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Original Article

Restless legs syndrome and central nervous system gamma-aminobutyric acid: preliminary associations with periodic limb movements in sleep and restless leg syndrome symptom severity

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ABSTRACT

Background: Previous research has demonstrated abnormalities in glutamate and *N*-acetyl aspartate (NAA) in the thalamus in individuals with restless legs syndrome (RLS) compared with healthy matched controls. However, levels of these transmitters in other RLS-related brain areas and levels of the most common inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), have not been assessed.
Methods: This study examined GABA, glutamate, and NAA levels in the dorsal anterior cingulate cortex (ACC), thalamus and cerebellum with the use of proton magnetic resonance spectroscopy (^{1}H-MRS) at 4 tesla (4 T) and Megapress difference-editing in 18 subjects with RLS and a matched control group without RLS. Actigraphy was performed on the nights before scans to assess periodic limb movements of sleep (PLMS).
Results: Levels of GABA, glutamate, and NAA were no different between RLS and control subjects in any of the three voxels of interest. However, GABA levels were positively correlated with both PLM indices and RLS severity in the thalamus and negatively with both of these measures in the cerebellum in RLS subjects. In addition, NAA levels were higher in the ACC in RLS than in controls.
Conclusion: Our preliminary data suggest that known cerebellar–thalamic interactions may modulate the intensity of RLS sensory and motor symptoms. In addition, anterior cingulate cortex may be associated with the affective components of the painful symptoms in this disorder.

1. Introduction

Restless legs syndrome (RLS) is characterized by an irresistible urge to move the legs, which is often associated with paresthesias. Central nervous systems (CNS) subserving sensory modulation, motor activity and pain appear to be altered in RLS [1]. In particular, leg discomfort and leg movements while awake in RLS are correlated with functional magnetic resonance imaging (fMRI)-related activation in the thalamus and cerebellum [2,3]. Similarly, enhanced dopaminergic binding [4] and a correlation of opioid binding with RLS disease severity [5] are seen in the thalamus in positron emission tomography (PET) studies in RLS. Ascending pain pathways project from the thalamus to the anterior cingulate cortex (ACC), which is involved in affective and cognitive aspects of pain [6]. ACC activity is abnormal in RLS by both fMRI [7,8] and PET [4,5].

Thalamic glutamate is 50% higher [9] and the neuronal marker *N*-acetyl aspartate (NAA) is reduced in the thalamus [10] in RLS using proton magnetic resonance spectroscopy (^{1}H-MRS). To date, no study has reported levels of gamma-aminobutyric acid (GABA), the major CNS inhibitory neurotransmitter, in patients with RLS.

The primary aim of this study is to quantify GABA, glutamate, and NAA levels in the ACC, thalamus and cerebellum in patients with RLS and matched controls. Levels of these transmitters/metabolites will be correlated to RLS severity and to objective measures of sleep and leg movement activity.

2. Methods

2.1. Participants

Adult (aged >18 years) subjects were recruited from the greater Boston, MA, area from May 2010 to April 2012. RLS subjects met diagnostic criteria from the International RLS Study Group (IRLSSG) [11], had a history of RLS symptoms at least 15 nights in the previous month, or, if treated, this frequency of symptoms before treatment was started, and had a history of significant sleep disturbance

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due to RLS as indicated by a score of at least 2 on question 5 on the RLS severity scale. Age- and sex-matched healthy control subjects without sleep complaints or a family history of RLS were also recruited. For RLS subjects, RLS-related medications were discontinued 48 h prior to PSG and benzodiazepines were discontinued a minimum of one week prior to PSG. Control subjects were not permitted to use CNS-active agents for two weeks prior to enrollment and for the duration of the study.

All subjects were evaluated with an unstructured clinical interview for history of sleep, psychiatric, and medical disorders. Baseline laboratories included urine toxicology and pregnancy testing (for female subjects). Exclusion criteria for all subjects included clinical evidence of any moderate-to-severe sleep disorder other than RLS (e.g. obstructive sleep apnea, insomnia, etc.); apnea–hypopnea index (AHI) >15 for all subjects; current or past (within the preceding year) diagnosis of alcohol or drug dependence/abuse; history of significant medical or neurologic illness including significant head trauma or loss of consciousness >30 min; body mass index >35 kg/m^2; consumption of >10 cigarettes/day, >2 caffeinated beverages/day, or >2 standard alcoholic drinks/day for a period >1 month within the preceding year; recent history of shift-work; contraindicated condition for MR scanning; and women who were pregnant, lactating, or planning to become pregnant during the study.

The study was approved by the Institutional Review Board of Partners Healthcare, the parent organization of Brigham and Women's Hospital and McLean Hospital, and carried out in accordance with the Declaration of Helsinki. All subjects received compensation for their participation in this study.

2.2. Actigraphy and polysomnography (PSG)

An actigraph (PAM-RL, Respironics, Pittsburgh, PA, USA) recorded limb movements in both legs from the subjects' bedtime until final wake time for four nights before, and the night of, the PSG. PSG channels included electroencephalograph (EEG), electrooculogram (EOG), submental electromyography (EMG), EMG of both anterior tibialis muscles (separate channels for each leg), oral/nasal airflow, nasal pressure, pulse oximetry, and respiratory effort. The PSG was analyzed for traditional sleep staging and PLM indices by the same experienced technologist.

2.3. Questionnaires and diary

All subjects were administered the Pittsburgh Sleep Quality Index (PSQI) [12] and the Beck Depression Inventory (BDI) [13] at the baseline visit. All subjects filled out diaries for two days prior to MRS scans, estimating sleep onset, time awake after sleep onset and total sleep time. RLS symptom severity on these two nights prior to MRS scans was assessed on this diary with a scale of 0 (no RLS symptoms) to 4 (very severe RLS symptoms). An IRLS severity scale [11], modified by adding the preface "since you stopped taking your RLS medication" for all questions, was filled out by RLS subjects on the morning of the MRS scan.

2.4. Magnetic resonance imaging

MRI was performed at 13:00–14:00 on the day following the PSG. After acquisition of spectral data from each of the three voxels during the MRS at 4 T, subjects were asked by study staff to verbally rate the level of RLS discomfort in their legs during the previous 20 min on a scale from 1 (no discomfort) to 10 (extreme discomfort).

Imaging and spectroscopy were performed on a whole-body 4 T magnetic resonance scanner (Varian/UNITYInova, Palo Alto, CA, USA) at McLean Hospital in Belmont, MA, USA. Data collection utilized a birdcage-design, radio-frequency (RF) head coil operating at 170.3 MHz for proton (XLR Imaging, London, Canada). Scout images confirmed optimal positioning, and unsuppressed water signal was shimmed to a global water line-width of ≤25 Hz. Subsequently, high-contrast T1-weighted anatomical images were taken in the sagittal and axial planes [echo time/repetition time, 6.2 s/11.4 ms; field-of-view, 24 × 24 × 8 cm (sagittal) and 22 × 22 × 16 cm (axial); read-out duration, 4 ms; receive band-width, ± 32 kHz; in-plane matrix size, 128 × 256 × 16 (sagittal) and 256 × 256 × 64 (axial); in-plane resolution, 0.94 × 1.9 mm (sagittal) and 0.94 × 0.94 mm (axial); read-out points, 512; slice thickness, 2.5 mm, flip-angle, 11°] for voxel positioning and image-based voxel tissue segmentation analysis.

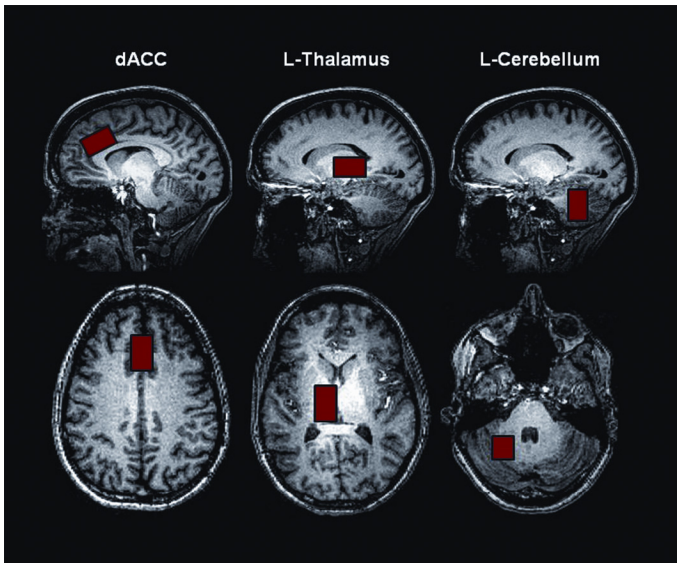

Fig. 1. Anatomical placement for dorsal anterior cingulate cortex (dACC), left thalamic (L-Thalamic) and left cerebellar (sub-vermis) (L-Cerebellum) proton magnetic spectroscopy voxels.

2.5. Proton MRS

The axial and sagittal high-resolution, T1-weighted anatomical images were used as a guide to systematically place single voxels in the left thalamic lobe (2 × 3 × 2 cm), bilateral dorsal ACC (3 × 2 × 2 cm), and left cerebellum (3 × 2 × 2 cm) (Fig. 1). Proton spectroscopy employed a GABA-optimized MEGA-PRESS sequence [14] for optimal measures of GABA using the difference-editing technique, as well as secondary measures of glutamate, NAA and total creatine (Cr) in the 68 ms sub-spectrum. Manual shimming of the magnetic field within each prescribed voxel achieved water line-widths ranging from 7 to 12 Hz. Following the automated optimization of water suppression power and tip angles, the transmitter frequency was set to the creatine resonance of 3.00 ppm to minimize chemical-shift displacement artifact for each spectral acquisition. The MEGA-PRESS sequence used the following acquisition parameters: TR, 2 s; TE, 68 ms; spectral band-width, 2 kHz; read-out duration, 512 ms; NEX, 384; and total scan duration, 13 min per voxel.

2.6. Proton MRS processing

All spectroscopic data processing and analyses were undertaken on a Linux workstation using reconstruction code written on-site (C-code) and commercial fitting software. In order to quantify difference-edited GABA with MEGA-PRESS data, the difference-edited spectra were fitted with LCModel [15,16] using basis sets acquired from phantoms at 4 T. All phase- and frequency-corrected 'on' and 'off' 68 ms sub-spectra were then averaged separately to