

AWARD NUMBER: W81XWH-13-1-0377

TITLE: Identifying Molecular Targets For PTSD Treatment Using Single Prolonged Stress

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14. ABSTRACT: Our statement of work proposed that in our first year of funding, we would examine whether SPS disrupts extinction retention by disrupting consolidation and/or retrieval of extinction memory, and/or enhancing fear memory reconsolidation or by disrupting contextual modulation of extinction retrieval. Data collection for all of these experiments, detailed in Specific Aim 1, has been completed and data analysis is currently underway. We proposed that work on Specific Aim 2, in which we examine if SPS enhancement in brain GR and β -AR expression alters glutamatergic and GABAergic function in neural circuits that mediate SPS-induced deficits in extinction retention, would also have been started during the first year and we have completed the behavior part and tissue collection for 5/8 experiments described in this aim. Scoring of behavioral data is underway for these experiments, with molecular assays planned for the coming year. In addition to completing the above, we have begun some of the work proposed in Specific Aims 3 and 4 that was timetabled for later in the funding period. In this report we detail findings from two of these experiments. We feel confident that we have exceeded the goals set out in our statement of work due to the large numbers of experiments for which data collection is complete, and the progress made on Specific Aims 3 and 4 ahead of schedule.					
15. SUBJECT TERMS PTSD, Single Prolonged Stress, Neurobiological Mechanisms					
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1. INTRODUCTION

Post-traumatic stress disorder (PTSD) is a chronic, debilitating psychiatric disorder that can emerge following exposure to a traumatic event. PTSD is characterized by a wide range of symptoms including hyperarousal, avoidance, intrusive memories and abnormalities in fear responses. While currently available therapies have some efficacy, they do not address the full range of PTSD symptoms, and they are not equally effective in all cases. Valid animal models are critical for understanding the neurobiological processes underlying psychopathology, for identification of novel therapeutic targets, and for development of treatment strategies. Over the past decade our laboratory has developed a validated animal model of PTSD, Single Prolonged Stress (SPS), which produces changes in physiology and behavior that are characteristic of PTSD. SPS animals have disruptions in the retention of extinction memories, a specific deficit exhibited by PTSD patients. In addition, we have demonstrated changes in glucocorticoid receptors (GR) in the hippocampus and prefrontal cortex (PFC) of SPS animals; and decreased levels of glutamate in the medial PFC, suggestive of decreased excitatory neurotransmission in a brain region critical for emotion regulation. Moreover, we have recently demonstrated lower spontaneous activity of noradrenergic neurons following SPS, as measured by single cell activity changes and tyrosine hydroxylase mRNA levels in the locus coeruleus. Based on our findings we hypothesize that SPS alters glucocorticoid and beta adrenergic receptor (β -AR) expression leading to changes in protein transcription that modulates glutamatergic and GABAergic function. In turn this could lead to aberrant excitatory and/or inhibitory neural transmission in extinction relevant brain regions leading to the expression of extinction retention deficits. In the proposed research we will test this novel causative model linking trauma exposure to abnormalities in fear memories by evaluating the following specific aims. #1: Determine if SPS disrupts extinction retention by altering extinction retrieval/consolidation, fear reconsolidation, and/or contextual modulation of extinction retrieval. #2: Demonstrate that SPS enhancement of brain GR and β -AR expression alters glutamatergic and GABAergic function in neural circuits that mediate SPS-induced deficits in extinction retention. #3: Demonstrate that pharmacological treatments that act at GR and β -AR, or normalize excitatory neural transmission, prevent SPS-induced extinction retention deficits. #4: Examine candidate vulnerability/resilience factors that interact with SPS exposure and demonstrate their effects on extinction retention and SPS-induced GR (and β -AR) upregulation. The research outlined in this proposal is a comprehensive and systematic examination of the biological basis of PTSD. Using the SPS model we may provide the necessary link between PTSD-specific behavioral changes and alterations in HPA axis, glutamatergic transmission and noradrenergic system activity. Using this foundation we will test novel pharmacological treatment strategies to prevent and reverse stress-induced change, and explore mechanisms of vulnerability and resilience.

2. KEYWORDS

Post-traumatic stress disorder, Single Prolonged Stress, Neurobiological Mechanisms

3. ACCOMPLISHMENTS

a. What were the major goals of the project?

The dates proposed for the completion of work in our statement of work (SOW) proposed a funding date of January 2013, however as the award was not received until Nov 2013, thus all timelines for the expected completion of work has been shifted by one year. For ease of reference, we have included a new column alongside our original proposed dates in the SOW to reflect this altered timeline. The SOW is provided in the Appendix.

In the first year of funding, we proposed that we would begin by completing the experiments outlined in Specific Aim 1. These experiments were designed to examine whether SPS disrupts extinction retention by disrupting consolidation and/or retrieval of extinction memory, and/or enhancing fear memory reconsolidation or by disrupting contextual modulation of extinction retrieval. The proposed timeline for completion of these studies was March 2014 – December 2014 (Specific Aim 1a) and December 2014 – July 2015 (Specific Aim 1b).

In addition, we proposed that work would begin on Specific Aim 2, in which we will examine if SPS enhancement in brain GR and β -AR expression alters glutamatergic and GABAergic function in neural circuits that mediate SPS-induced deficits in extinction retention. Work on Specific Aim 2a was proposed to begin in July 2014 and be completed by June 2015. Work on the rest of Specific Aim 2 (parts b and c) was scheduled to be completed in years 2-5 of the funding period.

b. What was accomplished under these goals?

For Specific Aim 1, data collection from 3 of the 4 experiments has been completed, and scoring and analysis of behavioral data is currently underway. According to the original/adjusted time table the first in (current) report would not include experimental data fully analyzed and ready to present, but we already have completed data collection for 3 of the 4 experiments proposed, and will continue scoring and analyzing these data in the coming months. Given that this work was scheduled for completion by July 2015, we feel very confident that we are making progress toward this goal.

For Specific Aim 2, behavior exposure/testing and brain tissue collection has been completed for experiments 5 of 8 experiments (including some from Aims 2a, b & c). Given that we have carried out behavioral testing and collected brains for 5 out of 8 experiments proposed in Specific Aim, we believe that good progress is being made towards completion of this part of the project. As with Specific Aim 1, during the first reporting period we have been collecting data for these initial experiments, and do not yet have findings to report. We are currently working on scoring and analyzing the behavioral data from these experiments, and plan to complete the associated molecular assays in the coming year. Given that the experiments in Specific Aim 2 were proposed to continue throughout 5 year of funding, and data collection on 5 of the 8 experiments outlined in our application, is underway we feel confident that we have made excellent progress toward our stated goals for work completed in the first year of funding.

In addition to completing data collection for many of the experiments from Specific Aims 1 & 2, we have also made significant progress on the experiments outlined in Specific Aims 3 and 4, which were not proposed to take place until later in the funding period.

For Specific Aim 3 we proposed to examine whether pharmacological compounds that prevent the sensitization of excitatory neurotransmission, administered during the SPS 7-day quiescent period, prevent SPS-induced extinction retention deficits. In order to investigate this we examined the effect of administration of phenytoin (PHE), which blocks voltage-dependent sodium channels, thereby reducing excitatory neural transmission, in the 7 days following exposure to SPS stressors. SPS (n = 24) and control (n = 24) rats were systemically administered phenytoin (vehicle, 20mg/kg, 40mg/kg) once per day for 7 days during the SPS 7-day quiescent period. One day after the last dose, animals were subjected to fear conditioning, fear extinction, and tested for extinction retention. In addition, we examined the expression of glucocorticoid receptors in the medial prefrontal cortex (mPFC), hippocampus (HPC) and

amygdala. We found that fear conditioning and extinction were unaffected by SPS or PHE (Figure 1), but SPS impaired extinction retention and both doses of PHE rescued this impairment (Figure 2). Similarly, SPS increased GR expression in the mPFC and dorsal HPC, and PHE prevented SPS-induced GR upregulation in the mPFC (Figure 3). These data demonstrate that PHE administration can prevent the development of extinction retention deficits and upregulation of GR. PHE exerts inhibitory effects on voltage-gated sodium channels, and decreases excitatory neural transmission via glutamate antagonism. If glutamate hyperactivity in the days following SPS contributes to SPS-induced deficits, then these data may suggest that the glutamatergic system constitutes a target for secondary prevention. These findings have been reported in a manuscript entitled “The effect of chronic phenytoin administration on single prolonged stress induced extinction retention deficits and glucocorticoid upregulation in the rat medial prefrontal cortex”, that was recently accepted for publication in the *Psychopharmacology* DOI: 10.1007/s00213-014-3635-x.

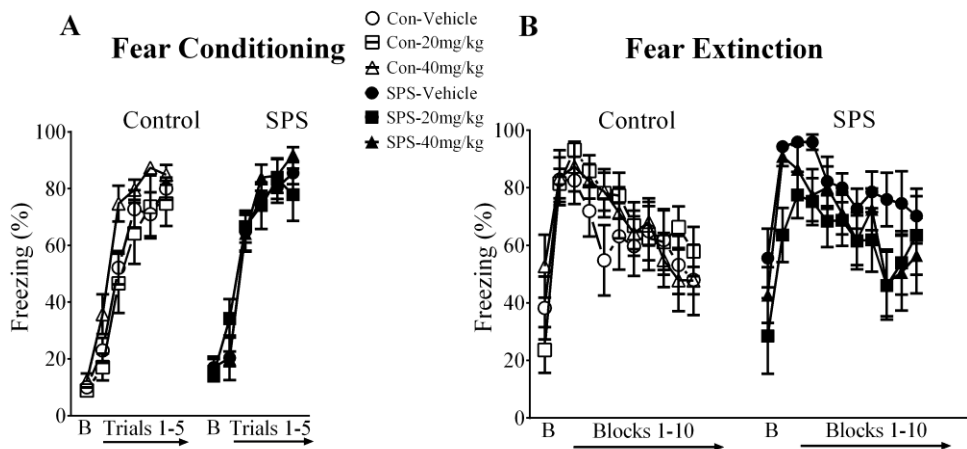


Fig. 1 Single prolonged stress (SPS) and phenytoin (PHE) effects on fear conditioning and fear extinction. A) Neither SPS nor PHE treatment had any effect on fear conditioning or B) fear extinction

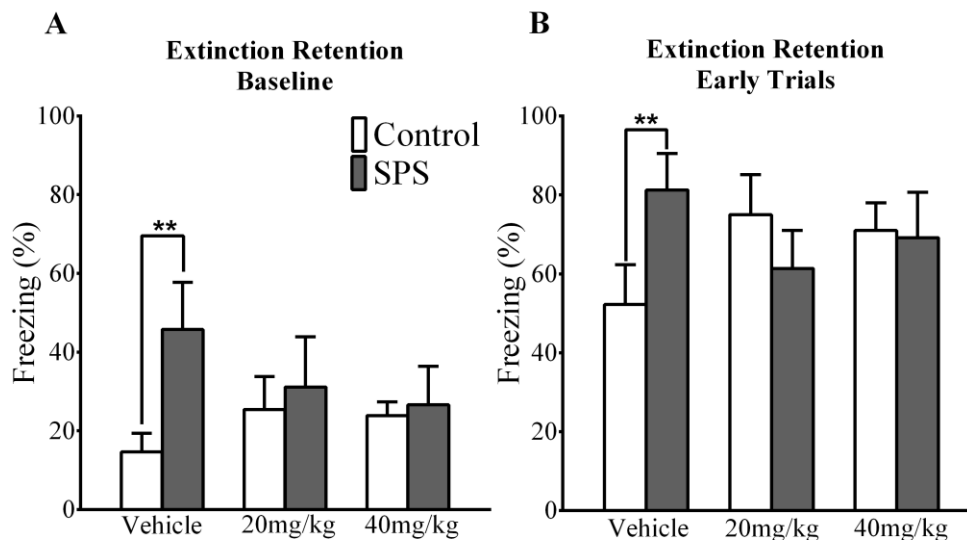


Fig. 2 Single prolonged stress (SPS) and phenytoin (PHE) effects on extinction retention. There was a significant interaction between SPS and PHE treatment during the extinction retention test session. A) During baseline, vehicle treated SPS animals froze more than vehicle treated controls and this difference

was not present following either dose of PHE. B) Similarly, during the early extinction retention trials vehicle treated SPS animals froze more than vehicle treated controls and this effect was not present following drug treatment with either dose of drug. No significant effects were found during late extinction retention trials. Planned comparison $^{**}P \leq .01$

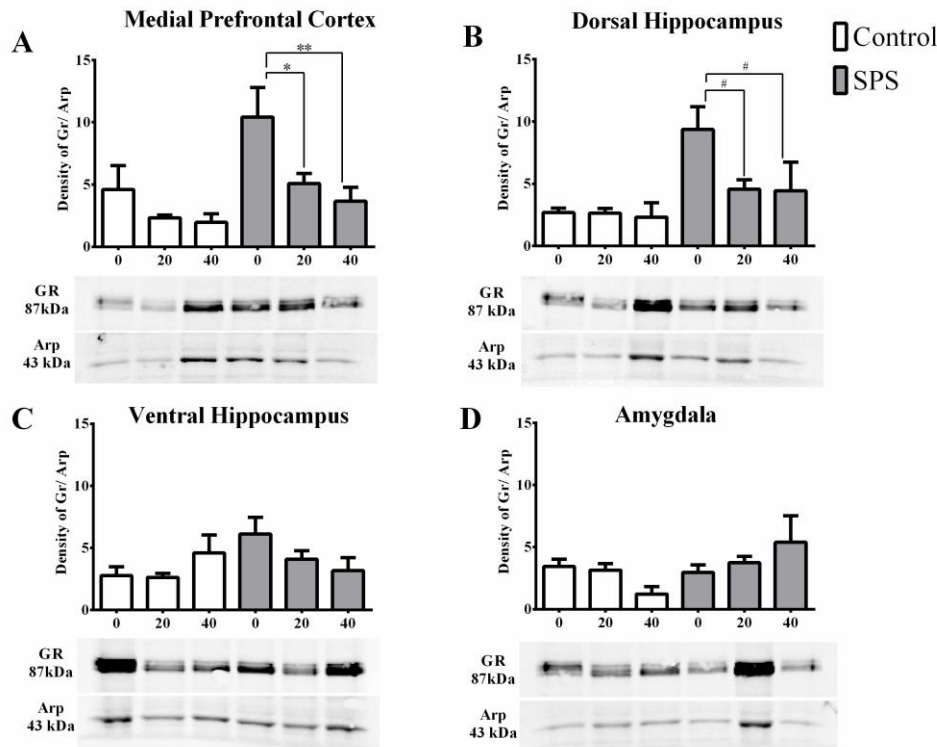


Fig. 3 Single prolonged stress (SPS) and phenytoin (PHE) effects on glucocorticoid receptor (GR) expression. Top panel: GR/actin-related protein (Arp) quantification; Bottom panel: Sample western blot probed for GR and Arp. A) SPS enhanced GR levels in the medial prefrontal cortex and chronic administration of both high (40 mg/kg) and low (20 mg/kg) dose PHE attenuated this, in SPS animals only. B) SPS enhanced GR levels in the dorsal hippocampus (HPC) and both doses of drug partially attenuated this effect. There were no significant effects in ventral HPC or amygdala. Planned comparison $^{\#}P \geq .10$; $^{*}P \geq .05$; $^{**}P \geq .01$

As a follow up to the above mentioned findings, we are now in the process of examining the effects of PHE at the same dosages on behavior and molecular targets when given for seven consecutive days *after* the quiescent period to examine the ability of this drug to reverse as well as prevent SPS-induced extinction retention deficits (experiment 16, as described in our application).

For Specific Aim 4, we proposed to investigate whether cognitive flexibility training, aimed at improving PFC function could be used as a way to attenuate stress-induced prefrontal dysfunction seen in SPS animals. Post-traumatic stress disorder (PTSD) is associated with neurocognitive impairments that have been attributed to functional deficits in prefrontal cortex (PFC). Accordingly, rats subjected to the single prolonged stress (SPS) model of PTSD show PFC-dependent fear associated learning deficits and decreased excitatory neurotransmission in the mPFC. It has been suggested that behavioral interventions that increase cognitive flexibility may attenuate PFC dysfunction. We therefore examined whether cognitive flexibility training (CFT) could prevent the development of extinction retention deficits in SPS rats. 64 male Sprague Dawley rats were exposed to CFT or a control lever training (LT) task. Animals were then matched for performance and subjected to SPS or control procedures. One week later, all rats underwent fear conditioning, extinction and extinction retention testing. One day later SPS animals will be fear conditioned, extinguished, and tested for extinction retention. Brains were harvested and will be

assayed for GR protein at a later date. CFT did not prevent the development of SPS-induced impairments in extinction retention. These findings suggest that pre-trauma CFT effects on enhancing PFC function do not extend to conveying resilience to the effects of SPS (Figure 4).

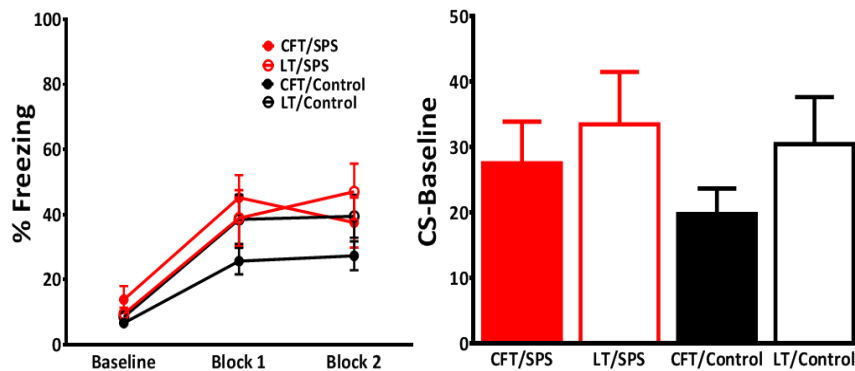


Fig. 4 Ability of Cognitive Flexibility Training (CFT) or Lever Training (LT) control to prevent Single Prolonged Stress-induced extinction retention deficits. All group effects were non-significant suggesting that CFT did not prevent the development of SPS-induced impairments in extinction retention.

c. What opportunities for training and professional development has the project provided?

During the first year of funding a post-doctoral fellow was recruited to join the project. This individual was a recent graduate from PhD training and came with expertise in animal behavioral tests of anxiety. During her first 6 months of work, she has developed her expertise on the disciplines related to the project, under the direction of the PI and Co-I. In the coming year, she will continue to develop the necessary skills to complete the program of work proposed, in particular focusing on learning to perform the molecular assays described in this project under the guidance of our laboratory manager and PI.

The Co-I has also continued her professional development, successfully gaining entry into the highly competitive Career Development Institute for Psychiatry, publishing several papers in peer-reviewed journals (*Biology of Mood and Anxiety Disorders*, 2013, 3,22; *Psychopharmacology*, 2014, DOI:10.1007/s00213-014-3635-x). Dr. George has recently been promoted within the University of Michigan's, Department of Psychiatry to the Research Investigator level.

Additionally, both the Co-I and the post-doctoral fellow working on this project work closely with undergraduate research assistants at the University of Michigan to complete the work described herein. Training for undergraduate research assistants involves them reading/learning background on PTSD and our animal model of this disorder, learning to implement and analyze results from the behavioral tests performed in the laboratory, performing animal husbandry tasks as needed, as well as molecular assays and other bench work.

The Co-I and PI have attended national conferences for continued professional development. In addition, all personnel working on the project attend locally available seminar series for continued professional development, for example The Department of Psychiatry Grand Rounds Series at the University of Michigan.

d. How were the results disseminated to communities of interest?

The findings from Specific Aim 3 and Specific Aim 4 detailed above have been disseminated to the wider scientific community through attendance by the PI and Co-I at national conferences, and presentation of the findings described in this report at those conferences. The Co-I has presented the results described above at the annual meeting for the Society of Biological Psychiatry and American College of Neuropsychopharmacology. Conference abstracts are detailed below in the relevant "Products" section.

e. What do you plan to do during the next reporting period to accomplish the goals?

During the next reporting period we plan to continue with the experiments outlined in Specific Aim 1, and to focus on completing the scoring of data, data analysis and presentation of the findings of the experiments that were conducted during this period. Similarly, we plan to complete the data analysis of behavior and molecular assay of the experiments described in Specific Aim 2 that have been started during this funding period. In addition, we plan to continue with the pharmacological intervention studies outlined in Specific Aim 3 as detailed in our statement of work for the second year of funding.

4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

a. What was the impact on the development of the principal discipline(s) of the project?

At this early stage in the project, we have nothing to report.

b. What was the impact on other disciplines? Nothing to Report.

c. What was the impact on technology transfer? Nothing to Report.

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

5. CHANGES/PROBLEMS:

a. Changes in approach and reasons for change Nothing to report

b. Actual or anticipated problems or delays and actions or plans to resolve them

Funding was not received until one year after the expected funding period therefore the work described herein has been delayed by one year. We have included a revised Statement of Work in the Appendix that shows the original anticipated completion dates for the Specific Aims, and the revised dates following this delay. Despite this delay, we believe we have made excellent progress in accomplishing the aims described in the SOW, completing not only data collection for 3/4 experiments in Specific Aim 1, but a significant number of experiments in Specific Aim 2, and some of the experiments from Specific Aims 3 and 4.

c. Changes that had a significant impact on expenditures Nothing to report

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

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

f. Significant changes in use or care of vertebrate animals. Nothing to report

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

6. PRODUCTS:

a. Publications, conference papers, and presentations.

Conference abstract: George, SA., Rodriguez-Santiago, M., Horvath, AP., Abelson, JL., Floresco, SB., Liberzon, I. Cognitive Flexibility Training and Fear Extinction Retention in the Single Prolonged Stress Model of PTSD. Society for Biological Psychiatry Annual Meeting. New York, NY.

Conference abstract: George, SA., Rodriguez-Santiago, M., Riley, J., Liberzon, I. Chronic phenytoin administration prevents single prolonged stress induced extinction retention deficits and glucocorticoid upregulation. American College of Neuropsychopharmacology Annual Meeting. Hollywood, FL.

b. Journal publications.

George, SA., Rodriguez-Santiago, M., Riley, J., Rodriguez, E., Liberzon, I. (2014) Chronic phenytoin administration prevents single prolonged stress induced extinction retention deficits and glucocorticoid upregulation in the medial prefrontal cortex. Psychopharmacology. DOI: 10.1007/s00213-014-3635-x. published, acknowledgement of federal support - Yes.

c. Books or other non-periodical, one-time publications. None

- d. **Other publications, conference papers, and presentations.** None
- e. **Website(s) or other Internet site(s)** None
- f. **Technologies or techniques** None
- g. **Inventions, patent applications, and/or licenses** None
- h. **Other Products** None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. What individuals have worked on the project?

Provide the following information for: (1) PDs/Pis; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name:	Israel Liberzon
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-4990-556X
Nearest person month worked:	2.4
Contribution to Project:	Dr Liberzon oversaw the completion of all research aims
Funding Support:	

Name:	Sophie George
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	0000-0002-3886-6411
Nearest person month worked:	12
Contribution to Project:	Dr George completed work under Specific Aims 1, 3 & 4
Funding Support:	

Name:	Chieh Chen
Project Role:	Post-doctoral fellow
Researcher Identifier (e.g. ORCID ID):	0000-0001-8480-7669
Nearest person month worked:	7
Contribution to Project:	Dr Chen completed work under Specific Aims 2 & 3
Funding Support:	

b. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Liberzon, PI

Pending Other Support Now Active

Stress Biomarkers: Attaching Biological Meaning to Field Friendly Salivary Measures

NIH R01 MH093486(Abelson, J)

07/01/12 – 6/31/17

Longitudinal MRI study of PTSD development from days to weeks after trauma
NIH subcontract to University of Toledo (Wang) 04/01/13 – 03/31/15

Neurobehavioral Moderators of Post-traumatic Disease Trajectories: Prospective MRI Study of Recent Trauma Survivors Role:
NIMH RO1 MH (Shalev) 04/1/2014 - 03/31/2019

Other support now closed

Using the neuroscience of parental behavior to inform the science of generosity
Research Foundation of SUNY Science of Generosity. University of Notre Dame (Swain)
12/01/10-07/31/12

Mental Health Education Grant (Liberzon)
NIMH R25MH63742 8/01/06-8/31/2012

Neurobiology of disrupted contextual odor discrimination in an animal model of PTSD
US-Israel Binational Science Foundation (Liberzon) 10/1/2008-9/30/2013

Medial Prefrontal Cortex And Hypothalamic-Pituitary-Adrenal Axis Roles, In Generation Of PTSD-Like, Symptoms, In SPS Model
CDMPR DoD PTSD Intramural PTSD Investigator Initiated Research Award
W81XWH-08-1-0661 DoD (Liberzon) 9/1/2008-09/30/2012

Mindfulness and Self-compassion Meditation for Coping with Deployment-Related Posttraumatic Stress Disorder: Randomized Controlled Trial for Combat
Army-DOD (PI: Liberzon) 09/22/2008- 10/21/2012
W81XWH-08-2-0208

George, Co-I

Other support now closed

Psychological modulation of the human HPA stress axis
NIMH R01 MH074852-01 (Abelson, J) 4/01/2007- 3/31/13 NCTX

Medial Prefrontal Cortex And Hypothalamic-Pituitary-Adrenal Axis Roles, In Generation Of PTSD-Like, Symptoms, In SPS Model

- c. What other organizations were involved as partners?**
Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

- a. COLLABORATIVE AWARDS:** n/a
b. QUAD CHARTS: n/a

9. APPENDICES:

Statement of work

Specific Aim #1: Determine if SPS disrupts extinction retention by altering extinction retrieval/consolidation, fear consolidation/reconsolidation, and/or contextual modulation of extinction retrieval.	# Anim als	Original Timeline	New Timeline
1a: SPS disrupts extinction retention by disrupting consolidation and/or retrieval of extinction memory, and/or enhancing fear memory re-consolidation. <i>Approach: Manipulate the times at which SPS is applied relative to fear extinction to isolate the specific memory process altered by SPS.</i>	64	3/1/13 – 11/30/13	3/1/14 – 11/30/14
1b: SPS disrupts extinction retention by disrupting contextual modulation of extinction retrieval. <i>Approach: Examine the effect of SPS on paradigms that probe contextual modulation of memory; fear renewal, spontaneous recovery.</i>	64	12/1/13 – 6/30/14	12/1/14 – 6/30/15
Specific Aim #2: Demonstrate that the SPS enhancement in brain GR and β-AR expression alters glutamatergic and GABAergic function in neural circuits that mediate SPS-induced deficits in extinction retention.			
2a: SPS disrupts glutamatergic and GABAergic function in the neural circuits that mediate SPS-induced extinction retention deficits. <i>Approach: Assay end measures and active processes of glutamatergic and GABAergic signaling in specific brain regions.</i>	80	7/1/13 – 5/31/14	7/1/14 – 5/31/15
2b: SPS disrupts glutamatergic and GABAergic function in neural circuits responsible for extinction retention deficits via a GR-induced genomic mechanism, which is driven by SPS-enhanced GR expression. <i>Approach: Examine the role of GRs in SPS-induced changes in glutamatergic and GABAergic function.</i>	256	5/1/14 – 7/31/15	5/1/15 – 7/31/16
2c: SPS disrupts glutamatergic and GABAergic function in neural circuits responsible for extinction retention deficit by enhanced β -AR expression. <i>Approach: Examine the role of β-ARs in SPS-induced changes in glutamatergic and GABAergic function.</i>	64	8/1/15 – 2/28/17	8/1/16 – 2/28/18
Specific Aim #3: Demonstrate that pharmacological treatments that act at GRs and β-ARs, or normalize excitatory neural processing, prevent SPS-induced extinction retention deficits.			
3a: Pharmacological treatments that prevent/reverse upregulation of GR and β -AR expression will prevent SPS-induced extinction retention deficits. <i>Approach: Administer compounds that act at GRs and β-ARs at select time points following SPS.</i>	384	6/1/14 – 1/31/16	6/1/15 – 1/31/17
3b: Chronic anti-kindling drug administration will normalize excitatory tone and thereby attenuate SPS-induced extinction retention deficits. <i>Approach: Administer anti-kindling compounds at select time points following SPS.</i>	96	2/1/16 – 6/30/17	2/1/17 – 6/30/18
Specific Aim #4: Examine candidate vulnerability/resilience factors that interact with SPS exposure and demonstrate their effects on extinction retention and SPS-induced GR (and β-AR) upregulation.			
4a: Stress exposure in rat “early adolescence” will lead to susceptibility to SPS. <i>Approach: Examine the effect of juvenile stress</i>	32	1/31/16 – 3/31/17	1/31/17 – 3/31/18

<i>(JS) on extinction retention deficit and GR expression.</i>			
4b: Cognitive/behavioral training will lead to resilience to SPS. Approach: Examine the effect of cognitive flexibility training (CFT) on extinction retention deficit and GR expression.	32	1/1/16 – 8/31/17	1/1/17 – 8/31/18
4c: Early adolescent experiences (JS or CFT) induce susceptibility or build resilience to SPS via GR (and β -AR) changes in extinction relevant brain regions. Approach: Examine changes in GR and β -AR expression following early adolescent manipulations.	0	7/1/17 – 11/30/17	