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Microglial density alters measures of axonal integrity and structural connectivity

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Abstract

Diffusion tensor imaging (DTI) has fundamentally transformed how we interrogate diseases and disorders of the brain in neuropsychiatric illness. DTI, and recently developed multi-compartment diffusion weighted imaging (MC-DWI) techniques such as NODDI, measure diffusion anisotropy presuming a static neuroglial environment; however, microglial morphology and density are highly dynamic in psychiatric illness and how alterations in microglial density might influence intracellular measures of diffusion anisotropy in DTI and MC-DWI brain microstructure is unknown. To address this question, DTI and MC-DWI studies of murine brains depleted of microglia were performed, which revealed significant alterations in axonal integrity and fiber tractography in DTI and in commonly used MC-DWI models. With accumulating evidence of the role of microglia in neuropsychiatric illness, our findings uncover the unexpected contribution of microglia to measures of axonal integrity and structural connectivity and provide unanticipated insights into the potential influence of microglia in diffusion imaging studies of neuropsychiatric disease.

Keywords

Diffusion weighted imaging; DTI; tractography; microglia; mouse; neuroinflammation

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DISCLOSURES

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INTRODUCTION

Magnetic resonance imaging (MRI) techniques, such as diffusion weighted imaging (DWI), are widely used to interrogate neuronal microstructure across a broad array of neurologic, neurocognitive, and neuropsychiatric diseases (1). The most widely applied DWI technique is diffusion tensor imaging (DTI), which provides measures of fractional anisotropy (FA) and mean diffusivity (MD) that have been interpreted as microstructural indices of white matter structural integrity (2,3). However, many features of tissue microstructure, both biophysical as well as neuropathological (4–8), can alter FA and MD in similar ways and thereby limit our ability to more specifically associate changes in white matter structural integrity to the specific molecular and cellular changes that underlie complex neurologic and neuropsychiatric disorders. In an effort to obtain greater specificity and to advance our understanding of white matter microstructure from the diffusion signal, modern DWI methods employ a multi-compartment approach that (*i*) considers the signal in a voxel to be the sum of contributions from several different compartments and (*ii*) models the signal from each compartment as biologically salient features of tissue microstructure such as a neuronal (intracellular) compartment and an extra-axonal (extracellular) compartment (9). By partitioning the diffusion signal into multiple tissue compartments, new multi-compartment diffusion weighted imaging (MC-DWI) models such as NODDI (neurite orientation dispersion and density imaging) (10), CHARMED (composite hindered and restricted model of diffusion) (11), and ActiveAx (12,13) have improved our ability to image axonal morphology, geometry, orientation, and dispersion (12–16) and have made significant contributions towards our understanding of brain connectivity and the role and performance of white matter pathways (13).

The application of DTI and emerging MC-DWI techniques to model white matter tracts in both health and disease is ubiquitous. A decrease in FA (or analogous compartments in MC-DWI) is broadly interpreted as being indicative of white matter injury with decreased white matter structural integrity, and, in the case of diffusion tractography, interpreted as decreased tract anisotropy, volume, and density that are representative of decreased structural connectivity. These interpretations and observations are based upon numerous validation studies performed in advanced *in silico* simulation data, MR phantom studies, and histopathological studies of axonal morphology and organization that have generally assumed a static glial state; however, mounting evidence has shown that both microglia and astrocytes are highly dynamic in neurologic and psychiatric illness (17,18). In particular, microglia and microglial-mediated neuroinflammation have been implicated across a broad range of acute and chronic neurologic disorders including ischemic stroke (19–21), traumatic brain injury (22–24), epilepsy (25,26), and chronic neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (27–32). Numerous neuropsychiatric disorders including schizophrenia (33–38), major depression (39–42), and autism spectrum disorder (36–38,43) have also been shown to exhibit hallmarks of neuroinflammation (33,44–47) where peripheral cellular (42,48) and humoral immunological abnormalities (40,42,48,49) are more prevalent in psychiatric patients than in healthy controls. Genome-wide studies in schizophrenia have also revealed an association with markers in the major histocompatibility complex region (MHC) (50,51) and post-mortem studies have provided

evidence of increased numbers of microglia and microglial activation in patients with schizophrenia, depression, and other affective disorders (35,52). In all cases, microglia have been shown to undergo striking morphological, functional, and molecular alterations; with glial cells such as microglia, astrocytes, and oligodendrocytes comprising nearly 50% of the total number of cells in the human brain (53–56), new insights and a more complete understanding how astrocytes, microglia, and oligodendrocytes contribute to quantitative diffusion measures of brain microstructure – independent of biophysical alterations to the neuron – is critical for the clinical translation of diffusion weighted imaging methodologies for improved diagnostic accuracy, development of targeted treatments, and therapeutic monitoring of neuropsychiatric illness.

Recent published work from our laboratory has demonstrated that the *extracellular/extra-neurite* compartment in NODDI (ODI) is sensitive to changes in microglial density and that increased microglial density is positively correlated with increased diffusion anisotropy in the extracellular/extra-neurite compartment (57). Our results mirror complementary work demonstrating that extra-axonal restricted diffusion is associated with reactive microglia (58). The purpose of this study is to extend these insights regarding microglial density and the extra-neurite compartment and quantify how alterations in microglial density, which is commonly encountered across a broad spectrum of neuropsychopathology, might impact *intracellular* diffusion measures of axonal integrity (such as FA) and structural connectivity in diffusion weighted imaging with selective elimination of microglia from the murine brain via CSF1R inhibition (59) and subsequent *ex vivo* imaging. With commonly used DWI techniques such as DTI and NODDI utilizing diffusion models that biophysically model static glial cell populations, we hypothesized that changes in microglial density would significantly impact intracellular measures of diffusion anisotropy corresponding to measures of axonal integrity (e.g., FA).

METHODS AND MATERIALS

Animals and Reagents

All experiments were performed in accordance with animal protocols approved by the Institutional Animal Care and Use Committee at our institution (Protocol #: M005899). 12-week-old C57BL/6J male mice (Charles River Laboratories, MA, USA) were used and were randomly assigned to control or experimental CSF1R inhibition cohorts ($n=12$ total; $n=6$ each cohort). Control animals were maintained on AIN-76A standard chow (Research Diets, NJ, USA); animals receiving CSF1R inhibition received AIN-76A compounded with the CSF1R inhibitor PLX5622 (Plexxikon, CA, USA; 1,200 mg/kg) as previously described (59). Animals receiving CSF1R inhibition were maintained on their admixed diet for 8-days. To allow for physiologic washout of PLX5622 and any potential unaccounted contribution of PLX5622 to the diffusion signal, mice from the control and the experimental groups were transcardially perfused with 4% PFA 1 day following withdrawal of CSF1R inhibition. All brains then cleanly dissected from the cranial vault for *ex vivo* imaging. These data have been previously published (57) and are presented herein as a secondary analysis.

MRI Acquisition

Ex vivo imaging and analysis, including standard data preprocessing, study template generation, and region of interest (ROI) analyses, was performed as previously described (60). Briefly, brains were extracted from the cranial vault and post fixed in 4% PFA. Imaged brains were placed in a custom-built holder immersed in Fluorinert (FC-3283, 3M, St. Paul, MN, USA) and imaged with a 4.7-T Agilent MRI system with a 3.5-cm diameter quadrature volume RF coil. Multi-slice, diffusion-weighted, spin echo images were used to acquire 10 nondiffusion weighted images ($b=0 \text{ s}\cdot\text{mm}^{-2}$) and 75 diffusion-weighted images (25: $b=800 \text{ s}\cdot\text{mm}^{-2}$, 50: $b=2,000 \text{ s}\cdot\text{mm}^{-2}$), using non-colinear diffusion-weighting directions. Diffusion imaging was performed with a TE/TR=24.17/2000-ms, FOV=30×30 mm², and a matrix=192×192 reconstructed to 256×256 for an isotropic voxel size of 0.25-mm over two signal averages. Raw data files were converted to NIfTI format and FSL was used to correct for eddy current artifacts. A DTI-based mouse brain atlas (61) was used as a template and to define regions of interest (ROI) including the left and right hippocampus, prefrontal cortex, globus pallidus, amygdala, nucleus accumbens, corpus callosum, and internal capsule. Multi-shell diffusion data were fit with the Microstructure Diffusion Toolbox (MDT) (62) to models of AxCaliber, NODDI, and ActiveAx. An additional compartment of isotropic restriction was included to account for potential fixative effects as recommended (12). FA, res (AxCaliber), NDI (NODDI), and ic (ActiveAx), which represent indices of axonal/intracellular diffusion in each diffusion model, were calculated in each ROI and compared between control and microglia-depleted animals. A Student's *t*-test was used to determine the significance ($p<0.05$) between ROI values between control and microglia-depleted animals; statistically significant differences were then determined after controlling for multiple comparisons with the Benjamini-Hochberg procedure (false discovery rate = .05).

Tractography

Tractography analysis was conducted using DSI Studio (<http://dsi-studio.labsolver.org>). A multi-shell diffusion scheme was used with *b*-values of 567.155, 1532.04 and 1878.53 s/mm² (75-directions total). The in-plane resolution was 0.125 mm and the slice thickness was 0.125 mm. After the diffusion tensor was calculated, a deterministic fiber tracking algorithm was used (63). Regions of interest were derived from a DTI-based mouse brain atlas (61) and seeding regions were placed in the left and right hippocampus (final volume size of 13 mm³). The angular threshold was 45 degrees and a step size of 0.12 mm was used. The anisotropy threshold was 0.2. The fiber trajectories were smoothed by averaging the propagation direction with 50% of the previous direction and tracts longer than 800 mm were discarded; a total of 10,000 seeds were placed. A Student's *t*-test was used to determine the significant difference ($p < 0.05$) between fiber number, thickness, and directionality in control versus CSF1R treated brains.

Immunofluorescent Staining

Immunofluorescent staining and quantification were completed as previously described (60). Primary antibodies used are anti-Iba-1 (rabbit Anti-Iba-1, dilution 1:2000, Abcam, Cambridge, MA, Cat # AB178847), and anti-NeuN (chicken Anti-NeuN, dilution 1:1500; EMD Millipore, Billerica, MA Cat# ABN91MI). Goat anti-rabbit conjugated to Alexafluor

488 (Abcam, Cambridge, MA, Cat# AB150077) and goat anti-chicken conjugated to Alexafluor 555 (Thermo Fisher Scientific, Waltham, MA former Invitrogen Cat# A-21437) were used as secondaries.

RESULTS

Mice were treated with PLX5622, a colony stimulating factor 1 receptor (CSF1R) inhibitor known to deplete microglia with no known secondary effects on neurons or other neuroglial cell lines (59) and we observed the expected reduction in microglial density in CSF1R treated animals compared to age- and sex-matched controls (Fig. 1A and B). Following imaging, we found statistically significant changes of decreased axonal integrity in both DTI and MC-DWI models across several ROIs in gray and white matter (Table 1). FA was significantly reduced in the right hippocampus and nucleus accumbens in animals treated with CSF1R when compared to controls, whereas the intra-axonal compartment of AxCaliber, res, demonstrated a significant increase in the left frontal anterior cortex, as well as in the right hippocampus, amygdala, and corpus callosum in animals depleted of microglia. Microglial depletion also contributed to a significant decrease in measured neurite density (NDI, NODDI) in the left frontal anterior cortex and nucleus accumbens, as well as the right hippocampus, frontal anterior cortex, amygdala, and corpus callosum. Finally, ActiveAx showed significant decrease in measures of intracellular diffusion in both left and right regions of the hippocampus, globus pallidus, amygdala, and nucleus accumbens (Table 1).

These changes in axonal integrity were also mirrored in calculated measures of brain structural connectivity. Deterministic fiber tractography of the right hippocampus identified markedly different tractography streamlines with clear visual differences in fiber orientation and organization between control (Fig. 1C) microglia-depleted animals (Fig. 1D). There was also a significant decrease in the number of fiber tracts ($p < 0.05$), mean tract length ($p < 0.05$) and tract volume ($p < 0.05$) in brains of animals treated with CSF1R inhibitor. The control samples averaged 2351 tracts with 1.39 mm mean length and 14.96 mm³ volume, whereas microglia depleted samples averaged 1962 tracts with 1.23 mm mean length and 13.68 mm³ volume. The left hippocampus showed no significant change in tract number (2219 control vs. 2197 CSF1R-treated), but mean tract length (1.58 mm vs. 1.46 mm) and tract volume (16.03 mm³ vs. 14.71 mm³) were both significantly decreased in microglia-depleted animals ($p < 0.05$) (Table 1, Supplemental Material).

DISCUSSION

Current interpretations of axonal integrity and white matter microstructural integrity infer that alterations in FA are representative of axonal changes such as (de)myelination and/or reflect alterations in axonal integrity, diameter, fiber density, or organization. These interpretations of DTI and of other multi-shell diffusion models, however, are predicated and based upon biophysical diffusion models that presume a static neuroglial environment in both health and disease. With increasing evidence both in translational animal models (64–68) and human clinical samples (67–70) that microglial density and morphology are highly dynamic in psychiatric illness, an understanding of how these changes in microglial density

and morphology may potentially affect quantitative indices of DTI and MC-DWI brain microstructure is critical for improved diagnostic accuracy, development of targeted treatments, and therapeutic monitoring in neuropsychiatric illness. Previous work from our group has shown that changes in microglial density can affect MC-DWI indices that pertain to the *extracellular/extra-neurite space*; in this study, we hypothesized that alterations of microglial density, which have been demonstrated in numerous neuropsychiatric disorders, would also affect *intracellular* measures of diffusion anisotropy commonly interpreted as axonal integrity and structural connectivity in both DTI and MC-DWI.

Following microglial depletion, we found that FA, and the intracellular indices of AxCaliber, NODDI, and ActiveAx representative of axonal integrity were all significantly affected in various regions of the brain. At the outset, we hypothesized that more highly compartmentalized DWI models (e.g. ActiveAx) would be less sensitive to changes in microglial density due to the incorporation of glial cells into the diffusion model; however, we found that of the four models analyzed, ActiveAx was the most sensitive to changes in microglial density, with NODDI the next most sensitive, and DTI being the least sensitive (Table 1). This finding was particularly unexpected as higher-ordered MC-DWI models ostensibly account for the presence of glial cells (not otherwise specified) and would thus be expected to more robustly quantify axonal morphology. DTI tractography and commonly employed deterministic streamline fiber tracking methods were also similarly sensitive to changes in microglial density, which was evidenced most strikingly by significant alterations and differences in the orientation and configuration of calculated white matter pathways.

A broad spectrum of neurologic, neurocognitive, and psychiatric illnesses spanning Alzheimer's disease, Huntington's disease, major depression, and schizophrenia are strongly associated with microglial neuroinflammation with resultant changes in microglial density throughout the brain. Our data provide compelling evidence that microglial density is able to significantly impact quantitative diffusion measures of axonal integrity and structural connectivity independent of biophysical alterations to the neuron and suggests a general reconsideration of the current interpretation of decreased diffusion anisotropy and structural connectivity as a marker of neuronal injury. Especially in the pre-clinical disease state where neuronal dysfunction and cognitive deficits are not clinically evident, our data suggests that not all reports of decreased anisotropy are necessarily representative of actual neuronal injury; instead, these changes may simply reflect a change in microglial density, especially in studies examining neuropathological processes in their earliest stages and those with a hypothesized neuroinflammatory/microglial component. Even in clinically significant disease states where there are significant measures of decreased white matter structural integrity and altered structural connectivity, neuronal injury may not be the prevailing neuropathological process present as other glial changes (such as decreased microglial density) may also be present and contribute to observed reductions in diffusion anisotropy. We might further speculate that changes in astrocyte density and morphology, which is also commonly seen in neuropsychiatric illness, may similarly contribute to altered measures of intracellular diffusion anisotropy as the number of astrocytes in the brain far outnumber that of microglia. Thus, a deeper understanding of how neuroglia – microglia as well as astrocytes and oligodendrocytes – contribute to the composite diffusion signal represents a

critical and vital step towards translating and implementing DWI imaging methodologies for the accurate and precise detection and monitoring of neurologic and psychiatric disease.

Potential limitations of our study include the use of *ex vivo* imaging, which were chosen in part as scan durations currently required of these imaging methods preclude the possibility of *in vivo* imaging. However, *ex vivo* techniques allow for higher SNR and spatial resolution required of this study and although tissue fixation may alter tissue diffusion MR measurements (71), dedicated *ex vivo* imaging analyses accounting for the presence of fixative are used (62) to address this issue.

Although our results represent a departure from common interpretations of FA and other intracellular diffusion parameters, our findings support a reexamination of our current understanding and interpretation of measures of axonal integrity and structural connectivity to additionally include the contribution of microglial density in quantitative measures of DTI and MC-DWI brain microstructure. Our study as well as previous work from our lab (57) illustrate how changes in microglial density are able to influence both intracellular and extracellular measures of diffusion anisotropy across a wide variety of diffusion imaging methodologies, and suggests the broad influence of microglia in diffusion imaging may be due to the diffusion models themselves as they generally underestimate the number of microglia present as well as the dynamic morphologic changes of microglia and other glial cells such as astrocytes (72). Interestingly, the magnitude of the effect of microglial density on intracellular diffusion parameters is similar to what we observed when examining the extracellular/extra-neurite compartment, which further suggests that our observations arise from unanticipated biological features that are not well described in the diffusion models themselves and not due to other potential spurious technical effects (e.g. optimizing shell parameters). Because the neuropathology of many neurologic and psychiatric diseases includes neuroinflammation with concomitant changes in microglial density and morphology, we need to consider these dynamic changes in microglial density and morphology when interpreting measures of DWI, rather than assuming changes of FA, res, NDI, and ic are related only to neuronal injury. The outcomes of our study also address longstanding questions regarding neuroimaging measures of diffusion anisotropy and the neurobiological basis of the diffusion signal. Particularly as new diffusion imaging techniques are developed and translated into research and clinical practice, our results advocate for novel neuroimaging procedures that explicitly account for the dynamic cellular changes now known to occur with microglia and astrocytes as an important consideration when imaging axonal integrity and defining structural connectivity throughout the brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1: CSF1R inhibition depletes microglia and influences estimations of white matter structural connectivity.

(A) Representative micrograph (10X) of a C57BL/6J mouse hippocampus fluorescently stained for neurons (anti-NeuN, red) and microglia (anti-Iba1, green) in control and (B) CSF1R inhibited animals ($n=6$, each group). As expected, CSF1R inhibition depletes microglia with no apparent change in neuron density. Scale bar=500 μm . (C) Representative examples of deterministic tractography of fibers following seeding in the right hippocampus in control and (D) CSF1R inhibitor treated animals. Microglial depletion yields markedly

different tractography streamlines with clear visual differences in fiber length, orientation, and organization with tract-based analyses of microglial depleted animals demonstrating significant reductions in the number, length, and volume of tracts modeled. Images are oriented via LPS anatomical coordinates (left, posterior, superior).

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Table 1:
Microglial depletion influences quantitative measures of intra-axonal integrity.

All values are mean $\pm$ s.e.m. Units of measure for FA, NDI, res and ic are [10^{-3} mm²/s]. Bolded and italicized p-values are statistically significant. Starred p-values are statistically significant after controlling the false discovery rate with the Benjamini-Hochberg procedure (false discovery rate = .05). Regions of interest (ROIs) are derived from the Center for *in vivo* Microscopy (CIVM) mouse brain atlas. $n = 6$ for both control and CSF1R inhibitor treated groups. *FA* fractional anisotropy, *NDI* neurite density index, *res* restricted compartment, *ic* intracellular compartment, *HC* hippocampus, *FAC* frontal anterior cortex, *GP* globus pallidus, *AM* amygdala, *Acc* accumbens, *CC* corpus callosum, *IC* internal capsule

DWI Measure	Hemi.	ROI	Mean $\pm$ SEM		p-value
			Control Male	CSF1R treated Male	
DTI (FA)	Left	HC	0.19716 $\pm$ 0.00522	0.20121 $\pm$ 0.00199	0.243
		FAC	0.16253 $\pm$ 0.00344	0.15418 $\pm$ 0.00507	0.101
		GP	0.19607 $\pm$ 0.00451	0.18885 $\pm$ 0.00442	0.140
		AM	0.17578 $\pm$ 0.00260	0.17271 $\pm$ 0.00216	0.193
		Acc	0.14813 $\pm$ 0.00529	0.14081 $\pm$ 0.00335	0.135
		CC	0.47080 $\pm$ 0.01312	0.47239 $\pm$ 0.01354	0.467
		IC	0.50706 $\pm$ 0.01453	0.53306 $\pm$ 0.01490	0.120
	Right	HC	0.20971 $\pm$ 0.00420	0.19347 $\pm$ 0.00286	<0.01*
		FAC	0.16674 $\pm$ 0.00252	0.16211 $\pm$ 0.00563	0.235
		GP	0.21106 $\pm$ 0.00204	0.21228 $\pm$ 0.00541	0.418
		AM	0.19457 $\pm$ 0.00666	0.18663 $\pm$ 0.00440	0.172
		Acc	0.15485 $\pm$ 0.00438	0.14302 $\pm$ 0.00422	<0.05
		CC	0.50562 $\pm$ 0.01498	0.51590 $\pm$ 0.01858	0.338
		IC	0.50847 $\pm$ 0.01125	0.51921 $\pm$ 0.01192	0.264
AxCaliber (res)	Left	HC	0.33137 $\pm$ 0.00323	0.33586 $\pm$ 0.00180	0.126
		FAC	0.33858 $\pm$ 0.01321	0.38078 $\pm$ 0.00948	<0.05
		GP	0.32410 $\pm$ 0.01083	0.30655 $\pm$ 0.00701	0.102
		AM	0.34347 $\pm$ 0.00718	0.34227 $\pm$ 0.00360	0.442
		Acc	0.32370 $\pm$ 0.00969	0.34308 $\pm$ 0.00773	0.074
		CC	0.41458 $\pm$ 0.00733	0.40948 $\pm$ 0.00514	0.291
		IC	0.43382 $\pm$ 0.00760	0.45314 $\pm$ 0.00907	0.067
	Right	HC	0.32055 $\pm$ 0.00319	0.33225 $\pm$ 0.00307	<0.05
		FAC	0.33596 $\pm$ 0.01471	0.35651 $\pm$ 0.00716	0.119
		GP	0.30639 $\pm$ 0.00506	0.31853 $\pm$ 0.00496	0.059
		AM	0.32737 $\pm$ 0.00622	0.34384 $\pm$ 0.00427	<0.05
		Acc	0.33943 $\pm$ 0.00875	0.34597 $\pm$ 0.00896	0.306
		CC	0.43376 $\pm$ 0.01067	0.47427 $\pm$ 0.00987	<0.01*
		IC	0.43825 $\pm$ 0.00834	0.44863 $\pm$ 0.01104	0.235
NODDI (NDI)	Left	HC	0.27892 $\pm$ 0.00378	0.28060 $\pm$ 0.00479	0.395

DWI Measure	Hemi.	ROI	Mean $\pm$ SEM		p-value	
			Control Male	CSF1R treated Male		
		FAC	0.26430 $\pm$ 0.01245	0.21564 $\pm$ 0.01181	<0.01*	
		GP	0.34014 $\pm$ 0.00959	0.32710 $\pm$ 0.00643	0.142	
		AM	0.27323 $\pm$ 0.01498	0.24658 $\pm$ 0.01045	0.088	
		Acc	0.29728 $\pm$ 0.01104	0.24386 $\pm$ 0.00903	<0.01*	
		CC	0.45193 $\pm$ 0.00453	0.46559 $\pm$ 0.01123	0.143	
		IC	0.48890 $\pm$ 0.01081	0.47998 $\pm$ 0.00424	0.230	
	Right	HC	0.29248 $\pm$ 0.00334	0.27484 $\pm$ 0.00459	<0.01*	
		FAC	0.27661 $\pm$ 0.01592	0.23105 $\pm$ 0.00924	<0.05	
		GP	0.35047 $\pm$ 0.01378	0.33119 $\pm$ 0.00826	0.129	
		AM	0.27667 $\pm$ 0.00535	0.24321 $\pm$ 0.00930	<0.01*	
		Acc	0.30272 $\pm$ 0.01162	0.28137 $\pm$ 0.00869	0.086	
		CC	0.52584 $\pm$ 0.00707	0.50085 $\pm$ 0.00899	<0.05	
		IC	0.47182 $\pm$ 0.00900	0.45647 $\pm$ 0.00923	0.131	
	ActiveAx (ic)	Left	HC	0.17479 $\pm$ 0.00441	0.16139 $\pm$ 0.00159	<0.01*
			FAC	0.16138 $\pm$ 0.00499	0.15842 $\pm$ 0.00399	0.326
			GP	0.15996 $\pm$ 0.00816	0.12757 $\pm$ 0.00505	<0.01*
			AM	0.16890 $\pm$ 0.00268	0.15387 $\pm$ 0.00565	0.05
			Acc	0.14249 $\pm$ 0.00582	0.11262 $\pm$ 0.00561	<0.01*
			CC	0.34932 $\pm$ 0.01184	0.35467 $\pm$ 0.01694	0.401
			IC	0.34061 $\pm$ 0.00592	0.34875 $\pm$ 0.01166	0.274
		Right	HC	0.17777 $\pm$ 0.00411	0.15786 $\pm$ 0.00218	<0.01*
			FAC	0.17160 $\pm$ 0.00401	0.16114 $\pm$ 0.00667	0.104
			GP	0.15920 $\pm$ 0.00500	0.13931 $\pm$ 0.00542	<0.05
			AM	0.17920 $\pm$ 0.00588	0.16436 $\pm$ 0.00433	<0.05
			Acc	0.14631 $\pm$ 0.00745	0.11569 $\pm$ 0.00286	<0.01*
			CC	0.38329 $\pm$ 0.01735	0.38160 $\pm$ 0.01226	0.469
			IC	0.33544 $\pm$ 0.00810	0.31822 $\pm$ 0.00719	0.072