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Exploring the Link Between Genetics, Chronic Stress, and Depression

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EXPLORING THE LINK BETWEEN GENETICS, CHRONIC STRESS, AND DEPRESSION

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ABSTRACT

Depression is a very debilitating mental illness that affects about 7% of the American Population [1] and up to about 350 million people worldwide [2]. Since the cause for depression and the reason why some individuals are more vulnerable than others are currently unclear, studying paradigms that model depression in animals, such as the learned helplessness paradigm, is useful to explore possible mechanisms and devise new treatments. To explore a possible link between genetics, chronic stress and depression, we have exposed mice vulnerable to stress to an inescapable forced swim paradigm. During the forced swim test, the mice were monitored to see how "helpless" they were via monitoring and recording movement. Afterwards, mice brains were collected, sliced and stained to assess neuronal activity and examined under a microscope. Increased immobility and reduced neuronal activation was observed in mice vulnerable to stress compared to their control littermates. Further quantification of neuronal activation will inform about brain regions responsible for the learned helplessness model of depression.

INTRODUCTION

Depression affects about 7% of the American Population [1] and up to about 350 million people worldwide [2]. Several studies have attempted to find genetic associations with depression in human populations [3]. A strong association between chronic uncontrollable stress and depression has been found [4].

In order to assess the effects of genes and stress in mice we have used genetically engineered mice in a learned helplessness paradigm.

cFos is a gene that is immediately expressed upon neuronal activation (Immediate Early gene). Comparison of cFos positive neuron numbers in similar brain regions in genetically modified (HET) and control mice (WT) can give insights into the brain regions responsible for the depressive phenotype [5]. Certain areas of the brain have been shown to be of higher interest in relation to learned helplessness, including: the medial prefrontal cortex, dorsal hippocampus, and the amygdala [6].

METHODS

- Male mice were genotyped via PCR from tail biopsies and split into two groups, HET (n=10) and WT (n=8) littermates .
- The forced swim test was modeled after Stone and Lin (2011) [7].
  - Each mouse was placed into a 44 x 24 x 21 cm tank, filled about 10 cm high with water at 32-34ºC.
- Mice were forced to swim for 15 minutes a day for a total of four days, and immobility time was recorded during these trials.
- On the fourth day, an hour and half after the mice finished the trial, they were deeply anesthetized and perfused with paraformaldehyde to preserve the brains.
- The brains were removed and sliced at 50um.
- Areas of interest were stained using an antibody against the cFos protein as described in Buhusi et al., 2016 [8].
- Stained brain slices were examined using a confocal microscope in order to tell where the cFos+ neurons are present and how many of neurons were activated.
- Images of specific brain regions were acquired and examined in photoshop to count the number of cFos activated cells. This is done by dividing pictures into square grids and counting number of activated cell per square.

RESULTS

Behavioral results:

Current results from the forced swim test show that HETs show more immobility time WT mice (Figure 1) (HET n = 10; WT n =8). T-test analysis of percent immobility of WT and HET mice revealed a p-value of 0.099, therefore the results are not statistically significant. More mice will be tested in the future in order to increase statistical power.

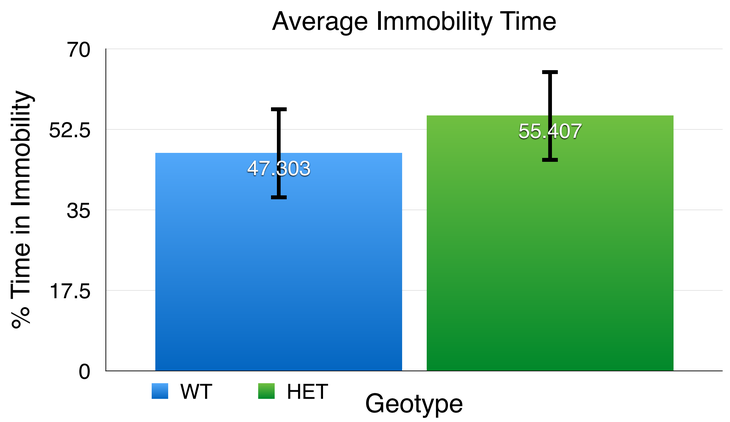

Figure 1

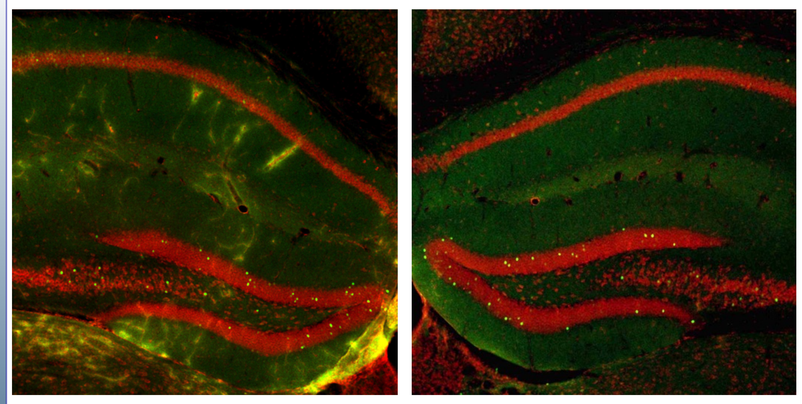

Figure 2. cFos expression (green) in the Mouse Dentate Gyrus during the Forced Swim Paradigm. Neurons are labeled red using Neurotrace.

cFos Activated Cells:

We have completed the analysis of cFos immunostaining in the dorsal hippocampus. Images were captured on a Zeiss 710 confocal microscope. cFos positive cells from the upper lip of the Dentate Gyrus of the mouse Hippocampus were counted (2 images per animal). The average activated cells in HETs was found to be 45.8 while in WTs 37.3 (Figure 2). A 2-tailed t-test was done on the two groups mice and found a p-value of 0.32. Therefore, there was no significant difference between the two genotypes in neuronal activation in the dentate gyrus region. Other sections of the brain, including the amygdala and the orbital frontal cortex, are currently being counted and analyzed.

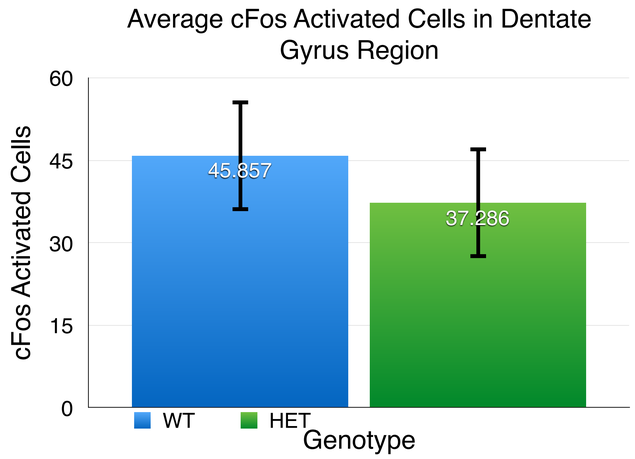

Figure 3

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REFERENCES

[1] Facts & Statistics. (2017). Retrieved October 06, 2017, Retrieved from https://adaa.org/about-adaa/press-room/facts-statistics.

[2] Pietrangelo, A. Depression and Mental Health by the Numbers: Facts, Statistics, and You. (2015). Retrieved October 06, 2017, Retrieved from https://www.healthline.com/health/depression/facts-statistics-infographic#1.

[3] Lin PY and Tseng P. Decreased glial cell line-derived neurotrophic factor levels in patients with depression: A meta-analytic study Author links open overlay panel. (2015). Journal of Psychiatric Research, 63, 20-27. Retrieved September 02, 2017, from http://www.sciencedirect.com/science/article/pii/S0022395615000369?via%3Dihub.

[4] Abelaira HM, Réus GZ, Quevedo J. Animal models as tools to study the pathophysiology of depression. (2013). Scieno Analytics, 35(2). Retrieved April 11, 2018, from http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-44462013000600007&lng=en&nrm=iso&tlng=en.

[5] Gaszner B, Kormos V, Kozicz T, Hashimoto H, Reglodi D, Helyes Z. The behavioral phenotype of pituitary adenylate-cyclase activating polypeptide-deficient mice in anxiety and depression tests is accompanied by blunted c-Fos expression in the bed nucleus of the stria terminalis, central projecting Edinger–Westphal nucleus, ventral lateral septum, and dorsal raphe nucleus. (2012). Cognitive, Behavioral, and Systems Neuroscience, 202, 283-299. Retrieved April 11, 2018, from https://www.sciencedirect.com/science/article/pii/S0306452211013339.

[6] Silva M, Aguiar DC, Diniz CRA, Guimarães FS, Joca SRL. Neuronal NOS Inhibitor and Conventional Antidepressant Drugs Attenuate Stress-induced Fos Expression in Overlapping Brain Regions. (2011). Cellular and Molecular Neurobiology, 32(3), 443-453. Retrieved October 08, 2017, from https://link.springer.com/article/10.1007%2Fs10571-011-9775-1.

[7]Stone E and Lin Y. Open-Space Forced Swim Model of Depression for Mice. (2011). Current Protocols in Neuroscience. Retrieved September 09, 2017, from http://onlinelibrary.wiley.com/doi/10.1002/0471142301.ns0936s54/abstract.

[8] Buhusi M, Olsen K, Yang BZ, Buhusi CV. Stress-Induced Executive Dysfunction in GDNF-Deficient Mice, A Mouse Model of Parkinsonism. (2016). F7ontiers in Behavioral Neuroscience. 10m 114. Retrieved October 05, 2017, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4914592/.