Obstructive sleep apnea is associated with low GABA and high glutamate in the insular cortex

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SUMMARY

The insular cortex is injured in obstructive sleep apnea (OSA) and responds inappropriately to autonomic challenges, suggesting neural reorganization. The objective of this study was to assess whether the neural changes might result from γ-aminobutyric acid (GABA) and glutamate alterations. We studied 14 OSA patients [mean age $\pm$ standard deviation (SD): 47.5 $\pm$ 10.5 years; nine male; apnea–hypopnea index (AHI): 29.5 $\pm$ 15.6 events h^{-1}] and 22 healthy participants (47.5 $\pm$ 10.1
years; 11 male), using magnetic resonance spectroscopy to detect GABA and glutamate levels in insular cortices. We localized the cortices with anatomical scans, and measured neurochemical levels from anterior to mid-regions. Left and right anterior insular cortices showed lower GABA and higher glutamate in OSA versus healthy subjects [GABA left: OSA $n = 6$: 0.36 $\pm$ 0.10 (mean $\pm$ SD), healthy $n = 5$: 0.62 $\pm$ 0.18; $P < 0.05$), right: OSA $n = 11$: 0.27 $\pm$ 0.09, healthy $n = 14$: 0.45 $\pm$ 0.16; $P < 0.05$; glutamate left: OSA $n = 6$: 1.61 $\pm$ 0.32, healthy $n = 8$: 0.94 $\pm$ 0.34; $P < 0.05$, right: OSA $n = 14$: 1.26 $\pm$ 0.28, healthy $n = 19$: 1.02 $\pm$ 0.28; $P < 0.05$]. GABA and glutamate levels were correlated only within the healthy group in the left insula (r: -0.9, $P < 0.05$). The altered anterior insular levels of GABA and glutamate may modify integration and projections to autonomic areas, contributing to the impaired cardiovascular regulation in OSA.

INTRODUCTION

The insular cortex in obstructive sleep apnea (OSA) shows structural and functional alterations, which likely contribute to adverse autonomic and affective symptoms characteristic of the syndrome (Harper *et al.*, 2003; Kumar *et al.*, 2012; Macey *et al.*, 2008). The altered functions include distorted insular functional magnetic resonance (fMRI) signals accompanying sympathetic nervous system challenges, and probably underlie the high sympathetic outflow in OSA, presumably contributing to the profuse sweating, cardiac arrhythmia and hypertension in the disorder. The insular injury in OSA also probably facilitates the development of depression and anxiety in the condition (Asghari *et al.*, 2012; Kurth *et al.*, 2010; Marin *et al.*, 2012). Mechanisms underlying insular dysfunction in OSA are unclear, but understanding the nature of the brain alterations could provide insights into symptoms that persist even with treatment (Marin *et al.*, 2012), as well as processes underlying partial or complete resolution with continuous positive airway pressure (CPAP) of other symptoms, such as high sympathetic tone (Fatouleh *et al.*, 2015).

Structural brain alterations in OSA could arise from multiple pathologies. Reduced mean diffusivity, apparent in many OSA brain regions (Kumar *et al.*, 2012), reflects an increase in barriers to intercellular water movement, which could arise from cell swelling due to inflammation or increased cell size due to glial activation. fMRI differences appear in the insular cortex during autonomic challenges (Harper *et al.*, 2003). The processes underlining those differences could be elucidated by determining the levels of the neurotransmitters

glutamate and γ-aminobutyric acid (GABA), which can now be measured non-invasively. Levels of these chemicals are sensitive to neural reorganization in response to injury, and can reflect different functional states (Arckens *et al.*, 2000). High levels of glutamate, in particular, are associated with excitotoxicity, a probable mechanism of injury in some brain areas in OSA.

The objective of this study was to assess GABA and glutamate in the anterior insular cortex in OSA patients, relative to healthy subjects. The anterior mid-insula was selected, as that subregion preferentially serves autonomic roles (Macey *et al.*, 2012). We hypothesized that, relative to healthy people, OSA patients would show elevated glutamate, based on the potential for hypoxia-induced excitotoxic conditions and altered GABA levels, based on impaired insular action.

METHODS

We studied 14 OSA patients [mean age: 47.5 ± 10.5 years; nine male; mean apnea–hypopnea index (AHI): 29.5 ± 15.6 events h^{-1}; SAO_2 min: 83 ± 9%] and 22 healthy subjects (mean age: 47.5 ± 10.1 years; 11 male). Patients were diagnosed at the UCLA Sleep Disorders Center. No participants had a history of head trauma or disease, current mental illness, use of psychoactive medications, cancer or cardiac disease. Patients were untreated, apart from two who had a prior history of CPAP use. Healthy subjects were screened to exclude sleep disorders. Procedures were approved by the UCLA Institutional Review Board, and participants provided written informed consent.

Neurotransmitters were measured with a magnetic resonance spectroscopy (MRS) method based on 'two-dimensional' MRS (Sarma *et al.*, 2014; see Supporting information). Neurochemicals changes were measured using compressed sensing (CS)-based four-dimensional (4D) echo-planar J-resolved spectroscopic imaging (EP-JRESI). High-resolution T1-weighted scans were acquired for localization of insular cortex. Group differences in GABA and glutamate concentrations (ratios with respect to creatine) in the anterior to mid-insular cortex and adjacent tissue were assessed with *t*-tests. The relative concentrations of 'Glx', a combination of glutamate and glutamine indicative of glutamate levels, were also assessed, as Glx is a more robust MRS measure than glutamate.

RESULTS

Neurotransmitter concentrations were recorded successfully from subsets of the 22 healthy and 14 OSA subjects (Table 1; example spectra Fig. 1). Differences between OSA and healthy subjects emerged for GABA (Fig. 2a) and glutamate (Fig. 2b) in the right and left insulae. The left insula showed poorer data quality in more subjects than the right (right successful measures: 69% GABA, 92% glutamate; left successful measures: 31% GABA, 39% glutamate). Success rates were similar for the OSA and healthy groups. When the two OSA patients with a prior history of CPAP were excluded the mean differences were similar, and the significant differences between OSA and healthy subjects remained, with the exception of glutamate in the right insula. However, Glx showed significantly higher levels in OSA versus healthy subjects in the right and left insulae, with and without excluding the two previously treated subjects (Table 1). Correlations between GABA and glutamate were significant only in healthy subjects in the left insula (*r*: −0.90, *P*: 0.04; Table 2; Fig. 2c,d).

DISCUSSION

The anterior insular cortex in OSA shows low GABA and high glutamate, conditions which would modify neural patterns within the structure. GABA normally acts as an inhibitory neurotransmitter, while glutamate is normally excitatory to neural processes. The influences of the insular cortices are complex, with both inhibitory and excitatory influences on hypothalamic, cingulate and frontal cortical

Table 1 Levels of GABA and glutamate in subsets of 14 OSA (12 never treated) and 22 healthy subjects

	Left insula					*Right insula*				
	OSA		*Healthy*			*OSA*		*Healthy*		
	n	*Mean ± SD*	n	*Mean ± SD*	P	n	*Mean ± SD*	n	*Mean ± SD*	P
GABA	6	0.36 ± 0.10	5	0.62 ± 0.18	0.03	11	0.27 ± 0.09	14	0.45 ± 0.16	< 0.01
Glutamate	6	1.61 ± 0.32	8	0.94 ± 0.34	< 0.01	14	1.26 ± 0.28	19	1.02 ± 0.28	0.02
Glx	6	1.92 ± 0.38	6	1.22 ± 0.44	0.01	14	1.75 ± 0.42	19	1.34 ± 0.42	0.01
	Never treated OSA					Never treated OSA				
GABA	5	0.35 ± 0.10	(as above)		0.03	9	0.27 ± 0.10	(as above)		< 0.01
Glutamate	5	1.50 ± 0.21	(as above)		< 0.01	12	1.20 ± 0.25	(as above)		0.08
Glx	5	1.79 ± 0.18	(as above)		0.01	12	1.69 ± 0.43	(as above)		0.04

P, *t*-test for group differences; Glx, glutamate + glutamine; GABA, γ-aminobutyric acid; OSA, obstructive sleep apnea; SD, standard deviation.

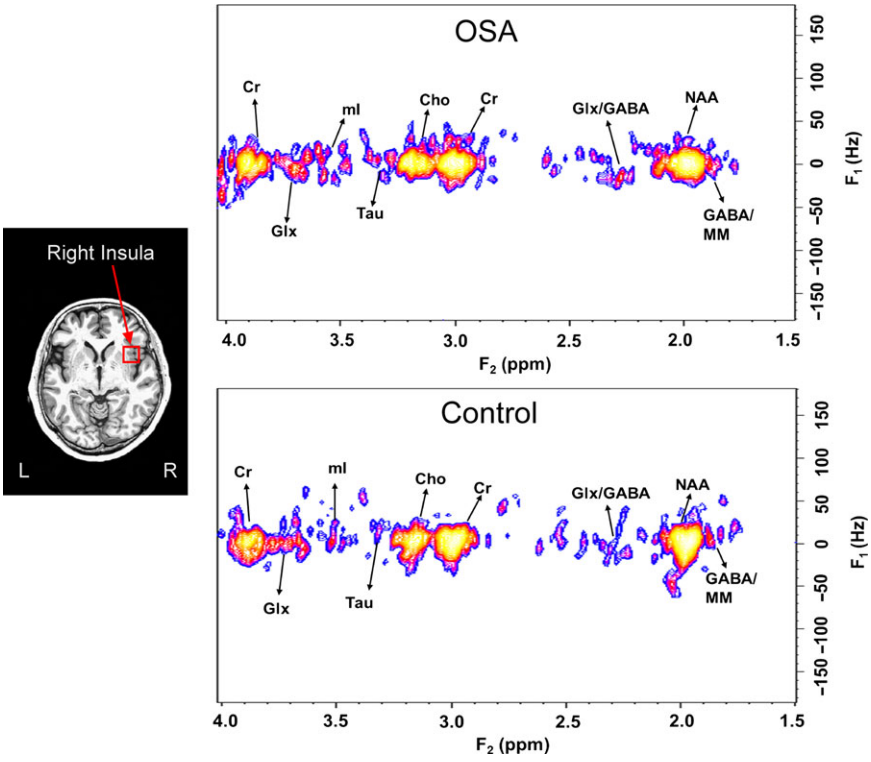
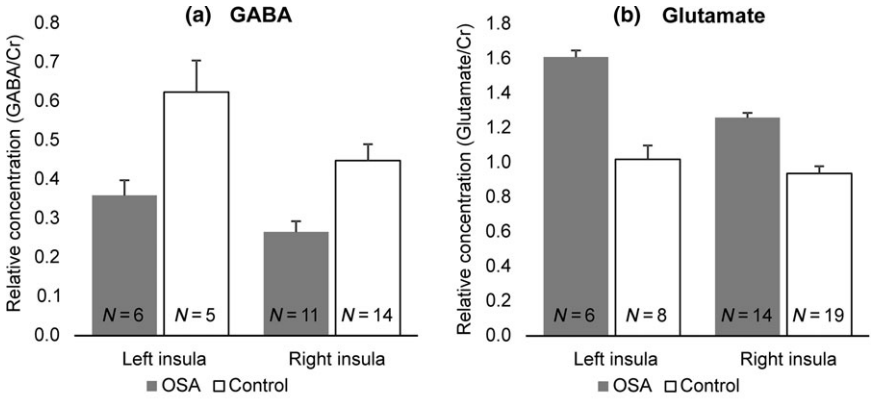


Figure 1. Left: example insula voxel is shown in red (15 mm^3) overlaid an individual anatomical scan. Right: example two-dimensional magnetic resonance spectroscopy (2D-MRS) spectra from right insulae in obstructive sleep apnea (OSA) (56-year-old female) and control (53-year-old male) subjects, with resonances of various neurochemicals indicated (Cho, choline; Cr, creatine; mI, myo-inisotol; MM, other macromolecules; NAA, N-acetylaspartate; Tau, taurine).



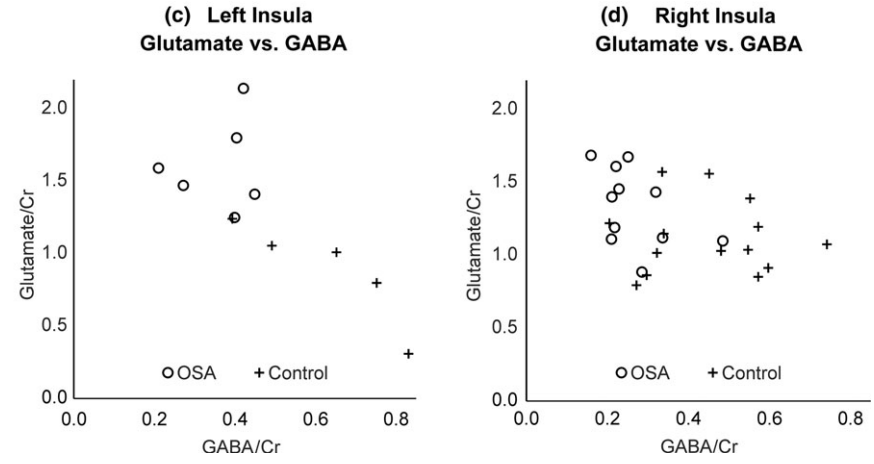


Figure 2. Insular (a) gamma aminobutyric acid (GABA) and (b) glutamate levels in subsets of 14 obstructive sleep apnea (OSA) and 22 healthy subjects [mean ± standard error of the mean (SEM)]. Scatterplots of glutamate versus GABA for subjects with both measures in the left (c) and right (d) insular cortices.

structures, among others. On a simplistic level, the combination of a reduction in the inhibitory GABA and increase in the excitatory glutamate could reflect a more active state overall; however, the complexity of the insular projections to multiple structures precludes such an exclusive generalization (Kurth *et al.*, 2010). For excitatory projections,

Table 2 Correlation table in subjects with GABA and glutamate measures

	Left insula						*Right insula*					
	OSA			*Healthy*			*OSA*			*Healthy*		
	n	r	P	n	r	P	n	r	P	n	r	P
Glutamate versus GABA	6	0.16	0.76	5	−0.90	0.04	11	−0.47	0.14	14	−0.04	0.90

P, correlation between γ-aminobutyric acid (GABA) and glutamate; OSA, obstructive sleep apnea.

enhanced glutamate could enhance insular influences, and the normal inhibition over hypothalamic structures could be reduced by the lower GABA levels, leading potentially to the exaggerated sympathetic tone in the condition. The altered neurotransmitter levels could also modify cingulate and hippocampal projections influencing mood, such as depression and anxiety, both of which are common in OSA. Low GABA in the insula has been associated with high levels of anxiety (Rosso *et al.*, 2014), and levels appear to be associated with processing of interoceptive stimuli, a function linked with depressive mechanisms (Wiebking *et al.*, 2014). The structures involved in anxiety and depression include the anterior cingulate, recipient of fibres from the cingulum and insular cortices. High glutamate has been found in these limbic areas in bipolar disorder (Soeiro-De-Souza *et al.*, 2015). The hypoxia exposure during apneic events probably leads to excessive excitation of the insular connecting fibres, and such excitotoxic processes are facilitated by glutamate. It is unknown whether the altered neurotransmitter levels revert to normal with CPAP treatment, but such knowledge would assist the selection of interventions and the determination of whether regional GABA and glutamate levels could be useful biomarkers of local neural tissue state in OSA.

The findings of reduced GABA and higher glutamate build upon an earlier demonstration of alterations in the anterior insular cortex in OSA, including changes in structure, function and metabolite levels. The insula shows impaired responses to autonomic challenges in OSA, especially during periods of sympathetic activity increases (Harper *et al.*, 2003). Resting state functional patterns also differ from heathy subjects when those patterns are correlated with other regions (Zhang *et al.*, 2015). Changes in water diffusivity reflect structural changes arising probably from glial activation or inflammation due to hypoxia, and damage to neurones or axons and myelin (Kumar *et al.*, 2012; Macey *et al.*, 2008). Increased myo-inositol in the left insula of OSA patients is also consistent with glial alterations (Yadav *et al.*, 2014). Increased glutamate levels may arise directly from astrocyte activation, as astrocytic glutamate is an energy source for neurones (Pellerin and Magistretti, 1994); if metabolism is altered by OSA in the insular cortex, glutamate from astrocytes may increase to compensate. The combined chemical, functional and structural findings confirm injury and reorganization of the insula in OSA, which probably contribute to clinical symptoms in the condition (Asghari *et al.*, 2012; Kurth *et al.*, 2010; Macey *et al.*, 2013; Marin *et al.*, 2012).

The high glutamate may reflect damaging excitotoxic processes arising from intermittent hypoxia (Jagadapillai *et al.*, 2014). Only 5 h of intermittent hypoxia simulating OSA effects can lead to cellular death (Pae *et al.*, 2005); thus, similar processes are probably operating in people with OSA. However, the high glutamate measured here was in whole tissue, and does not necessarily correspond to concentrations in extracellular fluid, where the neurochemical is key for excitotoxicity.

Interventions complementary to CPAP that influence neurotransmitter levels may help to normalize brain function in OSA. Both GABA and glutamate can be manipulated directly with pharmacological approaches and indirectly with behavioural changes. The measurements presented here could serve as a biomarker of any intervention targeting these brain chemicals.

Measurements were successful in 58% of instances, with the remainder not meeting quality criteria, especially in the left insula. Because OSA and healthy measurements were affected similarly, the low data quality is due probably to technical rather than biological reasons (e.g. poor shimming). The resolution of the MRS voxels was limited (example in Fig. 1), so the measures did not cover the entire anterior to mid-portion of the structure, and included adjacent tissue; the subregion studied will have varied slightly between subjects.

In conclusion, the anterior to mid-insular cortex shows lower GABA and higher glutamate levels in OSA compared with healthy subjects. These alterations probably reflect a functional neural reorganization that contributes to the autonomic and psychological symptoms in OSA, such as high sympathetic tone and refractory hypertension, and anxiety, depression and cognitive difficulties. High glutamate would facilitate excitotoxic processes. The altered neurotransmitter levels provide a biomarker of regional functional status of the OSA brain, and will allow insights into the neurological effects of treatment with CPAP or other interventions.

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AUTHOR CONTRIBUTIONS

Study design: PM and MAT; data collection: RA, PM, MS, RN and MAT; analysis: PM, MS, RN, PM, MS, RS, JMS, RN, RMH and MAT: interpretation and manuscript preparation: PM: MS, RN, RA and MAT.

REFERENCES

Arckens, L., Schweigart, G., Qu, Y. *et al.* Cooperative changes in GABA, glutamate and activity levels: the missing link in cortical plasticity. *Eur. J. Neurosci*., 2000, 12: 4222–4232.

Asghari, A., Mohammadi, F., Kamrava, S. K., Tavakoli, S. and Farhadi, M. Severity of depression and anxiety in obstructive sleep apnea syndrome. *Eur. Arch. Otorhinolaryngol*., 2012, 269: 2549–2553.

Fatouleh, R. H., Lundblad, L. C., Macey, P. M., McKenzie, D. K., Henderson, L. A. and Macefield, V. G. Reversal of functional changes in the brain associated with obstructive sleep apnoea following 6 months of CPAP. *Neuroimage Clin*., 2015, 7: 799–806.

Harper, R. M., Macey, P. M., Henderson, L. A. *et al.* fMRI responses to cold pressor challenges in control and obstructive sleep apnea subjects. *J. Appl. Physiol*., 2003, 94: 1583–1595.

Jagadapillai, R., Mellen, N. M., Sachleben, L. R. Jr and Gozal, E. Ceftriaxone preserves glutamate transporters and prevents intermittent hypoxia-induced vulnerability to brain excitotoxic injury. *PLoS ONE*, 2014, 9: e100230.

Kumar, R., Chavez, A. S., Macey, P. M., Woo, M. A., Yan-Go, F. L. and Harper, R. M. Altered global and regional brain mean diffusivity in patients with obstructive sleep apnea. *J. Neurosci. Res*., 2012, 90: 2043–2052.

Kurth, F., Zilles, K., Fox, P. T., Laird, A. R. and Eickhoff, S. B. A link between the systems: functional differentiation and integration within the human insula revealed by meta-analysis. *Brain Struct. Funct*., 2010, 214: 519–534.

Macey, P. M., Kumar, R., Woo, M. A., Valladares, E. M., Yan-Go, F. L. and Harper, R. M. Brain structural changes in obstructive sleep apnea. *Sleep*, 2008, 31: 967–977.

Macey, P. M., Wu, P., Kumar, R. *et al.* Differential responses of the insular cortex gyri to autonomic challenges. *Auton. Neurosci*., 2012, 168: 72–81.

Macey, P. M., Kumar, R., Woo, M. A., Yan-Go, F. L. and Harper, R. M. Heart rate responses to autonomic challenges in obstructive sleep apnea. *PLoS ONE*, 2013, 8: e76631.

Marin, J. M., Agusti, A., Villar, I. *et al.* Association between treated and untreated obstructive sleep apnea and risk of hypertension. *JAMA*, 2012, 307: 2169–2176.

Pae, E. K., Chien, P. and Harper, R. M. Intermittent hypoxia damages cerebellar cortex and deep nuclei. *Neurosci. Lett*., 2005, 375: 123–128.

Pellerin, L. and Magistretti, P. J. Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc. Natl Acad. Sci. USA*, 1994, 91: 10625–10629.

Rosso, I. M., Weiner, M. R., Crowley, D. J., Silveri, M. M., Rauch, S. L. and Jensen, J. E. Insula and anterior cingulate GABA levels in posttraumatic stress disorder: preliminary findings using magnetic resonance spectroscopy. *Depress. Anxiety*, 2014, 31: 115–123.

Sarma, M. K., Nagarajan, R., Macey, P. M. *et al.* Accelerated echo-planar j-resolved spectroscopic imaging in the human brain using compressed sensing: a pilot validation in obstructive sleep apnea. *AJNR Am. J. Neuroradiol*., 2014, 35: S81–S89.

Soeiro-De-Souza, M. G., Henning, A., Machado-Vieira, R. *et al.* Anterior cingulate glutamate–glutamine cycle metabolites are altered in euthymic bipolar I disorder. *Eur. Neuropsychopharmacol*., 2015, 25: 2221–2229.

Wiebking, C., Duncan, N. W., Tiret, B. *et al.* GABA in the insula—a predictor of the neural response to interoceptive awareness. *NeuroImage*, 2014, 86: 10–18.

Yadav, S. K., Kumar, R., Macey, P. M., Woo, M. A., Yan-Go, F. L. and Harper, R. M. Insular cortex metabolite changes in obstructive sleep apnea. *Sleep*, 2014, 37: 951–958.

Zhang, Q., Qin, W., He, X. *et al.* Functional disconnection of the right anterior insula in obstructive sleep apnea. *Sleep Med*., 2015, 16: 1062–1070.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Data S1. Supplemental methods: magnetic resonance spectroscopy.