Pure Award Final Report

Date of submission: September 24, 2018

Supervisor: Dr. P. H.

Introduction

Aging is a natural and inevitable process which results in many disadvantages such as reduction in long-term memory and neurodegenerative disorders such as Alzheimer' disease. There have been many attempts at explaining the process of aging. However, the heterogeneity and multifactorial characteristics of aging makes it a difficult to task to analyze.

During my summer research I examined mitochondrial functioning and its association with learning and memory. I looked at oxidative stress on membrane poly unsaturated fatty acids (PUFAs) and their involvement with long-term memory in *Lymnaea stagnalis*. Mitochondria play an important role in β-oxidation of PUFAs into their smaller counter parts (Liu et al., 2002). During this process, the PUFAs are transported into the mitochondria matrix using acyltransferases, Carnitine palmitoyltransferase 1 and 2 (CPT1 and 2). CPT1 is an integral membrane protein which is associated with the outer mitochondrial membrane. The CPT1 enzyme plays a crucial rule in the Carnitine palmitoyltransferase system by transferring the PUFAs across both mitochondrial membranes. Defects in CPT1 have been associated with a range of different disorders such as diabetes 2 and other age-related deficiencies (Cruciani-Guglielmacci et al., 2004).

In order to test the effects of CPT1 and mitochondrial functioning on learning and memory, I used etomoxir to selectively inhibit CPT1 (Schrauwen et al., 2002). My hypothesis stated that CPT1 dependent fatty acid transfer insufficiency is a key determinant of age and oxidative stress associated long-term memory failure.

Methods

Sucrose response testing

The snails were tested for their rasping ability one day following injections. This way used as a way of establishing whether or not the injections alter the ability of the snails to respond to sucrose. The snails were placed in separate cups containing 80 mL pond water. The initial rasping was measured for two minutes. They were then introduced to 10 mL pond water. The rasping was measured again for two minutes. This was followed by 10 mL of sucrose solution (40 mg/L concentration). The rasping was measured for two minutes.

Testing

In this experiment I used classical conditioning to train the snails and assess their long-term memory. I followed a four day protocol which included two day pre-testing, one day training and one day post-testing. During the pre-testing phase the snail's original response to a neutral stimulus, in this case amyl acetate, was assessed. During this phase, the sail was placed in a beaker and left for 15 minutes to acclimatize. Then, the snail's initial rasping was measured for two minutes. This was followed by addition of 10 mL of pond water to the beaker. The snail's rasping was measured again for two minutes. Then, 10 mL of solution containing amyl acetate (40μL/L concentration) was added to the beaker. The snail's rasping was measured again for two minutes. During pre-testing days, all snails were tested followed the above protocol. This was followed by testing. During the testing phase the snails were placed in their corresponding conditioned or unconditioned beakers. The conditioned snails were trained to pair amyl acetate (neutral stimulus) with sucrose (unconditioned stimulus). For the conditioned snails the training phase was consisted of addition of 50 mL of amyl acetate solution (40μL/L concentration) into

the training beaker followed by addition of 50 mL of sucrose solution (40mg/L concentration) 15 seconds after. This was repeated 5 times. The unconditioned group, however, were not trained to associated amyl acetate solution with sucrose. During the testing phase, the unconditioned snails received 50 mL of amyl acetate solution followed by 50 mL of pond water after 15 seconds. The training phase was followed by post-testing which assessed the snails' long-term testing. The post-testing phase followed the same protocol as the pre-testing phase. The data was collected and analyzed using Graph pad prism and Statistica software.

Injections

The experimental snails were injected with 0.1 mL of etomoxir solution (1.5 mM concentration) after pre-testing 2 and the control snails were injected with water. They were also deprived of food one day prior to training.

Results

The results for this experiment included that although there was no difference between groups in the sucrose response test, there was a significant difference between water injected and etomoxir injected conditioned groups following testing. The water injected snail demonstrated increased number of rasping following training which is representative of their long term learning to associate the neutral stimulus with the unconditioned stimulus. However, the etomoxir injected conditioned group did not demonstrate increased rasping in comparison to their unconditioned group or the pre-testing trial. Therefore, it can be concluded that the etomoxir injected conditioned group demonstrated a decrease in long-term memory and that etomoxir reduced the ability to generate learning and memory.

Knowledge gained

During this research experience I have improved my abilities to execute an independent research study. I gained a better understanding of mitochondrial involvement in learning and memory. The topic of oxidative stress and its association with long term memory is not fully investigated and this summer research provided a great opportunity for me to further explore this area. I have also expanded my knowledge and experience in animal behavioural studies. During this summer I was able to plan the process and increase my leadership skills and capabilities. In addition, my involvement in the lab helped me increase my communication skills.

Acknowledgements

I would like to thank the Undergraduate Research Committee (PURE) for the funding of this research. In addition, I would like to thank Dr. H and Dr. W for allowing me to be a part of their team and for helping me and teaching me along the way.

References

Cruciani-Guglielmacci, C., Hervalet, A., Douared, L., Sanders, N. M., Levin, B. E., Ktorza, A., & Magnan, C. (2004). Beta oxidation in the brain is required for the effects of non-esterified fatty acids on glucose-induced insulin secretion in rats. *Diabetologia*, *47*(11), 2032-2038.

Schrauwen, P., Hinderling, V., Hesselink, M. K., Schaart, G., Kornips, E., Saris, W. H., & Langhans, W. (2002). Etomoxir-induced increase in UCP3 supports a role of uncoupling protein 3 as a mitochondrial fatty acid anion exporter. *The FASEB Journal*, *16*(12), 1688-1690.

Liu, J., Head, E., Gharib, A. M., Yuan, W., Ingersoll, R. T., Hagen, T. M., & Ames, B. N. (2002). Memory loss in old rats is associated with brain mitochondrial decay and

RNA/DNA oxidation: partial reversal by feeding acetyl-L-carnitine and/or R-α-lipoic acid. *Proceedings of the National Academy of Sciences*, *99*(4), 2356-2361.