administration of a single dose of aripiprazole were measured in the same human subjects by PET with [^{11}C]raclopride and L-[β-^{11}C]DOPA, respectively, to determine changes in dopamine synthesis capacity by this antipsychotic in relation to the occupancy of dopamine D_2 receptors. Similar experimental protocol as previous our work with risperidone was used, and results were compared [14].

Results

The occupancies of dopamine D_2 receptors for each dose of aripiprazole as measured by PET with [^{11}C]raclopride ranged from 53% to 79% in the caudate and from 51% to 77% in the putamen (Table 1). Typical images of [^{11}C]raclopride for baseline and drug challenge studies are shown in Fig. 1. Reduced uptake of [^{11}C]raclopride in the striatum was observed after oral administration of aripiprazole.

The plasma concentrations of aripiprazole during [^{11}C]raclopride and L-[β-^{11}C]DOPA PET studies, averaged between the start and end of each scanning, were 12.0±2.1 ng/mL (mean ± SD) and 10.4±1.5 ng/mL for 3 mg of oral administration dose of aripiprazole, 29.0±2.1 ng/mL and 25.6±2.1 ng/mL for 6 mg, and 39.6–40.4 ng/mL and 38.2–39.7 ng/mL for 9 mg, respectively. The plasma concentrations of dehydroaripiprazole during [^{11}C]raclopride and L-[β-^{11}C]DOPA PET studies were 0.4±0.2 ng/mL (mean ± SD) and 0.5±0.2 ng/mL for 3 mg of oral administration dose of aripiprazole, 0.9±0.3 ng/mL and 1.1±0.4 ng/mL for 6 mg, and 1.1–1.6 ng/mL and 1.4–2.4 ng/mL for 9 mg, respectively.

The uptake rate constants k_i of L-[β-^{11}C]DOPA in the caudate and putamen, indicating the dopamine synthesis capacity for baseline and drug challenge studies, are shown in Table 2. No significant differences in k_i were observed between the two studies (paired t-test). Typical images of L-[β-^{11}C]DOPA for baseline study are shown in Fig. 2. Weighted sums of the natural neutral amino acids (NAAs) concentrations in plasma were 1170±142 nmol/mL for the baseline study and 1122±154 nmol/mL (mean ± SD) for the drug challenge study. The values showed no significant differences between the two studies (paired t-test).

Fig. 3 shows the relations between dopamine D_2 receptor occupancy and percentage changes in k_i by the drug challenge. There were no significant correlations. No dose dependency was observed in percentage changes in k_i by the drug challenge. The relations between k_i in the baseline study and percentage change in k_i by the drug challenge for each administration dose of aripiprazole are shown in Fig. 4. Significant negative correlations were observed among all administration dose (caudate: $P=0.005$, putamen: $P=0.027$).

Table 1. Dose of aripiprazole and ranges of occupancy of dopamine D_2 receptors.

Dose of aripiprazole (mg)	Occupancy (%)	
	Caudate	Putamen
3	53–61%	51–58%
	(57±4%)	(55±2%)
6	70–77%	66–72%
	(73±3%)	(69±3%)
9	77–79%	75–77%

(mean ± SD).
doi:10.1371/journal.pone.0046488.t001

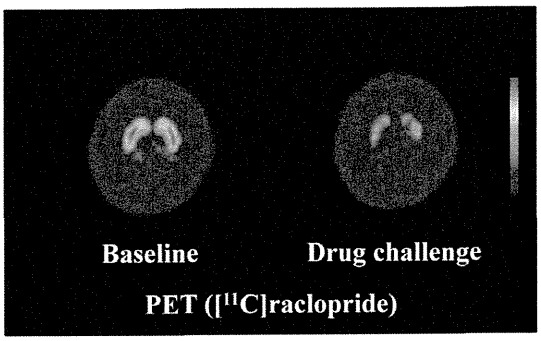

Figure 1. Typical PET summation images of frames between 32–60 min after intravenous injection of [^{11}C]raclopride for baseline and drug challenge (6 mg of aripiprazole) studies. The sections are transaxial at the level of putamen.
doi:10.1371/journal.pone.0046488.g001

Discussion

The present study was performed using similar experimental protocol as previous our work with the antipsychotic risperidone, an antagonist for dopamine D_2 receptors [14]. The effects of antipsychotics on presynaptic dopamine synthesis might be due to pharmacological action on dopaminergic autoreceptors [10] and by neural network regulation. While occupancy of dopamine D_2 receptors corresponding to the dose of aripiprazole was observed [15–18], the current study showed no significant changes in dopamine synthesis capacity by the administration of aripiprazole. There were also no significant correlations between the occupancy of dopamine D_2 receptors and changes in dopamine synthesis capacity by aripiprazole. These findings are similar to our previous observation in healthy human subjects using risperidone [14]. To our knowledge, this is the first study to investigate the effects of aripiprazole on dopamine synthesis capacity in humans using PET. Significant increases and decreases in dopamine synthesis capacities by antagonists and agonists, respectively, of dopamine D_2 receptors were observed in animal studies [7–9], indicating that pharmacological effects on dopaminergic autoreceptors and the neural network might cause changes in presynaptic dopamine synthesis capacity [10]. An increase in dopamine synthesis capacity by aripiprazole was observed in animal studies [11], although partial agonists for dopamine D_2 receptors might reduce presynaptic activity through feedback regulation [5,19]. However, no significant changes in dopamine synthesis capacity by a single administration of an antagonist or a partial agonist were observed

Table 2. Dopamine synthesis capacity k_i of both baseline and drug challenge studies.

	Caudate	Putamen
Baseline	0.0114±0.0022	0.0134±0.0014
Drug challenge	0.0111±0.0016	0.0136±0.0014

Values are mean ± SD.
Unit is min^{-1}.
No significant differences in k_i are observed between the two studies (paired t-test).
doi:10.1371/journal.pone.0046488.t002

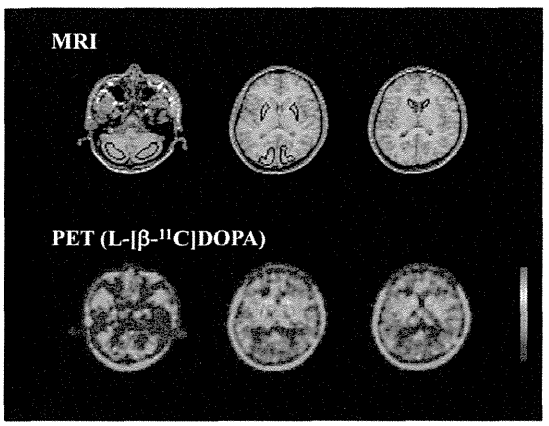

Figure 2. Regions of interest (ROIs) drawn on coregistered MR images. ROIs are defined for the cerebellar cortex, putamen, caudate head, and occipital cortex. Typical PET summation images of frames between 29–89 min after intravenous injection of L-[β-^{11}C]DOPA for baseline study are also shown.
doi:10.1371/journal.pone.0046488.g002

in healthy human subjects. The inconsistency of changes in dopamine synthesis capacity between the present study and previous animal studies might be due to differences in administration dose and way of aripiprazole.

In the present study, significant negative correlations were observed between baseline dopamine synthesis capacity and the percentage changes in dopamine synthesis capacity by aripiprazole. This indicates that aripiprazole administration causes either increase or decrease in dopamine synthesis capacity in subjects with low or high baseline dopamine synthesis capacity, respectively, and the degrees of increase and decrease in dopamine synthesis capacity depend on the baseline dopamine synthesis capacities. These findings are similar to our previous observation in healthy human subjects using the antagonist antipsychotic risperidone [14] and a previous report using the antagonist antipsychotic haloperidol [20]. In addition, the coefficients of variation of dopamine synthesis capacity were smaller in studies with the administration of aripiprazole than in baseline studies, the same as with risperidone [14]. These results indicate that the partial agonist antipsychotic aripiprazole can be assumed to stabilize dopamine synthesis capacity in the same way as antipsychotic drugs with antagonistic property. These also indicate that there are two groups in the healthy subjects with relatively high and low baseline dopamine synthesis capacities, however, we could not find any differences between the two groups. Although stabilizing effect of antipsychotic drugs on dopamine synthesis capacity were observed both in the antagonist and partial agonist antipsychotic drugs, its mechanism would be unknown. An abnormal responsivity in both phasic and tonic dopamine release, which might be related to the modulation of dopaminergic neurotransmission, has been considered in the pathophysiology of schizophrenia [21]. The therapeutic effects of aripiprazole might be related to stabilizing effects on such dopaminergic responsivity. It has also been reported that aripiprazole suppressed phasic dopamine release in methamphetamine-sensitized rat [22]. Although the occupancy of dopamine D_2 receptors ranged from about 50% to 80% in the present study, there might be some kind of threshold of occupancy by aripiprazole for the stabilizing effect of dopamine synthesis capacity to emerge. Further investigations about such threshold should be considered.

The occupancy of dopamine D_2 receptors in this study might be relatively lower than in previous reports regarding drug challenge studies being performed after daily administration of aripiprazole for more than ten days [15,18]. Because only an acute intervention was performed in the present study, the occupancy might actually be relatively lower. Aripiprazole treatment has been shown to be well tolerated with a dose up to 30 mg/day [23], and the optimal

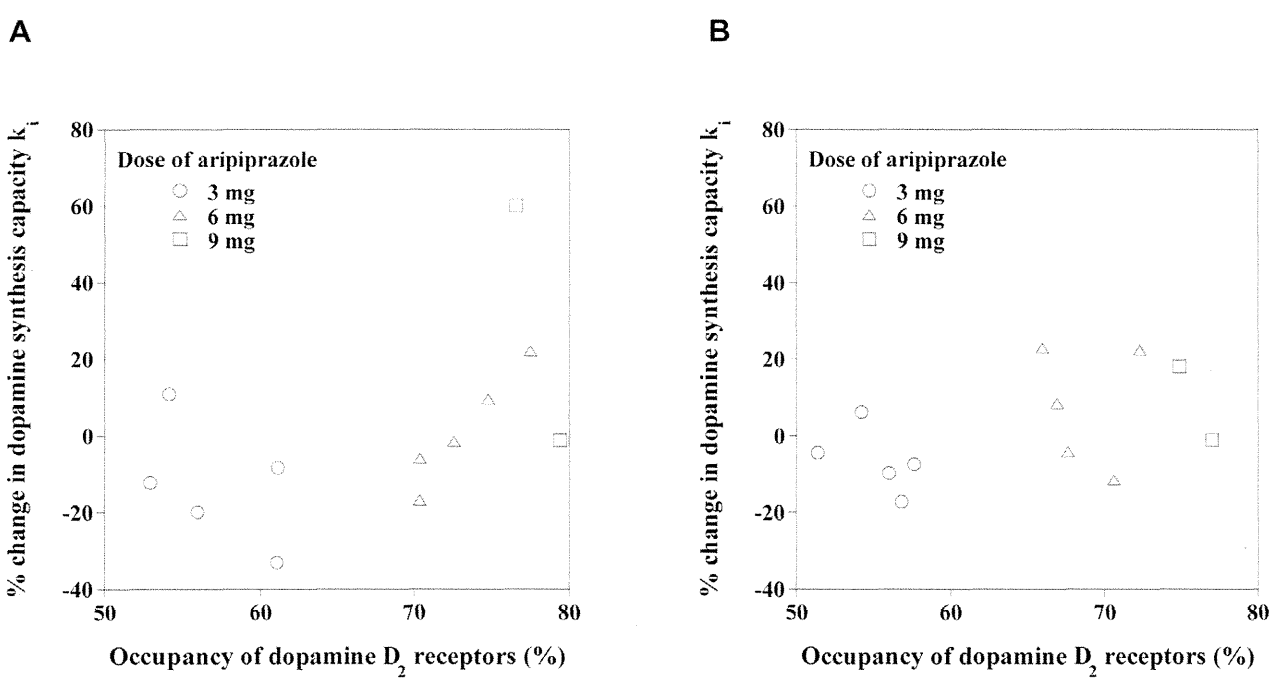

Figure 3. Relations between the occupancy of dopamine D_2 receptors and the percentage change in k_i by drug challenge with aripiprazole in the caudate (A) and putamen (B).
doi:10.1371/journal.pone.0046488.g003

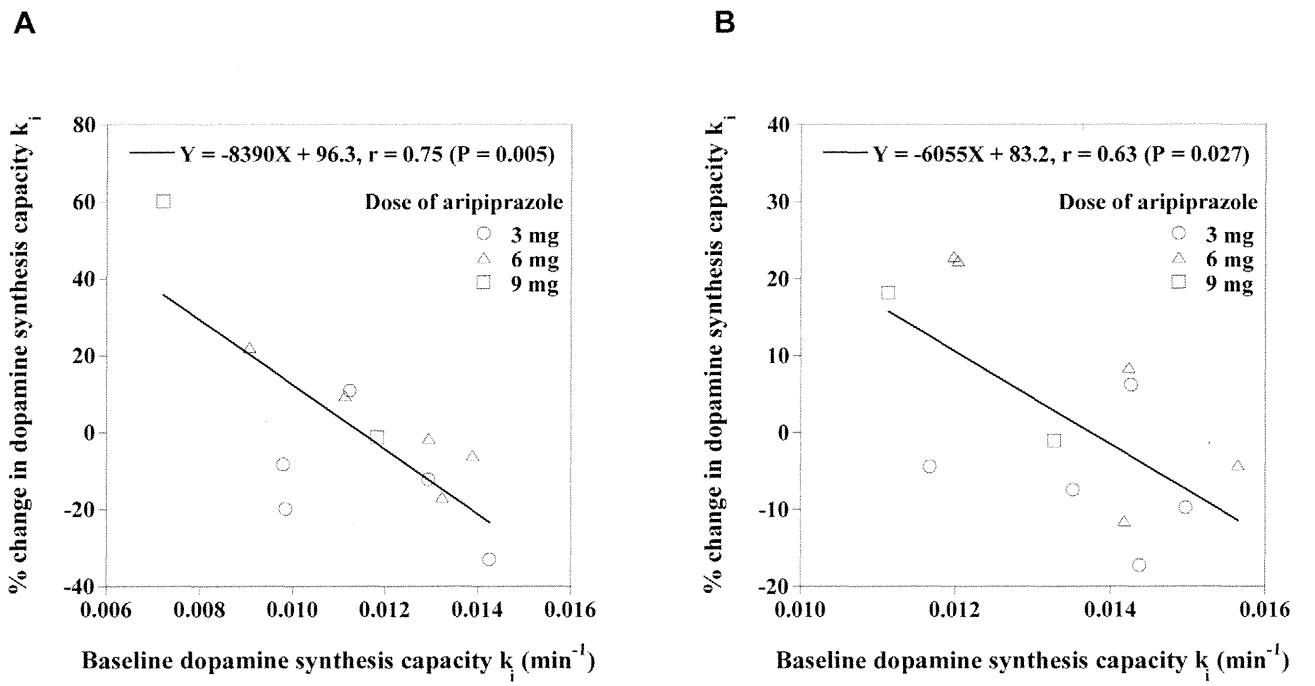

Figure 4. Relations between k_i in the baseline study and the percentage changes in k_i by drug challenge with aripiprazole in the caudate (A) and putamen (B).
doi:10.1371/journal.pone.0046488.g004

dose was reported to be 10 mg/day [4]. The doses of aripiprazole administered in this study (3–9 mg) were smaller than those doses. Since the starting dose of aripiprazole was set at 6–12 mg/day in Japan, from an ethical standpoint, a relatively small dose was used in the present study [24]. However, the chronic effects of relatively large doses of aripiprazole on dopamine synthesis capacity should be investigated in patients with schizophrenia in the future. In addition, the relation between changes in dopamine synthesis capacity and changes in clinical symptoms should also be investigated to confirm meaning of stabilizing effects of aripiprazole on dopamine synthesis capacity.

Aripiprazole also has an antagonistic action on serotonin 5-HT_{2A} receptors and a partial agonistic action on 5-HT_{1A} receptors with relatively high affinity [5]. The 5-HT_{2A} receptor antagonists have been reported to modulate endogenous dopamine release [25], and to reduce extrapyramidal side effects [26–28]. Since aripiprazole has an antagonistic action on 5-HT_{2A} receptors, it may modulate endogenous dopamine release. These reports suggest that changes in dopamine synthesis capacity by the administration of aripiprazole might be due not only to pharmacological effects on dopaminergic autoreceptors, but also on serotonin 5-HT_{2A} receptors similar to our previous report on risperidone [14]. To clarify this, additional studies using the same design and a selective antagonist for dopamine D_2 receptors, such as sulpiride, should be performed [14].

In conclusion, dopamine D_2 receptor bindings and dopamine synthesis capacities at resting condition and after oral administration of a single dose of the partial agonist antipsychotic aripiprazole were measured in the same human subjects. While dose-corresponding occupancy of dopamine D_2 receptors was observed, no significant changes in dopamine synthesis capacity by aripiprazole administration were observed. In addition, no significant correlation between occupancy of dopamine D_2 receptors and changes in dopamine synthesis capacity by aripiprazole was observed. On the other hand, a significant negative correlation was observed between baseline and aripiprazole-induced changes in dopamine synthesis capacities, indicating that the partial agonist antipsychotic aripiprazole can be considered as having a stabilizing effect on dopamine synthesis capacity, the same as antagonist antipsychotic drugs. This suggests that the therapeutic effects of aripiprazole in schizophrenia are possibly related to the stabilizing effects on dopaminergic neurotransmission responsivity.

Methods

Subjects

The study was approved by the Ethics and Radiation Safety Committees of the National Institute of Radiological Sciences, Chiba, Japan. Twelve healthy men (23–34 years of age, 24.1±3.2 years [mean ± SD]) were recruited and written informed consent was obtained. The subjects were free of somatic, neurological and psychiatric disorders according to their medical history and magnetic resonance (MR) imaging of the brain. No histories of current or previous drug abuse were revealed by interviews.

PET procedures

All PET studies were performed with a Siemens ECAT Exact HR+ system, providing 63 sections with an axial field of view of 15.5 cm [29]. Intrinsic spatial resolution was 4.3 mm in-plane and 4.2 mm full-width at half maximum (FWHM) axially. With a Hanning filter (cutoff frequency: 0.4 cycle/pixel), the reconstructed in-plane resolution was 7.5 mm FWHM. Data were acquired in three-dimensional mode. Scatter was corrected by a single scatter simulation technique [30]. A 10-min transmission scan using a ^{68}Ge-^{68}Ga line source was performed for attenuation correction. A head fixation device with thermoplastic attachments for individual fit was used to minimize head movement during the PET measurements.

PET studies were performed under resting condition (baseline study) and oral administration of aripiprazole (drug challenge study) on separate days. The interval between the 2 studies was 7 days in 7 subjects, and 14 days in 5 subjects. In each study, both PET scans with [^{11}C]raclopride and L-[β-^{11}C]DOPA were performed sequentially. Dynamic PET scanning was performed for 60 minutes following an intravenous rapid bolus injection of [^{11}C]raclopride. Then, one hour later, dynamic PET scanning was performed for 89 minutes after intravenous rapid bolus injection of L-[β-^{11}C]DOPA. The frame sequence consisted of twelve 20-sec frames, sixteen 1-min frames, and ten 4-min frames for [^{11}C]raclopride, and seven 1-min frames, five 2-min frames, four 3-min frames, and twelve 5-min frames for L-[β-^{11}C]DOPA. The radioactivity injected was 218–237 MBq and 364–392 MBq in the baseline studies, and 199–233 MBq and 364–415 MBq in the drug challenge studies for [^{11}C]raclopride and L-[β-^{11}C]DOPA, respectively. Specific radioactivity was 162–239 GBq/μmol and 24–124 GBq/μmol in the baseline studies, and 125–253 GBq/μmol and 17–273 GBq/μmol in the drug challenge studies for [^{11}C]raclopride and L-[β-^{11}C]DOPA, respectively. A venous blood sample was taken at the beginning of L-[β-^{11}C]DOPA PET scanning to measure natural neutral amino acid (NAA) concentration in plasma. The NAA concentration was measured by HPLC (L-8500 amino acid analyzer system, Hitachi Corp., Tokyo, Japan). The amino acids are phenylalanine, tryptophan, leucine, methionine, isoleucine, tyrosine, histidine, valine and threonine, which are transported via the same carrier at the blood-brain barrier as L-DOPA [31]. The weighted sum of the NAAs, which was the L-DOPA corresponding concentration of the nine NAAs for the carrier system, was calculated according to our previous work [32].

In the drug challenge studies, aripiprazole at 3–9 mg was orally administered 3.5 hours before the start of PET scanning with [^{11}C]raclopride. The aripiprazole dose was 3 mg in 5 subjects, 6 mg in 5 subjects, and 9 mg in 2 subjects. To estimate the plasma concentration of aripiprazole and its active metabolite, dehydroaripiprazole, venous blood sampling was performed at the start and end of each PET scan [33]. The plasma concentrations of aripiprazole and dehydroaripiprazole, which showed partial agonist effects similar to those of aripiprazole, were determined by the method of validated liquid chromatography coupled to mass spectrometry/mass spectrometry (LC-MS/MS) [34].

All MR imaging studies were performed with a 1.5-Tesla MR scanner (Philips Medical Systems, Best, The Netherlands). Three-dimensional volumetric acquisition of a T1-weighted gradient echo sequence produced a gapless series of thin transverse sections (TE: 9.2 msec; TR: 21 msec; flip angle: 30°; field of view: 256 mm; acquisition matrix: 256×256; slice thickness: 1 mm).

Regions of interest

All MR images were coregistered to the PET images with the statistical parametric mapping (SPM2) system [35]. Regions of interest (ROIs) were drawn manually on coregistered MR images and transferred to the PET images. ROIs were defined for the cerebellar cortex, putamen, caudate head, and occipital cortex (Fig. 2). Each ROI was drawn on three adjacent sections and data were pooled to obtain the average radioactivity concentration for the whole volume of interest. To obtain regional time-activity curves, regional radioactivity was calculated for each frame, corrected for decay, and plotted versus time. ROIs were drawn by in-house software. No software correction for head movement during PET measurements was applied to the dynamic PET images.

Calculation of occupancy of dopamine D_2 receptors

For PET studies with [^{11}C]raclopride, the binding potential (BP_{ND}) was calculated by the reference tissue model method [36,37], with which the time-activity curve in the brain region is described by that in the reference region with no specific binding, assuming that both regions have the same level of nondisplaceable radioligand binding:

$$C_i(t) = R_I \cdot C_r(t) + \left(k_2 - \frac{R_I \cdot k_2}{1 + BP_{ND}}\right) \cdot C_r(t) \otimes \exp\left(-\frac{k_2 \cdot t}{1 + BP_{ND}}\right),$$

where C_i is the radioactivity concentration in a brain region; C_r is the radioactivity concentration in the reference region; R_I is the ratio of K_1/K_1' (K_1, influx rate constant for the brain region; K_1', influx rate constant for the reference region); k_2 is the efflux rate constant for the brain region; ⊗ denotes the convolution integral. In this analysis, three parameters (BP_{ND}, R_I, and k_2) were estimated by non-linear least-squares curve fitting. The cerebellum was used as reference region. Dopamine D_2 receptor occupancy by aripiprazole was calculated as follows:

$$Occupancy(\%) = 100 \cdot \frac{BP_{ND(baseline)} - BP_{ND(drug)}}{BP_{ND(baseline)}},$$

where $BP_{ND(baseline)}$ is the BP_{ND} value in the baseline study, and $BP_{ND(drug)}$ is the BP_{ND} value in the drug challenge study.

Calculation of dopamine synthesis capacity

The uptake rate constant for L-[β-^{11}C]DOPA, indicating the dopamine synthesis capacity, was estimated by graphical analysis [38–40], which allows for calculation of the uptake rate constant k_i using time-activity data in a reference brain region with no irreversible binding. The k_i values can be estimated by simple linear least-squares fitting as follows:

$$\frac{C_i(t)}{C_i'(t)} = k_i \cdot \frac{\int_0^t C_i'(\tau)d\tau}{C_i'(t)} + F \qquad t > t*,$$

where C_i and C_i' are the total radioactivity concentrations in a brain region with and without irreversible binding, respectively, and t^* is the equilibrium time of the compartment for unchanged radiotracer in brain tissue. Plotting $C_i(t)/C_i'(t)$ versus $\int_0^t C_i'(\tau)d\tau/C_i'(t)$, after time t^*, yields a straight line with the slope k_i and intercept F. In the present study, the occipital cortex was used as reference region with no irreversible binding, because this region is known to have the lowest dopamine concentration [41] and least AADC activity [42]. The equilibrium time t^* was set to be 29 min, and data plots of 29 to 89 min were used for linear least-squares fitting [32,43]. The percentage change in k_i by oral administration of aripiprazole was calculated as follows:

$$\%\ change = 100 \cdot \frac{k_{i(drug)} - k_{i(baseline)}}{k_{i(baseline)}},$$

where $k_{i(baseline)}$ is the k_i value in the baseline study, and $k_{i(drug)}$ is the k_i value in the drug challenge study.

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Author Contributions

Conceived and designed the experiments: HI. Performed the experiments: HI H. Takano RA FK KT TN MS. Analyzed the data: HI H. Takano RA H. Takahashi FK KT TN MS. Contributed reagents/materials/analysis tools: HI H. Takano RA FK KT TN MS. Wrote the paper: HI H. Takano RA H. Takahashi TS.

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Association between Striatal Subregions and Extrastriatal Regions in Dopamine D_1 Receptor Expression: A Positron Emission Tomography Study

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Abstract

The mesencephalic dopamine (DA) system is the main DA system related to affective and cognitive functions. The system consists of two different cell groups, A9 and A10, which originate from different regions of the midbrain. The striatum is the main input from the midbrain, and is functionally organized into associative, sensorimotor and limbic subdivisions. At present, there have been few studies investigating the associations of DA functions between striatal subdivisions and extrastriatal regions. The aim of this study was to investigate the relationship of DA D_1 receptor (D_1R) expression between striatal subdivisions and extrastriatal regions in humans using positron emission tomography (PET) with voxel-by-voxel whole brain analysis. The PET study was performed on 30 healthy subjects using [^{11}C]SCH23390 to measure D_1R expression. Parametric images of binding potentials (BP_{ND}) were created using the simplified reference tissue model. Regions of interest were defined for striatal subdivisions. Multiple regression analysis was undertaken to determine extrastriatal regions that were associated with each striatal subdivision in BP_{ND} using statistical parametric mapping 5. The BP_{ND} values of associative, sensorimotor and limbic subdivisions were similarly correlated with those of multiple brain regions. Regarding the interrelationships among striatal subdivisions, mutual correlations were found among associative, sensorimotor and limbic subdivisions in BP_{ND} as well. The relationships in BP_{ND} between striatal subdivisions and extra-striatal regions suggest that differential striatal subdivisions and extrastriatal regions have a similar biological basis of D_1R expression. Different DA projections from the midbrain did not explain the associations between striatal subdivisions and extrastriatal regions in D_1R expression, and the DA-related neural networks among the midbrain, striatum and the other regions would contribute to a similar D_1R expression pattern throughout the whole brain.

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Introduction

The mesencephalic dopamine (DA) system is the main DA system, and it is related to affective and cognitive functions such as reward processing. The system is roughly divided into different groups, A9 and A10, whose cells are located in different regions of the midbrain, the substantia nigra (SN) and the ventral tegmental area (VTA), respectively. These different projections have been reported in rats, monkeys and humans [1,2,3]. The striatum provides the main input from the midbrain. Histologically, this region is not uniform, and it is functionally divided into striatal subdivisions termed associative (AST), sensorimotor (SMST) and limbic (LST), which process information related to cognitive, sensorimotor, and emotional functions, respectively [4]. The concept is based on neural networks termed "Cortico-striatal-thalamo-cortical loops" [5]. In brief, functionally different networks between each striatal subdivision and extrastriatal regions would exist through dopaminergic, glutaminergic and gamma-butylic amino acid (GABA) neurotransmissions, and these neurotransmissions interact with each other [6]. Regarding DA projections, A9 would project to the dorsal striatum (AST and SMST) and A10 to LST. A10 would have direct projections to cortical regions as well.

Ample literature describes the differential DA pathways and the distribution of DA receptors by *in vitro* methods, including distinct DA projections from the midbrain to the dorsal and ventral striatum [7], region-by-region differences of DA receptor distribution in the cortex [8], and alterations of DA projections in several neuropsychiatric illnesses [9,10]. Regarding neuroimaging studies, several reports have suggested the relationships between DA functions and cognitive functions [11,12], and the association of DA functions with the pathophysiology of neuropsychiatric illnesses [10,13]. Thus, it is worthwhile investigating DA functions in their relationships among different regions of the human brain by *in vivo* methods, especially between the DA receptor-rich regions (striatum) and other regions, which could provide new insights for studies of DA-

related cognitive functions and pathophysiologies of neuropsychiatric disorders.

However, at present, there have been few neuroimaging studies that directly demonstrated the relationship between striatal subdivisions and extrastriatal (ie., cortical) regions, which also have DA projections from the midbrain in DA receptor expressions. Clarification of this issue would lead to a better understanding of DA functions in region-by-region relationships, considering the manner of DA projections from the midbrain and the distinction of their targets, that is, the most DA-rich region, the striatum and cortical regions. In this sense, one possibility might be that DA receptor expressions are regulated differentially according to their origin of DA projection.

However, very recently, one positron emission tomography (PET) study has suggested that there was no relationship between cortical DA D_2 receptor (D_2R) densities and those of striatal regions [14]. Regarding another dopamine receptor subtype, DA D_1 receptor (D_1R), Rieckmann et al. reported that subdivisional striatal D_1R densities are similarly associated with those of multiple cortical regions, concluding that D_1R expressions in striatal and extrastriatal regions are not regulated independently, despite DA projections from different midbrain areas. In their study [15], interregional association of D_1R was assessed by a conventional method in terms of the analysis of PET images, that is, regions of interests (ROIs) were traced manually on each individual subject's image without spatial normalization. This method is potentially advantageous in preserving the information of raw images, but the results may partially depend on the rater's procedure. Thus, the conventional manual tracing method and another method, voxel-by-voxel analysis, could be expected to complement each other in respect to confirming their reliability and validity.

The aim of the present study was to investigate the relationship between striatal subdivisions and extrastriatal regions in DA D_1 receptor (D_1R) expression using PET in healthy humans by voxel-by-voxel analysis, a potentially more objective method than the manual ROI-tracing method used by Rieckmann et al. [15]. We hypothesized that D_1R availability of the striatum would be associated with the availability of extrastriatal regions regardless of its differential subdivisions, i.e., the D_1R expressions of AST, SMST and LST would be similarly correlated with the expressions of extrastriatal regions.

Methods

Ethics Statement

In accordance with the Helsinki Declaration of Human Rights (2000), written informed consent was obtained from all volunteers after detailed explanation of the study. This study protocol was approved by the Ethics and Radiation Safety Committees of the National Institute of Radiological Sciences, Chiba, Japan.

Subjects

A total of 30 healthy men (age = 25.4±5.9 [mean ± SD]) were recruited, and they gave their written informed consent for participation in this study. The subjects were free of somatic, neurological or psychiatric disorders on the basis of their medical history and magnetic resonance imaging (MRI) of the brain. They had no history of current or previous drug abuse.

PET Procedures

The PET system ECAT EXACT HR+(CTI-Siemens, Knoxville, TN) was used for all PET studies. The system provides 63 planes with a 15.5 cm axial field of view. The intrinsic spatial resolution was 4.3 mm in-plane and 4.2 mm full-width at half maximum (FWHM) axially. With a Hanning filter (cut-off frequency: 0.4 cycle/pixel), the reconstruct in-plane resolution was 7.5 mm FWHM. Data were acquired in three-dimensional mode. Scatter was corrected [16]. A head fixation device with thermoplastic attachments for individual fit minimized head movement during PET measurements. A 10-min transmission scan using a ^{68}Ge- ^{68}Ga line source was performed for correction of attenuation. After intravenous rapid bolus injection of [^{11}C]SCH23390, data were acquired for 60 min in a consecutive series of time frames. The frame sequences consisted of thirty 2-min frames. Injected radioactivity was 197–235 MBq and specific radioactivity was 23–81 GBq/μmol at the time of injection.

MRI Procedures

All MRI scanning was performed with a 1.5-T MR scanner (Philips Medical Systems, Best, The Netherlands). Three-dimensional volumetric acquisition of a T1-weighted gradient echo sequence produced a gapless series of thin transverse sections (TE: 9.2 ms; TR: 21 ms; flip angle: 30°; field of view: 256 mm; acquisition matrix: 256×256; slice thickness: 1 mm).

Calculation of Parametric Images

We used PMOD 3.1 software (PMOD Technologies Ltd., Zurich, Switzerland) for all the steps of the image processing and analysis. All MR images were coregistered to the PET images. MR images were transformed into standard brain size and shape by linear and non-linear parameters (anatomic standardization). The brain templates for anatomic standardization were Montreal Neurological Institute (MNI)/International Consortium for Brain Mapping (ICBM) 152 T1 templates as supplied with the PMOD software. All PET images were also transformed into standard brain size and shape by using the same parameters as for the MR images. Thus, brain images of all 30 subjects had the same anatomic format.

Binding potentials (BP_{ND}) were calculated by the reference tissue model method on a voxel-by-voxel basis [17,18]. BP_{ND} refers to the ratio of specifically bound radioligand to that of nondisplaceable radioligand in tissue at equilibrium. BP_{ND} is the typical measurement from reference tissue methods, as it compares the concentration of radioligand in receptor-rich to receptor-free regions [19]. In this study, parametric images, in which each voxel has its own BP_{ND} value, were generated using the cerebellum, a receptor-free region, as reference tissue.

Data Analysis

ROIs were drawn on a standardized and averaged MR image of all the subjects, and this ROI object map was applied to the parametric images of each of the 30 subjects, that is, only one ROI object map was applied to the parametric images of each of the 30 subjects completely in the same manner. Thus, this method is a more objective way to measure BP_{ND} values than that with different ROIs for each subject. Boundaries for ROIs of striatal functional subdivisions were defined for each striatal subregion [20,21]. The definition of the "functional subdivisions" was as follows: AST consisted of the precommissural dorsal caudate, precommissural dorsal putamen, and postcommissural caudate, and the BP_{ND} values of AST were calculated as the spatially weighted average of these three subregions; SMST and LST corresponded to the postcommissural putamen and ventral striatum, respectively (Figure 1).

Regarding the statistics, multiple regression analysis was performed by statistical parametric mapping (SPM5, Wellcome Trust Centre for Neuroimaging, London, UK) on a voxel-by-voxel

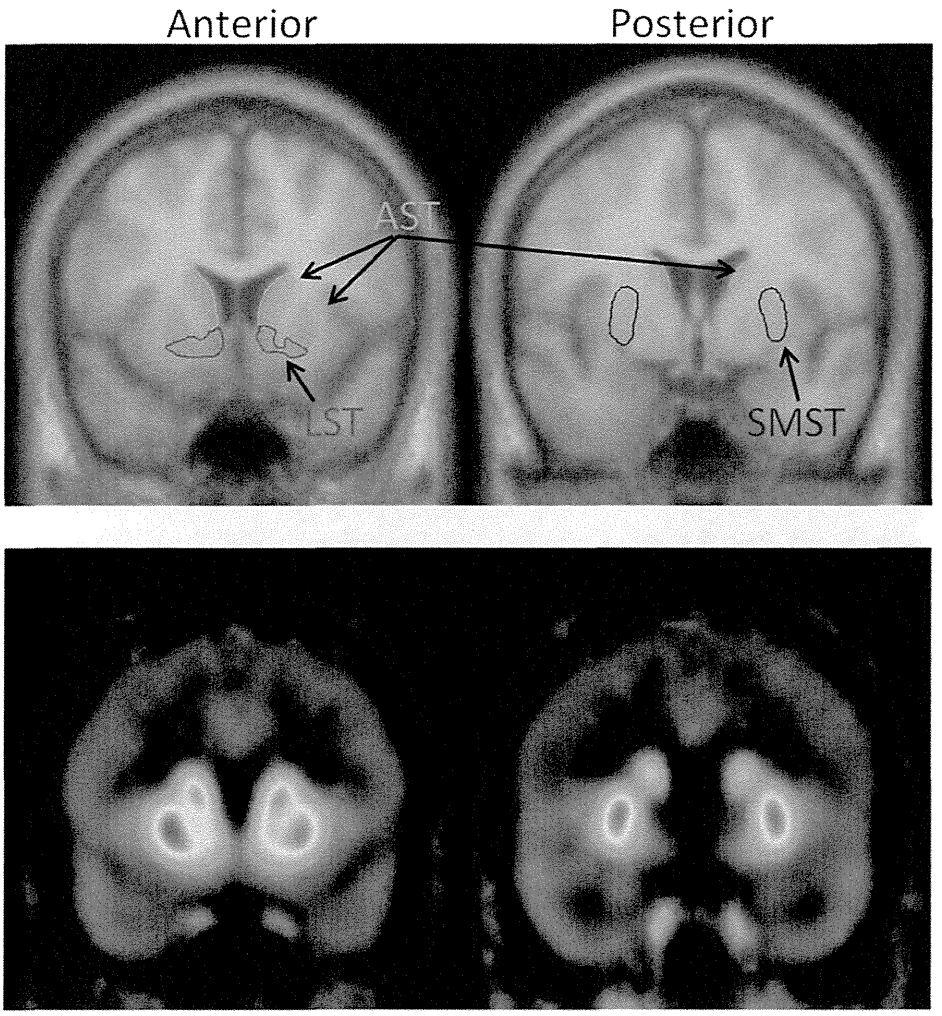

Figure 1. Definition of striatal functional subdivisions. Upper panel: MR images and regions of interest. Lower panel: parametric images corresponding to MR images in the upper panel.
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basis after the BP_{ND} values of the striatal subdivisions were obtained. The values of each striatal subdivision were used as covariates of interest in the design matrix to determine the regions correlating with each striatal subdivision in terms of their D_1R expression. Statistical thresholds were as follows: false recovery rate (FDR) $p<0.05$, extent threshold = 100 voxels. The results of the correlation were visualized in statistical parametric maps.

To confirm the result of voxel-by-voxel analysis with SPM5, Pearson's correlation coefficient was also calculated using the actual BP_{ND} values in extrastriatal regions with SPSS version 18.0. The ROIs of extrastriatal regions included thalamic, cingulate, prefrontal, temporal and occipital regions, and the boundaries for the ROIs were based on previous reports [21,22].

Results

The BP_{ND} values of AST, SMST, and LST were 1.61 ± 0.26, 1.70 ± 0.24, and 1.36 ± 0.19, respectively. The values were quite similar to our previous data for measuring D_1R in the striatum [22].

By voxel-by-voxel analysis, the values of each striatal subdivision (i.e., AST, SMST and LST) were positively correlated with those of multiple brain regions, i.e., frontal, temporal, parietal and occipital regions in a similar manner (Figure 2). Regarding the interrelationships among striatal subdivisions, mutual positive correlation was found among AST, SMST and LST in D_1R BP_{ND} (Figure 2).

In addition, the interregional positive correlations in BP_{ND} were revealed to be significant by the ROI analysis, that is, by the analysis using SPSS software with the actual BP_{ND} values of each ROI (Figure 3).

Discussion

The critical role of the DA system in cognitive functions has been suggested repeatedly, and abnormalities of the system have also been implicated in the pathophysiology of several neuropsychiatric disorders such as schizophrenia [13,23] and Parkinson's disease [10]. The main focus of those findings was restricted exclusively to D_1R functions or abnormalities in terms of their expression levels, distribution, and localization in several brain regions. In this sense, there is little evidence that refers to a direct association of a DA-rich region (the striatum) and extrastriatal regions, where DA receptors are present. It has been suggested that cognitive functions such as executive function would be associated with the manner of interregional relationship in D_1R

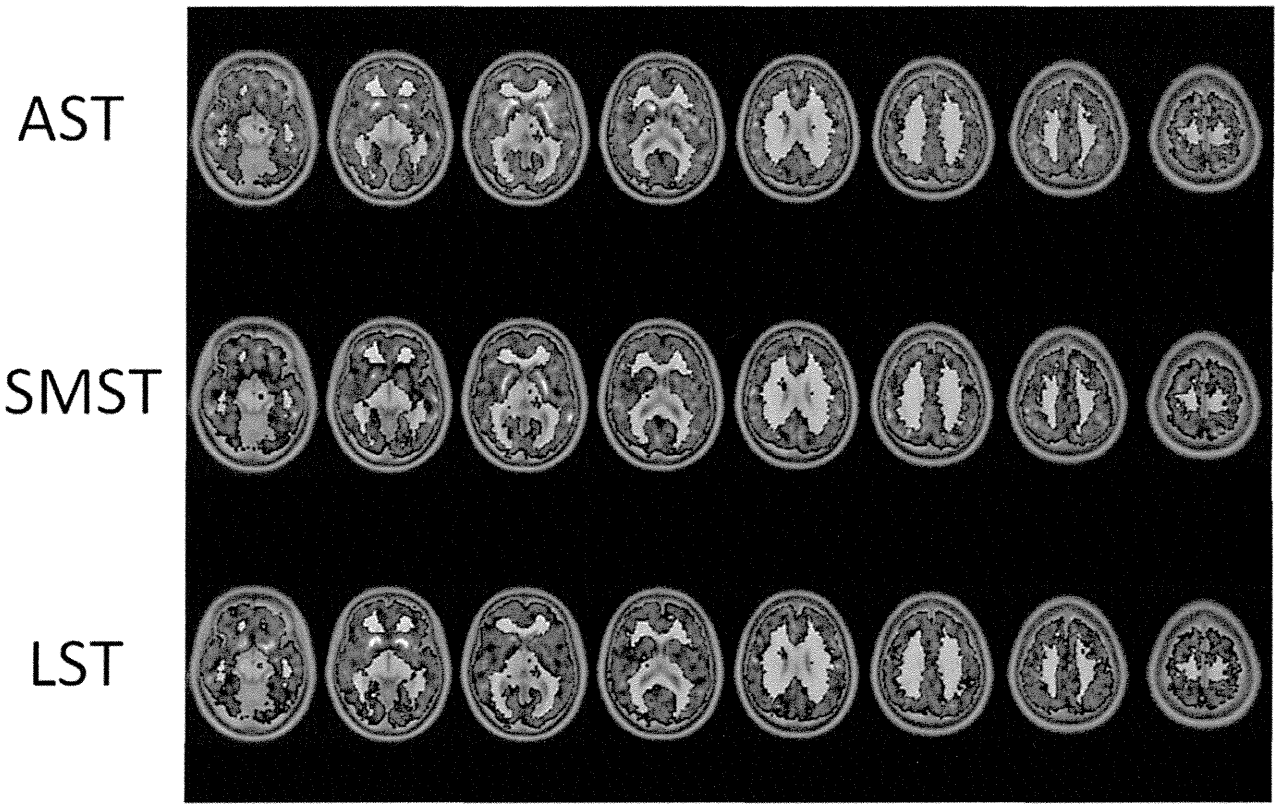

Figure 2. Correlation map of striatal functional subdivisions and extrastriatal regions, in addition to that of within striatal subdivisions. Correlations in brighter color (yellow) represent higher ones in terms of magnitude than those in red.
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expression [15], and therefore it would be worthwhile investigating the interregional patterns of bindings for studies of neuropsychiatric disorders in which cognitive dysfunction based on DA system dysregulation is considered to exist, such as schizophrenia and Parkinson's disease.

We could replicate the findings of a previous study by Rieckmann et al. [15] that demonstrated the association of striatal subdivisions and cortices by a conventional manual tracing method. The major findings of the present study were as follows: (a) BP_{ND} values of all striatal subdivisions (i.e., AST, SMST and LST) were significantly correlated with those of multiple brain regions on a voxel-by-voxel basis: (b) regarding the interrelationships among striatal subdivisions, they were also mutually correlated in their BP_{ND} values.

These results suggest that D_1R expressions in striatal subdivisions and extrastriatal regions are regulated uniformly. This could be explained by the complex connections of DA pathways throughout the whole brain. DA innervations from VTA and SN differentially project to striatal subdivisions as well as the cortical regions through A9 and A10, whereas glutaminergic innervations from cortical regions project to both VTA and SN via the striatum. Regarding the connection of the midbrain with the striatum, the midbrain has reciprocal projections both to (DA) and from (GABA) the striatum, with overlapping. Thus, DA pathways are connected via these pathways [4,24], and this would lead to similarity of the regulation of D_1R expressions among multiple brain areas, although differential DA projection from the midbrain (i.e., A9 and A10) is a part of the DA-related neural network.

Furthermore, striatal outputs to the cortex, which are altered by D_1R stimulation/blockade, would affect immediate-early gene expressions such as c-fos expression (as functional markers) in cortical regions [25]. If the D_1R function in each striatal

	AST	SMST	LST	ACC	MPFC	DLPFC	OFC	Temporal	Occipital	Parietal	Thalamus
AST		.643**	.587**	.436**	.361**	.247**	.507**	.637**	.188	.305**	.372**
SMST			.363**	.583**	.333**	.233**	.622**	.706**	.086	.338**	.410**
LST				.215**	.185*	.136*	.327**	.348**	.079	.162*	.228**

Figure 3. Correlations between striatum and extrastriatal regions and intercorrelations among striatal subdivisions in dopamine D_1 receptor BP_{ND}. **P<0.01, *P<0.05. Correlations in red: intercorrelations among striatal subdivisions. Correlations in blue: correlations between striatal subdivisions and extrastriatal regions. R^2 values are presented for the correlations.
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subdivision uniformly affects the expressions in cortical regions, each striatal subdivision and the cortical regions are mutually correlated in a similar manner. In this study, interrelationships among striatal subdivisions were found in their BP_{ND} values, thus providing a convincing explanation for the uniform relationships between different striatal subdivisions and cortical regions in their D_1R expression.

Finally, D_1R expressions throughout the whole brain might be generally (genetically) associated with each other, i.e., the larger the expressions in the striatum, the larger in the other regions as well. However, further investigations including postmortem and animal studies would be needed to clarify the genetic influence on the D_1R expression throughout the whole brain.

Several limitations need to be pointed out in the current study. First, the age of the participants was restricted to a relatively younger generation. Second, the subjects were all males. Further study with both male and female participants of a wider age range will be needed to give the findings a more generalized significance. Third, the reference tissue model has a potential limitation in terms of its theory, the assumption of the same non-specific binding throughout the whole brain, which might lead to a systemic bias in respect to between-region correlations of the bindings. To our knowledge, there has been no study that suggested a higher variation of receptor density corresponding to BP_{ND} in different regions compared with that of non-specific binding in the human population. However, the non-specific binding in tissue has been reported to be generally constant across species including humans [26]. Thus, the correlation of the current study would reflect the relationship in receptor density in itself rather than inter-individual variations of non-specific binding. Further theoretical and methodological improvements would be needed to assess interregional correlations of binding potentials more accurately, considering the influence of inter-individual variations in non-specific binding. Fourth, it could be argued that the interregional associations in the bindings in the current study may reflect the association in the serotonergic system in addition to the DA system because of the affinity of SCH23390 to 5-HT_{2A} receptors in cortical regions. However, this confounding effect of cortical 5-HT_{2A} binding would not be so critical in terms of the analysis of striatal and extrastriatal correlations because striatal binding reflects D_1R density only, whereas the bindings of cortical regions are significantly confounded by 5-HT_{2A} receptors [15]. In the present study, the BP_{ND} values of striatal and extrastriatal regions were highly correlated, and thus the correlations are not always considered to represent different receptor associations. Finally, in general, completely accurate image processing (namely, coregistration and normalization) is difficult in voxel-based analyses. In this study, the accuracy of image processing and ROI adjustment on parametric images was confirmed by visual inspection for each subject. However, at present, there is no absolute procedure in this regard because of the variation of the individual's brain in respect to its shape, size and sulcal anomaly. Further improvements in image processing technique would be necessary to raise the reliability of voxel-wise analysis.

Conclusions

In conclusion, differential striatal functional subdivisions could be associated with cortical regions in terms of D_1R expression in a similar manner. Although DA cell projections from VTA and SN innervate the striatum and extrastriatal regions via different DA pathways, DA-related neural networks throughout the whole brain including both striato-midbrain and cortical-striato connections would contribute to the association of the striatal subdivisions and extrastriatal regions in D_1R expression. Further study will be needed to clarify the mechanisms of D_1R expression regarding the interactions between DA and the other neurotransmitter systems such as glutamate, serotonin and GABA, and the mechanisms at molecular and genetic levels in the respective brain regions.

Author Contributions

Conceived and designed the experiments: HI TS HF. Performed the experiments: HI HT. Analyzed the data: HF YK FK. Wrote the paper: HF.

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Cross-cultural differences in the processing of non-verbal affective vocalizations by Japanese and Canadian listeners

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The Montreal Affective Voices (MAVs) consist of a database of non-verbal affect bursts portrayed by Canadian actors, and high recognitions accuracies were observed in Canadian listeners. Whether listeners from other cultures would be as accurate is unclear. We tested for cross-cultural differences in perception of the MAVs: Japanese listeners were asked to rate the MAVs on several affective dimensions and ratings were compared to those obtained by Canadian listeners. Significant Group × Emotion interactions were observed for ratings of Intensity, Valence, and Arousal. Whereas Intensity and Valence ratings did not differ across cultural groups for sad and happy vocalizations, they were significantly less intense and less negative in Japanese listeners for angry, disgusted, and fearful vocalizations. Similarly, pleased vocalizations were rated as less intense and less positive by Japanese listeners. These results demonstrate important cross-cultural differences in affective perception not just of non-verbal vocalizations expressing positive affect (Sauter et al., 2010), but also of vocalizations expressing basic negative emotions.

Keywords: montreal affective voices, emotion, voice, cross-cultural differences, social cognition

INTRODUCTION

Vocal affective processing has an important role in ensuring smooth communication during human social interaction as well as facial affective processing. Facial expressions are generally recognized as the universal language of emotion (Ekman and Friesen, 1971; Ekman et al., 1987; Ekman, 1994; Izard, 1994; Jack et al., 2012): however, several studies have demonstrated cross-cultural differences in facial expression between Western and Eastern groups (Ekman and Friesen, 1971; Ekman et al., 1987; Matsumoto and Ekman, 1989; Izard, 1994; Yrizarry et al., 1998; Elfenbein and Ambady, 2002; Jack et al., 2009, 2012). Whether such cross-cultural differences also exist in the recognition of emotional vocalizations is not clear.

Most previous cross-cultural studies of auditory perception have investigated the processing of emotional Valence using word stimuli (Scherer and Wallbott, 1994; Kitayama and Ishii, 2002; Ishii et al., 2003; Min and Schirmer, 2011). One important study demonstrated cross-cultural differences in the rating of Intensity when subjects recognized meaning of the words with major emotions such as joy, fear, anger, sadness, and disgust (Scherer and Wallbott, 1994). Another previous study examined cross-cultural differences in the perception of emotional words (Kitayama and Ishii, 2002). This study indicated that native English speakers spontaneously pay more attention to verbal content than to vocal tone when they recognize emotional words, whereas native Japanese speakers spontaneously attend more to vocal tone than to verbal content. The other study has shown that Japanese are more sensitive to vocal tone compared to Dutch participants in the experiment of the multisensory perception of emotion (Tanaka et al., 2010). Further, one other study demonstrated cross-cultural differences in semantic processing of emotional words (Min and Schirmer, 2011), but found no difference in the processing of emotional prosody between native and non-native listeners. These studies suggest cross-cultural differences in auditory recognition of emotional words.

Studies of affective perception in speech prosody are made complex, in particular, by the potential interactions between the affective and the linguistic contents of speech (Scherer et al., 1984; Murray and Arnott, 1993; Banse and Scherer, 1996; Juslin and Laukka, 2003). To avoid this interaction, some studies have controlled the processing of semantic content using pseudo-words (Murray and Arnott, 1993; Schirmer et al., 2005) or pseudo-sentences (Ekman and Friesen, 1971; Pannekamp et al., 2005; Schirmer et al., 2005). The other previous study has employed a set of low-pass filtered vocal stimuli to select the final set of emotional utterances (Ishii et al., 2003), i.e., non-verbal vocalizations often accompanying strong emotional states such as laughs or screams of fear. Non-verbal affective vocalizations are ideally suited to investigations of cross-cultural differences in the perception of affective information in the voice since they eliminate the need to account for language differences between groups.

A recent study compared the perception of such non-verbal affective vocalizations by listeners from two highly different cultures: Westerners vs. inhabitants of remote Namibian villages. Non-verbal vocalizations expressing negative emotions could be recognized by the other culture much better than those expressing

positive emotions, which lead the authors to propose that a number of primarily negative emotions have vocalizations that can be recognized across cultures while most positive emotions are communicated with culture-specific signals (Sauter et al., 2010). However this difference could be specific to English vs. Namibian groups, reflecting for instance different amounts of exposure to vocalizations through media or social interactions, and might not generalize to other cultures.

In the present experiment we tested for cross-cultural differences in perception of affective vocalizations between two cultures much more comparable in socio-economic status and exposure to vocalizations: Canadian vs. Japanese participants. Stimuli consisted of the Montreal Affective Voices (MAVs; Belin et al., 2008), a set of 90 non-verbal affect bursts produced by 10 actors and corresponding to emotions of Anger, Disgust, Fear, Pain, Sadness, Surprise, Happiness, and Pleasure. The MAVs have been validated in a sample of Canadian listeners and showed high inter-reliability in judgments of emotional Intensity, Valence, and Arousal as well as hit rates in emotional recognition (Belin et al., 2008). Here, we collected affective ratings using similar procedures in Japanese listeners and compared those ratings to those obtained in the Canadian listeners. Before the experiment, we predicted that ratings of negative emotion are culturally universal although cross-cultural differences would exist in ratings of positive emotion.

MATERIALS AND METHODS

SUBJECTS

Thirty Japanese subjects (male 15, female 15) participated in this study. The average age was 22.3 ± 1.4 years. The educational years of Japanese subjects were 14.1 ± 0.3. The data of Japanese subjects were compared with 29 Canadian subjects (male 14, female 15); average age: 23.3 ± 1.5 years (Belin et al., 2008). Both Japanese and Canadian participants consisted exclusively of undergraduate students.

After a thorough explanation of the study, written informed consent was obtained from all subjects, and the study was approved by the Ethics Committee of Nippon Medical School.

VOICE MATERIALS

The MAVs: 10 French-Canadian actors expressed specific emotional vocalizations and non-emotional vocalizations (neutral sounds) using "ah" sounds. The eight emotional vocalizations were angry, disgusted, fearful, painful, sad, surprised, happy, and pleased. The simple "ah" sounds were used to control the influence of lexical-semantic processing. Since each of the eight emotional vocalizations and the neutral vocalization were spoken by 10 actors, the total number of MAVs sounds was 90. The MAVs are available at: http://vnl.psy.gla.ac.uk/

EVALUATION SCALE

Each emotional vocalization was evaluated using three criteria: perceived emotional Intensity in each of the eight Emotions, perceived Valence, and perceived Arousal. Each scale had a range from 0 to 100.

The Valence scale represented the extent of positive or negative emotion expressed by the vocalization: 0 was extremely negative, and 100 was extremely positive. The Arousal scale represented the extent of excitement expressed by the vocalization: 0 was extremely calm, and 100 was extremely excited. The Intensity scale represented the Intensity of a given emotion expressed by the vocalization: 0 was not at all intense, and 100 was extremely intense. The Intensity scale was used for eight emotions: Anger, Disgust, Fear, Pain, Sadness, Surprise, Happiness, and Pleasure.

METHODS OF EVALUATION BY PARTICIPANTS

The MAVs vocalizations were played on a computer in a pseudo-random order. The subjects listened with headphones at a comfortable hearing level, and they evaluated each emotional vocalization for perceived Intensity, Valence, and Arousal using a visual analog scale in English on a computer (10 ratings per vocalization: 8 Intensity ratings, 1 Valence rating, 1 Arousal rating). Simultaneously, participants were given a printed Japanese translation of the scale labels, and by referring to this Japanese sheet, the test was performed using exactly the same procedure as in the Canadian study (Belin et al., 2008). All Japanese participants performed the experiment using a translation sheet with emotional words translated from English to Japanese. Based on previous studies (Scherer and Wallbott, 1994), the Japanese translation of English emotional labels was independently assessed by three clinical psychologists. Through their discussion, the appropriate emotional labels were determined.

STATISTICAL ANALYSIS

Statistical calculations were made using SPSS (Statistical Package for Social Science) Version 19.0. The Japanese data and the Canadian published data, with permission to verify, were statistically analyzed. A previous study demonstrated gender effects in Canadian participants using the MAV (Belin et al., 2008). Using the same methods to reveal the gender effects, an ANOVA with Emotion, Actor gender, and Participant gender as factors was calculated for ratings by the Japanese listeners. Further, to clarify the cross-cultural effect between Japanese and Canadian participants, three mixed two-way ANOVAs were calculated on ratings of Intensity, Valence, and Arousal. For each mixed ANOVA, to verify the equality of the variance of the differences by Emotions, Mauchly's sphericity was calculated. If the sphericity could not be assumed using Mauchly's test, Greenhouse–Geisser's correction was calculated.

RELIABILITY AND ACCURACY

First, we analyzed the inter-subject reliability of the ratings using Cronbach's alpha. Next, we examined the Intensity ratings for their sensitivity (hit rate, by Emotion) and specificity (correct rejection rate, by rating scale). Based on the previous report (Belin et al., 2008), the accuracy of emotional recognition was investigated using measures of sensitivity (hit rate, by Emotion) and specificity (correct rejection rate, by rating scale). For each vocalization, participants rated the perceived emotional Intensity along each of eight different scales (Anger, Disgust, Fear, Pain, Sadness, Surprise, Happiness, and Pleasure). To calculate sensitivity, for a given portrayed emotion, a maximum Intensity rating in the corresponding scale (i.e., if Intensity rating of Anger was highest when the subject listened to angry vocalization) was taken as a hit; otherwise, as a miss. In other words, emotions with high hit rates are those that

are well recognized, i.e., that scored highest on the scale of the intended emotion. Conversely, specificity relates to the extent to which the rating scale measures what it is intended to measure. To calculate specificity for a given rating scale, if the maximum score was obtained for the corresponding portrayed emotion across the eight vocalizations from one actor (i.e., when the subject listened to disgusted vocalization by actor 1, if rating of Disgust was highest in the eight emotional items), it was taken as a correct rejection; otherwise, as a false alarm. A highly specific rating scale is one rating scale for which the corresponding vocalization obtains the highest score. In other words, it is a measure of how a rating scale is specific to an emotion.

RESULTS

AFFECTIVE RATING

Inter-participant (30 participants) reliability across the 90 items [10 ratings scales: (Valence, Arousal, eight emotional Intensities) × (9 Emotional sounds) was analyzed: Cronbach's alpha = Japanese: 0.941, $F(89, 299) = 230.6$, $p < 0.001$]. Since this reliability for 30 subjects is very high, the ratings of 10 actors' vocalizations were averaged with the ratings of all 30 Japanese participants. [Canadian participants had an inter-participant reliability rating of 0.978 (Belin et al., 2008)]. **Table 1** shows the averaged ratings of Intensity, Valence, and Arousal for the present sample of Japanese participants and the Canadian participants in the study of Belin et al. (2008). **Figure 1** shows the distribution (average ± 2 SD) of ratings of 1-1. Intensity, 1-2. Valence, and 1-3. Arousal in Japanese and Canadian participants.

INTENSITY

A mixed two-way ANOVA with listeners' Group (Japanese, Canadian) and Emotion ($n = 8$) as factors was calculated on Intensity scores. A significant main effect was revealed between listener's Groups [$F(1, 57) = 20.828$, $p < 0.001$] as well as among the Emotions [$F(5.5, 313.5) = 40.520$, $p < 0.001$; Greenhouse–Geisser's test]. Crucially, a significant interaction between Group and Emotion was observed, $F(5.5, 313.5) = 9.137$, $p < 0.001$, (**Figure 1A**) indicating that rating differences between the two groups varied with the specific Emotion considered. *Post hoc* tests showed that Intensity ratings from Japanese listeners were significantly lower than ratings from Caucasian listeners for Anger, Disgust, Fear, Surprise, and Pleasure (t-test, $p < 0.05/8$: Anger, $t = -4.358$; Disgust, $t = -4.756$; Fear, $t = -3.073$; Surprise, $t = -2.851$; Pleasure, $t = -6.737$: **Table 1**; **Figure 1A**).

VALENCE

A mixed two-way ANOVA with listeners' Group (Japanese, Canadian) and Emotion ($n = 9$) as factors was calculated on Valence scores. There was a significant main effect of listeners' Group: $F(1, 57) = 5.920$, $p < 0.018$, as well as a significant main effect of Emotion $F(4.3, 244.3) = 224.926$, $p < 0.001$ (Greenhouse–Geisser's test). Crucially, a significant interaction between Group and Emotion was observed: $F(4.3, 244.3) = 25.101$, $p < 0.001$ (**Figure 1B**) indicating that rating differences between the two groups varied with the specific Emotion considered. *Post hoc* tests showed that Valence ratings from Japanese listeners were significantly higher than ratings from Caucasian listeners for Anger, Disgust, Fear

Table 1 | The mean (*M*) ratings of 1. Intensity, 2. Valence, and 3. Arousal for 10 actors' voices (5 male actors, 5 female actors) by 30 Japanese and 30 Canadian participants.

Vocal expression	1-1: Intensity					1-2: Valence					1-3: Arousal				
	Japan		Canada		*t*-test	Japan		Canada		*t*-test	Japan		Canada		*t*-test
	M	*SEM*	*M*	*SEM*	*P*	*M*	*SEM*	*M*	*SEM*	*P*	*M*	*SEM*	*M*	*SEM*	*P*
Neutral	–	–	–	–		46.7	1.2	47.6	1.7	n.s.	21.5	3.6	33.0	2.7	n.s.
Angry	55.0	3.5	74.7	2.9	<0.001*	39.2	2.9	16.6	1.7	<0.001*	78.3	1.6	73.0	2.1	n.s.
Disgusted	45.5	4.0	70.3	3.3	<0.001*	34.8	1.4	24.4	2.5	<0.001*	40.3	2.1	36.9	2.7	n.s.
Fearful	54.1	3.6	67.6	2.4	0.003*	34.0	2.7	24.3	1.3	0.002*	78.3	1.7	72.6	2.2	n.s.
Painful	52.4	3.7	57.8	3.0	n.s.	32.3	2.7	23.5	1.9	n.s.	69.3	2.0	66.4	2.1	n.s.
Sad	75.1	2.5	77.0	2.7	n.s.	21.7	2.2	19.3	1.6	n.s.	65.2	3.1	45.0	3.5	<0.001*
Surprised	65.4	3.4	77.2	2.4	0.006*	45.4	2.3	39.1	1.1	n.s.	70.5	2.3	71.2	1.9	n.s.
Happy	76.2	3.5	81.3	2.8	n.s.	84.9	2.0	85.2	2.1	n.s.	67.4	2.6	58.3	3.1	n.s.
Pleased	30.0	3.8	62.1	2.8	<0.001*	43.5	2.6	70.8	2.2	<0.001*	30.6	2.3	35.9	2.9	n.s.

*SEM is mean of standard deviation. A mixed two-way ANOVA demonstrated significant main effects of Group and Emotion in Intensity and Valence: $p < 0.001$; *represents significant difference of post hoc tests; Intensity: $p < 0.05/8$, Valence: $p < 0.05/9$, Arousal: $p < 0.05/9$.*

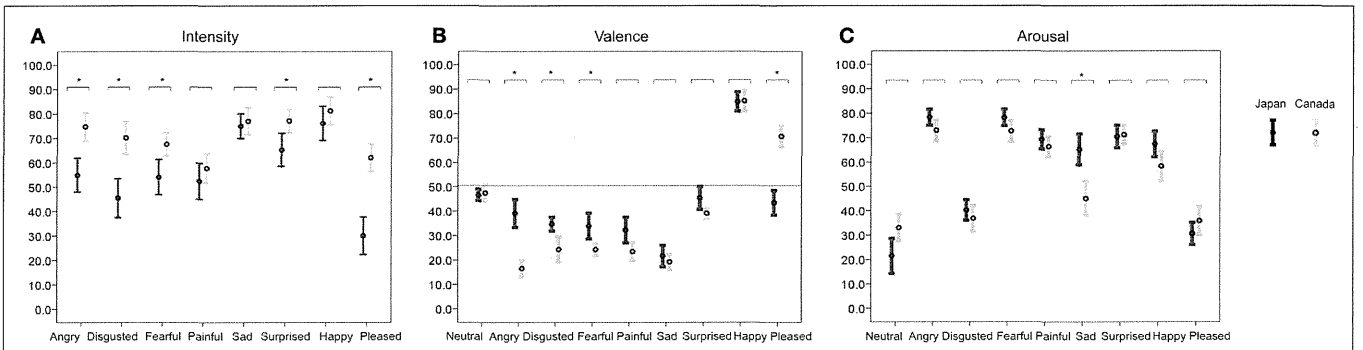

FIGURE 1 | Shows the distribution of ratings (error bar: mean ± SD) for each emotional sound judged by 30 Japanese and 30 Canadian participants for (A) Intensity, (B) Valence, and (C) Arousal. Each horizontal axis represents each rating score (0–100). Each vertical axis shows categories of emotional vocalizations. A mixed two-way ANOVA represents significant main effects of Subject group (Japanese and Canadian) and Emotion, respectively: $p < 0.001$; $*p < 0.05/8$ (Intensity), $*p < 0.05/9$ (Valence), $*p < 0.05/9$ (Arousal); *post hoc* t-test.

(t-test, $p < 0.05/9$: t-test, $p < 0.05/9$: Anger, $t = 6.696$, Disgust, $t = 3.608$; Fear, $t = 3.232$: **Table 1**; **Figure 1B**), whereas the Valence rating from Japanese listeners was significantly lower than ratings from Caucasian listeners for Pleasure (t-test, $p < 0.05/9$; Pleasure, $t = -8.121$; **Table 1**, **Figure 1B**).

AROUSAL

A mixed two-way ANOVA with listeners' Group (Japanese, Canadian) and Emotion ($n = 9$) as factors was calculated on Arousal scores. There was no significant main effect of Group: $F(1, 57) = 2.099$, $p > 0.05$, whereas there was a significant main effect of Emotion $F(4.4, 250.5) = 158.524$, $p < 0.001$ (Greenhouse–Geisser's test). Crucially, a significant interaction between Group and Emotion was observed: $F(4.4, 250.5) = 8.955$, $p < 0.001$ (**Figure 1C**), indicating that rating differences between the two groups varied with the specific Emotion considered. *Post hoc* tests showed that the Arousal ratings from Japanese listeners were significantly higher than ratings from Caucasian listeners for sad vocalizations (t-test, $p < 0.05/9$: sad, $t = 4.334$: **Table 1**; **Figure 1C**), whereas the other Emotions were not significantly different between Japanese and Canadian participants (t-test, $p > 0.05/9$: **Table 1**; **Figure 1C**).

SENSITIVITY AND SPECIFICITY

We evaluated the Intensity ratings for their sensitivity (hit rate, by Emotion) and specificity (correct rejection rate, by rating scale). A maximum Intensity rating in the scale corresponding to the portrayed emotion was considered as a hit; otherwise, as a miss. **Table 2** shows the Intensity ratings of portrayed emotions for Japanese and Canadian participants: means of hit rates by participants and means of correct rejection rates by participants.

A Mixed two-way ANOVA with listener's Group and Emotion ($n = 8$) as factors were calculated on the score of sensitivity and specificity, respectively. In both sensitivity and specificity, a significant main effect of Group was observed [sensitivity: $F(1, 57) = 51.6$, $p < 0.001$; specificity: $F(1, 57) = 44.8$, $p < 0.001$] as well as main effects of Emotion [sensitivity: $F(5.4, 310) = 38.0$, $p < 0.001$; specificity: $F(5.6, 320) = 41.5$, $p < 0.001$, Greenhouse–Geisser's test]. Interaction effects (Group × Emotion) for sensitivity and specificity were also observed sensitivity: $F(5.4, 310) = 9.0$, $p < 0.001$; specificity: $F(5.6, 320) = 11.0$, $p < 0.001$, indicating that rating differences between the two Groups varied with the specific Emotion considered.

There were significant differences in hit rates between Japanese and Canadian participants for angry, disgusted, fearful, painful, and pleased actors' vocalizations ($p < 0.05/8$, t-test): hit rates for these emotions were all lower in Japanese participants. In correct rejection rate, there were significant differences between Japanese and Canadian participants for Disgust and Fear ratings scales, with lower correct rejection rates in Japanese listeners ($p < 0.05/8$).

In Japanese participants, hit rates for each Emotion varied greatly, from 25% for fearful to 79% for sad. Hit rates and correct rejection rate to happy, sad, and surprised vocalizations were relatively high (more than 50%), whereas hit rates and correct rejection rate to angry, disgusted, fearful, painful, and pleased vocalizations were lower (less than 50%).

In **Table 2**, the maximum Intensity rating for each portrayed emotion is shown in bold. For fearful vocalizations only, the Emotion with a maximum score by Japanese participants was different from the portrayed emotion. Japanese listeners on average gave higher Intensity rating in the Surprise scale (66%) than the Fear scale (54%) in response to fearful vocalizations. For all other Emotions, Japanese participants gave the maximum ratings in the scale corresponding to the portrayed emotion, as did the Canadian listeners.

GENDER DIFFERENCES OF ACTOR AND PARTICIPANT

We examined the effects of participant's and actor's gender on hit rates in Japanese participants (**Figure 2**). A three-way mixed ANOVA was calculated with the factors of actor's gender and participant's gender as well as Emotion in Japanese participants. In addition to a significant effect of the emotion [$F(1, 56) = 70.285$, $p < 0.001$], a significant effect of actor's gender [$F(1, 56) = 4.003$, $p \leq 0.05$] was observed, whereas no significant effect was revealed in participant's gender [$F(1, 56) = 3.727$, $p > 0.05$] or interaction effect: emotion × actor's

Table 2 | Intensity ratings (0–100) averaged across all actors for each portrayed emotion and Intensity ratings scale in Japanese and Canadian participants.

Intensity rating scale		Portrayed emotion																Correct rejection rate (%)	
		Neutral		Angry		Disgusted		Fearful		Painful		Sad		Surprised		Happy		Pleased		Specificity	(Validity)
		M	SEM	*M*	SEM	*M*	SEM	*M*	SEM	*M*	SEM	*M*	SEM	*M*	SEM	*M*	SEM	*M*	SEM	*M*	SEM
Anger	Japan	9	1.1	**55**[bd]	5.6	18	4.5	25	5.1	33	5.4	14	4.3	21	5.0	7	2.5	12	3.4	36	6.6
	Canada	9	0.5	**75**[ac]	2.4	14	1.1	19	2.1	33	3.8	9	0.7	17	0.8	3	0.3	7	0.8	77	4.4
Disgust	Japan	12	1.3	49	5.7	**45**[ac]	5.9	48	6.3	48	6.2	33	6.2	33	6.0	8	2.9	30	5.8	44*	4.6
	Canada	10	0.5	23	0.7	**70**[ac]	2.7	21	1.6	26	2.7	9	0.6	24	1.4	4	0.3	8	0.7	73*	4.5
Fear	Japan	7	0.8	30	3.7	15	4.0	54	5.9	25	5.5	21	5.1	34	5.9	5	1.7	14	3.2	18*	4.7
	Canada	9	0.5	16	2.0	11	0.6	**68**[bc]	2.5	21	2.0	10	0.7	45	2.6	3	0.2	6	1.0	69*	3.0
Pain	Japan	6	0.8	30	5.7	22	4.7	31	6.1	**52**[d]	5.7	30	6.0	23	5.1	5	1.6	13	3.9	32	8.0
	Canada	9	0.9	24	1.6	11	1.1	31	3.1	**58**[ac]	3.6	26	1.8	21	1.0	3	0.2	7	0.4	62	4.0
Sadness	Japan	10	1.1	15	4.1	23	4.8	21	4.8	27	5.0	**75**[ac]	4.7	13	3.8	7	2.3	26	5.4	75	5.2
	Canada	11	0.8	13	1.2	9	0.8	13	1.2	15	1.5	**77**[ac]	3.6	11	0.4	3	0.2	5	0.3	89	2.5
Surprise	Japan	7	0.8	46	6.2	20	4.5	**66**[a]	5.2	36	6.0	17	4.4	**65**[ac]	5.3	17	4.4	17	4.1	66	7.5
	Canada	9	0.6	26	1.8	26	1.8	57	3.0	35	3.0	11	2.7	**77**[ac]	2.0	18	1.1	25	2.2	64	2.7
Happiness	Japan	7	0.8	12	3.2	13	3.2	9	2.8	9	2.7	13	3.5	15	4.0	**76**[ac]	4.6	25	5.0	59	3.4
	Canada	14	0.5	6	0.4	9	0.8	7	0.4	10	1.1	11	2.4	15	1.3	**81**[c]	1.2	54	3.3	76	3.0
Pleasure	Japan	6	0.8	10	2.9	13	3.3	8	2.4	8	2.4	10	2.7	12	3.2	64	5.6	**32**	6.0	29	5.2
	Canada	13	0.3	6	0.4	9	0.9	6	0.4	11	1.9	10	2.4	12	1.0	76	1.1	**62**	3.8	39	4.0
Hit rate (%)	Japan			44*	7.3	51*	5.1	25*	3.4	35*	7.9	79	4.6	72	6.8	69	3.2	34*	5.7		
	Canada			78*	5.0	81*	3.7	56*	3.0	51*	3.0	86	2.0	75	2.9	60	4.5	59*	3.8		

Boldface indicates maximum average rating. Note the high hit rates for most affective categories.

[a]$p < 0.001$. [b]$p < 0.05$, strongest rating on the scale corresponding to the portrayed emotion (columns). [c]$p < 0.001$. [d]$p < 0.05$, strongest rating for the portrayed emotion corresponding to the rating scale (rows; Fisher's protected least significance test).

**$p < 0.05/8$, t-test.*

gender [$F(1, 56) < 1$, $p > 0.05$], emotion × participant's gender [$F(1, 56) = 2.496$, $p > 0.05$], and emotion × actor's gender × participant's gender [$F(1, 56) < 1$, $p > 0.05$]. Hit rates were higher for vocalizations portrayed by the female actors irrespective of participant's gender (**Figure 2**).

Further, we investigated cultural effect on hit rates including Japanese and Canadian participants. A three-way ANOVA was calculated with the factors of listener's group, actor's gender, and participant's gender. A significant main effect was observed in listener's Group: $F(1, 110) = 83.211$, $p < 0.001$, and actor's gender $F(1, 110) = 11.675$, $p < 0.001$, and participant's gender $F(1, 110) = 8.396$, $p = 0.005 < 0.05$. Interaction effect showed no significant effect of listener's group × participant's gender, $F(1, 110) = 0.054$, $p > 0.05$, listener's group × actor's gender, $F(1, 110) = 0.428$, $p > 0.05$, actor's gender × participant's gender $F(1, 110) = 0.804$, $p > 0.05$, and listener's group × actor's gender × participant's gender, $F(1, 110) = 0.071$, $p > 0.05$. These results indicate that in hit rates, the effect of actor's gender exists regardless of cultures.

Gender differences were analyzed on ratings of Intensity, Valence, Arousal, and correct rejection rates as well as hit rates. A significant effect of actor's gender was observed in Intensity: $F(1, 55) = 136.712$, $p < 0.001$; Valence: $F(1, 55) = 14.551$, $p < 0.001$; Arousal: $F(1, 55) = 182.899$, $p < 0.001$; correct rejection rates: $F(1, 55) = 23.131$, $p < 0.001$. There was no significant effect of participant's gender in Intensity: $F(1, 55) = 0.002$, $p > 0.05$; Valence: $F(1, 55) = 1.289$, $p > 0.05$; Arousal: $F(1, 55) = 0.655$, $p > 0.05$. In correct rejection rate, a significant effect of participant's gender was observed: $F(1, 55) = 6.343$, $p = 0.015$, <0.05. No interaction between actor's gender and participant's gender was observed [Intensity: $F(1, 55) = 1.459$, $p > 0.05$, Valence: $F(1, 55) = 0.316$, $p > 0.05$, Arousal: $F(1, 55) = 2.191$, $p > 0.05$, Correct rejection rate: $F(1, 55) = 0.797$, $p > 0.05$].

DISCUSSION

We investigated cross-cultural differences between Japanese and Canadian participants in their perception of non-verbal affective vocalization using MAVs. The most intriguing finding is that significant Group × Emotion interactions were observed for all emotional ratings (Intensity, Valence, and Arousal). Ratings of Intensity and Valence for happy and sad vocalizations were not significantly different between Japanese and Canadian participants, whereas ratings for angry and pleased vocalizations were significantly different. Especially, for the Valence ratings in angry vocalizations, Japanese subjects rated less negative than Canadian subjects. Further, in the Valence ratings for pleasure vocalizations, Japanese subjects rated less positive than Canadian subjects.

CROSS-CULTURAL EFFECT FOR POSITIVE EMOTION

Correct rejection rates (validity) of Happiness and Pleasure were not significantly different between Caucasian and Japanese subjects (**Table 2**: Happiness: Canadian 76% vs. Japanese 56%, Pleasure: Canadian 39% vs. Japanese 29%). These findings suggest that these two items are valid beyond the culture. In our study, there was a significant difference in the ratings (Intensity and Valence) for pleased vocalizations between Japanese and Canadian participants, whereas no significant difference was observed in the ratings for happy vocalizations. Although Happiness (laughter) was well recognized across cultures, there were apparent cultural differences in the perception of Pleasure.

A recent study between Western participants and Namibian participants demonstrated that the positive vocalizations of achievement, amusement, sensual pleasure, and relief were recognized as culture-specific signals although happy vocalizations

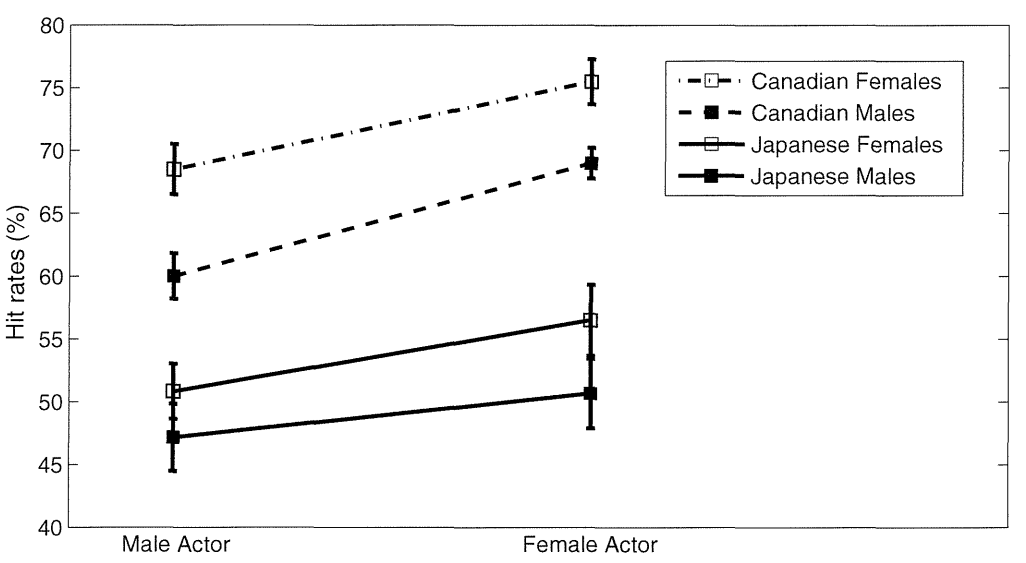

FIGURE 2 | Hit rates (percentage of test items with maximal rating on the scale corresponding to the portrayed emotion) split by actor's and participant's gender.

were recognized cross-culturally (Sauter et al., 2010). Our present result is similar to the findings of this previous study. Further, in accordance with our results, recent studies of facial expression have shown that happy facial expression is not cross-culturally different between Caucasian and Asian participants (Shioiri et al., 1999; Jack et al., 2009, 2012). Our results suggest that the happy emotion is universal in vocal recognition as well as facial recognition. On the other hand, in the vocal recognition, other positive emotions such as Pleasure can show culture-specific biases.

CROSS-CULTURAL EFFECT FOR NEGATIVE EMOTION

Correct rejection rates (validity) of Anger, Pain, Sadness and Surprise were not significantly different between Caucasian and Japanese subjects (**Table 2**). These findings suggest that these two items are valid beyond the culture. On the other hand, correct rejection rates of Disgust and Fear were significantly different between Caucasian and Japanese subjects (**Table 2**). These findings indicate that it is very difficult for Japanese to identify these two emotions when they listened to MAV.

A recent cross-cultural study between Western participants and Namibian participants suggested that primary basic negative emotions such as Anger, Disgust, Fear, Sadness, and Surprise can be recognized in both cultures (Sauter et al., 2010). We predicted that ratings of negative emotion are culturally universal. However, our results did not accord with that previous study, and we also observed cross-cultural differences in the recognition of Anger, Disgust, and Fear. **Figure 1** and **Table 1** show that Intensity ratings for angry, disgusted, fearful, and surprised vocalizations were significantly higher in the Canadian Group than in the Japanese Group. Valence ratings were higher in Japanese than in Canadians regarding some negative emotions (i.e., anger, disgust, and fear). These differences are consistent as higher perceived Intensity of a negative emotion is typically associated with lower (more negative) perceived Valence. These findings could reflect cross-cultural features of Intensity and Valence in negative emotion. Previous studies of facial expression have demonstrated that cross-cultural differences exist in the recognition of angry, disgusted, and fearful face (Shioiri et al., 1999; Jack et al., 2012). In agreement with these results, the recognition of Anger, Disgust, and Fear may reflect cross-cultural differences between Caucasian and Asian participants. On the other hand, the recognition of sad vocalizations (cries) was not significantly different, in agreement with Sauter et al. (2010). Previous studies of facial expression have shown cross-cultural differences in the recognition of sad expressions (Shioiri et al., 1999; Jack et al., 2012). This finding could reflect the fact that the recognition of sad vocalization could be more similar across cultures in comparison with the facial recognition. A previous study indicated that Japanese are severely affected by the meaning of words in recognition of Japanese emotions (Kitayama and Ishii, 2002). The other reason why Japanese find it difficult to differentiate negative emotional vocalizations may be that Japanese need more contextual information to recognize emotions than Canadians.

Concerning of ratings of negative vocalizations, **Table 2** shows that hit rates (accuracy) and specificity were lower in Japanese participants than in Canadian participants for ratings of angry, disgusted, fearful, and painful vocalizations. Especially, the strongest pattern of confusion was observed between fearful and surprised vocalizations in Japanese participants. This pattern is a typical pattern of confusion in Caucasian listeners as well (Belin et al., 2008). For both Japanese and Canadian participants, when listening to fearful vocalizations, the Intensity ratings for Surprise were high (Canadian: fearful 68 ± 2.5 vs. surprised 57 ± 3.0; Japanese: fearful 54 ± 5.9 vs. surprised 66 ± 5.2). These results suggest that it was difficult for Japanese participants to discriminate between fearful and surprised vocalizations. The hit rate of fearful vocalizations in Japanese participants was significantly lower than that in Canadian participants. In contrast, the hit rate of surprised vocalizations was not significantly different between Japanese and Canadian. This finding suggests that Japanese tend to be difficult to identify emotional intensity of fearful vocalizations from MAV.

A recent cross-cultural study between Japanese and Dutch participants demonstrated congruency effects displayed by happy face/voice and angry face/voice (Tanaka et al., 2010). This study indicated that, while listening to Anger voices by Dutch speakers, accuracy ratings of Japanese participants are significantly lower than Dutch participants. In agreement with this result, our study showed that ratings for angry vocalizations showed significantly less Intensity and less negative Valence in Japanese than in Canadian listeners.

THE EFFECTS OF PARTICIPANT'S AND ACTOR'S GENDER IN JAPANESE

Our present study has demonstrated a significant gender effect by actor in accordance with a previous Canadian study (Belin et al., 2008), and hit rates for female vocalizations are higher than for male vocalizations (**Figure 2**). In general, women are believed to be more emotionally expressive than are men (Fischer, 1993). A previous study of facial recognition also revealed that females had a higher rate of correct classification in comparison with males (Thayer and Johnsen, 2000). Our results suggest that Japanese as well as Canadians are also more accurate at recognizing female vocalizations.

A previous study demonstrated an effect of listener's gender in Canadian participants (Belin et al., 2008). In line with the previous study, in the analysis including Japanese and Canadian participants, the effect of participant's gender was replicated.

Our present study has at least two important limitations. First, stimuli consisted of acted vocalizations, not genuine expressions of emotion. Ideally, research on emotional perception would only use naturalistic stimuli. However, collecting genuine emotional expressions across different actors in comparable settings and for different emotions is very difficult and presents ethical problems. Second, in the present study, cross-cultural differences between Canadian and Japanese listeners were confirmed in the recognition of some emotional vocalizations. In the future, it will be necessary to develop a set of stimuli to increase cross-cultural validity.

In summary, we tested for cross-cultural differences between Japanese and Canadian listeners in perception of non-verbal affective vocalization using MAVs. Significant Group × Emotion interactions were observed for all ratings of Intensity, Valence, and Arousal in comparison with Japanese and Canadian participants of our present study. Although ratings did not differ across cultural groups for Pain, Surprise, and Happiness, they markedly differed for the angry, disgusted, and fearful vocalizations which were rated

by Japanese listeners as significantly less intense and less negative than by Canadian listeners; similarly, pleased vocalizations were rated as less intense and less positive by Japanese listeners. These results suggest, in line with Sauter et al. (2010), that there were cross-cultural differences in the perception of emotions through non-verbal vocalizations, and our findings further suggest that these differences are not necessarily only observed for positive emotions.

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Influence of contact with schizophrenia on implicit attitudes towards schizophrenia patients held by clinical residents

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Abstract

Background: Patients with schizophrenia and their families have suffered greatly from stigmatizing effects. Although many efforts have been made to eradicate both prejudice and stigma, they still prevail even among medical professionals, and little is known about how contact with schizophrenia patients affects their attitudes towards schizophrenia.

Methods: We assessed the impact of the renaming of the Japanese term for schizophrenia on clinical residents and also evaluated the influence of contact with schizophrenia patients on attitudes toward schizophrenia by comparing the attitudes toward schizophrenia before and after a one-month clinical training period in psychiatry. Fifty-one clinical residents participated. Their attitudes toward schizophrenia were assessed twice, before and one month after clinical training in psychiatry using the Implicit Association Test (IAT) as well as Link's devaluation-discrimination scale.

Results: The old term for schizophrenia, "Seishin-Bunretsu-Byo", was more congruent with criminal than the new term for schizophrenia, "Togo-Shitcho-Sho", before clinical training. However, quite opposite to our expectation, after clinical training the new term had become even more congruent with criminal than the old term. There was no significant correlation between Link's scale and IAT effect.

Conclusions: Renaming the Japanese term for schizophrenia still reduced the negative images of schizophrenia among clinical residents. However, contact with schizophrenia patients unexpectedly changed clinical residents' attitudes towards schizophrenia negatively. Our results might contribute to an understanding of the formation of negative attitudes about schizophrenia and assist in developing appropriate clinical training in psychiatry that could reduce prejudice and stigma concerning schizophrenia.

Keywords: Prejudice, Stigma, Implicit association test, Education, Schizophrenia

Background

Patients with schizophrenia and their families have suffered greatly from the stigmatizing effects and the educational, vocational, and interpersonal barriers resulting from negative social attitudes toward their conditions. Patients with schizophrenia have tremendous difficulties finding employment and acquiring living quarters, and they suffer from falsely pressed charges for violent crimes by these kinds of stigmatizing effects [1-7]. From this situation has arisen increasing interest in how these negative attitudes towards schizophrenia may interfere with their various abilities and also with their efforts to obtain effective treatment [8,9]. To reduce mental illness-related stigma (particularly regarding schizophrenia), various programs are underway internationally [1,10,11]. Although the view of schizophrenia has been changing according to advances in neurobiological understanding of the disorder, pharmacology and psychosocial treatments [12], prejudice and stigma are still prevalent even among

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