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14. ABSTRACT
The overall goal of the project was to develop an animal model of the focal dystonia benign essential blepharospasm. Consistent with the widely held view that dystonia results from an interaction between a predisposing condition and an environmental trigger, we proposed to use 7 Hz deep brain stimulation of the basal ganglia as the predisposing condition and dry eye as the environmental trigger. We hypothesized that the 7 Hz deep brain stimulation would exaggerate the blink adaptations to dry eye into spasms of lid closure characteristic of benign essential blepharospasm. Our preliminary data last year indicated that there were sex differences in the response to dry eye. This reporting year we completed the data analysis to show clear sex differences in the blink adaptations to dry eye. Our data suggest that dry eye increases the plasticity of female blink circuits relative to males, which provides an explanation of why blepharospasm is more common in females than in males.

Dystonia, benign essential blepharospasm, dry eye, motor plasticity, basal ganglia, deep brain stimulation, eyelids, blinking

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1. Introduction

The focal dystonia benign essential blepharospasm (**BEB**), arises from the convergence of a predisposing condition and an environmental trigger (Hallett et al. 2008). The overall goal of our project is to develop an animal model of BEB based on the hypothesis that hypersynchronized, 7 Hz neuronal oscillations of the basal ganglia create the predisposing condition and that eye irritation from dry eye is the environmental trigger. Our demonstration that hypersynchronized oscillations in the basal ganglia produced by 7 Hz deep brain stimulation of the subthalamic nucleus (**STN DBS**) exaggerate neural plasticity in normal male rats (Kaminer et al. 2014) provides a neural mechanism by which hypersynchronized basal ganglia activity could create a predisposing condition. From our studies showing that dry eye initiates neural plasticity in blink circuits to produce compensatory adaptations in blinking (Evinger et al. 2002; Kaminer et al. 2011; Peshori et al. 2001; Schicatano et al. 2002), we predict that combining 7 Hz STN DBS and dry eye will exaggerate neural plasticity and force the normally compensatory adaptive processes in response to dry eye to transform into the characteristics of BEB, *e.g.*, spasms of lid closure, excessive blinking, and trigeminal hyperexcitability. We propose two Specific Aims to test this hypothesis. The goal of the first Specific Aim is to show that synchronized theta oscillations in the basal ganglia exaggerate plasticity in the cerebellum and the excitability of trigeminal blink circuits as occurs in BEB patients. The Major Tasks to accomplish Specific Aim 1 are: 1) to investigate effects of synchronized basal ganglia oscillations on activity of the deep cerebellar nucleus neurons; and 2) to investigate the effects of synchronized basal ganglia oscillations on the activity of superior colliculus neurons. The purpose of the second Specific Aim is to demonstrate that synchronized 7 Hz oscillations established in the basal ganglia are sufficient to predispose mammals to develop BEB. The Major Tasks to accomplish Specific Aim 2 are: 1) to determine whether combining synchronized basal ganglia 7 Hz oscillations with corneal irritation is sufficient to develop spasms of lid closure and other characteristics of the focal dystonia BEB; and 2) to perform control experiments to determine that theta frequency is critical in enabling the development of spasms of lid closure.

2. Keywords

Dystonia, benign essential blepharospasm, dry eye, motor plasticity, basal ganglia, deep brain stimulation, eyelids, blinking

3. Accomplishments

Major goals of the project

The overarching goal of the planned investigations is to test our hypothesis that hypersynchronized 7 Hz oscillations in the basal ganglia create a predisposing condition that transforms the normally adaptive modifications initiated by dry eye into the spasms of lid closure, excessive blinking, and trigeminal hyperexcitability characteristic of individuals with the focal dystonia benign essential blepharospasm (**BEB**). To test this hypothesis, the 1st Specific Aim of the project is to demonstrate that synchronized 7 Hz (**theta**) oscillations in the basal ganglia exaggerate plasticity in the cerebellum and excitability of trigeminal blink circuits. Major Task 1 of Specific Aim 1 is to investigate effects of synchronized basal ganglia oscillations on the activity of the deep cerebellar nucleus neurons. Major Task 2 of Specific Aim 1 is to investigate the effects of synchronized basal ganglia oscillations on the activity of superior colliculus neurons. The 2nd Specific Aim of the project is to demonstrate that synchronized theta oscillations established in the basal ganglia are sufficient to predispose mammals to develop blepharospasm. Major Task 1 of Specific Aim 2 is to determine whether combining synchronized basal ganglia theta oscillations with corneal irritation is supports the development of spasms of lid closure and other characteristics of the focal dystonia BEB. Major Task 2 of Specific Aim 2 is to perform control experiments to determine that theta frequency is critical for the development of spasms of lid closure.

What was accomplished under these goals?

In this year, we began analyzing the data collected from Major Task 2 of Specific Aim 2 studies from this reporting period and continued data collection and analysis from Major Task 2 of Specific Aim 1.

Specific Aim 2, Major Task 2: Our previous work on our blepharospasm model exclusively used male rats (Evinger 2015; Schicatano et al. 1997). The current experiments, however, utilized both male and female rats. We anticipated that there would be no difference between the sexes before or after creating a dry eye condition by removal of the exorbital gland (Project Narrative, page 7). This assumption was incorrect. There were gender differences in blinking before exorbital gland removal. The threshold electrical current for stimulating the supraorbital branch of the trigeminal nerve to evoke a trigeminal reflex blink was higher for males than for females (Fig. 1B). To determine whether this sexual dimorphism was also present in humans, we reanalyzed data from our previous studies of normal human subjects (Peshori et al. 2001; Powers et al. 2010; Schicatano et al. 2002) to determine the thresholds for SO evoked blinks on the basis of gender. These data revealed that males required a significantly higher electrical current than females to evoke a trigeminal reflex blink (females 2.3 mA ± 0.18 (n=17); males 3.1 ± 0.19 (n=13); $t_{(28)}$ = 3.02, p < 0.01; Fig 2C). The high frequency stimulation (**HFS**) paradigm for depressing reflex blink gain (Mao and Evinger 2001; Ryan et al. 2014) (Project Narrative, page 8) produced a significantly larger gain reduction in male than in female rats (Fig. 2A;). Using data from our human study depressing trigeminal reflex blink amplitude with HFS (Mao and Evinger 2001), we separately considered the gain decrease for males and females. Although the number of subjects was too small to analyze statistically, the trend in these human data were the same as those in rodents (Fig. 2A). The average human gain decrease for the two male subjects was -0.27, whereas the gain change for the three female subjects was a 0.04 gain increase. Finally, the duration of OOemg activity, the length of lid closure (Evinger et al. 1991; VanderWerf et al. 2003) during spontaneous blinks, was longer in female than in male rats (Fig. 3B). Human spontaneous blinking also exhibited a similar sexual dimorphism in blink duration. Using eyelid position data from our previous study of human spontaneous blinking (Kaminer et al. 2011), showed that females exhibited longer duration lid closing during spontaneous blinks than did males for large blinks. Plotting lid closing duration as a function of spontaneous blink amplitude showed that blink duration increased along a power function for females, whereas male blink duration exhibited only small changes in duration as

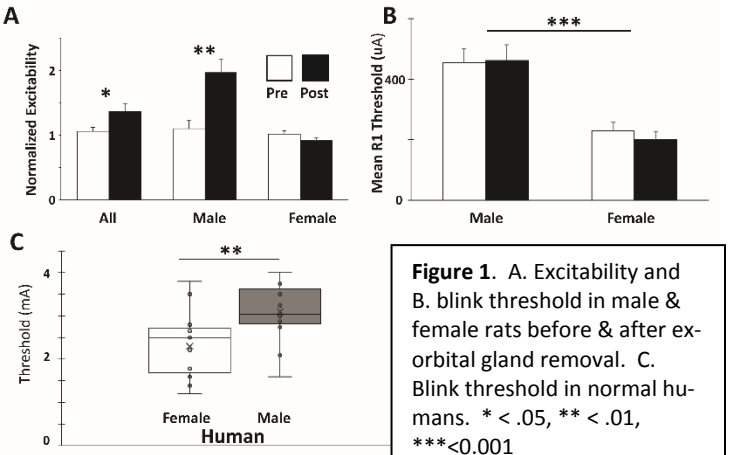

Figure 1. A. Excitability and B. blink threshold in male & female rats before & after exorbital gland removal. C. Blink threshold in normal humans. * < .05, ** < .01, ***<0.001

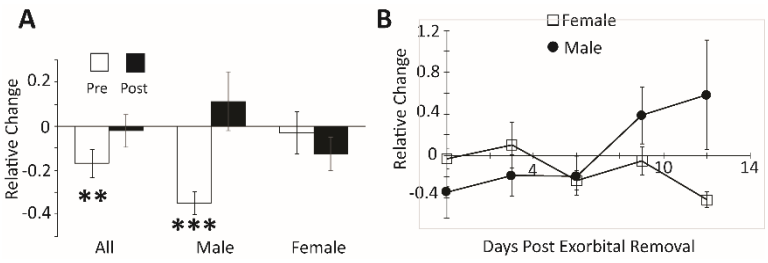

Figure 2. A. Gain changes pre & post exorbital gland removal in male & female rats. B Gain changes as a function of days after exorbital gland removal. ** <0.01, ***<0.001

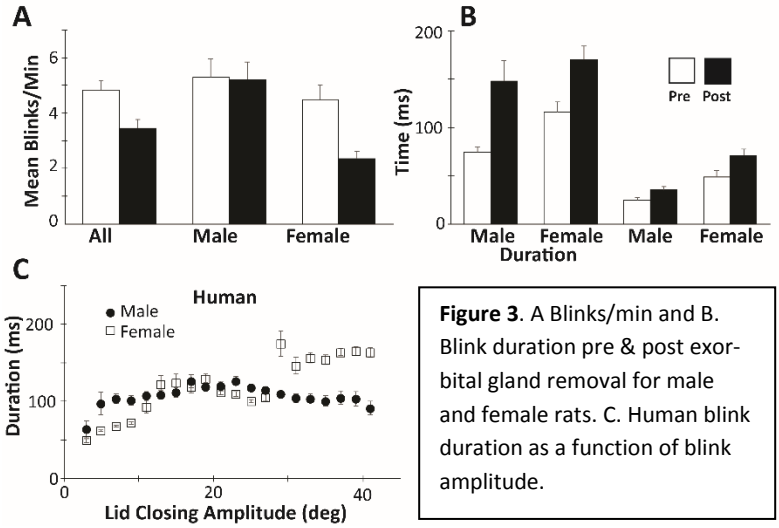

Figure 3. A Blinks/min and B. Blink duration pre & post exorbital gland removal for male and female rats. C. Human blink duration as a function of blink amplitude.

spontaneous blink amplitude increased (Fig. 3C). For spontaneous blinks over 30 deg, lid closure had a significantly longer duration in females than in males ($t_{(5)}$ = -15.8, p<0.00001). Thus, normal rodents and humans exhibited similar sexual dimorphisms in trigeminal reflex and spontaneous blinking.

Dry eye created by exorbital gland removal initiates several adaptations in blinking that minimize breaks in the corneal tear film, which cause dry eye discomfort. Because each blink reforms the corneal tear film by eliminating breaks in the tear film (Himebaugh et al. 2009), the trigeminal system adaptively increases the number of blinks evoked by a trigeminal stimulus (Fig. 4) and elevates the excitability of trigeminal reflex blinks (Fig. 1A). As we previously showed in humans (Evinger et al. 2002; Schicatano et al. 2002), dry eye increases the number of blinks evoked by a single trigeminal blink evoking stimulus, termed blink oscillations. Following exorbital gland removal, blink oscillations increased for all rats, but the increase was significantly larger for male than for female rats (Fig. 4). Blink excitability increased significantly for male rats with exorbital gland removal, whereas females changed insignificantly (Fig. 2A). Studies of humans with dry eye report that both males and females exhibit an increase in the spontaneous blink rate in subjects with dry eye relative to subjects without dry eye (Himebaugh et al. 2009; Nakamori et al. 1997). In contrast, following exorbital gland removal to create dry eye, male rats exhibited the expected increase in spontaneous blink rate, whereas females showed a significant *decrease* in spontaneous blink rate (Fig. 3A).

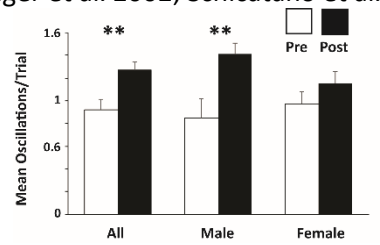

Fig. 4 Blink oscillations per trial before and after exorbital gland removal for male & female rats. **<0.01

A critical component of these sexual dimorphisms with dry eye is what happens with spinal trigeminal complex neuronal plasticity. The HFS paradigm measures this trigeminal blink circuit plasticity (Ryan et al. 2014). Averaged across all days post gland removal, neither male nor female rats showed the significant depression of blink reflex gain present before gland removal (Fig. 2A). This average obscured trends in the data that occurred after gland removal. Plotting gain change as a function of days after exorbital gland removal revealed that gain depression converted to gain potentiation in males, whereas gain depression increased steadily in females (Fig. 2B). Thus, the increased excitability of the trigeminal system created by dry eye disrupted plasticity mechanisms to cause blink potentiation, whereas trigeminal plasticity *increased* in females. Based on our prediction that combining 7 Hz STN DBS and dry eye exaggerates neural plasticity to force the normally compensatory adaptive processes of dry eye to transform into the characteristics of BEB, *e.g.*, spasms of lid closure, excessive blinking, and trigeminal hyperexcitability, our data from the last reporting period suggest that females should be more likely to develop blepharospasm than males. This preponderance of female BEB is exactly what occurs in humans (Asgeirsson et al. 2006; Defazio et al. 1999; Defazio et al. 2001; Hallett et al. 2008). From completing the work of Specific Aim 2, Task 2, we provide the first explanation for a preponderance of blepharospasm in females and underline the importance of investigating neural differences in between males and females in Specific Aim 1.

Specific Aim 1, Major Task 2: As described in the Project Narrative of the grant proposal (page 9), the goal of these experiments is to determine how different frequencies of STN DBS affect the activity of superior colliculus neurons and how changes in collicular activity modify trigeminal reflex blink excitability measured with the paired stimulus paradigm. Predictions about superior colliculus neuronal activity alterations with STN DBS come from the basal ganglia circuit that regulates reflex blink excitability through the superior colliculus (Basso and Evinger 1996; Basso et al. 1996; Gnadt et al. 1997). The substantia nigra pars reticulata inhibits superior colliculus neurons that excite nucleus raphe neurons. In turn, these raphe neurons inhibit spinal trigeminal blink circuits. In the paired stimulus paradigm that measures trigeminal blink excitability, the first reflex blink stimulus (Condition) activates trigeminal blink circuits to evoke the Condition blink and trigeminal complex neurons also send an excitatory drive to intermediate/deep layer collicular neurons. This colliculus neuron activation provides a transient excitatory drive onto the nucleus raphe magnus neurons that inhibits spinal trigeminal blink circuits (Basso and Evinger 1996). Thus, the second reflex blink stimulus (Test) in the paired stimulus paradigm occurs during trigeminal inhibition created by nucleus raphe magnus activation. Because of this inhibition, the Test blink is smaller than the Condition blink even though both are evoked by the same stimulus. We need to increase the number of female rats in these recording experiments to quantify gender differences of neuronal responses.

Changes to SOW timeline
Data analysis for Specific Aim 1, Major Task 2 continues into months 36 – 55 and Specific Aim 1, Major Task 1 data collection and analysis are projected for months 48-60. The new SOW is an appendix.

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

How were the results disseminated to communities of interest?
We are preparing a manuscript for publication on the data from Specific Aim 2 Major Task 2 and will complete a manuscript describing data from Specific Aim 1 Major Task 2 in the coming reporting period.

What are the plans for the next reporting period to accomplish the goals?
In addition to completing and submitting manuscripts from collected data, the work during the next reporting period will focus on finishing analysis of data collected and additional data collection from femlaes on Specific Aim1 Major Task 2, and to begin data collection on Specific Aim 1, Major Task 1 and analysis of those data.

Major Task 2 of Specific Aim 1 (Project Narrative page 9): A manuscript reporting these data is in preparation for submission.

Major Task 2 of Specific Aim 2 (Project Narrative pages 11-12): More data is being collected from female rats to enable analysis of the data based on sex. Initial progress is being made on a manuscript describing these studies.

Major Task 1 of Specific Aim 2 (Project Narrative pages 8-9):
The goal of these experiments is to determine how different frequencies of basal ganglia oscillations modify cerebellar interpositus (**IP**) activity and to correlate these changes with shifts in blink plasticity. As our data demonstrate that 7 Hz STN DBS exaggerates blink plasticity, 16 Hz STN DBS impairs blink plasticity, and 130 Hz STN DBS has no effect on the blink plasticity of normal rats (Kaminer et al. 2014), we will compare IP neural activity across all three frequencies. We will simultaneously record unitary activity and local field potentials (LFP) from blink related IP regions during SO stimulation before, during, and after our

trigeminal reflex blink gain paradigm (Project Narrative, page 8). We will compare the activity of individual IP neurons and LFP before the trigeminal reflex blink gain paradigm to their activity after the trigeminal reflex blink gain paradigm in male and female rats.

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 technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. Changes/Problems

Changes in approach and reasons for change

There were no changes in approach.

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

I have been unable to attract a postdoctoral fellow or a new graduate student to replace the one who graduated. An extensive teaching load in Spring 2019 significantly reduced my research productivity, which prevented me from completing the necessary experiments and data analysis. To increase the number of neurons recorded in each session and thereby speed up data, I am now recording with tetrodes instead of multiwire electrodes in Specific Aim 1.

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

6. Products

Journal publications

Matheis T, Evinger C, Schubert R, Mazzola S, Fels M, Kemper N, Reilmann R, Muratori L. "Biological motion perception in Huntington's disease" J. Huntington's Dis. 1-11, 2019

Books or other non-periodical, one time publications

None to Report

Other publications, conference papers, and presentations

None to Report

Website(s) or other internet site(s)

None to Report

Technologies or techniques

None to Report

Inventions, patent applications, and/or licenses

None to Report

Other Products

None to Report

7. Participants & other collaborating organizations

What individuals have worked on the project?

Name:	Leslie Craig Evinger
Project Role:	PI
Research Identifier:	0000-0002-0039-3348
Nearest Person Month Worked:	12
Contribution:	Experimental design, manuscript preparation, performing experiments, data analysis
Funding Support:	Current Grant

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

None to Report

What other organizations were involved as partners?

None to Report

8. Special reporting requirements

Not Applicable

9. Appendices

SOW

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STATEMENT OF WORK – Month/Day/Year
PROPOSED START DATE September 1, 2019

Site: Stony Brook University
Life Sciences Building
Department of Neurobiology & Behavior
Stony Brook, NY 11794-5230
PI: Leslie Craig Evinger, PhD

Specific Aim 2 (specified in proposal) **Demonstrate that synchronized theta oscillations established in the basal ganglia are sufficient to predispose mammals to develop blepharospasm**	**Timeline**	**Site 1**
Major Task 2: Determine whether combining synchronized basal ganglia theta oscillations with corneal irritation is sufficient to develop spasms of lid closure and other characteristics of the focal dystonia BEB	Months	
Subtask 1: Monitor blink frequency, blink reflex hyperexcitability, and spasms of lid closure in rats during 4 different conditions: 1) No DBS; 2) 7 Hz STN DBS alone; 2) 7 Hz STN DBS and dry eye; and 4) dry eye alone.	1-8	PI
Subtask 2: Data analysis of blink frequency, hyperexcitability, and spasms of lid closure and statistical comparison of data obtained in the 4 conditions.	6-9	PI
Milestone(s) Achieved: Published paper Evinger *J. Neuro-Ophthalmology*, 2015;35:374–379 describing animal model of blepharospasm.	11	PI
Major Task 2: Perform control experiments to determine that theta frequency is critical in enabling the development of spasms of lid closure		
Subtask 1: Repeat Subtask 1 of Major Task 1 using 16 Hz STN DBS or 130 Hz STN DBS.	9-18	PI
Subtask 2: Data analysis of blink frequency, hyperexcitability, and spasms of lid closure and statistical comparison with data obtained in Subtask 1 of Major Task 1.	15-19	PI
Subtask 3: Monitor blink frequency, blink reflex hyperexcitability, and spasms of lid closure in rats during 3 different conditions: 1) No DBS; 2) dry eye alone; and 3) 7 Hz STN DBS and dry eye.	18-24	PI
Subtask 4: Data analysis of blink frequency, hyperexcitability, and spasms of lid closure and statistical comparison with data obtained Subtask 1 of Major Task 1.	22-24	PI
Milestone(s) Achieved: Began work on manuscript describing results of Specific Aim 2 Major Task 2	45-50	PI
Specific Aim 1 **Demonstrate that synchronized theta oscillations in the basal ganglia exaggerate plasticity in the cerebellum and excitability of trigeminal blink circuits**		

Major Task 2: Investigate the effects of synchronized basal ganglia oscillations on activity of the superior colliculus neurons.		
Subtask 1: Record from superior colliculus neurons while rats participate in paired stimulus paradigm and receive 7 Hz, 16 Hz, 130 Hz, or no subthalamic deep brain stimulation.	36-52	PI
Subtask 2: Data analysis of single unit and local field potential data collected in Subtask 1 and correlation of neural activity with blink reflex excitability measures.	40-55	PI
Major Task 1: Investigate effects of synchronized basal ganglia oscillations on activity of the deep cerebellar nucleus neurons.		
Subtask 1: Record from interpositus neurons while rats participate in a blink plasticity paradigm and receive 7 Hz, 16 Hz, 130 Hz, or no subthalamic deep brain stimulation.	48-56	PI
Subtask 2: Data analysis of single unit and local field potential data collected in Subtask 1 and correlation of neural activity with brainstem plasticity measures.	49-57	PI
Milestone(s) Achieved: Submit a manuscript describing data from Specific Aim 1 Major Task 2 and a manuscript reporting data from Specific Aim 1 Major Task 2.	57-60	PI

If human subjects are involved in the proposed study, please provide the projected quarterly enrollment in the following table.

	Year 1				**Year 2**				**Year 3**
Target Enrollment (per quarter)	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**
Site 1									
Site 2									
Site 3									
Target Enrollment (cumulative)									

Note: The Government reserves the right to request a revised SOW format and/or additional information.