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TITLE: The Imprint of Psychogenic Nonepileptic Seizures on the Brain: A New Model and Imaging Biomarker

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of the project is to obtain evidence to support a new mechanism that assumes that PNES are caused by a predisposition for enhanced synchronization/overshoothing recruitment of brain regions involved in emotion control/processing of traumatic/stressful experiences. By assuming such a predisposition, the project implicitly assumes that PNES have a specific biological underpinning. The first year of the project was spent on finalizing questionnaires and report forms, obtaining IRB approval from participating institutions, implementation of the imaging protocol, processing pipelines and hiring and training of research personnel. The project was allowed to enroll patients from 05/18 on. The second year was spent on obtaining permission to add UCSF as additional referral site, analyzing incoming data, modifying the pipeline used to generate simulated data to simulate/characterize the fMRI signature of the PNES imprint and trying to maximize referral of suitable patients for the project.

15. SUBJECT TERMS

Psychogenic non-epileptic seizure, fMRI, overshooting, brain imprint, emotion, PTSD, trauma, stress

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1. INTRODUCTION:

Psychogenic non-epileptic seizures (PNES) are defined by the occurrence of seizure-like episodes that interfere with normal functioning but lack the characteristic ictal EEG manifestations of epileptic seizures. The overall goal of this application is to confirm a new PNES mechanism by identifying its characteristic signature in task-free fMRI data of PNES patients and demonstrating a relationship between PNES severity and the expression of this signature. The new mechanism assumes that PNES is associated with a predisposition for an overshooting recruitment. Overshooting recruitment describes a state that is characterized by an enhanced synchronization between brain regions normally involved in emotion control and by the additional recruitment of regions involved in abnormal emotion processing. Repeated or prolonged stress or traumatic experiences further reinforces this predisposition which renders the brain more susceptible for overshooting recruitment and leaves a characteristic imprint that is detectable in the individual's task-free fMRI even in the absence of stress. On the behavioral level, overshooting allows for aspects of pathological emotion processing, e.g. anxiety, to become apparent during mild stress and facilitates overshooting reactions severe enough to recruit the additional brain regions required to generate the individual's typical PNES, in moderate to high stress situations. The project is designed as a cross-sectional study and will enroll 40 PNES patients and 20 controls. All will undergo fMRI on a 3T magnet and a standardized assessment regarding PNES risk factors and psychiatric co-morbidities that will be used to calculate co-morbidity scores. A dynamic fMRI analysis approach will be used to capture the "overshooting signature" and to relate it to the severity of psychiatric comorbidity, seizure frequency and semiology at the group and individual level.

2. KEYWORDS

Psychogenic non-epileptic seizure, fMRI, overshooting, brain imprint, emotion, PTSD, trauma, stress.

3. ACCOMPLISHMENTS:

Scope of work (approved version)

Major Task 1 (Year 0-1). Project Initiation:
a. Hiring and training of study personnel
b. Setup databases and design study documents
c. Finalize imaging protocol
c. Writing IRB protocol
d. Obtain IRB approval UCSF, VA, DoD

Major Task 2 (Year 1-3): Patient Screening, Recruitment and Assessment incl imaging
a. Year 1: 8 PNES
b. Year 2: 26 PNES
c. Year 3: 6 PNES

Major Task 3 (Year 1-3): Control Screening, Recruitment and Assessment incl imaging
a. Year 1: 4 controls
b. Year 2: 13 controls
c. Year 3: 3 controls

Major Task 4 (Year 1-3): Data Processing
a. Compilation of clinical and psychiatric data, transfer into database
b. MRI preprocessing.

Major Task 5 (Year 2-3): Graph Analysis and cluster analysis to isolate PNES imprint
a. Data processing
b. Identification of PNES imprint/cluster

c. Correlation with psychiatric comorbidity score, seizure frequency, type
d. Imprint simulations
e. Additional analysis (requested during review): Proof that PNES imprint is not present in epilepsy patients. This subtask will use existing fMRI data from epilepsy patients and controls that has been acquired previously for another project.

Major Task 6 (Year 3): Stationary fMRI analysis to isolate PNES imprint
a. Data processing
b. Identification of PNES imprint/cluster
c. Correlation with psychiatric comorbidity score, seizure frequency, type

Major Task 7 (Year 3): Manuscript writing, result dissemination

What was accomplished under these goals?

Major Task 1 (Year 0-1). Project Initiation
Tasks outlined in 1 were all accomplished in Year 1. The project started the screening/enrolling of potential participants in 05/2018

Major Task 2 (Year 1-3): Patient Screening, Recruitment and Assessment incl imaging
At beginning of this reporting period it became clear that the patient recruitment was lacking behind the target numbers. Inclusion and exclusion criteria were reviewed with the so-investigators at the two referral centers without identifying a single/group of criteria that explained the low recruitment. To enhance recruitment, UCSF/ Dr.Garcia were added as referral center for the study.
UCSF IRB approved the addition on 09/12/2018
DoD HRPO approved the addition on 12/07/2018

As of 09/30/2019 8 PNES subjects were enrolled and had completed all study procedures/were completing study procedures. 2 additional patients were scheduled for informed consent.

Major Task 3 (Year 1-3): Control Screening, Recruitment and Assessment incl imaging
a. Year 2: Enrollment of controls was delayed due since it is intended to recruit controls who are matched to the patients re age, gender and socio-economic background.

Major Task 4 (Year 1-3): Data Processing
An imaging data pre-processing pipeline was developed and implemented and has been successfully deployed for the pre-processing of 6 data sets.

Major Task 5 (Year 2-3): Graph Analysis and cluster analysis to isolate PNES imprint
a. Data processing
e. Additional analysis (requested during review): Proof that PNES imprint is not present in epilepsy patients: Preliminary Analyses of 6 data sets were completed:

Preliminary Results

6 data sets (6 PNES subjects) were processed and underwent a preliminary (final data analysis requires control group) data analysis. This preliminary analysis was focused on demonstrating the overshooting predisposition and PNES imprint in these subjects.
Methods: Graph analysis and in particular "strength" and "modularity" were used. Strength allows to assess the degree of connectivity/synchronization of a region with increased strength indicating an enhanced synchronization with other brain regions. One of the defining criteria of "overshooting" is an enhanced synchronization between brain regions involved in stress/trauma processing and is therefore expected to be

characterized by increased strength. The second defining criteria of overshooting is the enhanced synchronization of brain regions involved stress/trauma processing with brain regions not typically involved in stress/processing in healthy subjects whose involvement reflects the predominant seizure type in PNES, e.g. motor and supplementary motor regions in PNES with minor and major motor seizures. Modularity a measure that identifies groups of regions preferentially interacting with each other but not with other regions as so-called "modules" is used to demonstrate this aspect of overshooting. It is expected that in PNES "typical stress" regions, e.g., amygdala, medial and dorso-lateral prefrontal regions, are assigned to the same module as "non-stress" but seizure defining brain regions, e.g. motor cortex. Finally, seizures are thought to represent a release phenomenon of overshooting, i.e. the overshooting has a maximum representation immediately before the seizure (maximum extent associated with maximum strength) and is absent/greatly reduced immediately after a seizure (similar to normal stress imprint, not associated with maximal strength fluctuations).

A dynamic fMRI analysis (1) is used to capture the expression of the PNES imprint/overshooting (strength/module configuration) over time. The following definitions are used:

Normal stress reaction imprint: ROIs in same module with amygdala over 50% of the resting state fMRI in 10 controls without PNES or PTSD. (control data from REAC/EF epilepsy project: PI Mueller)

PNES imprint: ROIs in same module with amygdala over 50% of resting state fMRI (total duration: 24 min) in individual PNES.

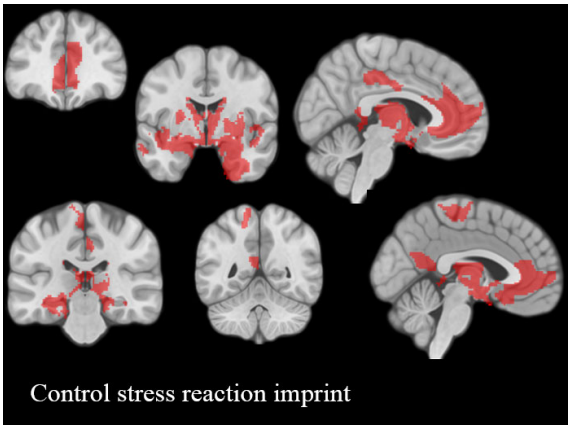

Figure 1

Results: Figure 1 shows the normal stress reaction imprint in controls encompassing ROIS in amygdala, hippocampus, anterior and mid cingulate, medial prefrontal, insula and pallidum/putamen, i.e. regions known to be involved in emotional control/ stress processing. The demographic/behavioral characteristics incl seizure semiology of the 6 PNES subjects is listed in

Table 1. NES003 had a patient-characteristic seizure immediately after the MR exam. NES006 had a seizure immediately before the MR exam.

Measure	NES001	NES002	NES003	NES004	NES005	NES006
Age/Gender	45/m	37/f	34/f	51/f	29/f	58/f
CAPS life/current	39/39	56/42	31/15	52/35	35/23	30/31
Dissociation*	43	22	11	5	17	0
PNES type	cat	min mot	cat, maj mot	cat, maj mot	min. & maj mot	cat, min mot
PNES frequency	2/month	2-10/day	2-10/month	2-3/week	3/week	10/day
Comment			mot seizure post MRI			mot seizure pre MRI

catatonic (cat): characterized by periods of motionless unresponsiveness, often accompanied by waxy flexibility
major motor (maj mot): brief four extremity asynchronous movements, spells with unusual behavior, e.g. thrashing, rocking, interspersed with motionless unresponsiveness.
minor motor (min mot): low-amplitude near-purposeful movements of the face or upper extremities, low amplitude rhythmic/synchronous bilateral movements.
subjective (sub): Sensory or emotional experiences generally with retained consciousness and behavior
*sum of DSPS lifetime scores

Table 1

Figure 2 shows the PNES imprints in these 6 subjects. The imprint of PNES with major motor PNES seizures includes large sections of the bilateral motor cortex. The PNES imprint in PNES with minor motor seizures is more confined, e.g. NES002, or even absent, e.g. NES006 who experienced a seizure immediately before the exam. The imprint PNES with catatonic seizures encompasses parts of the precuneus/posterior cingulate that are absent in the normal stress reaction imprint.

Table 2 shows the association between PNES imprint extent and connectivity fluctuations and Figure 3 the association between PNES imprint extent and connectivity fluctuations in the two subjects who experienced seizures before and after the MRI.

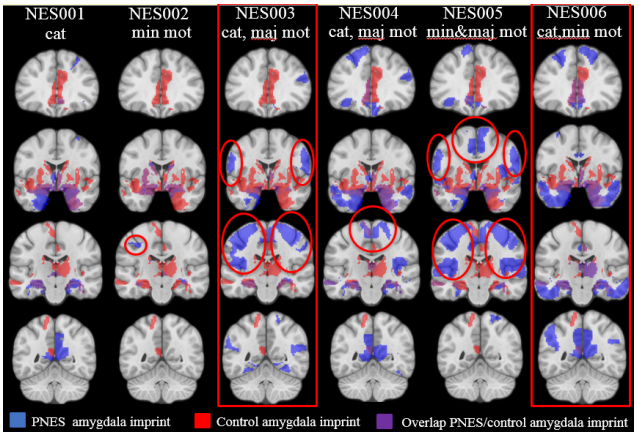

Figure 2

Conclusion: Although very preliminary the findings are consistent with the hypothesized PNES imprint/overshooting that is at the center of the new PNES model investigated by this project.

SubID	PNES imprint expression/connectivity	PNES imprint expression/intercommunity interactions
NES001	0.17	0.18
NES002	0.08	0.24
NES003	0.47	0.35
NES004	0.36	0.35
NES005	0.17	0.03
NES006	0.21	0.24

Table 2

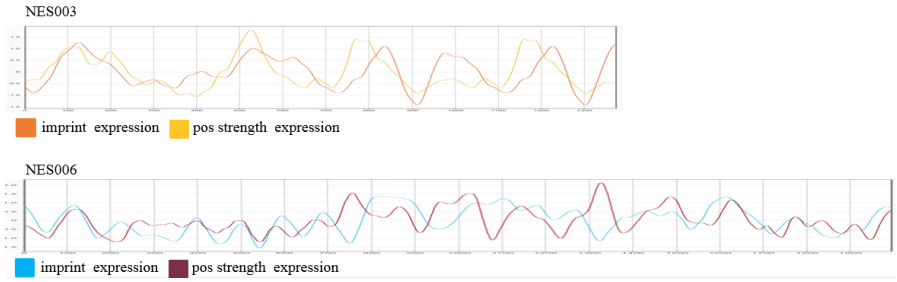

Figure 3

What opportunities for training and professional development has the project provided?
Nothing to report.

How were the results disseminated to communities of interest?
Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
Year 3 will focus on recruitment and assessment of eligible patients. The goal is to raise the number of patients that are enrolled in this period to meet the recruitment milestones specified in the SOW. The following is planned:
1. Revision of inclusion/exclusion criteria to identify those that were responsible for the high percentage of patients that were screened but not enrolled and eliminate/modify them.
2. Reach out to other epilepsy centers that are diagnosing/treating PNES..

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
Nothing to report.

What was the impact on other disciplines?
Nothing to report.

What was the impact on society beyond science and technology?
Nothing to report.

5. CHANGES/PROBLEMS

1. IRB approval (UCSF, VA, HRPO) took longer than expected.
2. Patient recruitment is lagging behind.

Actions taken:
1. The PI got in contact with epileptologists/psychiatrists diagnosing and treating PNES patients at:
a. Kaiser Permanente
b. Stanford Health
c. Sutter Health Sacramento

Terms of collaboration/IRB requirements are currently being investigated/discussed and IRB. It is intended to add these additional referral site by January 2020 latest.
2. The PI will apply for a no cost extension of the project

Actual or anticipated problems or delays and actions or plans to resolve them
Software up-grade (VD13A – VE11C) in Dec 2018. The development of the imaging protocol was adapted accordingly by choosing sequences that are available in both versions and were not modified in the new release.

Changes that had a significant impact on expenditures

CIND Leadership announced increase of MRI use costs by 14% ($572/h instead of original $500/h) starting 06/2019.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report

Significant changes in use or care of human subjects
Nothing to report

Significant changes in use of biohazards and/or select agents
Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**
  Nothing to report.

  Journal publications
  Nothing to report.

  Books or other non-periodical, one-time publications
  Nothing to report.

  Other publications, conference papers and presentations.
  Project presented by co-investigator Dr. Garga at the UCSF Epilepsy Annual Research Retreat on 01/24/2019.

  Website(s) or other Internet site(s)
  Nothing to report.

  Technologies or techniques
  Nothing to report.

Inventions, patent applications, and/or licenses
Nothing to report.

Other Products
Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: *Susanne Mueller*
Project Role: *PI*
Researcher Identifier *(ORCID ID): 0000-0002-5515-4432*
Nearest person month worked: *0.3*
Contribution to Project: *Development of IRB protocol, development of reporting documents, questionnaires, training of study personnel, setting up study logistics, finalizing imaging protocol, development of processing pipelines, testing of processing pipelines. Analysis of preliminary data, subject enrollment, scheduling of assessments.*

Name: *Thomas Neylan*
Project Role: *co-investigator*
Researcher Identifier (e.g. ORCID ID): *NA*
Nearest person month worked: *0.05*
Contribution to Project: *supervision psychiatric evaluation*

Name: *Nina Garga*
Project Role: *co-investigator*
Researcher Identifier (e.g. ORCID ID): *NA*
Nearest person month worked: *0.05*
Contribution to Project: *Screening and referral of PNES subjects*

Name: *Kenneth Laxer*
Project Role: *co-investigator*
Researcher Identifier (e.g. ORCID ID): *NA*
Nearest person month worked: *0.05*
Contribution to Project: *Screening and referral of PNES subjects*

Name: *Paul Garcia*
Project Role: *co-investigator*
Researcher Identifier: *NA*
Nearest person month worked: *0.05*
Contribution to Project *Screening and referral of PNES subjects*

Name: *Jennifer Hlavin*
Project Role: *study co-ordinator SFVAMC mental health*
Researcher Identifier (e.g. ORCID ID): *NA*
Nearest person month worked: *0.05*
Contribution to Project: *logistics of mental health assessment*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel
Nothing to report

What other organizations were involved as partners?
Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

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QUAD CHARTS: *Please see attachment*

APPENDICES:
Nothing to report