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The Effects of Obstructive Sleep Apnea Syndrome on the Dentate Gyrus and Learning and Memory in Children

(Running Title: Sleep apnea, dentate gyrus, and cognition)

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ABSTRACT

Obstructive sleep apnea syndrome (OSAS) is associated with intermittent hypoxia and sleep loss. In children, impairments of cognitive function are important manifestations, but underlying pathology is unknown. We hypothesized that OSAS would affect the dentate gyrus, a hippocampal subdivision essential to neurogenesis and cognition, and that this impact would further affect cognitive function in children. In children with OSAS (n=11) and control subjects (n=12; age and sex-matched), we performed diffusion tensor imaging and structural MRI, polysomnography, and neuropsychological assessments. We found that OSAS was associated with decreased mean diffusivity of the left dentate gyrus (P=0.002; FDR corrected; adjusting for sex, age, and BMI) showing a large effect size (partial η^2=0.491), but not with any other structural measures across the brain. Decreased dentate gyrus mean diffusivity correlated with a higher apnea hypopnea index (Spearman's r=-0.50, P=0.008) and a greater arousal index (r=-0.44, P=0.017). OSAS did not significantly affect neuropsychological measures (P's>0.5); however, a lower verbal learning score correlated with lower dentate gyrus mean diffusivity (r=0.54, P=0.004). Path analysis demonstrated that dentate gyrus mean diffusivity mediates the impact of OSAS on the verbal learning capacity. Finally, a diagnostic accuracy of a regression model based on dentate gyrus mean diffusivity reached 85.8% (cross validated). This study demonstrates a likely pathway of effects of OSAS on neurocognitive function in children, as well as potential utility of the dentate gyrus mean diffusivity as an early marker of brain pathology in children with OSAS.

Significance Statement

In this study we investigate the relationships between dentate gyrus structure, hippocampus-dependent cognition, and obstructive sleep apnea syndrome (OSAS). We demonstrate lower mean diffusivity of the dentate gyrus in children with OSAS, which correlates with a lower verbal learning and memory score. This study provides new evidence of disrupted microstructure of the dentate gyrus in children with OSAS that may help explain some of the neurocognitive deficits described in these children.

INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) in children is a common disorder characterized by recurrent events of partial or complete airway obstruction leading to perturbations in gas exchange and recurrent arousals (Marcus et al., 2012; Muzumdar and Arens, 2013). The disorder is also prevalent in adults, though the etiology may differ (Arens and Marcus, 2004). However, in both age groups similar consequences are noted (e.g., inflammatory, cardiometabolic derangements, neurocognitive deficits), suggesting common mechanisms leading to organ injury may be similar.

The neurological impairments described in children with OSAS include: a) impairments of behavioral regulation such as aggression, impulsivity and hyperactivity (Beebe et al., 2004; O'Brien et al., 2004) and b) impairments in neurocognitive function, particularly in the domains of attention, executive function, motor function, and learning and memory (O'Brien, 2009). However, not all studies support impairments in all neurocognitive domains (Jackman et al., 2012; O'Donoghue et al., 2012). A recent randomized control study known as the Childhood Adenotonsillectomy Trial (CHAT) of children 5-9 years of age with mild-moderate OSAS and mild hypoxemia, could not demonstrate impairments in the majority of the neuropsychological tests (Marcus et al., 2013), despite significant altered behavior. In fact, additional evidence of the negative impact of OSAS on neurocognitive function come from reports demonstrating poor academic achievements in children with mild forms of OSAS and in those with snoring alone (Gozal, 1998; Urschitz et al., 2003; Bourke et al., 2011) .

Intermittent hypoxia has been proposed as a key mechanism leading to the neurocognitive deficits described in children with OSAS (Gozal et al., 2001). Recurrent hypoxic events could lead to loss of neuronal integrity in regions regulating memory and learning

(Cervos-Navarro et al., 1991; Bartlett et al., 2004). Additional causes that should be considered and that may affect neurocognition in children are sleep fragmentation and sleep deprivation (Goel et al., 2009; Gevers et al., 2015; Hunter et al., 2016).

Recent neuroimaging studies focusing on the brain and using magnetic resonance imaging (MRI) and magnetic resonance spectroscopic imaging (MRSI) have shown significant changes in gray and white matter in both adults and children with OSAS (Macey et al., 2002; Morrell et al., 2010; Chan et al., 2014; Kumar et al., 2014), particularly in hippocampus and prefrontal cortex (Halbower et al., 2006; Torelli et al., 2011; O'Donoghue et al., 2012), which play important roles in memory, executive function, and motor regulation of breathing, respectively. However, the linkages between these findings, neurocognitive abnormalities, and OSAS remain understudied.

Here we have focused on the role of the hippocampus, particularly the dentate gyrus, in impact of childhood OSAS on neurocognitive function. The dentate gyrus is one of the few brain regions where neurogenesis occurs and it plays a key role in learning and memory, emotion, stress response, and spatial navigation (Sahay et al., 2007). Also, the fact that hippocampal neurons are known to be sensitive to oxidative stress (Wang and Michaelis, 2010) may raise an important question about the relationships between the dentate gyrus structure and OSAS. Addressing this may help identify a neural marker for brain pathology of OSAS.

In this study we tested the hypothesis that childhood OSAS impacts dentate gyrus structural integrity to a greater extent than other brain regions, and that the relationship between OSAS severity measured by either apnea hypopnea index (AHI), oxygen saturation nadir (SpO_2), or arousal index (AI) and neurocognitive function is mediated by measures of dentate gyrus integrity. To test this we investigated the microstructure (diffusion MRI) and macrostructure (structural MRI) of the dentate gyrus using combination of multimodal MRI and automated

115 hippocampal subdivision segmentation (Iglesias et al., 2015), and assessed learning and memory
116 using neuropsychological testing in children with or without OSAS. Additionally, the region-of-
117 interest (ROI) investigation was followed by whole brain analyses of gray and white matter
118 structure using diffusion and structural MRI for the completeness of analysis and comparison
119 with the existing literature.
120

METHODS

Participants

All participants were recruited from the population of patients referred for evaluation of sleep disordered breathing at the Sleep Disorders Center at Children's Hospital at Montefiore (CHAM). Inclusion criteria were: Ages of 13 to 18 years old. Exclusion criteria were: pre-existing neurological disorders such as seizures, developmental delay, brain tumor, degenerative brain diseases, any craniofacial and genetic syndromes with any CNS involvement, history of head trauma, and cyanotic heart disease or chronic lung disease with hypoxemia; history of vision or hearing impairment or any neurocognitive impairment; Non-English speakers; contraindications having a MRI (e.g., claustrophobia, cardiac pacemaker, orthodontics, or having a non-magnetic resonance compatible surgical/ferromagnetic implant)**.**

The HIPAA-compliant study was approved by the Institutional Review Board at Albert Einstein College of Medicine and Informed Consent was obtained from each patient and caregiver. We initially enrolled 14 children with OSAS and 13 controls. Of these, 11 OSAS (5 males and 6 females) and 12 controls (3 males and 9 females) completed all aspects of the study: polysomnography, brain MRI, and neuropsychological tests, and were included in final analyses.

Polysomnography

Participants were studied in the Sleep Disorders Center at CHAM in a quiet, darkened room. Participants slept under natural sleep conditions. The following parameters were recorded and stored on a computerized polysomnography acquisition and analysis system (*XLTEK*, Oakville, Ontario, Canada): chest and abdominal wall movement by respiratory inductance

plethysmography (Respitrace Systems; Ambulatory Monitoring Inc., Ardsley, NY); heart rate by ECG; inspired and expired end-tidal CO_2 tension ($P_{ET}CO_2$) by capnography (Capnogard 1265; Novametrix, Wallingford, CT); airflow by nasal pressure (Pro-Tech, Mukilteo, WA) and three-pronged thermistor, (Nihon Kohden, Tokyo, Japan); arterial oxygen saturation (SpO_2) by pulse oximetry (Masimo, Irvine, CA). In addition, we obtained the following electroencephalogram derivations (F_4-M_1, C_4-M_1, O_2-M_1, with back up electrodes at F_3, C_3 and O_1 and M_2), submental and tibial electromyograms, electrooculograms, and continuous infra-red video digital recording.

Sleep stage was performed using standard criteria (Iber C et al., 2007). Respiratory events were scored using pediatric standards (Iber C et al., 2007). Obstructive apnea was considered as cessation of airflow at the nose and mouth measured by thermal sensor associated with out-of-phase movement of the rib cage and abdomen lasting 2 breathing cycles. Hypopnea was defined as decrease of 30% or more in the amplitude of the nasal pressure transducer associated with a fall in 3% or more of basal oxygen saturation or with an arousal (Berry et al., 2012). OSAS was determined if the obstructive apnea index (AI) was >1 episodes/hour and/or AHI >5 episodes/hour. Hypoventilation was defined as 25% or more of total sleep time with end-tidal CO_2 > 50 mm Hg.

Magnetic Resonance Imaging Acquisition

Participants were scanned with a 3T Philips scanner at Gruss Magnetic Resonance Research Center of the Albert Einstein College of Medicine. For structural scans, T1-weighted images were acquired (axial 3D-MP-RAGE acquisition, FOV = 240 mm, TE = 4.6 ms, TR = 9.9 ms, voxel size = 1×1×1 mm, SENSE factor = 2.6). We collected dMRI using single shot spin echo

EPI (TE = 65, TR = 10000, FOV = 240 mm, voxel size = 2x2x2 mm, non-collinear diffusion sensitizing directions = 32, b = 800 s/mm^2, SENSE acceleration factor = 2.8).

Magnetic Resonance Imaging Analysis

Firstly, to test our hypothesis on the dentate gyrus structural disruption in childhood OSAS, we investigated the microstructure (diffusion MRI) and macrostructure (structural MRI) of the dentate gyrus using automated hippocampal subdivision segmentation. Secondly, we investigated gray and white matter structure across the whole brain using diffusion and structural MRI for the completeness of the study and comparison with the existing literature on effects of OSAS on children's brains. All neuroimaging analyses were conducted on High Performance Computing system in the Department of Systems Biology at Columbia University Medical Center.

Segmentation of Hippocampal Subdivisions (Dentate Gyrus): T1-weigted images were processed using an automated parcellation and segmentation method, Freesurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/; RRID:SCR_001847) (Fischl et al., 2002). We then segmented the hippocampal subdivisions, including the dentate gyrus - our structure of interest, using an automated method available in Freesurfer (version 6; RRID:SCR_005996) (Iglesias et al., 2015). This method combines a training dataset of manual labels based on human *ex vivo,* ultra-high resolution MRI (I.e., 0.13 mm isotropic resolution), neuroanatomy of neighboring structures (e.g., amygdala, parahippocampal gyrus) to estimate, and a novel atlas building algorithm based on Bayesian inference, to generate more reliable anatomical delineation of the hippocampal subdivisions.

Calculation of Diffusion Anisotropy in Dentate Gyrus and other Hippocampal Subregions: For preprocessing, diffusion weighted images were skull stripped, eddy current corrected, and registered to a structural scan in each individual affine registration using FSL's (Functional MRI of the Brain Software Library; RRID: SCR_002823) diffusion toolbox (Jenkinson et al., 2012). Using FSL's DTIFIT program, we calculated mean diffusivity and fractional anisotropy from diffusion weighted images. To avoid possibilities of suboptimal alignment, we chose not to register the images to a standard space (this was different from our earlier study (Cha et al., 2016)). Instead, using a diffusion image registered to a structural image in each participant, we calculated MD of the dentate gyrus and other hippocampal subdivisions. For intra-subject, inter-modality registration, we used linear affine registration in FSL (FMRIB's Linear Image Registration Tool or flirt; (Jenkinson et al., 2002).

Voxel-Based Morphometry: We performed a 'FSL-VBM' analysis of the gray matter across the whole brain. We segmented T1-weighted, skull-striped structural scans into gray matter. These gray matter images were then non-linearly registered using FNIRT (FMRIB's Non-linear Image Registration Tool) onto a template brain estimated from gray matter-segmented images across subjects (to avoid bias in template estimation due to the unbalanced numbers of subject across groups, we matched the numbers by randomly choosing 11 out of 12 HC; an estimated gray matter template was used to register the unchosen scan). The resulting registered (warped) gray matter images were smoothed with an isotropic Gaussian kernel with a sigma of 3 mm.

Tract-Based Spatial Statistics: We performed TBSS (Tract-Based Spatial Statistics) in FSL (Smith et al., 2006) to investigate associations of OSAS with white matter integrity (fractional anisotropy or FA) across the whole brain. Following the TBSS pipeline, we performed

registration of FA maps to the standard (e.g., Montreal Neurological Institute) space (using a combination of "tbss_2_reg –n" and "tbss_3_postreg –S"), resampling to 1-mm isotropic voxel space, skeletonisation (threshold FA = 0.2), calculation of distance map, and projection of the FA values onto the skeleton.

Neuropsychological Evaluation

Neuropsychological evaluation was performed in all subjects on the day of imaging studies. Premorbid IQ was assessed using the WRAT3 Reading subtest (Wilkinson, 1993). Cognitive assessment was obtained through administration of CogState (Collie et al., 2003; Maruff et al., 2009) (www.cogstate.com), a computer-administered battery of cognitive function that includes measures of both verbal and visual learning and memory.

Verbal learning and memory were assessed using the International Shopping List Task (ISLT). In the learning condition, the test administrator read the following instructions: “In this task, I am going to read you a shopping list. I would like you to remember as many items from this list as possible. Are you ready to start?” The test administrator then read a list of 16 words to the participant that were presented via the computer screen to the test administrator, but were not within the view of the participant. Words were presented to the participant at a rate of one word per second. A total of three learning trials were presented in this format. Responses were recorded by the test administrator using the computer-based interface. The primary variable of interest for this test was the total number of correct words recalled over 3 trials. In the delayed recall condition, the test administrator read the following instructions: “Tell me as many of the items from the shopping list as you can remember.” This occurred following a delay period of

238 approximately 15 minutes. Responses were again recorded by the test administrator using the
239 computer-based interface.
240
241 Visual learning and memory were measured using the Groton Maze Test (GMT). In the learning
242 condition, the participant was presented with a 10 x10 grid of square 'tiles' on a computer screen
243 and they were asked to learn a 28-step 'pathway' through the maze on the basis of trial and error
244 feedback. Participants were instructed that they could only move one tile at a time, they could
245 only move up, down, left, or right, and that they could not move diagonally or backwards. In
246 addition, participants were instructed to tap as quickly and as accurately as they could. A total of
247 5 learning trials were presented in this format. Responses were recorded using the computer
248 mouse and keyboard. In the delayed recall condition, the test administrator read the following
249 instructions: "You must now find the same hidden pathway that you learned before." The
250 participant was again presented with a 10x10 grid on the computer screen The participant
251 completed this delayed recall trial once 15 minutes after completion of the learning trials. The
252 primary variable of interest for both learning and memory conditions of this task was the number
253 of errors.
254
255 **Statistical Analysis**
256 Statistical analysis was done using SPSS (RRID:SCR_002865) and R (RRID:SCR_001905), and
257 randomise, a permutation test in FSL (for voxel-wise analysis) (Winkler et al., 2014). Statistical
258 significance was corrected for multiple comparison: e.g., in the dentate gyrus structural measures,
259 since we had no *a priori* hypothesis about laterality, we first assessed effects of OSAS on two
260 measures (for either MD or volumes) of the hemispheres in separate models, and then corrected
261 for two multiple comparisons using False Discovery Rate (FDR).

Neuropsychological testing: We factored in learning effects during the neuropsychological testing. A recent study with individuals with Alzheimer's disease reports a greater impairment of verbal learning and memory (assessed by ISLT) in a later trial (Thompson et al., 2011). This suggests that learning effects (e.g., sensitization) across multiple trials that are observed in healthy individuals may be disrupted in Alzheimer's disease. Based on this *a priori* knowledge, we factored in potential learning effects by multiple trials: we weighted a score in each trial by multiplying either 1, 2, or 3 (i.e., a trial order) to capture learning effects. We reasoned that this method might maximize any differences in the learning and memory measures between an optimal learner and a sub-optimal learner could be maximized (Thompson et al., 2011). In case of GMT, it is not clear whether such learning effects on non-verbal learning and memory could be detected; nevertheless, we have tested both unweighted and weighted scores for GMT as well.

General Linear Model: To test effects of OSAS on the neural and neuropsycological measures, we used generalized linear model (GLM). The primary outcome variable was either neural (e.g., dentate gyrus MD or volumes) or neuropsychological measures (ISLT or GMT); the predictor was diagnosis (OSAS/CONTROL); the covariates were age, sex, IQ, body mass index (BMI). Given the group difference in BMI, to avoid any collinearity between OSAS and BMI, we used group-mean centered BMI in these models.

Correlation: We used non-parametric methods (Spearman's rho) to test correlations of the dentate gyrus MD with clinical and neuropsychological variables among children with OSAS. To determine significance, permutation testing was used; conditional null distribution was

created based on Monte Carlo approximation using "coin" r package (https://cran.r-project.org/web/packages/coin) (Hothorn et al., 2008).

Mediation Analysis: We tested a hypothesis that a disrupted dentate gyrus MD mediates the effects of childhood OSAS on verbal learning and memory using mediation analysis ("mediation" R package) (Tingley et al., 2014). This method allows to assess a confidence interval of the mediation effect itself using rigorous sampling techniques with fewer assumptions of the data. Left dentate gyrus MD was used as the mediator; diagnosis, as the treatment variable; weighted ISLT score, as the outcome variable; age, sex, IQ, and BMI as the covariates. Average causal mediation effect (ACME) was determined using a non-parametric bootstrapping method (bias corrected and accelerated; 1,000 iterations).

Voxel-Wise Analysis (VBM and TBSS): Our primary interest was to test impact of OSAS on the gray matter volumes and white matter integrity (FA) using VBM and TBSS, respectively. General linear model containing diagnosis as the regressor, and age, sex, BMI, and IQ as the covariates was fitted using randomise in FSL.

As an exploratory analysis, we tested whether a decrease in the dentate gyrus MD was associated with volumetric changes in the neighboring brain regions. The model included the dentate gyrus MD as the regressor, diagnosis as the regressor-of-no-interest, and the same covariates as in the primary model above. We performed 10,000 Monte-Carlo sampling based random permutations, given a recent report of optimal permutation numbers in a VBM study (Dickie et al., 2015). Multiple comparison correction was done using threshold free cluster enhancement (TFCE) (Smith and Nichols, 2009).

309 Receiver-Operator Characteristics Analysis: We performed receiver-operator characteristics
310 (ROC) to test whether hippocampal microstructure measures can reliably predict OSAS using
311 binary logistic regression and leave one out cross validation. Once we obtained diagnostic
312 accuracy (area under curve or AUC), we calculated a minimum sample size based on alpha of
313 0.05 and beta of 0.8 using r package of pROC (Robin et al., 2011).
314

RESULTS

Subjects

Eleven children with OSAS (5 males and 6 females) and 12 controls (3 males and 9 females) completed polysomnography, neuropsychological tests, and brain MRI, and were included in the current analyses. Demographic data are presented in **Table 1**. No group differences in age, sex, or ethnicity were noted. However, children with OSAS had a higher BMI than the control subjects (P = 0.04), though both groups included overweight and obese subjects (3 overweight and 8 obese children in the OSAS group; 3 normal, 4 overweight, and 5 obese children in the control group). Given the literature on effects of obesity and some of the neural measures (e.g., hippocampal volumes (Hashimoto et al., 2015)) that we planned to test in this study, we further adjusted for BMI in all neural and neuropsychological measures in our statistical analyses.

Polysomnography

Polysomnography data with OSAS and controls are presented in **Table 2**. The OSAS group had moderate to severe OSAS and significant differences compared to the control group in regard to AHI (14.8 ± 7.1 vs 0.8 ± 1.1 events/hr; $P < 0.001$), AI (19.5 ± 11.0 vs 6.8 ± 2.7 events/hr; $P < 0.001$) and SpO_2 nadir (87.4 ± 11.7 vs 93.9 ± 1.4 %; $P = 0.003$).

Effects of OSAS on hippocampal microstructure

We found that OSAS was significantly associated with a decrease in left dentate gyrus MD with a large effect size ($t = 3.92$, $P_{FDR} = 0.002$, partial $\eta^2 = 0.491$; adjusting for age, sex, IQ, and BMI; no collinearity among the covariates was detected [variance inflation factor < 1.7]; the effects of OSAS were similarly significant without adjusting for BMI [P = 0.001]; **Figure 1A**). In a

separate hierarchical regression, BMI accounted no significant variance of left dentate gyrus MD without the presence of OSAS ($P = 0.118$), whereas, in the presence of OSAS, it accounted for a marginally significant variance ($P = 0.054$). No effects of OSAS were found on the right dentate gyrus ($P_{FDR} = 0.501$). Only MD showed the significant effect of OSAS; no significant effects of OSAS were found on volumes or fractional anisotropy of the dentate gyrus in either hemisphere (P's > 0.12). Dentate gyrus MD averaged across hemispheres showed no significant effects of OSAS ($P = 0.5$).

Across all participants, decreased dentate gyrus MD was significantly correlated with a higher AHI (r = -0.50, P = 0.008, one-sided Spearman's rank correlation with permutation; (**Figure 1D**) and with a greater arousal index (r = -0.44, P = 0.017). In children with OSAS only, the correlation remained significant with arousal index (r = -0.57, P = 0.033), but not with AHI (r = -0.39, P = 0.139). No significant correlations of dentate gyrus MD were found with total sleep time, sleep efficiency, awakening index, or SpO_2 nadir (P's > 0.24).

Exploratory analysis in other hippocampal subdivisions.

Of all the subdivisions of the hippocampus, OSAS was associated with a smaller MD or volume in the CA4, presubiculum, and fimbria with large effect size (partial η^2 of nearly 0.3) (**Figure 1B&C**).

Effects of childhood OSAS across the whole brain structure

In whole brain exploratory analyses, we found no significant impact of OSAS on the white matter integrity (measured by FA and MD; TBSS) or the gray matter volumes (VBM) at the whole brain corrected alpha of 0.05 (**Figure 3A**). No significant results were found in the white matter anisotropic measures.

Effects of childhood OSAS on neuropsychological measures

Effects of OSAS on ISLT (verbal) and GMT (non-verbal) learning memory did not reach significance (**Table 3**).

Across all participants, lower dentate gyrus MD was correlated with a lower ISLT "learning score" (number of correct responses across learning trials; weighted score) (r = 0.54, P = 0.004, one-sided Spearman's rank correlation with permutation; unweighted score: r = 0.42, P = 0.022; **Figure 1D**), but not with ISLT "delayed score" (number of correct responses on a delayed trial) (P = 0.36). In children with OSAS only, the correlation with ISLT "learning score" remained significant (r = 0.59, P = 0.028; unweighted score: r = 0.61, P = 0.023). ISLT scores showed no significant correlation with SpO_2 nadir or arousal index (P > 0.2) either in all participants or in children with OSAS. No significant correlations of GMT scores (weighted or unweighted) were found with neural or polysomnography measures (P's > 0.19).

Mediation of dentate gyrus MD between AHI and neuropsychological measures

We then tested a mechanistic hypothesis that dentate gyrus MD (left) mediates the effect of OSAS on learning and memory capacity. Although no significant *direct* effects of OSAS on learning and memory was observed, we reasoned this mediation analysis could reveal a plausible causal pathway, otherwise unobservable. We found a significant mediation effect of OSAS on a decrease in verbal memory capacity via a decrease in dentate gyrus MD (average causal mediation effect or ACME = -11.99, P = 0.022, bias-corrected & accelerated, **Figure 2**)

Correlation between dentate gyrus MD and volumes across regions.

We tested an exploratory hypothesis that, if a decrease in the dentate gyrus MD is related to childhood OSAS, this measure would also correlate with morphometric changes in other regions. We found that a decrease in left dentate gyrus MD significantly correlated with a decrease in gray matter integrity in the left hippocampus, amygdala, thalamus, striatum, and brainstem (whole brain corrected $P < 0.01$; **Figure 3B**). The regions showing the correlation were subcortical and cortical regions surrounding the hippocampus, such as, the parahippocampal gyrus or the ventral visual stream. The correlation was more apparent in the left hemisphere than in the right hemisphere. Of note, in the earlier VBM analysis, no effects of OSAS were shown in those regions.

Prediction of childhood OSAS based on dentate gyrus MD

Lastly, we tested whether dentate gyrus MD can reliably predict diagnosis of OSAS in a given individual. Binary logistic regression using dentate gyrus MD only showed modest diagnostic power with diagnostic accuracy of 69.8% with sensitivity of 81.8%, specificity of 72.7%, and diagnostic odds ratio of 12 (leave one out cross validation). A model with dentate gyrus MD and BMI—an important risk factor for OSAS (Peppard et al., 2000)—showed a greater diagnostic power with diagnostic accuracy of 85.8%, sensitivity of 90%, specificity of 72.7%, diagnostic odds ratio of 24.11. Adding demographic information, such as age and sex decreased diagnostic accuracy to 78% (**Figure 4**). Demographic information and BMI without dentate gyrus MD showed diagnostic accuracy of 62.9%. For the model with MD and BMI, a minimum sample size based on alpha of 0.05 and beta of 0.8 was estimated as 9 in each group.

DISCUSSION

In this study, we found significant associations of childhood OSAS specifically with microstructure (i.e., mean diffusivity) of the dentate gyrus, but not with macrostructural measure (i.e., volume) of the dentate gyrus or in other brain regions. A decrease in the dentate gyrus MD mediated the effect of OSAS on verbal memory capacity, and correlated with a decrease in volumes of the neighboring gray matter (such as the hippocampus, and parahippocampal gyrus). Taken together, this study suggests that disrupted dentate gyrus microstructure may be central to understanding the effects of OSAS on neurocognitive development. The association between OSAS and dentate gyrus microstructure appeared to be independent of BMI. In addition, no significant relationships between BMI and neuropsychological measures were found. Because obesity is an important risk factor for OSAS, it is difficult to disentangle whether the effects are driven largely by obesity or OSAS, or both. Future studies with a larger sample size and a longitudinal design may be able to distinguish between these effects.

Our study extends the literature of the impact of OSAS on neurocognition. Prior human and animal studies demonstrate atrophy of widespread brain regions (Macey et al., 2002; Morrell et al., 2003; Huynh et al., 2014), hippocampal damage (Bartlett et al., 2004; Fung et al., 2007), and cognitive deterioration (Decary et al., 2000; Kielb et al., 2012) in OSAS. An early study in children with OSAS (aged 6-16yr) reports a decrease in neuronal metabolites (N-acetyl aspartate/choline ratio) in the hippocampus and decrements in neuropsychological measures (ref. brain-behavioral relationships were not reported in this study) (Halbower et al., 2006). Likewise, a decrease in diffusion anisotropic measures in widespread brain structures, including the hippocampus is reported in a recent study with adults with OSAS (Kumar et al., 2014). In addition to these, the present study also suggests a role of childhood OSAS that may be specific

to the dentate gyrus microstructure, with no association with gray matter volumes (VBM) and white matter integrity (TBSS). However, given the small sample size, the study may have been underpowered to detect small effects between OSAS, on gray and white matter. The decrease in the dentate gyrus MD may indicate an early neural impact of childhood OSAS that may also be associated with subsequent effects on neurocognition. This may be also supported by our exploratory analysis suggesting that the decrease in the dentate gyrus MD may mediate the impact of OSAS on verbal learning and memory; and the correlation of the decrease in the dentate gyrus MD with a decrease in hippocampal/parahippocampal gray matter volumes. However, because of the cross-sectional nature of the study, this mediational analysis needs to be interpreted with caution and confirmed in a larger and prospective study.

What are the neurobiological underpinnings of the dentate gyrus diffusivity? Diffusion MRI characterizes microstructural properties of tissues by measuring the displacement of water molecules in the tissues. In an anisotropic structure, such as gray and white matter (cf. an isotropic structure, i.e., CSF), the key factor of degrees of diffusion is permeability of cellular membranes (Assaf and Cohen, 2009). Since anisotropy contrast is poor in the gray matter, mean diffusivity is considered a better metric of gray matter tissue properties than fractional anisotropy. For example, in neurodegenerative disorders such as Alzheimer's disease (Rose et al., 2006), Mild Cognitive Disorder (Muller et al., 2005; Rose et al., 2006), an increase—rather than a decrease—in hippocampal mean diffusivity correlates with a decrease in hippocampal volume and a cognitive deficit. Although these studies suggest hippocampal diffusivity as a useful metric of pathological microstructural properties, its interpretation however requires a careful consideration of the underlying physiology of the hippocampus in the developing brain: for example, neurogenesis. In children's brains, neurogenesis may occur at a higher rate than in adults' brains. (Spalding et al., 2013). Since neurogenesis couples with angiogenesis and

vascular remodeling, greater degrees of neurogenesis in children's brains than in adults' brains may reflect a greater magnitude of neurovascular change. Thus, microstructure of the hippocampus, indexed by mean diffusivity in this study, in children's brains is likely to be different from that of adults' brains; but to our knowledge, this has yet to be tested. This physiological difference with regard to neurogenesis should be considered when interpreting diffusion metrics.

We speculate that the decrease in the dentate gyrus MD in childhood OSAS may be related to a change in dentate gyrus microstructure—perhaps resulting from a decrease in hippocampal neurogenesis and the related changes in tissue properties indexed by MD. This may reconcile the results of this study and the literature. First, as learning and memory is often thought to involve dentate gyrus neurogenesis, in our study, decreased dentate gyrus MD in OSAS correlated with a poorer verbal learning score (ISLT) perhaps indicating altered neurogenesis in the dentate gyrus. This is in line with the recent conceptualization of linking hippocampal neurogenesis to hippocampus-dependent cognition in normal (Deng et al., 2010) and pathophysiology (Sahay et al., 2007). Second, the correlation between a greater OSAS severity (AHI) and a smaller dentate gyrus MD may reflect an association between a greater intermittent hypoxia in OSAS and a reduced neurogenesis-related microstructural integrity in the dentate gyrus.

Alternative explanations of the decrease in the dentate gyrus MD in OSAS merit consideration. Let us consider neurodegeneration. Hypoxia causes neurodegeneration (Nakajima et al., 2000). One might think that intermittent hypoxia in OSAS may initiate an apoptotic process in the hippocampus. This may explain the correlation with a gray matter volume decrease, and the mediation effect on cognitive function. Nevertheless, this account directly conflicts with the existing literature reporting a correlation between hippocampal

neurodegeneration and an increase, not a decrease, in hippocampal mean diffusivity in older adults (Muller et al., 2005; Rose et al., 2006).

Another consideration may be a compensatory mechanism in response to intermittent hypoxia. Some animal studies report acceleration of neurogenesis in response to intermittent hypoxia (Zhu et al., 2005; Zhu et al., 2010). However, it remains to be tested whether the magnitude of intermittent hypoxia used in the laboratory experiments with rodent models is comparable to that of OSAS. Furthermore, the compensatory mechanism does not explain either the correlation with the gray matter volume decrease or the mediation effects on verbal learning and memory. Therefore, neither alternative account may reconcile our data or the literature.

The dentate gyrus MD may confer clinical utility. Our ROC analysis with leave-one-out cross validation showed the decrease in the dentate gyrus MD along with BMI, a known risk factor of OSAS (Peppard et al., 2000), predicted diagnosis of childhood OSAS with 90% sensitivity and 72% specificity. Although it remains to be replicated in a larger sample, this may open a possibility of neuroimaging-based biomarker of diagnosis or treatment strategies of childhood OSAS. For example, a short (less than 15 min) MRI session could offer a quick, reliable, alternative biomarker for the diagnosis of OSAS.

There are limitations worth considering in this study. First, the inferences drawn in this cross-sectional study need to be confirmed with a longitudinal study to formally test the mediation effects of microstructure of the dentate gyrus. Second, potentially suboptimal delineation (particularly for pediatric samples) of the dentate gyrus and the limited spatial resolution of DTI ($2mm^3$) used in this study could be sources of error (e.g., partial volume effect). Third, we interpret the findings within the OSAS group showing a stronger and statistically significant correlation of dentate gyrus MD with arousals and not with AHI with caution. Based on the moderate association between the dentate gyrus MD and AHI, it is likely that the study

503 may have been underpowered to distinguish whether these effects were driven by arousals alone
504 or also by AHI. Thus, our results remain to be replicated in a larger sample to better refine these
505 effects and the effects of obesity in these children.

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677 ## FIGURE LEGENDS

678 **Figure 1. Significant effects of OSAS on dentate gyrus microstructure and cognition in**
679 **children. A**, Violin plot of mean diffusivity (MD) of the dentate gyrus (left hemisphere) in
680 OSAS and Controls (box represents interquartile range; white dot, median; thin line, 95%
681 confidence interval; density plot width, frequency). **B**, Representative segmentation of
682 hippocampal subdivisions based on T1-weighted images using an automated segmentation
683 method. **C**, An exploratory analysis showing effects (effect sizes, partial eta squares) of OSAS
684 on hippocampal mean diffusivity and volumes across subdivisions. **D**, For all subjects (OSAS
685 and Controls) a decrease in dentate gyrus mean diffusivity correlates with an increase in apnea
686 hypopnea index, an increase in arousal index, and a decrease in verbal learning (International
687 Shopping List Test). AHI, apnea hypopnea index; DG, dentate gyrus; HT, hippocampal tail; Fim,
688 fimbria; HATA, hippocampus–amygdala-transition-area; Sb, subiculum; Pre-s, presubiculum;
689 Par-s, parasubiculum.
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693 **Figure 2. Path analysis shows that decreased left dentate gyrus mean diffusivity mediates**
694 **effect of OSAS on learning and memory capacity.** Parameter estimates are shown with P
695 values (in brackets). For OSAS/Control, diagnosis was used; for verbal learning and memory,
696 ISLT. Red dotted arrow denotes average causal mediation effect (ACME). Covariates in the
697 mediation model are not shown (age, sex, and IQ).

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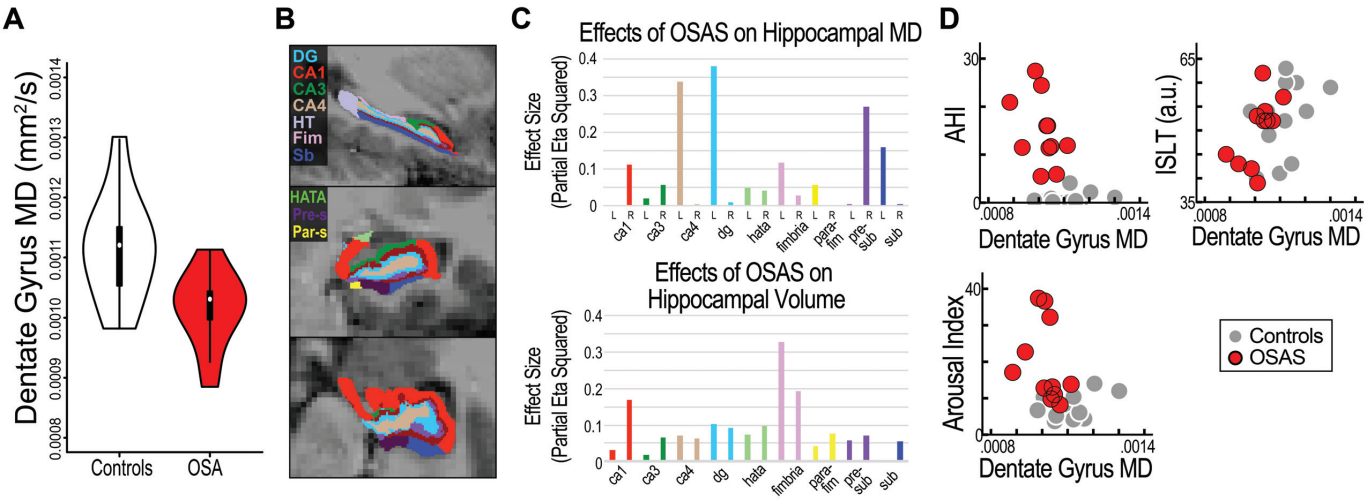
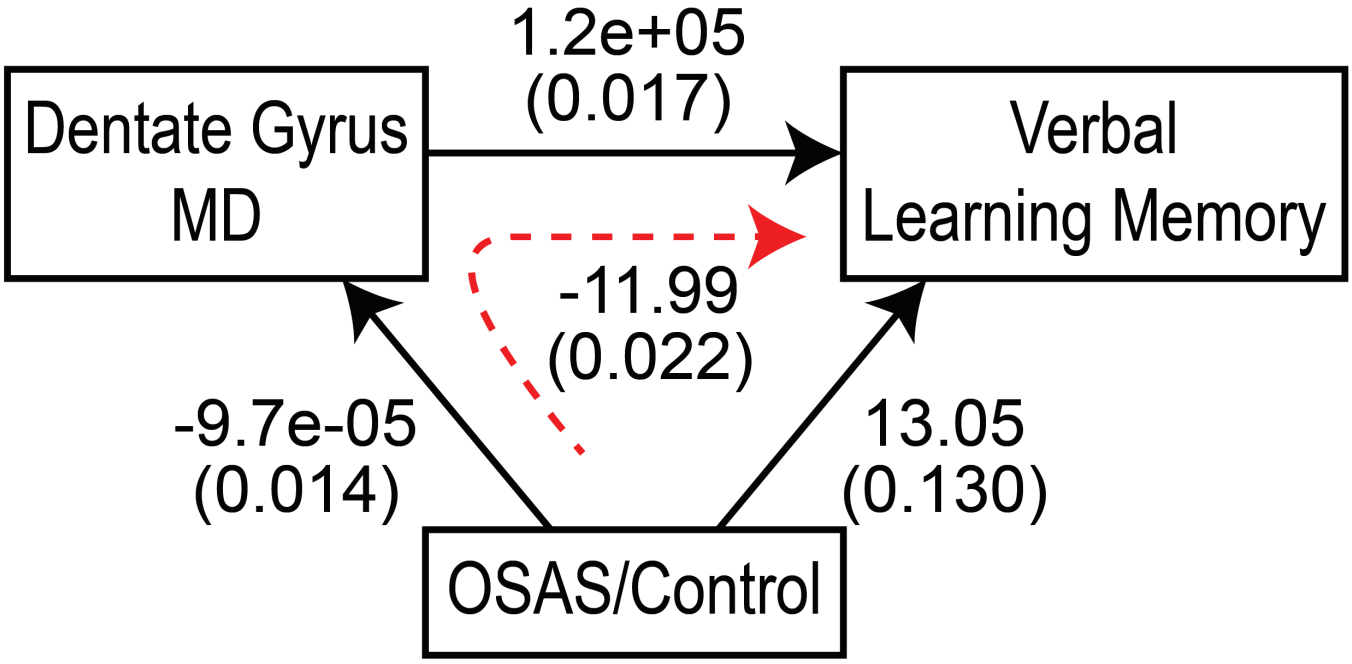
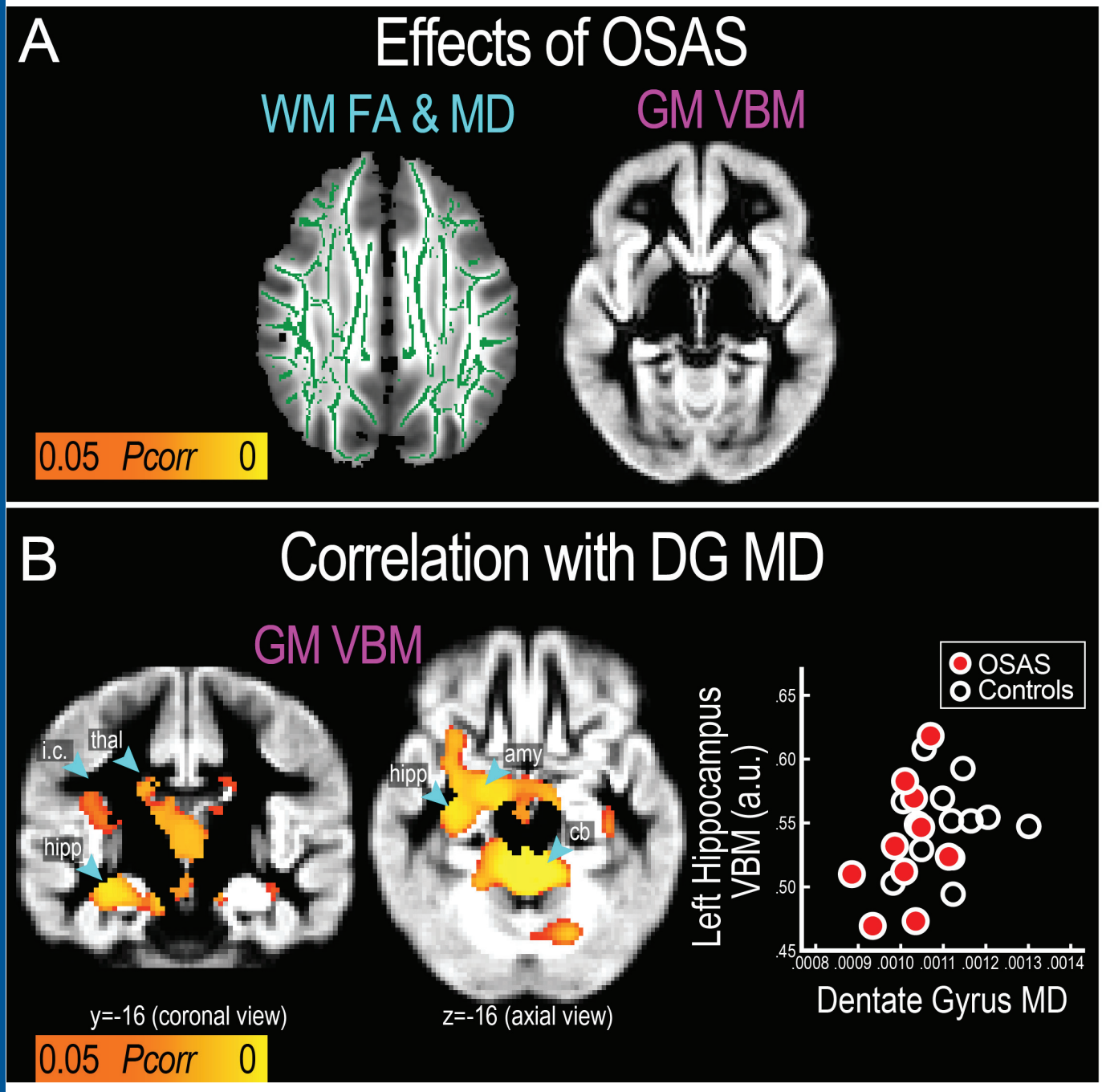
Figure 3. Voxel-Wise Whole-Brain Analyses on Gray and White Matter. ***A,*** No significant group differences were found in fractional anisotropy or mean diffusivity of the white matter (track-based spatial statistics; green represents a group-mean white matter "skeleton" map) or in gray matter intensity (voxel-based morphometry) across the whole brain at corrected $P < 0.05$. ***B,*** Voxel-based morphometry analysis showed that a less dentate gyrus mean diffusivity correlates with a smaller volume in the left hippocampus, amygdala, striatum, and thalamus. The scatter plot is based on relative signal intensity (in arbitrary unit) extracted from the ventral hippocampus around the dentate gyrus from VBM analysis. Amyg, amygdala; cb, cerebellum; FA, fractional anisotropy; GM, gray matter; hipp, hippocampus; i.c., insula cortex; MD, mean diffusivity; TBSS, tract-based spatial statistics; thal, thalamus; VBM, voxel-based morphometry.

Figure 4. Dentate gyrus mean diffusivity predicts diagnosis of OSAS. Receiver-Operator Characteristics (ROC) plot shows sensitivity and specificity in prediction of diagnosis using a logistic regression model with dentate gyrus mean diffusivity and BMI. ROC analysis was cross-validated (leave one out cross validation).







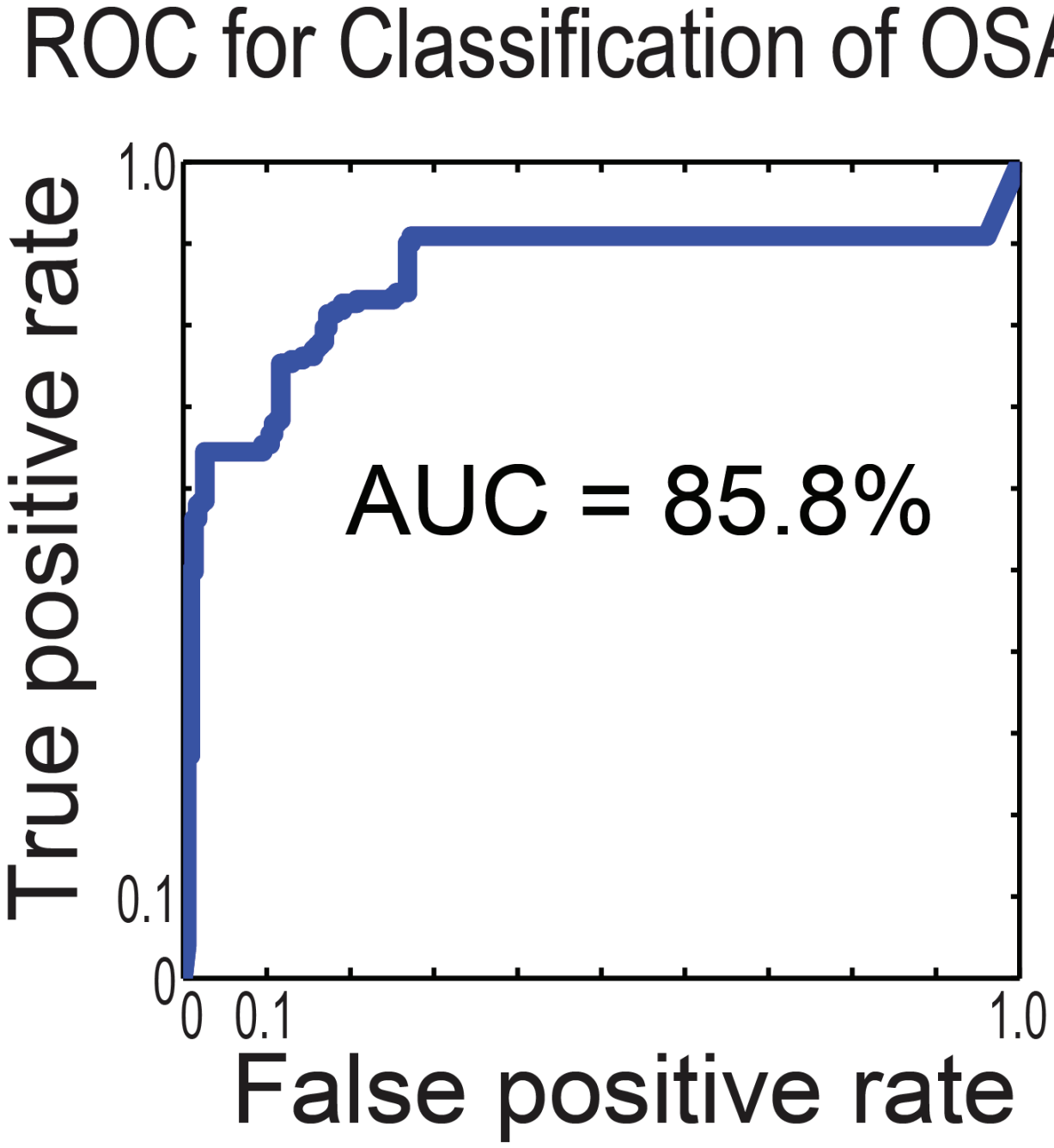


TABLE 1. Demographic Data

	OSAS (n=11)	Control (n=12)	Test Statistics	P value
Age (years)	14.8 ± 1.5	15.1 ± 1.4	Mann-Whitney U = 63	0.88
Height (cm)	164.3 ± 8.0	160.1 ± 6.8	Mann-Whitney U = 58	0.62
Weight (kg)	101.5 ± 23.9	76.0 ± 20.8	Mann-Whitney U = 24	0.01
BMI (kg/m2)	38.0 ± 10.5	29.6 ± 7.6	Mann-Whitney U = 101	0.03
Males (%)	45	25	$\chi 2=0.44$	0.51
Ethnicity	2 AA, 9 H	1 AA, 1 C, 1 A, 9 H	$\chi 2=0.95$	0.62
Prior adenotonsillectomy	4	1	$\chi 2=1.25$	0.26
Handedness	11 right	11 right, 1 left	$\chi 2=0.96$	0.32
IQ	92.6 ± 14	98.3 ± 10.8	Mann-Whitney U = 47	0.42

Mean ± standard deviation. BMI, Body mass index; AA, African American; C, Caucasian; A, Asian; H, Hispanic.

TABLE 2: Polysomnography

	OSAS (n=11)	Control (n=12)	Test Statistics	P value
Apnea - Hypopnea Index (events/hr)	14.8 ± 7.1	0.8 ± 1.1	Mann-Whitney U = 0	< 0.001
Arousal Index (events/hr)	19.5 ± 11.0	6.8 ± 2.7	Mann-Whitney U = 7	< 0.001
Total Sleep Time (hrs)	5.7 ± 0.9	5.7 ± 1.2	Mann-Whitney U = 66	0.98
Sleep Efficiency (%)	80.1 ± 13.2	81.0 ± 15.3	Mann-Whitney U = 56	0.56
Awakening Index (events/hr)	6.3 ± 4.0	3.9 ± 1.8	Mann-Whitney U = 42	0.14
Baseline SpO2 (%)	99.7 ± 0.5	99.4 ± 0.9	Mann-Whitney U = 55	0.52
SpO2 Nadir (%)	87.4 ± 11.7	93.9 ± 1.4	Mann-Whitney U = 18	0.003
Baseline ETCO2 (mmHg)	40.4 ± 3.8	39.5 ± 4.8	Mann-Whitney U = 23	0.81
Peak ETCO2 (mmHg)	47.6 ± 7.6	45.2 ± 4.3	Mann-Whitney U = 44	0.28

Mean ± standard deviation. SpO_2, arterial oxygen saturation; $ETCO_2$, end-tidal carbon dioxide

TABLE 3: Neuropsychological Performance

		OSAS (n=11)	Control (n=12)	Test Statistic (*F**)	P value
ISLT Learning (# items correct/16)	Trial 1	6.0±1.3	6.3 ± 1.4	0.11	0.74
	Trial 2	7.9 ± 2.0	8.3 ± 2.1	0.08	0.77
	Trial 3	9.5 ± 1.4	9.8 ± 1.3	0.59	0.45
	Total	23.3 ± 3.2	24.4 ± 3.9	0.001	0.93
	Weighted†	50.1 ± 7.3	52.4 ± 7.7	0.234	0.63
ISLT Delay (# items correct/16)		9.2 ± 1.6	8.8 ± 2.5	1.031	0.32
GMT Learning (# errors)	Trial 1	11.8 ± 4.6	17.5 ± 4.4	0.36	0.55
	Trial 2	11.6 ± 2.8	11.3 ± 4.0	0.05	0.82
	Trial 3	9.1 ± 3.1	9.5 ± 4.3	0.09	0.76
	Trial 4	7.2 ± 4.3	9.2 ± 5.2	2.50	0.13
	Trail 5	6.4 ± 2.2	9.4 ± 7.2	1.43	0.24
	Total	51.1 ±11.9	56.8 ± 20.1	1.00	0.33
	Weighted†	127.9 ± 32.4	152.2 ± 70.4	1.048	0.32
GMT Delay (# errors)		5.5 ± 4.0	6.9 ± 4.6	0.77	0.39
WRAT3 Reading (standardized score)		92.6 ± 14.0	98.3 ± 10.9	0.23	0.63

Mean ± standard deviation. ISLT=International Shopping List Test; GMT=Groton Maze Test; WRAT3=Wide Range Achievement Test – 3 Reading subtest. *Effects of OSAS using a general linear model adjusting for age, sex, BMI (group-mean centered), and IQ. †Weighted by trial order to account for learning effects (Thompson et al., 2011).