GABA and Glutamate Levels in Occlusal Splint-Wearing Males with Possible Bruxism

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Introduction

The repetitive jaw-muscle activity generally known as bruxism (clenching and grinding of teeth during both sleep and while awake) (1) is commonly encountered by clinicians in dentistry, neurology, and psychiatry. Sleep bruxism (SB) has been reclassified in recent years as a sleep-related movement disorder; this category also includes periodic limb movement and rhythmic movement disorders (2). SB has been estimated at 10-20% of the pediatric population, 5-8% of the adult population, with a decrease to 3% in geriatric populations; no sex differences have been documented to date (3-5). SB is more frequent in smokers, those with high consumption of caffeine or alcohol, and in individuals taking neuroactive chemicals that affect the CNS (6-10). Clenching and grinding of teeth activities are often seen in individuals with stress and anxiety disorders (11-13) and are comorbid with restless leg syndrome, sleep apnea, oromandibular myoclonus, rapid eye movement behavior disorder, and other parasomnias (10, 14). Iatrogenic secondary causes of such activities may

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include delivery/cessation of neuroactive medications (15), certain dental procedures, and treatment for temporomandibular disorder (TMD) (16-18). A high occurrence of TMD pain has been documented in persons exhibiting (1) the behaviors of both SB and daytime clenching (awake bruxism), and (2) the sleep disorders of sleep apnea, insomnia, and bruxism (10, 15, 19-24).

A link between emotion-induced brux-like activities and group I temporomandibular disorder (TMD) was proposed long ago (25-27), although the mechanism underpinning this association is still unclear. Recent neuroimaging studies of bruxism have identified the involvement of the Hypothalamic–Pituitary–Adrenal (HPA) axis system, which is also implicated in TMD and Post-Traumatic Stress Disorder (PTSD). Currently, it is thought that bruxism, PTSD, and other stress-related psychiatric disorders, are due to a dysfunction of a circuit involving the medial prefrontal/anterior cingulate cortical region, dorsolateral prefrontal cortex (DLPFC), hippocampus, and amygdala.

The role of neurochemicals in anxiety-related behaviors such as bruxism has been and continues to be of intense interest for some time now (9, 28-34). The exact neurochemical mechanisms that cause certain selective serotonin reuptake inhibitors (SSRIs) to manifest sleep bruxism is a focus of research efforts (9, 31, 33) as are those involved in the important comorbid factors of sleep regulation, endocrine systems, autonomic functions, stress/anxiety, and motor control (14, 15, 35-37). As demonstrated by the bruxism-ameliorating effects of the drugs gabapentin, tiagabine, gamma-hydroxybutyrate, diazepam, and lorazepam, the major neurotransmitter γ-aminobutyric acid (GABA) is suggested to play a critical role in bruxism (9).

Magnetic resonance spectroscopy (MRS) techniques allow for a noninvasive examination of *in vivo* brain function by assessing regional concentrations of neurotransmitter metabolites (38). As determined by recent MRS studies (39, 40), GABA plays an important role in the pathophysiology of human anxiety disorders such as panic disorder and PTSD (41). Goddard et al. discovered lower than normal cortical GABA levels in panic disorder individuals (42, 43). The etiology of oral dysfunctions such as bruxism and TMD is multifactorial and psychological factors are considered a major component in the initiation and progression of these disorders (21), which suggests that GABA neuronal system may also be critical in the manifestation of bruxism. The increased incidence of anxiety and depression in these patients (26, 44-46) has led to a theory that psychological factors, such as anxiety, predispose patients to TMD/bruxism by increasing tooth grinding and clenching behaviors, which may produce masticatory muscle fatigue and soreness (25-27). We hypothesized that the stress-related behavioral disorder of bruxism and anxiety-related disorders share similar underlying mechanisms involving the inhibitory neurotransmitter GABA as well as the metabolites N-acetylaspartate (NAA), creatine, choline-containing compounds, myo-inositol, glutamate and glutamine (47). To study this cross-link between brux-like behaviors and anxiety-related disorders, we performed a proton (^{1}H) MRS study for metabolite quantification in anxiety-related regions of the brain involved in the HPA axis system. HPA axis dysfunction plays a major role in the anxiety disorders reported by patients who clench and grind their teeth and suffer with TMDs (48). We focused on two HPA-axis brain regions, the right hippocampus and right thalamus and selected the right

hemisphere because of the documented laterality in stress-regulatory components of the HPA axis. In addition, we also investigated the DLPFC because of its role in anxiety-related disorders (49) and a dorsal anterior cingulate cortex/pre-supplementary motor area (dACC/ preSMA) involved in motor planning (50, 51). The dACC has also been implicated in anxiety behavioral disorders such as PTSD (52, 53) .

In this study we sought to identify parallels in metabolic and neurotransmitter changes between the manifestation of brux-like behavior and reported changes in anxiety disorders. The long term goal of this imaging-based, noninvasive research of the neurochemical mechanisms affecting the manifestation of oral behaviors such as bruxism is to provide improved treatment strategies in the clinical population.

Materials and Methods

Subject Recruitment

Subject group classification (see Appendix A) was based on an interview that was conducted after self-reported tooth clenching and grinding history, followed up by evaluation of each subject's protective occlusal splint and positive responses on the TMD history questionnaire (54). Examination of subjects' occlusal splint confirmed that subjects indicating a history of possible brux-like behavior did in fact have an occlusal splint, which they were currently using and which was prescribed by a dentist specifically for the purpose of protecting the dentition from possible brux-like behaviors. The TMD questionnaire documented subjects' perception of pain, loss of function, and possible brux-like behavior. Responses to the questions that best assessed possible brux-like behavior are presented in Appendix A, which shows not only the responses of both occlusal splint (OCS) and control (CON) subjects to selected questionnaire items, but also their occlusal splint use as prescribed and fabricated by dental clinicians. Based on this classification, 8 male subjects (age: 28.6±3.0 years; mean ± SD) were recruited and classified in the OCS group. Subjects were classified to exhibit possible brux-like behavior if currently reporting active clenching and grinding of teeth and wearing of a protective occlusal splint, being right-handed, 20-45 years old, not currently under medication for migraine headaches, without previous history of brain injury or psychiatric problems, magnetic surgical implants, false teeth, retainers, or magnetic braces, having normal hearing sensitivity by self-report, and not being claustrophobic by self-report. The control (CON) group consisted of 9 age-matched (25.5±1.9 years) healthy men with the inclusion criteria identical to the OCS group except for possible brux-like behavior and occlusal splint use. In this study, the diagnosis of sleep bruxism was deemed "possible" because of OCS subject self-report and questionnaire use (1). Written informed consent approved by the Indiana University Institutional Review Board was obtained from all subjects prior to participation and all procedures conformed to international STROBE guidelines.

MRS data acquisition and analysis

^{1}H MRS data were acquired on a 3 T Siemens Magnetom Tim-Trio MR scanner (Siemens Healthcare, Erlangen, Germany) using a 32-channel head array coil. Both single voxel short echo time Point RESolved Spectroscopy (PRESS) spectra (TE=30 ms, TR=1500 ms, 128

averages) and GABA-edited spectra (TR=1500 ms, TE=68 ms) using MEGA-PRESS (55-57) were obtained from four volumes of interest (VOIs): thalamus ($25\times25\times25$ mm^3, 392 averages), hippocampus ($17\times40\times17$ mm^3, 512 averages), DLPFC ($25\times30\times22$ mm^3, 392 averages) and dACC/preSMA ($25\times35\times25$ mm^3, 392 averages). A reference spectrum without water suppression was obtained in each brain region for phase and frequency correction. Placements of the VOIs and representative spectra from each brain region are illustrated by Figure 1.

The post-processing and quantification of all spectra was performed with LCModel (v6.2-0R) (58). PRESS spectra were analyzed for the major metabolites N-acetyl aspartate (NAA), choline (Cho), total creatine (tCr), myo-inositol (mIns) and glutamate (Glu), whereas GABA levels were obtained from the GABA-edited spectra. Quantification results are expressed in institutional units (i.u.) and only the NAA, tCr, Glu, and mIns metabolites from the PRESS spectrum with LCModel fitting standard deviation below 20% were used for further statistical analysis. Since the GABA resonance at 3.0 ppm also contains some signal from macromolecules, GABA results are reported as GABA+ (GABA + macromolecules). More detailed descriptions of the MRS data acquisition and analysis methods are provided in Appendix B.

Statistical Analysis

All statistical calculations were performed with SPSS (Version 20.0, IBM Corp.). The questionnaire data were grouped into three categories: depression, anxiety and pain with an overall (summed) score calculated for each category. The scores in each category were compared between groups using the Wilcoxon–Mann–Whitney two-sample rank-sum test. Spearman's rank correlations were computed for the questionnaire scores in each of the categories and regional metabolite estimates. A Group (2; OCS and CON) × Region (4; hippocampus, DLPFC, thalamus and preSMA) × Metabolite (5; NAA, Glu, mIns, tCr, and GABA+) repeated measures ANOVA was performed with a post-hoc ANOVA F-test conducted where effects of Group × Region interaction were significant. In addition, questionnaire scores were examined in the regression analysis or as covariates when showing significant effects of Group.

Results

TMD questionnaire

As shown in Appendix A, questionnaire data from both groups indicated that all occlusal splint subjects reported experiencing daytime and night time tooth clenching/grinding, morning jaw soreness /stiffness, and the use of a protective occlusal splint obtained from a dentist. Control subjects had negative responses to all of the aforementioned questionnaire items shown in Appendix A.

Anxiety and depression scores in all subjects were significantly correlated (Spearman's $r=$ 0.736, $p<0.01$, two-sided). There were no significant group differences in the scores of depression (Mann-Whitney $U=29$, $p=0.54$, two-tailed) or pain (Mann-Whitney $U=24.5$, $p=0.28$, two-tailed), while a trend was present for anxiety (Mann-Whitney $U=18.5$, OCS

mean=3.88, CON mean=1.00, p=0.09, two-tailed). This trend-level anxiety score difference was in the anticipated direction (e.g. higher for the OCS). Therefore, anxiety scores were added as a covariate in the MRS data analysis to test whether anxiety contributed to the metabolite group differences reported below.

MRS

The multivariate tests in the repeated-measures ANOVA showed significant Group × Region interaction (Wilk's lambda=0.38, F=3.36). In the repeated-measures ANOVA, Group × Region interaction was significant for two metabolites, GABA+ ($F(3,55)$=6.66, p=0.001) and Glu ($F(3,55)$=3.22, p=0.031). Between-group post-hoc ANOVA showed significant effects only in the DLPFC, where lower levels of GABA+ ($F(1,12)$=14.01, p=0.003) and higher levels of Glu ($F(1,13)$=14.71, p=0.002) were observed in OCS. These GABA+ and Glu group differences in the DLPFC were reduced but remained significant (GABA+, $F(1,13)$=5.17,p=0.049; Glu; $F(1,13)$=5.829, p=0.039) after the inclusion of anxiety as a covariate. Furthermore, GABA+ and Glu levels in the DLPFC showed a significant negative relationship (Pearson's r=−0.754, p=0.003 two-sided) as illustrated by Figure 2. While no group differences in GABA+ and Glu were present in the hippocampus, these two metabolites did show positive relationship (Pearson's r=0.783, p=0.004 two-sided; see Figure 3).

Discussion

While the focus of previous neurochemical studies was on sleep bruxism (SB) (15), there is some evidence that the variability of bruxism symptoms in *both* diurnal and nocturnal forms may have a neurochemical basis, involving different brain regions such as the ventral tegmental area and the distribution of striatal dopaminoceptor-2 (D_{2R}) receptors (59). In this study, lower GABA$^+$ levels in occlusal splint DLPFC subjects suggest that anxiety-related circuits (49, 60) that may affect possible bruxism were less inhibited than in controls (Table 1). Decreased frontal lobe GABA levels have also been detected in panic disorder individuals (42) albeit in the medial rather than dorsolateral prefrontal cortex.

A review of earlier studies of DLPFC metabolite levels in anxiety subjects indicates that the DLPFC plays an important role in responses to threatening stimuli, particularly in anxiety disorders (60-63). It is currently thought that the DLPFC guides control of tasks by providing excitatory feedback to pools of neurons that process task-relevant aspects of anxiety-provoking stimuli; in this case the DLPFC may enhance the manifestation of bruxism by channeling anxiety-associated stimuli to those brain regions actually causing the behavior. Increased DLPFC activity during an emotional Stroop task suggests that the DLPFC is important for task control mechanisms in the face of emotional distraction (64-66). The documented decrease of bruxism with increased age may also be related to an age-related decrease of DLPFC mechanisms regarding early perceptual features (67).

Hippocampus metabolite levels in anxiety subjects have been reported to show higher Glx, myo-inositol, and Cr, and to be correlated with psychiatric symptoms and mitochondrial disorders (68). Alterations in hippocampal activity and volume have also been documented in anxiety disorders (52, 69). In our study, no significant group differences were found for

any of the reported metabolites in the hippocampus. However, a significant positive correlation between GABA+ and Glu emerged. Interestingly, these two metabolites were negatively related in the DLPFC, which may reflect the documented bidirectional interactions between the hippocampus and the DLPFC (70). In this sense, our finding might indicate the presence of a negative feedback circuit between hippocampus and DLPFC, which may play an important role in regulating the manifestation of bruxing behaviors (71-73). The activity dynamics between the DLPFC and hippocampus in retrieval of facts during problem solving (70) and our findings may also suggest a role for both the DLPFC and hippocampus in motor memory systems (74, 75) that might be involved in bruxism behaviors. It has also been suggested that epileptic seizures involving limbic structures within the temporal lobe (hippocampus) may activate masticatory central pattern generators that help cause bruxism behaviors (76). A recent case report documented a 5-year old male with a cystic lesion compressing the left hippocampus and exhibiting teeth grinding behavior during sleep (77). It has been found that DLPFC of idiopathic generalized epilepsy patients demonstrate increased levels of glutamine and GABA compared with controls (57). This differs from our study in that DLPFC of OCS subjects showed significantly lower levels of GABA+ and higher levels of Glu (see Table 1). The precise neurochemical mechanisms and interactive relationships between epilepsy and brux behaviors need to be investigated further.

Thalamus metabolite levels in restless legs syndrome individuals demonstrate significantly higher levels of Glx/Cr than control subjects (78). In the present study, the thalamic GABA showed only trend-level group differences (see Table 1) that might be suggestive of another nondopaminergic neurologic system that plays a role in the manifestation of bruxism. It has also been noted that thalamus activity is decreased in PTSD, an important anxiety disorder (79-81).

In this study, we detected no metabolic group differences in the preSMA/dACC; this despite our earlier fMRI findings that the preSMA/dACC may play an important role in oral brux-like behaviors such as tooth grinding and clenching (50, 51). This discrepancy may be due to the passive nature of the MRS scans in the present study, while our earlier fMRI studies employed the active, physical tasks of jaw clenching and tooth grinding.

Limitations of this study include: (1) modest sample size, and (2) occlusal splint wearer inclusion criteria that were based on self-report and were non-specific in bruxing classification. In addition, continuing analysis of possible sleep bruxism and pain data in the occlusal splint wearers was not performed. In this study, subject gender selection was necessarily driven by a small sample so we focused on males due to the higher incidence of PTSD and TBI in men (82, 83). In the future, we intend to include women subjects and make gender ratios similar and more representative of possible sleep bruxism behaviors prevalence. Polysomnography (PSG), the current gold standard for determination of bruxism (15, 84) was not used in this study because of limited research monies available to us. We hope to include PSG analyses in future studies.

Larger voxel sizes were chosen to compensate for the low signal-to-noise ratio of GABA MRS. The geometric limitations of the MRS VOIs preclude complete sampling of some

neuroanatomical regions or include small contributions of adjacent non-targeted regions. The measured GABA levels include some contribution from co-edited macromolecules (MM30) at 3 ppm and a small contribution from homocarnosine and are hence reported as GABA+ (GABA+MM30). However, changes in macromolecules have not been reported for anxiety-related disorders to date.

Conclusions

These results in our proof-of-concept study are the first indications of the disturbances in GABAergic and glutamatergic systems of possible sleep bruxers. Future research in larger samples should improve sensitivity of quantifying GABA and other pertinent metabolites and detecting group differences. In addition, results of this study indicate a need for a more comprehensive MRS investigation with an emphasis on the coupling of anxiety-related and limbic regions with executive control brain networks. The relevance of such research is supported by the observed differences between HPA anxiety-related brain areas as indicated by our finding of negative feedback between the hippocampus and DLPFC. Careful further investigations may reveal not only the neurochemical mechanisms underlying bruxism behaviors and their interactions with other anxiety disorders, but also myofascial TMD as recently documented by Gerstner et al. (85).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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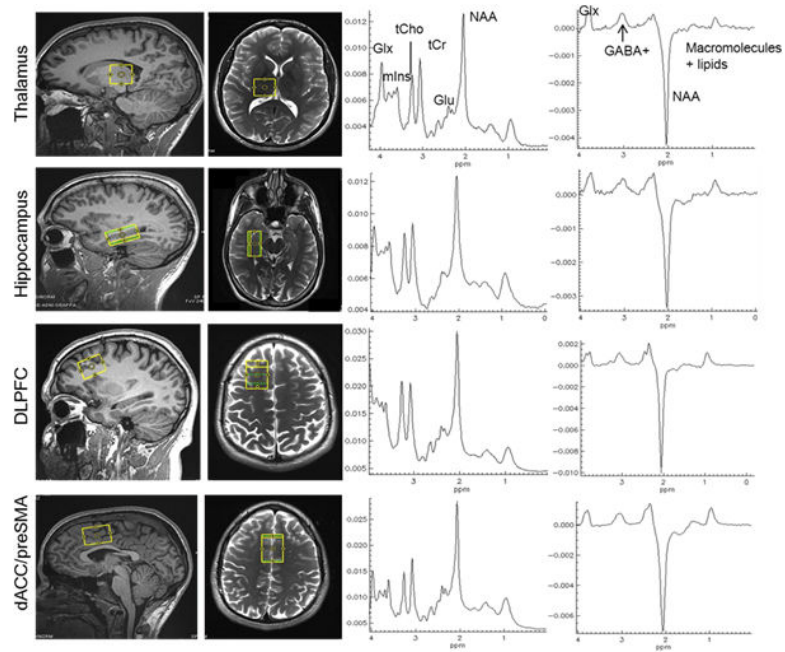
Figure 1.
Sagittal and axial views showing representative VOI placement in the thalamus, hippocampus, DLPFC and dACC/preSMA (left column) and the respective short TE single voxel ^{1}H PRESS spectra (middle column) and MEGA-PRESS GABA spectra (right column). The GABA+ peak at 3 ppm is very prominent for all four regions of interest. VOI = Volume Of Interest.
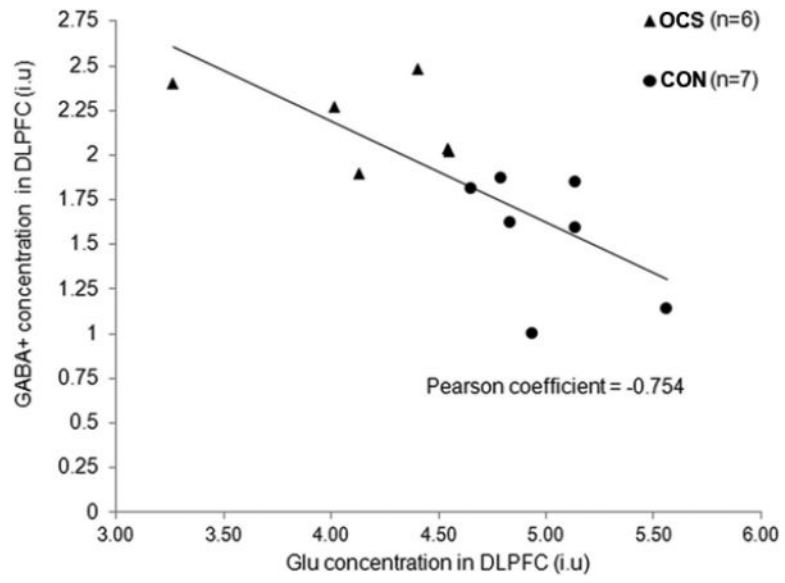
Figure 2.
In the DLPFC, a significant negative correlation (Pearson coefficient=−0.754, p=0.003) is present between GABA+ and Glu concentrations.
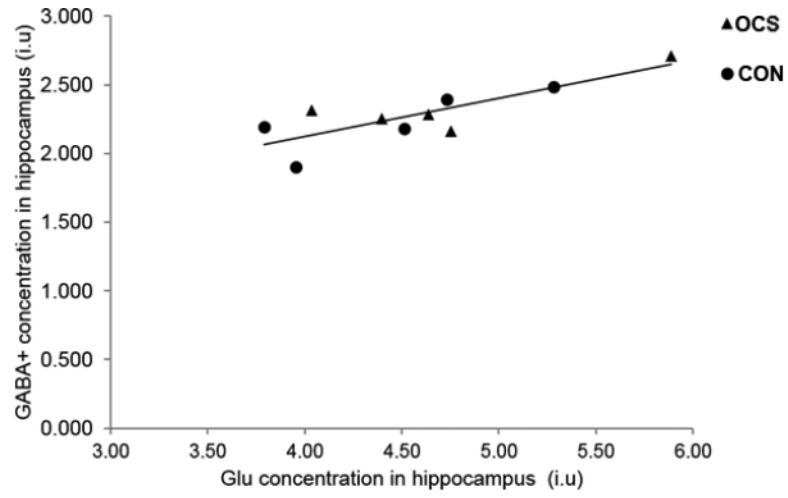
Figure 3.
In the hippocampus, a significant positive correlation (Pearson coefficient=0.783, *p*=0.004) is present between GABA+ and Glu concentrations.

Table 1

Metabolite concentrations (in i.u.) are listed for four brain regions and both groups.

Group	Region		NAA	Glu	mIns	tCr	GABA
CON	Hipp	Mean	3.83	4.64	4.62	3.66	2.32
		Standard Deviation	0.70	0.60	1.02	0.75	0.20
		N	7	7	7	7	7
	DLPFC	Mean	5.20	**4.15**‡	3.78	3.93	**2.18**‡
		Standard Deviation	0.41	0.49	0.23	0.37	0.24
		N	6	6	6	6	6
	Thal	Mean	5.49	4.45	3.59	4.01	**2.86***
		Standard Deviation	0.77	1.03	0.86	0.62	0.36
		N	7	6	7	7	7
	dACC/preSMA	Mean	5.87	6.01	4.68	4.71	2.81
		Standard Deviation	0.57	0.67	0.43	0.17	0.25
		N	9	9	9	9	9
OCS	Hipp	Mean	3.96	4.46	3.40	3.21	2.40
		Standard Deviation	0.94	0.60	0.79	0.92	0.32
		N	6	5	6	6	8
	DLPFC	Mean	5.21	**4.97**‡	3.80	4.25	**1.55**‡
		Standard Deviation	0.48	0.31	0.59	0.22	0.35
		N	8	8	8	8	7
	Thal	Mean	5.24	3.78	3.55	4.33	**3.23***
		Standard Deviation	0.48	0.60	0.48	0.36	0.38
		N	8	8	8	8	8
	dACC/preSMA	Mean	6.10	6.02	4.73	4.84	2.80
		Standard Deviation	0.55	0.66	0.40	0.43	0.10
		N	8	8	8	8	8

Significant (‡, $p<0.01$) or trend-level (*, $0.05 \leq p \leq 0.10$) differences between occlusal splint-wearer (OCS) and control (CON) groups are indicated. Hipp = Hippocampus, DLPFC = Dorsolateral prefrontal cortex, Thal = Thalamus, dACC/preSMA = dorsal anterior cingulate cortex/pre-supplementary motor area.