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Empathy and Judging Other's Pain: An fMRI Study of Alexithymia

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Because awareness of emotional states in the self is a prerequisite to recognizing such states in others, alexithymia (ALEX), difficulty in identifying and expressing one's own emotional states, should involve impairment in empathy. Using functional magnetic resonance imaging (fMRI), we compared an ALEX group (n = 16) and a non-alexithymia (non-ALEX) group (n = 14) for their regional hemodynamic responses to the visual perception of pictures depicting human hands and feet in painful situations. Subjective pain ratings of the pictures and empathy-related psychological scores were also compared between the 2 groups. The ALEX group showed less cerebral activation in the left dorsolateral prefrontal cortex (DLPFC), the dorsal pons, the cerebellum, and the left caudal anterior cingulate cortex (ACC) within the pain matrix. The ALEX group showed greater activation in the right insula and inferior frontal gyrus. Furthermore, alexithymic participants scored lower on the pain ratings and on the scores related to mature empathy. In conclusion, the hypofunction in the DLPFC, brain stem, cerebellum, and ACC and the lower pain-rating and empathy-related scores in ALEX are related to cognitive impairments, particularly executive and regulatory aspects, of emotional processing and support the importance of self-awareness in empathy.

Keywords: anterior cingulate cortex, dorsolateral prefrontal cortex, emotion regulation, empathy, self-awareness

Introduction

The construct of empathy refers to the ability to identify with and vicariously share the feelings and thoughts of others. This naturally occurring subjective experience of similarity between the feelings of self and others is an important aspect of building interpersonal relationships. However, there are several essential aspects of empathy: 1) an affective response to another person, which often, but not always, entails sharing that person's emotional state (affective component); 2) a cognitive capacity to take the perspective of the other person (cognitive component); and 3) some regulatory mechanisms that keep track of the origins of self and other feelings (Decety and Jackson 2004). An integrative model of empathy was proposed by Preston and de Waal (2002). This model draws on that the perception of actions or emotions automatically activates the neural mechanisms that are responsible for the generation of those actions or emotions. Such a system prompts the observer to resonate with the emotional state of another individual, as a result of the observer activating the motor representations and associated autonomic and somatic responses that stem from the observed target.

In support of this perception–action integrative model, recent functional neuroimaging studies revealed shared neuronal substrates for empathy to the pain of others (Morrison et al. 2004; Singer et al. 2004; Botvinick et al. 2005; Jackson et al. 2005, 2006; Lamm et al. 2007; Saarela et al. 2006). These studies have indicated that watching others in painful situations taps into the neural mechanisms that mediate the affective-motivational component of pain processing. Notably, the anterior cingulate cortex (ACC) and anterior insula are similarly activated by the experience of pain in the self and by the observation of others in painful situations.

Self-awareness is a fundamental aspect of empathy because the individual's recognition of their own feelings is the basis for identification with the feelings of others (Gallup 1998; Decety and Jackson 2004). Individuals with alexithymia (ALEX) are typically unable to identify, understand, or describe their own emotions. Psychiatric and psychosomatic patients with ALEX are unable to talk about feelings due to a lack of emotional self-awareness (Sifneos 1972, 1996). ALEX has been repeatedly found in psychiatric disorders that have deficits in the recognition of feelings belonging to the self and identification with others, such as autism and Asperger syndrome (Frith 2004; Hill et al. 2004; Berthoz and Hill 2005), schizophrenia (Stanghellini and Ricca 1995; Cedro et al. 2001), and borderline personality disorder (Guttman and Laporte 2002). ALEX has also been found in psychopathic personality disorder, where there is a deficit in empathy (Haviland et al. 2004).

Although the concept of ALEX was originally used to describe the characteristics of psychosomatic patients, recently it has been used to refer to deficits in emotional functioning in broader populations (Taylor et al. 1997; Taylor and Bagby 2004). Some researchers hypothesized that ALEX is associated with brain abnormalities (Hoppe and Bogen 1977; Nemiah 1977; Buchanan et al. 1980). Neuroimaging studies found that ALEX may be associated with a higher level cognitive deficit in estimating emotional inputs—in which the ACC plays a crucial role—rather than a lack of neuronal response in structures representing lower level processing of emotional stimuli (Berthoz et al. 2002; Kano et al. 2003). ALEX has also been found to be related to dysfunction in the posterior cingulate cortex during various mental imagery conditions (Aleman 2005; Mantani et al. 2005). Lane et al. (1997) stressed the core feature of ALEX as a deficit in conscious awareness of emotions (e.g.,

differentiating, symbolizing emotions, and appreciating complexity in the experience of self and other). Thus, ALEX refers to an impairment in not only affective but also cognitive emotional processing.

To our knowledge, the concept of ALEX itself does not explicitly include deficits in empathy. However, the lack of knowledge of their own emotional experiences should be associated with a lack of empathy in alexithymics (e.g., Krystal 1979; see also levels of emotional awareness in Lane and Schwartz 1987). Vorst and Bermond (2001) argued that an important aspect of ALEX is "operative thinking" (i.e., preoccupation with "things" at the expense of object relations), which covers many aspects of ALEX including the lack of empathy.

The notion of "shared representations" between self and other accounts for the functional computational properties that emerge from the direct link between perception and action (Decety and Sommerville 2003; Decety and Jackson 2004, 2006; Sommerville and Decety 2006). Because empathy relies on vicarious sharing of the feelings and thoughts of others, this common representational network between the self and others in conjunction with self-other awareness provides the basic mechanism for empathy (Decety and Sommerville 2003; Decety and Jackson 2004, 2006; Decety and Grèzes 2006). From this perspective, we propose that ALEX (which is a deficit in identifying emotional states in oneself) may be associated with (or lead to) an impairment in empathy (connecting to other's emotional states). In line with this idea, some studies demonstrated that individuals with ALEX show poor performance in identifying the emotional values of facial expressions (e.g., Parker et al. 1993; Lane et al. 1996). Only a few studies, however, have focused on the relationship between ALEX and empathetic ability (Rastam et al. 1997; Guttman and Laporte 2002). Moreover, their results are not conclusive as to whether a deficit in empathetic ability is an essential component of ALEX.

The purpose of the present study was to explore whether individuals with ALEX have deficit in empathetic ability, and if so, what aspect of empathy is impaired. We measured the neurohemodynamic activity with functional magnetic resonance imaging (fMRI) in participants with ALEX as compared with non-alexithymic controls, in potentially empathic situations involving both cognitive and affective aspect of pain-processing network (response to pictures depicting human hands and feet in potentially painful situations and judging the degree of pain in those situations; cf., Jackson et al. 2005). In addition, we compared the scores assessing the empathy-related abilities in the 2 groups. We hypothesized that the ALEX group would score lower on pain- and empathy-related scores and show different neural response in pain-related regions demonstrated by previous neuroimaging studies about pain processing, for example, the primary and secondary somatosensory cortices, the posterior insula, the ACC, the middle and anterior insula, thalamus, brain stem, and lateral prefrontal cortex (for reviews, Davis 2000; Peyron et al. 2000; Rainville 2002).

Methods and Materials

The study was approved by the local Ethics Committees (National Center of Neurology and Psychiatry in Japan, National Institute of Mental Health) and conducted in accordance with the Declaration of Helsinki.

Subjects

Three hundred and ten college students completed the 20-item Toronto Alexithymia scale (TAS-20; Taylor et al. 2003). Individuals with high and low TAS-20 total scores ($n = 20$, top quartile score > 60; $n = 18$, bottom quartile score < 39) were selected in order to obtain a sample with as large a variance on ALEX as possible. Thirty-seven students gave informed written consent and participated in the experiment (Table 1). Participants were interviewed using the mini international neuropsychiatric interview (Sheehan et al. 1998). No subject had any history of neurological, major medical, or psychiatric disorder. All participants were right handed, as assessed by the Edinburgh handedness inventory (Oldfield 1971). The participants were the same as reported in our previous study about the association between ALEX and mentalizing (Moriguchi et al. 2006). However, the present studies were conducted in a completely different setting. In the present study, we focus only on the analyses of the other's pain perception paradigm.

The whole sample described above ($n = 37$) was divided into 2 groups based on the cutoff scores on the TAS-20: ALEX (TAS > 60) and non-alexithymia (non-ALEX; TAS < 39) groups. The structured interview, modified edition, of the Beth Israel hospital psychosomatic questionnaire (SIBIQ; Arimura et al. 2002) was used to further confirm the presence or absence of ALEX. Four participants with high TAS-20 and low SIBIQ scores, and 3 with low TAS-20 and high SIBIQ scores, were discarded. Table 1 gives comparative information about the resulting ALEX group ($n = 16$) and non-ALEX group ($n = 14$).

Psychological Instruments

The TAS-20 (Taylor et al. 2003; the Japanese version by Komaki et al. 2003) is a 20-item self-administered questionnaire. The items are scored on a 5-point scale from strongly disagree to strongly agree. The TAS-20 has a 3-factor structure. Factor 1 assesses difficulty in identifying feelings. Factor 2 assesses difficulty in describing feelings. Factor 3 assesses externally oriented thinking.

The SIBIQ for ALEX (Arimura et al. 2002) is based on the Beth Israel hospital psychosomatic questionnaire (Sriram et al. 1988), used mainly with psychosomatic patients. The SIBIQ was developed for patients with some physical or psychiatric symptoms, and they were asked to describe how they perceived their own symptoms. For interviewing nonpatients with no symptoms, we modified the SIBIQ by adding questions about their feelings in response to bad/sad/difficult (negative) or happy/good/satisfying (positive) events they had experienced. If they replied that they had no equivalent life events, we added "if" questions in which they were asked to imagine some situations that are generally supposed to cause emotional responses (similar to the Alexithymia-provoked response questionnaire [Krystal et al. 1986]) and required them to answer in terms of their own emotions. The testers rated these answers on the scale of the SIBIQ. The SIBIQ was conducted by 2 medical doctors, who were acquainted clinically with ALEX, and their 2 scores were averaged for each subject. There is no standard cutoff point on the SIBIQ. We set the thresholds as the top quartile of the SIBIQ scores (equivalent to >47) as "high" SIBIQ and the lowest quartile (<25) as "low" SIBIQ.

Table 1
Appearance of TAS-20 and SIBIQ scores in the 2 groups

	Whole	Non-ALEX	ALEX
n (Male/female)	37 (7/30)	14 (2/12)	16 (3/13)
Age, mean (SD) (years)	20.4 (0.94)	20.8 (0.89)	20.2 (1.0)
TAS-20	Minimum–maximum, mean (SD)		
Total	26–74, 51.2 (16.5)	26–38, 34.1 (3.7)	61–74, 66.1 (4.5)
F1	7–32, 18.0 (8.1)	7–19, 10.6 (3.7)	19–32, 24.7 (3.9)
F2	5–25, 15.4 (6.2)	5–18, 9.6 (3.9)	15–24, 20.1 (2.4)
F3	9–30, 17.9 (5.1)	9–21, 13.9 (3.3)	13–30, 21.4 (4.0)
SIBIQ total	18–70, 42.2 (16.7)	18–56, 31.5 (11.8)	25–70, 52.2 (14.1)

Note: F1 (factor 1), difficulty in identifying feeling; F2 (factor 2), difficulty in describing feeling; F3 (factor 3), externally oriented thinking; SD, standard deviation. The whole sample ($n = 37$) is introduced to analysis of main effect of painful picture tasks and correlation analysis between neural activations and psychological measurements. Non-ALEX ($n = 14$) and ALEX ($n = 16$) groups were obtained from this whole sample excluding the participants with discrepancy between TAS-20 and SIBIQ scores (cf., **Materials and Methods**).

The emotional empathy scale (EES; Mehrabian and Epstein 1972; Japanese version developed by Kato and Takagi 1980) is a self-administered questionnaire that measures the ability of "emotional empathy," defined as an affective response to somebody else's emotional experience. Mehrabian and Epstein (1972) had made the items of EES with expectation of multiple subscales of EES, but no subscales were extracted, although the Japanese version was subdivided into 3 components (Kato and Takagi 1980) in the Japanese population as follows: 1) Emotional warmth; a tender and compassionate attitude toward other's feelings. People with emotional warmth are impressionable in response to art, novel, and movies, as well as other's sorrow and distress, and sometimes participate in voluntary activities. 2) Emotional chill; an apathetic and sometimes disfavoring attitude toward other's feeling like sorrow, distress, and joy etc. Such people always keep others at a distance. 3) Emotional affectedness; a tendency to be easily influenced by other's feelings. It is almost the same as "emotional contagion."

The interpersonal reactivity index (IRI; Davis 1983; Japanese version developed by Aketa 1999) was another self-administered questionnaire measuring the empathetic ability of the participants. The IRI consists of 4 scales, each measuring a distinct component of empathy: 1) empathic concern, feeling emotional concern for others and 2) perspective taking, cognitively taking the perspective of another, related to social competence. The factors (1) and (2) were characterized as desirable interpersonal styles. 3) fantasy, emotional identification with characters in books, films, etc. and 4) personal distress, negative feelings in response to the distress of others.

The stress coping inventory (SCI; Lazarus and Folkman 1984; Japanese version developed by the Japanese Institute of Health Psychology 1996) was used to investigate the participants' character and coping style in response to emotional stimuli. The SCI has 2 major factors: 1) cognitive coping strategy and 2) emotional coping strategy. There are 8 subscales on the SCI: 1) confrontational, 2) distancing, 3) self-controlling, 4) seeking social support, 5) accepting responsibility, 6) escape-avoidance, 7) problem solving, and 8) positive reappraisal.

The Japanese version of these psychological scales (the TAS-20, EES, IRI, and SCI) were the ones that have been translated into Japanese using back-translation method, and the factor analyses of these Japanese versions showed the same factor components as the original English versions except for the EES. However, the concurrent validity and reliability in each psychological measurement have been confirmed, indicating that the Japanese version of each psychological test measures the same aspects as the original one.

Picture Stimuli

The picture stimuli had been previously developed and validated by Jackson et al. (2005) and were used with their permission. The picture stimuli consisted of a series of digital color pictures that showed right hands and right feet in painful and nonpainful situations, shot from angles that facilitate a first-person perspective (i.e., no mental rotation of the limb is required for the observer). All situations depicted familiar events that can happen in everyday life. Various types of pain (mechanical, thermal, and pressure) were represented. The target persons in the pictures varied in gender and age (between 8 and 56 years), and their limbs and arms were smoothed in order to avoid any influences of age and gender on judgments. For each painful situation, there was a corresponding neutral picture, which involved the same setting without any painful component. The 96 painful pictures used in this study were selected from a larger sample, on the basis of the pain intensity ratings of 20 independent subjects. All pictures were edited to the same size and resolution (600 × 600 pixels).

Scanning Method and Procedure

Participants took part in one fMRI session. The session consisted of 26 blocks. The participants were asked to watch and assess the pictures depicting right hands or feet in painful situations as a task condition (12 blocks) and right hands or feet in neutral situations as a control condition (12 blocks). The baseline trials showed a static cross (2 blocks at the middle and end of the session). The order of conditions was randomized within the session. No picture was presented more than once throughout the whole experiment. Each task or control block consisted of eight 4-s trials of the same condition. Each picture was shown for 2 s, followed for 2 s by a modified faces pain-rating scale (Wong and Baker 1988) that illustrated the 4-point Likert-type pain scale (no pain [0], a little pain [1], moderate pain [2], and worst possible pain [3]). In the baseline trials, subjects were asked to passively look at the central cross for 4 s and were not shown the pain-rating scale. In the task and control conditions, subjects were instructed to rate the intensity of pain they thought the person in the picture would feel in each situation. At the end of each task and control trial, they used a 4-button response box under their right hand to select the rating (thumb = 0, index = 1, middle finger = 2, and fourth finger = 3). The participants were required to press the button in every trial in the task and the control condition along the scale, thereby controlling for the motor output involved in the rating process across the 2 conditions. Participants were provided with several training trials prior to the scanning session in order to be acquainted with the rating scale and the task within the allotted time. The pictures used in the training trials were different from those used as stimuli for the fMRI measurements.

Data Acquisition and Analyses

Magnetic resonance imaging data were acquired on a 1.5-T Siemens Magnetom Vision Plus System. Changes in blood oxygenation level-dependent T_2*-weighted magnetic resonance (MR) signal (Ogawa et al. 1990) were measured using a gradient echo-planar imaging (EPI) sequence (repetition time [TR] = 4000 ms, echo time [TE] = 40 ms, field of view [FOV] = 220 mm, flip angle = 90 degree, 64 × 64 matrix, 40 slices per slab, slice thickness 3.0 mm, 0.3 mm gap, voxel size = 3.44 × 3.44 × 3.3 mm). For each scan session, a total of 213 EPI volume images were acquired along the AC–PC plane. Structural MR images were acquired with a magnetization-prepared rapid gradient echo sequence (TE/TR, 4.4/11.4 ms; flip angle, 15 degree; acquisition matrix, 256 × 256; 1 NEX FOV, 31.5 cm; slice thickness, 1.23 mm). The first 5 volumes of EPI images were discarded because of instability of magnetization; therefore, we obtained 208 volumes of EPI for analysis.

The stimuli were projected onto a screen, ~50 cm from the subject's head. The participants viewed the screen through a mirror attached to the head coil.

Image processing was carried out using Statistical Parametric Mapping software (SPM2, Wellcome Department of Imaging Neuroscience, London, UK). The EPI images were realigned and coregistered to the subjects' T_1-weighted MR images. Then the T_1 images were transformed to the anatomical space of a template brain whose space is based on the MNI (Montreal Neurological Institute) stereotactic space. The parameters for the transformation were applied to the coregistered EPI images. The normalized images were smoothed by a 6-mm full-width half-maximum Gaussian kernel. A first fixed level of analysis was computed subjectwise using the general linear model with hemodynamic response function modeled as a boxcar function whose length covered the 8 successive pictures of the same type.

To test the hypotheses about regionally specific effects in the painful picture condition, the estimates were compared by means of linear contrasts for each epoch (painful picture epoch as task condition versus neutral picture epoch as control). The resulting set of voxel values for each contrast constituted a statistical parametric map (SPM) of the t-statistic SPM (t). Anatomic localization was presented as MNI coordinates, and to check the localization of the Brodmann area (BA), the Talairach coordinates (Talairach and Tournoux 1988) were used. First-level contrasts were introduced in a second-level random-effect analysis (Friston et al. 1999) to allow for population inferences.

Main effects for watching painful pictures were computed using 1-sample tests for each ALEX (n = 16) and non-ALEX group (n = 14) separately and subsequent conjunction analysis of both 1-sample tests to show overlapping activations between 2 groups. The analyses were done for each of the contrasts of interest, which yielded a SPM of the t-statistic (SPM [t]), subsequently transformed to the unit normal distribution (SPM [Z]). A voxel and cluster level threshold of $P < 0.05$ corrected for multiple comparisons (false discovery rate; t = 2.26 for non-ALEX group, 2.42 for ALEX group, 2.58 for conjunction analysis) was used to identify other pain-related regions, compared against the null hypothesis.

To compare the differences in neural activity between the ALEX group (n = 16) and the non-ALEX group (n = 14), 2-sample tests were

used. The height and extent thresholds were set at $Z = 3.09$ ($P < 0.001$ uncorrected) and $k = 20$, respectively. For the areas with an a priori pain-related hypothesis (derived from Singer et al. 2004; Jackson et al. 2005, 2006), we applied more lenient height and extent thresholds; $Z = 2.6$ ($P < 0.005$ uncorrected) and $k = 20$, respectively (adopted from Raij et al. 2005) within the regions activated in the 1-sample group tests and conjunction analysis to reduce the risk of false negatives. If the regions with significant differences were included in an a priori pain matrix confirmed by the previous studies (Peyron et al. 2000; Morrison et al. 2004; Singer et al. 2004; Jackson et al. 2005, 2006; Raij et al. 2005), we confirmed them as group effects on pain-related activations. The a priori regions were obtained from regions that had been emphasized as important components and frequently reported in the literature, that is, the primary and secondary somatosensory cortices, the posterior insula, the caudal ACC, the middle and anterior insula, thalamus, brain stem, and lateral prefrontal cortex (Davis 2000; Peyron et al. 2000). To further clarify the characteristics of regions with group differences in the a priori pain matrix, we made regions of interest (ROIs) consisted of 20 voxels centered on each peak coordinate found in the group comparisons in the present study and calculated individual mean contrast values (task minus control) for each ROI using Marsbar software (http://marsbar.sourceforge.net). The correlation coefficients between pain ratings and neural responses within pain-related regions were calculated. (ROI corrected $P < 0.05$). The correlation coefficients between these ROI mean contrast values and psychological measurement scores were also calculated to investigate the features of the regions with group differences.

Results

Behavioral Measures

In the one sample, the individual ratings of painful pictures were significantly higher than those of neutral control pictures (paired *t*-test: mean [standard deviation (SD)] score of sum of task pictures' ratings in each subject; 34.2[3.6], control pictures' ratings; 12.2[2.0], $T = 343$, $P < 10^{-28}$). Table 2 compares the scores for the pain ratings, IRI, EES, and the SCI between the ALEX ($n = 16$) and the non-ALEX groups ($n = 14$). Alexithymic participants showed lower pain ratings than non-alexithymics, indicating that they attributed lower levels of pain to the people depicted in the painful situation pictures. They scored lower on the IRI scales assessing "perspective taking" and "empathic concern," suggesting that they were less able to take the perspective of another and had less empathy. On the EES, alexithymics scored less on "warmth." Alexithymics scored lower on the SCI scales of "cognitive," "problem solving," and "positive reappraisal," indicating that they were less likely to use these approaches to manage emotional stimuli. On the other hand, alexithymics had significantly higher "personal distress" scores on the IRI.

Table 2
Comparison of psychological measurements in the ALEX and non-ALEX groups

	Mean (SD)		
	Non-ALEX ($n = 14$)	ALEX ($n = 16$)	T
Pain ratings	23.8 (3.0)	21.0 (4.3)	2.08*
IRI			
Fantasy	19.9 (6.7)	17.7 (5.6)	1.01
Perspective taking	18.5 (4.9)	14.6 (3.4)	2.61*
Empathic concern	20.0 (3.7)	16.1 (4.9)	2.48*
Personal distress	12.5 (3.7)	15.8 (4.1)	−2.31*
EES			
Warmth	58.0 (3.2)	49.2 (7.9)	3.93**
Chill	29.3 (10.2)	35.6 (8.6)	−1.89
Affectedness	21.0 (7.1)	22.0 (3.0)	−0.53
SCI			
Cognitive	36.9 (12.4)	26.3 (10.7)	2.57*
Emotional	27.7 (8.1)	23.9 (7.4)	1.30
Problem solving	10.7 (4.7)	7.4 (4.0)	2.11*
Confrontational	5.9 (2.1)	5.5 (2.5)	0.55
Seeking social support	6.9 (3.8)	4.6 (3.7)	1.74
Accepting responsibility	10.6 (3.8)	8.4 (4.4)	1.49
Self-controlling	8.1 (3.9)	6.9 (3.4)	0.91
Escape–avoidance	6.1 (2.6)	4.8 (1.7)	1.61
Distancing	4.7 (2.9)	4.9 (2.1)	−0.21
Positive reappraisal	11.6 (4.0)	7.7 (4.1)	2.63*

Note: SD, standard deviation.
*$P < 0.05$.
**$P < 0.001$.

The fMRI Data

One-Sample Analyses and Conjunction Analysis

Tables 3–5 and Figures 1–3 give the results of 1-sample tests (one for each group) throughout the whole brain related to higher activations in response to the painful pictures than the neutral pictures and conjunction analysis of both groups. Tables 3–5 give representative coordinates in pain-related regions; all the coordinates are listed in Table 1 in the Supplementary Materials. In each group and conjunction analysis, a similar activity pattern was found. Significant signal changes were detected in the dorsal ACC (Lt > Rt, BA 24/32), anterior insula (Lt > Rt, BA 13), middle/inferior lateral prefrontal cortices (Lt > Rt, BA 9/10/11/44–47), and postcentral/superior parietal cortices (Lt > Rt, BA 2; Rt > Lt, BA 1/2/3/5/7) adjacent to inferior parietal lobule (BA 40), thalamus (Rt > Lt), brain stem (dorsal pons/midbrain), and cerebellums (Rt > Lt). Additionally, activations were also found in the visual-related/fusiform areas/uncus (BA 18/19/20), superior/middle frontal gyrus (BA 6), and inferior frontal gyrus (BA 44/46). The only exception is that no significant activity was found in the pons in the ALEX group in contrast to high activity in this region in the non-ALEX group; also there was no activation in the pons in the conjunction analysis.

We calculated the correlation between neural activations in response to painful pictures and the individual pain ratings in all participants. Within the activated areas identified in the previous and present studies of perception of others in pain network, we found positive correlations between the rating scales and neural activities in the following areas: ROIs on the right caudal ACC (BA 32, center [x, y, z] [mm] = [10, 28, 40], $r = 0.44$, $P = 0.00312$), sensory association cortex (BA 7 [(28, −68, 54), $r = 0.59$, $P = 0.00006$], BA 40 [(40, −50, 50), $r = 0.63$, $P = 0.00002$]), left lateral prefrontal cortex (BA 9, [−20, 52, 34], $r = 0.41$, $P = 0.00587$), right dorsal pons ([12, −34, −40], $r = 0.52$, $P = 0.00055$), left thalamus ([−8, −12, 4], $r = 0.40$, $P = 0.00769$), and right cerebellum ([18, −60, −16], $r = 0.54$, $P = 0.00031$).

Group Comparison Analysis

We compared the ALEX ($n = 16$) group with the non-ALEX ($n = 14$) group, examining group effects on neuronal activity in response to painful pictures controlled with neutral pictures (Table 6, Fig. 4). We found lower hemodynamic activity in the ALEX group compared with non-ALEX group in the left dorsolateral prefrontal cortex (DLPFC) (BA 8/9/10) in the posterior lobes of cerebellar cortices, dorsal pons, left middle/superior frontal gyrus (BA 6/8), and right middle temporal gyrus ($P < 0.001$ uncorrected, $k = 20$). Although the ACC did not show a significant difference with the chosen threshold, we found

Table 3
Coordinates and Z and T scores for the pain-related brain areas activated in response to painful picture stimuli in a 1-sample test for the non-ALEX group

Area	BA	MNI x, y, z (mm)	T	Z
ACC				
Lt	24	−10, 2, 52	4.9	4.13***
	32	−8, 14, 48	5.74	4.63***
Rt	24	8, −2, 36	3.61	3.24**
	32	12, 16, 42	3.92	3.47*
Cerebellum				
Lt anterior culmen	—	−32, −34, −38	9.06	6.14****
Rt posterior declive	—	26, −64, −28	9.39	6.26****
DLPFC				
Lt inferior frontal	9	−54, 10, 32	8.3	5.85****
	10	−50, 44, 0	6.68	5.12****
	45	−56, 14, 2	4.03	3.55*
	46	−46, 36, 14	8.17	5.8****
Lt middle frontal	9	−42, 36, 40	4.56	3.91***
	10	−42, 50, 16	6.69	5.13****
	11	−44, 54, −14	5.25	4.34***
Lt superior frontal	9	−20, 56, 34	6.69	5.13****
	10	−34, 56, 20	5.95	4.74*
Rt inferior frontal	9	56, 10, 32	5.83	4.68***
	45	56, 10, 26	5.39	4.43*
Rt middle frontal	11	50, 50, −16	2.27	2.16**
	46	54, 32, 30	4.66	3.97***
	47	52, 48, −8	4.04	3.55**
Rt superior frontal	10	24, 72, 4	2.95	2.73**
Insula				
Lt anterior	13	−30, 16, 8	3.51	3.17*
	—	−40, −10, 0	2.3	2.18**
Rt anterior/inferior frontal	13	44, 24, 12	3.42	3.1*
Midbrain				
Lt	—	0, −32, 0	5.87	4.7***
Lt substantia nigra	—	−10, −20, −16	4.06	3.57*
Rt	—	6, −18, −22	2.74	2.56*
Pons	—	−2, −38, −42	5.37	4.42***
Primary somatosensory cortex				
Lt inferior parietal lobule	40	−40, −50, 58	8.06	5.75****
Lt postcentral gyrus	3	−34, −36, 48	5.03	4.21*
	1	−60, −28, 42	8.72	6.01*
Rt postcentral gyrus	5	36, −46, 58	5.47	4.48*
	2	52, −28, 44	9.54	6.32****
Secondary somatosensory cortex				
Lt postcentral gyrus	40	−62, −20, 22	3.76	3.35*
Rt inferior parietal lobule	40	68, −36, 36	2.41	2.28**
Rt postcentral gyrus	3/40	62, −20, 36	7.76	5.62****
Thalamus				
Lt/ventral lateral nucleus	—	−14, −14, 10	5.72	4.62***
Rt/ventral anterior nucleus	—	16, −6, 12	4.54	3.9***

Note: Lt; left, Rt; right.
*$P < 0.05$ false discovery rate (FDR) corrected in each ROI.
**$P < 0.05$ FDR corrected (height threshold: $t = 2.26$).
***$P < 0.001$ FDR corrected (height threshold: $t = 4.28$).
****$P < 0.05$ family wise error (FWE) corrected (height threshold: $t = 6.39$).

Table 4
Coordinates and Z and T scores for the pain-related brain areas activated in response to painful picture stimuli in a 1-sample test for the ALEX group

Area	BA	MNI x, y, z (mm)	T	Z
ACC				
Lt	24	0, 6, 28	4.05	3.6**
	32	−8, 24, 40	4.96	4.2***
Rt	24	6, 24, 16	2.62	2.5**
	32	6, 8, 52	4.93	4.2***
	8/32	6, 16, 48	4.98	4.2*
Cerebellum				
Lt posterior pyramis/vermis	—	0, −74, −38	5.18	4.3***
Rt posterior uvula	—	12, −74, −44	7.18	5.4****
DLPFC				
Lt inferior frontal	47	−52, 18, −6	4.1	3.6*
	45	−58, 20, 24	4.44	3.8*
	46	−46, 34, 12	6.79	5.2****
	9	−56, 8, 32	6.95	5.3****
	44	−56, 8, 20	6.98	5.3*
Lt middle frontal	11	−46, 52, −12	3.58	3.2*
	10	−32, 60, 10	5.44	4.5***
Rt inferior frontal	47	56, 22, −6	3.73	3.3*
	45	54, 28, 6	5.14	4.3***
	9	56, 8, 32	6.29	4.9***
	10	34, 40, 24	3.02	2.8*
Rt middle frontal	46	38, 28, 20	4.69	4***
Insula				
Lt	13	−30, 26, 0	3.08	2.8**
	13	−38, −6, 8	4.51	3.9*
Rt	—	36, 20, 2	4.42	3.8*
Midbrain				
Rt substantia nigra	—	18, −18, −6	4.61	3.9**
Primary somatosensory cortex				
Lt postcentral	3	−30, −38, 48	4.34	3.8*
	5	−42, −44, 66	6.84	5.2****
	1	−60, −28, 42	7.85	5.7*
	2	−68, −24, 30	8.2	5.8*
Rt inferior parietal lobule	40	38, −34, 42	6.18	4.9*
Rt postcentral	5	34, −52, 70	9.03	6.1****
	2	52, −28, 44	9.49	6.3*
Secondary somatosensory cortex				
Lt inferior parietal lobule	40	−68, −26, 30	8.27	5.8****
Lt postcentral gyrus	40	−52, −26, 20	3.57	3.2*
Rt postcentral gyrus	3/40	62, −20, 38	8.08	5.8****
Thalamus				
Lt	—	−14, −18, 10	5.08	4.2***
Rt	—	8, −26, −4	3.83	3.4*

Note: Lt, left; Rt, right.
*$P < 0.05$ false discovery rate (FDR) corrected in each ROI.
**$P < 0.05$ FDR corrected (height threshold: $t = 2.42$).
***$P < 0.001$ FDR corrected (height threshold: $t = 4.53$).
****$P < 0.05$ family wise error (FWE) corrected (height threshold: $t = 6.39$).

reduced activation for the ALEX group in the left ACC (BA 24/32) when using a more lenient threshold ($P < 0.005$, $k = 20$) within the a priori pain-related region. The ALEX group showed stronger signal change compared with the non-ALEX group in the right anterior insula (BA 13) and the inferior frontal gyrus (BA 45) within a pain matrix and additionally bilateral ventral anterior cingulate gyri, right superior frontal gyrus, and right superior/middle temporal gyrus. ALEX group also showed increased activity in the right posterior insula (BA 13) compared with non-ALEX although activation in this area was not found in the conjunction analysis. Correlation coefficients between the hemodynamic activation in each ROI and the psychological measurement scores are shown in Table 7 for the pain-related regions found in the group comparison (i.e., right DLPFC [peak] [x, y, z] = [−20, 56, 34]; left ACC [−12, 2, 52]; left dorsal pons [−2, 38, −42]; left cerebellum [−14, −64, −32]; right inferior frontal gyrus [Rt IFG] [54, 22, 4]; right anterior insula [38, 14, 2]; and right posterior insula [38, −30, 18]). The DLPFC did not show any significant correlations with the psychological scores. The left dorsal ACC showed a significant positive correlation coefficient with "self-controlling" on the SCI. The brain stem (dorsal pons) showed a negative correlation with "personal distress" on the IRI and a positive correlation with "cognitive" on the SCI. The left cerebellum showed a positive correlation with "warmth" on the EES and "problem solving" on the SCI. The right anterior insula correlated positively with "affectedness" on the EES and negatively with "cognitive" and "problem solving" on the SCI. The right posterior insula had positive correlation with "personal distress" on the IRI and negative correlation with "cognitive," "seeking social support," "accepting responsibility," and "positive reappraisal." The Rt IFG showed a negative correlation with "warmth" on the EES and "positive reappraisal" on the SCI.

Discussion

The results of the present experiment support previous neuroimaging studies of empathy for pain, showing selective

Table 5
Coordinates and *Z* and *T* scores for the pain-related brain areas activated in response to painful picture stimuli in conjunction analysis of 1-sample tests on both groups

Area	BA	MNI *x*, *y*, *z* (mm)	*T*	*Z*
ACC				
Lt	24	0, 0, 40	3.26	2.98**
	32	−8, 24, 40	4.54	3.9*
Rt	8/32	2, 18, 48	4.48	3.86*
Cerebellum				
Lt posterior tonsil	—	−30, −36, −40	3.48	3.15**
DLPFC				
Lt inferior frontal	47	−48, 44, −12	3.84	3.41*
	44	−56, 8, 22	5.43	4.45*
	46	−46, 34, 12	6.79	5.18*
	9	−56, 8, 32	6.95	5.25*
Lt middle frontal	10	−34, 60, 8	5.22	4.33*
Rt inferior frontal	44	54, 8, 20	2.76	2.58**
	9	56, 10, 32	5.64	4.57**
Rt middle frontal	46	42, 28, 20	4.14	3.63**
Insula				
Lt	13	−42, −2, 4	2.75	2.57**
	13	−32, 16, 10	3.31	3.02**
Midbrain				
Lt midbrain	—	−2, −36, −4	2.68	2.51**
Lt substantia nigra	—	−14, −22, −8	3.33	3.03**
Primary somatosensory cortex				
Lt postcentral	3	−38, −28, 58	4.36	3.78*
	5	−42, −46, 62	5.33	4.39*
	2	−62, −24, 36	7.13	5.34*
	1	−60, −28, 42	7.85	5.66*
Rt inferior parietal lobule	40	38, −34, 42	6.18	4.87*
Rt postcentral	5	38, −48, 60	4.87	4.11*
	2	52, −28, 44	9.49	6.3**
Lt inferior parietal lobule	40	−54, −32, 46	8.17	5.8*
Secondary somatosensory cortex				
Lt	40	−62, −20, 22	3.76	3.35*
Rt	3/40	62, −20, 38	7.5	5.51*
Thalamus				
Lt	—	−14, −18, 10	4.73	4.02**
Rt	—	4, −32, −4	3.29	3**

Note: Lt; left, Rt; right.
*$P < 0.05$ false discovery rate (FDR) corrected in each ROI.
**$P < 0.05$ FDR corrected (height threshold: $t = 2.58$).

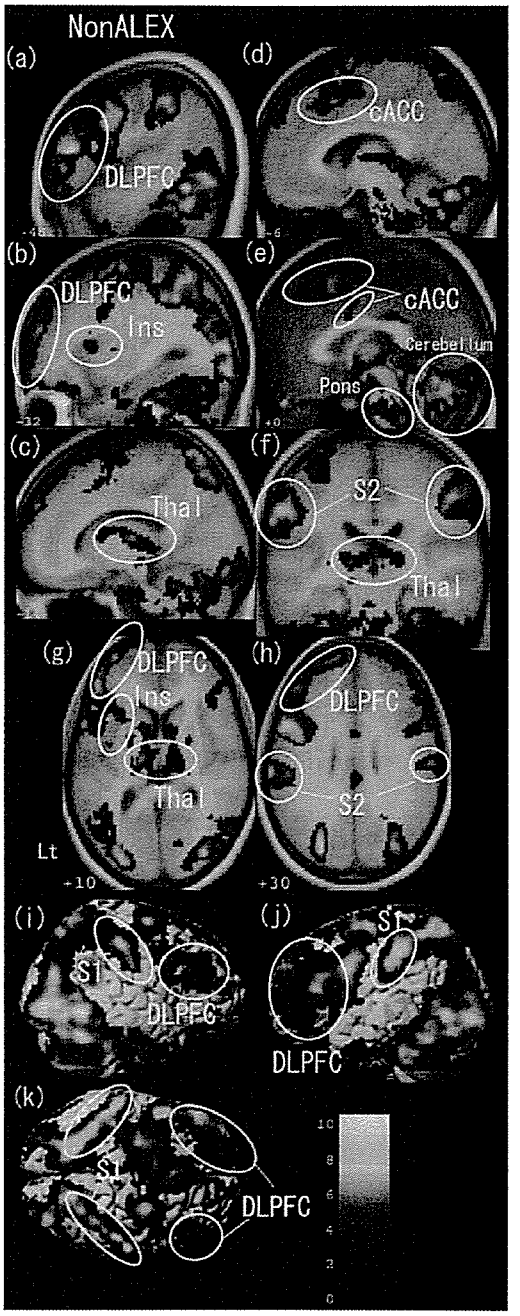

Figure 1. Brain images of the higher regional cerebral activation in response to the other's painful pictures compared with control pictures in the non-alexithymic sample. The brain images illustrate the clusters with neural activities in response to the other's pain task (contrasted with no-pain control pictures) within pain-related regions using 1-sample tests for the non-ALEX group ($n = 14$). The white circles on the brain images indicate the notable clusters related to the pain network. The bar on the lower right shows the range of *t* scores for SPM. The height threshold for illustrating the clusters was $P < 0.05$ corrected (false discovery rate). (*a–e*) sagittal view; (*f*) coronal view; (*g*, *h*) axial view; (*i*) right side; (*j*) left side; (*k*) top. Ins, insula; Thal, thalamus; cACC, caudal anterior cingulate cortex; S2, secondary sensory cortex; S1, primary sensory cortex.

activation of the neural network mediating the perception of other's pain (Morrison et al. 2004; Singer et al. 2004; Botvinick et al. 2005; Jackson et al. 2005, 2006; Lamm et al. 2007; Saarela et al. 2006). Interestingly, individuals with ALEX rated the painful stimuli as less painful than individuals without ALEX. Furthermore, fMRI measures showed lower signal change in the left lateral prefrontal cortex, left ACC, cerebellum, and dorsal pons in the ALEX group than in the non-ALEX group in response to viewing pictures of painful situations.

The behavioral measures revealed that the ALEX group showed lower scores for pain ratings and on questionnaires assessing empathetic qualities. This indicates that ALEX is associated with not only difficulty in representing one's own emotional state but also the emotions of others. It is worth noting that Guttman and Laporte (2002) reported behavioral results very similar to ours: alexithymic participants had higher levels of IRI personal distress and lower levels of perspective taking and fantasy. Personal distress scale has clearly different features from other scales on IRI: perspective taking and fantasy were significant and positively related to empathic concern, whereas a significant inverse relationship was found between perspective taking and personal distress (Davis 1983). Personal distress involves the experiences of another's distress as if it were one's own due to incapability of distinguishing the self-other difference. It is generally considered as a primitive form of empathic response in developmental science because the infant imitates the emotional distress of another but without an awareness of the other's situation or condition (Eisenberg 2000; Decety 2007; Lamm et al. 2007). Davis (1996) noted that personal distress as a mere reactive response to another's condition, rather than a direct representation of another's

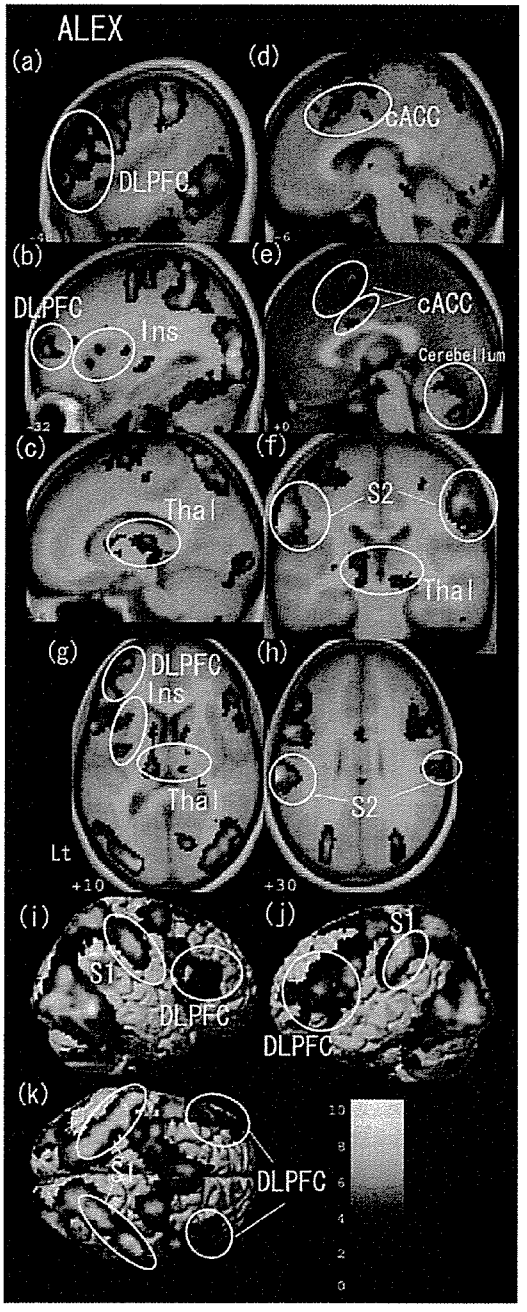

Figure 2. Brain images of the higher regional cerebral activation in response to the other's painful pictures compared with control pictures in the alexithymic sample. The brain images illustrate the clusters with neural activities in response to the other's pain task (contrasted with no-pain control pictures) within pain-related regions using 1-sample tests for the ALEX group (n = 16). The white circles on the brain images indicate the notable clusters related to the pain network. The bar on the lower right shows the range of t scores for SPM. The height threshold for illustrating the clusters was $P < 0.05$ corrected (false discovery rate). (*a–e*) sagittal view; (*f*) coronal view; (*g, h*) axial view; (*i*) right side; (*j*) left side; (*k*) top. Ins, insula; Thal, thalamus; cACC, caudal anterior cingulate cortex; S2, secondary sensory cortex; S1, primary sensory cortex.

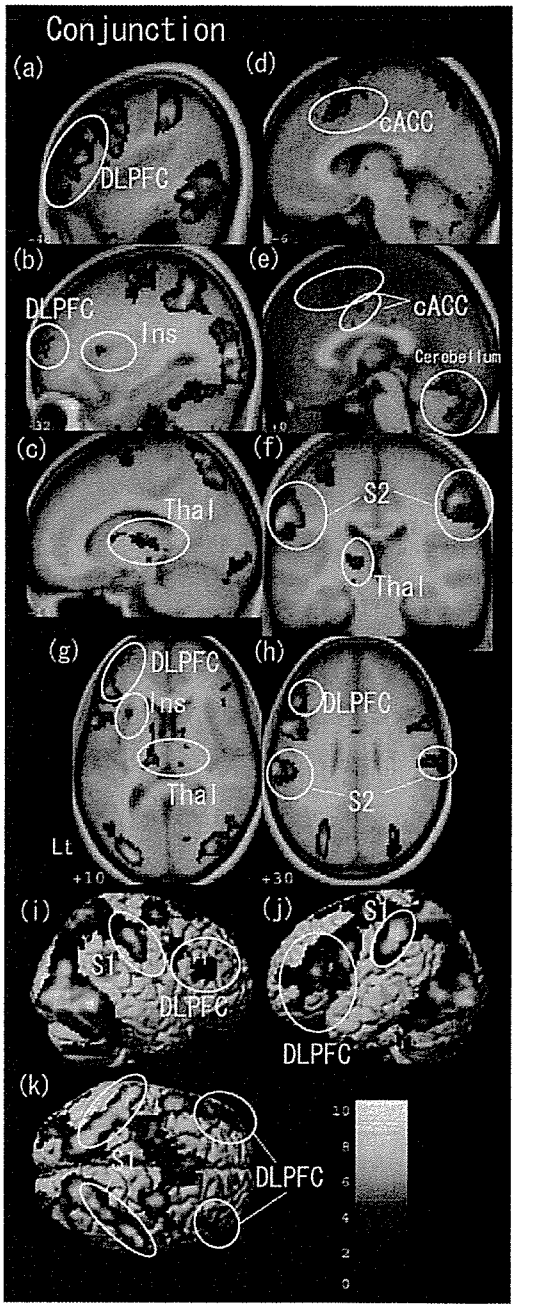

Figure 3. Brain images of the higher regional cerebral activations in response to the other's painful pictures compared with control pictures in conjunction analysis of both groups. The brain images illustrate the clusters with neural activities in response to the other's pain task (contrasted with no-pain control pictures) within pain-related regions in conjunction analysis that shows overlapping areas using two 1-sample tests (ALEX group [n = 16] and non-ALEX group [n = 14]). The white circles on the brain images indicate the notable clusters related to the pain network. The bar on the lower right shows the range of t scores for SPM. The height threshold for illustrating the clusters was $P < 0.05$ corrected (false discovery rate). (*a–e*) sagittal view; (*f*) coronal view; (*g, h*) axial view; (*i*) left side; (*j*) right side; (*k*) top. Ins, insula; Thal, thalamus; cACC, caudal anterior cingulate cortex; S2, secondary sensory cortex; S1, primary sensory cortex.

affect, characterized by a negative affective tone and self-oriented thought processes. Such individuals experiencing personal distress as a reaction to another's distress tend to feel more anxious and uncomfortable regardless of the state of mind of the other. Personal distress scale is associated with high levels of social dysfunction, fearfulness, uncertainty, emotional vulnerability, shyness, and social anxiety. High personal distress was characterized by their concern with how others evaluate them and with lowered concern for others (Davis 1983). Thus,

Table 6
Coordinates and *Z* and *T* scores for the brain areas differently activated between the ALEX and non-ALEX groups; group comparison using 2-sample tests

Area	BA	MNI *x*, *y*, *z*(mm)	*T*	*Z*	Cluster *k*
ALEX < non-ALEX					
Lt lateral prefrontal cortex	9	−20, 56, 34	4.73	4.02	113
	9	−12, 60, 32	4.31	3.74	
	8	−12, 52, 44	4.07	3.57	
Cerebellum					
Lt anterior dentate	—	−14, −64, −32	4.98	4.18	133
Lt anterior culmen	—	−18, −50, −24	4.95	4.16	
	—	−10, −54, −28	4.33	3.76	
Lt posterior declive	—	−4, −70, −20	4.73	4.02	115
Rt posterior declive	—	6, −76, −28	4.02	3.54	
	—	22, −70, −28	3.91	3.46	26
Lt posterior cerebellar tonsil	—	−32, −34, −40	4.6	3.94	100
Lt brain stem pons	—	−22, −32, −36	4.5	3.87	
	—	−2, −38, −42	4.34	3.76	70
Lt dorsal anterior cingulate gyrus[a]	24	−12, 2, 52	3.42	3.1	25
	24	−14, 4, 56	3.28	2.99	
Rt middle temporal gyrus	38	32, 2, −32	4.83	4.08	21
Lt superior frontal gyrus	8	−20, 12, 46	4.09	3.59	20
Lt middle frontal gyrus	6	−32, 10, 58	4.04	3.55	28
ALEX > non-ALEX					
Rt anterior insula[a]	13	38, 14, 2	3.49	3.15	65
Rt posterior insula	13	38, −30, 18	4.26	3.71	46
	40	48, −24, 16	4.08	3.58	
Rt IFG	45	54, 26, 6	5.48	4.48	40
Rt ventral anterior cingulate	24	6, 26, 14	5.33	4.39	53
Rt superior frontal gyrus	9	20, 42, 34	5.16	4.29	71
Rt middle temporal gyrus	21	62, −6, −6	4.55	3.9	100
	21	60, 2, −8	4.16	3.64	
Rt superior temporal gyrus	22	62, −26, −2	4.49	3.86	20
Lt ventral anterior cingulate	—	−8, 38, 4	4.15	3.63	22

Note: Height and extent threshold: $T = 3.41$ ($P = 0.001$ uncorrected) and $k = 20$ voxels. Lt, left; Rt, right.
[a] $T = 2.76$, ($P = 0.005$ uncorrected) and $k = 20$ voxels.

personal distress is regarded as a less mature aspect of empathy and is related to impairments in cognitive aspects of empathy. Higher levels of personal distress in alexithymics in the present study indicate that ALEX may be related to immature forms of empathy (Guttman and Laporte 2000, 2002). We also found significantly lower scores in the ALEX group on the EES for warmth and on the SCI for cognitive, "planful problem solving," and positive reappraisal, reflecting their less cognitive strategies on the occasion of coping with emotional stress. ALEX has been found to be associated with low "emotional intelligence" (Fukunishi et al. 2001), which has a factor of empathy in terms of recognizing and understanding emotions in others (Goleman 1995). Therefore, we consider that alexithymic individuals, who have difficulty in identifying their own feelings, are also poor at representing and evaluating other's mental states, especially in terms of their cognitive aspects.

The fMRI experiment showed that the main effect of watching painful stimuli was associated with activation in the somatosensory (SI/SII), thalamus, ACC, anterior insula, cerebellum, lateral prefrontal cortex, and brain stem. Consistent with previous neuroimaging studies, activation in these areas involved in empathy for pain was replicated, without physical sensation of actual pain stimulation. Furthermore, we found a relationship between the evaluation of painful pictures and activation in the lateral prefrontal cortex, pons, cerebellum, and right caudal ACC, as previously reported (Singer et al. 2004; Jackson et al. 2005). In our study, sensory inputs and motor outputs were controlled, so these activations derived from only visual input and processing these stimuli, not sensory feedback as a result of pressing the response buttons. It is possible that to accurately estimate pain in others, participants might further engage almost the whole pain matrix, not only the affective component within the pain network, notably the rostral ACC and anterior insula. Interestingly, a recent transcranial magnetic stimulation study demonstrated the sensorimotor side of empathy for pain by showing a reduction in excitability of hand muscles during the observation of painful stimuli (Avenanti et al. 2005). Together with our results, this points to the implication of regions other than those implicated in the affective component of empathy for pain.

The group comparison analyses indicated lower activation in the left lateral prefrontal cortex, dorsal pons, cerebellum, and ACC in the ALEX group as compared with the non-ALEX group. These regions have been demonstrated to be activated in association with the perception of other's pain (Singer et al. 2004; Jackson et al. 2006; Lamm et al. 2007) and in other pain-related studies (Davis 2000; Peyron et al. 2000; Raij et al. 2005). Reportedly, the interregional correlation of midbrain and medial thalamic activity was reduced during high left DLPFC activity (Lorenz et al. 2003). This indicates that the DLPFC exerts active control of pain perception by modulating cortico-subcortical and corticocortical pathways. Furthermore, the locus of the region in the present study is close to that activated by empathic and forgiveness tasks (Farrow et al. 2001), chronic facial pain contrasted with the pain-free condition after thalamic stimulation (Kupers et al. 2000), and rating the valence and intensity of affective pictures (Grimm et al. 2005). In summary, the DLPFC was associated with cognitive (especially executive and/or regulatory) processing of visual stimuli. These results are consistent with the hypothesis proposed by Taylor and Bagby (2004) of a hypofunction of the prefrontal cortex in individuals with ALEX, referring to the neuroimaging study by Hariri et al. (2000). In addition, it has been suggested that the DLPFC, reciprocally connected to many other neocortical areas, including the ACC, as well as the basal ganglia and the brain stem, regulates the functions that utilize emotional feelings for a survival function like planning and initiative. This includes the capacity to harmonize current behavior with the demands of the environment. Hence, it would be expected that selective lesions in this neural network may result in alexithymic features (Bermond 1997). Empathy requires emotional regulation (Eisenberg 2000; Decety and Jackson 2006; Decety 2007), and the DLPFC is key region implicated in this process (Ochsner and Gross 2005). It is thus logical to suggest that lateral prefrontal hypoactivity in ALEX is associated with a deficit in cognitive (particularly executive/regulating) function in empathizing and evaluating other's pain.

Moreover, the caudal ACC (cCZ [caudal cingulate zone]; Picard and Strick 1996, posterior part of 24b′; Vogt and Peters 1981, Vogt et al. 1996) showed less activation in alexithymics than in non-alexithymics. TAS-20 total scores have been reported to be correlated with the size of the normalized surface area of the right ACC (Gundel et al. 2004). The ACC has been associated with conscious awareness of emotion (Lane et al. 1997). The locus of the ACC that was less activated in ALEX in our study corresponds to the cognitive subdivision of the ACC that is involved in second-order representation or awareness (Lane 2000; Berthoz et al. 2002). Alexithymic individuals have been reported to show less activation in the ACC in response to the emotionally laden (e.g., anger) components of facial expressions (Kano et al. 2003). Interestingly, Vogt et al. (1996) argued that

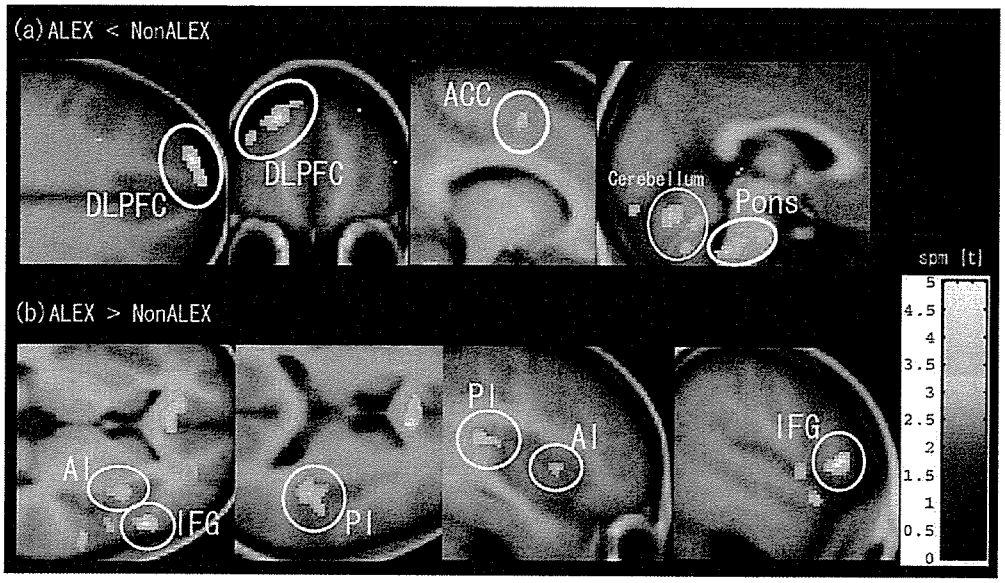

Figure 4. Brain images of the different regional cerebral activations between individuals with and without ALEX in response to the other's painful picture task. The orthogonal views of brain images illustrate the clusters with different neural activities in response to the other's pain task (contrasted with no-pain control pictures) within pain-related regions. The bar on the lower right shows the range of t scores for SPM. The height and extent threshold for illustrating were $Z = 2.6$ ($T = 3.33$), ($P < 0.005$ uncorrected) and $k = 20$, respectively. (*a*) The figures for the notable clusters with less activation in the ALEX group compared with non-ALEX group. Peak MNI coordinates (x, y, z) = (−20, 56, 34); cACC, caudal anterior cingulate cortex (−12, 2, 52); brain stem (dorsal pons) (−2, 38, −42); and cerebellum (−14, −64, −32). (*b*) The figures for the clusters with more activation in the ALEX group than the non-ALEX group. AI, anterior insula (38, 14, 2); PI, posterior insula (38, −38, 18); and IFG, inferior frontal gyrus (54, 22, 4).

Table 7
Correlation coefficients between the mean neural activity in ROIs found in group comparisons for each psychological measurement

	Lt DLPFC	Lt cACC	Pons	Lt cerebellum	Rt AI	Rt PI	Rt IFG
EES							
Warmth	0.22	0.24	0.28	**0.36***	−0.12	−0.11	**−0.36***
Chill	−0.07	0.19	−0.14	−0.23	−0.14	−0.05	0.02
Affectedness	−0.08	−0.11	−0.18	0.1	**0.36***	0.24	0.21
IRI							
Fantasy	0.27	0.14	0	−0.02	−0.05	0.19	−0.12
Perspective taking	0.17	0.14	0.06	0.11	−0.31	−0.09	−0.14
Empathic concern	0.2	0.07	0.19	0.31	−0.14	−0.06	−0.19
Personal distress	−0.15	−0.05	**−0.44***	0.01	0.28	**0.42***	0.07
SCI							
Cognitive	0.15	0.24	**0.37***	0.3	**−0.35***	**−0.48***	−0.33
Emotional	−0.07	0.18	0	0.04	−0.2	−0.32	−0.3
Problem solving	0.16	0.17	0.3	**0.37***	**−0.36***	−0.26	−0.3
Confrontational	0.02	−0.12	0.12	−0.02	−0.29	−0.11	−0.18
Seeking social support	0.09	0.04	0.14	0.23	−0.09	**−0.37***	−0.32
Accepting responsibility	0.1	0.26	0.26	0.03	−0.25	**−0.34***	−0.15
Self-controlling	−0.07	**0.35***	0.15	0.32	−0.15	−0.28	−0.02
Escape-avoidance	0.05	0.05	−0.03	−0.06	−0.1	−0.19	−0.25
Distancing	−0.15	0.09	−0.16	−0.24	−0.05	−0.12	0.02
Positive reappraisal	0.11	0.24	0.29	0.22	−0.29	**−0.53***	**−0.47***

Note: cACC, caudal anterior cingulate cortex; AI/PI, anterior/posterior insula; IFG, inferior frontal gyrus; Lt, left; Rt, right.
Bold type *$P < 0.05$.

different parts of cingulate cortex are engaged in different processing levels of nociceptive information and that area 24b′ is involved in the controlling aspect of pain processing like response selection. A meta-analysis concluded that mid-ACC hemodynamic activations detected in the first-hand experience of pain reflect the cognitive dimension of pain experience, including the awareness and response selection to pain stimuli (Peyron et al. 2000). The location of the caudal ACC activation, observed in group comparison analysis, is more posterior than the rostral ACC region associated with affective reaction to pain. Therefore, we suggest that ALEX may be related to some impairment in the cognitive-motivational aspects of pain processing. It is important to note that the motivational dimension of pain processing includes the selection and preparation of movements of aversion (Morrison et al. 2004). Reportedly, activation in the mid-ACC is related to processes regarding pain in self, such as somatic monitoring, negative stimulus evaluation, and the selection of appropriate skeletomuscular movements of aversion (Isomura and Takada 2004; Jackson et al. 2006). Considering the participants with ALEX scored lower on pain and empathy scales, they would not be concerned by other's pain, so there should be less need for them to prepare their own organisms for a negative threatening experience. That might result in the less activation in caudal ACC in alexithymics in the present study. Interestingly, neural activities in this region had a positive correlation with pain ratings, which alexithymic participants estimated as lower. Activity in this region is associated with self-control ability in response to painful picture tasks. These results are fairly consistent with the ACC deficit model of ALEX (Lane et al. 1997; Berthoz et al. 2002).

The dorsal pons and cerebellum were found to be less activated in the ALEX group in the present study. Although monoaminergic projections from the brain stem to the prefrontal cortex are well known (Porrino and Goldman-Rakic 1982), there are sizable and highly ordered inputs to the pons from the DLPFCs, which are then relayed to the cerebellum (Schmahmann and Pandya 1997). Hence, this area is an integral node in the distributed cortical-subcortical neural circuitry supporting cognitive operations (Schmahmann and Pandya 1995). In order to evaluate painful situations in others without actually experiencing pain, people probably also rely on high-order cognitive functions to access minor changes in their physical state as a tool for estimating the stimulus input. Cerebellum abnormality is related to a broad range of psychiatric

disorders (Konarski et al. 2005). Furthermore, in our study, neural activity in the pons was associated with the cognitive coping strategy scale and negatively correlated with personal distress. Neural activation in the cerebellum correlated with problem solving coping style, which suggests that the subtentorial structures may be engaged in cognitive control aspects of empathy for other's pain.

We found more activation in the anterior insula in the ALEX group. The anterior insula, known to be closely connected to the amygdala and ventral ACC, plays an important role in responding to emotional stimuli as "ventral prelimbic" areas, and these regions are often synchronized with each other (Mayberg 1997). The prelimbic areas were found to be suppressed (or biased against) during cognitively demanding tasks like a counting stroop task (Bush et al. 1998, 2000). Furthermore, reciprocal changes involving the prelimbic area and prefrontal cortex were also found. Hemodynamic increases in the prelimbic area and decreases in the prefrontal cortex were reported in response to sadness, although these 2 areas demonstrated the inverse correlation as a person recovered from a depressive state (Mayberg et al. 1999). If an individual engages less cognitive processing for the painful pictures, the suppression of activation in the anterior insula would be decreased. The ALEX group, which has more impairment in cognitive aspects, may have had more activation in the anterior insula compared with the non-alexithymics as a result of decreased suppression. In contrast to our study, Kano et al. (2003) found reduced activation in the anterior insula in response to emotional faces. The reason for this discrepancy might be that our study required participants to judge other's pain cognitively, whereas the paradigm used by Kano and colleagues involved the passive observation of emotional stimuli, less cognitively demanding. Thus, the present study might tap into more cognitive processing than the study by Kano and colleagues. Furthermore, the finding in the present study that neural activity in the insula was associated with more personal distress and emotional affectedness and less cognitive and less problem solving coping styles supports these inferences.

ALEX group also showed increased neural activity in the right posterior insula, although this region was not extracted by the conjunction analysis. Craig (2003) noted that the dorsal posterior insula involves the primary (not metarepresentational) interoceptive representation of the inputs of physiological condition from all tissues of the body, including pain, temperature, itch, sensual touch, muscular and visceral sensations, vasomotor activity, hunger, thirst, and "air hunger." Thus, the posterior insula is related to lower level representation of the physical state. Considering that neural activity in this region positively correlated with the personal distress scale and negatively with cognition-related stress coping scales, the result of stronger activity in the posterior insula in the ALEX group indicates that individuals with ALEX might be stuck in lower level representation of one's own physical state. Interestingly, a recent neuroscience research, including intracranial electrophysiological stimulations in neurological patients, indicates that distinct subregions of the insula contribute to different aspects of empathy (Decety and Lamm 2006). The posterior insula is associated with personal distress (self-oriented response), whereas the anterior insula is associated with empathy (other oriented emotional responses).

In the present study, the neural activity in the Rt IFG ($x = 54$, $y = 22$, $z = 4$) was stronger in ALEX group than non-ALEX group. Eisenberger et al. (2003) and Eisenberger Lieberman (2003, 2004) noted that the right ventral prefrontal cortex activation (RVPFC [$x = 42$, $y = 27$, $z = 11$], near the Rt IFG in the present study [$x = 42$, $y = 27$, $z = 11$]) was associated with less dorsal ACC activation and less self-reported distress across participants, suggesting that the RVPFC might serve a self-regulatory suppressive function by disrupting the pain distress. One possible interpretation is that the individuals with ALEX might try to deny and suppress the negative emotional aspects of the painful picture stimuli, resulting in their discreet evaluation about pain in the task pictures. Relationship between ALEX and a suppressive aspect of emotional processing remains to be solved.

A limitation of our study is that multiple correlation analyses were computed between hemodynamic ROI activation and psychological measurements, which might induce a significant result due to chance in each correlation analysis. However, adopting a more conservative corrected alpha level could increase false negative results. Although the present correlation study is useful to check the features of hemodynamic activation in each ROI in an exploratory way, one should acknowledge that the present results are only suggestive values and need further reconfirmations in future experiments. Another limitation is that the perception of pain in others with the use of pictures of limbs does not account for the full construct of empathy, but only part of it. According to several recent neurocognitive models of empathy, this capacity includes emotional contagion (that can lead to personal distress), sympathy, cognitive empathy, helping behavior etc., which all share aspects of their underlying process and cannot be totally disentangled (Preston and de Waal 2002; Decety and Jackson 2004, 2006; Lawrence et al. 2006). Further studies are needed in the future with the task to focus and discriminate each more specific aspect of empathy. Moreover, it has not been concluded whether the degree of ALEX does not influence thresholds for experimentally induced pain (de Zwaan et al. 1996; Nyklicek and Vingerhoets 2000; Jackson et al. 2002), so the results of the group comparisons for empathy to pain in the present study might be affected by actual tolerances for pain. Relationship between pain perception and ALEX remains to be clarified.

In conclusion, the present study demonstrates that individuals with ALEX showed diminished pain ratings, less mature empathy scores, and decreased neural activity associated with cognitive empathy to other's pain, notably in the lateral prefrontal and caudal anterior cingulate areas rather than in affective components like the anterior insula. The pain-related areas like the pons, ACC, and cerebellum showing decreased neural activities in ALEX were associated with cognitive aspects of empathy and coping style questionnaires. Empathy is comprised of a number of components such as taking others' perspectives and emotional regulation (including identifying, describing, and objectifying inner feelings) based on continuous self-awareness (Decety and Jackson 2004, 2006). Any organism capable of self-recognition would have an introspective awareness of its own mental state and the ability to ascribe mental states to others (Humphrey 1990). The emergence of a self-representation in psychological development is crucial for the empathic process (e.g., Lewis et al. 1989). Taken together with our results, the impaired cognitive (particularly executive/regulatory) aspects of empathy could be a part of the core deficit in ALEX, which is associated with impaired emotional regulation and also highlights the importance of self-awareness in empathy.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

Notes

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References

Aketa H. 1999. Structure and measurement of empathy: Japanese version of Davis's Interpersonal Reactivity Index (IRI-J). Psychol Rep Sophia Univ. 23:19-31.

Aleman A. 2005. Feelings you can't imagine: towards a cognitive neuroscience of alexithymia. Trends Cogn Sci. 9:553-555.

Arimura T, Komaki G, Murakami S, Tamagawa K, Nishikata H, Kawai K, Nozaki T, Takii M, Kubo C. 2002. Development of the structured interview by the modified edition of Beth Israel hospital psychosomatic questionnaire (SIBIQ) in Japanese edition to evaluate alexithymia. Jpn J Psychosom Med. 42:259-269.

Avenanti A, Bueti D, Galati G, Aglioti SM. 2005. Transcranial magnetic stimulation highlights the sensorimotor side of empathy for pain. Nat Neurosci. 8:955-960.

Bermond B. 1997. Brain and alexithymia. In: Vingerhoets AJM, van Bussel FJ, Boelhouwer AJW, editors. The (non)expression of emotions in health and disease. Tilburg (The Netherlands): Tilburg University Press. p. 115-129.

Berthoz S, Artiges E, Van De Moortele PF, Poline JB, Rouquette S, Consoli SM, Martinot JL. 2002. Effect of impaired recognition and expression of emotions on frontocingulate cortices: an fMRI study of men with alexithymia. Am J Psychiatry. 159:961-967.

Berthoz S, Hill EL. 2005. The validity of using self-reports to assess emotion regulation abilities in adults with autism spectrum disorder. Eur Psychiatry. 20:291-298.

Botvinick M, Jha AP, Bylsma LM, Fabian SA, Solomon PE, Prkachin KM. 2005. Viewing facial expressions of pain engages cortical areas involved in the direct experience of pain. Neuroimage. 25:312-319.

Buchanan DC, Waterhouse GJ, West SC Jr. 1980. A proposed neurophysiological basis of alexithymia. Psychother Psychosom. 34:248-255.

Bush G, Luu P, Posner MI. 2000. Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn Sci. 4:215-222.

Bush G, Whalen PJ, Rosen BR, Jenike MA, McInerney SC, Rauch SL. 1998. The counting Stroop: an interference task specialized for functional neuroimaging—validation study with functional MRI. Hum Brain Mapp. 6:270-282.

Cedro A, Kokoszka A, Popiel A, Narkiewicz-Jodko W. 2001. Alexithymia in schizophrenia: an exploratory study. Psychol Rep. 89:95-98.

Craig AD. 2003. Interoception: the sense of the physiological condition of the body. Curr Opin Neurobiol. 13(4):500-505.

Davis KD. 2000. Studies of pain using functional magnetic resonance imaging. In: Casey KL, Bushnell MC, editors. Pain imaging (Progress in pain research and management, Vol 18). Seattle (WA): IASP press. p. 195-210.

Davis MH. 1983. Measuring individual differences in empathy: evidence for a multidimensional approach. J Pers Soc Psychol. 44:113-126.

Davis MH. 1996. Empathy: a social psychological approach. Boulder (CO): Westview Press.

de Zwaan M, Biener D, Bach M, Wiesnagrotzki S, Stacher G. 1996. Pain sensitivity, alexithymia, and depression in patients with eating disorders: are they related? J Psychosom Res. 41:65-70.

Decety J. 2007. A social cognitive neuroscience model of human empathy. In: Harmon-Jones E, Winkielman P, editors. Fundamentals of social neuroscience. New York: Guilford Publications. p. 9246-9270.

Decety J, Grèzes J. 2006. The power of simulation: imaging one's own and other's behavior. Brain Res. 1079:4-14.

Decety J, Jackson PL. 2004. The functional architecture of human empathy. Behav Cogn Neurosci Rev. 3:71-100.

Decety J, Jackson PL. 2006. A social neuroscience perspective of empathy. Curr Dir Psychol Sci. 15:54-58.

Decety J, Lamm C. 2006. Human empathy through the lens of social neuroscience. Sci World J. 6:1146-1163.

Decety J, Sommerville JA. 2003. Shared representations between self and other: a social cognitive neuroscience view. Trends Cogn Sci. 7:527-533.

Eisenberg N. 2000. Emotion, regulation, and moral development. Annu Rev Psychol. 51:665-697.

Eisenberger NI, Lieberman MD. 2004. Why rejection hurts: a common neural alarm system for physical and social pain. Trends Cogn Sci. 8:294-300.

Eisenberger NI, Lieberman MD, Williams KD. 2003. Does rejection hurt? An FMRI study of social exclusion. Science. 302:290-292.

Farrow TF, Zheng Y, Wilkinson ID, Spence SA, Deakin JF, Tarrier N, Griffiths PD, Woodruff PW. 2001. Investigating the functional anatomy of empathy and forgiveness. Neuroreport. 12:2433-2438.

Friston KJ, Holmes AP, Worsley KJ. 1999. How many subjects constitute a study? Neuroimage. 10:1-5.

Frith U. 2004. Emanuel Miller lecture: confusions and controversies about Asperger syndrome. J Child Psychol Psychiatry. 45:672-686.

Fukunishi I, Wise TN, Sheridan M, Shimai S, Otake K, Utsuki N, Uchiyama K. 2001. Association of emotional intelligence with alexithymic characteristics. Psychol Rep. 89:651-658.

Gallup GG. 1998. Self-awareness and the evolution of social intelligence. Behav Processes. 42:239-247.

Goleman D. 1995. Emotional intelligence: why it matters more than IQ. New York: Bantam Books.

Grimm S, Schmidt CF, Bermpohl F, Heinzel A, Dahlem Y, Wyss M, Hell D, Boesiger P, Boeker H, Northoff G. 2006. Segregated neural representation of distinct emotion dimensions in the prefrontal cortex—an fMRI study. Neuroimage. 30(1):325-340.

Gundel H, Lopez-Sala A, Ceballos-Baumann AO, Deus J, Cardoner N, Marten-Mittag B, Soriano-Mas C, Pujol J. 2004. Alexithymia correlates with the size of the right anterior cingulate. Psychosom Med. 66:132-140.

Guttman H, Laporte L. 2002. Alexithymia, empathy, and psychological symptoms in a family context. Compr Psychiatry. 43:448-455.

Guttman HA, Laporte L. 2000. Empathy in families of women with borderline personality disorder, anorexia nervosa, and a control group. Fam Process. 39:345-358.

Hariri AR, Bookheimer SY, Mazziotta JC. 2000. Modulating emotional responses: effects of a neocortical network on the limbic system. Neuroreport. 11:43-48.

Haviland MG, Sonne JL, Kowert PA. 2004. Alexithymia and psychopathy: comparison and application of California Q-set prototypes. J Pers Assess. 82:306-316.

Hill E, Berthoz S, Frith U. 2004. Brief report: cognitive processing of own emotions in individuals with autistic spectrum disorder and in their relatives. J Autism Dev Disord. 34:229-235.

Hoppe KD, Bogen JE. 1977. Alexithymia in twelve commissurotomized patients. Psychother Psychosom. 28:148-155.

Humphrey N. 1990. The uses of consciousness In: Brockman J, editor. Speculations: the reality club. New York: Prentice Hall. p. 67-84.

Isomura Y, Takada M. 2004. Neural mechanisms of versatile function in primate anterior cingulate cortex. Rev Neurosci. 15:279-291.

Jackson PL, Brunet E, Meltzoff AN, Decety J. 2006. Empathy examined through the neural mechanisms involved in imagining how I feel versus how you feel pain. Neuropsychologia. 44(5):752-761.

Jackson PL, Meltzoff AN, Decety J. 2005. How do we perceive the pain of others? A window into the neural processes involved in empathy. Neuroimage. 24:771-779.

Jackson PL, Rainville P, Decety J. 2006. To what extent do we share the pain of others? Insight from the neural bases of pain empathy. Pain. 125(1-2):5-9.

Jackson T, Nagasaka T, Fritch A, Gunderson J. 2002. Alexithymia is not related to tolerance for cold pressor pain. Percept Mot Skills. 94:487-488.

Japanese Institute of Health Psychology. 1996. Lazarus type stress coping inventory. Tokyo (Japan): Jitsumu Kyoiku Shuppan.

Kano M, Fukudo S, Gyoba J, Kamachi M, Tagawa M, Mochizuki H, Itoh M, Hongo M, Yanai K. 2003. Specific brain processing of facial expressions in people with alexithymia: an H2 15O-PET study. Brain. 126:1474–1484.
Kato T, Takagi H. 1980. A trait of the emotional empathy in adolescence. Stud Psychol Tsukuba Univ. 2:33–42.
Komaki G, Maeda M, Arimura T, Nakata A, Shinoda H, Ogata I, Shimura M, Kawamura N, Kubo C. 2003. The reliability and factorial validity of the Japanese version of the 20-item Toronto Alexithymia Scale. J Psychosom Res. 55(2):143.
Konarski JZ, McIntyre RS, Grupp LA, Kennedy SH. 2005. Is the cerebellum relevant in the circuitry of neuropsychiatric disorders? J Psychiatry Neurosci. 30:178–186.
Krystal H. 1979. Alexithymia and psychotherapy. Am J Psychother. 33:17–31.
Krystal JH, Giller EL Jr, Cicchetti DV. 1986. Assessment of alexithymia in posttraumatic stress disorder and somatic illness: introduction of a reliable measure. Psychosom Med. 48:84–94.
Kupers RC, Gybels JM, Gjedde A. 2000. Positron emission tomography study of a chronic pain patient successfully treated with somatosensory thalamic stimulation. Pain. 87:295–302.
Lamm C, Batson CD, Decety J. Forthcoming. The neural basis of human empathy. Effects of perspective-taking and cognitive appraisal. J Cogn Neurosci.
Lane RD. 2000. Neural correlates of conscious emotional experience. In: Nadel L, editor. Cognitive neuroscience of emotion. Oxford: Oxford University Press. p. 345–370.
Lane RD, Ahern GL, Schwartz GE, Kaszniak AW. 1997. Is alexithymia the emotional equivalent of blindsight? Biol Psychiatry. 42:834–844.
Lane RD, Schwartz GE. 1987. Levels of emotional awareness: a cognitive-developmental theory and its application to psychopathology. Am J Psychiatry. 144:133–143.
Lane RD, Sechrest L, Reidel R, Weldon V, Kaszniak A, Schwartz GE. 1996. Impaired verbal and nonverbal emotion recognition in alexithymia. Psychosom Med. 58:203–210.
Lawrence EJ, Shaw P, Giampietro VP, Surguladze S, Brammer MJ, David AS. 2006. The role of 'shared representations' in social perception and empathy: an fMRI study. Neuroimage. 29:1173–1184.
Lazarus RS, Folkman S. 1984. Stress, appraisal, and coping. New York: Springer.
Lewis M, Sullivan MW, Stanger C, Weiss M. 1989. Self development and self-conscious emotions. Child Dev. 60:146–156.
Lorenz J, Minoshima S, Casey KL. 2003. Keeping pain out of mind: the role of the dorsolateral prefrontal cortex in pain modulation. Brain. 126:1079–1091.
Mantani T, Okamoto Y, Shirao N, Okada G, Yamawaki S. 2005. Reduced activation of posterior cingulate cortex during imagery in subjects with high degrees of alexithymia: a functional magnetic resonance imaging study. Biol Psychiatry. 57:982–990.
Mayberg HS. 1997. Limbic-cortical dysregulation: a proposed model of depression. J Neuropsychiatry Clin Neurosci. 9:471–481.
Mayberg HS, Liotti M, Brannan SK, McGinnis S, Mahurin RK, Jerabek PA, Silva JA, Tekell JL, Martin CC, Lancaster JL, et al. 1999. Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. Am J Psychiatry. 156:675–682.
Mehrabian A, Epstein N. 1972. A measure of emotional empathy. J Pers. 40:525–543.
Moriguchi Y, Ohnishi T, Lane RD, Maeda M, Mori T, Nemoto K, Matsuda H, Komaki G. 2006. Impaired self-awareness and theory of mind: an fMRI study of mentalizing in alexithymia. Neuroimage. 32(3):1472–1482.
Morrison I, Lloyd D, di Pellegrino G, Roberts N. 2004. Vicarious responses to pain in anterior cingulate cortex: is empathy a multisensory issue? Cogn Affect Behav Neurosci. 4:270–278.
Nemiah JC. 1977. Alexithymia. theoretical considerations. Psychother Psychosom. 28:199–206.
Nyklicek I, Vingerhoets AJ. 2000. Alexithymia is associated with low tolerance to experimental painful stimulation. Pain. 85:471–475.
Ochsner KN, Gross JJ. 2005. The cognitive control of emotion. Trends Cogn Sci. 9:242–249.
Ogawa S, Lee TM, Kay AR, Tank DW. 1990. Brain magnetic resonance imaging with contrast dependent on blood oxygenation. Proc Natl Acad Sci USA. 87:9868–9872.
Oldfield RC. 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia. 9:97–113.
Parker JD, Taylor GJ, Bagby RM. 1993. Alexithymia and the recognition of facial expressions of emotion. Psychother Psychosom. 59:197–202.
Peyron R, Laurent B, Garcia-Larrea L. 2000. Functional imaging of brain responses to pain. A review and meta-analysis (2000). Neurophysiol Clin. 30:263–288.
Picard N, Strick PL. 1996. Motor areas of the medial wall: a review of their location and functional activation. Cereb Cortex. 6:342–353.
Porrino LJ, Goldman-Rakic PS. 1982. Brainstem innervation of prefrontal and anterior cingulate cortex in the rhesus monkey revealed by retrograde transport of HRP. J Comp Neurol. 205:63–76.
Preston SD, de Waal FB. 2002. Empathy: its ultimate and proximate bases. Behav Brain Sci. 25:1–20.
Raij TT, Numminen J, Narvanen S, Hiltunen J, Hari R. 2005. Brain correlates of subjective reality of physically and psychologically induced pain. Proc Natl Acad Sci USA. 102:2147–2151.
Rainville P. 2002. Brain mechanisms of pain affect and pain modulation. Curr Opin Neurobiol. 12:195–204.
Rastam M, Gillberg C, Gillberg IC, Johansson M. 1997. Alexithymia in anorexia nervosa: a controlled study using the 20-item Toronto alexithymia scale. Acta Psychiatr Scand. 95:385–388.
Saarela MV, Hlushchuk Y, Williams AC, Schurmann M, Kalso E, Hari R. 2006. The compassionate brain: humans detect intensity of pain from another's face. Cereb Cortex. Advance Access published February 22, 2006, doi:10.1093/cercor/bhj141.
Schmahmann JD, Pandya DN. 1995. Prefrontal cortex projections to the basilar pons in rhesus monkey: implications for the cerebellar contribution to higher function. Neurosci Lett. 199:175–178.
Schmahmann JD, Pandya DN. 1997. Anatomic organization of the basilar pontine projections from prefrontal cortices in rhesus monkey. J Neurosci. 17:438–458.
Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC. 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. J Clin Psychiatry. 59(Suppl 20):22–33.
Sifneos PE. 1972. Short-term psychotherapy and emotional crisis. Cambridge (MA): Harvard University Press.
Sifneos PE. 1996. Alexithymia: past and present. Am J Psychiatry. 153:137–142.
Singer T, Seymour B, O'Doherty J, Kaube H, Dolan RJ, Frith CD. 2004. Empathy for pain involves the affective but not sensory components of pain. Science. 303:1157–1162.
Sommerville JA, Decety J. 2006. Weaving the fabric of social interaction: articulating developmental psychology and cognitive neuroscience. Psychon Bull Rev. 13(2):179–200.
Sriram TG, Pratap L, Shanmugham V. 1988. Towards enhancing the utility of Beth Israel hospital psychosomatic questionnaire. Psychother Psychosom. 49:205–211.
Stanghellini G, Ricca V. 1995. Alexithymia and schizophrenias. Psychopathology. 28:263–272.
Talairach J, Tournoux P. 1988. Co-planar stereotaxic atlas of the human brain. New York: Thieme.
Taylor GJ, Bagby RM. 2004. New trends in alexithymia research. Psychother Psychosom. 73:68–77.
Taylor GJ, Bagby RM, Parker JD. 2003. The 20-Item Toronto alexithymia scale. IV. Reliability and factorial validity in different languages and cultures. J Psychosom Res. 55:277–283.
Taylor GJ, Bagby RM, Parker JDA. 1997. Disorders of affect regulation: alexithymia in medical and psychiatric illness. Cambridge (UK): Cambridge University Press.
Vogt BA, Peters A. 1981. Form and distribution of neurons in rat cingulate cortex: areas 32, 24, and 29. J Comp Neurol. 195(4):603–625.
Vogt BA, Derbyshire S, Jones AK. 1996. Pain processing in four regions of human cingulate cortex localized with co-registered PET and MR imaging. Eur J Neurosci. 8(7):1461–1473.
Vorst HCM, Bermond B. 2001. Validity and reliability of the Bermond-Vorst alexithymia questionnaire. Pers Individ Dif. 30:413–434.
Wong DL, Baker CM. 1988. Pain in children: comparison of assessment scales. Pediatr Nurs. 14:9–17.

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The Val66Met polymorphism of the brain-derived neurotrophic factor gene affects age-related brain morphology

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Abstract

We investigated the effects of the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism on age-associated changes of brain morphology in 109 Japanese healthy subjects using MRI with optimized voxel-based morphometry technique. A significant age-related volume reduction was found in the dorsolateral prefrontal cortices (DLPFC), anterior cingulate cortices, and temporal and parietal cortices in all subjects. Further analysis revealed a significantly negative correlation between age and the volume of the bilateral DLPFC only in the Met-BDNF carriers, and a significant interaction between the polymorphism and age-associated volume changes in the bilateral DLPFC. Furthermore, Met-carriers showed a significant interaction ($p < 0.0001$) between the gender and the genotype on the gray matter volume in the DLPFC, and female Met-carriers showed more widespread age-associated volume reduction in DLPFC than male Met-carriers. Our data suggest that the Val66Met polymorphism may impact on age-related changes of the brain, which might be associated with the functional variance of neuroprotective effects of the BDNF. Furthermore, we suggest that genotype effects of the BDNF gene on brain morphology might differ in female from in male.

Keywords: Brain-derived neurotrophic factor; Val66Met polymorphism; Magnetic resonance imaging; Voxel-based morphometry; Dorsolateral prefrontal cortex; Aging

Brain-derived neurotrophic factor (BDNF), a member of neurotrophin family, has important roles in hippocampal plasticity and hippocampal-related learning and memory through long-term potentiation [15]. It also plays an important role in preventing death of neurons during development and protecting cholinergic neurons of the basal forebrain and the hippocampus from induced death in the adult brain [21].

A common missense polymorphism of the BDNF gene producing a valine to methionine amino acid substitution (Val66Met) affects the activity dependent secretion of BDNF in neurons and affects memory function [6,8]. Neuroimaging studies revealed that this polymorphism affected memory-related neuronal activities measured by functional magnetic resonance imaging (MRI) and macroscopic morphology of the hippocampus [8,12,23,28]. Regarding the brain morphology in normal individuals, Pezawas et al. [23] reported that Met-BDNF carriers had smaller volumes of the hippocampi and the prefrontal cortices as compared to individuals with homozygous Val-BDNF. This result was recently replicated in another mixed study of healthy and schizophrenic subjects [28]. Although several neuroimaging studies have indicated that environmental factors considerably impact on human brain structures even in normal adult brains [18], these data suggest that genetic factors such as polymorphism of BDNF might also strongly affect human brain morphology, and contribute to individual differences of brain morphology.

Aging is another factor which strongly affects brain morphology in human. There are several studies that demon-

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strated morphological changes associated with normal aging in vivo [10,24]. A general trend in the in vivo volumetric studies of healthy volunteers points to the prefrontal cortex as the cortical region in which the largest age-related volume reduction is observed. Considering the previous findings that BDNF is expressed abundantly in the prefrontal cortex [25] and that BDNF has a neuroprotective effect, Val66Met polymorphism might have some impacts on age-related morphological changes. However, there is no datum whether this polymorphism is associated with age-related morphological changes.

To clarify whether the BDNF polymorphism impacts on morphological changes associated with aging, we analyzed structural MR images in 109 normal individuals using optimized voxel-based morphometry (VBM) technique.

One hundred and thirty healthy subjects participated in the study. Written informed consent was obtained from all subjects in accord with ethical guidelines in place at local ethical committee. All of the subjects were recruited from local advertisements and underwent a Japanese version of National Adult Reading Test (JART) that is essentially the same as National Adult Reading Test [22] and MRI scanning. We employed JART as a convenient tool to measure IQ for each participant because previous study reported that it showed high correlation with IQ in healthy subjects [20]. All subjects were screened by a questionnaire regarding medical history and excluded if they had neurological, psychiatric or medical conditions that could potentially affect the central nervous system, such as substance abuse or dependence, atypical headache, head trauma with loss of consciousness, asymptomatic or symptomatic cerebral infarction detected by T2 weighted MRI, hypertension, chronic lung disease, kidney disease, chronic hepatic disease, cancer, or diabetes mellitus. Template creation for the optimized VBM was based on a sample of the 120 subjects, aged 36.2 ± 12.1 years (range 20–72). All subjects were Japanese. Since single nucleotide polymorphism (SNP) genotyping, described in the next section, was done successfully in 109 subjects, the MR images of these 109 subjects were used for subsequent analyses. According to the polymorphism, subjects were categorized into the following three groups: a homozygous Val-BDNF group ($n = 41$), a Val/Met-BDNF group ($n = 51$), or a homozygous Met-BDNF group ($n = 17$). The genotype distribution of this SNP was not deviated with Hardy–Weinberg equilibrium (chi-square = 0.03, $p = 0.86$). Because of the small number of subjects with homozygous Met-BDNF, the Val/Met-BDNF group and homozygous Met-BDNF group were treated as one group, the Met-BDNF carriers ($n = 68$). The demographic data of these groups are the following; the homozygous Val-BDNF comprised 26 females and 15 males, two were left-handed, aged 36.9 ± 13.0 years (range 21–68), and the mean education period and JART score were 16.2 ± 2.8 years (range 12–24) and 75.5 ± 13.3 (equivalent to 108.8 ± 9.55 for full scale IQ (range 50–96; equivalent to 90.5–123.6 for full scale IQ), respectively. The Met-BDNF carriers comprised 45 females and 23 males, three were left-handed, aged 35.8 ± 11.6 years (range 20–72), and their mean education period and JART score were 16.9 ± 3.0 years (range 12–28) and 78.0 ± 11.6 (equivalent to 110.7 ± 8.3) for full scale IQ (range 45–99; equivalent to 86.9–125.8 for full scale IQ), respectively. The mean age, gender ratio, handedness, education period, or JART score did not differ between the two groups (two sample t-test, data not shown).

The detail process of genotyping of BDNF Val66Met SNP (dbSNP accession: rs6265) was described previously [13]. Primers and probes for detection of the SNP (TaqMan SNP Genotyping assays on demand) were purchased from Applied Biosystems (ABI, Foster City, CA, USA). PCR cycling conditions were: at 95 °C for 10 min, 50 cycles of 92 °C for 15 s and 60 °C for 1 min.

All MR studies were performed on a 1.5 T Siemens Magnetom Vision plus system. A three dimensional volumetric acquisition of a T1-weighted gradient echo sequence produced a gapless series of 144 sagittal sections using an MPRage sequence (TE/TR, 4.4/11.4 ms; flip angle, 15°; acquisition matrix, 256 × 256; 1NEX, field of view, 31.5 cm; slice thickness, 1.23 mm).

Data were analyzed with Statistical Parametric Mapping 2 (SPM2) (http://www.fil.ion.ucl.ac.uk/spm/; Wellcome Department of Imaging Neuroscience, London, UK) running on MATLAB 6.5 R1 (MathWorks, Natick, MA). Before analyses, each image was confirmed by a neuroradiologist to eliminate images with artifacts, and then anterior commissure–posterior commissure line was adjusted. First, we made a customized anatomical T1 template and prior probability images from the sample of 120 brains [10]. Then, images were processed using an optimized VBM script (dbm.neuro.uni-jena.de/vbm.html). The detail of this process is described elsewhere [2,10]. The normalized segmented images were modulated by multiplication with Jacobian determinants of the spatial normalization function to encode the deformation field for each subject as tissue density changes in the normal space. Finally, images were smoothed using a 12 mm full width half maximum of isotropic Gaussian kernel. Statistical analyses were performed with SPM2, which implemented a General Linear Model. Proportional scaling was used to achieve global normalization of voxel values between images. First, we used a two-sample t-test to test regional population effect on gray matter volume. For this analysis, we set $p < 0.005$ without a correction for multiple comparisons, followed by applying small volume correction to each cluster with a false discovery rate (FDR) < 0.05. For the small volume correction, spheres with radius 10 mm around the peak were set as regions of interest (ROIs). The resulting sets of t-values constituted the statistical parametric maps {SPM (t)}. Anatomic localization was according to both MNI coordinates and Talairach coordinates, obtained from M. Brett's transformations (http://www.mrc-cbu.cam.ac.uk/Imaging/Common/mnispace.shtml) and presented as Talairach coordinates. Since a previous study with Caucasians demonstrated a significant reduction of volumes in the hippocampi and the frontal cortices in Met-BDNF carriers, we applied an additional hypothesis-driven ROI method to test regional population effects in these regions by using the Wake Forest University PickAtlas [19].

The genotype effects on age-related morphological changes were tested using a single subject condition and covariate model. Since several studies reported gender different age-related mor-

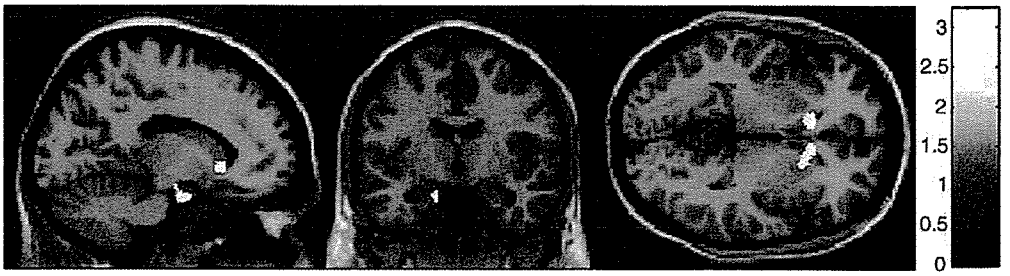

Fig. 1. The volume reduction of Met-BDNF carriers compared to that of individuals with homozygous Val-BDNF ($p < 0.05$, small volume correction with FDR). A significant reduction of volumes of the left parahippocampal gyrus (t-value: 2.92, Talairach coordinates (TAL): −12, −3, −19) and the bilateral heads of caudate nucleus (left: t-value; 3.23, TAL: −9, 22, −3, right: t-value; 3.02, TAL: 10, 21, −4) in the Met-BDNF carriers was noted.

phological changes in the brain [7], we additionally examined genotype effects on age-related morphological changes in each gender, separately. Orthogonalized first order polynomial expansion of age was treated as a covariate of interest to determine the linear effects of age [5]. Since second- and third-order polynomial expansions did not contribute to the age effect model of our sample, we removed them from a design matrix. Considering the possible association between IQ and brain morphology, we treated JART score as a nuisance variable. For this analysis, we applied $p < 0.001$, corrected for multiple comparisons with FDR < 0.05 as a statistical threshold [9]. MarsBar program (marsbar.sourceforge.net/) was also used to extract data from the regions of interest.

Fig. 1 shows a significant reduction of gray matter volumes of the left parahippocampal gyrus (Brodmann area (BA) 34), and bilateral heads of the caudate nucleus in Met-BDNF carriers when compared to homozygous Val-BDNF individuals. Even in hypothesis-driven ROI approach with a lenient statistical threshold (uncorrected $p = 0.05$), we could not find any significant differences of hippocampal nor prefrontal cortical volumes between the two groups. The results were essentially unchanged even when the restricted samples of subjects (female group, male group, or young group aged under 40 years old) were analyzed (data not shown).

Fig. 2 shows morphological changes related to normal aging. A significant negative correlation between age and the gray matter volumes was noted in the bilateral dorsolateral prefrontal cortices (DLPFC; BA9, 46), right superior temporal gyrus (STG; BA22), bilateral insulae (BA13), bilateral caudate nuclei, left anterior cingulate gyrus (BA24), bilateral inferior parietal lobules (BA40), bilateral precunei (BA7), and bilateral fusiform gyri (BA37) in all subjects. In homozygous Val-BDNF individuals, a significant age-related volume reduction was found in the bilateral insulae (BA13) and right STG (BA22). On the other hand, Met-BDNF carrier showed an additional negative correlation of the gray matter volumes in the bilateral DLPFC (BA9, 46) and right dorsal premotor area (BA6) with age. Additional analyses in each gender revealed a significant interaction ($p < 0.0001$) in Met-carriers between the gender and the genotype on the gray matter volume in the DLPFC, and female Met-carriers showed more widespread age-associated volume reduction in DLPFC than male Met-carriers. Male Met-carrier also showed volume

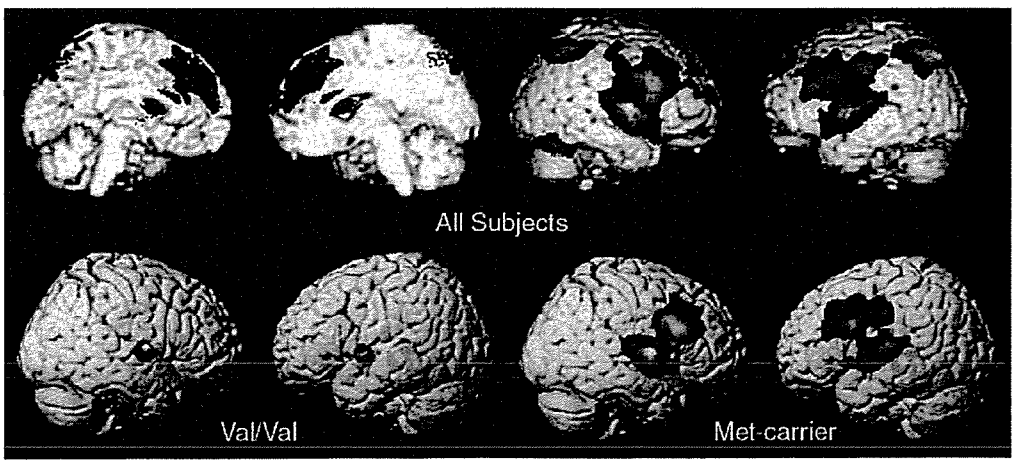

Fig. 2. (Top) The volume reduction associated with normal aging in all subjects ($p < 0.05$, FDR corrected). All subjects showed negative correlation with age in the bilateral DLPFC, right STG, bilateral insulae, bilateral caudate nuclei, left anterior cingulate gyrus, bilateral inferior parietal lobules, bilateral precunei, and bilateral fusiform gyri. (Bottom) The volume reduction associated with normal aging in each genotypic group ($p < 0.05$, FDR corrected). (Left) Results of individuals with homozygous Val-BDNF. Individuals with homozygous Val-BDNF showed negative correlation with age in the bilateral insulae (right: t-value: 4.36, TAL: 42, −2, 4; left: t-value: 4.52, TAL: −43, −2, 4) and the right superior temporal gyrus (t-value: 4.57, TAL: 47, 9, −4). (Right) Results of Met-BDNF carriers. The Met-BDNF carriers showed negative correlation with age in the bilateral dorsolateral prefrontal cortices (right: t-value: 6.5, TAL: 52, 21, 26; left: t-value: 6.12, TAL: −48, 19, 32) as well as the bilateral insulae and the superior temporal gyri.

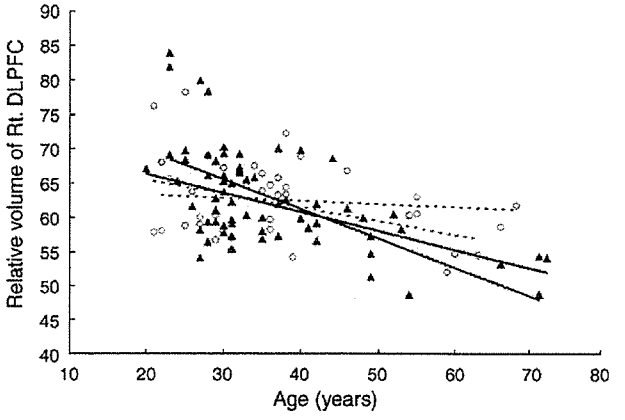

Fig. 3. Scatter plot of relative gray matter volume of the right DLPFC against age in each genomic group. The Met-BDNF carriers showed more significant volume reduction with normal aging compared to homozygous Val-BDNF subjects in the bilateral DLPFC in each gender (right: male Met-BDNF carriers: $y=-0.27x+71.8$, $r=-0.71$, $p<0.0001$, male homozygous Val-BDNF subjects: $y=-0.046x+64.2$, $r=-0.12$, $p=0.67$, female Met-BDNF carriers: $y=-0.43x+78.4$, $r=-0.56$, $p<0.001$, female homozygous Val-BDNF subjects: $y=-0.20x+69.5$, $r=-0.41$, $p=0.03$; left: male Met-BDNF carriers: $y=-0.20x+67.2$, $r=-0.53$, $p=0.01$, male homozygous Val-BDNF: $y=-0.11x+65.3$, $r=-0.25$, $p=0.367$, female Met-BDNF carriers: $y=-0.48x+77.0$, $r=-0.71$, $p<0.0001$, female homozygous Val-BDNF: $y=-0.14x+65.3$, $r=-0.27$, $p=0.18$). Due to limitations of space, only the plot at the right DLPFC in each gender is shown. Blue stands for male subjects and red stands for female subjects. Open circle: homozygous Val-BDNF; closed triangle: Met-BDNF carrier. Dotted lines are the regression line of homozygous Val-BDNF, whereas solid lines are those of Met-BDNF carrier.

reduction in the right inferior parietal lobules (BA40, t-value: 3.86, Talairach coordinates: 40, −43, 53). We found a significant interaction effect (male: $p=0.003$, female: $p<0.0001$) between the aging effect and the genotype on the gray matter volume in the DLPFC in each gender. (right: male Met-BDNF carriers: $r=-0.71, p<0.001$, male homozygous Val-BDNF subjects: $r=-0.12, p=0.67$; female Met-BDNF carriers: $r=-0.56$, $p<0.001$, female homozygous Val-BDNF subjects: $r=-0.41$, $p=0.03$; left: male Met-BDNF carriers: $r=-0.53$, $p=0.01$, male homozygous Val-BDNF subjects: $r=-0.25$, $p=0.367$, female Met-BDNF carriers: $r=-0.71$, $p<0.0001$, female homozygous Val-BDNF subjects: $r=-0.27$, $p=0.18$) (Fig. 3).

This is the first study which investigated the impacts of BDNF Val66Met polymorphism on age-associated brain morphological changes in normal individuals. We found an exaggerated age-related volume reduction of the DLPFC in the Met-BDNF carriers.

Several studies demonstrated morphological changes associated with normal aging in the STG, insula, inferior parietal lobules, motor cortex, ACC, and DLPFC [10,24]. In consistent with previous studies, our data also showed age-related volume reduction in similar regions in all subjects' analysis of each gender. Further analysis revealed that the Met-BDNF carriers showed a stronger negative correlation between age and gray matter volume in the DLPFC and right precentral gyrus when compared to individuals with homozygous Val-BDNF. Though the mechanisms underlying the predilection of the prefrontal cortex for age-related volume reduction are still unclear, the prefrontal cortex exhibits the greatest age-related alteration of GABA and glutamate [11], and glucose metabolism and age-related declines in regional cerebral blood flow [4]. Though there has been no study investigating the relationship between Val66Met SNP and vulnerability to age-related changes, BDNF protein itself is reported to be associated with aging. Amounts of BDNF protein in hippocampal pyramidal neurons and dentate granule cells are decreased during aging in monkeys [14]. Further, several studies demonstrated neuroprotective effects of BDNF [3,29]. Our data suggest that the Met-BDNF carriers, particularly females carrying Met-BDNF allele, may be more vulnerable to aging than individuals with homozygous Val-BDNF. Considering the fact that prefrontal cortex is one of the regions in which BDNF is expressed abundantly [25], we suggest that the Val66Met polymorphism may be associated with functional variances of neuroprotective and stress resistant effects of BDNF, which results in different effects on age-related morphological changes. Furthermore, we found a reduction of the striatal volumes in met-BDNF carriers as compared to individuals with homozygous Val-BDNF. It has been postulated that enhancement of BDNF in the cortex may be involved in protection of striatal neurons against damage via anterograde transport because BDNF exerts neuroprotective effects against excitoxicity in the striatum [1,16]. The result, reduced volumes in the striatum in met-BDNF carriers, may again suggest the reduced neuroprotective effects of met-BDNF. Since there has been no direct evidence of differential regulation of vulnerability to neurodegenerative process by BDNF Val66Met polymorphism, further study such as investigating how Val66Met SNP affects cell survival in a cellular model is required to clarify our speculation.

Although we could not replicate results of the previous studies, the smaller hippocampus in the Met-BDNF carriers [23,28], our data also suggest that BDNF polymorphism should have impacts on brain morphology associated with episodic memory. The discrepancy between our results and those of the previous studies could be partially explained by the racial difference. Binding its receptor TrkB, BDNF activates several pathways including the PI3-kinase/Akt, the mitogen-activated protein kinase, and PLC-gamma1 pathway [15]. These signals are known to be critical for survival of neuron, suggesting that not only Val66Met polymorphism of BDNF, but also interaction of polymorphism of each signal or molecule has effects on brain morphology. Racial differences might be related to such interactions, resulting in the different findings. This may partially contribute to the discrepancy in associations between BDNF polymorphism and the prevalence of neuropsychiatric diseases in Asian and Caucasian populations [17,27].

Finally, we mention a limitation of this study. To explore the association between aging effects on the brain morphology and the Val66Met polymorphism, we performed a cross-sectional study. There is a secular bias, which can be resolved by a longitudinal study. In this context, our data may be considered preliminary rather than conclusive. However, a recent longitudinal MR study of normal aging demonstrated that cross-sectional and longitudinal estimates of atrophy rates were similar [26].

In conclusion, we found that Val66Met polymorphism of BDNF had impacts on age-associated morphological changes in Japanese subjects. Our data suggest that Val66Met polymorphism of BDNF may play important roles for vulnerability to age-related morphological changes as well as the efficiency of plasticity, especially in DLPFC. Furthermore, we suggest that genotype effects of the BDNF gene on brain morphology might differ in female from in male.

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References

[1] C.A. Altar, N. Cai, T. Bliven, M. Juhasz, J.M. Conner, A.L. Acheson, R.M. Lindsay, S.J. Wiegand, Anterograde transport of brain-derived neurotrophic factor and its role in the brain, Nature 389 (1997) 856–860.
[2] J. Ashburner, K.J. Friston, Voxel-based morphometry—the methods, Neuroimage 11 (2000) 805–821.
[3] Z.C. Baquet, J.A. Gorski, K.R. Jones, Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor, J. Neurosci. 24 (2004) 4250–4258.
[4] M. Bentourkia, A. Bol, A. Ivanoiu, D. Labar, M. Sibomana, A. Coppens, C. Michel, G. Cosnard, A.G. De Volder, Comparison of regional cerebral blood flow and glucose metabolism in the normal brain: effect of aging, J. Neurol. Sci. 181 (2000) 19–28.
[5] C. Buchel, R.J. Wise, C.J. Mummery, J.B. Poline, K.J. Friston, Nonlinear regression in parametric activation studies, Neuroimage 4 (1996) 60–66.
[6] Z.Y. Chen, P.D. Patel, G. Sant, C.X. Meng, K.K. Teng, B.L. Hempstead, F.S. Lee, Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons, J. Neurosci. 24 (2004) 4401–4411.
[7] C.E. Coffey, J.F. Lucke, J.A. Saxton, G. Ratcliff, L.J. Unitas, B. Billig, R.N. Bryan, Sex differences in brain aging: a quantitative magnetic resonance imaging study, Arch. Neurol. 55 (1998) 169–179.
[8] M.F. Egan, M. Kojima, J.H. Callicott, T.E. Goldberg, B.S. Kolachana, A. Bertolino, E. Zaitsev, B. Gold, D. Goldman, M. Dean, B. Lu, D.R. Weinberger, The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function, Cell 112 (2003) 257–269.
[9] C.R. Genovese, N.A. Lazar, T. Nichols, Thresholding of statistical maps in functional neuroimaging using the false discovery rate, Neuroimage 15 (2002) 870–878.
[10] C.D. Good, I.S. Johnsrude, J. Ashburner, R.N. Henson, K.J. Friston, R.S. Frackowiak, A voxel-based morphometric study of ageing in 465 normal adult human brains, Neuroimage 14 (2001) 21–36.
[11] I.D. Grachev, A. Swamkar, N.M. Szeverenyi, T.S. Ramachandran, A.V. Apkarian, Aging alters the multichemical networking profile of the human brain: an in vivo (1)H-MRS study of young versus middle-aged subjects, J. Neurochem. 77 (2001) 292–303.
[12] A.R. Hariri, T.E. Goldberg, V.S. Mattay, B.S. Kolachana, J.H. Callicott, M.F. Egan, D.R. Weinberger, Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance, J. Neurosci. 23 (2003) 6690–6694.
[13] R. Hashimoto, T. Okada, T. Kato, A. Kosuga, M. Tatsumi, K. Kamijima, H. Kunugi, The breakpoint cluster region gene on chromosome 22q11 is associated with bipolar disorder, Biol. Psychiatry 57 (2005) 1097–1102.
[14] M. Hayashi, F. Mistunaga, K. Ohira, K. Shimizu, Changes in BDNF-immunoreactive structures in the hippocampal formation of the aged macaque monkey, Brain Res. 918 (2001) 191–196.
[15] E.J. Huang, L.F. Reichardt, Neurotrophins: roles in neuronal development and function, Annu. Rev. Neurosci. 24 (2001) 677–736.
[16] Z. Kokaia, G. Andsberg, Q. Yan, O. Lindvall, Rapid alterations of BDNF protein levels in the rat brain after focal ischemia: evidence for increased synthesis and anterograde axonal transport, Exp. Neurol. 154 (1998) 289–301.
[17] H. Kunugi, Y. Iijima, M. Tatsumi, M. Yoshida, R. Hashimoto, T. Kato, K. Sakamoto, T. Fukunaga, T. Inada, T. Suzuki, N. Iwata, N. Ozaki, K. Yamada, T. Yoshikawa, No association between the Val66Met polymorphism of the brain-derived neurotrophic factor gene and bipolar disorder in a Japanese population: a multicenter study, Biol. Psychiatry 56 (2004) 376–378.
[18] E.A. Maguire, D.G. Gadian, I.S. Johnsrude, C.D. Good, J. Ashburner, R.S. Frackowiak, C.D. Frith, Navigation-related structural change in the hippocampi of taxi drivers, Proc. Natl. Acad. Sci. (2000) 4398–4403.
[19] J.A. Maldjian, P.J. Laurienti, R.A. Kraft, J.H. Burdette, An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets, Neuroimage 19 (2003) 1233–1239.
[20] K. Matsuoka, Y. Kim, H. Hiro, Y. Miyamoto, K. Fujita, K. Tanaka, K. Koyama, N. Kazuki, Development of Japanese Adult Reading Test (JART) for Predicting Premorbid IQ in Mild Dementia, Seishinigaku 44 (2002) 503–511.
[21] J.K. Morse, S.J. Wiegand, K. Anderson, Y. You, N. Cai, J. Carnahan, J. Miller, P.S. DiStefano, C.A. Altar, R.M. Lindsay, Brain-derived neurotrophic factor (BDNF) prevents the degeneration of medial septal cholinergic neurons following fimbria transection, J. Neurosci. 13 (1993) 4146–4156.
[22] H.E. Nelson, A. O'Connell, Dementia: the estimation of premorbid intelligence levels using the New Adult Reading Test, Cortex 14 (1974) 234–244.
[23] L. Pezawas, B.A. Verchinski, V.S. Mattay, J.H. Callicott, B.S. Kolachana, R.E. Straub, M.F. Egan, A. Meyer-Lindenberg, D.R. Weinberger, The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology, J. Neurosci. 24 (2004) 10099–10102.
[24] N. Raz, F. Gunning-Dixon, D. Head, K.M. Rodrigue, A. Williamson, J.D. Acker, Aging, sexual dimorphism, and hemispheric asymmetry of the cerebral cortex: replicability of regional differences in volume, Neurobiol. Aging 25 (2004) 377–396.
[25] M. Sandrini, S.F. Cappa, S. Rossi, P.M. Rossini, C. Miniussi, The role of prefrontal cortex in verbal episodic memory: rTMS evidence, J. Cogn. Neurosci. 15 (2003) 855–861.
[26] R.I. Scahill, C. Frost, R. Jenkins, J.L. Whitwell, M.N. Rossor, N.C. Fox, A longitudinal study of brain volume changes in normal aging using serial registered magnetic resonance imaging, Arch. Neurol. 60 (2003) 989–994.
[27] P. Sklar, S.B. Gabriel, M.G. McInnis, P. Bennett, Y.M. Lim, G. Tsan, S. Schaffner, G. Kirov, I. Jones, M. Owen, N. Craddock, J.R. DePaulo, E.S. Lander, Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Brain-derived neutrophic factor, Mol. Psychiatry 7 (2002) 579–593.
[28] P.R. Szeszko, R. Lipsky, C. Mentschel, D. Robinson, H. Gunduz-Bruce, S. Sevy, M. Ashtari, B. Napolitano, R.M. Bilder, J.M. Kane, D. Goldman, A.K. Malhotra, Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation, Mol. Psychiatry 10 (2005) 631–636.
[29] H. Yanamoto, I. Nagata, M. Sakata, Z. Zhang, N. Tohnai, H. Sakai, H. Kikuchi, Infarct tolerance induced by intra-cerebral infusion of recombinant brain-derived neurotrophic factor, Brain Res. 859 (2000) 240–248.

Behavioral/Systems/Cognitive

Psychostimulant-Induced Attenuation of Hyperactivity and Prepulse Inhibition Deficits in *Adcyap1*-Deficient Mice

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Psychostimulants, including amphetamine, act as antihyperkinetic agents in humans with hyperkinetic disorder such as attention-deficit hyperactivity disorder and are known to be effective in enhancing attention-related processes; however, the underlying mechanisms have not been adequately addressed. Mice lacking the *Adcyap1* gene encoding the neuropeptide pituitary adenylate cyclase-activating polypeptide (*Adcyap1*$^{-/-}$) display psychomotor abnormalities, including increased novelty-seeking behavior and hyperactivity. In this study, *Adcyap1*$^{-/-}$ mice showed sensory-motor gating deficits, measured as deficits in prepulse inhibition (PPI), and showed normal PPI in response to amphetamine. Amphetamine also significantly decreased hyperlocomotion in *Adcyap1*$^{-/-}$ mice, and this paradoxical antihyperkinetic effect depended on serotonin 1A (5-HT$_{1A}$) receptor signaling. c-Fos-positive neurons were increased in the prefrontal cortex in amphetamine-treated *Adcyap1*$^{-/-}$ mice, suggesting increased inhibitory control by prefrontal neurons. Additionally, amphetamine produced an antihyperkinetic effect in wild-type mice that received the 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin. These results indicate that *Adcyap1*$^{-/-}$ mice act as a model of hyperlocomotion and PPI deficits and suggest that 5-HT$_{1A}$-mediated pathways are important determinants of the psychostimulant-elicited, rate-dependent effects that are in a negative function of the baseline rate of activity.

Key words: neuropeptide; knock-out mice; psychostimulant; hyperactivity; prepulse inhibition; serotonin 5-HT$_{1A}$ receptor

Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide originally isolated from ovine hypothalamus based on its ability to stimulate adenylate cyclase in rat anterior pituitary cell cultures and a member of the vasoactive intestinal peptide (VIP)/secretin/glucagon family. It exerts multiple activities as a neurotransmitter or neuromodulator via three G-protein-linked receptors, one PACAP-specific (PAC$_1$) receptor and two receptors that are shared with VIP (VPAC$_1$ and VPAC$_2$) (Arimura, 1998; Vaudry et al., 2000; Hashimoto et al., 2006). Our recently developed mice lacking the *Adcyap1* gene encoding the neuropeptide PACAP (*Adcyap1*$^{-/-}$) have marked phenotypes, including behavioral abnormalities (Hashimoto et al., 2001; Shintani et al., 2002; Kawaguchi et al., 2003; Tanaka et al., 2004). *Adcyap1*$^{-/-}$ mice are born in the expected Mendelian ratios but show a high early mortality rate before weaning. The surviving *Adcyap1*$^{-/-}$ females exhibit reduced fertility, which is partly attributable to reduced mating frequency, and inadequate maternal behavior. Furthermore, *Adcyap1*$^{-/-}$ mice display remarkable behavioral changes, including hyperlocomotion and jumping behavior in an open field, and increased novelty-seeking behavior. These salient phenotypes may be attributable to, at least in part, perturbed monoamine neurotransmission, because serotonin (5-HT) metabolism is slightly decreased in the cerebral cortex and striatum of *Adcyap1*$^{-/-}$ mice, and hyperactive behavior is ameliorated by the antipsychotic drug haloperidol (Hashimoto et al., 2001). However, the mechanisms involved and the pathophysiological significance still remain unclear.

It is commonly accepted that changes in dopaminergic tone highly correlate with alterations in locomotor activity. Psychostimulants such as amphetamine and methylphenidate are indirect agonists that facilitate the action of catecholamines including dopamine (DA), and their effects on motor activity have been hypothesized as being rate dependent. Low baseline rates of activity are increased by stimulants, whereas higher rates are increased to a lesser extent, or even decreased, as a result of drug treatment, such that stimulant-induced change is a negative linear function of the baseline rate of activity (Solanto, 1998, 2002). However, the underlying mechanism remains essentially unknown.

Prepulse inhibition (PPI) is the phenomenon in which a weak prepulse stimulus attenuates the response to a subsequent startling stimulus, providing an operational measure of sensorimo-

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tor gating and a cross-species form of information processing, deficient in patients with schizophrenia and some other neuropsychiatric disorders, including comorbid attention-deficit hyperactivity disorder (ADHD) and Tourette's syndrome (Castellanos et al., 1996; Geyer et al., 2001). Although psychostimulants reduce PPI in normal subjects, they are known to be effective in enhancing attention-related processes (Solanto, 2002) as well as attending PPI (Hawk et al., 2003) in ADHD children. However, stimulant effects on PPI have not been adequately assessed clinically, and with respect to stimulant response of PPI, there is no animal model showing a positive response. To date, understanding of the therapeutic action mechanisms of stimulants is still in its infancy.

In the present study, we demonstrated that *Adcyap1*$^{-/-}$ mice showed psychopathological aspects of hyperactivity and PPI deficits, as well as beneficial responses to amphetamine. Our results give new insights into the mechanisms underlying the therapeutic effects of psychostimulants.

Materials and Methods

Animals. All animal experiments were performed in accordance with protocols approved by the Animal Care and Use Committee of Graduate School of Pharmaceutical Sciences, Osaka University. Generation of *Adcyap1*$^{-/-}$ mice by a gene-targeting technique has been reported previously (Hashimoto et al., 2001). The null mutation was backcrossed onto an Institute of Cancer Research mouse background. Wild-type and *Adcyap1*$^{-/-}$ mice used were obtained from the intercross of heterozygous animals.

PPI analysis. Acoustic startle responses were measured in a startle chamber (SR-LAB; San Diego Instruments, San Diego, CA) using standard methods described previously (Sakaue et al., 2003). The testing session started with a 5 min acclimatization to the startle chamber in the presence of 65 dB background white noise. Testing consisted of 40 120-dB pulses alone and 10 pulses preceded (100 ms) by a prepulse of 66, 68, 71, or 77 dB. Pulses were randomly presented with an average of 15 s between pulses. Twelve no-stimulus trials were included to assess spontaneous activity during testing that was routinely observed to be 10–20 (arbitrary unit). For drug treatments, animals were placed in the startle chamber just after intraperitoneal injection of amphetamine or 30 min after intraperitoneal injection of haloperidol. PPI was calculated as a percentage score: PPI (%) = (1 − [(startle response for pulse with prepulse)/(startle response for pulse alone)]) × 100.

Locomotor activity. Locomotor activity was quantified in plastic activity monitoring boxes (30 × 30 × 30 cm) for 90 min using an infrared photocell beam detection system Acti-Track (Panlab, Barcelona, Spain) after intraperitoneal injection of drug or saline. The number of jumps was scored for 90 min using video recordings by experienced observers blinded to the mouse genotypes.

Measurement of rectal temperature. Rectal temperature was recorded with a Physitemp Bat 12 digital thermometer (Physitemp Instruments, Clifton, NJ) before and after intraperitoneal drug injection.

Immunohistochemistry. After intraperitoneal injection of amphetamine or saline, mice were placed back into their boxes. Two hours after injection, mice were deeply anesthetized with pentobarbital, perfused transcardially with saline, followed by a solution of 4% paraformaldehyde in PBS. Frontal sections (30 μm) containing medial prefrontal cortices (prelimbic cortex and infralimbic cortex) at +1.42 mm from the bregma and dorsomedial striatum at +0.50 mm from the bregma (Franklin and Paxinos, 1997) were cut and processed for immunohistochemistry with anti-c-Fos rabbit polyclonal primary antibody (sc-52; Santa Cruz Biotechnology, Santa Cruz, CA) and biotin-labeled anti-rabbit IgG secondary antibody (Nichirei, Tokyo, Japan). c-Fos-positive nuclei were counted manually by experienced observers blinded to the mouse genotypes.

Statistical analysis. Statistically significant differences were assessed by ANOVA, followed by *post hoc* Mann–Whitney *U* test or Tukey's multiple comparison test, where applicable.

Results

PPI deficits in *Adcyap1*$^{-/-}$ mice

To investigate a possible role of PACAP in sensorimotor gating, PPI was measured in *Adcyap1*$^{-/-}$ mice and their wild-type littermate controls. There was no significant difference in startle amplitudes elicited at 100 or 120 dB between the two groups. Pulse intensities of 65 and 77 dB, selected as background noise and the highest prepulse intensity, respectively, elicited negligible startle when not paired with the startle stimulus in both groups (Fig. 1*A*, bottom). *Adcyap1*$^{-/-}$ mice showed diminished PPI at 71 and 77 dB prepulse intensities compared with wild-type mice (Fig. 1*A*, top). To examine the developmental changes of PPI deficits in *Adcyap1*$^{-/-}$ mice, PPI was tested at postnatal weeks 4, 6, and 8. There was no significant difference in startle amplitudes between the two groups at all studied ages (Fig. 1*B*, bottom). At postnatal week 4, PPI levels were similar between the two groups (Fig. 1*B*, top). Although wild-type mice showed an age-dependent increase in PPI, there was no significant increase in PPI in *Adcyap1*$^{-/-}$ mice from 4 to 8 weeks of age. At postnatal weeks 6 and 8, PPI levels were lower in *Adcyap1*$^{-/-}$ mice when compared with wild-type mice by 44 and 35%, respectively. PPI deficits in *Adcyap1*$^{-/-}$ mice were also seen at 14 weeks of age (data not shown).

Effects of psychostimulants on PPI deficits in *Adcyap1*$^{-/-}$ mice

Dopaminergic systems have been postulated to be involved in the control of PPI (Swerdlow and Geyer, 1998); therefore, the effects of the D_2 dopamine receptor blocking antipsychotic haloperidol on PPI deficits were assessed in *Adcyap1*$^{-/-}$ mice. Haloperidol (0.02 or 0.2 mg/kg) failed to improve PPI deficits in *Adcyap1*$^{-/-}$ mice (Fig. 1*C*, top) and showed no significant effect on startle amplitudes in both groups (Fig. 1*C*, bottom). We assessed possible effects of psychostimulants on the PPI deficits in *Adcyap1*$^{-/-}$ mice and found that amphetamine, at a clinically relevant dose range (2 mg/kg) (Gainetdinov and Caron, 2000), reversed PPI deficits to the control level in wild-type mice (Fig. 1*D*, top). Consistent with a previous report (Ralph et al., 2001b), a higher dose of amphetamine (10 mg/kg) tended to reduce PPI in wild-type mice, although there were no significant changes in PPI levels between the two groups. Startle amplitudes were reduced in *Adcyap1*$^{-/-}$ mice that received 10 mg/kg amphetamine (Fig. 1*D*, bottom).

Effects of haloperidol and psychostimulants on abnormal jumping behavior in *Adcyap1*$^{-/-}$ mice

We examined the effects of haloperidol and psychostimulants on explosive jumping behavior in *Adcyap1*$^{-/-}$ mice. Haloperidol effectively reduced the number of jumps in *Adcyap1*$^{-/-}$ mice (Fig. 2*A*). Interestingly, amphetamine and methylphenidate also reduced the number of jumps in *Adcyap1*$^{-/-}$ mice (Fig. 2*B*,*C*).

Amphetamine-evoked paradoxical antihyperkinetic effect

Adcyap1$^{-/-}$ mice maintained high initial levels of locomotor activity with reduced thigmotaxis or wall-hugging behavior, an index of anxiety, during the open field test (Hashimoto et al., 2001). As expected, wild-type mice responded to amphetamine (2 mg/kg) with increased locomotor activity (Fig. 3*A*); however, in sharp contrast, amphetamine (2 mg/kg) paradoxically attenuated hyperlocomotion in *Adcyap1*$^{-/-}$ mice (Fig. 3*B*). Likewise, 10 mg/kg amphetamine increased locomotor activity in wild-type mice and still produced antihyperkinetic effects in

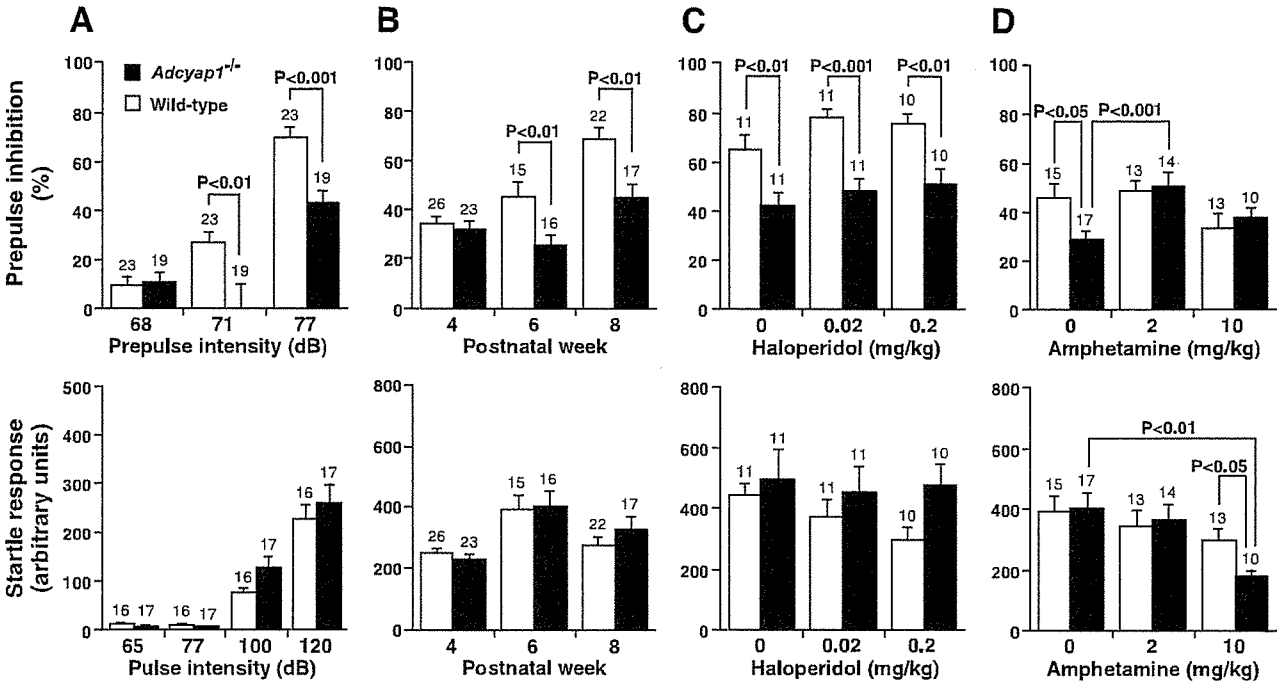

Figure 1. Startle amplitudes without prepulses and PPI of the startle reflex in *Adcyap1*$^{-/-}$ mice. ***A–D***, PPI levels (top) and startle responses (bottom) at different pulse intensities in 8-week-old mice (***A***), at 77 dB prepulse followed by 120 dB startle pulses in mice at postnatal weeks 4, 6, and 8 (***B***), or in 8-week-old mice after pretreatment with haloperidol (***C***) or amphetamine (***D***). The number of wild-type (open bars) and *Adcyap1*$^{-/-}$ (closed bars) mice are indicated above the bars. Data are expressed as means ± SEM.

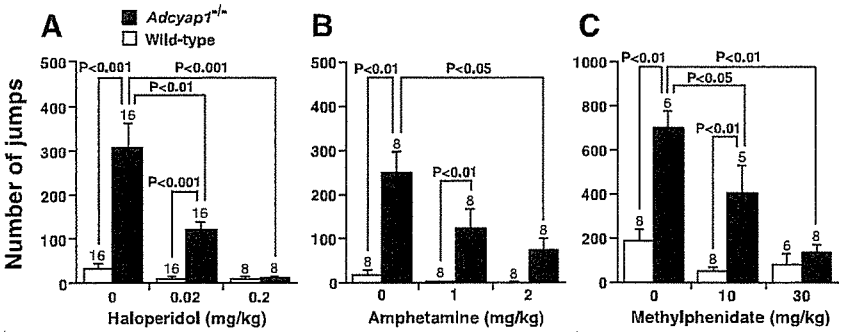

Figure 2. ***A–C***, Number of jumps in *Adcyap1*$^{-/-}$ mice after pretreatment with haloperidol (***A***), amphetamine (***B***), and methylphenidate (***C***). The number of wild-type (open bars) and *Adcyap1*$^{-/-}$ (closed bars) mice are indicated above the bars. Data are expressed as means ± SEM.

Adcyap1$^{-/-}$ mice (data not shown). *Adcyap1*$^{-/-}$ mice entered the center region more often than wild-type mice. Amphetamine (2 mg/kg) inhibited such aberrant behavior and, instead, increased thigmotaxis as seen in wild-type mice (Fig. 3*C*).

Possible involvement of 5-HT$_{1A}$ receptor signaling in the psychobehavioral changes

We explored possible neurochemical alterations relevant to psychobehavioral changes in *Adcyap1*$^{-/-}$ mice and found reduced hypothermic response to 5-HT$_{1A}$ agonists. The 5-HT$_{1A}$ agonists 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) or buspirone significantly decreased rectal temperature in wild-type mice, whereas the response was markedly attenuated in *Adcyap1*$^{-/-}$ mice (Fig. 4). Therefore, we examined the possible involvement of 5-HT$_{1A}$ signaling in the amphetamine-evoked antihyperkinetic effect in *Adcyap1*$^{-/-}$ mice. The selective 5-HT$_{1A}$ receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY-100635) (0.3 mg/kg) blocked the action of amphetamine (Fig. 5*B*). We also found that amphetamine produced paradoxical calming effects in wild-type mice that received 8-OH-DPAT (0.05 mg/kg) (Fig. 5*A*). Neither WAY-100635 nor 8-OH-DPAT alone influenced locomotor activity in wild-type and *Adcyap1*$^{-/-}$ mice (Fig. 5*C*,*D*).

c-Fos-positive neurons were increased in prefrontal cortex in amphetamine-treated *Adcyap1*$^{-/-}$ mice

Presynaptic 5-HT$_{1A}$ autoreceptors have been shown to mediate the hypothermic response, and receptor density correlates with the hypothermic response to 5-HT$_{1A}$ agonists (Aguirre et al., 1998). Therefore, we performed reverse transcription-PCR analysis to quantify 5-HT$_{1A}$ and other 5-HT receptor mRNA levels, as well as microarray analysis. However, to date, we have not confirmed changes in expression of mRNA for 5-HT receptors and other genes that are probably responsible for altered psychomotor functions in *Adcyap1*$^{-/-}$ mice (data not shown).

There is evidence implicating the prefrontal cortex in the pathophysiology of motor dysregulation as well as PPI deficits (Swerdlow and Geyer, 1998; Goldman-Rakic et al., 2000). Therefore, we examined c-Fos expression as a marker for postsynaptic activity to define the pattern of neurons excited by amphetamine (Fig. 6). The number of c-Fos-positive neurons increased in the medial prefrontal cortex in amphetamine-treated *Adcyap1*$^{-/-}$