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TITLE: Post-Injury Sleep Disruption Alters the Inflammatory Response to Traumatic Brain Injury

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14. ABSTRACT

Background: Traumatic brain injury (TBI) alters baseline neuroendocrine function and the process to restore homeostasis following a stressor. We predict that this altered post-injury stress response is a key mediator in long-term recovery. Sleep disruption (SD) is an environmental stressor that increases neuroinflammation, neuronal injury and loss, and behavioral impairment. To date, the role of post-injury SD is unclear and no studies have considered the interrelationship between TBI, SD, and neuropathology associated with Alzheimer's disease (AD). We provide preliminary data showing that TBI reduces the corticosterone (CORT)-mediated stress response to acute SD in TBI mice. Mice exposed to SD displayed increased neuroinflammatory cytokine expression expressed by microglia/macrophages (CCL2, Trem2, TNF, IL1) and increased leukocyte accumulation in the brain. Finally, post-injury SD increased phosphorylation of microtubule associated protein tau (MAPT, tau) in TBI mice compared to all other groups, which occurred in close spatial proximity to reactive microglia/macrophages suggesting the two events are related. *Hypothesis: SD after TBI impairs neuroendocrine (stress) signaling resulting in increased neuroinflammation, impaired functional recovery, and enhanced neurodegenerative pathology reflecting key features of AD.*

15. SUBJECT TERMS
Traumatic brain injury (TBI), Alzheimer's disease (AD), neuroinflammation, stress, sleep disruption

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1. **Introduction:** Although a TBI occurs in a matter of milliseconds, the biological consequences of a brain injury may last a lifetime. We propose that the brain's stress response to an immune challenge is compromised following a single TBI. As a result, subsequent life stressors that further challenge the immune system worsen long-tern recovery from TBI. Mounting evidence shows that chronic sleep/wake disruption is a significant stressor that promotes inflammation resulting in brain dysfunction and behavioral impairment. Thus, we predict that post-injury sleep disruption (SD) is a significant and understudied stressor that largely influences outcome through inflammatory pathways. The proposed studies will examine the effects of SD after TBI, including the development of AD-like brain pathology in an experimental model. We propose that SD after TBI impairs neuroendocrine (stress) signaling resulting in increased inflammation, impaired functional recovery (behavior) and enhanced neurodegenerative pathology reflecting key features of AD. To date, no studies have examined the effects of post-injury SD pertaining to development of AD-like pathological features. Thus the experiments included in this proposal will provide novel insight to the interrelationship between TBI, sleep/wake, stress, and AD. This project is particularly relevant to high risk TBI populations such as military personnel where sleep/wake disruption is potentially enhanced following return to duty.
2. **Key Words:** traumatic brain injury, sleep disruption, stress, corticosterone, neuroinflammation, microglia, Alzheimer's disease
3. **Accomplishments:**
    a. What were the major goals of the project?
        i. Major Task 1: Approvals; 1-4 months; 100% complete
        ii. Major Task 2: Training of Staff and Equipment Setup; 1-4 months; 100% complete
        iii. Major Task 3: Complete studies for Specific Aim 1, Define the immediate and delayed effects of post-injury SD; 4-12 months; 80% complete
        iv. Major Task 4: Complete studies for Specific Aim 2, Test if glucocorticoid receptor (GR)-mediated signaling influences CORT response to daily post-injury SD; 15-25 months; 20% complete
        v. Major Task 5: Complete studies for Specific Aim 3, Determine if microglia mediate pathological tau accumulation in TBI mice exposed to SD; 22-36 months; 0% complete
    b. What was accomplished under these goals?
        i. Major Task 1: Upon receipt of award notification, we updated our existing IACUC protocol at Ohio State to include all procedures for the proposed experiments. Once these procedures were approved by Ohio State, we submitted the ACURO protocol for review. We received feedback from the ACURO review on October 2, which required us to amend of Ohio State IACUC protocol again. Once those amendment were approved, we submitted a response to the ACURO review on October 22. An updated IACUC approval letter was also provided on November 21. We received ACURO approval to begin experiments on December 18. We began data collection in January 2020. Our SOW allocated 1-4 months for approvals, which was a realistic expectation.
        ii. Major Task 2: We purchased 4 PiezoSleep Chambers for sleep/wake analysis from Signal Solutions. Data collection occurred January-March 2020. Based on recommendations from our collaborators, Drs. Lifshitz and Rowe, we upgraded our PiezoSleep system in Summer 2020. The Adapt-A-Base upgrade included a customized base from Signal Solutions that fits underneath the sleep fragmentation (SF) chambers. This allowed us to keep mice housed in SF chambers. Also, we can generate sleep/wake data continuously for up to 30 DPI. Our SOW allocated 1-4 months for training staff and equipment setup, which was attainable; however, this was extended because of the unforeseen need to upgrade the system. We have been successfully using the Adapt-A-Base system for about one year.
        iii. Major Task 3: To date, we have completed all live animal experiments for Sub Tasks 1-3 resulting in approximately 70% total progress on Major Task 3. Specifically, multiple cohorts of mice were generated for the 14 and 30 DPI time points. Briefly, mice were exposed to daily, transient SF for 14 days. Some cohorts were terminated at 14 DPI (approximately, N = 48 total mice) to study the immediate effects of SF. Other cohorts were returned to standard housing and terminated at 30 DPI (approximately, N = 80 total mice) to study the delayed effects of SF. We have brain tissue from control sham, control TBI, SF sham, and SF TBI mice at both time points. Brain tissue was collected for immunohistochemical, biochemical, and transcriptional analysis. Sleep/wake

behavior was monitored for 30 DPI. During recovery after injury, all mice completed behavioral analysis, including open field, y maze, water maze, and fear conditioning. Final behavioral data are included in the appendix. Now all effort is dedicated to generating data from collected tissue, which will then be organized into figures for future manuscript submission. The SOW included up to 21 months for the first manuscript to be published, which has already passed, There was no way to predict the COVID-19 pandemic at the time of submission, which is ultimately what delayed progress in this project. Nonetheless, we feel confident in the progress made to date and look forward to preparing two manuscripts describing data from Specific Aim 1 in 2022.

iv. Major Task 4: To date, we have accomplished approximately 40% of Sub Task 1. We decided to incorporate jugular catheter mice for Specific Aim 2 dexamethasone studies. Briefly, we purchase mice from Charles River that have a jugular catheter implanted before shipping to Ohio State. Upon arrival, the mice receive sham or TBI following by control housing or exposure to SF. At 7, 14, 21, and 30 DPI half of the mice in each group receive dexamethasone and the other half receive vehicle at 5am. Repeated blood samples are collected at 1 hour, 1 hour 45 minutes, and 2 hours 15 minutes after injection to evaluate plasma CORT. Right now we have approximately N = 2-5/ experimental group. Unfortunately, approximately 40% of mice reach early exclusion criteria before 30 DPI and have to be removed from the study. Therefore, several more cohorts are needed to complete the dexamethasone studies. We recently initiated studies for Sub Task 2 that examine the effects of mifepristone after TBI. These experiments will continue in the next quarter.

c. What opportunities for training and professional development has the project provided?
    i. As expected, new equipment from Signal Solutions provided an opportunity for students and staff in the lab to learn a new software program for sleep/wake analysis. More importantly, though, this equipment really forced us to evaluate our housing space for mice during sleep/wake analysis. Much effort has been spent on ensuring that the sleep/wake data collected is valid and accurate. This required us to run several “test” mice to make sure that environmental stimuli like air flow and vibration did not confound the data being collected.
    ii. Students ran behavioral tests independently, which provided learning opportunities for optimizing open field, y maze, and fear conditioning paradigms. Furthermore, we decided to shift our behavioral testing to the active phase so that we did not unnecessarily disrupt sleep with behavioral testing during the inactive phase. We had many lab discussions on this change, which was very educational for the students and staff.
    iii. We have never worked with jugular catheter mice before; however, they have been a valuable tool in measuring the plasma CORT response to SF. Zach Zimomra has been leading these studies, which requires careful handling of the mice and repeated injections over 30 days. In addition, Zach has to flush the catheters every couple days to ensure patency.
d. How were the results disseminated to communities of interest?
    i. Over the last year, a graduate student in my lab presented data from this project in three virtual poster sessions at Ohio State.
    ii. Houle S, Tapp Z, Cotter C, Zimomra Z, Robertson S, Reyes Y, J, Godbout J, Kokiko-Cochran O.N (2021). “Sleep Fragmentation Following Traumatic Brain Injury Impairs Cognition and Has Lasting Impacts on Sleep/Wake Function” *2021 Institute for Behavioral Medicine Research Day, 2021 Chronic Brain Injury Research Day, 2021 Department of Neuroscience Research Day*
e. What do you plan to do during the next reporting period to accomplish the goals?
    i. Our primary goals center on completing the 14 and 30 DPI projects. Therefore, effort will be spent on completing sleep/wake analysis, immunohistochemistry, and western blot analysis. A sub-group of cortical brain tissue will also be used for NanoString multiplex gene expression analysis.

ii. We will continue working on dexamethasone and mifepristone experiments for Major Task 4, which includes experiments for Specific Aim 2. We expect at 1-2 cohorts for each Sub Task to be completed in the next quarter.
iii. In order to initiate studies for Major Task 5, we need to purchase PLX5622. After receipt of the drug, we will begin Sub Task 1.

4. Impact:

a. What was the impact on the development of the principal discipline(s) of the project?
   i. Nothing to Report
b. What was the impact on other disciplines?
   i. Nothing to Report
c. What was the impact on technology transfer?
   i. We decided to upgrade our PiezoSleep system from Signal Solutions. The company introduced a customizable adapt-a-base system that could be designed to fit any non-standard sized home cage. This is really fantastic for our work because now we do not have to move our mice to a new home cage for sleep/wake analysis. As with any new system, we have had to work closely with both Signal Solutions as well as Lafayette Instruments (the manufacturer of our SF chambers) to ensure the two pieces of equipment are compatible. This optimization will likely influence the way other investigators are taught to use the equipment.
d. What was the impact on society beyond science and technology?
   i. Nothing to Report

5. Changes/Problems:

a. Changes in approach and reasons for change?
   i. As mentioned in previous sections, we upgraded our sleep/wake analysis system. We also expanded the system to be able to analyze 8 animals at once instead of 4. This upgrade will streamline data collection while decreasing stress associated with cage change in our mice.
   ii. We incorporated mice with a jugular catheter to facilitate multiple blood draws in the dexamethasone experiments. This approach allows us to document the plasma CORT response to daily SF for up to 30 DPI. We can document how the stress response changes within any given mouse as well as within an experimental group over time. Because we expect to see a sex-specific stress response, we initiated these experiments with male mice only. Follow up studies can be completed in female mice.
b. Actual or anticipated problems or delays and actions or plans to resolve them
   i. The lab was forced to shut down on March 16, 2020, because of international spread of COVID-19. The Ohio State University as well as the state of Ohio placed strict guidelines on us regarding research and even coming to campus. On March 20, 2020, we were asked to not start any new experiments. On March 23, 2020, the Governor of the state of Ohio initiated a stay at home order through the end of May. Following guidelines for a statewide reopening of businesses, The Ohio State University provided guidelines for returning to campus. All key personnel worked from home until at least June 1, 2020. During that time, all bench work and live animal work was cancelled. We used the time to review the literature, prepare manuscripts for submission at a later time, and analyze the data that had already been generated. The pandemic was beyond our direct control and therefore we could not do anything personally to move the project forward without the support of our state government and University leadership.
c. Changes that had a significant impact on expenditures
   i. The lab has been open since June 2020, but we were forced to follow strict social distancing guidelines through June 2021. For example, limited people could be in a lab space at once, and no undergraduate students were allowed in the lab until June 2021. Lab members were encouraged to work from home when possible and any exposure to a positive COVID-19 case resulted in strict quarantine guidelines. Therefore, even though the lab was open, it was still difficult to make substantial progress. Social distancing in shared spaces made it difficult to schedule days for surgeries and imaging. Fall semester began in August, 2021, at Ohio State. With the COVID-19 vaccine, social

distancing guidelines eased, and lab work has continued to pick up. We are still experiencing some delays due to product shortage and long shipping times. Nonetheless, we remain enthusiastic as moving the project forward and do not anticipate any additional delays. Realistically though, we are probably at least six months behind our expected schedule.

d. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
    i. Nothing to Report
e. Significant changes in use or care of human subjects
    i. Nothing to Report
f. Significant changes in use or care of vertebrate animals
    i. Nothing to Report
g. Significant changes in use of biohazards and/or select agents
    i. The cost of PLX5622 has substantially changed since the proposal was submitted for this project. Our most recent quote was for approximately $20,000, which is about 10x higher than what was originally expected. We will likely have to examine fewer time points in Aim 3 so that less drug is needed.

6. Products:

a. Publications, conference papers, and presentations
    i. Houle S, Tapp Z, Cotter C, Zimomra Z, Robertson S, Reyes Y, J, Godbout J, Kokiko-Cochran O.N (2021). "Sleep Fragmentation Following Traumatic Brain Injury Impairs Cognition and Has Lasting Impacts on Sleep/Wake Function" *2021 Institute for Behavioral Medicine Research Day, 2021 Chronic Brain Injury Research Day, 2021 Department of Neuroscience Research Day*
b. Website(s) or other Internet site(s)
    i. Nothing to Report

c. Technologies or techniques
    i. Nothing to Report
d. Inventions, patent applications, and/or licenses
    i. Nothing to Report
e. Other Products
    i. Nothing to Report

7. Participants & Other Collaborating Organizations:

a. What individuals have worked on this project?

Name	**Project Role**	**Researcher Identifier (era commons)**	**% Effort**	**Contribution to Project**
Olga Kokiko-Cochran	PI	Kokikoo	13	Assisted with data analysis and interpretation; facilitated discussions with collaborators
Jonathan Godbout	Co-I	Godbout2	3	Provided consultation in initial design and interpretation of data collected
Juan Peng	Co-I	JuanPeng	5	Assisted with experimental design; will play a larger role in assistance with data analysis and interpretation as it is generated
Zachary Zimomra	Key Personnel	N/A	30	Performed animal studies and analyzed data
Samuel Houle	Neuroscience	N/A	0	Performed animal studies

	Graduate Student			and analyzed data

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?
   i. I received an Alzheimer's supplement for my R01 from the NIH.
   ii. I received a Diversity supplement for my R01 for the NIH
   iii. My collaborator, Dr. Godbout, received a R01, which includes some salary support for me as a co-investigator.

R01NS109585-02S1 Kokiko-Cochran (PI) 8/15/2020-3/31/2021
Agency: National Institutes of Health/National Institute of Neurological Disorders and Stroke
Title: Characterizing sleep disruption as a post-injury stressor
Goal: This supplement aims to define the role of brain injury-induced neuroinflammation in promoting Alzheimer's disease-like pathology.
Role: PI

R01NS109585-03S1 (Kokiko-Cochran, O.N. Overall PI) 7/1/2021-3/31/2024
Agency: NIH/NINDS, Research supplement to promote diversity
Title: Characterizing sleep disruption as a post-injury stressor
Goal: This supplement aims to support a graduate student in the lab, who is working on the Alzheimer's disease supplement.
Role: mentor

R01NS109954 (Godbout J.G. Overall PI) 4/1/2021-3/31/2026
Agency: NIH/NINDS
Title: Chronic and evolving inflammation after traumatic brain injury: Microglial priming and neuropsychiatric complications
Goal: The goal is to provide consultation in brain injury, inflammation, and behavioral studies.
Role: Co-I

c. What other organization were involved as partners?
   i. Nothing to Report

8. Special Reporting Requirements

a. Collaborative awards
   i. Nothing to Report
b. Quad Charts
   i. Nothing to Report

9. Appendices

Adult C57BL/6 mice were either injured by lateral fluid percussion injury (TBI, n=23), or uninjured (sham, n=22). Following injury, mice were singularly housed in Lafayette Instruments, sleep fragmentation chambers for 30 days post-injury (DPI). TBI and Sham mice either received 5 hours of mechanical sleep fragmentation (SF) daily from 5am-10am for the first 14 DPI and then recovered till 30 DPI or did not receive SF as control (con) groups. This resulted in 4 groups; Sham con (n=10), Sham SF (n=12), TBI con (n=11), and TBI SF (n=12). At 7 DPI mice were tested with Y maze to measure working spatial memory, at 9-14 DPI mice spatial memory and spatial learning were assessed with Morris Water Maze. From 27-29 DPI contextual memory was assessed via contextual fear conditioning.

<u>Figure 1:</u> Post-TBI SF Impairs Spatial Learning and Memory 14 DPI.
Mouse working spatial memory was assessed at 7 DPI via Y maze **(A)**. Groups displayed no differences in total number of arm entries **(B)** or percentage of spontaneous alternations, indicated no effect of TBI or post-injury SF on mouse working spatial memory 7 DPI **(C)**. 9-14DPI mice spatial memory was tested using Morris water maze. TBI SF mice displayed significant impaired learning over the 5 testing days and no differences in swim speed **(D-F)**. Over this testing TBI SF mice used more

non-spatially directed search strategies and did not adapt over the course of the week **(G)**. ($p < 0.05$) (Mean ± SEM).

<u>Figure 2:</u> TBI Impacts Mouse Performance in Contextual Fear Conditioning Paradigm 29 DPI. Mouse contextual memory was assessed via contextual fear conditioning (FC) 27-29 DPI **(A)**. TBI significantly increased the amount of time mice spent freezing during the acquisition phase of FC regardless of prior SF exposure **(B-C)**. During the extinction phase no tone is played to measure mice reactions to reintroduction to the environment from the acquisition day. TBI significantly increased the amount of time that mice spent freezing during the extinction phase of FC regardless of prior SF exposure **(D-E)**. During the retention phase mouse recognition of the cued tone is assessed. All groups froze significantly longer than sham control mice **(F-G)**. Differential freezing on Day 1 of the contextual FC paradigm indicates that TBI mice may remain sensitized to tone-shock pairing even after a period of recovery from SF. ($p < 0.05$) (Mean ± SEM).

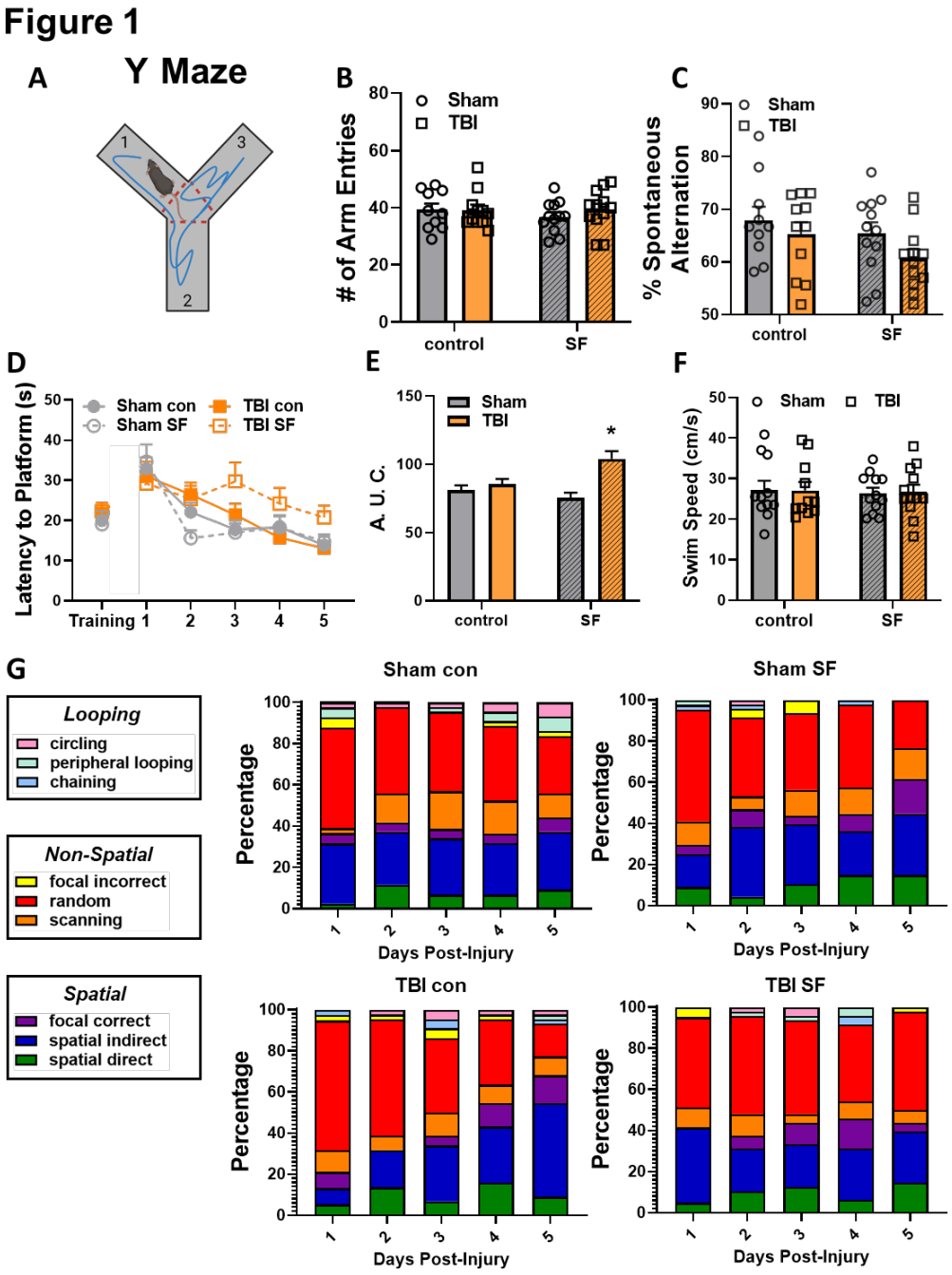
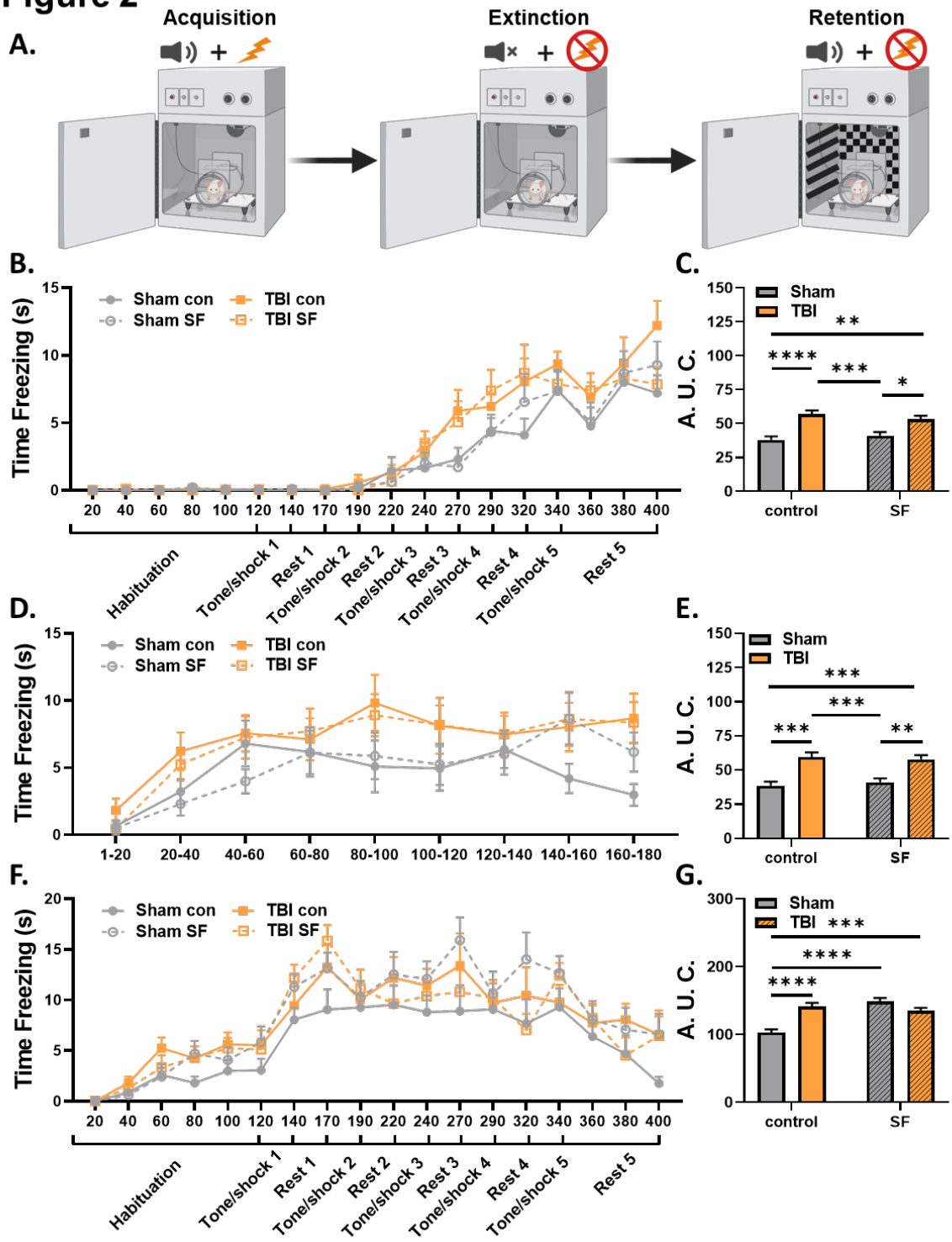


Figure 2


A.
Acquisition
Extinction
Retention
B.
Time Freezing (s)
Sham con
Sham SF
TBI con
TBI SF
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Habituation
Tone/shock 1
Rest 1
Tone/shock 2
Rest 2
Tone/shock 3
Rest 3
Tone/shock 4
Rest 4
Tone/shock 5
Rest 5
C.
A. U. C.
Sham
TBI
control
SF
D.
1-20 20-40 40-60 60-80 80-100 100-120 120-140 140-160 160-180
E.
F.
G.