

# Treadmill-Exercise Increases Antioxidant Homeostasis And Decreases Oxidative Stress-Induced Anxiety in Rats



Nada Sarraj, Manish Taneja, Gaurav Chugh, Kaustuv Saha and Samina Salim  
Dept. Of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, TX

## INTRODUCTION

Oxidative stress is a critical regulator of anxiety and physical exercise is reported to improve anxiety. However, the mechanism of the effect of exercise in this phenomenon is not known. In the present study, we investigated whether *i)* oxidative stress increases anxious behavior and *ii)* exercise increases antioxidant enzymes levels and reduces oxidative stress-induced anxious behavior of rats.

## MOTIVATION AND BACKGROUND

Anxiety is a fundamental emotion required to cope with potential threatening stimuli. In general, anxiety is protective, but excessive anxiety can prove disabling and could manifest in anxiety disorders. Overall, anxiety disorders affect an estimated 40 million people in the U.S. Although effective treatments for anxiety disorders are available, a vast majority of anxiety patients are unresponsive to classical anti-anxiety medications and also experience side effects. The failure to effectively treat these patients due to the poorly understood underlying biology of anxiety, costs \$42 billion a year in lost productivity (1). Therefore, improving the understanding of neural mechanisms of anxiety would create improved treatments and reduce the individual and societal costs of anxiety disorders. In this study we have investigated the involvement of oxidative stress in anxiety, and a protective role of treadmill exercise against oxidative stress in the treatment of anxiety and propose exercise as a better intervention over drugs with their unavoidable side-effects.

Antioxidant proteins, glyoxalase1 (Glo1) and glutathione reductase1 (Gsr1) have been reported as modulators of anxiety (2). Glo1 and Gsr1 are part of a counter-regulatory mechanism, the antioxidant system that neutralizes adverse effects of reactive oxygen species (ROS) including superoxide anion, hydroxyl radical, hydrogen peroxide and peroxynitrite anion that cause tissue damage and loss of cellular function. This balance between antioxidation and oxidation is critical in maintaining a healthy biological system. Oxidative stress is caused by an imbalance between the formation of ROS and the cellular antioxidant defense system and the brain is considered extremely sensitive to oxidative stress (3).

Moreover, treadmill training, known as a general mood elevator, is reported to reduce oxidative stress in the brain (4). Our data suggests that prior long-term exercise training mitigates oxidative stress-mediated anxious behavior of rats. Using behavioral tests, we have evaluated region specific association of Glo1, and Gsr1 with anxiety in locus coeruleus (LC), hippocampus and amygdala (brain areas implicated in anxiety response) (5).

## TECHNIQUES AND APPROACH

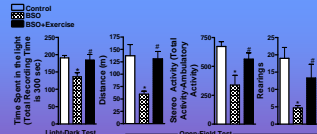
**Our animal model of oxidative stress:** Male Sprague-Dawley rats (200-250 g) were acclimatized for one week before treatment. The rats were subjected to treadmill exercise protocol for 4 weeks for 30 min daily for 1 week and then 60 min of exercise for 3 additional weeks at a speed of 15 m/min. The rats were given a rest period of 5 min between each 15 min the first week and then after 30 min in the last three weeks. Rats had free access to standard rodent chow and water. At the end of three weeks of exercise, rats were injected with L-buthionine-(S,R)-sulfoximine (BSO) (300 mg/kg, i.p.) once daily for 7 days before behavior testing. BSO is considered a pro-oxidant as it is reported to increase oxidative stress markers in brain regions involved in anxiety (5). Control group received vehicle injections (sodium carbonate and saline mixture used to dissolve BSO).

**Light-Dark Test:** Rodents are nocturnal and prefer darker areas, so the decrease in the exploratory activity in a light area is indicative of increased anxiety (2,5). The dark-light box consists of: light compartment (27x27x27 cm) and a dark compartment (black colored surrounding walls and floor, 27x18x27) separated by a partition with an opening for passage from one compartment to the other. The rats were placed at the center of the light compartment. Total time spent in the illuminated part was recorded during 5 min period. A rat was defined to have entered the lit or dark box when both front paws and front shoulders were inside the respective compartment.

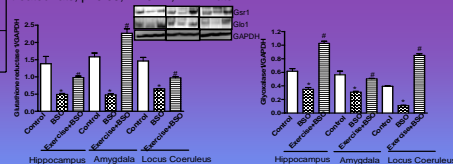
**Open Field Test:** The open field test analyzes free locomotion and exploratory behavior of rats 24 h after the last BSO injection. The open field task was carried out in 60x40 cm open field surrounded by 50 cm high walls. The animals were placed at the center and were left free to explore the arena for 15 min and analyzed by a computer-based system (Optomax, Columbus Instruments; OH): number of rearing, horizontal activity (distance traveled), and time spent freezing.

## RESULTS AND ANALYSIS

Indices of Oxidative Stress	Control (Exercise)	BSO (7Days)	Exercise+BSO
8-Isoprostane urine, pg/mg Creatinine	0.901 ± 0.02	2.11 ± 0.02*	1.01 ± 0.04#
8-Isoprostane serum, fg/mg Creatinine	44.2 ± 1.1	62.4 ± 2.0*	43.2 ± 4.0#
MDA, nmol/mg of protein (Hippocampus)	0.20 ± 0.01	0.42 ± 0.02*	0.22 ± 0.01*
MDA, nmol/mg of protein (Amygdala)	0.18 ± 0.01	0.33 ± 0.02*	0.20 ± 0.01*
Glutathione, nmol/mg protein (Hippocampus)	2.02 ± 0.2	0.82 ± 0.1 *	2.2 ± 0.2#
Glutathione, nmol/mg protein (Amygdala)	1.41 ± 0.2	0.44 ± 0.1 *	1.3 ± 0.2#



Four weeks of treadmill exercise reversed BSO-induced anxiety in rats. In light-dark test, BSO-treated rats spent more time in dark areas than control (exercise only) or exercise plus BSO rats and display reduced movement (A), activity (B) and rearing (C) in the open field-test while exercised plus BSO rats behave just as control. \*significantly different from control, # from BSO-treated rats, p<0.05, ANOVA, N=12 rats.



BSO decreased Gsr1 and Glo1 proteins while prior treadmill exercise reversed BSO-induced decrease in these proteins. Brain homogenates were subjected to SDS-PAGE and immunoblotted using Gsr1, Glo1 and GAPDH (internal control) antibodies. \*significantly different from control, #significantly different from BSO treated rats, p<0.05, ANOVA, N=3.

## CONCLUSION

- ★ BSO increased the levels of oxidative stress markers in the brain regions implicated in the anxiety response (hippocampus, amygdala, locus coeruleus).
- ★ This was accompanied by decrease in Gsr1 and Glo1 levels, the antioxidant proteins implicated in anxiety.
- ★ Anxious behavior tests (light-dark exploration and open field tests) revealed that BSO treated rats were more anxious than control rats.
- ★ Prior treatment with 4-week exercise training in rats attenuated BSO-induced increase in oxidative stress markers, decrease in Gsr1 and Glo1 levels and anxious behavior.
- ★ Our studies suggest a potential role of antioxidant enzymes in anxiety phenotype and support exercise as an intervention in the treatment of anxiety.

**Future Direction:** To investigate the mechanism responsible for oxidative stress mediated anxious behavior of rats and a beneficial effect of exercise in this process.

## REFERENCES

- (1) Kessler et al. 2005 (2) Horvath et al. 2005 (3) Halliwell and Gutteridge, 1999 (4) Colman and Berchtold (2002) (5) Masood et al. 2008

## ACKNOWLEDGEMENTS

Grant support: UH GEAR grant to S.S and SURF award to N.S

