ASSESSING CHANGES IN THE MECHANOSENSITIVITY OF PRIMARY DISSOCIATED DORSAL ROOT GANGLION SENSORY NEURONS WITH THE CHEMOTHERAPEUTIC DRUG PACLITAXEL.

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INTRODUCTION

Paclitaxel is a widely administered chemotherapy for the treatment of breast cancer. The most reported side effect of paclitaxel treatment is the development of neuropathy, characterized by numbness and pain in the extremities [3]. PTX-induced neuropathy cause a sequela of sensory symptoms that can be associated with different classes of cutaneous sensory neurons. Large diameter neuron dysfunction leads to a loss of vibration sense, while symptom like dysesthesia, cold and mechanical allodynia can be attributed to dysfunction in small and medium diameter neurons [1,2]. A hallmark sign of PTX neuropathy is the development of mechanical allodynia and dysfunction of cutaneous mechanoreceptors [4]. Raising the question as to whether the pathological mechanism is associated with a reduction in the expression of mechanosensitive ion channels, or a change in the mechanical force required to generate membrane depolarization. In this study, we aimed to elucidate a potential mechanism by conducting a gene plex of mechanosensitive ion channels in dissociated neuron cultures and assess the mechanical sensitivity of dissociated neurons after PTX.

MATERIALS and METHODS and AIMS

Dissociated neurons were treated with 10nM paclitaxel or vehicle DMSO. To assess if paclitaxel changed the expression of mechanosensitive ion channels, Nanostring N-counter was used to quantify the expression of mechanosensitive ion channels. To investigate if paclitaxel changes the mechanosensitivity of neurons, neurons were cultured on micropillar arrays and treated with paclitaxel, then loaded with a fluorescent indicator, and an individual pilus from beneath the neuron was deflected to apply mechanical stimulation via the cell-substrate interface. Activation was measured by changes in fluorescence.

Investigate if 10nM PTX treatment changes expression of mechanosensitive ion channels in dissociated DRG neurons

Assess if PTX treatment alters the mechanosensitivity of dissociated DRG neurons to deflections of micro pili

Identify if PTX induced changes in mechanosensitivity is localized to subset of dissociated DRG neurons.

CONCLUSION

A classic feature in PTX neuropathy is the development of lingering numbness and mechanical allodynia. To investigate the mechanosensory changes documented in PTX neuropathy, we looked to determine if there were expression changes in sensory neurons or whether PTX induces functional changes in the mechanosensitivity of dissociated primary sensory neurons. There were no significant changes in the expression of any of the ion channels of interest with PTX treatment. This is not conclusive to rule out channel expression as a potential mechanism, as there was large variability between replicates and channels. While this demonstrates the neuron is producing mechanosensitive ion channels, further work needs to be conducted to ensure they are being expressed at the cell membrane. PTX treatment decreased the mechanosensitivity and reduced the variability in response of medium diameter neurons to stimuli. A decrease in the sensitivity is not consistent with the development of mechanical allodynia but may be a potential mechanism for the development of numbness. PTX may act on the sensory neurons in a manner that decreases sensitivity to mechanical stimuli upstream of the expression of mechanically sensitive ion channels. It may be likely that PTX effects the accessory tissues around cutaneous mechanoreceptors *in vivo,* altering the transduction of stimuli to the cutaneous mechanoreceptor, which may lead to increased sensitivity and the development of allodynia..

REFERENCES

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RESULTS

Table 3. Nanostring expression counts from dissociated neurons whole cell lysis

	DMSO VEHICLE *mean ± sem*	10nM PTX *mean ± sem*	Significance
ASIC 2	958.6 ± 136.6	799.2 ± 94.81	*ns*
ASIC 3	821.4 ± 62.72	659.5 ± 95.96	*ns*
Peizo 2	1918 ± 592.2	1342 ± 178.9	*ns*
Stoml 3	81.46 ± 16.21	69.3 ± 27.1	*ns*
TMEM 120A	1054 ± 80.48	1132 ± 94.11	*ns*
TMEM 150C	266.2 ± 49.37	331.8 ± 56.64	*ns*
TRPV4	2119 ± 201.2	1864 ± 166.0	*ns*
TRPV1	349.1 ± 79.12	206.6 ± 31.66	*ns*

PTX and DMSO neurons had changes in fluorescence with 390nm and 492nm pilus deflections.

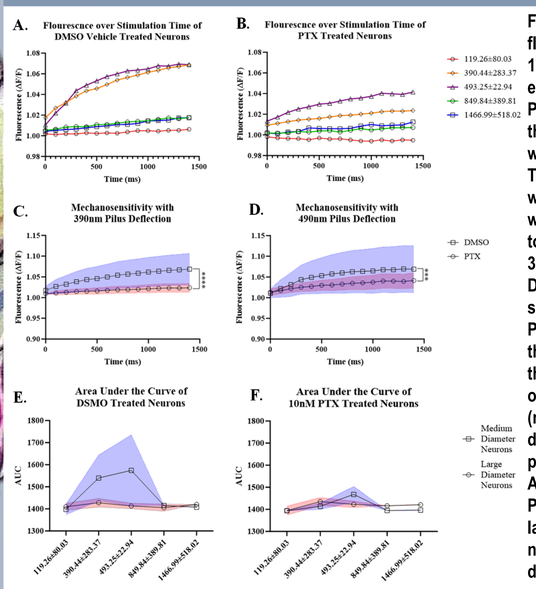


Figure 2. A. DMSO fluorescence over the 1500ms stimulation with each pilus deflection. B. PTX fluorescence over the 1500ms stimulation with each deflection. C. The DMSO fluorescence with 390nm deflection was statistically different to PTX fluorescence with 390nm deflection. D. DMSO fluorescence was significantly different to PTX fluorescence with the 493nm deflection. E. the AUC of fluorescence of DMSO treated medium (n=5) and large (n=8) diameter neurons with pilus deflection. F. The AUC of fluorescent of PTX medium (n=9) and large (n=10) diameter neurons with pilus deflection.

10nM PTX treatment alters the mechanosensitivity of medium diameter sensory neurons on micropillar arrays

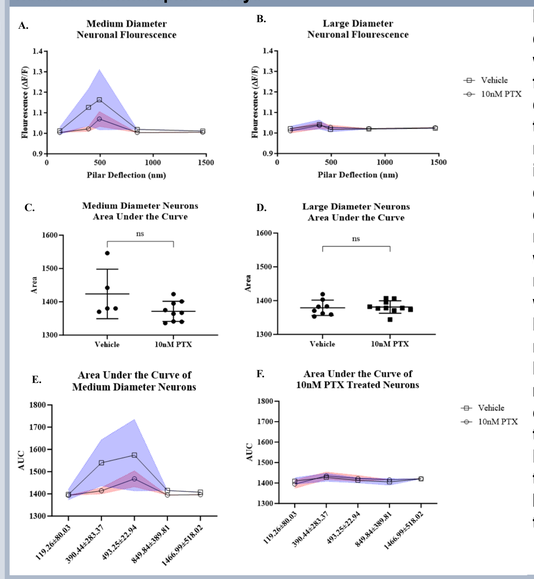
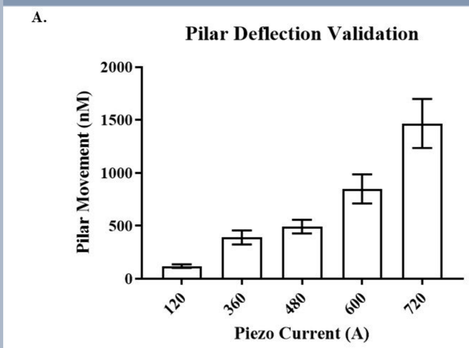


Figure 3. A. Medium diameter neuron treated with 10nM PTX had less fluorescence with pilus deflection (ns). B.PTX treated large diameter neuron had no difference in fluorescence with pilus deflection. C and D. The difference in the AUC of neuronal fluorescence with pilus deflection was not significantly different with 10nM PTX treatment. E. Medium diameter neurons treated with 10nM PTX had reduced mechanosensitivity, compared to DMSO treated medium neurons. F. There was no change in the mechanosensitivity of large diameter neurons treated with 10nM PTX.

The Piezo electric stimulation was converted to nm pilus deflection.



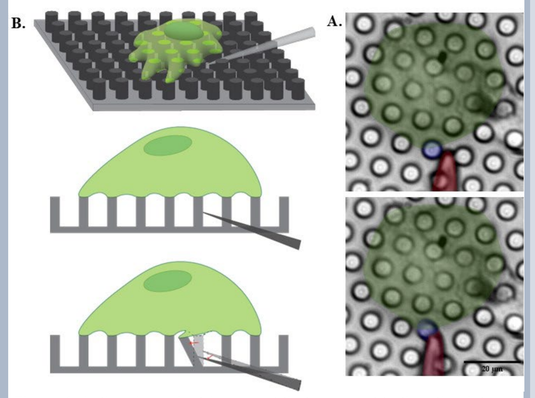


Figure 1. A. the piezo electric current that was sent to the stimulator as the nm deflection of the pilus of interest. With increased current there was increased nm deflection of the pilus. B. A pictographic representation of the deflection of the pilus by the glass pipette during stimulation. C. A dissociated neuron on micropilli (green), the pilus of interest (blue), with the glass pipette (Red) before stimulation and during the stimulation.

Medium diameter have sensory neurons are mechanosensitive to 390nm and 493nm pilus deflections

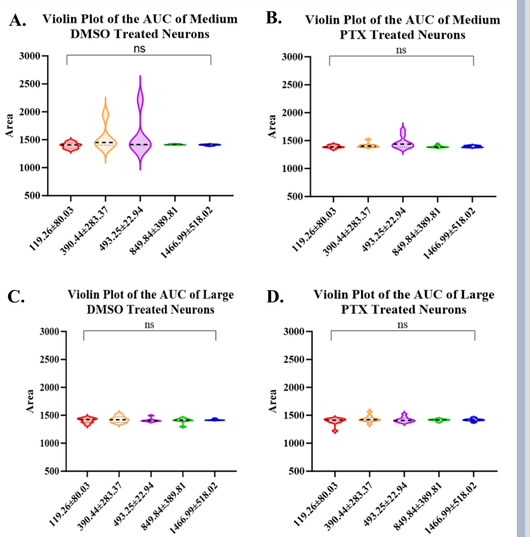


Figure 4. A, B, C and D. Violin plots of the AUC of neuronal fluorescence of medium and large diameter sensory neurons with increasing pilus deflections, treated with either 10nM PTX or DMSO vehicle.