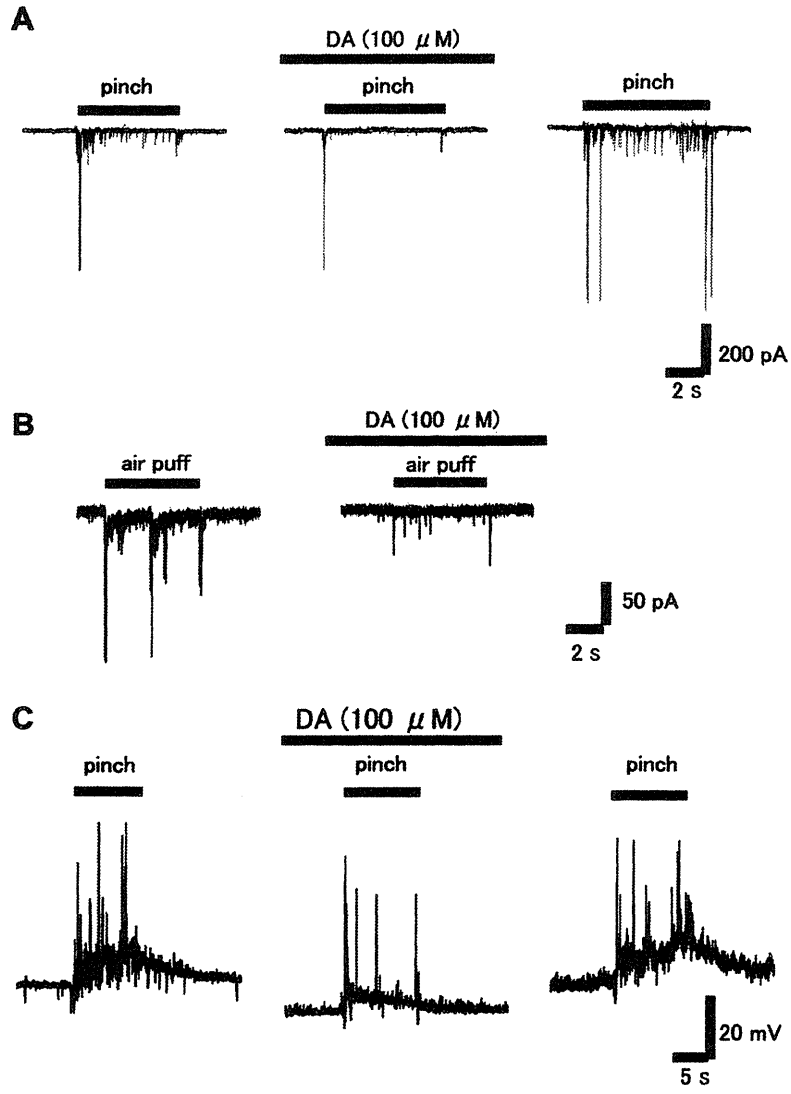

Fig. 6. Reduction of pinch and air puff evoked firing in SG neurons by DA. (A) EPSCs elicited by pinching in voltage-clamp mode ($V_H = -70$ mV) in the control (left). DA (100 μM) suppressed repeated EPSCs during pinching in a reversible manner without affecting amplitude of large EPSCs evoked at the beginning and end of pinch stimulus (middle and right). (B) EPSCs elicited by air puff in voltage-clamp mode ($V_H = -70$ mV) in the control (left). DA (100 μM) suppressed repeated EPSCs during air puff (right). (C) Pinch stimuli applied to the ipsilateral hindlimb produced a barrage of EPSPs accompanied by action potentials under a current-clamp condition (left). DA (100 μM) hyperpolarized membrane of SG neuron and inhibited the action potentials in a reversible manner (middle and right).

(Figs. 6B, 7B). In the current-clamp mode, pinch stimuli elicited a barrage of excitatory postsynaptic potentials (EPSPs), some of which were accompanied by an action potential (Fig. 6C). The evoked EPSPs disappeared within 1 s after the stimulation, and did not show any desensitization of the responses. Perfusion with DA caused a slight hyperpolarization of the membrane and suppressed action potentials in a reversible manner (Fig. 6C). Evaluation by frequency and area was also used in the current-clamp mode. DA decreased the area of action potentials or EPSPs during pinching the skin in all the neurons tested (42.7 ± 6.9% of the control; $n = 9$, $P < 0.01$) and the frequency of action potentials or EPSPs with pinch decreased in 7 of 9 neurons tested (70.2 ± 12.4% of the control; $n = 9$, $P < 0.05$) (Fig. 7B).

3.5. Effect of electrical stimulation of A11

The region of A11 is the principle source of descending dopaminergic pathways. We investigated whether antinociceptive actions were induced in SG neurons by focal electrical stimulation (ES) of A11. Schematic diagrams of A11-ES are shown in Fig. 8A. A11-ES induced outward currents in 36 of 50 (72%) neurons recorded (Fig. 8B), with an average amplitude of 7.5 ± 1.6 pA ($n = 36$). Moreover, A11-ES decreased the frequency and amplitude of EPSCs (Fig. 8C). Our definition of significance was if the frequency or amplitude of EPSCs decreased by more than 10% compared with those of the control. The frequency was decreased in 39 of 46 (84.7%) neurons (average of 61.9 ± 3.0%, $n = 39$, of the controls) by A11-ES. The amplitude was decreased in 24 of 46 (52.2%) neurons (average 82.2 ± 1.5%, $n = 24$, of the control) by A11-ES. We also examined whether the D2-like antagonist, sulpiride, blocked these A11-ES effects (Fig. 8D). In the presence of sulpiride (100 μM), the A11-ES induced outward current was blocked, and its average amplitude was only 1.6 ± 0.6 pA ($n = 16$). The frequency and amplitude by A11-ES under sulpiride were 91.3 ± 4.7% and 98.7 ± 3.3% ($n = 17$), respectively, of the controls. These results were significantly lower than that in the absence of sulpiride ($P < 0.01$).

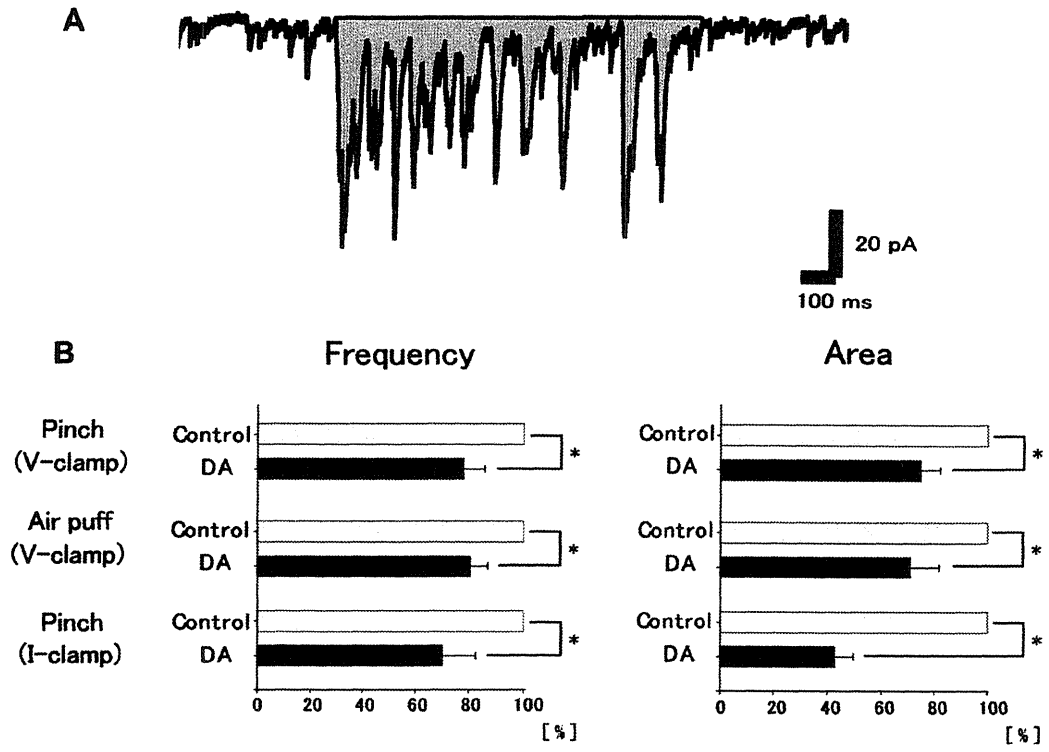

Fig. 7. Analysis of the responses to the noxious and innocuous stimuli. (A) Schematic diagrams of area. Analysis of area surrounded by the baseline and border of EPSCs was done by using software (clampfit10). (B) Analysis about the frequency and area of EPSCs, EPSPs and action potentials, which were induced by noxious and innocuous stimuli to the ipsilateral hindlimb. The frequency and area significantly decreased during DA perfusion compared with the controls in the absence of DA. These results were showed when either noxious or innocuous stimuli to the skin were given.

4. Discussion

The descending inhibitory system of nociceptive information is important for the control of excessive pain. Compared with the large quantity of literature on NA and 5-HT as the descending inhibitory systems, there is little on the analgesic effect of DA. Several reports have described the direct actions of supraspinal DA on nociceptive responses in regions of the brain [39], such as the striatum [1,31,38] and in review, [19], the basal ganglia [17], the nucleus accumbens [25,49], the anterior insular cortex [8], the anterior cingulated cortex [30] and the ventrolateral orbital cortex [41]. However, there have been no pharmacological studies using agonists and antagonists for DA receptor subtypes of the superficial dorsal horn in whole-cell patch-clamp recordings under *in vivo* conditions. In the present study, the effects of DA on SG neurons were tested using the *in vivo* patch-clamp recording technique that enabled us to examine the effect of drugs on synaptic responses evoked by noxious (pinch) and innocuous (air puff) stimuli applied to the skin. In addition, we investigated whether antinociceptive actions were induced in SG neurons by A11-ES.

4.1. Pre- and postsynaptic actions and character of D2-like receptors in SG neurons

The application of DA induced outward currents or hyperpolarization in about 70% of SG neurons tested in the present study. The DA-induced outward current is observed in the presence of TTX (Fig. 3C). DA also significantly decreases the frequency of mEPSCs in the presence of TTX. Furthermore, mEPSCs are completely blocked by CNQX (Fig. 3D), but the DA-induced outward current is observed in the presence of CNQX. These results suggest that DA has both presynaptic and postsynaptic actions to inhibit synaptic transmission in SG neurons. The DA-induced outward current was completely blocked by GDP-β-S in the pipette solution (Fig. 4B). The perfusion of a non-selective K^+ channel blocker, Ba^{2+}, also inhibited the outward currents induced by DA (Fig. 4A). Since these DA-induced outward currents were mimicked by a selective D2-like receptor agonist, quinpirole, and attenuated by a selective D2-like receptor antagonist, sulpiride (Fig. 5A, 5C), it is suggested that the DA-induced outward current is mediated by G-protein-activated K^+ channels through D2-like receptors. Thus, DA acts directly on D2-like receptors in the postsynaptic membrane of SG neurons. The DA-induced outward currents and hyperpolarization were induced by D2-like receptor-mediated activation of K^+ channels, which have been reported in various neurons other than SG neurons, such as the substantia nigra pars compacta [27], striatal neurons [18,51], ventral tegmental area [34] and pituitary cells [7,11]. A previous study has shown that glutamatergic fibers that terminate onto ventral tegmental dopaminergic neurons possess D2-like receptors, activation of which inhibits glutamate release by reducing Ca^{2+} influx [24]. Similarly, these presynaptic D2-like receptors activate glutamate release onto postsynaptic neurons. However, further investigations will be required to clarify the role of presynaptic dopaminergic receptors in the spinal dorsal horn.

In the present study, an inward current was observed in 19 neurons of 219 recorded SG neurons (9%). D1-like receptor-mediated postsynaptic excitation by inhibiting K^+ conductance or activating cation conductance has been reported in striatal cholinergic interneurons [2]. Although an autoradiographic study has demonstrated that both D1-like and D2-like receptors are densely localized in the superficial laminae of the spinal dorsal horn [28], the effect of D1-like receptor agonist on membrane currents was not detected in the present study. Thus, further investigations will

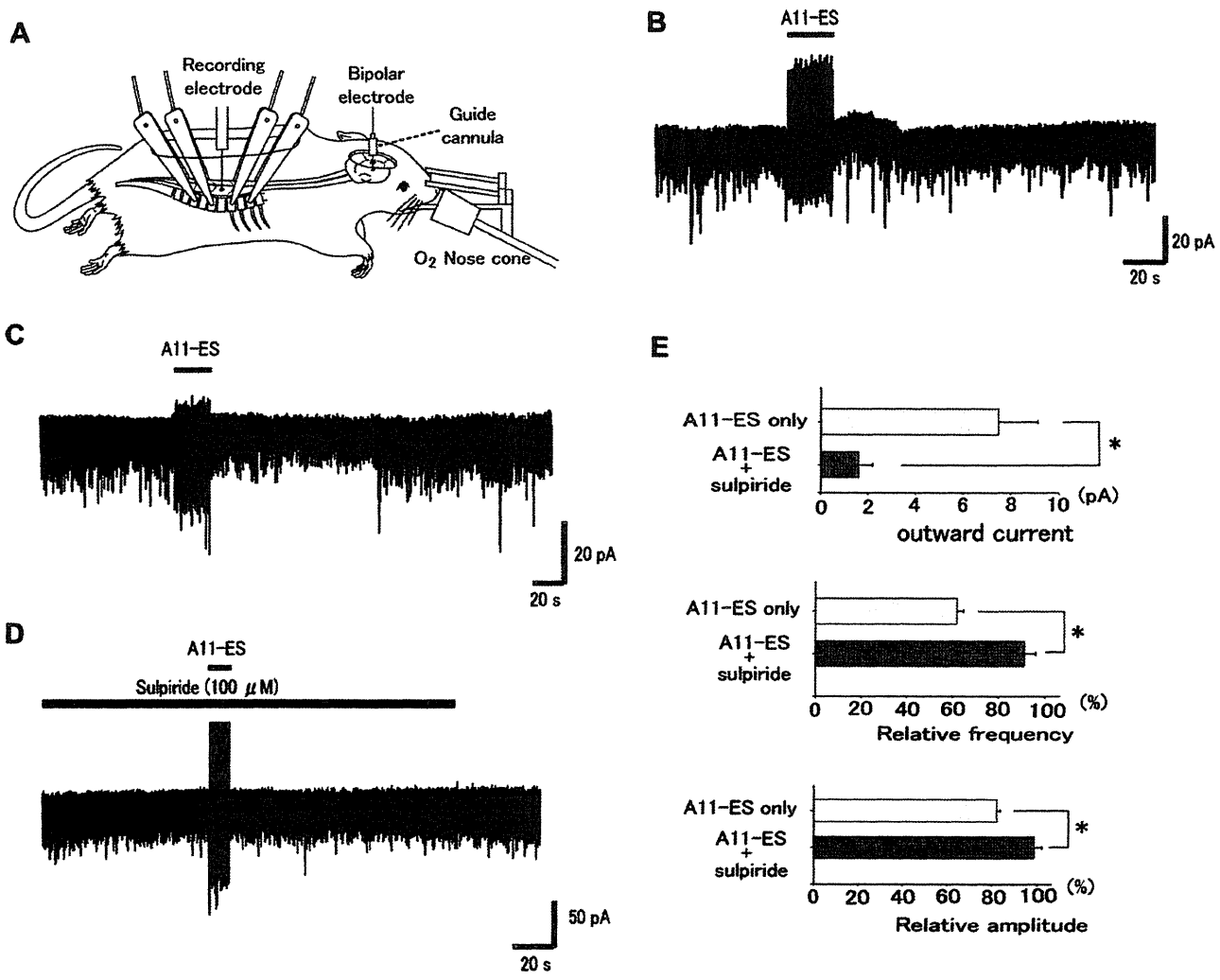

Fig. 8. A11-ES induced antinociceptive effects through activation of D2-like receptor in SG neurons. (A) Schematic diagrams of A11-ES during *in vivo* patch-clamp recordings on SG neurons. (B) A11-ES induced outward currents immediately in 36 of 50 (72%) neurons recorded. The average amplitude was 7.5 ± 1.6 pA; $n = 36$. (C) A11-ES decreased the frequency and amplitude of EPSCs. The frequency was decreased in 39 of 46 (84.7%) neurons (61.9 ± 3.0% of the control; $n = 39$). The amplitude was decreased in 24 of 46 (52.2%) neurons (82.2 ± 1.5% of the control; $n = 24$). (D) In the presence of sulpiride (100 μM), A11-ES induced outward current was blocked, and its average amplitude was only 1.6 ± 0.6 pA ($n = 16$). The frequency and amplitude by A11-ES under sulpiride ($n = 17$) were 91.3 ± 4.7% and 98.7 ± 3.3%, respectively, of the control. These results were significantly lower than those in the absence of sulpiride ($P < 0.01$).

be required to clarify the role of D1-like receptors in the spinal dorsal horn.

4.2. DA produced analgesic effects in SG neurons for both noxious and innocuous stimuli to the skin

In this study, the pinch-evoked action potentials and EPSPs were attenuated in frequency and area by the application of DA, probably due to the hyperpolarization of membrane (Figs. 6C, 7B). In the voltage-clamp mode, the pinch-induced EPSCs were also markedly attenuated in frequency and area by the application of DA (Fig. 7B). The EPSCs evoked by an innocuous (air puff) mechanical stimuli were decreased in frequency and area by the application of DA, as with the pinch stimuli (Fig. 7B). This shows that DA has inhibitory effects in the spinal cord to both noxious and innocuous stimuli to the skin. As shown in Fig. 2A, spontaneous EPSCs are observed in a substantial number of SG neurons *in vivo*, while spontaneous EPSCs are hardly recorded in slice preparations [56]. Both the frequency and amplitude of EPSCs observed in the *in vivo* preparations are markedly depressed by DA (Fig. 2B). These results may be due to the membrane hyperpolarization in interneurons or presynaptic depression of transmitter release by DA acting at the terminals of the primary afferents. It is suggested that the DA-induced blockade of noxious transmission is at least partly mediated by the actions of DA on the presynaptic site, suppressing the evoked EPSCs, thereby reducing the number of action potentials in SG neurons during pinching.

4.3. DA in the dorsal horn is projected from dopaminergic neurons in the region of A11

Previous studies have demonstrated that the intrathecal administration of DA and/or D2-like agonist have attenuated pain related behavior which was induced by thermal stimuli [3,22,29]. Similarly, intrathecal administration of DA induces mechanical antinociception when assessed by withdrawal thresholds [48]. This antinociceptive action of DA agonists is selectively blocked by D2-like antagonists, but not by D1-like antagonists [3,29]. Furthermore, it was previously reported that intrathecal administration of not only D2-like receptor agonists but also high doses of a D1-like

receptor agonist produced the analgesic effect in carrageenan induced chronic inflammation [15,16], or haloperidol-induced dopamine-supersensitivity [4]. Thus, D2-like receptors within the spinal dorsal horn may play a significant regulatory role in a variety of pain sensations and D1-like receptors could have some of an analgesic action affecting morbidity after chronic inflammation or dopamine-supersensitivity.

The dopaminergic A11 neurons in the periventricular, posterior region of the hypothalamus are a major source of descending dopaminergic projections to the spinal cord [5,20,43]. Focal electrical stimulation of A11 neurons produce an antinociceptive effect in the spinal dorsal horn, and which is reversed by the spinal administration of a D2-like receptor antagonist [12,52]. A D2-like agonist administered onto A11 neurons produces attenuation of neuropathic hypersensitivity, and this effect is reversed by intrathecal administration of an α_2-adrenoceptor antagonist or a 5-HT_{1A} receptor antagonist but not by a D2-like antagonist [52]. These findings suggest that activation of the dopamine D2 receptor in A11 neurons may selectively suppress neuropathic hypersensitivity, owing to mechanisms that involve descending noradrenergic pathways acting on spinal α_2-adrenoceptor, and 5-HT_{1A} receptor. This action is probably different from the antinociception induced by electrical stimulation of A11 neurons, because our results confirm a direct inhibition of SG neurons in the spinal cord by activation of dopamine D2-like receptors.

Dopaminergic innervation of the spinal cord is largely derived from the brain. A previous study reported that there are no dopaminergic cell bodies in the spinal cord and that only fibers and terminals are immunoreactive for DA [21]. Therefore, the potential origin of endogenous DA appears to be from dopaminergic neurons in the region of A11. On the other hand, it has been reported that there is a small population of DRG neurons that exhibits a clear DA-immunoreactivity [53]. However, in that report, the amount of DA in DRG is about 10-fold lower than the levels found in dorsal horn of spinal cord. Moreover, in another recent report, tyrosine hydroxylase (TH) was expressed in small DRG neurons in the adult mouse, but aromatic amino acid decarboxylase was rare and did not colocalize with TH [6]. In addition, our previous study showed that stimulating a dorsal root either singly or repetitively does not evoke a slow current in SG neurons [35]. Therefore, it is possible that DA is not released from the central terminal of primary afferents innervating SG neurons. Although the possibility cannot be completely excluded that DA is released from the peripheral nerve terminals, it is reasonable to consider that most of dorsal horn DA arises from projections of dopaminergic neurons in the region of A11. Since we demonstrate that A11-ES induced antinociceptive effects in SG neurons are similar to those following perfusion of DA in the spinal cord, it is suggested that DA in the dorsal horn originates from dopaminergic neurons projected from the A11 region as a descending inhibitory system.

In conclusion, the present study using the *in vivo* patch-clamp technique indicates that the antinociceptive effects of DA originating from the descending dopaminergic pathway are mediated by the actions on both presynaptic and postsynaptic sites, reducing glutamate release onto SG neurons. The postsynaptic SG neurons induce hyperpolarizations by interacting with D2-like receptors and G-protein-mediated activation of K^+ channels. These findings suggest that the dopaminergic descending pathway has an antinociceptive effect and it may be of use in the understanding of intractable pain.

5. Conflict of interest statement

There are no conflicts of interest regarding this study, for any of the authors.

Acknowledgements

This work was supported in part by MEXT KAKENHI 2279139 to W.T. and MEXT KAKENHI 22591647 to T.N.

References

[1] Ansah OB, Leite-Almeida H, Wei H, Pertovaara A. Striatal dopamine D2 receptors attenuate neuropathic hypersensitivity in the rat. Exp Neurol 2007;205:536–46.
[2] Aosaki T, Kiuchi K, Kawaguchi Y. Dopamine D1-like receptor activation excites rat striatal large aspiny neurons in vitro. J Neurosci 1998;18:5180–90.
[3] Barasi S, Duggal KN. The effect of local and systemic application of dopaminergic agents on tail flick latency in the rat. Eur J Pharmacol 1985;117:287–94.
[4] Barasi S, Ben-Sreti MM, Clatworthy AL, Duggal KN, Gonzalez JP, Robertson J, Rooney KF, Sewell RD. Dopamine receptor-mediated spinal antinociception in the normal and haloperidol pretreated rat: effects of sulpiride and SCH 23390. Br J Pharmacol 1987;90:15–22.
[5] Björklund A, Skagerberg G. Evidence for a major spinal cord projection from the diencephalic A11 dopamine cell group in the rat using transmitter-specific fluorescent retrograde tracing. Brain Res 1979;177:170–5.
[6] Brumovsky P, Villar MJ, Hökfelt T. Tyrosine hydroxylase is expressed in a subpopulation of small dorsal root ganglion neurons in the adult mouse. Exp Neurol 2006;200:153–65.
[7] Castelletti L, Memo M, Missale C, Spano PF, Valerio A. Potassium channels involved in the transduction mechanism of dopamine D2 receptors in rat lactotrophs. J Physiol 1989;410:251–65.
[8] Coffeen U, López-Avila A, Ortega-Legaspi JM, del Angel R, López-Muñoz FJ, Pellicer F: Dopamine receptors in the anterior insular cortex modulate long-term nociception in the rat. Eur J Pain 2008;12:535–43.
[9] D'Mello R. Dickenson AH: Spinal cord mechanisms of pain. Br J Anaesth 2008;101:8–16.
[10] Commissiong JW, Galli CL, Neff NH. Differentiation of dopaminergic and noradrenergic neurons in rat spinal cord. J Neurochem 1978;30:1095–9.
[11] Einhorn LC, Gregerson KA, Oxford GS. D2 dopamine receptor activation of potassium channels in identified rat lactotrophs: whole-cell and single-channel recording. J Neurosci 1991;11:3727–37.
[12] Fleetwood-Walker SM, Hope PJ, Mitchell R. Antinociceptive actions of descending dopaminergic tracts on cat and rat dorsal horn somatosensory neurones. J Physiol 1988;399:335–48.
[13] Furue H, Narikawa K, Kumamoto E, Yoshimura M. Responsiveness of rat substantia gelatinosa neurons to mechanical but not thermal stimuli revealed by *in vivo* patch-clamp recording. J Physiol 1999;521:529–35.
[14] Fürst S. Transmitters involved in antinociception in the spinal cord. Brain Res Bull 1999;48:129–41.
[15] Gao X, Zhang Y, Wu G. Effects of dopaminergic agents on carrageenan hyperalgesia in rats. Eur J Pharmacol 2000;406:53–8.
[16] Gao X, Zhang Y, Wu G. Effects of dopaminergic agents on carrageenan hyperalgesia after intrathecal administration to rats. Eur J Pharmacol 2001;418:73–7.
[17] Greco R, Tassorelli C, Armentero MT, Sandrini G, Nappi G, Blandini F. Role of central dopaminergic circuitry in pain processing and nitroglycerin-induced hyperalgesia. Brain Res 2008;1238:215–23.
[18] Greif GJ, Lin YJ, Liu JC, Freedman JE. Dopamine-modulated potassium channels on rat striatal neurons: specific activation and cellular expression. J Neurosci 1995;15:4533–44.
[19] Hagelberg N, Jääskeläinen SK, Martikainen IK, Mansikka H, Forssell H, Scheinin H, Hietala J, Pertovaara A. Striatal dopamine D2 receptors in modulation of pain in humans: a review. Eur J Pharmacol 2004;500:187–92.
[20] Hökfelt T, Phillipson O, Goldstein M. Evidence for a dopaminergic pathway in the rat descending from the A11 cell group to the spinal cord. Acta Physiol Scand 1979;107:393–5.
[21] Holstege JC, Van Dijken H, Buijs RM, Goedknegt H, Gosens T, Bongers CM. Distribution of dopamine immunoreactivity in the rat, cat and monkey spinal cord. J Comp Neurol 1996;376:631–52.
[22] Jensen TS, Yaksh TL. Effects of an intrathecal dopamine agonist, apomorphine, on thermal and chemical evoked noxious responses in rats. Brain Res 1984;296:285–93.
[23] Kato G, Yasaka T, Katafuchi T, Furue H, Mizuno M, Iwamoto Y, Yoshimura M. Direct GABAergic and glycinergic inhibition of the substantia gelatinosa from the rostral ventromedial medulla revealed by in vivo patch-clamp analysis in rats. J Neurosci 2006;26:1787–94.
[24] Koga E, Momiyama T. Presynaptic dopamine D2-like receptors inhibit excitatory transmission onto rat ventral tegmental dopaminergic neurones. J Physiol 2000;523:163–73.
[25] Koyanagi S, Himukashi S, Mukaida K, Shichino T, Fukuda K. Dopamine D2-like receptor in the nucleus accumbens is involved in the antinociceptive effect of nitrous oxide. Anesth Analg 2008;106:1904–9.
[26] Kumazawa T, Perl ER. Excitation of marginal and substantia gelatinosa neurons in the primate spinal cord: indications of their place in dorsal horn functional organization. J Comp Neurol 1978;177:417–34.
[27] Lacey MG, Mercuri NB, North RA. Dopamine acts on D2 receptors to increase potassium conductance in neurones of the rat substantia nigra zona compacta. J Physiol 1987;392:397–416.

[28] Levant B, McCarson KE. D_3 dopamine receptors in rat spinal cord: implications for sensory and motor function. Neurosci Lett 2001;303:9–12.
[29] Liu QS, Qiao JT, Dafny N. D2 dopamine receptor involvement in spinal dopamine-produced antinociception. Life Sci 1992;51:1485–92.
[30] López-Avila A, Coffeen U, Ortega-Legaspi JM, del Angel R, Pellicer F. Dopamine and NMDA systems modulate long-term nociception in the rat anterior cingulate cortex. Pain 2004;111:136–43.
[31] Magnusson JE, Fisher K. The involvement of dopamine in nociception: the role of D(1) and D(2) receptors in the dorsolateral striatum. Brain Res 2000;855: 260–6.
[32] Millan MJ. Descending control of pain. Prog Neurobiol 2002;66:355–474.
[33] Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. Physiol Rev 1998;78:189–225.
[34] Momiyama T, Todo N, Sasa M. A mechanism underlying dopamine D1 and D2 receptor-mediated inhibition of dopaminergic neurones in the ventral tegmental area in vitro. Br J Pharmacol 1993;109:933–4.
[35] Nakatsuka T, Ataka T, Kumamoto E, Tamaki T, Yoshimura M. Alteration in synaptic inputs through C-afferent fibers to substantia gelatinosa neurons of the rat spinal dorsal horn during postnatal development. Neuroscience 2000;99:549–56.
[36] Narikawa K, Furue H, Kumamoto E, Yoshimura M. In vivo patch clamp analysis of IPSCs evoked in rat substantia gelatinosa neurons by cutaneous mechanical stimulation. J Neurophysiol 2000;84:2171–4.
[37] Paxinos G, Watson C. The rat brain in stereotaxic coordinates, Ed 6th. Amsterdam: Academic Press; 2007.
[38] Pertovaara A, Martikainen IK, Hagelberg N, Mansikka H, Någren K, Hietala J, Scheinin H. Striatal dopamine D2/D3 receptor availability correlates with individual response characteristics to pain. Eur J Neurosci 2004;20:1587–92.
[39] Potvin S, Grignon S, Marchand S. Human evidence of a supra-spinal modulating role of dopamine on pain perception. Synapse 2009;63:390–402.
[40] Sandkühler J. The organization and function of endogenous antinociceptive systems. Prog Neurobiol 1996;50:49–81.
[41] Sheng HY, Qu CL, Huo FQ, Du JQ, Tang JS. D2-like but not D1-like dopamine receptors are involved in the ventrolateral orbital cortex-induced antinociception: a GABAergic modulation mechanism. Exp Neurol 2009;215:128–34.
[42] Skagerberg G, Bjorklund A, Lindvall O, Schmidt RH. Origin and termination of the diencephalo-spinal dopamine system in the rat. Brain Res Bull 1982;9: 237–44.
[43] Skagerberg G, Lindvall O. Organization of diencephalic dopamine neurones projecting to the spinal cord in the rat. Brain Res 1985;342:340–51.
[44] Sonohata M, Furue H, Katafuchi T, Yasaka T, Doi A, Kumamoto E, Yoshimura M. Actions of noradrenaline on substantia geltatinosa neurones in the rat spinal cord revealed by *in vivo* patch recording. J Physiol 2003;555:515–26.
[45] Spike RC, Todd AJ. Ultrastructural and immunocytochemical study of lamina II islet cells in rat spinal dorsal horn. J Comp Neurol 1992;323:359–69.
[46] Sugiura Y, Lee CL, Perl ER. Central projection of identified, unmyelinated (C) afferent fibers innervating mammalian skin. Science 1986;234:358–61.
[47] Swanson LW, Kuypers HG. The paraventricular nucleus of the hypothalamus: cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labeling methods. J Comp Neurol 1980;194:555–70.
[48] Tamae A, Nakatsuka T, Koga K, Kato G, Furue H, Katafuchi T, Yoshimura M. Direct inhibition of substantia gelatinosa neurons in the rat spinal cord by activation of dopamine D2-like receptors. J Physiol 2005;568:243–53.
[49] Taylor BK, Joshi C, Uppal H. Stimulation of dopamine D2 receptors in the nucleus accumbens inhibits inflammatory pain. Brain Res 2003;987: 135–43.
[50] Vallone D, Picetti R, Borrelli E. Structure and function of dopamine receptors. Neurosci Biobehav Rev 2000;24:125–32.
[51] Waszczak BL, Martin LP, Greif GJ, Freedman JE. Expression of a dopamine D2 receptor-activated K^+ channel on identified striatopallidal and striatonigral neurons. Proc Natl Acad Sci USA 1998;95:11440–4.
[52] Wei H, Viisanen H, Pertovaara A. Descending modulation of neuropathic hypersensitivity by dopamine D2 receptors in or adjacent to the hypothalamic A11 cell group. Pharmacol Res 2009;59:355–63.
[53] Weil-Fugazza J, Onteniente B, Audet G, Philippe E. Dopamine as trace amine in the dorsal root ganglia. Neurochem Res 1993;18:965–9.
[54] Yoshimura M, Furue H. Mechanisms for the anti-nociceptive actions of the descending noradrenergic and serotonergic systems in the spinal cord. J Pharmacol Sci 2006;101:107–17.
[55] Yoshimura M, Jessell TM. Primary afferent-evoked synaptic responses and slow potential generation in rat substantia gelatinosa neurons in vitro. J Neurophysiol 1989;62:96–108.
[56] Yoshimura M, Nishi S. Blind patch-clamp recordings from substantia gelatinosa neurons in adult rat spinal cord slices: pharmacological properties of synaptic currents. Neuroscience 1993;53:519–26.
[57] Yoshimura M, Nishi S. Primary afferent-evoked glycine- and GABA-mediated IPSPs in substantia gelatinosa neurones in the rat spinal cord in vitro. J Physiol (Lond) 1995;482:29–38.

PAIN RESEARCH 26 (2011) 11–18

Original Article

Examination of subjective sensations and vasomotor reaction to environmental temperature changes in chronic pain patients with impaired cold sensation

Hiroki Sakurai[1,2], Jun Sato[2,3], Takahiko Yoshimoto[2], Yusuke Ohmichi[2]
Atsuko Morimoto[2], Kenichi Arai[2], and Takahiro Ushida[2]

[1]Faculty of Health Sciences, Hamamatsu University
[2]Multidisciplinary Pain Center, Aichi Medical University
[3]Research Institute of Environmental Medicine, Nagoya University

Abstract

Symptoms of pain in chronic pain patients are known to influence by ambient temperature (AT) change and some of these patients also show temperature unadaptability. These clinical features suggest that alteration of autonomic thermoregulatory systems might be underlying in development and maintenance of chronic pain. In this study, we examined effects of AT change exposure to the pain intensity and autonomic thermoregulatory function in chronic pain patients with impaired cold sensitivity. Two upper extremity neuropathic pain patients who have impaired cold sensitivity in whole body were participated in the present study. The subject sat a chair in an artificial climate chamber (27°C, 50% RH) during the experiment. After the baseline measurements, room temperature was decreased to 15°C in 25 min (cold exposure) and then increased to 40°C in 15 - 20 min (heat exposure). Pain intensity and thermal sensation (cold–cool–warm–hot feeling) during the exposure were frequently measured on a numerical rating scale. Skin temperature, skin blood flow (SBF), and sweating in both hands, and tympanic temperature were also measured continuously throughout the experimental period. In one patient, SBF showed lower baseline value in the affected limb than the healthy limb. During cold exposure, both patients showed increase in their pain intensity in the affected limb, but not expressed any cold feeling in spite of lowering tympanic temperature. Cold exposure decreased SBF in both hands of patients. During heat exposure, both patients expressed hot feeling as an ambient temperature gradually rises, but did not show any change in pain intensity. Heat exposure increased sweating, SBF in both hands of patients. The present study indicated that lowering ambient temperature increased pain intensity in the affected limb of two patients irrespective of impaired cold sensitivity. In these subjects, however, SBF and sweating responded in a different manner to the ambient temperature changes. This could reflect differences in autonomic thermoregulatory function between these patients.

Keywords
Environmental change; Chronic pain; Thermoregulatory function

Received: 17 January 2011
Accepted: 9 February 2011

寒さを感じなくなった慢性痛患者に対する環境温曝露試験

櫻井 博紀[1,2]／佐藤 純[2,3]／吉本 隆彦[2]／大道 裕介[2]／森本 温子[2]／新井 健一[2]／牛田 享宏[2]

[1]浜松大学 保健医療学部，[2]愛知医科大学医学部 学際的痛みセンター，[3]名古屋大学 環境医学研究所

はじめに

慢性痛患者では痛みだけでなく運動機能や発汗，自律系機能の障害も伴っていることが多く，天気の変わり目や寒くなると症状が悪化するといった訴えを経験する。気象変化の影響を受けやすい運動器の慢性痛としては関節リウマチ[1,2,3,4]，腰痛症[5,6]，線維筋痛症[7,8]の報告例が多い。腰痛症において天気と痛みの関係を6ヵ月間にわたり調査したものでは，天気に敏感な患者群では気温と気圧が低い時に痛みの訴えが強いことが示されている[9]。これらの報告以外にも，神経絞扼症候群[10]，幻肢痛[11]，骨関節炎[1]，片頭痛[12]，ヘルペス後神経痛[13]，反射性交感神経性ジストロフィ[10,14]などの多くの慢性痛疾患において，気象変化と疼痛の程度に有意な相関性が示されている。

また，慢性痛モデル動物であるCCIモデルで寒冷曝露することにより疼痛行動が増加するといった報告もある[15]。

そこで，本研究では慢性痛の発症に伴って寒さを感じなくなったと訴える患者に対して，人工的に気温を変化させることで気象痛が再現されるかどうか検討し，そのときの自律神経系動態についても解析した。

方　法

対象は外傷後に上肢の神経障害性疼痛をきたし，寒さを感じなくなったと訴える慢性痛患者2名およびコントロール（健常者）1名である(Table 1)。慢性痛患者のうち被験者Aは右上肢のCRPS（複合性局所疼痛症候群：complex regional pain syndrome）のため広汎脊髄後根進入部遮断術（DREZotomy）と後根切断術(Rhisotomy)を行った患者である。手術の影響もあり，症状として自発痛，感覚脱失，運動麻痺を認めている（罹患期間13年)。被験者Bは自動二輪での事故による右腕神経叢引き抜き損傷(全型損傷)で，罹患期間4ヵ月，症状として自発痛，感覚脱失，運動麻痺がある。

本研究は愛知医科大学倫理委員会および浜松大学倫理委員会の承認のもとに行った。プロトコルに沿って，被験者には本研究の趣意を十分に説明し，文書にて同意を得たのちに行った。

Table 1 Clinical characteristics of subjects

	Sex	Age	Disease	Affected limb	Duration	Symptoms
Chronic pain patient A	M	64	Postoperative disorder on CRPS	Right upper limb	13 Y	spontaneous pain, anesthesia, motor paralysis
Chronic pain patient B	M	36	Brachial plexus injury	Right upper limb	4 M	spontaneous pain, anesthesia, motor paralysis
Healthy subject	M	39	—	—	—	—

実験は，温・湿度が制御可能な人工気候室内(これまでに我々の人工気候室で行った論文参照)にて，全身の寒冷・暑熱曝露を行い，主観的感覚の変化と血行動態，発汗機能を解析した。被験者は人工気候室内でハーフパンツのみ着用し，椅子に座った状態で計測を行った。

寒冷曝露試験では被験者に27℃，50%の定常状態から25分程度で15℃，50%に寒冷曝露を行い，その後5分間保持した。また，暑熱曝露試験では27℃，50%の定常状態から15分程度で40℃，50%に暑熱曝露を行い，30分間保持した。寒冷・暑熱曝露に伴う主観的感覚の変化として，患部の自発痛をNRS(numerical rating scale) にて，また，全身の温度感覚として快適さと温度感覚のカテゴリースケール[16] (Table 2) を用いて，5分ごとに聴取した。自律神経系動態のパラメータとして皮膚温をサーモグラフィ(Avio: Neo Thermo)で5分ごとに測定し，また，サーミスタで鼓膜温 (深部温)，レーザードップラー血流計(ADVANCE: ALF21)で上肢皮膚血流量を同時に温度曝露中連続測定した。暑熱曝露においてはこれらのパラメータとともに上肢の発汗量を換気カプセル法にて測定した。換気カプセル法は，機密性の小型のカプセルを皮膚に装着し，カプセル内に乾燥ガスを流し，汗の蒸発による湿気の変化分を湿度検知器で発汗量を連続的に測定する方法である[17]。

Table 2 Category scale of temperature sensation

Thermal sensation
7. hot
6. warm
5. slightly warm
4. neutral
3. slightly cool
2. cool
1. cold

結　果

(1) 27℃, 50%の定常状態の評価

健常者では痛みの訴えはなく，皮膚血流では示指にて左右差はなく，皮膚温に関しても左右差はなかった。一方，被験者Aは痛み感覚はNRSで10段階中2〜3であった。患側の手関節以遠は視診にて健側に比べて皮膚色は白っぽく，筋も萎縮しておりアトロフィー状にみえた。皮膚血流は患側の不随意運動のため前腕での測定としたが，患側0.13 (AU)，健側0.14 (AU) であり，左右差はみられなかった。皮膚温は健側に比べて患側の手関節以遠が低かっ

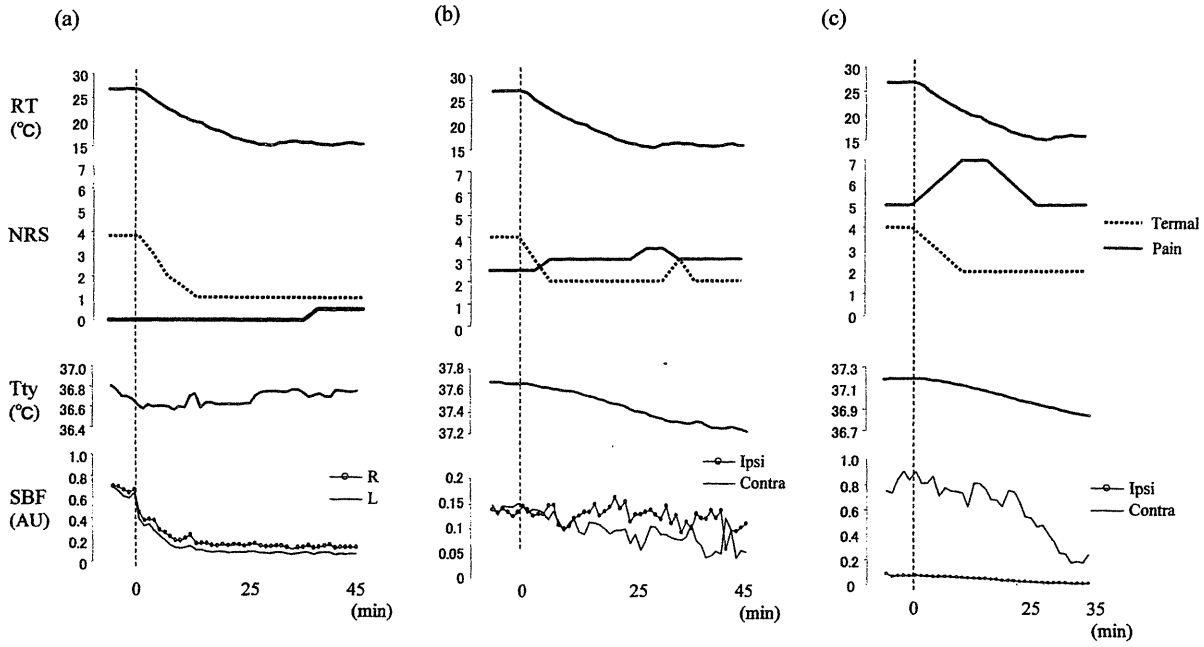

Fig.1 The time courses of changes in each parameters during cold exposure.

(a) Healthy subject, (b) chronic pain patient (subject A), (c) chronic pain patient (subject B).
RT = room temperature; NRS = numerical rating scale (Thermal, Pain); Tty = tympanic temperature; SBF = skin blood flow.

た。被験者Bでは痛み感覚はNRSで10段階中5であった。患側の手関節以遠は視診にて健側に比べて皮膚色は赤黒く，むくんでいた。皮膚温は健側に比べて患側が低く，皮膚血流も患側0.08(AU)，健側0.78(AU)と患側が健側に比べて約1/10であった (Fig.1)。

(2) 寒冷曝露試験

健常者では寒冷曝露開始後15分程度で温度感覚として寒さを感じるようになった。寒冷曝露によって痛み感覚は変化なくNRSで0と痛みなしのままであった。鼓膜温は36.7℃で変化なしであった。皮膚温は全体に低下がみられた。皮膚血流は寒冷曝露開始からすぐに左右同期して急速な減少がみられ，変化の程度としては寒冷曝露開始後25～30分の5分間の平均では，曝露前に対して左側88%，右側79%の減少率であった。

被験者Aは寒冷曝露開始10分後から涼しいという温度感覚となったが寒いという温度感覚にはならず，むしろ心地よいという表現であった。痛み感覚は寒冷曝露開始10分後からNRSで2から3に変化し，痛みの増強がみられた。鼓膜温は37.7℃から37.3℃への0.4℃の低下であった。皮膚温は全体に低下がみられた。皮膚血流は寒冷曝露によって緩徐な減少がみられた。変化の程度としては寒冷曝露開始後25～30分の5分間の平均では，曝露前に対して患側4%，健側35%の減少率であった。

被験者Bは寒冷曝露開始10分後から涼しいという温度感覚となったが寒いという温度感覚にはならず，むしろ心地よいという表現であった。痛み感覚は寒冷曝露開始後からNRSで5から徐々に上昇し曝露10分後では7となった

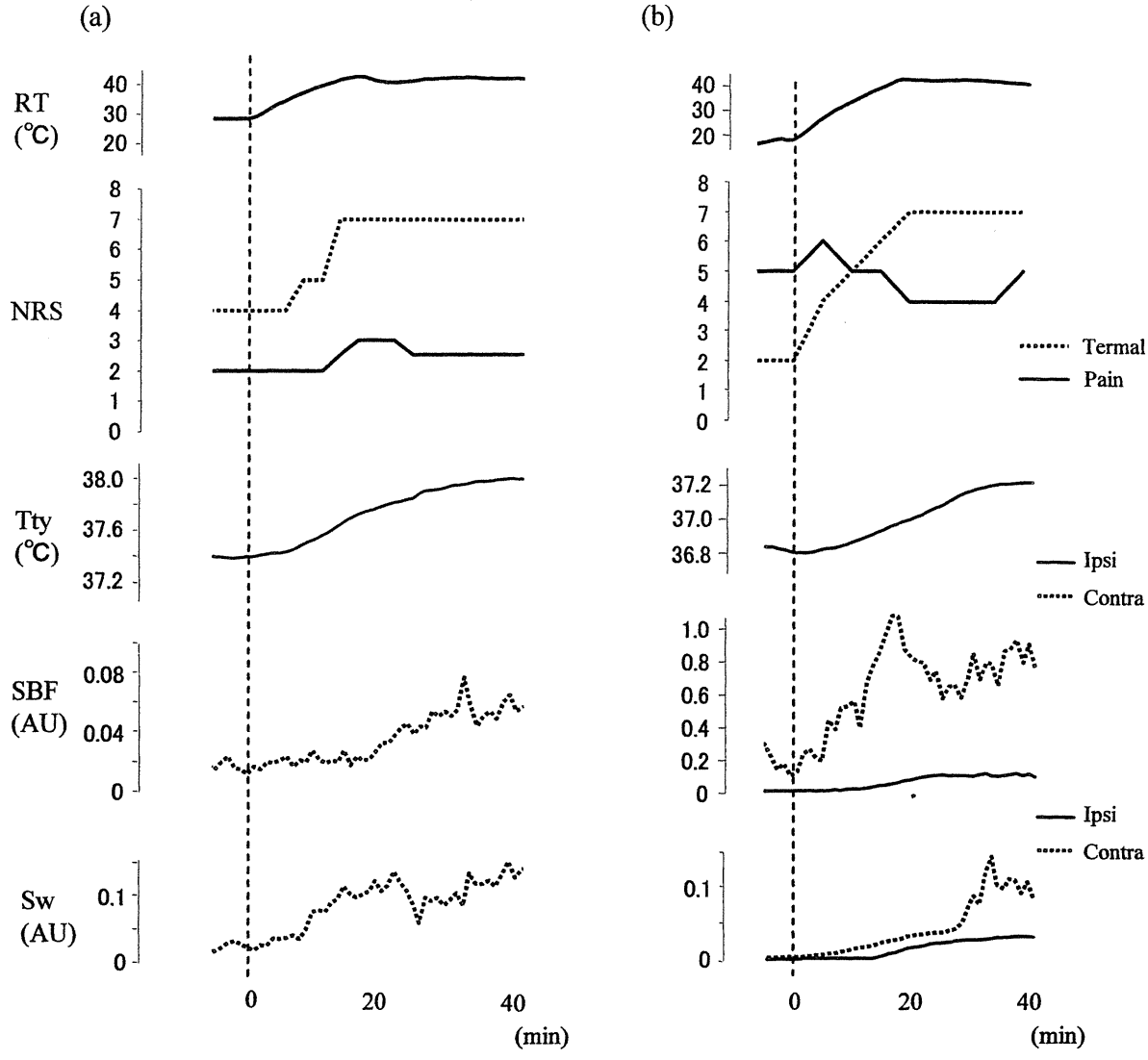

Fig.2 The time courses of changes in each parameters during heat exposure in chronic pain patients.
(a) subject A, (b) subject B.
RT = room temperature; NRS = numerical rating scale (Thermal, Pain); Tty = tympanic temperature; SBF = skin blood flow; Sw = sweating.

が，曝露15分後から再び低下しはじめ25分後には最初の5に戻った。鼓膜温は37.2℃から36.9℃への0.3℃の低下であった。皮膚温は全体に低下がみられた。皮膚血流は寒冷曝露によって緩徐な減少がみられた。変化の程度としては寒冷曝露開始後25～30分の5分間の平均では，曝露前に対して患側78%，健側69%の減少率であった（Fig.1）。

(3) 暑熱曝露試験

被験者Aでは暑熱曝露開始15分後から暑いという温度感覚となり不快感を訴えた。痛み感覚は暑熱曝露開始20分後からNRSで2から3に変化し，痛みの増強がみられた。鼓膜温は

37.3℃から37.9℃への0.6℃の上昇であった。皮膚温は全体に上昇がみられた。皮膚血流は暑熱曝露によって緩徐な増加がみられた。変化の程度としては，暑熱曝露開始後15～45分での40℃保持の30分間の平均では，曝露前に対して患側53%，健側135%の増加率であった。発汗は健側のみの測定ではあるが，暑熱曝露前から若干の発汗がみられ，暑熱曝露によって緩徐な増加がみられ，暑熱曝露45分間での発汗量は3.66 mg/cm^2/minであった。

被験者Bでは暑熱曝露開始20分後から暑いという温度感覚となり不快感を訴えた。痛み感覚は暑熱曝露開始5分後にNRSで5から6に増加したが，その後は4と減少し，40分後では5に戻った。鼓膜温は36.8℃から37.2℃への0.4℃の上昇であった。皮膚温は全体に上昇がみられた。皮膚血流は暑熱曝露によって緩徐な増加がみられた。変化の程度としては，暑熱曝露開始後15～45分での40℃保持の30分間の平均では，曝露前に対して患側330%，健側60%の増加率であった。発汗も暑熱曝露によって緩徐な増加がみられ，暑熱曝露45分間での発汗量は患側0.68 mg/cm^2/min，健側2.81 mg/cm^2/minであった (Fig.2)。

考　察

今回，寒さを感じない慢性痛患者2名に対して人工的な寒冷曝露を行った結果，いずれも寒さを感じることは無かった。一方で，痛みは気温低下時に増強する現象を再現することができた。また，病態により差異はあるものの，自律神経系動態のパラメータにおいて患側の変化が少ないなどの非対称性がみられた。

気温が低下した際に痛み，しびれ感などの症状が悪化する慢性痛患者は多く経験する。一般に寒冷環境において痛みが増強する要因として，気温低下による自律神経系活動の変化が考えられる。今回の研究では，健常者においても寒冷曝露では皮膚血流が低下し，交感神経活動による皮膚血管収縮が起こっていることが示唆されている。慢性痛の中には，交感神経依存性疼痛 (SMP: sympathetically-maintained pain) などのように交感神経系が深く関わっている病態が存在することが知られている[18]が，慢性炎症時や神経因性疼痛の病態時には少なからず交感神経と痛覚線維間の組織学的・機能的な異常連絡が出現し，交感神経活動が直接的に痛覚線維を興奮させ痛みを引き起こすメカニズムが駆動することが知られている[19,20]。

ここで，寒さを感じなくなった慢性痛患者において，皮膚血流が健側では健常者と慢性痛患者がほぼ同じであること，また，寒冷曝露により皮膚血流は健常者，慢性痛患者ともに低下し，健常者では左右同期して寒冷曝露開始後急速に低下したのに対し，慢性痛患者では緩徐な低下であり，さらに，被験者Aでは健側に対して患側の変化が小さかったことから，血管収縮反応が慢性痛患者では小さいことが示唆され，交感神経系の状態自体は逆に同等もしくは低下している可能性が考えられる。CRPS患者の患側でアドレナリンレベルが低下しているとの報告[21]もあることから，今回の慢性痛患者においても同様のことが言えるかもしれない。

一方，他の慢性痛患者と異なり，今回の慢性痛患者では15℃と健常者では寒さを感じる環境温度においても寒さを感じず，訴えが再現された。この寒さを感じない要因として，末梢の受容器の機能低下や中枢での体温調節機能の変容が考えられる。寒冷曝露により温度感覚とし

て「中立」から「涼しい」に変化したことや皮膚血流の低下がみられており，寒冷に対する温度検出系は末梢・中枢とも消失しているわけではない。「涼しい」という温度感覚であったことから，温度閾値に変容が生じて寒いと判断するまで到達していないことが考えられ，また，鼓膜温が寒冷曝露により健常者に比べて低下が大きい傾向もみられており，体温調節発現の設定点(セットポイント)が低体温側に移動し，寒冷順化が生じている可能性が考えられる。

また，暑熱曝露によって発汗はみられたが，被験者Bでは健側に比べ患側では発汗が生じるのが遅く，発汗量も少なかったことから患側末梢における発汗交感神経活動の低下が考えられ，また，健側では27℃の定常状態でも発汗がみられており，中枢での発汗のセットポイントが低温側に移動している可能性が考えられる。すなわち，寒さを感じにくくなった慢性痛患者においては，寒冷・暑熱変化に対する体温調節機構におけるセットポイントが全体に低い温度領域に移動している可能性が推察される。この要因としては，冷感受性線維群の感作現象[22]やTRPM8といった温度感受性チャネルなど末梢の受容器の感受性変化[23]や，中枢での温・冷ニューロンの感受性変化，セットポイント調節機構の変化が考えられるが，どの部分が重要な役割をしているかは今後さらなる検討が必要である。

結　語

本研究における寒さを感じなくなった慢性痛患者においては，交感神経系活動の低下，セットポイントの低温側への移動が生じている可能性が示唆されたことから，疼痛系の異常だけでなく，気温変化に対する適応能力の変容として温度受容器などを介した感覚系，さらには交感神経系や体温調節系の変化が様々な形で生じている可能性が考えられた。今後，慢性痛への自律神経系や体温調節系の関与についてさらなる検討が必要と考えられる。

文　献

1) Hollander, J.L., Environment and musculoskeletal diseases, Archiv. Eniviron. Health., 6 (1963) 89-98.
2) Rasker, J.J., Peters, H.J., et al., Influence of weather on stiffness and force in patients with rheumatoid arthritis, Scand. J. Rheumatol., 15 (1986) 27-36.
3) Guedj, D., Weinberger, A., Effect of weather conditions on rheumatic patients, Ann. Rheum. Dis., 49 (1990) 158-159.
4) Gorin, A.A., Smyth, J.M., et al., Rheumatoid arthritis patients show weather sensitivity in daily life, but the relationship is not clinically significant, Pain, 81 (1999) 173-177.
5) Shutty, M.S. Jr., Cundiff, G., et al., Pain complaint and the weather: weather sensitivity and symptom complaints in chronic pain patient, Pain, 49 (1992) 199-204.
6) Hendler, N.H., Jamison, R.N., et al., The relationship of diagnoses and weather sensitivity in chronic pain patients, J. Neuromuscloskeletal. System., 3 (1995) 10-15.
7) Yunus, M., Masi, A.T., et al., Primary fibromyalgia (Fibrositis): Clinical study of 50 patients with matched normal controls, Seminars in Arthritis and Rheumatism, 11 (1981) 151.
8) Fors, E.A., Sexton, H., Weather and the pain in fibromyalgia: are they related? Ann. Rheum. Dis., 61 (2002) 247-250.
9) McGorry, R.W., Meteorological conditions and self-report of low back pain, Spine, 23 (1998) 2096-2102.
10) Hendler, N.H., Jamison, R.N., et al., The relationship of diagnoses and weather sensitivity in chronic pain patients, J. Neuromuscloskeletal. System., 3 (1995) 10-15.

11) Mitchell, S.W., The relations of pain to weather, being a study of the natural history of a case of traumatic neuralgia, Am. J. Med. Sci., 146(1877) 305-329.

12) Anderson, B. Jr., Heyman, A., et al., Migraine-like phenomena after decompression from hyperbaric environment, Neurology, 15(1965) 1035-1040.

13) Nurmikko, T., Bowsher, D., Somatosensory findings in postherpetic neuralgia, J. Neurol., 53(1990)135-141.

14) Hooshmand, H., Chronic pain: reflex sympathetic dystrophy, Prevention and management, CRC Press, Inc., Florida, 1993.

15) Sato, J., Morimae, H., et al., Effects of lowering ambient temperature on pain-related behaviors in a rat model of neuropathic pain, Exp. Brain. Res., 133(2000)442-449.

16) Stolwijk, J.A.J., Hardy, J.D., Partitional calorimetric studies of responses of man to thermal transients, J. Appl. Physiol., 21(1966) 967-977.

17) 大橋俊夫, 温熱性・精神性発汗試験, 日本自律神経学会 編, 自律神経機能検査第4版, 文光堂, 東京, 2007, pp224-229.

18) McMahon, S.B., Mechanisms of sympathetic pain, Br. Med. Bull., 47(1991)584-600.

19) Sato, J., Perl, E.R., Adrenergic excitation of cutaneous pain receptors induced by peripheral nerve injury, Science., 29(1991)1608.

20) Sato, J., Suzuki, S., et al., Adrenergic excitation of cutaneous nociceptors in chronically inflamed rats, Neurosci. Lett., 164(1993)225.

21) Drummond, P.D., Finch, P.M., et al., Reflex sympathetic dystrophy: the significance of differing plasma catecholamine concentrations in affected and unaffected limbs, Brain, 114(1991) 2025-2036.

22) Takahashi, K., Sato, J., et al., Response of C-fiber low threshold mechanoreceptors and nociceptorsto cold were facilitated in rats persistently inflamed and hypersensitive to cold, Neurosci. Res., 47(2003)409.

23) Tominaga, M., Caterina, M.J., Thermosensation and pain, J. Neurobiol., 61(2004)3-12.

Address for correspondence: Hiroki Sakurai
Faculty of Health Sciences, Hamamatsu University
Hamamatsu, Shizuoka 431-2102, Japan
Tel: 053-428-3511 / Fax: 053-428-1202
E-mail: hsaku@hamamatsu-u.ac.jp

The World Journal of Biological Psychiatry, 2011; Early Online, 1–7

ORIGINAL INVESTIGATION

The *KCNH2* gene is associated with neurocognition and the risk of schizophrenia

RYOTA HASHIMOTO[1–3], KAZUTAKA OHI[2,3], YUKA YASUDA[2,3], MOTOYUKI FUKUMOTO[2,3], HIDENAGA YAMAMORI[2–4], KOUZIN KAMINO[2,5], TAKASHI MORIHARA[2], MASAO IWASE[2], HIROAKI KAZUI[2] & MASATOSHI TAKEDA[1,2]

[1]*Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, Osaka, Japan,* [2]*Department of Psychiatry, Osaka University Graduate School of Medicine, Osaka, Japan,* [3]*CREST (Core Research for Evolutionary Science and Technology) of JST (Japan Science and Technology Agency), Saitama, Japan,* [4]*Department of Molecular Neuropsychiatry, Osaka University Graduate School of Medicine, Osaka, Japan, and* [5]*Shoraiso National Hospital, Yamatokoriyama, Nara, Japan.*

Abstract

Objectives. A genetic variant (rs3800779; M30) in the *KCNH2* gene has been associated with schizophrenia, a lower intelligence quotient (IQ) and processing speed scores, altered brain functions and increased *KCNH2-3.1.* mRNA levels in the hippocampus. The aims of this study were to investigate whether the *KCNH2* polymorphism is associated with schizophrenia-related neurocognitive deficits and to confirm the association between the variant and schizophrenia. *Methods.* The effects of the risk genotype on IQ and seven neurocognitive batteries were examined by the analysis of covariance in 191 healthy subjects. We performed a meta-analysis of the association between M30 and schizophrenia using five independent ethnic groups (1,720 cases; 2,418 controls). *Results.* Consistent with the previous study, we provided evidence that subjects with the risk T carriers had significantly lower IQ scores than those with the G/G genotype ($P = 0.048$). Of the seven neurocognitive batteries, subjects with the risk genotype demonstrated lower performances on attention/vigilance ($P = 0.0079$) and working memory ($P = 0.0066$) relative to subjects with the G/G genotype. Meta-analysis demonstrated evidence for an association between M30 and schizophrenia without showing heterogeneity across studies (odds ratio = 1.18; $P = 0.0017$). *Conclusions.* These data suggest that the *KCNH2* polymorphism could be associated with schizophrenia-related neuropsychological deficits and the risk of developing schizophrenia.

Key words: *schizophrenia, KCNH2 (potassium channel, voltage-gated subfamily H, member 2), intelligence quotient (IQ), single nucleotide polymorphism (SNP), meta-analysis, neurocognition*

Introduction

Schizophrenia is a common, complex psychiatric disease characterized by both clinical and genetic heterogeneity. There are strong genetic components of the disease with an estimated heritability of approximately 80% (Cardno and Gottesman 2000; Tsuang 2000). Attempts have been made to minimize this heterogeneity and to clarify the genetic architecture. One strategy for gene discovery proposes using quantitative neurobiological traits as intermediate phenotypes instead of relying on the diagnosis of schizophrenia alone to identify cases for investigation (Meyer-Lindenberg and Weinberger 2006; Tan et al. 2008a). This strategy has the potential to reduce clinical and genetic heterogeneity by applying alternative phenotypes that better reflect the underlying genetic vulnerability than does diagnostic categorization. Neurocognitive deficits, a core component of schizophrenia (Green 2006), are considered promising intermediate phenotypes for gene

Correspondence: Ryota Hashimoto, Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, D3, 2-2, Yamadaoka, Suita, Osaka, 565-0871, Japan. Tel: +81 6 6879 3074. Fax: +81 6 6879 3059. E-mail: hashimor@psy.med.osaka-u.ac.jp

(Received 22 February 2011; accepted 7 July 2011)
ISSN 1562-2975 print/ISSN 1814-1412 online © 2011 Informa Healthcare
DOI: 10.3109/15622975.2011.604350

discovery in schizophrenia (Snitz et al. 2006; Husted et al. 2009). There is substantial evidence suggesting that most cognitive abilities have a genetic basis (Chen et al. 1998; Posthuma et al. 2001; Berrettini 2005; Husted et al. 2009). The estimated heritabilities of processing speed, attention/vigilance, verbal intelligence quotient (IQ) and performance IQ are 33–48, 48–62, 85 and 69%, respectively.

Recently, Huffaker et al. identified a potential schizophrenia susceptibility (human ether-a-go-go-related) gene, *KCNH2,* which encodes a voltage-activated potassium channel (Huffaker et al. 2009). The *KCNH2* gene contains 15 exons spanning 33 kb on chromosome 7q35–q36. A genetic variant (rs3800779; M30) in the *KCNH2* gene predicts lower IQ and processing speed scores, decreased hippocampal volume, altered memory-linked hippocampal functions and working memory-linked prefrontal functions. It also predicts increased expression levels of a primate- and brain-specific *KCNH2*-3.1 isoform in the hippocampus (Huffaker et al. 2009). Expression of *KCNH2*-3.1 in rodent cortical neurons causes a marked alteration in KCNH2 channel physiology resulting in high-frequency, nonadapting neuronal firing patterns (Huffaker et al. 2009). In this study, we examined the effects of the M30 genotype on IQ and seven neurocognitive functions shown to be associated with genetic liability in schizophrenia. We then conducted a meta-analysis of M30 in previously reported samples added to a Japanese sample to establish further evidence for an association between the *KCNH2* gene and schizophrenia.

Methods and materials

Subjects

Neurocognitive test data were available for 191 Japanese healthy individuals (49.2% males (94/97); mean age ± SD: 36.0 ± 11.5 years; years of education ± SD: 15.5 ± 2.4 years). Data from different number of subjects were available in each test (general IQ 143 subjects, speed of processing 188, attention/vigilance 191, working memory 190, Verbal Learning and Memory 190, Visual Learning and Memory 190, Reasoning and problem solving 150, and Social cognition 86). Demographic variables for subjects included in each cognitive test are shown in Supplementary Table I (available online). Although we attempted to examine all neurocognitive tests from all subjects as much as we could, all tests data were available for 83 subjects. Because an association between an SNP in the *KCNH2* gene and cognitive function was observed in healthy controls, we attempted to replicate the previous association finding in healthy controls (Huffaker et al. 2009). The use of healthy subjects to investigate an association between a genetic variant and neurocognitive function avoids the potential confounders related to the duration of illness and medical treatment. Healthy controls were recruited by local advertisements in Osaka, Japan. Psychiatrically, medically and neurologically healthy controls were evaluated using the Structured Clinical Interview for DSM-IV-Non-Patient Edition (SCID-I/NP) to exclude individuals who had received psychiatric medications. Subjects were also excluded from this study if they had neurological or medical conditions that could potentially affect the central nervous system, such as atypical headaches, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, active cancer, cerebrovascular disease, epilepsy, seizures, substance-related disorders or mental retardation. We excluded any control subjects with neurological disorders or first- or second-degree relatives with psychiatric disorders using an unstructured interview. All subjects were biologically unrelated Japanese individuals.

The subjects for the genetic association study consisted of 478 unrelated patients with schizophrenia (48.3% males (231/247); mean age ± SD: 48.4 ± 15.7 years) and 640 unrelated healthy controls (46.3% males (296/344); mean age ± SD: 58.9 ± 21.4 years). All subjects used in this analysis are unrelated Japanese, as described previously (Ohi et al. 2009b, 2010). Cases were recruited from both outpatients and inpatients at Osaka University Hospital and the psychiatric hospitals. Each subject with schizophrenia had been diagnosed by at least two trained psychiatrists based on an unstructured clinical interview; diagnoses were made based on the criteria of the DSM-IV. Controls were recruited through local advertisements. Psychiatrically healthy controls were evaluated using unstructured interviews to exclude individuals who had current or past contact with psychiatric services. 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 was approved by the Research Ethical Committee of Osaka University.

SNP selection and SNP genotyping

We selected rs3800779 (M30) in the *KCNH2* gene because this SNP has been associated with schizophrenia, as described in the introduction (Huffaker et al. 2009). 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 custom-designed

TaqMan 5'-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, CA, USA), as described previously (Hashimoto et al. 2007). No deviation from Hardy–Weinberg equilibrium in the examined SNP was detected in patients with schizophrenia or in controls ($P > 0.05$).

Neurocognitive testing

General intellectual function was derived from the Full Scale IQ portion of the Wechsler Adult Intelligence Scale-Third Edition (WAIS-III) (Wechsler 1997). The Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Neurocognition Committee selected seven neurocognitive domains from all available factor-analytic studies of cognitive performance in schizophrenia patients (Green et al. 2004; Nuechterlein et al. 2004). Seven neurocognitive batteries were selected based upon previous studies to assess the following seven domains (Nuechterlein et al. 2004, 2008): (1) speed of processing, (2) attention/vigilance, (3) working memory, (4) verbal learning and memory, (5) visual learning and memory, (6) reasoning and problem solving, and (7) social cognition. The speed of processing was assessed using the Category Fluency Test (total number of animals named in 60 s) (Sumiyoshi et al. 2004). Attention/vigilance was evaluated using the Continuous Performance Test-Identical Pairs version (*d'*) (Cornblatt et al. 1988). Working memory was measured using the Wechsler Memory Scale-Revised (WMS-R) digit span subtest (number of correct trials) (Sugishita 2001). Verbal learning and memory was assessed using the immediate recall portion of the Rey Auditory Verbal Learning Test (Lezak 1995) in which the participants were asked to recall a list of 15 words spoken by a tester. The procedure was repeated five times (sessions 1–5), and the sum of the recalled words from sessions 1 to 5 was used for the analysis. If the participants scored 15/15, we treated the scores of the participant as 15 after the session; possible scores range from 0 to 75. Visual learning and memory was evaluated using the visual reproduction I subtest of the WMS-R (number of correct trials) (Sugishita 2001). Reasoning and problem solving was measured using the tower of Hanoi task (number of correct trials) (Ohi et al. 2009a). Social cognition was assessed using the Emotion Recognition test (correct rate of the Facial Emotion Labeling Test (FELT)) (Sekiyama et al. 2008). The subjects included in this analysis were assessed by trained clinical psychologists to obtain scores on the WAIS-III Full Scale IQ and the seven schizophrenia-related neurocognitive batteries.

Meta-analysis

The studies included in the meta-analysis were obtained using PubMed using the search terms "KCNH2" and "schizophrenia". The analyzed data encompassed all publications up to October 2010. Additionally, references cited in the publications obtained were examined to identify additional potentially relevant studies that might not be listed in PubMed. Studies were included in the meta-analysis if they met the following criteria: (1) published in a peer-reviewed journal in English and (2) included a genetic association study between the *KCNH2* gene and schizophrenia. Our meta-analysis included allele frequency data from all available case–control studies only and did not include the original family-based dataset that provided strong evidence for the positive association in the original report by Huffaker et al. (Huffaker et al. 2009). We calculated each number of alleles from the allele frequency and the odds ratio data for each study.

Statistical analyses

Statistical analyses were performed using the PASW Statistics 18.0 software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and controls or between genotypes were analyzed using χ^2-tests for categorical variables and the Mann–Whitney *U*-test for continuous variables. Based on the assumption that demographic variables such as age and education years might not be fitted to a normal distribution, we used the nonparametric Mann–Whitney test arbitrary to assess the demographic variables. The presence of Hardy–Weinberg equilibrium was examined using the χ^2-test for goodness of fit. To control for confounding factors such as age, sex and years of education, we used a one-way analysis of covariance (ANCOVA) for neurocognitive tests, based on the assumption that the neurocognitive variables could be fitted to a normal distribution. The effect of the *KCNH2* genotype on IQ was analyzed by a one-way ANCOVA with sex and years of education as covariates because the IQ scores were already corrected for age. The effects on the seven neurocognitive domains were analyzed by a one-way ANCOVA with age, sex and years of education as covariates. Bonferroni correction was applied for multiple testing on seven domains to avoid type I errors. Standardized effect sizes were indicated using Cohen's *d* and η^2.

The meta-analysis was performed using the Comprehensive Meta-Analysis software (Version 2.0, BIOSTAT, Englewood Cliffs, NJ, USA). Cochran's χ^2-based *Q*-statistical test was performed to assess possible heterogeneity among studies. The fixed-effect

Table I. Impact of M30 in the *KCNH2* gene on IQ and on seven cognitive batteries.

Variables		Healthy subjects					ANCOVA		
	n	*n*	T carrier	*n*	G/G	Cohen's *d*	*F*	*P* values	η^2
General intellectual function	143	29	107.5 ± 15.4	114	110.3 ± 10.9	−0.21	3.98	0.048	0.028
Speed of processing	188	35	22.4 ± 5.4	153	21.2 ± 4.7	0.24	0.76	0.39	0.004
Attention/vigilance	191	36	3.63 ± 0.51	155	3.75 ± 0.47	−0.24	7.20	0.0079	0.037
Working memory	190	36	15.3 ± 3.9	154	16.8 ± 3.7	−0.39	7.55	0.0066	0.039
Verbal learning and memory	190	36	56.4 ± 9.0	154	57.9 ± 7.9	−0.17	2.02	0.16	0.011
Visual learning and memory	190	36	38.8 ± 2.9	154	39.3 ± 2.1	−0.20	2.49	0.12	0.013
Reasoning and problem solving	150	31	13.2 ± 7.5	119	13.8 ± 7.1	−0.08	0.95	0.33	0.006
Social cognition	86	19	58.8 ± 13.1	67	62.3 ± 13.1	−0.27	2.49	0.12	0.030

IQ, intelligence quotient; ANCOVA, analysis of covariance. Means ± SD are shown. The effect sizes are typically categorized as small ($d = 0.20$, $\eta^2 = 0.01$), medium ($d = 0.50$, $\eta^2 = 0.06$) or large ($d = 0.80$, $\eta^2 = 0.14$). To control for confounding factors, the effect of the *KCNH2* genotype on IQ was analyzed by one-way ANCOVA with sex and years of education as covariates because the IQ scores were already corrected for age. The effects on seven neurocognitive domains were analyzed by one-way ANCOVA with age, sex and years of education as covariates.

model described by Mantel-Haenszel was applied in the absence of heterogeneity ($p > 0.05$). The significance of the pooled odds ratio (OR) was assessed using a *z*-test. The significance level for all statistical tests was set at two-tailed $P < 0.05$.

Results

The effect of the KCNH2 risk polymorphism on IQ and on seven neurocognitive batteries

There were no differences in demographic variables – age, sex, or years of education – between genotype groups in each cognitive test (Supplementary Table I available online). As shown in Table I, we found a significant genotype effect on general intellectual function ($F_{1,139} = 3.98$, $P = 0.048$). Additionally, we found significant genotype effects on attention/vigilance ($F_{1,186} = 7.20$, $P = 0.0079$) and working memory ($F_{1,185} = 7.55$, $P = 0.0066$) from the seven batteries. The effect sizes (η^2) of IQ, attention/vigilance and working memory were 0.028, 0.037 and 0.039, respectively. Subjects with the risk T carriers had lower performances on these tests than did those with the G/G genotype (Figure 1). The genotype effect on working memory remained positive after the correction for multiple tests (corrected $P = 0.046$), while the genotype effect on attention/vigilance did not reach statistical significance after the correction (corrected $P = 0.055$). No significant genotype effect was found in any other cognitive batteries ($P > 0.05$).

Association between a genetic variant in the KCNH2 gene and schizophrenia by meta-analysis

The frequency of the T allele of M30 was higher in patients (11.0%) than in controls (9.7%) in the Japanese population used in this study. The direction

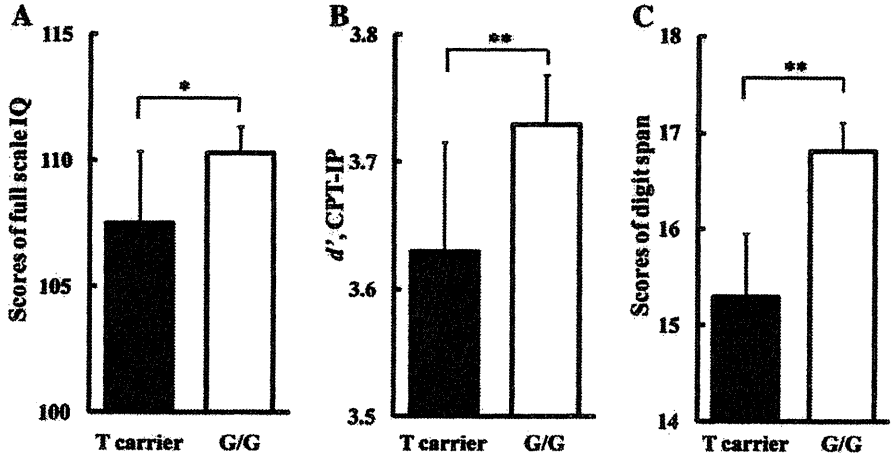

Figure 1. Association between the *KCNH2* risk genotype and IQ, attention/vigilance and working memory. IQ (A), attention/vigilance (B) and working memory (C). The *x*-axis represents T carriers and individuals with the G/G genotype. The *y*-axis represents scores of each test. Error bars represent the standard error of the mean. *$P < 0.05$, **$P < 0.01$.

Table II. Demographics of the combined studies.

	Authors	Ethnicities	Patients	Controls	Diagnostic criteria
	Case–control studies				
1	Huffaker et al. (2009)	German	905	1323	DSM-IV and ICD-10
2	Huffaker et al. (2009)	Armenian	161	161	ICD-10
3	Huffaker et al. (2009)	Italian	92	220	DSM-IV
4	Atalar et al. (2010)	Turkish	84	74	DSM-IV
5	Hashimoto et al. (present study)	Japanese	478	640	DSM-IV
	Family-based studies				
6	Huffaker et al. (2009)	USA (CBDB)	296 Caucasian families		DSM-IV
7	Huffaker et al. (2009)	USA (NIMH-GI)	71 Caucasian families		DSM-IIIR

CBDB, Clinical Brain Disorders Branch; NIMHGI, National Institute of Mental Health-Genetics Initiative.
We selected five independent case–control and two family-based data sets from previous and present studies. Two family-based samples (studies 6 and 7) were excluded from the present study because the published genotype data (affecteds and unaffecteds) were not available and the family-based samples with a family history of schizophrenia were not representative of the general population. Because we simply examined the association in the case–control samples, we included four independent case–control samples (studies 1, 2, 3, 4 and 5) (1,720 cases; 2,418 controls).

of the difference in allele frequency between patients and controls is consistent with previous studies (Huffaker et al. 2009; Atalar et al. 2010); however, the results did not represent a statistically significant difference between the groups ($z = 0.99$, $P = 0.32$, OR (95% confidence interval) = 1.15 (0.87–1.51)]. Our study size of 478 cases and 640 controls in a Japanese population had insufficient power (< 0.80) to detect as small an effect as an OR of 1.12, as described in the previous genome-wide association study (O'Donovan et al. 2008). Thus, we performed a meta-analysis to provide enough power to detect such a small effect. We included five independent case-control samples, as described in Table II (1,720 cases, 2,418 controls) (Huffaker et al. 2009; Atalar et al. 2010). The meta-analysis of M30 in all available schizophrenia data sets provided evidence for an association with schizophrenia ($z = 3.14$, $P = 0.0017$, OR (95% confidence interval) = 1.18 (1.06–1.31)] and no evidence for heterogeneity across studies ($Q = 3.55$, $P = 0.47$) (Table III, Figure 2). A sensitivity analysis revealed that the evidence for the association was not dependent upon the inclusion of any one data set (Supplementary Figure 1 available online).

Discussion

In this study, we replicated the association between the risk genotype *KCNH2* and IQ, and we further demonstrated the associations of the genotype with attention/vigilance and working memory in healthy Japanese subjects. We provided evidence that subjects with the risk T carriers had lower performances on these cognitive tests than did those with the G/G genotype. The effect sizes of the differences in these tests between individuals with T carriers and those with the G/G genotype were small to medium. Huffaker et al. reported a significant association between the M30 genotype and performance on IQ testing and on processing speed, which was extracted as a factor in healthy subjects (Huffaker et al. 2009). We did not find an association between processing speed and the risk genotype; however, we found associations between attention/vigilance and working memory and the risk genotype. To assess genotype

Table III. Comparison of allele frequencies of the *KCNH2* polymorphism (M30) in the combined samples.

	SCZ, Number of alleles (%)			CON, Number of alleles (%)			Statistics for each study		
M30 (rs3800779)	*T*	*G*	Sum	*T*	*G*	Sum	*P* value (*z*)	OR (95% CI)	Weight (fixed)
German	615 (34.0)	1195 (66.0)	1810	820 (31.0)	1826 (69.0)	2646	**0.035 (2.10)**	1.15 (1.01–1.30)	65.4
Armenian	105 (32.5)	217 (67.5)	322	87 (27.0)	235 (73.0)	322	0.13 (1.51)	1.30 (0.93–1.82)	9.2
Italian	51 (27.9)	133 (72.1)	184	114 (26.0)	326 (74.0)	440	0.63 (0.48)	1.10 (0.75–1.62)	7.1
Turkish	55 (32.7)	113 (67.3)	168	31 (20.9)	117 (79.1)	148	**0.020 (2.33)**	1.84 (1.10–3.06)	4.1
Japanese	105 (11.0)	851 (89.0)	956	124 (9.7)	1156 (90.3)	1280	0.32 (0.99)	1.15 (0.87–1.51)	14.1
Pool	931 (27.1)	2509 (72.9)	3440	1176 (24.3)	3660 (75.7)	4836	**0.0017 (3.14)**[a]	1.18 (1.06–1.31)	

SCZ, patients with schizophrenia; CON, healthy controls.
[a]Test of heterogeneity: $Q = 3.55$, df(Q) = 4, $P(Q) = 0.47$, $I^2 = 0$.

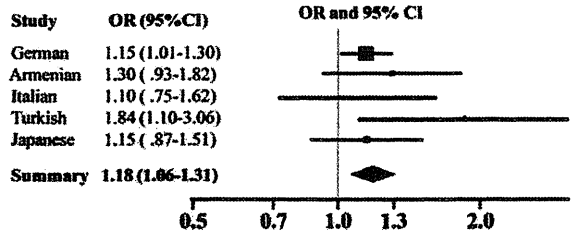

Figure 2. Forest plot of M30 results in the *KCNH2* gene based upon all combined populations. Solid squares and horizontal lines indicate the weighted odds ratios and 95% confidence intervals. The overall results are shown by the diamond. The results of the meta-analysis shown here are under the fixed-effects model.

effects on cognitive function, we measured seven domains based on MATRICS, a method different from the factor analyses-derived cognitive dimensions used in the previous study. Thus, the discrepancy between studies might be due to the differences in the cognitive dimensions, the methodology of the cognitive data analysis (direct measurements versus factors calculated by several measurements) and/or ethnic difference between European and Japanese individuals.

Second, we provide further evidence for an association between M30 and schizophrenia in combined case-control samples having now added a Japanese population. T allele frequencies of M30 in Japanese and European were different (~0.11 vs. ~0.27) based on previous and present genome dataset. However, there was no evidence for heterogeneity across studies in the meta-analysis, suggesting that there was no obvious population stratification in the combined case–control samples. It is important to note that our meta-analysis did not include the family-based sample that showed strong evidence for association in the original report. A leave-one-out sensitivity analysis revealed that the significant meta-analysis results were not being driven by a single data set. Removal of any one data set did not negate the significance of the association from the meta-analysis. As expected, the effect size observed in this study was quite small (1.16), consistent with the results from a GWAS report (O'Donovan et al. 2008). Our data are consistent with the concept that many susceptibility risk alleles for schizophrenia come from common variants of small effect. Our data also suggest that a common allele could have a stronger influence on intermediate phenotypes than on the diagnosis of schizophrenia. Despite the importance of cognitive deficits in schizophrenia, no drug has been approved for the treatment of this aspect of schizophrenia. Some antipsychotics bind and inhibit KCNH2 with affinities comparable to their affinities for the dopamine D2 receptors (Kongsamut et al. 2002). Further research will be required to clarify the role of *KCNH2* in the pathophysiology of schizophrenia. This research might potentially lead to new targets for antipsychotic medications.

There were several limitations to this study. We examined only M30 in the *KCNH2* gene, based on evidence that the variant predicts cognition, brain structure and function, and the gene expression level. We did not examine other markers of *KCNH2* gene or other genes to identify the association between those phenotype and schizophrenia. The lack of such association makes it unclear whether our results are directly linked with M30 or with other polymorphisms in linkage disequilibrium with this genetic variant. In addition, the neurocognitive tests batteries used in this study measure several complex functions (such as executive functions), not only associated with the one gene. Large number of researches show significant importance of genes connected with dopaminergic neurotransmitter system and other genes may interact with dopaminergic system (Tan et al. 2008b). Further study to investigate not only the single marker M30 but also these SNP/gene interactions is required.

Acknowledgments

We thank all individuals who participated in this study. We also appreciate Daniel R. Weinberger for critical comments on the manuscript. This work was supported in part by Grants-in-Aid from the Japanese Ministry of Health, Labor and Welfare (H18-kokoro-005, H19-kokoro-002, Comprehensive Research on Disability Health and Welfare, and the Research Committee of System Development for Clinical Trials in Psychiatry and Neurology), the Japanese Ministry of Education, Culture, Sports, Science and Technology (18689030), CREST of JST, and Japan Foundation for Neuroscience and Mental Health.

Statement of Interest

None to declare.

References

Atalar F, Acuner TT, Cine N, Oncu F, Yesilbursa D, Ozbek U, et al. 2010. Two four-marker haplotypes on 7q36.1 region indicate that the potassium channel gene HERG1 (KCNH2, Kv11.1) is related to schizophrenia: a case control study. Behav Brain Funct 6:27.

Berrettini WH. 2005. Genetic bases for endophenotypes in psychiatric disorders. Dialogues Clin Neurosci 7:95–101.

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

Chen WJ, Liu SK, Chang CJ, Lien YJ, Chang YH, Hwu HG. 1998. Sustained attention deficit and schizotypal personality features

in nonpsychotic relatives of schizophrenic patients. Am J Psychiatry 155:1214–1220.
Cornblatt BA, Risch NJ, Faris G, Friedman D, Erlenmeyer-Kimling L. 1988. The Continuous Performance Test, identical pairs version (CPT-IP): I. New findings about sustained attention in normal families. Psychiatry Res 26:223–238.
Green MF, Nuechterlein KH, Gold JM, Barch DM, Cohen J, Essock S, et al. 2004. Approaching a consensus cognitive battery for clinical trials in schizophrenia: the NIMH-MATRICS conference to select cognitive domains and test criteria. Biol Psychiatry 56:301–307.
Green MF. 2006. Cognitive impairment and functional outcome in schizophrenia and bipolar disorder. J Clin Psychiatry 67(Suppl 9):3–8; discussion 36–42.
Hashimoto R, Hashimoto H, Shintani N, Chiba S, Hattori S, Okada T, et al. 2007. Pituitary adenylate cyclase-activating polypeptide is associated with schizophrenia. Mol Psychiatry 12:1026–1032.
Huffaker SJ, Chen J, Nicodemus KK, Sambataro F, Yang F, Mattay V, et al. 2009. A primate-specific, brain isoform of KCNH2 affects cortical physiology, cognition, neuronal repolarization and risk of schizophrenia. Nat Med 15:509–518.
Husted JA, Lim S, Chow EW, Greenwood C, Bassett AS. 2009. Heritability of neurocognitive traits in familial schizophrenia. Am J Med Genet B Neuropsychiatr Genet 150B:845–853.
Kongsamut S, Kang J, Chen XL, Roehr J, Rampe D. 2002. A comparison of the receptor binding and HERG channel affinities for a series of antipsychotic drugs. Eur J Pharmacol 450:37–41.
Lezak MD. 1995. Neuropyschological assessment. 3rd ed. New York: Oxford University Press.
Meyer-Lindenberg A, Weinberger DR. 2006. Intermediate phenotypes and genetic mechanisms of psychiatric disorders. Nat Rev Neurosci 7:818–827.
Nuechterlein KH, Barch DM, Gold JM, Goldberg TE, Green MF, Heaton RK. 2004. Identification of separable cognitive factors in schizophrenia. Schizophr Res 72:29–39.
Nuechterlein KH, Green MF, Kern RS, Baade LE, Barch DM, Cohen JD, et al. 2008. The MATRICS Consensus Cognitive Battery, part 1: test selection, reliability, and validity. Am J Psychiatry 165:203–213.
O'Donovan MC, Craddock N, Norton N, Williams H, Peirce T, Moskvina V, et al. 2008. Identification of loci associated with schizophrenia by genome-wide association and follow-up. Nat Genet 40:1053–1055.
Ohi K, Hashimoto R, Yasuda Y, Kiribayashi M, Iike N, Yoshida T, et al. 2009a. TATA box-binding protein gene is associated with risk for schizophrenia, age at onset and prefrontal function. Genes, Brain and Behavior 8:473–480.
Ohi K, Hashimoto R, Yasuda Y, Yoshida T, Takahashi H, Iike N, et al. 2009b. Association study of the G72 gene with schizophrenia in a Japanese population: a multicenter study. Schizophr Res 109:80–85.
Ohi K, Hashimoto R, Yasuda Y, Yoshida T, Takahashi H, Iike N, et al. 2010. The chitinase 3-like 1 gene and schizophrenia: evidence from a multi-center case-control study and meta-analysis. Schizophr Res 116:126–132.
Posthuma D, de Geus EJ, Boomsma DI. 2001. Perceptual speed and IQ are associated through common genetic factors. Behav Genet 31:593–602.
Sekiyama R, Iwase M, Takahashi H, Nakahachi T, Takahashi K, Ikezawa K, et al. 2008. Perception of emotional and neutral facial expression correlates with social functioning in chronic schizophrenia. Seishin Igaku 50:337–344.
Snitz BE, Macdonald AWr, Carter CS. 2006. Cognitive deficits in unaffected first-degree relatives of schizophrenia patients: a meta-analytic review of putative endophenotypes. Schizophr Bull 32:179–194.
Sugishita M. 2001. Japanese Wechsler Memory Scale-Revised. Tokyo: Nihonbunkakagakusha.
Sumiyoshi C, Sumiyoshi T, Matsui M, Nohara S, Yamashita I, Kurachi M, et al. 2004. Effect of orthography on the verbal fluency performance in schizophrenia: examination using Japanese patients. Schizophr Res 69:15–22.
Tan HY, Callicott JH, Weinberger DR. 2008a. Intermediate phenotypes in schizophrenia genetics redux: is it a no brainer? Mol Psychiatry 13:233–238.
Tan HY, Nicodemus KK, Chen Q, Li Z, Brooke JK, Honea R, et al. 2008b. Genetic variation in AKT1 is linked to dopamine-associated prefrontal cortical structure and function in humans. J Clin Invest 118:2200–2208.
Tsuang M. 2000. Schizophrenia: genes and environment. Biol Psychiatry 47:210–220.
Wechsler D. 1997. Manual for the Wechsler Adult Intelligence Scale-III. San Antonio, TX: The Psychological Corporation.

Supplementary material available online

Supplementary Table I. Demographic variables for subjects included in the neurocognitive battery analysis.
Supplementary Figure 1. A sensitivity analysis of M30.