Neuronal inhibition through DmFLYC1 mechanosensitive channel with magnetic stimulation

Lee Jiyoun, Oh Myoungjae, Park Sangmin

Yonsei IBS CNM Science Factory.

Abstract

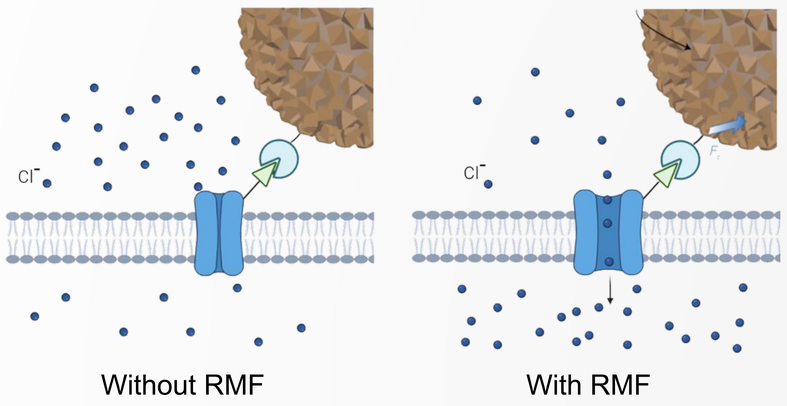
Continuing research from Y-iBS, *Non-contact long-range magnetic stimulation of mechanosensitive neurons in freely moving animals (2021)*, here we show an attempt to silence neurons through a novel mechanosensitive chloride ion channel, DmFLYC1, instead of the well known Piezo1. Lentiviral transduction is an effective way to introduce the DmFLYC1 gene into HEK293 kidney cells. Its fluorescence is stronger compared to results from lipofection.

Application of magnetic force onto m-torquer attached HEK293 cells opens the DmFLYC1 channel, inducing a chloride ion influx through a chemical gradient.

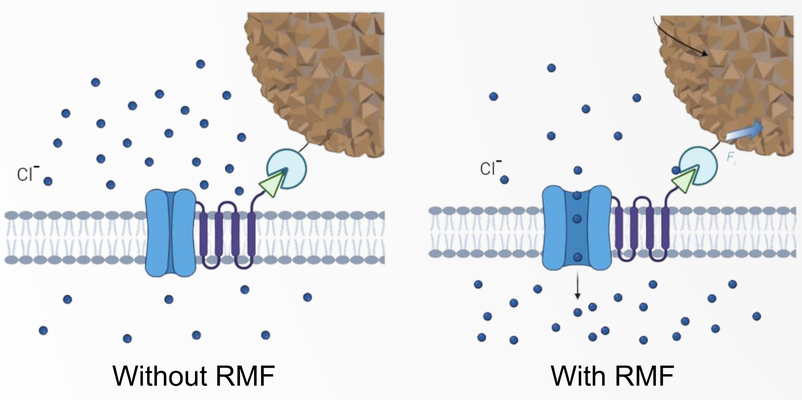
mClY chloride ion indicator shows that after rotational magnetic force is applied to m-torquer, the fluorescence bleaches rapidly after contact with the ion influx. While some portray intended results, the others are extremely variant. Yet, this experiment did present the potential of DmFLYC1's application to neuronal inhibition and ultimately allowing remote silencing of neuron signals.

Introduction

DmFLYC1-H293 Schematic



DmFLYC1-PDGFRβ Schematic



Neurons have a resting membrane potential of -70mV. When an accumulation of stimuli increases its potential to a certain threshold, ligand-gated cation channels open and sodium ion influx occurs. This depolarization of membrane potential is how neurons fire signals. After sending signals, membrane potential becomes repolarized rapidly to a hyperpolarized state. This is even below the cell's resting potential. Silencing neurons induce this hyperpolarization so that cells are more unlikely to start an action potential.

DmFLYC1 is a mechanosensitive chloride ion channel which can be activated with mechanical force. To apply adequate mechanical force to this channel, a magnetic nanoparticle called m-torquer was used. M-torquer is a 500 nm-sized nanoconstruct of octahedral nanoparticles designed with weak ferromagnetism and magnetic anisotropy. Under 20 - 50 mT, m-torquer can produce 2 - 10pN of torque force that can activate mechanosensitive ion channels without disrupting the cell membrane.

M-torquer is specific to myc tags, but the optimal myc position is yet known. Two possible locations can be an extension of the intracellular C-terminal to the matrix with a transmembrane protein PDGFRβ to attach the tag distant from the channel (DmFLYC1-PDGFRβ), or addition of the sequence in the middle extracellular region of the transmembrane channel (DmFLYC1-H293).

mClY is a YFP based chloride sensor which bleaches upon chloride contact. To observe chloride influx, mClY was introduced to HEK293 human embryonic kidney cells(control), DmFLYC1-H293, and DmFLYC1-PDGFR-β cells through lipofection. Using mClY, chloride influx resulting from DmFLYC1 channel opening through m-torquer and rotating magnetic field (RMF) can be verified. Quantifying the rate of bleach change is used to predict chloride influx rate.

Methods

Lentiviral vector production	DmFLYC1-H293 and DmFLYC1-PDGFRβ gene cloned in lentiviral vector.
Lipofection into Lenti-x-293T	Lentiviral vector transfection into Lenti-x-293T to harvest lentivirus with DmFLYC1 gene.
Transduction into HEK293	Lentivirus transduction into mammalian HEK293 cells for steady expression of DmFLYC1-H293 and DmFLYC1-PDGFRβ.
MNP attachment	Anti-myc-m-torquer (MNP) addition to cells.
Cl^- movement imaging	mClY lipofection into HEK293-DmFLYC1, and observation of Cl^- movement with and without rotating magnetic field(RMF) through epifluorescence.

Results

1) Lentiviral vector production

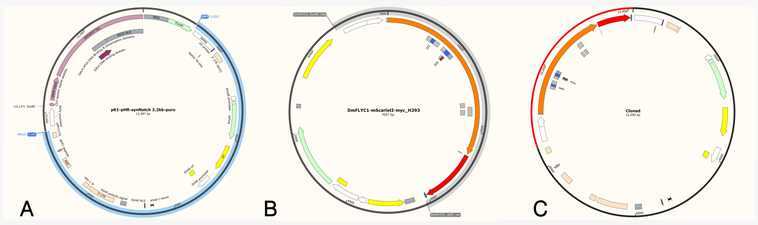


Figure.1 Ligation of insert and backbone for lentiviral vector production.
(A) pHR lentiviral backbone (B) DmFLYC1-mScarletI insert (C) cloned DNA

2) DmFLYC1-H293 expression in HEK293 cells

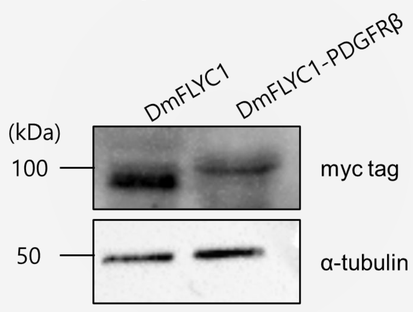


Figure.2 Expression of DmFLYC1-H293 through lipofection into HEK 293 cells.
(A) myc (B) α-tubulin

3) Comparison of DmFLYC1 expression level between lentiviral-delivered and lipofectamin-delivered

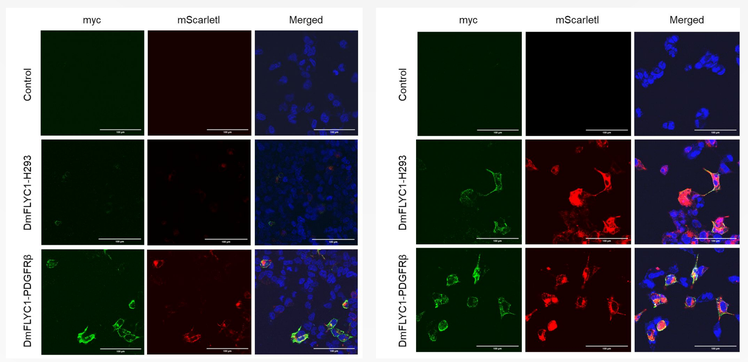


Figure.3 Expression of DmFLYC1-H293, and DmFLYC -PDGFRβ observed with mScarletI. (left) lipofectamin-delivered (right) lentiviral-delivered.
Lentiviral-delivered show more expression.

4) MNP treatment to DmFLYC1 expressing HEK293 cells

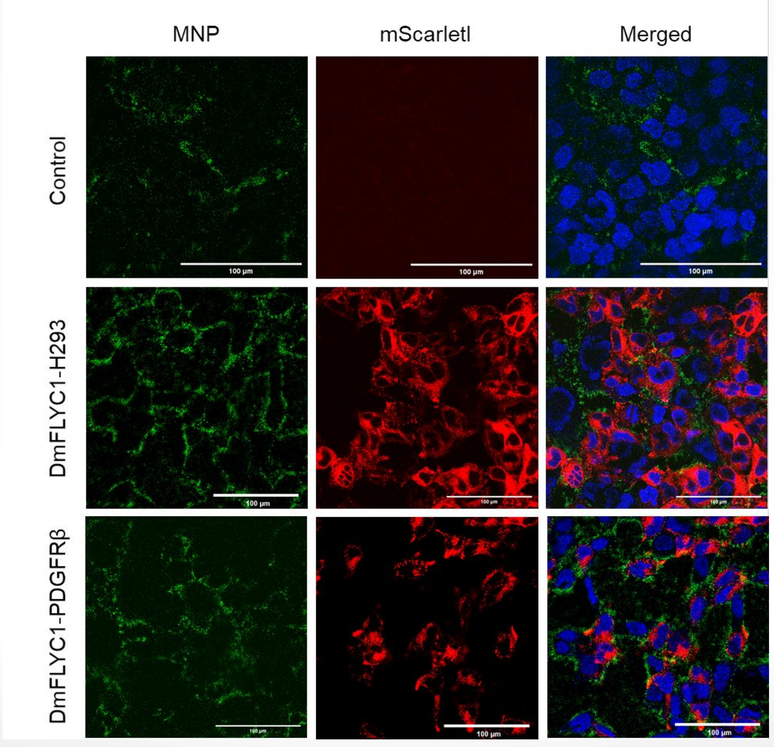


Figure.4 MNP binding to DmFLYC1-H293 and DmFLYC1-PDGFRβ expressing cells.

5) mCIY imaging for cell inactivation

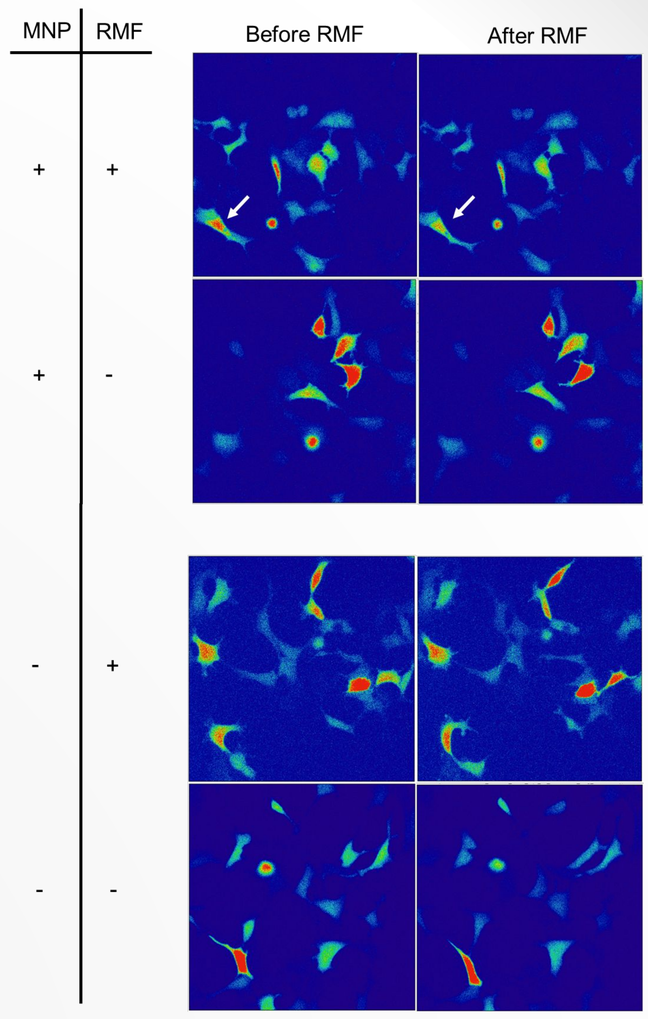


Figure.5 Epifluoresence image of DmFLYC1-H293 with various MNP and RMF conditions. Arrow in MNP+, RMF+ indicates the mCIY bleaching in MNP attached cells after RMF.

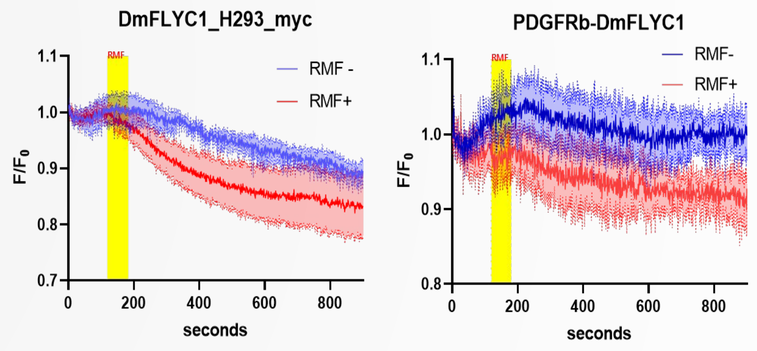


Figure. 6 Cl- concentration change measured with fluorescence change after RMF exposure

Discussion

It is possible that m-torquer bound DmFLYC1 did not have enough time to completely transport chloride ions during RMF applied period. This would cause extracellular chloride concentration to be higher than the intracellular chloride concentration. Since ion channels are heavily dependent on the electrochemical gradient, intracellular chloride ions would fail to move out from HEK293 as the movement of chloride ions is against the gradient. To examine this hypothesis, a further experiment of measuring intracellular and extracellular chloride ion concentration is required along with a longer RMF exposure time.

Since chloride ions have to be pumped out from the cell, they require an active transport mechanism. It is possible that more energy from whether ATP or torque energy from the m-torquer is required for complete efflux of chloride ions from the cell. Designing additional experiments with cells with varying ATP concentration and varying RMF exposure time would be helpful to analyze whether this is the case.

From an electrical and mechanical perspective, due to a distant attachment of m-torquer, DmFLYC1 did not receive enough force to fully open the channel and only a few amounts of chloride ions passed through. According to the law of action and reaction, only a small force repels the influx of chloride ions. Therefore, the recovery time is measured to be longer than expected. To analyze whether this is true, measuring the membrane potential of the cell before and after RMF exposure and calculating the difference is required.

After analyzing incomplete recovery of intracellular chloride ion concentration, experiment with neuron cell instead of HEK293 is suggested. Successful hyperpolarization of neurons through DmFLYC1 will allow precise deactivation of neurons, which would aid in treating diseases such as seizures and convulsions.