Mimicking nAChR using the prokaryotic homolog ELIC

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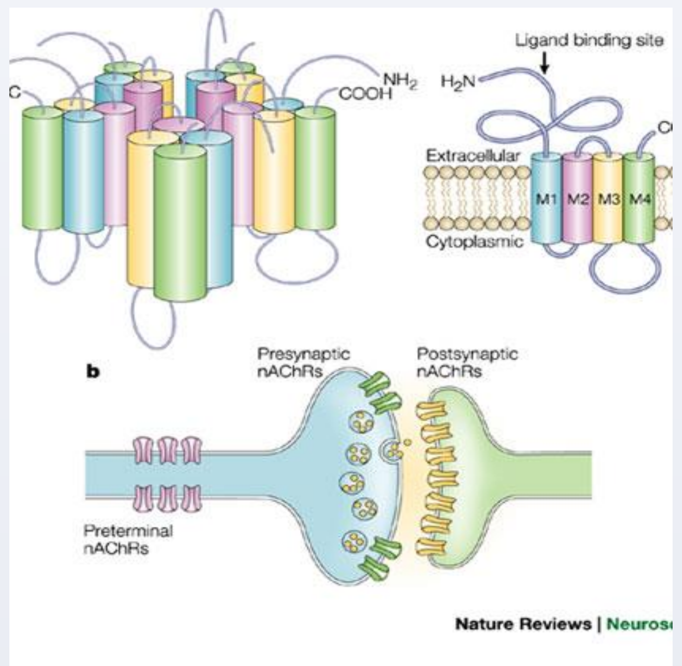
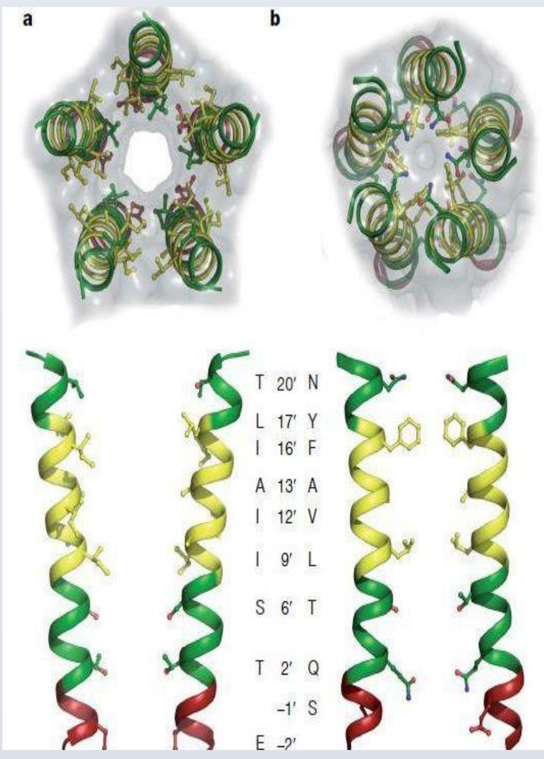
INTRODUCTION



AChRs, short for acetylcholine receptors, are found in the neuromuscular system, the central nervous system, as well as the peripheral nervous system, more specifically the autonomic nervous system. It is part of a superfamily of proteins called pLGICs (pentameric ligand-gated ion channels), which also include receptors for serotonin, amino butyric acid, and glycine. There are two types of AChRs, which are nAChRs, short for nicotinic acetylcholine receptors, and mAChRs, short for muscarinic acetylcholine receptors. As the names suggest, nAChRs are responsive to nicotine whereas mAChRs are responsive to muscarine. We are particularly interested in the nAChRs, which can be located at neuromuscular junctions and at synapses between neurons. They are ligand-gated ion channels. When acetylcholine, the natural agonist, is bound to the nAChR, a flux of ions is permitted to cross the cellular membrane, which can result in a depolarization and therefore a muscle contraction or a cognitive function.



Presently, we are only provided with a cryoEM structure for nAChR, which has a lower resolution then x-ray crystallography. This makes studying nAChR much more difficult. However, the x-ray structures for GLIC and ELIC were recently discovered. As such, we are trying to use these prokaryotic homologs to understand the mechanisms and functions that cannot be probed with nAChR. While GLIC and ELIC are both pLGICs, the study focuses on the use of ELIC. ELIC comes from the bacteria Erwinia chrysanthemi and offers many advantages. For one, it is easy to work with and we know much about it, but it is also easier to mutate, which allows us to study the effects of specific mutations.

Both nAChR and ELIC have four transmembrane domains ranging from M1 to M4. Previous studies suggest that the M4 domain is the furthest from the M2 channel forming and that mutations in the M4 domain affect not only channel gating, but also the extent to which the channel opens. In the M4 domain, there is a gene at the C418 position in the average person. However, a mutation replacing the cysteine by a tryptophan (C418W) results in patients with congenital myasthenic syndromes (CMSs). We found that the equivalent position for this gene, in ELIC, is at L308.

PURPOSE

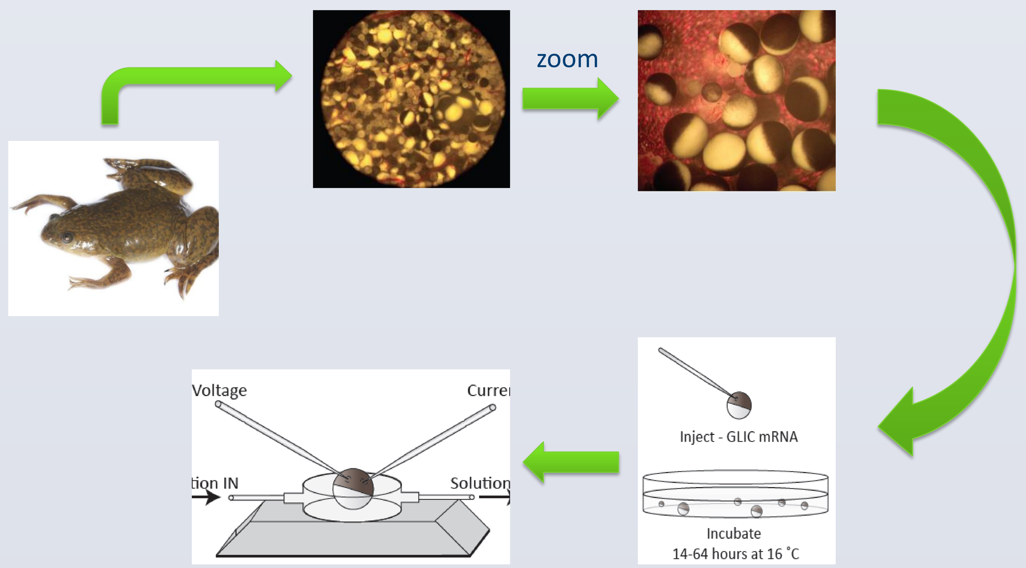
We hope to reproduce the cysteine (L308C) and tryptophan (L308W) version of this gene in ELIC in order to better study and compare the two. This allows us to determine if ELIC mimics nAChR.

MATERIAL & METHODS

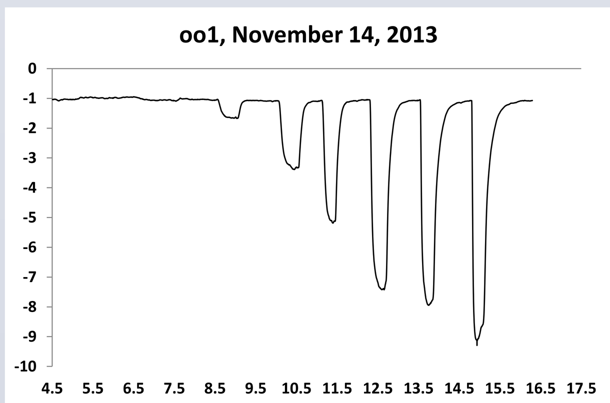
Oocyte preparation: Fresh oocytes are collected from *Xenopus laevis* frogs and kept in Petrie dishes. A collagenase treatment is then performed in order to release said oocytes from the lobe-sac and to remove the follicular layer of cells that surrounds them. They are then kept in a solution of ND96+.

RNA injection into oocytes: A HEKA temperature controlled pipette puller is used to pull a glass pipette in order to obtain two fine tipped pipettes. Next, tweezers are used to break the tips in order to create a small hole. Following this, mineral oil is inserted into the pipette using a syringe the pipette is placed on a nanoject II micro-injector. Lastly, a specific RNA solution is drawn in to fill the pipette and it is then injected into the oocytes, which are incubated for at least a day.

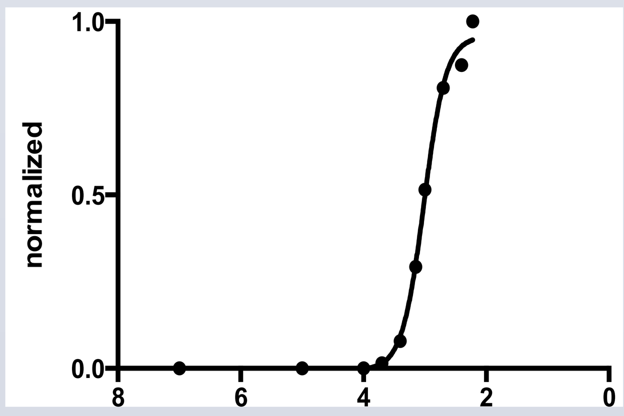
Measurement of pLGIC whole cell current in oocytes: A 10x stock filtered MES buffer is prepared using 10x 140mM NaCl, 10x 2.8mM KCl, and 10x 2mM $MgCl_2$. This buffer is then fixed to pH 7 and used to create various solutions of cysteamine ranging in concentrations from 0.01mM to 6mM. These solutions are then used to fill all the syringes attached to the two-electrode voltage clamp (TEVC) system. Once this is prepared, the pipette puller is used to pull large tip pipettes. These tips are filled with a 3M KCl stock solution and fitted onto the voltage and current electrodes of the TEVC system. At this point, an oocyte is placed in the flow chamber and is impaled by the pipettes. Lastly, the buffers are allowed into the flow chamber one by one and, using lab scribe, the oocyte's membrane potential is measured.



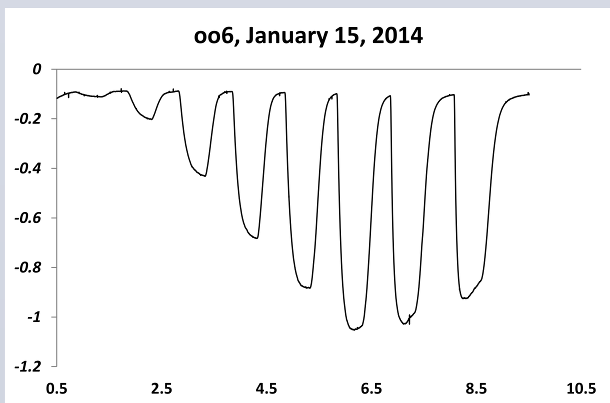
RESULTS



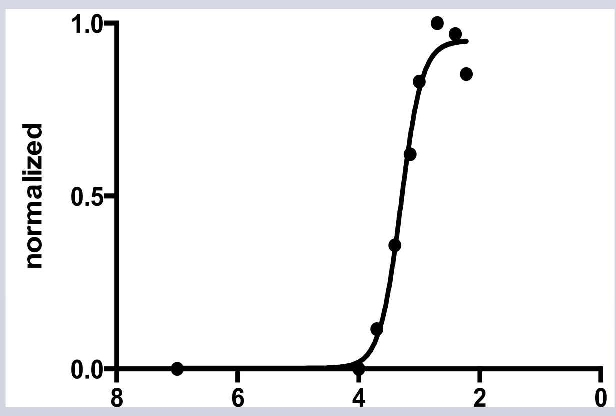
Graph 1.1: Reading of oocyte 1 WT, November 14, 2013, showing peaks in Ia corresponding to the respectful concentration of cysteamine.



Graph 1.2: Normalized graph of oocyte 1 WT, November 14, 2013, showing an EC50 of 3.014 corresponding to a cysteamine concentration of 0.97mM.



Graph 4.1: Reading of oocyte 6 L308W, January 15, 2014, showing peaks in Ia corresponding to the respectful concentration of cysteamine.



Graph 4.2: Normalized graph of oocyte 6 L308W, January 15, 2014, showing an EC50 of 3.302 corresponding to a cysteamine concentration of 0.50mM.

DISCUSSION

Following the results obtained during this UROP placement, we are inclined to believe that the L308W mutation found in the M4 domain, in ELIC, causes a left shift. This is demonstrated as we notice oocytes carrying this specific mutation require lower concentrations of cysteamine in order to open their ion channels. As shown in the results, we see this gain of function as we begin seeing peaks for mutated oocytes around the 0.2mM cysteamine concentration mark whereas the wild type oocytes only begin showing peaks at the 0.4mM cysteamine concentration mark.

Due to a lack of time, I was unable to test oocytes carrying the L308C mutation. Doing so would allow us to fully determine if ELIC mimics nAChR regarding the mutation and could be done in a future study.

CONCLUSION

In conclusion, we were able to recreate the C418W mutation in ELIC, which became L308W. In doing so, we were able to determine that ELIC does mimic nAChR regarding the tryptophan mutation.

REFERENCES

1. Bocquet, N., Nury, H., Baaden, M., Le Poupon, C., Changeux, J.-P., Delarue, M., et al. (2009). X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. *Nature* , 111-114.
2. Corringer, P.-J., Baaden, M., Bocquet, N., Delarue, M., Dufresne, V., Nury, H., et al. (2010). Atomic structure and dynamics of pentameric ligand-gated ion channels: new insight from bacterial homologues. *The Physiological Society* , 565-572.
3. Laviolette, S. R., & Kooy, D. v. (2004). The neurobiology of nicotine addiction: bridging the gap from molecules to behaviour. *Nature* , 55-65.
4. Shen, X.-M., Deymeer, F., Sine, S. M., & Engel, A. G. (2006). Slow-Channel Mutation in Acetylcholine Receptor aM4 Domain and Its Efficient Knockdown. *American Neurological Association* , 128-136.
5. Image of Xenopus laevis frog taken from: http://flickr.com/photo/19731486@N07/8325732255

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