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Cortical Surface Based Threshold Free Cluster Enhancement and Cortex-wise Mediation

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Abstract

Threshold-free cluster enhancement (TFCE) is a sensitive means to incorporate spatial neighborhood information in neuroimaging studies without using arbitrary thresholds. The majority of methods have applied TFCE to voxel-wise data. The need to understand the relationship among multiple variables and imaging modalities has become critical. We propose a new method of applying TFCE to vertex-wise statistical images as well as cortex-wise (either voxel- or vertex-wise) mediation analysis. Here we present TFCE_mediation, a toolbox that can be used for cortex-wise multiple regression analysis with TFCE, and additionally cortex-wise mediation using TFCE. The toolbox is open source and publicly available (https://github.com/trislett/TFCE_mediation). We validated TFCE_mediation in healthy controls from two independent multimodal neuroimaging samples (N=199; N=183). We found a consistent structure-function relationship between surface area and the first independent component (IC1) of the N-back task, that white matter fractional anisotropy is strongly associated with IC1 N-back, and that our voxel-based results are essentially identical to FSL randomise using TFCE (all $P_{FWE}<0.05$). Using cortex-wise mediation, we showed that the relationship between white matter FA and IC1 N-back is mediated by surface area in the right superior frontal cortex ($P_{FWE}<0.05$). We also demonstrated this same mediation model is present using vertex-wise mediation ($P_{FWE}<0.05$). In conclusion, cortex-wise analysis with TFCE provides an effective analysis of multimodal neuroimaging data. Further, cortex-wise mediation analysis may identify or explain a mechanism that underlies an observed relationship among a predictor, intermediary and dependent variables in which one of these variables is assessed at a whole brain scale.

Introduction

Cortical thickness and surface area are distinct neuroanatomical phenotypes that together compose cortical grey matter volume. Both phenotypes are highly heritable (h^2>80%), but the genetic contribution is essentially distinct suggesting that they should be considered separately in structural neuroimaging studies (Chen, et al., 2012; Hawrylycz, et al., 2012; Panizzon, et al., 2009). However, to date the majority of studies apply voxel-based morphometry (VBM) treating cortical thickness and surface area as the same entity, or they exclusively focus on cortical thickness or surface area without little regard for the their relationship. This separation extends to the analysis of other neuroimaging measures acquired using magnetic resonance imaging (MRI) modalities (e.g., functional activation, and white matter structure). There is a significant need for statistical methodologies that connect multimodal imaging with clinical, cognitive, and functional phenotypes, especially since rich neuroimaging and phenotypic databases, such as the Human Connectome Project and the Alzheimer's Disease Neuroimaging Initiative are available (Petersen, et al., 2010; Van Essen, et al., 2012),. Moreover, methodological heterogeneity in examining different MRI modalities leads to difficulties in comparing the results across different studies. For example, smoothing and thresholding techniques, applied to neuroimaging studies to improve sensitivity by incorporating spatial neighborhood information, can be arbitrary and are inconsistent between studies. The varying thresholds for these settings can have important differential effects on statistical analyses that favor the survival of focal or wide spread effects at thresholds of statistical significance (Genovese, et al., 2002).

Threshold free cluster enhancement (TFCE) has been shown to robustly improve sensitivity among a wide range of signal shapes and varying levels of signal-to-noise ratio (Smith and Nichols, 2009). TFCE is a summation of cluster size at various significance levels, and as such solves the problem of determining a primary significance level for cluster size-based statistics (Smith and Nichols, 2009). It is currently employed in FSL's (FMRIB Software Library) VBM, tract-based spatial statistics (TBSS), and subcortical shape analyses (Jenkinson, et al., 2012; Smith, et al., 2004). However, to date, TFCE has mostly been applied to analyses of voxel-wise data. Only few studies have applied TFCE to vertex-based analyses of surface area and cortical thickness, and no statistical analysis toolbox has been published examining the validity and utility (Hill, et al., 2010). In addition, TFCE is generally not applied to more complex statistical models linking neuroimaging and behavioral outcome measures.

Taken together, there is a need to develop a fast and effective means to perform TFCE on brain-wide vertex data, surface area and cortical thickness, as well as integrate neuroimaging phenotypes with behavioral measures using TFCE. Cortex-wise (used henceforth to denote both vertex- or voxel-wise) mediation with TFCE may infer statistically causal relationships among multimodal imaging data. At a cortex-wise scale, mediation provides additional spatial information of an association beyond statistical causal inference. For instance, it could be determined which voxels or vertices are mediating the effect of genotype on a neurocognitive relevant phenotype. If significant cortex-wise mediation occurs, relationships between specific genotypes and subsets of vertices or voxels that also predict the neurocognitive functioning. In this way, cortex-wise mediation could be considered a data reduction strategy and a powerful way to connect seemingly disparate biological and behavioral measures. Furthermore, cortex-wide mediation with TFCE can be applied broadly across all neuroimaging modalities including, but not limited to, voxel-based analyses of diffusion tensor imaging (DTI), and vertex-based analyses of cortical thickness and surface area. Therefore, TFCE may be a powerful means for cortex-based analyses where voxel-wise mediation can be performed.

Here, we introduce novel, a freely available statistical toolbox TFCE_mediation for: 1) analysis of cortical surfaces at a vertex-wise scale as well as incorporating mediation analysis, and 2) voxel-wise mediation analysis of white matter structure. Importantly, TFCE_mediation employs a fast and generalizable algorithm for TFCE. We first describe in detail the application of TFCE to surface-based analyses and to white matter fraction anisotropy (FA). Next, we describe cortex-wise mediation with TFCE for cortical surfaces and white matter FA. Then, we validate and assess the utility of TFCE_mediation in two independent samples of healthy controls in well-studied as well as neurocognitive phenotypes.

Materials and Methods

General description

TFCE_mediation were developed as statistical front-ends for already established MRI processing platforms. The main focus of our software is to provide TFCE-based mediation analysis which, to the best of our knowledge, no other software packages include. The software provides a unique combination of (1) a fast least-squares solution for multiple regression analyses, (2) a cortex-wise mediation analysis, (3) efficient memory optimization to reduce the amount of RAM for large scale analyses, 4) creation of the midthickness surface, (5) a customizable definition of adjacency of the vertices via geodesic distance, and (6) an optimized TFCE algorithm that can work with all data types. To improve accessibility and allow continued development from the neuroimaging community, both were written in python and cython using publicly available software packages including NumPy, SciPy, and Nibabel. TFCE_mediation is publicly available under GPL 3.0 license (https://github.com/trislett/TFCE_mediation). For surface-based analysis, TFCE_mediation uses vertex-wise data applying standard procedures from Freesurfer Software Suite (http://surfer.nmr.mgh.harvard.edu/). For voxel-based analyses, any standard processing may be used for a four-dimensional strandarized NifTi image and binarized mask. Beyond conventional multiple regression strategies, TFCE_mediation is able to perform cortex-wise mediation analysis with TFCE enhancement. We have recently shown that voxel-wise mediation with TFCE is a potentially useful method demonstrating statistically causal relationships among genetic variants, white matter FA, and working memory performance (Lett, et al., 2016).

Although we here use FSL and Freesurfer processing in our analyses, TFCE_mediation can be used with other software including: ANTS, CIVET, AFNI, and SPM. The core components after the initial Freesurfer surface creation are novel and have not been copied or adapted from any source code in Freesurfer and FSL. Freesurfer is only necessary for creating the cortical thickness or surface area template of all subjects. We also provide a number of tools to perform additional analyses which may be useful without employing TFCE_mediation including: a fast and parallelizable Box-Cox transformation on vertex data, efficient calculation of TFCE, and defining the adjacency of vertices. TFCE_mediation was designed to be fast, embarrassingly parallel (parallelized in independent blocks), modular, resumable, and with low random-access memory (RAM) usage. Therefore, they are ideal for large scale computing platforms, as well as for use on personal computers. Furthermore, we reduced the statistical 'black-box' by having our source code easily accessible to any user with moderate programming experience.

TFCE_mediation

Cluster size calculations are clearly defined in volume-based analyses, as the adjacency of two voxels (i.e., if they belong to the same cluster) is defined by voxel geometry. However, the adjacency of vertices is less defined on surfaces. Compared to voxel-based analyses where adjacency of voxels is defined by the size of the voxels, vertices can have any number of neighbors at varying distances (i.e., a number of triangles connected to a single point). A surface-based equivalent of TFCE is used to determine statistical significance.

For TFCE_mediation voxel-based analyses, any four dimensional NifTi volume (three image for each subject registered to a common template by the number of subjects) and a binarized mask are used as input. In contrast, TFCE_mediation vertex-based analyses require initial processing using Freesurfer (http://surfer.nmr.mgh.harvard.edu/). Freesurfer's recon-all is performed on each subjects T1-weight images for preprocessing and creation of surface for analysis (Dale, et al., 1999). TFCE_mediation uses a script that creates, a template of all subjects is created using standard Freesurfer methods. Therefore, Freesurfer is only necessary for creating the cortical thickness or surface area template of all subjects.

A list of subjects is submitted to create a template with an option for either cortical thickness or surface area. For each subject, surface data is then resampled to the 'fsaverage' using surface-based registration where the cortical manifold is inflated to a sphere and homologous neuroanatomical features are matched (Fischl, et al., 1999a; Fischl, et al., 1999b). After registration, all subjects are merged into a single image separately for each hemisphere. The images are smoothed using full-width half maximum (FWHM) of 3mm. The smoothing is performed to ensure proper fit of surface values (Figure 1). Next, mean surface-based data are created for each hemisphere. Since it has been suggested that surface area follows roughly a lognormal distribution (Winkler, et al., 2012), as an additional step, the unsmoothed images can also be normalized using a fast multivariate power (Box-Cox) transformation tool supplied in TFCE_mediation (Box and Cox, 1964).

Next, cortex-wise statistical analysis is applied. All statistical analysis are completely independent of any other neuroimaging analyses packages. There are two methods of analysis to choose from: multiple regression or mediation. The common steps are explained below. The predictor (independent) variables and covariates of no interest are dummy coded as separate files. A two-step least squares regression is performed. The covariates are regressed against the cortex-wise data (e.g., for vertex-based analysis: 163842 vertices * number of subjects* 2 hemispheres) to produce a corrected residual image. The predictor variables are then regressed against the residual image to produce T-statistic images. All vectorized least-squared regression analyses are performed using a fast, compiled cython algorithm (approximately 180 ms for a large array, 163842 vertices * 382 subjects * 2 hemispheres, on a single 2.4 GHz Intel Xeon E5-2620 v3 Processor). The two-step least square regression is common technique in structural equation modelling, and is required for mediation analysis. For multiple regression analysis, TFCE_mediation has the option to perform a single-step regression. Nevertheless, the two-step procedure offers a number of advantages. For randomization (permutation analysis), it reduces the size of the matrices, and consequently, the RAM and computation time required. It also reduces potential bias that can be introduced during randomization from combining datasets. The latter is particularly important for cortex-wise mediation, as a confounding variable may only be relevant to parts of the model. If the predictor variable is not completely independent from covariates, 'regressor-tools' supplied with TFCE_mediation has the option to regress out the effect of the covariates from the predictor variable and return the residuals, to ensure the results would be the same using a single multivariate analysis with multiple covariates. Alternatively, 'regressor-tools' may be used to orthogonalize the predictor variable and covariates which would also produce similar results to a single multivariate analysis.

The T-statistic images then undergo TFCE. Our TFCE algorithm is generalizable to all types of neuroimaging modalities. Prior to analysis, an adjacency set for each cortex-wise measure needs to be defined. We provide two strategies for calculating the adjacency of vertices either via a simple calculation of the triangular mesh, or through the exact geodesic distance on the fsaverage midthickness projection (Mitchell, et al., 1987; Surazhsky, et al., 2005). The latter can be performed using the supplied adjacency lists (1mm, 2mm or 3mm geodesic distance) or created using 'vertex-create-adjac-list' tool included with TFCE_mediation. We chose the cortical midthickness as a surface to undergo TFCE since each square millimeter of the surface area represents approximately the same cortical volume irrespective of gyral or sulcal location (Van Essen, 2005). Thus, the extent clustering performed in TFCE is less biased based on cortical folding. The default adjacency distance used in TFCE_mediation is 3mm (for comparisons, see Figure 1; Figures S1-S2); however, both the surface projection distance and adjacency distance can be customized using 'vertex-create-adjac-list'. We use an efficient TFCE algorithm based on the disjoint sets data structure (approximately 200 ms for each hemisphere, ~300000 vertices, on a single 2.4 GHz Intel Xeon E5-2620 v3 Processor). The T-statistic images undergo TFCE with the same setting recommended in FSL's recommendation for 2D data (E=1, H=2) (Smith, et al., 2006; Smith and Nichols, 2009). It should be noted that the 2D data setting have not been previously validated, and they constitute a trade-off between sensitivity and false-discovery rate by weighting the analysis on either strength of statistic (i.e., H), and the degree of connectivity (E; Figure 2, Table S1). For voxel and vertex based analyses, the TFCE setting and connectivity are fully customizable.

Significance of the TFCE transformed T-statistic image can only be assessed via permutation testing. The entire multivariate process is repeated with the order of the subjects being permutated. From each permutated TFCE image, the maximum TFCE value from both hemispheres is extracted and written to a text file. After all permutations are completed, the maximum TFCE values from each permutation are ordered and the family-wise error rate corrected p-value (P_{FWE}) is determined by its rank on the ordered list. For example, the 500th highest value from 10000 permutations corresponds to P_{FWE}=0.05. Finally, one minus P_{FWE} images are produced by finding the nearest ranked value corresponding to each vertex TFCE value. The output is analogous to the corrected p-value images outputted by FSL randomise (Winkler, et al., 2014).

Similarly, for employing cortex-wise mediation, significance of the Z-statistic cortex-wise measure is assessed using TFCE and randomization (maximum TFCE values from 10000 permutations), and subsequently P_{FWE} images are produced. It should be noted that randomization strategies to assess significance of the Sobel equation are considered to be a better alternative than parameter tests that impose distribution assumptions (Preacher and Hayes, 2008). For further details regarding the cortex-wise mediation analysis see the Supporting Information.

Validation of Vertex-based TFCE using receiver-operator characteristic (ROC)

Validation of TFCE has previously been performed on voxel-based statistics (Smith and Nichols, 2009). We performed a complementary analysis instead using vertex-based statistics. We used a real surface based T-statistic image as the 'ground truth' as compared to the same image with added Gaussian noise (mean=0, unit variance). All T-statistic images underwent TFCE, and P_{FWE} images were produced using non-parametric testing as described in the previous sections. To assess the sensitivity (i.e., true positive rate) and false positive rate for each ROC curve, 1000 P_{FWE} image with random Gaussian noise were created. All images were binarized at $P_{FWE}<0.05$ threshold, a score was produced for each vertex (i.e., mean number of significant values among the 1000 images with Gaussian noise), and the score was tested against the 'ground truth' $P_{FWE}<0.05$ binarized image. The resulting ROC curves were then plotted, and the area under the curve (AUC) was calculated (Figure S1, Figure S2). We examined the ROC curves among different FWHM values and geodesic distances used in the TFCE adjacency sets (Figure S1). We followed-up our analysis using a FWHM of 3mm and a geodesic distance of 3mm, except now we examined the ROC curves at increasing levels of noise from one to three times the Gaussian added than in the previous analysis (Figure S2). That is, we first examined the ROC curves among different surface based parameters, and then used ROC curves to assessed TFCE at increasing levels of noise.

Validation of vertex-based TFCE using a simulated effect on a known cluster size

We examined the simulated effects of a known effect and cluster size on vertex-wise surface area from 200 subjects. The subjects were randomly selected and equally divided into two groups of 100. Using bilateral masks that are approximately equivalent to Brodmann area 44, one hundred subjects had an added mean difference (from the other 100 subjects) that was Gaussian distributed (mean=0.05, standard deviation=0.179) that corresponds to the average power (beta) equal to 80% among all vertices of the masks. The power was determined via mean and standard deviation of the simulated effect with an equal sampling ratio, and for a sample size of 200. Multiple regression analysis was then performed at different TFCE setting with age, sex, and site as covariates (Figure 2; Table S1). We then plot 1-P_{FWE} images for various TFCE setting and uncorrected outlining the original masks (Figure 2). To assess the sensitivity, specify, accuracy, and false discovery rate for each TFCE setting we compared P_{FWE}<0.05 binarized images after 10000 permutations to the 'ground truth' of the bilateral masks.

Validation of TFCE_mediation vertex- and voxel-based analyses

To validate TFCE_mediation we a) compared the results obtained by vertex-based analysis between two independent samples and b) compared the results obtained of voxel-based analysis to FSL randomise. We hypothesized that TFCE_mediation will produce replicating results and that a meaningful mediation would exist. Thus we expected to demonstrate that the basic statistical framework is correct and expands on existing tools by using mediation analysis among imaging modalities. We expected TFCE_mediation to perform faster with lower RAM usage compared to other tools. It should be stressed we chose multiple strategies of validation: analysis of results that are established, replication across both samples, and demonstration of utility among different neuroimaging phenotypes. Specifically, we aimed to 1) examine sex-specific differences in cortical thickness consistently reported in the literature (Im, et al., 2006; Luders, et al., 2006; Lv, et al., 2010), 2) examine if cortical surfaces predict functional connectivity consistently across two samples, 3) show that TFCE_mediation voxel-wise multiple regression and FSL's randomise obtain the same results regarding the association between white matter FA and functional connectivity during the N-back task, and 4) demonstrate integration of neuroimaging phenotypes by showing that the relationship between cortical surfaces and functional connectivity is mediated by white matter FA using TFCE_mediation voxel-wise and vertex-wise analyses.

Participants

Two healthy control samples were used to assess the validity and utility of TFCE_mediation. All subjects were healthy German volunteers with parents and grandparents of European origin (self-report) who were recruited in Berlin, Bonn and Mannheim. Sample characteristics are described in Table 1. All participants gave prior written informed consent. No participant reported a lifetime or family history of schizophrenia or affective disorder. The study was approved by the local ethics committees of the universities of Heidelberg, Bonn, and Charité Berlin.

Image Acquisition and Preprocessing

See the Supporting Information for details of the neuroimaging acquisition and preprocessing of the T1-weighted, BOLD fMRI, and diffusion-weighted MRI scans for the MooDS and NGFN_PLUS samples.

Statistical Analysis

All analyses were performed using multiple regression as discussed in detail in the previous sections. Cortex-wise statistical analyses were significant at an alpha less than 0.05 after correction for FWE determined by the maximum TFCE values from 10000 permutations. Multiple comparisons correction across neuroimaging modalities were not performed.

To assess the utility of TFCE_mediation we first examined the effect of sex on cortical thickness. Next, to evaluate structure-functional relationships, we looked for association among cortical surfaces and a general measure of brain network activation during concurrent fMRI and a visuospatial N-back task.

We performed cortex-wise mediation analysis since we observed a significant association among IC1 N-back and surface area, and as well as among IC1 N-back and white matter FA. Our general model included FA as the independent variable, surface area as the mediator, and IC1 N-back as the dependent variable. Our first model used voxel-wise FA as the independent variable, the mean right superior frontal surface area from the Desikan-Killiany Atlas (Desikan, et al., 2006) as the mediator, and IC1 N-back as the dependent variable. We follow-up up our analysis with surface as the independent variable and FA as the dependent variable to test the directionality of the association between FA and surface area.

We applied the same cortex-wise mediation model except we used the significant cluster mean FA values from the voxel-based mediation analysis as a region of interest, and surface area as vertex-wise variable. The purpose of this analyses was to demonstrate that mediation at the vertex-wise level using vertex-based mediation analyses produces similar results to voxel-wise analysis. The expected results would be cortex-wise mediation at the right superior frontal surface area.

Results

Selection of proper smoothing levels and surface adjacency definition

Two dimensional smoothing is performed to ensure proper inter-subject registration. The minimal FWHM should be selected since TFCE is performed downstream on the T-statistic midthickness surface. We examined the effect on surface area at four FWHM thresholds: no smoothing, 1 mm, 2 mm, and 3 mm FWHM. Without FWHM there is a clear, poor fit among the midthickness vertices (Figure 1). A 3mm FWHM is roughly equivalent to smoothing among each voxel and its neighbor in all directions, and there seems to be proper fit among the midthickness surface (Figure 1). After introducing random Gaussian noise (mean=0, unit variance) to the T-statistic images, compared to the T-statistic images without noise the ROC curves were remarkably similar among all parameters (Figure S1). The AUC values were all greater than 0.97 suggesting TFCE is robust again noise at all adjacency definitions and all FWHM (Figure S2). At a FWHM of 3mm and 3mm geodesic distance, the vertex-based TFCE was relatively resilient against increasing levels of noise (all AUC>0.97; Figure S2). The ROC curves are similar to voxel-based TFCE (Smith and Nichols, 2009), suggesting that vertex-based TFCE is similarly robust again noise.

Validation of surface-based TFCE analysis using a simulated effect on real data

Surface-based TFCE provided significant results with high specify and accuracy among all of the different values of H or E examined; however, differences in sensitivity and false discovery rate are present with minor changes to these TFCE parameters (all $P_{FWE}<0.05$; Figure 2). In general, the effect of H weights the TFCE value towards the strength of the association (i.e., the t-value), and the effect of E weights the TFCE value on the level of connectivity (as expected, see (Smith and Nichols, 2009)). Against the 'ground-truth' bilateral Broadmann area 44 mask, the FSL recommended settings for two-dimensional surfaces (H=2, E=1) had a high degree of sensitivity (right: 0.798, left: 0.666), but also had a high degree of false positive rate (right: 0.293; left: 0.702; Table S1). The setting with greatest accuracy was H=2, and E=2/3 (right: 0.981; left: 0.977) had relatively high specificity (right: 0.638; left: 0.192), and low false discovery rate (right: 0.063; left: 0.110 Table S1).

Vertex analysis of sex differences in cortical surfaces

Across both samples, we observed significantly greater cortical thickness in the motor and associative cortices, and less cortical thickness in the right temporal region of females ($P_{FWE}<0.05$; Figure S3). Moreover, females had greater cortical thickness independently in each sample ($P_{FWE}<0.05$, Figure S4). On a single 2.4 GHz Intel Xeon E5-2620 v3 Processor, the TFCE_mediation randomization for a block of 200 permutations elapsed ~ 7.3 minutes using approximately with 384 subjects using 1.5 GB of RAM. Using TFCE_mediation parallel processing with 12 cores, 10000 permutations took ~50 minutes using approximately 20.5 GB of RAM.

Vertex analysis of cortical surfaces and N-back functional activation

Our ICA analysis revealed eight independent components with the first independent component (IC1) explaining 16.5% of variance, robustly fitting the 2-back greater than 0-back contrast (model: $F_{1,124}$=2269.0, $p<1.0 \times 10^{-5}$; contrast [2>0]; z=19.1,$p<1.0 \times 10^{-5}$), and fitting the spatial activation pattern of the N-back task (Figure S5) (Owen, et al., 2005). Among both samples, there was a significant association among N-back IC1 and surface area ($P_{FWE}<0.05$, Figure 3) after correcting for age, sex, sample, and site. Importantly, there was significant association among N-back IC1 and surface area in sample 1 ($P_{FWE}<0.05$), and independently in sample 2 ($P_{FWE}<0.05$) with overlap in the right DLFPC, left medial temporal lobe, as well as sections of the precuneus and occipital regions (Figure S6). There was no significant association with cortical thickness.

Voxel analysis of white matter FA and N-back activation with comparison to FSL randomise

TFCE_mediation has the option perform a two-step least squares regression. This approach can be directly compared to results from FSL randomise for the TBSS skeleton. In sample 1, the white matter FA skeleton strongly predicted N-back IC1 using both TFCE_mediation and FSL randomise ($P_{FWE}<0.05$), particularly in the anterior corpus callosum (Figure 4). Furthermore after 10000 permutations, the corrected 1-P value images are virtually identical (Figure 4; Dice's coefficient [$P_{FWE}<0.1$] = 0.999; Dice's coefficient [$P_{FWE}<0.05$] = 1.00; Dice's coefficient [$P_{FWE}<0.01$] = 1.00). On a single 2.4 GHz Intel Xeon E5-2620 v3 Processor, the TFCE_mediation randomization performed approximately two times faster than FSL's randomise (approximately 120000 voxels; TFCE_mediation: ~ 2.6 minutes per 200 permutations with 199 subjects compared to FSL randomise:

~ 6 minutes per 200 permutations); moreover, the RAM required was much less (TFCE_mediation: ~400 MB per core compared to FSL randomise: ~7 GB per core). Using TFCE_mediation parallel processing with 12 cores, 10000 permutations took ~20 minutes and used approximately 4.5 GB of RAM.

Cortex-wise mediation

Voxel-wise FA predicted right superior frontal surface area particularly in the right superior corona radiata and right fornix (Path A: $P_{FWE} < 0.05$; Figure S7). Furthermore, the right superior frontal surface area was associated with IC1 N-back (Path B; t=2.1, p=0.04). In the previous section we demonstrated that voxel-wise FA predicts IC1 N-back (Path C; Figure 4). After 10000 permutations, there was significant voxel mediation particularly in right superior corona radiata, and the right fornix ($P_{FWE}<0.05$; Figure 5). There was no significant voxel-wise mediation with surface area as the independent variable, FA as the independent variable, and IC1 N-back as the dependent variable ($P_{FWE}>0.05$).

For our vertex-based model, we used the mean FA as the independent variable, whole brain surface area as the mediator, and IC1 N-back as the dependent variable. Mean FA strongly predicted surface area throughout the cortex, particularly in the anterior cingulate cortices, precuneus, and superior frontal regions (Path A, $P_{FWE}<0.05$; Figure S8). In the previous section, we demonstrated that cortical surface area is associated with IC1 N-back (Path B; Figure 3, Figure S6), and mean FA strongly predicted IC1 N-back (Path C; t=3.7, $P = 2.7 \times 10^{-4}$). There was significant mediation particularly in the right superior frontal cortex, although other regions were also significant including the left superior frontal cortex ($P_{FWE}<0.05$, Figure 6). There was no significant vertex-wise mediation with surface area as the independent variable ($P_{FWE}>0.05$).

Discussion

This study had multiple objectives: (1) to demonstrate TFCE_mediation is a useful tool to perform whole brain analyses of cortical thickness and surface area employing TFCE, (2) to show that the two step regression technique, provides the same results as FSL's randomise for the TBSS white matter FA skeleton, (3) to establish the utility of cortex-wise mediation analysis, and (4), release TFCE_mediation as freely available and open-source software. We showed that TFCE_mediation showed similar sex differences in cortical thickness. Furthermore, surface area predicted IC1 N-back after TFCE in both samples. Whole brain FA also predicted N-back IC1, and the two-step regression procedure of TFCE_mediation gave effectively identical results as FSL randomise. Last, we demonstrated that the association between surface area and N-back IC1 is mediated by white matter FA using voxel-wise statistics for both surface area and skeletonized white matter FA. Therefore, we provided a potential relationship among three distinct neuroimaging modalities suggesting that TFCE_mediation may be an efficacious and valid means to assess cortical structure and mediating factors at a cortex-wise scale.

In addition to our primary goal to demonstrate the validity and utility of TFCE_mediation, we have provided some potentially interesting neuroscientific findings. We observed in two independent samples larger bilateral cortical thickness in the primary motor and associative cortices of females compared to males, which replicates previous findings (Im, et al., 2006; Luders, et al., 2006; Lv, et al., 2010). Furthermore, we demonstrated that functional activation during the N-back visuospatial working memory task is consistently associated with surface area in the right DLPFC and left medial temporal lobe independently in both samples. To our knowledge, the association between N-back fMRI activation and whole brain surface area has not been previously examined although the regions found are typically associated with working memory functioning. Since cortical thickness alone was not associated with IC1 in the N-back test, our results suggest both surface area as well as cortical thickness need to be investigated. We found a strong association between white matter FA throughout the brain with IC1 N-back. This structure-function relationship is in-line with previous research demonstrating an association between white matter FA and working memory dysfunction (Lett, et al., 2016; Lett, et al., 2014; Takeuchi, et al., 2010; Vestergaard, et al., 2011). Furthermore, we demonstrated that white matter FA terminating in the right superior frontal cortex mediates the association of IC1 N-back between average surface area in that region, that may provide link among three distinct neuroimaging modalities. Mediation was not present when surface area was the independent variable that could suggest a directionality to results that highlights the need for a priori hypotheses of the cortex-wise mediation models. Moreover, our mediation analysis was conducted primarily to validate our voxel- and vertex-wise mediation. Therefore, these are secondary findings, and should be treated as such.

For surface based analyses, we found the FWHM of 3mm to spatially smooth the vertex data seems to provide the correct balance among over-smoothing, and thus protecting against spurious surface values and over estimating the extent cluster of TFCE. This transformation implies that there is some data loss. However, we were able to demonstrate both focal effects of greater cortical thickness in the motor cortex of females, as well as more widespread association among surface area and IC1 N-back suggesting the benefits of TFCE outweigh the data lost from smoothing. According to our ROC curves, the adjacency definition performed well among all parameter, and it was best when selecting a triangular mesh (i.e., the immediately adjacent vertices) and the 3mm geodesic distance. We recommend using the 3mm geodesic distance as opposed to the triangular mesh since it incorporates the most information from neighboring vertices, and matches the FWHM smoothing. Further, the mesh is not uniform among vertices so TFCE may be biased where vertices are close together such as in sulci regions. We also observe that different values settings are sensitive enough produce significant results from a simulated effect on real surface area data. However, using different E and H values will provide a trade-off between sensitivity and false discover rate. Nevertheless, TFCE on vertex-wise statistic images was able to detect true associations at multiple levels of added noise and setting. Altogether, our analyses show that TFCE on cortical surfaces is robust against noise mirroring what is observed for TFCE on voxel surfaces (Smith and Nichols, 2009).

To best of our knowledge, TFCE_mediation is the only tool that determines the adjacency of vertices based on geodesic distance, as well as at the midthickness projected surface. At the midthickness projection each square millimeter of the surface area represents approximately the same cortical volume irrespective of gyral or sulcal location (Van Essen, 2005) suggesting at the midthickness skeleton the spatial connectivity is not biased based on brain topography. Furthermore, Box-Cox power transformation gives approximately the same results and max TFCE null distribution as the untransformed surface data. Therefore, these similar results suggest that the difference among variance and distribution normality among gyri and sulci do not have an appreciable effect at the midthickness surface after TFCE.

Mediation is a meaningful method to combine performance (or clinical) data with brain structure, and thus, potentially provides functional significance to brain structure associations. In particular, cortex-wise mediation may be sensitive to detect latent effects than only multiple regression analyses because of the randomization procedure. That is, significant mediation effects are less likely to occur by chance than correlation among two variables. This poses a challenge during permutation testing since randomizing of all variables would underestimate the null distribution. To ensure significance is not over represented, we indexed the randomization so that the relationship among the two non-cortex-wise variables is held constant. Furthermore, in our two-step regression procedure, nuisance variables are regressed prior to statistical analyses and randomization. Taken together, this produces a null distribution free of bias from design. Moreover, it should be noted that even though the Sobel Z statistic tends to be conservative (Mackinnon, et al., 1995), confounding variables can lead to significant mediation where there is no relationship. For instance, sex is a massive confounder on surface area that may drive a significant indirect effect. Therefore sex should be considered in all cortex-wise surface area analyses. In general, the same assumptions of the mediation outlined by Baron and Kenny should be followed for cortex-wise analyses (Baron and Kenny, 1986).

There are additional caveats that should be considered for cortex-wise mediation analysis. Mediation implies a causal association, but it must be stressed that this is a statistical association. It is statistically impossible to resolve the direction of mediator and dependent variable associations (Baron and Kenny, 1986). Therefore, it is important that directionality is guided by *a priori* hypotheses. For instance, we have previous shown that the effect of a genetic variant in the glutamate decarboxylase 1 gene on digit-span working memory performance is mediated by frontal white matter FA (Lett, et al., 2016). In this example, genotype, FA and cognitive performance are clearly independent and the direction of effects is clear. In contrast, when combining variables that are not clearly distinct, such as different neuroimaging modalities, the direction of effect can only be statistically inferred. Second, cortex-wise mediation should be used as a guide for further analyses. The TFCE values can only provide significance, and not effect size. Therefore, *post hoc* analyses should be performed to determine the degree of mediation that has occurred on Z-statistic masks that have not been TFCE transformed. Third, adequate sample size is necessary for mediation. Although it is difficult performing a power analysis for the indirect effect after TFCE, it has been argued that structural equation models ideally require a sample size of around 200 subjects (Fan, et al., 1999). As a general guideline, if any steps in the mediation are underpowered then cortex-wise mediation is also underpowered.

We have highlighted the use of multiple regression and mediation analyses; however, other statistical models could be incorporated such as mixed-model regression of longitudinal data, and genome-wide association study of cortex-wise data. The low RAM usage of our software allows cortex-wise analyses on very large datasets (e.g., N>1000) such as neuroimaging consortia which may be difficult with other software.

Conclusion

TFCE_mediation is a novel method for examining cortical thickness and surface area using TFCE. Furthermore, since TFCE_mediation cortex-wise mediation analyses can incorporate multimodal, biologically relevant data in a single analysis, they may be more powerful than either approach alone.

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Tables

Table 1. Characteristics of the samples

	Berlin	Bonn	Mannheim	Female:Male	Age (Mean±SD)
MooDS	N=56	N=67	N=76	92:107	34.2±9.8
NGFN_PLUS	N=55	N=70	N=58	82:101	42.6±12.2

SD, standard deviation; NGFN_PLUS = "NGFN PLUS - Genetics of Alcohol Addiction" (http://www.ngfn.de/en/alkoholabh__ngigkeit.html); MooDS = "NGFN PLUS - MooDS: Systematic Investigation of the Molecular Causes of Major Mood Disorders and Schizophrenia" (http://www.ngfn.de/en/schizophrenie.html)

Figure Legends

Figure 1. Raw T-statistic images transformed with TFCE for surface area ranging from full-width half maximum (FWHM) values from 0 to 3mm and adjacency values ranging from the triangular mesh to a distance of 3mm at the midthickness surface. Red to yellow vertices correspond to 50% of the vertex-wise maximum TFCE value to the maximum TFCE value. The images are overlaid on the midthickness projected surface.

Figure 2. The significant association of added simulated mean difference with a Gaussian distribution and standard deviation equivalent to beta of 80% for each vertices. The simulated effect was added to randomly real data (N=200) to labeled region of Brodmann Area 44 for each hemisphere. TFCE_mediation regression analysis was performed and P_{FWE} images were produced after 10000 permutations. There were significant associations among all 'H' and 'E' setting (P_{FWE}<0.05, red-yellow). The bars correspond to P_{FWE}<0.05 and lower. The images are overlaid on the midthickness projected surface. Covariates included age, sex, and site of the randomly selected subjects.

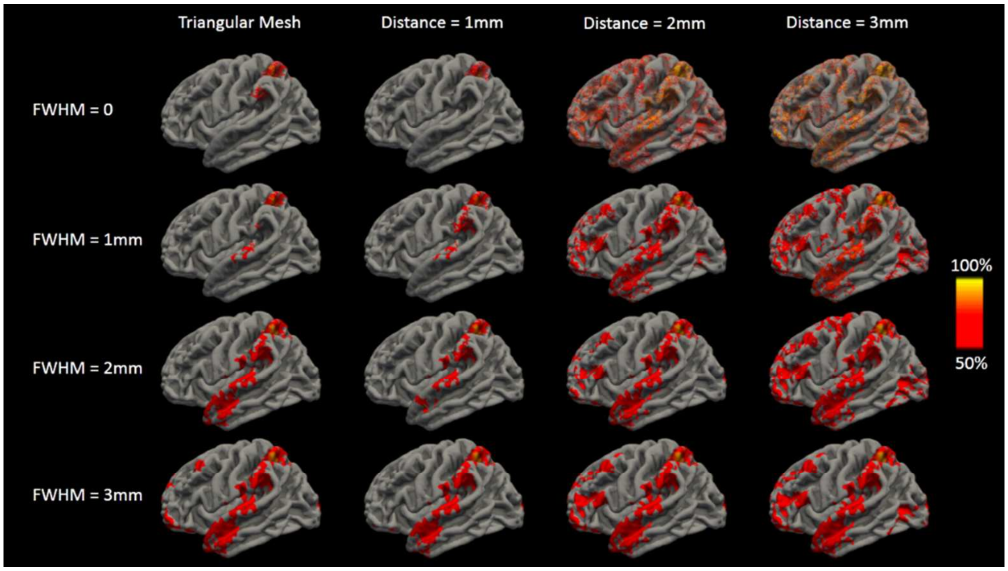
Figure 3. The association among cortical surface area and N-Back IC1 across both samples (N=382). Left: surface area predicted N-back IC1 across the cortex after TFCE P_{FWE}<0.05 after 10000 permutations. The red and blue bars correspond to P_{FWE} < 0.05 and lower. Right: partial correlations from the mean surface area values from significant clusters predicting N-Back IC1. The mean of the maximum left hemisphere cluster (17585.0 mm^2, max P_{FWE}<0.001) predicted IC1 N-back (partial R^2=0.056, $F_{1,374}$=22.3, p = 5.7 x 10^{-8}), and the mean of the maximum right hemisphere (22470.9 mm^2, max P_{FWE} =0.003) predicted IC1 N-back (partial R^2=0.033, $F_{1,374}$=12.7, p =4.1 x 10^{-4}). Covariates

included age, sex, site, and sample.

Figure 4. Association among white matter skeleton FA and IC1 (first independent component) of the N-Back working memory task in the MooDS sample. FA was strongly associated with N-Back IC1 across the TBSS skeleton particular in the corpus callosum. This result was consistent when statistical analysis was independently performed using: (a) FSL randomise, and (b) TFCE_mediation. Mean FA is transparent, and the red-yellow color bar represents significant ($P<0.05$) $1-P_{FWE}$ values corresponding to each voxel. Covariates included age, sex, and site.

Figure 5. Voxel-wise mediation analysis to examine the indirect effect of right superior frontal surface area mediating the correlation among FA and IC1 N-Back. There was significant mediation predominately from the right cingulum bundle ($P_{FWE}<0.05$). The red-yellow bars correspond to $1-P_{FWE}$ values ranging from p = 0.05 and lower of TFCE transformed Sobel Z statistics. Covariates included age, sex, and site.

Figure 6. Vertex-wise mediation analysis to examine the indirect effect of surface area mediating the correlation among mean cluster FA and IC1 N-Back. The red-yellow bar correspond to TFCE transformed Sobel Z statistics with $P_{FWE} < 0.05$ and lower. Covariates included age, sex, and site.



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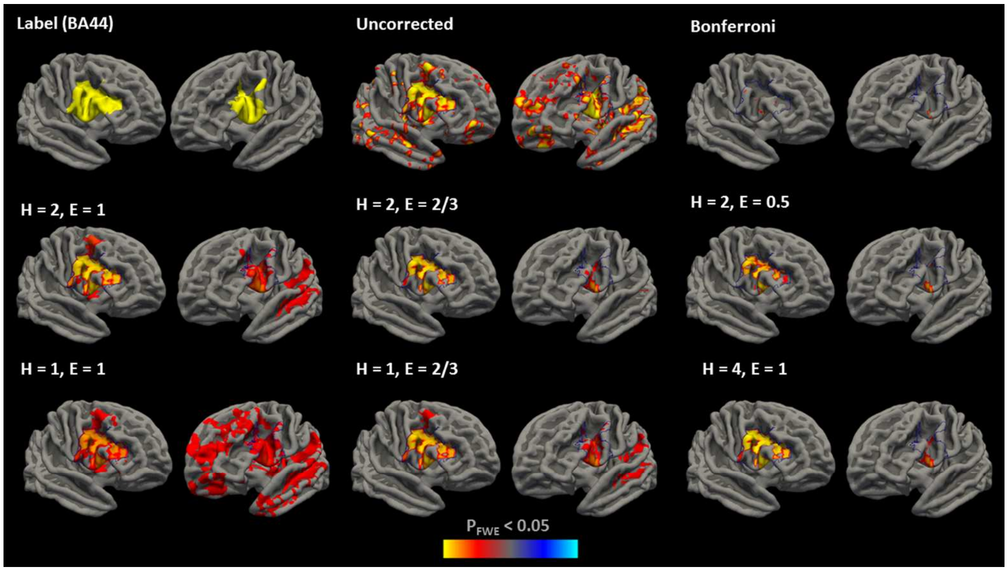
175x98mm (150 x 150 DPI)



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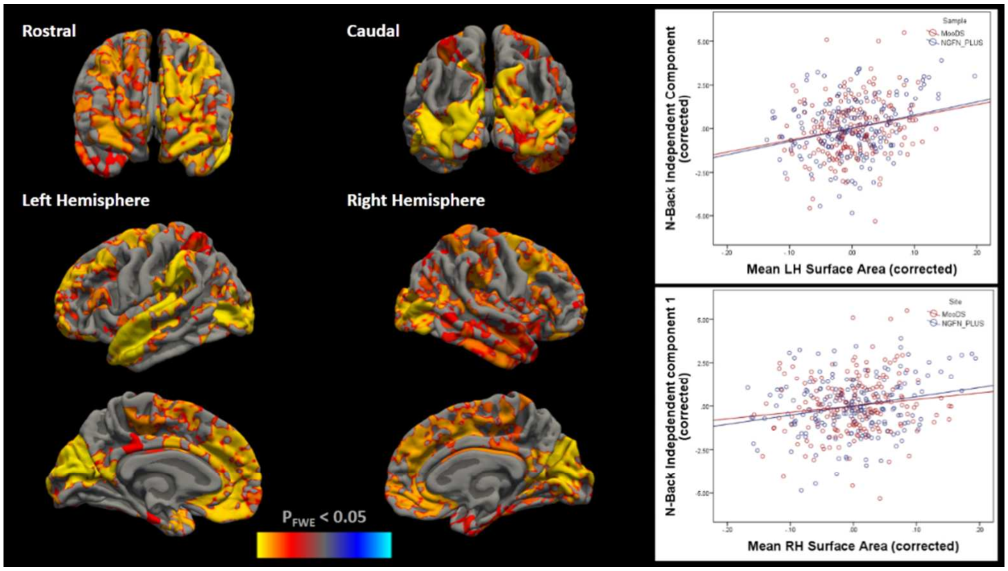
338x190mm (96 x 96 DPI)



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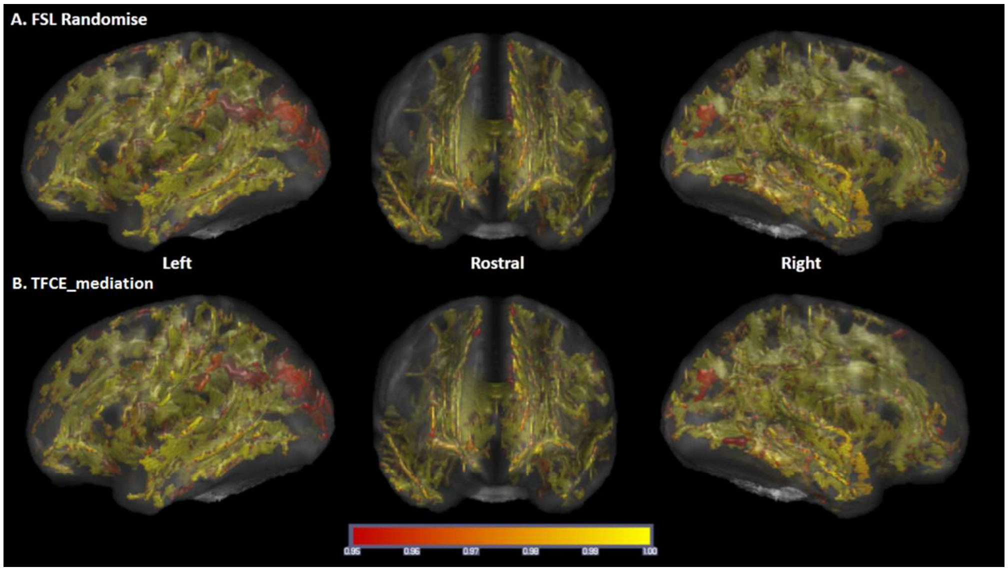
175x98mm (150 x 150 DPI)



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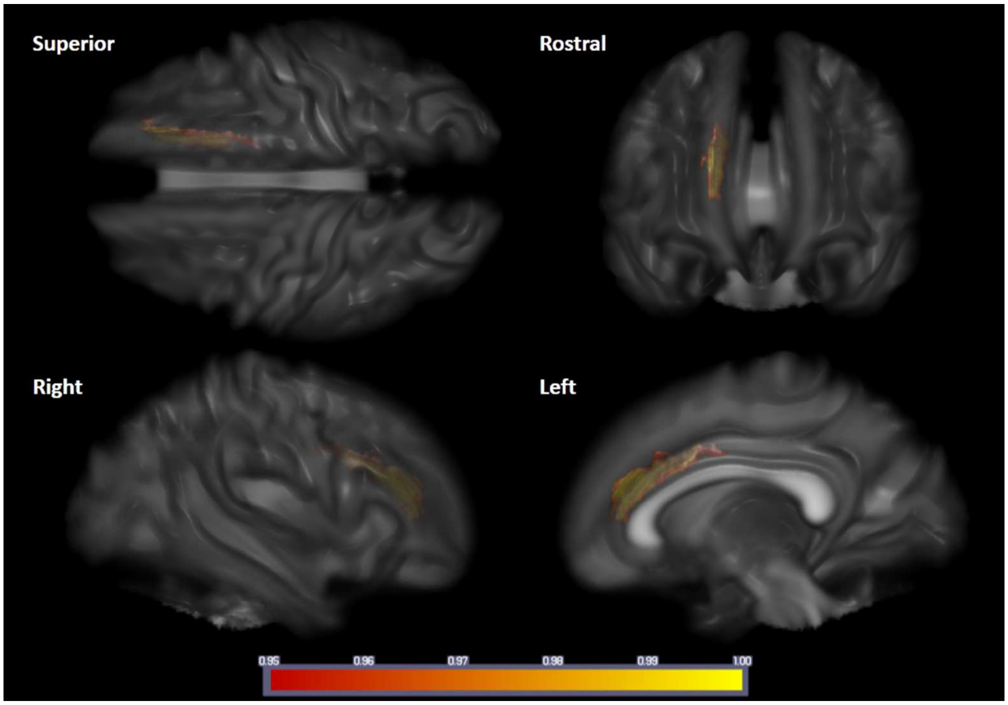
274x154mm (96 x 96 DPI)



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273x189mm (96 x 96 DPI)

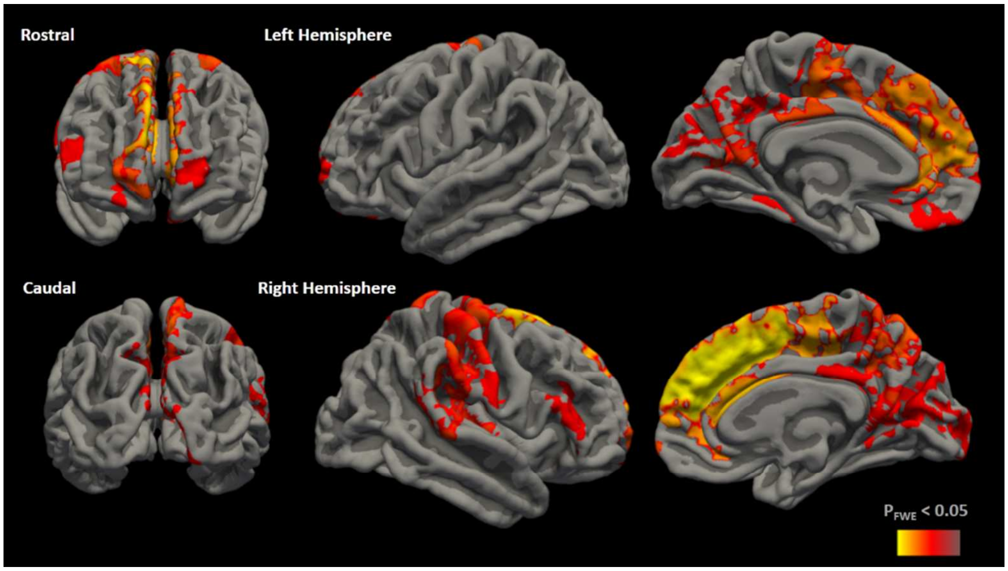

Figure 6. Vertex-wise mediation analysis to examine the indirect effect of surface area mediating the correlation among mean cluster FA and IC1 N-Back. The red-yellow bar correspond to TFCE transformed Sobel Z statistics with PFWE < 0.05 and lower. Covariates included age, sex, and site.

175x98mm (150 x 150 DPI)