

Figure 10. Conductances associated with the action of NPY in the type II dBNST neurons. **A, D**, Representative steady-state current responses to voltage ramp pulses from -30 to -120 mV recorded from type II neurons in the periods of pre-NPY (gray) and post-NPY (black) ($1 \mu\text{M}$, 2 min) application in the absence (**A**) or presence (**D**) of ZD7288 ($10 \mu\text{M}$). The insets show the subtraction of the currents (NPY-induced net currents). Axis titles in **A** and **D** apply in the insets. **B, E**, Average traces of NPY-induced currents in type II neurons in the absence (**B**; $n = 8$) or presence (**E**; $n = 8$) of ZD7288. Gray shadows represent SEM. Vertical dashed lines show the reversal potential. **C, F**, Effects of NPY on the holding currents at -70 mV (upper) and the input resistance (lower). Gray lines represent the data obtained from individual neurons, and black symbols and lines show the averaged data obtained from eight neurons. * $p < 0.05$, ** $p < 0.01$ compared with pre-NPY application (paired t test).

2.75 ($df = 7$), $p < 0.05$, paired t test, $n = 8$), indicating that I_h constitutively depolarizes membrane potentials in type II neurons. In the presence of ZD7288, NPY did not affect either the holding currents (-11.07 ± 8.92 and -16.54 ± 7.40 pA before and after NPY application, respectively; $t = 1.47$ ($df = 7$), $p > 0.05$, paired t test; Fig. 10F, upper) or the input resistance (662.9 ± 95.8 and 791.8 ± 183.9 MΩ before and after NPY application, respectively; $t = 2.05$ ($df = 7$), $p > 0.05$, paired t test; Fig. 10F, lower). In the presence of ZD7288, the reversal potential of NPY-associated currents shifted to a more negative potential (-85.3 ± 6.2 mV, $n = 8$; Fig. 10E) compared with the reversal potential in the absence of ZD7288 (-60.4 ± 6.0 mV). These results indicate that NPY-induced hyperpolarization was mediated primarily by suppression of I_h in type II dBNST neurons. The remaining ZD7288-resistant component, which showed a negative slope conductance with the reversal potential of approximately -85 mV, might be mediated, at least in part, by closing potassium channels.

Effects of CRF and NPY on the firing activity in type II dBNST neurons

The effects of CRF and NPY on the firing activity in type II dBNST neurons were examined. A 2 min bath application of $1 \mu\text{M}$ CRF gradually depolarized membrane potentials, as observed

in the above experiments, and then induced spikes (Fig. 11A), which persisted over 30 min. The number of spikes significantly increased from 12.2 ± 7.1 spikes/1.5 min in the period before CRF application to 206.8 ± 68.1 spikes/1.5 min in the period after CRF application ($t = 3.35$ ($df = 5$), $p < 0.05$, $n = 6$, paired t test; Fig. 11B). On the other hand, NPY tended to suppress the firing activity (Fig. 11C). The number of spikes decreased from 28.5 ± 26.5 in the period before NPY application to 15.0 ± 12.3 spikes/1.5 min in the period during NPY application (Fig. 11D). In the presence of NPY, no significant increase in the spikes was observed after the application of $1 \mu\text{M}$ CRF (35.8 ± 30.8 spikes/1.5 min; $t = 0.21$ ($df = 5$), $p > 0.05$ vs control (before NPY application) and $t = 0.62$ ($df = 5$), $p > 0.05$ vs NPY alone (during NPY application), $n = 6$, paired t test; Figure 11D).

Discussion

In the current study, using a conditioned place paradigm and an *in vivo* microdialysis technique, we demonstrated that enhanced neurotransmission via CRF receptors within the dBNST plays a key role in the negative affective component of pain. Additionally, we showed that intra-dBNST injection of NPY suppressed pain-induced CPA. To our knowledge, these findings are the first reported providing evidence of the opposing roles of CRF and NPY within the dBNST in the negative affective component of pain. We also demonstrated that intra-dBNST CRF injection produced CPA even in the absence of formalin-

evoked noxious stimulation and that coadministration of NPY suppressed CRF-induced CPA. These results suggest that NPY injected into the dBNST may suppress formalin-induced CPA through inhibition of CRF-mediated neurotransmission.

Behavioral studies using a CPA test have successfully elucidated the neural substrates and mechanisms underlying the affective component of pain (Johansen et al., 2001; Tanimoto et al., 2003; Deyama et al., 2008). Because this test was based on associative learning between a noxious stimulus-induced aversive affect and a neutral environmental context, whether the attenuation of CPA was due to impairment of associative learning or suppression of the primary aversive affect is difficult to determine. In the present study, we demonstrated that intra-dBNST CRF injection produced CPA even in the absence of formalin-induced noxious stimulus. This indicates that the activation of CRF receptors within the dBNST is sufficient to produce the negative affective states. Therefore, the attenuation of CPA by inhibition of intra-dBNST CRF-mediated neurotransmission may be due to the reduction of the primary aversive affect.

To investigate the cellular mechanisms of CRF-induced CPA and the counteracting effect of NPY, we examined the effects of CRF and NPY on neuronal excitability in dBNST neurons. Interestingly, we found that only type II, not type I or III, neurons responded to both CRF and NPY in the dBNST. Bath application of CRF depolarized

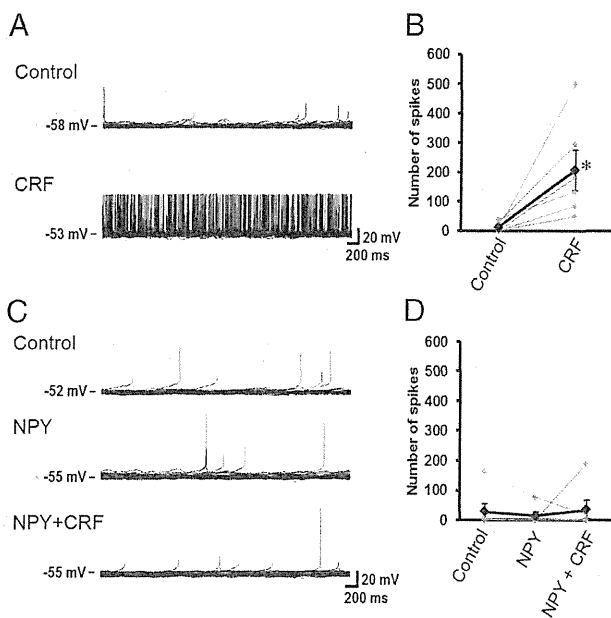


Figure 11. Effects of CRF on firing activity of type II dBNST neurons in the absence (*A, B*; $n = 6$) and presence (*C, D*; $n = 6$) of NPY. *A, C*, Representative traces (36 overlaid) from current-clamp recordings in type II dBNST neurons before and after application of peptides. *B, D*, Effects of CRF and NPY on the number of spikes in type II dBNST neurons. Gray symbols and lines show data obtained from individual neurons, and black symbols and lines show averaged data obtained from six neurons. * $p < 0.05$ compared with pre-CRF application control (paired t test).

membrane potentials significantly in type II neurons. In contrast, NPY-induced hyperpolarization in type II neurons. Additionally, coapplication of NPY extinguished the CRF-induced depolarization. Similar results were observed in firing activities of type II dBNST neurons. Specifically, CRF increased firing activities of type II neurons significantly, and coapplication of NPY suppressed them. These results demonstrated opposing effects of CRF and NPY at the cellular level, as observed at the behavioral level. These counteracting effects of CRF and NPY on neuronal excitability in type II dBNST neurons may explain the opposing roles of these peptides in the negative affective component of pain.

Analyses of $I-V$ relationships in the absence and presence of CRF demonstrate that there may be at least two classes of type II dBNST neurons. The steady-state current in type IIa neurons was reduced by CRF and reversed its polarity at approximately -95 mV, suggesting the involvement of potassium channels. Jedema and Grace (2004) reported the direct action of CRF on locus ceruleus neurons, increasing neuronal excitability by decreasing a potassium conductance in a cAMP-dependent manner. A similar mechanism may be involved in the depolarizing effect of CRF on type IIa dBNST neurons. On the other hand, CRF increased steady-state current and its reversal potential was approximately -50 mV in type IIb neurons, implying the contribution of nonselective cation current (Takano et al., 1996; Yang and Ferguson, 2002; Kaneko et al., 2008). In support of this hypothesis, CRF has been reported to activate nonselective cationic conductances in the pituitary gland (Takano et al., 1996). The reversal potentials observed in the type IIa and IIb neurons were slightly deviated from the predicted (-109.3 mV for potassium current) or reported (> -45 mV for nonselective cation current) (Takano et al., 1996; Ito and Dulon, 2002; Yang and Ferguson, 2002; Kaneko et al., 2008) values, suggesting that both channels express in both type IIa and IIb neurons with different levels,

rather each channel exclusively expresses in either type IIa or IIb neurons. Further studies are necessary to characterize the detailed physiological properties of these two types of neurons.

NPY hyperpolarized type II dBNST neurons with an increase in input resistance. $I-V$ relationship analyses revealed that NPY reduced steady-state currents, the reversal potential of which was approximately -60 mV, indicating suppression of cationic conductance(s). Consistent with this finding, ZD7288, an I_h blocker, inhibited the hyperpolarizing effect of NPY and shifted the reversal potential of NPY-associated currents toward a more negative potential, demonstrating that NPY-induced hyperpolarization was mediated by blocking I_h channels. Similar hyperpolarizing effect of NPY via inhibition of I_h channels has been reported in BLA pyramidal neurons (Giesbrecht et al., 2010).

A previous study suggested that the majority of type II BNST neurons were GABAergic interneurons, which may innervate output neurons within the BNST (Hammack et al., 2007). Thus, CRF-induced activation of type II neurons could result in the inhibition of output neurons. Indeed, in the ventrolateral BNST, Dumont and Williams (2004) demonstrated that activation of neurons exhibiting a depolarizing sag (type I or II neurons) enhanced GABA_A-IPSC in the output neurons projecting to the ventral tegmental area (VTA). Georges and Aston-Jones (2001, 2002) have reported that the BNST sends excitatory drive to VTA dopaminergic neurons. Recently, we have demonstrated that this excitatory drive from the BNST to VTA dopaminergic neurons is mainly composed of two GABAergic neurons (Kudo et al., 2012). Specifically, most VTA-projecting BNST neurons are GABAergic neurons, which preferentially innervate VTA GABAergic neurons. Therefore, activation of VTA-projecting BNST output neurons is predicted to promote VTA dopaminergic neuron activity through a disinhibition mechanism. Pain-induced increase in CRF release within the BNST may activate the type II BNST neurons, which could suppress VTA-projecting BNST output neurons, thereby attenuating the excitatory drive from the BNST to the VTA dopaminergic neurons. The attenuation of VTA dopaminergic neuron activity through this mechanism may be involved in pain-induced aversion. In support of this hypothesis, it has recently been reported that optogenetic inhibition of VTA dopaminergic neurons causes CPA (Tan et al., 2012).

Although several lines of evidence suggest the critical role of CRF₁ receptors in CRF-induced negative emotion such as anxiety and fear, there is still continued controversy over the role of CRF₂ receptors in these emotional states (Takahashi, 2001; Bale and Vale, 2004). Regarding the BNST, Sahuque et al. (2006) reported that anxiety-like behaviors induced by the intra-BNST CRF injection were prevented by coadministration of a CRF₁, but not a CRF₂, receptor antagonist. On the other hand, either CRF₁ or CRF₂ receptor antagonist prevented CPA induced by intra-BNST CRF injection, consistent with our current results showing that both CRF₁ and CRF₂ receptor antagonists suppressed pain-induced CPA. These findings suggest that distinct neuronal pathways may be involved in different negative emotional states such as anxiety and aversion. Further studies using neuronal pathway-specific methods such as optogenetic approaches are necessary to address this issue.

A large body of literature suggests that anxiolytic and anti-aversive effects of NPY are mediated primarily by Y₁ receptors (Heilig, 1995; Kask et al., 1997; Nakajima et al., 1998; Primeaux et al., 2005). Additionally, there are reports that Y₂ receptors also mediate the suppression of negative affect (Sørensen et al., 2004). In contrast, Y₂ receptors have been reported to mediate negative emotions, such

as anxiety (Nakajima et al., 1998; Sajdyk et al., 2002). Thus, in the present study, we examined the involvement of Y_1 and Y_5 subtypes in the inhibitory effect of intra-dBNST NPY on CRF-induced CPA and found that both Y_1 and Y_5 receptors play important roles in this effect of NPY. In this study, we revealed the heterogeneity of type II dBNST neurons. Further electrophysiological experiments using Y_1 - and Y_5 -specific agonists are needed to clarify the neuronal populations expressing these receptors.

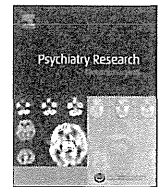
Increasing data have demonstrated opposing effects of CRF and NPY. Intracerebroventricular injection of Y_1 receptor antagonist BIBP3226 induced an anxiogenic effect in the elevated plus maze test in rats, which was blocked by pretreatment with a nonselective CRF receptor antagonist (Kask et al., 1997). Sajdyk et al. (2006) reported that urocortin I, a CRF receptor agonist, injected into the BLA-induced CPA, which was reversed by coadministration of NPY. Electrophysiological analysis revealed opposing actions of CRF and NPY on the excitability of BLA neurons (Giesbrecht et al., 2010). In the ventrolateral BNST, it has been reported that NPY and CRF have opposite modulating effects on GABA_A-IPSC (Kash and Winder, 2006). The present study has added pain-induced aversion to the list of negative emotional states in which opposing effects of CRF and NPY play important roles.

Further studies are necessary to determine the neuronal circuits involved in the induction of negative affect associated with pain stimulation, and the molecular mechanisms operating the ion channels involved in the opposing actions of CRF and NPY on dBNST neuronal excitability. Nevertheless, we have uncovered some of the neuronal mechanisms underlying the negative affective component of pain by showing the opposing roles of intra-dBNST CRF and NPY in pain-induced aversive behaviors and the opposing actions of these peptides on neuronal excitability converging on the same target, type II neurons, within the dBNST.

References

- Bale TL, Vale WW (2004) CRF and CRF receptors: role in stress responsivity and other behaviors. *Annu Rev Pharmacol Toxicol* 44:525–557. CrossRef Medline
- Deyama S, Nakagawa T, Kaneko S, Uehara T, Minami M (2007) Involvement of the bed nucleus of the stria terminalis in the negative affective component of visceral and somatic pain in rats. *Behav Brain Res* 176:367–371. CrossRef Medline
- Deyama S, Katayama T, Ohno A, Nakagawa T, Kaneko S, Yamaguchi T, Yoshioka M, Minami M (2008) Activation of the β -adrenoceptor-protein kinase A signaling pathway within the ventral bed nucleus of the stria terminalis mediates the negative affective component of pain in rats. *J Neurosci* 28:7728–7736. CrossRef Medline
- Deyama S, Katayama T, Kondoh N, Nakagawa T, Kaneko S, Yamaguchi T, Yoshioka M, Minami M (2009) Role of enhanced noradrenergic transmission within the ventral bed nucleus of the stria terminalis in visceral pain-induced aversion in rats. *Behav Brain Res* 197:279–283. CrossRef Medline
- Deyama S, Ide S, Kondoh N, Yamaguchi T, Yoshioka M, Minami M (2011) Inhibition of noradrenaline release by clonidine in the ventral bed nucleus of the stria terminalis attenuates pain-induced aversion in rats. *Neuropharmacology* 61:156–160. CrossRef Medline
- Dumont EC, Williams JT (2004) Noradrenaline triggers GABA_A inhibition of bed nucleus of the stria terminalis neurons projecting to the ventral tegmental area. *J Neurosci* 24:8198–8204. CrossRef Medline
- Georges F, Aston-Jones G (2001) Potent regulation of midbrain dopamine neurons by the bed nucleus of the stria terminalis. *J Neurosci* 21:RC160. Medline
- Georges F, Aston-Jones G (2002) Activation of ventral tegmental area cells by the bed nucleus of the stria terminalis: a novel excitatory amino acid input to midbrain dopamine neurons. *J Neurosci* 22:5173–5187. Medline
- Giesbrecht CJ, Mackay JP, Silveira HB, Urban JH, Colmers WF (2010) Countervailing modulation of I_{h1} by neuropeptide Y and corticotrophin-releasing factor in basolateral amygdala as a possible mechanism for their effects on stress-related behaviors. *J Neurosci* 30:16970–16982. CrossRef Medline
- Gutman AR, Yang Y, Ressler KJ, Davis M (2008) The role of neuropeptide Y in the expression and extinction of fear-potentiated startle. *J Neurosci* 28:12682–12690. CrossRef Medline
- Hammack SE, Mania I, Rainnie DG (2007) Differential expression of intrinsic membrane currents in defined cell types of the anterolateral bed nucleus of the stria terminalis. *J Neurophysiol* 98:638–656. CrossRef Medline
- Hara Y, Nakaya H (1997) Dual effects of extracellular ATP on the muscarinic acetylcholine receptor-operated K^+ current in guinea-pig atrial cells. *Eur J Pharmacol* 324:295–303. CrossRef Medline
- Heilig M (1995) Antisense inhibition of neuropeptide Y (NPY)- Y_1 receptor expression blocks the anxiolytic-like action of NPY in amygdala and paradoxically increases feeding. *Regul Pept* 59:201–205. CrossRef Medline
- Ito K, Dulon D (2002) Nonselective cation conductance activated by muscarinic and purinergic receptors in rat spiral ganglion neurons. *Am J Physiol Cell Physiol* 282:C1121–1135. Medline
- Jedema HP, Grace AA (2004) Corticotropin-releasing hormone directly activates noradrenergic neurons of the locus ceruleus recorded *in vitro*. *J Neurosci* 24:9703–9713. CrossRef Medline
- Johansen JP, Fields HL (2004) Glutamatergic activation of anterior cingulate cortex produces an aversive teaching signal. *Nat Neurosci* 7:398–403. CrossRef Medline
- Johansen JP, Fields HL, Manning BH (2001) The affective component of pain in rodents: direct evidence for a contribution of the anterior cingulate cortex. *Proc Natl Acad Sci U S A* 98:8077–8082. CrossRef Medline
- Kaneko K, Tamamaki N, Owada H, Kakizaki T, Kume N, Totsuka M, Yamamoto T, Yawo H, Yagi T, Obata K, Yanagawa Y (2008) Noradrenergic excitation of a subpopulation of GABAergic cells in the basolateral amygdala via both activation of nonselective cationic conductance and suppression of resting K^+ conductance: a study using glutamate decarboxylase 67-green fluorescent protein knock-in mice. *Neuroscience* 157:781–797. CrossRef Medline
- Kash TL, Winder DG (2006) Neuropeptide Y and corticotropin-releasing factor bi-directionally modulate inhibitory synaptic transmission in the bed nucleus of the stria terminalis. *Neuropharmacology* 51:1013–1022. CrossRef Medline
- Kask A, Rago L, Harro J (1997) Alpha-helical CRF(9–41) prevents anxiogenic-like effect of NPY Y_1 receptor antagonist BIBP3226 in rats. *Neuroreport* 8:3645–3647. CrossRef Medline
- Kosten TA (1994) Clonidine attenuates conditioned aversion produced by naloxone-precipitated opiate withdrawal. *Eur J Pharmacol* 254:59–63. CrossRef Medline
- Kudo T, Uchigashima M, Miyazaki T, Konno K, Yamasaki M, Yanagawa Y, Minami M, Watanabe M (2012) Three types of neurochemical projection from the bed nucleus of the stria terminalis to the ventral tegmental area in adult mice. *J Neurosci* 32:18035–18046. CrossRef Medline
- Lee Y, Davis M (1997) Role of the hippocampus, the bed nucleus of the stria terminalis, and the amygdala in the excitatory effect of corticotropin-releasing hormone on the acoustic startle reflex. *J Neurosci* 17:6434–6446. Medline
- Morin SM, Ling N, Liu XJ, Kahl SD, Gehlert DR (1999) Differential distribution of urocortin- and corticotropin-releasing factor-like immunoreactivities in the rat brain. *Neuroscience* 92:281–291. CrossRef Medline
- Murai Y, Akaike T (2005) Orexins cause depolarization via nonselective cationic and K^+ channels in isolated locus coeruleus neurons. *Neurosci Res* 51:55–65. CrossRef Medline
- Nakagawa T, Yamamoto R, Fujio M, Suzuki Y, Minami M, Satoh M, Kaneko S (2005) Involvement of the bed nucleus of the stria terminalis activated by the central nucleus of the amygdala in the negative affective component of morphine withdrawal in rats. *Neuroscience* 134:9–19. CrossRef Medline
- Nakajima M, Inui A, Asakawa A, Momose K, Ueno N, Teranishi A, Baba S, Kasuga M (1998) Neuropeptide Y produces anxiety via Y_2 -type receptors. *Peptides* 19:359–363. CrossRef Medline
- Parker RM, Herzog H (1999) Regional distribution of Y-receptor subtype mRNAs in rat brain. *Eur J Neurosci* 11:1431–1448. CrossRef Medline
- Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates, Ed 4. San Diego, CA: Academic.
- Primeaux SD, Wilson SP, Cusick MC, York DA, Wilson MA (2005) Effects

- of altered amygdalar neuropeptide Y expression on anxiety-related behaviors. *Neuropsychopharmacology* 30:1589–1597. CrossRef Medline
- Rafeian-Kopaei M, Gray AM, Spencer PS, Sewell RD (1995) Contrasting actions of acute or chronic paroxetine and fluvoxamine on morphine withdrawal-induced place conditioning. *Eur J Pharmacol* 275:185–189. CrossRef Medline
- Sahuque LL, Kullberg EF, Mcgeehan AJ, Kinder JR, Hicks MP, Blanton MG, Janak PH, Olive MF (2006) Anxiogenic and aversive effects of corticotropin-releasing factor (CRF) in the bed nucleus of the stria terminalis in the rat: role of CRF receptor subtypes. *Psychopharmacology* 186:122–132. CrossRef Medline
- Sajdyk TJ, Schober DA, Smiley DL, Gehlert DR (2002) Neuropeptide Y-Y₂ receptors mediate anxiety in the amygdala. *Pharmacol Biochem Behav* 71:419–423. CrossRef Medline
- Sajdyk TJ, Fitz SD, Shekhar A (2006) The role of neuropeptide Y in the amygdala on corticotropin-releasing factor receptor-mediated behavioral stress responses in the rat. *Stress* 9:21–28. CrossRef Medline
- Sakanaka M, Shibasaki T, Lederis K (1986) Distribution and efferent projections of corticotropin-releasing factor-like immunoreactivity in the rat amygdaloid complex. *Brain Res* 382:213–238. CrossRef Medline
- Sørensen G, Lindberg C, Wörtwein G, Bolwig TG, Woldbye DP (2004) Differential roles for neuropeptide Y Y₁ and Y₅ receptors in anxiety and sedation. *J Neurosci Res* 77:723–729. CrossRef Medline
- Takahashi LK (2001) Role of CRF₁ and CRF₂ receptors in fear and anxiety. *Neurosci Biobehav Rev* 25:627–636. CrossRef Medline
- Takano K, Yasufuku-Takano J, Teramoto A, Fujita T (1996) Corticotropin-releasing hormone excites adrenocorticotropin-secreting human pituitary adenoma cells by activating a nonselective cation current. *J Clin Invest* 98:2033–2041. CrossRef Medline
- Tan KR, Yvon C, Turiault M, Mirzabekov JJ, Doehner J, Labouèbe G, Deisseroth K, Tye KM, Lüscher C (2012) GABA neurons of the VTA drive conditioned place aversion. *Neuron* 73:1173–1183. CrossRef Medline
- Tanimoto S, Nakagawa T, Yamauchi Y, Minami M, Satoh M (2003) Differential contributions of the basolateral and central nuclei of the amygdala in the negative affective component of chemical somatic and visceral pains in rats. *Eur J Neurosci* 18:2343–2350. CrossRef Medline
- Tzschentke TM (1998) Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Prog Neurobiol* 56:613–672. CrossRef Medline
- Van Pett K, Viau V, Bittencourt JC, Chan RK, Li HY, Arias C, Prins GS, Perrin M, Vale W, Sawchenko PE (2000) Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. *J Comp Neurol* 428:191–212. CrossRef Medline
- Walter A, Mai JK, Lanta L, Görös T (1991) Differential distribution of immunohistochemical markers in the bed nucleus of the stria terminalis in the human brain. *J Chem Neuroanat* 4:281–298. CrossRef Medline
- Yang B, Ferguson AV (2002) Orexin-A depolarizes dissociated rat area postrema neurons through activation of a nonselective cationic conductance. *J Neurosci* 22:6303–6308. Medline



Impaired integrity of the brain parenchyma in non-geriatric patients with major depressive disorder revealed by diffusion tensor imaging

Khin K. Tha ^{a,*}, Satoshi Terae ^{b,*}, Shin Nakagawa ^c, Takeshi Inoue ^c, Nobuki Kitagawa ^c, Yuki Kako ^c, Yasuya Nakato ^c, Kawser Akter Popy ^b, Noriyuki Fujima ^b, Yuri Zaitso ^b, Daisuke Yoshida ^b, Yoichi M. Ito ^d, Tamaki Miyamoto ^e, Tsukasa Koyama ^c, Hiroki Shirato ^a

^a Hokkaido University Graduate School of Medicine, Department of Radiobiology and Medical Engineering, N-15, W-7, Kita-ku, Sapporo 060-8638, Japan

^b Department of Diagnostic Radiology, Hokkaido University Graduate School of Medicine, N-15, W-7, Kita-ku, Sapporo 060-8638, Japan

^c Department of Psychiatry, Hokkaido University Graduate School of Medicine, N-15, W-7, Kita-ku, Sapporo 060-8638, Japan

^d Department of Biostatistics, Hokkaido University Graduate School of Medicine, N-15, W-7, Kita-ku, Sapporo 060-8638, Japan

^e Department of Health Sciences, Hokkaido University Graduate School of Medicine, N-15, W-7, Kita-ku, Sapporo 060-8638, Japan

ARTICLE INFO

Article history:

Received 19 July 2011

Received in revised form

16 June 2012

Accepted 11 July 2012

Keywords:

Fractional anisotropy

Mean diffusivity

Voxel-based analysis

Histogram

ABSTRACT

Diffusion tensor imaging (DTI) is considered to be able to non-invasively quantify white matter integrity. This study aimed to use DTI to evaluate white matter integrity in non-geriatric patients with major depressive disorder (MDD) who were free of antidepressant medication. DTI was performed on 19 non-geriatric patients with MDD, free of antidepressant medication, and 19 age-matched healthy subjects. Voxel-based and histogram analyses were used to compare fractional anisotropy (FA) and mean diffusivity (MD) values between the two groups, using two-sample *t* tests. The abnormal DTI indices, if any, were tested for correlation with disease duration and severity, using Pearson product-moment correlation analysis. Voxel-based analysis showed clusters with FA decrease at the bilateral frontal white matter, anterior limbs of internal capsule, cerebellum, left putamen and right thalamus of the patients. Histogram analysis revealed lower peak position of FA histograms in the patients. FA values of the abnormal clusters and peak positions of FA histograms of the patients exhibited moderate correlation with disease duration and severity. These results suggest the implication of frontal-subcortical circuits and cerebellum in MDD, and the potential utility of FA in evaluation of brain parenchymal integrity.

© 2012 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Major depressive disorder (MDD) is a common disorder with a chronic pattern of recurrence and a lifetime prevalence of 16.2% (Kessler et al., 2003). It is the fourth most disabling medical condition worldwide based on disability-adjusted life years (Greenberg et al., 1993). The neurobiology of MDD is not completely understood. A number of previous studies involving morphometric analysis using optical disector methodology, voxel-based morphometry using magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT), and positron emission tomography (PET) have reported a reduction in cortical thickness and the density of cortical neurons, as well as alterations in cerebral blood flow or glucose metabolism of cerebral cortices in MDD. These results are suggestive of gray

matter pathology (Graff-Guerrero et al., 2004; Uranova et al., 2004; Egger et al., 2008; Fujimoto et al., 2008). On the other hand, recent converging evidence based on the findings of diffusion tensor imaging (DTI) is suggestive of abnormalities of white matter or frontal-subcortical circuits (e.g., Tekin and Cummings, 2002; Bae et al., 2006; Nobuhara et al., 2006; Shimony et al., 2009).

DTI is an MRI technique which can quantify white matter integrity noninvasively and in vivo (Moseley, 2002). This technique enables quantification of white matter integrity through its ability to detect motion of water molecules. Many studies suggest that DTI can uncover microstructural white matter abnormalities that cannot be detected by other MRI techniques (e.g., Nagesh et al., 2008; Tha et al., 2010). It has also been reported that the abnormalities revealed by DTI correlate significantly with clinical severity in various white matter pathologies (e.g., Della Nave et al., 2007; Tha et al., 2010). Two major indices, fractional anisotropy (FA) and mean diffusivity (MD), are usually used to quantify microstructural white matter integrity by DTI (Moseley, 2002). FA quantifies the degree of directional coherence, whereas MD quantifies the degree of magnitude of water diffusion.

* Corresponding author. Tel.: +81 11 706 5977; fax: +81 11 706 7876.

E-mail addresses: kktha@med.hokudai.ac.jp (K.K. Tha), saterae@med.hokudai.ac.jp (S. Terae).

The major DTI indices are usually evaluated by either one or more of the following techniques: region-of-interest (ROI)-based analysis, tract (tractography)-based analysis, histogram analysis, and voxel-based analysis (Jones et al., 2005; Taoka et al., 2007). ROI-based and tract (tractography)-based analyses are usually chosen if there is a known targeted anatomical area or tract to be evaluated. The latter two techniques are better suited if the area of involvement is not known, or when an a priori spatial selection and hypothesis are not made. In addition, the latter two techniques do not involve the delineation of ROIs—which requires an operator with expertise in neuroanatomy and involves some inherent subjectivity (Marquez de la Plata et al., 2011). Previous DTI studies on MDD have been performed using ROI-based analysis, voxel-based analysis, and/or tract (tractography)-based analysis (e.g., Taylor et al., 2004; Malykhin et al., 2008; Korgaonkar et al., 2011; Wu et al., 2011). Regarding selection of patients, the majority of these studies were performed on geriatric patients and patients being treated with antidepressant medication (e.g., Alexopoulos et al., 2002; Yang et al., 2007; Alexopoulos et al., 2008; Zou et al., 2008). The results of these studies revealed impaired white matter integrity in these patients. However, it is possible that the findings of these studies were confounded by age-related pathology and/or the effect of antidepressant medication (Korgaonkar et al., 2011). With aging, the FA values of all white matter tracts decrease and their MD values increase (Sala et al., 2012). There have also been a few reports about the effect of antidepressant medication on white matter integrity (Yoo et al., 2007; Sijens et al., 2008; Taylor et al., 2011). Normalization of FA and MD values of cerebral white matter after treatment with antidepressants such as citalopram, fluoxetine, and sertraline, as well as a decrease in the FA values of the right posterior thalamic radiation following treatment with citalopram, have been documented—suggestive of the modification of white matter integrity by antidepressant medication. The number of DTI studies performed on non-geriatric patients who were free of antidepressant medication is limited (Li et al., 2007; Ma et al., 2007; Korgaonkar et al., 2011; Ouyang et al., 2011; Wu et al., 2011; Zhu et al., 2011). Although the results of these preliminary studies suggest impaired white matter integrity in MDD, the location of abnormalities is inconsistent among the studies, and the results have not been replicated (Korgaonkar et al., 2011; Wu et al., 2011)—calling for the need for further investigations.

This study was aimed to evaluate white matter integrity in non-geriatric (e.g., < 65 years) patients with MDD who were free of antidepressant medication for at least 6 months, by using voxel-based and histogram analyses of DTI. It was hypothesized that these patients would have impaired integrity of white matter that could be depicted by DTI.

2. Methods

2.1. Participants

This prospective study was approved by the local institutional review board. Written informed consent was obtained from all participants.

The patients were recruited during a 35-month period (August, 2007 to March, 2010), at the Department of Psychiatry, Hokkaido University Hospital. Inclusion criteria for the patients were age between 20 and 64 years (Waxman et al., 1982; Korgaonkar et al., 2011), diagnosis of MDD according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR), and being free of antidepressant medication for at least 6 months. Exclusion criteria were absolute contraindications for MRI, comorbid axis I or II disorders, history of electroconvulsive therapy, history of diseases that might affect white matter integrity (e.g., infarct, hemorrhage, migraine), and significant abnormality on conventional MRI sequences (i.e., T2-weighted imaging (T2WI) and fluid-attenuated inversion recovery (FLAIR) imaging sequences). Of 25 patients who fit the inclusion criteria, 19 patients were eligible for the study. No patients had any psychotic features. Eleven patients had never been treated with antidepressants. The remaining eight patients had a previous history of medication with one or more antidepressant agents, but had been free of the medication for at least 6 months.

To obtain normal control data, MRI was also performed on 19 age-matched normal subjects. Exclusion criteria were absolute contraindications for MRI, axis I or II disorders, history of diseases that might affect white matter integrity, and any obvious abnormality on the conventional MRI sequences. Psychiatric diseases were excluded through a brief structured diagnostic interview (Mini-International Neuropsychiatric Interview; MINI) (Sheehan et al., 1998).

The demographic characteristics of the patients and control subjects are summarized in Table 1.

2.2. MRI

In all patients, MRI was performed on the day of first clinical consultation for the current episode or the day on which the diagnosis was made. There was no undue delay in the prescription of antidepressant medication for the purpose of this study.

MRI was performed using a 1.5 T imager and a standard head coil. The participants were asked not to move their heads during the examination, and foam pads were used to minimize involuntary head motion. An axial single-shot spin-echo echo-planar imaging sequence was used for DTI. Parameters included the following: repetition time (TR)/echo time (TE), 5100/139 ms; *b* value (*b*), 1000 s mm⁻²; diffusion-encoding gradients, 12 directions; number of signals acquired, two; field of view (FOV), 240 × 240 mm; matrix size, 128 × 128 (interpolated into 256 × 256); pixel size, 1.875 × 1.875 mm; intersection gap, 1.5 mm; section thickness, 5 mm; and section number, 23. Echo-planar images with no diffusion weighting (*b* = 0 s mm⁻²) were also obtained for use in spatial normalization and coregistration.

In addition to DTI, axial fast spin-echo T2WI (TR/TE, 4540/96 ms; effective echo train length (ETL_{eff}), 7; FOV, 180 × 240 mm; matrix size, 185 × 448; pixel size, 0.973 × 0.536; intersection gap, 1.5 mm; section thickness, 5 mm; and section number, 23), axial fast FLAIR imaging (TR/TE, 9000/104 ms; inversion time (TI), 2500 ms; FOV, 180 × 240 mm; matrix size, 192 × 256; pixel size, 0.938 × 0.938; intersection gap, 1.5 mm; section thickness, 5 mm; and section number, 23), and three-dimensional T1-weighted imaging with a magnetization-prepared rapid acquisition gradient-echo (MPRAGE) sequence (TR/TE, 1900/3.9 ms; TI, 1100 ms; flip angle, 15°; FOV, 250 × 250 mm; matrix size, 256 × 256;

Table 1
The demographic characteristics of the patients and normal subjects.

	Patients (n=19)	Control subjects (n=19)	P-value
Age	38.6 ± 13.5 years (20–61) ^a	36.5 ± 12.5 years (22–60) ^a	0.613 ^b
Gender (men/women)	12/7	13/6	0.729 ^c
Previous history of antidepressant medication (present/absent)	8/11	–	–
Previous episodes (present/absent)	5/14	–	–
Total disease duration	18.37 ± 28.17 months (1–96) ^a	–	–
Duration of current episode	5.89 ± 5.69 months (1–24) ^a	–	–
HDRS-17	19.00 ± 4.00 (11–26) ^a	–	–
GAF	43.79 ± 9.90 (28–58) ^a	–	–
MADRS	26.21 ± 5.57 (20–39) ^a	–	–
CGI-S	4.68 ± 0.89 (3–6)	–	–

^a Data are presented in mean ± standard deviation (range).

^b The statistical significance was evaluated by using two-sample *t* test.

^c Or χ^2 test.

pixel size, 0.978×0.978 ; gapless; section thickness, 1 mm; imaging plane, coronal; and section number, 240) were also acquired.

2.3. Image processing and analysis

2.3.1. Construction of FA and MD maps

FA and MD maps were constructed on a workstation from the diffusion tensor images, according to the methods of Basser and Pierpaoli (1996) (Dr. View/LINUX R2.5.0; AJS, Tokyo, Japan). To ensure computation only of tensors inside the brain rather than the surrounding air, a brain mask, computed based on the signal intensity of diffusion tensor images, was applied to the diffusion tensor images.

2.3.2. Voxel-based analysis

The steps involved all closely followed those of a previous report (Tha et al., 2010). First, the customized FA and MD templates were built from the FA and MD maps of the control subjects. For this purpose, the echo-planar images with no diffusion weighting of each control subject were first warped to the standard echo-planar template of Statistical Parametric Mapping software (SPM5, www.fil.ion.ucl.ac.uk), by using default parameters. This transformation information was then applied to the FA and MD maps of each subject. The warped FA and MD maps were averaged and smoothed with a 6-mm full-width half-maximum (FWHM) Gaussian kernel, to form the customized FA and MD templates. Visual review of the output images was performed to ensure that no obvious registration error was encountered.

Next, the native FA and MD maps of all patients and control subjects were warped to the customized FA and MD templates, respectively. The parameters applied were the same as those described previously. Visual review of all warped images was performed to ensure that no obvious registration error was encountered. Individual maps were then smoothed with a 6-mm FWHM Gaussian kernel.

Warped and smoothed FA and MD maps of the patients and control subjects were next compared voxel-by-voxel by using a two-sample *t* test. The analysis was restricted to the area covered by a mask (void of cortical gray matter and ventricles)—which was developed from the customized FA template by discarding voxels with an FA value of less than 0.2 (MRICron, www.cabiatl.com/mricron/mricron). An uncorrected *P* value of less than 0.001 and a cluster size of greater than 50 voxels were considered significant. The FA and MD values of the clusters in the patients which differed significantly from the control subjects, if any, were measured (MarsBaR, www.marsbar.sourceforge.net).

2.3.3. Histogram analysis

The steps involved were the same as those of a previous report (Mori et al., 2008), except that white matter masks were used in this study instead of whole-brain binary masks. In brief, the axial sections of individual MPRAGE images of each participant were coregistered to the echo-planar images with no diffusion weighting (SPM5). Visual review of the output images was performed to ensure that no obvious registration error was encountered. From the axial sections of the MPRAGE images, white matter was automatically segmented by using the default parameters of SPM5. Each segmented image was checked to ensure accuracy. The segmented white matter (the segmented area also included the thalami and part of the basal ganglia) was used to serve as individual white matter masks. Each white matter mask was applied to the FA and MD maps of the corresponding participant (MRICron). Histograms were then calculated for the masked FA and MD maps of each participant (ImageJ, www.rsb.info.nih.gov/ij). The bin width of the FA histograms was set as 0.01 (between 0.0 and 1.0), and that of MD histograms was set as 5×10^{-5} (between 0.0 and 0.0055). To correct skewness, the natural logarithmic transformation of FA and MD bins was used. To correct for individual differences in the brain volume, each histogram was normalized by the total number of voxels contributing to the histogram. From each histogram, the peak height and location were extracted. These histogram parameters were then compared between the two groups with a two-sample *t* test. A *P* value of less than 0.05 was set to determine statistical significance.

2.3.4. Correlation between the altered DTI indices and clinical variables

The absolute values of altered DTI indices of the patients (for voxel-based analysis, FA or MD values of the clusters which differed significantly from the control subjects; for histogram analysis, the peak height or position of FA or MD histograms which differed significantly from the control subjects), if any, were tested for correlation with total disease duration, duration of current disease episode, scores on scales assessing clinical severity (17-item Hamilton Depression Rating Scale (HDRS-17; Williams, 1988), Global Assessment of Functioning (GAF; Hall, 1995), Montgomery-Åsberg Depression Rating Scale (MADRS); (Montgomery and Åsberg, 1979), Clinical Global Impression-Severity (CGIS; Guy, 1976), and age, by using Pearson product-moment correlation analysis. The difference in the absolute values of altered DTI indices between the gender groups was evaluated with a two-sample *t* test. For all conditions, a *P* value of less than 0.05 was considered to indicate a significant difference. Correction for multiple comparisons was not performed.

3. Results

3.1. Voxel-based analysis

The results of voxel-by-voxel comparison of FA values between the two groups are shown in Fig. 1. Clusters with a significant decrease in FA values were observed in the bilateral frontal white matter, the anterior limbs of the internal capsule, the left putamen, the mediodorsal nucleus of the right thalamus, and the anterior and superior aspect of bilateral cerebellar hemispheres of the patients. These clusters persisted even after controlling for age and gender. There were no significant clusters with an increase in the FA or altered MD values in the patients.

3.2. Histogram analysis

The mean FA and MD histograms of the patients and control subjects are shown in Fig. 2 and Fig. 3, respectively. The peak position of the FA histograms of the patients was significantly lower than that of the control subjects ($P=0.00097$). The results revealed a tendency toward a higher peak height in the MD

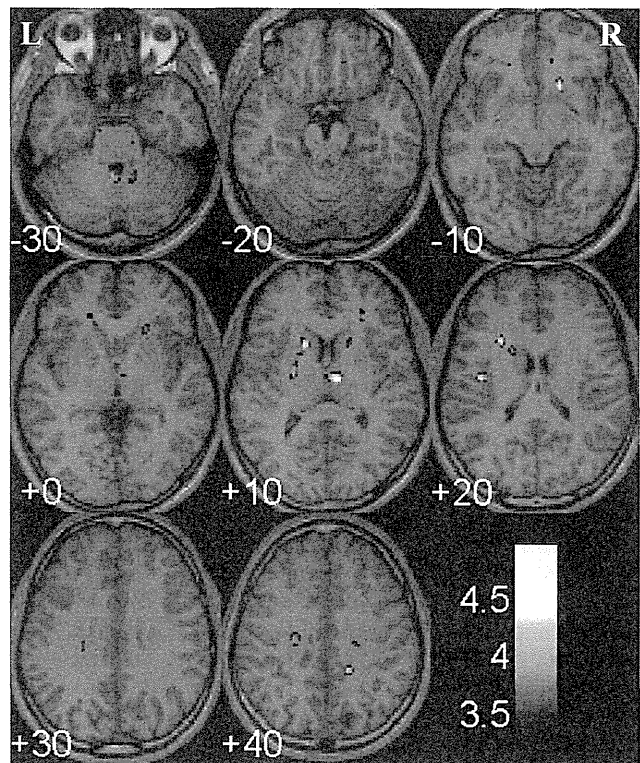


Fig. 1. The results of voxel-by-voxel comparison of FA values between the patients and control subjects, superimposed on a T1-weighted template. Clusters with significant decrease in FA values (uncorrected $P < 0.001$, minimum cluster size = 50 voxels) are observed in the patients, in the right frontal white matter ((i) MNI coordinates: $x=12, y=43, z=-18$, cluster size = 70 voxels, $t=4.34$); (ii) MNI coordinates: $x=37, y=-19, z=59$, cluster size = 100 voxels, $t=4.43$), left frontal white matter (MNI coordinates: $x=-24, y=-21, z=34$, cluster size = 135 voxels, $t=4.53$), right anterior limb of internal capsule (MNI coordinates: $x=17, y=18, z=-9$, cluster size = 111 voxels, $t=4.63$), left anterior limb of internal capsule (MNI coordinates: $x=-14, y=14, z=9$, cluster size = 135 voxels, $t=4.62$), left putamen (MNI coordinates: $x=-24, y=-7, z=7$, cluster size = 74 voxels, $t=4.12$), the mediodorsal nucleus of right thalamus (MNI coordinates: $x=7, y=-14, z=8$, cluster size = 138 voxels, $t=4.98$), right cerebellar hemisphere (MNI coordinates: $x=22, y=-48, z=-27$, cluster size = 83 voxels, $t=4.14$), and left cerebellar hemisphere (MNI coordinates: $x=-3, y=-49, z=-25$, cluster size = 50 voxels, $t=4.17$). Look-up table represents the *t* values. *L* and *R* represent the left and right sides, respectively.

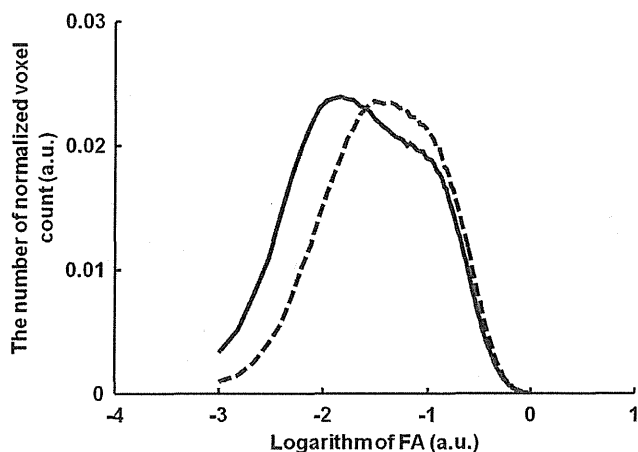


Fig. 2. The mean FA histograms of the patients (solid line) and control subjects (dotted line). The average number of normalized voxels is plotted against the logarithm of the FA values. The peak position of FA histogram of the patients is significantly lower than that of normal subjects ($P=0.00097$). The peak height of FA histograms does not vary significantly.

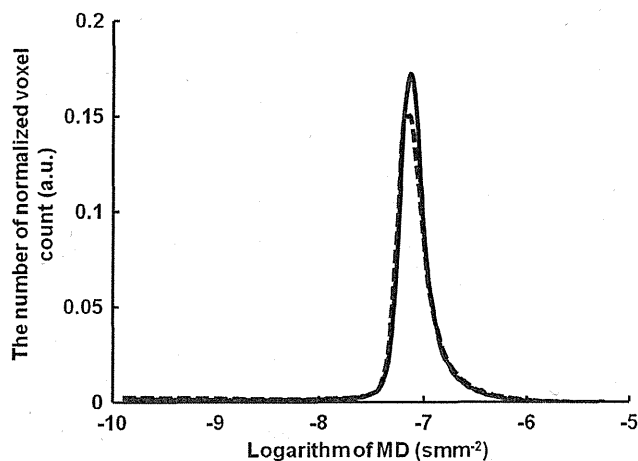


Fig. 3. The mean MD histograms of the patients (solid line) and control subjects (dotted line). The average number of normalized voxels is plotted against the logarithm of the MD values. No significant difference in the peak position or height of MD histograms is observed.

histograms of the patients, but this alteration was not statistically significant ($P=0.11615$). The peak height of the FA histograms and the peak position of the MD histograms did not vary significantly between the two groups ($P=0.79527$ and $P=1$, respectively).

3.3. Correlation between the altered DTI indices and clinical variables

The results of the tests of correlation between the altered DTI indices of the patients and clinical variables are summarized in Table 2 and Fig. 4. Of clusters with a significant FA decrease, the FA value of the right frontal white matter ($x=12$, $y=43$, $z=-18$) revealed a moderate negative correlation with duration of current disease episode. The right frontal white matter ($x=37$, $y=-19$, $z=59$) and the right anterior limb of the internal capsule revealed moderate negative correlation with the GAF. The left anterior limb of the internal capsule exhibited a moderate positive correlation with total disease duration. The right cerebellar hemisphere exhibited a moderate positive correlation with the HDRS-17. The peak position

of the FA histogram had a moderate positive correlation with total disease duration and the duration of the current disease episode. The FA value of the left cerebellar hemisphere revealed a moderate positive correlation with age. There was no significant difference in the regional FA values or the peak positions of FA histograms between the gender groups ($P > 0.220$).

4. Discussion

This study evaluated white matter integrity in non-geriatric patients with MDD by using DTI. Two semiautomated/automated methods—voxel-based and histogram analyses—were used for the analysis. Both methods were able to identify abnormalities in the patients. Voxel-based analysis showed clusters with a significant decrease in FA at the bilateral frontal white matter and the anterior limbs of the internal capsule. In addition to cerebral white matter, clusters with a significant decrease in the FA were also observed in the subcortical gray matter (including the left putamen and the mediodorsal nucleus of the right thalamus) and bilateral cerebellar hemispheres. In histogram analysis, a lower peak position (i.e., “shift toward the left”) of the FA histograms of the patients was observed, indicative of inclusion in the patients’ images of a greater number of voxels with low FA values compared to normal subjects.

Decrease in the FA is reflective of an impaired directional coherence of the brain microstructures (Moseley, 2002). The histological correlates of decrease in the FA, as revealed by the reports of autopsies and biopsies of various diseases of the brain and experimental models, include larger axonal spacing, a decrease in axon count, diameter and density, and myelin loss (Beaulieu, 2011). The autopsy reports of patients with MDD are scarce. Limited evidence has suggested myelin pallor in the white matter underlying prefrontal cortex (Regenold et al., 2007). Taken together with the knowledge that myelin pallor results from demyelination, incomplete myelination of axons, axonal loss, or axonal agenesis, our finding of FA decrease in the bilateral frontal white matter and anterior limbs of the internal capsule may reflect axon and/or myelin abnormality. Histological proof regarding the changes of the putamen, thalamus, and cerebellar hemispheres in MDD is lacking. The exact pathological process that occurs in these structures is thus not known. Nonetheless, from the findings of neuronal loss in some subcortical structures such as the nucleus basalis, substantia nigra, and raphe nucleus (Tsopelas et al., 2011), it is possible that similar pathological changes are encountered. Axon and/or myelin abnormalities are also possible, as axon and myelin are the components of these structures.

Human behaviors (executive functions, social behavior, and motivational states) are mediated mainly by three parallel frontal-subcortical circuits (Tekin and Cummings, 2002). These circuits originate from the dorsolateral prefrontal region, lateral orbitofrontal region, and anterior cingulate portion of the frontal cortex; and form connections to the striatum, basal ganglia, and thalamus. From the thalamus, fibers of these circuits loop back to the cortex of origin. In addition to forming closed loops, these circuits form open connections with the other areas of the frontal lobe, parietal and temporal lobes, amygdala, hippocampus, substantia nigra, subthalamic nucleus, hypothalamus, and the brainstem. Our findings of decrease in the FA at the bilateral frontal white matter, anterior limbs of the internal capsule, left putamen and right thalamus are suggestive of impaired integrity or dysfunctioning of these circuits. Although mediation of human behavior by these circuits is well established, emerging evidence is suggestive of participation of the cerebellum in the regulation of mood and cognition as well (Diamond, 2000). The cerebellum

Table 2

Tests of correlation between the abnormal DTI indices and the clinical variables.

	Correlation coefficients (P value)						
	Total disease duration	Duration of current episode	HDRS-17	GAF	MADRS	CGIS	Age
Clusters with significant decrease in FA							
Right frontal white matter ($x=12, y=43, z=-18$)	−0.296 (0.219)	−0.482 (0.036)*	0.084 (0.732)	−0.012 (0.960)	−0.117 (0.634)	−0.006 (0.980)	0.188 (0.440)
Right frontal white matter ($x=37, y=-19, z=59$)	0.080 (0.746)	0.318 (0.184)	0.135 (0.583)	−0.462 (0.046)*	0.413 (0.079)	0.412 (0.080)	−0.392 (0.097)
Left frontal white matter	0.247 (0.308)	0.087 (0.723)	0.160 (0.512)	−0.367 (0.122)	0.351 (0.141)	0.377 (0.112)	0.109 (0.657)
Right anterior limb of internal capsule	0.335 (0.160)	0.163 (0.506)	−0.117 (0.634)	−0.479 (0.038)*	0.229 (0.345)	0.363 (0.127)	−0.070 (0.775)
Left anterior limb of internal capsule	0.483 (0.036)*	−0.011 (0.964)	0.078 (0.750)	−0.410 (0.081)	0.384 (0.105)	0.359 (0.131)	−0.308 (0.200)
Left putamen	−0.112 (0.648)	−0.191 (0.433)	−0.026 (0.915)	−0.276 (0.252)	0.120 (0.626)	0.327 (0.171)	−0.397 (0.092)
Mediodorsal nucleus of the right thalamus	0.434 (0.063)	−0.116 (0.635)	0.052 (0.831)	−0.248 (0.307)	0.152 (0.535)	0.326 (0.173)	−0.424 (0.071)
Right cerebellar hemisphere	−0.073 (0.767)	−0.034 (0.891)	0.525 (0.021)*	−0.383 (0.106)	0.272 (0.260)	0.345 (0.148)	0.258 (0.286)
Left cerebellar hemisphere	0.168 (0.492)	0.214 (0.380)	0.434 (0.063)	−0.274 (0.257)	0.252 (0.297)	0.177 (0.470)	0.498 (0.030)*
Peak position of FA histogram	0.576 (0.010)*	0.550 (0.015)*	−0.020 (0.936)	−0.087 (0.723)	0.402 (0.088)	0.040 (0.869)	0.219 (0.368)

Abbreviations: HDRS-17=17-item Hamilton Depression Rating Scale; GAF= Global Assessment of Functioning; MADRS=Montgomery-Åsberg Depression Rating Scale; CGI-S=Clinical Global Impression-Severity.

* Indicates statistical significance ($P < 0.05$).

forms anatomical and functional connections with the prefrontal cortex, subcortical limbic structures, and monoamine-producing brainstem nuclei (Konarski et al., 2005; Strick et al., 2009). Altered cerebellar levels of glial fibrillary acidic proteins (GFAP) have been observed in the patients with MDD (Fatemi et al., 2004). PET and functional MRI studies have also uncovered distinct variations in cerebellar activity between the patients with MDD and normal subjects (e.g., Dolan et al., 1992; Bearegard et al., 1998; Videbech et al., 2001). Our finding of decrease in the FA in the bilateral cerebellar hemispheres is also supportive of implication of the cerebellum in the neuropathology of MDD.

Previous DTI studies on non-geriatric patients with MDD who were free of antidepressant medication have reported decreases in the FA in the white matter of the superior longitudinal fasciculus, cingulate, sagittal stratum, posterior thalamic radiation, anterior limb and retrolenticular part of the internal capsule, external capsule, splenium of the corpus callosum, stria terminalis, and the other subcortical and deep white matter of the frontal, parietal and temporal lobes (Li et al., 2007; Ma et al., 2007; Korgaonkar et al., 2011; Ouyang et al., 2011; Wu et al., 2011; Zhu et al., 2011). Added to these are our findings of the decrease in the FA in the left putamen, the mediodorsal nucleus of the right thalamus, and the bilateral cerebellar hemispheres. Taken together, these findings are suggestive of heterogeneity in the areas of the brain that are involved in MDD. It is possible that such heterogeneity arises from a variation in the circuits that are severed among the study population. From our finding of abnormalities in the frontal white matter and mediodorsal nucleus of the thalamus, it is thought that the dorsolateral prefrontal or orbitofrontal circuit is at least involved in our patients (Tekin and Cummings, 2002). Further stratification of analysis based on clinical symptoms may render more consistent results; however, variation in the exact anatomical location of the abnormalities along a single circuit (i.e., in terms of MNI or Talairach coordinates) would remain unsolved (Blood et al., 2010).

In this study, histogram analysis of FA and MD was also performed, as a supplemental analysis to voxel-based analysis. To our knowledge, this is the first study that has applied a histogram analysis of DTI in psychiatric diseases. This technique

involves the creation of a frequency distribution showing the proportion of voxels in an image within a given range of signal intensity (Cercignani, 2011). Two major indices, peak height and location, are usually evaluated in histogram analysis. While the former reflects a change in a portion of the voxels that contribute to the maximum frequency, the latter is suggestive of rather a global change in voxels which contribute to the maximum frequency (Cercignani et al., 2001). This might suggest that there remained voxels with low FA values left undiscovered by the voxel-based analysis. Additional indices that can be evaluated include skewness (the magnitude of symmetry of the histograms) and kurtosis (peakness of histograms) (Mori et al., 2008). Histogram analysis of DTI has been successfully used in brain tumor classification (Wang et al., 2012), investigation of clinical correlates in leukoariorosis (Della Nave et al., 2007) and multiple sclerosis (Cercignani et al., 2001), differentiation between ischemic and non-ischemic patients with moyamoya disease (Mori et al., 2008), evaluation of traumatic brain injury (Marquez de la Plata et al., 2011), and so on. In this study, histogram analysis enabled a discrimination between the patients and normal subjects by its peak position, which is suggestive of its applicability in the evaluation of integrity of brain parenchyma in MDD. When the cut-off value for the peak position of an FA histogram is 0.2, the sensitivity and specificity in distinguishing the patients from normal subjects are 0.89 and 0.74, respectively, as derived from ROC analysis (www.statsdirect.com). The strengths of histogram analysis include its ability to test many thousands of voxels as well as its robust statistical power (Della Nave et al., 2007). Its drawback is limited topographic information.

Because this study is an exploratory study, tests of correlation were performed without correction for multiple comparisons. A number of significant correlations between the FA values and the clinical variables were observed. The finding of positive correlation between the FA values and the clinical variables, especially clinical severity, is unusual. It is possible that the results of tests of correlation are inflated by Type I error associated with multiple comparisons (Gordis and Khamis, 2004). If corrected for

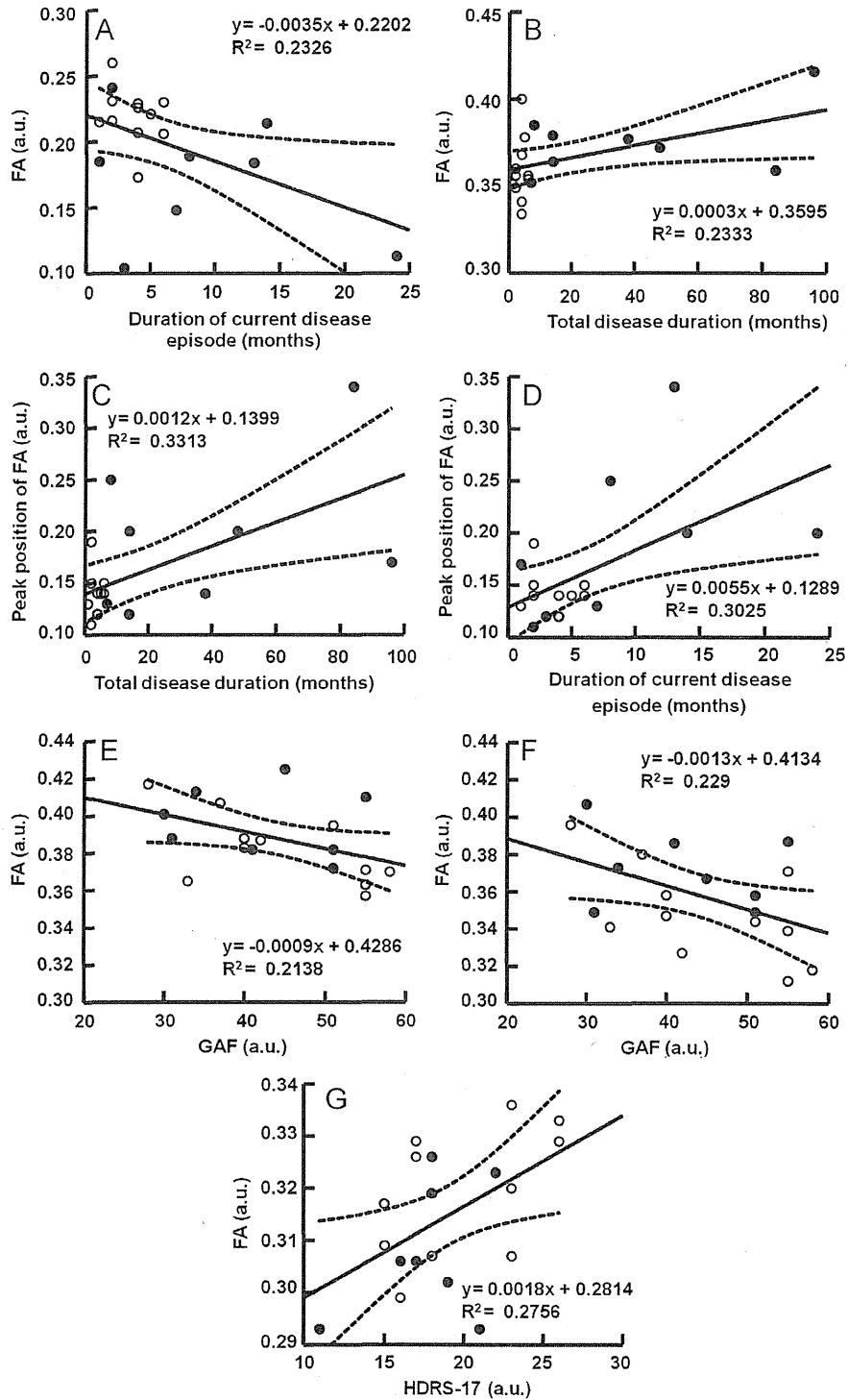


Fig. 4. Scatterplots showing correlation between the abnormal DTI indices and the clinical variables. Correlations between (A) the FA values of right frontal white matter ($x=12, y=43, z=-18$) and the duration of current disease episode, (B) the FA values of the left anterior limb of internal capsule and total disease duration, (C) the peak positions of FA histograms and total disease duration, (D) the peak positions of FA histograms and the duration of current disease episode, (E) the FA values of the right frontal white matter ($x=37, y=-19, z=59$) and the GAF, (F) the FA values of the right anterior limb of internal capsule and the GAF, and (G) the FA values of the right cerebellar hemisphere and the HDRS-17, are shown. White circles represent the medication-naïve patients. Black circles represent the patients who had a previous history of antidepressant medication. The solid lines represent the mean, and the dashed lines represent 95% confidence interval for the mean predicted value. Abbreviations: a.u.=arbitrary unit; FA=fractional anisotropy; GAF=Global Assessment of Functioning; HDRS-17=17-item Hamilton Depression Rating Scale.

multiple comparisons, no pairs with significant correlation would persist. Added to this, the slope of the regression lines might have been modified, and the correlation coefficient values might have

been artificially increased, by the outliers (Hatch and Prihoda, 1992). On the other hand, if these correlations are considered as true correlations, the observation of difference in the pattern of

correlation among the regions would suggest variation in the pattern of injury among the regions. Negative correlation between the duration of current disease episode and the FA value of the right frontal white matter ($x=12$, $y=43$, $z=-18$) would be indicative of impaired frontal white matter integrity with disease duration; and may reflect advancement of axon and/or myelin injury with time. Positive correlation between disease duration and the regional FA values may be due to alteration in the local microenvironment by the long disease process or previous antidepressant medication. First, patients with long disease duration are exposed to chronic stress. Chronic stress has been demonstrated to contribute to frontostriatal regeneration and reductions in the length and branch numbers of apical dendrites, particularly in the anterior cingulate cortex (Radley et al., 2004). As dendrite formation and increased synaptic density are associated with decrease in the FA, the dendritic changes induced by chronic stress could in turn present with a relative increase in the FA (Baloch et al., 2009; Taylor et al., 2011). However, owing to difference in histological composition between the two structures, the finding involving the anterior cingulate cortex may not plausibly explain the positive correlation between the FA value of the left anterior limb of the internal capsule and disease duration. Second, in this study, eight patients (those with long disease duration) had a previous history of antidepressant medication. Considering the half-lives of antidepressants, these patients were determined as being free of medication effects. However, the possibility that these patients' tissue microenvironment was modified and/or aggressively treated by previous medication cannot be completely excluded. The significant positive correlation between the regional FA values and clinical severity might be due to astrocytic hypertrophy. Although the evidence is still limited, a recent autopsy report that investigated the white matter underlying anterior cingulate cortex of depressed patients who committed suicide revealed an increase in the volume of astrocytic processes (representative of astrocytic hypertrophy) in the white matter (Torres-Platas et al., 2011). If it is assumed that astrocytic hypertrophy also occurs in the other white matter regions involved in MDD and that the degree of hypertrophy is severe enough to induce an increase in the regional FA values, the finding of FA increase with disease severity would be explained.

A few limitations of this study need to be addressed. First, correction for eddy current-induced distortion was not performed. This resulted in an imperfect match between the white matter masks constructed from the anatomical images (MPRAGE) and the FA and MD maps. Estimation of the average displacement between the MPRAGE images and the FA maps revealed an average shift of 0.91, 2.18, and 0.30 mm along the x , y , and z directions, respectively. Nevertheless, the magnitude of displacement did not vary significantly between the patients and control subjects, so as to maintain validity of the analytical method for evaluation of global brain parenchymal or white matter abnormalities. Second, the slice thickness used for DTI was 5 mm, and the interslice gap was 1.5 mm. These parameters were chosen so as to allow acquisition of all necessary sequences in clinically acceptable scan time. Ideally, thinner slices without interslice gap are desired. Third, DSM-IV-TR was used to diagnose MDD in the patients, whereas the MINI was used to exclude psychiatric diseases in normal subjects. Hence, there might be a question about potential bias arising from the use of different diagnostic assessment methods between the two groups. However, the possibility is considered unlikely as the MINI was designed to be used to rule out psychiatric diseases, and is compatible with DSM-IV-TR (Sheehan et al., 1998).

In conclusion, this study evaluated the integrity of the brain parenchyma in non-geriatric patients with MDD by using two different analytical methods of DTI. The findings suggest an

impaired integrity of the frontal-subcortical circuits and cerebello-cerebellar connections, which are known or thought to mediate human behavior. Consistency in the distribution of abnormalities is lacking among the DTI studies of MDD, including the current report, which may suggest spatial heterogeneity of the abnormalities in MDD. The peak position of the FA histograms may be used as supplementary to voxel-based analysis, in evaluation of the brain parenchymal integrity in MDD.

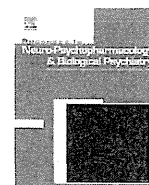
Acknowledgement

The authors are greatly indebted to the anonymous reviewers for the invaluable comments. This study was supported by (1) Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program of Project for Developing Innovation Systems of the Ministry of Education, Culture, Sports, Science and Technology, the Japanese Government, and (2) the Grant-in-aid for Scientific Research by the Japan Society for Promotion of Science (Project number 20591430).

References

- Alexopoulos, G.S., Kiosses, D.N., Choi, S.J., Murphy, C.F., Lim, K.O., 2002. Frontal white matter microstructure and treatment response of late-life depression: a preliminary study. *American Journal of Psychiatry* 159, 1929–1932.
- Alexopoulos, G.S., Murphy, C.F., Gunning-Dixon, F.M., Latoussakis, V., Kanellopoulos, D., Klimstra, S., Lim, K.O., Hoptman, M.J., 2008. Microstructural white matter abnormalities and remission of geriatric depression. *American Journal of Psychiatry* 165, 238–244.
- Bae, J.N., MacFall, J.R., Krishnan, K.R., Payne, M.E., Steffens, D.C., Taylor, W.D., 2006. Dorsolateral prefrontal cortex and anterior cingulate cortex white matter alterations in late-life depression. *Biological Psychiatry* 60, 1356–1363.
- Baloch, S., Verma, R., Huang, H., Khurd, P., Clark, S., Yarowsky, P., Abel, T., Mori, S., Davatzikos, C., 2009. Quantification of brain maturation and growth patterns in C57BL/6 J mice via computational neuroanatomy of diffusion tensor images. *Cerebral Cortex* 19, 675–687.
- Basser, P.J., Pierpaoli, C., 1996. Microstructural and physiological features of tissues elucidated by quantitative-diffusion tensor MRI. *Journal of Magnetic Resonance B* 111, 209–219.
- Beaulieu, C., 2011. What makes diffusion anisotropic in the nervous system?. In: Jones, D.K. (Ed.), *Diffusion MRI: Theory, Methods, and Applications*. Oxford University Press, New York, pp. 92–109.
- Beaugard, M., Leroux, J.M., Bergman, S., Arzoumanian, Y., Beaudoin, G., Bourgouin, P., Stip, E., 1998. The functional neuroanatomy of major depression: an fMRI study using an emotional activation paradigm. *Neuroreport* 14, 3253–3258.
- Blood, A.J., Iosifescu, D.V., Makris, N., Perlis, R.H., Kennedy, D.N., Dougherty, D.D., Kim, B.W., Lee, M.J., Wu, S., Lee, S., Calhoun, J., Hodge, S.M., Fava, M., Rosen, B.R., Smoller, J.W., Gasic, G.P., Breiter, H.C., 2010. Microstructural abnormalities in subcortical reward circuitry of subjects with major depressive disorder. *PLoS One* 11, e13945.
- Cercignani, M., Ingles, M., Pagani, E., Comi, G., Filippi, M., 2001. Mean diffusivity and fractional anisotropy histograms of patients with multiple sclerosis. *American Journal of Neuroradiology* 22, 952–958.
- Cercignani, M., 2011. Strategies for patient-control comparison of diffusion MR data. In: Jones, D.K. (Ed.), *Diffusion MRI: Theory, Methods, and Applications*. Oxford University Press, New York, pp. 485–499.
- Della Nave, R., Foresti, S., Pratesi, A., Ginestroni, A., Inzitari, M., Salvadori, E., Giannelli, M., Diciotti, S., Inzitari, D., Mascalchi, M., 2007. Whole-brain histogram and voxel-based analyses of diffusion tensor imaging in patients with leukoariosis: correlation with motor and cognitive impairment. *American Journal of Neuroradiology* 28, 1313–1319.
- Diamond, A., 2000. Close interrelation of motor development and cognitive development and of the cerebellum and prefrontal cortex. *Child Development* 71, 44–56.
- Dolan, R.J., Bench, C.J., Brown, R.G., Scott, L.C., Friston, K.J., Frackowiak, R.S., 1992. Regional cerebral blood flow abnormalities in depressed patients with cognitive impairment. *Journal of Neurology, Neurosurgery & Psychiatry* 55, 768–773.
- Egger, K., Schocke, M., Weiss, E., Auffinger, S., Esterhammer, R., Goebel, G., Walch, T., Mechtcheriakov, S., Marksteiner, J., 2008. Pattern of brain atrophy in elderly patients with depression revealed by voxel-based morphometry. *Psychiatry Research: Neuroimaging* 164, 237–244.
- Fatemi, S.H., Laurence, J.A., Aragi-Niknam, M., Stary, J.M., Schulz, S.C., Lee, S., Gottesman, I.I., 2004. Glial fibrillary acidic protein is reduced in cerebellum of subjects with major depression, but not schizophrenia. *Schizophrenia Research* 69, 317–323.

- Fujimoto, T., Takeuchi, K., Matsumoto, T., Fujita, S., Honda, K., Higashi, Y., Kato, N., 2008. Metabolic changes in the brain of patients with late-onset major depression. *Psychiatry Research: Neuroimaging* 164, 48–57.
- Gordji, T., Khamis, H., 2004. Simple solution to a common statistical problem: interpreting multiple tests. *Clinical Therapeutics* 26, 780–786.
- Graff-Guerrero, A., González-Olvera, J., Mendoza-Espinosa, Y., Vaugier, V., García-Reyna, J.C., 2004. Correlation between cerebral blood flow and items of the Hamilton rating scale for depression in antidepressant-naïve patients. *Journal of Affective Disorders* 80, 55–63.
- Greenberg, P.E., Stiglin, L.E., Finkelstein, S.N., Berndt, E.R., 1993. The economic burden of depression in 1990. *Journal of Clinical Psychiatry* 54, 405–418.
- Guy, W., 1976. *ECDEU Assessment Manual for Psychopharmacology*. Department of Health, Education, and Welfare, Rockville, MD: U.S.
- Hall, R.C.W., 1995. Global Assessment of Functioning: a modified scale. *Psychosomatics* 36, 267–275.
- Hatch, J.P., Prihoda, T.J., 1992. The effect of influential outliers on parameter estimation in regression analysis. *Biofeedback and Self-Regulation*, 17, 153–156.
- Jones, D.K., Symms, M.R., Cercignani, M.C., Howard, R.J., 2005. The effect of filter size on the outcome of VBM analyses of DT-MRI data. *Neuroimage* 26, 546–554.
- Kessler, R.C., Berglund, P., Demler, O., Jin, R., Koretz, D., Merikangas, K.R., Rush, A.J., Walters, E.E., Wang, P.S., 2003. National comorbidity survey replication. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). *The Journal of American Medical Association* 289, 3095–3105.
- Konarski, J.Z., McIntyre, R.S., Grupp, L.A., Kennedy, S.H., 2005. Is the cerebellum relevant in the circuitry of neuropsychiatric disorders? *Journal of Psychiatry and Neuroscience* 30, 178–186.
- Korgaonkar, M.S., Grieve, S.M., Koslow, S.H., Gabrieli, J.D., Gordon, E., Williams, L.M., 2011. Loss of white matter integrity in major depressive disorder: evidence using tract-based spatial statistical analysis of diffusion tensor imaging. *Human Brain Mapping* 32, 2161–2171.
- Li, L., Ma, N., Li, Z., Tan, L., Liu, J., Gong, G., Shu, N., He, Z., Jiang, T., Xu, L., 2007. Prefrontal white matter abnormalities in young adult with major depressive disorder: a diffusion tensor imaging study. *Brain Research* 1168, 124–128.
- Ma, N., Li, L., Shu, N., Liu, J., Gong, G., He, Z., Li, Z., Tan, L., Stone, W.S., Zhang, Z., Xu, L., Jiang, T., 2007. White matter abnormalities in first-episode, treatment-naïve young adults with major depressive disorder. *American Journal of Psychiatry* 164, 823–826.
- Malykhin, N., Concha, L., Seres, P., Beaulieu, C., Coupland, N.J., 2008. Diffusion tensor imaging tractography and reliability analysis for limbic and paralimbic white matter tracts. *Psychiatry Research: Neuroimaging* 164, 132–142.
- Marquez de la Plata, C.D., Yang, F.G., Wang, J.Y., Krishnan, K., Bakhadirov, K., Paliotta, C., Aslan, S., Devous, M.D., Moore, C., Harper, C., McColl, R., Munro Cullum, C., Diaz-Arrastia, R., 2011. Diffusion tensor imaging biomarkers for traumatic axonal injury: analysis of three analytic methods. *Journal of the International Neuropsychological Society* 17, 24–35.
- Montgomery, S.A., Åsberg, M., 1979. A new depression scale designed to be sensitive to change. *British Journal of Psychiatry* 134, 382–389.
- Mori, N., Miki, Y., Fushimi, Y., Kikuta, K., Urayama, S., Okada, T., Fukuyama, H., Hashimoto, N., Togashi, K., 2008. Cerebral infarction associated with moyamoya disease: histogram-based quantitative analysis of diffusion tensor imaging—a preliminary study. *Magnetic Resonance Imaging* 26, 835–840.
- Moseley, M., 2002. Diffusion tensor imaging and aging: a review. *NMR in Biomedicine* 15, 553–560.
- Nagesh, V., Tsien, C.I., Chenevert, T.L., Ross, B.D., Lawrence, T.S., Junick, L., Cao, Y., 2008. Radiation-induced changes in normal-appearing white matter in patients with cerebral tumors: a diffusion tensor imaging study. *International Journal of Radiation Oncology, Biology, Physics* 70, 1002–1010.
- Nobuhara, K., Okugawa, G., Sugimoto, T., Minami, T., Tamagaki, C., Takase, K., Saito, Y., Sawada, S., Kinoshita, T., 2006. Frontal white matter anisotropy and symptom severity of late-life depression: a magnetic resonance diffusion tensor imaging study. *Journal of Neurology, Neurosurgery and Psychiatry* 77, 120–122.
- Ouyang, X., Tao, H.J., Liu, H.H., Deng, Q.J., Sun, Z.H., Xu, L., Liu, Z.N., Xue, Z.M., 2011. White matter integrity deficit in treatment-naïve adult patients with major depressive disorder. *East Asian Archives of Psychiatry* 21, 5–9.
- Radley, J.J., Sisti, H.M., Hao, J., Rocher, A.B., McCall, T., Hof, P.R., McEwen, B.S., Morrison, J.H., 2004. Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. *Neuroscience* 125, 1–6.
- Regenold, W.T., Phatak, P., Marano, C.M., Gearhart, L., Viens, C.H., Hisley, K.C., 2007. Myelin staining of deep white matter in the dorsolateral prefrontal cortex in schizophrenia, bipolar disorder, and unipolar major depression. *Psychiatry Research* 151, 179–188.
- Sala, S., Agosta, F., Pagani, E., Copetti, M., Comi, G., Filippi, M., 2012. Microstructural changes and atrophy in brain white matter tracts with aging. *Neurobiology of Aging* 33, 488–498.
- Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The mini-international neuropsychiatric interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *The Journal of Clinical Psychiatry* 59, 22–33.
- Shimony, J.S., Sheline, Y.I., D'Angelo, G., Epstein, A.A., Benzinger, T.L., Mintun, M.A., McKinstry, R.C., Snyder, A.Z., 2009. Diffuse microstructural abnormalities of normal-appearing white matter in late life depression: a diffusion tensor imaging study. *Biological Psychiatry* 66, 245–252.
- Sijens, P.E., Mostert, J.P., Irwan, R., Potze, J.H., Oudkerk, M., Keyser, J.D., 2008. Impact of fluoxetine on the human brain in multiple sclerosis as quantified by proton magnetic resonance spectroscopy and diffusion tensor imaging. *Psychiatry Research: Neuroimaging* 164, 274–282.
- Strick, P.L., Dum, R.P., Fiez, J.A., 2009. Cerebellum and nonmotor function. *The Annual Review of Neuroscience* 32, 413–434.
- Taoka, T., Kin, T., Nakagawa, H., Hirano, M., Sakamoto, M., Wada, T., Takayama, K., Wuttikul, C., Iwasaki, S., Ueno, S., Kichikawa, K., 2007. Diffusivity and diffusion anisotropy of cerebellar peduncles in cases of spinocerebellar degenerative disease. *Neuroimage* 37, 387–393.
- Taylor, W.D., MacFall, J.R., Boyd, B., Payne, M.E., Sheline, Y.I., Krishnan, K.R., Doraiswamy, P.M., 2011. One-year change in anterior cingulate cortex white matter microstructure: relationship with late-life depression outcomes. *American Journal of Geriatric Psychiatry* 19, 43–52.
- Taylor, W.D., MacFall, J.R., Payne, M.E., McQuoid, D.R., Provenzale, J.M., Steffens, D.C., Krishnan, K.R., 2004. Late-life depression and microstructural abnormalities in dorsolateral prefrontal cortex white matter. *American Journal of Psychiatry* 161, 1293–1296.
- Tekin, S., Cummings, J.L., 2002. Frontal-subcortical neuronal circuits and clinical neuropsychiatry: an update. *Journal of Psychosomatic Research* 53, 647–654.
- Tha, K.K., Terae, S., Yabe, I., Miyamoto, T., Soma, H., Zaito, Y., Fujima, N., Kudo, K., Sasaki, H., Shirato, H., 2010. Microstructural white matter abnormalities of multiple system atrophy: in vivo topographic illustration by using diffusion-tensor MR imaging. *Radiology* 255, 563–569.
- Torres-Platas, S.G., Hercher, C., Davoli, M.A., Maussion, G., Labonté, B., Turecki, G., Mechawar, N., 2011. Astrocytic hypertrophy in anterior cingulate white matter of depressed suicides. *Neuropsychopharmacology* 36, 2650–2658.
- Tsopelas, C., Stewart, R., Sawa, G.M., Brayne, C., Ince, P., Thomas, A., Matthews, F.E., Medical Research Council Cognitive Function and Ageing Study, 2011. Neuro-pathological correlates of late-life depression in older people. *The British Journal of Psychiatry* 198, 109–114.
- Uranova, N.A., Vostrikov, V.M., Orlovskaya, D.D., Rachmanova, V.I., 2004. Oligodendroglial density in the prefrontal cortex in schizophrenia and mood disorders: a study from the Stanley neuropathology consortium. *Schizophrenia Research* 67, 269–275.
- Videbech, P., Ravnikilde, B., Pedersen, A.R., Egander, A., Landbo, B., Rasmussen, N.A., Andersen, F., Stødkilde-Jørgensen, H., Gjedde, A., Rosenberg, R., 2001. The Danish PET/depression project: PET findings in patients with major depression. *Psychological Medicine* 31, 1147–1158.
- Wang, S., Kim, S., Zhang, Y., Wang, L., Lee, E.B., Syre, P., Poptani, H., Melhem, E.R., Lee, J.Y., 2012. Determination of grade and subtypes of meningiomas by using histogram analysis of diffusion-tensor imaging metrics. *Radiology* 262, 584–592.
- Waxman, H.M., Carner, E.A., Dubin, W., Klein, M., 1982. Geriatric psychiatry in the emergency department: characteristics of geriatric and non-geriatric admissions. *Journal of the American Geriatrics Society* 30, 427–432.
- Williams, J.B., 1988. A structured interview guide for the Hamilton depression rating scale. *Archives of General Psychiatry* 45, 742–747.
- Wu, F., Tang, Y., Xu, K., Kong, L., Sun, W., Wang, F., Kong, D., Li, Y., Liu, Y., 2011. White matter abnormalities in medication-naïve subjects with a single short-duration episode of major depressive disorder. *Psychiatry Research: Neuroimaging* 191, 80–83.
- Yang, Q., Huang, X., Hong, N., Yu, X., 2007. White matter microstructural abnormalities in late-life depression. *International Psychogeriatrics* 19, 757–766.
- Yoo, S.Y., Jang, J.H., Shin, Y.H., Kim, D.J., Park, H.J., Moon, W.J., Chung, E.C., Lee, J.M., Kim, I.Y., Kim, S.I., Kwon, J.S., 2007. White matter abnormalities in drug-naïve patients with obsessive-compulsive disorder: a diffusion tensor study before and after citalopram treatment. *Acta Psychiatrica Scandinavica* 116, 211–219.
- Zhu, X., Wang, X., Xiao, J., Zhong, M., Liao, J., Yao, S., 2011. Altered white matter integrity in first-episode, treatment-naïve young adults with major depressive disorder: a tract-based spatial statistics study. *Brain Research* 1369, 223–229.
- Zou, K., Huang, X., Li, T., Gong, Q., Li, Z., Ou-Yang, L., Deng, W., Chen, Q., Li, C., Ding, Y., Sun, X., 2008. Alterations of white matter integrity in adults with major depressive disorder: a magnetic resonance imaging study. *Journal of Psychiatry & Neuroscience* 33, 525–530.



The effects of the co-administration of the α_1 -adrenoreceptor antagonist prazosin on the anxiolytic effect of citalopram in conditioned fear stress in the rat

Naoki Takamura^{a,b}, Takahiro Masuda^{a,b}, Takeshi Inoue^{a,*}, Shin Nakagawa^a, Tsukasa Koyama^a

^a Department of Psychiatry, Hokkaido University Graduate School of Medicine, Sapporo, Japan

^b Pharmacology Research Laboratories, Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan

ARTICLE INFO

Article history:

Received 20 February 2012

Received in revised form 10 May 2012

Accepted 23 May 2012

Available online 30 May 2012

Keywords:

Anxiety

Conditioned fear

Prazosin

Selective serotonin reuptake inhibitor

ABSTRACT

Several studies have shown that the α_1 -adrenoreceptor is involved in controlling extracellular serotonin levels. The administration of the α_1 -adrenoreceptor antagonist prazosin was shown to decrease extracellular serotonin levels in the hippocampus, the prefrontal cortex and the raphe nucleus, while the administration of the α_1 -adrenoreceptor agonist cirazoline was shown to increase serotonin levels. Furthermore, the elevation of serotonin levels induced by the selective serotonin reuptake inhibitor (SSRI) citalopram was attenuated by prazosin. Thus, α_1 -adrenoreceptor antagonists may affect SSRI-induced increases in extracellular serotonin levels and their antidepressive and anxiolytic effects. However, little is known about the influence of α_1 -adrenoreceptor antagonists on the behavioral pharmacological effects of SSRIs. The conditioned fear stress-induced freezing behavior is an animal model of anxiety and can detect the anxiolytic effect of SSRIs. To clarify whether an α_1 -adrenoreceptor antagonist affects the anxiolytic action of SSRIs, we examined the effects of the co-administration of the α_1 -adrenoreceptor antagonist prazosin and the SSRI citalopram using the contextual conditioned fear stress model. Low-dose prazosin (0.03 mg/kg) significantly attenuated the citalopram (3 mg/kg)-induced decrease in conditioned freezing. Moreover, high-dose (0.5 mg/kg), but not low-dose (0.03 mg/kg), prazosin significantly attenuated citalopram (10 mg/kg)-induced decreases in conditioned freezing. These drugs did not affect the spontaneous motor activity of the rats. Therefore, these results suggest that blocking the α_1 -adrenoreceptor decreases the anxiolytic effect of citalopram.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Selective serotonin reuptake inhibitors (SSRIs) are prescribed for many disorders, including major depressive disorder and various anxiety disorders (generalized anxiety disorder, panic disorder, post-traumatic stress disorder, etc.) (Zohar and Westenberg, 2000). Brain microdialysis studies have shown that SSRIs increase the extracellular serotonin concentrations 2–5 fold in the raphe nucleus, the hypothalamus and the cerebral cortex (Fuller, 1994). These long-term changes in extracellular serotonin concentrations induce receptor reorganization, hippocampal neurogenesis and other changes (Santarelli et al., 2003; Stahl, 1998). These changes are thought to be relevant to the efficacy of SSRIs in depressive and anxiety disorders.

Several *in vivo* brain microdialysis studies suggest that the α_1 -adrenoreceptor is involved in controlling extracellular serotonin levels. The administration of prazosin, a selective and centrally active

α_1 -adrenoreceptor antagonist (Menkes et al., 1981), was shown to decrease extracellular serotonin levels in the hippocampus, the prefrontal cortex and the raphe nucleus (Gobert et al., 1998; Hjorth et al., 1995; Pudovkina et al., 2003; Rouquier et al., 1994). Conversely, the administration of the α_1 -adrenoreceptor agonist cirazoline increased extracellular serotonin levels in the raphe nucleus (Amargos-Bosch et al., 2005; Pudovkina et al., 2003). The elevation of serotonin levels induced by citalopram in the raphe nucleus, the prefrontal cortex and the hippocampus was attenuated by the concurrent administration of prazosin (Rea et al., 2010).

Furthermore, antipsychotic drugs, which are used as augmentation therapy for treatment-resistant depression and anxiety disorders, and most tricyclic antidepressants have α_1 -adrenoreceptor antagonistic effects (Bandelow et al., 2008; Gillman, 2007; Lam et al., 2009; Pudovkina et al., 1993; Nasrallah, 2008; Richelson and Souder, 2000). Earlier studies have shown that the antipsychotic drug olanzapine, which has a significant affinity for the α_1 -adrenoreceptor, attenuated the increase in extracellular serotonin levels induced by fluoxetine (an SSRI) as measured by *in vivo* microdialysis studies (Koch et al., 2004; Zhang et al., 2000). This result suggests that these α_1 -adrenoreceptor antagonistic drugs affect SSRI-induced increases in extracellular serotonin levels and therefore modulate their antidepressant and anxiolytic effects. However, little is known

Abbreviations: ANOVA, analysis of variance; SSRI, selective serotonin reuptake inhibitor; SERT, serotonin transporter; NET, norepinephrine transporter.

* Corresponding author at: Department of Psychiatry, Hokkaido University Graduate School of Medicine, North 15, West 7, Sapporo 060-8638, Japan. Tel.: +81 11 706 5160; fax: +81 11 706 5081.

E-mail address: tinoue@med.hokudai.ac.jp (T. Inoue).

about the influence of α_1 -adrenoreceptor antagonists on the behavioral pharmacological effects of SSRIs.

The conflict test and the elevated plus-maze test are generally used as animal models of anxiety to evaluate the efficacy of benzodiazepines, a class of anxiolytic drugs. However, these traditional animal models of anxiety failed to demonstrate the anxiolytic effect of SSRIs (Borsini et al., 2002). Thus, it has proven difficult to study the mechanism of anxiolytic actions of SSRIs using these animal models. The conditioned fear stress-induced freezing model can be used as an animal model of anxiety. Our previous studies have shown that the freezing behavior is attenuated by both benzodiazepines and serotonergic anxiolytic drugs, including serotonin1A receptor agonists and SSRIs (Hashimoto et al., 1996; Inoue et al., 1996, 2004, 2011; Nishitsuji et al., 2006). Thus, the conditioned fear stress model is thought suitable to evaluate drugs affecting serotonergic neurotransmission. Based on our previous studies, SSRIs are thought to mitigate anxiety in the contextual conditioned fear stress model by increasing extracellular serotonin levels by blocking the serotonin transporter and enhancing serotonergic neural transmission (Inoue et al., 2011). Taken together with the previous studies described above concerning the role of α_1 -adrenoreceptors in controlling serotonin release, it is hypothesized that the α_1 -adrenoreceptor antagonists interfere with the effect of SSRIs on the conditioned fear stress-induced freezing behavior by inhibiting the increase in extracellular serotonin levels effected by SSRIs.

In this study, to verify the hypothesis that antagonism of the α_1 -adrenoreceptor affects the anxiolytic action of SSRIs, we examined the effects of the co-administration of the α_1 -adrenoreceptor antagonist prazosin with the SSRI citalopram in the contextual conditioned fear stress model. These results showed that the α_1 -adrenoreceptor antagonist prazosin attenuated the inhibitory effects of citalopram on conditioned freezing. In addition, this effect was independent of any non-specific effects on motor activity. These results suggest that α_1 -adrenoreceptors play an important role in SSRI-induced anxiolytic effects on conditioned fear in rats.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (250–350 g) were obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan). The rats were housed in groups of four in a temperature-controlled environment ($22 \pm 1^\circ\text{C}$) with free access to food and water. The subjects were maintained on a 12 h light/dark cycle (light phase: 06:30–18:30). The experiments began after a 1 week acclimatization period. The rats were tested between 08:30 and 11:30.

All experiments were approved by the Hokkaido University School of Medicine Animal Care and Use Committee and were in compliance with the Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Citalopram hydrobromide was donated by the Dainippon Sumitomo Pharma Co., Ltd. Prazosin hydrochloride was obtained from the Sigma-Aldrich Corp. (St. Louis, MO, USA). Citalopram was dissolved in 0.9% NaCl solution (saline) and administered by subcutaneous injection (s.c.) at a volume of 1 ml/kg 60 min prior to testing. Prazosin was dissolved in 0.5% methylcellulose and administered by intraperitoneal injection (i.p.) at a volume of 1 ml/kg 75 min prior to testing. The doses of citalopram and prazosin used in this study were based on our previous experiments and previous reports by other investigators (Inoue et al., 2011; Kakui et al., 2009; Rouquier et al., 1994; Selken and Nichols, 2007).

2.3. The contextual conditioned fear stress model

The rats were individually subjected to 150 s of inescapable electric footshock (five 2.5 mA scramble footshocks, pulse wave, 30 s duration) that were delivered at intershock intervals of 35–80 s (mean 60 s) in a shock chamber with a grid floor ($19 \times 22 \times 20$ cm, Medical Agent Co., Kyoto, Japan). The electric shocks were applied by a Model SGS-02D Shock Generator (Medical Agent Co.). A day later, the rats were placed in the shock chamber without any footshock and observed for 5 min. Their behavior was videotaped and later scored by human observation. During the observation period (test period), the duration of freezing behavior was recorded using a modified time-sampling procedure, as previously described (Inoue et al., 2004). Every 10 s, the behavior in which the subject was currently engaged was classified as either 'freezing' or 'activity'. Freezing was defined as the absence of any observable movement of the skeleton and the vibrissae, except that related to respiration. All other behavior was scored as activity. The subject was classified as either freezing or active according to its behavior throughout the entire 10-s period.

The percentage scores for the duration of freezing behavior (% freezing) were calculated for each 5-min observation period.

2.4. The assay of motor activity

To exclude the possibility that citalopram and prazosin administration affect freezing nonspecifically by affecting spontaneous activity, we measured rat spontaneous activity for 5 min, the same period used to monitor conditioned freezing. In detail, the motor activity of unshocked rats was measured after the administration of prazosin (0.03 and 0.5 mg/kg, i.p.), citalopram (3 and 10 mg/kg, s.c.) and after the co-administration of both drugs. The rats were individually habituated in their home cages to the testing room for one day. Prazosin was administered 75 min prior to testing, and citalopram was administered 60 min prior to testing. Rats were individually placed in a testing cage. Their motor activity was automatically recorded for 5 min as previously described by Ohmori et al. (1994) using an infrared sensor that detected thermal radiation. Horizontal movement was responsible for most of the count. Other body movements also contributed when they included a substantial horizontal component.

2.5. Data analysis

All data are presented as the mean \pm SEM of the individual values for each rat among the groups. Statistical analyses of the freezing behavior and motor activity data were performed using a one-way analysis of variance (ANOVA) followed by a Fisher's LSD test for parametric multiple comparisons. In the event that the data were not normally distributed or homogenous for variance, a Kruskal–Wallis analysis followed by a Scheffe test for non-parametric analyses was performed to detect any significant effects of treatments.

3. Results

3.1. The effect of the co-administration of prazosin (0.03 mg/kg) with acute low-dose citalopram (3 mg/kg) treatment on conditioned freezing

The SSRI citalopram (3 mg/kg) significantly reduced the duration of conditioned freezing. Although the administration of prazosin (0.03 mg/kg) by itself effected no remarkable change in conditioned freezing, the co-administration of prazosin reversed the freezing inhibition induced by citalopram [$F(3, 56) = 4.12, P < 0.05$, one-way ANOVA] (Fig. 1).

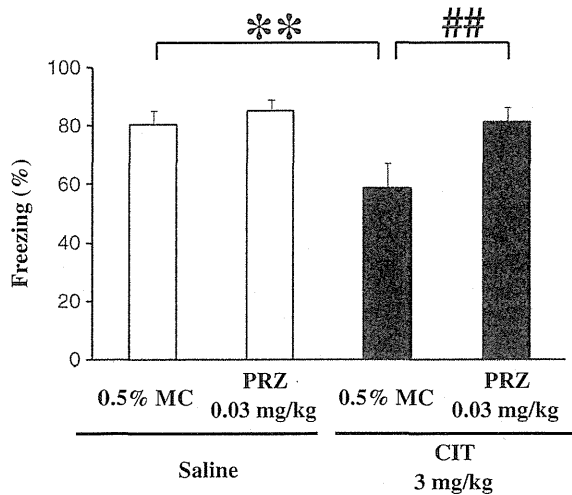


Fig. 1. The effect of the co-administration of prazosin with acute low-dose citalopram treatment (3 mg/kg) on conditioned fear in rats. One day after footshocks, vehicle or prazosin (PRZ; 0.03 mg/kg, i.p.) and saline or citalopram (CIT; 3 mg/kg, s.c.) were administered 75 min and 60 min before testing, respectively. The data are presented as the mean \pm SEM ($n = 14$ – 16). ** $P < 0.01$ versus vehicle control and ## $P < 0.01$ versus citalopram alone (Fisher's PLSD test). MC, methylcellulose.

3.2. The effect of the co-administration of prazosin (0.03 mg/kg and 0.5 mg/kg) with acute high-dose citalopram (10 mg/kg) treatment on conditioned freezing

We next investigated the effect of prazosin (0.03 mg/kg) on the high-dose citalopram (10 mg/kg)-induced anxiolytic effect. Citalopram (10 mg/kg) alone significantly reduced the conditioned freezing response. However, prazosin (0.03 mg/kg) failed to antagonize the citalopram-induced inhibition of freezing behavior [$F(3, 28) = 4.50$, $P < 0.05$, one-way ANOVA] (Fig. 2a). Because 0.03 mg/kg prazosin did not antagonize the anxiolytic effect of citalopram (10 mg/kg) (Fig. 2a), we next investigated whether 0.5 mg/kg of prazosin affected the anxiolytic effect of citalopram (10 mg/kg). While the administration of prazosin (0.5 mg/kg) alone did not affect conditioned freezing, the co-administration of prazosin completely reversed the anxiolytic effect of citalopram (10 mg/kg) [$H = 13.20$, $P < 0.01$, Kruskal–Wallis test] (Fig. 2b).

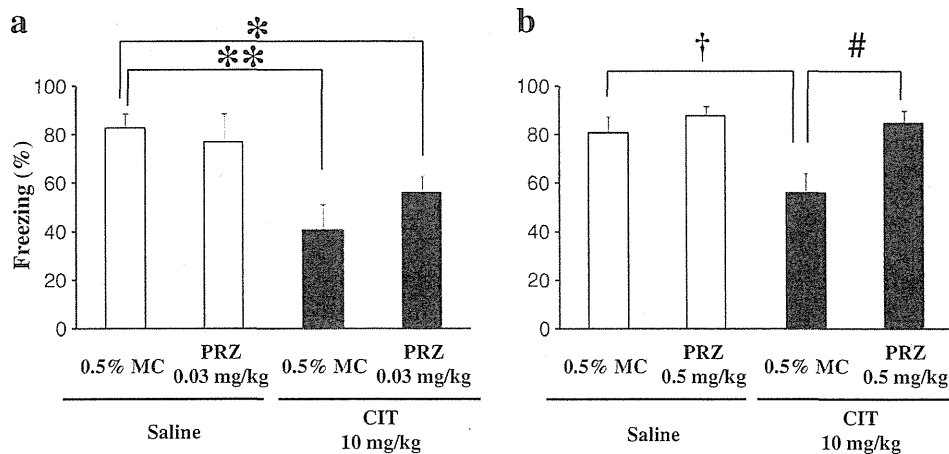


Fig. 2. The effect of the co-administration of prazosin with acute high-dose citalopram treatment (10 mg/kg, i.p.) on conditioned fear in rats. (a) Vehicle or prazosin (PRZ; 0.03 mg/kg, i.p.) and saline or citalopram (CIT; 10 mg/kg, s.c.) were administered 75 min and 60 min before testing, respectively. (b) Vehicle or prazosin (PRZ; 0.5 mg/kg, i.p.) and saline or citalopram (CIT; 10 mg/kg, s.c.) were administered 75 min and 60 min before testing, respectively. The data are presented as the mean \pm SEM ($n = 16$) (a) * $P < 0.05$, ** $P < 0.01$ versus vehicle control (Fisher's PLSD test), (b) † $P < 0.05$ versus vehicle control and # $P < 0.05$ versus citalopram alone (Scheffé test).

3.3. Motor activity

Citalopram and prazosin had no effect on the motor activity of the rats. Furthermore, the co-administration of prazosin and citalopram also had no effect on locomotor activities (Table 1).

4. Discussion

In the present study, the α_1 -adrenoreceptor antagonist prazosin attenuated the inhibitory effects of citalopram on conditioned freezing. Prazosin itself had no effect on conditioned freezing. The administration of citalopram alone, prazosin alone or their combination did not influence rat locomotor activities. Therefore, the effect of the co-administration of citalopram and prazosin on conditioned freezing was likely not due to non-specific effects on motor activities. Thus, these results suggest the importance of the α_1 -adrenoreceptor in the citalopram-induced anxiolytic effect in the rat contextual conditioned fear model.

SSRIs increase extracellular serotonin levels by binding to serotonin transporters and suppressing serotonin reuptake (Fuller, 1994). While SSRIs do not exhibit an anxiolytic effect in traditional anxiety models such as the conflict test or the elevated plus maze (Borsini et al., 2002), our previous studies showed that the SSRIs exhibited an anxiolytic effect in the conditioned fear stress-induced freezing model consistent with clinical findings that SSRIs are effective in the treatment of anxiety disorders; we also showed that the increase in extracellular serotonin levels induced by SSRIs decreases conditioned freezing (Inoue et al., 2011). Thus, this model is suitable to evaluate the mechanism of the anxiolytic effect of SSRIs, and the results from this model may be extrapolated to human anxiety disorders with the consideration of some limitations.

Previous investigations have demonstrated that noradrenergic neurons project to the dorsal raphe nucleus and the serotonergic neurons in the raphe nucleus are under the α_1 -adrenoreceptor-mediated tonic facilitatory influence (Baraban and Aghajanian, 1980, 1981; Lejeune et al., 1994; Vandermaelen and Aghajanian, 1983). Therefore, changes in the noradrenergic activities of the raphe nucleus may modulate the serotonergic neurotransmission in several brain regions relevant to anxiety, including the frontal cortex, the hippocampus and the amygdala. In fact, α_1 -adrenoreceptor antagonists suppress the firing activity of serotonergic neurons in the raphe nucleus (Lejeune et al., 1994; Vandermaelen and Aghajanian, 1983), and serotonin release was inhibited by antagonizing

Table 1

The effect of the co-administration of prazosin (PRZ; 0.03 mg/kg or 0.5 mg/kg) and citalopram (CIT; 3 mg/kg or 10 mg/kg) on spontaneous motor activity.

Drug treatment (mg/kg)	Motor activity (counts/5 min)
0.5% MC + saline	10.4 ± 8.7
PRZ (0.03) + saline	0 ± 0
0.5% MC + CIT (3)	4.0 ± 2.6
PRZ (0.03) + CIT (3)	9.8 ± 6.7
0.5% MC + saline	15.3 ± 9.1
PRZ (0.5) + saline	2.1 ± 1.9
0.5% MC + CIT (10)	24.5 ± 8.6
PRZ (0.5) + CIT (10)	1.1 ± 0.8

All data are represented as the mean ± SEM (n = 8). MC, methylcellulose.

the α_1 -adrenoreceptors of the rat hippocampus, the prefrontal cortex and the raphe nucleus (Gobert et al., 1998; Hjorth et al., 1995; Pudovkina et al., 2003; Rouquier et al., 1994). Moreover, citalopram increased extracellular serotonin levels in the raphe nucleus, the prefrontal cortex and the hippocampus that was attenuated by the co-administration of prazosin (Rea et al., 2010). Citalopram has a selectively high affinity for the serotonin transporter (SERT) and low affinity for the norepinephrine transporter (NET) and various monoamine receptors including the α_1 -adrenoreceptor (Millan et al., 2001). Moreover, the doses of citalopram used in the present study significantly increased extracellular serotonin levels but do not significantly affect the extracellular levels of noradrenaline or dopamine (Bymaster et al., 2002; Millan et al., 2001). Thus, our results were not caused by a direct effect of prazosin on citalopram-induced changes in noradrenergic neural transmission. Taken together with the previous studies, our results support the hypothesis that α_1 antagonism attenuates serotonin release, thereby interfering with the anxiolytic effect of SSRIs on conditioned freezing.

In this study, low-dose prazosin (0.03 mg/kg) failed to attenuate the anxiolytic effect of high-dose citalopram (10 mg/kg). However, high-dose prazosin (0.5 mg/kg) attenuated the anxiolytic effect of high-dose citalopram (10 mg/kg) (Fig. 2). Rouquier et al. (1994) showed that systemic administration of prazosin at doses of 0.03, 0.1 and 0.4 mg/kg [intravenous injection (i.v.)] decreased the extracellular serotonin levels in the rat hippocampus in a dose-dependent manner (50%, 60% and 65%, respectively, 60 min after drug administration). They also showed that the systemic administration of 0.4 mg/kg (i.v.) prazosin decreased the extracellular serotonin levels (a 35% decrease 60 min after administration) in the striatum, although 0.03 mg/kg prazosin did not affect serotonin release. Therefore, it can be concluded that high-dose prazosin (0.5 mg/kg) was necessary to inhibit the anxiolytic effect of high-dose citalopram (10 mg/kg). Our data support this conclusion because high-dose prazosin (0.5 mg/kg) is more potent at inhibiting serotonin release than low-dose prazosin (0.03 mg/kg). Thus, these results suggest that the more antagonistic a drug is toward the α_1 -adrenoreceptor, the more potently the drug attenuates the anxiolytic effect of SSRIs.

Earlier *in vivo* microdialysis studies examining the co-administration of an antipsychotic drug (with an affinity for α_1 -adrenoreceptors) and SSRIs support a role for α_1 -adrenoreceptors in the modulation of extracellular serotonin levels. The acute administration of the atypical antipsychotic drug olanzapine attenuated the increase in extracellular serotonin induced by fluoxetine (Koch et al., 2004; Zhang et al., 2000). These results might be explained by the affinity of olanzapine for the α_1 -adrenoreceptor. The results of our study and previous studies by other investigators suggest that the use of α_1 -adrenoreceptor antagonists in conjunction with SSRIs may weaken the clinical efficacy of the SSRIs. Clinically, such α_1 antagonistic types of antidepressants and antipsychotics are widely used to treat depressive and anxiety disorders (Bandelow et al., 2008; Lam et al., 2009). Our results suggest that we should consider whether the drugs to be co-prescribed with SSRIs are α_1 -adrenoreceptor antagonists because such drugs may attenuate the antidepressive and anxiolytic effects of SSRIs.

Clinically, however, SSRIs exhibit their antidepressive and anxiolytic effects only after long-term treatment (Den Boer and Westenberg, 1988;

Oehrberg et al., 1995). Thus, we next plan to examine the chronic effect of prazosin and citalopram co-administration in future experiments. Moreover, we also plan to investigate the efficacy of the combined administration of various antipsychotics and SSRIs on conditioned freezing. The results of these further studies may provide useful information to guide clinical anti-anxiety therapy.

5. Conclusion

In conclusion, the findings of this study show that prazosin attenuates the citalopram-induced anxiolytic effect. This study demonstrates that the citalopram-induced suppression of freezing in the conditioned freezing model was significantly attenuated by the administration of the α_1 -adrenoreceptor antagonist prazosin. Antagonists of the α_1 -adrenoreceptor may play an important role in SSRI-induced anxiolytic effects on conditioned fear in rats.

Conflicts of interest

Dr. Tsukasa Koyama has received honoraria from GlaxoSmithKline, Astellas and Eli Lilly and has received research/grant support from Dainippon Sumitomo Pharma, Astellas and GlaxoSmithKline; he is a member of the advisory board of GlaxoSmithKline and Mitsubishi Tanabe Pharma. The other authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported in part by Grants-in-Aid for Scientific Research No. 21591478 (T. Inoue) from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

References

- Amargos-Bosch M, Artigas F, Adell A. Effects of acute olanzapine after sustained fluoxetine on extracellular monoamine levels in the rat medial prefrontal cortex. *Eur J Pharmacol* 2005;516:235–8.
- Bandelow B, Zohar J, Hollander E, Kasper S, Moller HJ, Allgulander C, et al. World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for the pharmacological treatment of anxiety, obsessive-compulsive and post-traumatic stress disorders – first revision. *World J Biol Psychiatry* 2008;9:248–312.
- Baraban JM, Aghajanian GK. Suppression of firing activity of 5-HT neurons in the dorsal raphe by alpha-adrenoceptor antagonists. *Neuropharmacology* 1980;19:355–63.
- Baraban JM, Aghajanian GK. Noradrenergic innervation of serotonergic neurons in the dorsal raphe: demonstration by electron microscopic autoradiography. *Brain Res* 1981;204:1–11.
- Borsini F, Podhorna J, Marazziti D. Do animal models of anxiety predict anxiolytic-like effects of antidepressants? *Psychopharmacology* 2002;163:121–41.
- Bymaster FP, Zhang W, Carter PA, Shaw J, Chernet E, Phebus L, et al. Fluoxetine, but not other selective serotonin uptake inhibitors, increases norepinephrine and dopamine extracellular levels in prefrontal cortex. *Psychopharmacology (Berl)* 2002;160:353–61.
- Den Boer JA, Westenberg HG. Effect of a serotonin and noradrenaline uptake inhibitor in panic disorder; a double-blind comparative study with fluvoxamine and maprotiline. *Int Clin Psychopharmacol* 1988;3:59–74.
- Fuller RW. Uptake inhibitors increase extracellular serotonin concentration measured by brain microdialysis. *Life Sci* 1994;55:163–7.
- Gillman PK. Tricyclic antidepressant pharmacology and therapeutic drug interactions updated. *Br J Pharmacol* 2007;151:737–48.
- Gobert A, Rivet JM, Audinot V, Newman-Tancredi A, Cistarelli L, Millan MJ. Simultaneous quantification of serotonin, dopamine and noradrenaline levels in single frontal cortex dialysates of freely-moving rats reveals a complex pattern of reciprocal auto- and heteroreceptor-mediated control of release. *Neuroscience* 1998;84:413–29.
- Hashimoto S, Inoue T, Koyama T. Serotonin reuptake inhibitors reduce conditioned fear stress-induced freezing behavior in rats. *Psychopharmacology (Berl)* 1996;123:182–6.
- Hjorth S, Bengtsson HJ, Milano S, Lundberg JF, Sharp T. Studies on the role of 5-HT1A autoreceptors and alpha 1-adrenoceptors in the inhibition of 5-HT release—I. *BMJ* 1995;34:615–20.
- Inoue T, Hashimoto S, Tsuchiya K, Izumi T, Koyama T. Effect of citalopram, a selective serotonin reuptake inhibitor, on the acquisition of conditioned freezing. *Eur J Pharmacol* 1996;311:1–6.
- Inoue T, Kitaichi Y, Koyama T. SSRIs and conditioned fear. *Prog Neuropsychopharmacol Biol Psychiatry* 2011;35:1810–9.
- Inoue T, Li XB, Abekawa T, Kitaichi Y, Izumi T, Nakagawa S, et al. Selective serotonin reuptake inhibitor reduces conditioned fear through its effect in the amygdala. *Eur J Pharmacol* 2004;497:311–6.

- Kakui N, Yokoyama F, Yamauchi M, Kitamura K, Imanishi T, Inoue T, et al. Anxiolytic-like profile of mirtazapine in rat conditioned fear stress model: functional significance of 5-hydroxytryptamine 1A receptor and α_1 -adrenergic receptor. *Pharmacol Biochem Behav* 2009;92:393–8.
- Koch S, Perry KW, Bymaster FP. Brain region and dose effects of an olanzapine/fluoxetine combination on extracellular monoamine concentrations in the rat. *Neuropharmacology* 2004;46:232–42.
- Lam RW, Kennedy SH, Grigoriadis S, McIntyre RS, Milev R, Ramasubbu R, et al. Canadian Network for Mood and Anxiety Treatments (CANMAT) clinical guidelines for the management of major depressive disorder in adults. III. Pharmacotherapy. *J Affect Disord* 2009;117(Suppl. 1):S26–43.
- Lejeune F, Audinot V, Gobert A, Rivet JM, Spedding M, Millan MJ. Clozapine inhibits serotonergic transmission by an action at alpha 1-adrenoceptors not at 5-HT1A receptors. *Eur J Pharmacol* 1994;260:79–83.
- Leysen JE, Janssen PM, Schotte A, Luyten WH, Megens AA. Interaction of antipsychotic drugs with neurotransmitter receptor sites *in vitro* and *in vivo* in relation to pharmacological and clinical effects: role of 5HT2 receptors. *Psychopharmacology (Berl)* 1993;112:S40–54.
- Menkes DB, Baraban JM, Aghajanian GK. Prazosin selectively antagonizes neuronal responses mediated by alpha1-adrenoceptors in brain. *Naunyn Schmiedeberg Arch Pharmacol* 1981;317:273–5.
- Millan MJ, Gobert A, Lejeune F, Newman-Tancredi A, Rivet JM, Auclair A, et al. S33005, a novel ligand at both serotonin and norepinephrine transporters: I. Receptor binding, electrophysiological, and neurochemical profile in comparison with venlafaxine, reboxetine, citalopram, and clomipramine. *J Pharmacol Exp Ther* 2001;298:565–80.
- Nasrallah HA. Atypical antipsychotic-induced metabolic side effects: insights from receptor-binding profiles. *Mol Psychiatry* 2008;13:27–35.
- Nishitsuji K, To H, Shimizu T, Yanase Y, Yamada T, Hara C, et al. The pharmacokinetics and pharmacodynamics of tandospirone in rats exposed to conditioned fear stress. *Eur Neuropsychopharmacol* 2006;16:376–82.
- Oehrberg S, Christiansen PE, Behnke K, Borup AL, Severin B, Soegaard J, et al. Paroxetine in the treatment of panic disorder. A randomised, double-blind, placebo-controlled study. *Br J Psychiatry* 1995;167:374–9.
- Ohmori T, Abekawa T, Muraki A, Koyama T. Competitive and noncompetitive NMDA antagonists block sensitization to methamphetamine. *Pharmacol Biochem Behav* 1994;48:587–91.
- Pudovkina OL, Cremers TIFH, Westerink BHC. Regulation of the release of serotonin in the dorsal raphe nucleus by α_1 and α_2 adrenoceptors. *Synapse* 2003;50:77–82.
- Rea K, Folgering J, Westerink BHC, Cremers TIFH. α_1 -Adrenoceptors modulate citalopram-induced serotonin release. *Neuropharmacology* 2010;58:962–71.
- Richelson E, Souder T. Binding of antipsychotic drugs to human brain receptors focus on newer generation compounds. *Life Sci* 2000;68:29–39.
- Rouquier L, Claustre Y, Benavides J. Alpha 1-adrenoceptor antagonists differentially control serotonin release in the hippocampus and striatum: a microdialysis study. *Eur J Pharmacol* 1994;261:59–64.
- Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 2003;301:805–9.
- Selken J, Nichols DE. α_1 -Adrenergic receptors mediate the locomotor response to systemic administration of (\pm)-3,4-methylenedioxymethamphetamine (MDMA) in rats. *Pharmacol Biochem Behav* 2007;86:622–30.
- Stahl SM. Mechanism of action of serotonin selective reuptake inhibitors. Serotonin receptors and pathways mediate therapeutic effects and side effects. *J Affect Disord* 1998;51:215–35.
- Vandermaelen CP, Aghajanian GK. Electrophysiological and pharmacological characterization of serotonergic dorsal raphe neurons recorded extracellularly and intracellularly in rat brain slices. *Brain Res* 1983;289:109–19.
- Zhang W, Perry KW, Wong DT, Potts BD, Bao J, Tollefson GD, et al. Synergistic effects of olanzapine and other antipsychotic agents in combination with fluoxetine on norepinephrine and dopamine release in rat prefrontal cortex. *Neuropsychopharmacology* 2000;23:250–62.
- Zohar J, Westenberg HG. Anxiety disorders: a review of tricyclic antidepressants and selective serotonin reuptake inhibitors. *Acta Psychiatr Scand Suppl* 2000;403:39–49.

RESEARCH ARTICLE

Open Access

Utility and limitations of PHQ-9 in a clinic specializing in psychiatric care

Takeshi Inoue*, Teruaki Tanaka, Shin Nakagawa, Yasuya Nakato, Rie Kameyama, Shuken Boku, Hiroyuki Toda, Tsugiko Kurita and Tsukasa Koyama

Abstract

Background: The Patient Health Questionnaire-9 (PHQ-9), despite its excellent reliability and validity in primary care, has not been examined for administration to psychiatric patients. This study assesses the accuracy of PHQ-9 in screening for major depressive episode and in diagnosing major depressive episode in patients of a psychiatric specialty clinic.

Methods: We compared operational characteristics of PHQ-9 as a screening and diagnostic instrument to *DSM-IV-TR* diagnosis by a trained psychiatrist as a reference standard. The reference criteria were "current major depressive episode" or "current major depressive episode with major depressive disorder". PHQ-9 was used with two thresholds: diagnostic algorithm and summary scores (PHQ-9 ≥ 10). The optimal cut-off points of PHQ-9 summary scores were analyzed using a receiver operational characteristics (ROC) curve.

Results: For "current major depressive episode", PHQ-9 showed high sensitivity and high negative predictive value at both thresholds, but its specificity and positive predictive value were low. For "current major depressive episode with major depressive disorder", PHQ-9 also showed high sensitivity and high negative predictive value at both thresholds, but the positive predictive value decreased more than that for "current major depressive episode". The ROC analysis showed the optimal cut-off score of 13/14 for "current major depressive episode".

Conclusions: PHQ-9 is useful for screening, but not for diagnosis of "current major depressive episode" in a psychiatric specialty clinic.

Background

The Patient Health Questionnaire (PHQ) was developed in 1999 as a self-report version of the Primary Care Evaluation of Mental Disorders (PRIME-MD), which was designed for criteria-based diagnosis of several mental disorders that are commonly encountered in primary care [1]. The diagnostic validity of the depression module of the PHQ (PHQ-9) has been reported in a study involving 3000 patients in eight primary care clinics. Moreover, PHQ-9 for the diagnosis of major depressive disorder and any mood disorder showed moderate agreement with the diagnosis of the Structured Clinical Interview for *DSM-III-R* (SCID) by mental health professionals [1]. The validity for screening against the *DSM-IV* diagnosis of major depression, reliability, and

feasibility of PHQ-9 is regarded as excellent [2]. PHQ-9 consists of the nine diagnostic criteria items of *DSM-IV* major depressive episode. Therefore, it has good logical validity for the *DSM-IV* diagnosis of major depressive episode.

Two recent meta-analyses have shown good pooled sensitivity (0.80 and 0.77, respectively) and specificity (0.92 and 0.94, respectively) for PHQ-9 against the *DSM-IV* diagnosis of major depressive disorder or major depressive episode in the settings of primary care clinics and clinics other than psychiatric clinics [3,4]. However, the utility and validity of PHQ-9 for the screening for major depressive episode or major depressive disorder has not been examined in a psychiatric specialty clinic. Although one might argue that such a brief self-report screening test is not necessary for specialist psychiatrists, various self-administered questionnaires are often used before a psychiatric interview, even in clinics specializing in psychiatric treatment. Above all, the screening for

* Correspondence: tinoue@med.hokudai.ac.jp
Department of Psychiatry, Hokkaido University Graduate School of Medicine,
North 15, West 7, Kita-ku, Sapporo 060-8638, Japan

major depressive episode, which has a high prevalence, using a self-administered questionnaire such as PHQ-9 is useful to prevent underdiagnosis in patients with several other psychiatric disorders that might have comorbidity with major depressive episode. Therefore, the utility and limitation of PHQ-9 for patients with psychiatric disorders in psychiatric specialty clinics must be studied before its application to psychiatric practice.

For this study, a mood disorder specialist psychiatrist administered PHQ-9 to patients of psychiatric specialty clinics of a university hospital, and compared its diagnostic capability with that of *DSM-IV-TR* for diagnosis of major depressive episode and major depressive disorder. In earlier studies in primary care clinics and clinics other than psychiatric clinics, the *DSM-IV* diagnosis was done using SCID by trained interviewers or mental health professionals, but not by psychiatrists [1,5,6], which might be a disadvantage of earlier studies because psychiatric diagnosis, especially of hypomanic episodes, a history of which has a major impact on the accuracy of PHQ-9 screening for major depressive disorder, is extremely difficult, demanding many years of training. Additionally, we evaluated and discussed diagnostic factors contributing to false-positive diagnosis.

Methods

Subjects

From February 2008 to July 2009, 153 outpatients, who visited the Department of Psychiatry, Hokkaido University Hospital as new patients, were consecutively included in the study. Each had been diagnosed using the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR)* [7] by a mood disorder specialist psychiatrist (T.I.) who was blinded to the PHQ-9 results and who had more than 20 years of clinical experience in this field of psychiatry. The Japanese version of PHQ-9 [8] was administered to patients during their waiting time as a routine clinical task. This study was performed in accordance with the Declaration of Helsinki and was approved by the institutional review board of Hokkaido University Hospital.

PHQ-9

The PHQ-9 was self-completed by the patient in written form. Major depressive episodes were diagnosed in two ways using the PHQ-9: diagnostic algorithm and a summary score. The diagnostic algorithmic threshold for diagnosing major depressive episode was regarded as fulfilled if the answer to question #1a or question #1b and five or more of questions #1a-#1i was at least "more than half the days" (question #1i was counted if present at all) [1]. The threshold $PHQ \geq 10$ signified that the summary score of questions #1a-#1i (range = 0-27) must be 10 or higher. This cutoff point was chosen because it

has been reported and recommended the most consistently [3,4,6].

Psychiatric evaluations

DSM-IV-TR diagnoses of various psychiatric disorders including mood disorders (major depressive disorder, minor depressive disorder, bipolar disorder, dysthymic disorder, and cyclothymic disorder) were made by a psychiatrist specializing in mood disorder (T.I.) using the *Quick Reference to Diagnostic Criteria from the DSM-IV-TR* on the same day when the patients answered PHQ-9. The average interview duration was 60 min. In each case, the presence of a current major depressive episode, which can appear in patients with major depressive disorder or bipolar disorder, was identified. In most patients with major depressive disorder, the 17-item Hamilton Depression Rating Scale (HDRS) and the Montgomery-Åsberg Depression Rating Score (MADRS, 10 items) were administered to evaluate the severity of depression in routine clinical work [9,10]. We also used the global assessment of functioning (GAF) scale, which reflects the overall level of psychological, social, and occupational functioning of individuals [7]. Some patients, however, did not receive the HDRS, MADRS, or GAF evaluation because of the limited time of the regular clinical interview.

Data analysis

With respect to criterion validity, we investigated sensitivity, specificity, positive predictive value, negative predictive value, and the overall accuracy for the diagnostic algorithmic threshold and summary score threshold ($PHQ \geq 10$). The *DSM-IV-TR* diagnoses of "current major depressive episode" and "current major depressive episode with major depressive disorder" were the criterion standard.

Receiver Operating Characteristics (ROC) analysis was used to ascertain an optimal cut-off point of PHQ-9 summary scores for screening for major depressive episode in the setting of a psychiatric specialty clinic. This curve shows "sensitivity" versus "1-specificity" for every possible cut-off point. The area under the curve (AUC) was calculated as an indicator of the discriminative property of the scale. A maximal "Youden Index" (sensitivity + specificity - 1) was calculated and suggested as an optimal cut-off point for PHQ-9 [11].

Pearson's correlation coefficients between PHQ-9 scores and GAF or MADRS or HDRS scores of patients with major depressive disorder were calculated to assess their validity for severity measures of depressive symptoms.

All continuous data are presented as means with standard deviations or 95 % confidence intervals (CIs).

Results

Demographic characteristics and DSM-IV-TR diagnosis of subjects

Demographic characteristics and *DSM-IV-TR* diagnoses of 153 subjects are presented in Table 1. The most common diagnosis was major depressive disorder with subsequent bipolar disorder: 45 % of subjects were diagnosed with mood disorders. Two patients with major depressive disorder had comorbid psychiatric disorders: mild dementia and social anxiety disorder. Among them, only one patient with social anxiety disorder had a current major depressive episode that fulfilled its diagnostic criteria.

Validity of PHQ-9 for the screening for current major depressive episode

Table 2 (left half) presents operating characteristics of PHQ-9 for "current major depressive episode" for the PHQ-9 diagnostic algorithmic threshold and for the cut-

Table 1 Characteristics and *DSM-IV-TR* diagnoses of 153 patients

Characteristic	Value
Sex	
Female, n (%); male, n (%)	96 (63); 57 (37)
Age, mean ± SD (yr)	44.6 ± 18.3
Range	14–82
<i>DSM-IV-TR</i> diagnosis, n (%)	
Major Depressive Disorder	50 (32.7)
Minor Depressive Disorder	2 (1.3)
Dysthymic Disorder	1 (0.7)
Bipolar Disorder	16 (10.5)
Developmental Disorders	2 (1.3)
Dementia	10 (6.5)
Mental Disorders due to a General Medical Condition	7 (4.6)
Substance-Related Disorders	3 (2.0)
Schizophrenia	8 (5.2)
Schizoaffective Disorder	2 (1.3)
Panic Disorder	10 (6.5)
Other Anxiety Disorders	10 (6.5)
Somatoform Disorders	2 (1.3)
Eating Disorders	6 (3.9)
Insomnia	11 (7.2)
Adjustment Disorders	7 (4.6)
Premenstrual Dysphoric Disorder	1 (0.7)
None	5 (3.3)
Comorbidity with Major Depressive Disorder, currently met for a Major Depressive Episode	
Social Anxiety Disorder	1 (0.7)

off point (threshold) of summary score of PHQ-9 ≥ 10 , which has been recommended most consistently as the cut-off point [3,4,6]. Both thresholds had satisfactory sensitivity and negative predictive value: the summary score threshold (PHQ ≥ 10) had slightly higher sensitivity (0.94) and a more negative predictive value (0.94) than the diagnostic algorithmic threshold did, although the former had slightly lower specificity and positive predictive value than the latter did.

Among 73 positive cases for the PHQ-9 diagnostic algorithmic threshold, 34 cases (46.6 %) were falsely positive for having a current major depressive episode, as inferred from low specificity and positive predictive value. The *DSM-IV-TR* diagnoses of false-positive cases were schizophrenia in 4 cases, panic disorder in 4 cases, adjustment disorder in 5 cases, eating disorders in 5 cases, dementia in 2 cases, and insomnia in 5 cases. The false-positive patients showed high PHQ-9 summary scores comparable to those of the true-positive patients (data not shown) and exhibited depressed mood, but their depressive symptoms were not continuous during more than 2 weeks, as defined in *DSM-IV-TR*. Only 2 patients were diagnosed with other depressive disorders (minor depressive disorder and dysthymia).

False-negative cases were far fewer than false-positive cases. This tendency was particularly pronounced for the threshold of the summary score of PHQ-9 ≥ 10

Validity of PHQ-9 for the screening for current major depressive episode with major depressive disorder

Table 2 (right half) presents operating characteristics of PHQ-9 for "current major depressive episode with major depressive disorder" for the PHQ-9 diagnostic algorithmic threshold and for the cut-off point (threshold) of summary score of PHQ-9 ≥ 10 . Both thresholds had satisfactory sensitivity and negative predictive value: the summary score threshold (PHQ ≥ 10) had slightly better sensitivity (0.93) and negative predictive value (0.94) than the diagnostic algorithmic threshold did, although the former had slightly lower specificity and positive predictive value than the latter did.

Compared with false-positive cases for the PHQ-9 diagnostic algorithmic threshold against "current major depressive episode", the number of those against "current major depressive episode with major depressive disorder" increased by 7 patients. That is to say, the positive predictive value was decreased more than that for "current major depressive episode". All of these additional false-positive cases were bipolar disorder patients: 1 bipolar I and 6 bipolar II patients. As expected, bipolar depressed patients were positive for PHQ-9 because depressive symptoms of major depressive disorder and bipolar disorder show only subtle differences [7,12].