The Impact of Social Defeat on NF-κB p-65 Activation in Liver: A Study in C57BL/6J Male Mice

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Abstract

An increase in mood and anxiety disorders causes a demand to invent new medications and treatment strategies to reduce neuro-inflammation in the brain and liver. The drug of interest, β-funaltrexamine (β-FNA) has shown to reduce jauntiness behavior in mice from a previous experiment. We currently are determining if Social Stress increases when a different strain or same strain of mouse is introduced into the community of mice. This project, Repeated Social Defeat (RSD) has 2 test groups, Single Bout of Social Defeat and RSD to analyze different stress levels through NF-κB p65 Activation levels. This activation level was determined by using Western Blot Analysis. The Results of this experiment showed that the community of mice had increased stress levels regardless of the strain of mice. This experiment will be footstep for the upcoming implementation of β-FNA) as a therapeutic drug.

Introduction

Background: About 30 million people in the United States suffer from mood and anxiety disorders, thus creating personal and economical strain. Developing new medications and treatment strategies is imperative for those who suffer with these disorders and those who do not respond to available treatments.[3-5] A key component that plays an important role in brain and liver disorders is neuroinflammation. Unfortunately, there are only a few drugs on the market currently that target neuroinflammation. For Example, β-funaltrexamine (β-FNA), a variant of naltrexone, inhibits the activation of astrocytes. [3-7] This has shown to reduce the jauntiness behavior caused by exposure to a bacterial product[7-8]. The interest of this study is to seek the therapeutic relevance of β-FNA as a neuroprotective agent in cases of extreme social stress or bullying.[3] An efficient model for implementing extreme social stress is the Repeated Social Defeat (RSD). This involves in a community of mice (C57BL/6J) being disturbed by adding an aggressor or "bully" mouse that is larger and darker than the community of mice.[7] Also, another group of mice received only a single bout of social defeat. They were used as a control to refer if a different strain of mouse causes a higher level of stress than an addition of the same strain.

Purpose: The overall purpose of this experiment is to identify the effects of RSD on neuroinflammation, and develop an experiment for β-FNA in relation to neuroinflammation.

Analysis: Western Blot Analysis[1] was executed to identify NF-κB p65 levels in the liver of mice that underwent social defeat[8]. Mice, Both Novel C-57 and Aggressor, which were exposed to a single bout of social defeat, had higher levels of p65 when comparing to the control mice p65 levels. Also, the RSD Mice expressed similar results as the Single Bout of Social Defeat Mice.

Conclusion: This highlights that the community of mice had extreme stress when any mice was added to their community regardless of strain, color, or aggressiveness. We hypothesize that these results and experiments will be helpful and informational to attain neuroinflammation drugs for therapeutic purposes.

Methods & Materials

- 48 Male C57BL/6J Mice (6 Weeks Old) (Jackson Lab) were separated into 3 testing groups (Control, Novel, and Aggressor) and allowed to acclimate to the new environment.
- Novel C-57 Mice: Any Mouse that is from the same species (C57BL/6J) of the testing mouse but, from a different testing environment.
- Aggressor Mice: A Different strain of Mice (CD-1) were used to Model as a "Bully" (Retired Breeding Male) and shifted into the testing environment with C57BL/J6 Mice.
- Different Types of Treatment
  - ***Control:*** A group of mice were sacrificed after 2 hour bout within the same community.
  - ***Single Bout:*** A group of mice were sacrificed after a 2 hour bout of social defeat and interactions with a relative mouse (same species).
  - ***Repeated Social Defeat:*** A group of mice were sacrificed after 12 hours after the final bout of 2 hour bouts of social defeat for 6 days and interactions with a foreign aggressive mouse (different species).
- Liver Tissue Samples were collected, Homogenized, and then stored at -80 °C
- Western Blots
  - Using the BCA Assay (Thermo Scientific Pierce Reagent A (Ref#23228), Pierce Reagent B (Ref#23224)) Total Protein Concentration was determined and Samples were prepared (50µg/25 µL).
  - After 7.5% Acrylamide Gels (Bio-Rad) were molded, Proteins were loaded into wells and separated by electrophoresis, transferred to membrane, and blocked with 5% BSA.
  - Antibodies need to be prepared and labeled to be applied onto the Membrane (PDVF).
    - Phospho-Related p65 (Cell Signaling) (Ref#3033)
    - Total-p65 (Cell Signaling) (Ref#4764)
    - ß-Tubulin - Loading Control (Cell Signaling) (Ref#2146)
  - Membranes need to be coated with ECF Substrate (GE Healthcare Amersham ECF Substrate) (Vendor#RPN-5785) and then imaged.(Typhoon Scanner).
- IQTools: Used to convert .gel files to .tif files.
- Adobe Photoshop and ImageJ (NIH Resource: Free): Used to obtain the integrated density values for the bands of interest.
- GraphPad Prism (Version 8): Used to obtain visual aid and identifying significant values by completing various tests (ANOVA, Tukey, Kruskal-Wallis, and Unpaired-T-Test).

Schematic of Social Defeat

Test Group 1

Single Bout

Sacrificed after 2 hours of bout.

Test Group 2

Repeated Social Defeat

Sacrificed after 12 hours after final 2 hours of bout for 6 consecutive days.

Figure 1.
C57BL/6J mice were separated into 3 testing groups and allowed to acclimate to their new environment. Also, A CD-1 Mouse is Introduced as a "Bully" to the C57Bl/6J Mice.

Results

Single Bout of Social Defeat

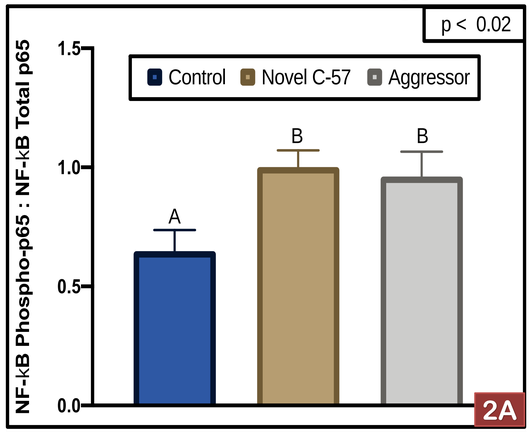

Figure 2A.
The Effect of NF-κB p-65 Activation (Single Bout of Social Defeat) Determined By Using Western Blots.

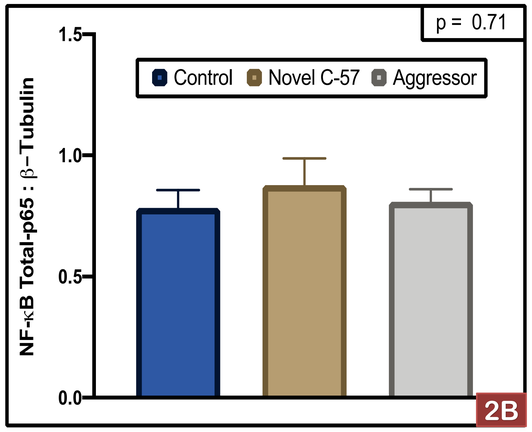

Figure 2B.
The Effect of NF-κB p-65 Expression in Single Bout of Social Defeat. Determined By Using Western Blots.

Repeated Social Defeat

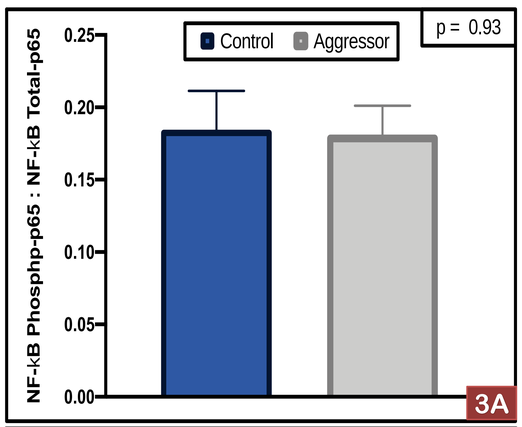

Figure 3A.
The Effect of NF-κB p-65 Activation in Repeated Social Defeat. Determined By Using Western Blots.

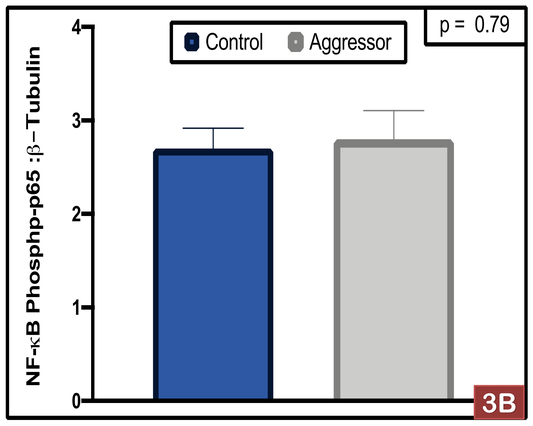

Figure 3B.
The Effect of NF-κB p-65 Expression in Repeated Social Defeat. Determined By Using Western Blots.

Conclusion

As expressed in figure 2A, the addition of the novel C-57 and the aggressor in the single bout of social defeat had an increase in NF-κB p65 activation, thus an increase in inflammatory signaling. On the other hand, the activation levels in the repeated social defeat aggressor did not increase as significant as the single bout for social defeat.

- These results indicate that when an unfamiliar mouse is introduced into an established community, the induced stress leads to an increase in inflammatory signaling.
- This also shows that the amount of time the mice are interacting with the other mouse has no effect on the stress level of the residing mice community.
- A drug with anti-inflammatory capabilities such as the µ-opioid antagonist ß-FNA, could be characterized as a therapeutic treatment for mood and anxiety disorders.

Future Directions

1. Investigate expressions levels with other Relative Inflammatory Mediators.
2. Investigate expression values of NF-κB-p65 immediately after the final bout of Repeated Social Defeat.
3. Investigate Inflammatory Mediators in the Central Nervous System (CNS) and the Peripheral Nervous System (PNS)
4. **Investigate The Action Of ß-Funaltrexamine (ß-FNA), A µ-Opioid Antagonist That Previous Studies Have Shown Inhibits Neuro-Inflammation**
5. Repeat Previous Experiment with Female Mice (NF-κB-p65) under same conditions.

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