# Understanding How Mechanical Stress Affects Calcium Release in Co-Cultured Neuronal and Smooth Muscle Cells

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Jacqueline Novak

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Approved by:

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Prof. Suzanne F. Scarlata, Advisor

# ABSTRACT

The neuromuscular junction serves to connect neurons to muscle fibers allowing for brain signals, that code voluntary and involuntary movement, to be transmitted to muscle fibers that will then contract. This contraction is through calcium. The mechanism for calcium influx in both neuronal and smooth muscle cells is mediated by G-Protein Coupled Receptors (GPCR), that bind the neurotransmitters on the cell membrane and trigger a cascade, eventually releasing intracellular calcium. The goal of this study is to investigate how mechanical stress affects the combined calcium response in neuronal and smooth muscle cells when they are forming a neuromuscular junction model and compare the response of each cell type individually. This is achieved by co-culturing WKO-3M22 smooth muscle cells with PC12 neuronal cells and mechanically stressing them on polydimethylsiloxane polymer dishes. The cell's calcium response was imaged using a fluorescent sensor over time and graphed to view trends. We find that the calcium response increases in the cells forming the neuromuscular junction model when stressed mechanically and stimulated by the neurotransmitter bradykinin. Understanding how the intracellular response of calcium release is affected by formation of the neuromuscular junction, mechanical stress, and neurotransmitter stimulation will further our knowledge of how intracellular calcium response could be affected in an abnormal neuromuscular junction produced by various diseases, as well as the response that might be affected by the action of a drug on GPCRs.

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# BACKGROUND

### The Neuromuscular Junction

The neuromuscular junction (NMJ) serves to connect motor neurons to muscle fibers allowing the transfer of brain signals that code for muscle contraction (Fig. 1). Physiologically, brain signals work through changes in the membranes voltage potential, also known as an action potential. Once an action potential reaches the final motor neuron in the nerve fiber, the action potential propagates down the cell and causes voltage gated calcium channels to open causing influx at the axon terminal. The calcium influx triggers synaptic vesicles containing neurotransmitters to fuse with the axon terminal membrane, causing the exocytosis of neurotransmitters into the synaptic cleft. A significant release of neurotransmitters into the synaptic cleft, ensures the binding of neurotransmitters to receptors on the motor end plate. The neurotransmitter will then influx causing an action potential that will propagate down the muscle cell, leading to the influx of calcium. The influx of calcium will be one of the final steps before muscle cell contraction (Omar et al., 2022).

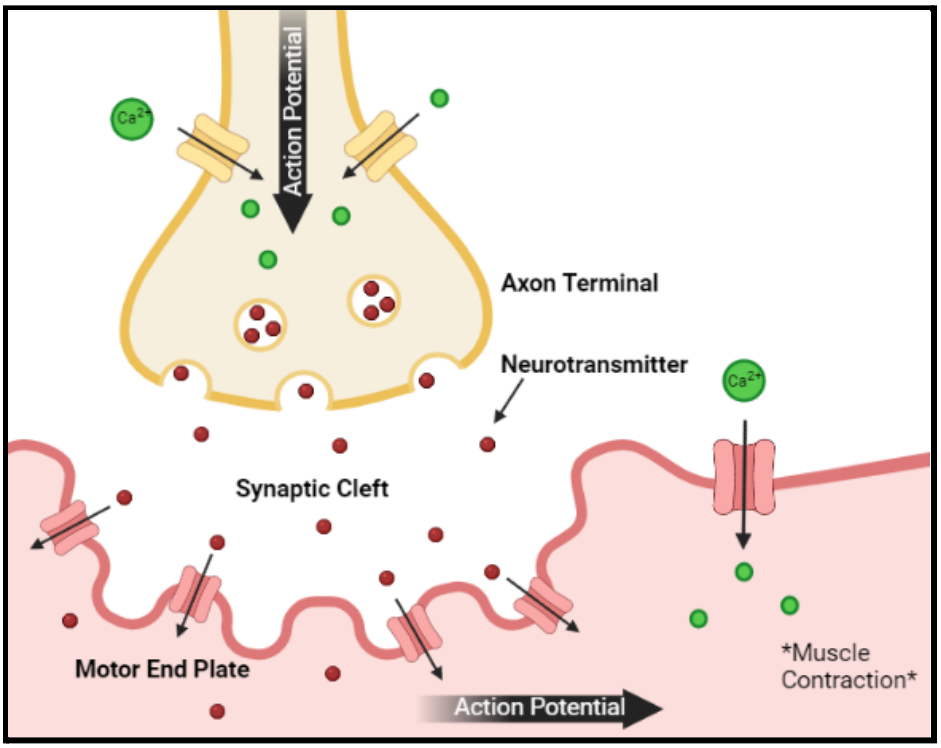


**Figure 1.** *Diagram of the NMJ outlining the simplified process by which a nerve action potential is transferred to a muscle cell, leading to muscle cell contraction.*

The NMJ is an area of research interest because it is responsible for converting intent to move into movement action. When there is an abnormality in the NMJ, the ability to pass signals from neurons to muscle fibers is either reduced or completely ceased (paralysis). There are many diseases and disorders that affect the NMJ that require more research for alternative treatments such as myasthenia gravis, botulism, and Lambert–Eaton myasthenic syndrome (Hirsch, 2007). Calcium response directly affects how much neurotransmitter is released into the synaptic cleft, which will determine if an action potential is delivered to muscle fibers and if contraction will occur. Understanding how intracellular calcium release is affected by stress and stimulation will provide insight to how the calcium response could be altered in abnormal NMJs.

**G-Protein Coupled Receptor Pathway**

Cells are able to communicate with their external environment through proteins that are embedded in the cell membrane. These membrane proteins allow the cell to receive stimuli that initiate a cascade to relay the signal intracellularly (Alberts et al., 2002). Calcium responses in the NMJ are mediated by G-protein coupled receptors. GPCRs are a family of membrane receptors that are linked to multiple pathways, involving G-proteins, that relay signals from the outside of the cell to the inside. The phosphatidylinositol ($PIP_2$) signal pathway results in the mobilization of calcium. This pathway begins when an extracellular ligand, such as a neurotransmitter, binds to the GPCR, which is bound to the $G_q$ protein consisting of three subunits (alpha, beta, and gamma). The alpha subunit exchanges its bound GDP molecule for a GTP molecule, causing the alpha subunit to lose its affinity for the beta and gamma subunit complex. The alpha subunit is then free to move along the membrane where it eventually binds to phospholipase Cβ (PLCβ) and activates it. Upon activation, PLCβ catalyzes the hydrolysis of $PIP_2$ into inositol (1,4,5) trisphosphate ($IP_3$) and diacylglycerol (DAG). $IP_3$ then binds to a ligand gated calcium channel receptor on the membrane of the endoplasmic reticulum. The channel will open and allow calcium to flow down its concentration gradient and increase the cytosolic concentration of calcium (Dhyani et al., 2020).

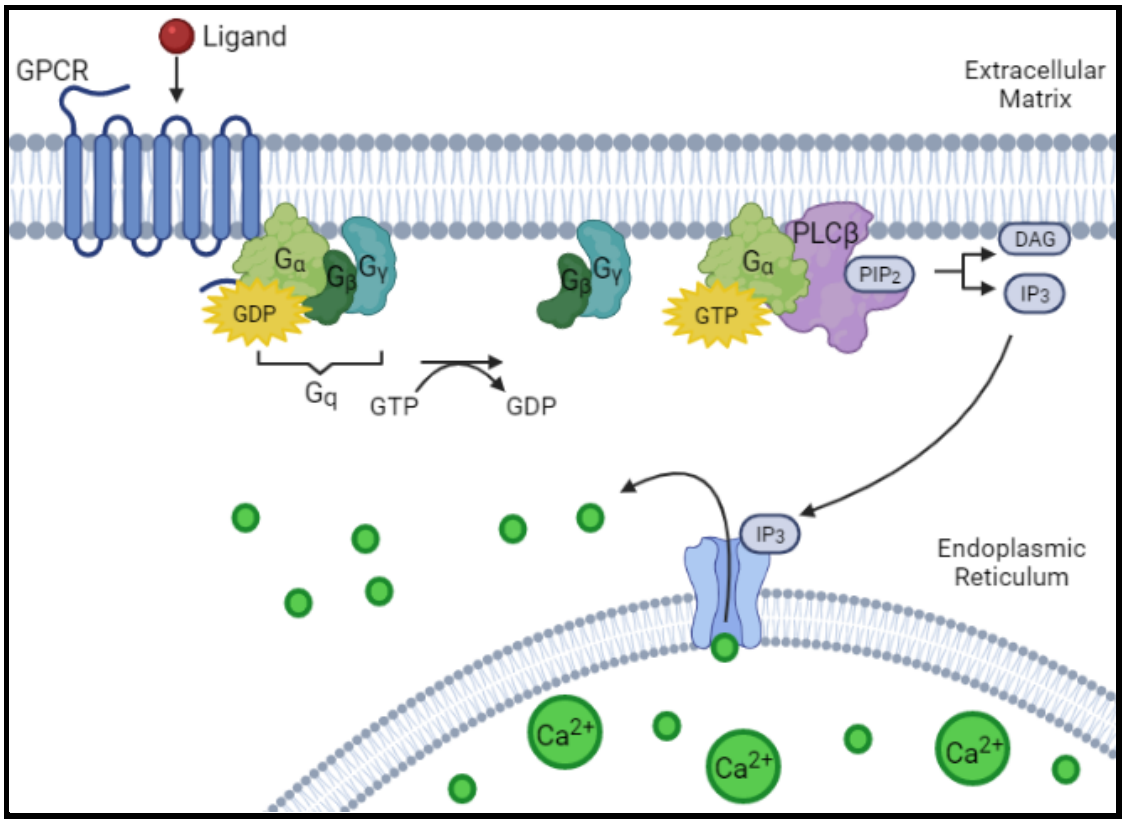


**Figure 2.** *Diagram of GPCR phosphatidylinositol pathway leading to the increase of cytosolic calcium.*

GPCRs and the many pathways that are linked to them are common targets for pharmaceutical drugs. Approximately 40% of all FDA approved drugs aim to target this family of membrane receptors because of their involvement in many biological processes (Alhosaini et al., 2021). It is advantageous to understand how the phosphatidylinositol ($PIP_2$) signal pathway's calcium response reacts to different conditions and how those conditions might alter the effectiveness of a GPCR target drug. In this study, we investigate calcium signals in a model of the neuromuscular junction, which is subjected to mechanical and chemical changes.

**Experimental Rationale**

To thoroughly understand the calcium behavior in co-cultured cells, stimulation of the GPCR phosphatidylinositol signal pathway is necessary. Studies have shown that the GPCR phosphatidylinositol signal pathway can be activated using different stimulating agents such as bradykinin peptide and carbachol neurotransmitter (Appell et al., 1989; Calizo et al., 2012; Predescu et al., 2019; Qifti et al., 2021). Their stimulation is found to increase cytosolic calcium

levels in PC12 neuronal cells (Appell et al., 1989; Pozzan et al., 1986; Qifti et al., 2021). Suggesting that PC12 cells would be a suitable neuronal cell choice to be able to efficiently chemically stimulate our cells in our experimentation. PC12 cells have been previously used to mimic human neurons and model the neuromuscular junction in vitro by co-culturing with C2C12 myoblasts, as well as with WKO-3M22 (Arifuzzaman et al., 2019; Skinner, 2021). The WKO-3M22 cell line has shown to produce significant response to carbachol stimulation with osmotic stress, leading to the choice to use PC12 and WKO-3M22 cells to model the NMJ. Mechanical stress has been explored in co-cultured PC12 and WKO cell lines when stimulated with osmotic stress and has shown to affect the calcium behavior (Skinner, 2021). Thus, we chose to implement mechanical stress in addition to our chemical stimulation. Our goal is to investigate how mechanical stress affects the combined calcium response in neuronal and smooth muscle cells when they are forming a neuromuscular junction model and compare the response of each cell type individually.

# METHODS

### Cell Culture

PC12 neuronal cells, taken from adrenal medulla pheochromocytoma of the Wistar Kyoto rat, were cultured in Dulbecco's modified Eagle medium (DMEM) containing high glucose complete with 10% heat inactivated horse serum, 5% fetal bovine serum, and 1% penicillin-streptomycin. WKO-3M22 smooth muscle cells, taken from the Wistar Kyoto rat, were cultured in DMEM containing high glucose complete with 10% fetal bovine serum, 1% L-glutamine, 1% penicillin-streptomycin, and 1% sodium pyruvate. Both cell lines were incubated at 37 degrees Celsius with 5% $CO_2$. Each cell line was washed with phosphate buffered saline (PBS) before being split into glass bottom dishes. To help the WKO cell line detach from the dish, 2 mL of Trypsin was added.

### PDMS Plate Preparation

Polydimethylsiloxane polymer (PDMS) plates (Fig. 3) were made from a special mold that would allow the plates to fit in a stretch device from Professor Kristen Billiar's lab. The PDMS was made from the Sylgard 184 Silicone Elastomer kit . The plates were sterilized by soaking in 70% ethanol under an ultraviolet (UV) light for 15 minutes. The plates were then coated in 20 µL of sulfosuccinimidyl 6-(4'-azido-2'-nitrophenylamino) hexanoate (Sulfo-SANPAH) diluted in 2 mL of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES buffer) under a UV light for 15 minutes. Next, the plates were washed with PBS 3 consecutive times (5 minutes, 3 minutes, and 2 minutes). Then, the plates were coated in 2 mL of collagen type I diluted in 0.02 M acetic acid for 24 hours in the fridge. The next day the plates were coated in 2 mL of an antibiotic solution containing 100 µg/mL Pen-Strep, 2.5 µg/mL Amphotericin B, 10 µg/mL Ciprofloxacin, and 100 µg/mL Gentamycin for 24 hours in the fridge (Skinner, 2021).

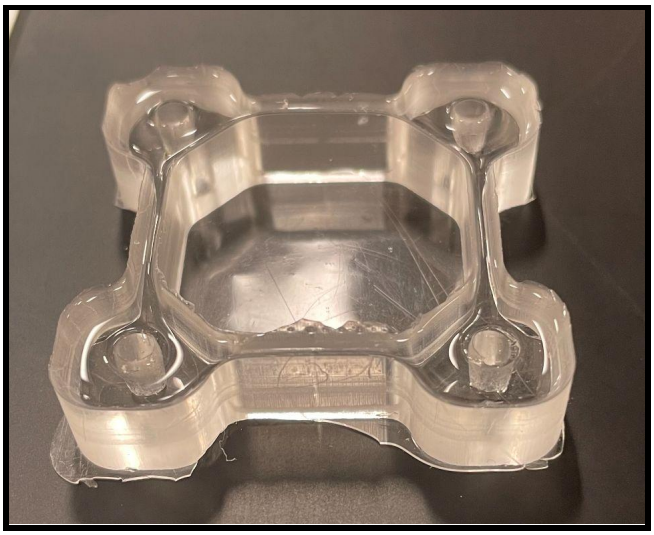

**Figure 3.** *PDMS dish before sterilization.*

### PDMS Cell Plating

The cell lines were seeded on the PDMS dishes 72 hours before experimentation. Plates were washed with 2 mL of PBS and then received 2 mL of DMEM containing high glucose complete with 10% heat inactivated horse serum, 5% fetal bovine serum, and 1% penicillin-streptomycin. The PC12 cells were seeded at a concentration of $5.00*10^4$ cells/mL and the WKO cells were seeded at a concentration of $5.25*10^3$ cells/mL. After seeding, the plates were placed in the incubator (Skinner, 2021).

### Cell Differentiation

Once the cells had adhered to the PDMS plates their media was replaced with differentiation media made of DMEM complete with 1% penicillin-streptomycin, 2% horse serum, and 2% fetal bovine serum. Neuronal growth factor was added to each plate at a concentration of 1.5 μL/mL. Cells were allowed to differentiate in the incubator for 48 hours.

### Calcium Labeling

After differentiating for 48 hours, the cells were washed with 1 mL of Hank's Balanced Salt Solution (HBSS). Then, 8 μL of fluorescent Calcium Green (Thermo Fisher, Invitrogen, Catalog No.C3012) indicator diluted in 1mL of HBSS was added to each plate and left in the incubator for 1 hour covered in tinfoil to protect the Calcium Green from the light. Before imaging, the

Calcium Green solution was removed and the cells were washed with 1 mL HBSS and then while stretching and imaging, 2 mL of HBSS was placed in each plate.

**Cell Stretching**

The experiments that received cell stretching were stretched and then immediately used to image controls, resulting in a 20-30 minute recovery between stretch and stimulation. Professor Billiar's stretch device was used to stretch the PDMS dish the cells were adhered to. The stretch that was implemented to the cells was bi-direction at either 5% or 10% stretch for 5 minutes, for one oscillation per second.

**Cell Imaging and Stimulation**

The PDMS plate was placed on the stage of a Zeiss LSM 510 confocal microscope. A 488 nm laser was used while the cells were imaged with a 10X objective lens. The cells were imaged by video of 1 frame per 3.39 seconds with a total of 200 frames per video, resulting in a 13.3 minutes capture of the cells' calcium response. On frame 10, 5 μM of stimulation was added. Both a brightfield channel and a fluorescent Calcium Green channel were recorded.

**Analysis**

The brightfield and fluorescent Calcium Green videos were analyzed in Fiji image processing software (Fig. 4). Each individual cell was selected using the brightfield image and the fluorescent Calcium Green intensities were recorded for each cell over the time series. The intensities of the fluorescent Calcium Green channel for each cell was pasted into Microsoft Excel and normalized to the first frames intensity. The average of the normalized intensities were calculated for each condition as well as the population standard deviation and error. The average normalized intensities were plotted against time in SigmaPlot 14.5. Each condition was statistically analyzed using the Mann-Whitney Rank Sum Test.

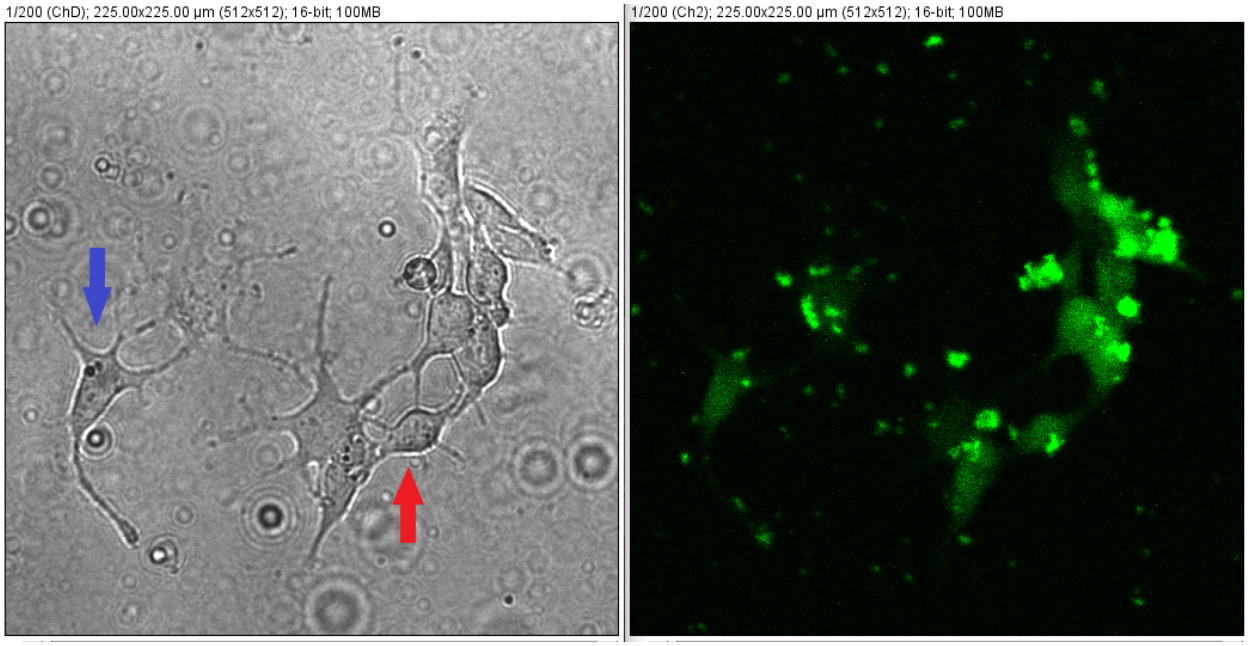


**Figure 4.** *Brightfield image (left) and fluorescent Calcium Green image (right) of differentiated co-cultured PC12 (red arrow) and WKO cells (blue arrow) on Fiji software.*

# RESULTS

To understand the effects of how the calcium response of co-cultured neuronal PC12 cells and smooth muscle WKO cells is affected by stimulation, mechanical stretch, and forming a model of the neuromuscular junction, we cultured each cell line individually and then co-cultured them before experimentation. The cells were co-cultured on PDMS plates coated with collagen and antibiotics and then differentiated 48 hours before experimentation. A fluorescent Calcium Green indicator (Thermo Fisher) was added to the cells to visualize the calcium. The calcium intensities were then graphed for each experimental condition and compared.

### Calcium Behavior With and Without Stimulation

Co-cultured PC12 and WKO cells were imaged with no stimulation for a control. This control will serve as a reference for many conditions to compare the variable that is being changed (i.e. stimulation or stretch). The control was compared to the co-culture that was subjected to carbachol stimulation and co-culture subjected to bradykinin stimulation (Fig. 5). The ratio of WKO to PC12 cells is 8:10.

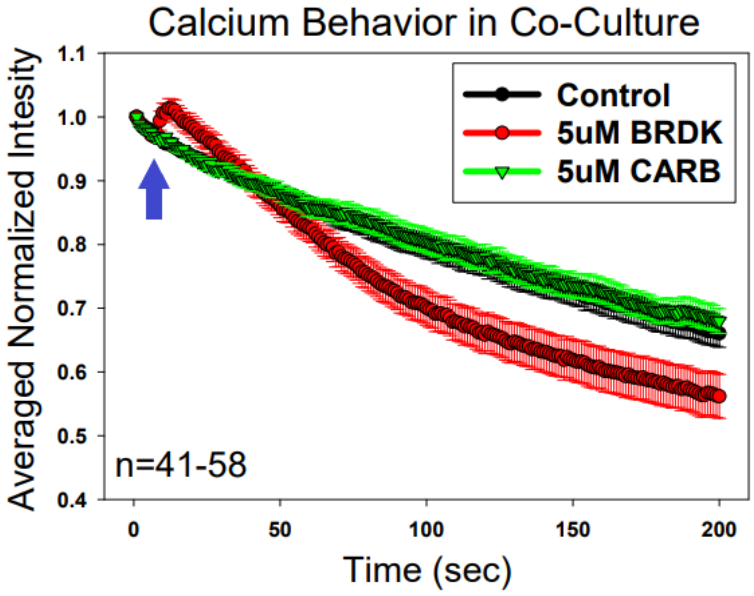


**Figure 5.** *Co-cultured PC12 and WKO cells with 5uM stimulation of bradykinin (BRDK) and 5uM stimulation of carbachol (CARB) compared to no stimulation. Addition of stimulation is indicated by the blue arrow. The n number indicates the number of individual cells that were analyzed in the different conditions. Note that the control data almost completely overlaps the carbachol data.*

The calcium response, after carbachol is added, is not altered. The difference in the median values between the control group and the carbachol stimulated group is not great enough to exclude the possibility that the difference is due to random sampling variability (P = 0.331). The calcium response after bradykinin is added peaks transiently and has an overall faster recovery of the intracellular calcium. There is a statistically significant difference between the control group and the bradykinin stimulated group (P = <0.001). Due to the fact that bradykinin stimulated the cells better than carbachol, bradykinin was used for the following experiments when stimulation was part of the experimental condition.

The calcium response of PC12 cells cultured individually with and without stimulation was compared to the PC12 cells' calcium response when they are forming the co-culture model of the NMJ with and without stimulation.

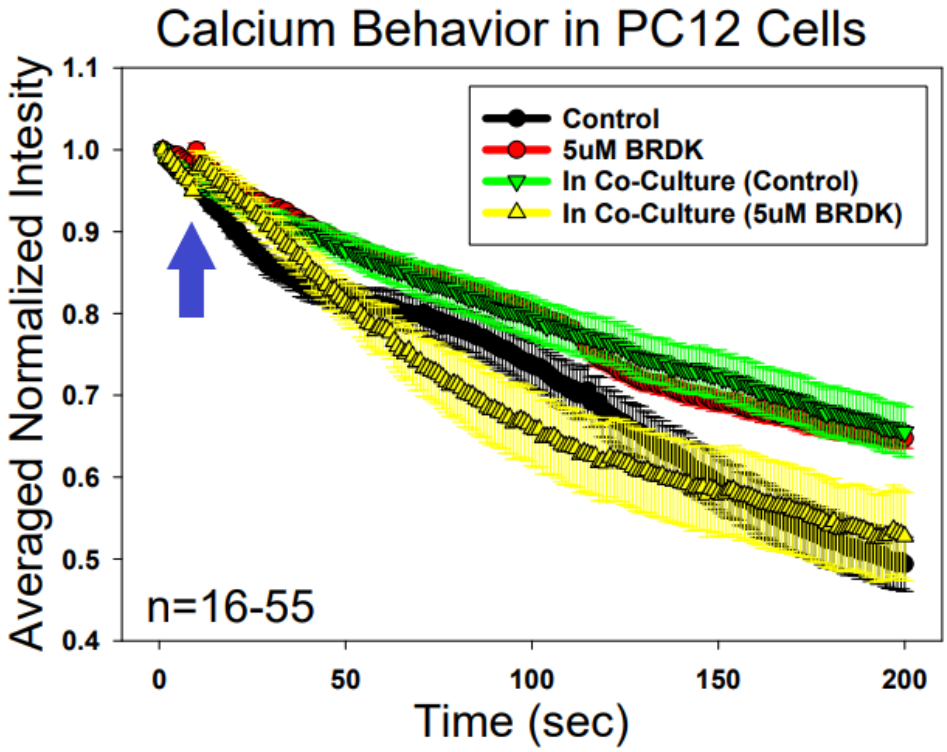


**Figure 6.** *Individually cultured PC12 with and without 5uM bradykinin stimulation compared to co-cultured PC12 cells with and without 5uM bradykinin stimulation. Addition of stimulation is indicated by the blue arrow. The n number indicates the number of individual cells that were analyzed in the different conditions.*

The calcium response in PC12 cells that were cultured individually with stimulation compared to without stimulation showed the calcium peaking transenty after stimulation was added and having a slower calcium recovery (P = <0.001). The calcium response in the PC12 cells that were co-cultured with stimulation compared to without stimulation showed a transient peak after stimulation was added and a faster recovery (P = <0.001).

The calcium response of WKO cells cultured individually with and without stimulation was compared to the WKO cells' calcium response when they are forming the co-culture model of the NMJ with and without stimulation.

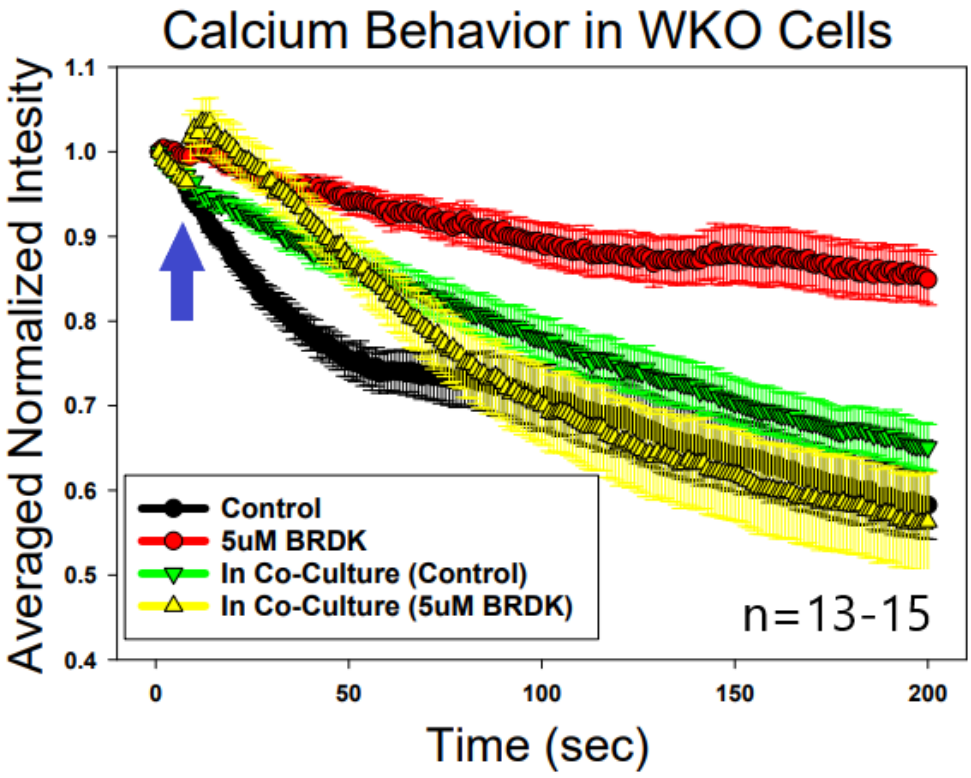


**Figure 7.** *Individually cultured WKO cells with and without 5uM bradykinin stimulation compared to co-cultured WKO cells with and without 5uM bradykinin stimulation. Addition of stimulation is indicated by the blue arrow. The n number indicates the number of individual cells that were analyzed in the different conditions.*

The same calcium responses were seen in the WKO cells as the PC12 cells. The calcium response in WKO cells that were cultured individually with stimulation compared to without

stimulation, also showed the calcium peaking transenty after stimulation was added and having a slower calcium recovery (P = <0.001). The calcium response in the WKO cells that were co-cultured with stimulation compared to without stimulation, also showed a transient peak after stimulation was added and a faster recovery (P = <0.001).

**Calcium Behavior In Co-Culture When Stretched**

The co-culture was mechanically stretched at two different stretch percentages for 5 minutes and their calcium responses were compared to the co-culture control with no stretch.

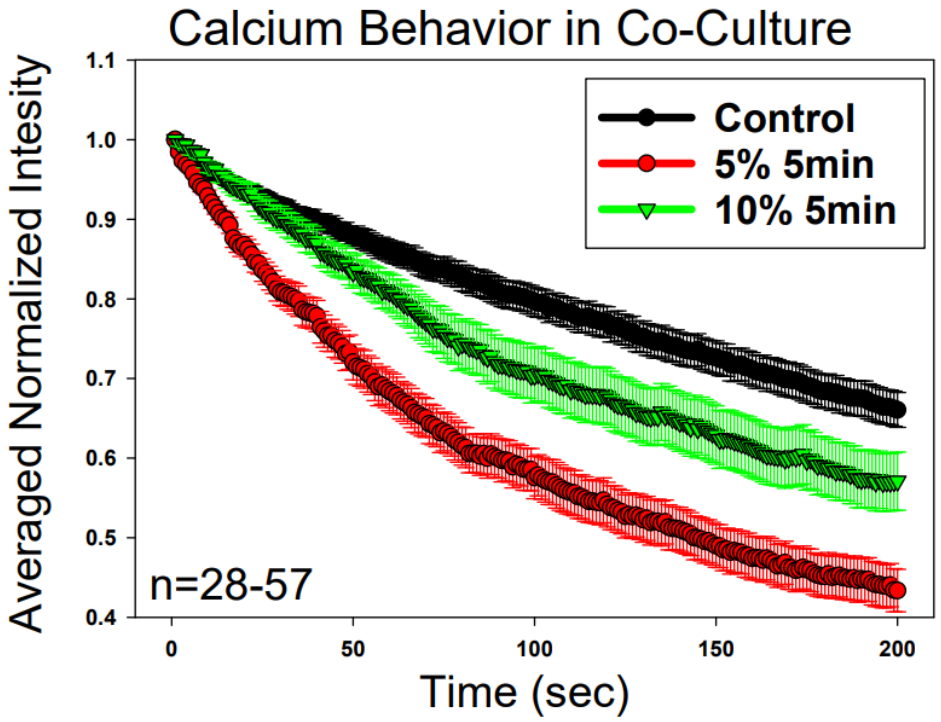


**Figure 8.** *Co-cultured WKO and PC12 cells with 5% stretch for 5 minutes and 10% stretch for 5 minutes compared to the co-cultured cells with no stretch. The n number indicates the number of individual cells that were analyzed in the different conditions.*

The calcium response of the co-cultured cells after receiving 5% stretch for 5 minutes showed a faster recovery than the control (P = <0.001). Similarly, the calcium response after 10% stretch for 5 minutes showed a faster recovery than the control (P = <0.001). Interestingly, the 5% stretch for 5 minutes showed a faster calcium recovery than the 10% stretch for 5 minutes (P = <0.001).

The same stretch percentages for 5 minutes were performed but with the addition of bradykinin stimulation. These conditions were compared to the control of co-culture cells with bradykinin stimulation.

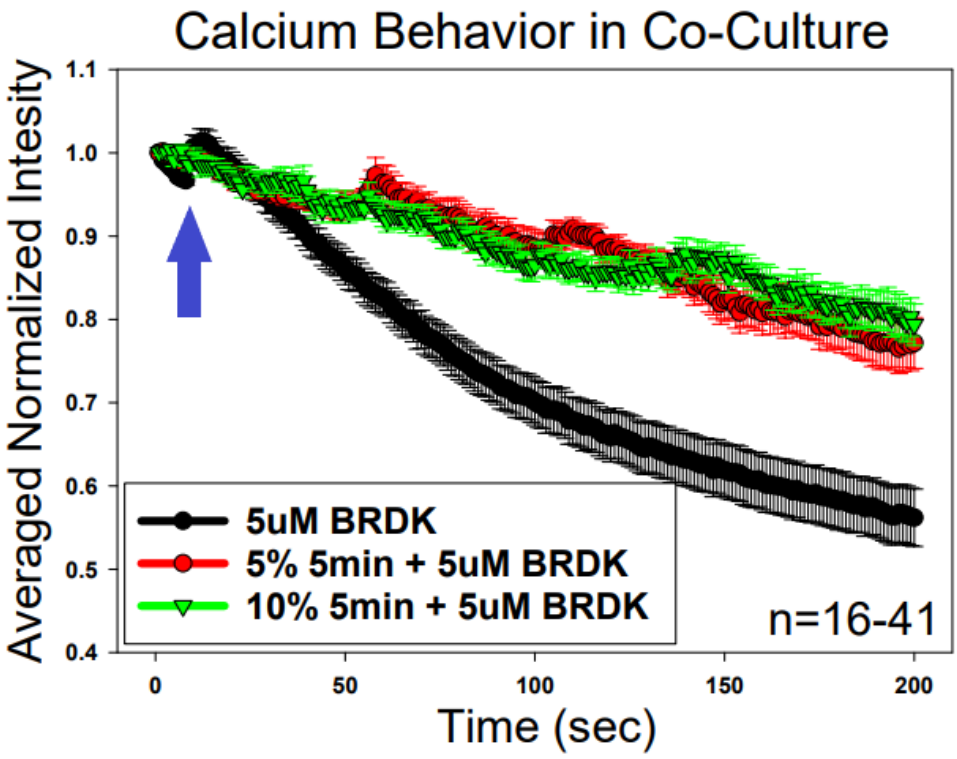


**Figure 9.** *Co-cultured WKO and PC12 cells with 5% stretch for 5 minutes, with bradykinin stimulation, and 10% stretch for 5 minutes, with bradykinin stimulation, compared to the co-cultured cells with no stretch and bradykinin stimulation. The addition of stimulation is indicated by the blue arrow. The n number indicates the number of individual cells that were analyzed in the different conditions.*

The calcium response that is observed after 5% stretch for 5 minutes with stimulation and 10% stretch for 5 minutes with stimulation showed there is a significant increase in calcium release compared to co-cultured cells with stimulation and no stretch ($P = <0.001$).

The calcium release in co-culture of cells with 5% stretch for 5 minutes with stimulation, where the stimulation occurs 20-30 minutes after stretch is compared to co-cultured cells that have 5% stretch for 5 minutes with stimulation but the stimulation occurs directly after stretch (no recovery).

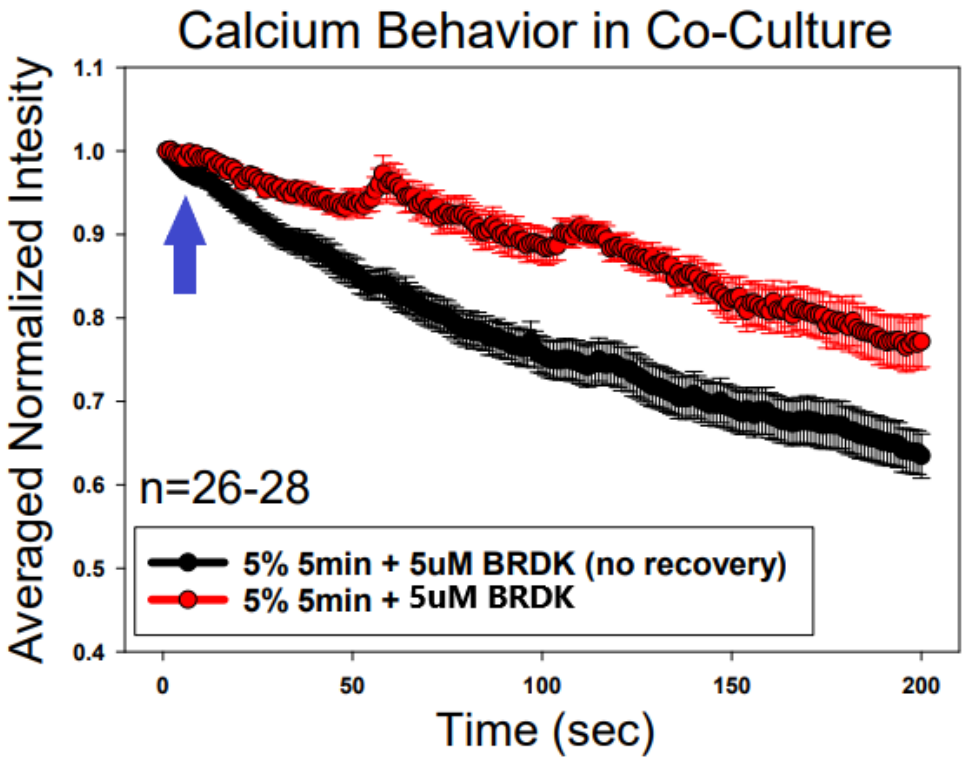


**Figure 10.** *Co-cultured WKO and PC12 cells with 5% stretch for 5 minutes, with bradykinin stimulation after 20-30 minute stretch recovery, compared to 5% stretch for 5 minutes, with bradykinin stimulation after no stretch recovery. The addition of stimulation is indicated by the blue arrow. The n number indicates the number of individual cells that were analyzed in the different conditions.*

The calcium response of the cells that were stretched then stimulated with no recovery compared to the calcium response of cells that were stretched and stimulated with a 20-30 minute stretch recovery showed a faster calcium recovery ($P = <0.001$).

# DISCUSSION

In this study, our goal was to compare calcium responses of different conditions relating to stimulating and stretching our model of the NMJ. First, we wanted to see the effects of stimulation on co-cultured cells' calcium response. Carbachol was used as a stimulant but upon analysis it was seen that the carbachol did not alter the calcium response relative to the control. Then bradykinin was used as a stimulant because it has previously been shown to activate the GPCR phosphatidylinositol signal pathway in another rat smooth muscle cell line (A10) (Calizo et al., 2012). The bradykinin stimulation was compared to the carbachol stimulation and we found that the stimulation had a greater effect on the calcium response. This behavior is most likely due to there being more $B_2R$ bradykinin receptors present in the WKO cells than carbachol receptors (Calizo et al., 2012). We find that with bradykinin stimulation there is also faster calcium recovery. This phenomenon is also found in both the PC12 and WKO cells when they are co-cultured but analyzed separately, suggesting that the PC12 and WKO cells provide similar calcium responses that equally contribute to the overall calcium response when stimulated in co-culture. The cell lines when individually cultured were shown to be stimulated by bradykinin but the fast recovery seen in co-cultures is not seen here. Individually, both cell lines have a slower calcium recovery than their individually cultured controls. This comparison suggests that the faster recovery after stimulation of the co-cultured cells is caused by being *in* the co-culture and forming the model NMJ.

Stretch conditions were applied to the co-cultured cells to compare how the stretch affects their calcium response compared to the co-cultured cells that have not been stretched. First, 5% and 10% stretch were compared to no stretch. The stretch caused calcium to recover faster than the control. There was a notable difference between the recovery of the 5% stretch and the 10% stretch. The 5% stretch caused a faster recovery than the 10% stretch. We are unsure why the smaller stretch percentage caused a faster calcium recovery than the larger stretch percentage considering the deviation from the control is greater in the smaller stretch percentage. When the stretch conditions are repeated with bradykinin stimulation, it is seen that the calcium has a significant increase compared to the stimulated control. This increase is caused by the cells being stimulated after stretching because we do not see this calcium increase when the cells are

stretched but not stimulated. When the 5% stretch for 5 minutes with stimulation condition is compared to the 5% stretch for 5 minutes with stimulation but there is no recovery between the stretch and the stimulation, the calcium response has a faster recovery. This shows that the calcium is recovering faster closer to when the cells are stretched .

# CONCLUSION

Our results show that there are significant effects on calcium behavior produced by bradykinin stimulation, co-culturing PC12 and WKO cells, varying percentages of bi-directional stretch, and altering the time that cells recover after stretch and before stimulation. Future works related to this study should focus on repeating conditions with a shorter recovery time between stretch and stimulation, allowing us to see if the stretch and simulation time gap affects the calcium trends noticed in this study. In addition, stretch should be applied to PC12 and WKO cells when they are individually cultured to see how each cell type responds to stretch and compare that to each cell type stretched in co-culture.

# REFERENCES


Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). Membrane Proteins. *Molecular Biology of the Cell. 4th Edition*. https://www.ncbi.nlm.nih.gov/books/NBK26878/

Alhosaini, K., Azhar, A., Alonazi, A., & Al-Zoghaibi, F. (2021). GPCRs: The most promiscuous druggable receptor of the mankind. *Saudi pharmaceutical journal : SPJ : the official publication of the Saudi Pharmaceutical Society*, *29*(6), 539–551. https://doi.org/10.1016/j.jsps.2021.04.015

Appell, K. C., & Barefoot, D. S. (1989). Neurotransmitter release from bradykinin-stimulated PC12 cells. Stimulation of cytosolic calcium and neurotransmitter release. The Biochemical Journal, 263(1), 11–18. https://doi.org/10.1042/bj2630011

Arifuzzaman, M., Ito, A., Ikeda, K., Kawabe, Y., & Kamihira, M. (2019). Fabricating Muscle-Neuron Constructs with Improved Contractile Force Generation. *Tissue Engineering. Part A*, *25*(7–8), 563–574. https://doi.org/10.1089/ten.TEA.2018.0165

Calizo, R. C., & Scarlata, S. (2012). A Role for G-Proteins in Directing G-Protein-Coupled Receptor–Caveolae Localization. Biochemistry, 51(47), 9513–9523. https://doi.org/10.1021/bi301107p

Dhyani, V., Gare, S., Gupta, R. K., Swain, S., Venkatesh, K. V., & Giri, L. (2020). GPCR mediated control of calcium dynamics: A systems perspective. *Cellular signalling*, *74*, 109717. https://doi.org/10.1016/j.cellsig.2020.109717

Hirsch, N. P. (2007). Neuromuscular junction in health and disease. *Br J Anaesth*, 99 (2007), pp. 132-138. https://doi.org/10.1093/bja/aem144

Omar, A., Marwaha, K., & Bollu, P. C. (2022). Physiology, Neuromuscular Junction. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK470413/

Pozzan, T., Di Virgilio, F., Vicentini, L. M., & Meldolesi, J. (1986). Activation of muscarinic receptors in PC12 cells. Stimulation of Ca2+ influx and redistribution. The Biochemical journal, 234(3), 547–553. https://doi.org/10.1042/bj2340547

Predescu, D. V., Crețoiu, S. M., Crețoiu, D., Pavelescu, L. A., Suciu, N., Radu, B. M., & Voinea, S. C. (2019). G Protein-Coupled Receptors (GPCRs)-Mediated Calcium Signaling in Ovarian Cancer: Focus on GPCRs activated by Neurotransmitters and Inflammation-Associated Molecules. *International journal of molecular sciences*, *20*(22), 5568. https://doi.org/10.3390/ijms20225568

Qifti, A., Jackson, L., Singla, A., Garwain, O., & Scarlata, S. (2021). Stimulation of phospholipase Cβ1 by Gαq promotes the assembly of stress granule proteins. *Science Signaling*, *14*(705), eaav1012. https://doi.org/10.1126/scisignal.aav1012

Skinner, C. (2021). *The Effects of Stretch on Intracellular Ca2+ Signaling in Cocultured Neuronal and Smooth Muscle Cells.* : Worcester Polytechnic Institute.