http://dx.doi.org/10.5665/sleep.2228

Sex Differences in White Matter Alterations Accompanying Obstructive Sleep Apnea

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Study Objectives: Females with obstructive sleep apnea (OSA) show different psychological and physiological symptoms from males, which may be associated with sex-related variations in neural injury occurring with the disorder. To determine whether male- or female-specific brain injury is present in OSA, we assessed influences of sex on white matter changes in the condition.

Design: Two-group factorial.

Setting: University medical center.

Patients or Participants: 80 subjects total, with newly diagnosed, untreated OSA groups of 10 female (age mean ± SE: 52.6 ± 2.4 years, AHI 22.5 ± 4.1 events/h) and 20 male (age 48.9 ± 1.7, AHI 25.5 ± 2.9) patients, and 20 female (age 50.3 ± 1.7) and 30 male (age 49.2 ± 1.4) healthy control subjects.

Interventions: None.

Measurements and Results: Brain fiber integrity was assessed with fractional anisotropy (FA), a diffusion tensor imaging-derived measure. Sleep quality, daytime sleepiness, depression, and anxiety were assessed with questionnaires. We identified regions of differing injury in male versus female OSA patients by assessing brain regions with significant interaction effects of OSA and sex on FA. Areas of sex-specific, OSA-related FA reductions appeared in females relative to males, including in the bilateral cingulum bundle adjacent to the mid hippocampus, right stria terminalis near the amygdala, prefrontal and posterior-parietal white matter, corpus callosum, and left superior cerebellar peduncle. Females with OSA showed higher daytime sleepiness, anxiety and depression levels, and reduced sleep quality.

Conclusions: Sex differences in white matter structural integrity appeared in OSA patients, with females more affected than males. These female-specific structural changes may contribute to or derive from neuropsychological and physiological symptom differences between sexes.

Keywords: Magnetic resonance imaging, diffusion tensor imaging, hypoxia, autonomic nervous system, cingulum, superior cerebellar peduncles, stria terminalis

Citation: Macey PM; Kumar R; Yan-Go FL; Woo MA; Harper RM. Sex differences in white matter alterations accompanying obstructive sleep apnea. *SLEEP* 2012;35(12):1603-1613.

INTRODUCTION

Obstructive sleep apnea (OSA), characterized by repeated obstructions of the upper airway with intermittent hypoxic exposure, is associated with multiple detrimental physiological and psychological consequences, and occurs in at least 5% of the population.[1] The disorder is less common in females than males, with women showing an OSA prevalence less than half of that in men.[2] Furthermore, OSA characteristics differ between sexes; women with OSA typically report different sleep-related complaints from males, and have a higher incidence of chronic pulmonary disease, vascular issues, including heart failure and possibly related tissue fluid retention, and hypothyroidism, while male OSA patients show higher levels of cardiovascular disease and arrhythmia than females.[3-5] Other physiological differences appear in overnight blood pressure changes and upper airway muscle tone.[6-9] Disease severity, as measured by apneic events per hour (apnea/hypopnea index [AHI]), tends to be lower in females, but women are symptomatic at lower AHI levels.[5] The incidence of certain neuropsychological characteristics in OSA, including depression and anxiety symptoms, is higher in women.[3,4,10-13] The mechanisms underlying these sex differences in symptoms are unclear, but many physiological and psychological consequences may result from neural dysfunction arising from the injury in the condition.

Alterations in neural structure, reflected as gray matter loss, changes in metabolite levels, and alterations in water diffusion, appear in OSA.[14-27] These alterations are accompanied by functional consequences,[28-32] which likely contribute to the deleterious characteristics of the sleep disorder, including psychological and physiological comorbidities. These differences could result, in part, from sex-related variation in neural alterations from hypoxia, since animal models of intermittent hypoxia show relative neuroprotection in females.[33,34] Estrogen may play a protective role, as shown in animal models,[35,36] and as reflected in the postmenopause rapid increase in incidence of OSA in women, which approaches that in men.[37] However, the postmenopausal elevation of prevalence of OSA might develop from an array of processes other than direct neural influences, such as upper airway morphological changes accompanying cervical vertebrae alterations,[38] although those anatomical changes may also be hormonally based. Direct evidence of sex differences in central nervous system injury related to OSA is lacking.

Diffusion tensor imaging (DTI) is a magnetic resonance imaging (MRI) procedure sensitive to changes in water diffusion associated with alterations in structure. One DTI-derived measure is fractional anisotropy (FA), which is an index of axonal structural integrity.[39,40] The FA measure reflects the directionality of water movement in tissue; in regions of dense, closely aligned, well-myelinated fiber tracts, water movement is relatively constrained along the direction of those tracts, but movement perpendicular to the fibers is impeded. The FA index

Submitted for publication January, 2012
Submitted in final revised form May, 2012
Accepted for publication June, 2012
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reflects that directionality, being the ratio of diffusivity along the direction of easiest movement (termed axial direction) to the diffusivity across the plane perpendicular to the axial direction. Lower FA values reflect lower impedance in the plane perpendicular to the axial direction, which can result from less axonal white matter myelin, thus reflecting reduced axonal integrity. Obstructive sleep apnea patients show extensive structural changes as indicated by lower FA,[15] and the procedure has been used elsewhere to demonstrate other sex-related white matter changes.[41-44] The purpose of this study was to test the hypothesis that male-female differences in axonal injury are present in OSA, as measured with FA. Psychological symptoms were also measured to assist interpretation of white matter findings, and specifically to determine whether potential brain changes occurred independently of psychological factors. Further studies will be needed to establish interactions of injury with psychological variables, since this study was designed only to determine whether structural brain changes differ by sex.

METHODS

Subjects

We studied 10 female and 20 male OSA patients recruited from the UCLA Sleep Laboratory, and 20 female and 30 male control subjects recruited from the Los Angeles community. These subjects were a subset of those used in a previous study.[15] No OSA subjects had started treatment for the sleep disorder, and all were recently diagnosed; they were referred to the study at the time of their diagnostic overnight polysomnographic sleep assessment and were typically scanned within 4 weeks. OSA patients were included if they were diagnosed as moderate or severe, based on AHI being ≥ 15 events/h.[45] Calculated parameters included AHI, mean and nadir oxygen saturation, and classification as REM-dominant OSA if inspection illustrated apneas occurring exclusively (or almost so) in REM sleep. After verification of inclusion and exclusion criteria, the control subjects were interviewed for the presence of symptoms of OSA (e.g., daytime sleepiness, snoring, non-restorative sleep, bed partner report of disturbed breathing). Control subjects who reported such symptoms were scheduled for a full sleep study to verify their status. Five subjects in total were screened, 2 of whom were included in the study (AHI 2 and 3), one of whom was ultimately diagnosed with OSA, and the remaining 2 of whom were diagnosed with mild OSA (5 < AHI < 15) and excluded from the study. Male and female OSA groups were matched for severity, and all 4 groups (control male and female, OSA male and female) were age-matched. No subjects had a history of psychiatric disorders, cardiovascular disease, stroke, or other major illness, and no subjects were taking psychotropic or cardiovascular medications. Scanner limitations precluded patients with metallic implants or weight over 125 kg. All procedures were in accordance with the Declaration of Helsinki and approved by the UCLA Institutional Review Board, and subjects provided written informed consent.

Psychological Symptoms

We assessed daytime sleepiness with the Epworth Sleepiness Scale (ESS),[46] and sleep quality with the Pittsburgh Sleep Quality Index (PSQI).[47] We also assessed depressive symptoms with the Beck Depression Inventory-II (BDI)[48,49] and anxiety symptoms with the Beck Anxiety Inventory (BAI).[50] The ESS is scored between 0 and 24; a score ≥ 10 is considered above normal. The PSQI is a 19-item questionnaire assessing self-reported sleep quality and sleep times, with a score ≥ 5 indicating clinically meaningful disturbed sleep. The BAI is a 21-question instrument with a maximum score of 63, where scores ≥ 8 indicate higher than normal levels of anxiety. Similarly, the BDI is a 21-item questionnaire, with scores ≥ 10 considered mild or greater levels of depressive symptoms, although higher cutoffs are sometimes used.

MRI Scanning

We collected DTI and other anatomical scans with a 3.0 Tesla MRI scanner (Siemens Magnetom Tim-Trio, 8-channel head coil). Diffusion tensor imaging data consisted of 4 separate series using a single-shot multi-section spin-echo echo-planar pulse sequence (repetition time [TR] = 10,000 ms; echo-time [TE] = 87 ms; flip angle = 90°) in the axial plane, with a 128 × 128 matrix size, 230 × 230 mm field of view (FOV), 2.0 mm slice thickness, 75 slices and no interslice gap, and a readout bandwidth of 1346 Hz/pixel. Diffusion gradients were applied along 12 directions with b = 700 s/mm^2, in addition to the b = 0 s/mm^2 (b0) images. An acceleration factor of 2 was applied using the parallel imaging generalized autocalibrating partially parallel acquisition technique. To enable correction for field-related distortions, we also collected phase-difference and magnitude images (1st TE = 4.98 ms, 2nd TE = 7.44 ms; TR = 880 ms; flip angle = 90°; matrix size = 64 × 64; FOV = 192 × 192 mm; slice thickness = 3.0 mm; number of slices = 36). High-resolution 3-dimensional T1-weighted anatomical scans were collected using a magnetization-prepared rapid-acquisition-gradient-echo pulse sequence (TR = 2200 ms; TE = 2.2 ms; inversion time = 900 ms; flip angle = 9°; matrix size = 256 × 256; FOV = 230 × 230 mm; slice thickness = 1.0 mm; number of slices = 176).

Analysis: Preprocessing

The 3 later DTI series were realigned to the first series using the non-diffusion weighted (b0) images; the diffusion gradient direction vectors of the images were rotated accordingly. Corrections for eddy-current related distortions were performed using an affine coregistration.[51,52] Phase distortions due to magnetic field inhomogeneities were corrected using fieldmaps calculated from the phase-difference and magnitude images, with software from the SPM5 "Fieldmap" toolbox.[53] The diffusion tensor was calculated at each voxel using a linear model, and FA derived using the SPM5 "Diffusion" toolbox. The b0 images were spatially normalized to the Montreal Neurological Institute (MNI) template, and the FA maps had the same transformation applied (see Appendix A in supplemental material for details). Finally, the spatially normalized FA maps were smoothed (isotropic Gaussian filter with full-width half-maximum = 10 mm).

Analysis: Statistical Modeling

We implemented a factorial model in SPM5[54]; this earlier version of the SPM software was used to maintain consistency with our previous findings.[15] As FA is a quantitative measure, we implemented a model to assess absolute means rather than the SPM5 default of relative means (as used in functional mag-

Table 1—Subject information across groups, with adjusted means and standard errors (± SE)

	Female		Male		4-Group ANOVA	OSA Main Effects		2-Group ANOVA	Sex Main Effects		2-Group ANOVA
	Control	OSA	Control	OSA	P (F-test)	Control	OSA	P (F-test)	Female	Male	P (F-test)
N	20	10	30	20		50	30		30	50	
Age (years)	50.3 ± 1.7	52.6 ± 2.4	49.2 ± 1.4	48.9 ± 1.7	0.6	49.6 ± 1.1	50.2 ± 1.5	0.8	51.1 ± 1.5	49.1 ± 1.0	0.3
BMI (kg/m^2)	22.3 ± 0.8	33.0 ± 2	24.8 ± 0.7	28.7 ± 0.8	< 0.001	23.8 ± 0.4	30.1 ± 0.9	< 0.001	25.9 ± 1.2	26.4 ± 0.5	0.7
PSQI	4.4 ± 0.7	10.6 ± 1.1	3.9 ± 0.6	7.7 ± 0.8	< 0.001	4.1 ± 0.4	8.7 ± 0.8	< 0.001	6.4 ± 0.8	5.4 ± 0.5	0.3
Epworth SS	7.3 ± 0.8	11.0 ± 1.1	5.1 ± 0.7	9.5 ± 0.8	< 0.001	6.0 ± 0.5	10.0 ± 0.8	< 0.001	8.5 ± 0.7	6.9 ± 0.6	0.09
BDI (depression)	4.05 ± 1.4	14.0 ± 2.0	3.8 ± 1.1	6.1 ± 1.4	< 0.001	3.9 ± 0.6	8.7 ± 1.7	0.002	7.4 ± 1.7	4.7 ± 0.7	0.1
BAI (anxiety)	5 ± 2.0	20.6 ± 2.9	3.5 ± 1.7	7.5 ± 2.0	< 0.001	4.1 ± 0.7	11.9 ± 2.7	0.001	10.2 ± 2.4	5.1 ± 1.2	0.03
OSA Parameters					2-Group ANOVA						
AHI (events/h)		22.5 ± 4.1		25.5 ± 2.9	0.2						
SpO_2 nadir		86.8 ± 2.3		78.4 ± 1.7	0.01						
SpO_2 baseline		94.5 ± 0.7		95 ± 0.5	0.8						
					P (χ^2)						
REM Dominant OSA		50%		15%	0.04						

Group effects were tested by four-way ANOVA across male and female OSA, and male and female control groups for demographic and psychological measures, and by two-way ANOVA across male and female OSA for polysomnographic parameters. BMI, body mass index; REM, rapid-eye movement sleep.

netic resonance imaging analyses). We created a linear model with sex and OSA terms and an interaction variable. We also included a constant term. We created a mask for the statistical analysis to include all regions with large axonal groups including white matter, and to exclude non-brain regions and areas with very low FA, which could have high variability. The purpose was to restrict analysis to areas which likely included axonal groups, but with a conservative approach to ensure all brain areas of potential interest were included. The mask was created based on a threshold of 0.15 applied to an FA map of the mean of all subjects' normalized images; the threshold level was relatively low (0.2 or 0.3 is used on some studies of healthy people[55,56]) to ensure inclusion of all regions with axonal groups, and to allow for inclusion of regions with reduced FA which would occur with injury. We first assessed the effects of interest (sex and OSA status) across the whole brain using an F test at a threshold of 0.05 false discovery rate (FDR), to be consistent with previous findings.[15] We performed post hoc testing in brain regions showing significant effects to determine the direction and magnitude of changes.

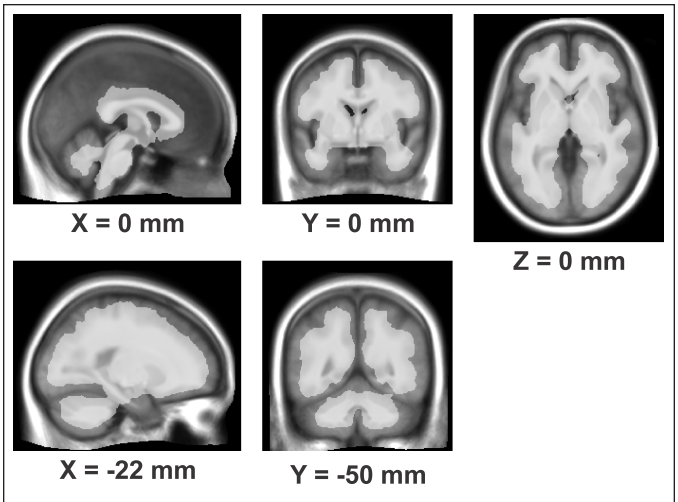

Figure 1—Regions (white semi-transparent overlay) meeting the criterion of FA > 0.15, overlaid onto average of 80 subjects' anatomical images normalized to MNI space. Locations of slices are in MNI coordinates.

RESULTS

Subjects

Detailed characteristics of the subjects are shown in Table 1. All subgroups were age-matched (overall age mean ± SD = 49.8 ± 7.6 years), and the disease severity was similar for male and female OSA subgroups (overall AHI = 23.8 ± 12.7 events/h). Significant group differences appeared in all symptoms (Table 1), with OSA females showing the highest levels of each symptom (BAI, BDI, ESS, and PSQI). Physiologically, the oxygen saturation nadir was lower in males, and REM-related apneas dominated in 50% of females versus 15% of males.

White Matter Structural Changes

An absolute FA threshold of 0.15 led to the exclusion of non-brain regions and outer cortical areas from the analysis, and the inclusion of all white matter, and mixed regions (Figure 1). The inclusion of some ventricular and purely gray matter regions likely reflects variations in spatial normalization, but the consequence of these additional regions would be only a very modest reduction in sensitivity resulting from a more stringent multiple comparisons threshold due to the slight increase in number of voxels in the search area.[57] (See Figure S1 in supplemental material for statistical maps of the F-test of the entire model at 2 FA thresholds, 0.015 and 0.05, along with an average of the 80 subjects' anatomical scans for reference.)

Overall Effects

The model showed significance in the F-test of the effects of interest across a wide number of brain regions (Figure 2A), with 0.297 liters (approximately 18% of brain volume) showing effects at the 0.05 level (FDR correction). Significant differences were present across many brain regions for the previously

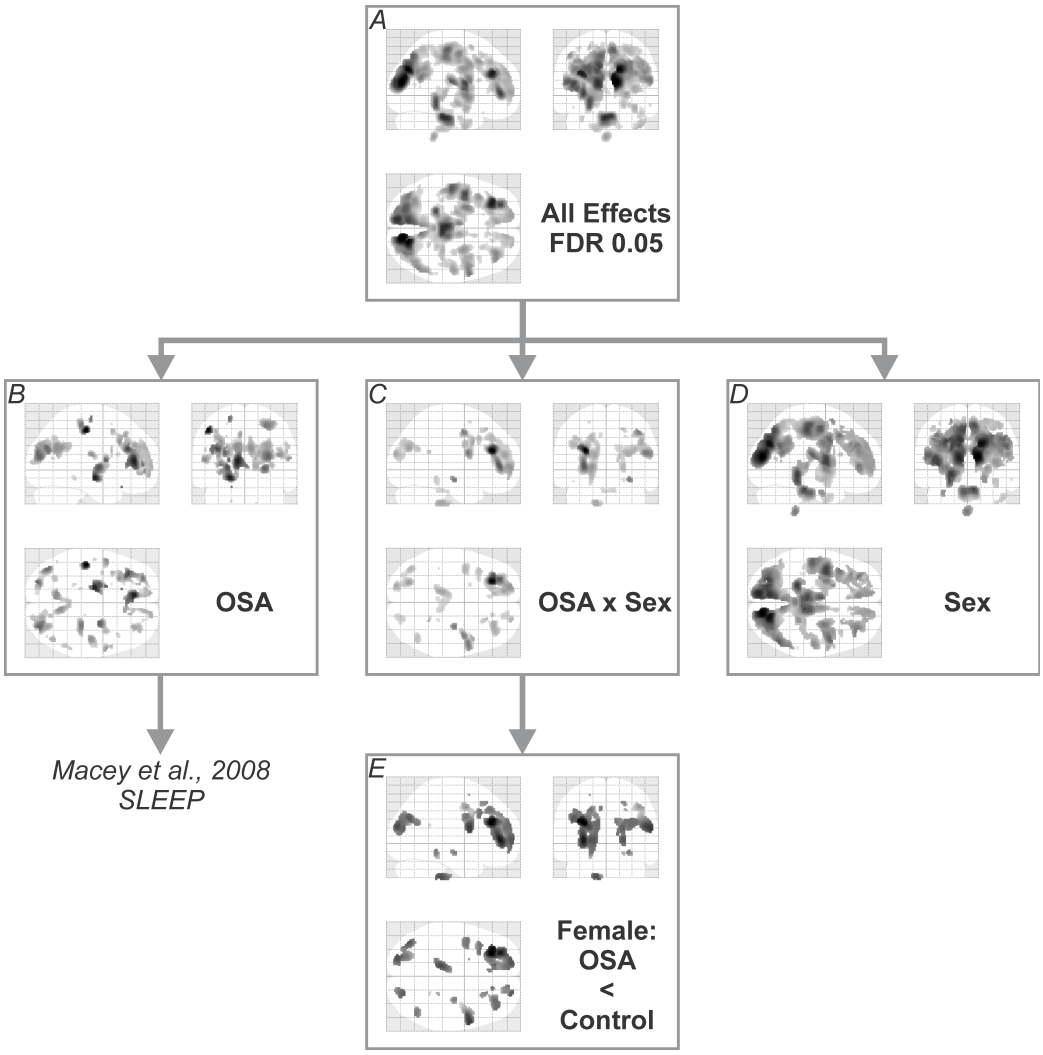

Figure 2—Glass brain view of areas showing significant effects (F test, FDR < 0.05) for **A:** all effects (sex, OSA, and interaction); **B:** OSA effects, i.e., the equivalent to our previous report;[15] **C:** interaction of sex and OSA; **D:** sex effects. **E:** post hoc test of OSA × sex interaction illustrating regions of significantly lower FA in female OSA versus female control subjects (ANCOVA, t statistic at FDR 0.05). (In the glass brain view, all regions of significance throughout the volume are projected onto sagittal, coronal and axial views, giving a visual indication of overall extent and approximate location of significant effects.)

reported[15] OSA effect (Figure 2B), a sex-by-OSA interaction (Figure 2C), and a direct sex effect (Figure 2D).

Interaction of Sex and OSA

Regions showing an interaction effect (Figure 2C) were tested across conditional means with post hoc tests to characterize differences of interest. In these areas, male OSA patients did not show effects relative to male controls at this statistical threshold. In contrast, female OSA patients showed lower FA than healthy female subjects in many white matter structures (Figures 3-5). These areas included fibers projecting to prefrontal regions (Figure 3, coronal and sagittal views), the bilateral forceps minor (Figure 3, axial z = 3, 20, and 22) with greater impact on the left (Figure 3, axial z = 3, 10, 20), and projections from the anterior corona radiata (Figure 3, axial z = 20, 22) with greater differences on the left (Figure 3, axial z = 3, 10). Transverse projections to the lateral frontal cortex on the right, in the region of the postcentral gyrus, showed lower FA (Figure 3, axial z = 20 and 22). Bilaterally, the posterior end of the forceps major extending into the parietal cortex was affected (Figure 3, axial z = 20 and 22). A bilateral region of the inferior aspect of the cingulum bundle, in the region of the mid-to-posterior hippocampus, was affected (Figure 4), with greater extent of change on the left. The right stria terminalis near its terminus in the amygdala was also affected (Figure 4). The brainstem showed an area of significantly reduced FA in the left superior cerebellar peduncle (Figure 5).

DISCUSSION

Changes in white matter in OSA differ by sex, with injury appearing specifically in female OSA subjects. The white matter injury here is inconsistent with female neuroprotection from hypoxia injury in animal models,[33,34] and to stroke in humans,[58,59] but the changes may help explain symptom differences between the sexes. Alternatively, the symptom differences in anxiety and depression in particular may contribute to the neural injury, perhaps in spite of female neuroprotection. Affected axonal bundles included interconnecting fibers to limbic structures, such as the bilateral cingulum and right stria terminalis, and axons to bilateral frontal and parietal cortices and the left

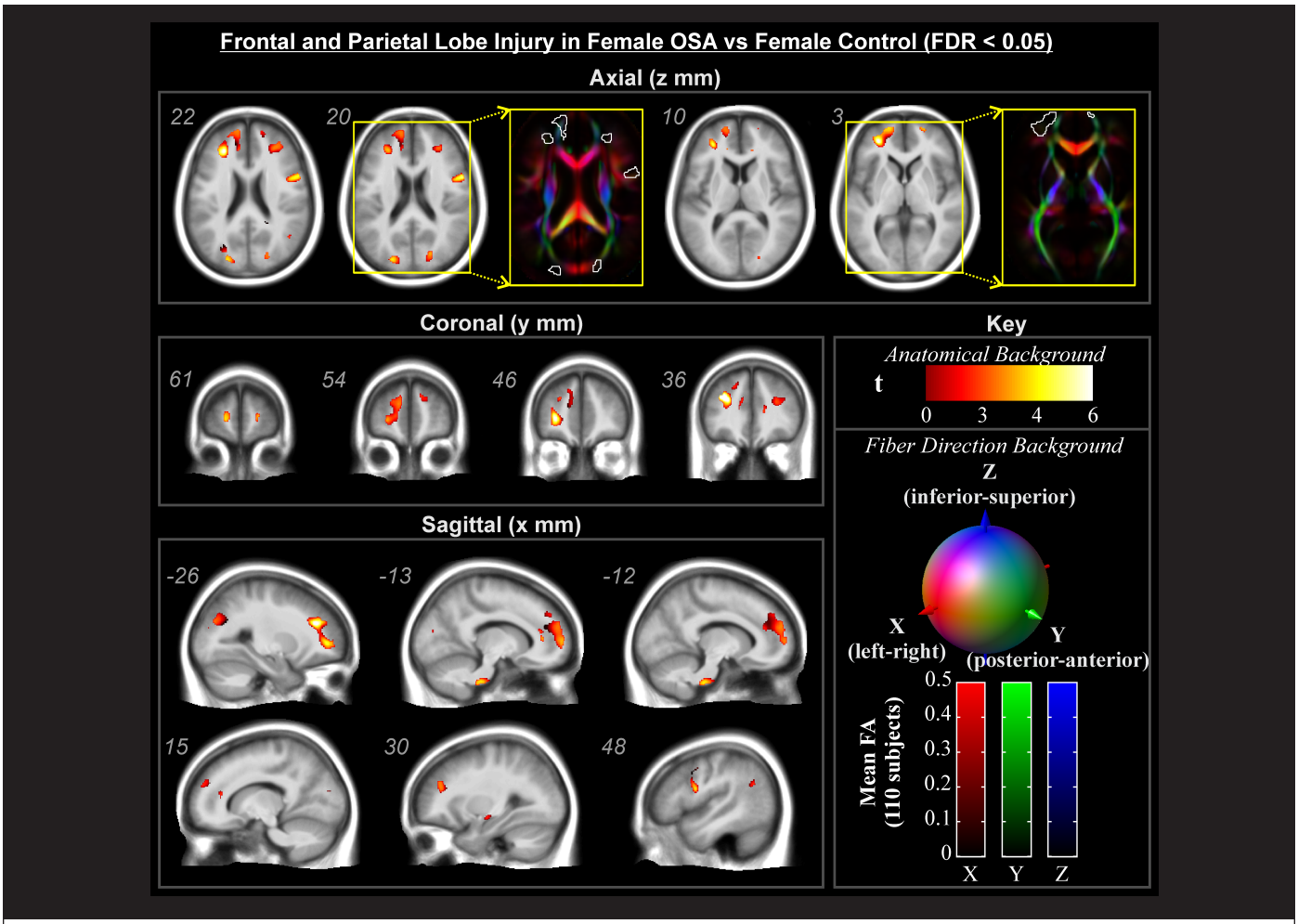

Figure 3—Forebrain regions showing significant interaction effects (Figure 2E) with post-hoc tests of significant female-OSA compromised structure (lower FA) relative to female-control (FDR < 0.05), color-coded according to significance level; numbers indicate locations in MNI coordinates (mm). Backgrounds are the average of 80 subjects' spatially normalized T1 anatomical volumes (grayscale), and DTI-derived tensor 1st eigenvector direction images (color).

superior cerebellar peduncle. Although the origin of the injury cannot be established, the compromised white matter may mediate aspects of the increased psychological symptoms in OSA females over males, particularly anxiety and depression. Limbic and cerebellar areas are also involved in autonomic regulation; thus, the sex differences may play a role in the variations in cardiovascular symptoms between males and females. The findings add white matter injury to the range of characteristics that differ between the males and females with OSA, and reinforce the importance of considering sex as a factor in studies of the central nervous system in the sleep disorder.

Potential Mechanisms of Neural Alterations

Hypoxic and ischemic injury associated with obstructive events likely plays a major role in the changes found in brain structures in OSA. Animal models of intermittent hypoxia show neural injury, with sex-related variations.[33,34,60] Resting blood flow is higher in females,[61,62] which presumably is protective against ischemic conditions, such as may occur with large blood pressure swings that occur with obstructive apneic events.[63] However, whether this vascular advantage is present in female OSA subjects is unknown. Estrogen also confers neuroprotection in females, at least in animal models[35,36]; thus, lower levels of this hormone in post-menopausal females might place those subjects at greater risk relative to their younger counterparts. Other factors may contribute to the structural brain changes, including hypertension and chronic stress and immune system activation. The demonstration of very high levels of type 2 diabetes in OSA patients raises the possibility that additive effects of metabolic disturbances and OSA may contribute to neural injury, at least in general OSA populations,[64] as diabetes alone is associated with structural brain changes,[65,66] but the patterns of neural injury in diabetes across sexes have not been examined. Obesity is another factor associated with neural changes,[67,68] presumably from a combination of metabolic and inflammatory factors contributing to the pathophysiology that injures the brain, although many other factors are likely involved.[69] The presence of psychological symptoms of depression and anxiety in females is likely associated with increased sympathetic and chronic inflammatory activation,[70-72] but the extent of neural injury resulting from these comorbid conditions is also unknown.

Relationship of Neural Changes with Physiological Symptoms

The left superior cerebellar peduncle was affected in female OSA subjects. Those fibers normally communicate between cerebellar nuclei, such as the fastigial autonomic nucleus, to more

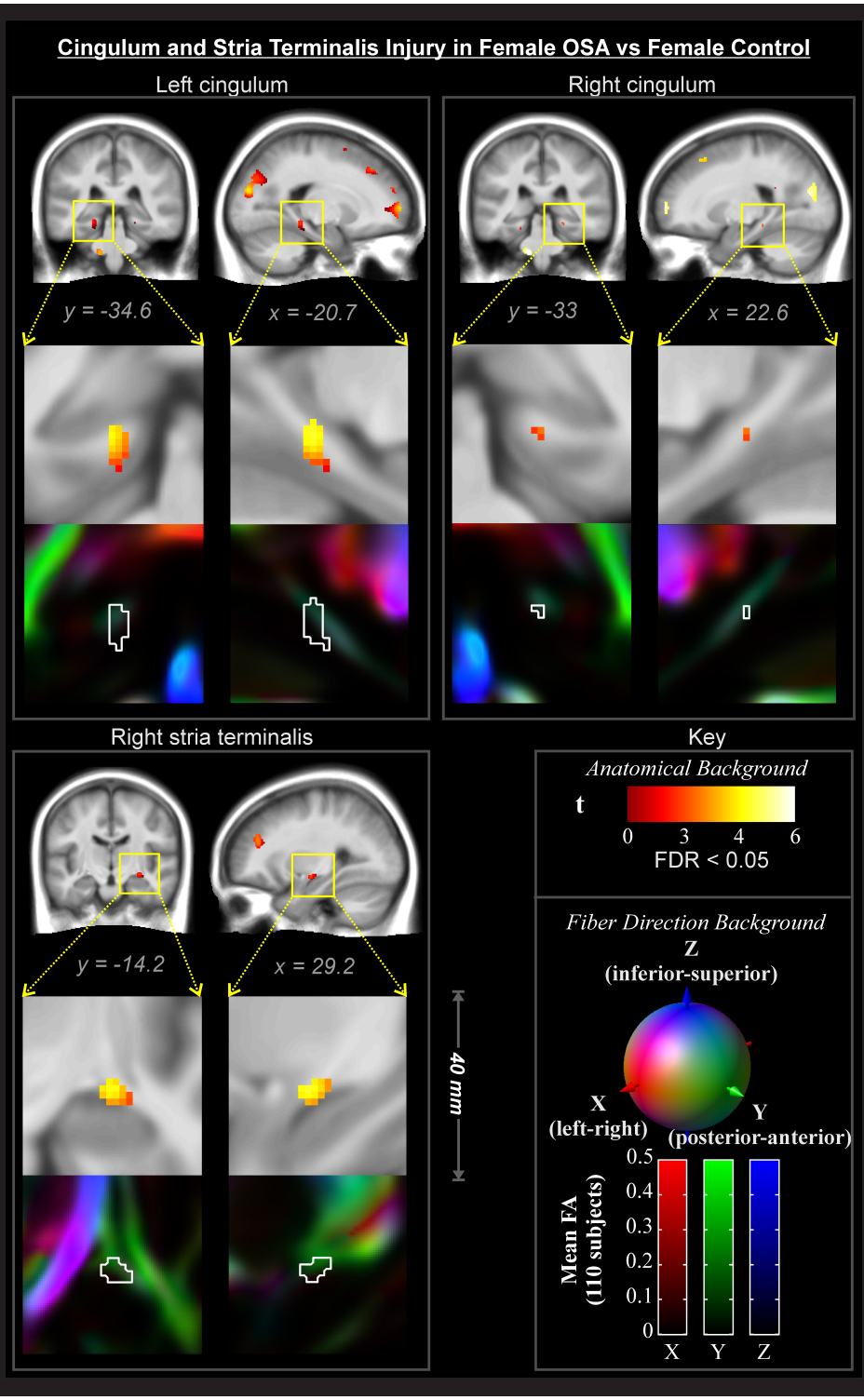

Figure 4—Bilateral cingulum and right stria terminalis regions showing significant interaction effects (Figure 2E) with post hoc tests of significant female-OSA compromised structure (lower FA) relative to female-control (FDR < 0.05), color-coded according to significance level; numbers indicate locations in MNI coordinates (mm). Backgrounds are the average of 80 subjects' spatially normalized T1 anatomical volumes (grayscale), and DTI-derived tensor 1st eigenvector direction images (color).

rostral brain areas, including cardiovascular regulatory areas.[73-77] Autonomic functions are altered in combined male-female OSA populations,[78,79] with elevated sympathetic activity a particular concern, potentially leading to the development of hypertension[80] and cardiac arrhythmias.[81,82] The findings here may relate to the sex-specific variations in cardiovascular symptoms.

Male-female differences in upper airway muscle tone and chemical ventilatory drive have been described in sleep disordered breathing populations,[83-89] and healthy females show reduced upper airway collapsibility over males, an effect associated with higher tongue genioglossal activity.[90,91] Resolution of brainstem areas was too low to determine if other, smaller

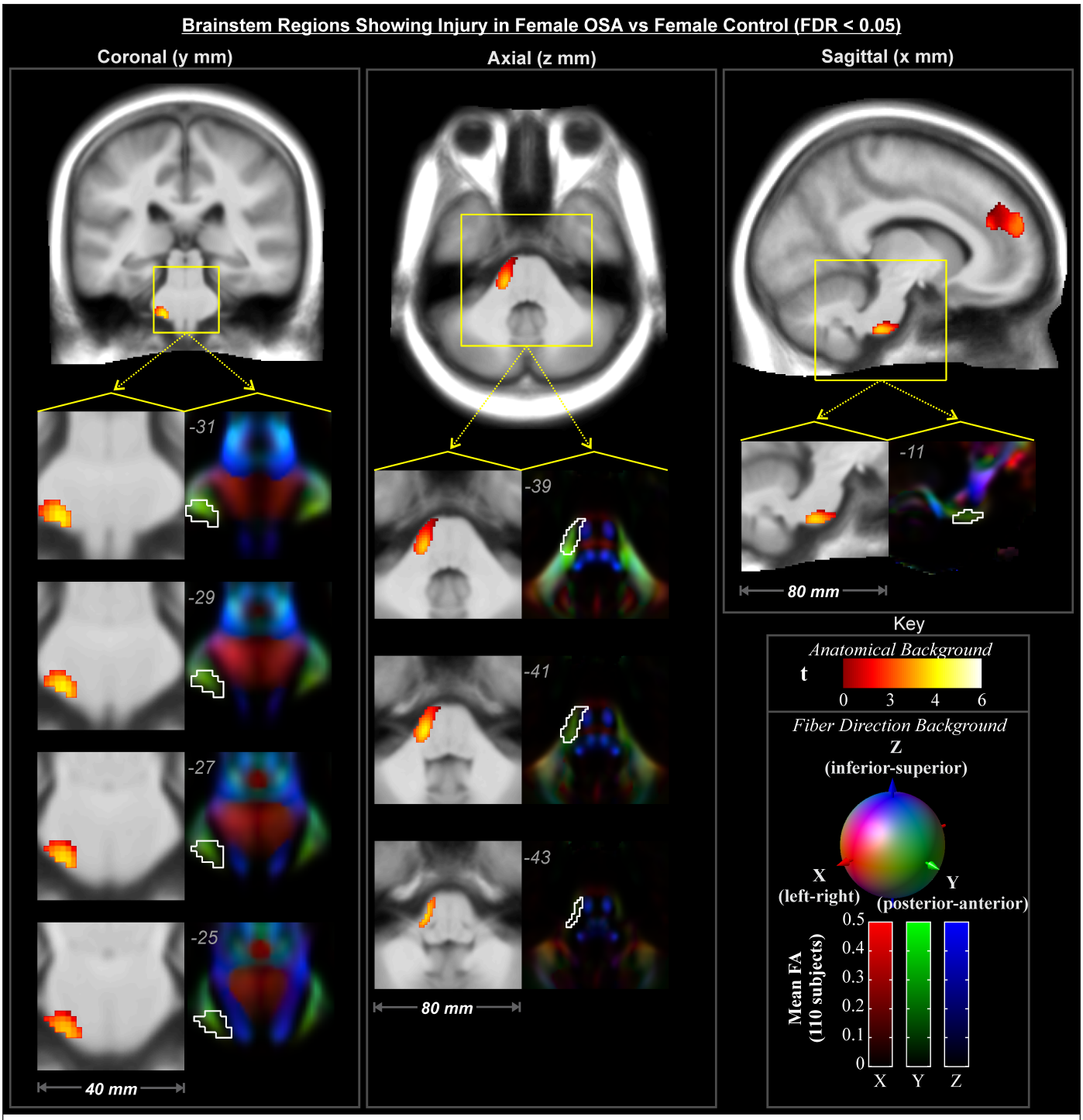

Figure 5—Brainstem regions showing significant interaction effects (Figure 2E) with post-hoc tests of significant female-OSA compromised structures (lower FA) relative to female-control (FDR < 0.05), color-coded according to significance level; numbers indicate locations in MNI coordinates (mm). Backgrounds are the average of 80 subjects' spatially normalized T1 anatomical volumes (grayscale), and DTI-derived tensor 1st eigenvector direction images (color).

structures were specifically affected in females, such as serotonergic projections from the raphe which project to the hypoglossal nucleus. Those raphe projections show functional sex-related differences in rats,[92,93] which may also be present in humans.

Age differences were not significant, although the female OSA group was slightly older than the control and male OSA groups (from 2.3 to 3.7 years older). Since age was a covariate in the model, simple aging effects were likely factored out in the findings. The more complex age-related influence of menopausal status could not be addressed, but the sample included pre-, peri-, and post-menopausal females, so the levels of the potentially neuroprotective[35,36] estrogen hormones will likely have been highly variable in this sample.[94] Furthermore, any beneficial effects would have been present for the duration of the disorder, which would likely be many years prior to their recent diagnosis.[95]

The sleep parameter variations between the sexes do not explain the neural changes. A polysomnography parameter that

differed between OSA males and females was the SpO_2 nadir, but the males were substantially lower than females, suggesting that, if anything, the women patients experienced a more modest level of OSA. Although the oxygen saturation nadir is a crude indicator of hypoxic exposure, the finding suggests that female OSA patients did not experience hypoxia to the extent males did, and therefore, hypoxic exposure is unlikely to be a major factor in the neural differences. An additional parameter showing differences was the greater incidence of apneas in REM sleep in females, but the relevance of REM-only versus all sleep state events is unknown.

Relationship of Neural Changes with Psychological Symptoms

Depression is more common in women with OSA than men,[4,13,96,97] a finding replicated in the present study. The locations of the structural changes are consistent with this higher prevalence in women, including injury in the cingulum bundle adjacent to the hippocampus, and the stria terminalis near its terminus in the amygdala. We earlier showed that the anterior cingulate, hippocampus, and amygdala all showed more injury in depressed OSA subjects than OSA subjects without depression (not partitioned by sex),[98] and the cingulum bundle carries fibers between all three structures.[99,100] Communication between the cerebellum and limbic regions could additionally be affected by the injury to the superior cerebellar peduncle.[101,102] Fibers to prefrontal regions adjacent to the genu of the anterior cingulate cortex, also injured in depressed OSA subjects, were affected in OSA females, and could be associated with impaired mood regulation. The cingulate areas showing female OSA injury are similar to those areas affected in depression independent of OSA.[103-105] Other psychological symptoms, including anxiety and daytime fatigue, tend to be higher in female OSA patients.[4,88] Earlier, we found that anxiety (not partitioned by sex) was associated with enhanced injury in OSA patients in particular regions which included the cingulum bundle as well as targets of the bundle, including the hippocampus and amygdala.[106] The findings here, especially in the cingulum bundle and stria terminalis, the latter carrying fibers from the caudate to the amygdala, two areas having major roles in both depression and anxiety,[107-110] suggest that females may have contributed to a major portion of the earlier description of anxiety and depression-related findings of structural brain changes in OSA. However, whether depression and anxiety are triggered by OSA-induced brain changes or whether such symptoms interact with OSA patterns to induce injury is unclear.

Considerations

The measure used here, FA, is an indirect measure of brain injury that reflects structure, specifically the parallel organization of brain tissue.[39] The index is high in large axonal tracks, but low in gray matter (and close to zero in cerebrospinal fluid). Reductions in FA appear in the presence of fiber injury, including demyelination, shrinkage, and cell death.[111] Other pathologies that lead to reduced FA include fluid accumulation from micro-vascular disease or larger lesions. Additional measures, such as specialized MRI procedures which can partition myelin from axonal and neuronal injury (e.g., magnetization transfer imaging,[112] axial and radial diffusivity,[113] axial and radial kurtosis[114]) may provide further understanding of the structural changes, and will be useful in determining whether the injury developed from hypoxia and other accompaniments of OSA, or preexisted the condition.

The groups in the present sample showed significant differences in BMI. One pattern that could potentially have contributed to the white matter findings is the greater difference in BMI between OSA and controls in females as opposed to males (i.e., 10.7 versus 3.9 kg/m^2). Obesity-related contributions to neural changes cannot be isolated in the present study, and other known factors, especially chronic inflammatory action which is increased with obesity, as well as elevated blood pressure, will need to be considered to distinguish the source of neural pathology in obese OSA patients.[68,115-117]

Although the male and female OSA groups were matched for AHI, the OSA characteristics differed between sexes, consistent with other studies (e.g., Resta et al.[118]). The sleep characteristics, including sleep onset time, awakenings, and time in different sleep stages likely differed between sexes, based on the PSQI and depressive symptom scores (the BDI includes disturbed sleep symptoms). Oxygen desaturation was less severe in females, and REM-dominant OSA was present in significantly more female that male OSA patients. The impact on neural injury of these characteristics is unclear, but what is apparent is that the exposure to OSA phenomena differed between male and female subjects, and these sleep physiology differences could be related to the brain structural differences reported here.

CONCLUSIONS

Male and female OSA patients show marked differences in disease characteristics and comorbidities between sexes; those differences are accompanied by substantial differences in extent and location of white matter injury. Females with OSA show several defined frontal, limbic, and cerebellar axonal structures with reduced integrity, which likely relate to the symptom differences between the sexes, especially variations in psychological symptoms of depression and anxiety, but potentially also physiological differences in cardiovascular characteristics. Whether the psychological symptoms contribute to the injury as opposed to the injury triggering the onset of such symptoms is unknown, and evidence exists for both possibilities. Within the OSA population, the findings suggest females are more affected than males with respect to white matter alterations, which has implications for the importance of early detection and treatment of the disorder in females.

ABBREVIATIONS

AHI, apnea/hypopnea index
BAI, Beck Anxiety Inventory
BDI, Beck Depression Inventory-II
BMI, body mass index
CSF, cerebrospinal fluid
DTI, diffusion tensor imaging
ESS, Epworth Sleepiness Scale
FA, fractional anisotropy
FDR, false discovery rate
FOV, field of view
MNI, Montreal Neurological Institute
MRI, magnetic resonance imaging
OSA, obstructive sleep apnea

PSQI, Pittsburgh Sleep Quality Index
REM, rapid eye movement
SpO_2, oxygen saturation
SE, standard error
TE, echo time
TR, repetition time

ACKNOWLEDGMENTS

The authors thank Ms. Rebecca K. Harper for support with recruitment and data collection, and Drs. Jennifer A. Ogren and Heidi L. Richardson for editorial assistance. Work for this study was performed at the University of California at Los Angeles. Financial support was provided by the National Institutes of Health, HL-60296 and NR-011230.

DISCLOSURE STATEMENT

This was not an industry supported study. The authors have indicated no financial conflicts of interest.

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Appendix A: Process of spatial normalization of DTI images to MNI space

Using standard spatial normalization techniques results in errors with DTI data due to noise and distortions inherent in those EPI-based images. These distortions are distinct from the diffusion-weighted eddy-current effect warping that is corrected with affine coregistration. We developed a process to perform the b0-to-MNI spatial normalization to account for these noise and distortions, based on matching the DTI image to the T1 anatomical scan, and applying the T1 normalization parameters to the DTI data. The adapted normalization process consists of the following steps:

1. The T1 anatomical image was segmented into gray and white matter and cerebrospinal fluid (CSF) using the "unified segmentation" procedure in SPM[119]; the output of this step is three probability maps of the likelihood of brain regions being each particular compartment type. This procedure also produces a transformation from the T1 space to the MNI template.
2. The b0 image was coregistered to the T1 scan using a warping transformation. This process involved applying the SPM unified segmentation/spatial normalization procedure to the b0 image while using the subjects' gray and white matter and CSF probability maps as the "tissue probability maps" (TPM) in the procedure. The TPM define the template space to which the unified segmentation attempts to warp the target image; thus, this step spatially "normalizes" the b0 image to the T1 while allowing for some warping (i.e., undistortion). The output of this step is a transformation from DTI to T1 space.
3. The DTI images (FA maps) are transformed and resliced into T1 space.
4. The T1-space FA maps are transformed and resliced into MNI space, based on the T1 spatial normalization parameters.

We visually checked this process on over 100 adult and 50 pediatric subjects using the "Check Reg" SPM tool, which allows comparison of normalized DTI, T1, and MNI template images. We found the spatial normalization to be consistently improved over standard approaches, with the improvements most notable in the brainstem and prefrontal cortex regions.

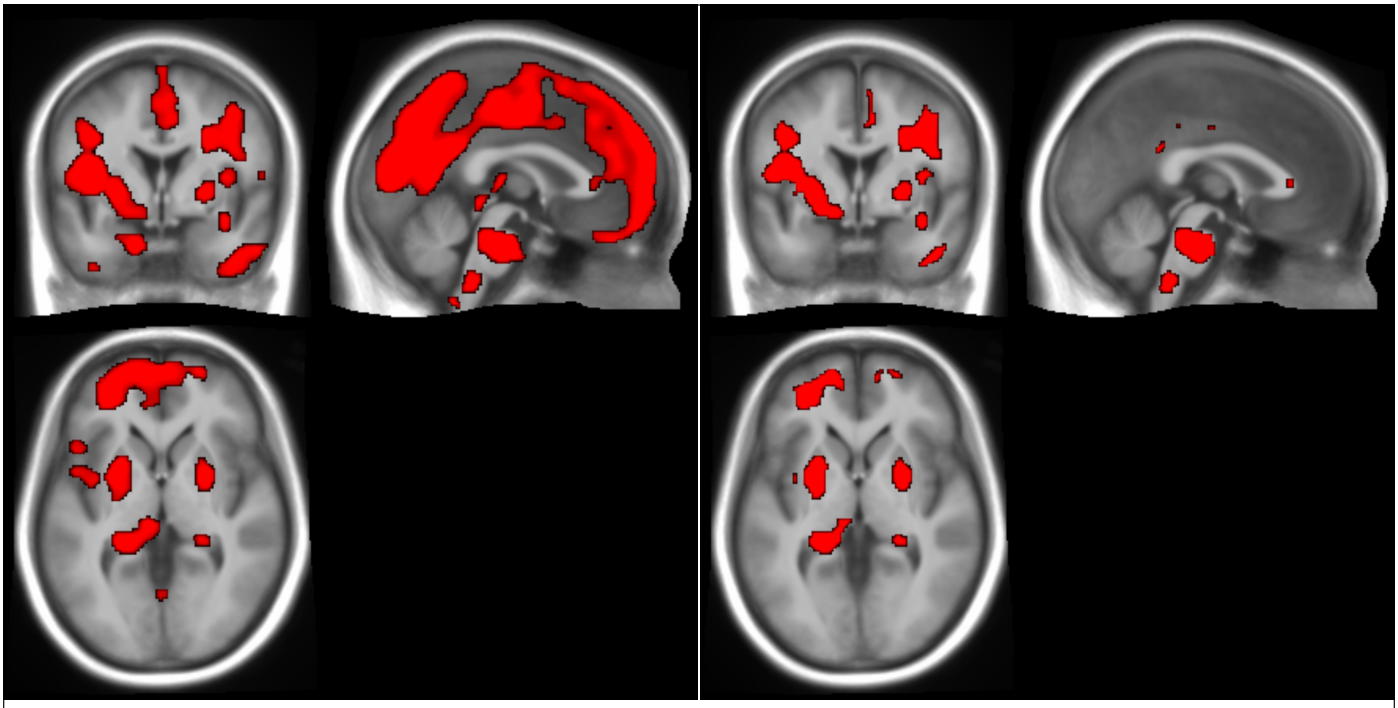

Figure S1—Two FA masks based on alternative thresholds (left: threshold = 0.15, as used in manuscript; right: threshold = 0.05), with the mask in red on a background of the mean anatomical image. These are the coronal (top left), sagittal (top right), and axial (bottom left) views centered on the MNI origin. These were created by overlaying the thresholded FA maps on the mean anatomical background in mricron software.