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TITLE: Measuring Glial Metabolism in Repetitive Brain Trauma and Alzheimer's Disease

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CONTRACTING ORGANIZATION: Brigham and Women's Hospital Inc.  
Boston, MA 02115

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<b>14. ABSTRACT</b> The most significant finding in this report was the application of denoising methods to multinuclear spectroscopy data which showed greatly improved signal-to-noise ratios of up to 3.4 times that of the original data. Six methods: Single value decomposition (SVD), wavelet, sliding window, sliding window with Gaussian weighting, spline and spectral improvements with Fourier thresholding were tested to determine which provided optimal performance. The sliding window with Gaussian weighting showed the most consistent performance across all levels of SNR with the best performance using spline and unweighted sliding window at low SNR data. This analysis will be incorporated into our post-processing pipeline for the <sup>13</sup> C spectroscopy data that will be acquired in this study after human subjects approval.					
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## 1. INTRODUCTION:

Glutamate is a key compound in cellular metabolism with its most important role as a neurotransmitter with which the brain utilizes 80% of energy consumption to maintain this important cycle. Elevated levels of glutamate have been shown to be predictive of outcome in severe traumatic brain injury and our preliminary data from existing studies, have shown that glutamate remains elevated in the chronic stages of repetitive brain trauma as well. Current methods of measure brain glutamate using proton spectroscopy is not specific to different cell types or the dynamic changes that undergo metabolism. We have developed a novel, non-invasive, quantitative method of measuring the dynamic rates of glutamate using  $^{13}\text{C}$ -labeled acetate, the primary fuel for glial cells, which can be tracked through the cerebral glutamate synthesis cycle using  $^{13}\text{C}$  magnetic resonance spectroscopy. Our goal is to utilize infusion of  $^{13}\text{C}$ -labeled acetate in our existing cohort of retired NFL athletes with and without increased glutamate, subjects with Alzheimer's disease, military veterans with a history of traumatic brain injury, and age-matched controls to measure the effect of repetitive brain trauma upon glutamate metabolism. Our hypothesis is that increased glutamate found in these players, will be reflected in up-regulation of glial pathways. The result of the study would be to identify the dysfunctional pathways that underlie glutamate excitotoxicity in sports-related brain trauma. These dysfunctions will provide precise targets for existing glutamate medications that are known to modulate specific pathways. Therefore we anticipate not only providing a better understanding of the metabolic mechanisms of sports-related head injury but also to provide data that will be useful for the development of much needed treatments for this devastating disease.

## 2. KEYWORDS:

Repetitive brain trauma, glial metabolism, glutamate, multinuclear spectroscopy, chronic traumatic encephalopathy, sports-related brain injury, military-related brain injury, Alzheimer's disease,  $^{13}\text{C}$  acetate

## 3. ACCOMPLISHMENTS:

### 3.1. Major Goals

Our overall aim will be to better understand glial metabolism within the context of RBT and AD and its potential findings in veterans. Specifically, we will:

Aim 1: Determine the mechanism (excitotoxicity?) that results in increased cerebral Glx levels by comparing glial metabolic rates in NFL athletes with the highest levels of Glx and those with the lowest levels.

Aim 2: Determine the mechanism (neurodegeneration?) that results in decreased cerebral Glx levels by comparing glial metabolic rates in NFL athletes with the lowest levels of Glx and AD patients.

Aim 3: Identify the specific metabolic pathway that results in alternations of cerebral Glx levels in military and healthy controls as well as in comparison with NFL and AD subjects.

Aim 4: Correlate the glial and glutamate metabolic rates with additional measures obtained in the parent studies including of a) serum, CSF, and genetic biomarkers in the NFL subjects and b) neurocognitive

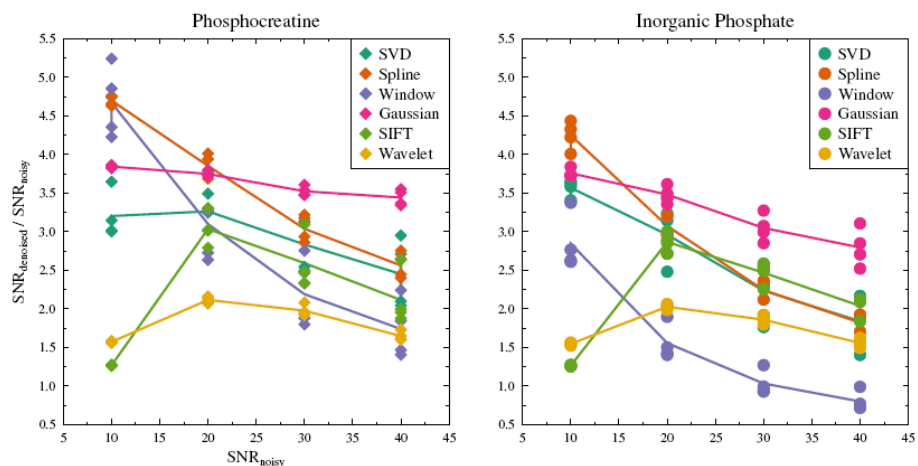
measures in all cohorts. The result of the study would be to identify the underlying physiological changes in glial metabolism in RBT such as neuroinflammation and glutamate excitotoxicity thus providing targets for much needed treatments as well as provide a safe, non-radioactive test to monitor these treatments.

### 3.2. Goal Accomplishments

Major Task 1 under Aim 1 is to submit local IRB protocol of amend existing study protocols and to amend existing material transfer agreements to include the new protocol. Initially, our intent was to amend an existing HRPO-approved protocol (A-15247.31: Neurochemical and Multimodal Biomarkers for Chronic Traumatic Encephalopathy) with the new procedures to be used in this proposal however given that both the methodology and population will differ, it was recommended by our local IRB to submit a new proposal. The protocol was submitted for review and returned based on concerns regarding the safety of using  $^{13}\text{C}$ -labeled acetate in human subjects.  $^{13}\text{C}$ -acetate has been used safely across a large number of studies, including my own studies in a previous lab however we wanted to ensure that we include the details that would be best for review at Partners. I therefore contacted Dr. Gerald Berry at Children's Hospital Boston, who conducts  $^{13}\text{C}$ -acetate studies in children. He kindly shared his IRB protocol so that I could use the appropriate wording to satisfy the concerns of the IRB committee. In addition, I obtained additional information from my former lab that could be used as evidence of the safety of the method. The IRB protocol was then resubmitted on February 16th for review. It was then returned asking for approval from the Magnetic Resonance Imaging Safety Committee for the use of the  $^{13}\text{C}$  coil which was developed in our laboratory. We met with the safety committee and they requested safety testing. While we have used the coil for previous IRB-approved studies, we have conducted those tests to ensure that there are no risks to the subject. The results were submitted to the committee for their review and was approved. The proposal has been resubmitted and we await the outcome.

During this time we have been working on improving the post-processing methods. Previous studies from our lab have shown that applying denoising techniques in the indirect temporal domain can greatly improve the accuracy of metabolite quantification for individual spectra, reducing the need for averaging and improving resolution. We have extended this further by comparison of a range of different denoising methods for dynamic MRS. Six denoising methods were considered: Single value decomposition (SVD), wavelet, sliding window, sliding window with Gaussian weighting, spline and spectral improvements with Fourier thresholding (SIFT). The spline fitting method consistently performed best for input signals with low SNR, but its performance deteriorated as the input signal improved. By contrast the SIFT method performed

relatively poorly on both low and high SNR inputs, but performed much better for intermediate values. Surprisingly the wavelet method gave consistently poor results, only improving the SNR by a factor of around 2. Both the SVD and Gaussian weighted sliding window give



**Figure 1.** Statistical comparison of the improvement in SNR as a function of initial input SNR for the 6 different denoising methods, applied to phosphocreatine (left) and inorganic phosphate (right).

consistently high denoising across the range of input noise levels, but the Gaussian window is undoubtedly the most successful method for the forms of data considered here, offering an average 3.5 times improvement in SNR. These results will now be integrated into the post-processing pipeline for the study.

### 3.3. Training and Professional Development

While there study does not have a component for training and professional development, Dr. Stern and I co-supervise and mentor Dr. Michael Alosco through an F32 Kirchstein Post-doctoral award. While Dr. Alosco does not contribute directly to the study, he has observed the work that has been done and has been part of our scientific discussions.

### 3.4. Results Dissemination

The denoising methods described above was submitted as an abstract to the 24<sup>th</sup> Annual Meeting and Exhibition of the International Society of Magnetic Resonance in Medicine in Singapore where it was presented as a plenary talk.

### 3.5. Next Reporting Period Plan

After local IRB approval is obtained, documentation will be sent to HRPO for approval. As soon as we receive HRPO approval, we will begin recruitment of subjects. During the next quarter as we wait for the approval, we will work on preparing for the study including preparation for the FITBIR upload of our data.

## 4. IMPACT:

### 4.1. Principle Discipline

Our dynamic  $^{13}\text{C}$  MR studies will track the transfer of labeled  $^{13}\text{C}$  from injected acetate to glutamate and glutamine through the TCA cycle by serial acquisition of spectra with a repetition time of 1 second over 30 minutes. Due to the low SNR, in the past we used an average of 5 minutes of data (300 acquisitions) to adequately measure the time course of the  $^{13}\text{C}$  metabolites which reduced the temporal resolution of the data. The denoising methods provide a great improvement to the SNR of  $^{13}\text{C}$  spectroscopy data and improvement of temporal resolution by using the Gaussian sliding window. All of this comes at no-cost to the experiment as all of it is done in the data post-processing.

### 4.2. Other Disciplines

The denoising methods, while specific to spectroscopy, have potential applications across any serially collected data of low signal to noise.

### 4.3. Technology Transfer

There is a strong potential that the  $^{13}\text{C}$  spectroscopy data collection and denoising methods may be licensed by BrainSpec, Inc., a startup company that was recently spun out of my laboratory at the Center for Clinical Spectroscopy. We recently submitted a Small Business Technology Transfer proposal (R43) through the National Institutes of Health last month for this technology to be used for treatment monitoring in substance abuse disorders. There are likely similar opportunities in the DOD SBIR/STTR portfolio which we will explore in the near future.

## Society

At this time, there is nothing to report.

## **5. CHANGES/PROBLEMS:**

### **5.1. Changes in approach**

Our collaborators at the BU CSTE and Dr. Robert Stern received U01 funding from NIH (DIAGNOSE-CTE) that will widen the basis for subject recruitment for new subjects in addition to the ones that have been recruited for the previous R01-funded study (DETECT). This doesn't necessitate any changes to our proposal or protocol but will allow us to recruit subjects more rapidly.

There has also been concern regarding the upload of the non-spectroscopy related measures (serum, CSF, and genetic biomarkers as well as neurocognitive measures) in our study to the FITBIR database. It is our understanding that the DETECT cohort will not have their data uploaded to FITBIR as the study pre-dates the FITBIR mandate. The DIAGNOSE cohort will have their data uploaded to FITBIR but that it will be embargoed until 2025, at the conclusion of the study. I have discussed this with Dr. Stern and he has reassured me that all of the DETECT and DIAGNOSE data will be available for our study for correlation analysis to the spectroscopy measures as proposed in Aim 4 of our study.

However, we also understand the agency's desire for data to be uploaded to FITBIR and will take responsibility for the upload of the non-spectroscopy related measures to the FITBIR database from both DETECT and DIAGNOSE subjects that we recruit. We are in further discussion with Dr. Stern about how this should be handled but are confident that this issue can be resolved. We do want to emphasize that this does not change or impact our original proposal. There may be additional effort on our part to collect and upload the data as it was anticipated that it would be uploaded through the original studies, however this data will be available to us for analysis and completion of our aims.

### **5.2. Actual or anticipated problems**

The IRB approval has taken longer than anticipated. We recognize the impact of the delays on our timeline and have been working with the Boston University Center for Study of Traumatic Encephalopathy whose role is to recruit our subjects. Access to the aforementioned U01 study cohort will significantly widen our database of individuals that can be recruited for the study. Dr. Stern has been working with his staff to ensure that those subjects will be available for our study as well. This will ensure that we can recruit subjects at double the projected rate for the study.

### **5.3. Impact on expenditures**

We have been judicious with funds to ensure that if necessary, there will be sufficient funds for the study. At this time we do not anticipate increased costs for the additional FITBIR integration as discussed above but will report to the agency if the efforts exceed our expectations.

### **5.4. Significant changes in use or care of human subjects**

None.

## **6. PRODUCTS:**

### **6.1. Publications, conference papers, and presentations**

Rowland BC, Lin AP. A comparison of denoising methods in dynamic MRS. Proc Int Soc Magn Reson Med (2016) 24:261

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name: Alexander Lin  
Project Role: Principal Investigator  
Research Identifier: orcid.org/0000-0001-8236-880X  
Nearest Person Month Worked: 4  
Contribution to Project: Dr. Lin has been addressing the concerns of the IRB and MR safety committees by conducting tests of the 13C coil for safety purposes. The results of the tests have been sent to the committee for evaluation.

Name: Huijin 'Vicky' Liao  
Project Role: Study Coordinator  
Research Identifier: <https://www.linkedin.com/in/huijun-vicky-liao-3b682451>  
Nearest Person Month Worked: 4  
Contribution to Project: Ms. Liao has assisted Dr. Lin with the acquisition of the 13C spectroscopy data and assisted with the submission of the IRB documentation.

Name: Ben Rowland  
Project Role: Postdoc  
Research Identifier: n/a  
Nearest Person Month Worked: 6  
Contribution to Project: Dr. Rowland has been preparing for the start of the project by improving the software required for the data analysis by developing six different denoising methods. He also assisted with the testing of the 13C coil.

Name: Robert Stern  
Project Role: Subaward PI  
Research Identifier: orcid.org/0000-0002-5008-077X  
Nearest Person Month Worked: 1  
Contribution to Project: Dr. Stern has assisted with the recruitment process as described above.

Name: Johnny Jarnagin  
Project Role: Subaward Study Coordinator  
Research Identifier: n/a  
Nearest Person Month Worked: 2  
Contribution to Project: Mr. Jarnagin has a list of subjects that he has identified for the study. He has also discussed the concept of augmented studies to the U01 with participants and they have been willing to do so.

### 7.1. Change in Personnel

Nothing to Report.



## 7.2. Other Organizations

Organization Name: Boston University School of Medicine

Location of Organization: Boston, Massachusetts

Partner's contribution to the project:

- Collaboration: Dr. Robert Stern and Johnny Jarnagin are our collaborators.
- Personnel exchanges: As described above, Dr. Michal Alosco has been an observer in our lab from Boston University and while not directly contributing to this project, has been involved in the analysis of other MR spectroscopy data in the same cohort of subjects.

## 8. SPECIAL REPORTING REQUIREMENTS

Nothing to Report.

## 9. APPENDICES:

Please see attached abstract.

# A comparison of denoising methods in dynamic MRS

Benjamin C Rowland<sup>1</sup> and Alexander P Lin<sup>1</sup>

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## Synopsis

**MR spectroscopy is often used to study dynamic systems, such as muscle energetics using <sup>31</sup>P. The need to perform temporal averaging to improve signal to noise ratios can compromise the temporal resolution of the measurements. Indirect time domain denoising can help to resolve this issue. In this study we evaluate six potential denoising approaches for dynamic MRS.**

## Purpose

In MR spectroscopy it is often interesting to observe a dynamic system as it varies in time, for example phosphocreatine depletion and resynthesis in exercising muscle. To achieve an adequate signal-to-noise ratio (SNR), particularly at lower field strengths, sequential spectra are typically averaged, compromising the achievable temporal resolution. Previous studies have shown that applying denoising techniques in the indirect temporal domain can greatly improve the accuracy of metabolite quantification for individual spectra, reducing the need for averaging and improving resolution<sup>1</sup>. This study forms the first comparison of a range of different denoising methods for dynamic MRS.

To assess the performance of any denoising technique, it is necessary to start with a "ground-truth" signal to which random noise can be added before the technique is applied. Synthetic timecourses often lack subtle features or use mathematically exact shapes which may be biased towards certain denoising methods. In order to accurately capture real-world nuances and variations, we chose to derive our ground truth signals from experimental data.

## Methods

Six denoising methods were considered: SVD, wavelet, sliding window, sliding window with Gaussian weighting, spline and SIFT.

We selected four exams measuring <sup>31</sup>P MRS in exercising muscle, covering a representative range of clinical cases: a young female, young male, middle-aged male (all healthy volunteers) and a male patient with peripheral artery disease. From these exams the noisy timecourses of the PhosphoCreatine (PCr) and inorganic Phosphate (Pi) peaks were extracted. In each case, our smooth ground truth was obtained by applying the SVD, Gaussian window, spline and SIFT de-noising methods and averaging the results. Combining these four methods produces a smooth timecourse which is not biased towards a particular model representation (sinusoids, splines etc.). An example of this process is shown in figure 1. It is important to remember that this derived ground truth is not required to exactly match the true underlying signal of the source data, but merely to create a timecourse with similar characteristics.

Randomly generated white Gaussian noise was added to each ground truth at four different SNRs (10, 20, 30, 40), covering the typical range observed experimentally. For each initial time course and noise level a Monte Carlo approach was adopted where 400 noisy signals were created and denoised by all six methods, in order to assess the average performance of each method.

## Results

It was determined that the averaging method for producing the ground truth did not introduce significant bias towards any model, by applying each method to the plain denoised signal.

The results of the denoising are shown in figure 2. For each set of 400 noisy signals the average standard deviation between the denoised signal and the ground truth was used to calculate the final SNR. This was then divided by the initial value to show the proportional improvement in SNR, enabling comparison of the results for different initial levels of noise.

The spline fitting method consistently performed best for input signals with low SNR, but its performance deteriorated as the input signal improved. By contrast the SIFT method performed relatively poorly on both low and high SNR inputs, but performed much better for intermediate values. Surprisingly the wavelet method consistently gave relatively poor results, only improving the SNR by a factor of around 2.

Both the SVD and Gaussian weighted sliding window give reliably high denoising across the range of input noise levels, but the Gaussian window is undoubtedly the most successful method for the forms of data considered here, offering an average 3.5 times improvement in SNR.

#### Conclusion

A wide variety of denoising methods can be applied to improve SNR in dynamic MR spectroscopy, leading to improved concentration estimates and temporal resolution and reduced uncertainties. For signals found in 31P muscle spectroscopy, this study determined a simple sliding window method with Gaussian weighting to perform best.

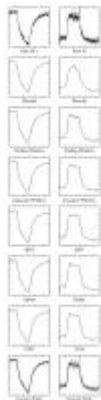
#### Acknowledgements

This study was funded in part by the Osher Center for Integrative Medicine.

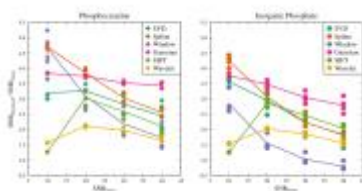
#### References

- 1 Rowland B, Merugumala S, Liao H et al. Magn Reson Med 2015

#### Figures



The in vivo measured time courses for phosphocreatine and inorganic phosphate (top row) are denoised by applying six different techniques (rows 2 to 7). These individual smooth time courses are combined to produce a single ground truth (bottom row) containing characteristics of all methods while retaining the features of the raw data (shown in grey on the bottom row).



A statistical comparison of the improvement in SNR as a function of initial input SNR for the 6 different denoising methods, applied to phosphocreatine (left) and inorganic phosphate (right).