 AJRCCM Articles in Press. Published on October 29, 2010 as doi:10.1164/rccm.201005-0693OC

Obstructive Sleep Apnea: brain structural changes and neurocognitive function before and after treatment

Running title: sleep apnea, brain changes, treatment

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The paper assesses the effect of obstructive sleep apnea on brain structure and cognitive performance, and the changes after treatment with Continuous Positive Airway Pressure. The main finding is that cognitive impairment is associated with a decrease of grey-matter volume in specific cerebral regions, and that these can be reversed by treatment with an increase of grey-matter volume in specific hippocampal and frontal brain regions. These changes are significantly correlated with the improvement in specific neuropsychological tests (executive-functioning and short-term memory), underlining the importance of early diagnosis and treatment of sleep apnea. Specific neuropsychological measures represent valuable tools for the assessment of therapy success, and can offer the evidence that adherence to treatment can lead not only to clinical, but also to brain-structural, recovery.

Acknowledgments: Study supported by Respironics Foundation, Pittsburgh, USA

Word count: 3410

"This article has an Online Data Supplement which is accessible from this issue's table of content online at www.atsjournals.org"

Abstract

Rationale: Obstructive Sleep Apnea (OSA) is commonly associated with neurocognitive impairments that, however, have not been consistently related to specific brain structure abnormalities. Knowledge of the brain structures involved in OSA and the corresponding functional implications could provide clues to the pathogenesis of cognitive impairment and its reversibility in this disorder.

Objectives: To investigate the cognitive deficits and the corresponding brain morphology changes in OSA, and the modifications after treatment, using combined neuropsychological testing and Voxel-Based-Morphometry.

Methods: 17 treatment-naïve sleep apnea patients and 15 age-matched healthy controls. All underwent a sleep study, cognitive tests and magnetic resonance imaging. After three-months treatment, cognitive and imaging data were collected to assess therapy efficacy.

Measurements and Main Results: Neuropsychological results in pre-treatment OSA showed impairments in most cognitive areas, as well as in mood and sleepiness. These impairments were associated with *focal* reductions of grey-matter volume in the left hippocampus (enthorinal cortex), left posterior parietal cortex and right superior frontal gyrus. After treatment, we observed significant improvements involving memory, attention and executive-functioning that paralleled grey-matter volume increases in hippocampal and frontal structures.

Conclusions: The cognitive and structural deficits in obstructive sleep apnea may be secondary to sleep deprivation and repetitive nocturnal intermittent hypoxemia. These negative effects may be recovered by consistent and throughout treatment. Our findings highlight the importance of early diagnosis and successful treatment of this disorder.

Word count: 226

Key Words: obstructive sleep apnea; brain structure; neurocognitive function; treatment

Introduction

Obstructive Sleep Apnea (OSA) is a common sleep-disorder, affecting at least two to four percent of middle-aged individuals. OSA is associated with neurocognitive and cardiovascular morbidities[1], reduced quality of life[2], impaired work-performance, and increased risk of vehicular and industrial accidents[3].

OSA patients demonstrate several neuropsychological impairments[4,5], but the link between these deficits and localized brain dysfunction is debated[6-8]. Repeated apneic and hypopneic events during sleep result in intermittent hypoxemia, hypercapnia, cortical and sympathetic nervous system arousal, and sleep-fragmentation[6,9]. Intermittent hypoxemia is associated with increased sympathetic vasoconstriction and decreased vascular protective mechanisms that can contribute to structural and functional changes in the vasculature of the brain[4,7,9].

Neuroimaging studies may aid in identifying patients at greatest risk for poor outcome by examining relationships between brain integrity and functional response to treatment. The available structural imaging studies are largely inconsistent because of methodological and sample variability[7,8]. Results of structural studies investigating Grey-Matter (GM) density or volume changes are also heterogeneous, although hippocampal involvement is reported frequently[10-14].

Voxel-Based-Morphometry (VBM[15,16]) studies have demonstrated a GM-density reduction in frontal, parietal, temporal, hippocampal and cerebellar regions of OSA patients[10]. Similarly, Yaouhi at al.[12] reported GM-loss in frontal and temporo–parieto–occipital cortices, hippocampal and cerebellar regions, despite minor memory and motor impairments. Only hippocampal and parahippocampal GM-loss was observed by other

investigators[11]. O'Donoghue et al.[17] found GM-density loss in the posterior hippocampal cortex and the left insular region before treatment only with an uncorrected statistical threshold, and no region showing a significant GM-increase after treatment using a corrected threshold. They suggested that the variability in results across studies was related to VBM-methods and statistical threshold settings.

We hypothesized that neuropsychological changes in OSA patients may be associated with localized brain changes, and that these changes may be at least partially reversed by effective treatment. We tested the hypotheses that structural brain differences would exist a) between OSA patients and controls, and b) in OSA patients before and after three-months of treatment.

Preliminary results of this study have been previously reported in the form of an abstract[18].

Methods

Participants

Seventeen severe treatment-naïve male OSA patients (age-range = 30-55) and 15 male age-and-education-matched healthy controls were studied. Inclusion criteria for OSA: apnea/hypoapnea index (AHI) > 30. Inclusion criteria for controls: AHI < 5, free of psychiatric and medical disorders. Exclusion criteria: symptoms of cognitive deterioration (Mini-Mental < 24), sleep disorders other than OSA, hypertension (>160/100), diabetes, use of psychoactive medications, structural brain abnormalities (additional details are provided in the Online Data Supplement). There was no significant demographic difference between patients and controls (Table 1). Participants provided written informed consent to the experimental procedure, that was approved by the local Ethical Committee of Vita-Salute

San Raffaele University, Milan, Italy. Participants were evaluated at baseline (BL) and after three-months treatment with CPAP (with C-Flex, Respironics, M-series). One OSA participant dropped out due to low adherence to CPAP treatment. Full nocturnal-polysomnography (PSG) was performed at BL and after treatment. Apnea events were defined as any 80% drops of respiratory amplitude lasting > 10 seconds; hypopneas were defined as any 30% drops of respiratory amplitude lasting > 10 seconds associated with > 3% desaturation, or with arousal[19]. Apnea-hypopnea index was calculated as an index of the number of apnea and hypopnea events per hour of sleep. All participants underwent magnetic resonance imaging session two to three hours after waking the morning after sleep study.

Neuropsychological evaluation

All participants underwent a neuropsychological evaluation[20] of short and long-term memory, executive functions, constructional abilities, vigilance, attention and abstract reasoning (further details are provided in the Online Data Supplement). Additionally, participants completed the self-report Epworth Sleepiness Scale to evaluate daytime somnolence, the Beck Depression Inventory to evaluate mood and SF-36 to assess overall quality of life. Tests were administered in Italian and scored according to the published procedures[20]. Group differences were investigated using non-parametric two-sample (Mann-Whitney U test) and paired (Wilcoxon signed rank test) t-tests.

Magnetic-resonance-imaging data acquisition

T1-weighted magnetic-resonance images were acquired with a 3-Tesla Philips Achieva scanner.

VBM data pre-processing and statistical analysis

Image pre-processing and statistical analyses were performed using SPM5 (http://www.fil.ion.ucl.ac.uk/spm) on Matlab v7.4 (Mathworks-Inc., Sherborn, MA), and the VBM5.1-toolbox (http://dbm.neuro.uni-jena.de) (additional details are provided in the online data supplement).

Focal GM-volume differences between pre-treatment OSA and controls, and between pre- and post-treatment OSA, were investigated using two-sample and paired t-tests, respectively, with age as nuisance variable. Unless otherwise stated, the statistical threshold was $p < 0.05$ Family-wise-Error (FWE) corrected at the cluster-level[21] (primary threshold at the voxel-level of $p < 0.005$ uncorrected). In a separate analysis we replicated the methods of O'Donoghue et al.[17] to directly compare our results with their negative data.

Cerebral regions showing significant effects were identified using the cytoarchitectonic-mapping implemented in the SPM-Anatomy-Toolbox[22].

Regions-Of-Interest (ROIs) analyses with the SPM5-toolboxes Marsbar (http://marsbar.sourceforge.net/) and Anatomy-Toolbox[22] highlighted group differences in GM-volume, and correlations between the latter, disease severity (apnea-hypopnea index and hypoxia) and performance in neurocognitive tests showing a significant behavioral effect, in both a) the clusters resulting from VBM-analyses, and b) cytoarchitectonic (anatomically independent) subdivisions of the hippocampus[23]. Here the threshold was $p < 0.05$ corrected for multiple comparisons using False-Discovery-Rate (FDR).

Results

Neurocognitive data

Before treatment patients and controls were similar on demographic measures, whereas they differed significantly in Body Mass Index and sleepiness (OSA participants higher than controls) (Table 1), and on all neurocognitive measures (OSA patients poorer than controls) (Table 2). After treatment patients showed a significant improvement in sleepiness and in all cognitive tests, except for total time on Stroop test (executive function), false-positives at Rey list recognition (long-term memory) and Trail Making Test B (executive function). Also mood (even if always in normal ranges) and quality of life (SF-36) significantly improved after treatment.

Brain structural changes before CPAP treatment

A significant *reduction* of GM-volume in pre-treatment patients, compared with controls, was observed in the left posterior-parietal cortex and right superior-frontal gyrus (see Figure 1, Table 3 and Table E1 in the Online Data Supplement). We observed no GM-volume increase in patients compared with controls, even when employing an uncorrected p-value ($p < 0.005$). Motivated by a-priori hypotheses of OSA-related hippocampal abnormalities[10-14], we focused on the hippocampus with an uncorrected threshold of $p < 0.005$. We found reduced GM-volume in OSA participants, compared to controls, in the left parahippocampal gyrus (enthorinal-cortex). In all of these regions, GM-volume was negatively correlated with errors at the Stroop test (executive function). GM-volume in the left posterior-parietal cortex was also positively correlated with performance in the Raven test (abstract reasoning) and negatively correlated with sleepiness score (Table 4 and Table E2 in the Online Data Supplement). With regard to disease severity, we observed a significant negative correlation between AHI and time with SaO2 < 90% and GM-volume in the left posterior parietal cortex

($p < 0.05$), as well as a trend towards statistical significance between AHI and GM-volume in the right superior frontal gyrus ($p = 0.067$) (Table 5).

Neither overall GM-volume nor WM-volume or Total-Intracranial-Volume significantly differed between pre-treatment OSA patients and controls (two-sample t-test, two-tailed, $p > 0.05$), although we observed a trend for reduced GM-volume in the former group ($p = 0.09$) (see Table E3 in the Online Data Supplement).

Since patients and controls differed significantly in Body Mass Index, specific analyses were run to test for its potential effect on brain structure. No significant correlation between GM-volume and BMI was found (see additional details, Table E4 and Figure E1 in the Online Data Supplement).

Brain structural changes after CPAP treatment

No specific brain region showed a significant GM-reduction after treatment, even when employing an uncorrected p-value ($p < 0.005$). Instead, specific hippocampal (left subiculum and bilateral enthorinal-cortex) and frontal (superior and middle-frontal gyri, and medial-orbitofrontal cortex) clusters demonstrated a GM-volume increase (see Figure 1, Table 3 and Table E1 in the Online Data Supplement). The GM-volume increase in the left hippocampus was correlated with the improvement on the Stroop test (executive function) (Table 4 and Table E2 in the Online Data Supplement). Among the above cerebral regions, OSA severity (AHI and time with SaO2 < 90% before treatment) was positively correlated with hippocampal GM-volume increase *after* treatment in the right enthorinal cortex and in the left subiculum ($p < 0.05$) (Table 5). There was also a trend for a positive correlation between AHI and GM-volume in the right middle/superior frontal gyrus ($p = 0.061$) and left enthorinal cortex ($p = 0.052$).

After treatment with CPAP a significant increase of overall GM-volume was observed in OSA patients ($p < 0.05$), despite no significant increase of Total-Intracranial-Volume ($p > 0.5$). Indeed, the average amount of GM-volume increase was associated with a comparable, yet non statistically significant ($p = 0.013$), reduction in cerebro-spinal-fluid (CSF) volume (see Table E3 in the Online Data Supplement).

Hippocampal structural changes before and after treatment (CPAP)

A conjunction analysis (Conjunction-null test)[24] revealed brain regions demonstrating group effects *both* before (OSA versus controls) and after (with-treatment OSA versus treatment-naïve OSA) CPAP treatment. This analysis highlighted the left anterior parahippocampal gyrus (enthorinal-cortex; Figure 1, Table 3 and Table E1 in the Online Data Supplement), where GM-volume increase after treatment was correlated with improved performance in the Digit-Span Forward (short-term memory), Corsi (short-term memory), and Stroop test (executive function) (Figure1, Table 4 and Table E2 in the Online Data Supplement). Additionally, we observed in this region a trend towards a significant positive correlation ($p = 0.072$) between GM-volume and level of hypoxia (time with $SaO2 < 90\%$ and nadir SaO2) (Table 5).

Pre- and post-treatment Grey-Matter changes in hippocampal cytoarchitectonic subdivisions

The hippocampus is a complex structure including several subdivisions that are associated with different functions and pathological conditions[25,26]. We explored whether such anatomical segregation enlightened interpretation of the correlations noted above. Based on a-priori hypotheses[10-14] we carried out a small volume correction, regions-of-interest

(ROIs) analyses and correlations with cognitive performance in the cytoarchitectonic subdivisions of the hippocampus[23](Table 4; additional details in Tables E2, E3, E5 and Figure E2 in the Online Data Supplement).

The results confirmed a significant GM-volume reduction in pre-treatment OSA participants, compared with controls, in the right cornu-ammonis and the enthorinal-cortex bilaterally (see Table E3 in the Online Data Supplement). The amount of GM-volume in the left enthorinal-cortex was negatively correlated with errors in the Stroop test (executive function) (see Table 4 and Table E2 in the Online Data Supplement). After treatment, we observed a significant bilateral GM-volume increase in the cornu-ammonis, enthorinal-cortex, fascia-dentata and subiculum (see Table E3 in the Online Data Supplement), and GM-increase in the left cornu-ammonis correlated with improvement in the Stroop test (executive function) (see Table 4 and Table E2 in the Online Data Supplement).

As for disease severity, AHI before treatment was negatively correlated with hippocampal GM-volume in the bilateral cornu-ammonis, enthorinal-cortex and fascia-dentata ($p < 0.05$). AHI before treatment was also positively correlated with hippocampal GM-volume increase *after* treatment in the bilateral cornu-ammonis, enthorinal-cortex, fascia-dentata and subiculum ($p < 0.05$) (see Table E5 in the Online Data Supplement for a complete description of the observed correlations in the cytoarchitectonic subdivisions of the hippocampus[23]).

Re-analysis of the data with the "optimized-VBM"

Similarly to previous results[17], when we used the "optimized"-VBM in SPM2 no regions survived a threshold of $p < 0.05$ corrected for multiple comparisons, be it with False-Discovery-Rate (FDR) at the voxel-level, or Family-Wise-Error (FWE) at the cluster-level as in

our analysis. At $p < 0.001$ uncorrected we observed, in pre-treatment patients compared with controls, scattered regions of GM-volume reduction and increase. At the same threshold, in post-, compared with pre-treatment patients, we found bilateral increases of GM-volume in the left middle-frontal gyrus and postcentral gyrus, and no region in the opposite comparison (see Table E6 in the Online Data Supplement).

Discussion

The aims of this study were to: a) investigate the neuropsychological deficits in severe OSA and their association with structural brain changes, and b) assess whether any cognitive improvement in OSA patients after three-months of CPAP treatment reflected a change in the underlying cerebral structure.

Cognitive and structural deficits at BL

Neuropsychological results demonstrated impairments in memory, attention, executive functions and constructional abilities, as well as higher sleepiness and lower score in the BDI (mood), in untreated OSA patients. These impairments were associated with *focal* GM volume reductions in the left hippocampal enthorinal cortex, in the left posterior parietal cortex, and in the right superior frontal gyrus.

Alterations in hippocampal structures have been previously reported in OSA patients, using VBM[10,11,12], quantitative-magnetic-resonance-imaging[13], and spectroscopy[14]. The location of the hippocampal cluster is consistent with previous results[10], and region-of-interest analyses highlighted a significant GM-volume decrease in the same region (right cornu-ammonis) where a GM-density reduction was previously reported[11]. The hippocampus is extremely sensitive to hypoxic damage, and, in rats exposed to intermittent hypoxia

during sleep, impaired spatial learning is associated with increased apoptosis within region CA1[6]. Gale and Hopkins[13] reported hippocampal atrophy, associated with memory impairment both in OSA patients and patients with carbon-monoxide poisoning. The hippocampal changes in OSA may also be associated with the attentional and executive impairment displayed by neuropsychological tests, as the connections between the prefrontal cortex and the thalamus are extensive[27]. The attentional, executive and constructional deficits may also reflect the GM-volume decrease in superior parietal and frontal regions involved in attention[28] and working-memory[29]. Reduced cerebral activity in the right superior-frontal gyrus has been reported in OSA patients compared with healthy controls when performing a specialized task to assess working-memory[30,31].

Overall, the significant correlation between the amount of localized GM volume reduction (left posterior-parietal cortex, right superior-frontal gyrus and the left enthorinal-cortex, as well as the left enthorinal-cortex hippocampal cytoarchitectonic-subdivision) and performance on the Stroop test supports the hypothesis that executive dysfunction is a core component of the OSA neuropsychological syndrome.

Cognitive and structural improvement following treatment

After three-months of treatment we observed a significant improvement in all cognitive domains. This improvement was related to a GM-volume increase in the hippocampus (left subiculum and bilateral enthorinal cortex), the medial orbitofrontal cortex, and the rostral portion of the right superior frontal gyrus. When focusing on the cytoarchitectonic subdivision of the hippocampus[22,23], a GM-volume increase was observed bilaterally in the cornu ammonis, enthorinal cortex, fascia dentata and subiculum after treatment. These results are strengthened by the significant correlation between reduction of errors at the

Stroop test and GM-volume increase in the left subiculum in the VBM analysis, and in the cornu ammonis in the region-of-interest analysis. In the case of the enthorinal-cortex, the amount of GM-increase after treatment was correlated with improvement in verbal and visuo-spatial short-term memory and attention/executive functioning, indicating a direct correlation between the GM changes and cognitive improvement.

Our results contradict the negative findings reported by O'Donoghue et al.[17] of no significant GM-difference between pre-treatment OSA and controls, and no changes after therapy. These findings are conflicting with those reported in other related VBM studies where significant grey-matter reductions were shown in OSA patients pre-treatment[10-12]. O'Donoghue et al.[17] interpreted their negative results in terms of different pre-processing of the data and different statistical analyses/threshold. However, it is crucial to take into consideration the continuous improvement of neuroimaging softwares, leading to more sensitive analyses and more robust results[16]. Indeed, by using the same argument we demonstrated that the use of more recent VBM-methods (VBM5-toolbox and SPM5-unified segmentation) compared with those previously employed by O'Donoghue et al.[17] (the SPM2-optimized-VBM approach) enhanced sensitivity and specificity of our results (see additional information in the Online Data Supplement).

The mechanisms leading to structural changes in VBM are a topic of intensive debate, in light of the increasing literature on brain structural plasticity highlighted by morphometric analyses[16,32]. The human hippocampus retains its ability to generate neurons throughout life[33], and there is evidence that regular practice improves the rate of adult neurogenesis and fosters the preservation of newly generated neurons[34.] Frontal and hippocampal structural plasticity due to environmental enrichment has been shown in animal models[35].

These results may then suggest a scenario in which the hippocampus, due to its sensitivity to hypoxia and innervation of small vessels, is the region that is most strongly and quickly affected by hypoxic and hypercapnic episodes[6]. The ensuing structural hippocampal damage results in cognitive deficits involving not only memory, but also attention and executive functioning, either directly (due to the role of hippocampus in these functions[36]) or indirectly (due to altered functional connectivity with the parietal and prefrontal cortex, which is affected as well[27]). Yet, the hippocampal plasticity[35] allows for a structural recovery locally and in connected brain areas.

These results provide important clues on the debate over the pathogenesis of cognitive impairment in OSA and its reversibility. We propose a number of potential origins for the deficits demonstrated in our current findings. Both hypoxemia and sleep-fragmentation have been proposed as potential contributors. The reversibility of structural changes has been suggested to indicate that they are secondary to sleep fragmentation, repetitive nocturnal hypoxemia or disturbances in autonomic activity, rather than to pre-existing cortical damage[17]. To date, the lack of evidence for the reversibility of structural alterations in OSA (if any) has left this issue unresolved. Regardless of the origin of the deficit, the mechanism of brain change could be either neurogenic or vasogenic. We propose it to be vasogenic. We believe that the pattern of neuropsychological changes in OSA is similar to that seen in cases with mild cerebrovascular disease (most commonly small vessel disease). The microvascular model of OSA has been posited previously and supported in some studies. Moreover, this model lends itself nicely to further evaluation using more sophisticated methods to assess microbleeds and other small vessel abnormalities in the

brain. Finally, this explanation is parsimonious with many of the positive cardiovascular studies in OSA. The true origin and mechanism, however, are yet to be uncovered.

There are many potential implications for the results presented herein. If the cause of impairment is vasogenic, there are treatment approaches (e.g., steroid therapy) that could mediate the long-term effects of vasogenic edema in the brain. Whatever the cause, identifying the underlying mechanism could open doors for treatment approaches to avoid the brain-related changes demonstrated through our study. The association between the VBM findings and severity of disease may also be important. Such findings, if replicated, can serve as a marker of long-term negative outcomes associated with OSA. This could initiate more intensive follow-up or additional testing to identify early indicators of brain involvement (e.g., neuroimaging or neuropsychological testing). Finally, these data could serve as motivators for patients to be adherent to treatment. If such findings are shared with patients, risk perception of non-adherence could be increased thus increasing adherence to treatment. It should be noted, however, that these findings must first be replicated and taken to more depth regarding their causative factors.

A limitation of our study is that healthy controls were not re-evaluated at three months, a restriction due to practical considerations of study cost and participant availability for repeated scanning. In principle, the absence of this control would not allow to exclude either an effect of learning on cognitive performance or of “spontaneous” brain changes. However, several considerations speak against this interpretation. Our neuropsychological battery included tests that are unlikely to be prone to significant learning effects, except for the Rey list learning, for which an alternate yet equivalent form was used at the three-months assessment. As for the post-treatment neural changes observed in patients, none of the

regions showing a GM-volume increase in the present study has been associated with an age-related enlargement. Global GM-volume linearly decreases with age[15], and age-related loss of GM concentration has been reported in several regions[15] including the hippocampus[37]. Finally, the significant correlation between cognitive improvement in specific tests and the GM-volume increase in specific regions strengthens our conclusions. No spontaneous improvement in test performance has been reported with sham treatment[38]. Additionally, it is worth to highlight that our sample included severe hypoxic patients (% of time spent with SaO2 < 90% = 30.4 ±13.4). It is warrant to plan a study with non-hypoxic sleepy patients.

In summary, this study provides the first evidence that structural brain abnormalities exist in regions susceptible to hypoxemia, and that they can change with treatment. These results suggest that even the negative neurological effects of hypoxemia may reverse with consistent and thorough treatment. Therefore, adherence to treatment can lead not only to clinical, but also to brain-structural recovery. It must be underlined that the patients in this trial showed a positive response to treatment. The MRI changes may thus represent a marker of treatment response.

Acknowledgments

We acknowledge the help of Miss Antonella Iadanza MSc (Neuroradiology Unit, San Raffaele Scientific Institute, Milan, Italy) for technical support, and Mr Daniele Bizzozero MSc, Mr Antonio Massimo MSc and Miss Cristina Martinelli MSc (Sleep Disorders Center, San Raffaele Scientific Institute, Milan, Italy) for polysomnographic recordings.

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Figure Legends

Figure I. Structural brain changes in OSA before and after CPAP treatment.

From top to bottom, a) regions showing a GM-volume decrease in untreated OSA patients compared to controls ($p < 0.05$ corrected for multiple comparisons based on cluster-extent)[20]; b) regions showing a GM-volume increase in post-treatment, compared to pre-treatment, OSA patients ($p < 0.05$ corrected for multiple comparisons based on cluster-extent)[20]; c) left hippocampal enthorinal-cortex showing both a GM-volume reduction before treatment and a GM-volume increase after treatment ($p < 0.001$ uncorrected for multiple comparisons based a-priori hypotheses[10-14]), as well as the correlation between GM-volume increase in this region and cognitive improvement after treatment ($p < 0.05$ corrected for multiple comparisons with false-discovery rate). Significant clusters are projected on representative slices of a brain-template in stereotaxic space. The distance (in mm) of each slice from the anterior commissure is also shown.

Table 1. Demographic and clinical data

	Baseline			Follow-up	
	Controls (n = 15)	OSA (n = 17)	*p-value*	OSA (n = 16) ‡	*p-value*
Demographic data					
Age (years)	42.15 (6.64)	44 (7.63)	ns	43.37 (7.41)	ns
Education (years)	13.23 (3.09)	12.24 (2.70)	ns	12.31 (2.77)	ns
Clinical data					
Body-Mass-Index (kg/m2)	26.10 (2.50)	31.24 (4.35)	0.01	31.09 (4.45)	ns
Apnea/Hypoapnea-Index	1.6 (1.5)	55.83 (19.08)	0.001	2.5 (2.4)	0.001
Mean SaO2 (%)	93.1 (1.5)	70.41 (9.13)	0.012	91.4 (1.9)	0.016
Time SaO2 below 90% (min.)	0.3 (1.2)	30.42 (13.4)	0.001	0.8 (0.5)	0.001
CPAP use (min./night)				349.36 (34.15)	
Days of CPAP use > 4h (%)				82.5 (9.78)	
Mean CPAP Pressure (cmH2O)				11.5 (2.9)	
Blood Pressure – systolic (mmHg)	121.32 (3.99)	123.85 (10.44)	ns	120.89 (9.23)	ns
Blood Pressare – diastolic (mmHg)	81.00 (4.31)	83.41 (5.82)	ns	82.69 (6.65)	ns
Beck Depression Inventory	1.46 (2.16)	3.76 (3.94)	*0.007*	1.75 (2.95)	*0.01*
Epworth Sleepiness Scale	3 (1.25)	11.94 (5.47)	*<0.001*	2.81 (2.78)	*<0.001*
SF36 (quality of life, total score)	80.89 (9.37)	68.91 (15·43)	*0.01*	80.35 (15.09)	*0.002*

Comparison of demographic and clinical data between controls and OSA patients pre-treatment, and between OSA patients pre- and post- treatment

‡ one drop-out for low treatment compliance

ns = non statistically significant

Table 2. Neuropsychological data

	Baseline			Follow-up	
	Controls (n = 15)	OSA (n = 17)	*p-value*	OSA (n = 16) ‡	*p-value*
Neuropsychological scores					
Global Cognitive Functions					
Mini Mental State Evaluation	30.00 (0.00)	29.35 (1.05)	*ns*	29.75 (0.57)	*ns*
Raven	34.6 (1.29)	31.70 (3.90)	*0.002*	33.25 (2.46)	*0.03*
Short Term Memory					
Digit-span forward	6.93 (0.70)	5.58 (1.00)	*<0.001*	6.56 (0.81)	*0.002*
Corsi	6.53 (0.91)	5.11 (1.05)	*0.001*	6.18 (0.83)	*0.002*
Long Term Memory					
Rey-list (learning)	58 (7.01)	48.70 (9.67)	*0.006*	58.18 (7.92)	*<0.001*
Rey-list (recall)	13 (1.96)	10.58 (2.47)	*0.003*	13.12 (2.24)	*0.002*
Rey-list (recognition)	14.86 (0.51)	14.29 (1.10)	*0.03*	14.87 (0.34)	*0.01*
Rey-list (false positives)	0.13 (0.35)	1 (1.17)	*0.02*	0.37 (0.61)	*ns*
Attention and Executive Functions					
Digit-span backward	5.6 (0.91)	4.17 (1.01)	*0.001*	5.12 (0.88)	*0.004*
Stroop (time)	23.06 (8.13)	39.12 (21.88)	*0.009*	34.73 (17.57)	*ns*
Stroop (errors)	0.73 (1.03)	5.31 (3.57)	*<0.001*	0.86 (1.35)	*<0.001*
Trail-making test (A)	22.73 (5.72)	26.82 (4.50)	*0.02*	23.18 (7.29)	*0.03*
Trail-making test (B)	59.4	82.35	*0.005*	78.87	*ns*

	(14.16)	(24.19)		(21.79)	
Paced Auditory Serial Addition Test – (errors)	5.13 (3.58)	21.52 (10.07)	*<0.001*	7.31 (7.17)	*<0.001*

Comparison of neuropsychological data between controls and OSA patients pre-treatment, and between OSA patients pre- and post- treatment

‡ one drop-out for low treatment compliance

ns = non statistically significant

Table 3. Voxel-Based-Morphometry results

Hemisphere	Anatomical region (Brodmann-area/structure)	K	Z-score
Controls > Pre-treatment			
Right	Superior frontal gyrus (6)	8625	4.43
Left	Inferior parietal lobule	5966	3.88
Left	Parahippocampal gyrus (Enthorinal cortex*)	798	3.28**
Post-treatment > Pre-treatment			
Left	Hippocampus (Subiculum*)	3807	4.41
Left	Parahippocampal gyrus (Enthorinal cortex*)	1600	3.89
Right	Hippocampus (Enthorinal cortex)	1684	3.82
Right/Left	Mid orbital gyrus (11/10)	1666	4.03
Right	Middle/Superior frontal gyrus (46/10)	2333	3.99
Pre-treatment & Post-treatment (Conjunction analysis)			
Left	Parahippocampal gyrus (Enthorinal cortex*)	149	3.76**

From top to bottom, cerebral regions showing a significant GM-volume change at baseline (controls > OSA pre-treatment), at follow-up (OSA post-treatment > OSA pre-treatment), and both at baseline and follow-up, in the main VBM-analysis. $P < 0.05$ corrected for multiple comparisons based on cluster-extent. K = cluster extent in number of voxels (1 x 1 x 1 $mm^{3;}$ see Table E1 in the Online Data Supplement for the complete cytoarchitectonic labeling of the observed clusters with the SPM-Anatomy-Toolbox[22]).

* probabilistic assignment by the Anatomy-Toolbox[22]

** uncorrected threshold of $p < 0.001$ based on a-priori hypotheses[10-14]

Table 4. Correlation between neuro-structural and cognitive changes in the VBM-clusters and hippocampal cytoarchitectonic subdivisions

Averaged GM-volume in VBM-clusters					
	Raven	Digit forward	Corsi	Stroop-errors	ESS
Pre-treatment OSA: GM-volume and performance					
Right Superior frontal gyrus	ns	ns	ns	-0.621 *0.01**	ns
Left Posterior parietal cortex	0.604 *0.01**	ns	ns	-0.661 *0.005**	-0.610 *0.009**
Left Hippocampus (Enthorinal cortex)	ns	ns	ns	-0.704 *0.002**	ns
Post-treatment OSA-patients: GM-volume increase and cognitive improvement					
Left Hippocampus (Subiculum)	ns	ns	ns	-0.725 *0.002**	ns
Pre- & Post-treatment OSA-patients (Conjunction analysis): GM-volume and performance change					
Left Hippocampus (Enthorinal cortex)	ns	0.726 *0.001**	0.554 *0.02**	-0.676 *0.005**	ns
Averaged GM-volume in cytoarchitectonic hippocampal ROIs from the Anatomy-Toolbox					
	Raven	Digit forward	Corsi	Stroop-errors	ESS
Pre-treatment OSA: GM-volume and performance					
Left Enthorinal cortex	ns	ns	ns	-0.802 *0·0001**	ns
Post-treatment OSA-patients: GM-volume increase and cognitive improvement					
Left Cornu ammonis	ns.	ns	ns	-0.736 *0.001**	ns

Significant correlations between GM-volume and scores at neurocognitive tests for the clusters resulting from the main VBM-analysis (top), and the cytoarchitectonic subdivisions of the human hippocampus[22-23] (bottom), before and after treatment. Within each cell, the number at the top indicates the value of the correlation, the one at the bottom (*in italic)*

indicates the corresponding p-value. All reported correlations are significant at $p < 0.05$ corrected for multiple comparisons with False-Discovery-Rate (FDR) (see Table E2 in the Online Data Supplement for the complete list of correlations significant at $p < 0.05$ uncorrected for multiple comparisons).

GM = Grey-Matter, VBM = Voxel-Based-Morphometry, ESS = Epworth-Sleepiness-Scale, ROIs = Regions-Of-Interest, ns = non statistically significant

Table 5. Correlation between disease severity (AHI and hypoxia) and GM-volume in the VBM clusters

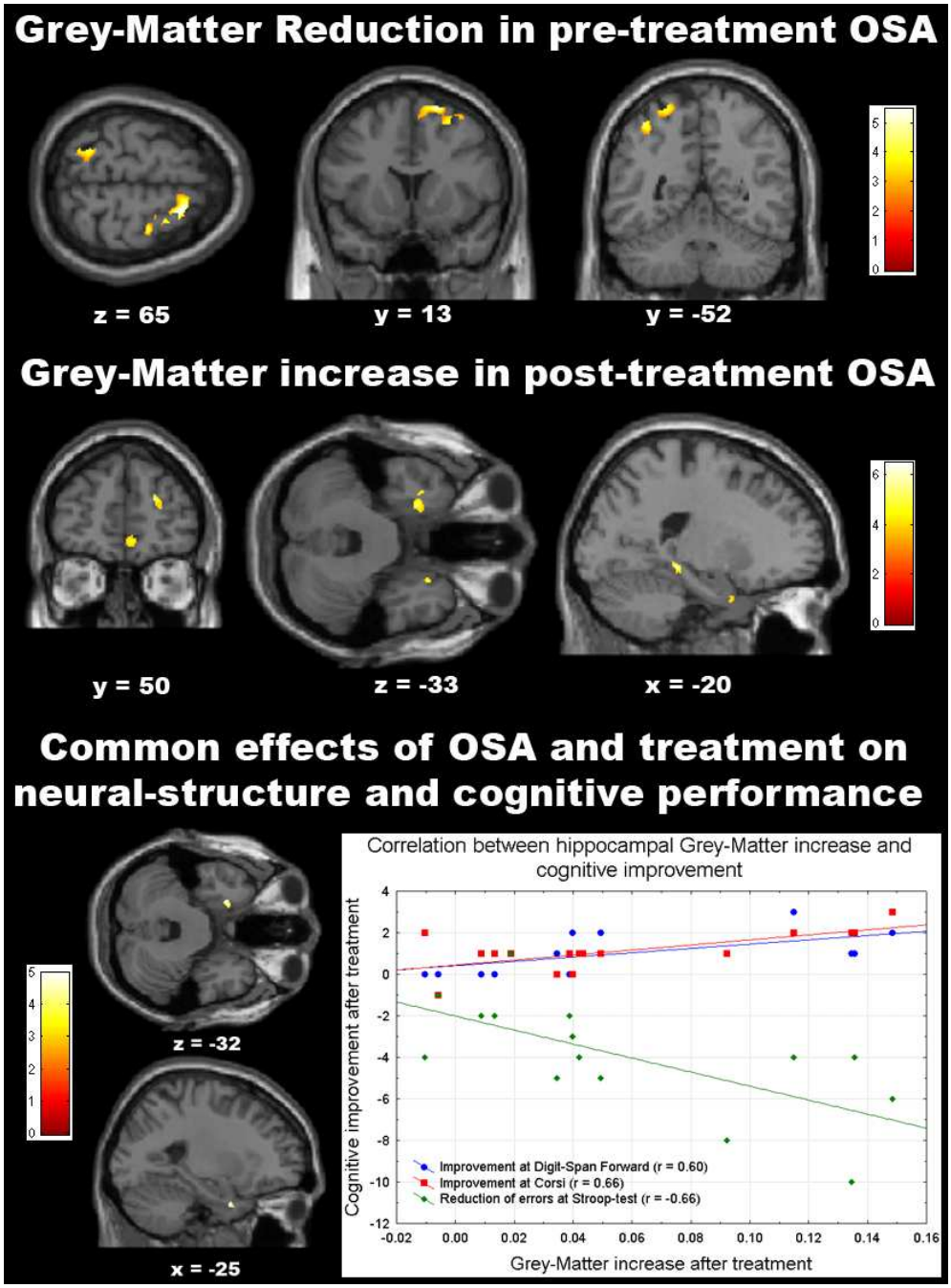
Brain region	AHI	Hypoxia (% time with SaO2< 90%)
Pre-treatment		
Right Superior frontal gyrus	-0.46 *0.067*	-0.34 *0.188*
Left Inferior parietal lobule	-0.69 *0.002**	-0.58 *0.015**
Left Parahippocampal gyrus (Enthorinal cortex)	-0.39 *0.123*	-0.39 *0.121*
Post-treatment		
Right/Left Mid orbital gyrus	0.41 *0.114*	0.26 *0.33*
Right Middle/Superior frontal gyrus	0.48 *0.061*	0.16 *0.55*
Right Hippocampus (Enthorinal cortex)	0.59 *0.015**	0.57 *0.02**
Left Hippocampus (Subiculum)	0.64 *0.007 **	0.51 *0.045**
Left Parahippocampal gyrus (Enthorinal cortex)	0.49 *0.052*	0.45 *0.083*
Pre-treatment & Post-treatment		
Left Parahippocampal gyrus (Enthorinal cortex)	-0.39 *0.133*	-0.46 *0.072*

From top to bottom, correlations between disease severity (Apnea-Hypopnea-Index and time with SaO2 < 90%) before treatment and a) GM-volume before treatment, b) GM volume increase after treatment in the cerebral regions highlighted by the VBM analysis, and c) in the parahippocampal region where GM-volume was both reduced before treatment and increased after treatment. Within each cell, the number at the top indicates the value of

the correlation, the one at the bottom (*in italic)* indicates the corresponding p-value. Significant correlations ($p < 0.05$) are marked by an asterisk. See Table E5 in the Online Data Supplement for a complete description of the observed correlations between GM-volume and AHI in the cytoarchitectonic subdivisions of the hippocampus[23].

AHI = Apnea-Hypopnea-Index

Figure 1


Grey-Matter Reduction in pre-treatment OSA
z = 65
y = 13
y = -52
Grey-Matter increase in post-treatment OSA
y = 50
z = -33
x = -20
Common effects of OSA and treatment on neural-structure and cognitive performance
z = -32
x = -25
Correlation between hippocampal Grey-Matter increase and cognitive improvement
Cognitive improvement after treatment
Grey-Matter increase after treatment
Improvement at Digit-Span Forward (r = 0.60)
Improvement at Corsi (r = 0.66)
Reduction of errors at Stroop-test (r = -0.66)

Obstructive Sleep Apnea: brain structural changes and neurocognitive function before and after treatment

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Online Data Supplement

Methods

Polysomnography

All OSA patients underwent full nocturnal polysomnography (PSG) the night before MRI scanning. Standard electroencephalograms, electrooculogram, chin electromyogram, electrocardiogram, airflow, thoracic and abdominal excursions, oximetry and tibialis electromyogram to screen for periodic leg movements were recorded. Apnea was defined as a $\geq$ 80% drop of respiratory amplitude, lasting at least 10 seconds. Hypopnea was defined as a 50% drop of respiratory amplitude, lasting at least 10 seconds, associated with repeated respiratory effort and arousals or oxygen saturation drops $\geq$ 3%. The apnea-hypopnea index (AHI) was defined as an index of the number of apnea and hypopnea events per hour of sleep. The lowest nocturnal oxygen saturation (SaO_2) value and the percentage of time with SaO_2 below 90% during total sleep were also recorded. Records were scored for sleep stages according to the criteria of Rechtshaffen and Kales[E1].

All participants, including healthy controls, reported regular sleep-wake schedules based on daily sleep diaries with an average total sleep time of 6.9 $\pm$ 1.1 hours in the 4 days prior the study.

Neuropsychological evaluation

All participants underwent a neuropsychological assessment[E2] including Mini Mental State Evaluation (general cognitive function), Digit-span forward (short-term memory) and backward (working-memory), Corsi (visuo-spatial short-term memory), Rey-list (learning, recall, and recognition; long-term memory), Trail making test (divided attention), Stroop test (executive functions, inhibition, selective attention), Paced-Auditory-Serial-Addition Test (PASAT; vigilance and executive functions), and Raven progressive matrices (abstract reasoning). An alternative equivalent version of the Rey-list learning test was employed at follow-up, to avoid practice effects. Administration of the neuropsychological test battery lasted approximately 30 min.

Positive Airway Pressure (PAP) treatment

Patients were fully adherent to manual titration night of PAP. They were all sent home with fixed PAP with C-Flex for 3 months (Respironics, M series). Adherence at home was objectively reported by Encore software and patients with compliance lower than 4 hours/night and <80% of days of usage were excluded (n=1).

Voxel-Based-Morphometry

Voxel-based-morphometry (VBM) is a whole-brain, unbiased technique for characterizing regional cerebral volume and tissue concentration differences in structural magnetic resonance images, involving voxel-wise statistical analysis of pre-processed structural Magnetic-Resonance (typically

T1-Weighted) images[E3]. Two basic types of analyses can be run within the framework of VBM, in which local grey-matter (GM) volume and/or concentration can be compared between subjects-groups (e.g. patients versus controls) or correlated with a given continuous or discrete parameter (e.g. performance in a given test). VBM has become increasingly widely used as a tool to examine patterns of brain change in healthy aging[E4] or neurodegenerative disease[E5], and neuroanatomical correlates of behavioral or cognitive deficits[E6] and skills[E7].

Magnetic-resonance-imaging data acquisition

T1-weighted magnetic-resonance images (150 slices, TR=600 ms, TE=20 ms, in-plane resolution 1x1x1 mm^3) were acquired with a 3-Tesla Philips Achieva scanner (Philips Medical Systems, Best, NL).

VBM data pre-processing and statistical analysis

Image pre-processing and statistical analyses were performed using SPM5 (http://www.fil.ion.ucl.ac.uk/spm) on Matlab v7.4 (Mathworks-Inc., Sherborn, MA), and the VBM5.1-toolbox (http://dbm.neuro.uni-jena.de).

Images were bias-corrected, segmented into GM, white-matter (WM) and cerebro-spinal fluid (CSF) components and normalized[E8] to stereotaxic space (http://Loni.ucla.edu/ICBM/ICBM_TissueProb.html). The normalised GM-maps were modulated with the Jacobian determinants of the normalisation deformation parameters (modulation of non-linear effects only), and smoothed with an 8-mm Full-Width-Half-Maximum (FWHM) Gaussian-kernel. We excluded all voxels with a GM-value<0.2 (maximum = 1) to avoid edge effects at the border between GM and WM.

Focal GM-volume differences between pre-treatment OSA and controls, and between pre- and post-treatment OSA, were investigated using two-sample and paired t-tests, respectively, with age as nuisance variable. Unless otherwise stated, the statistical threshold was $p<0.05$ Family-wise-Error (FWE) corrected at the cluster-level[E9] (primary threshold at the voxel-level of $p<0.005$ uncorrected). In a separate analysis we replicated the methods of O'Donoghue et al.[E10] to directly compare our results with their negative data.

Cerebral regions showing significant effects were identified using the cytoarchitectonic-mapping implemented in the SPM-Anatomy-Toolbox[E11].

Regions-Of-Interest (ROIs) analyses with the SPM5-toolboxes Marsbar (http://marsbar.sourceforge.net/) and Anatomy-Toolbox[E11] highlighted group differences in GM-volume, and correlations between the latter, disease severity (AHI and hypoxia) and performance in neurocognitive tests showing a significant behavioral effect, in both a) the clusters resulting from VBM-analyses, and b) cytoarchitectonic (anatomically independent) subdivisions of the hippocampus[E12] (see Figure E2 in the Online Data Supplement for the visual description of the cytoarchitectonic hippocampal subdivisions). Here the threshold was set at $p<0.05$ corrected for multiple comparisons using False-Discovery-Rate (FDR).

Re-analysis of the data with the Optimized-VBM in SPM2

Our results contradict the negative findings reported by O'Donoghue et al.[E10] of no significant GM-difference between pre-treatment OSA and controls, and no changes after therapy. These findings are conflicting with those reported in other related VBM studies where significant grey-matter reductions were shown in OSA patients pre-treatment[E13, E14, E15]. O'Donoghue et al.[E10] interpreted their negative results in terms of different pre-processing of the data and different statistical analyses/threshold. However, it is crucial to take into consideration the continuous improvement

of neuroimaging softwares, leading to more sensitive analyses and more robust results[E16]. Indeed, by using the same argument we demonstrated that the use of more recent VBM-methods (VBM5-toolbox and SPM5-unified segmentation) compared with those previously employed by O'Donoghue et al.[E10] (the SPM2-optimized-VBM approach) enhanced sensitivity and specificity of our results. Indeed, when re-analyzing our data with the Optimized-VBM in SPM2 we did not observe any significant difference between controls and patients before PAP-treatment, nor a difference between pre- and post-treatment OSA patients (only for exploratory purposes, see Table E6 for the clusters observed when using a lenient $p < 0.001$ uncorrected statistical threshold).

Correlation between Grey-Matter Volume and BMI

Specific whole-brain and regions-of-interest (ROIs) analyses were run to test for potential effects of Body Mass Index (BMI) on brain structure. Whole-brain analyses failed to show significant correlations between grey-matter volume and BMI (only for exploratory purposes, see Figure E2 for the clusters observed when using a lenient $p < 0.001$ uncorrected statistical threshold). Additionally, ROIs analyses showed a non significant negative correlation between BMI and grey-matter volume in the clusters resulting from the controls > pre-treatment-OSA comparison in the main VBM analyses (see Table E4).

Table E1. VBM results with stereotaxic coordinates and cytoarchitectonic labeling

H	Anatomical region (BA/structure)	ATp	K	Cluster labeling		MNI		Z-score
					x	y	z	
Controls > Pre-treatment								
R	Superior frontal gyrus (6)		8625	36.1% in R area 6	22	10	65	4.43
	Superior frontal gyrus (6*)	50*			33	-11	64	3.98
	Middle frontal gyrus (6)				27	14	56	3.42
L	Inferior parietal lobule (SPL7A*/hIP3)	40*/30	5966	49.7% in L SPL (7A)	-38	-52	54	3.88
	Superior parietal lobule (7A*/7PC)	70*/30		8.9% in L SPL (7PC)	-24	-57	64	3.84
				5% in L SPL (7P)				
				3.8% in L hIP1				
				3.4% in L hIP2				
L	Parahippocampal gyrus (EC*)	90*	798	53.8% in L hippocampus (EC)	-22	2	-32	3.28**
				3.8% in L hippocampus (SUB)				
Pre-treatment > Controls								

Pre-treatment > Post-treatment								

Post-treatment > Pre-treatment								
L	Hippocampus (SUB*)	100%	3807	97.4% in L hippocampus (SUB)	-20	-33	-9	4.41
	Hippocampus (SUB*)	90%		1.3% in L hippocampus (CA)	-22	-29	-14	3.77
	Hippocampus (SUB*)	80%			-25	-27	-17	3.27
L	Parahippocampal gyrus (EC*)	50*	1600	37.1% in L hippocampus (EC)	-25	2	-33	3.89
				6.3% in L amygdala (LB)	-34	5	-33	3.65
					-31	4	-20	3.39
R	Hippocampus (EC)	10%	1684	5.3% in R hippocampus	29	10	-33	3.82
					33	-6	-31	3.35
R/L	Mid orbital gyrus (11/10)		1666		8	47	-10	4.03

R	Middle/Superior frontal gyrus (46/10)		2333		22	50	20	3.99
					27	54	14	3.88
					26	49	15	3.28
					22	57	22	3.20
Pre-treatment & Post-treatment (Conjunction analysis)								
L	Parahippocampal gyrus (EC*)	50*	149	62.4% in L hippocampus (EC)	-25	3	-32	3.76**

Cerebral regions showing a significant GM-volume change before (controls > pre-treatment-OSA) or after (post-treatment-OSA > pre-treatment-OSA) treatment in the main VBM-analysis ($p < 0.05$ corrected for multiple comparisons based on cluster-extent), localised with cytoarchitectonic mapping as implemented in the SPM-Anatomy-Toolbox[E11].

H = Hemisphere, R = Right, L = Left, BA = Broadmann Area, ATp = probability of the voxel belonging to the indicated BA, K = cluster extent in number of voxels (1 x 1 x 1 mm^3), SPL = Superior Parietal Lobule, hIP = human Intra-Parietal, EC = Enthorinal Cortex, SUB = Subiculum, CA = Cornu-Ammonis, LB = Latero-Basal complex. A single asterisk (*) indicates probabilistic assignment by the Anatomy-Toolbox[E11]. A double asterisk (**) beside the Z-value indicates an uncorrected $p < 0.001$ threshold based on a-priori hypotheses.

Table E2. Correlation between neuro-structural and cognitive changes in the VBM-clusters and hippocampal cytoarchitectonic subdivisions

Averaged GM-volume in VBM-clusters										
	Raven	Digit forward	Digit backward	Corsi	Rey-Recall	Stroop-time	Stroop-errors	PASAT	Trail-B	ESS
Pre-treatment OSA (GM-volume and performance)										

R SFG							-0.621 *0.01**			
L IPL	0.604 *0.01**						-0.661 *0.005**			-0.610 *0.009* *
L EC				0.579 *0.01*		-0.532 *0.03*	-0.704 *0.002**			
Post-treatment OSA-patients (GM-volume increase and cognitive improvement)										
R mOG		0.663 *0.005*					-0.524 *0.04*			
R EC		0.541 *0.03*					-0.544 *0.03*			
L SUB							-0.725 *0.002**			
L EC		0.504 *0.04*		0.594 *0.01*			-0.695 *0.004*			
Pre- & Post-treatment OSA-patients (Conjunction analysis, GM-volume and performance change)										
L EC		0.726 *0.001**		0.554 *0.02**			-0.676 *0.005**			
<u>Averaged GM-volume in cytoarchitectonic hippocampal ROIS from the Anatomy-Toolbox</u>										
	Raven	Digit forward	Digit backward	Corsi	Rey Recall	Stroop-time	Stroop-errors	PASAT	Trial-B	ESS
Pre-treatment OSA (GM-volume and performance)										
L CA							-0.731 *0.001*			
R CA							-0.577 *0.02*	0.496 *0.04*		
L EC							-0.802 *0.0001**			

R EC		0.531 *0.03*					-0.680 *0.003*			
L FD							-0.570 *0.02*			
R FD							-0.647 *0.006*			
L HATA							-0.551 *0.026*			
L SUB							-0.504 *0.04*		0.537 *0.02*	
R SUB							-0.568 *0.021*			
Post-treatment OSA-patients (GM-volume increase and cognitive improvement)										
L CA							-0.736 *0.001**			
R CA							-0.644 *0.009*			
L EC							-0.711 *0.002*			
R EC		0.594 *0.01*					-0.700 *0.003*			
L FD							-0.521 *0.04*			
R FD			-0.501 *0.04*							
L HATA					-0.609 *0.01*					

R HATA		0.541 *0.03*								
L SUB		0.579 *0.01*								
R SUB		0.535 *0.03*								

Significant correlations between GM-volume and scores at neurocognitive tests for the clusters resulting from the main VBM-analysis (top), and the cytoarchitectonic subdivisions of the human hippocampusE[12] (bottom), before and after treatment. Within each cell, the number at the top indicates the value of the correlation, the one at the bottom (in italic) indicates the corresponding p-value. All reported correlations are significant at $p<0.05$. Asterisks (*) indicate correlations surviving a False-Discovery-Rate (FDR) correction for multiple comparisons as shown in the main text.

GM = Grey-Matter, VBM = Voxel-Based-Morphometry, PASAT = Paced-Auditory-Serial-Addition Test, ESS = Epworth-Sleepiness-Scale, L = Left, R =Right, SFG = Superior Frontal Gyrus, IPL = Inferior Parietal Lobule, EC = Enthorinal Cortex, mOG = mid Orbital Gyrus, Cortex, SUB = Subiculum, ROIs = Regions-Of-Interest, CA = Cornu Ammonis, FD = Fascia Dentata, HATA = Hippocampus-Amygdala-Transition-Area.

Table E3. The effects of OSA and treatment (CPAP) on brain-structure

	Baseline			Follow-up	
	Controls	Pre-treatment OSA	*p-value*	Post-treatment OSA	*p-value*
Global Brain Volumes (mm^3)					
GM-volume	610.96±57.04	568.05±78.80	*0.09*	591.60±48.26	*0.04**
WM-volume	545.65±48.36	523.31±51.31	*0.22*	522.87±50.12	*0.85*
CSF-volume	610.12±55.11	633.18±96.94	*0.41*	613.43±71.56	*0.13*
TIV-volume	1766.74±102.01	1724.55±136.64	*0.33*	1727.91±138.90	*0.71*
Percent GM-signal change (%, averaged over voxels)					
	Controls minus Pre-treatment OSA			Post- minus pre-treatment OSA	
Regions resulting from the main VBM analysis (Controls >Pre-treatment OSA)					
Right Superior frontal gyrus		17.28%	*<0.001**	2.71%	*0.07*
Left Inferior parietal lobule		13.50%	*<0.001**	2.29%	*0.03**
Left Parahippocampal gyrus (Enthorinal cortex)		9.87%	*0.001**	4.15%	*0.004**
Regions resulting from the main VBM analysis (Post-treatment > Pre-treatment OSA)					
Right Mid orbital gyrus		-	-	8.65%	*0.001**
Right Middle/Superior Frontal gyrus		-	-	9.03%	*<0.001**
Right Hippocampus (Enthorinal cortex)		-	-	4.84%	*0.001**
Left Hippocampus (Subiculum)		-	-	2.55%	*<0.001**
Left Parahippocampal gyrus (Enthorinal cortex)		-	-	8.89%	*0.001**

Cytoarchitectonic hippocampal ROIS from the Anatomy-Toolbox					
Left Cornu ammonis		2.40%	*0.21*	5.12%	*0.01**
Right Cornu ammonis		4.28%	*0.05**	5.57%	*0.01**
Left Enthorinal cortex		6.56%	*0.04**	7.11%	*0.005**
Right Enthorinal cortex		5.66%	*0.04**	6.99%	*0.009**
Left Fascia dentata		-1.00%	*0.37*	3.48%	*0.01**
Right Fascia dentata		1.14%	*0.33*	4.26%	*0.04**
Left Hippocampus-Amygdala-Transition-Area		1.40%	*0.37*	3.44%	*0.13*
Right Hippocampus-Amygdala-Transition-Area		3.71%	*0.21*	2.62%	*0.11*
Left Subiculum		3.95%	*0.08*	4.54%	*0.01**
Right Subiculum		2.18%	*0.21*	4.30%	*0.01**

From top to bottom, structural differences concerning global-brain-volumes (mean ± standard-deviation), GM-signal change (percentage) in the clusters resulting from the main VBM analysis, and GM-signal change in the cytoarchitectonic subdivisions of the human hippocampus[E12] between a) controls and pre-treatment OSA patients, and b) pre- and post-treatment OSA patients. GM-signal change values represent the *difference* between groups. Significant comparisons ($p < 0.05$) are marked by an asterisk.

GM = Grey-Matter, WM = White-Matter, CSF = Cerebro-Spinal-Fluid, TIV = Total-Intracranial-Volume, GM = Grey-Matter, ROIs = Regions of Interest.

Table E4. Correlation between Grey-Matter volume and Body Mass Index (BMI)

	Correlation between GM-volume and BMI in pre-treatment OSA	
	Pre-treatment OSA	*p-value*
R Superior frontal gyrus	-0.30	*0.25*
L Inferior parietal lobule	-0.24	*0.36*
L Parahippocampal gyrus (Enthorinal cortex)	-0.32	*0.21*

Correlation between Body Mass Index (BMI) and grey-matter volume in the cerebral regions showing significant grey-matter volume reduction in pre-treatment OSA than controls.

BMI = Body Mass Index, L =left, R = right.

Table E5. Correlation between disease severity (AHI) and Grey-Matter volume in the cytoarchitectonic subdivisions of the hippocampus

	Before treatment	After treatment
Left Cornu ammonis	-0.5535 *0.026**	0.5409 *0.031**
Right Cornu ammonis	-0.5007 *0.048**	0.5823 *0.018**
Left Enthorinal cortex	-0.4811 *0.05**	0.5379 *0.032**
Right Enthorinal cortex	-0.6075	0.6510

	*0.013**	*0.006**
Left Fascia dentata	-0.5192 *0.039**	0.4872 *0.05**
Right Fascia dentata	-0.6113 *0.012**	0.5746 *0.020**
Left Hippocampus-Amygdala-Transition-Area	-0.3562 *0.176*	0.3244 *0.220*
Right Hippocampus-Amygdala-Transition-Area	-0.2335 *0.384*	0.3931 *0.132*
Left Subiculum	-0.4216 *0.104*	0.5858 *0.017**
Right Subiculum	-0.3686 *0.160*	0.5759 *0.020**

Significant correlations between Apnea-Hypopnea-Index(AHI) before treatment and a) GM-volume before treatment (left), b) GM-volume increase after treatment (right) in the cytoarchitectonic subdivisions of the human hippocampus[E12]. Within each cell, the number at the top indicates the value of the correlation, the one at the bottom (in italic) indicates the corresponding p-value. Significant correlations ($p < 0.05$) are marked by an asterisk.

Table E6. Re-analysis of the data with the "Optimized-VBM"

H	Anatomical region (BA/structure)	K		MNI		Z-score
			X	y	z	
Controls > Pre-treatment						
L	Middle frontal gyrus (6)	481	-33	2	54	3.78
	Precentral gyrus (6)		-30	-5	60	3.45
L	Precentral gyrus (6/4a)	324	-37	-16	60	4.51
L	Superior frontal gyrus (6)	185	-19	5	66	3.61
L	Superior frontal gyrus (6)	62	-16	-6	70	3.35
R	Middle frontal gyrus	39	35	17	47	3.38
	Superior frontal gyrus (6)	29	21	8	68	3.23
R	Precentral gyrus (6)	94	33	-13	63	3.73
R	Precentral gyrus (6)	41	37	0	51	3.37
L	Anterior cingulate cortex	16	-6	28	16	3.48
R	SMA (6)	25	11	2	65	3.34
L	Postcentral gyrus (4p/3b/4a)	181	-34	-30	56	3.67
L	Postcentral gyrus (2/1/3b)	22	-48	-25	52	3.20
Pre-treatment > Controls						
L	IFG pars opercularis (44/45)	18	-57	16	24	3.54
L	IFG pars triangularis	10	-39	31	21	3.38
L	Precuneus (SPL 7a)	182	-9	-65	39	3.49
R	Precuneus (SPL 7m/7p/7a)		2	-64	42	3.15
L	Lingual gyrus (18/17)	70	-8	-84	-7	3.49
Pre-treatment > Post-treatment						

Post-treatment > Pre-treatment						
L	Middle frontal gyrus	7	-32	30	36	3.14
L	Postcentral gyrus (3a/3b/4p)	154	-46	-15	32	4.00

Cerebral regions showing a change of GM-volume before or after CPAP treatment when using the "*Optimized-VBM approach*" with SPM2 and a threshold of $p < 0.001$ uncorrected for multiple comparisons. No region survived any correction for multiple comparisons (either $p < 0.05$ with False Discovery Rate (FDR) or $p < 0.05$ Family-Wise-Error (FWE) based on cluster-extent[E9]), in any contrast.

H = Hemisphere, R = Right, L = Left, BA = Broadmann Area, K = cluster extent in number of voxels (1x1x1 mm^3), SMA = Supplementary Motor Area, IFG = Inferior Frontal Gyrus.

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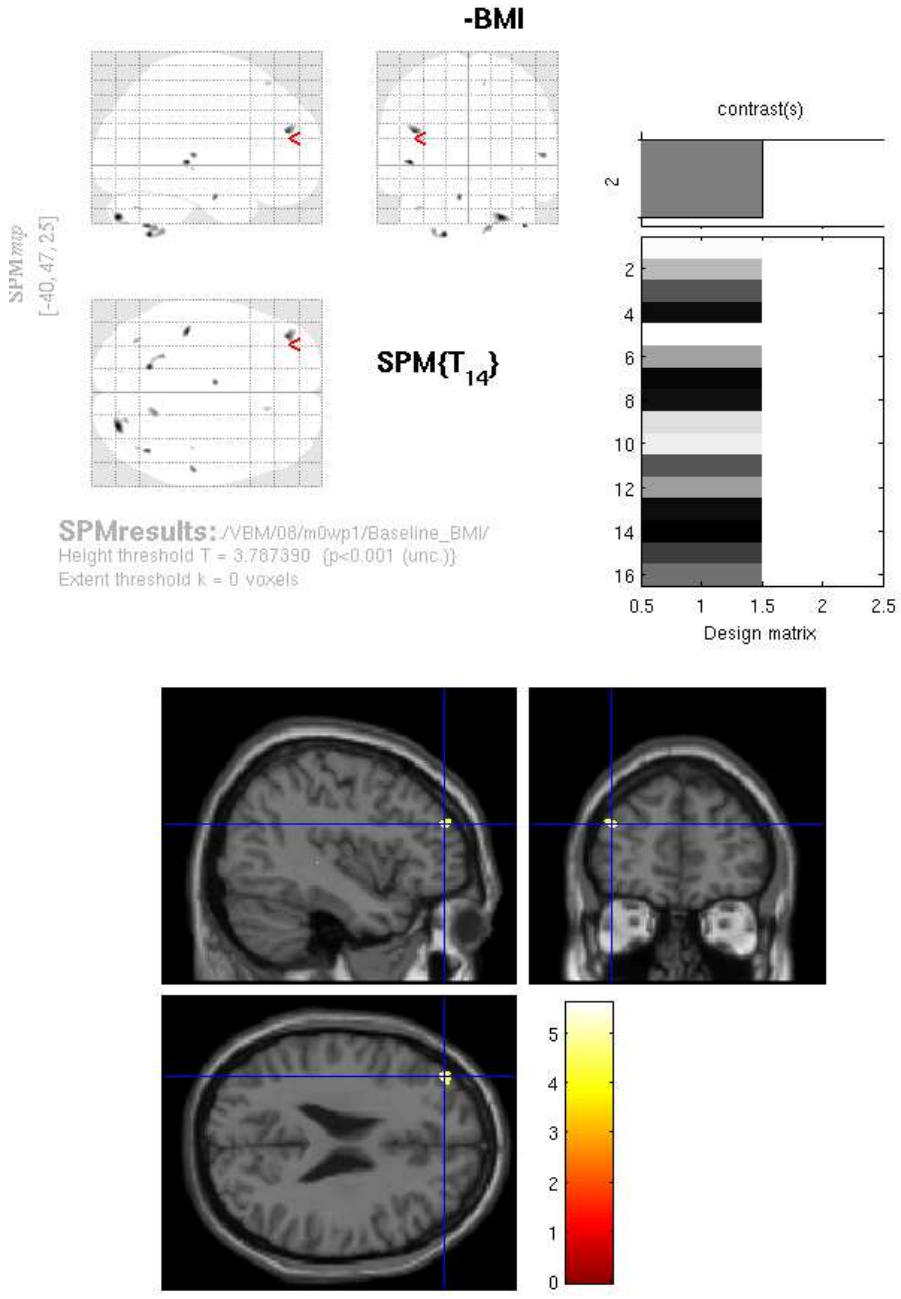
Figure E1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6
8
10
12
14
16
0.5
1
1.5
2
2.5
Design matrix
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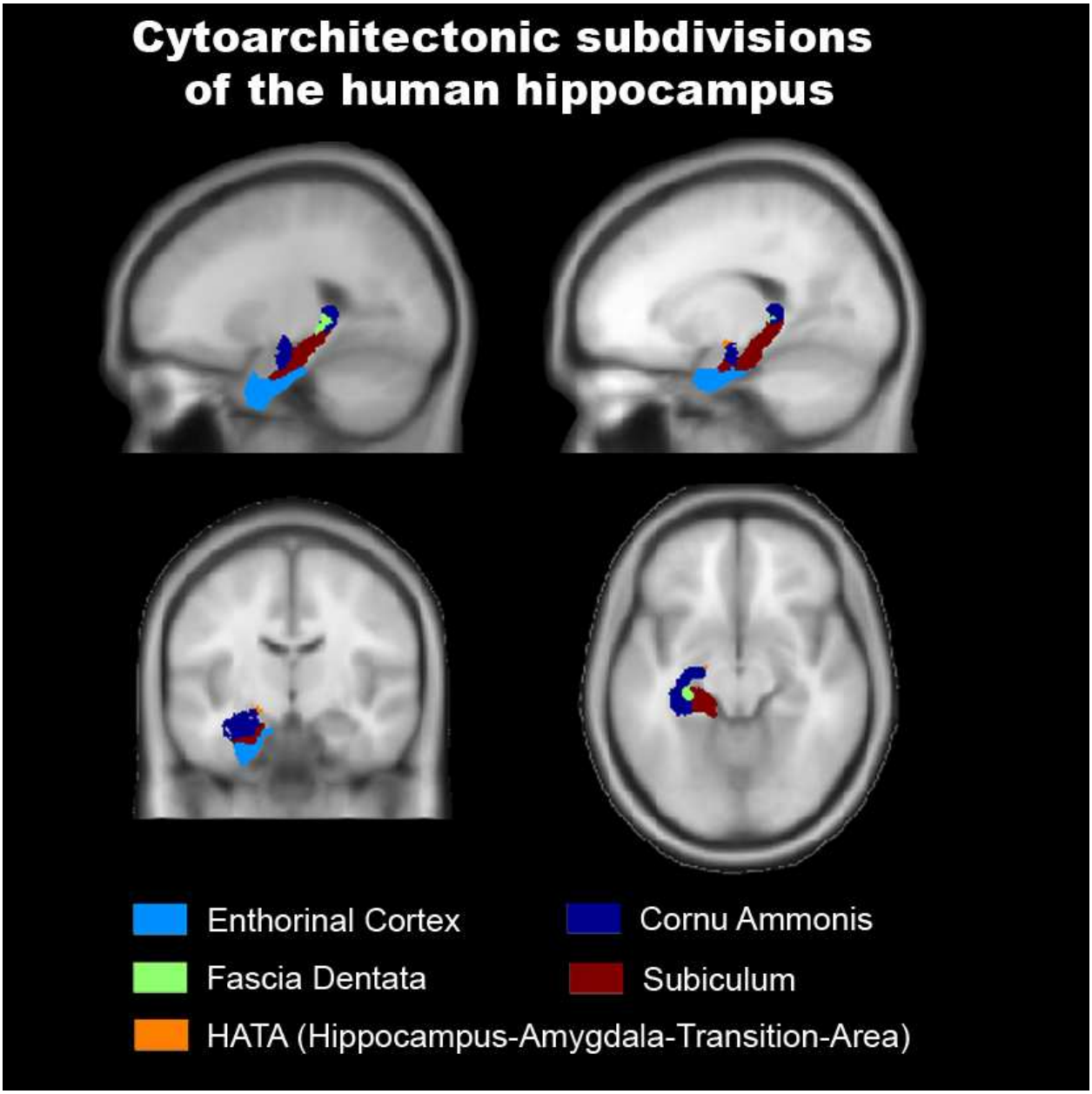
4
3
2
1
0

Figure E2


Cytoarchitectonic subdivisions
of the human hippocampus
Enthorinal Cortex
Cornu Ammonis
Fascia Dentata
Subiculum
HATA (Hippocampus-Amygdala-Transition-Area)