DOES EXPOSURE TO CHRONIC STRESS IN RODENTS ALTER THE LEVEL OF SIRT1 IN THE NUCLEUS ACCUMBENS?

A thesis submitted to the University of Arizona College of Medicine – Phoenix
in partial fulfillment of the requirements for the Degree of Doctor of Medicine

Sandy Peoples
Class of 2020

Mentor: Deveroux Ferguson, PhD

Does exposure to chronic stress in rodents alter the level of SIRT1 in the nucleus accumbens?

Sandy Peoples, B.S., University of Arizona-College of Medicine-Phx
Deveroux Ferguson, Ph.D., University of Arizona-College of Medicine-Phx

Email: sandypeoples@email.arizona.edu

Abstract

In this study we show that chronic social defeat stress, an ethologically validated model of depression in mice, stably induces SIRT1 levels in the nucleus accumbens, an area of the brain that is associated with motivation and reward. We exposed rodents to chronic social defeat stress for a period of ten days and then assessed the rodents on a social interaction test to determine stress response. Rodents were then classified as susceptible or resilient to chronic stress. SIRT1 mRNA and protein levels were then measured in the nucleus accumbens. Results showed that SIRT1 mRNA and protein levels were increased in susceptible rodents but not control or resilient rodents. This supports our hypothesis that SIRT1 levels are associated with depression and anxiety-like behaviors induced by chronic stress and may identify a novel signaling pathway for the treatment of major depressive disorders.

Significance Statement

Current antidepressant medications such as monoamine oxidase inhibitors (MAOIs) and selective serotonin reuptake inhibitors (SSRIs) function by targeting the inhibition of serotonin, norepinephrine and/or dopamine reuptake in the brain.[15] Although there are a variety of pharmacological treatment options, they do not always work quickly or effectively. According to Slattery " approximately 30-40% of patients do not respond to any therapy."[14] Therefore there is a great need to find new and better therapeutic targets for treating depression and anxiety. If SIRT1 can be proven to play a role in the NAc related to anxiety and depression-like behaviors a novel signaling pathway could be introduced as a target for the development of antidepressant drugs.[16]

Introduction

SIRT1

Sirtuins (silent information regulator 2) related enzymes are responsible for a variety of functions in the mammalian brain.[1] They are histone deacetylases or ADP-ribosyl transferases. While initially their function was thought to be mostly in transcription silencing, it has been discovered that they also function to deacetylate nonhistone proteins- greatly increasing their scope of impact.[2] They are regulator proteins that are thought to play a role in certain types of stress responses.[3] SIRT1 is found in the cell nucleus and functions in metabolism, inflammation and neurodegeneration. It has been associated with p53 (a tumor suppressor protein)- over-expression of SIRT1 has been shown to inhibit p53 and retard muscle differentiation. SIRT1 also targets PGC-1alpha which is the master regulator of mitochondrial biogenesis. SIRT1 may also serve a role in protecting axons in the nervous system.[1]

SIRT1 has been linked to Major Depressive Disorder in a 2015 study published in Nature. Low-coverage whole-genome sequencing of 5,303 Chinese women with recurrent Major Depressive Disorder identified two loci contributing to risk of MDD on chromosome 10; one was located near the SIRT1 gene.[19]

Nucleus Accumbens

The nucleus accumbens (NAc) is located in the ventral striatum and is responsible for, among other things, motivation and reward in the vertebrate brain.[2] There are many neurotransmitters that function within the NAc, including serotonin, dopamine, and GABA. Due to it being a connection between the limbic and motor systems the NAc is thought to be a component in survival and reproduction drives. The NAc is also thought to regulate positive emotional responses in the brain.[4] The NAc receives input from the ventral tegumental area, the substantial nigra, the subiculum, amygdala, hippocampus, thalamus, pre limbic and prefrontal cortex which are brain regions implicated in depression and anxiety. For example, the hippocampus has a high density of stress hormone receptors and research shows that stress inhibits neurogenesis in this area of the brain. Inhibited neurogenesis has been implicated in the pathogenesis of depression

and anxiety.[5] Additionally, the amygdala plays a role in the conditioning of fear responses, associating non threatening stimuli with threatening stimuli when presented at the same time.[6]

Chronic Stress

Stress is a normal biological response to certain environmental factors. Stress is beneficial at times and can promote adaptation to a changing environment. However chronic stress has been shown to lead to what McEwen[7] refers to as "allostatic load." He goes on to state that "allostatic load leads to impaired immunity, atherosclerosis, obesity, bone demineralization, and atrophy of nerve cells in the brain." These are all processes that are seen in depression and anxiety disorders.[7]

Animal models of disease

To study how chronic stress influences the development of depression, it is critical to have validated animal models for depression.[8] Three methods to develop animal models of depression are currently used: "(1) genetic manipulation, (2) selective breeding for behavioral extremes, and (3) environmental, physical or pharmacological manipulations or a combination of the above."[9] For this study the third method is utilized by using chronic social defeat stress, which is an ethnologically validated model of depression in rodents.[10,11,12] Conditioned behavior in these rodents is then measured with behavioral tests that have been designed under the premise that the basic physiological mechanisms underlying fear in animals, in this case rodents, and humans are similar. Some examples of these tests are the open field test, the elevated maze test, the light-dark exploration test, the social interaction test and the forced swim test, a task designed to measure features of anxiety and depression in humans.[13]

Materials and Methods

This is a randomized controlled trial where rodents are exposed to chronic social defeat stress in ten minute increments for a period of ten days and then observed and measured on several tests. The nucleus accumbens (NAc) is then harvested and SIRT1 mRNA and protein levels are tested at 48 hrs and ten days following harvestation.

The rodents tested in this study are male C57BL/6J mice at 7-9 weeks old obtained from Jackson Laboratory. They will be housed on a 12 hour light-dark cycle with free access to food and water. They will be acclimated to the facility for one week prior to experimentation. The aggressor species is male CD1 retired breeder mice at 9-13 months old obtained from Charles River Laboratories. "All animal procedures were approved by Mount Sinai School of Medicine and University of Arizona Medical School Institutional Animal Care and Use Committees."[16]

The test mice will be exposed to an aggressive unknown CD1 retired breeder mouse for ten minutes per day for up to ten days. After this exposure the test mouse will remain in the cage but will be separated from the breeder mouse by a barrier that allows sensory but no physical

exposure to the aggressor mouse. A sub-maximal social defeat paradigm will also be performed to test whether an experimental manipulation may change the animal's susceptibility to stress. In the sub-maximal defeat test the animal is exposed to the CD1 aggressor for five minutes followed by a fifteen minute break. This is repeated two more times with a different CD1 aggressor each time. After the last social defeat interaction the test mice will be observed and measured in the open-field test to measure stress response and divided into susceptible or resilient phenotypes.[17]

In the *open-field test* the mice are exposed to the CD1 retired breeder in a cage and EthoVision video tracking based methods (Noldus) are used to record how much time was spent by the mice in different areas of the enclosure.[16]

Control mice will be housed in the same conditions but with animals of the same strain to replicate normal interaction. They will then be measured on the same tests as the test mice before NAc collection.

After the animals have undergone social defeat and social interaction testing the animal will be euthanized and the NAc will be harvested. mRNA and protein levels will be measured at 48 hours and ten days using the following methods:

Immunoblotting (also known as western blot) will be used to measure NAc tissue protein levels.[18]

Immunohistochemistry also measures tissue proteins levels but is performed on tissue sections thereby allowing determination of the topographical expression profile of a protein.[18]

SIRT1 activity assay will be performed with a fluorescent assay kit (Cayman) according to the manufacturer's instructions.

RNA isolation and PCR[18]

Chronic stress is expected to induce SIRT1 mRNA levels in the NAc of susceptible mice 48 hrs after the last social defeat. Levels are expected to remain elevated ten days later. Increased SIRT1 protein expression in the NAc of susceptible mice at both 48 hrs and ten days is also expected. Changes are not expected in the levels of SIRT1 mRNA and protein expression in the the NAc of resilient animals at 48 hrs or ten days. Also no difference is expected in SIRT1 mRNA levels in the NAc after sub-maximal defeat.

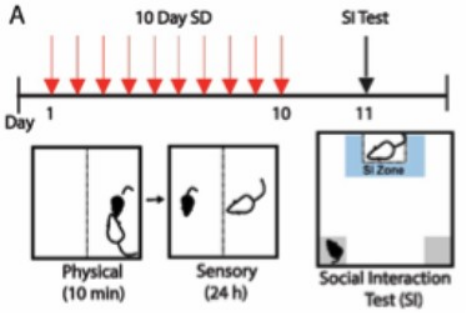
A one way Anova will be performed to determine significance. 27 animals will be needed in the control group and the test group to detect a 25% difference in protein expression for 80% statistical power with an alpha of 0.05. Software used is Stata version 14 (College Station, TX).

Results will be processed by Graphpad (Graphpad Software, Inc).

Results

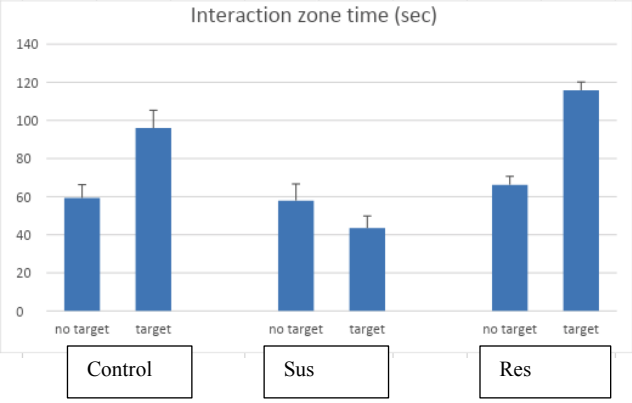
Male C57BL/6J mice rodents were first subjected to chronic social defeat stress by male CD1 retired breeder mice for a period of ten days (Figure 1). They were then measured on a social interaction test (open field test) to determine stress response. This response determined whether the mice would be classified as susceptible or resilient. A control group not exposed to social defeat stress was also measured on the social interaction test.

Figure 1



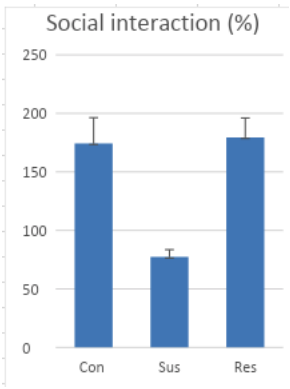
We then used a Wilcoxon Signed-ranks test to determine statistical significance of the time spent interacting with the target in the social interaction test (Figure 2). In the control group the interaction time spent with the target was statistically higher than with no target ($Z = -2.380$, $p < 0.0173$). In the susceptible group the interaction time spent with the target was statistically lower than with no target. ($Z = 2.366$, $p < 0.0180$). In the resilient group the interaction time spent with the target was statistically higher than with no target. ($Z = -2.201$, $p < 0.0277$)

Figure 2



We then wanted to determine whether there was a difference between the percentage of time spent in the social interaction zones between the control, susceptible and resilient groups. We observed a significant difference in the percentage between the control and resilient groups versus the susceptible group (Figure 3, $F=12.913$, $p = 0.0016$). Mean ± SEM

Figure 3



We then used real-time PCR to measure SIRT1 mRNA expression in the nucleus accumbens 48 hours after the last social defeat episode. We also used immunoblotting to measure SIRT1 protein expression for the same time period.

We observed that chronic social defeat stress induced SIRT1 mRNA levels in the NAc of susceptible mice at 48 hours (Figure 4, F= 9.75, p = 0.001) Mean ± SEM, and 10 days (Figure 5, F = 5.99, p = 0.01) Mean ± SEM. No change was seen in resilient mice.

Figure 4

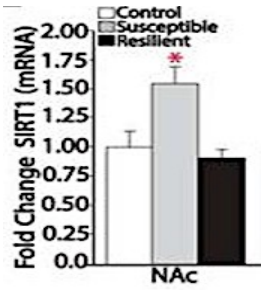
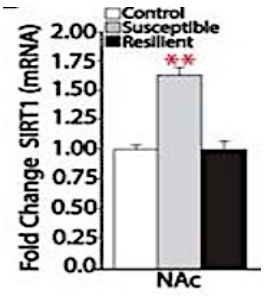
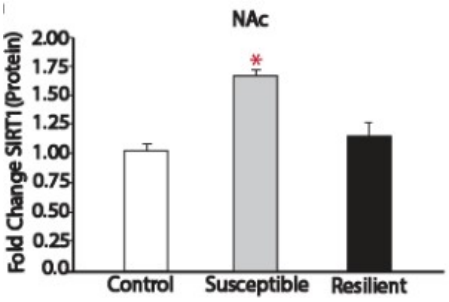


Figure 5



We also observed that the induction of SIRT1 mRNA in the NAc at 48 hours was paralleled by increased protein expression in the same region in susceptible mice but not resilient mice (Figure 6, $F = 4.13$, $p = 0.03$). Mean ± SEM

Figure 6



Discussion

Our results demonstrate that chronic social defeat stress stably induces SIRT1 expression in the nucleus accumbens of susceptible mice. A recent publication in Nature identified a reproducible association of the SIRT1 locus in individuals with Major Depressive Disorder.[19] This correlates with our results demonstrating that SIRT1 levels in the nucleus accumbens are associated with depression and anxiety-like behaviors induced by chronic stress.

Additional support for the significance of these findings comes from Libert et al. (2011)[22] who identified SNPs (rs10997870) in the SIRT1 gene associated with the risk of anxiety in humans. Similar findings were demonstrated using samples from 9000 adult Caucasian twins studied in the Virginia Adult Twin Study of Psychiatric and Substance Use Disorders (VATSPSUD).[20] Another study of Japanese subjects also found a significant association between another SIRT1 SNP (rs10997875) and MDD.[21] These results contribute to our hypothesis that SIRT1 plays a role in regulating depression and anxiety related behaviors and could possibly introduce a new pharmacological target for the treatment of MDD.

References

1. *Yamamoto H, Schoonjans K, Auwerx J. Sirtuin Functions in Health and Disease. Molecular Endocrinology. 2007;21(8):1745-1755. doi:10.1210/me.2007-0079.*

2. *Shirayama Y, Chaki S. Neurochemistry of the Nucleus Accumbens and its Relevance to Depression and Antidepressant Action in Rodents. Current Neuropharmacology. 2006;4(4):277-291. doi:10.2174/157015906778520773.*

3. *Kelly G. A Review of the Sirtuin System, its Clinical Implications, and the Potential Role of Dietary Activators like Resveratrol: Part 1. Alternative Medicine Review. 2010;3:245-63.*

4. *Salgado S, Kaplitt M. The Nucleus Accumbens: A Comprehensive Review. Stereotactic and Functional Neurosurgery. 2015;93(2):75-93. doi:10.1159/000368279.*

5. *Snyder J, Soumier A, Brewer M, Pickel J, Cameron H. Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. Nature. 2011;476(7361):458-461. doi:10.1038/nature10287.*

6. *Nestler E, Barrot M, DiLeone R, Eisch A, Gold S, Monteggia L. Neurobiology of Depression. Neuron. 2002;34(1):13-25. doi:10.1016/s0896-6273(02)00653-0.*

7. *MCEWEN B. Protection and Damage from Acute and Chronic Stress: Allostasis and Allostatic Overload and Relevance to the Pathophysiology of Psychiatric Disorders. Annals of the New York Academy of Sciences. 2004;1032(1):1-7. doi:10.1196/annals.1314.001.*

8. *Molet J, Maras P, Avishai-Eliner S, Baram T. Naturalistic rodent models of chronic early-life stress. Dev Psychobiol. 2014;56(8):1675-1688. doi:10.1002/dev.21230.*

9. *Neumann I, Wegener G, Homberg J, et al. Animal models of depression and anxiety: What do they tell us about human condition? Progress in Neuro-Psychopharmacology and Biological Psychiatry 2011;35(6):1357–1375.*

10. *Berton O. Essential Role of BDNF in the Mesolimbic Dopamine Pathway in Social Defeat Stress. Science 2006;311(5762):864–868.*

11. *Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nature Neuroscience Nat Neurosci 2006;9(4):519–525.*

12. *Krishnan V, Han M-H, Graham DL, et al. Molecular Adaptations Underlying Susceptibility and Resistance to Social Defeat in Brain Reward Regions. Cell 2007;131(2):391–404.*

13. *Bailey K, Crawley J. Anxiety-Related Behaviors in Mice. CRC Press/Taylor & Francis. 2009. Available at: http://www.ncbi.nlm.nih.gov/books/NBK5221/. Accessed March 31, 2016.*

14. *Slattery D, Cryan J. The Ups and Downs of Modelling Mood Disorders in Rodents. ILAR Journal. 2014;55(2):297-309. doi:10.1093/ilar/ilu026.*

15. *Yeragani V, Ramachandraih C, Subramanyam N, Bar K, Baker G. Antidepressants: From MAOIs to SSRIs and more. Indian Journal of Psychiatry. 2011;53(2):180. doi:10.4103/0019-5545.82567.*

16. *Ferguson D, Kim H, Hesterman J et al. SIRT1 mediates depression-like behaviors in the Nucleus Accumbens.*

17. *Krishnan V et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions.*

18. *Ferguson D, Koo JW, Feng J, et al. Essential role of SIRT1 signaling in the nucleus accumbens in cocaine and morphine action. The Journal of Neuroscience 2013;33:16088–16098.*

19. *Cai N, Bigdeli TB, Kretzschmar W, et al. Sparse whole-genome sequencing identifies two loci for major depressive disorder. Nature 2015;523(7562):588–591. doi:10.1038/nature14659*

20. *KENDLER, K., GARDNER, C. and PRESCOTT, C. (2001). Panic syndromes in a population-based sample of male and female twins. Psychological Medicine, 31(6), pp.989-1000.*

21. *Kishi, T., Yoshimura, R., Kitajima, T., Okochi, T., Okumura, T., Tsunoka, T., Yamanouchi, Y., Kinoshita, Y., Kawashima, K. and Fukuo, Y. (2010). SIRT1 gene is associated with major depressive disorder in the Japanese population. Journal of Affective Disorders, 126(1-2), pp.167-173.*

22. *Libert, S., Pointer, K., Bell, E., Das, A., Cohen, D., Asara, J., Kapur, K., Bergmann, S., Preisig, M., Otowa, T., Kendler, K., Chen, X., Hettema, J., van den Oord, E., Rubio, J. and Guarente, L. (2011). SIRT1 Activates MAO-A in the Brain to Mediate Anxiety and Exploratory Drive. Cell, 147(7), pp.1459-1472.*