatry (online publication).

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