The Effect on Memory If Arc Loses its Function of Removing AMPAR

Yuchen Wang*

8328 Georgetown Pike, The Madeira School, McLean, VA 22102

ABSTRACT: This work analyzes arc protein's ability in balancing Long-Term Potentiation and Long-Term Depression and inquires on unrestricted LTP's impact. The purpose of this analysis is to investigate the effect on memory if arc protein loses its function of removing AMPA receptors. By dividing mice into the wild type group and the LTD deficit group, this work emphasizes on the important of LTD in relieving the saturation point of LTP.

1 Introduction

Arc (Arc protein and its translation process.) can modify synaptic plasticity, both Long-Term Potentiation (LTP) and Long-Term Depression (LTD). It is known to modulate LTP via facilitating dendritic spine growth and induce LTD by AMPAR endocytosis, and so on [10]. Arc helps to strengthen dendritic spines via reforming and reorganizing actin polymers and its proteins aid the growth of the size of dendritic spines. The dendritic spines' cytoskeleton is made up of actin filaments, which are formed through polymerization of actin subunits into polymers. Actin polymerization happens at the same site as the localization of Arc mRNA [3]. Arc proteins increase the number of actin polymers and allow the spine to mature quicker. A mature dendritic spine has a larger surface area, which facilitates its synaptic strength as it allows more receptors on its surface [6]. Hence, more ions can enter the synapse to promote potentiation.

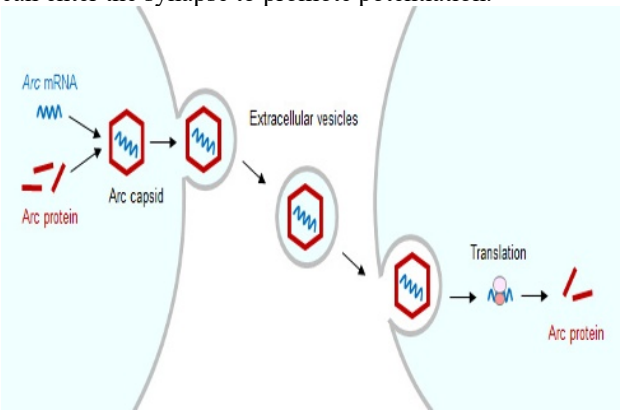

Figure 1: Arc protein translation process

Arc induces LTD by removing AMPA receptors. Arc mRNA is transported to activated dendritic regions and translated into Arc protein which reduces the amplitude of synaptic currents (Long Term Potentiation and Long-Term Depression) mediated by AMPA-type glutamate receptors (AMPARs) [9]. Arc removes AMPARs which are composed of GluR2 and GluR3 (GluR2/3) in the hippocampal slice. "This effect is prevented by RNAi knockdown of Arc, by deleting a region of Arc known to interact with endophilin 3 or by blocking clathrin-coated endocytosis of AMPARs" [5]. Arc reduces the number of AMPAR which in turn leads to a decrease in AMPA-mediated synaptic currents and causes LTD.

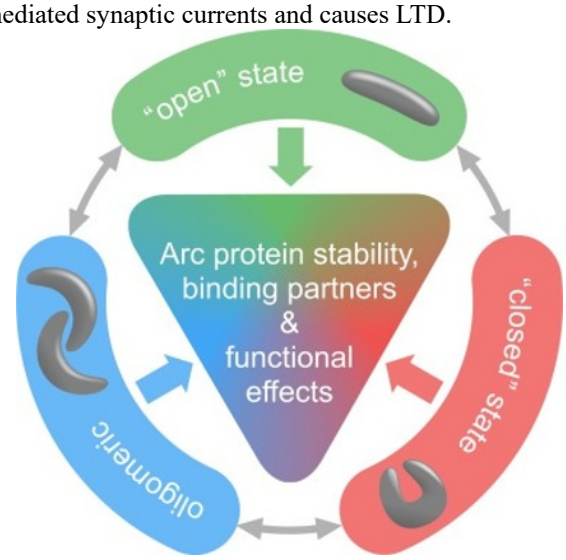

Figure 2: Arc protein's different state

LTD happens when the connections of synapses between neurons become weaker. It is important to memorize the formation. It can be caused by prolonged low-intensity stimulation. "This low stimulation is not enough depolarization to cause a large amount of removal of NMDA receptors, but it can let some calcium ions flow into the cell and activate a cellular cascade that leads to the removal of AMPA receptors" [2]. Therefore, it can reduce the number of receptors on the neuron and weaken the synapses.

AMPA endocytosis causes LTD. Arc protein located at the synapses bind endophilin 1 and dynamin 2 to recycle AMPA-R. Arc together with other proteins removes

*cwang@madeira.org

AMPA-R via creating Microvesicles (MVEs) [8]. There are four genes responsible for the endocytosis of AMPAR. "DNM2 gene, which makes the protein dynamin 2. Sh3gl1 gene makes the protein Endophilin-A2 [7]. Sh3gl3 gene makes the protein Endophilin-A3. AP50 gene makes the protein Clathrin adaptor protein. Amino acids number 91-100 in the Arc protein are responsible for AMPAR endocytosis" [1].

We wonder if Arc's the ability to play a role in both helping balance LTP and LTD in order to prevent an excessive amount of either. This leads to an inquiry on the impact of unrestricted LTP. Thus, we derive the question: what is the effect on memory if Arc loses its function of removing AMPAR?

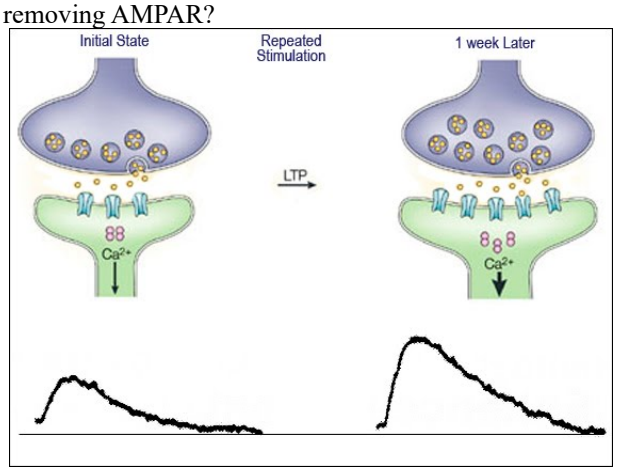

Figure 3: Long term potentiation stimulation

2 Experiment

Before the experiment starts, we need to prepare the material and experiment groups. In order to produce mice that do not have the function of removing AMPA receptor in their arc protein by endocytosis, we produce a small mutation in the 91-100 amino acid of the arc protein, which makes the arc protein unable to bind to the endophilin and therefore the AMPA receptor cannot be removed. We also breed 2 types of mice, one type of which is wild type mice, and the other type is LTD deficit mice. LTD deficit mice cannot remove their AMPA receptors while the wild type is the normal mice.

Figure 4: Wild-type mice Control Test 1

The first is a control experiment to determine whether the mutation is successful or not, that is, whether all other functions of Arc are preserved in the mutant mice. We can use RNA in situ hybridization to determine whether the arc of mutant mice is located on synapses. These tests are performed using sections of the hippocampus of the LTD-deficit mice. We also measure the ratio of NMDA to AMPA receptors in both groups of mice to see if the mutation can successfully block AMPA receptor circulation. What's more, this ratio helps to indicate that we expect to have more AMPA in LTD-deficit mice than in wild-type mice. This ratio can be detected by analyzing the voltage variation of the stimulated axon. All voltage changes are caused by AMPA receptors, as the drug inhibitors are added to inhibit NMDA receptors.

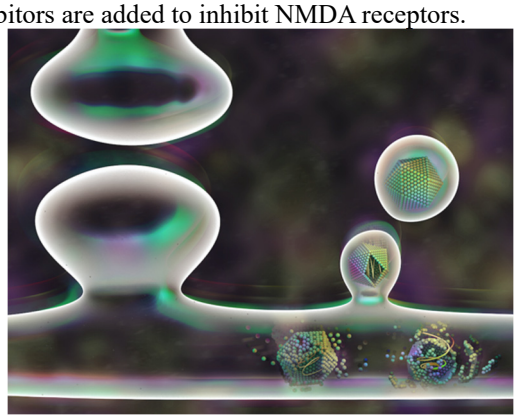

Figure 5: Arc protein and its shape Control Test 2

The second control experiment examines the ability of the LTD-deficit mice to maintain LTP and whether their functions are normal. The Potassium chloride is added to the culture medium to induce cell enhancement. We measure whether LTP occurs in the process.

Since the results of both experiments show that the LTD-deficit mice have all the other functions of arc and can have LTP as well, we continue to conduct behavioral tests.

Control Behavioral Test

The third experiment is a behavior test to see the ability of mice to form new memories with saturated LTP studied. We induce LTP in two groups of mice by optogenetics. Mice see the flash, which leads to a rapid discharge of neurons in the Dentate Gyrus so that the synapses between DG and CA3 are fully potentiated. Then, we start a fear conditioning experiment on mice. They are put into two environments, and one has a shock, while the other does not. After a few hours, they are returned to the environment previously associated with the shock. If the mice show freezing behavior, it means that the memory has been formed, otherwise, the memory is not formed. The main experiment investigates whether AMPA-endocytosis LTD impacts mice's ability to form new memories with a saturated amount of LTP. LTP is induced in both groups of mice via optogenetics. This method is chosen due to its preciseness in controlling the location of potentiation, and the relatively small amount of time required to create a saturated level of LTP.

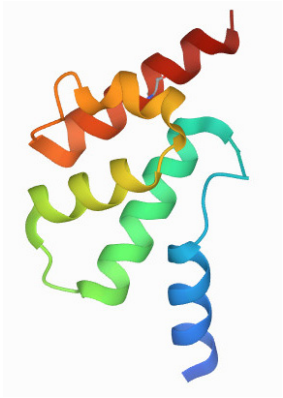

Figure 6: Arc protein and its structure

3 Conclusion

After the experiment, we predict four different kinds of results from the two (2) different experiment groups.

Both Groups Learn

We believe that if both groups, namely the wild type of group and the LTD deficit group, learn the LTD does not matter to the formation of new memories as it is extremely likely that there is no LTP saturation point that prevents the formation of memory. Besides, it is also possible that other mechanisms can induce LTD other than AMPA endocytosis, and this mechanism may help to relieve saturation of LTP and thus help to form new memories.

LTD-Deficit Group Learn

The second result is that the LTD-deficit group learns, and then the LTD is a major blockage of the formation of memory as LTP can continuously build up and form memories if there is no LTD. In our opinion, this is unlikely to happen because of the limit of LTP.

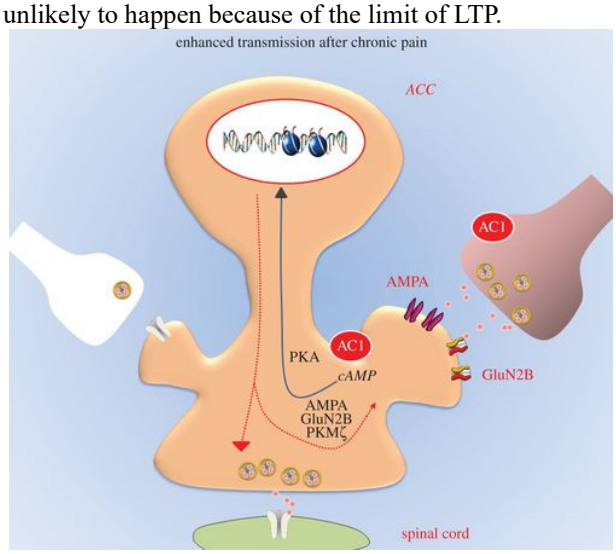

Figure 7: AMPA receptors and enhanced transmission

Wild-Type Group Learn

The third prediction is our hypothesis, which is: if wild type mice group learns, then LTD plays a huge role in relieving the saturation of LTP because LTP can be saturated to a point which new memories cannot be formed, and both LTD and LTP shall exist to form new memories. new memories cannot be formed, and both LTD and LTP shall exist to form new memories. However, it also would imply that the LTD-deficit mice may have an alternative mechanism that rapidly induces LTD, given that the AMPA-endocytosis LTD cannot relieve the situation.

Neither Group Learn

The fourth prediction is if both groups do not learn, then LTD does not play a significant role in relieving the saturation of LTP because ARC induced LTD does not relieve saturation of LTP quickly enough and the occurrence of LTD is slower and it is not proportional to the occurrences of LTP. Therefore, LTD that happens naturally does not cancel out LTP.

The significance of this experiment is to discover the importance of LTD. Since it is hypothesized that LTD deficit group now learn, we believe that LTD plays a huge role in relieving the saturation of LTP and it is important to learning and forming new memories. Several adjustments can be fixed if the experiment is repeated. One potential issue with this experiment design would be the adult LTD-deficit mice. Even if it has been proven that the group has no other differences than wild type mice, it might be possible for the LTP to have reacted saturation as the mice grow to adulthood. Another possible control experiment would be to have multiple behavioral tests with different length of time between the optogenetics-induced LTP and the beginning of training. This would produce results that demonstrate the time it requires for LTD to occur. Then, it will be applied to the main experiment.

Future Directions

We also come up with the following questions to be answered: 1. We believe that AMPA is the only other way that can cause LTD through this experiment, so we wonder what other receptors can cause LTD? 2. What is the effect of overload glutamate receptors on synapses or neurons? 3. Given that LTP and LTD are balanced, this indicates that LTP will saturate and a mechanism to balance LTD is needed in this case. However, LTP occurs at synapses so it is possible tha

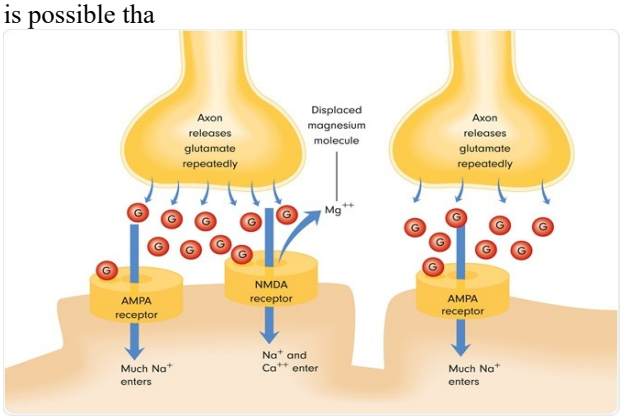

Figure 8: NMDA and AMPA receptors t if a person has more neurons or a larger size of hippocampus,

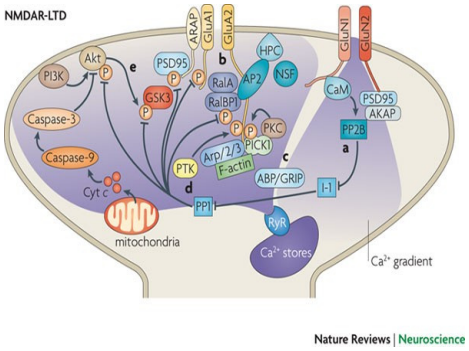

Figure 9: Long term depression in a synapse

then the person can form more LTP before becoming saturated. Therefore, does an increase in the number of hippocampal neurons allow more LTP formation?

REFERENCES

1. DNM2 Gene - Genetics Home Reference - NIH." U.S. National Library of Medicine, National Institutes of Health, 17 Aug. 2020, ghr.nlm.nih.gov/gene/DNM2.
2. Franco, H. 2-Minute Neuroscience: Long-Term Potentiation (LTP). 14 Mar. 2018, youtu.be/-mHgPfXHzJE.
3. Huang, F., et al. "Actin Polymerization and ERK Phosphorylation Are Required for Arc/Arg3.1 MRNA Targeting to Activated Synaptic Sites on Dendrites." Journal of Neuroscience, vol. 27, no. 34, 2007, pp. 9054–9067., doi:10.1523/jneurosci.2410-07.2007.
4. Kerrigan, Talitha L., and Andrew D. Randall. "A New Player in the 'Synaptopathy' of Alzheimer's Disease – Arc/Arg 3.1." Frontiers in Neurology, vol. 4, 2013, doi:10.3389/fneur.2013.00009.
5. Verde, Emiliano M. Rial, et al. "Increased Expression of the Immediate-Early Gene Arc/Arg3.1 Reduces AMPA Receptor-Mediated Synaptic Transmission." Neuron, vol. 52, no. 3, 2006, pp. 461–474., doi: 10.1016/j.neuron.2006.09.031.
6. Fukata, Yuko, et al. "Molecular Constituents of Neuronal AMPA Receptors." The Journal of Cell Biology, vol. 169, no. 3, 2005, pp. 399–404, http://www.jstor.org/stable/3658218.
7. Dong, Erbo, et al. "A Reelin-Integrin Receptor Interaction Regulates Arc MRNA Translation in Synaptoneurosomes." Proceedings of the National Academy of Sciences of the United States of America, vol. 100, no. 9, National Academy of Sciences, 2003, pp. 5479–84, http://www.jstor.org/stable/3139737.
8. Bolshakov, Vadim Y., and Steven A. Siegelbaum. "Postsynaptic Induction and Presynaptic Expression of Hippocampal Long-Term Depression." Science, vol. 264, no. 5162, American Association for the Advancement of Science, 1994, pp. 1148–52, http://www.jstor.org/stable/2885136.
9. Guzowski, J. F., Lyford, G. L., Stevenson, G. D., Houston, F. P., McGaugh, J. L., Worley, P. F., & Barnes, C. A. (2000). Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. The Journal of Neuroscience, 20(11), 3993–4001. https://doi.org/10.1523/jneurosci.20-11-03993.2000
10. Fucile, Sergio, et al. "Effects of Cyclothiazide on GluR1/AMPA Receptors." Proceedings of the National Academy of Sciences of the United States of America, vol. 103, no. 8, National Academy of Sciences, 2006, pp. 2943–47, http://www.jstor.org/stable/30049518.
11. Synaptic plasticity (long term potentiation and depression) - mcauliffe neur 493. Google Sites. (n.d.). Retrieved October 10, 2021, from https://sites.google.com/site/mcauliffeneur493/home/synaptic-plasticity.
12. Biolaboratories, arigo. (2021, October 10). Viral-like capsids, new trans-synaptic mrna transport mechanism - news - company. Arigo biolaboratories. Retrieved October 10, 2021, from https://www.arigobio.com/news/arc-capsid.