

INVESTIGATION OF TUF 1 IN CHRONIC PAIN AND DEREGULATION OF THE LHPA AXIS

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Introduction

Chronic pain is considered a form of chronic stress that over time may cause deregulation of the LHPA (limbic hypothalamic-pituitary adrenocortical) axis accompanied by disturbances in the limbic system (1). These disturbances may increase the probability of developing a mental disorder. TUF 1 is a novel protein that is robustly expressed in the limbic and hypothalamic regions in the brain, suggesting an involvement in the molecular mechanisms underlying these processes.

Given these findings, we hypothesize that TUF 1 may be involved in the molecular modulation of chronic pain and LHPA axis deregulation. The von-Frey filament test was used to measure mechanical allodynia (pain in response to a previously innocuous stimulus) which occurs in cases of chronic hyperalgesia, and the Hargreaves test which was used as a secondary measure of pain sensitivity (2). These experiments will help evaluate if presence or absence of TUF 1 protein regulate response to chronic pain and processes of the LHPA axis (3).

Methods

Animals:

This experiment used 4 wildtype and 11 knockout animals for both the Hargreaves and von-Frey filament test.

Von-Frey Filament Test:

In the electronic Von-Frey test, pressure was applied in varying degrees to the animals hind paw and a digital readout determined the pressure to which the animal withdrew their paw. Each mouse was placed in an elevated plastic mesh floor with a clear Plexiglas chamber (5 x 5 x 8cm), and allowed to acclimate for 20-30 minutes. The filament was applied to the hind paws to the point when the animal withdrew their paw. Sensitivity to the von-Frey test was measured by their withdrawal response upon filament application. An ascending level of force was applied in order to invoke withdrawal of the paw. The threshold that involved a withdrawal response was then recorded in millinewtons (mN). Each animal was tested five times in an ascending order of force. Once a force was determined to be the threshold of the animal's sensitivity, no greater force was applied (4).

Hargreaves Test:

The Hargreaves test involved placing mice in Plexiglas chambers that allow free movement. The mice were acclimated to the test chambers for 20 minutes. During the testing period, a focused infrared source is moved under the hind paw of the mouse when it is not moving. Application of the focused infrared beam comes from a button press. when the mouse senses the heat intensity grow, it withdrew its paw, causing a photo-sensor in the source to stop a timer. This time indicates the latency from heat onset to withdrawal of the paw (4).

Results

Figure 1. Force Threshold for Von-Frey Filament Test

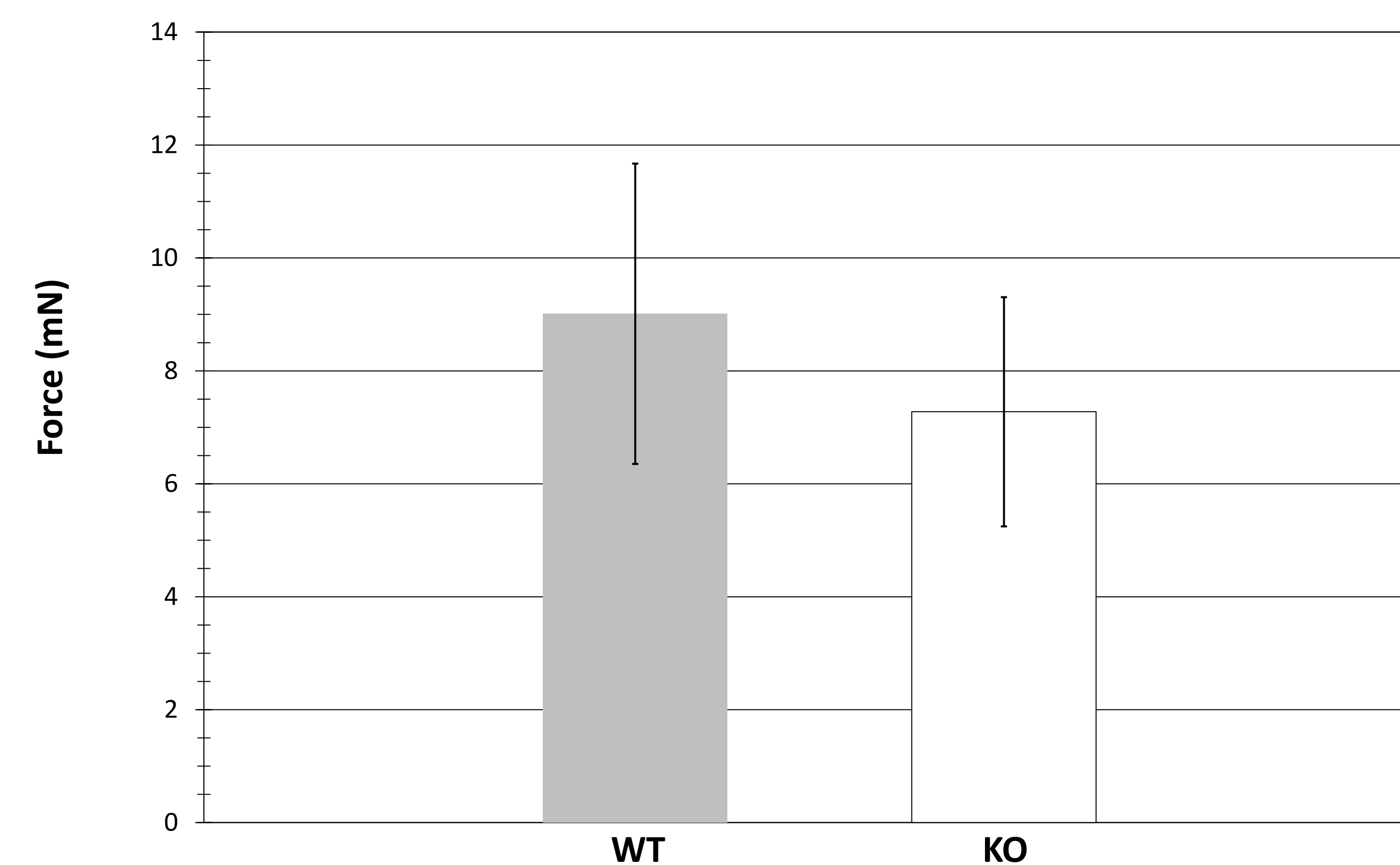
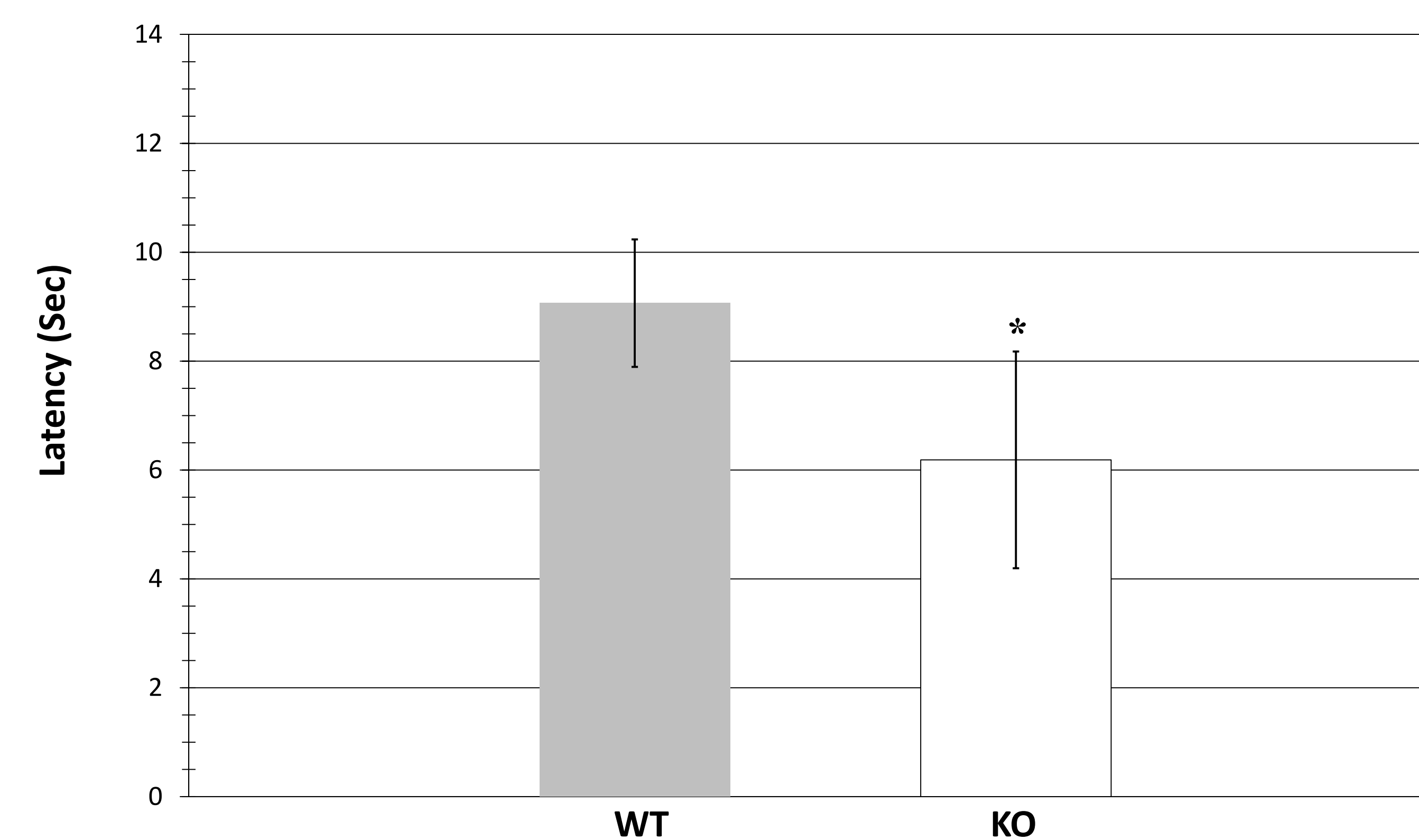


Figure 2. Average Latency for Hargreaves Test



- In both the von Frey filament (Fig. 1) and Hargreaves test (Fig. 2), KO animals showed a general trend of decreased withdrawal latency and force threshold compared to their WT counterparts. However, results obtained from conducting a Student's t-test signify a significant difference in the average withdrawal latency for KO animals in the Hargreaves test (Fig 2). This decreased withdrawal latency in KO mice indicate a possible interaction between the absence of TUF 1 genes and the animals increased sensitivity to nociceptive stimuli.
- Force threshold for KO animals in the von-Frey filament test did not show significance, but the general increased sensitivity indicated through a decreased threshold force in KO mice suggests the presence of mechanical allodynia.

Conclusion

Absence of the TUF 1 gene, in KO animals, lead to a decrease in withdrawal latency in the Hargreaves test, indicating a possible involvement of TUF 1 in mediating some forms of pain. In the Hargreaves test, the withdrawal latency is associated with hyperalgesia, with an decreased latency demonstrating an exaggerated pain response. Although KO mice did not display an exaggerated reaction to the von-Frey filament test, it is possible that absence of the TUF 1 gene may only affect specific pain modalities. However, due to the small number of WT animals it is possible that the results obtained may be different with a larger control group. Possible implications of this research would be further supported through more extensive experimentation.

References

- 1) Rotzinger, S., Lovejoy, D., Tan, Laura., (2010). Behavioral effects of neuropeptides in rodent models of depression and anxiety. *Peptides*, 736-756.
- 2) Xie, W., (2011). Assessment of Pain in Animals. *Neuromethods*, 1-21.
- 3) Yvonne, M,U., Xie, W., Meij, J., Dolgas, M., Yu, L., Herman, J. (2006). Limbic and HPA axis function in an animal model of chronic Neuropathic pain. *Physiology and Behavior*
- 4) Pitcher, G., Ritchie, J., Henry, J. (1998). Paw withdrawal threshold in the von-Frey hair test is influenced by the surface on which the rat stands. *Journal of Neuroscience Methods*, 185-193.

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