Elucidating pain-related activity in the EEG from the rat spinal nerve ligation model (SNL) of neuropathic pain

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BACKGROUND

In both clinical settings and in preclinical models, specific electroencephalographic (EEG) frequencies increase during high pain states. In collaboration with the NIH HEAL Initiative Preclinical Screening Platform for Pain (PSPP) PsychoGenics conducted a longitudinal evaluation of the EEG for pain signatures in the spinal nerve ligation (SNL) model of pain. We investigated male and female Sprague Dawley rats for a period of 120 days following SNL surgery and compared Naïve, Sham and SNL rats for changes in EEG power in the Theta, Low Gamma and High Gamma frequency ranges.

METHODS

60 male and 60 female Sprague-Dawley (SD) rats were implanted for longitudinal EEG recordings. Rats were separated into three groups: Naïve, Sham and SNL. Twenty rats were implanted per group to achieve a target of n=15/group. Head mounted implants (8239, Pinnacle Technologies, Inc, Lawrence, KS) were used to record 2-EEG/1-EMG through the 8200 series rat recording system (Pinnacle Technologies, Inc, Lawrence, KS).

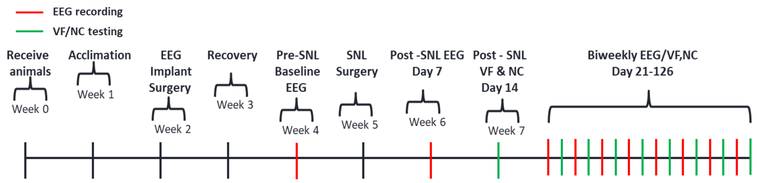
Screw electrodes were placed at S1 hindlimb area contralateral to injured side (2 mm posterior and 2 mm lateral from Bregma) and PFC (contralateral to injured side; 3.5 mm anterior and 1 mm lateral from Bregma). Both the SI and PFC electrodes were referenced to cerebellar electrode.

Rat EEG recording system

Spinal nerve ligation (SNL) surgery: Ligation of the L5 and L6 spinal nerves was performed as described in Kim and Chung (1992). Male and female EEG implanted rats either received ligation of the L5 and L6 spinal nerves (SNL group), a sham surgery where the spinal nerves were not ligated (Sham group) or did not receive any additional surgical procedures (Naïve group). Animals were tested 7 days post-op, and every other week thereafter, for hind paw hypersensitivity and gait analyses. Paw withdrawal thresholds were determined with von Frey filaments as described by Chaplan et al. (1994). Gait was analyzed using NeuroCube (NC). Gait analyses and paw withhold threshold are reported elsewhere.

Absolute Theta (4-8 Hz), Low Gamma (30-50 Hz), and High Gamma (65-100 Hz) power was extracted from wake periods using a MATLAB program by Neurotecnix (Saab et. al, 2012, 2013). For normalization, we selected a period with stable quiet waking (the first 6 h of the dark phase) and generated mean pre-SNL EEG power. To minimize inter-subject variability, each subject's post-SNL frequency power data was normalized by their corresponding pre-SNL baseline.

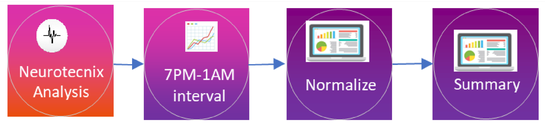
PSPP study timeline



Schematic design for longitudinal EEG, VF, and NC pre- and post-SNL surgery

EEG was evaluated biweekly starting on day 7 post-SNL for up to 120 days. Von Frey (VF) and gait assessments using NC were performed on these rats longitudinally on opposite weeks for up to 120 days as well.

EEG data processing



Absolute Theta and Gamma power analysis on each Wake-epoch EDF for the 22-h recording using software developed by Neurotecnix group.

Determining theta and gamma powers for interval from 7PM to 1AM (the first 6 hours of the dark phase) for each recording

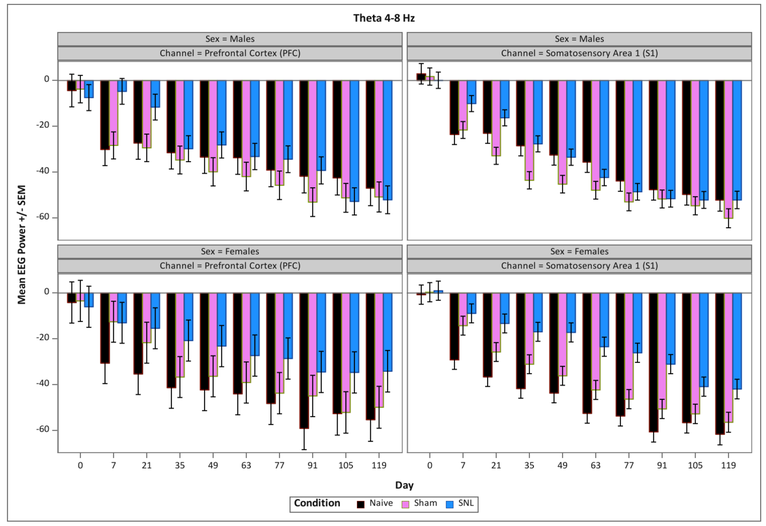
Calculating within-subject percent change (Normalization) for post-SNL in comparison with baseline (pre-SNL) for each animal

Summarizing results over day of experiment, animal's sex, and experimental condition

Statistical analysis using linear mixed model (SAS Proc Mixed) with a random intercept, and subject nested in treatment

RESULTS

Theta (4-8Hz) Power in Male and Female SNL Rats



Male Prefrontal Cortex : Theta power			
Day	Treatment	_Treatment	Significance
7	SNL	Sham	*
7	SNL	Naïve	*

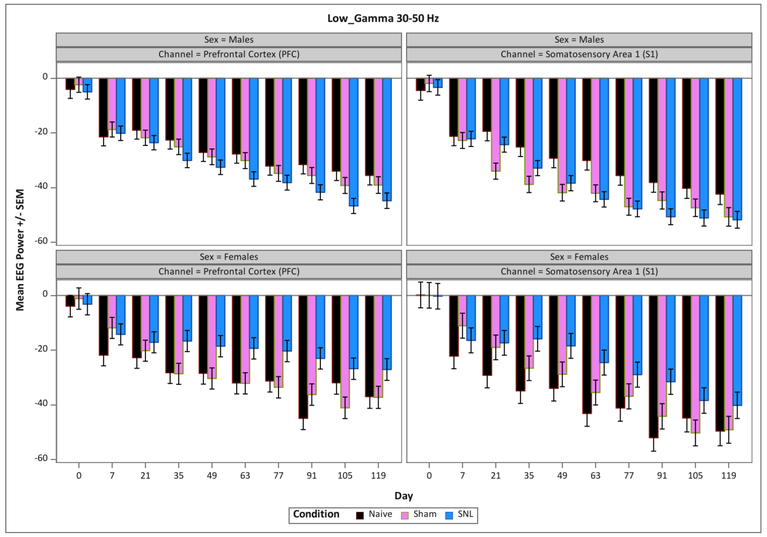
Male Somatosensory Area (S1) : Theta power			
Day	Treatment	_Treatment	Significance
7	SNL	Sham	*
21	SNL	Sham	**
35	SNL	Sham	**
35	Sham	Naïve	*

Female Somatosensory Area (S1) : Theta power			
Day	Treatment	_Treatment	Significance
7	SNL	Naïve	**
7	Sham	Naïve	*
21	SNL	Naïve	***
35	SNL	Sham	*
49	SNL	Sham	**
49	SNL	Naïve	****
63	SNL	Sham	**
63	SNL	Naïve	****
77	SNL	Sham	**
77	SNL	Naïve	***
91	SNL	Sham	**
91	SNL	Naïve	****
105	SNL	Naïve	*
119	SNL	Naïve	**

Naïve n=12-19
Sham n=15-19
SNL n=16-19

Significance :
*p<0.05
**p<0.01;
***p<0.001;
****p<0.0001

Low Gamma (30-50Hz) Power in Male and Female SNL Rats



Male Somatosensory Area (S1) : Low gamma power			
Day	Treatment	_Treatment	Significance
21	SNL	Sham	*
21	Sham	Naïve	**
35	Sham	Naïve	*
49	Sham	Naïve	*
63	SNL	Naïve	**
63	Sham	Naïve	*
77	SNL	Naïve	*
77	Sham	Naïve	*
91	SNL	Naïve	*

Male Prefrontal Cortex : Low gamma power			
Day	Treatment	_Treatment	Significance
105	SNL	Naïve	*

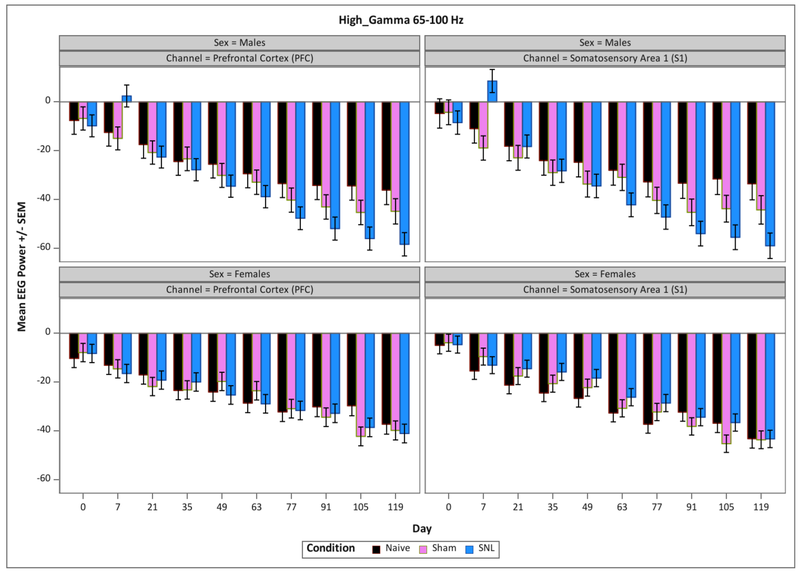
Female Prefrontal Cortex : Low gamma power			
Day	Treatment	_Treatment	Significance
77	SNL	Sham	*
91	SNL	Sham	*
91	SNL	Naïve	***
105	SNL	Sham	*

Female Somatosensory Area (S1) : Low gamma power			
Day	Treatment	_Treatment	Significance
35	SNL	Naïve	*
91	SNL	Naïve	*

Naïve n=12-19
Sham n=15-19
SNL n=16-19

Significance :
*p<0.05
**p<0.01;
***p<0.001;

High Gamma (65-100Hz) Power in Male and Female SNL Rats



Male Prefrontal Cortex : High gamma power			
Day	Treatment	_Treatment	Significance
7	SNL	Sham	*
105	SNL	Naïve	*
119	SNL	Naïve	*

Male Somatosensory Area (S1) : High gamma power			
Day	Treatment	_Treatment	Significance
7	SNL	Naïve	*
91	SNL	Naïve	*
105	SNL	Naïve	*
119	SNL	Naïve	**

Naïve n=12-19
Sham n=15-19
SNL n=16-19

Significance :
*p<0.05
**p<0.01;

SUMMARY AND CONCLUSIONS

- Significant and sustained changes in S1 EEG theta power were observed in female SNL rats as compared to the Naïve and Sham rats.
- In male SNL rats, S1 EEG theta power was significantly different from Sham rats though day 35 but was not sustained through later weeks.
- Theta power in the prefrontal cortex in male SNL rats decreased compared to both Sham and Naïve rats for day 7 following SNL surgery but not during any further days. Theta power in the female prefrontal cortex failed to reach significance.
- Both low and high gamma show some significant changes in the SNL rats, primarily compared to Naïve rats.
- These initial efforts with NIH's PSPP program has provided evidence of an objective electrophysiological biomarker in the EEG of SNL rats that could be useful for screening potential novel compounds for treating pain.

References

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PSPP is currently accepting assets for evaluation
For eligibility and participation inquiries, contact:

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For more information about PSPP, visit (or scan the QR):
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