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RESEARCH ARTICLE

Brain mitochondrial dysfunction and driving simulator performance in untreated obstructive sleep apnea

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Abstract

It is challenging to determine which patients with obstructive sleep apnea (OSA) have impaired driving ability. Vulnerability to this neurobehavioral impairment may be explained by lower brain metabolites levels involved in mitochondrial metabolism. This study compared markers of brain energy metabolism in OSA patients identified as vulnerable vs resistant to driving impairment following extended wakefulness. 44 patients with moderate-severe OSA underwent 28hr extended wakefulness with three 90min driving simulation assessments. Using a two-step cluster analysis, objective driving data (steering deviation and crashes) from the 2nd driving assessment (22.5 h awake) was used to categorise patients into vulnerable (poor driving, $n = 21$) or resistant groups (good driving, $n = 23$). ^{1}H magnetic resonance spectra were acquired at baseline using two scan sequences (short echo PRESS and longer echo-time asymmetric PRESS), focusing on key metabolites, creatine, glutamate, *N*-acetylaspartate (NAA) in the hippocampus, anterior cingulate cortex and left orbito-frontal cortex. Based on cluster analysis, the vulnerable group had impaired driving performance compared with the resistant group and had lower levels of creatine (PRESS p = ns, APRESS $p = 0.039$), glutamate, (PRESS $p < 0.01$, APRESS $p < 0.01$), NAA (PRESS $p = 0.038$, APRESS $p = 0.035$) exclusively in the left orbito-frontal cortex. Adjusted analysis, higher glutamate was associated with a 21% (PRESS) and 36% (APRESS) reduced risk of vulnerable classification. Brain mitochondrial bioenergetics in the frontal brain regions are impaired in OSA patients who are vulnerable to driving impairment following sleep loss. These findings provide a potential way to identify at risk OSA

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phenotype when assessing fitness to drive, but this requires confirmation in larger future studies.

KEYWORDS
accident risk, driving impairment, extended wakefulness, MRS, OSA, spectroscopy

1 | INTRODUCTION

Obstructive sleep apnea (OSA) is a common sleep disorder with community prevalence rising over last 30 years to over 20% most probably due to increased global obesity and more sensitive OSA measurement techniques (Benjafield et al., 2019). Common symptoms of OSA include excessive daytime sleepiness, impaired alertness and neurobehavioral dysfunction leading to a more than two-fold increased risk of traffic and workplace accidents (Ellen et al., 2006). OSA-related motor vehicle accidents cost $15.9 billion annually in the United States (Sassani et al., 2004) and exceed $3 billion in Australia (Hillman, Murphy, Antic, & Pezzullo, 2006).

Although the prevalence of OSA is high and the cost due to accidents is substantial, this is driven predominantly by a minority of patients at higher risk of driving impairment (Vakulin et al., 2014). Identifying at-risk patients is currently difficult due to the large heterogeneity in OSA symptoms and lack of strong and consistent relationships between OSA severity indices (apnea hypopnea index (AHI), hypoxemic dips, and arousals from sleep) with measures of neurobehavioural dysfunction and impaired driving ability (Vakulin et al., 2014). Consequently, universal limitation of driving in OSA is not feasible or appropriate. Therefore, there is a pressing need to find novel markers that may better explain the disease heterogeneity in daytime impairment and identify OSA patients at greater risk of driving performance impairment (e.g. drowsy driving and crash risk; Vakulin et al., 2014).

The brain is impacted by multiple insults in untreated OSA (Rosenzweig et al., 2015). Acutely, sleep architecture is fragmented. The brain is subjected to repeated hypopneas/apneas with associated dips in oxygen saturation. Chronically, OSA is linked with cardiovascular and metabolic impairment that may negatively impact brain function and cerebrovascular autoregulation (Dredla & Castillo, 2019). These multifaceted impacts of OSA further complicate efforts to identify those who are vulnerable to driving impairment. Extended wakefulness for 24 h (sleep deprivation) results in lower global and regional cerebral metabolism of the mandatory brain fuel, glucose (Thomas et al., 2000). Forty hours of extended wakefulness impacts brain energy levels for several days, despite two nights recovery sleep (Plante et al., 2014). Chronic sleep loss as seen in insomnia with short sleep duration (<5 h) is linked to reduced levels of brain metabolites associated with energy metabolism, including key markers of brain bioenergetics and metabolism, aspartate, glutamine and creatine (Miller et al., 2017).

Brain energy metabolism is impaired in OSA patients relative to healthy controls (Xia et al., 2016). In particular, OSA appears to affect the most abundant and important markers of cell viability and energy metabolism, including creatine, glutamate and *N*-acetylaspartate (NAA). One small study has shown that OSA patients had lower creatine levels in the hippocampal region relative to controls (Bartlett et al., 2004). The authors also noted that lower creatine levels correlated with greater number of attentional lapses on the psychomotor vigilance test (PVT). Another, more recent study of 15 patients with severe OSA using ^{31}P MRS, found that higher inorganic phosphate/adenosinetriphosphate ratio (Pi/ATP), indicative of lower phosphorylation potential, was associated with worse PVT and driving simulator performance (D'Rozario et al., 2018). The most recent review of MRS studies in OSA found that there was considerable heterogeneity in the outcomes, exacerbated by the complexity of the disorder and the range of impacts it can have (Xia et al., 2016).

Neurobehavioral function is significantly impaired in OSA, particularly in the domains of vigilance and sustained attention, memory and executive function, which are largely driven by the pre-frontal cortex and associated attentional networks (Beebe, 2005). These brain regions use the highest proportion of energy during wakefulness and are particularly suceptible to the sleep fragmentation and nocturnal hypoxemia associated with OSA (Beebe, 2005). There are significant inter-individual differences in vulnerability to sleep deprivation and the consequent functional impairment (Van Dongen, Baynard, Maislin, & Dinges, 2004). Evidence from blood oxygen level dependent (BOLD) functional brain imaging suggest that vulnerability to performance impairment following extended wakefulness may relate to baseline resting brain activity. Those exhibiting higher activity in the BOLD response to a baseline task were resistant to cognitive performance decrements following extended wakefulness (Mu et al., 2005). There is a strong coupling between resting activity as measured by the BOLD response and brain metabolism (Aubert & Costalat, 2002). However, it is not known if this relationship holds in those with OSA, particularly given the impact of sleep fragmentation and intermittent hypoxia on brain activity (Li et al., 2015) and the documented reduction in cerebrovascular reactivity in this population (Placidi, Diomedi, Cupini, Bernardi, & Silvestrini, 1998).

Identifying OSA patients who are vulnerable to cognitive impairment following extended wakefulness is an important and currently unresolved clinical challenge, paricularly in the context of fitness to drive and accident risk (Vakulin et al., 2014). The primary aim of this study was to compare brain metabolite profiles measured at baseline between OSA patients characterised as vulnerable versus resistant to driving impairment following extended wakefulness. We hypothesised that OSA patients who were vulnerable to the effects

of extended wakefulness, evidenced by poor driving performance, would show impaired brain energy metabolism as measured by MRS, compared with resistant patients.

2 | METHODS

This study was funded by the National Health and Medical Research Council of Australia GNT1028624. All study procedures were approved by the Sydney Local Health District (RPAH Zone) Protocol No. X12-00828 and HREC/12/RPAH/40, and the University of New South Wales Human Research Ethics Committee, Ref No. HC121213. The study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12613001171707).

2.1 | Patient recruitment and screening

All patients were recruited from sleep clinics at the Woolcock Institute of Medical Research and the Royal Prince Alfred Hospital, Sydney. Prospective OSA patients potentially eligible to take part in the study were contacted by phone in the first instance to assess initial eligibility. *Inclusion Criteria*: Males and females; Age 25–70; Polysomnography confirmed OSA (AHI > 10 events/h), Oxygen Desaturation Index (ODI) ≥ 3% > 8 events/h; Not treated for OSA (have not previously used continuous positive airway pressure therapy or mandibular advancement splint) for longer than 3 months and been off treatment for at least six months prior to participation in the study. *Exclusion Criteria*: Clinically significant co-morbidity; uncontrolled medical conditions e.g. cardiac failure, hypertension, hypercapnia; claustrophobia or unable to have MRI/MRS due to metal fragments or other foreign bodies; history of head injury or psychiatric/neurological disorder (including stroke); use of central nervous system active agents; heavy alcohol consumption (>40 g daily); current shift-worker; professional drivers.

2.2 | Introductory visit and consent

All OSA patients who expressed interest in participating in this study attended a face to face introduction and screening session at the Woolcock Institute of Medical Research (see Figure 1 for flow diagram of recruitment). During this session, the study protocol and all procedures were explained, and informed consent was obtained. The patients were asked to complete a detailed health and sleep background questionnaire. The questionnaires assessed the following components: health and background including medical conditions and medication use; sleep quality, using the Pittsburgh Sleep Quality Index (PSQI) daytime sleepiness, using the Epworth Sleepiness Scale (ESS) insomnia symptoms, using the insomnia severity index (ISI) depression and anxiety, using the depression, anxiety and stress scale (DASS) and driving history. Patients completed practice runs on all neurobehavioral tests and the driving simulation task to become familiar with all tests, minimise the impact of practice effects and assess susceptibility to motion sickness which is exclusionary (particularly important for driving simulation). All subsequent experimental visits were scheduled at least 5 days after the introductory screening visit to allow enough time for pre-laboratory assessments consisting of sleep diary and actigraphy.

2.3 | Pre-laboratory assessment

All patients were issued a sleep diary and an actigraph (Philips Respironics, Actiwatch 2, Bend) during the introductory screening session to monitor habitual sleep and wake habits and activities leading up to the experimental laboratory protocol. Actigraphy monitors were worn for 5–7 days prior to the extended wakefulness protocol. Participants were instructed to maintain a regular sleep pattern.

2.4 | Extended wakefulness protocol

All patients were scheduled to attend the main experimental laboratory visit consisting of a sleep study and 28 h of an extended wakefulness challenge (EWC) with repeated neurobehavioral, electrophysiological and physiological assessments (Figure 2). All patients were asked to arrive at the sleep laboratory at 5 pm. Lighting in the laboratory was kept stable at 0 lux during sleep period and between 40 and 50 lux for all wake assessments, verified by a calibrated luxometer (MT940, Major Tech, Elandsfontein, R.S.A.). The ambient room temperature was kept constant at 22–24°C throughout the entire protocol.

2.5 | Baseline polysomnography

Full attended PSG was performed at the ACCESS sleep laboratory, Woolcock Institute of Medical Research using Embletta Titanium acquisition hardware (Embla Systems). The set up included an extended EEG montage with the EEG activity recorded using referential derivations at scalp positions F3-M2, Fz-(M1+M2/2) F4-M1, C3-M2, Cz-(M1/+M2/2), C4-M1, Pz-(M1/+M2/2), O1-M2, Oz-(M1/+M2/2), O2-M2 according to the International 10–20 System of Electrode Placement and left lateral, right lateral and right supraocular electrooculogram (EOG), submental electromyogram (EMG), nasal cannula to measure nasal pressure, limb movement sensors, inductive plethysmography for thoraco-abdominal motion, lead II electrocardiography and arterial oxygen saturation (finger pulse oximetry). All EEG signals were digitized, sampled and stored at a rate of 512 Hz. Sleep staging and manual scoring of arousal and respiratory events was performed using standard criteria according to the 2007 American Academy of Sleep Medicine (AASM) (alternative) criteria (Iber, Ancoli-Israel, Chesson, & Quan, 2007). Apneas were defined as cessations of nasal flow lasting ≥10 s. Hypopneas were defined

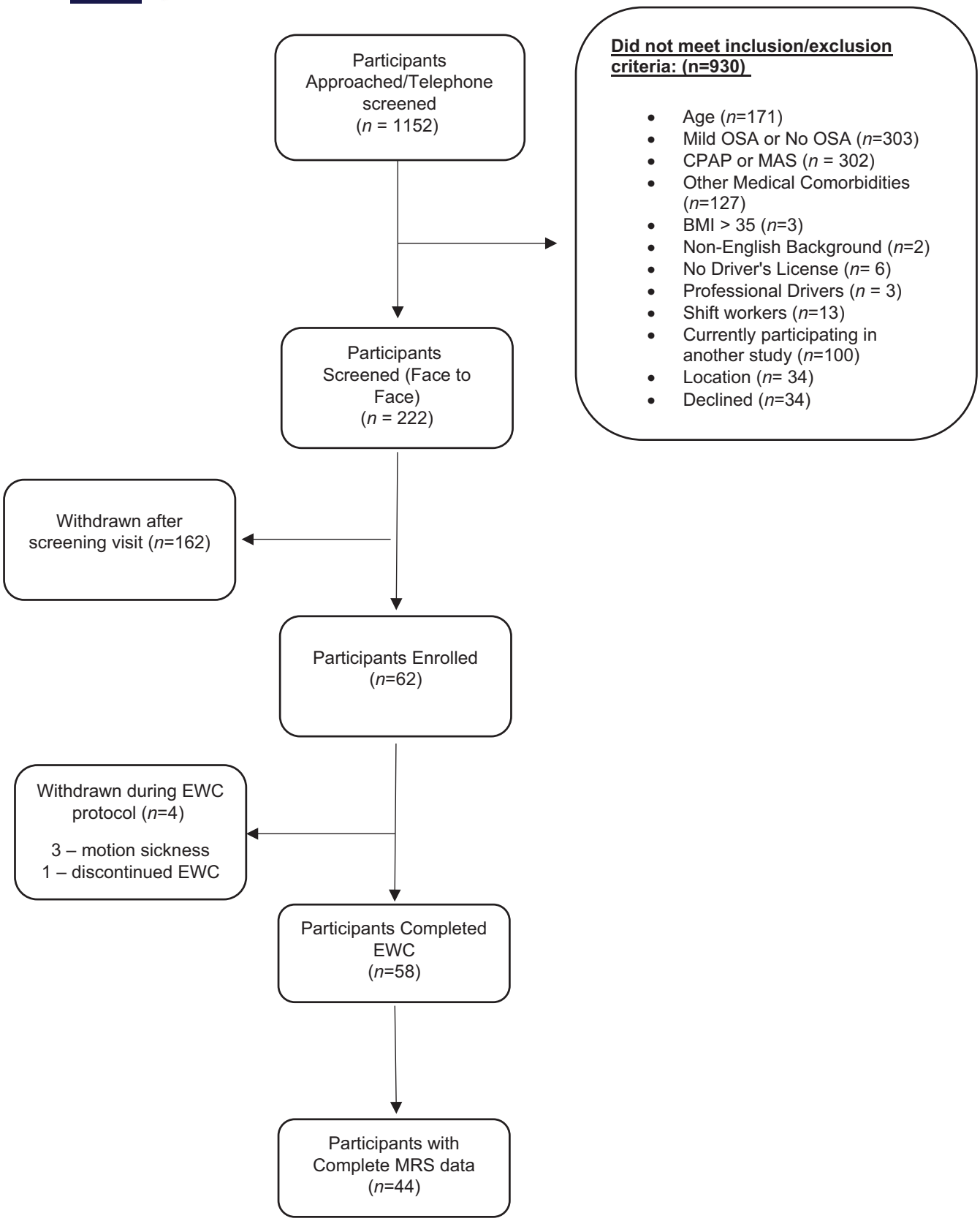


FIGURE 1 Flow diagram of patient screening and recruitment

as a >50% decrease in nasal flow (or in both thoracic and abdominal excursions) and associated with either a ≥3% oxygen desaturation or an EEG arousal. An AHI of at least 10 events/h of sleep by these criteria has been shown to be approximately equivalent to an AHI of at least 5/h of sleep used in earlier studies (Ruehland et al., 2009), and so was the definition used in this study.

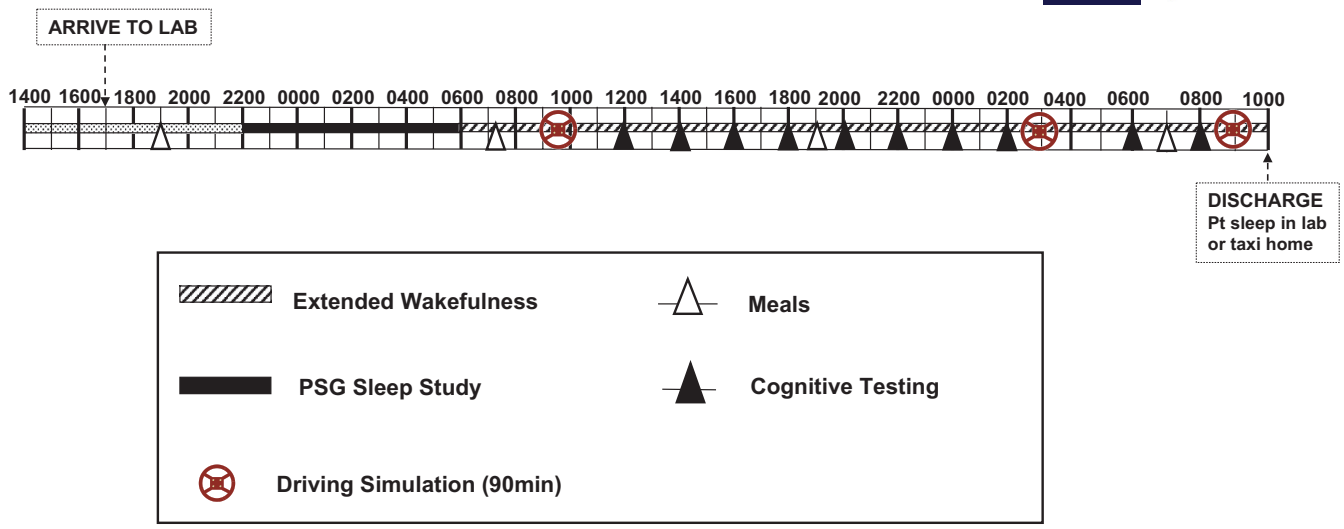
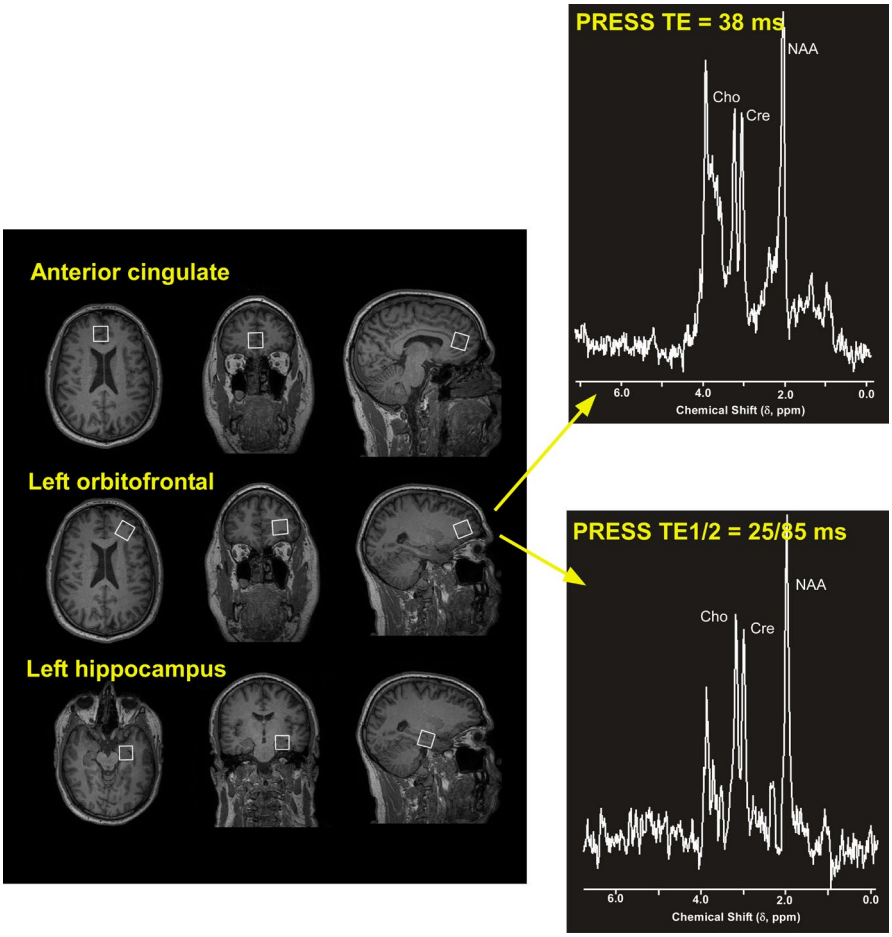


FIGURE 2 Extended Wakefulness Challenge Protocol

FIGURE 3 Location of MR spectra. Coronal, saggital and axial slices from a 3D-TFE image (TR/TE 5.9/2.7 ms, 0.9 mm isotropic) showing placement of 2 cm^3 voxels for PRESS (TE = 38 ms) and asymmetric PRESS (TE1 = 25 ms TE2 = 85 ms) spectra



2.6 | AusEd driving simulator assessment

The AusEd driving simulator task (Woolcock Institute of Medical Research, Sydney, Australia; Desai et al., 2007) simulates driving on a country road at night. The task was performed on a desktop computer with a Logitech MOMO steering wheel and pedals for acceleration and braking and involved a 90 min monotonous country driving scenario as described previously (Vakulin et al., 2014). In brief, steering deviation was measured from the average deviation in centimeters from the driver's median lane position (each lane

was 360 cm wide) sampled at 30Hz. Participants were instructed to maintain speed within 60–80 km/h, but to apply the brakes as quickly as possible whenever a slow moving truck was presented ahead in the driving lane. This occurred a total of seven times during the drive. Crashes occurring throughout the driving task were defined as: car left the road (all four wheels completely off the road), collision with a truck, or if the car was stationary for >3 s. The AusEd task has been shown to be sensitive to impairment in performance associated with sleep loss, circadian effects, alcohol and obstructive sleep apnea (Desai, Marks, Jankelson, & Grunstein, 2006; Vakulin et al., 2009). The driving test was administered at three time points during the EWC protocol: at baseline 9.00 am–10.30 am and following extended wakefulness 2.30 am–4.00 am (22.5 h of wakefulness) and 8.00 am–9.30 am (26.5 h of wakefulness). The primary driving data was derived from the 2nd driving test following 22.5 h of wakefulness when group driving performance decrements were highest. The main outcome measures were average steering deviation from the median lane position which represents continuous driving performance measure, and the number of simulator crash events, which represents driving simulator performance failure as most of these incidents are the result of microsleeps or fall asleep events (Vakulin et al., 2009).

2.7 | Magnetic resonance spectroscopy

At least a week prior, or at least a week following the laboratory extended wakefulness protocol, baseline MR data were acquired from the brain of each subject at 3T (Philips Achieva TX) using a 32 channel head coil. T1-weighted images (3D-TFE, Act TR/TE = 5.9/2.7ms, shot length = 1519 ms. voxel size 0.9 mm isotropic, 250 slices, SENSE 3.5 in slice direction) were used to place voxels for spectroscopy acquisition (2 cm^3) in the left orbitofrontal cortex, left hippocampus and anterior cingulate cortex (Figure 3). These brain regions have previously been shown to have altered metabolites in OSA compared to healthy participants (Xia et al., 2016) and are an important part of driving and attention related neural networks (Calhoun & Pearlson, 2012). Following field adjustment (shimming) and optimization of water suppression for each voxel, two 1H spectra were collected as follows: 1 x PRESS spectrum (TE 38ms, TR 2000 ms, 32 averages, 1024 complex data points) and 1 x asymmetric PRESS spectrum (TE1 = 25, TE = 85, TR 2000 ms, 32 averages, 1024 complex data points). The asymmetric PRESS acquisition is optimized for detection of glutamine, but also shows utility for other major metabolites apart from *myo*-inositol (Rae, Geng, & Williams, 2012). The asymmetric PRESS sequence has a longer echo time compared to the short-echo PRESS, meaning lower signal to noise but minimal interference from baseline distortions due to macromolecules as well as differences in the ability to detect changes in the creatine/phosphocreatine ratio (Rae, 2014). The number of acquisitions was kept short (32) to minimize the opportunity for introducing movement artefact. Water reference spectra were also acquired concomitantly.

After spectroscopy acquisition we also obtained high angular resolution diffusion-weighted acquired with diffusion weighting (b = 2400 s/mm^2) along 61 non-collinear directions for structural white matter assessment, and an echo planar imaging resting state time series.

All spectra were analysed using jMRUI (v4.0 build162). Following removal of the residual water resonance using a Hankel Lanczos Singular Values Decomposition filter, the spectra were fitted in the time domain using the QUEST algorithm (Ratiney et al., 2005) with a basis set generated using the NMRScope tool based on standard chemical shift and coupling constant information (Govindaraju, Young, & Maudsley, 2000). The PRESS metabolite basis set included aspartate, creatine, glutamate, glutamine, GSH, glycerophosphocholine, *myo*-inositol *and N*-acetylaspartate. Although values for aspartate, glutamine and GSH were included in the fitting process for completeness of the spectral model, they were not included in any analyses. The asymmetric PRESS basis set similarly included all the above metabolites plus lactate, which is also resolved well with this sequence although it, as well as aspartate and GSH were not included in analyses. Metabolite values were expressed relative to the unsuppressed water reference (Wilson et al., 2019). Spectroscopy ROIs were assessed for relative contributions of grey and white matter using a tool developed by Dr. Nia Goulden and Dr. Paul Mullins of Bangor University for partial volume estimation of Philips MRI data (Gasparovic et al., 2006).

2.8 | Statistical analyses

Defining vulnerable and resistant OSA patients

All data analysis was performed using IBM SPSS Statistics 23 software package and data figures produced using GraphPad Prism 8 software. Two-step cluster analysis was used to group OSA patients into either a vulnerable or resistant group based on their driving impairment following 22.5 h of extended wakefulness. Two-step cluster analysis is an approach which helps to identify homogeneous groups of cases based on the distribution of the input variables (Kent, Jensen, & Kongsted, 2014). In this study we used two key objective driving simulator performance parameters (steering deviation and number of crashes) from the driving test following 22.5 h of extended wake (period of highest sleep propensity and vulnerability to impaired driving performance) as the two inputs into the two-step cluster analysis. The log-likelihood distance measure and Bayesian (BIC) clustering criteria was applied with automatically determined number of clusters, with a silhouette coefficient of >0.5 which is defined by SPSS as good data partitioning (see Figure 4, extended wake condition). All data were checked for normality and were log transformed in cases where the data were not normally distributed. Unpaired samples *t*-tests were used to examine the mean differences in anthropometrics, PSG sleep study results and MRS outcomes, between the vulnerable and resistant OSA groups, with a level of significance defined

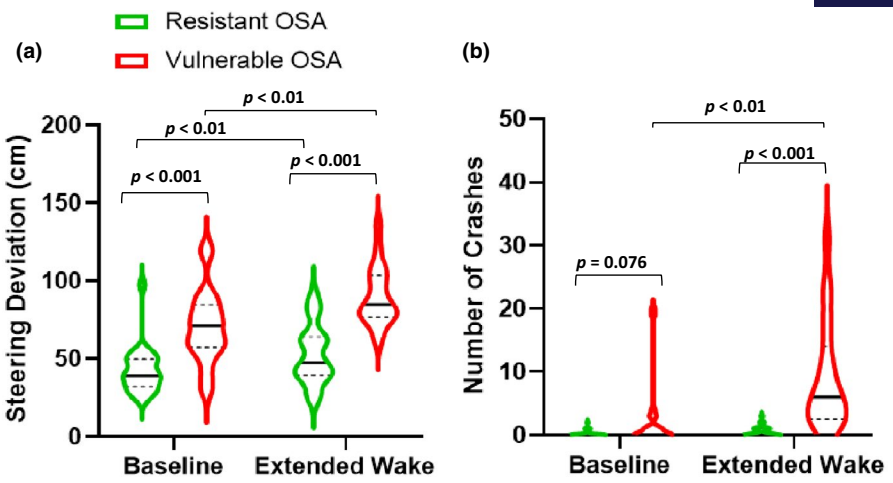


FIGURE 4 Violin plots showing the median and interquartile range for simulator steering deviation (A) and number of crashes (B) data under baseline conditions (3 h of wakefulness) and following extended wake (22.5 h of wakefulness) in OSA patients who were defined through cluster analysis as Resistant (Green) to driving impairments, exhibiting small and insignificant deterioration in driving simulator performance, and those who are Vulnerable (Red) and show marked performance decrements as a result of extended wakefulness

as $p < 0.05$. Logistic regression models were used to test for independent associations between the metabolites of interest while adjusting for baseline characteristics that significantly differed between the vulnerable and resistant groups. All covariates were kept constant with separate models ran for the 3 MRS metabolites for both PRESS and APRESS sequence data.

Analysis of MRS variables was limited to the metabolites of interest. No adjustments for multiple comparisons were made due to the difficulty in doing these adjustments when the variables are not independent with resultant generation of Type 2 errors. Instead, we made no mathematical adjustments but considered whether the overall metabolic pattern was consistent with systemic changes i.e. were the all changes in bioenergetic variables consistent or not? In addition, we have quoted affect sizes for all the driving and MRS variables in each region so that the reader can make their own assessment of the robustness of the findings. This approach has been recommended as more useful than corrections for multiple comparisons (Nakagawa, 2004).

3 | RESULTS

Fifty eight patients with OSA successfully completed the EWC protocol. Four patients withdrew before completing the study due to motion sickness (three patients) and unwillingness to continue beyond the first 8 h (one patient). There were 44 patients with complete MRS and driving simulator data (see Figure 1 for flow diagram of recruitment). There were no significant demographic, anthropometric or sleep study differences between the 44 patients included in this analysis and the 14 patients who were excluded due to incomplete data. This report focuses on these 44 patients with their characteristics, questionnaire and PSG results shown in Table 1. Cluster analysis identified two distinct clusters (groups), consisting of patients with excessive steering deviation and crashes (vulnerable OSA group, $n = 21$) and those with minimal steering deviation and crashes (resistant group, $n = 23$), see Figure 4. There were no differences in sex distribution between the vulnerable vs resistant groups ($\chi^2 = 0.14$, $p = 0.71$). Overall, compared with the resistant OSA group, the vulnerable OSA group had shorter habitual sleep duration based on average nightly sleep duration from actigraphy records prior to EWC protocol, although it is important to note this data comes from 35 out of the 44 patients (20 resistant OSA and 15 vulnerable OSA) due to device failure or insufficient data due to patients failing to wear the device. Furthermore, there were no correlations between total sleep time with driving simulator or MRS outcomes. Vulnerable OSA patients exhibited more severe OSA based on higher average AHI overall and in NREM sleep, as well as a greater degree of hypoxemia as measured by ODI > 3%, average oxygen desaturations and oxygen desaturation nadir. There were no significant differences between vulnerable and resistant OSA groups in any other variables including questionnaire outcomes on chronotype, sleep quality, insomnia symptoms, daytime sleepiness or depression, anxiety, and stress.

3.1 | Differences in driving performance between vulnerable and resistant OSA patients

Based on cluster analysis using the steering deviation and crash events from the driving simulator test following 22.5 h extended wakefulness, the vulnerable OSA patients showed significantly worse driving simulator performance compared with resistant patients (Figure 4). Vulnerable OSA patients demonstrated significantly more steering deviation at baseline (Cohen's d_s 1.4, $p < 0.001$) and following extended wakefulness (Cohen's d_s 1.9,

TABLE 1 Demographic, sleep study and questionnaire data

Variable	All OSA	Resistant OSA	Vulnerable OSA
N	44	23	21
Sex (M/F)	39/5	20/3	19/2
Age (year)	51.5 [43.8–55.3]	50.0 [42.0–54.0]	53.0 [46.0–58.0]
Body mass index	30.1 [27.8–33.0]	30.0 [28.0–31.0]	32.0 [27.0–35.0]
Epworth sleepiness score	9.0 [6.8–11.0]	8.0 [6.0–10.5]	11.0 [7.0–14.0]
Sleep onset latency (min)	4.6 [1.4–8.5]	4.9 [1.5–10.3]	4.0 [1.2–5.7]
Wake after sleep onset (min)	46.4 [35.2–65.7]	45.5 [39.1–65.9]	46.5 [25.3–64.5]
Total sleep time, PSG (min)	411.0 [384.0–431.7]	403.5 [378.5–423.8]	418.0 [392.7–432.2]
Total sleep time, actigraphy (min)[b]	414.4 [355.6–453.2]	430.0 [392.0–468.1]	351.5 [332.0–427.9][a]
Sleep efficiency (%)	88.5 [83.4–91.2]	88.2 [82.1–90.9]	88.9 [83.6–94.4]
% Stage N1 sleep	3.9 [2.6–5.9]	5.6 [3.3–6.6]	3.2 [2.4–4.4][a]
% Stage N2 sleep	59.9 [55.6–68.7]	57.8 [55.0–62.5]	61.4 [59.0–75.1]
% Stage N3 sleep	15.6 [9.1–20.2]	15.3 [11.6–18.8]	15.6 [7.3–21.8]
% REM sleep	19.0 [14.8–22.9]	20.4 [15.8–24.4]	16.3 [14.3–20.3]
Apnea hypopnea index (events/h)	34.1 [17.7–55.2]	30.1 [13.6–46.1]	42.3 [21.2–71.9][a]
Oxygen desaturation Index >3% (events/h)	23.7 [10.1–45.1]	18.2 [8.4–35.1]	28.5 [13.0–55.7][a]
EEG arousal index (events/h)	26.4 [15.7–44.7]	23.3 [17.7–32.0]	36.9 [14.9–55.4]
% time <90% SaO2	0.2 [0.0–2.1]	0.0 [0.0–1.5]	0.5 [0.1–2.0]
Morningness eveningness questionnaire	28.5 [25.0–31.3]	27.0 [25.0–31.5]	29.0 [26.0–31.0]
Depression score (DASS)	3.0 [2.0–8.0]	4.0 [2.0–12.0]	2.0 [2.0–6.0]
Anxiety score (DASS)	2.0 [0.0–4.0]	2.0 [0.0–5.0]	2.0 [2.0–4.0]
Stress score (DASS)	6.0 [2.0–12.0]	8.0 [4.0–12.0]	6.0 [2.0–10.0]
Pittsburgh sleep quality index	6.0 [5.0–11.3]	7.0 [5.0–9.5]	6.0 [4.0–12.0]
Insomnia severity index	9.5 [7.0–13.0]	9.0 [7.5–13.0]	10.0 [7.0–13.0]

Note: Values are Medians and Inter quartile ranges. Group Comparison *p*-values represents the comparison between the Resistant versus Vulnerable OSA groups with unpaired *t*-test *p*-values shown for normally distributed variables and Mann Whitney-*U* test shown for non-normally distributed variables. Gender compared using Chi^2 test.

[a]Significant difference between vulnerable and resistant groups, $p < 0.05$.

[b]Actigraphy sleep data available only in 35 out of the 44 patients (20 for resistant OSA patients and 15 for vulnerable OSA patients).

$p < 0.001$). Number of crash events were not statistically different between the vulnerable vs resistant OSA patients at baseline (Cohen's d_s 0.47, $p = 0.076$), but vulnerable patients had significantly more crashes following extended wakefulness (Cohen's d_s 1.24, $p < 0.001$). Extended wakefulness significantly impaired driving performance in both vulnerable and resistant OSA patients. In the resistant OSA patients, steering deviation increased from baseline to extended wake condition (mean ± SD, baseline 42.5±15.2 cm vs. extended wake 52.8 ± 18.9 cm, Cohen's d_s 0.6, $p < 0.01$), while the number of crashes did not change (median and IQR, baseline 0.0 [0.0–0.0] vs. extended wake 0.0 [0.0–1.0], Cohen's d_s 0.49, $p = 0.09$). In the vulnerable OSA patients, steering deviation increased from baseline to extended wake condition (mean ± SD, baseline 70.3 ± 23.4 cm vs. extended wake 89.7 ± 18.7 cm, Cohen's d_s 0.92, $p < 0.01$). The vulnerable OSA patients also showed a significant increase in the number of driving simulator crashes from baseline to extended wake condition (median and IQR, baseline 0.0 [0.0–1.0] vs. extended wake 6.0 [3.0–12.0], Cohen's d_s 0.92, $p < 0.01$).

3.2 | Brain energy metabolites in vulnerable and resistant OSA patients

Compared to resistant OSA patients, the vulnerable patients showed overall decreased levels of key brain metabolites using both types of MRS sequences (APRESS and PRESS). We found lower levels of glutamate and NAA in the vulnerable OSA group in the left orbitofrontal cortex using the APRESS sequence (Cohen's d_s for Glu 0.98 and NAA 0.65). This result was corroborated by the PRESS sequence, which also showed lower levels of glutamate and NAA (Cohen's d_s for Glu 0.87 and NAA 0.67) (Figure 5) as well as creatine. There were no significant group differences in these brain metabolites in the hippocampus or the anterior cingulate cortex.

3.3 | Logistic regression model results

The adjusted associations between MRS metabolites from APRESS and PRESS sequence with OSA patients grouping status is shown in

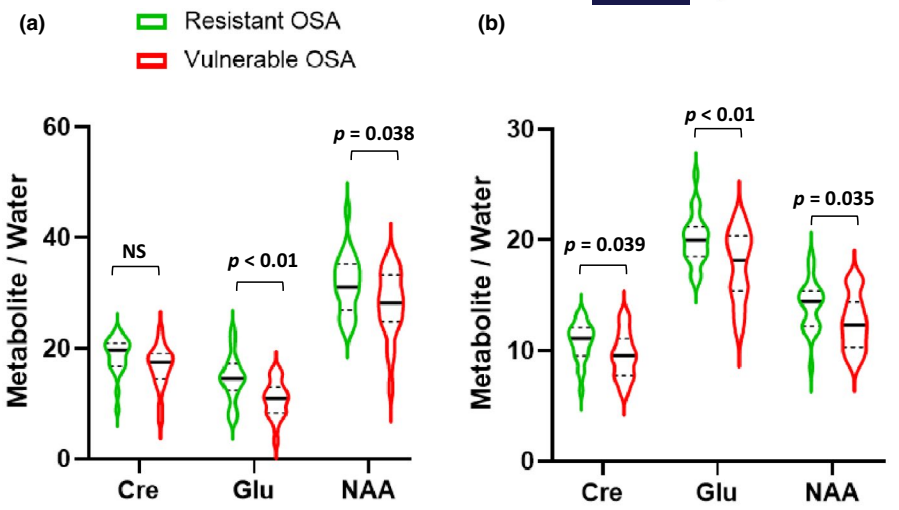


FIGURE 5 Violin plots showing the median and interquartile range for Ratios (to water) of creatine (Cre), glutamate (Glu) and *N*- acetylaspartate (NAA) in the left orbitofrontal cortex for the Vulnerable (Red) and Resistant (Green) OSA patients acquired using asymmetric PRESS (A) and PRESS (B) sequences

Table 2. All logistic models were adjusted for age, ESS, AHI and the MRS metabolite of interest. Higher level of glutamate was independently associated with a 21% (PRESS, B = −0.21, 95% CI 0.66–0.99, $p = .043$) and 36% (APRESS, B = −0.35, 95% CI 0.51–0.98, $p = 0.035$) reduced odds of being classified into the vulnerable group. Levels of creatine or NAA were not significantly associated with vulnerability groupings in the adjusted models. None of the baseline characteristics were significantly associated with the groupings. Separate models were ran including the oxygen desaturation index and arousal index due to collinearity with the AHI and have shown similar results.

4 | DISCUSSION

This study showed significantly lower levels of glutamate, creatine, NAA in the left orbitofrontal cortex in those with OSA who displayed vulnerability to impaired performance on a driving simulator task following extended wakefulness compared to those who did not. In particular higher glutamate levels were independently associated with a reduced risk of being vulnerability to driving impairment following extended wakefulness when adjusting for baseline factors. Importantly, these findings were consistently demonstrated using two different acquisitions with different echo-times, both illustrating a moderate to large effect size, reinforcing the robustness of the result.

This study builds on the existing case control literature which established that relative to healthy matched controls, participants with OSA have impaired brain bioenergetics as assessed using MRS by our team (Bartlett et al., 2004) and others (Xia et al., 2016), as well as significantly impaired driving performance using the same driving simulator assessment (Vakulin et al., 2009, 2014). Consequently, the primary aim of this study was to further investigate the underlying mechanism of the driving impairment by comparing the baseline brain metabolite profiles of OSA patients characterised as vulnerable versus those characterised as resistant to driving impairment following extended wakefulness. The metabolites we measured are known markers of energy metabolism.

NAA is a marker of mitochondrial energetics and is related to activity of the malate aspartate shuttle (von Jonquieres et al., 2018) which transports reducing equivalents between the cytosol and mitochondrion. This allows function of the electron transport chain with subsequent generation of ATP, as well as maintaining cytosol redox balance for driving cytosolic redox reactions, including those in glycolysis. Inhibition of mitochondrial activity has been shown to result in acute reduction of NAA (Bates et al., 1996). Alternative interpretations of a reduction of NAA infer loss of neurons. A recent meta-analysis found decreased grey matter in the orbito-frontal cortex in OSA compared to healthy controls (Huang, Tang, Lyu, Yang, & Chen, 2019), However, this is unlikely in this instance to be of significant impact as there was no significant change in grey/white matter in this voxel between vulnerable and resistant OSA patients and no reduction in choline levels (also a marker of cell density; Miller et al., 1996).

Creatine levels are directly related to brain function and ATP turnover (Rae & Broer, 2015) through creatine kinase systems located in both the cytosol and mitochondrion. Increased creatine levels are known to relate to improved cognitive function (Avgerinos, Spyrou, Bougioukas, & Kapogiannis, 2018) and supplementation has been suggested to decrease mental fatigue (Watanabe, Kato, & Kato, 2002). Here, the level of creatine change reached statistical significance in the PRESS spectrum but not in the APRESS spectrum (Figure 5). As explained above, the creatine resonance is derived from both creatine and phosphocreatine, with the latter having a shorter transverse relaxation time (T2) than creatine. This means that at longer echo times, such as that used in the asymmetric PRESS acquisition (110 ms) the resonance is dominated by creatine with less contribution from phosphocreatine than would be seen in the short-echo PRESS (38 ms). This is consistent with there being relatively less phosphocreatine in the vulnerable compared to resistant OSA patients. Brain phosphocreatine levels have been shown to decline following wakefulness in both humans (Gordji-Nejad et al., 2018) and animals (Dworak, McCarley, Kim, Kalinchuk, & Basheer, 2010).

TABLE 2 Logistic Regression Models adjusting for main baseline characteristics

	B	SE	*p*-value	Exp(B)	95% CI for EXP(B)	
Asymmetric PRESS metabolite models						
Age	0.079	0.044	0.072	1.082	0.993	1.179
ESS	0.067	0.085	0.426	1.070	0.906	1.263
AHI	0.019	0.015	0.207	1.019	0.990	1.049
Creatine	−0.105	0.122	0.390	0.900	0.709	1.144
Age	0.061	0.046	0.185	1.063	0.971	1.164
ESS	0.037	0.090	0.684	1.037	0.869	1.238
AHI	0.021	0.015	0.160	1.021	0.992	1.052
Glutamate	−0.214	0.106	0.043	0.807	0.656	.994
Age	0.072	0.044	0.102	1.074	0.986	1.171
ESS	0.070	0.084	0.406	1.072	0.910	1.264
AHI	0.019	0.015	0.208	1.019	0.989	1.050
N-acetylaspartate	−0.057	0.074	0.441	0.945	0.817	1.092
Asymmetric PRESS metabolite models						
Age	0.070	0.044	0.114	1.072	0.983	1.169
ESS	0.071	0.081	0.381	1.074	0.915	1.260
AHI	0.021	0.016	0.195	1.021	0.989	1.054
Creatine	−0.183	0.215	0.396	0.833	0.546	1.270
Age	0.063	0.046	0.174	1.065	0.973	1.167
ESS	0.022	0.092	0.807	1.023	0.854	1.225
AHI	0.034	0.016	0.036	1.035	1.002	1.068
Glutamate	−0.351	0.166	0.035	0.704	0.508	0.976
Age	0.062	0.045	0.174	1.064	0.973	1.163
ESS	0.072	0.082	0.376	1.075	0.916	1.262
AHI	0.024	0.015	0.113	1.024	0.994	1.055
N-acetylaspartate	−0.152	0.167	0.363	0.859	0.620	1.191

Abbreviations: AHI, Apnea hypopnea index; ESS, Epworth sleepiness score.

Although we observed univariate group difference in the levels of NAA and creatine between the vulnerable and resistant OSA groups, when we adjust for baseline characteristics (age, ESS, and AHI) the association between NAA and creatine levels with vulnerability status was attenuated. More research in larger samples is necessary to further examine the relationship between baseline NAA and creatine levels with sleepiness related driving impairment.

Glutamate is the major excitatory brain neurotransmitter, with levels of glutamate also indicative of metabolic activity, being directly associated with Krebs cycle rates (Rae, 2014). Lower glutamate levels can therefore be indicative of decreased mitochondrial respiration or decreased neural activity. Indeed, we have observed significant differences in baseline levels of glutamate between vulnerable and resistant groups. Furthermore, we found that higher glutamate was independently associated with a reduced risk of driving impairment and being vulnerable to extended wakefulness when adjusting for key baseline characteristics.

Only a few studies have specifically examined the relationship between OSA severity, cognitive function and brain metabolites across various brain regions including insular cortex (Kang, Tian, & Li, 2018), mid-brain (Macey et al., 2017), frontal regions (O'Donoghue et al., 2012; Pereira et al., 2017) and hippocampus (Bartlett et al., 2004; O'Donoghue et al., 2012; Pereira et al., 2017). In one small early study using ^{1}H MRS spectroscopy, Bartlett and colleagues found that lower creatine levels correlated with worse performance on the psychomotor vigilance task (PVT) and greater OSA severity including respiratory disturbance index, arousal index and average oxygen desaturation (Bartlett et al., 2004). Tonon et al. (2007) found that baseline NAA levels in OSA were associated with hypoxemia (lowest SpO2 saturation) and daytime sleepiness (MSLT). Another study reported significant correlations between NAA/Cho ratios in frontal regions and Cho/Cre ratios in the hippocampus with the arousal index and hypoxemia but found no correlations with cognitive function using a broad range of tests of memory, vigilance (PVT), IQ, inhibition and set-shifting as well as subjective sleepiness using the ESS (O'Donoghue et al., 2012). Pereira et al. (2017), also found associations between prefrontal cortex levels of GABA and glutamate with the AHI and hypoxemia (O_2 nadir) but no significant correlations were observed in the hippocampus, nor was any cognitive function assessed in that study.

The above literature highlights that overall, there are consistent findings of reduced levels of key brain metabolites in OSA relative to controls and these were associated with the degree of OSA severity and cognitive function in some studies. Here, we specifically examined whether these baseline bioenergetics deficits were worse in those with OSA who were vulnerable to impaired driving performance compared to those who were resistant.

Studies on the effect of intermittent hypoxia in humans are limited and difficult to interpret. It is plausible that exposure to intermittent hypoxia both promotes and mitigates apnea (Mateika & Narwani, 2009) and in turn affects brain bioenergetics and metabolism. For example, a study of brain energy levels using dynamic ^{31}P magnetic resonance spectroscopy (MRS) showed that brain ATP levels decreased rapidly during apnea in patients with OSA but returned to normal upon restoration of normoxia (Rae et al., 2009). Certainly the type of intermittent hypoxia a person is subjected to can produce remarkably divergent outcomes (Serebrovskaya, Manukhina, Smith, Downey, & Mallet, 2008) and those with OSA have been shown to have poorer cerebral blood flow responses to hypoxic challenge(s) (Jensen, Vestergaard, Tonnesen, Larsson, & Jennum, 2018). While intermittent hypoxia may have both protective and deleterious effects, it has been suggested that the degree of severity of OSA related hypoxia might determine which outcome it produces, with more severe hypoxia related to worse outcomes (Navarrete-Opazo & Mitchell, 2014). Indeed, we have found in this study that the oxygen desaturation index was significantly higher in the vulnerable vs resistant OSA group based on driving performance following extended wakefulness. However, we did not observe significant univariate or adjusted associations between hypoxemia and brain metabolites. Future research is needed to further examine the links between hypoxemia, brain metabolites and vulnerability to performance impairment under high sleep pressure conditions.

Actigraphy data in a sub-group of patients suggested that vulnerable patients had shorter habitual sleep compared with the resistant patients at the group level. However, sleep duration was not correlated with either driving performance or MRS outcomes. Given these data was only available in sub-group of patients the role of habitual sleep duration is unclear and deserves future investigation.

Driving is a complex task compared to the PVT and recruits multiple cognitive elements and interconnected neural circuits referred to as the driving functional connectome (Calhoun & Pearlson, 2012). The only study that has previously examined vigilance (PVT) and driving simulator performance (AusEd) used ^{31}P MRS in 15 male patients with severe OSA. It found that decreased mitochondrial phosphorylation potential (ratio of inorganic phosphate to ATP) in the temporal lobe was associated with poorer driving performance and vigilance function in both rested and sleep deprivation condition (D'Rozario et al., 2018). This is broadly in agreement with our findings of lower levels of markers of energy and mitochondrial metabolism and respiration. Evidence suggests that mitochondrial, rather than cytosolic bioenergetics bears the brunt of the hypoxic load in apnea (Rae et al., 2009) and as observed in this study, mitochondrial bioenergetics, particularly in frontal brain regions in OSA patients may help differentiate patients who are vulnerable from those who are resistant to poor driving performance during a vigilance demanding driving simulator task.

There are some potential limitations as well as notable strengths of this study. The lack of a healthy non OSA control group precludes our ability to compare metabolite profiles and driving performance. However, the main objective of the current study was not to compare metabolites and driving performance between patients with OSA and controls, which is well established by our group (Bartlett et al., 2004; Vakulin et al., 2009, 2014) and others (Xia et al., 2016). Rather we explored differenced in key brain metabolites measured at baseline in OSA patients who are subsequently identified as vulnerable vs resistant to driving task impairment following extended wakefulness. The lack of controls does not impact on our ability in addressing this important clinical question. Strengths of this study include the larger sample size relative to previous MRS studies. Further our participants were a highly characterised clinical sample of OSA regarding objective sleep and clinical evaluation and driving task assessment to ensure we identified patients who are vulnerable vs resistant following extended wakefulness using clustering approach based on objective driving outcome data. The use of two MRS sequences further adds to the robustness of the findings.

In conclusion, we have found that brain metabolites associated with mitochondrial bioenergetics are lower in the frontal brain regions of OSA patients who are more susceptible to sleep loss and consequent driving simulator performance impairment. These findings provide new insights that may help explain the vast heterogeneity in driving performance in OSA and represent a novel way to identify at risk phenotype of OSA when assessing fitness to drive. This requires confirmation in larger future studies with test evaluation methodology focusing specifically on driving performance outcomes.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTION

A.V. provided intellectual input into study design, has led the data collection, sleep data processing and statistical analysis of all data, data interpretations and write up of the manuscript. M.A.G. has processed all magnetic resonance spectroscopy data and contributed to manuscript drafting and revisions. A.J.D. has provided intellectual input into study design, sleep data processing and analysis, results interpretation and manuscript drafting. D.S. has contributed to sleep and MRS data collection and manuscript drafting. H.O. has contributed to sleep and MRS data collection and manuscript drafting. D.B. has provided intellectual input into study design results interpretation and manuscript drafting. K.W. has provided intellectual input into study design results interpretation and manuscript drafting. R.D.M. has provided intellectual input into study design results interpretation and manuscript drafting. R.R.G and C.D.R. have played a key senior roles study design, sleep analysis plan, results interpretation and manuscript drafting.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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