Cerebral Cortex, 2018; 1–10

doi: 10.1093/cercor/bhy271
Original Article

ORIGINAL ARTICLE

Cultural variation in the gray matter volume of the prefrontal cortex is moderated by the dopamine D4 receptor gene (*DRD4*)

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Abstract

Recent evidence suggests a systematic cultural difference in the volume/thickness of prefrontal regions of the brain. However, origins of this difference remain unclear. Here, we addressed this gap by adopting a unique genetic approach. People who carry the 7- or 2-repeat (7/2-R) allele of the dopamine D4 receptor gene (*DRD4*) are more sensitive to environmental influences, including cultural influences. Therefore, if the difference in brain structure is due to cultural influences, it should be moderated by *DRD4*. We recruited 132 young adults (both European Americans and Asian-born East Asians). Voxel-based morphometry showed that gray matter (GM) volume of the medial prefrontal cortex and the orbitofrontal cortex was significantly greater among European Americans than among East Asians. Moreover, the difference in GM volume was significantly more pronounced among carriers of the 7/2-R allele of *DRD4* than among non-carriers. This pattern was robust in an alternative measure assessing cortical thickness. A further exploratory analysis showed that among East Asian carriers, the number of years spent in the U.S. predicted increased GM volume in the orbitofrontal cortex. The present evidence is consistent with a view that culture shapes the brain by mobilizing epigenetic pathways that are gradually established through socialization and enculturation.

Key words: *DRD4*, Gene x culture interaction, medial prefrontal cortex, orbitofrontal cortex, self-construal

Are there differences in brain volume across cultural, ethnic, and ancestry groups? In the past, brain volume was often erroneously seen as the ultimate measure of general intelligence and moral character (Gould 1996). Given the racist overtone of previous work, the possible cultural and ethnic variation in brain volume has understandably alarmed many scientists and humanists. Nevertheless, recent work presents initial evidence of systematic differences in the gray matter (GM) volume of the brain as a function of culture and related social psychological variables (Chee et al. 2011; Kitayama et al. 2017; Wang et al. 2017). This line of research differs from the past work in its focus on regionally specific GM volume as well as on a general hypothesis that the group difference in brain volume is due partly to experience, rather than only to genetic ancestry.

In the current work, we seek to shed light on the possible contribution of experience to a previously reported cultural difference in the GM volume in prefrontal regions including the orbitofrontal cortex (OFC) and the medial prefrontal cortex (mPFC) (Chee et al. 2011). To do so, we drew on recent gene x environment interaction research. In particular, certain polymorphic genetic variations, including a varying number tandem repeat (VNTR) variation in the dopamine D4 receptor gene (*DRD4*), modulate sensitivity to environmental influences (Belsky and Pluess 2009; Kim and Sasaki 2014; Kitayama et al.

2014, 2016; Silveira et al. 2016). Accordingly, if the cultural variation in GM volume is due, in part, to cultural experience, it is likely to be moderated by some of these genetic polymorphisms including the *DRD4* VNTR polymorphisms.

The present study is informed by prior work documenting psychological differences between European Americans and East Asians (Triandis 1989; Markus and Kitayama 1991; Nisbett et al. 2001; Kitayama and Uskul 2011; Kitayama and Salvador 2017). Compared to European Americans, East Asians are thought to be more interdependent or less independent in social orientation (Markus and Kitayama 1991). Much of this work is based on both self-report questionnaires and behavioral experimental tasks, although more recent work shows similar cultural differences in functional brain activations (Kitayama and Uskul 2011; Han and Ma 2014; Kitayama and Salvador 2017). Despite the advancement over the last two decades, however, rarely has this work tested structural properties of the brain including GM volume across the cultural groups. One important exception is a study by Chee and colleagues (2011). They acquired structural brain images of 70 Singaporeans and 70 European Americans and tested regionally specific GM volume and thickness. Several striking cultural variations were observed among young adults (in their early 20 s). In particular, the GM volume and thickness was greater in the prefrontal regions including OFC and mPFC among European Americans than among East Asians.

Converging evidence emerged in two more recent structural imaging studies, which showed that the GM volume in both OFC and mPFC is linked to the construal of the self as interdependent – one of the dimensions commonly used to explain the cultural difference between European Americans and East Asians. Kitayama et al. (2017) scanned 135 Japanese adults in early and middle adulthood (20–39 years of age) and found that interdependent self-construal (SC) was inversely associated with the GM volume of the bilateral OFC. In another recent study, Wang and colleagues (2017) scanned 265 Chinese young adults (mean age of 23 years) and observed that interdependent SC was associated with reduced GM volume of mPFC.

While certain unknown genetic factors could be linked to both interdependent SC and GM volume, this association could also be due to experience. Specifically, less interdependently oriented individuals may be more likely to engage in psychological activities that recruit certain prefrontal regions. For example, prior work links OFC to value-based judgment ("What do I like?") (O'Doherty 2011), the ability to sustain transitivity in personal preferences (Fellows 2011), and more generally, goal-oriented behaviors (Rolls and Grabenhorst 2008). All these activities are likely linked to lower levels of interdependence (Kitayama and Park 2014). Further, mPFC is implicated in the development of a clear personal self (Kelley et al. 2002; Northoff and Bermpohl 2004; Heatherton et al. 2006; van der Meer et al. 2010; Chua et al. 2011), another function likely inversely related to interdependent orientations (Campbell et al. 1996). In light of a growing body of work documenting effects of sustained engagement in scripted behaviors on GM volume (Maguire et al. 2000; Draganski et al. 2004; Driemeyer et al. 2008), the culturally sanctioned chronic recruitment of both OFC and mPFC functions might explain the inverse association between interdependent SC and the OFC and mPFC GM volume.

To test this proposition, we drew on recent evidence that certain genetic polymorphisms both support and modulate environmental influences including cultural influences (Belsky and Pluess 2009; Sasaki et al. 2013; Kim and Sasaki 2014; Kitayama et al. 2014). Of particular interest are genes coding the functions of dopamine, a neurotransmitter that plays a key role in reward processing (Forbes et al. 2009). Reward processing is central to some distinct psychological operations involved in reinforcement-based learning, such as computation of reward prediction errors and abstraction of rules governing reward contingencies (Set et al. 2014). It therefore may play a key role in cultural acquisition (Kitayama and Uskul 2011; Kitayama et al. 2016; Kitayama and Salvador 2017). Hence, if a given cultural difference is caused by reinforcement-based social learning of different cultural values and norms, the extent of the cultural difference should vary as a function of genetic polymorphisms of genes that regulate the strength of dopaminergic signaling in the central nervous system (Kitayama et al. 2014, 2016).

Among numerous genes that are potentially relevant (Set et al. 2014), one gene, the dopamine D4 receptor gene (*DRD4*), is particularly promising (Sasaki et al. 2013; Kitayama et al. 2014, 2016; Silveira et al. 2016; Tompson et al. 2018). Compared to a common allelic variant (the 4-repeat allele of *DRD4*), two evolutionarily more recent VNTR variants that are thought to have emerged over the last 50 000 years (Wang et al. 2004), the 7-repeat and 2-repeat alleles, are associated with increased dopamine signaling capacity and hence heightened sensitivity to environmental influences (Sheese et al. 2007; Belsky and Pluess 2009; Bakermans-Kranenburg and van IJzendoorn 2011). Indeed, it has been found that the developmental outcomes of European and European American children carrying the 7-repeat allele of *DRD4* are dependent on parental quality, whereas those not carrying this allele appear less sensitive to the quality of parenting (Windhorst et al. 2015; King et al. 2016) (the 2-repeat is more common in East Asians, with very few European Americans and Europeans carrying this variant). As may be expected from this body of evidence, the aforementioned cultural difference in social orientation (with East Asians being more interdependent or less independent than European Americans) is highly reliable among those who carry the 7- or 2-repeat (7/2-R) allele of *DRD4*, but not among those who carry neither (Kitayama et al. 2014). This *DRD4* x culture interaction extends to emotional experience. Prior work suggests the existence of emotion norms encouraging the experience of positive emotions in lieu of negative emotions in Western cultures; but the corresponding emotion norms in East Asia emphasize harmonious balance between positive and negative emotions (Kitayama et al. 2000). A recent study finds that the pattern of emotional experience that conforms to the varying cultural norms of emotion is observed among carriers of the 7/2-R allele of *DRD4*, but not among non-carriers (Tompson et al. 2018).

Building on this evidence, we hypothesize that if the cultural difference in GM volume of the prefrontal regions (particularly, OFC and mPFC) were due in part to cultural experience, this difference would be more pronounced among the carriers of the 7/2-R allele of *DRD4* than among non-carriers. In addition, if the 7/2-R allele of *DRD4* sensitizes the carriers of these alleles to cultural experience, they may be more influenced by new cultural norms when they immigrate to a new country. We explored this possibility by taking advantage of the fact that our East Asian participants had been born in Asia, had subsequently moved to the U.S., and had stayed there for variable durations. A possible increase of the prefrontal GM volume due to exposure to U.S. culture could be moderated by *DRD4* such that these effects were more pronounced among the carriers of the 7/2-R allele of *DRD4* than among non-carriers.

Method

Participants

One hundred and thirty-two healthy right-handed undergraduate students at University of Michigan were paid to participate in the study. All those participants were part of a larger study of cross-cultural variation (n = 635). Participants in the present study were recruited from this large pool of subjects based on their *DRD4* status (7/2-R vs. other alleles), such that roughly equal number of carriers of 7/2-R and non-carriers were tested. Sixty-six participants were European Americans who were born and raised in the U.S. (45 females and 21 males; age range 18–23 years; mean age of 20.2 years), and 66 were Asian-born East Asians who were born in an East Asian country (i.e., China, Japan, Korea, and Taiwan) and had been in the U.S. for less than 10 years (40 females and 26 males; age range 18–27 years; mean age of 21.2 years). Six of them were excluded from further analysis due to poor quality of their structural magnetic resonance imaging (MRI) scans which prevented optimal tissue segmentation, leaving us with 63 in each cultural group for a total of 126 participants. Data and structural brain images of the present study are available at: https://osf.io/wpfuv/. The study was approved by the Internal Review Board of University of Michigan, and all participants in the study provided written informed consent.

Image Acquisition

Scanning was performed using a Philips three Tesla MRI scanner (Phillips Medical Systems, Andover, MA). A high-resolution T1-weighted structural image was acquired from all participants (echo time = 4.6 ms, repetition time = 9.8 ms, 256 × 200 matrix, flip angle = eight degrees, field of view = 256 × 256 × 180 (mm), 180 contiguous 1 mm sagittal slices per volume).

Genotyping

The genotyping procedure has been described in detail in Kitayama et al. (2014). An Oragene saliva kit (OG-500) was used for saliva collection (DNA Genotek, Kanata, Ontario, Canada). Genomic DNA was extracted using a high-capacity membrane-based column (QuickGene810, AutoGen, Inc., Holliston, MA) and was quantitated using an A260/A280 ratio with a NanoDrop spectrophotometer (ThermoScientific, Inc., Wilmington, DE) and agarose gel electrophoresis. The *DRD4* VNTR polymorphism was amplified, with 0.2 μM of *DRD4* forward primer 5′-GCGACTACGT GGTCTACTCG and 0.2 μM of *DRD4* reverse primer 5′-AGGACCCT CATGGCCTTG (Lichter et al. 1993), using the Roche GC-Rich PCR System amplification buffer (Roche Applied Science, Inc., Mannheim, Germany) and 20 ng of genomic DNA in a volume of 25 μl. The samples were heated in a Stratagene thermocycler (Life Technologies, Inc., Grand Island, NY) at 95 °C for 3 min, then cycled 40 times at 95 °C for 20 s, 57 °C for 20 s, and 72 °C for 1 min, followed by 72 °C for 3 min. Polymerase chain reaction products were separated and visualized on a 2% agarose gel (type 1-A, Sigma, St. Louis, MO) stained with ethidium bromide.

Among the 126 participants that were included in the brain image analysis, frequencies of the *DRD4* VNTR alleles were: for European American participants, 10.3% 2 R, 4.0% 3 R, 65.9% 4 R, 0.8% 5 R, 16.7% 7 R, and 2.4% 8 R; for East Asian participants, 22.2% 2 R, 0.8% 3 R, 76.2% 4 R, and 0.8% 5 R. As in previous work (Reist et al. 2007; Sasaki et al. 2013; Kitayama et al. 2014), carriers of 7 R and 2 R alleles were compared (32 European Americans and 26 East Asians) with non-carriers of these alleles (mostly 4 R/4 R, together with more infrequent variants including the 3 R, 5 R, and 8 R alleles; 31 European Americans and 37 East Asians).

Questionnaires: Self-Construal (SC) Scale

The SC scale was composed of 30 items designed to assess the degree of each participant's SC as independent (15 items) or interdependent (15 items) (Singelis 1994). Sample items for independent SC include, "I enjoy being unique and different from others in many respects" and "I feel it is important for me to act as an independent person". Sample items for interdependent SC include, "I will sacrifice my self interest for the benefit of the group I am in" and "My happiness depends on the happiness of those around me". Participants rated each item on a scale from 1 (strongly disagree) to 7 (strongly agree). Internal consistency of the independent SC and the interdependent SC were adequate for both European American participants (α = 0.65 and α = 0.62, respectively) and East Asian participants (α = 0.68 and α = 0.72, respectively). The scale was administered during the pre-scanning survey, which occurred approximately two weeks prior to the scanning session.

VBM Analysis

Image processing and measurement

Participants' brain images were preprocessed using voxel-based morphometry (VBM) implemented in Statistical Parametric Mapping (SPM) software (SPM8; Wellcome Department of Cognitive Neurology, London, UK). VBM is a fully-automated and unbiased MRI-based image analysis technique that identifies the amount of GM of the structural scan (Ashburner and Friston 2000), frequently used to detect regionally specific differences in brain structure (Good et al. 2002). Each participant's T1-weighted image was examined, and the orientation and origin point were manually adjusted when necessary in order to better match the template. Then the images were segmented into different tissue types including GM, white matter (WM), and cerebrospinal fluid (CSF). After this, the "Diffeomorphic Anatomical Registration using Exponentiated Lie algebra" (DARTEL) algorithm was applied to create a study-specific template of GM, which was then affine-registered to Montreal Neurological Institute (MNI) space. All individually segmented GM images of the participants were then nonlinearly warped to match the space of the DARTEL template. A modulation step was performed as well by multiplying the warped tissue probability maps by the Jacobian determinant of the warp on a voxel-by-voxel basis, which represents the relative volume ratio before and after the warping. As a final preprocessing step, all segmented, normalized, and modulated images were smoothed with a 10-mm full-width half-maximum Gaussian kernel. The global volumes of GM, WM, and CSF for each scan were estimated as the total number of voxels of each tissue type multiplied by the voxel size. Total intracranial volume (TIV) of each image was calculated by summing the total GM volume, total WM volume, and total CSF volume.

Region of interest definition

Based on our hypotheses, two anatomical regions of interest (ROI), OFC and mPFC, were defined for VBM analysis using the WFU PickAtlas toolbox (Maldjian et al. 2003). For OFC ROI, we used bilateral middle OFC defined by Automated Anatomical Labeling atlas (Fig. 1-A). The same anatomical OFC ROI was used in the Kitayama et al. (2017) study and thus included the area linked to interdependent SC in that study. For mPFC ROI,

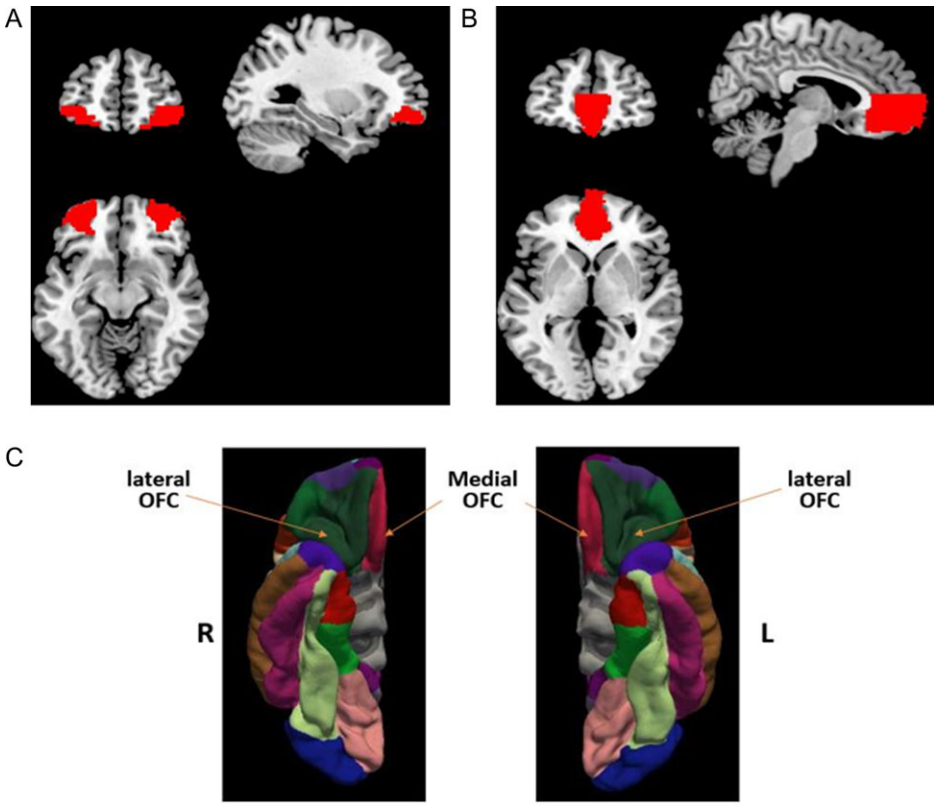

Figure 1. The region of interest (ROI) for the present analysis. A. A coronal (top left), sagittal (top right), and axial (bottom left) view of the middle OFC ROI for VBM analysis. B. A coronal (top left), sagittal (top right), and axial (bottom left) view of the mPFC ROI for VBM analysis. C. Medial and lateral OFC ROI for Freesurfer analysis from a ventral viewpoint.

we followed Wang et al. (2017) by combining the bilateral medial frontal gyrus, cingulate region, and medial orbitalfrontal gyrus voxels in the 71-segmentation version of the IBASPM (Individual Brain Atlas using Statistical Parametric Mapping) and then cropping to the following parameters: x = –15 mm to 15 mm, y > 30 mm, and z < 10 mm (Fig. 1-B).

Statistical analysis

Statistical analysis was performed on the pre-processed GM images. We examined cultural difference as well as the predicted moderation by *DRD4* on cultural difference at the OFC and mPFC GM volume by extracting normalized and smoothed voxel values of the OFC ROI and the mPFC ROI from each participant and analyzing them in the framework of general linear model. TIV was included as a covariate to control for overall head size. Age and sex were also covaried because they may confound GM volume. Correlation with SC, as well as correlation with the number of years in the U.S. for East Asian participants were tested with the same OFC and mPFC ROI values and the same set of covariates. Statistical significance for the ROI analysis was set at $p < 0.05$ (uncorrected).

We also carried out whole brain voxel-level analyses to explore whether additional regions show a significant cultural difference or a significant interaction between culture and *DRD4* status in the GM volume. Because of the potential edge effects at the border of GM and WM, all voxels with a GM value of < 0.2 were excluded to retain only those voxels that were relatively homogeneous. Nonstationary cluster extent correction, which corrects for nonisotropic smoothness of VBM data, was also applied (Hayasaka and Nichols 2004). We used the full factorial design implemented in SPM8 to test three contrasts, 1) GM volume for European Americans > GM volume for East Asians, 2) GM volume for European Americans < GM volume for East Asians, and 3) the cultural difference (European Americans > East Asians) in carriers > the cultural difference (European Americans > East Asians) in non-carriers. TIV, age, and sex were included as covariates in the model, and the threshold of significance was set at $p < 0.05$ at the voxel level (FWE-corrected).

Freesurfer Analysis

One concern of cross-cultural research using neuroimaging is that the shape of the brain differs between Caucasians and Asians (Zilles et al. 2001). In VBM, standard templates used during the spatial normalization are based on European Americans (Tang et al. 2010). In applying such templates to individual brains, care is taken to warp the brains to create voxel-for-voxel correspondence. Moreover, additional steps are taken to adjust for regional stretching/constriction caused by the spatial normalization so as to preserve the original volume (Ashburner and Friston 2000). Nevertheless, a concern could remain since there are very few cross-cultural comparisons on structural MRI scans. We therefore used another neuroimaging technique, Freesurfer, to replicate our cross-cultural comparisons. One advantage of Freesurfer that is especially pertinent in the present work is that unlike VBM, which can only measure structural properties of brains that have been normalized to the standard template, Freesurfer can generate regional measurements of each individual brain itself. That is, for each single brain,

Freesurfer can extract the cortex, parcellate the cortex into different anatomical regions (which can serve as ROIs) based on gyral and sulcal information derived from manually traced training set, and produce detailed measurements on each region (Fischl and Dale 2000; Fischl et al. 2004).

Image processing

The brain images were processed with Freesurfer 5.3.0 (Martinos Imaging Centre). Each image was processed by running the "recon-all" command with the default setting. The image processing involves intensity non-uniformity correction, automated Talairach transformation, intensity normalization, removal of non-brain tissue using a hybrid watershed algorithm, linear and non-linear transformations to a probabilistic brain atlas, and parcellation and labeling of cortical and subcortical structures based on gyral and sulcal information derived from manually traced training set. Each pial surface and gray-white junction mesh was carefully reviewed by a single researcher (QY) and manually edited if necessary.

ROI definition and statistical analysis

Statistical analysis was conducted on neuroanatomical regions produced by Freesurfer's individual-based automated surface parcellation using the Desikan-Killiany Atlas (Desikan et al. 2006). For OFC, we used four ROIs from this atlas – medial and lateral OFC of the left and right hemisphere (Fig. 1-C). Thickness measure of these regions was extracted from the stat files that Freesurfer produced using the "aparcstats2table" command, after the medial OFC and lateral OFC of each hemisphere were combined using the "mri_mergelabels" command. After that, the OFC of each hemisphere was averaged in thickness to produce a single OFC ROI for each participant. Note that cortical thickness was not tested for mPFC per se since this region does not have its own parcellation in Freesurfer's built-in atlas (Desikan-Killiany Atlas and Destrieux Atlas) (Desikan et al. 2006; Destrieux et al. 2010), and the OFC ROI in the Desikan-Killiany Atlas included substantial portion of mPFC. To examine the cultural difference as well as the predicted moderation by *DRD4* on cultural difference at the OFC thickness, the OFC thickness was analyzed with the general linear model, with age and sex included as covariates. Correlational analyses were carried out with the same OFC ROI and the same set of covariates.

Results

Demographics and Questionnaire Data

As expected, East Asians were more interdependent in their SC than European Americans ($t_{(124)} = -2.565$, $p = 0.012$); however, the two groups did not differ on the independent SC ($t_{(124)} = 0.380$, $p = 0.705$). See Table 1 for demographics, self-construal mean scores, and *DRD4* genotype information for both East Asians and European Americans.

VBM ROI Analysis

We first tested OFC GM volume as a function of both culture and *DRD4* status. With TIV, age, and sex as covariates, a general linear model showed a significant main effect of culture, with OFC GM volume being greater for European Americans than for East Asians ($B = 0.016$, $t_{(119)} = 7.253$, $p < .001$, 95% CI = [0.012, 0.020]). Importantly, the 2-way interaction between culture and *DRD4* status proved significant ($B = -0.005$, $t_{(119)} = -2.585$, $p = .011$, 95% CI = [−0.010, −0.001]) (Fig. 2-A). The cultural difference was significantly larger for *DRD4* 7/2 R carriers ($B = 0.021$, $t_{(119)} = 6.800$, $p < .001$, 95% CI = [0.015, 0.027]) than for non-carriers, although it was significant even for non-carriers ($B = 0.010$, $t_{(119)} = 3.591$, $p = .003$, 95% CI = [0.005, 0.016]).

Next, we tested whether the OFC GM volume would be associated with interdependent SC. After controlling for TIV, age, and sex, the OFC GM volume was inversely correlated with participants' interdependent SC ($r = -.197$, $t_{(121)} = -2.212$, $p = .029$) (Fig. 3-A). The same relationship existed within each cultural group (*rs* = −.115 and −.134, for European Americans and East Asians, respectively), although it did not reach statistical significance ($t_{(58)} = -.885$, $p = .380$ and $t_{(58)} = -1.033$, $p = .306$, respectively), plausibly due to the reduced power. As in Kitayama et al. (2017), there was no significant correlation between the OFC volume and independent SC ($r = .059$, $t_{(121)} = .645$, $p = .520$).

We carried out the same set of analyses on the GM volume of mPFC. When the mPFC GM volume was analyzed as a function of both culture and *DRD4* status after controlling for TIV, sex, and age, the main effect of culture was significant such that the volume was significantly greater for European Americans than for East Asians ($B = 0.023$, $t_{(119)} = 8.670$, $p < .001$, 95% CI = [0.018, 0.028]). The 2-way interaction between culture and *DRD4* status was marginally significant ($B = -0.004$, $t_{(119)} = -1.717$, $p = .088$, 95% CI = [−0.009, 0.001]) (Fig. 2-B). This cultural difference tended to be larger for carriers ($B = 0.027$, $t_{(119)} = 7.213$, $p < .001$, 95% CI = [0.020, 0.035]) than for non-carriers, although the cultural difference was significant even for non-carriers ($B = 0.019$, $t_{(119)} = 5.275$, $p < .001$, 95% CI = [0.012, 0.026]). In addition, as in Wang et al. (2017), the mPFC GM volume was inversely correlated with interdependent SC albeit marginally, controlling for TIV, age, and sex ($r = -.172$, $t_{(121)} = -1.920$, $p = .057$) (Fig. 3-B). There was no correlation with independent SC ($r = .037$, $t_{(121)} = .402$, $p = .688$).

To assess the robustness of all our findings presented above, we also carried out voxel-level analysis with small volume correction using SPM, based on the OFC and mPFC ROI defined. This analysis yielded consistent results. Detailed method and results of this analysis are included in the Supplementary Materials.

Table.1. Descriptive statistics of demographics, self-construal scores, and *DRD4* status information

	European Americans	East Asians	Significance
n	63	63	
Mean age (SD)	20.2 (1.61)	21.2 (1.65)	$t = -3.498$, $p = .001$
DRD4 (7/2-R vs. other alleles)	32/31	26/37	$\chi^2 = 1.150$, $p = .284$
Sex (male vs. female)	20/43	24/39	$\chi^2 = 0.559$, $p = .455$
Self-construal			
Independent: mean (SD)	4.87 (0.56)	4.83 (0.59)	$t = 0.380$, $p = .705$
Interdependent: mean (SD)	4.85 (0.53)	5.10 (0.56)	$t = -2.565$, $p = .012$

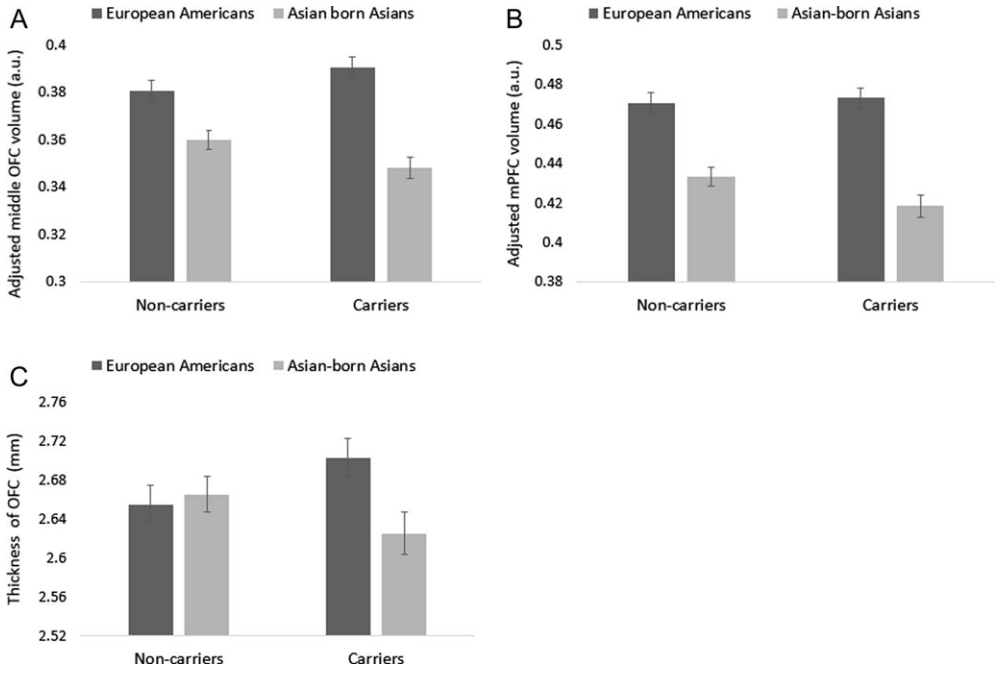

Figure 2. Mean value of GM volume/thickness as a function of culture and *DRD4* status. Error bars represent ± 1SE. A. Mean value of GM volume of the OFC ROI. B. Mean value of GM volume of the mPFC ROI. C. Mean value of thickness of the OFC ROI.

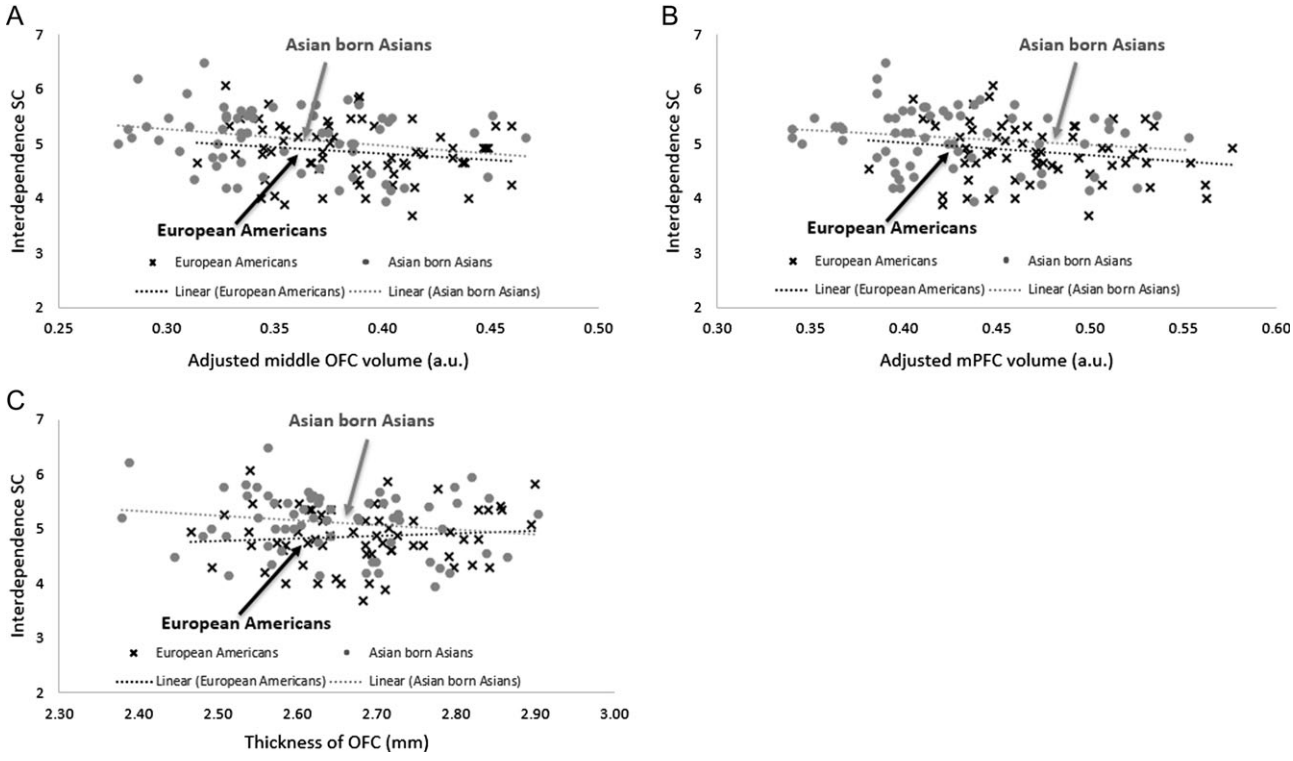

Figure 3. Correlation with interdependent self-construal (SC). A. A scatter plot showing relationship between interdependence SC and GM volume of the OFC ROI. B. A scatter plot showing relationship between interdependence SC and GM volume of the mPFC ROI. C. A scatter plot showing relationship between interdependence SC and thickness of the OFC ROI.

Freesurfer ROI Analysis

With age and sex as covariates, the Freesurfer analysis performed on the OFC/mPFC thickness showed a significant 2-way interaction between culture and *DRD4* status (B = –0.022, $t_{(120)}$ = –2.265, p = .025, 95% CI = [–0.041, –0.003]) (Fig. 2-C). The OFC/mPFC thickness was significantly greater for European

Americans compared to for East Asians among *DRD4* 7/2-R carriers (B = 0.039, $t_{(120)}$ = 2.666, p = .009, 95% CI = [0.010, 0.068]). The same contrast was not statistically significant for non-carriers (B = 0.005, $t_{(120)}$ = .385, p = .701, 95% CI = [−0.022, 0.032]). In this analysis, the cultural main effect at the OFC thickness did not achieve statistical significance (B = 0.017, $t_{(120)}$ = 1.649, p = .102, 95% CI = [−0.003, 0.037]). Unlike in the VBM, there was no significant relationship between OFC/mPFC thickness and interdependent SC after controlling for age and sex (r = −.069, $t_{(122)}$ = −.766, p = .445) (Fig. 3-C). Nor was there any significant association between OFC thickness and independent SC controlling for age and sex (r = −.009, $t_{(122)}$ = −.096, p = .923).

Exploratory Analyses

Effect of the years in the U.S. for East Asians

As mentioned above, East Asian participants were born in East Asia and moved to the U.S. at a later point. This enabled us to test whether the number of years in the U.S. would predict the OFC and mPFC GM volume/thickness among the East Asians. We first tested this prediction using the VBM data. After controlling for TIV, age, and sex, a significantly positive relationship between number of years and OFC GM volume was found for *DRD4* 7/2 R carriers (r = .447, $t_{(21)}$ = 2.289, p = .033). This relationship was negligible for non-carriers (r = .110, $t_{(32)}$ = .626, p = .536). Caution is due because the interaction between number of years and *DRD4* status was not significant (B = 0.003, $t_{(56)}$ = 1.133, p = .262, 95% CI = [−0.002, 0.007]) (Fig. 4-A). As for the mPFC volume, there was no effect of the time in the U.S. for East Asian carriers (r = .295, $t_{(21)}$ = 1.417, p = .171) or non-carriers (r = .039, $t_{(32)}$ = .219, p = .828) (Fig. 4-B). A replication of these findings using voxel-level analysis with small volume correction is included in Supplementary Materials. When the analysis was performed on Freesurfer generated OFC/mPFC cortical thickness, the association between the number of years in the U.S. and the OFC/mPFC thickness was positive, albeit marginally, for carriers, controlling for age and sex (r = .345, $t_{(22)}$ = 1.726, p = .098) (This correlation became highly significant when a single data point that deviated substantially from the predicted regression line (with the Cook's distance greater than the conventional cut-off, 4/N, indicated by the arrow in Fig. 3-D) was removed (r = .536, $t_{(21)}$ = 2.906, p = .008)). This association was negligible for non-carriers (r = .124, $t_{(33)}$ = .720, p = .477). Caution is warranted because the interaction between number of years and *DRD4* status was not significant (B = 0.009, $t_{(57)}$ = .849, p = .399, 95% CI = [−0.013, 0.031]) (The interaction between *DRD4* status and number of years in the U.S. was marginally significant when the potential outlier was excluded (B = 0.020, $t_{(56)}$ = 1.807, p = .076, 95% CI = [−0.002, 0.041])) (Fig. 4-C).

Whole brain voxel-level analysis

The whole brain analysis was performed with VBM (see Tables S1-S3 and Fig. S1-S2 in Supplementary Materials). Of note, a large area of the prefrontal cortex was greater in GM volume for European Americans than for East Asians (Table S1 and Fig. S1-A). This area was centered at the right anterior prefrontal cortex and extended to both the bilateral OFC including the area identified by Kitayama et al. (2017) and more medial regions including the one identified by Wang et al. (2017). This finding also replicates the earlier finding by Chee and colleagues (2011). In addition, the left visual association area showed a similar cultural difference. Regions that showed greater GM volume for East Asians than for European Americans are summarized in Table S2 and Fig. S1-B. There was little correspondence to the Chee et al. (2011) study. We did not find any region showing a significant Culture x *DRD4* interaction. However, with a more liberal voxel-level threshold of p < .001 (uncorrected), a few voxels centered at right anterior OFC and left anterior cingulate cortex emerged as significant (Table S3 and Fig. S2).

Discussion

Our finding is the first to show that VNTR polymorphisms of the *DRD4* moderate a cultural difference in the GM volume and cortical thickness of the prefrontal areas including OFC and mPFC. Since these polymorphisms likely modulate the effectiveness of reinforcement-based learning, our result suggests that the cultural difference in brain volume is mediated in part by cultural experience. Specifically, we first replicated the early cross-cultural study by Chee and colleagues (2011) and found that the GM volume of both mPFC and OFC is larger for European Americans than for East Asians. Moreover, this group difference was more pronounced for the carriers of the 7/2-R of *DRD4* than for non-carriers. This finding is unlikely to suffer from the potential brain shape confound insofar as it was also evident in Freesurfer-based analysis.

Although the Culture x *DRD4* status interaction was statistically significant in all three cases (GM volume of mPFC, GM volume of OFC, and cortical thickness of OFC/mPFC), some nuanced differences are also evident across the measures. The cultural difference was apparent even for non-carriers in the measure of GM volume of both mPFC and OFC, but it ceased to be significant in the cortical thickness of OFC/mPFC. It might be the case that brain shape confounds caused some biases in the GM volume measure such that the GM volume was underestimated for East Asians compared to European Americans. The fact that the measure that is free from this confound showed no cultural difference among non-carriers is consistent with this conjecture. It would seem desirable to use both VBM and Freesurfer in all cross-cultural or cross-ethnic comparisons on structural properties of the brain in future work. We also wish to note that the current analysis yielded no significant within-culture contrasts between carriers and non-carriers. Future work should test a larger number of subjects in each culture to test the prediction that carriers are more likely than non-carriers to show a cultural-typical pattern of GM volume (i.e., increased or decreased prefrontal GM volumes for European Americans and East Asians, respectively).

Our work is consistent with recent theorizing (Kitayama and Salvador 2017) that when certain behaviors involving personal decision making or goal pursuit are positively reinforced, relevant connections in all neural circuitries implicated in these behaviors, including the prefrontal regions, may also be strengthened. This positive reinforcement may be assumed to increase neural activities of the regions, which in turn result eventually in gradual expansion of the GM volume or thickness of the region. It is plausible that the structural change caused by the reward contingencies in culture may be mediated or stabilized by epigenetic pathways that are gradually established through socialization (Meaney 2001; Cole 2014).

Also consistent with our cultural hypothesis is the increase of the GM volume as a function of the length of exposure to U.S. culture among East Asians with the 7/2-R allele of *DRD4*. Although important, the finding should be regarded as preliminary. First, we are possibly underpowered in this analysis (N = 63, around 30 in each group). Future work with greater sample size would be needed to replicate this finding. Second, since all participants were around the same age (20 years of age) when

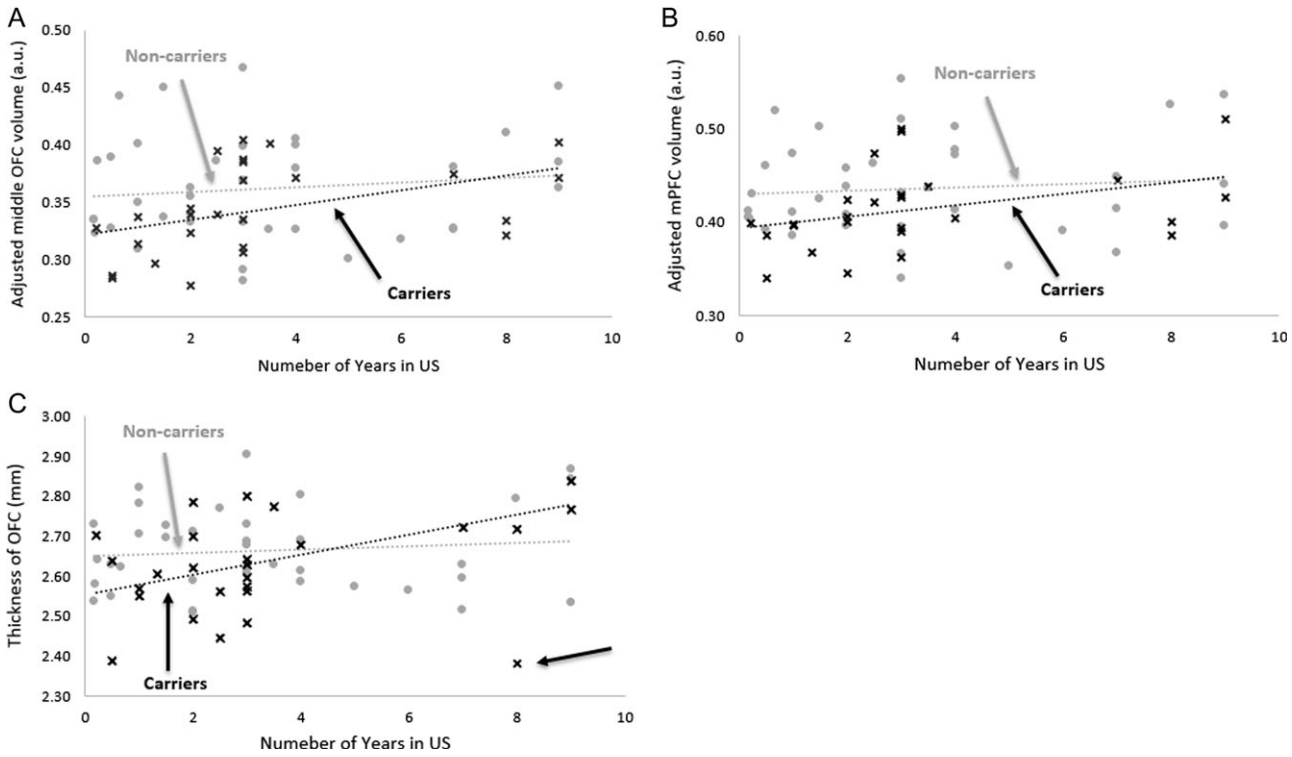

Figure 4. Correlation with number of years in the U.S. among East Asians who either carried or did not carry the 7- or 2-repeat allele of *DRD4*. A. A scatterplot showing GM volume of OFC as a function of number of years in the U.S. among East Asians. B. A scatterplot showing GM volume of mPFC as a function of number of years in the U.S. among East Asians. C. A scatterplot showing thickness of OFC as a function of number of years in the U.S. among East Asians (A potential outlier is indicated by the arrow).

tested, the number of years in the U.S. is confounded with how old or young they were when they moved to the U.S ($r = -.82$). Our data are consistent with the view that those who moved to the U.S. during their puberty years are more influenced especially if they were the carriers (Cheung et al. 2011). Third, one important function of OFC is to monitor and track reward contingencies in the environment (Rolls and Grabenhorst 2008). Hence, it remains possible that moving to new cultures may engage OFC functions more as one is learning the new cultural values and norms. If so, the same effect of exposure to a new culture may also be observed in anyone, regardless of cultural backgrounds, who goes to foreign countries.

Last, but not least, it is noteworthy that we replicated the inverse association between interdependent SC and GM volume of both mPFC (Wang et al. 2017) and OFC (Kitayama et al. 2017) in a new sample. This association is consistent with the hypothesis that interdependence is related to down-regulation of prefrontal functions such as making decisions based on preferences and forming clear self-concepts. We should hasten to add that while interdependent SC is higher for East Asians than for European Americans as predicted, this cultural difference in the interdependence SC did not mediate the cultural difference in the prefrontal cortical volume. Evidently, then, the sense of "me" as interdependent (as reflected in higher scores in the interdependent SC scale) is only one of many aspects of interdependence that differentiate the two cultural groups. These aspects may include daily routines, conventions, and practices, as well as tacit knowledge and implicit assumptions or lay theories that are shared in each group. They are likely to influence the cortical volume above and beyond the influences interdependent SC might have on it.

Some limitations of the current work warrant discussion. First, although our use of *DRD4* as a marker of environmental influence is novel, the evidence is still correlational and thus indirect. Future work must use a longitudinal design to investigate causal influences of cultural environments. Second, we focused exclusively on one gene, *DRD4*. This choice was based on a series of studies documenting the moderation of cultural influences by this gene (Sasaki et al. 2013; Kitayama et al. 2014; Silveira et al. 2016; Tompson et al. 2018). Nevertheless, the current evidence must be extended to other genes involved in dopamine signaling in the central nervous system (Forbes et al. 2009; Set et al. 2014; Kitayama et al. 2016). Only future work can tell whether *DRD4* is unique in some way as a potent moderator of cultural influence. Third, specific mechanisms involved in the change of cortical volume or thickness have yet to be fully understood. Nor do we know whether there might be any functional consequences of this change. These questions must be addressed in future work to better understand the role of brain structure as a mediating element of cultural influences on behavior.

Last, we postulated that the carriers of the 7/2-R allele of *DRD4* perform normative tasks more, which in turn, results in corresponding variations in the prefrontal GM volume. However, this hypothesis must be extended in more specific terms. One intriguing possibility involves culturally sanctioned food habits that reflect long-standing ecological niches of the respective cultural groups (Talhelm et al. 2014). In particular, foods that are available and sanctioned in Western cultures (e.g., red meat and milk) might have higher calories than foods that are common and sanctioned in East Asian cultures (e.g., grain and fish), which could yield differences in GM volume. If those who internalize

cultural values, including the carriers of the 7/2-R allele of *DRD4*, should eat culturally sanctioned foods more than non-carriers (Levine et al. 2016; Silveira et al. 2016), the observed cultural difference could be mediated by culturally sanctioned eating behavior.

For a long time, consideration of racial and ethnic differences in brain volume and shape has been tainted with charge of racism and genetic determinism (Gould 1996). Our work addressed the topic of local volumetric differences in brain structure, but with different methods and theoretical orientation. Methodologically, we controlled for TIV and focused on regionally specific GM volume and cortical thickness. Theoretically, we used a measure of self-construal (one critical dimension of culture) and specific *DRD4* polymorphisms as tools to illuminate the extent of cultural influences and thereby underscore the intriguing potential significance of experience in shaping brain differences across cultures.

Supplementary Material

Supplementary material is available at *International Journal for Quality in Health* Care online.

Funding

This work was supported by grants from National Science Foundation (BCS 0717982 and SES 1325881)

Notes

Conflict of Interest: None declared

References

Ashburner J, Friston KJ. 2000. Voxel-based morphometry – the methods. Neuroimage. 11(6):805–821.

Bakermans-Kranenburg MJ, Van Ijzendoorn MH. 2011. Differential susceptibility to rearing environment depending on dopamine-related genes: New evidence and a meta-analysis. Dev Psychopathol. 23(1):39–52.

Belsky J, Pluess M. 2009. Beyond diathesis stress: differential susceptibility to environmental influences. Psychol Bull. 135 (6):885–908.

Campbell JD, Trapnell PD, Heine SJ, Katz IM, Lavallee LF, Lehman DR. 1996. Self-concept clarity: Measurement, personality correlates, and cultural boundaries. J Pers Soc Psychol. 70(1):141–156.

Chee MWL, Zheng H, Goh JOS, Park D, Sutton BP. 2011. Brain structure in young and old East Asians and Westerners: Comparisons of structural volume and cortical thickness. J Cogn Neurosci. 23(5):1065–1079.

Cheung BY, Chudek M, Heine SJ. 2011. Evidence for a sensitive period for acculturation: Younger immigrants report acculturating at a faster rate. Psychol Sci. 22(2):147–152.

Chua HF, Ho SS, Jasinska AJ, Polk TA, Welsh RC, Liberzon I, Strecher VJ. 2011. Self-related neural response to tailored smoking-cessation messages predicts quitting. Nat Neurosci. 14(4):426–427.

Cole SW. 2014. Human social genomics. PLoS Genet. 10(8): e1004601.

Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, Buckner RL, Dale AM, Maguire RP, Hyman BT, et al. 2006. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage. 31(3):968–980.

Destrieux C, Fischl B, Dale A, Halgren E. 2010. Automatic parcellation of human cortical gyri and sulci using standard anatomical nomenclature. Neuroimage. 53(1):1–15.

Draganski B, Gaser C, Busch V, Schuierer G, Bogdahn U, May A. 2004. Neuroplasticity: Changes in grey matter induced by training. Nature. 427(6972):311–312.

Driemeyer J, Boyke J, Gaser C, Büchel C, May A. 2008. Changes in gray matter induced by learning – revisited. PLoS One. 3(7):e2669.

Fellows LK. 2011. Orbitofrontal contributions to value-based decision making: evidence from humans with frontal lobe damage. Ann N Y Acad Sci. 1239:51–58.

Fischl B, Dale AM. 2000. Measuring the thickness of the human cerebral cortex from magnetic resonance images. Proc Natl Acad Sci USA. 97(20):11050–11055.

Fischl B, van der Kouwe A, Destrieux C, Halgren E, Ségonne F, Salat DH, Busa E, Seidman LJ, Goldstein J, Kennedy D, et al. 2004. Automatically parcellating the human cerebral cortex. Cereb Cortex. 14(1):11–22.

Forbes EE, Brown SM, Kimak M, Ferrell RE, Manuck SB, Hariri AR. 2009. Genetic variation in components of dopamine neurotransmission impacts ventral striatal reactivity associated with impulsivity. Mol Psychiatry. 14(1):60–70.

Good CD, Scahill RI, Fox NC, Ashburner J, Friston KJ, Chan D, Crum WR, Rossor MN, Frackowiak RS. 2002. Automatic differentiation of anatomical patterns in the human brain: Validation with studies of degenerative dementias. Neuroimage. 17(1):29–46.

Gould SJ. 1996. The mismeasure of man. New York (NY): W. W. Norton & Company. p. 352.

Han S, Ma Y. 2014. Cultural differences in human brain activity: A quantitative meta-analysis. Neuroimage. 99:293–300.

Hayasaka S, Nichols TE. 2004. Combining voxel intensity and cluster extent with permutation test framework. Neuroimage. 23(1):54–63.

Heatherton TF, Wyland CL, Macrae CN, Demos KE, Denny BT, Kelley WM. 2006. Medial prefrontal activity differentiates self from close others. Soc Cogn Affect Neurosci. 1(1):18–25.

Kelley WM, Macrae CN, Wyland CL, Caglar S, Inati S, Heatherton TF. 2002. Finding the self? An event-related fMRI Study. J Cogn Neurosci. 14(5):785–794.

Kim HS, Sasaki JY. 2014. Cultural neuroscience: Biology of the mind in cultural contexts. Annu Rev Psychol. 65:487–514.

King AP, Muzik M, Hamilton L, Taylor AB, Rosenblum KL, Liberzon I. 2016. Dopamine receptor gene DRD4 7-Repeat Allele X maternal sensitivity interaction on child externalizing behavior problems: Independent replication of effects at 18 Months. PLoS One. 11(8):e0160473.

Kitayama S, King A, Hsu M, Liberzon I, Yoon C. 2016. Dopamine-system genes and cultural acquisition: The norm sensitivity hypothesis. Curr Opin Psychol. 8:167–174.

Kitayama S, King A, Yoon C, Tompson S, Huff S, Liberzon I. 2014. The dopamine D4 receptor gene (DRD4) moderates cultural difference in independent versus interdependent social orientation. Psychol Sci. 25(6):1169–1177.

Kitayama S, Markus HR, Kurokawa M. 2000. Culture, emotion, and well-being: Good feelings in Japan and the United States. Cogn Emot. 14(1):93–124.

Kitayama S, Park J. 2014. Error-related brain activity reveals self-centric motivation: Culture matters. J Exp Psychol Gen. 143(1):62–70.

Kitayama S, Salvador C. 2017. Culture embrained: Going beyond the nature-nurture dichotomy. Perspect Psychol Sci. 12(5): 841–854.

Kitayama S, Uskul AK. 2011. Culture, mind, and the brain: Current evidence and future directions. Annu Rev Psychol. 62:419–449.

Kitayama S, Yanagisawa K, Ito A, Ueda R, Uchida Y, Abe N. 2017. Reduced orbitofrontal cortical volume is associated with interdependent self-construal. Proc Natl Acad Sci USA. 114(30):7969–7974.

Levine CS, Miyamoto Y, Markus HR, Rigotti A, Boylan JM, Park J, Kitayama S, Karasawa M, Kawakami N, Coe CL, et al. 2016. Culture and healthy eating: The role of independence and interdependence in the United States and Japan. Pers Soc Psychol Bull. 42(10):1335–1348.

Lichter JB, Barr CL, Kennedy JL, Van Tol HHM, Kidd KK, Livak KJ. 1993. A hypervariable segment in the human dopamine receptor D4 (DRD4) gene. Hum Mol Genet. 2(6):767–773.

Maguire EA, Gadian DG, Johnsrude IS, Good CD, Ashburner J, Frackowiak RS, Frith CD. 2000. Navigation-related structural change in the hippocampi of taxi drivers. Proc Natl Acad Sci USA. 97(8):4398–4403.

Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH. 2003. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage. 19(3): 1233–1239.

Markus HR, Kitayama S. 1991. Culture and the self: Implications for cognition, emotion, and motivation. Psychol Rev. 98(2): 224–253.

Meaney MJ. 2001. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu Rev Neurosci. 24:1161–1192.

Nisbett RE, Peng K, Choi I, Norenzayan A. 2001. Culture and systems of thought: Holistic versus analytic cognition. Psychol Rev. 108(2):291–310.

Northoff G, Bermpohl F. 2004. Cortical midline structures and the self. Trends Cogn Sci. 8(3):102–107.

O'Doherty JP. 2011. Contributions of the ventromedial prefrontal cortex to goal-directed action selection. Ann N Y Acad Sci. 1239:118–129.

Reist C, Ozdemir V, Wang E, Hashemzadeh M, Mee S, Moyzis R. 2007. Novelty seeking and the dopamine D4 receptor gene (DRD4) revisited in Asians: Haplotype characterization and relevance of the 2-repeat allele. Am J Med Genet B Neuropsychiatr Genet. 144B(4):453–457.

Rolls ET, Grabenhorst F. 2008. The orbitofrontal cortex and beyond: From affect to decision-making. Prog Neurobiol. 86(3):216–244.

Sasaki JY, Kim HS, Mojaverian T, Kelley LDS, Park IY, Janusonis S. 2013. Religion priming differentially increases prosocial behavior among variants of the dopamine D4 receptor (DRD4) gene. Soc Cogn Affect Neurosci. 8(2):209–215.

Set E, Saez I, Zhu L, Houser DE, Myung N, Zhong S, Ebstein RP, Chew SH, Hsu M. 2014. Dissociable contribution of prefrontal and striatal dopaminergic genes to learning in economic games. Proc Natl Acad Sci USA. 111(26):9615–9620.

Sheese BE, Voelker PM, Rothbart MK, Posner MI. 2007. Parenting quality interacts with genetic variation in dopamine receptor D4 to influence temperament in early childhood. Dev Psychopathol. 19(4):1039–1046.

Silveira PP, Gaudreau H, Atkinson L, Fleming AS, Sokolowski MB, Steiner M, Kennedy JL, Meaney MJ, Levitan RD, Dubé L. 2016. Genetic differential susceptibility to socioeconomic status and childhood obesogenic behavior: Why targeted prevention may be the best societal investment. JAMA Pediatr. 170(4):359–364.

Singelis TM. 1994. The measurement of independent and interdependent self-construals. Pers Soc Psychol Bull. 20(5): 580–591.

Talhelm T, Zhang X, Oishi S, Shimin C, Duan D, Lan X, Kitayama S. 2014. Large-scale psychological differences within China explained by rice versus wheat agriculture. Science. 344(6184):603–608.

Tang Y, Hojatkashani C, Dinov ID, Sun B, Fan L, Lin X, Qi H, Hua X, Liu S, Toga AW. 2010. The construction of a Chinese MRI brain atlas: A morphometric comparison study between Chinese and Caucasian cohorts. Neuroimage. 51(1): 33–41.

Tompson SH, Huff ST, Yoon C, King A, Liberzon I, Kitayama S. 2018. The dopamine D4 receptor gene (DRD4) modulates cultural variation in emotional experience. Cult. Brain. 65:1157.

Triandis HC. 1989. The self and social behavior in differing cultural contexts. Psychol Rev. 96(3):506–520.

van der Meer L, Costafreda S, Aleman A, David AS. 2010. Self-reflection and the brain: A theoretical review and meta-analysis of neuroimaging studies with implications for schizophrenia. Neurosci Biobehav Rev. 34(6):935–946.

Wang E, Ding YC, Flodman P, Kidd JR, Kidd KK, Grady DL, Ryder OA, Spence MA, Swanson JM, Moyzis RK. 2004. The genetic architecture of selection at the human dopamine receptor D4 (DRD4) gene locus. Am J Hum Genet. 74(5):931–944.

Wang F, Peng K, Chechlacz M, Humphreys GW, Sui J. 2017. The neural basis of independence versus interdependence orientations: A voxel-based morphometric analysis of brain volume. Psychol Sci. 28(4):519–529.

Windhorst DA, Mileva Seitz VR, Linting M, Hofman A, Jaddoe VW, Verhulst FC, Tiemeier H, van IJzendoorn MH, Bakermans-Kranenburg MJ. 2015. Differential susceptibility in a developmental perspective: DRD4 and maternal sensitivity predicting externalizing behavior. Dev Psychobiol. 57(1):35–49.

Zilles K, Kawashima R, Dabringhaus A, Fukuda H, Schormann T. 2001. Hemispheric shape of European and Japanese brains: 3-D MRI analysis of intersubject variability, ethnical, and gender differences. Neuroimage. 13(2):262–271.